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EVOLUTION ALONG WINEMAKING AND COMPARISION BETWEEN RIOJA AND BORDEAUX WINES.

NOELIA PRIETO PEREA
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# Anthocyanin derivatives and tannins of red wine by HPLC-DAD-MS/MS. Evolution along winemaking and comparision between Rioja and Bordeaux wines

Memoria presentada para optar al grado de
Doctor Internacional en Ciencias Químicas

**Noelia Prieto Perea**

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A mi padre y mi hermano, Antonio y Javier,
a Iñaki y sobre todo a ti,
que me cuidas desde donde estés.

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*Se tú, e intenta ser feliz,*
*pero sobre todo, se tú..*

**Charles Chaplin**

# Abbreviations, acronyms and symbols

| | |
|---|---|
| A | Anthocyanin |
| a* | Chromaticity red/green |
| AA | Aminoacids |
| Ac | Acetic Acid, acethyl |
| APcI | Atmospheric pressure chemical ionization |
| API | Atmospheric pressure ionization |
| ar | aromatic |
| arm | harmonics |
| as | asymmetry |
| AT | Acidez total (Total Acidity) |
| caff | Caffeic Acid, caffeoyl |
| b* | Chromaticity yellow/blue |
| Cat or C | (+)-Catechin |
| C* | Colour saturation |
| ºC | Celsius degrees |
| CC | Column chromatography |
| CCC | Countercurrent chromatography |
| $CH_3CN$ | Acetonitrile |
| CID | Collision induced dissociation |
| CIE | Commission Internationale de l'Eclariage |

| | |
|---|---|
| CDA | Computerized data analysis |
| CE | Collision Energy |
| comb | combination |
| CP | Phenolic compounds |
| CVE | Cross validation error |
| CV | Cone voltage |
| Cy | Cyanidin |
| Cy-3-glc | Cyanidin-3-glucoside |
| CZA | Crianza |
| DAD | Diode array detector |
| DC | Direct current |
| DOCa | Denominación de Origen Calificada |
| Dp | Delphinidin |
| Dp-3-glc | Delphinidin-3-glucoside |
| Dp-3-(6-Ac)-glc | Delphinidin-3-(6-acetyl)-glucoside |
| Epicat or EC | (-)-Epicatechin |
| Epigallocat or EGC | Epigallocat |
| (epi)cat | (+)-Catechin or (-)-Epicatechin, unknown isomer |
| (epi)gallocat | (+)-Gallocatechin or (-)-Epigallocatechin, unknown isomer |
| ESI | Electrospray ionization |
| ESI(-), ESI- | Electrospray ionization, negative mode |
| ESI(+), ESI+ | Electrospray ionization, positive mode |
| ESI-CID-MS/MS | Electrospray ionization-collision induced dissociation-tandem mass spectrometry |
| ESI-MS | Electrospray ionization-mass spectrometry |

| | |
|---|---|
| et | ethyl |
| EtOH | Ethyl alcohol |
| eV | Electronvoltio |
| F | Flavanol |
| FT-IR | Fourier transformed infrared |
| fur | Furfuryl |
| gal | Galactose, galactosyl, galactoside |
| Gallocat or G | (+)-Gallocatechin |
| GC | Gas chromatography |
| glc | Glucose, glucosyl, glucoside |
| H* | Hue |
| $H_2O$ | water |
| HAc | Acetic acid |
| HCl | Hydrochloric acid |
| HPLC | High performance liquid chromatography |
| IE | Electronic impact |
| IT | Ionic trap |
| IR | Infrared spectroscopy |
| L* | Brightness |
| LC | Liquid chromatography |
| LLE | Liquid-liquid extraction |
| LOD | Limit of detection |
| LOQ | Limit of quantification |
| $[M]^+$ | Molecular ion |
| $[M-H]^+$ | Protonated molecule |
| $[M-H]^-$ | Deprotonated molecule |
| m/z | Mass-charge ratio |

| | |
|---|---|
| MALDI | Matrix assisted laser desorption ionization |
| MeOH | Methanol |
| MRM | Experiment of MS/MS in which is monitorized the intensity resulting from a selected fragmentation called transition |
| MS | Mass spectometry |
| MS/MS | Tandem mass spectrometry |
| $MS^n$ | Esperiments to monitor mass fragmentation in more than two stages |
| Mv | Malvidin |
| Mv-3-glc | Malvidin-3-glucoside |
| Mv-3-(6-Ac)-glc | Malvidin-3-(6-acethyl)-glucoside |
| Mv-3-(6-caff)-glc | Malvidin-3-(6-caffeoyl)-glucoside |
| Mv-3-(6-*p*-coum)-glc | Malvidin-3-(6-*p*-coumaroyl)-glucoside |
| n | Number of samples |
| nd | Not detected |
| $N_2$ | Nitrogen (gas) |
| NaOH | Sodium hydroxide |
| $Na_2HPO_4$ | Sodium hydrogenphosphate |
| $NaH_2PO_4$ | Sodium dihydrogen phosphate |
| NMR | Nuclear Magnetic Resonance |
| OEMV | Observatorio Español del Mercado del vino |
| PCA | Principal component analysis |
| PCA2 | Procyanidin A2 |
| PCB1 | Prodelphinidin B1 |
| PCB2 | Prodelphinidin B2 |
| PCB3 | Prodelphinidin B3 |
| PCC1 | Procyanidin C1 |

| | |
|---|---|
| *p*-coum | *p*-coumaric acid |
| PC's | Principal components |
| $PC_1$ | First principal component |
| $PC_2$ | Second principal component |
| $PC_3$ | Third principal component |
| PLS | Partial least squares |
| Pn | Peonidin |
| Pn-3-glc | Peonidin-3-glucoside |
| Pn-3-(6-Ac)-glc | Peonidin-3-(6-acethyl)-glucoside |
| Pn-3-(6-*p*-coum)-glc | Peonidin-3-(6-*p*-coumaroyl)-glucoside |
| Pt | Petunidin |
| Pt-3-glc | Petunidin-3-glucoside |
| Pt-3-(6-Ac)-glc | Petunidin-3-(6-acethyl)-glucoside |
| Pt-3-(6-*p*-coum)-glc | Petunidin-3-(*p*-coumaroyl)-glucoside |
| PTFE | Polytetrafluoroethylene |
| *q* | Total electric charge |
| Q1 | First quadrupole |
| Q2 | Second quadrupole |
| Q3 | Third quadrupole |
| QqQ | Triple quadrupole analyzer |
| $r^2$ | Regression coefficient |
| RDA | Mechanisms of Retro Diels Alder fragmentation |
| ref | Referencia |
| RMN | Resonancia Magnética Nuclear |
| RSD | Relative standard deviation |
| RVA | Reserva |
| $R^2$ | Squared correlation coefficient |

| | |
|---|---|
| s | Standard deviation |
| SEC | Standard error of calibration or molecular size exclusion chromatography |
| SIM | Monitoring a single mass/charge ratio, mass experiment |
| sim | simetric |
| SIR | Monitoring a single mass/charge ratio, mass experiment |
| SPE | Solid phase extraction |
| SRM | Experiment of MS/MS in which the resulting intensity of a selected fragmentation called transition is monitored |
| T | Colour scheme |
| TFA | Trifluoroacetic acid |
| TIC | Total ionic current |
| TOF | Time of flight analyzer |
| $t_R$ | Retention time |
| *u* | Atomic mass unit |
| UV | Ultraviolet |
| UV-vis | UV-visible absortion spectroscopy |
| V | Variance or voltio |
| v | vinyl |
| μ | Dipolar moment |
| μg | Microgram |
| $\lambda_{max}$ | UV-vis maximum absortion wavelength |

# Índice

# CAPÍTULO 1

## INTRODUCCIÓN

# Capítulo I

# INTRODUCCIÓN

## I.1. HISTORIA DEL VINO

No se puede conocer de forma certera el origen del vino, ya que se produce de forma natural cuando el zumo de la uva entra en contacto con levaduras, como la conocida *Saccharomy cescerevisiae*. Hace miles de años, dicho microorganismo no se encontraba entre la flora de las distintas variedades de vid, sino que estaba presente en especies arbóreas como el roble. Sin embargo, las vides silvestres tenían una gran tendencia a trepar por las ramas de los árboles, lo que pudo originar una espontánea inoculación en las uvas y la consecuente fermentación de su jugo, que no pudo pasar inadvertida a las primeras civilizaciones. El hecho de que el vino sea una bebida que fermente sola, determinó, para algunos historiadores, que los pueblos primitivos lo consideraran una bebida milagrosa. No obstante el origen de su forma más elaborada surge con el refinamiento del proceso natural por parte del ser humano a lo largo de la historia[1].

La etimología conocida de la palabra española vino procede de la latina *vinum*, y ésta de la griega *oἶνος*, aunque se considera que la raíz se encuentra próxima a la palabra sánscrita *vana* (amor), que también dio origen a las palabras *Venus* y *Venera*.

[1]Togores, H.; *Tratado de Enología*; Tomo I; Ed. Mundi-Prensa, Madrid, **2002**, 31-81.

Esta relación semántica estaría dada por la antigua creencia en los poderes afrodisíacos del vino.

Su creación está rodeada de leyendas, quizá una de lasmás conocida sea la leyenda persa que cuenta que un ave dejó caer a los pies del rey *Djemchid* unas semillas de las que nacieron plantas que dieron abundantes frutos. Al beber el oscuro jugo fermentado de dichos frutos, se durmió profundamente y al despertar se sintió curado y feliz y por ello decidió llamarlo "*DarouéShah*" (el remedio del rey). Cuando su descendiente *Cambises* fundó Persépolis, los viticultores plantaron viñas alrededor de la ciudad, dando origen al célebre vino de Shiraz, ciudad próxima a Persépolis.

Sin embargo, numerosas pruebas han demostrado que la vid, tanto silvestre como vinífera, existe desde la Era Terciaria, puesto que se han encontrado hojas registradas en las piedras y semillas en asentamientos prehistóricos, en tumbas, en pirámides y en pequeñas ánforas en las ruinas de cientos de ciudades. Todo ello no hace más que atestiguar la gran antigüedad de este cultivo, extendido por todo el hemisferio norte, desde el Himalaya hasta lo que es actualmente el territorio de los Estados Unidos. Cuando se produjeron las glaciaciones, en la era Cuaternaria, y el hemisferio norte se cubrió de hielo, desaparecieron gran parte de las plantaciones. Sin embargo, algunas plantas se salvaron, en lo que se conoce como los refugios climáticos. El más importante de estos refugios, el Refugio Caucásico, se encontraba en Asia y es allí donde se conservó la mayor cantidad de especies vegetales, siendo considerado por muchos botánicos del mundo como el lugar donde se originó y luego se distribuyó hacia el mundo la mayor parte de las especies frutales actuales, entre ellas la vid.

Las especies procedentes de estas zonas avanzaron hacia las riberas del Mediterráneo, mezclándose con otras especies como la *Vitis silvestris* de la Europa del norte, dando origen a las actuales variedades de uva. Estos cruzamientos entre variedades tuvieron como consecuencia la aparición de viníferas con granos de uvas más pequeños convirtiéndose en las predecesoras de las actuales variedades tintas, como la *Cabernet Sauvignon*, *Merlot*, *Tempranillo*, etc…, de las que se obtienen en la actualidad vinos tintos de gran calidad.

Seguramente, el primer vino que aprendieron a hacer los seres humanos al exprimir un racimo entre sus manos fue el vino rosado. Era seguramente el vino de los fenicios y de los egipcios, como se deduce observando escenas de vendimia que adornan los bajorrelieves de algunos monumentos erigidos en la época. En esas escenas se observan personas recolectando racimos, pisando uvas; también, un

prensador, elaborado con una gran bolsa de yute, que es retorcida por esclavos; y, finalmente, se ven escenas en las que algunos esclavos llenan tinajas con el jugo recogido (Figura I.1). Dichas tinajas estaban recubiertas en su interior con brea y una vez llenas de vino se sellaban con barro, de tal forma que el vino se conservaba durante años en tales recipientes.

Analizando la escena con ojos enológicos, es técnicamente imposible obtener otra cosa que no sea vino rosado, al no hacerse la suficiente maceración de los hollejos que requiere el vino tinto.

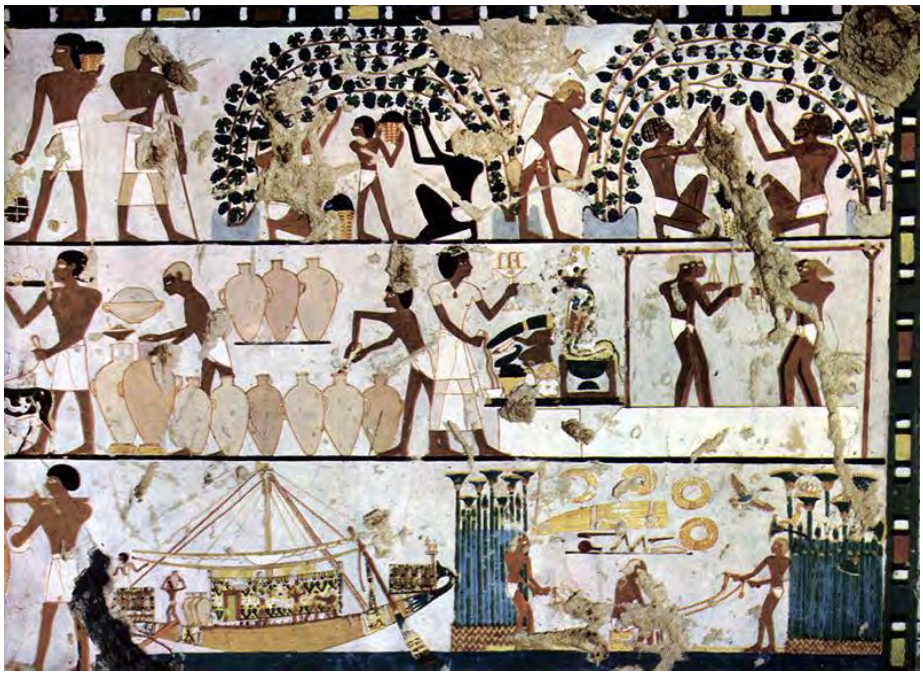

**Figura I.1** Escenas egipcias que representan el proceso de elaboración del vino.

En esta época, el vino se consideraba un lujo que únicamente los sacerdotes y los nobles podían beber, aunque en los periodos festivos lo escanciaban hasta los egipcios de las clases más bajas[2,3].

Gracias al desarrollo del comercio marítimo en el Mediterráneo, el vino comenzó a expandirse hacia el oeste de la mano de los mercaderes fenicios y griegos. Más tarde, los griegos serían los maestros de los romanos, quienes a su vez instruyeron a los íberos.

[2]Flinders, W.M.; *Social Life in Ancient Egypt*, Kessinger Publishing, Whitefish (Montana, USA), **2004**, ISBN1417977264.
[3]Guasch, J.; Maria, R.; Andrés-Lacueva, C.;Jáuregui, O.;Lamuela-Raventós, R. M.; *The origin of the ancient Egyptian drink Shedeh revealed using LC/MS/MS*, J. Archaeolog.Sci. **2006**, 33 (1),98-101.

Tras la conquista romana, el cultivo de la vid se generalizó en todo el territorio del Imperio y la elaboración de vinos se convirtió en una fuente de riqueza, especialmente en el sur y este de Francia y en el *Hermitage*, sobre el Ródano, convirtiéndose las Galias en el centro del intercambio y venta de vinos hacia todas las zonas europeas.

Durante el renacimiento comienza una nueva etapa en la historia del vino, porque es en los siglos XV y XVI cuando se mejoran los sistemas de vinificación y los vinos de Borgoña, Champaña y Burdeos, en Francia, comienzan a adquirir la fama que los hará célebres. Sin olvidar a Dom Perignon, que descubre el modo de preparar el vino espumoso.

A lo largo de toda la historia, el vino ha estado muy bien considerado por la alta sociedad occidental, siendo testigo imprescindible en cualquier acontecimiento o banquete de importancia y alrededor de él se han firmado los grandes tratados y acontecimientos históricos de Occidente.

El vino posee ciertos atributos que inciden de forma grata en la mayoría de los sentidos; los aromas afectan a los sentidos del olor, los diferentes sabores del vino al gusto y los colores a la vista. Todos ellos tienen un origen químico que se ha ido identificando poco a poco a lo largo de finales del siglo XX y comienzos del XXI.

## I.2. ELABORACIÓN DEL VINO TINTO

La elaboración del vino ha ido añadiendo cada vez más elementos tecnológicos a medida que el ser humano ha ido experimentando y adquiriendo cada vez más conocimiento acerca de los diferentes procesos que tienen lugar durante su elaboración.

Las técnicas de elaboración del vino juegan un papel muy importante en la extracción de polifenoles de las uvas y su posterior estabilización en el vino: el tiempo de maceración y fermentación en contacto con las pieles y las pepitas de las uvas, el prensado, el envejecimiento en barrica y en botella,…

El color del vino está fuertemente influenciado por la variedad de uva y las distintas prácticas enológicas, la forma de elaborar el vino, la temperatura y el tiempo de almacenaje y la exposición al oxígeno[4].

[4]Atanasova, V.; Fulcrand, H.; Cheynier, V.; Moutonet, M.; *Effect of oxygenation on polyphenol changes occurring in the course of wine-making*, Anal.Chim.Acta**2002**, 458, 15-27.

### I.2.1. Vendimia

Los factores que más inciden en la calidad del vino son principalmente la materia prima, es decir la uva, y la metodología de elaboración, existiendo un tercer factor de notable importancia, la operación de vendimia y el transporte de las uvas a la bodega, ya que los racimos deben llegar a la bodega lo más intactos posible. Una rotura de las uvas durante la vendimia se traduce en una pérdida de mosto y en una posible fermentación alcohólica prematura e indeseable[5].

### I.2.2. Maceración y fermentación alcohólica

Muchos vinos se echaron a perder hasta que se comprendió que la vinificación era un proceso puramente anaeróbico, es decir, sin la presencia de oxígeno.

Antiguamente se procedía al prensado de las uvas directamente tras la vendimia, con el objeto de obtener el mosto. Lo habitual es que lo hicieran personas descalzas pisando las uvas recolectadas en recipientes perforados en el fondo, obteniéndose de esta forma el primer mosto. Este método proporcionaba una producción en pequeña escala. Posteriormente se desarrollaron las prensas en forma de husillo, que permitían controlar la presión. En la actualidad se emplean prensas neumáticas herméticamente cerradas.

El prensado tiene que ser suave para extraer el mosto respetando la estructura del hollejo y sin aplastar las pepitas. Es conveniente no sólo respetar su integridad, sino también rasgar el hollejo en una mayor longitud, mediante un aplastamiento rápido pero no violento de los granos de uva. De este modo se consigue aumentar la superficie de maceración y activar el intercambio de sustancias entre las fases sólida y líquida de la vendimia[6].

En vinos de Rioja se utilizan dos metodologías diferentes de maceración y fermentación alcohólica, un procedimiento conocido como "despalillado" de las uvas y otro de maceración carbónica.

- ***"Despalillado"***: El primer paso de este procedimiento es una operación previa de limpieza en el que se eliminan la vegetación y los raspones. Esta

[5]Hidalgo Togores, J.;*Vendimia. Recepción de uva en la bodega*. En Tratado de enología. Tomo I. Ediciones Mundi-Prensa, Madrid, **2002**, pp. 203-295.

[6]Hidalgo Togores, J.; *Tratamientos mecánicos de la vendimia*. En Tratado de enología. Tomo I. Ediciones Mundi-Prensa, Madrid, **2002**, pp. 297-384.

operación (despalillado) se realiza en tambores metálicos perforados que giran a gran velocidad y las uvas salen enteras por las perforaciones del tambor.

El despalillado debe respetar la integridad de la uva, es decir, no puede provocar roturas o trituración de la uva, y en particular no debe partir, aplastar o dañar las pepitas o semillas[7].

Una vez que se ha producido la separación de la uva de la parte vegetal, tiene lugar el llamado estrujado de las uvas. La función principal de este estrujado es provocar la rotura de las bayas por presión radial y así evitar que se rompan, dañen o aplasten las pepitas de la uva. Si las pepitas de las uvas llegaran a romperse, liberarían ciertos ácidos grasos que, en presencia de oxidasas y aire, darían lugar a compuestos con seis carbonos como el hexanal o el cis-3-hexanal. Estos compuestos pueden otorgar al futuro vino un sabor herbáceo[7].

Además, el estrujado tiene un papel de aceleración en las primeras fases de la maceración. Es sobre todo en maceraciones en vino tinto, donde la difusión precoz de antocianinas libres (que tiene lugar en las primeras etapas de la maceración) tiene un efecto importante sobre las reacciones entre dichos compuestos y los taninos que serán extraídos más tarde.

Después de separar la uva del racimo y haber roto los granos de uva, se inician de forma inmediata y simultánea dos fenómenos, por un lado la maceración o intercambio de sustancias entre el mosto y la parte sólida y, por otro lado, una serie de transformaciones bioquímicas producidas por las enzimas de las uvas.

Los fenómenos de maceración se desarrollan con una permanencia más o menos prolongada del mosto junto a las partes sólidas de la vendimia. Las sustancias contenidas en la parte sólida pasan al mosto y son en su mayor parte sustancias apreciadas, como antocianinas, taninos, compuestos nitrogenados, polisacáridos, etc; pero también pueden transferirse otras sustancias indeseables para el vino.

La fermentación alcohólica, llevada a cabo hoy en día en recipientes de acero inoxidable, es la parte principal del proceso de la elaboración del vino; en realidad el vino no puede elaborarse de forma alguna sin ella. Tiene como

[7]Flanzy, C.; *Vinificaciones: principales operaciones*. En Enología: fundamentos científicos y tecnológicos. Ediciones Mundi-Prensa, Madrid, **2003**, pp. 418-442.

principal efecto la conversión de los azúcares del mosto en alcohol etílico. Los organismos capaces de llevar a cabo esta fermentación son las levaduras del género *Saccharomyces*.

Durante la fermentación alcohólica de la uva tinta, el gas carbónico resultante empuja hacia arriba los hollejos, formando una barrera natural llamada "sombrero". Se pueden aplicar operaciones de remontado, que consisten en remojar el "sombrero" con el mosto, encaminadas principalmente a activar la maceración de los hollejos con el mosto. El hollejo también puede ser removido periódicamente en una operación que se llama bazuqueo[8].

El descube es la operación de vaciado del depósito que contiene la vendimia fermentada. Una vez escurrido el vino, dentro del depósito se encuentra una mezcla de hollejos y pepitas fermentadas, también llamados orujos, y una buena cantidad de vino que lo embebe, por lo que es necesario realizar un prensado de ese orujo para terminar de extraer el vino que contienen[8].

- ***Maceración carbónica***: La maceración carbónica consiste en dejar fermentar las uvas enteras, sin estrujar ni despalillar, es decir, sin quitarles el raspón. Debido al peso y al manejo de los racimos, parte de los granos de uva se rompen, comenzando la fermentación alcohólica de este mosto, que ocupa el fondo del lagar. El $CO_2$ producido desplaza al $O_2$, lográndose de este modo una atmósfera libre de oxígeno[9]. En estas condiciones anaeróbicas, tiene lugar en el interior de los granos enteros lo que se conoce como fermentación intracelular. Cuando la concentración de alcohol en el interior del grano llega a unos 2º, el grano se rompe. En una maceración carbónica estricta, todos los granos de uva permanecen enteros y con una apariencia normal, aunque en su interior se desarrollan diferentes procesos metabólicos sin la intervención de ningún microorganismo.

  Tras recoger el vino "de lágrima" o "escurrido", los racimos son volteados y, posteriormente prensados, obteniéndose de este modo otras dos fracciones, llamadas vino "de corazón" y vino "de prensa". Lo habitual es mezclar las tres fracciones en un depósito donde, sin presencia ya de hollejos, continúa la

[8]Hidalgo Togores, J.; *Elaboración de vinos tintos y claretes*. En Tratado de enología. Tomo II. Ediciones Mundi-Prensa, Madrid, **2002**, pp. 757-820.

[9]Flanzy, C.; *Vinificación en tinto*. En Enología: Fundamentos Científicos y Tecnológicos. AMV Ediciones y Ediciones Mundi-Prensa, Madrid, **2003**, pp. 462-496.

fermentación alcohólica hasta que todos los azúcares se transforman en alcohol y $CO_2$.

Los vinos elaborados por maceración carbónica presentan habitualmente un potencial aromático elevado y suelen ser más afrutados y algo más suaves en boca (menos astringentes y ácidos) que los elaborados mediante el procedimiento de despalillado[10].

Esta metodología de elaboración se asocia principalmente a vinos jóvenes, mientras que para los vinos que van a ser destinados a crianza se suele optar por la otra técnica, el despalillado, ya que los vinos elaborados por maceración carbónica carecen de la estabilidad suficiente para ser envejecidos durante largos periodos.

La elaboración de vendimias tintas por el sistema de maceración carbónica se utiliza especialmente en las zonas de Rioja Alta y Rioja Alavesa con la variedad Tempranillo[11].

### I.2.3. Fermentación maloláctica

Durante la elaboración del vino tiene lugar otro proceso llamado fermentación maloláctica, en la que actúan las bacterias lácticas presentes de forma natural en la uva para convertir el ácido málico en ácido láctico, reduciendo de este modo la acidez del vino[12].

Los efectos que produce la fermentación maloláctica sobre los vinos tintos pueden ser deseables o beneficiosos en algunos casos, mientras que en otros no lo son en absoluto. Entre los primeros se encuentran los vinos que se destinan a la crianza, donde es imprescindible obtener una buena estabilidad biológica, que garantice su conservación en el tiempo; o bien en otros vinos en los que la acidez málica puede ser tan elevada que la fermentación maloláctica es la única solución posible, aún a costa de perder potencial aromático, como sucede en algunos vinos jóvenes.

Las consecuencias del desarrollo de una fermentación maloláctica pueden ser:

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[10]Etaio, I. (Ed.); *¿Por qué específicamente los vinos de Rioja Alavesa?*. En Guía para la evaluación sensorial de la calidad de los vinos tintos de Rioja Alavesa. Servicio Central de Publicaciones del Gobierno Vasco, San Sebastián, **2007**, pp. 29-36.

[11]Ruiz Hernández, M.; *Estrujado de la vendimia*. En Tratado de vinificación en tinto. AMV Ediciones y Ediciones Mundi-Prensa, Madrid, **2004**, pp. 104-113.

[12]Hidalgo Togores, J.; *Fermentación maloláctica*. En Tratado de enología. Tomo II. Ediciones Mundi-Prensa, Madrid, **2002**, pp. 839-856.

- Disminución de la intensidad del color: debido a una modificación del pH del vino durante la fermentación maloláctica se produce una modificación de los antocianos, sobre todo por una destrucción de los mismos a causa de una posible hidrólisis de la molécula producida por el complejo enzimático de las bacterias lácticas en la búsqueda de la parte azucarada de los antocianos.
- Modificación del aroma: debido en parte a una disminución de los aromas varietales por degradación o hidrólisis de los compuestos aromáticos de la uva y, por otra parte, a una atenuación o desaparición de las sustancias aromáticas formadas durante la fermentación alcohólica. Pero el compuesto aromático más característico de la fermentación maloláctica es el diacetilo (butano-2,3-diona), formado a partir de la degradación de ácido cítrico con un inconfundible olor a mantequilla, aunque en concentraciones pequeñas (<5mg/L) puede contribuir positivamente en el aroma del vino.

### I.2.4. Adición de dióxido de azufre

Las propiedades del dióxido de azufre ($SO_2$) como conservante de los vinos son conocidas desde hace siglos, pero su utilización en las operaciones prefermentativas de las vendimias es bastante más reciente, remontándose a finales del siglo XIX. Las propiedades positivas del dióxido de azufre superan ampliamente a las negativas, que también existen, siendo todavía hoy un instrumento indispensable en la tecnología de elaboración y conservación de los vinos. Entre las propiedades positivas cabe destacar su efecto antioxidante, sus propiedades antimicrobianas, la intensa acción degradante sobre los hollejos que permite una mayor maceración en las vinificaciones en tinto, su papel en la mejora y mantenimiento de los aromas de los vinos, etc. Las propiedades negativas se asocian con las dosis elevadas en las vendimias o en los vinos, pudiendo aparecer olores defectuosos o suponiendo un posible riesgo para la salud humana por la ingestión de este compuesto que acompaña a los vinos[13].

### I.2.5. Maduración y envejecimiento

El origen de la crianza del vino nació de su necesidad de transportarlo. Los envases de transporte evolucionaron desde las tinajas de barro, que tenían el problema de la fragilidad, hasta el uso de pellejos u odres, fabricados con cueros

[13]Hidalgo Togores, J.; *El anhídrido sulfuroso y otros compuestos complementarios*. En Tratado de enología. Tomo I. Ediciones Mundi-Prensa, Madrid, **2002**, pp. 421-454.

curtidos e impermeabilizados con resinas o "pez", con gran resistencia al transporte, pero que deformaban las características del vino con olores y sabores extraños. Sin embargo, en los países del norte, los depósitos en los que se elaboraban y almacenaban los vinos eran de madera, y construyeron recipientes más pequeños para el transporte utilizando la misma madera. Esta forma de transporte se extendió de manera que los vinos llegaban hasta el consumidor después de haber permanecido cierto tiempo en barriles de madera, llegándose a apreciar de este modo las características que ésta aportaba al vino.

La maduración en barricas es una especie de oxidación lenta del vino. En la actualidad el vino se almacena en barricas de roble procedente de América y de Francia, contribuyendo el roble francés más a la aportación de extractos sólidos y compuestos fenólicos; además su vida útil es mayor que la del roble americano.

Durante la permanencia del vino en las barricas se produce una decantación e insolubilización de diversas sustancias que contiene, pudiendo estos sedimentos tener un efecto negativo en la calidad del vino. La eliminación de los sedimentos se realiza periódicamente mediante una operación conocida como trasiego, que consiste en cambiar el vino de una barrica a otra para separar el vino de los sedimentos o lías acumulados en la parte inferior. El trasiego permite disminuir la posibilidad de reactivaciones de microorganismos, eliminar del vino un exceso de dióxido de carbono, homogeneizar el vino de crianza y corregir el nivel de dióxido de azufre.

La situación más favorable antes de iniciar la etapa de envejecimiento en barrica es tener una concentración de antocianinas equilibrada con la de flavanoles, en una relación antocianina/flavanol de 1/4. Esta situación es difícil de conseguir; si no es posible, es preferible una situación de mayor concentración de flavanoles con el fin de que todas las antocianinas disponibles puedan participar en reacciones de polimerización, eliminando el exceso de flavanoles después de la crianza para evitar una condensación entre ellos, que proporcionaría un sabor con exceso de astringencia en el vino.

Un vino pobre en flavanoles, en el que la concentración de antocianinas sea mayor que la de flavanoles es el menos apto para la crianza, debido a que el exceso de antocianinas reacciona por oxidación originando ácidos fenólicos incoloros, ocasionándose una importante destrucción del color.

La utilización de barricas nuevas o seminuevas que permitan una suave oxidación del vino, así como un régimen de temperaturas suave y constante entre

12-15ºC y una humedad relativa del 70-80% es el mejor procedimiento de crianza de vinos en barrica.

El envejecimiento en madera es un procedimiento que se usa para estabilizar el color y enriquecer las características sensoriales del vino, probablemente debido a los efectos beneficiosos de los compuestos que se extraen de la madera, como los elagitaninos o los aldehídos de la madera[14]. Además, la permeación de oxígeno a través de la madera favorece la formación de derivados de antocianinas y flavanoles[15].

El embotellado es una operación relativamente reciente en la historia del vino. Se empezó a realizar cuando era posible elaborar vidrios más robustos y asequibles. Las botellas de vidrio primitivas tenían una forma abombada y las actuales, entre las que destaca la Bordelesa, tienen un volumen estándar de 0.75 L. Un elemento importante en el embotellado es la encapsulación, que puede emplear tapones de materiales naturales (corcho), semisintéticos, sintéticos o cápsulas metálicas. Antes del embotellado se realizan operaciones de clarificación, estabilización y filtración para eliminar residuos del proceso de elaboración y conseguir que el vino sea un líquido limpio.

Las condiciones de crianza de un vino dentro de la botella son generalmente reductivas, aunque puede producirse una entrada de aire dentro de la misma a través del corcho, especialmente durante las primeras semanas. Durante este periodo las antocianinas prácticamente llegan a desaparecer, aunque se mantienen sus formas combinadas, de este modo la intensidad de color puede permanecer estable. El color de los vinos puede evolucionar hacia tonos teja, debido en parte a la formación de compuestos de tipo piranoantociánico, de marcada tonalidad teja y resistentes a oxidaciones, al dióxido de azufre y a variaciones de pH.

No todos los vinos están preparados para largos periodos de almacenamiento, la gran mayoría es aconsejable que se consuma en el propio año. Por regla general, los vinos tintos se conservan mejor que los blancos. Los vinos de crianza donde primen las uvas *Cabernet Sauvignon* o *Tempranillo* suelen tener una larga vida, por lo que las cepas de estas uvas son las predominantes para la elaboración de vinos de Burdeos y de Rioja, respectivamente.

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[14]Cano-López, M.; López-Roca, J. M.; Pardo-Minguez, F.; Gómez-Plaza, E.; *Oak barrel maturation vs micro-oxigenation: effect on the formation of anthocyanin-derived pigments and wine colour*, Food Chem. **2010**, 119, 191-195.

[15]De Rosso, M.; Panighel, A.; Dalla-Vedova, A.; Stella, L.; Flamini, R.; *Changes in chemical composition of a red wine aged in acacia, cherry, chestnut, mulberry and oak barrels*, J. Agric. Food Chem., **2009**, 57, 1915-1920.

# I.3. DENOMINACIÓN DE ORIGEN RIOJA

El vino ya aparece documentado en La Rioja durante la época de la dominación romana, ya que encontraron en este territorio las condiciones idóneas para su cultivo y elaboración. Los suelos predominantemente arcillo-calcáreos tienen un efecto beneficioso sobre el aporte hídrico a la vid y, en consecuencia, sobre la maduración de la uva.

La región vitivinícola de la Rioja se encuentra en el Valle del Ebro, con influencia de los climas atlántico y mediterráneo. Esta situación geográfica, las condiciones climatológicas y la constitución del suelo la convierten en una zona privilegiada para el cultivo de la vid.

La gran diversidad del terreno y el clima hace que esta región vitivinícola se divida en tres subzonas: Rioja Alta, Rioja Alavesa y Rioja Baja (Figura I.2).

**Rioja Alta**: Situada en el margen derecho del Ebro, es la subzona con mayor superficie de la denominación, la cual representa el 40% de la producción. La variedad de sus suelos, predominantemente de arcilla ferrosa, y unas precipitaciones moderadas, permiten obtener vinos de calidad, moderadamente alcohólicos y coloreados, aptos para el envejecimiento en barricas. Las variedades de uva predominantes son *Tempranillo* (tinto) y *Viura* (blanco).


**Figura I.2** Mapa geográfico de las distintas subzonas pertenecientes a la Denominación de Origen Calificada de Rioja.

**Rioja Alavesa**: Se encuentra en la margen izquierda del Ebro, aguas arriba de Logroño. Aunque en esta subzona de Rioja, el clima es poco lluvioso, sus suelos calcáreos permiten obtener vinos de calidad, con un contenido moderado de alcohol y, también, una acidez moderada, aptos para el consumo y la crianza en barrica. De esta subzona se obtienen el 22% de los vinos de la denominación y las variedades predominantes son *Tempranillo* (tinto) y *Viura* (blanco).

**Rioja Baja**: Situada principalmente en la margen derecha del río Ebro, aguas abajo de Logroño. El 38% de los vinos de Rioja proceden de esta subzona, caracterizada principalmente por suelos aluviales y unas precipitaciones moderadas. En los últimos años ha habido un aumento en el uso de uvas de la variedad *Tempranillo* (tinto), pero sigue estando muy presente la variedad *Garnacha* (tinto) y *Viura* (blanco).

Durante la segunda mitad del siglo XIX el vino de Rioja consiguió la posición que tiene en la actualidad. A mediados de este siglo la plaga de *filoxera* afectaba a las principales regiones vitivinícolas europeas, por lo que numerosos bodegueros franceses llegaron a La Rioja en búsqueda de nuevos proveedores para satisfacer la demanda e implantar el método de elaboración bordelés, que consiste en la crianza de los vinos. De este modo, de la combinación de una destacada materia prima y de las técnicas francesas de elaboración, el vino de Rioja alcanza una calidad y prestigio universal.

En 1926 se creó el Consejo Regulador, primero de España, con los objetivos de delimitar la zona de producción del vino de Rioja, controlar la expedición del precinto de garantía y establecer medidas legales para proteger al vino de Rioja de falsificadores. Pero no sería hasta abril de 1991 cuando, mediante una Orden Ministerial, se le otorga el carácter de "Denominación de Origen Calificada", siendo la primera en España que posee este rango[16]. En 2004 se aprueba su reglamento (modificado por última vez el 26 de septiembre del 2012)[17] , en el cual están recogidos los requisitos de la denominación como: las variedades de vid autorizadas para la elaboración de los vinos protegidos, los requisitos para el uso de las menciones de

[16]Orden del 3 de abril de 1991 por la que se otorga el carácter de Denominación de Origen Rioja y se aprueba su Reglamento y el de su Consejo Regulador (B.O.E. 85 de 9 abril).

[17]Orden AAA/2127/2012 del 26 de septiembre, por la que se modifica el Reglamento de la Denominación de Origen Calificada Rioja y de su Consejo Regulador, aprobado por Orden APA/3465/2004, de 20 de octubre. (BOE no 242 de 8 de octubre de 2012).

*Crianza*, *Reserva* y *Gran Reserva*, la indicación de la subzona y las características analíticas de los vinos protegidos por la denominación Rioja[18].

Los vinos tintos amparados bajo esta Denominación de Origen pueden ser elaborados a partir de determinadas variedades de uva autorizadas, como *Tempranillo, Garnacha, Mazuelo* y *Graciano*, admitiéndose pequeños porcentajes de uva blanca de las variedades *Viura, Garnacha blanca* y *Malvasía de Rioja*.

La variedad de uva *Tempranillo* es la variedad tinta española de mayor calidad y fama, siendo originaria de la zona alta del Ebro entre La Rioja y Navarra. El nombre de *Tempranillo* procede de su maduración temprana, produciendo cuando está bien cultivada unos tintos de importante carga polifenólica y un inconfundible aroma a frutos negros y a regaliz, siendo muy adecuados para su crianza. Los vinos de Rioja Alavesa, tanto jóvenes como crianzas, se asocian a la variedad *Tempranillo*.

El uso de las calificaciones "*Crianza*", "*Reserva*" y "*Gran Reserva*" está igualmente regulado, pudiendo utilizar las distintas indicaciones los vinos que cumplan los siguientes requisitos:

**Vinos de *Crianza***: Deben cumplir un proceso de maduración de dos años naturales, con una permanencia de un año como máximo en barrica de roble, seguido y complementado con envejecimiento en botella.

Las calificaciones de *Reserva* y *Gran Reserva* las podrán utilizar únicamente los vinos de añadas concretas, que hayan adquirido una armonía en el conjunto de sus cualidades organolépticas y una riqueza aromática destacada, y deberán cumplir un proceso de envejecimiento específico en cada caso.

**Vinos de *Reserva***: Envejecimiento en barrica de roble y en botella durante un periodo total de 36 meses como mínimo, con una estancia en barrica de roble de al menos 12 meses.

**Vinos de *Gran Reserva***: Envejecimiento en barrica de roble durante un periodo mínimo de 24 meses, seguido y complementado con un envejecimiento en botella de 36 meses como mínimo.

[18]Orden APA 3465/2004 de 20 de Octubre, por la que se aprueba el Reglamento de la Denominación de Origen Calificada Rioja y de su Consejo Regulador, así como las Normas para la calificación de los vinos con derecho a dicha Denominación. (B.O.E. no 259 de 27 de octubre de 2004).

# I.4. LA REGIÓN VITIVINÍCOLA DE BURDEOS

Al igual que la región vitivinícola de Rioja, la historia del vino en la región bordelesa comienza durante la ocupación romana en Saint Emilion.

La región vinícola de Burdeos se extiende por el oeste de Francia, en la región de Aquitania y es la segunda región vinícola más grande de Francia.

La principal razón del éxito de la viticultura en la región bordelesa es el excelente ecosistema y el suelo calizo que tiene para el crecimiento adecuado de la vid. El estuario de la Gironda domina las regiones a lo largo con sus afluentes, los ríos Garona y Dordoña, y juntos irrigan la tierra y proporcionan un clima oceánico para la región. Estos dos ríos son los que definen las principales subdivisones geográficas de la región (Figura I.3). Las principales variedades de uvas empleadas en la región vitivinícola bordelesa son *Cabernet Sauvignon* y *Merlot*, aunque la variedad *Cabernet Franc*, no muy usada en esta región, también está autorizada.

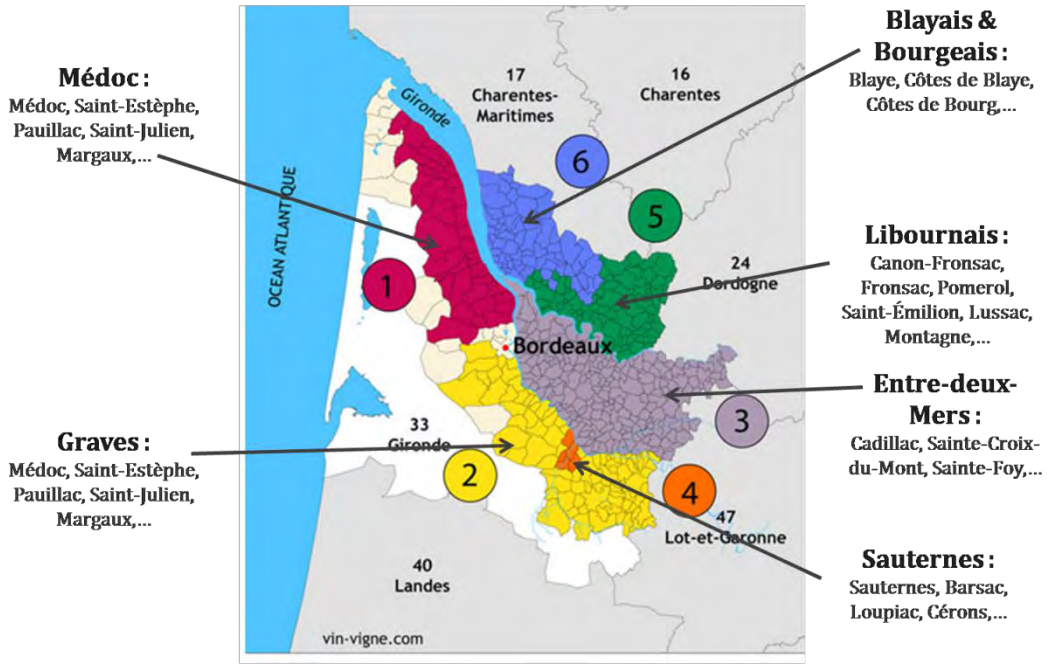


**Figura I.3** Mapa geográfico de las distintas subzonas pertenecientes a la regiónvitivinícola de Burdeos

**"La orilla derecha"**: se encuentra situada en la ribera derecha del Dordoña, en las regiones septentrionales de la región, alrededor de la ciudad de Libourne.

**"Entre-deux-Mers"**: expresión francesa que significa "entre dos mares". Esta región se encuentra entre los ríos Dordoña y Garona, en el centro de la región.

**"La orilla izquierda"**: esta zona se sitúa en la ribera izquierda del Garona, en el oeste y sur de la región bordelesa.

Siete son las subregiones más destacadas de la región vitivinícola bordelesa: Médoc, Graves, Sauternes, Saint Emilion, Pomerol, Entre-deux-Mers y Fronsac. Junto a ellas, hay cuatro de menor importancia: Saint Macaire, Premières côtes de Bordeaux, Blayais & Bourgeais.

Aunque popular en el ámbito doméstico, el vino francés rara vez se exportaba, y las regiones cubiertas por viñas y el volumen de vino producido era bajo. Sin embargo, en el siglo XII, la popularidad de los vinos de Burdeos se incrementó con el matrimonio entre Enrique II y Leonor de Aquitania.

Conforme se incrementaba la popularidad del vino de Burdeos, otras regiones de Francia comenzaron a cultivar sus propios vinos ya etiquetarlos como productos de Burdeos. Por ello los productores de vino de la región de Aquitania demandaron que el gobierno impusiera una ley declarando que sólo el producto de Burdeos podía etiquetarse con ese nombre. El *INAO* o *Institut National des Appellations d'Origine* fue creado con este propósito. Sin embargo no fue hasta 1936 cuando el gobierno respondió a las peticiones de los productores de vino y afirmó que todas las regiones de Francia tenían que nombrar sus vinos por el lugar en el que se habían producido. Por ello, a partir de este momento, los productos eran etiquetados con el sello aprobado de la Appellation d'Origine Contrôlée (AOC) Bordeaux (denominación de origen de Burdeos). Esta ley más tarde se extendió a otros productos como el queso, aves de corral y hortalizas.

Las57 AOC de Burdeos y los estilos de vino que representan se categorizan normalmente en seis familias, cuatro tintos y dos blancos, basadas en las subregiones:

**Tinto de *Bordeaux y Bordeaux Superieur***: Son los tintos de Burdeos "básicos", que se permite producir por toda la región. Estos vinos tienden a ser frutales y se producen en un estilo que pretende ser bebido como un vino joven. Sin embargo, algunos viticultores producen vinos de Burdeos Superior (Bordeaux Superieur) que son vinos con un estilo más similar al de otras familias de tintos.

**Tinto Côtes de Bordeaux**: Son vinos que en su mayoría se producen mediante mezcla de variedades, usualmente dominada por la variedad *Merlot*. Estos vinos tienden a ser intermedios entre el tinto de burdeos y burdeos superior y las más

famosas apelaciones de las riberas izquierda y derecha tanto en estilo como en calidad.

**Tinto Libourne, o "Vinos de la orilla derecha"**: Son vinos dominados por *Merlot*, con muy poco porcentaje de la variedad de *Cabernet Sauvignon*, siendo los dos más famosos Saint Emilion y Pomerol. Estos vinos a menudo tienen una gran concentración frutal, taninos más suaves y que perduran más tiempo.

**Tinto Graves y Médoc o "vinos de la orilla izquierda"**: Al norte y al sur de Burdeos se encuentran las zonas más clásicas del vino bordelés en donde se producen vinos dominados por la variedad de *Cabernet Sauvignon*, pero a menudo con una porción significativa de *Merlot*. Estos vinos son concentrados, tánicos, de larga vida y la mayor parte de ellos se elaboran para ser conservados en bodega antes de beberse.

**Vinos blancos secos**: Vinos blancos secos se hacen por toda la región mediante una mezcla de *Sauvignon blanc* y *Semillon*, siendo los de Graves los más conocidos. Las mejores versiones tienden a tener una significativa influencia de roble.

**Vinos blancos dulces**: En varias zonas de toda la región, el vino blanco dulce se hace con uvas S*emillon*, *Savignon blanc* y *Muscadelle*. Los más conocidos de estas apelaciones son de Sauternes, donde se producen algunos de los más famosos vinos dulces.

## I.5. LOS COMPONENTES QUÍMICOS DEL VINO TINTO:LOS POLIFENOLES

El mosto antes de la fermentación se compone principalmente de agua y azúcares, así como de los ácidos málico y tartárico, además de otros componentes químicos en menor medida, responsables todos ellos de la composición final del vino. La fermentación alcohólica transforma gran parte de los azúcares del mosto en alcohol, pero dejará otros compuestos interesantes. Algunos de estos compuestos dan un cierto carácter a la cata de un vino, entre los que destacan los compuestos polifenólicos.

Los polifenoles constituyen uno de los grupos más amplios y diversos de productos naturales distribuidos universalmente en las plantas. Son una de las clases

de metabolitos secundarios más importantes, derivados de la ruta biosintética del siquimato y del metabolismo fenilpropanoico[19,20,21].

Los polifenoles son abundantes en el vino y son uno de los componentes que le proporcionan mayores atributos. Son el tercer tipo de compuestos más importante, por detrás de los azúcares y los ácidos, y se encuentran principalmente en la piel y las pepitas de las uvas. Los polifenoles contribuyen a muchas de las características organolépticas de los vinos, como son el color, la astringencia, el amargor y el aroma[22]; es por esta razón que los viticultores cuidan en detalle su evolución durante las fases de vinificación. La concentración de polifenoles depende en gran parte de la variedad de *Vitis vinífera* empleada en la elaboración del vino y del clima en el que se haya cultivado.

Debido a sus propiedades antioxidantes y antiinflamatorias, los polifenoles se han asociado con varios efectos fisiológicos beneficiosos para la salud, y el consumo moderado de vino tinto con una menor incidencia de enfermedades cardiovasculares, un fenómeno conocido como la *"paradoja francesa"*[23,24].

La estructura básica de los compuestos fenólicos es un anillo bencénico sustituido por uno o varios grupos hidroxilo. Su reactividad es debida al carácter ácido de las funciones fenólicas y al carácter nucleófilo del anillo bencénico. Se clasifican según el esqueleto básico carbonado que presentan, en compuestos no-flavonoides y flavonoides (verdaderos "polifenoles" en su estructura química).

Las uvas contienen compuestos no-flavonoides principalmente en la pulpa mientras que los flavonoides se localizan en la piel, la pepita y el tallo. Los compuestos no-flavonoides más importantes en la uva y el vino son los ácidos fenólicos (ácidos benzoicos e hidroxicinámicos, que estrictamente pueden no ser considerados "polifenoles") (Figura I.4) y los estilbenos[25] (Figura I.5).

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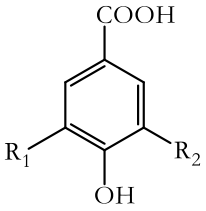
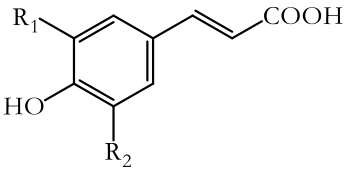
[24]Tufarelli, V.;Casalino, E.; D'Alessandro, A. G.; Laudadio, V.; *Dietary Phenolic Compounds: Biochemistry, Metabolism and Significance in Animal and Human Health*, Curr. DrugMetab. **2017**, 18 (10), 905-913.

[25]Zamora, F.; *El color del vino tinto*. En Elaboración y crianza del vino tinto. Aspectos científicos y prácticos. AMV Ediciones y Ediciones Mundi-Prensa, Madrid, **2003**, pp. 13-52.

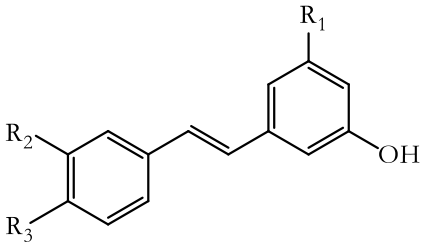



| Ácido benzoico | $R_1$ | $R_2$ |
|---|---|---|
| Ácido gálico | -OH | -OH |
| Ácido p-hidroxibenzoico | -H | -H |
| Ácido siríngico | $-OCH_3$ | $-OCH_3$ |




| Ácido hidroxicinámico | $R_1$ | $R_2$ |
|---|---|---|
| Ácido p-cumárico | -H | -H |
| Ácido cafeico | -OH | -H |
| Ácido ferúlico | $-OCH_3$ | -H |
| Ácido sinápico | $-OCH_3$ | $-OCH_3$ |

**Figura I.4** Estructuras químicas de los ácidos benzoicos y de los ácidos hidroxicinámicos.




| Estilbeno | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| Resveratrol | -OH | -H | -OH |
| Piceida | -OGlc | -H | -OH |
| Astringina | -OGlc | -OH | -OH |
| Picetanol | -OH | -OH | -OH |

**Figura I.5** Estructura química de los estilbenos.

Los flavonoides están constituidos básicamente por dos anillos aromáticos unidos por una cadena de tres átomos de carbono, presentando una estructura tipo $C_6$-$C_3$-$C_6$. Los anillos aromáticos se denominan A y B y al heterociclo piránico central presente en algunas familias de flavonoides se le asigna la letra C (FiguraI.6).

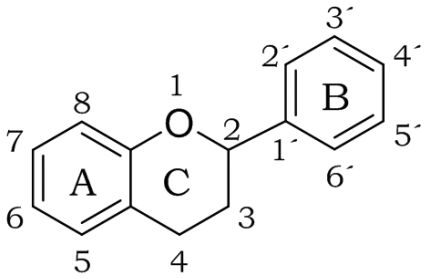
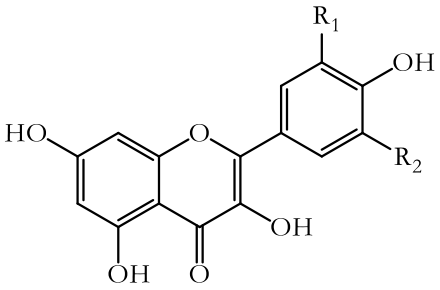
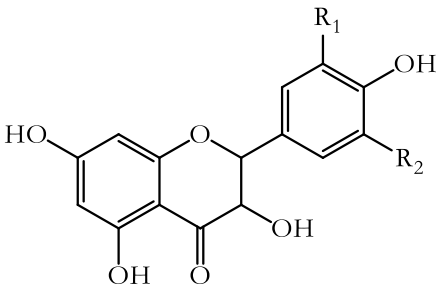


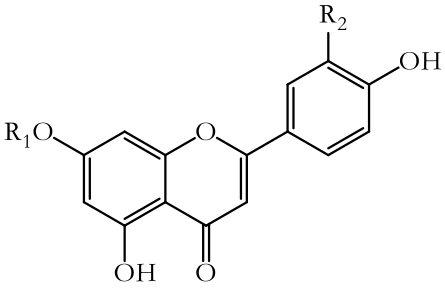

**Figura I.6** Estructura química básica de los flavonoides.




| Flavonol | $R_1$ | $R_2$ |
|---|---|---|
| Kamferol | -H | -H |
| Quercitina | -OH | -H |
| Miricetina | -OH | -OH |
| Isoramnetina | $-OCH_3$ | -H |




| Flavanonol | $R_1$ | $R_2$ |
|---|---|---|
| 2,3-dihidrokamferol | -H | -H |
| 2,3-dihidroquercitina | -OH | -H |




| Flavona | $R_1$ | $R_2$ |
|---|---|---|
| Apigenina | -H | -H |
| Luteolina | -H | -OH |

**Figura I.7** Estructura química de los flavonoles, flavanonoles y flavonas.

Las principales familias de flavonoides presentes en uvas del género Vitis variedad vinifera, y en los correspondientes vinos elaborados con estas uvas, son los flavanoles y las antocianinas, aunque también se pueden encontrar otros tipos de flavonoides en menor grado, como flavonoles[28], flavanonoles y flavonas. En la Figura I.7 se muestran las estructuras básicas de estos últimos[29], que dentro de una misma familia difieren en los grupos hidroxilo y metoxilo del anillo B. Estas estructuras básicas pueden presentar glicosilación en el carbono C3 en el caso de flavonoles y flavanonoles y, en ocasiones, los glicósidos aparecen acilados.

### I.5.1. FLAVANOLES

Los flavanoles o flavan-3-oles son flavonoides que se caracterizan por presentar una cadena saturada de 3 carbonos (heterociclo C) y por ser la única familia de flavonoides presente en su forma aglicona, es decir, no unida a ningún resto glicosídico.

Se encuentran en las partes sólidas de la uva (pepita, piel y tallo) y pueden aparecer como monómeros, oligómeros o polímeros; éstos dos últimos tipos también son conocidos como proantocianidinas o taninos condensados[30].

Los monómeros de flavanol encontrados en uvas *Vitis vinífera* son(+)-catequina, (-)-epicatequina, (+)-galocatequina y(-)-epigalocatequina[31]. Los dos primeros son ortodifenoles hidroxilados en las posiciones 3´y 4´ del anillo B y los dos últimos son trihidroxilados en las posiciones 3´, 4´y 5´, como puede verse en la FiguraI.8.

Estas moléculas presentan dos centros de asimetría en los carbonos C2 y C3, de tal manera que los cuatro monómeros se agrupan en dos pares de diastereoisómeros cuya configuración ha sido determinada como (2R, 3S), para (+)-catequina y (+)-galocatequina, y (2R, 3R), para (-)-epicatequina y (-)-epigalocatequina[32].

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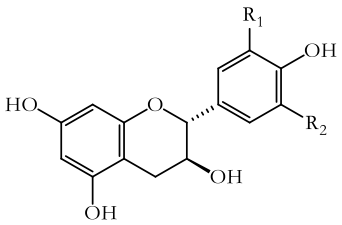
[28]Souquet, J. M.; Labarbe, B.; Le Guernevé, C.;Cheynier, V.; Moutounet, M.; *Phenolic composition of grape stems,* J. Agric. Food Chem. **2000**, 48, 1076-1080.

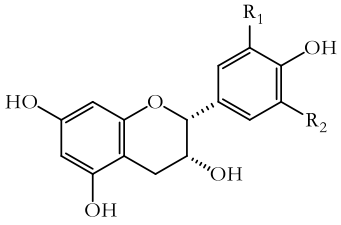
[29]Monagas, M.; Bartolomé, B.; Gómez-Cordovés, C.; *Updated knowledge about the presence of phenolic compounds in wine*, Crit. Rev. Food Sci. Nutr. **2005**, 45, 85-118.

[30]Escarpa, A.; González, M. C.; *An overview of analytical chemistry of phenolic compounds in foods*, Crit. Rev.Anal.Chem.**2001**, 31, 57-139.

[31]Revilla, E.; Bourzeix, M.; Alonso, E.; *Analysis of catechins and proanthocyanidins in grape seeds by HPLC with photodiode array detection*, Chromatographia **1991**, 31, 465-468.

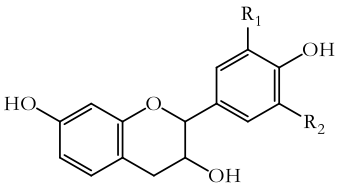
[32]Macheix, J. J.; Fleuriet, A.; Billot, J.; *The main phenolics of fruit.*En Fruit Phenolics. Press: Boca Ratón (FL, USA), **1990**, pp. 1-103.



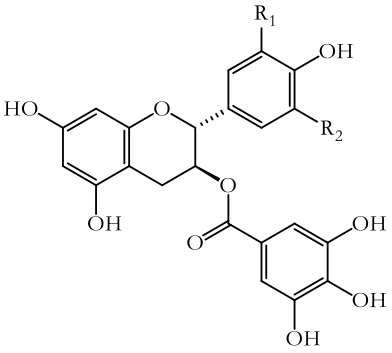

| Flavan-3-ol | R1 | R2 |
|---|---|---|
| (+)-Catequina | H | OH |
| (+)-Galocatequina | OH | OH |
| (+)-Afzelequina | H | H |

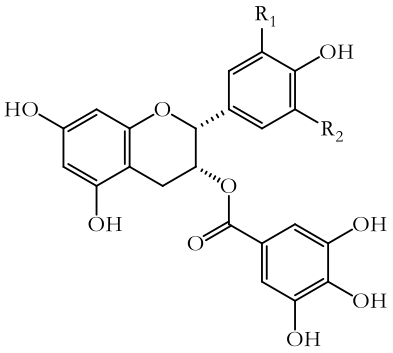
| Flavan-3-ol | R1 | R2 |
|---|---|---|
| (-)-Epicatequina | H | OH |
| (-)-Epigalocatequina | OH | OH |
| (-)-Epiafzelequina | H | H |




| Flavan-3-ol | R1 | R2 |
|---|---|---|
| (Epi)fisetinidol | OH | H |
| (Epi)robinetidol | OH | OH |

**Figura I.8** Estructuras químicas de los flavan-3-oles.




| Flavan-3-ol galoilado | R1 | R2 |
|---|---|---|
| (+)-Catequin-3-O-galato | H | OH |
| (+)-Galocatequin-3-O-galato | OH | OH |
| (+)-Afzelequin-3-O-galato | H | H |

| Flavan-3-ol galoilado | R1 | R2 |
|---|---|---|
| (-)-Epicatequin-3-O-galato | H | OH |
| (-)-Epigalocatequin-3-O-galato | OH | OH |
| (-)-Epiafzelequin-3-O-galato | H | H |

**Figura I.9** Estructura química de los flavan-3-oles galoilados.

Por otro lado, los flavan-3-oles también pueden encontrarse glicosilados; sin embargo, no existen amplios estudios sobre estos compuestos. En la naturaleza pueden aparecer 3-, 5- y 7-O-glicosilados, además de también haberse detectado 6- y 8-C-glicosilados[36]. Dichos compuestos han sido detectados en uvas[37] y en la madera de algunos árboles[38], así como en algunas plantas[39,40], legumbres[41]e incluso en el licor de cacao[42]. A modo de ejemplo, en la Figura I.10 se muestran la estructura de la (epi)catequina 3- y 7-O-glicosilada con glucosa.

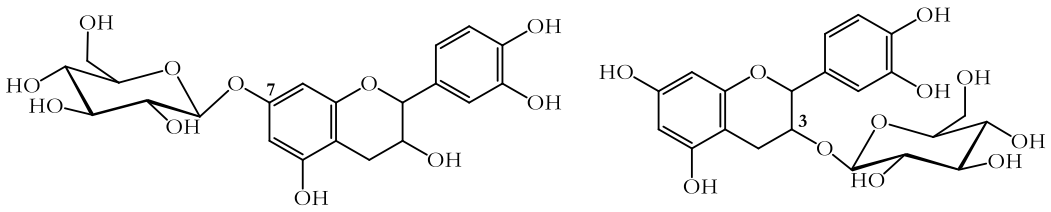


**Figura I.10** Estructura química (de izquierda a derecha) del (epi)catequin-7-O-glucósido y del (epi)catequin-3-O-glucósido.

### I.5.2. ANTOCIANINAS

Las antocianinas son los pigmentos responsables del color de las uvas y se localizan principalmente en la piel, excepto en las variedades tintoreras, en las que aparecen también en la pulpa. Las antocianinas se extraen al mosto durante la vinificación proporcionando el color rojo a los vinos tintos jóvenes y son las precursoras de los pigmentos derivados formados durante el envejecimiento del vino[43,44].

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[36]Friedrich, W.; Galensa, R.; *Identificationof a new flavanolglucoside from barley (Hordeumvulgare L.) and malt*, Eur. Food Res. Technol. **2002**, 214, 388-393.

[37]Vodnar, D. C.; Florina, L.; Vasile, F.; Crişan, G.; Socaciu, C.*; Identification of the bioactive compounds and antioxidant, antimutagenic and antimicrobial activities of thermally processed agro-industrial waste*, Food Chemistry **2017**, 231, 131-140.

[38]Bekker, M.; Bekker, R.; Brandt, V.E.; T*wo flavonoid glicosides and a miscellaneous flavan from the bark of Guibourtiacolesperma*, Phytochem. **2006**, 67, 818-823.

[39]Karioti, A.; Bilia, A. R.; Messori, L.; Skaltsa, H.; *Proanthocyanidin glycosides from the leaves of Quercus ilex L. (Fagaceae)*, Tetrahedron Let. **2009**, 50, 1771-1776.

[40]Cheng, X.; Guo, C.; Yang, Q.; Tang, X.; Zhang, C.; *Isolation and identification of radical scavenging components of seeds of Desmodiumstyracifolium*, Chemistry of Natural Compounds **2017**, 53, 36-39.

[41]Cui, E. J.; Song, N. Y.; Shrestha, S.; Chung, I. S.; Kim, J. Y.; Jeong, T. S.; Baek, N. I.; Flavonoid glycosides from cowpea seeds (Vignasinensis K.) inhibit LDL oxidation, Food Sci. and Biotechnology **2012**, 21 (2), 619-624.

[42]Hatano, T.; Miyatake, H.; Natsume, M.; Osakabe, N.; Takizawa, T.; Ito, H.; Yoshida, T.; *Proanthocyanidins glycosides and related polyphenols from cacao liquor and their antioxidant effects*,Phytochem.**2002**, 59, 749-758.

[43]Castañeda-Ovando, A.; Pacheco-Hernández, M. L.; Páez-Hernández, M. E.; Rodríguez, J. A.; Galán-Vidal, C. A.; *Chemical studies of anthocyanins: A review*, Food Chem. **2009**, 113, 859–871.

[44]Fernandes, I.; Faria, A.; Calhau, C.; de Freitas, V.; Mateus, N.; *Bioavailability of anthocyanins and derivatives*, J. Funct. Foods**2014**, 7, 54–66.

Son derivados hidroxilados o metoxilados del catión 3, 5, 7, 4´-tetrahidroxiflavilio (fenil-2-benzopirilio) (Figura I.11).

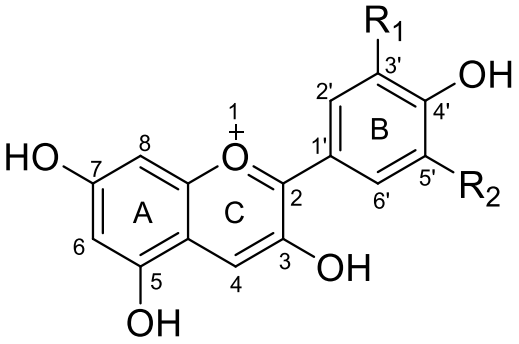



**Figura I.11** Estructura química del catión 3, 5, 7, 4´-tetrahidroxiflavilio.

La deficiencia del electrón del catión flavilio hace que las agliconas libres (antocianidinas) sean altamente reactivas[45] y no aparezcan libres en la naturaleza[46], sino que aparezcan en forma de glicósidos. Las antocianinas identificadas en uvas *Vitisvinifera* y en vinos elaborados con estas uvas son 3-monoglucósidos de cinco antocianidinas: delfinidina, cianidina, petunidina, peonidina y malvidina, diferenciándose entre ellas por el número y la posición de los grupos hidroxilo y metoxilo en el anillo B de las agliconas. Aparecen también derivados acilados de estos monoglucósidos, formados por esterificación en el carbono 6 de la glucosa con los ácidos acético, *p*-cumárico o cafeico. Un hombro en el espectro de absorción UV-vis en la zona de 320 nm es característico de una función acilo de un ácido hidroxicinámico (ácidos *p*-cumárico y cafeico). Las estructuras de las antocianinas, así como de los compuestos acilados se pueden observar en la Figura I.12.

Los compuestos monoglucósidos de las cinco antocianidinas, así como acetilglucósidos, *p*-cumaroilglucósidos y cafeoilglucósidos se han identificado en uvas y en vinos[47,48].También se ha identificado el isómero *cis* de lamalvidín-3-O-6-p-

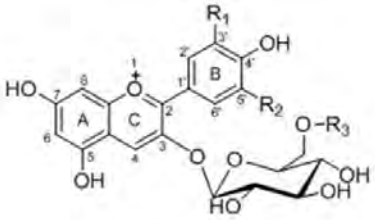
[45]Jackman, R. L.; Yada, R. Y.; Tung, M.; Speers, R. A.; *Anthocyanins as food colorants-A review*, J. Food Biochem.**1987**, 11, 201-247.

[46]Strack, D.; Wray, V.; Advances in research since 1986, Harborne (Ed.), Chapman & Hall, Londres**1993**, pp. 1-63.

[47]Chinnici, F.; Sonni, F.; Natali, N.; Galassi, S.; Riponi, C.;*Colour features and pigment composition of Italian carbonic macerated red wines*, Food Chem. **2009**, 113, 651-657.

[48]Revilla, I.; Pérez-Magariño, S.; González-SanJosé, M. L.; Beltrán, S.;*Identification of anthocyanin derivatives in grape skin extracts and red wines by liquid chromatography with diode array and mass spectrometric detection*, J.Chromatogr.A**1999**, 847, 83-90.

cumaroil glucósido (Mv-3-(6-p-coum)-glc) en uvas del género *Vitis* y vinos, cuya configuración espacial le hace más polar que el isómero *trans*[49,50].




| Antocianidina | $R_1$ | $R_2$ |
|---|---|---|
| Delfinidina | -OH | -OH |
| Cianidina | -OH | -H |
| Petunidina | $-OCH_3$ | -OH |
| Peonidina | $-OCH_3$ | -H |
| Malvidina | $-OCH_3$ | $-OCH_3$ |

| $R_3$ | |
|---|---|
| No acilada | H |
| acetil | OH $CH_3$ |
| p cumaroil | —OH—CH=CH— OH |
| cafeoil | —OC-HC=HC— OH, OH |

**Figura I.12** Estructura química de las antocianinas.

Las antocianinas presentan un espectro de absorción con dos bandas diferenciadas, una de ellas en la zona del visible (banda I) y otra banda en la zona UV (banda II).

El máximo de absorción en el visible está relacionado con el modelo de sustitución del anillo B, cuanto mayor es el número de sustituyentes oxigenados, mayor es la intensidad relativa Banda I/Banda II (efecto hipercrómico).

En las antocianinas trisustituidas, delfinidín-3-glucósido (Dp-3-glc), petunidín-3-glucósido (Pt-3-glc) y malvidín-3-glucósido (Mv-3-glc), el máximo de absorción está desplazado 10 nm respecto a las disustituidas, cianidín-3-glucósido (Cy-3-glc) y peonidín-3-glucósido (Pn-3-glc). La metilación del grupo hidroxilo no parece tener incidencia en la longitud de onda, no apreciándose el desplazamiento hipsocrómico típico de flavonoides metoxilados, probablemente debido a que la metilación se da en las posiciones 3´ y 5´, que afectan menos al cromóforo al no conjugarse efectivamente.

Las antocianinas tienen varias posiciones que pueden reaccionar con compuestos nucleófilos y electrófilos. Pueden sufrir un ataque nucleófilo en los

[49]García-Beneytez, E.; Cabello, F.; Revilla, E.; *Analysis of grape and wine anthocyanins by HPLC-MS.* J. Agric. Food Chem. **2003**, 51, 5622-5629.

[50]Li, S. Y.; He, F.; Zhu, B. Q.; Xing, R. R.; Reeves, M J.; Duan, C. Q.; *A systematic analysis strategy for accurate detection of anthocyanin pigments in red wines*, Rapid Commun. Mass Spectrom.**2016**, 30, 1619–1626.

carbonos C2 y C4 del anillo piránico C, la hidratación del C2 lleva a la forma hemiacetálica incolora. También pueden reaccionar con compuestos electrófilos por los grupos hidroxilos y los carbonos C6 y C8 del anillo floroglucinol (anillo A). La existencia del grupo hidroxilo en posición 5 es muy importante para la reactividad[51].

La deficiencia de carga está repartida en toda la molécula, no solo se encuentra en el oxígeno del anillo C, por lo tanto, es un catión ampliamente estabilizado por resonancia donde el anillo B es coplanar y también está involucrado en la deslocalización de la carga. Las distancias de enlaces de los anillos A y C son bastante similares, es decir, el anillo C tiene una estabilidad similar al sistema aromático, siendo la familia de flavonoides con el anillo C más estable y difícil de fragmentar. La ausencia de grupo ceto en la posición C4, presente en otras familias de flavonoides, hace que no tengan posiciones de fácil fragmentación.

En uvas que no sean de la especie *Vitis vinífera* se pueden encontrar antocianinas con un segundo resto glucosídico unido al grupo hidroxilo en la posición C5[52], sin embargo hay algunos estudios que muestran que pequeños niveles de antocianinas diglucosiladas pueden estar presentes en uvas *Vitis vinifera*[53].

El color de las antocianinas también cambia con el pH del medio. Así, las antocianinas se caracterizan por el hecho de que en disolución existen en equilibrio diversas especies ácido-base[54]. Las diferentes formas químicas de las antocianinas en función del pH del medio se muestran en la Figura I.13.

[51]De Freitas, V.; Mateus, N.; *Chemical transformations of anthocyanins yielding a variety of colours (Review)*, Environ.Chem.Lett.**2006**, 4, 175-183.

[52]Mazzuca, P.; Ferranti, P.; Picariello, G.; Chianese, L.; Addeo, F.; *Mass spectrometry in the study of the anthocyanins and their derivatives: differentiation of Vitisvinifera and hybrid grapes by liquid chromatography/electrospray ionization mass spectrometry and tandem mass spectrometry*, J. Mass Spectrom. **2005**, 40, 83-90.

[53]Baldi, A.; Romani, A.; Mulinacci, N.; Vincieri, F. F.; Casetta, B.; *HPLC/MS application to anthocyanins of Vitisvinifera L*, J. Agric. Food Chem. **1995**, 43, 2104-2109.

[54]Borkowski, T.; Szymusiak, H.; Gliszczyńska-Swiglo, A.; Tyrakowska, B.; *The effect of 3-O-β-glucosylation on structural transformations on anthocyanidins*, Food Res. Int. **2005**, 38, 1031-1037.

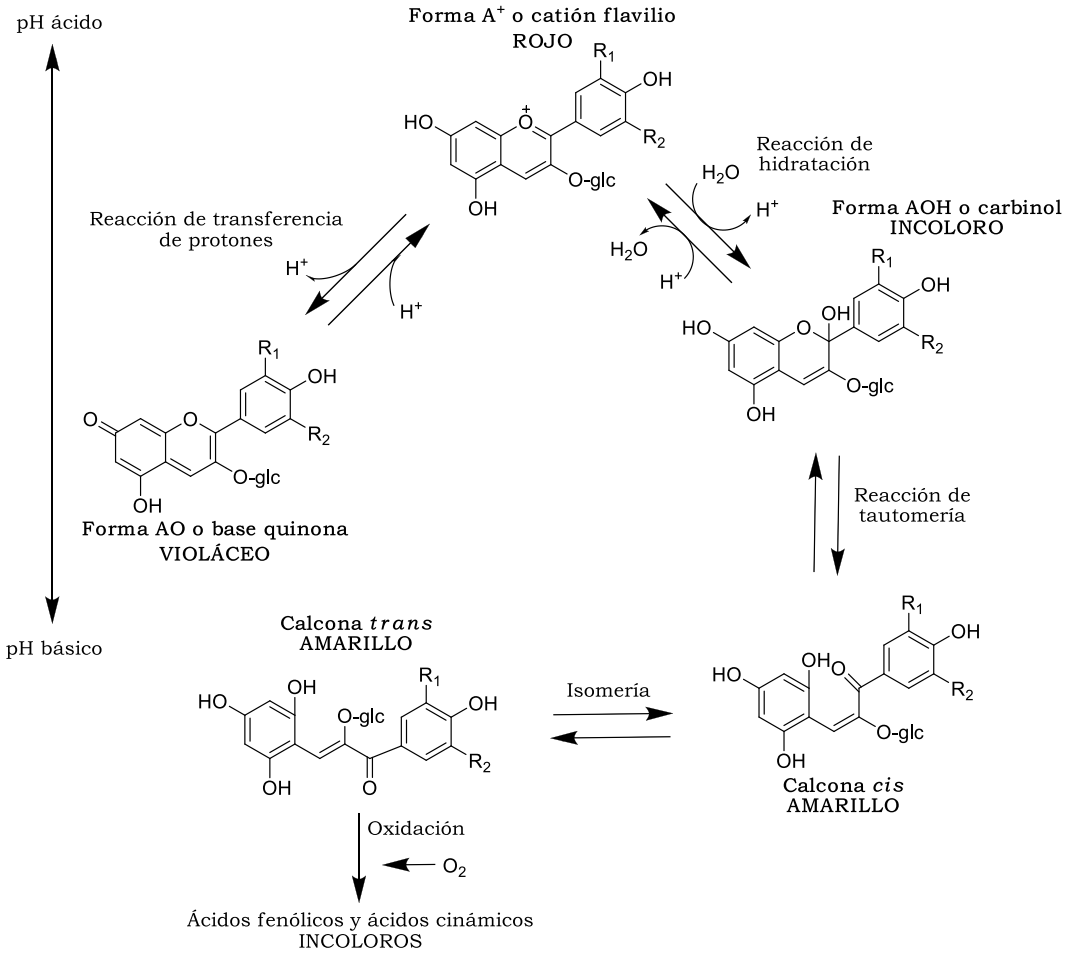



**Figura I.13** Equilibrios de las antocianinas en función del pH.

Al pH del vino, las antocianinas coexisten en equilibrio en formas diferentes, como catión flavilio ($A^+$), de color rojo, como base quinona (AO), de color violáceo, y como la forma hemiacetal incolora (AOH), siendo esta última la forma predominante al pH del vino[55,56]. Sólo el 20-30% de las antocianinas se encuentra en la forma coloreada flavilio a este pH, de manera que sólo ese porcentaje contribuye al color del vino.

Estos tipos de equilibrios ácido-base pueden desplazarse en función de las interacciones de las antocianinas con otras moléculas del medio, en un fenómeno conocido como copigmentación.

[55]Timberlake, C. F.; *Anthocyanins-occurrence, extraction and chemistry,* Food Chem. **1980**, 5, 69-80.

[56]Durner, D.; Carle, R; Schweiggert, R. M.; *Improvement and Stabilization of Red Wine Color*, J. Food Sci. Technol. **2016**, 295, 239-264.

### I.5.3. COPIGMENTACIÓN

La copigmentación consiste en un desplazamiento del equilibrio de hidratación de las antocianinas como resultado de la interacciónπ-π entre la estructura plana de la antocianina y otras moléculas que actúan como copigmentos.

Las moléculas que pueden actuar como copigmentos requieren una disposición plana, debido a que es necesario un apilamiento con el plano de la antocianina[57].

Las antocianinas en formas coloreadas, es decir, en forma flavilio ($A^+$) y base quinoidal (AO) pueden dar lugar a este tipo de interacción no covalente con otras moléculas del medio. Estas interacciones provocan desplazamientos del equilibrio de las antocianinas hacia formas coloreadas[58], limitando de esta forma el efecto decolorante de una acidez débil.

Este tipo de complejos adopta una configuración tipo sándwich que protege el grupo cromóforoflavilio del ataque nucleófilo del agua, reduciendo de esta forma la proporción de las formas hemiacetal y chalcona. El resultado final es una mayor intensidad de la proporción de color de la que cabría esperar en función del pH del medio.

Existen diferentes tipos de compuestos que pueden actuar como copigmentos, entre ellos aminoácidos, nucleótidos, hidratos de carbono y compuestos fenólicos, incluso los cromóforos de las antocianinas pueden actuar entre ellos, así como residuos aromáticos de la propia molécula (copigmentación intramolecular)[59]. La copigmentación intramolecular es más eficiente en la protección frente al ataque nucleófilo del agua que la intermolecular.

Entre los compuestos fenólicos, los flavonoles y los ácidos hidroxicinámicos presentan una mayor inclinación a actuar como copigmentos[60] durante la

[57]Álvarez, I.; Aleixandre, J. L.; García, M. J.; Lizama, V.; *Effect of the prefermentative addition of copigments on the polyphenolic composition of Tempranillo wines after malolacticfermentation,* Eur. Food Res. Technol. **2009**, 228, 501-510.

[58]González-Manzano, S.; Mateus, N.; de Freitas, V.; Santos-Buelga, C.; *Influence of the degree of polymerisation in the ability of catechins to act as anthocyanin copigments*, Eur.Food Res. Technol. **2008**, 227, 83-92.

[59]Figueiredo, P.; George, F.; Tatsuzawa, F.; Toki, K.; Saito, N.; Brouillard, R.; *New features of intramolecularcopigmentation by acylatedanthocyanins*, Phytochem. **1999**, 51, 125-132.

[60]Escott, C.; Morata, A.; Loira, I.; Tesfaye, W.; Suarez-Lepe, J. A.; *Characterization of polymeric pigmetns and pyranoanthocyanins formed in microfermentations of non-Saccharomyces yeasts*, J. Appl. Microbiol. **2016**, 121, 1346-1356.

fermentación, aunque parece estar relacionado con la variedad de la uva[61]. Aunque su proporción en el vino sea considerablemente mayor, los flavanoles presentan menor inclinación, atribuyéndose esta poca tendencia a una estructura no plana que impide el acercamiento a la antocianina[62].

La presencia de etanol en el medio es un factor limitante de esta interacción, debido a que la presencia de disolventes orgánicos debilita las interacciones hidrofóbicas intermoleculares, ocasionando la desestabilización de los complejos formados[63].

Se ha observado que un aumento del grado de metoxilación del anillo B de la antocianina aumenta la magnitud de estas interacciones. Este tipo de interacciones aumenta la intensidad del color de los vinos tintos.

El estudio de estos procesos de copigmentación es de gran importancia para establecer la relación entre la composición de la uva y el color del vino. Se piensa incluso que este tipo de interacciones son el primer paso en la formación de nuevos pigmentos que determinan el color de los vinos envejecidos[64].

### I.5.4. DERIVADOS ANTOCIÁNICOS

La concentración de las antocianinas empieza a descender en los primeros pasos de la elaboración del vino debido a la actividad de las levaduras; y después, por reacciones de condensación, polimerización, oxidación y precipitación.

Algunas de estas reacciones implican la degradación de la antocianina, pero otras llevan a la formación de pigmentos que proporcionan diferentes matices al vino. Los cambios de color del vino que tienen lugar durante el envejecimiento del vino se

[61]García-Marino, M.; Hernández-Hierro, J. M.; Rivas-Gonzalo, J. C.; Escribano-Bailón, M. T.; *Colour and pigment composition of red wines obtained from comaceration of Tempranillo and Graciano varieties*, Anal. Chim.Acta**2010**, 660, 134-142.

[62]Gónzalez-Manzano, S.; Dueñas, M.; Rivas-Gonzalo, J. C.; Escribano-Bailón, M. T.; Santos-Buelga, C.; *Studies on the copigmentation between anthocyanins and flavan-3-ols and their influence in the colour expresión of red wine*, Food Chem. **2009**, 114, 649-656.

[63]Gónzalez-Manzano, S.; Santos-Buelga, C.; Dueñas, M.; Rivas-Gonzalo, J. C.; Escribano-Bailón, T.; *Colour implications of self-association processes of wine anthocyanins*, Eur.Food Res. Technol. **2008**, 226, 483-490.

[64]Hermosín-Gutiérrez, I.; Sánchez-Palomo-Lorenzo, E.; Vicario-Espinosa, A.; *Phenolic composition and magnitude of copigmentation in young and shortly aged red wines made from the cultivars Cabernet Sauvignon, Cencibel and Syrah*, Food Chem. **2005**, 92, 269-283.

atribuyen a reacciones que tienen lugar entre las antocianinas y otras moléculas presentes en el medio y que llevan a la formación de nuevos pigmentos más estables[65].

Estos pigmentos se pueden clasificar en dos grandes grupos, los pigmentos formados por condensaciones de antocianinas con flavanoles, que pueden tener lugar de forma directa o mediante acetaldehído y las piranoantocianinas, producto de las reacciones de cicloadición de las antocianinas con otras moléculas del medio, como ácido pirúvico, acetaldehído, ácidos hidroxicinámicos, vinilflavanoles, etc.

Se pueden diferenciar dos tipos de pigmentos en base a su comportamiento con el cambio de pH del medio[66]: un primer tipo con un comportamiento similar al de las antocianinas, de este tipo serían los compuestos formados por condensación directa flavanol-antocianina; la otra clase de pigmentos se caracteriza por su alta resistencia a la hidratación y a la decoloración por bisulfito, a este grupo pertenecen las piranoantocianinas y los pigmentos formados por condensación antocianina-flavanol mediada por acetaldehído, que son más resistentes que las antocianinas precursoras[67], aumentando de este modo la estabilidad del color de los vinos envejecidos.

### I.5.4.1. Condensaciones de antocianinas con flavanoles

Los pigmentos formados por condensaciones entre antocianinas y flavanoles pueden darse de forma directa[68,69,70] o mediada por acetaldehído[71,72,73], generándose un puente de etilideno en la estructura.

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[65]Marquez, A.; Serratosa, M. P.; Merida, J.; *Influence of bottle storage time on colour, phenolic composition and sensory properties of sweet red wines*, Food Chemistry **2014**, 146, 507-514.

[66]Es-Safi, N. E.; Fulcrand, H.; Cheynier, V.; Moutounet, M.*; Studies on the acetaldehyde-induced condensation of (-)-epicatechin and malvidin-3-O-glucoside in a model solution system,* J. Agric. Food Chem. **1999**, 47, 2096-2102.

[67]Salas.C.; Le Guernevé, C.; Fulcrand, H.; Poncet-Legrand, C.; Cheynier, V.; *Structure determination and colour properties of a new directly linked flavanol-anthocyanin dimer*, TetrahedronLett.**2004**, 45, 8725-8729.

[68]Ribereau-Gayon, P. in: P. Markakis (Ed), Anthocyanins as Food Colors, Academic Press Inc., London **1982**, p. 209.

[69]Remy, S.; Fulcrand, H.; Labarbe, B.; Cheynier, V.; Moutounet, M.; *First confirmation in red wine of products resulting from direct anthocyanin-tannin reactions*, J. Sci. Food Agric. **2000**, 80, 745-751.

[70]Willemse, C. M.; Stander, M. A.; Vestner, J.; Tredoux, A. G. J.; de Villiers, A.; *Comprehensive two dimensional hydrophilic interaction chromatography (HILIC) × reversed-phase liquid chromatography coupled to high-resolution mass spectrometry (RP-LC-UV-MS). Analysis of anthocyanins and derived pigments in red wine*, Analytical Chemistry **2015**, 87, 12006-12015.

[71]Rivas-Gonzalo, J. C.; Bravo-Haro, S.; Santos-Buelga, C.; *Detection of compounds formed through the reaction of malvidin-3-monoglucoside and catechin in the presence of acetaldehyde*, J. Agric. Food Chem. **1995**, 43, 1444-1449.

[72]Francia-Aricha, E. M.; Guerra, M. T.; Rivas-Gonzalo, J. C.; Santos-Buelga, C.; *New anthocyanin pigments formed after condensation with flavanols*, J. Agric. Food Chem. **1997**, 45, 2262-2266.

**a) Condensación directa antocianina-flavanol**

Existen dos mecanismos para la formación de pigmentos formados por condensación directa antocianina-flavanol que dan lugar a pigmentos del tipo F-$A^+$ o $A^+$-F, según la posición relativa del flavanol y la antocianina en la estructura del compuesto.

En la formación de pigmentos del tipo F-$A^+$ tiene lugar una catálisis ácida del enlace interflaváníco de una proantocianidina, formándose el carbocatión intermedio $F^+$, que actúa como electrófilo reaccionando con la forma hemiacetálica hidratada de la antocianina AOH, que actúa como nucleófilo. Esta reacción da lugar a un compuesto incoloro del tipo F-AOH, que se deshidrata y se obtiene el pigmento en forma flavilio F-$A^+$ rojo[74]. El esquema de formación de este tipo de compuestos se puede observar en la FiguraI.14.

La ruptura ácida del enlace interflaváníco de la proantocianidina está lógicamente favorecida a pH bajo, pero se ha demostrado que también ocurre a pH=3.2, por lo que este tipo de compuestos pueden formarse en el vino[75].

El enlace flavanol-antocianina en compuestos de tipo F-$A^+$ puede tener lugar entre el carbono C4 de la unidad superior del pigmento y los carbonos C6 o C8 de la unidad inferior, siendo los compuestos que presentan la unión C4-C8 más estables. Se ha detectado un posible derivado de (+)-catequin-Mv-3-glc con el enlace interflaváníco en las posiciones C4-C6 en disoluciones modelo[76]. La espectrometría de masas no permite distinguir si la unión entre el flavanol y la antocianina se produce entre los carbonos C4-C8 o C4-C6.

[73]Lee, D. F.; Swinny, E. E.; Jones, G. P.;*NMR identification of ethyl-linked anthocyanin-flavanol pigments formed in model wine ferments*, Tetrahedron Lett.**2004**, 45, 1671-1674.

[74]Remy-Tanneau, S.; Le Guernevé, C.; Meudec, E.; Cheynier, V.; *Characterization of a colorless anthocyanin-flavan-3-ol dimer containing both carbon-carbon and ether interflavanoid linkages by NMR and mass spectrometry*, J. Agric. Food Chem. **2003**, 51, 3592-3597.

[75]Vidal, S.; Cartadale, J.; Souquet, H.; Fulcrand, V.; Cheynier, V.*; Changes in proanthocyanidin chain length in winelike model solutions*, J. Agric. Food Chem. **2002**, 50, 2261-2266.

[76]Salas, E.; Atanasova, V.; Poncet-Legrand, C.; Meudec, E.; Mazauric, J. P.; Cheynier, V.;*Demonstration of the occurrence of flavanol-anthocyanin adducts in wine and in model solutions*. Anal.Chim.Acta**2004**, 513, 325-332.

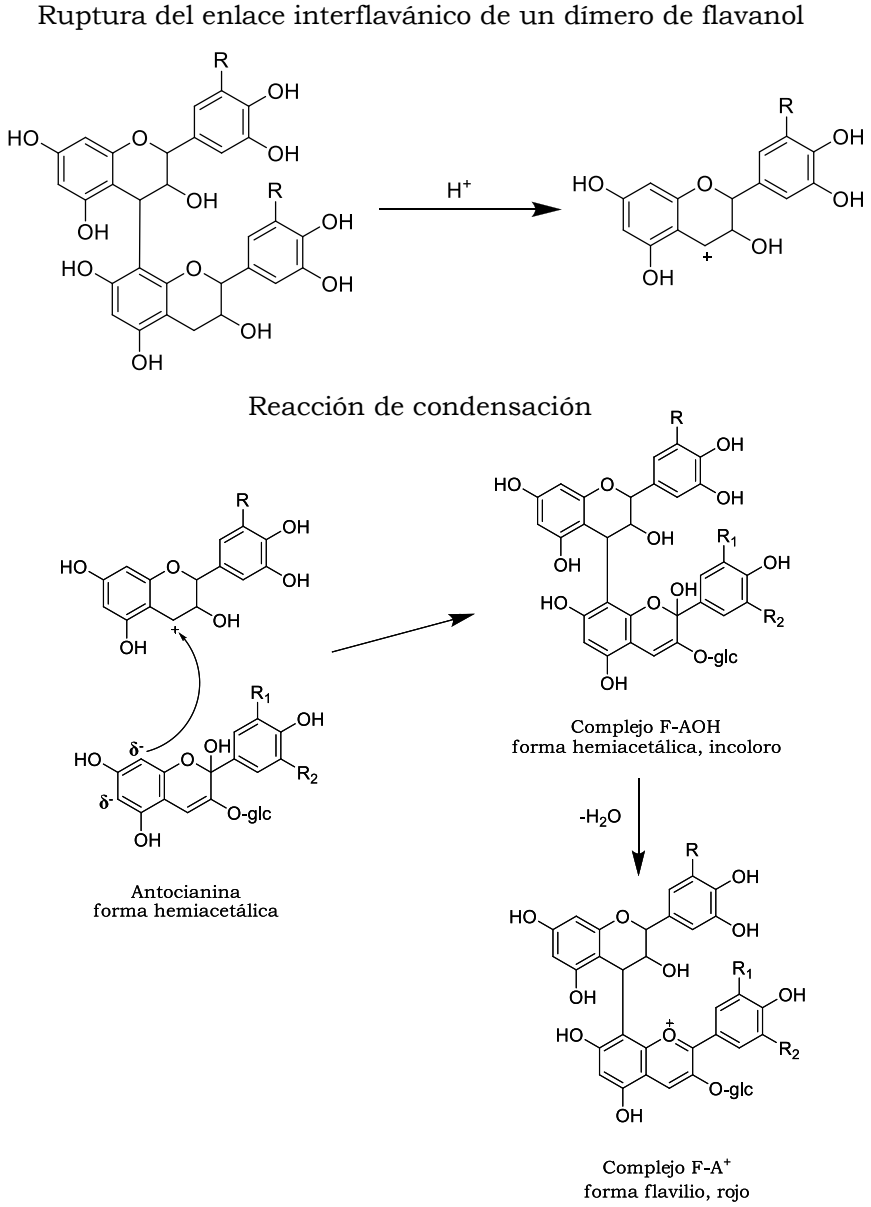


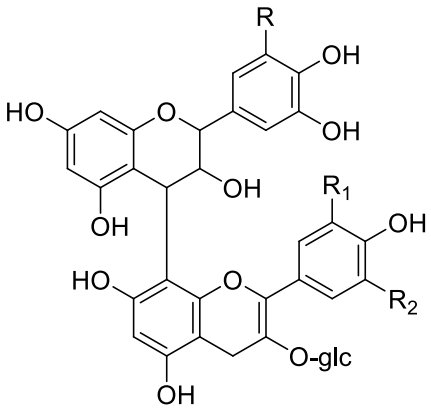

**Figura I.14** Esquema de formación de compuestos de tipo F-A+, a partir de un dímero de flavanol.

Este tipo de compuestos se han detectado en vino, tanto en forma flavilio como flaveno incolora[77,78], indicando que la forma flaveno de la antocianina también presenta carácter nucleófilo y tiene lugar la reacción con el carbocatión del flavanol, al

[77] Sánchez-Ilárduya, M. B.; Sánchez-Fernández, C.; Viloria-Bernal, M.; Lopez-Marquez, D. M.; Berrueta, L. A.; Gallo, B.; Vicente, B.; *Mass spectrometry fragmentation pattern of coloured flavanol-anthocyanin and anthocyanin-flavanol derivatives in aged red wines of Rioja*, Aust. J. Grape Wine Res. **2012**, 18(2), 203-214.

[78] Sánchez-Ilárduya, M. B.; Sánchez-Fernández, C.; Garmón-Lobato, S.; Abad-García, B.; Berrueta, L. A.; Gallo, B.; Vicente, B.; *Detection of non-coloured anthocyanin-flavanol derivatives in Rioja aged red wines by liquid chromatography-mass spectrometry*, Talanta**2014**, 121, 81-88.

igual que ocurría con la antocianina en forma hidratada[79]. Si los pigmentos se encuentran en forma flaveno, su peso molecular es dos unidades mayor que el correspondiente a la forma flavilio. La estructura de este tipo de compuestos en los que la antocianina se encuentra en forma flaveno se muestra en la Figura I.15.




Complejo F-A
forma flaveno, incoloro

**Figura I.15** Estructura química de compuestos de tipo F-A, en el que la antocianina se encuentra en la forma incolora flaveno.

Para la formación de pigmentos de tipo $A^+$-F, la antocianina está en forma flavilio ($A^+$) y actúa como electrófilo. Por otro lado, los grupos hidroxilo de los carbonos C5 y C7 del flavanol tienen efecto mesomérico y le confieren al flavanol carácter nucleófilo en los carbonos C6 y C8. Por lo tanto, tiene lugar una adición nucleófila del flavanol sobre el catión flavilio, dando lugar a un complejo en el que la antocianina se encuentra en la forma incolora flaveno. Este complejo se puede oxidar a la forma coloreada flavilio y posteriormente a una sal xantilio amarilla, o evolucionar a un producto de condensación cíclico incoloro con un enlace de tipo A [A-(C4-C8), (C2-O-C7)-F][80,81], cuya estructura se ha caracterizado mediante RMN[82], siendo también posible que el enlace éter se forme entre los carbonos C2 y C5 de la antocianina y el flavanol, respectivamente. El mecanismo de formación de condensaciones

[79]Salas, E.; Atanasova, V.; Poncet-Legrand, C.; Meudec, E.; Mazauric, J. P.; Cheynier, V.; *Demonstration of the occurrence of flavanol-anthocyanin adducts in wine and in model solutions*, Anal. Chim.Acta**2004**, 513, 325-332.

[80]Flamini, R.; *Mass Spectrometry in grape and wine chemistry. Part I: Polyphenols*. Mass Spectrom.Rev.**2003**, 22, 218-250.

[81]Bishop, P. B.; Nagel, C. W.; *Characterization of the condensation product of malvidin-3,5-diglucoside and catechin*, J. Agric. Food Chem. **1984**, 32, 1022-1026.

[82]Remy-Tanneau, S.; Le Guernevé, C.; Meudec, E.; Cheynier, V.; *Characterization of a colorless anthocyanin-flavan-3-ol dimer containing both carbon-carbon and ether interflavanoid linkages by NMR and mass spectrometry*, J. Agric. Food Chem. **2003**, 51, 3592-3597.

antocianina-flavanol con enlace de tipo A podría ocurrir desde el intermedio flaveno mediante una adición intramolecular del grupo hidroxilo del flavanol al doble enlace C2-C3. Un esquema de esta ruta de formación se puede observar en la Figura I.16.

Estos compuestos en los que se forma un enlace bicíclico de tipo A son resistentes a la tiolisis, a diferencia de los pigmentos en los que la antocianina se encuentra en forma flaveno[83]. Se han detectado compuestos incoloros de este tipo en extractos de vino[84]. El peso molecular de los compuestos que presentan en su estructura un enlace de tipo A es dos unidades mayor que el correspondiente a compuestos en los que la antocianina se encuentra en forma flavilio.

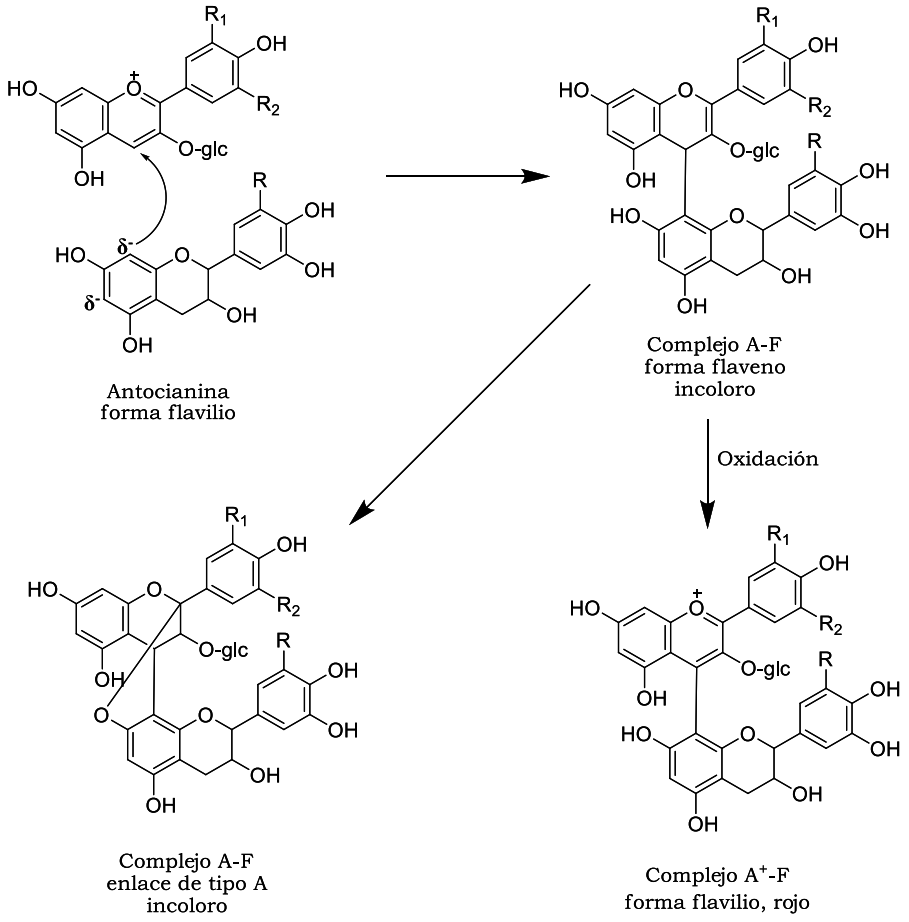



**Figura I.16** Esquema de formación de compuestos de tipo A-F.

[83]Salas, E.; Dueñas, M; Schwarz, M.; Winterhalter, P.; Cheynier, V.; Fulcrand, H.;*Characterization of pigments from different high speed countercurrent chromatography wine fractions,* J. Agric. Food Chem. **2005**, 53, 4536-4546.

[84]Remy, S.; Fulcrand, H.; Labarbe, B.; Cheynier, V.; Moutounet, M.; *First confirmation in red wine of products resulting from direct anthocyanin-tannin reactions*, J. Sci. Food Agric. **2000**, 80, 745-751.

Al pH del vino, las antocianinas reaccionan simultáneamente como agentes nucleófilos y electrófilos. Ambos tipos de reacciones son dependientes del pH, ya que dependen de la proporción de formas catiónicas, $A^+$ o $F^+$, que aumenta con la acidez.

Los elagitaninos de la madera pueden favorecer la formación de productos de condensación directa entre antocianinas y flavanoles, además el $O_2$ favorece la ruptura de las proantocianidinas, alcanzando éstas sus niveles más altos de concentración en los primeros meses de envejecimiento en barrica.

Este tipo de derivados presenta desplazamientos batocrómicos en la longitud de onda de máxima absorción del espectro UV-vis, es decir, hacia valores mayores, aportando al vino tonalidades violáceas[85].

**b) Condensación antocianina-flavanol mediante acetaldehído**

El acetaldehído, generado por la oxidación de etanol[86] o por el metabolismo de las levaduras, está presente de forma natural en el vino. Se forma en mayor concentración en condiciones oxidantes, por eso los mecanismos en los que participa están favorecidos en estas condiciones.

La formación de pigmentos resultantes de la condensación entre antocianinas y flavanoles mediada por acetaldehído requiere pH ácido, para que el acetaldehído esté en forma catiónica. Una vez que se ha producido la protonación del acetaldehído, tiene lugar la adición del catión resultante a una posición nucleófila del anillo A del flavanol, que pueden ser los carbonos C6 o C8 del anillo floroglucinol, formándose un producto inestable que se protona dando lugar al catión etil-flavanol. Este catión sufre el ataque nucleófilo de la antocianina para dar lugar al pigmento derivado antocianina-etil-flavanol[87,88,89]. El ataque nucleófilo entre los dos anillos floroglucinol

[85]Macz-Pop, G. A.; González-Paramás, A. M.; Pérez-Alonso, J. J.; Rivas-Gonzalo, J. C.; *Newflavanol-anthocyanin condensed pigments and anthocyanin composition in Guatemala beans*. J. Agric. Food Chem. **2006**, 54, 536-542.

[86]Francia-Aricha, E. M.; Rivas-Gonzalo, J. C.; *Effect of malvidin-3-monoglucoside on the browning of monomeric and dimericflavanols*. Z. Lebensm. Unters.Forsch.A**1998**, 207, 223-228.

[87]Pissarra, J.; Lourenco, S.; González-Paramás, A. M.; Mateus, N.; Santos-Buelga, C.; Silva, A. M. S.; de Freitas, V.; Structural *Characterization of new malvidin-3-glucoside-catechin aryl/alkyl-linked pigments*, J. Agric. Food Chem. **2004**, 52, 5519-5526.

[88]Sheridan, M. K.; Elias, R. J.; *Reaction of Acetaldehyde with Wine Flavonoids in the Presence of Sulfur Dioxide*, J. Agric. Food Chem. **2016**, 64 (45), 8615-8624.

[89]Dipalmo, T.; Crupi, P.; Pati, S.; Clodoveo, M- L.; Di Luccia, A.; *Studying the evolution of anthocyanin-derived pigments in a typical red wine of Southern Italy to assess its resistance to aging*, LWT-Food Sci. Technol. **2016**, 71, 1-9.

A (flavanol y antocianina) puede ser por los carbonos C6 o C8, pero es más probable que tenga lugar por la posición C8 porque se estabiliza mejor la carga negativa[90].

El catión etil-flavanol puede sufrir una pérdida de agua dando lugar al vinilflavanol, que puede reaccionar también con las antocianinas proporcionando otros derivados de tipo piranoantociánico que se describirán posteriormente.

El esquema de formación de este tipo de derivados se puede observar en la FiguraI.17.

Este tipo de compuestos se han detectado tanto en vino como en disoluciones modelo[91,92].Entre los compuestos formados por condensación mediada por acetaldehído se pueden formar diferentes isómeros, por un lado diastereoisómeros (R, S) debido al carbono asimétrico del puente de etilideno y, por otro lado, regioisómeros (C8-C8 o C8-C6) debido a las diferentes posiciones de unión de la unidad de flavanol a la antocianina[93].

Su formación y pérdida es rápida[94], más que la reacción de formación de productos de condensación directa. Estos compuestos son más estables frente a cambios de pH y decoloración por $SO_2$, sin embargo, son menos estables en disoluciones acuosas debido a la ruptura del puente de etilideno que une la antocianina al flavanol96.

Estos pigmentos son rojizo-azulados y proporcionan matices violáceos al vino, en especial inmediatamente después de terminar la fermentación. En general, el enlace alquil-interflavonoide produce desplazamientos batocrómicos de unos 15 nm, si bien la naturaleza del alquilo no parece influir.

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[90]De Freitas, V.; Mateus, N.; *Chemical transformations of anthocyanins yielding a variety of colours (Review)*, Environ. Chem. Lett. **2006**, 4, 175-183.

[91]He, F.; Liang, N. N.; Mu, L.; Pan, Q. H.; Wang, J.; Reeves, M. J.; Duan, C. Q.; *Anthocyanins and their variation in red wines II. Anthocyanin derived pigments and their color evolution*, Molecules **2012**, 17(2), 1483-1519.

[92]Dipalmo, T.; Crupi, P.; Pati, S.; Clodoveo, M. L.; Di Luccia, A.; *Studying the evolution of anthocyanin-derived pigments in a typical red wine of Southern Italy to assess its resistance to aging*, LWT-Food Sci. Technol**2016**, 71, 1-9.

[93]Marquez, A.; Serratosa, M. P.; Merida, J.; *Influence of bottle storage time on color, phenolic composition and sensory properties of sweet*, Food Chem. **2014**, 146, 507-514.

[94]Cano-López, M.; Pardo-Mínguez, F.; Schmauch, G.; Saucier, C.; Teissedre, P. L.; López-Roca, J. M.; Gómez-Plaza, E.;*Effect of micro-oxygenation on color and anthocyanin-related compounds of wines with different phenolic contents*,J. Agric. Food Chem. **2008**, 56, 5932-5941.

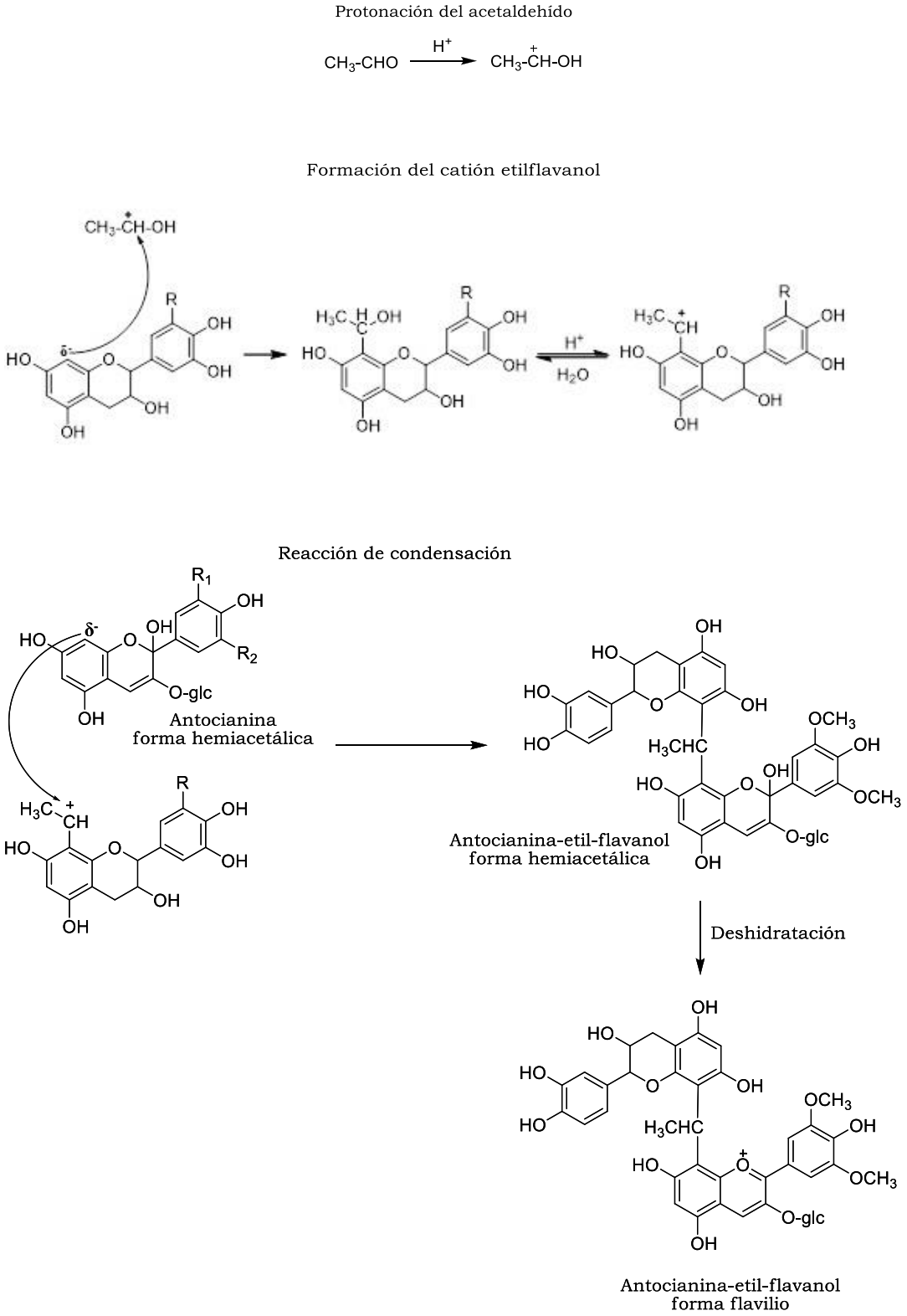


**Figura I.17** Esquema de formación de compuestos de tipo A-etil-F.

### I.5.4.2. Piranoantocianinas

El otro gran grupo de derivados antociánicos se forma por una reacción de cicloadición nucleófila de compuestos presentes en el vino a la antocianina en forma de catión flavilio, dando lugar a la formación de un nuevo anillo piránico en la estructura entre el carbono C4 y el grupo hidroxilo en la posición 5 de la antocianina[99]. Debido a la formación de este nuevo anillo piránico, este tipo de compuestos recibe el nombre de piranoantocianinas, cuya estructura básica se muestra en la FiguraI.18, siendo R diferente tipo de sustituyente para cada familia de derivado piranoantociánico distinta.

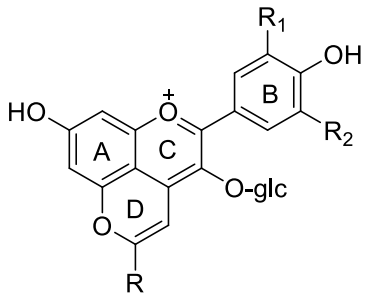


**Figura I.18** Estructura química básica de las piranoantocianinas.

Inicialmente se pensaba que este tipo de pigmentos se formaban por condensación directa entre antocianinas y flavanoles o a través de una molécula de acetaldehído. Sin embargo en los últimos años se ha demostrado que las antocianinas pueden reaccionar con otros compuestos de bajo peso molecular como el ácido pirúvico[100], acetaldehído[101], ácido acetoacético[102], ácidos hidroxicinámicos[103], y vinilflavanoles[104], obteniéndose así esta familia de derivados antociánicos a los que se les llamó piranoantocianinas. Esta familia incluye una gran cantidad de compuestos

[99]De Freitas, V.; Mateus, N.; *Formation of pyranoanthocyanins in red wines: a new and diverse class of anthocyanin derivatives*, Anal. Bioanal. Chem. **2011**, 401(5), 1467-1477.

[100]Dipalmo, T.; Crupi, P.; Pati, S.; Clodoveo, M. L.; Di Luccia, A.; *Studying the evolution of anthocyanin-derived pigments in a typical red wine of Southern Italy to assess its resistance to aging*, LWT-Food Sci. Technol. **2016**, 71, 1-9.

[101]Benabdeljalil, C.; Cheynier, V.; Fulcrand, H.; Hakiki, A.; Mosaddak, M.; Moutounet, M.; *Evidence of new pigments resulting from reaction between anthocyanins and yeast metabolites*, Sci. Aliments **2000**, 20, 203-220.

[102]Lu, Y.; Foo, L. Y.; *Unusual anthocyanin reaction with acetone leading to pyranoanthocyaninformation*, Tetrahedron Lett. **2001**, 42, 1371-1373.

[103]De Villiers, A.; Cabooter, D.; Lynen, F.; Desmet, G.; Sandra, P.; *High-efficiency high performance liquid chromatographic analysis of red wine anthocyanins*, J. Chromatogr. A **2011**, 1218 (29), 4660-4670.

que pueden reaccionar de nuevo produciendo otra generación de compuestos[105], donde los precursores son los derivados antociánicos[106], como el caso de las portisinas[107], llamadas así porque la mayoría de compuestos de este tipo se han detectado en vinos de Oporto.

Estos derivados antociánicos se forman durante la fermentación y envejecimiento del vino y contribuyen al cambio progresivo del color rojo-púrpura de los vinos jóvenes a un color más anaranjado. Sin embargo, esta no es la única característica importante de este tipo de compuestos. Mientras las antocianinas pierden casi el 80% de la intensidad de color cuando se incrementa el pH[108] como resultado de la formación de la base incolora carbinol, el color de las piranoantocianinas prácticamente no varía debido a que la reacción de cicloadición C4/C5 proporciona gran estabilidad química al compuesto ya que protege al C4 del ataque nucleófilo del agua evitando la formación de la base incolora carbinol y protege de la decoloración por $SO_2$, además de proporcionar resistencia a la oxidación[109].

La gran estabilidad del nuevo anillo piránico hace que en los espectros de masas de este tipo de derivados sólo se observen las pérdidas de la glucosa o de la glucosa acilada, en su caso y en el caso de derivados de antocianinas con vinilflavanoles se observa también el ión fragmento producto de la ruptura retro Diels-Alder del flavanol; es decir, en este tipo de experimentos no se rompe el nuevo anillo piránico formado. Tampoco se observa su fragmentación en experimentos de MS/MS, presentando los pigmentos derivados de este tipo el mismo patrón de fragmentación que la correspondiente antocianina precursora, pudiéndose establecer de este modo una especie de huella dactilar de cada antocianina.

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[104] Blanco-Vega, D.; Gómez-Alonso, S.; Hermosín-Gutiérrez, I.; *Identification, content and distribution of anthocyanins and low molecular weight anthocyanin-derived pigments in Spanish commercial red wines*, Food Chem. **2014**, 158, 449-458.

[105] Marquez, A.; Serratosa, M. P.; Merida, J.; *Pyranoanthocyanin derived pigments in wine: structure and formation during winemaking. A review*, J. Chem. **2013**, ID 713028, 1-15.

[106] Oliveira, J.; Azevedo, J.; Silva, A. M. S.; et al.; *Pyranoanthocyanin dimers: a new family of turquoise blue anthocyanin-derived pigments found in port wine*, J. Agric. Food Chem. **2010**, 58(8), 5154-5159.

[107] Carvalho, A.; Oliveira, J.; de Freitas, V.; Mateus, N.; Melo, A.; *Unusual color change of Vinylpyranoanthocyanin-phenolic pigments*, J. Agric. Food Chem. **2010**, 58, 4292-4297.

[108] Bakker, B.; Timberlake, C. F..; *Isolation, identification and characterization of new color-stable anthocyanins occurring in some red wines*, J. Agric. Food Chem. **1997**, 45, 35-43.

[109] Morata, A.; Gómez-Cordovés, M. C.; Calderón, F.; Suárez, J. A.; *Effects of pH, temperature and SO2 on the formation of pyranoanthocyanins during red wine fermentation with two species of Saccharomyces*, Int. J. Food Microbiol. **2006**, 106(2), 123-129.

Este tipo de compuestos, exceptuando a los pigmentos conocidos como portisinas, presenta desplazamientos hipsocrómicos en la longitud de onda de máxima absorción del espectro UV-vis, proporcionando al vino una tonalidad teja[110].

Una posible explicación para este efecto hipsocrómico observado puede ser que la carga del anillo C podría estar deslocalizada por resonancia y estar parcialmente situada en el átomo de oxígeno del nuevo anillo D formado. Se observa también una pequeña banda en la zona de 370 nm, característica de las antocianinas 4-sustituidas[111].

**a) Derivados con ácido pirúvico, acetaldehído y ácido acetoacético**

El ácido pirúvico es el intermedio metabólico más importante, proviene del metabolismo de la levadura durante la fermentación alcohólica y también por la actividad de la bacteria láctica durante la fermentación maloláctica[112].

Se han postulado dos estructuras para este tipo de piranoantocianinas, por un lado, Bakker y Timberlake[113] propusieron una estructura en base a experimentos de RMN para un nuevo compuesto derivado de Mv-3-glc llamado *Vitisina A*, que no estaba presente en uvas frescas, sólo en uvas almacenadas o en vinos envejecidos. Esta estructura consistía en un núcleo de Mv-3-glc con un anillo piránico adicional con un sustituyente ceto y un grupo hidroxietileno, que se puede observar en la Figura I.19

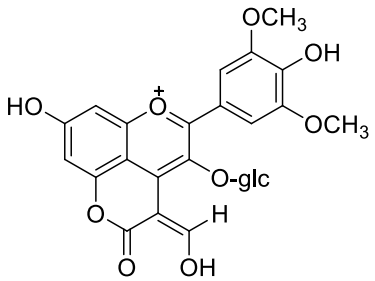



**Figura I.19** Estructura química del derivado de la antocianina con ácido pirúvico propuesta por Bakker y Timberlake.

[110]Morata, A.; Calderón, F.; González, M. C.; Gómez-Cordovés, M. C.; Suárez, J. A.; *Formation of the highly stable pyranoanthocyanins (Vitisins A and B) in red wines by the addition of pyruvic acid and acetaldehyde*, Food Chem. **2007**, 100(3), 1144-1152.

[111]Oliviera, J.; Fernandes, V.; Miranda, C. et al.; *Color properties of four cyanidin-pyruvic acid adducts*, J. Agric. Food Chem. **2006**, 54, 6894-6903.

[112]Morata, A.; Calderón, F.; González, M. C.; Gómez-Cordovés, M. C.; Suárez, J. A.; *Formation of highly stable pyranoanthocyanins (vitisins A and B) in red wines by the addition of pyruvic acid and acetaldehyde*, Food Chem. **2007**, 100, 1144-1152.

[113]Bakker, J.; Timberlake, C. J.; *Isolation, identification and characterization of new color-stable anthocyaninsocurring in some red wines*, J.Agric.Food Chem. **1997**, 45, 35-43.

Este mismo compuesto fue aislado por Fulcrand*et al*[114], que propusieron un mecanismo de formación y una estructura diferente. Se realizaron distintos experimentos con disoluciones modelo que demostraron que la reacción tenía lugar entre la antocianina y el ácido pirúvico. La estructura propuesta en este caso consistía en un núcleo de Mv-3-glc con un anillo piránico que tiene un sustituyente carboxílico y es, debido a esto, que este tipo de compuestos reciben también el nombre de 5-carboxipiranoantocianinas y también aductos vinilfórmicos. Esta última estructura, que se puede observar en laFiguraI.20, ha sido confirmada por varios autores por experimentos de RMN[115] y espectrometría de masas[116].

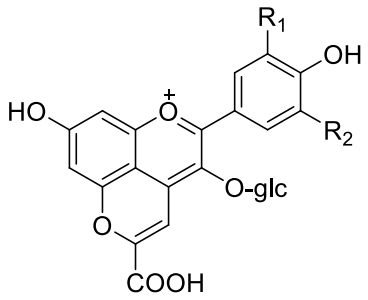



**Figura I.20** Estructura química de derivados de la antocianina con ácido pirúvico propuesta por Fulcrand et al.

La formación de este tipo de compuestos podría ser el resultado de una reacción de cicloadición en la que participan el C4 y el grupo hidroxilo del carbono C5 de la antocianina y un enlace etilénico de la otra molécula. El enlace inicial que se forma entre los carbonos C4 de la antocianina y uno de los carbonos del doble enlace de la otra molécula se debe a la naturaleza fuertemente electrófila del catión flavilio y a la naturaleza nucleófila del doble enlace de la molécula enolizable. El intermedio resultante reacciona con el grupo hidroxilo del carbono C5 de la antocianina formándose de esta manera un nuevo anillo piránico en la estructura. Tiene lugar a continuación un proceso de deshidratación y posterior oxidación para dar lugar al derivado de tipo piranoantociánico en el que la conjugación de los electrones π permite la aromatización del nuevo anillo formado y la deslocalización de la carga. En un medio ácido como es el vino, es la forma enólica del ácido pirúvico la que reacciona con la antocianina (Figura I.21). Como se ha mencionado previamente, esta reacción

[114]Fulcrand, H.; Benabdeljalil, C.; Rigaud, J.; Cheynier, V.; Moutounet, M.*; A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins*, Phytochem.**1998**, 47, 1401-1407.

[115]Mateus, N.; Silva, A. M.; Vercauteren, J.; de Freitas, V.; *Occurrence of anthocyanin-derived pigments in red wines*, J.Agric.Food Chem. **2001**, 49, 4836-4840.

[116]Oliveira, J.; Alhimho da Silva, M.; Teixeira, N.; De Freitas, V.; Salas, E.; *Screening oh anthocyanins and anthocyanin-derived pigments in red wine grape pomace using LC-DAD/MS and MALDI-TOF techniques*, J. Agric. Food Chem. **2015**, 63 835), 7636-7644.

aumenta la estabilidad del producto obtenido, especialmente a altos valores de pH, frente a $SO_2$ y frente a aumentos de temperatura.

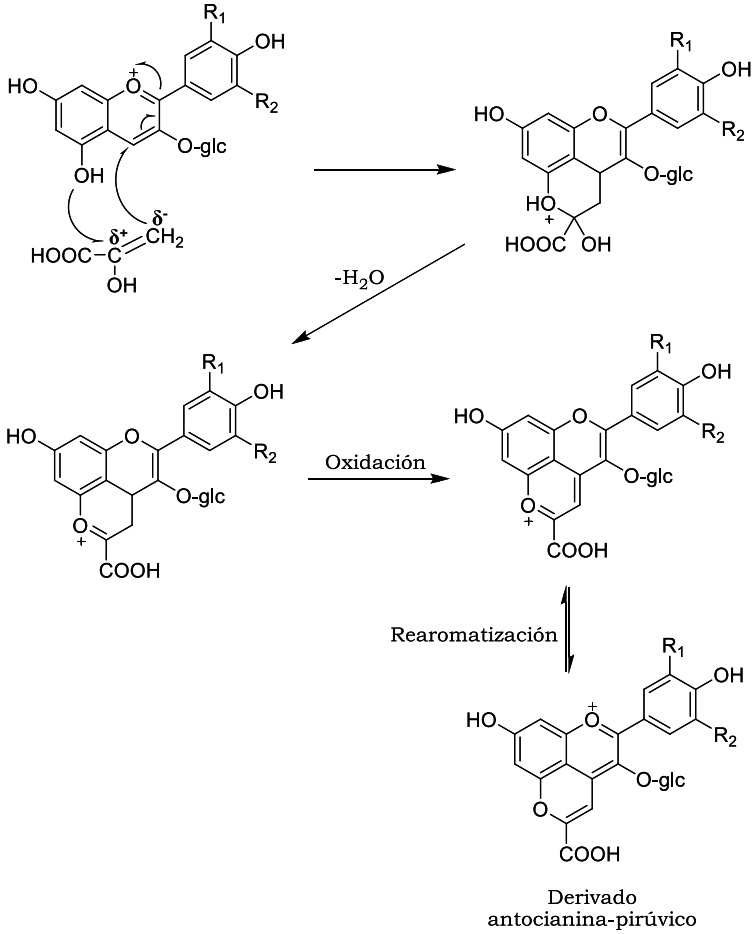



**Figura I.21** Esquema de formación de derivados de tipo antocianina-pirúvico.

Parece que la permanencia del vino en barrica favorece la formación de este tipo de derivados, debido a que su formación requiere condiciones oxidantes[117], no el $O_2$ en sí mismo, sino especies con él.

Como se ha mencionado previamente, este tipo de reacciones de cicloadición se pueden dar también con otros metabolitos secundarios, como acetaldehído y ácido acetoacético. El correspondiente derivado de la antocianina Mv-3-glc y acetaldehído se conoce también como *Vitisina B*.

El acetaldehído del vino tiene dos orígenes, por un lado, el metabolismo de la levadura durante la fermentación alcohólica y, por otro lado, la oxidación del etanol en

[117]Dipalmo, T.; Crupi, P.; Pati, S.; Clodoveo, M. L.; Di Luccia, A.; *Studying the evolution of anthocyanin-derived pigments in a typical red wine of Southern Italy to assess its resistance to aging*, LWT-Food Sci. Technol. **2016**, 71, 1-9.

presencia de polifenoles durante el envejecimiento[118]. Las mayores cantidades de *Vitisina B* se encuentran en vinos de Oporto[119] debido a que se añade acetaldehído durante el proceso de elaboración del vino. A este tipo de derivados de antocianinas con acetaldehído se les conoce también como vinilaductos.

Se han detectado este tipo de compuestos en vinos elaborados con uvas de la variedad *Tempranillo*[120], Garnacha[121], Cabernet Sauvignon[122]. Esta reacción también puede tener lugar con la acetona, pero no se da en la naturaleza.

Los productos de las reacciones de cicloadición de las antocianinas con acetaldehído y ácido acetoacético presentan las estructuras mostradas en la FiguraI.22. Estos derivados siguen la misma ruta de formación que los derivados de antocianinas con ácido pirúvico, con un paso de descarboxilación adicional en el caso de los derivados de antocianinas con ácido acetoacético.

Como se ha comentado previamente, este tipo de compuestos presenta desplazamientos hipsocrómicos de la longitud de onda de máxima absorción del espectro UV-vis. La *vitisina A* (Mv-3-glc-pirúvico) desplaza el máximo a 503-510nm y la *Vitisina B* (Mv-3-glc-acetaldehído) lo desplaza hasta 490-494nm[123]. El correspondiente derivado de Mv-3-glc con ácido acetoacético (Mv-3-glc-4-vinilmetilo) tiene un espectro UV-vis similar a la *Vitisina B*, pero con el máximo de absorción desplazado a longitudes de onda aún menores ($\lambda_{máx}$=478 nm).

[118]Escott, C.; morata, A.; Loira, I.; Tesfaye, W.; Suarez-Lepe, J. A.; *Characterization of polymeric pigments and pyranoanthcoyanins formed in microfermentations of non-Saccharomyces yeasts*, J. Appl. Microbio. **2016**, 121(5), 1346-1356.

[119]Morata, A.; Calderón, F.; González, M. C.; Gómez-Cordovés, M. C.; Suárez, J. A.;*Formation of highly stable pyranoanthocyanins (vitisins A and B) in red wines by the addition of pyruvic acid and acetaldehyde*, Food Chem. **2007**, 100, 1144-1152.

[120]Sánchez-Ilárduya, M. B.; Sánchez-Fernández, C.; Garmón-Lobato, S.; Viloria-Bernal, M.; Abad-García, B.;Berrueta, L. A.; Gallo, B.; Vicente, B.; *Tentative identification of pyranoanthocyanins in Rioja aged red wines by high-performance liquid chromatography and tandem mass spectrometry*, Aust. J. Grape Wine Res. **2014**,20, 31-40.

[121]Blanco-Vega, D.; Gómez-Alonso, S.; Hermosín-Gutiérrez, I.; *Identification, content and distribution of anthocyanins and low molecular weight anthocyanin-derived pigments in Spanish commercial red wines*, Food Chem. **2014**, 158, 449-458.

[122]Aguirre, M. H.; Isaacs, M.; Matsuhiro, B.; Mendoza, L.; Santos, L.; Torres, S.; *Anthocyanin composition in aged Chilean Cabernet Sauvignon red wines*, Food Chem. **2011**, 12, 514-519.

[123]Blanco-Vega, D.; Lopez-Bellido, F. J.; Alia-Robledo, J. M.; Hermosin-Gutierrez, I.; *HPLC-DAD-ESI-MS/MS characterization of pyranoanthocyanins pigments formed in model wine*, J. Agric. Food Chem. **2011**, 59(17), 9523-9531.

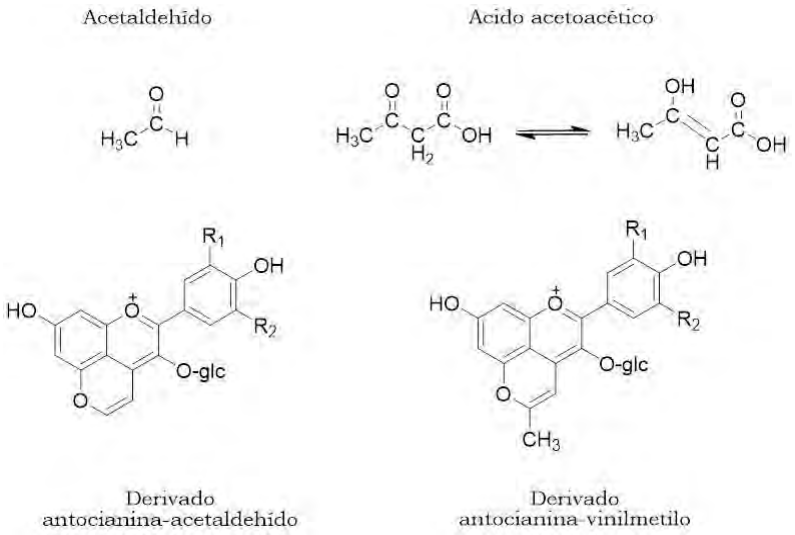



**Figura I.22** Estructura química del acetaldehído y del ácido acetoacético y de sus correspondientes derivados con antocianinas.

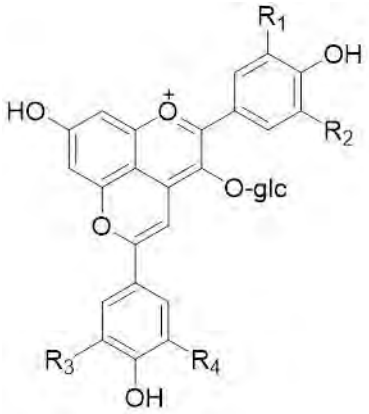
### b) Derivados con ácidos hidroxicinámicos

Los compuestos volátiles vinilfenol, vinilcatecol, vinilguaiacol y vinilsiringol son los derivados descarboxilados de los ácidos hidroxicinámicos *p*-cumárico, cafeico, ferúlico y sinápico, respectivamente. Estos volátiles se asocian con aromas que dan sensaciones desagradables al vino[125]. Los ácidos hidroxicinámicos y algunos de sus derivados descarboxilados pueden reaccionar con las antocianinas para dar lugar a la formación de los derivados antocianina-vinilfenol, antocianina-vinilcatecol, antocianina-vinilguaiacol y antocianina-vinilsiringol[126] (Figura I.23).

Como en el caso de los pigmentos descritos previamente, son más resistentes que las antocianinas precursoras al ataque de nucleófilos, como el agua y el $SO_2$, por la ocupación del C4. Como son más resistentes que los monoglucósidos, el vino al envejecer adquiere tonalidades teja, además, su formación reduce el contenido de fenoles volátiles en los vinos tintos. Los ácidos hidroxicinámicos pueden contribuir a la dureza y al amargor, si participan en este tipo de reacciones de formación de piranoantocianinas, disminuyen la dureza y el amargor en el vino.

[125]Etievant, P.; *Volatile phenol determination in wine*, J. Agric. Food Chem. **1981**, 29, 65-67.

[126]Mateus, N. Oliveira, J.; Pissarra, J.; González-Paramás, A. M.; Rivas-Gonzalo, J.; Santos-Buelga, C.; Silva, A. M. S.; de Freitas, V.*; A new vinyl pyranoanthocyanin pigment occurring in aged red wine*, Food Chem. **2006**, 689-695.




| Derivado | $R_3$ | $R_4$ |
|---|---|---|
| Vinilfenol | -H | -H |
| Vinilcatecol | -H | -CH |
| Vinilguaicol | -H | $-OCH_3$ |
| Vinilsiringol | $-OCH_3$ | $-OCH_3$ |

**Figura I.23** Estructura química de los derivados de antocianinas con ácidos hidroxicinámicos.

Se han descrito dos mecanismos de formación para este tipo de derivados, por un lado las antocianinas pueden reaccionar con los productos de descarboxilación de los ácidos hidroxicinámicos[127], que tiene lugar debido a la actividad de la cinamato-descarboxilasa de la levadura *Saccharomycescerevisiae*, para dar lugar a los correspondientes antocianina-4-vinilfenol y antocianina-4-vinilguaiacol, ya que la actividad de esta levadura es específica para los ácidos *p*-cumárico y ferúlico y no lleva a cabo la descarboxilación de los ácidos cafeico y sinápico[128]. Este mecanismo de formación, que se muestra en la Figura I.24, es rápido e importante en los primeros momentos del envejecimiento[129].

Por otro lado, se puede dar una reacción de cicloadición entre la antocianina y el ácido hidroxicinámico, seguida de una posterior descarboxilación para dar lugar a los diferentes derivados (Figura I.25), incluyendo en este caso también la formación de los derivados antocianina-4-vinilcatecol y antocianina-4-vinilsiringol, siendo este mecanismo lento e importante durante la maduración y el envejecimiento[130].

[127]Schwarz, M.; Winterhalter, P.; A *novel synthetic route to substituted pyranoanthocyanins with unique colour properties*, Tetrahedron Lett. **2003**, 44, 7583-7587.

[128]Chatonnet, P.; Dubourdieu, D.; Boidron, J. N.; Lavigne, V.; *Synthesis of volatile phenols by Saccharomyces cerevisiae in wines*, J. Sci. Food Agric. **1993**, 62, 191-202.

[129]Dipalmo, T.; Crupi, P.; Pati, S.; Clodoveo, M. L.; Di Luccia, A.; *Studying the evolution of anthocyanin-derived pigments in a typical red wine of Southern Italy to assess its resistance to aging*, LWT-Food Sci. Technol. **2016**, 71, 1-9.

[130]Wang, H.; Race, E. J.; Shrikhande, J.; *Anthocyanin transformation in Cabernet Sauvignon wine during aging*, J.Agric. Food Chem. **2003**, 51, 7989-7994.

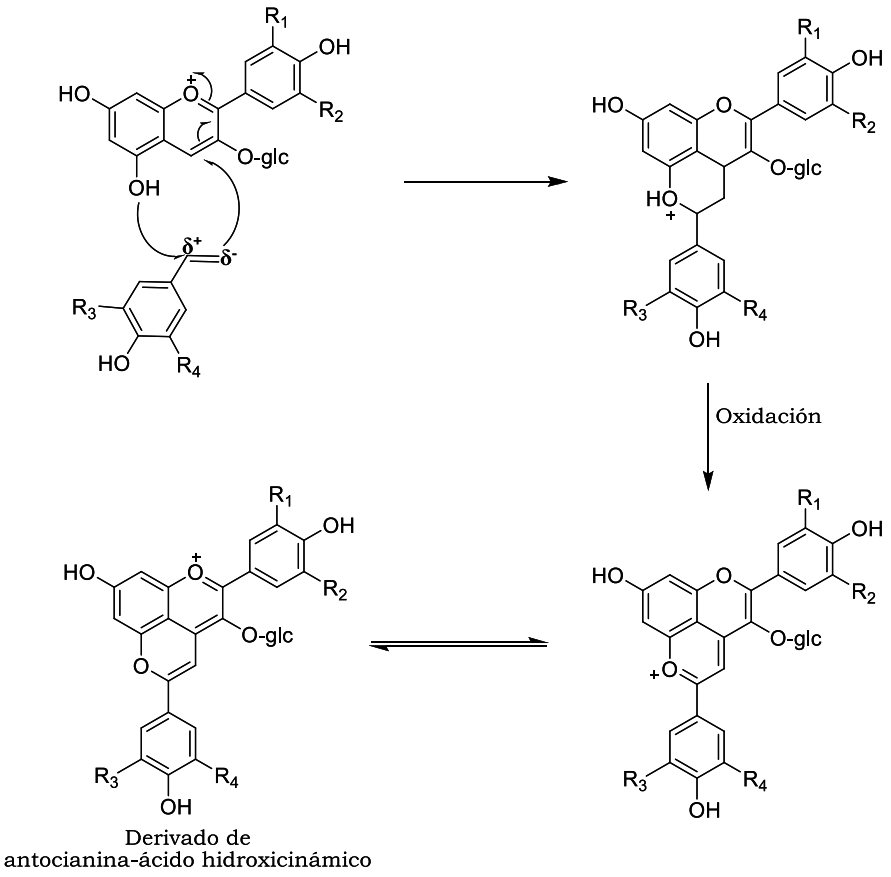



**Figura I.24** Esquema de reacción de antocianinas con derivados descarboxilados de ácidos hidroxicinámicos.

La reacción directa requiere que el anillo aromático del ácido hidroxicinámico tenga sustituyentes dadores para estabilizar el intermedio iónico carbenio formado.

Como se ha mencionado previamente, este tipo de derivados presenta desplazamientos hipsocrómicos en el espectro UV-vis. Por ejemplo, las longitudes de onda de máxima absorción del espectro aparecen desplazadas hasta 500-503 nm en caso de derivados con vinilfenol[137].

Se han detectado este tipo de compuestos en vinos elaborados con uvas de la variedad *Tempranillo*[138], Garnacha[139], Cabernet Sauvignon[140], especialmente de los

[137]Blanco-Vega, D.; López-Bellido, F. J.; Alía-Robledo, J. M.; Hermosín-Gutiérrez, I.; *HPLC-DAD-ESI-MS/MS characterization of pyranoanthocyanins pigments formed in model wine*, J. Agric. Food Chem. **2011**, 59, 9523-9531.

[138]Sánchez-Ilárduya, M. B.; Sánchez-Fernández, C.; Garmón-Lobato, S.;Viloria-Bernal, M.; Abad-García, B.;Berrueta, L. A.; Gallo, B.; Vicente, B.; *Tentative identification of pyranoanthocyanins in Rioja aged red wines by high-performance liquid chromatography and tandem mass spectrometry*, Aust. J. Grape Wine Res. **2014**, 20, 31-40.

derivados con vinilfenol y vinilguaiacol[141]. Después de un incremento inicial de la concentración, ésta permanece estable durante los dos primeros años de envejecimiento. Debido a que no se produce la reacción entre las antocianinas y el producto de descarboxilación de los ácidos cafeico y sinápico, en estos primeros meses los derivados antocianina-vinilcatecol y antocianina-vinilsiringol aparecen en baja concentración.

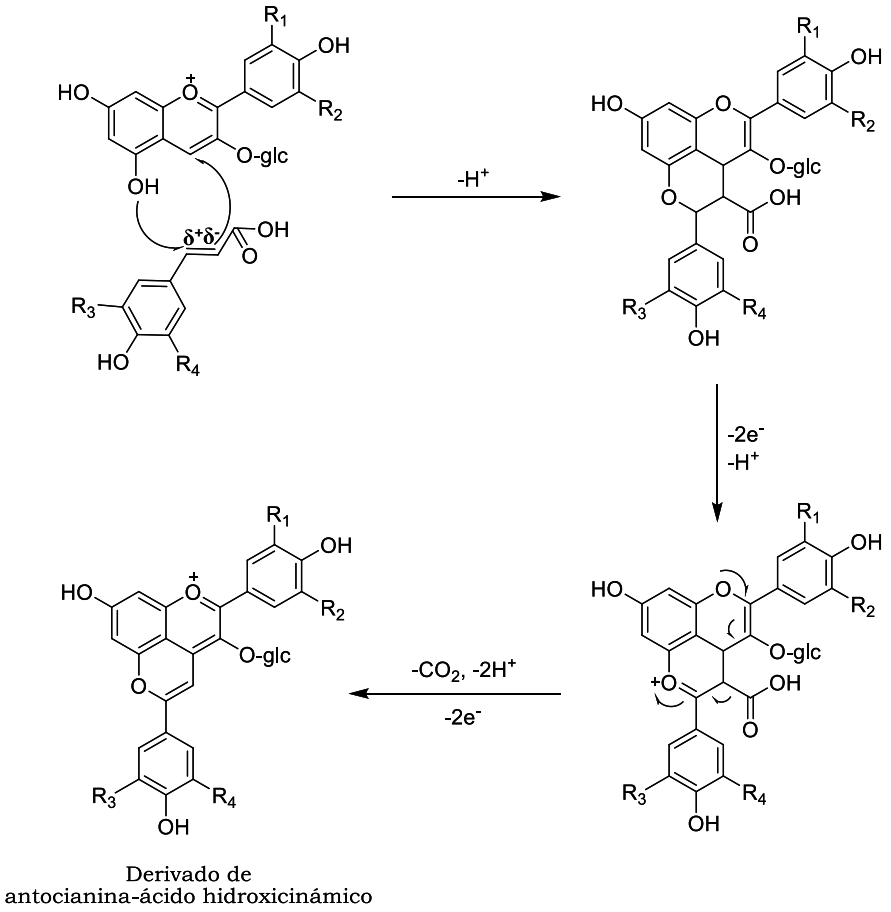



**Figura I.25** Esquema de reacción de antocianinas con ácidos hidroxicinámicos.

[139]Blanco-Vega, D.; Gómez-Alonso, S.; Hermosín-Gutiérrez, I.; *Identification, content and distribution of anthocyanins and low molecular weight anthocyanin-derived pigments in Spanish commercial red wines*, Food Chem. **2014**, 158, 449-458.

[140]Aguirre, M. H.; Isaacs, M.; Matsuhiro, B.; Mendoza, L.; Santos, L.; Torres, S.; *Anthocyanin composition in aged Chilean Cabernet Sauvignon red wines*, Food Chem. **2011**, 12, 514-519.

[141]Dipalmo, T.; Crupi, P.; Pati, S.; Clodoveo, M. L.; Di Luccia, A.; *Studying the evolution of anthocyanin-derived pigments in a typical red wine of Southern Italy to assess its resistance to aging*, LWT-Food Sci. Technol. **2016**, 71, 1-9.

Después de dos o tres años de envejecimiento, se puede observar otro aumento en la concentración de estos derivados; este incremento es debido a la reacción directa de las antocianinas con los ácidos hidroxicinámicos[143].

**c) Derivados con vinilflavanoles**

Las antocianinas reaccionan con vinilflavanoles para dar lugar a derivados que también presentan en su estructura el nuevo anillo piránico, pudiendo estar los vinilflavanoles constituidos por uno o más monómeros de flavanol.

Este tipo de compuestos puede presentar estereoisómeros por dos motivos, por un lado, el flavanol puede ser (+)-(galo)catequina o (-)-epi(galo)catequina, y por otro lado, la posición de unión antocianina-flavanol, que puede ser por los carbonos C6 o C8 del flavanol[144].

La formación de este tipo de derivados comienza, como en el caso de los derivados de antocianinas con flavanoles formados por condensación mediada por acetaldehído, una vez que se ha producido la protonación del acetaldehído. Tiene lugar a continuación la adición del catión resultante a una posición nucleófila del anillo A del flavanol, que pueden ser los carbonos C6 o C8 del anillo floroglucinol, formándose un producto inestable que se protona dando lugar al catión etil-flavanol. El catión etil-flavanol puede sufrir una pérdida de agua dando lugar al vinilflavanol (Figura I.26). Los vinilflavanoles también se pueden formar por la ruptura de pigmentos del tipo antocianina-etil-flavanol[145].

Una vez formado el vinilflavanol tiene lugar una reacción de cicloadición nucleófila con la antocianina, en un mecanismo similar al descrito previamente para otros tipos de derivados piranoantociánicos y que se puede observar en la Figura I.27.

La presencia de este tipo de compuestos indica la existencia de otra vía de interacción entre flavanoles y antocianinas durante el envejecimiento del vino.

Esta adición oxidativa provoca que los nuevos pigmentos estén ampliamente conjugados, permitiendo la aromatización del nuevo anillo piránico D.

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[143]Gao, Y.; Tian, Y.; Liu, D.; Li, Z.; Zhang, X.; Li, J.; Huang, J.; Wang, J.; Pan, Q.; *Evolution of phenolic compounds and sensory in bottled red wines and their co-development*, Food Chem. **2015**, 173, 565-574.

[144]Pati, S.; Losito, I.; Gambacorta, G.; La Notte E.; Palmisano, F.; Zambonin, P. G.; *Simultaneous separation and identification of oligomericprocyanidins and anthocyanin-derived pigments in raw red wine by HPLC-UV-ESI-MS$^n$*, J. Mass Spectrom. **2006**, 41, 861-871.

[145]Weber, F.; Wintherhalter, P.; *Synthesis and structure elucidation of ethyliden-linked anthocyanin- Flavan-3-ol olifomers*, Food Res. Int. **2013**, 65, 69-76.

Este tipo de pigmentos aportan al vino una tonalidad teja, presentando longitudes de onda de máxima absorción en el espectro UV-vis a 500-511 nm. Debido a la sustitución en el C4, son más resistentes a la decoloración por el pH del medio y $SO_2$ que los pigmentos con puente de etilideno. Este tipo de compuestos con sustitución en el C4 presentan un pico de absorción en el espectro en la zona de 350-370 nm[146].

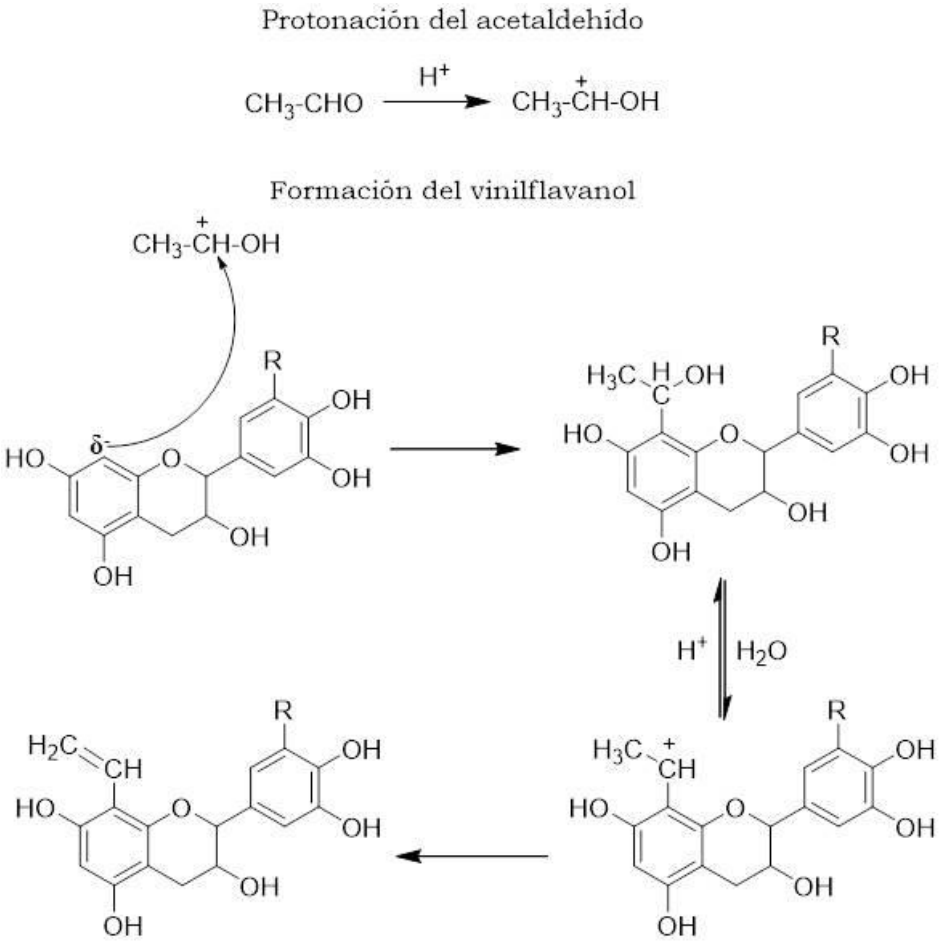



**Figura I.26** Protonación del acetaldehído y formación del vinilflavanol.

Si el flavanol es dímero el pigmento presenta un desplazamiento batocrómico de aproximadamente 9 nm respecto a los que contienen un monómero. Con este hecho se pone de manifiesto la importancia del flavanol en el color del pigmento, sugiriendo que ocurre un fenómeno de copigmentación intramolecular entre el resto del flavanol y el grupo cromóforo de la piranoantocianina. La acilación con ácido p-cumárico no parece tener influencia en la longitud de onda de máxima absorción, debido a que está fuera

[146]Gómez-Cordovés, C.;*New pigments produced in red wines via different enological processes*. En Red wine color.American Chemical Society, Nueva Orleans, **2004**, pp. 89-124.

de la estructura que forman la antocianina y el flavanol, como han demostrado experimentos de RMN y espectrometría de masas[147].

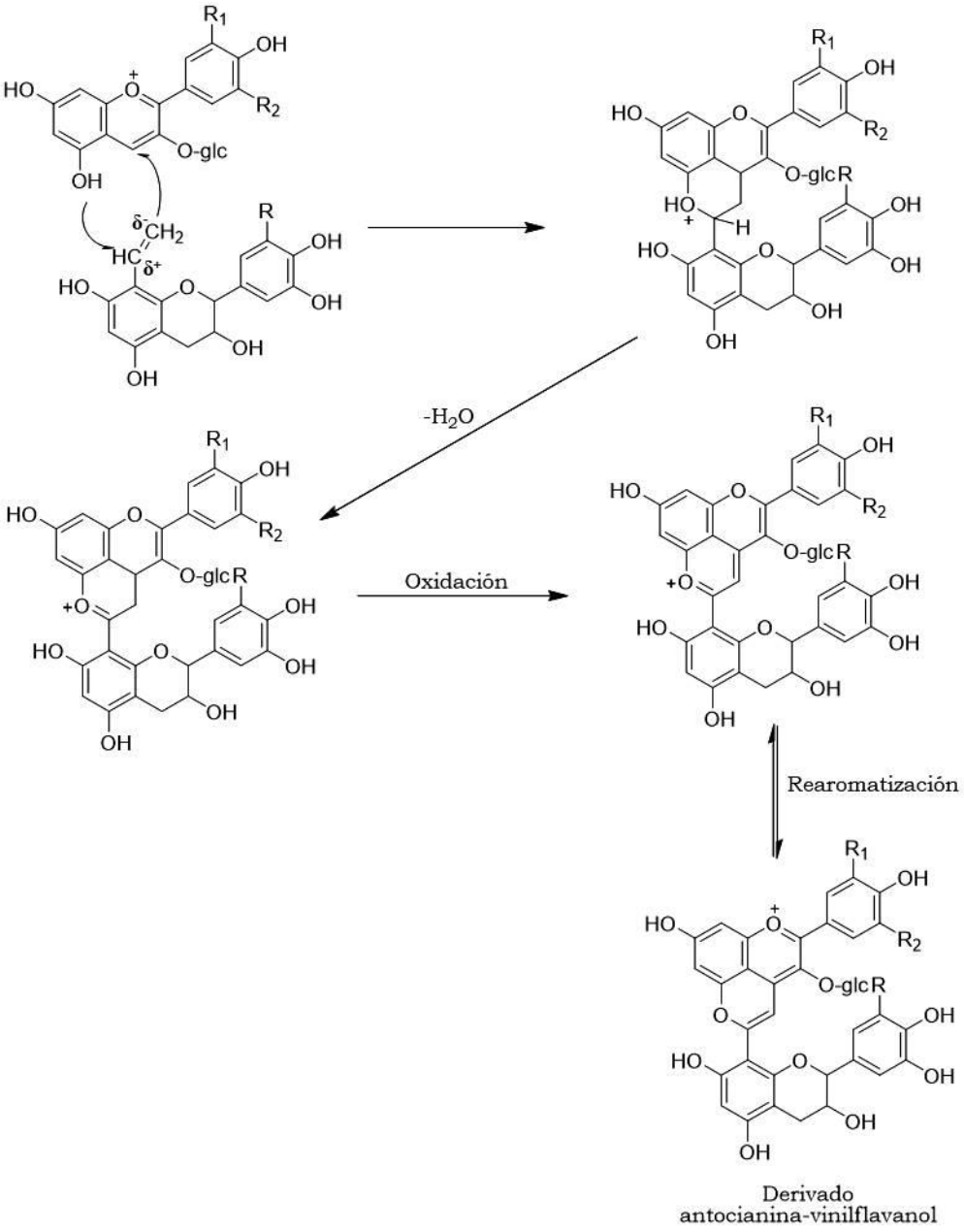



**Figura I.27** Esquema de reacción de la antocianina con el vinilflavanol para dar lugar a los derivados de tipo antocianina-vinilflavanol.

[147]Nave, F.; Teixeira, N.; Mateus, N.; de Freitas, V.; *Hemisynthesis and structural characterization of flavanol-vitisins by mass spectrometry*, Rapid Commun. Mass Spectrom.**2010**, 24, 1964-1970.

**d) Portisinas**

Este tipo de derivados son resultado de interacciones entre los derivados de las antocianinas con ácido pirúvico y los vinilflavanoles o con los derivados de los ácidos hidroxicinámicos[148].

El mecanismo de formación de estos pigmentos supone un ataque nucleófilo del doble enlace olefínico del vinilflavanol o del derivado del ácido hidroxicinámico al carbono C10 del derivado de la antocianina con ácido pirúvico, que es electrófilo, seguido de la pérdida de ácido fórmico y oxidación[149].Los esquemas de formación de los compuestos formados por reacción de los derivados de antocianinas con ácido pirúvico y vinilflavanoles y por otro lado, las reacciones con derivados de ácidos hidroxicinámicos se pueden observar en las Figuras I.28 y I.29, respectivamente.

La amplia conjugación de electrones π le proporciona una alta estabilidad al pigmento, presentando desplazamientos batocrómicos en la longitud de onda de máxima absorción del espectro UV-vis, aportando al vino tonalidades violáceas. El aumento de la hidroxilación en el anillo fenólico de las portisinas contribuye a este desplazamiento batocrómico. La longitud de onda de máxima absorción del espectro se sitúa en los 572 nm para los derivados de Mv-3-glc[150,151] de este tipo.

Como en los casos anteriores, este tipo de derivados presenta una alta resistencia a la decoloración debida a la alta protección del grupo cromóforo frente a un ataque nucleófilo del agua, que daría lugar a la forma hemiacetálica, o del $SO_2$.

[148]Oliveira, J.; Azevedo, J.; Silva, A. M. S.; Teixeira, N.; Cruz, L.; Mateus, N.; de Freitas, V.; *Pyranoanthocyanin dimers: a new family of turquoise blue anthocyanin-derived pigments found in port wine*, J. Agric. Food Chem. **2010**, 58, 5154-5159.

[149]Mateus, N.; Silva, A. M.; Rivas-Gonzalo, J. C.; Santos-Buelga, C.; de Freitas, V.; *A new class of blue anthocyanin-derived pigments isolated from red wines*, J. Agric. Food Chem. **2003**, 51, 1919-1923.

[150]Mateus, N.; Oliveira, J.; Santos-Buelga, C.; Silva, A. M. S.; de Freitas, V. A. P.;*NMR structure characterization of a new vinylpyranoanthocyanin-catechinpigment*, Tetrahedron. Lett. **2004**, 45, 3455-3457.

[151]De Freitas, V.; Mateus, N.; *Chemical transformations of anthocyanins yielding a variety of colours (Review)*, Environ. Chem. Lett. **2006**, 4, 175-183.

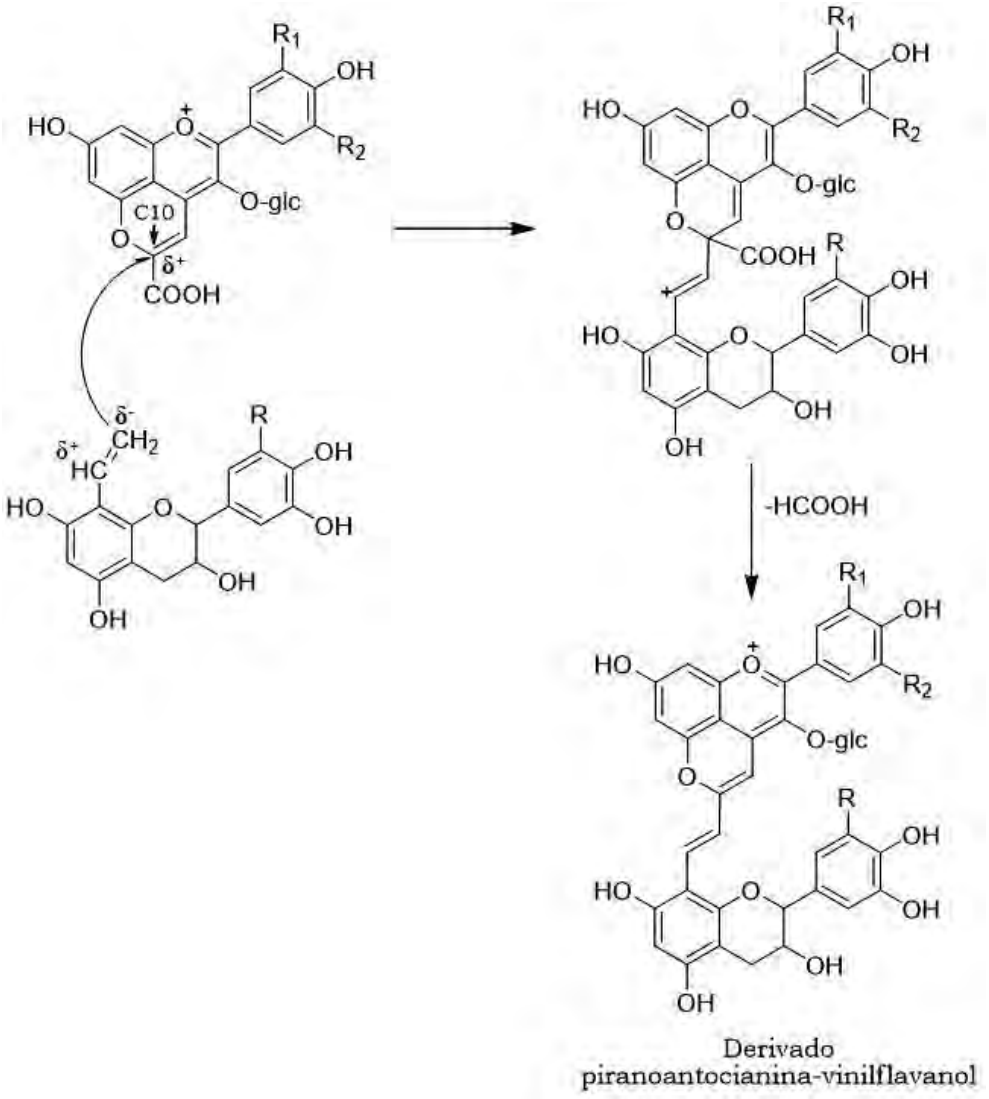



**Figura I.28** Esquema de reacción del derivado antocianina-pirúvico con un vinilflavanol para dar lugar a un compuesto de tipo piranoantocianina-vinilflavanol.

Esta resistencia es mayor en el caso de derivados con vinilflavanoles, debido a que el ácido hidroxicinámico es un grupo pequeño que no protege al cromóforo tan eficientemente como el vinilflavanol del ataque nucleófilo del agua o del bisulfito[152].

[152]Oliveira, J. de Freitas, V. Silva, A. M. S.; Mateus, N.; *Reaction between hydroxycinnamic acids and anthocyanin-pyruvic acid adducts yielding new portisins*, J. Agric. Food Chem. **2007**, 55, 6349-6356.

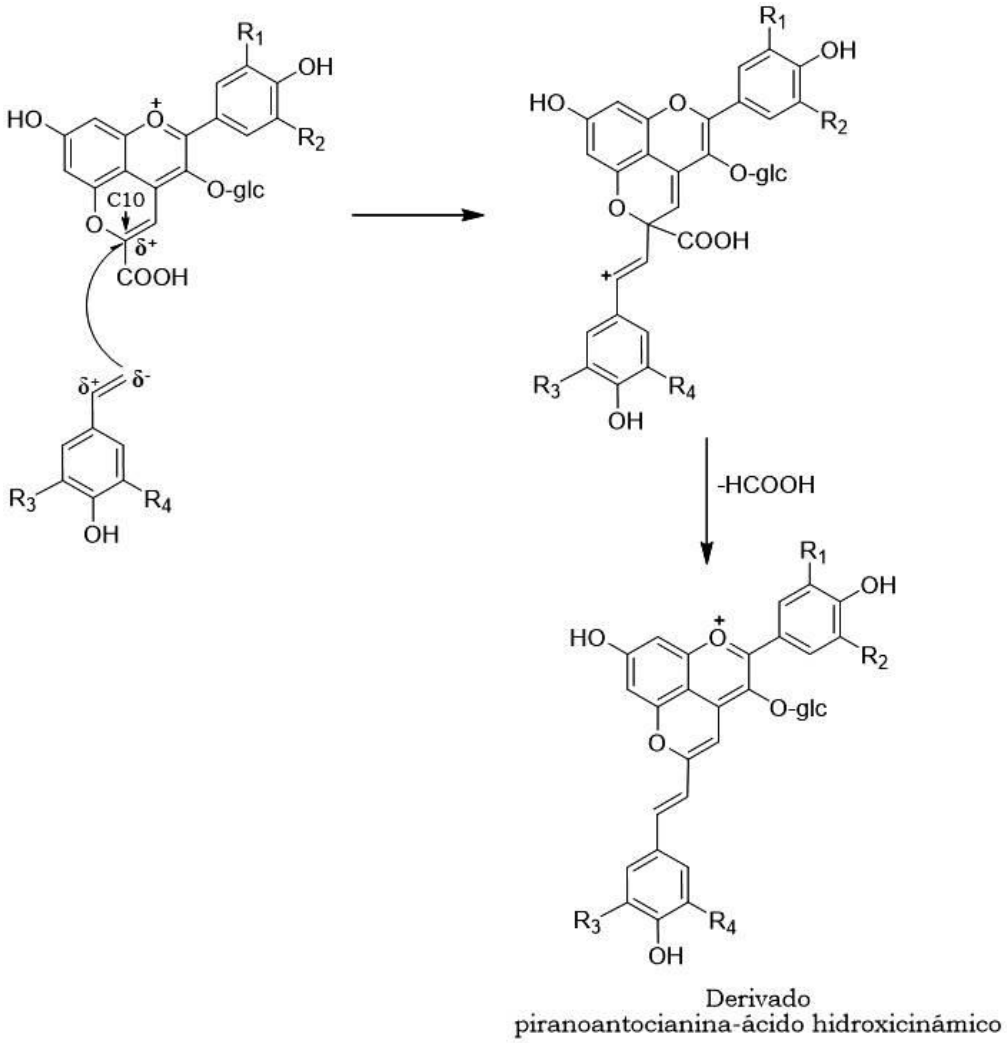



**Figura I.29** Esquema de reacción del derivado antocianina-pirúvico con un derivado de ácido hidroxicinámico para dar lugar a un compuesto de tipo piranoantocianina-ácido hidroxicinámico.

### I.5.5. TANINOS CONDENSADOS

Los taninos condensados resultan de las polimerizaciones de los flavan-3-oles y se encuentran entre los polifenoles más importantes. Son capaces de producir combinaciones estables con las proteínas y con otros polímeros como por ejemplo los polisacáridos. En consecuencia, son los responsables de la sensación de astringencia que produce el vino en contacto con la saliva[153].

[153]Delcambre, A.; Saucier, C.; *Identification of new flavan-3-ol monoglycosides by UHPLC-ESI-QTOF in grapes and wine*, J. Mass. Spectrom. **2012**, 47, 727-736.

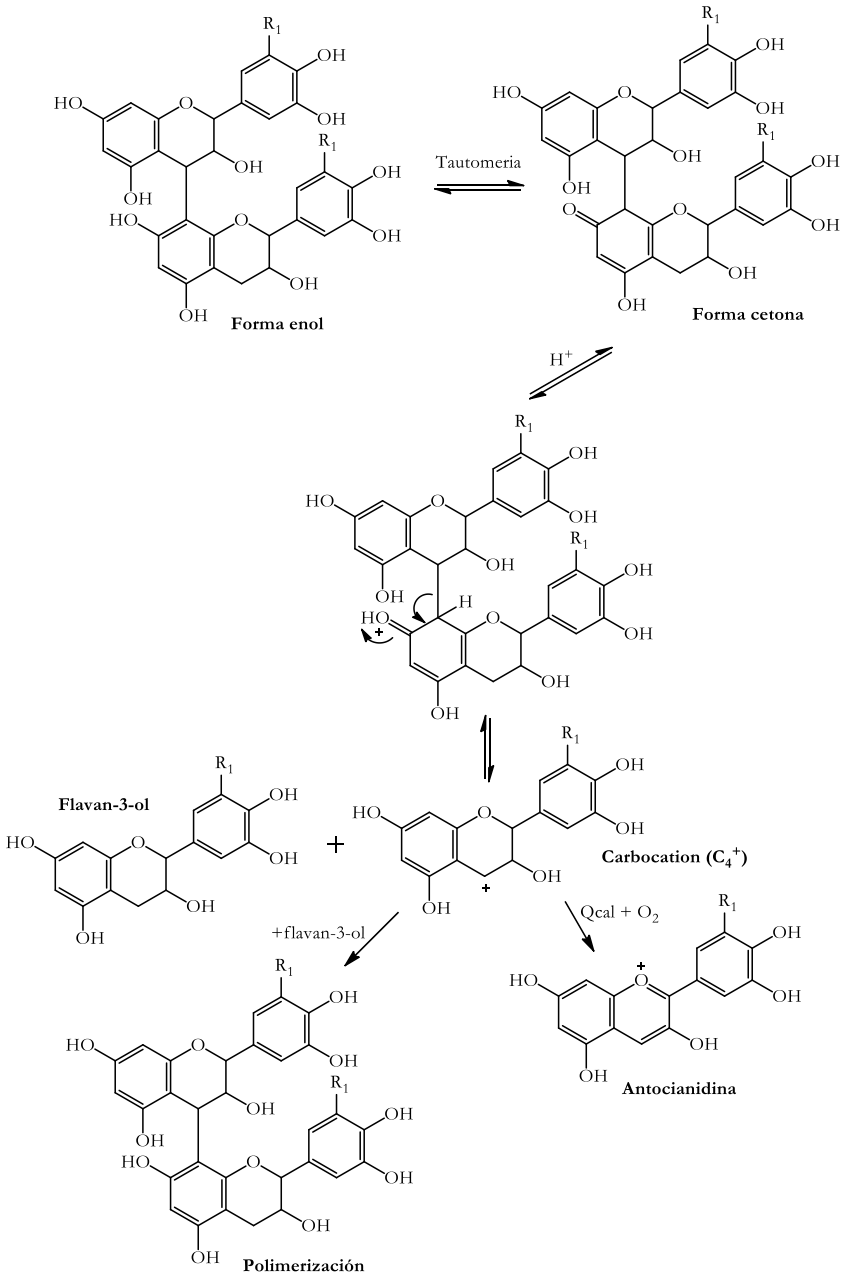



**Figura I.30** Productos de las procianidinasdiméricas por hidrólisis ácida.

Están compuestos de monómeros de catequinas y epicatequinas formando las procianidinas o por monómeros de galocatequinas y epigalocatequinas, formando las prodelfinidinas. Estos nombres son debidos a que en medio fuertemente ácido se hidrolizan para dar lugar a las antocianidinas cianidina o delfinidina, respectivamente

(Figura I.30)[155]. Además, también existen taninos condensados formados por unidades tanto de (epi)catequina y (epi)galocatequina, a las cuales se les suele denominar procianidinas mixtas, si la unidad superior es (epi)catequina, o prodelfinidinas mixtas, si la unidad superior es (epi)galocatequina.

Estos compuestos se encuentran presentes en la uva y se han detectado tanto en la piel como en los hollejos.

Tanto los monómeros de flavanol como las procianidinas y prodelfinidinas, presentan una clara tendencia a la polimerización. Las reacciones que conducen a la polimerización pueden llevarse a cabo tanto de forma directa como indirecta. Entre los taninos formados por condensación directa se diferencian los que presentan un enlace interflavanoide de tipo B y los de tipo A y entre los formados por condensación indirecta, se diferencian las condensaciones indirectas mediadas por acetaldehído, ácido glioxílico y furfural[153].

#### I.5.5.1. Taninos formados por condensación directa: enlace de tipo B

Las procianidinas de tipo B son aquellas que están unidas mediante un único enlace interflavonoide[156]. Este se dará entre dos o más flavan-3-oles, formándose de esta manera desde dímeros, trímeros o tetrámeros hasta taninos que pueden llegar a contener 30 unidades de flavan-3-ol, alcanzando relaciones masa/carga (m/z) mayores de 6000.

Los dímeros de procianidinas de tipo B más conocidos son 8, los cuales se diferencian en su configuración espacial y en las posiciones del enlace interflavonoide.

En primer lugar en las procianidinas B1, B2, B3 y B4 (Figura I.31) el enlace interflavonoide se forma entre los carbonos 4 y 8, sin embargo en las procianidinas B5 a la B8 es entre los carbonos 4 y 6 (Figura I.32). Las prodelfinidinas B1 a B8 son iguales, pero con dos (epi)galocatequinas en lugar de dos (epi)catequinas. Y también se pueden formar dímeros mixtos con una (epi)catequina y una (epi)galocatequina[157].

Tal y como se observa en las Figuras I.31 y I.32, la procianidina B1 tiene una estructura epicatequina-(4β→8)-catequina; la B2,epicatequina-(4β→8)-epicatequina; la B3, catequina-(4α→8)-catequina;la B4, catequina-(4α→8)-epicatequina; la B5,

[155]Teixeira, N.; Azevedo, J.; Mateus, N.; de Freitas, V.; *Proanthocyanidin screening by LC-ESI-MS- of Portuguese red wines made with teinturier grapes*, Food Chem. **2016**, 190, 300-307.

[156]Teixeira, N.; Azevedo, J.; Mateus, N.; de Freitas, V.; *Proanthocyanidin screening by LC-ESI-MS- of Portuguese red wines made with teinturier grapes*, Food Chem. **2016**, 190, 300-307.

[157]Zhang, S.; Li, L.; Cui, Y.; Luo, l.; Li, Y.; Zhou, P.; Sun, B.; *Preparative high-speed counter-current chromatography separation of grape seed proanthocyanidins according to degree of polymerization*, Food Chem. **2017**, 219, 399-407.

epicatequina-(4β→6)-epicatequina; la B6, catequina-(4α→6)-catequina; la B7, epicatequina-(4β→6)-catequina y la procianidina B8, catequina-(4α→6)-epicatequina.

Por su lado la procianidina C1 es un trímero que presenta una estructura epicatequina-(4β→8)-epicatequina-(4β→8)-epicatequina.

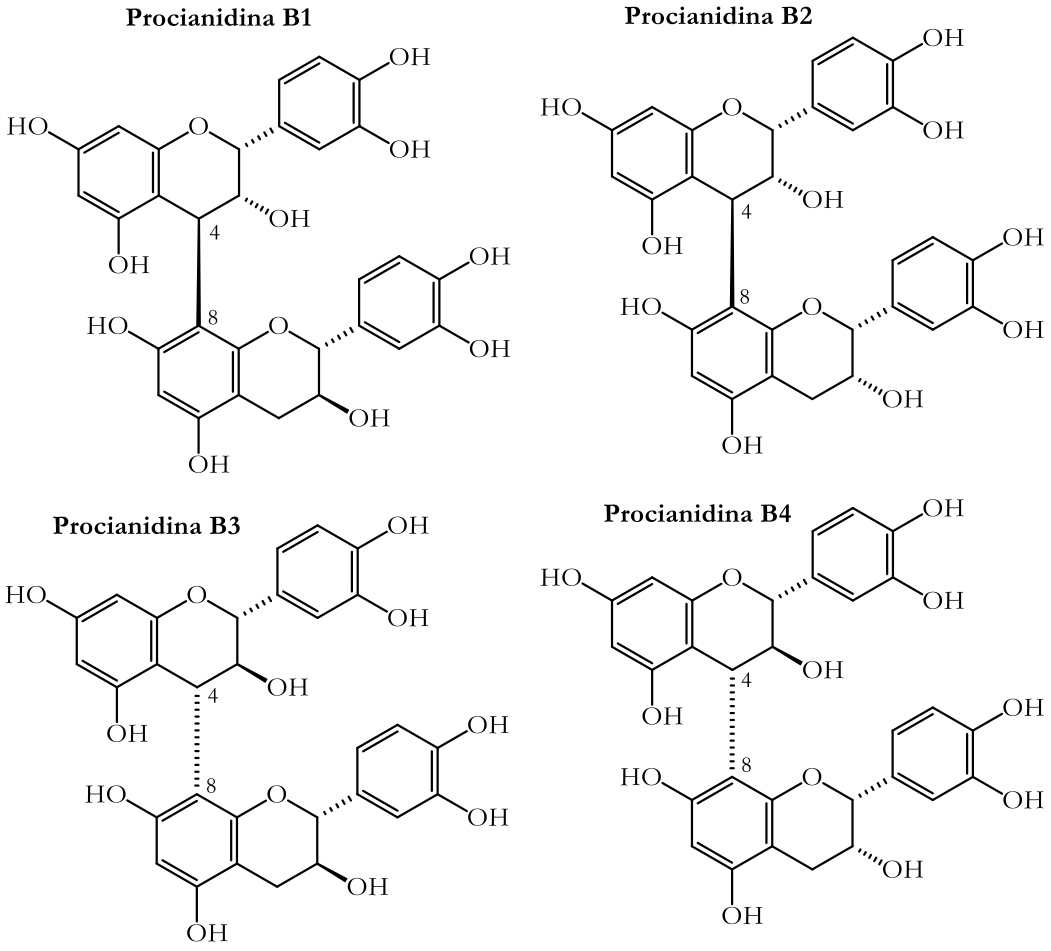



**Figura I.31** Estructura química de las procianidinas B1 a la B4.

El mecanismo de polimerización para la formación de las proantocianidinas no está claro[158], aunque todos los mecanismos de condensación propuestos coinciden en que se da la adición nucleófila de las posiciones C8 o C6 del flavan-3-ol inferior (o terminal) a la posición C4 del flavan-3-ol superior (o de extensión), el cual se

[158]Xie, D. Y.; Dixon, R. A.; *Proanthocyanidin biosynthesis-still more questions than answere?,* Phytochem. **2005**, 66, 2127-2144.

encuentra en un estado electrofílico tras su conversión en diferentes intermedios de reacción, tal y como se muestra en la Figura I.33.[159]

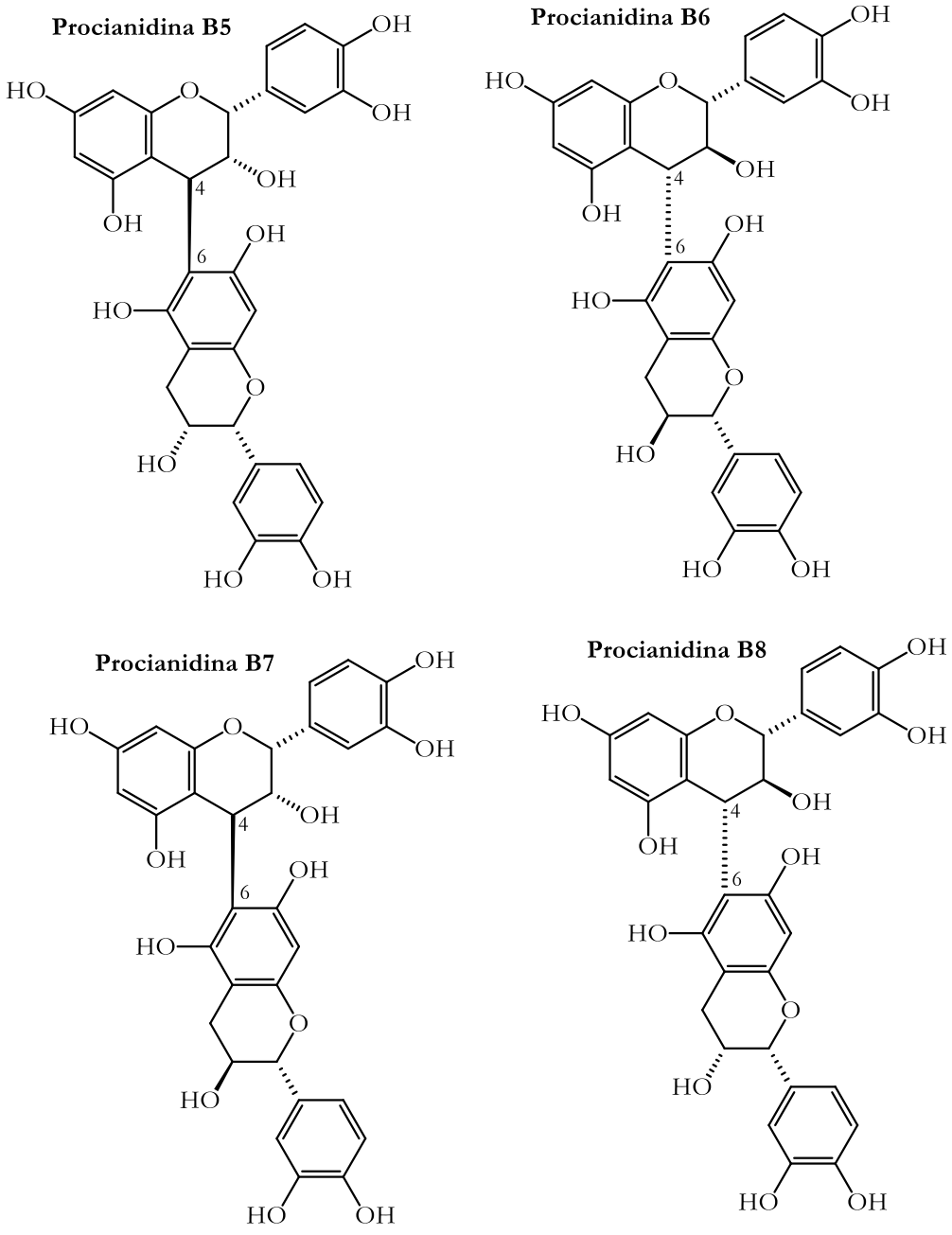



**Figura I.32** Estructura química de las procianidinas B5 a la B8.

[159]He, F.; Pan. Q-H.; Duan, C-Q.; *Biosynthesis and Genetic Regulation of Proanthocyanidins in Plants,* Molecules **2008**, 13, 2674-2703.

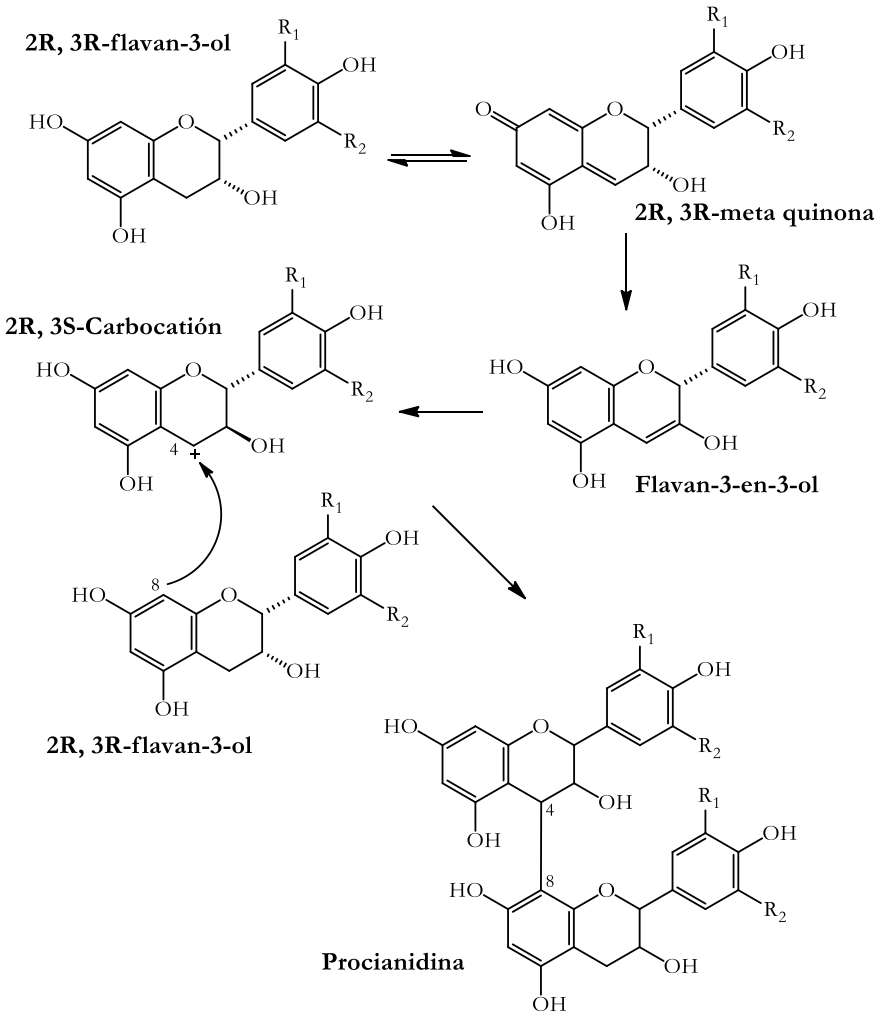


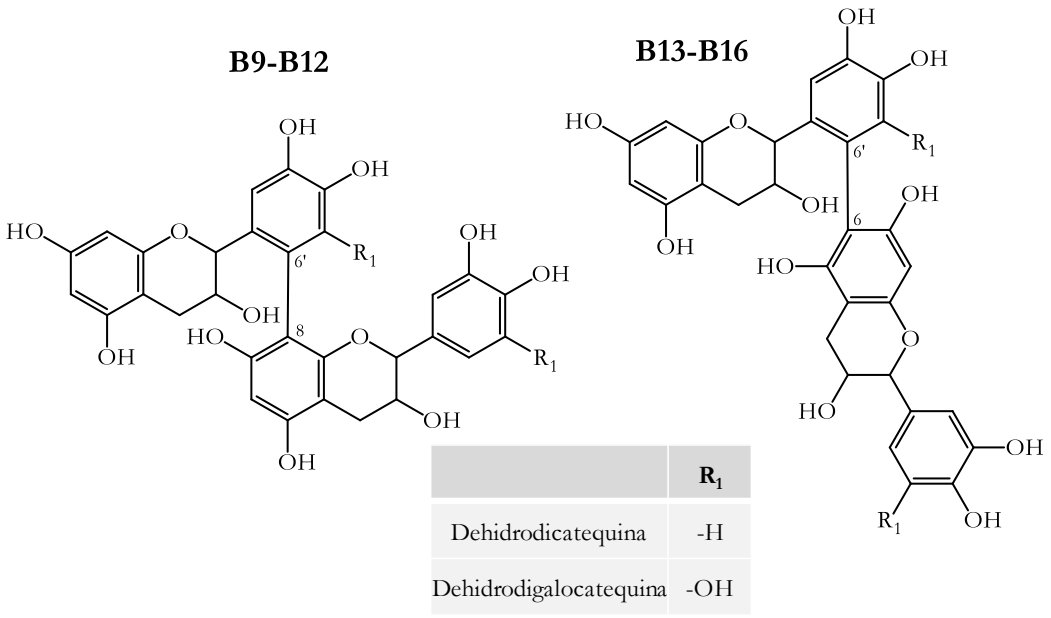

**Figura I.33** Ruta propuesta para la condensación de flavan-3-oles.

Estos compuestos se encuentran sobre todo en las semillas de la uva, pero también se pueden encontrar en las pieles y tallos de la uva[160].

Por otro lado, a las procianidinas B9 a la B16 se les conoce con el nombre de dehidrodicatequinas, ya que son el resultado de una oxidación enzimática, química o una auto-oxidación de los flavan-3-oles. Estos productos de oxidación presentan enlaces entre los carbonos 6´ y 8 ó 6´ y 6 (Figura I.34), resultado de la reacción de condensación entre el anillo B del flavan-3-ol superior y el anillo A del inferior[161]. Al igual que en los casos anteriores, se diferencian entre sí por la configuración espacial, es decir, por los diferentes diastereoisómeros que las forman.

[160]Spranger, I.; Sun, B.; Mateus, A. M.; de Freitas, V.; Ricardo da Silva, J. M.; *Chemical characterization and antioxidant activities of oligomeric and polymeric procyanidin fractions from grape seeds*, Food Chem. **2008**, 108(2), 519-532.

[161]He, F.; Pan, Q-H.; Shi, Y.; Zhang, X-T.; Duan, C-Q.; *Identification of autoxidation oligomers of flavan-3-ols inmodel solutions by HPLC-MS/MS,* J. Mass. Spectrom. **2009**, 44, 633–640.




| | $R_1$ |
|---|---|
| Dehidrodicatequina | -H |
| Dehidrodigalocatequina | -OH |

**Figura I.34** Estructura química de las dehidrodicatequinas B9 a la B16.

### I.5.5.2. Taninos formados por condensación directa: enlace de tipo A

La diferencia principal entre las procianidinas de tipo B y las de tipo A (Figura I.35) es que éstas presentan un enlace inteflavonoide (IFL) adicional de tipo éter, entre las posiciones C2-O-C5 o entre las posiciones C2-O-C7. Este tipo de procianidinas han sido detectadas en varias fuentes naturales como, por ejemplo, en algunas plantas[162,163], en la piel de los cacahuetes[164], en caquis, ciruelas[165], arándanos[166], granos de cacao[167], en uvas[168,169] y, en consecuencia, en el vino[170].

[162]Porter, L. J.; *Flavans and proanthocyanidins.* In The Flavonoids, Advances in research since 1980, J. B. Harborne (Ed.). Chapman and Hall: London **1988**, 21.

[163]Lazarus, S. A.; Adamson, G. E.; Hammerstone, J. F.; Schmitz, H. H.; *High performance liquid chromatography/mass spectrometry analysis of proanthocyanidins in foods and beverages*, J. Agric. Food Chem. **1999**, 47, 3693-3701.

[164]Dong, X. Q.; Zou, B.; Zhang, y.; Ge, Z. Z.; Du, J.; Li, C. M.; *Preparation of A-type proanthocyanidin dimmers from peanut skins and persimmon pulp and comparison of the antioxidant activity of A-type and B-type dimmers*, Fitoterapia **2013**, 91, 128-139.

[165]Tomas-Barberan, F. A.; Gil, M. I.;Cremin, P.; Waterhouse, A. L.; Hess-Pierce, B.; Kader, A.; A. *HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches, and plums,* J. Agric. Food Chem. **2001**, 49, 4748-4760.

[166]Tarascou, I.; Mazauric, J. P.; Meudec, E.; Souquet, J. M.; Cunningham, D.;*Cheynier, V.; Fulcrand, H.; Characterisation of genuine and derived cranberry proanthocyanidins by LC-ESI-MS*, Food Chem. **2011**, 128(3), 802-810.

[167]Esatbeyoglu, T.; Wray, V.; Winterhalter, P.; *Isolation of dimeric, trimeric, tetrameric and pentamericprocyanidins from unroasted cocoa beans (Theobroma cacao L.) using countercurrent chromatography*, Food Chem. **2015**, 179, 278-289.

[168]Passos, C. P.; Cardoso, S. M.; Domingues, M. R. M.; Domingues, P.; Silva, C. M.; Coimbra, M. A.; *Evidence for galloylated type-A procyanidins in grape seeds*, Food Chem. **2007**, 105, 1457-1567.

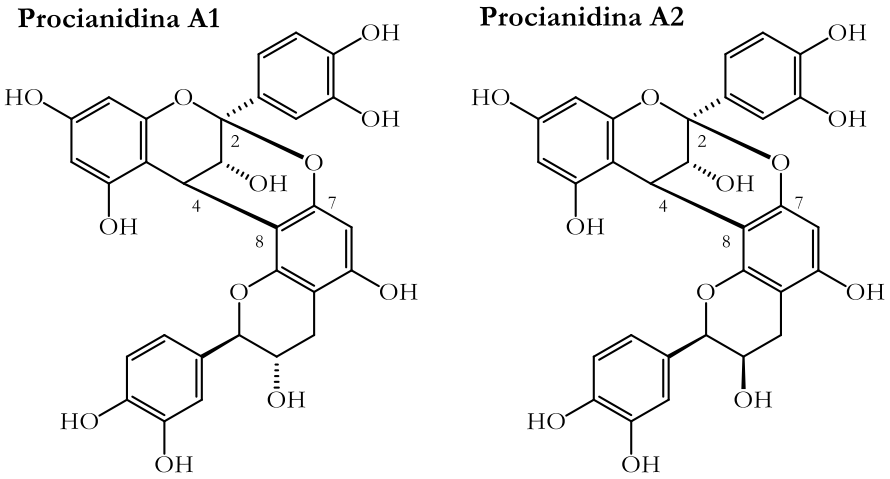



**Figura I.35** Estructura química de las procianidinas A1y A2.

La formación de este segundo enlace que da el nombre a las procianidinas de tipo A, proviene de un acoplamiento intramolecular a partir de su precursora de tipo B; así, la procianidina A2 provendría de la procianidina B2, cuando en ésta se produce un acoplamiento intramolecular entre las posiciones C2-O-C7[172], y lo mismo ocurriría con el resto de procianidinas de tipo B descritas anteriormente.

### I.5.5.3. Trímeros con enlace de tipo A y de tipo B

Se ha detectado la existencia de trímeros con un enlace interflavonoide de tipo A y dos enlaces IFL de tipo B (Figura I.36); a estos trímeros también se les denomina procianidinas de tipo A en algunas referencias bibliográficas[173]. No han sido detectados este tipo de compuestos en el vino, posiblemente porque, por lo general, han sido estudiados mediante LC-MS los derivados generados tras una reacción de tiólisis, que rompe los polímeros, con lo que no se estudia la estructura completa de los compuestos, sino únicamente el grado de polimerización[174]. Sin embargo, ha sido

[169]Wang, Z.; Ashraf-Khorassani, M.; Taylor L. T.*; Feasibility Study of Online Supercritical Fluid Extraction–Liquid Chromatography– UV Absorbance–Mass Spectrometry for the Determination of Proanthocyanidins in Grape Seeds*, J. Chrom. Sci. **2005**, 43, 109-115.

[170]Vivas de Gaulejac, N.; Vivas, N.; Absalon, C.; Nonier, M.F.; *Identification du procyanidole A2 dans le raisin et le vin de Vitisvinifera L. cv. Merlot noir et Cabernet Sauvignon*, J.Internat. des Sciences de la Vigne et du Vin **2001**, 35, 51-56.

[172]Poupard, P.; Sanoner, P.; Baron, A.; Renard, C. M. G. C.; Guyot, S.; *Characterization of procyanidin B2 oxidation products in an apple juice model solution and confirmation of their presence in apple juice by high-performance liquid chromatography coupled to electrospray ion trap mass spectrometry,* J. Mass. Spectrom. **2011**, 1186-1197.

[173]Flamini, R.; *Mass Spectrometry in grape and wine Chemistry. Part I: Polyphenols*, Mass Spectrom. Rev. **2003**, 22, 218-250.

[174]Passos, C. P.; Cardoso, S. M.; Domingues, M. R. M.; Domingues, P.; Silva, C. M.; Coimbra, M. A.; *Evidence for galloylated type-A procyanidins in grape seeds*, Food Chem. **2007**, 105, 1457-1567.

ampliamente estudiada su estructura en un amplio rango de materiales: en hojas de te[175],la piel de los cacahutes[176,177], arándanos[178] o en la piel de las almendras[179].

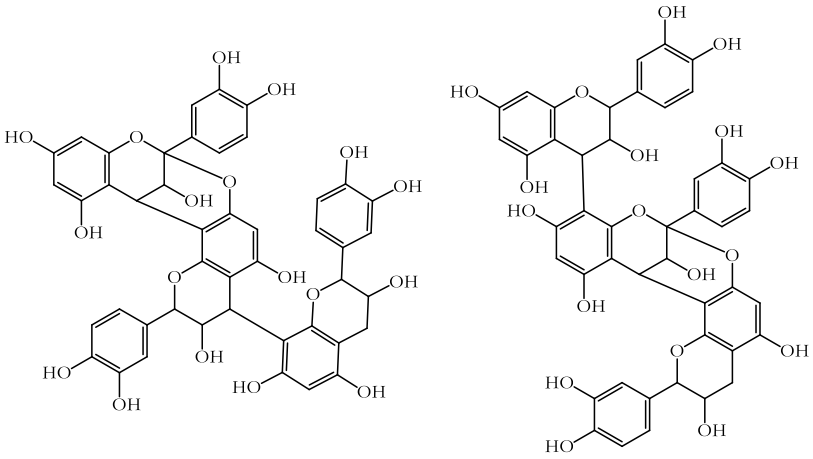


**Figura I.36** Estructura química de los trímeros de tipo A.

### I.5.5.4. Taninos formados por condensación indirecta mediada por acetaldehído

Estos compuestos unidos por un puente de etilideno se han encontrado en vino tinto y sistemas de vino modelo[180].

Durante la vinificación y el envejecimiento del vino, los taninos condensados sufren modificaciones químicas o enzimáticas y algunas de esas modificaciones dan como resultado nuevos puentes interflavanoicos[181].Los taninos formados por condensación indirecta son aquellos en los que la condensación de dos o más flavan-3-oles se da a través de la intervención química de otra molécula, como es el caso de la condensación mediada por acetaldehído. En la Figura I.37 se muestra como se

[175]Xu, C.; Yang, B.; Zhu, W.; Li, X.; Tian, J.; Zhang, L.; *Characterisation of polyphenol constituents of Linderae aggregate leaves using HPLC fingerprint analysis and their antioxidant activities*, Food Chem. **2015**, 186, 83-89.

[176]Sarnoski, P. J.; Johnson, J. V.; Reed, K. A.; Tanko, J. M.; O´Keefe, S. F.; *Separation and characterization of proanthocyanidins in Virginia type peanut skins by LC-MS*$^n$, Food Chem. **2012**, 131, 927-939.

[177]Dong, X. Q.; Zou, B.; Zhang, y.; Ge, Z. Z.; Du, J.; Li, C. M.; *Preparation of A-type proanthocyanidin dimmers from peanut skins and persimmon pulp and comparison of the antioxidant activity of A-type and B-type dimmers*, Fitoterapia **2013**, 91, 128-139.

[178]Tarascou, I.; Mazauric, J. P.; Meudec, E.; Souquet, J. M.; Cunningham, D.;*Cheynier, V.; Fulcrand, H.; Characterisation of genuine and derived cranberry proanthocyanidins by LC-ESI-MS*, Food Chem. **2011**, 128(3), 802-810.

[179]Prodanov, M.; Garrido, I.; Vacas, V.; Lebrón-Aguilar, R; Dueñas, M.; Gómez-Cordovés, C.; Bartolomé, B.; *Ultrafiltration as alternative purification procedure for the characterization of low and high molecular-mass phenolics from almond skins.*, Anal. Chem. Acta.**2008**, 609, 241-251.

[180]Sonni, F.; Moore, E. G.; Clark, A. C.; Chinnici, F.; Riponi, C.; Scollary, G. R.;*Impact of glutathione on the formation of methylmethine and carboxymethine-bridged (+)-catechin dimers in a model wine system*, J. Agric. Food Chem. **2011**, 59(13), 7410−7418.

[181]Drinkine, J.; Lopes, P.; Kennedy, J. A.; Teissedre, P. L.; Saucier, C.; *Ethylidene-Bridged Flavan-3-ols in Red Wine and Correlation with Wine Age*, J. Agric. Food Chem. **2007**, 55, 6292-6299.

produce la adición nucleófila de la posición 8del flavan-3-ol al carbocatión del acetaldehído, seguidamente el aducto pierde una molécula de agua para la formación de un nuevo carbocatión, seguida de una nueva adición nucleófila de la posición 8de otra molécula de flavan-3-ol al acetaldehído unido ya al primer flavan-3-ol[182]. Además, esta reacción también puede ocurrir entre las posiciones 6 y 8 ó 6 y 6, dando lugar a diferentes isómeros de posición.

Por otro lado, se ha confirmado en bibliografía[183] la presencia de aductos del carbocatión del acetaldehído con dímeros y trímeros de flavan-3-oles, con lo que la polimerización puede continuar siguiendo el mismo mecanismo descrito anteriormente.

Por último, hay que destacar que para el tanino con el puente de etilideno formado entre los carbonos 6 y 8, el carbono del puente es asimétrico, con lo que este compuesto presentará dos isómeros (R y S), frente a las otras dos opciones (8 y 8; y, 6 y 6), en las que ese carbono no es asimétrico.

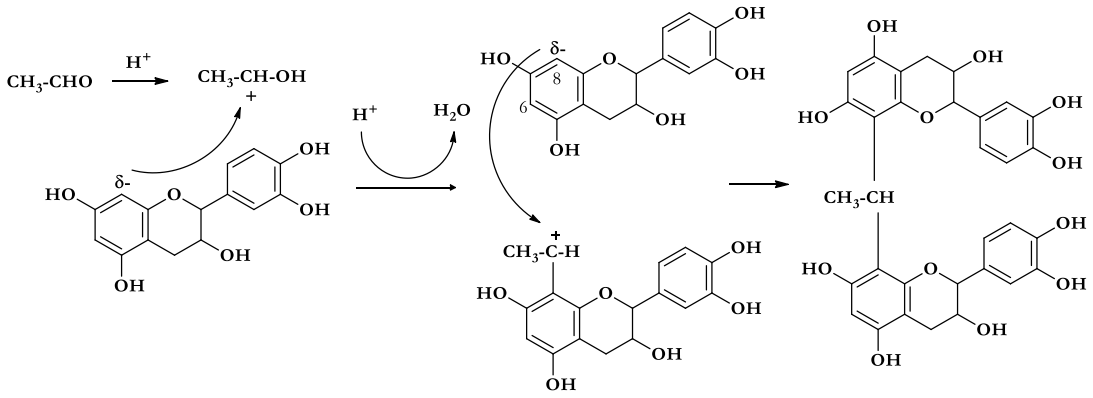


**Figura I.37** Mecanismo para la formación de los taninos formados por condensación indirecta mediada por acetaldehído.

Por otro lado, la despolimerización de los taninos con puente de etilideno o la deshidratación de los aductos etil alcohol-flavan-3-oles, formados durante el proceso de condensación mediada por acetaldehído para dar taninos con puente de etilideno, puede dar lugar a la formación de vinil-flavan-3-oles[184] (Figura I.38).

[182]Sheridan, M. K.; Elias, R. J.; *Reaction of acetaldehyde with wine flavonoids in the presence of sulfur dioxide*, J. Agric. Food Chem. **2016**, 64, 8615-8624.

[183]Mateus, N.; Silva, A. M.; Vercauteren, J.; de Freitas, V.; *Occurrence of anthocyanin-derived pigments in red wines*, J.Agric.Food Chem. **2001**, 49, 4836-4840.

[184]Saucier, C.; Guerra, C.; Pianet, I.; Laguerres, M.; Glories, Y.; *(+)-Catechin-acetaldehyde condensation products in relation to wine-ageing*, Phytochem.**1997**, 46(2), 229-234.

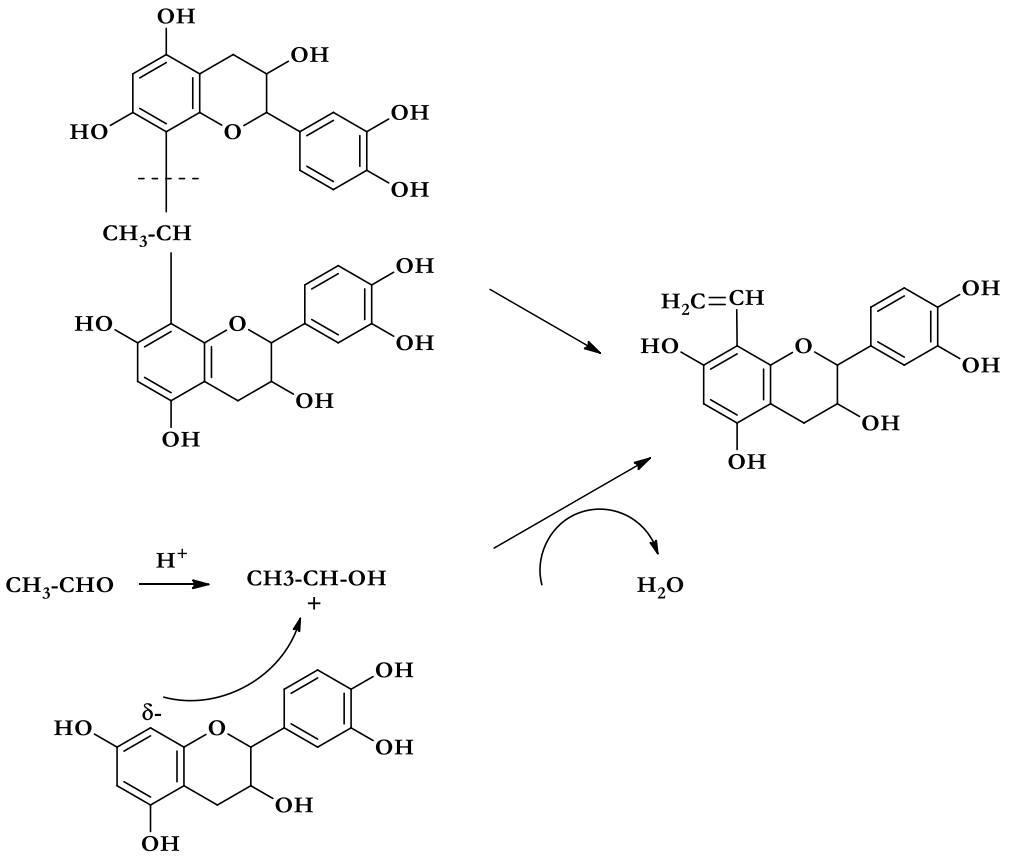



**Figura I.38** Rutas de formación del compuesto vinil(epi)catequina.

### I.5.5.5. Taninos formados por condensación indirecta mediada por ácido glioxílico

El ácido glioxílico es producto de la oxidación del ácido tartárico. El mecanismo de formación de los taninos mediados por ácido glioxílico es muy similar al visto anteriormente, la única diferencia es que en este caso el puente se forma con el ácido en lugar de con el acetaldehído; por lo que, a lo largo de la vinificación y del proceso de envejecimiento del vino, habrá una competición entre ambos por la condensación con los flavan-3-oles[185].

En la Figura I.39, se muestra como se produce la adición nucleófila de la posición 8del flavan-3-ol al carbocatión del ácido glioxílico. A continuación, el aducto del ácido pierde una molécula de agua para la formación de un nuevo carbocatión, seguida de una nueva adición nucleófila de la posición 8de otra molécula de flavan-3-ol al ácido glioxílico unido ya al primer flavan-3-ol. Y, al igual que para el acetaldehído, esta reacción también puede ocurrir entre las posiciones 6 y 8 ó 6 y 6,

[185]Drinkine, J.; Saucier, C.; Glories, Y.;*(+)-Catechin-aldehyde condensations: Competition between acetaldehyde and glyoxylicacid*,J. Agric. Food Chem. **2005**, 53, 7552-7558.

dando lugar a diferentes isómeros de posición, además de darse los isómeros R y S debidos al carbono asimétrico cuando el puente se forma entre los carbonos 6 y 8.

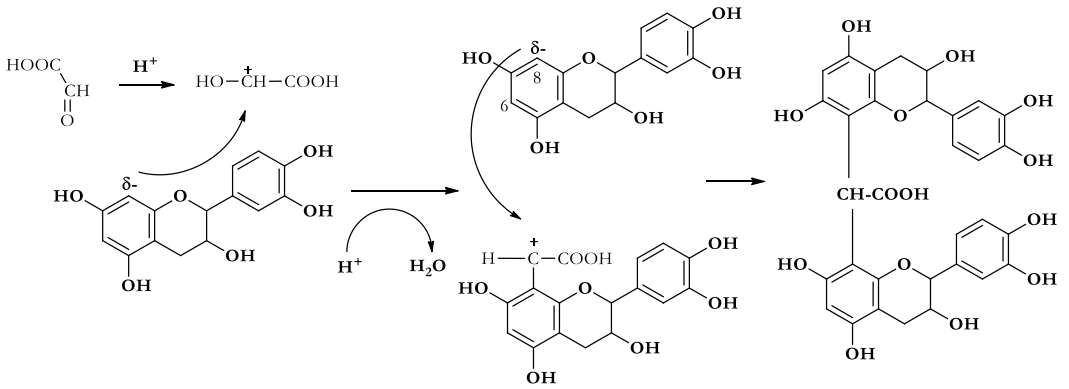


**Figura I.39** Mecanismo para la formación de los taninos formados por condensación indirecta mediada por ácido glioxílico.

### I.5.5.6. Taninos formados por condensación indirecta mediada por furfural

Los furfurales no son compuestos deseados, debido a que producen el pardeamiento que aparece en las frutas, produciendo además un sabor desagradable en los zumos[186]. La formación de estos compuestos y el aumento de su concentración durante el almacenamiento de los zumos de frutas se considera un indicador del deterioro de la calidad de los mismos, que puede ser debido a un calor mayor del debido durante el almacenamiento de los zumos. Por ello, el 5-(hidroximetil)furfural y el furfural se usan para evaluar el grado de pardeamiento no-enzimático en alimentos[187].

A pesar de que los furfurales no estén presentes en las uvas, su formación se produce debido a las condiciones usadas durante el proceso de elaboración de los zumos[188]. Además, estos compuestos también provienen de la madera de las barricas vía transferencia al vino durante el periodo de envejecimiento del mismo, lo cual contribuye directamente al aroma y sabor del vino[189]. Debido a esta presencia de aldehídos, en el vino ocurren reacciones con los flavanoles y/o con las antocianinas,

[186]Es-Safi, N. E.; Cheynier, V.; Moutounet, M.; *Interactions between cyanidin 3-O-glucoside and furfural derivatives and their impact on food color changes*, J. Agric. Food Chem. **2002**, 50, 5586-8895.

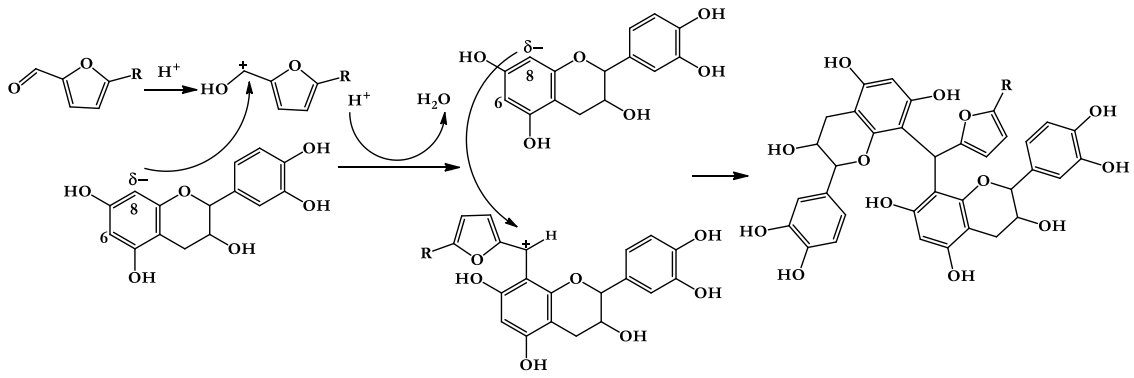
[187]Nonier, M. F.; Pianet, i.; Laguerre, M.; Vivas de Gaulejac, N.; *Condensation products derived from flavan-3-ol oak wood aldehydes reaction*, Anal. Chim. Acta **2006**, 563, 76-83.

[188]Es-Safi, N-E.; Cheynier, V.; Moutounet, M.*; Study of the Reactions between (+)-Catechin and Furfural Derivatives in the Presence or Absence of Anthocyanins and Their Implication in Food Color Change*, J. Agric. Food Chem. **2000**, 48, 5946-5954.

[189]Nonier, M. F.; Vivas, N.; Absalon, C.; Vitry, C.; Fouquet, E.; Vivas de Gaulejac, N.; *Structural diversity of nucleophilic adducts from flavanols and oak wood aldehydes.*, Food Chem. **2008**, 107, 1494-1505.

dando lugar a productos de condensación enlazados mediante un puente diferente en función del aldehído con el que se dé la reacción. Estos compuestos intervienen en la evolución del color y de la astringencia del vino[190].

En la Figura I.40, se muestra en mecanismo para la formación de los taninos con puente de furfural. El mecanismo es igual que el descrito en los apartados anteriores y se puede dar la formación del puente entre los carbonos 8 y 8 (como se muestra en la figura) o entre las posiciones 6 y 8 ó 6 y 6, dando lugar a diferentes isómeros de posición, además de darse los isómeros R y S debidos al carbono asimétrico cuando el puente se forma entre los carbonos 6 y 8.



| | **R** |
|---|---|
| Furfural | -H |
| 5-(hidroximetil)furfural | $-CH_2-OH$ |

**Figura I.40** Mecanismo para la formación de los taninos formados por condensación indirecta mediada por furfurales.

### I.5.5.7. Sales xantilium

Los taninos formados por condensación indirecta pueden sufrir una deshidratación, seguida de un proceso de oxidación dando lugar a las sales xantilium, las cuales producen un amarilleamiento de las disoluciones y en consecuencia su

[190]Sousa, C.; Mateus, N.; Perez-Alonson, J.; Santos-Buelga, C.; De Freitas, V.;*Preliminary study of oaklins, a new class of brick-red catechin-pyrylium pigments resulting from the reaction between catechin and wood aldehydes.,* J. Agric Food Chem. **2005**, 53, 9249-9256.

máximo de absorción es de 446-460 nm[191]. En la Figura I.41, se muestra el mecanismo de formación para estos compuestos, ya descritos en bibliografía[192].

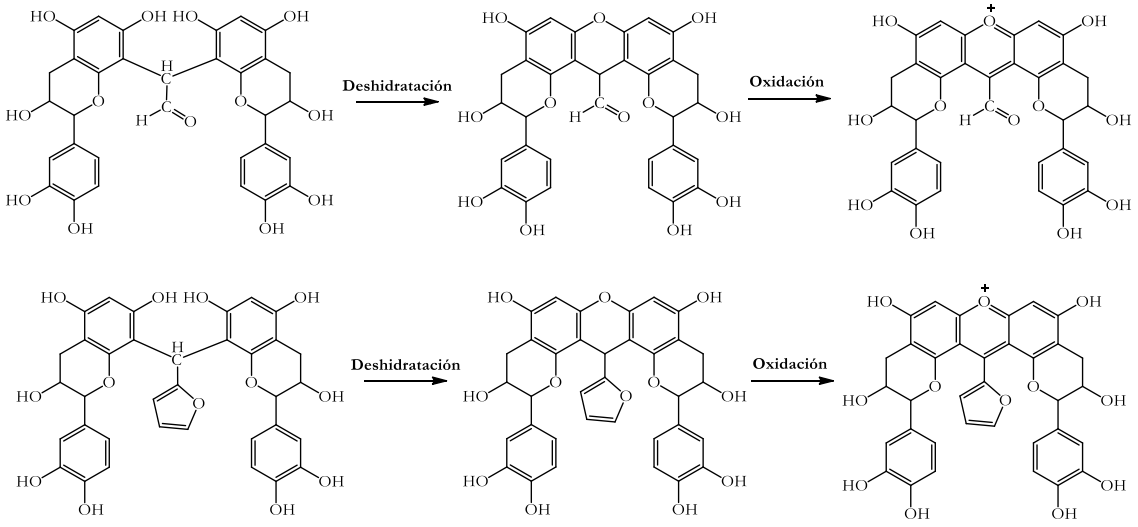



**Figura I.41** Mecanismo de formación de las sales xantilium a partir de los taninos formados por condensación indirecta mediada por (de arriba abajo) ácido glioxíloco o furfural.

### I.5.6. TANINOS HIDROLIZABLES

Los taninos hidrolizables son poliésteres de derivados de azúcar (D-glucosa), que están formados por condensación de varias unidades de ácido gálico o ácido elágico(Figura I.42), esterificados a uno o varios azúcares. Se denominan hidrolizables porque en medio ácido se rompen mediante hidrólisis de los enlaces éster[193]. Dependiendo de si es el ácido gálico o el elágico el que está formando los enlaces ésteres, se distinguen los galotaninos y los elagitaninos.

[191]Es-Safi, N-E.; Le Guernevé, C.; Cheynier, V.; Moutounet, M.*; New Phenolic Compounds Formed by Evolution of (+)-Catechin and Glyoxylic Acid in Hydroalcoholic Solution and Their Implication in Color Changes of Grape-Derived Foods.*, J. Agric. Food Chem. **2000**, 48, 4233-4240.

[192]Es-Safi, N-E.; Le Guernevé, C.; Fulcrand, H.; Cheynier, V.; Moutounet, M.*; NewPolyphenolic Compounds with Xanthylium Skeletons Formed through Reaction between (+)-Catechin and GlyoxylicAcid,* J. Agric. Food Chem. **1999**, 47, 5211-5217.

[193]Hagerman, A.E.; Butler, L.G.; *Tannins and Lignins.*En: Rosenthal, G.; Berenhaum, M. (Eds).;*Herbivores their interactions with secondary plant metabolites.* **1991**, 355-388.

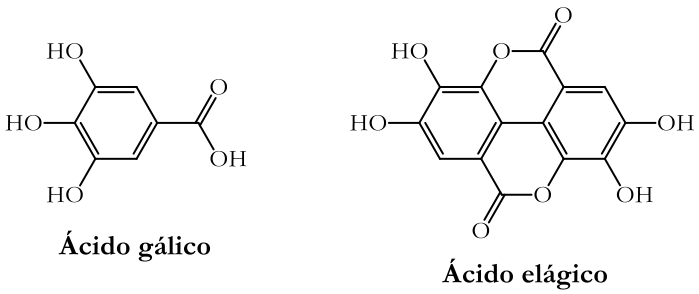



**Figura I.42** Estructura química de los ácidos gálico y elágico.

Los taninos hidrolizables se encuentran presentes en la madera de roble[194]. Las barricas más utilizadas para la estancia de los vinos en ellas son las de madera de roble americano *Quercus alba L.* y de roble europeo *Quercus robur L*[195]. La mayor diferencia entre estas maderas de roble es que el europeo, normalmente tiene más fenoles por unidad de material extractable no volátil que el americano; sin embargo el americano se cree que contribuye más al sabor por unidad de taninos.

De los fenoles extraíbles con etanol de la madera de roble, el 90% son no flavonoides; entre ellos están las ligninas, el ácido gálico, ácido elágico, ácidos aromáticos, aldehídos y taninos hidrolizables[196]. Los taninos hidrolizables no volátiles suponen de un 5 a un 15 % del peso seco de la madera de roble. No obstante, este porcentaje también varía en función del tostado de la barrica[197].

Los galotaninos, que son los taninos hidrolizables más simples, son ésteres poligaloilados de glucosa (Figura I.43) que se encuentran presentes en las agallas del roble y, además, se pueden adicionar al vino mediante productos comerciales.

Los elagitaninos tienen el alcohol polihídrico (D-glucosa) con estructura básica y los grupos OH se esterifican con ácido gálico, dándose así su estructura. Se cree que los elagitaninos se forman a partir de los galotaninos por acoplamiento oxidativo entre dos grupos galoilo próximos[198]. Los dos elagitaninos más comunes son la vescalagina y la castalagina (isómeros), que al hidrolizarse se transforman en vescalina y

[194]Saucier, C.; Jourdes, M.; Glories, Y.; Quideau, S.; Extraction, *Detection, and Quantification of Flavano-Ellagitanins and Ethylvescalagin in a Bordeaoux Red Wine Aged in Oak Barrels*, J. Agric. Food Chem. **2006**, 54, 7349-7354.

[195]Glabasnia, A.; Hofmann, T.; Sensory-Directed *Identification of Taste-Active Ellagitannins in American (Quercus alba L.) and European Oak Wood (Quercusrobur L.) and Quantitative Analysis in Bourbon Whiskey and Oak-Matured Red Wines,* J. Agric. Food Chem. **2006**, 54, 3380-3390.

[196]Jarauta, I.; Cacho, J.; Ferreira, V.; *Concurrent Phenomena Contributing to the Formation of the Aroma of Wine during Aging in Oak Wood: An Analytical Study*, J. Agric. Food Chem. **2005**, 53, 4166-4177.

[197]Glabasina, A.; Hofman, T.; *Identification and Sensory Evaluation of Dehydro- and Deoxy-ellagitannins Formed upon Toasting of Oak Wood (Quercusalba L.)*, J. Agric. Food Chem. **2007**, 55, 4109-4118.

[198]Maatta-Riihinen, K.R.: Kamal-Eldin,A.;Riitta,A.; *Identification and Quantification of Phenolic Compounds in Berries of Fragaria and Rubus Species (Family Rosaceae)*, J. Agric. Food Chem. **2004**, 52, 6178-6187.

castalina, respectivamente (Figura I.44); y, además, pueden dar acoplamientos intermoleculares con otros taninos hidrolizables.

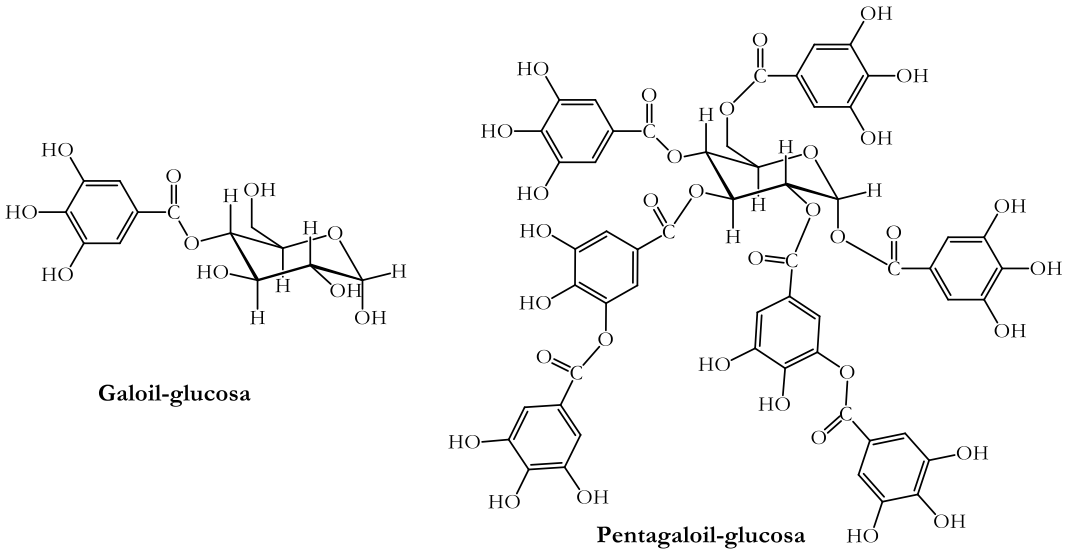



**Figura I.43** Estructura química de los galotaninos: galoil-glucosa y pentagaloil-glucosa.

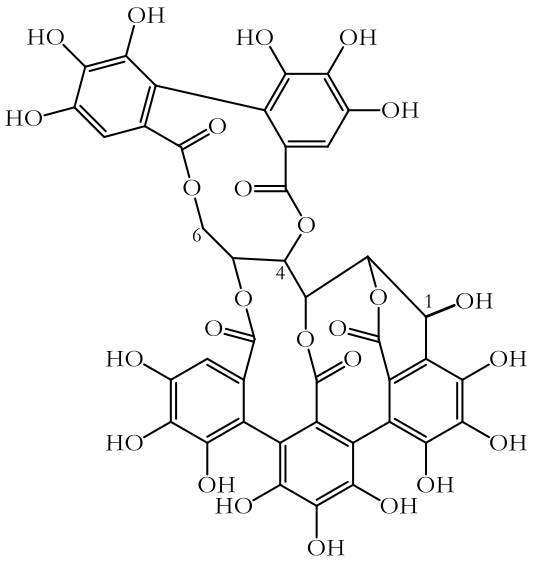
Estos taninos influyen en la estructura de los compuestos fenólicos y en el color del vino tinto por un aumento de la velocidad en la condensación en las procianidinas, mientras limita la degradación de las mismas mediante oxidación y precipitación[200,201]. La presencia de varios grupos hidroxilo (OH) en posición orto provoca que estos compuestos se vean envueltos en reacciones de oxidación en los vinos tintos; además, debido a que son hidrosolubles, se disuelven rápidamente en el vino. Cuando se compara el consumo de oxígeno entre elagitaninos como la castalagina y flavan-3-oles como la catequina, este consumo es mucho mayor cuando el vino contiene elagitaninos. Esto es debido a que en la catequina hay dos grupos hidroxilo en posición orto, frente a los 15 OH de la castalagina[202,203], por lo que protegen al resto de compuestos de ser oxidados.

[200] García-Estévez, I.; Alcalde-Eon, C.; Puente, V.; Escribano-Bailón, T.; *Enological tannin effect on red wine color and pigment composition and relevance of the yeast fermentation products*, Molecules **2017**, 22, 2046-2061.

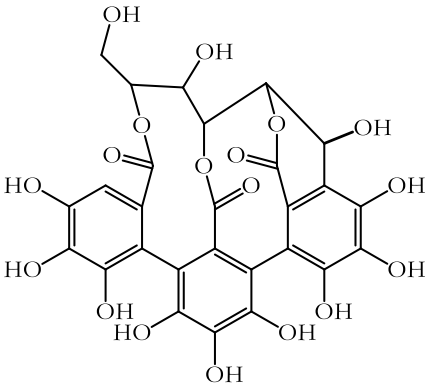
[201] García-Estévez, I.; Escribano-Bailon, T.; Rivas-Gonzalo, J. C.; Alcalde-Eon, C. *Development of a fractionation method for the detection and identification of oak ellagitannins in red wines*. Anal. Chem. Acta. **2010**, 660, 171-176.

[202] García-Estévez, I.; Alcalde-Eon, C.; Puente, V.; Escribano-Bailón, T.; *Enological tannin effect on red wine color and pigment composition and relevance of the yeast fermentation products*, Molecules **2017**, 22, 2046-2061.

[203] Vivas, N.; Glories, Y. *Role of Oak Wood Ellagitanins in the Oxidation Process of Red Wines during Aging*. Am. J. Enol. Vitic.**1996**, 47 (1), 103-107.



**Vescalagina/Castalagina**



**Vescalina/Castalina**

**Figura I.44** Estructura química de los elagitaninosvescalagina/castalagina (isómeros) y vescalina/castalina (isómeros).

# I.6. MÉTODOS DE ANÁLISIS DE POLIFENOLES

La caracterización estructural de los polifenoles, y de sus derivados, requiere el empleo de métodos rápidos y fiables para el análisis y la identificación de estos compuestos naturales presentes en el vino.

El desarrollo de técnicas de fraccionamiento y aislamiento de antocianinas, derivados antociánicos y taninos es un requisito para lograr su identificación y conocer mejor sus propiedades, especialmente en el estudio de mezclas muy complejas.

Dada la gran complejidad de la matriz, se han utilizado procedimientos de cromatografía de exclusión por tamaño molecular (SEC), para lo que se han utilizado distintas fases estacionarias, siendo las más utilizadas las fases comerciales Sephadexy Toyopearl[204]. Se han utilizado también otras técnicas como la extracción en fase sólida (SPE)[205].

[204]Sun, B.; Fernandes, T. A.; Spranger, M. I.; *A new class of anthocyanin-procyanidin condensation products detected in red wine by electrospray ionization multi-stage mass spectrometry analysis,* Rapid Commun. Mass Spectrom. **2010**, 24, 254-260.

[205]Tomaz, I.; Maslov, L.; *Simultaneous determination of phenolic compounds in different matrices using phenyl-hexyl stationary phase*, Food Anal.Method.**2016**, 9(2), 401-410.

El nacimiento de la cromatografía liquida de alta resolución (HPLC) supuso un avance para el análisis de alimentos y hoy en día es la técnica más empleada para la identificación y cuantificación de antocianinas[206] y taninos.

La cromatografía en fase reversa utilizando columnas C18 permite excelentes separaciones en mezclas de antocianinas con otros compuestos polifenólicos y un tiempo de análisis relativamente corto[207]. También es una técnica que se utiliza en los estudios de perfiles tánicos de uvas y vinos.

Como tipos de detección acoplados a la cromatografía liquida de alta resolución, empleada en el análisis de polifenoles, se encuentran los del tipo espectrométrico: espectroscopía UV-visible, espectro fluorimetría, espectrometría de masas y RMN, aunque en algunas investigaciones se han utilizado también los de detección electroquímica[208].

El estudio del espectro UV-vis de los compuestos proporciona información acerca de la sustitución del anillo B y del tipo de derivado antociánico de que se trata[209], pero no permite distinguir en ocasiones entre compuestos del mismo tipo. Por lo tanto, es necesario complementar la información obtenida con otras técnicas que proporcionan más información estructural, por ejemplo, la espectrometría de masas (MS)[210] o la resonancia magnética nuclear (RMN)[211]. Así, la espectrometría de masas y la resonancia magnética nuclear son actualmente las técnicas más utilizadas en la identificación de polifenoles.

La espectrometría de masas es un modo de detección muy sensible, que permite elucidar estructuras por diferenciación del peso molecular y el patrón de

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[206]Versari, A.; Boulton, R. B.; Parpinello, G. P.; *A comparison of analytical methods for measuring the color components of red wines.,* Food Chem. **2008**, 106, 397-402.

[207]Sáez, V.; Gayoso, C.; Riquelme, S.; Pérez, J.; Vergara, C.; Mardones, C.; von Baer, D.; *C18 column with in series absorbance and fluorescence detection for simultaneous monitororing changes in stillbenoid and proanthocyanidin concentrations during grape cane storage*, J. Chromatogr. B **2018**, 1074, 70-78.

[208]Novak, I.; Janeiro, P.; Seruga, M.; Oliveira-Brett, A.M.; *Ultrason extracted flavonoids from four varieties of Portuguese red grape skins determined by reverse phase high performance liquid chromatography with electrochemical detection*, Anal. Chim. Acta. **2008**, 630, 207-115.

[209]Ribeiro, L. F.; Ribani, R. H.; Francisco, T. M. G.; Soares, A. A.; Pontarolo, R.; Haminiuk, C. W. I.;*Profile of bioactive compounds from grape pomace (Vitisvinifera and Vitislabrusca) by spectrophotometric, chromatographic and spectral analyses*, J Chromatogr B AnalytTechnol Biomed Life Sci**2015**, 1007, 72-80.

[210]Abad-García, B.; Berrueta, L. A.; Garmón-Lobato, S.; Gallo, B.; Vicente, F.; *A general analytical strategy for the characterization of phenolic compounds in fruit juices by high-performance liquid chromatography with diode array detection coupled to electrospray ionization and triple quadrupole mass spectrometry*, J. Chromatogr. A **2009**, 1216, 5398-5415.

[211]Ferreira-Lima, N.; Vallverdú-Queralt, A.; Meudec, E.; Mazauric, J-P.; Sommerer, N.; Bordignon-Luiz, M. T.; Cheynier, V.; Le Guernevé, C.; *Synthesis, identification and structure elucidation of adducts formed by reactions of hydroxycinnamic acids*, J. Nat. Prod. **2016**, 79(9), 2211-2222.

fragmentación[212,213], permite estudiar los cambios que tienen lugar durante el envejecimiento del vino y las posibles polimerizaciones y reacciones con otros flavonoides.

Por otro lado, el empleo de un sistema de detección como el RMN permite no sólo la elucidación estructural de los polifenoles, sino que también proporciona información sobre los posibles mecanismos de formación de los derivados antociánicos y taninos[211] o permite la identificación de productos de reacción de antocianinas con otros compuestos tales como derivados de ácidos cinámicos, catequinas o flavonoles.

En el caso de las estructuras poliméricas de los taninos también se emplean con profusión métodos de despolimerización previa a la separación cromatográfica. Los más empleados hoy en día consisten en la conversión de las proantocianidinas en sus unidades constitutivas por medio de catálisis ácida en presencia de exceso de un nucleófilo (tiólisis[214,215] y degradación con floroglucinol[216,217]. Mediante estos métodos se obtiene información sobre el grado medio de polimerización y la composición de las unidades terminales y de extensión[218]; sin embargo, no aportan información individual para cada uno de los taninos que puedan existir en el vino.

Como se ha mencionado previamente, los compuestos polifenólicos son los principales responsables del sabor y del color del vino, y a lo largo del envejecimiento del mismo tienen lugar diferentes reacciones entre las antocianinas y los taninos del medio que dan lugar a otros pigmentos que modifican el color del vino. El conocimiento de las estructuras antociánicas es muy importante para predecir el comportamiento de un vino durante el envejecimiento e intentar mejorar la estabilización del color. Además, los polifenoles son los compuestos más importantes

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[212]Pati, S.; Liberatore, M.T.; Gambacorta, G.; Antonacci, D.; La Notte, E.; *Rapid screening for anthocyanins and anthocyanin dimers in crude grape extracts byhigh performance liquid chromatography coupled with Diode array detection and tandem mass spectrometry*, J. Chromatogr. A. **2009**, 1216, 3864-3868.

[213]Huang, Z.; Wang, B.; Williams, P.; D. Pace, R.; *Identification of anthocyanins in muscadine grapes with HPLC-ESI-MS*, Food Sci. Technol. **2009**, 42, 819-824.

[214]Cadot, Y.; Caillé, S.; Samson, A.; Barbeau, G.; Cheynier, V.; *Sensory representation of typicality of Cabernet franc wines related to phenolic composition: Impact of ripening stage and maceration time*, Anal. Chim. Acta. **2012**, 732, 91-99.

[215]Mazerolles, G.; Preys, S.; Bouchut, C.; Meudec, E.; Fulcrand, H.; Souquet, J. M.; Cheynier, V.: *Combination of several mass spectrometry ionization modes: A multiblock analysis for a rapid characterization of the red wine polyphenolic composition*, Anal. Chim. Acta. **2010**, 678(2) 195-202.

[216]Kemp, B. S.; Harrison, R.; Creasy, G. L.; *Effect of mechanical leaf removal and its timing on flavan-3-ol composition and concentrations in Vitisvinifera L. cv. Pinot Noir wine*, Aust. J. Grape Wine Research. **2011**, 17(2), 270-279.

[217]Chira, K.; Pacella, N.; Jourdes, M.; Teissedre, P. L.; *Chemical and sensory evaluation of Bordeaux wines (Cabernet-Sauvignon and Merlot) and correlation with wine age*, Food Chem. **2011**, 126(4), 1971-1977.

[218]Nonier, M. F.; Vivas, N.; Vivas de Gaulejac, N.; Fouquet, E.; *Development of direct-size exclusion chromatography separation for the determination of molar mass of native procyanidins in the phenolate form*, J. Chrom. A. **2005**, 1089, 263-269.

relacionados con los beneficios del consumo del vino en el tratamiento de desórdenes circulatorios[219].

El acoplamiento de la cromatografía líquida con la espectrometría de masas (HPLC-MS) resulta ser una técnica muy adecuada para el estudio de los polifenoles en extractos de uvas y vino, debido a que proporciona información estructural en línea de compuestos que se encuentran en baja concentración en mezclas complejas; más aún, si se cuenta con la posibilidad de realizar experimentos de masas en tándem (MS/MS).

### I.6.1. FUNDAMENTOS DE LA ESPECTROMETRÍA DE MASAS

La espectrometría de masas se basa en la separación de iones (positivos o negativos) generados a partir de moléculas en función de su relación masa/carga (m/z), obteniéndose de esta manera un espectro de masas.

Es una técnica que se fundamenta en los principios clásicos del movimiento de iones a través de campos magnéticos y eléctricos y se emplea principalmente para identificar compuestos desconocidos, cuantificar sustancias conocidas y elucidar las propiedades estructurales y físicas de los iones[220].

Las partes más importantes de un espectrómetro de masas son la fuente de ionización, el analizador y el detector.

En espectrometría de masas es un requisito esencial la formación de iones de la muestra en fase gas. En el caso del acoplamiento de la cromatografía líquida de alta resolución con la espectrometría de masas, se deben transformar las moléculas de analito que se encuentran en disolución en la fase móvil en iones en fase gaseosa, sin que se produzca la degradación térmica; y, también, eliminar la gran cantidad de gas y vapor procedente de la fase móvil antes de entrar en la región de alto vacío del analizador. Es en la fuente de ionización donde se generan estos iones y se aceleran hacia el analizador.

Los iones en fase gas se separan en el analizador de masas dependiendo de su relación masa/carga (m/z), aprovechando sus diferentes propiedades magnéticas y eléctricas. Por último, los iones impactan en el detector, donde el flujo se convierte en una corriente eléctrica proporcional. Un sistema de recogida de datos registra la

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[219]Esteban-Fernandez, A.; Zorraquin-Pena, I.; Gonzalez de Llano, D.; Bartolome, B.; Moreno-Arribas, M. V.; *The role of wine and food polyphenols in oral health*, Trends Food Sci. Technol. **2017**, 69, 118-130.

[220]Vita Di Stefano, G. A.; Bongiorno, D.; Cunsolo, V.; Muccilli, V.; Sforza, S.; Dossena, A.; Drahos, L.; Vékey, K.; *Applications of liquid chromatography–mass spectrometry for food analysis*, J. Chromatogr. A **2012**, 1259, 74– 85.

magnitud de estas señales eléctricas en función de la relación m/z y convierte esta información en un espectro de masas.

Una amplia mayoría de las aplicaciones analíticas de la técnica HPLC-MS utiliza fuentes de ionización a presión atmosférica(API), tanto de tipo electrospray (ESI) como ionización química a presión atmosférica (APcI). No es fácil en ocasiones conocer de antemano cuál de las dos sondas es más adecuada para un determinado analito, sin embargo, se suele utilizar la sonda ESI para compuestos de polaridad media-alta y la sonda APcI para aquellos con una polaridad baja-media[221].

Una fuente API consta de cuatro componentes básicos:

1.- Dispositivo de introducción de muestra o sonda.

2.- Cámara de ionización, donde se generan los iones mediante ionización por electrospray (ESI) o ionización química a presión atmosférica (APcI).

3.- Cono de muestreo, que es el orificio de entrada de iones.

4.- Sistema de transferencia de iones, en el que éstos son transportados a la región de alto vacío del espectrómetro.

La trasmisión desde la región a presión atmosférica hasta la de alto vacío es la etapa en la que se produce la pérdida de la mayor parte del analito. Se han diseñado diferentes geometrías de fuentes API, entre las que se encuentra la geometría Z-spray, en la que los iones son extraídos en una trayectoria ortogonal a la que se generan en la fuente, obteniéndose de esta manera una mayor sensibilidad debido a que se eliminan moléculas neutras del spray, disminuyendo ruido de fondo y favoreciéndose la eliminación de los aductos en la cámara de ionización.

La interfase ESI, utilizada en este trabajo, consiste en una fuente de geometría Z-spray. El efluente del sistema cromatográfico entra en la interfase a través de la sonda, al aplicar un alto voltaje entre el capilar de entrada de muestra y el cono de muestreo y con la ayuda de un flujo coaxial de $N_2$, el efluente se convierte en un aerosol cargado. El flujo coaxial de $N_2$ mejora la producción del spray en situaciones de elevados caudales de flujo, sin pérdida de sensibilidad. Un segundo flujo de $N_2$ calentado (gas de desolvatación) ayuda a la evaporación del disolvente. En este tipo de fuente Z-spray existe otro tercer caudal de $N_2$, que sale por el cono de muestreo en dirección contraria al spray (gas de cono). En la nebulización, la mayor parte de la

[221]Ardrey, B.; *Applications of High Performance Liquid Chromatography-Mass Spectrometry*. En Liquid Chromatography-Mass Spectrometry. An Introduction. Ed: Ando, D. J., Consultant, Dartford, Kent, UK, **2003**, pp. 129-232.

materia del spray es arrastrada a desecho, mientras que una pequeña parte es arrastrada a través del orificio del cono de muestreo. Diferencias de presión y de voltaje dirigen los iones a través del cono de muestreo, del cono de extracción y de las lentes rf en dirección al analizador[222]. Un esquema de este tipo de fuente de ionización se muestra en la Figura I.45.

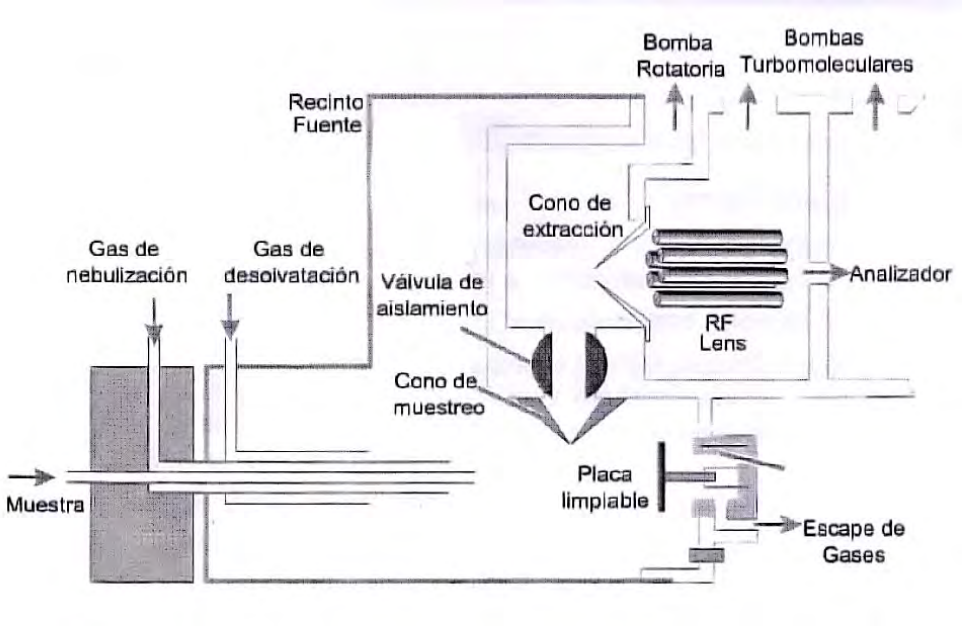


**Figura I.45** Esquema general de la sonda de electrospray empleada por Waters-Micromass.

Cuando se aplica un voltaje al capilar del electrospray, los iones de la misma polaridad migran en el líquido hacia el extremo del capilar, alargándose la superficie del líquido y formándose el llamado "cono de Taylor"[223]. Cuando la acumulación del exceso de iones de la misma polaridad en la superficie del líquido hace que la fuerza de repulsión culómbica supere a la tensión superficial (se alcanza el límite de Rayleigh), tiene lugar la explosión culómbica y se emiten desde el capilar multitud de minúsculas gotas (aprox 1 μm), cargadas con iones de la misma polaridad. Las gotas emitidas disminuyen su tamaño como consecuencia de la evaporación del disolvente, mientras que la carga de la gota permanece constante, produciéndose la fisión de la gota repetidas veces hasta formar gotas muy pequeñas (FiguraI.46).

[222]Lemière, F. Interfaces for LC-MS. Guide to LC-MS. LC-GC, Europe **2001**.

[223]Meher, A. K.; Cheng, Y-C.; *Polarization induced electrospay ionization mass spectrometry for the analysis of liquid, viscous and solid samples*, J Mass Spectrom. **2015**, 50(3), 444-450.

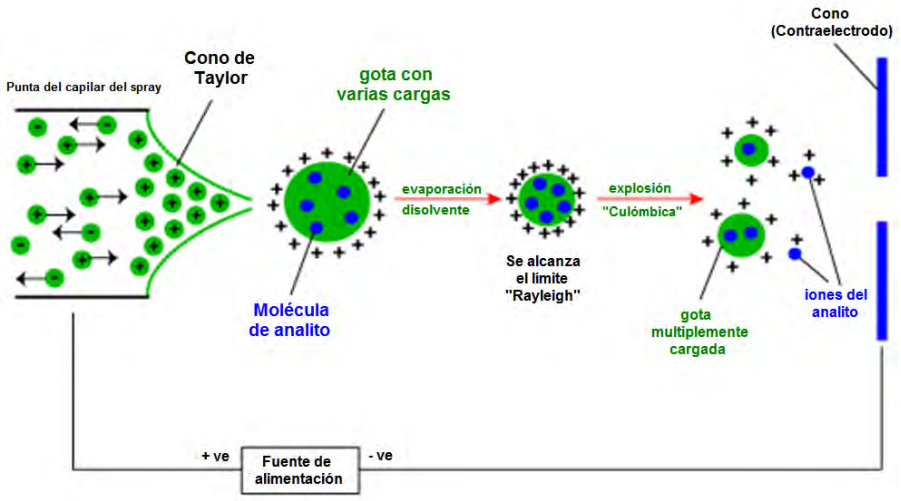


**Figura I.46** Mecanismo de formación de iones en fase gaseosa en la interfase ESI.

Se han descrito dos modelos, el modelo de vaporización iónica y el de carga residual, para explicar la formación de iones a partir de gotas muy pequeñas y altamente cargadas. El modelo de vaporización iónica propone que en las gotas con radio inferior a 10 nm, debido a las repulsiones de carga, prima la evaporización iónica en la superficie de la gota frente a la fisión de la gota y los iones pasan a fase gaseosa. El modelo de carga residual defiende que la fisión de la gota produce gotas extremadamente pequeñas que contienen un solo ión. La evaporación del disolvente de éstas produce iones en fase gaseosa.

Los iones formados se "extraen" del spray gracias a la diferencia de potencial existente entre el capilar y el cono de muestreo. Al entrar en la cámara de presión intermedia las moléculas neutras y los aductos iónicos del disolvente se eliminan gracias a una corriente de gas en sentido contrario (gas de cono), que impide la entrada de dichas sustancias, y a la aceleración de los iones hacia la zona de baja presión del espectrómetro. Si se aumenta la aceleración de los iones (aumentando la diferencia de potencial entre los dos electrodos) se puede producir la ruptura de los iones por colisión con moléculas de gas residual procedente de la fase móvil y del $N_2$ utilizado como gas de nebulización. Los fragmentos producidos por este fenómeno, denominado disociación por colisión inducida (CID) en la fuente, pueden ser utilizados para la elucidación estructural de moléculas.

El analizador es uno de los componentes más importantes en un espectrómetro de masas, debido a que su función es clasificar los iones procedentes de la fuente de ionización en función de su relación m/z y de él dependen en principal medida la

resolución, sensibilidad, rango de masas y tiempo medio de residencia de los iones en el espectrómetro.

Los analizadores más utilizados en espectrometría de masas son los cuadrupolos, fundamentalmente por su bajo coste y robustez. El cuadrupolo consiste en cuatro filtros paralelos de molibdeno alineados paralelamente entre sí y equidistantes de un eje central imaginario situado sobre el eje Z cartesiano. Se puede observar esta disposición en la Figura I.47.

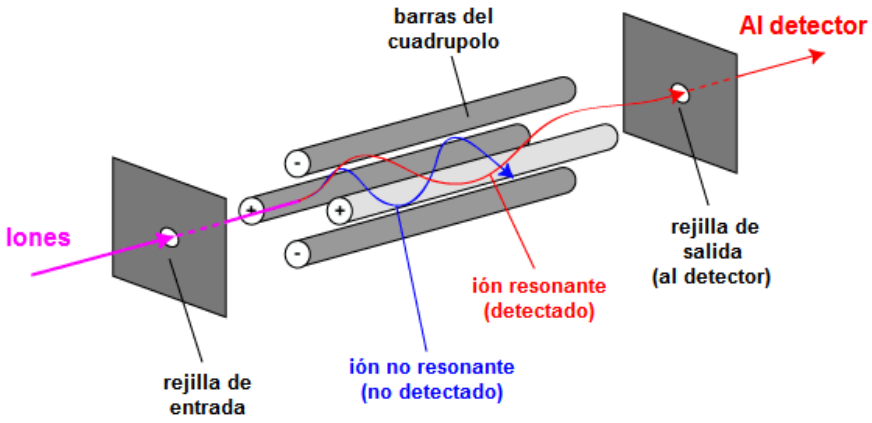


**Figura I.47** Esquema de un analizador de tipo cuadrupolo. Se muestran las trayectorias de los iones.

Mediante la aplicación a cada pareja de barras opuestas de voltajes variables de corriente continua (DC) y de radiofrecuencia (RF) superpuestos, se consigue que los iones de masas determinadas pasen por el "túnel" formado por las cuatro barras polares, siguiendo trayectorias oscilantes estables que les conducen al detector, mientras que las demás masas, al ser inestables sus trayectorias, no alcanzan el detector y son desviadas fuera del conjunto de barras. Variando los voltajes podemos enfocar de modo sucesivo las diferentes masas presentes y obtener de este modo el espectro de masas correspondiente[224].

La pareja de electrodos situados en el plano XZ actúa como filtro de masas bajas, ya que para determinados valores de potencial de corriente continua y potencial de radiofrecuencia, sólo consiguen pasar con trayectorias estables las masas mayores de un determinado valor. Por otro lado, los electrodos situados en el plano YZ actúan como un filtro que elimina las masas altas, rechazando las mayores de un cierto valor. La combinación de ambas parejas de electrodos produce como resultado que, para

[224]Ardrey, B.; *Mass Spectrometry*. En Liquid Chromatography-Mass Spectrometry. An Introduction. Ed: Ando, D. J., Consultant, Dartford (Kent, UK) **2003**, pp. 33-74.

unas determinadas condiciones de operación, sólo puedan pasar los iones de una masa concreta. De este modo, variando los valores de potencial de corriente continua y potencial de radiofrecuencia, se pueden conseguir de modo sucesivo trayectorias estables para todo el rango de masas estudiado.

Otros analizadores utilizados también en espectrometría de masas son la trampa iónica (IT), que confina a los iones en orbitas cerradas mediante radiofrecuencias y que, empleando pulsos de radiofrecuencias permiten realizar barridos y los analizadores de tiempo de vuelo (TOF), que determinan la relación m/z en función del tiempo que necesita el ion para recorrer una distancia[225].

Los iones generados en la ionización son, por regla general, suficientemente estables para alcanzar el detector, por lo que no tiene sentido acoplar analizadores en serie si no es con el objetivo de inducir fragmentación entre ellos. La técnica más habitual es la fragmentación en vuelo inducida por colisión con partículas de alta o baja energía, en un procedimiento totalmente análogo al que tiene lugar en la fuente de ionización, pero que se realiza en celdas de colisión especializadas con un gas de colisión, generalmente argón, a una presión controlada.

Las celdas de colisión son generalmente cuadrupolos, hexapolos u octapolos de transmisión, es decir, se aplican únicamente radiofrecuencias. La extensión y la energía de las fragmentaciones en estas celdas de colisión se controlan de forma bastante precisa, a diferencia de las fragmentaciones en el cono. La eficacia de estas fragmentaciones puede ser muy alta, compensando la ionización en general muy blanda de la interfase. Una imagen representativa de una celda de colisión se muestra en la FiguraI.48.

[225]Fjeldsted, J. C.; *Accurate mass measurements with orthogonal axis time-of-flight mass spectrometry*.En Liquid Chromatography Time-of-Flight Mass Spectrometry.John Wiley & Sons Inc, Nueva Jersey **2009**, pp. 3-16.

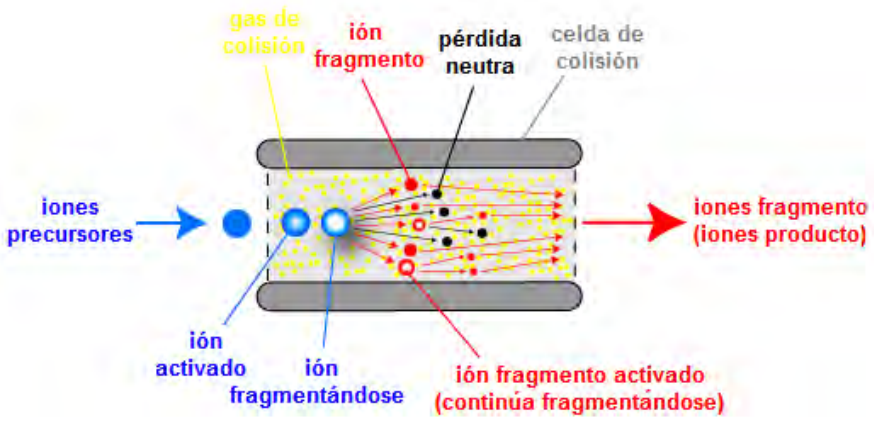


**Figura I.48** Celda de colisión de un espectrómetro de masas, en la que se puede observar la fragmentación de un ion precursor en varios iones fragmento.

Existen muchos tipos de configuraciones de MS en tándem, en nuestro caso se ha utilizado una configuración de tipo triple cuadrupolo (QqQ)[226], que se muestra en la Figura I.49, en la que el segundo cuadrupolo actúa como celda de colisión, siendo ésta la configuración más habitual para el análisis de trazas y el *screening* de productos naturales. Estos equipos son fácilmente automatizables, emplean bajos voltajes, presentan una resolución adecuada para la mayoría de las aplicaciones y unos niveles de sensibilidad y robustez muy buenos.

En una configuración de tipo triple cuadrupolo, el primer y tercer cuadrupolo funcionan como analizadores de iones y se les aplica tanto corrientes de voltaje continuo (DC) como de radiofrecuencia (RF), mientras que el cuadrupolo intermedio funciona como celda de colisión, en el que únicamente se aplican corrientes de radiofrecuencias. Esta configuración es una disposición de MS/MS, sin embargo generalmente la ionización se optimiza para ajustar la ionización producida en la fuente, con lo que se puede llegar a obtener un espectro de $MS^2$.

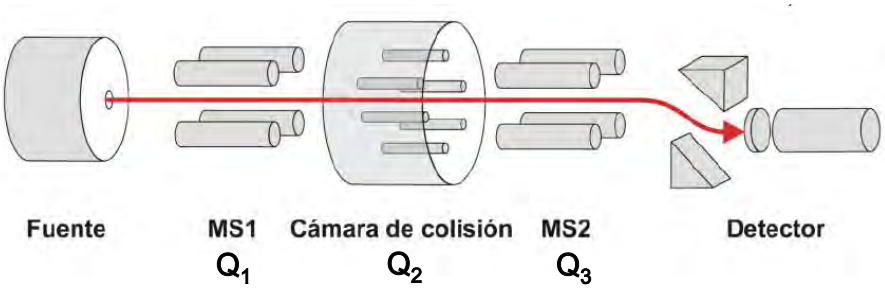


**Figura I.49** Configuración de tipo triple cuadrupolo (QqQ) de un espectrómetro de masas.

[226]Flamini, R.; Traldi, P. *MS/MS Methodologies*.En Mass Spectrometry in grape and wine chemistry.John Wiley & Sons Inc. Nueva Jersey, **2010**, pp. 76-93.

En este tipo de equipos HPLC-MS/MS se pueden realizar tanto experimentos de MS como de MS/MS.

En los experimentos de MS únicamente funciona como analizador el primer cuadrupolo ($Q_1$), permaneciendo cerrada la válvula del gas de colisión y actuando la celda de colisión ($Q_2$) y el tercer cuadrupolo ($Q_3$) en modo de trasmisión. Se pueden realizar experimentos en modo *scan*, en los que el $Q_1$ se encuentra barriendo y se obtienen los espectros de masas, o experimentos en modo SIR o SIM (*single or selected ion recording or monitoring*), en los que el $Q_1$ está fijado en una relación m/z, consiguiéndose de este modo mayor sensibilidad. Una representación de este tipo de experimentos se puede observar en la Figura I.50.

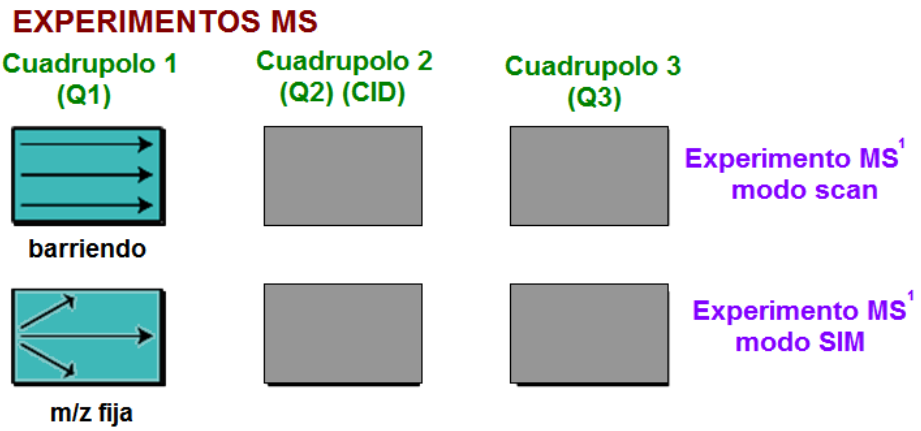


**Figura I.50** Experimentos de MS que se realizan en una configuración de tipo triple cuadrupolo.

En los experimentos MS/MS la celda de colisión se activa, hay gas en su interior, y se induce la disociación de las moléculas mediante colisiones con un gas inerte, generalmente argón (mecanismo CID). En una configuración de este tipo, se pueden llevar a cabo diferentes experimentos MS/MS[227,228,229], en los que los cuadrupolos $Q_1$ y $Q_3$ actúan como analizadores, que se describen a continuación:

- **Barrido de iones producto (*Productor daughter ion scan*)**: Se selecciona un ion precursor en el $Q_1$, desviándose el resto de iones procedentes de la ionización. Este ion precursor se rompe en la celda de colisión con una energía determinada y el

[227]Ardrey, B.; *Mass Spectrometry*. En Liquid Chromatography-Mass Spectrometry. An Introduction. Ed. Ando, D. J., Consultant, Dartford (Kent, UK) **2003**, pp. 33-74.

[228]Li, S. Y.; He, F.; Zhu, B. Q.; Xing, R. R; Reeves, M. J.; Duan, C. Q.;*A systematic analysis strategy for accurate detection of anthocyanin pigments in red wines*, *Rapid Commun. Mass Spectrom.***2016**, 30 (13), 1619-1626.

[229]Ji, M.; Li, C.; Li, Q.;*Rapid separation and identification of phenolics in crude red grape skin extracts by high performance liquid chromatography coupled to diode array detection and tandem mass spectrometry*, J. Chromatogr. A **2015**, 1414, 138-146.

$Q_3$ funciona en modo barrido para obtener el espectro de los iones fragmento procedentes del ion precursor seleccionado. Este tipo de espectros es muy habitual en el análisis estructural, ya que origina espectros de gran calidad y permite la caracterización estructural de los compuestos estudiados.

- **Barrido de iones precursores (*Precursor or parent ion scan*)**: En este tipo de experimentos se estudian los precursores que se fragmentan originando un ion fragmento determinado. En este caso el $Q_1$ realiza un barrido, los iones se fragmentan en la celda de colisión y el $Q_3$ deja pasar un único ion fragmento, con lo que el espectrómetro registra señal sólo en el caso de que en el $Q_1$ se esté dejando pasar un ion precursor que se fragmente dando lugar al ion fragmento seleccionado en el $Q_2$.

- **Barrido de pérdida neutra (*Constant neutral loss scan*)**: En este caso se estudian todos los iones que se fragmentan perdiendo un mismo fragmento neutro. Los cuadrupolos $Q_1$ y $Q_3$ trabajan en modo barrido, pero con un desfase entre ellos igual a la pérdida neutra estudiada. Se observa señal cuando el $Q_1$ deja pasar un ión que al fragmentarse sufre la pérdida de un fragmento neutro con una relación m/z igual al desfase entre los cuadrupolos, originando de este modo el ion fragmento que el $Q_3$ está dejando pasar.

- **SRM (*Selected reaction monitoring*) o MRM (*Multiple reaction monitoring*):** Se estudia la fragmentación de un ion precursor seleccionado que produce un ion fragmento determinado. El $Q_1$ selecciona un único ion precursor que se fragmenta en la celda de colisión, el $Q_3$ sólo deja pasar un ion fragmento determinado, por lo que se monitoriza la intensidad originada por una fragmentación específica, denominada transición. Los dos cuadrupolos están fijos, de modo que la sensibilidad es muy alta, además sólo originarían señal otros compuestos que se fragmenten dando lugar a la misma transición, con lo que la selectividad y especificidad de la técnica es muy alta, permitiendo análisis muy sensibles incluso en matrices complejas. Es una técnica que aporta grandes ventajas para la cuantificación de los analitos.

Un esquema de estos cuatro tipos de experimentos MS/MS se puede observar en la FiguraI.51.

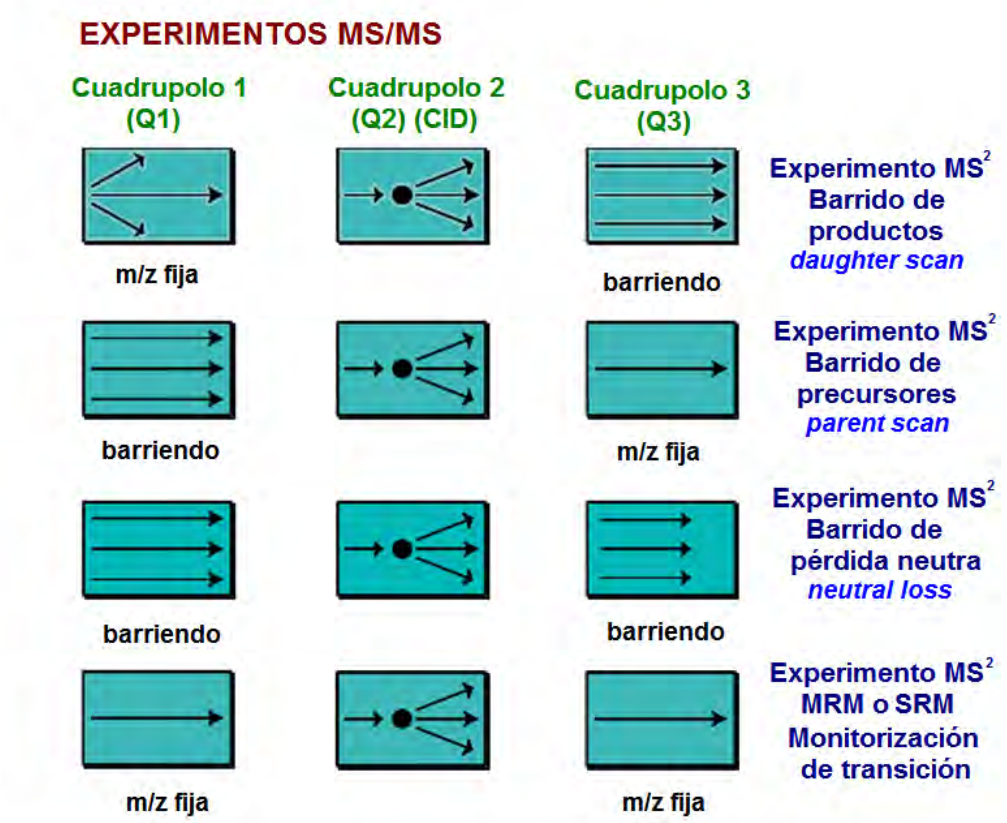


**Figura I.51** Experimentos de MS/MS que se realizan en una configuración de tipo triple cuadrupolo.

## I.7. MEDIDA DEL COLOR

El color es uno de los aspectos organolépticos y de los parámetros de calidad más importantes de un vino, determinante en la evolución sensorial del mismo.

El color de un vino evoluciona progresivamente a lo largo del envejecimiento y puede dar una idea de su edad, de su estado de conservación e incluso de algunos de los defectos que luego se notarán al beberlo. Está fuertemente influenciado por el contenido antociánico de las uvas, así como también por los diferentes procedimientos de elaboración y conservación del vino.

Las antocianinas son las responsables del color rojo de las uvas[230] y de los vinos jóvenes, pero a lo largo del envejecimiento del vino tienen lugar diferentes reacciones entre las antocianinas y otras moléculas del medio que dan lugar a otros pigmentos que modifican el color del vino.

[230]Dugo, P.; Favoino, O.; Lo Presti, M.; Luppino, R.; Dugo, G.; Mondello, L.; *Determination of anthocyanins and related components in red wines by micro and capillary HPLC*, J. Sep. Sci. **2004**, 27, 1458-1466.

Bajo el punto de vista receptivo, el color es la sensación producida en el observador humano cuando su retina es estimulada por energía radiante[231].Se denomina espectro visible a la región del espectro electromagnético que el ojo humano es capaz de percibir (Figura I.52).

Los colores rojo, amarillo y azul son los colores primarios, a partir de los que se puede reproducir cualquier otro color. De la suma de los colores primarios obtenemos el naranja, el verde y el violeta, que se denominan colores secundarios.

Los colores complementarios son aquellos que se encuentran uno frente al otro en el círculo cromático (Figura I.53). Se obtiene mediante la contraposición de un primario con el color secundario formado por los otros dos primarios. Así el complementario del color rojo es el verde, el del amarillo es el violeta y el del azul es el naranja.

Cuando la luz incide sobre un objeto, su superficie absorbe ciertas longitudes de onda y refleja otras. Sólo las longitudes de onda reflejadas pueden ser vistas por el ojo humano, de manera que el cerebro sólo percibe esos colores. La superficie de los objetos refleja en mayor medida las longitudes de onda del color complementario al que absorben principalmente, es decir, el cerebro humano percibe el color complementario del absorbido por cada objeto.

La longitud de onda de máxima absorción de las antocianinas se sitúa alrededor de 525 nm, es decir absorben mayoritariamente luz verde y el ojo humano percibe el color rojo.

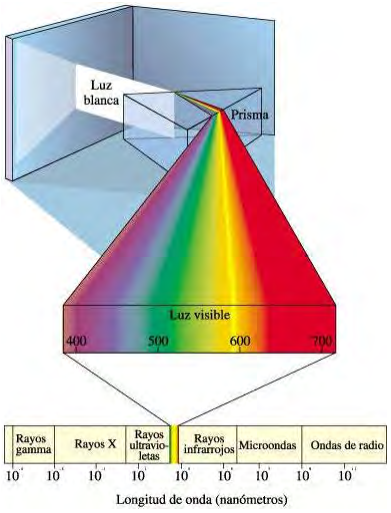


**Figura I.52** Espectro visible.

[231]Ruiz Hernández, M.; *La vista y el vino*. En La cata y el conocimiento de los vinos. AMV Ediciones y Ediciones Mundi-Prensa, **2003**, pp. 125-184.


**Figura I.53** Círculo cromático.

El desplazamiento batocrómico de la longitud de onda de máxima absorción del espectro UV-vis que mostraban los pigmentos formados por condensaciones de antocianinas con flavanoles, tanto de forma directa como mediadas por acetaldehído, ocasionado por una mayor absorción a longitudes de onda mayores (hacia el amarillo) se traduce en el aporte de tonos violáceos al vino, debido a que el ojo humano percibe el color complementario, el violeta. De la misma manera, la formación de derivados de tipo piranoantociánico produce desplazamientos hipsocrómicos en la longitud de onda de máxima absorción, es decir, hacia una mayor absorción del color azul, que es percibido por el ojo humano como tonalidad naranja, traduciéndose en el aporte de esta tonalidad al vino.

Se han desarrollado diferentes metodologías para medir el color de un vino, de los cuales se describen a continuación las dos más utilizadas: la medición de los parámetros estándar y de las coordenadas cromáticas.

### I.7.1. PARÁMETROS ESTÁNDAR

Los parámetros estándar se determinan según la metodología descrita por Glories[232,233].

Este procedimiento consiste en la medición de las absorbancias a 420, 520 y 620 nm de cada muestra. La Intensidad Colorante refleja cuánto color posee el vino y se calcula sumando cada uno de los componentes medidos.

[232]Glories, Y.: *The color of red wines. Part 1. Anthocyanin and tannin equilibriums.* Connaissance Vigne Vin **1984**, 18, 195-217.

[233]Glories, Y.; *The color of red wines. Part 2. Measurement, origin and interpretation.* Connaissance Vigne Vin **1984**, 18, 253-271.

$$IC = A_{420}+A_{520}+A_{620}$$

La Tonalidad indica la importancia relativa del amarillo sobre el rojo, expresa en tanto por ciento, y se calcula de la siguiente manera:

$$\text{Tonalidad (T)} = (A_{420}/A_{520}) \times 100$$

Las componentes amarilla, roja y azul del color se determinan como el cociente de cada una de ellas respecto a la Intensidad Colorante (IC), expresadas también en tanto por ciento. Estos parámetros indican la importancia relativa de cada uno de estos colores en el color global del vino.

$$\text{Componente amarilla } (A_{420}\%) = (A_{420}/IC) \times 100$$
$$\text{Componente roja } (A_{520}\%) = (A_{520}/IC) \times 100$$
$$\text{Componente azul } (A_{620}\%) = (A_{620}/IC) \times 100$$

### I.7.2. COORDENADAS CROMÁTICAS. ESPACIO CIELAB.

En 1976 la *Commission Internationale de l'Éclairage* (CIE) estableció unas normas, aceptadas internacionalmente[234,235,236], destinadas a la correcta definición del color. Estas normas hacen referencia a las características de los iluminantes, a las condiciones de observación y a las curvas espectrales de sensibilidad del ojo normal para tres estímulos luminosos convencionales denominados X, Y, Z.

Estos valores triestímulo representan los tres colores base (X: rojo virtual, Y: verde virtual y Z: azul virtual) con los que se puede reproducir, mediante su combinación, la totalidad de los colores existentes. Las coordenadas X, Y, y Z son en realidad una expresión numérica que representa la proporción relativa de cada uno de los colores base necesaria para reproducir, en el ojo del observador, el color concreto del objeto analizado.

[234]Esparza, I.; Santamaría, C.; Calvo, I.; Fernández, J. M.; *Significance of CIELAB parameters in the routine analysis of red wines*, J. Food **2009**, 7, 189-199.

[235]Monagas, M., Martín-Álvarez, P. J.; Gómez-Cordovés, C.; Bartolomé, B.; *Time course of the colour of young red wines from Vitisvinifera L. during ageing in bottle*, Int. J. Food Sci. Technol. **2006**, 41, 892-899.

[236]Wrolstad, R. E.; Durst, R. W.; Lee, J.;*Trackingcolor and pigment changes in anthocyanin products,* Trends Food Sci. Technol. **2005**, 16, 423-428.

El cálculo simple de estos valores triestímulo se obtiene mediante la medición de la transmitancia a las longitudes de onda 450, 520, 570 y 630 nm de la muestra de vino y aplicando las siguientes ecuaciones:

$$X = 19.717\ T_{450} + 1.884\ T_{520} + 42.539\ T_{570} + 32.474\ T_{630} - 1.841$$
$$Y = 7.950\ T_{450} + 34.764\ T_{520} + 42.736\ T_{570} + 15.759\ T_{630} - 1.180$$
$$Z = 103.518\ T_{450} + 4.190\ T_{520} + 0.251\ T_{570} - 1.831\ T_{630} + 0.818$$

La CIE definió en 1976 el espacio CIELAB, en el que se trata de representar en el espacio la totalidad de los colores. Mediante unas coordenadas L*, a* y b* se puede definir un color dentro del espacio CIELAB. Los ejes representan las graduaciones entre colores opuestos, la coordenada L* va desde el blanco al negro, a* desde el verde al rojo y b* desde el amarillo al azul, como se observa en la Figura I.54.

El cálculo de L*, a* y b* se determina a partir de los valores triestímulo X, Y y Z de acuerdo con las siguientes ecuaciones:

$$L^* = 116\ (Y/100)^{1/3} - 16$$
$$a^* = 500\ [(X/94.825)^{1/3} - (Y/100)^{1/3}]$$
$$b^* = 200\ [(Y/100)^{1/3} - (Z/107.383)^{1/3}]$$

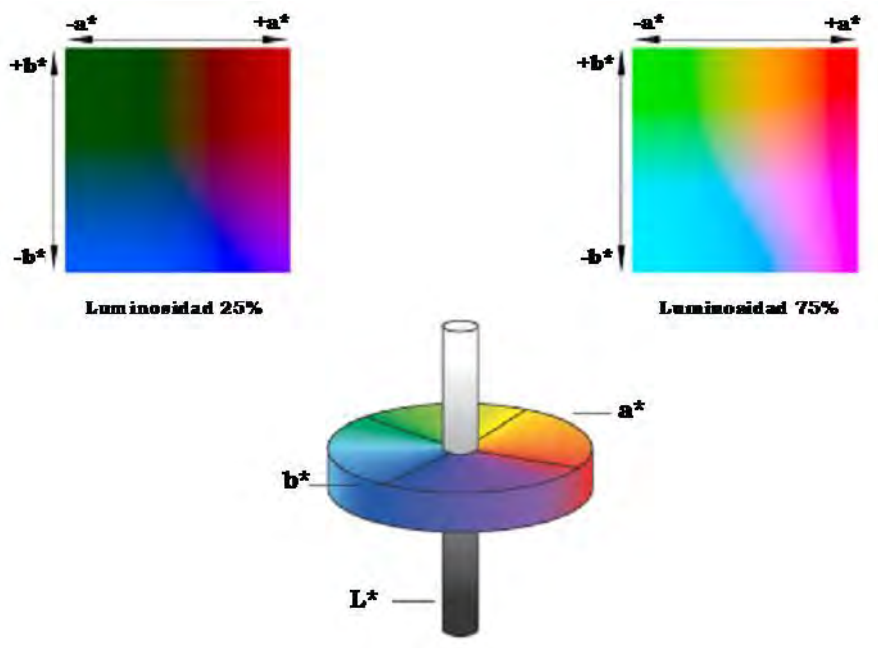


**Figura I.54** Coordenadas cromáticas.

Para simplificar más la expresión del color, las coordenadas a* y b* del espacio CIELAB pueden transformarse en las coordenadas esféricas H* y C* de acuerdo con las siguientes ecuaciones:

$$H^* = \text{arctg}\,(b^*/a^*)$$

$$C^* = (a^{*2} + b^{*2})^{1/2}$$

De esta manera, el color de cualquier objeto queda definido dentro del espacio CIELAB por tres coordenadas denominadas L*: Claridad, H*: TonoyC*: Croma o Saturación, como se puede observar en la Figura I.55.

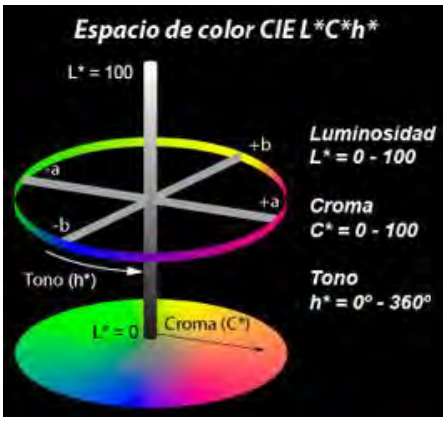


**Figura I.55** Espacio del color CIE. Coordenada cromática L* y coordenadas esféricas H* y C*.

## I.8. QUIMIOMETRÍA

En la actualidad, la automatización y versatilidad de las técnicas utilizadas en los laboratorios analíticos permiten disponer de una gran cantidad de información para cada una de las numerosas muestras que es posible analizar en un tiempo relativamente corto, haciéndose necesario el empleo de procedimientos matemáticos y estadísticos para extraer la información útil de todo el conjunto de datos.

La Quimiometría es una disciplina química que utiliza procedimientos matemáticos, estadísticos y de lógica formal para diseñar o seleccionar procedimientos experimentales óptimos de medida, extraer la máxima información química relevante mediante el análisis de datos químicos y obtener conocimiento a partir de sistemas químicos.

Abarca todos los aspectos del procedimiento analítico tales como el muestreo, el diseño de experimentos, la estimación de parámetros, el análisis de agrupamientos, la optimización y resolución de mezclas, el procesado de señales, la búsqueda de

patrones de comportamiento en los resultados experimentales, etc. Por este motivo, se ha introducido en un gran número de campos de la investigación química, como la química ambiental, la geoquímica, la química clínica, la química de los alimentos[237,238,239,240,241,242,], etc.

En el caso del vino, son muchos los parámetros que pueden influir en la composición del mismo, como la variedad de la uva, las características del suelo y el clima de la zona de producción, el proceso de elaboración, la duración de la permanencia del vino en barrica y del envejecimiento en botella, etc. La composición de cada vino determina, por otro lado, sus características organolépticas como el sabor, el color y el olor. Las diferentes variables químicas son el intermedio entre las causas y los efectos y permite establecer las conexiones entre ambas partes.

Como consecuencia del elevado número de descriptores de las muestras, se hacen necesarias herramientas de análisis estadístico-matemático para establecer relaciones causa-efecto a partir de las variables químicas medidas. La Quimiometría suministra estas herramientas y además, facilita la posibilidad de utilizar el mínimo número de variables necesarias para encontrar la solución al problema analítico planteado.

Con fines de caracterización de alimentos se utilizan los procedimientos conocidos como *técnicas de reconocimiento de modelos*, que incluyen técnicas de visualización y reducción de la dimensionalidad de los datos, procedimientos de agrupamiento y técnicas de clasificación.

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[237]Miaw, C.; Sheng, W.; Assis, C.; Silva, A.; Rangel.C. S.; Cunha, M. L.; Sena M. M.; de Souza, S.; Vitorino, C.; *Determination of main fruits in adulterated nectars by ATR-FTIR spectroscopy combined with multivariate calibration and variable selection methods*, Food Chem. **2018**, 254, 272-280.

[238]De Lerma, N. L.; Peinado, R. A.; Puig-Pujol. A.; Mauricio, J. C.; Moreno, J.; Garcia-Martinez, T.; *Influence of two yeast strains in free, bioimmobilized with alginate forms on the aromatic profile of long aged sparkling wines*, Food Chem. **2018**, 250, 22-29.

[239]Gad, H. A.; Bouzabata, A.; *Application of chemometrics in quality contol of Turmeric (Curcuma longa) based on ultra-violet, fourier transform-infrared and H-1 NMR spectroscopy*, Food Chem. **2017**, 237, 857-864.

[240]Winkler-Moser, J. K.; Singh, M.; Rennick, K. A.; Bakota, E. L.; Jham, G.; Liu, S. X.; Vaughn, S. F.; *Detection of corn adulteration in Brazilian coffee (coffea Arabica) by tocopherol profiling and near-infrared (NIR) spectroscopy*, J. Agric. Food Chem. **2015**, 63(49), 10662-10668.

[241]Iglesias-Rodríguez, R.; Fernández-Delgado, M.; Barciela-García, J.; Pena-Crecente, R. M.; García-Martín, S.; Herrero-Latorre, C.; *Comparison of several chemometric techniques for the classification of orujo distillate alcoholic samples from Galicia (northwest Spain) according to their certified brand of origin*, Anal. Bioanal. Chem. **2010**, 397, 2603-2614.

[242]Ni, Y.; Wang, Y.; Kokot, S.; *Simultaneous kinetic spectrophotometric analysis of five synthetic food colorants with the aid of chemometrics*, Talanta **2009**, 78, 432-441.

### I.8.1. TÉCNICAS DE RECONOCIMIENTO DE MODELOS

Las técnicas de reconocimiento de modelos (pattern recognition) relacionan el espacio químico con el espacio de las causas, asignando una muestra alimentaria a un grupo o categoría. Éste ha sido uno de los campos de mayor aplicación de la quimiometría en el ámbito de la química alimentaria, puesto que permite la diferenciación de muestras por su origen y posibilita la detección de fraudes alimentarios[243,244,245].

El conjunto de medidas empleadas para caracterizar una determinada muestra se denomina vector de datos (pattern vector). Cuando únicamente se miden dos variables, el vector de datos para cada muestra se representa gráficamente por un punto en un espacio de 2 dimensiones. Las coordenadas de dicho punto son los valores de las dos variables medidas para esa muestra concreta, y constituyen un vector de dos dimensiones, llamado vector de datos[246].

Las técnicas de reconocimiento de modelos se basan en que los vectores que representan muestras similares se disponen en una misma zona del espacio definido por las variables. Sin embargo, cuando una muestra se caracteriza mediante *n* variables, dicha muestra se define a través de un vector de datos que representa un punto en un espacio *n*-dimensional, siendo necesarios métodos estadísticos multivariantes para poder llevar a cabo la identificación de los posibles grupos.

#### I.8.1.1. Técnicas de preprocesamiento: Escalado

En general, previamente a la aplicación de las técnicas de análisis multivariante, es necesario un preprocesamiento de los datos. En ocasiones, éste se realiza debido a que forma parte de la técnica quimiométrica utilizada.

El pretratamiento de los datos más habitual consiste en una normalización de los mismos para eliminar la influencia que sobre las técnicas de clasificación puede ejercer el diferente tamaño y escala de los datos. En principio, todas las variables tienen igual importancia con fines de clasificación, por lo que debe evitarse un mayor

[243]Maracajá, T.;Belmiro, C.;Fernandes Pereira, C.;Silveira, A. P.;*Paim Red wines from South America: Content of phenolic compounds and chemometric distinction by origin*, Microchem. J. **2017**, 133, 114–120.

[244]Winkler-Moser, J. K.; Singh, M.; Rennick, K. A.; Bakota, E. L.; Jham, G.; Liu, S. X.; Vaughn, S. F.; *Detection of corn adulteration in Brazilian coffee (coffea Arabica) by tocopherol profiling and near-infrared (NIR) spectroscopy*, J. Agric. Food Chem. **2015**, 63(49), 10662-10668.

[245]Abad-Garcia, B.; Garmon-Lobato, S.; Sanchez-Ilarduya, M. B.; Berrueta, L. A.; Gallo, B.; Vicente, F.; Alonso-Salces, R. M.; *Polyphenolic contents in Citrus fruit juices: authenticity assessment*, Eur. Food Res. Technol. **2014**, 238(5)803-818.

[246]Esbensen, K. H.; *An introduction to multivariate data analysis and experimental design*, Ed. CAMO, **2001**, 1-112.

"peso" de las variables con valores numéricos superiores frente a aquellas con valores inferiores. Este proceso denominado "escalado" tiene un papel importante en el tratamiento estadístico.

El autoescalado y escalado por rango son los métodos más habituales, aunque existen otros de más reducida aplicación.[247]

En el procedimiento de autoescalado, el valor de una muestra *i* para una variable *j*, considerada como $x_{ij}$, se sustituye por un nuevo valor, $z_{ij}$, obtenido al restar la media de la variable, $x_j$, y dividiendo por la desviación estándar de la misma, $s_j$:

$$z_{ij} = \frac{x_{ij} - \bar{x}_j}{s_j}$$

En el procedimiento de autoescalado por rango, las variables son escaladas de manera que el valor mínimo de la variable sea igual a cero y el valor máximo, igual a la unidad:

$$z_{ij} = \frac{x_{ij} - \min(x_{ij})}{\max(x_{ij}) - \min(x_{ij})}$$

Con ambos procedimientos de autoescalado, se obtienen las variables medidas en una misma escala, evitando la influencia de los diversos tamaños de las variables en los resultados obtenidos.

#### I.8.1.2. Técnicas de visualización y reducción de la dimensionalidad: Análisis de Componentes Principales (PCA)

Dentro de los métodos estadísticos multivariantes existen técnicas de visualización y reducción de la dimensionalidad, que son procedimientos en los que mediante la reducción de la dimensionalidad de los datos se permite una visualización de éstos con la pérdida de la mínima cantidad de información posible. Ésta es la base del Análisis de Componentes Principales (PCA), que se utilizará en este trabajo, siendo una de las técnicas de análisis multivariante más utilizadas.

El Análisis de Componentes Principales consiste en la transformación de las variables originales en unas nuevas variables no correlacionadas entre sí denominadas *Componentes Principales*, cada una de las cuales es una combinación lineal de las variables originales. Esta técnica busca un conjunto de ejes ortogonales que representen las direcciones de máxima varianza de los datos, consiguiendo de este

[247]Meloun, M.; Forina, M.; Militky, J.; *Scaling, weighting and transforms*. En Chemometrics for analytical chemistry.Vol. I; Ellis Horwood: Chichester (UK) **1992**, 212-226.

modo analizar la estructura de los mismos y reducir la dimensionalidad de los vectores que caracterizan las muestras.

En una matriz de datos $X_{ij}$, donde *j* es el número de variables para *i* individuos, el objetivo del Análisis de Componentes Principales es resumir la información contenida encontrando *p* factores que sustituyan a las variables, siendo $p<j$, con la pérdida de la mínima cantidad de información[248].

Se calculan las componentes principales de la matriz de datos $X_{ij}$. Las direcciones sobre las que se realiza la nueva proyección representan nuevas variables $U_{ip}$, (*p* componentes principales), que son combinación lineal de las variables originales.

Los llamados *loadings* son los coeficientes de las combinaciones lineales sobre las variables originales que definen las nuevas variables. Cada muestra es descrita ahora por unas nuevas coordenadas con respecto a los componentes principales, llamadas *scores*[249,250].

Se debe determinar el número de componentes principales con los que puede obtenerse una descripción adecuada de los datos originales[251]. Para ello, a partir de los *loadings* se calculan los autovalores, que indican cuál es el porcentaje de la varianza total explicada por cada uno de los componentes principales. Los componentes principales se ordenan de manera decreciente según la varianza explicada por cada uno de ellos. Uno de los criterios utilizados para seleccionar el número adecuado de componentes principales es el criterio de Kaiser, que selecciona aquellos componentes principales con autovalor mayor que la unidad.

Se analizan a continuación los *scores*, es decir, las nuevas coordenadas con respecto a los componentes principales que describen las muestras estudiadas y los coeficientes de correlación, con el fin de buscar grupos de muestras similares, muestras anómalas, variables relevantes, etc.

Una vez que se ha completado el Análisis de Componentes Principales, se pueden realizar rotaciones adicionales de los mismos con el fin de aumentar su

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[248]Adams, M. J.; *The principles of multivariate data analysis*. En Analytical methods for food authentication. Blackie Academic & Professional, Londres**1998**, pp. 308-336.

[249]Cserháti, T.; *Fundamentals*. En Multivariate methods in chromatography. A practical guide. John Wiley & Sons Ltd, Chichester**2008**, pp. 1-8.

[250]Leardi, R.; *Chemometrics in data analysis*. En Food authenticity and traceability. Woodhead Publishing Limited, Cambrigde**2000**, pp.299-320.

[251]Ramis Ramos, G.; García Álvarez-Coque, M. C. (Ed); *Análisis de Componentes Principales*. En Quimiometría, Síntesis, Madrid **2001**, pp. 157-182.

relación con las variables originales más importantes. Esta rotación se realiza mediante algoritmos que incrementan la correlación entre los componentes y las variables que forman grupos. La rotación facilita el análisis de datos, puesto que las variables que constituyen cada factor subyacente se pueden identificar con mayor nitidez, distinguiéndolas de las que no contribuyen al mismo.

El Análisis de Componentes Principales ha sido una técnica ampliamente utilizada en el análisis de productos alimentarios[252,253,254] con el objetivo de diferenciar entre distintos tipos vinos o zumos, variedades de uva, manzanas o granos de cafe, y de establecer relaciones entre los parámetros que afectan a la calidad del mismo.

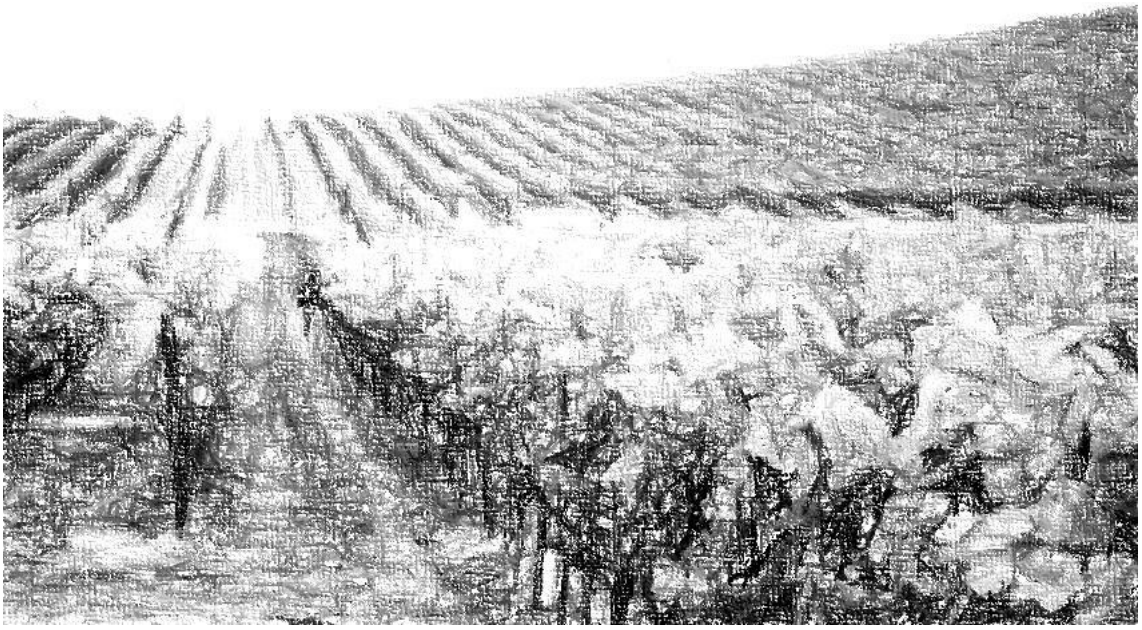
[252]Diaz, R.; Gallart-Ayala, H.; Sancho, J. V.; Nunez, O.; Zamora, T.; Martins, C. P. B.; Hernandez, F.; Hernandez-Cassou, S.; Saurina, J.; Checa, A.;*Told through the wine: A liquid chromatography-mass spectrometry interplatform comparison reveals the influence of the global approach on the final annotated metabolites in non-targeted metabolomics*, J. Chromatogr. A **2016**, 1433, 90-97.

[253]García-Marino, M.; Hernández-Hierro, J. M.; Rivas-Gonzalo, J. C.; Escribano-Bailón, M. T.; *Colour and pigmentcomposition of red winesobtainedfromco-maceration of Tempranillo and Graciano varieties*, Anal. Chim. Acta **2010**, 660, 134-142.

[254]Budić-Leto, I.; Vrhovšek, U.; Gajdoš-Kljusurić, J.; Lovrić, T.; *Anthocyanin pattern of skin extracts from the Babić and Plavac Mali grapes and anthocyanin pattern of the produced wine*, Acta Alimentaria **2009**, 38, 67-75.

# CHAPTER II

## OBJECTIVES



# Chapter II

## OBJECTIVES

This work has three main objectives:

- Firstly, the identification of some new anthocyanin derivatives formed during the winemaking by high perfomance liquid chromatography with diode array and tandem Mass Spectrometric detector. This analytical methodology was developed by our group previously.
- Secondly, study the evolution of the anthocyanin derivatives and tannins during the making and ageing of red wine from Rioja. Quantification of anthocyanin derivatives and tannins, which had already been identified, in different samples along alcoholic fermentation, malolactic fermentation and barrel ageing of red wines from Rioja will be carried out by High Performance Liquid Chromatography with tandem Mass Spectrometric detection (HPLC-DAD-MS/MS) in Multiple Reaction Monitoring (MRM) mode, simplifying the wine matrix using solid phase extraction (SPE).
- And last, look for differences in the anthocyanic and tannic composition of red wines samples from Rioja and Bordeaux. Quantification of free anthocyanins, anthocyanin derivatives and tannins in different final wine samples from Rioja and Bordeaux will be performed by HPLC-DAD-MS/MS with previous SPE.

# CHAPTER III

## ANALYTICAL METHODOLOGY



# Chapter III

## ANALYTICAL METHODOLOGY

Analytical methodologies used for the identification and quantification of several anthocyanins, anthocyanin-derived pigments and tannins in red wines, which had been identified by our group in previous works[255,256], will be presented in this chapter.

The separation and determination of anthocyanin derived pigments and tannins is a hard task, due to their great number and chemical diversity present in wine and in most cases to their low concentrations. Moreover, the presence of other chemical components in wine make necessary a first step before the anthocyanin derivatives and tannins assay to simplify the sample matrix.

### III.1. STANDARDS, SOLVENTS AND REAGENTS

Malvidin-3-O-glucoside and (+)-catechin, used for calibration purposes, and other anthocyanin, flavanol and tannin standards used for structural identification were purchased as indicated in Table III.1.

[255]Sánchez-Ilárduya, M.B. *Pigmentos derivados antociánicos de los vinos tintos de la Rioja: Estudio analítico, influencia en el color y evolución durante la crianza*, PhD Thesis, University of Basque Country, Spain, España, **2010**.

[256]Sánchez-Fernández, C.; *Búsqueda de marcadores de tipo tanino en vinos tintos de Rioja: Estudio cualitativo y cuantitativo por HPLC-MS/MS*, PhD Thesis, University of Basque Country, Spain, **2012**.

**Table III.1.** Standard of anthocyanins and tannis.

| Standard | Supplier | Reference | CAS |
|---|---|---|---|
| **Cyanidin-3,5-O-diglucoside** | Extrasynthèse | 0932S | [2611-67-8] |
| **Cyanidin-3-O-galactoside** | Extrasynthèse | 0923S | [27661-36-5] |
| **Cyanidin-3-O-glucoside** | Extrasynthèse | 0915S | [7084-24-4] |
| **Cyanidin-3-O-rutenoside** | Extrasynthèse | 0914S | [18719-76-1] |
| **Cyanidin** | Extrasynthèse | 0909S | [528-58-5] |
| **Pelargonidin-3,5-diglucoside** | Extrasynthèse | 903 | [17334-58-6] |
| **Pelargonidin-3-O-glucoside** | Extrasynthèse | 0907S | [18466-51-8] |
| **Pelargonidin** | Extrasynthèse | 0912S | [134-04-3] |
| **Peonidin-3-O-glucoside** | Extrasynthèse | 929 | [6906-39-4] |
| **Peonidin** | Extrasynthèse | 0906S | [134-01-0] |
| **Malvidin-3,5-O-diglucoside** | Extrasynthèse | 0930S | [16727-30-3] |
| **Delphinidin** | Extrasynthèse | 0904S | [528-53-0] |
| **Malvidin-3-galactoside** | Extrasynthèse | 0931S | [30113-37-2] |
| **Malvidin-3-O-glucoside** | Extrasynthèse | 0911S | [7228-78-6] |
| **Malvidin** | Extrasynthèse | 0913S | [643-84-5] |
| **(+)-Catechin** | Extrasynthèse | 0976S | [154-23-4] |
| **(-)-Epicatechin** | Extrasynthèse | 0977S | [490-48-0] |
| **(+)-Galocatechin** | Chroma Dex | 07044-303 | [3371-27-5] |
| **(-)-Epigalocatechin** | Extrasynthèse | 0979S | [970-74-1] |
| **PCB1** | Extrasynthèse | 983 | [20315-25-7] |
| **PCB2** | Extrasynthèse | 984 | [29106-49-8] |
| **PCC1** | Chroma Dex | 16232-1453 | [37064-30-5] |
| **PCA2** | Extrasynthèse | 0985S | [41743-41-3] |
| **(+)-Catechin-gallate** | Chroma Dex | 03315-133 | [130405-40-2] |
| **(-)-Epicatechin-gallate** | Extrasynthèse | 0978S | [970-74-1] |
| **(+)-Galocatechin-gallate** | Chroma Dex | 07045-183 | [4233-96-9] |
| **(-)-Epigalocatechin-gallate** | Extrasynthèse | 0981S | [989-51-5] |

Methanol and acetonitrile (Romil Chemical Ltd, Heidelberg, Germany) were of HPLC grade. Water was purified on a MilliQ system (Millipore, Bedford, MA, USA). Trifluoroacetic acid (Merck, Darmstdt, Germany) was of spectroscopy grade. Acetic acid (Merck, Darmstdt, Germany ), hydrochloric acid (32%, Merck, Darmstdt, Germany), L-(+)-tartaric acid (Sigma-Aldrich, Steinheim, Germany) and reagents $NaH_2PO_4$ and $Na_2HPO_4$ (Fluka, Steinheim, Germany) were of analytical grade.

All solvents used were previously filtered through 0.45 μm nylon membranes (Lida, Kenosha, WI, USA).

## III.2. SAMPLES

*Evolution (fermentations and ageing) sampling*: Different samples were taking from several red wine elaborations of 2012 harvests at some Rioja wineries along alcoholic and malolactic fermentations and barrel ageing, in order to study the formation and evolution of each anthocyanin derivatives and tannins.

*Rioja and Bordeaux 2014 sampling:* A total of 190 wines were analyzed, 96 of them were from Rioja (from the three different zones of Rioja: 32 wines from Rioja Alavesa, 32 wines from Rioja Alta and 32 wines from Rioja Baja) and the other 94 samples were from different zones of Bordeaux (36 wines from Médoc, 13 wines from Graves, 5 wines from Blayais & Bourgeais, 27 wines from Libournais) and from the generic denominations of Bordeaux (13 wines of Bordeaux and Bordeaux Supérieur).

Within Rioja wines, 24 wines were qualified as young, 24 as *Crianza* (at least 12 months of ageing in oak barrel), 24 as *Reserva* (36 months of total ageing (barrel+bottle), with at least 12 months of ageing in oak barrel) and 24 as *Gran reserva* (at least 24 months of ageing in oak barrel followed by 36 months of bottle ageing).

*Rioja 2015 sampling*: 15 Rioja red wines samples with different winemaking conditions concerning grape cultivars, oak origin and barrel ageing period were sampled, in order to study their influence on anthocyanin derivatives and tannins.

## III.3. QUANTIFICATION OF ANTHOCYANINS BY HPLC-DAD

The quantification of anthocyanins in red wines was carried out by High Performance Liquid Chromatography (HPLC) with a diode array detector (DAD). The HPLC-DAD conditions used in the analysis of wine samples had been previously optimized and employed in our group[257] and were slightly modified[258]. The anthocyanins which were analyzed appear on Table III.2.

[257] Abad-García, B.; Berrueta, L. A.; Garmón-Lobato, S.; Gallo, B.; Vicente, F.; *A general analytical strategy for the characterization of phenolic compounds in fruit juices by high performance liquid chromatography with diode array detection coupled to electrospray ionization and triple quadrupole mass spectrometry*, J Chromatogr. A **2009**, 1216, 5398-5415.

[258] Rasines-Perea, Z.; Prieto-Perea, N.; Romera-Fernandéz, M.; Berrueta, L. A.; Gallo, B.; *Fast determination of anthocyanins in red grape musts by Fourier transform mi-infrared spectroscopy and partial least squares regression*, Eur. Food Res. Technol. **2015**, 240, 897-908.

**Table III.2.** Relative retention times for the 13 anthocyanins analyzed in this research.

| | Compound | Abbreviation | $t_R'$ (min) | $t_R'$ (min) |
|---|---|---|---|---|
| **1** | **Delphinidin-3-O-glucoside** | **Dp-3-glc** | 0.43 | - |
| **2** | **Cyanidin-3-O-glucoside** | **Cy-3-glc** | 0.58 | - |
| **3** | **Petunidin-3-O-glucoside** | **Pt-3-glc** | 0.67 | - |
| **4** | **Peonidin-3-O-glucoside** | **Pn-3-glc** | 0.88 | - |
| **5** | **Malvidin-3-O-glucoside** | **Mv-3-glc** | 1.00 | - |
| **6** | **Delphinidin-3-O-(6-acetyl)-glucoside** | **Dp-3-(6-Ac)-glc** | - | 0.71 |
| **7** | **Petunidin-3-O-(6-acetyl)-glucoside** | **Pt-3-(6-Ac)-glc** | - | 0.80 |
| **8** | **Peonidin-3-O-(6-acetyl)-glucoside** | **Pn-3-(6-Ac)-glc** | - | 0.86 |
| **9** | **Malvidin-3-O-(6-acetyl)-glucoside** | **Mv-3-(6-Ac)-glc** | - | 0.87 |
| **10** | **Malvidin-3-O-(6-caffeoyl)-glucoside** | **Mv-3-(6-caff)-glc** | - | 0.92 |
| **11** | **Petunidin-3-O-(6-p-coumaroyl)-glucoside** | **Pt-3-(6-p-coum)-glc** | - | 0.94 |
| **12** | **Peonidin-3-O-(6-p-coumaroyl)-glucoside** | **Pn-3-(6-p-coum)-glc** | - | 0.99 |
| **13** | **Malvidin-3-O-(6-p-coumaroyl)-glucoside** | **Mv-3-(6-p-coum)-glc** | - | 1.00 |

The HPLC-DAD analysis was performed in a Hewlett-Packard (Palo Alto, CA, USA) series 1100 chromatograph equipped with a vacuum degasser, a quaternary pump, an automatic and thermostated injector, an oven for the column and a diode array detector. A reverse-phase Phenomenex (Torrance, CA, USA) Luna C18(2) column (150 mm x 1.6 mm I.D., 3 μm) at 30 ºC, protected with a guard column Phenomenex C18 (4 mm x 3.0 mm I.D.), was used. Data processing was performed by HP Chemstation v. 6. 01.

The solvents used were 0.5% (v/v) aqueous solution of trifluoroacetic acid as mobile phase A and acetonitrile as mobile phase B, and the elution gradient used was: lineal gradient from 12 to 15%B, 0-15 min; isocratic at 15%B, 15-25 min; lineal gradient from 15 to 25%B, 25-40 min, lineal gradient from 25 to 30%B, 40-50 min; lineal gradient from 30 to 100%B, 50-55 min; washing with 100% B and re-equilibration of the column.

The flow rate was 0.8 mL/min and the injection volume was 50 μL. The sample vials in the automatic injector were thermostated at 4 ºC to guarantee the preservation of the samples. Before to the injection into de HPLC instrument, the wines samples were filtered through an Acrodisc CR13 0.45 μm PTFE membrane (Pall, Port

Washington, NY) and amber flask vials were used to avoid the light degradation of the anthocyanins.

UV-Vis spectra were recorder from 250 to 600 nm to confirm that the chromatographic peak being quantifies had an absorption spectrum of anthocyanic type, and the quantification was carried out at 530 nm.

The 13 anthocyanins were identified by their relative retention times using malvidin-3-O-glucoside as reference peak for non-acylated ones and Mv-3-(6-p-coum) as reference for acylated anthocyanins (see Figure III.1) and by comparison of their UV-vis absorption spectra with those of some available anthocyanin commercial standards (Figure III.2). In addition, these identifications had been confirmed using a Micromass (Milford, MA, USA) Quattro Micro triple quadrupole mass spectrometer, coupled to the exit of the diode array detector and equipped with a Z-spray ESI source (HPLC-DAD-MS/MS). Table III.3 shows m/z values for each anthocyanin.

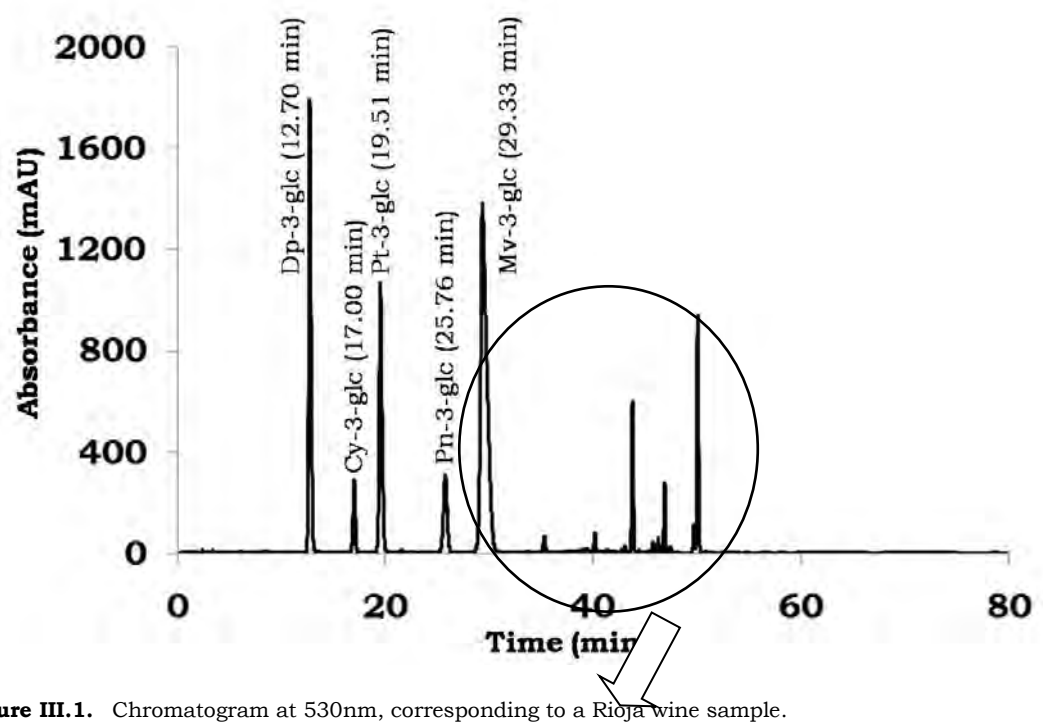



**Figure III.1.** Chromatogram at 530nm, corresponding to a Rioja wine sample.

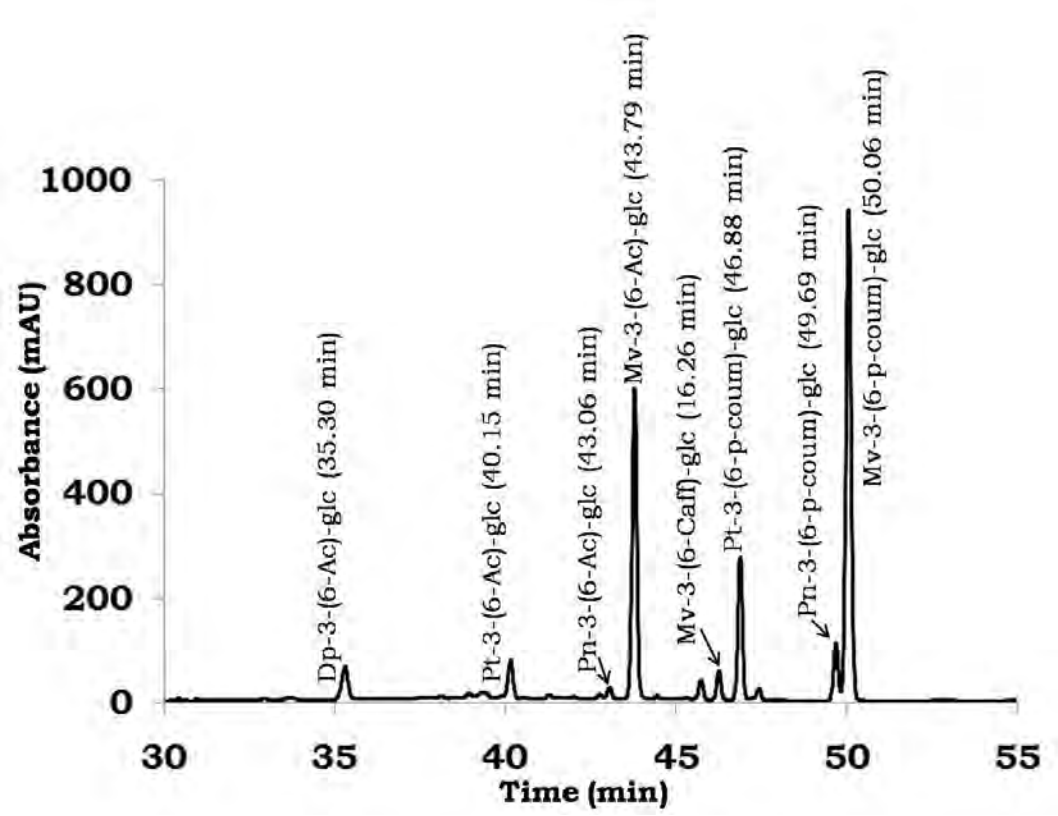



**Figure III.1.** Cont.

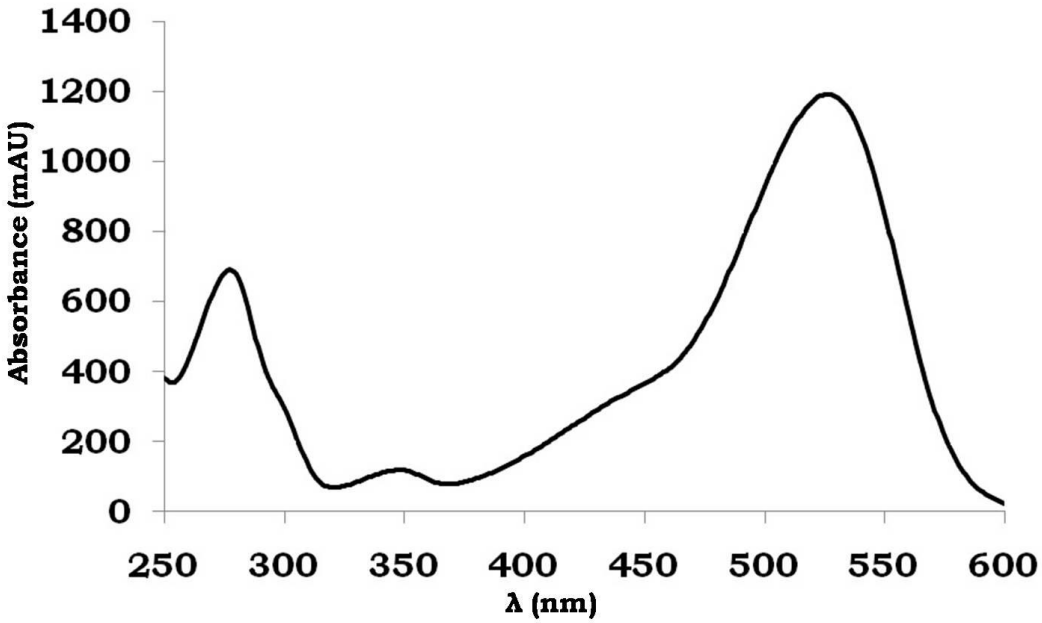



**Figure III.2.** Example of UV-vis absorption spectra corresponding to a malvidin-3-O-glucoside standard solution of 50 mg/L.

**Table III.3.** Values of m/z ratios for molecular ion $M^+$ and for the fragment ion related to the aglycone ($Y_0^+$) for each identified anthocyanin.

| Anthocyanin | $M^+$ | $Y_0^+$ |
|---|---|---|
| **Dp-3-glc** | 465 | 303 |
| **Cy-3-glc** | 449 | 287 |
| **Pt-3-glc** | 479 | 317 |
| **Pn-3-glc** | 463 | 301 |
| **Mv-3-glc** | 493 | 331 |
| **Dp-3-(6-Ac)-glc** | 507 | 303 |
| **Pt-3-(6-Ac)-glc** | 521 | 317 |
| **Pn-3-(6-Ac)-glc** | 505 | 301 |
| **Mv-3-(6-Ac)-glc** | 535 | 331 |
| **Mv-3-(6-caff)-glc** | 655 | 331 |
| **Pt-3-(6-*p*-coum)-glc** | 625 | 317 |
| **Pn-3-(6-*p*-coum)-glc** | 609 | 301 |
| **Mv-3-(6-*p*-coum)-glc** | 639 | 331 |

Quantification of all anthocyanins was performed against the same external standard and thus all concentrations are expressed as mg/L equivalents of malvidin-3-O-glucoside. Precision calculated as %RSD was lower than 5%, and limit of quantification was 0.01 mg/L.

The stock solution and the intermediate and standard solutions were prepared using methanol with 0.1% (v/v) hydrochloric acid as solvent to ensure stability of the pattern. The standard solutions were not prepared with more than 20% of methanol in order to not distort chromatographic peak. All standard solutions were preserved in dark vials at 4 ºC. Standard solutions were injected in the range 0.01-300 mg/L.

Table III.4 collects as an example slope, intercept and regression coefficient for a calibration performed during this investigation, which was carried out using 16 different concentration levels and 3 replicates for each level. Due to the wide concentration range, it was decided to divide it into several parts.

**Table III.4.** Linear model for the five different ranges of Mv-3-glc calibration.

| Anthocyanin | Linear Range | Slope | Intercept | $R^2$ |
|---|---|---|---|---|
| Mv-3-glc | 0.01-0.10 | 152.35±0.23 | -0.43±0.01 | 0.9999 |
| | 0.10-1.0 | 137.46±0.42 | 0.4±1.0 | 0.9997 |
| | 1.0-10.0 | 157.3±2.4 | -23±13 | 0.9996 |
| | 10.0-100.0 | 172.3±1.9 | (-12±11)·10 | 0.9998 |
| | 100.0-300.0 | 146.8±1.6 | (242±37)·10 | 0.9998 |

Detection limit was calculated as three times the average height of noise, while quantification limit was obtained by multiplying this average height by ten. The repeatability, expressed as percent relative standard deviation (%RSD (n=3)), was determined for each calibration level. All these results are collected in Table III.5.

**Table III.5.** Repeatability in each calibration level for the Mv-3-glc, expressed as %RSD (n=3), and detection and quantification limits.

| Anthocyanin | Concentration level (mg/L) | RSD (%) | Detection limit (mg/L) | Quantification limit (mg/L) |
|---|---|---|---|---|
| Mv-3-glc | 0.01 | 4.8 | 0.004 | 0.01 |
| | 0.10 | 0.8 | | |
| | 1.0 | 0.6 | | |
| | 10.0 | 0.8 | | |
| | 100.0 | 0.1 | | |
| | 300.0 | 0.5 | | |

## III.4. IDENTIFICATION OF NON-COLOURED AND COLOURED ANTHOCYANIN DERIVATIVES BY HPLC-DAD-ESI(+)-CID-MS/MS

The separation and determination of anthocyanin derivatives is a hard task, due to their great number and chemical diversity present in wine and in most cases to their low concentrations. Moreover, the presence of other chemical components in wine make necessary a first step before the anthocyanin derivatives and tannins assay to simplify the sample matrix.

Two analytical methodologies, (fractionation by column chromatography (CC) and Solid Phase Extraction followed by CC (SPE + CC)) were performed to simplify the sample matrix and to preconcentrate analytes before the identification by HPLC-DAD-

ESI(+)-CID-MS/MS. This pretreatment was optimized and validated by our group previously[259].

**Fractionation by column chromatography (CC)**

Ten millilitres of wine were filtered through a 0.45 mm Pall (Ann Arbor, Michigan, USA) acrodisc Polytetrafluoroethylene (PTFE) filter and were injected using a six-way Rheodyne valve (Cambridge, UK) in a 250 x 15 mm Internal diameter (i.d.) glass column packed with Toyopearl HW-40S (Tosoh Bioscience, Stuttgart, Germany). This stationary phase is a modified methacrylate polymer with a hydrophilic surface due to ether and hydroxyl groups The particle size of the stationary phase is 20-40 μm, pore size is 50 Å and exclusion limit is 3000 Da.

Methanol was used as elution solvent at a flow rate of 0.5 mL/ min using a HPLC pump and 30 fractions of 6 mL for each wine were collected. These fractions were concentrated to dryness under a stream of nitrogen in a Zimark TurboVap-LV evaporator (Hopkinton, Massachusetts, USA) at 25°C, redissolved in 400 mL of the initial HPLC mobile phase solution (88% of a TFA:$H_2O$ 0.5:99.5 v/v solution and 12% acetonitrile) and filtered through a 0.45-mm PTFE membrane before analysis by HPLC-DAD-ESI(+)-CID-MS/MS.

**Solid Phase Extraction followed by fractionation by column chromatography (SPE + CC)**

In order to preconcentrate the wine sample, an SPE procedure was adapted from the literature[260]. Two empty commercial cartridges (IST, Hengoed Mid, Glam, UK, 150 mL capacity) were filled with 40 g of C18-modified silica (IST, particle size 60 mm). Both top and bottom of solid phase were covered with 20 mm polyethylene frits (IST).The cartridges were activated with 120 mL of methanol, washed with 2 x 120 mL of ultrapurified water and preconditioned with 120 mL of phosphate buffer (1 mol/L, pH = 7.0). Two hundred millilitres of red wine were de-alcoholized in a Büchi (Büchi Labortechnik AG, Flawil, Switzerland) R200 rotary evaporator at 25°C during 30 min. Two 60 mL aliquots of de-alcoholized wine were loaded in two preconditioned cartridges.

[259] Sánchez-Ilárduya, M.B. *Pigmentos derivados antociánicos de los vinos tintos de la Rioja: Estudio analítico, influencia en el color y evolución durante la crianza*, PhD Thesis, University of Basque Country, Spain, España, **2010**.

[260] Sun, B.; Leandro, M. C.; de Freitas, V.; Spranger, M. I.; *Fractionation of red wine polyphenols by solid-phase extraction and liquid chromatography*, J. Chromatogr. A **2006**, 1128, 27-38.

Elution began with 200 mL of 0.125 mol/L phosphate buffer (pH = 7.0) to elute phenolic acids, followed by 200 mL of ethyl acetate for the elution of monomer and oligomer flavanols and, finally, 250 mL of methanol for the elution of anthocyanin derivatives. The two methanolic extracts from both cartridges were mixed and evaporated to dryness in the rotary evaporator and redissolved in 15 mL of methanol. The rest of the procedure continued as the previous one (CC procedure), loading 10 mL of the redissolved extract in the glass column packed with Toyopearl HW-40S.

After these methodologies, the identification of compounds was carried out in a Alliance 2695 liquid chromatography system from Waters (Milford, USA), equipped with a vacuum degasser, a quaternary pump, an automatic and thermostated injector and an oven for the column.

The system is coupled to a diode array detector (DAD) Waters 2996 and a triple quadrupole mass spectrometer Micromass Quatro micro (Manchester, UK) with a Z-spray source which is coupled to an electrospray sounding line. The software we used was MassLynx v. 4.0.

The column used was a Phenomenex Luna C18 (2) (Torrance, USA) (150 x 4.6 mm) and the particle size is 3 µm, with a precolumn of Waters Nova-Pack C18 (Barcelona, Spain) (10 x 3.9 mm) and the particle size is 4 µm.

The solvents used in the chromatographic separation for the identification of non-coloured and coloured anthocyanin derivatives were TFA:$H_2O$ (0.5:99.5 v/v) as mobile phase A and acetonitrile as mobile phase B. The elution gradient used was: 0-45 min, lineal gradient, 12-15% B; 45-100 min, lineal gradient, 15-25% B; 100-140 min, lineal gradient, 25-40% B; 140-145 min, isocratic, 40% B; 145-150 min, lineal gradient, 40-100% B; followed by a column cleaning and reconditioning. The column temperature was 30  C and the automatic injector was thermostated at 4 ºC. The injection volume was 50 µL and the flow was 0.8 mL/min. The triple quadrupole is couple to the output of the diode array detector by a *T* union which divides the flow. This union allows the entry of 0.25 mL into the spectrometer, which avoids the ionization of analytes in the electrospray source.

The parameters of the electrospray interface were as follows: capillar voltage was 3.2 kV in positive mode, cone voltage was 3 V and RF lens voltage was 0 V. The temperature of the source was 120º C. Nitrogen was used as desolvation gas and its flow and temperature was 450 L/h and 300º C, respectively.

The acquisition in MS scan was performed in centroid mode in a range from 50 to 1500 u in positive mode, collecting the mass spectra at different voltages (10, 20, 30, 40 and 60 V). The scan time was 1.5 seconds with an interscan time of 0.1 seconds. Study of MS spectra was used for structure elucidation.

## III.5. QUANTIFICATION OF NON-COLOURED AND COLOURED ANTHOCYANIN DERIVATIVES BY HPLC-DAD-ESI(+)-CID-MS/MS

Firstly, before carrying out the non-coloured and coloured anthocyanin derivatives assay, a solid phase extraction (SPE) was performed to simplify the sample matrix and avoid the ionic saturation during the subsequent sample analysis by HPLC-DAD-ESI(+)-CID-MS/MS. This pretreatment was optimized and validated by our group previously[261]. SPE conditions are detailed in Figure III.3.

Once the sample cleaning is made, 21 compounds, derivative from Malvidin-3-*O*-glucoside, and other 22 compounds, which were optimized during this study, were analyzed by HPLC-DAD-ESI(+)-CID-MS/MS in multiple reaction monitoring (MRM) mode. In this technique of MS/MS, the precursor ion mass and the most intense fragmented ion are fixed, so that only compounds with that mass transition lead signal in the detector, achieving there by a high selectivity.

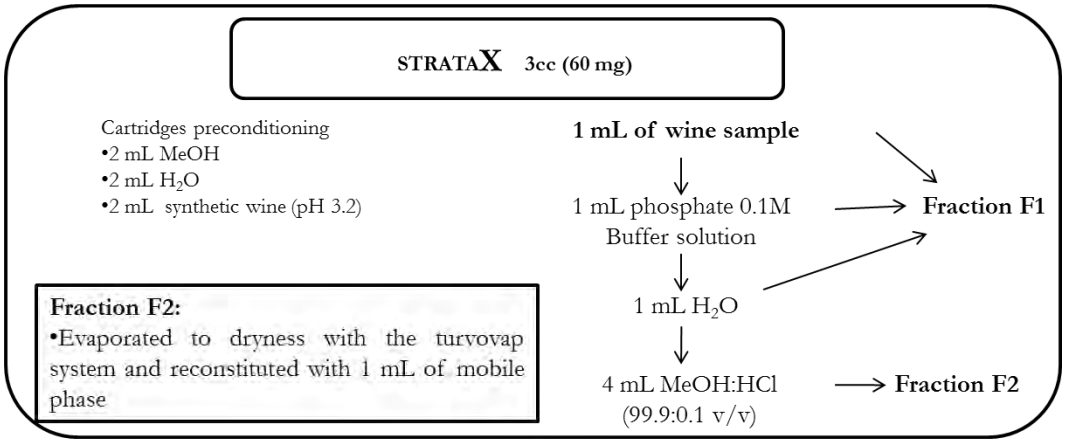



**Figure III.3.** Scheme for solid phase extraction of all wine samples for further quantitative analysis of anthocyanin derivatives by HPLC-DAD-ESI(+)-CID-MS/MS.

[261] Sánchez-Ilárduya, M.B. *Pigmentos derivados antociánicos de los vinos tintos de la Rioja: Estudio analítico, influencia en el color y evolución durante la crianza*, PhD Thesis, University of Basque Country, Spain, España, **2010**.

The solvents used in the chromatographic separation for the quantification of non-coloured and coloured anthocyanin derivatives were TFA:$H_2O$ (0.5:99.5 v/v) as mobile phase A and acetonitrile as mobile phase B.

The column used was a Phenomenex Onyx Monolithic C18 (100 x 3.0 mm), that allows to perform faster[262], and the elution gradient used was: 0-0.29 min, isocratic, 12% B; 0.29-4.29 min, lineal gradient, 12-15% B; 4.29-9.17 min, lineal gradient, 15-25% B; 9.17-12.72 min, lineal gradient, 25-40% B; 12.72-13.17 min, isocratic, 40% B; 13.17-13.63 min, lineal gradient, 40-100% B; 13.63-21.63 min, isocratic, 100% B, after that, a column reconditioning was made. The column temperature was 30 C and the automatic injector was thermostated at 4 ºC. The injection volume was 50 µL and the flow was 0.3 mL/min.

The 21 anthocyanin derivatives selected to use them as markers of Rioja alavesa wines and the 22 compounds optimized during this research are listed below (Table III.6), where it can be observed the corresponding transitions, the collision energies (CE) and the optimum cone voltages (CV) and Figures III.4 to III.9 show an example of the MRM chromatograms achieved.

[262]Rostagno, M.; Palma, M.; García-Barroso, C.; *Fast analysis of soy isoflavones by high-performance liquid chromatography with monolithic columns*, Anal. Chim. Acta **2007**, 582, 243-249.

**Table III.6.** Anthocyanin derivatives and their corresponding retention time, mass transitions, cone voltages (CV) and collision energies (CE).

| | Compound | | $t_R$ (min) | Transition | CV (V) | CE (eV) |
|---|---|---|---|---|---|---|
| *Direct condensation Flavanol-Anthocyanin. Flavylium form* | **14** | Catechin-Mv-3-glc | 4.7 | 780.9→618.9 | 35 | 25 |
| | **15** | Epicatechin-Mv-3-glc | 5.9 | 780.9→618.9 | 35 | 25 |
| | **16** | Catechin-Mv-3-(6-p-coum)-glc | 12.76 | 926.9→618.9 | 35 | 25 |
| | **17** | Epicatechin-Mv-3-(6-p-coum)-glc | 13.98 | 926.9→618.9 | 35 | 25 |
| | **18** | (Epi)Gallocatechin-Mv-3-glc | 3.36 | 796.9→634.9 | 35 | 25 |
| | **19** | (Epi)Gallocatechin-Mv-3-(6-p-coum)-glc | 10.54 | 942.9→634.9 | 35 | 25 |
| | **20*** | Di-(Epi)Catechin-Mv-3-glc | 5.06 | 1069.3→618.8 | 50 | 30 |
| | **21*** | (Epi)Catechin-(Epi)Gallocatechin-Mv-3-glc | 4 | 1085.3→634.9 | 50 | 30 |
| *Direct condensation Anthocyanin-Flavanol. Flavene form (type 1)* | **22*** | Mv-3-glc-Catechin | 7.3 | 783.2→469.1 | 30 | 20 |
| | **23*** | Mv-3-glc-Epicatechin | | | | |
| *Direct condensation Flavanol-Anthocyanin. Flavene form (type 2)* | **24*** | Catechin-Mv-3-glc | 6.2 | 783.2→469.1 | 30 | 20 |
| | **25*** | Epicatechin-Mv-3-glc | | | | |
| *Direct condensation Anthocyanin-Flavanol. A-type biciclyc bond (type 3)* | **26*** | Mv-3-glc-A-Catechin | 10.7 | 783.2→469.1 | 30 | 20 |
| | **27*** | Mv-3-glc-A-Epicatechin | | | | |
| *Flavanol-A-Flavanol-Anthocyanin* | **28*** | (Epi)Catechin-Mv-3-glc-A-(Epi)Catechin 1 | 3.6 | 1071.3→783.2 | 20 | 20 |
| | **29*** | (Epi)Catechin-Mv-3-glc-A-(Epi)Catechin 2 | 4.3 | 1071.3→783.2 | 20 | 20 |
| | **30*** | (Epi)Gallocatechin-Mv-3-glc-A-(Epi)Catechin 1 | 3.4 | 1087.3→631.2 | 30 | 30 |
| | **31*** | (Epi)Gallocatechin-Mv-3-glc-A-(Epi)Catechin 2 | | | | |
| *Flavanol-A-Flavanol-Anthocyanin* | **32*** | (Epi)Catechin-A-(Epi)Catechin-Mv-3-glc | 4.4 | 1071.3→781.2 | 20 | 10 |
| *Anthocyanin-Flavanol-A-Flavanol* | **33*** | Mv-3-glc-(Epi)Catechin-A-(Epi)Catechin | 5.4 | 1071.3→781.2 | 20 | 10 |

*: The cone voltages and collision energies were optimized during this study.

**Table III.6.** (Continue)

| | | Compound | $t_R$ (min) | Transition | CV (V) | CE (eV) |
|---|---|---|---|---|---|---|
| *Anthocyanin with ethylidene-bridged* | **34** | Mv-3-glc-8-ethyl-Catechin 1 | 12.1 | 808.9→518.9 | 35 | 25 |
| | **35** | Mv-3-glc-8-ethyl-Catechin 2 | 12.6 | 808.9→518.9 | 35 | 25 |
| | **36** | Mv-3-glc-8-ethyl-Epicatechin | 12.9 | 808.9→518.9 | 35 | 25 |
| | **37** | Mv-3-(6-p-coum)-glc-8-ethyl-(Epi)Catechin 1 | 15 | 954.9→664.9 | 35 | 25 |
| | **38** | Mv-3-(6-p-coum)-glc-8-ethyl-(Epi)Catechin 2 | 16.1 | 954.9→664.9 | 35 | 25 |
| *Vitisins B* | **39** | Mv-3-glc-acetaldehyde | 11.1 | 516.9→354.9 | 25 | 25 |
| | **40** | Mv-3-(6-p-coum)-glc-acetaldehyde | 14.6 | 662.9→354.9 | 35 | 25 |
| *Vitisins A* | **41** | Mv-3-glc-pyruvic | 10.5 | 560.9→398.9 | 25 | 25 |
| | **42** | Mv-3-(6-p-coum)-glc- pyruvic | 13.6 | 706.9→398.9 | 35 | 25 |
| *Derivative with acetoacetic acid* | **43** | Mv-3-glc-vinylmetyl | 12.5 | 530.9→368.9 | 25 | 25 |
| *Derivatives with hydroxycinnamic acids* | **44** | Mv-3-glc-4-vinylphenol | 16.1 | 608.9→446.9 | 25 | 25 |
| | **45** | Mv-3-(6-p-coum)-glc-4-vinylphenol | 17.1 | 754.9→446.9 | 35 | 25 |
| | **46** | Mv-3-glc-4-vinylcatechol | 15.6 | 624.9→462.9 | 35 | 25 |
| | **47** | Mv-3-glc-4-vinylguaiacol | 16.3 | 638.9→476.9 | 35 | 25 |
| | **48** | Mv-3-(6-p-coum)-glc-4-vinylguaiacol | 17.2 | 784.9→476.9 | 35 | 35 |
| *Derivatives with vinylflavanols* | **49*** | Mv-3-glc-4-vinylcatechin | 14.7 | 805.2→643.1 | 60 | 30 |
| | **50*** | Mv-3-glc-4-vinylepicatechin | 15.5 | 805.2→643.1 | 60 | 30 |
| | **51*** | Mv-3-(6-*p*-coum)-glc-4-vinylcatechin | 15.7 | 951.2→643.1 | 60 | 30 |
| | **52*** | Mv-3-(6-*p*-coum)-glc-4-vinylepicatechin | | | | |
| | **53*** | Mv-3-glc-4-vinylgallocatechin | 13.5 | 821.2→659.1 | 40 | 30 |
| | **54*** | Mv-3-glc-4-vinylepigallocatechin | 14.4 | 821.2→659.1 | 40 | 30 |
| | **55*** | Mv-3-(6-*p*-coum)-glc-4-vinyl(Epi)Gallocatechin 1 | 14.9 | 967.2→659.1 | 50 | 30 |
| | **56*** | Mv-3-(6-*p*-coum)-glc-4-vinyl(Epi)Gallocatechin 2 | | | | |

*: The cone voltages and collision energies were optimized during this study.

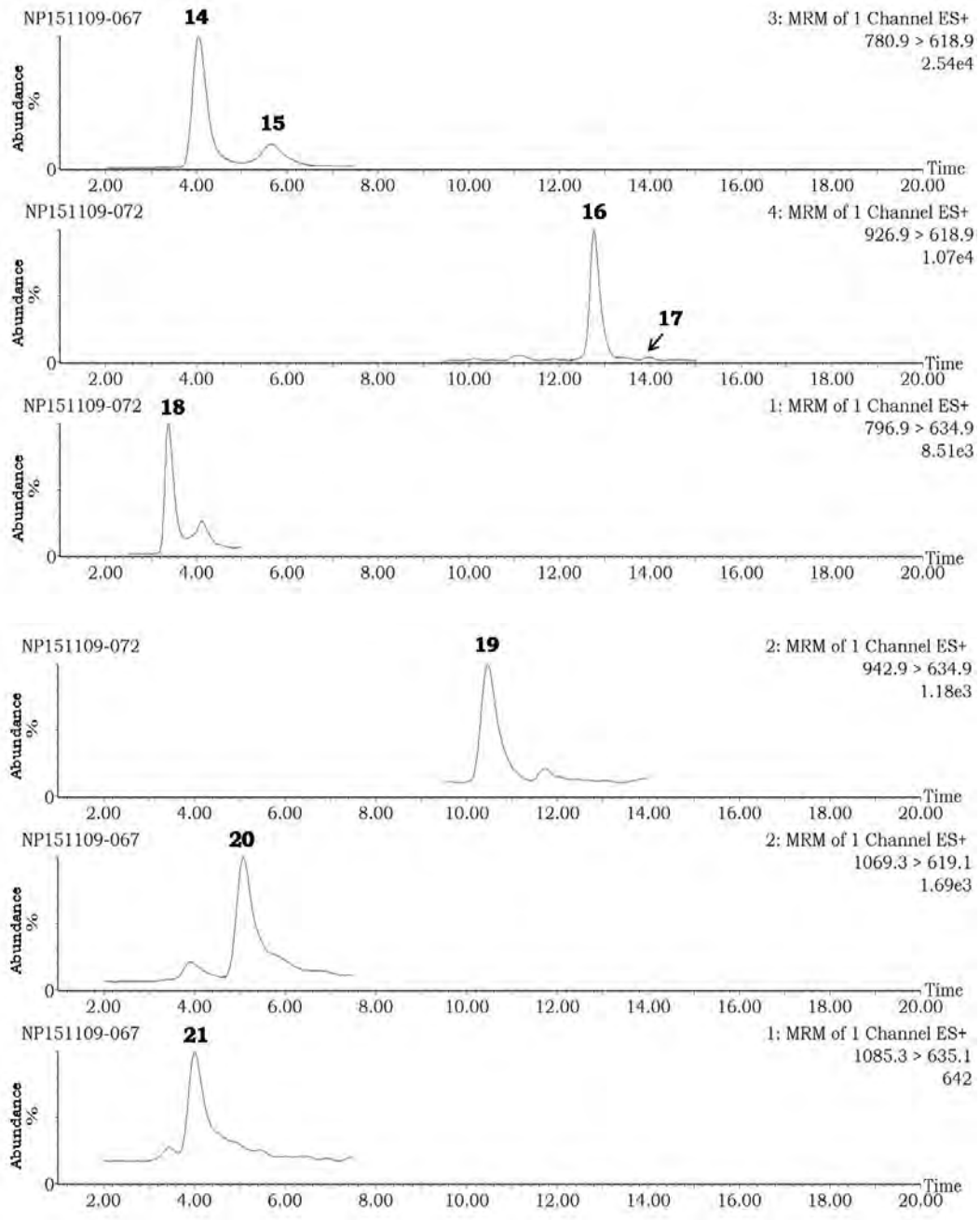



**Figure III.4.** MRM chromatograms achieved of condensation of anthocyanins in flavylium form with flavanols.

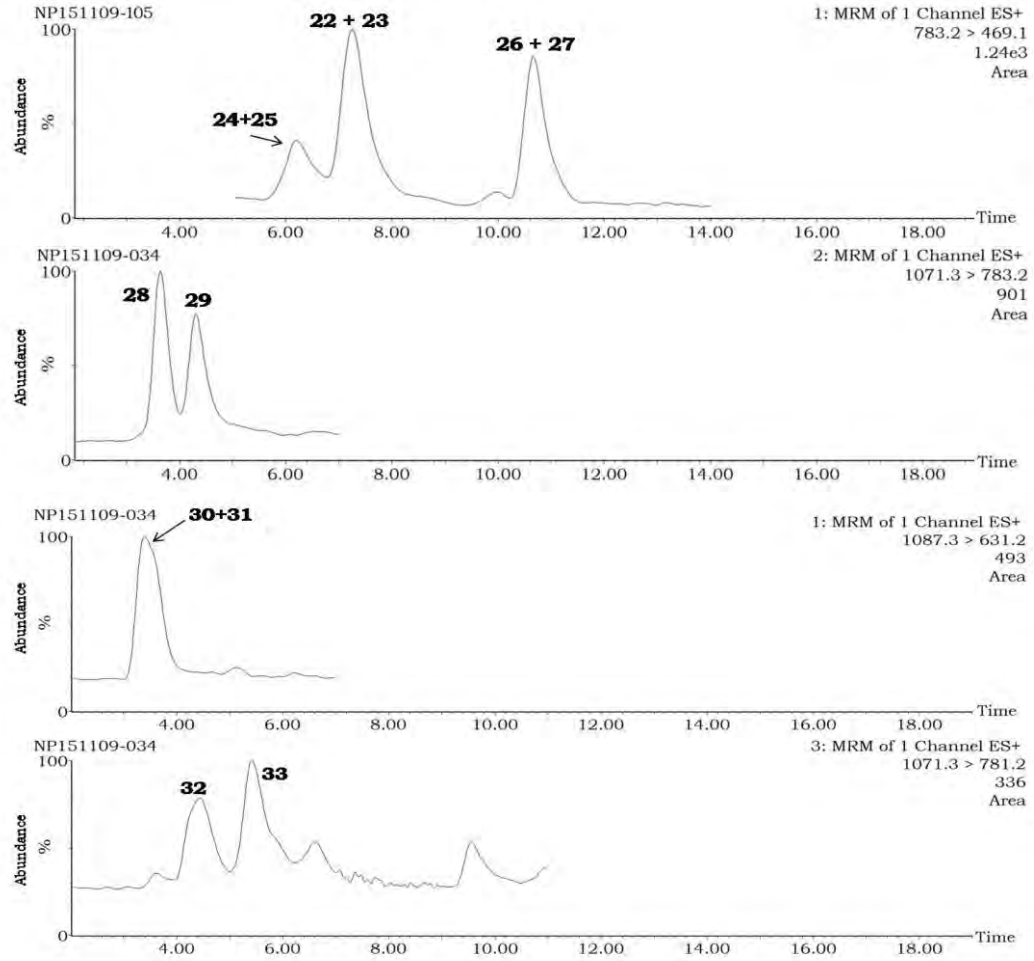



**Figure III.5.** MRM chromatograms achieved of condensation of anthocyanins in flavene form and with A bond with flavanols.

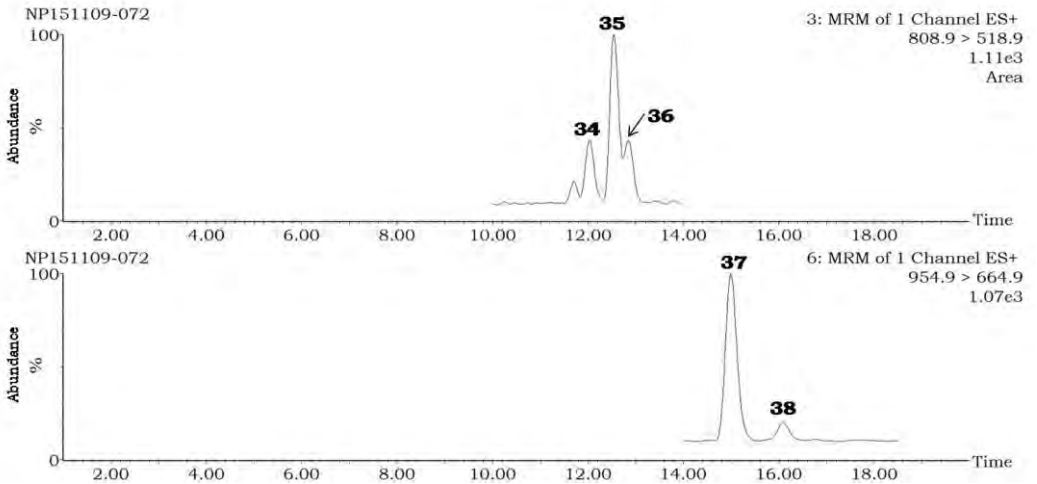



**Figure III.6.** MRM chromatograms achieved of ethylidene-bridge anthocyanin-flavanol condensation derivatives.

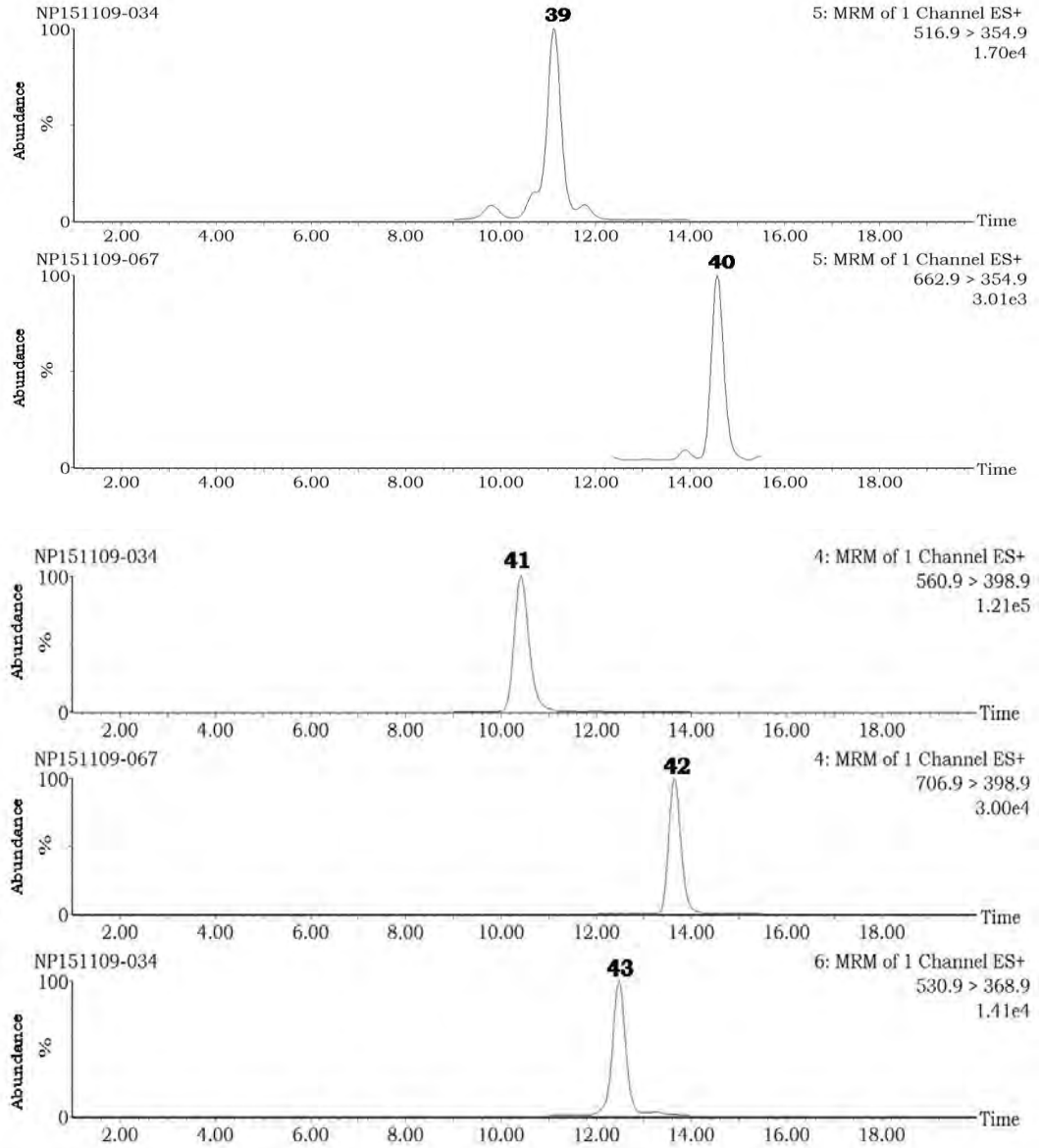



**Figure III.7.** MRM chromatograms achieved of pyranoanthocyanins derivatives with acetaldehyde, pyruvic acid and acetoacetic acid.

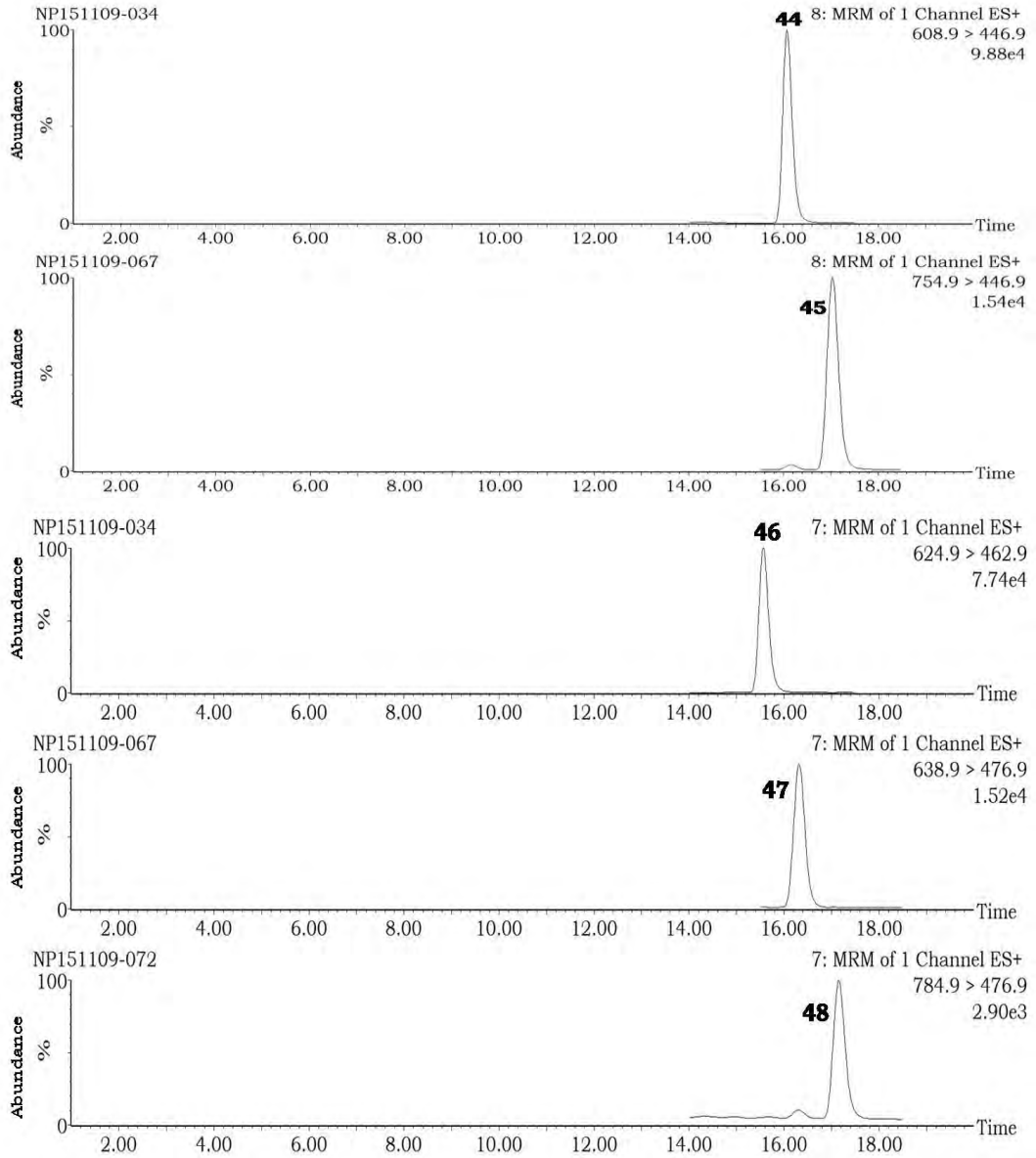



**Figure III.8.** MRM chromatograms achieved of anthocyanin derivatives with hydroxycinnamic acids.

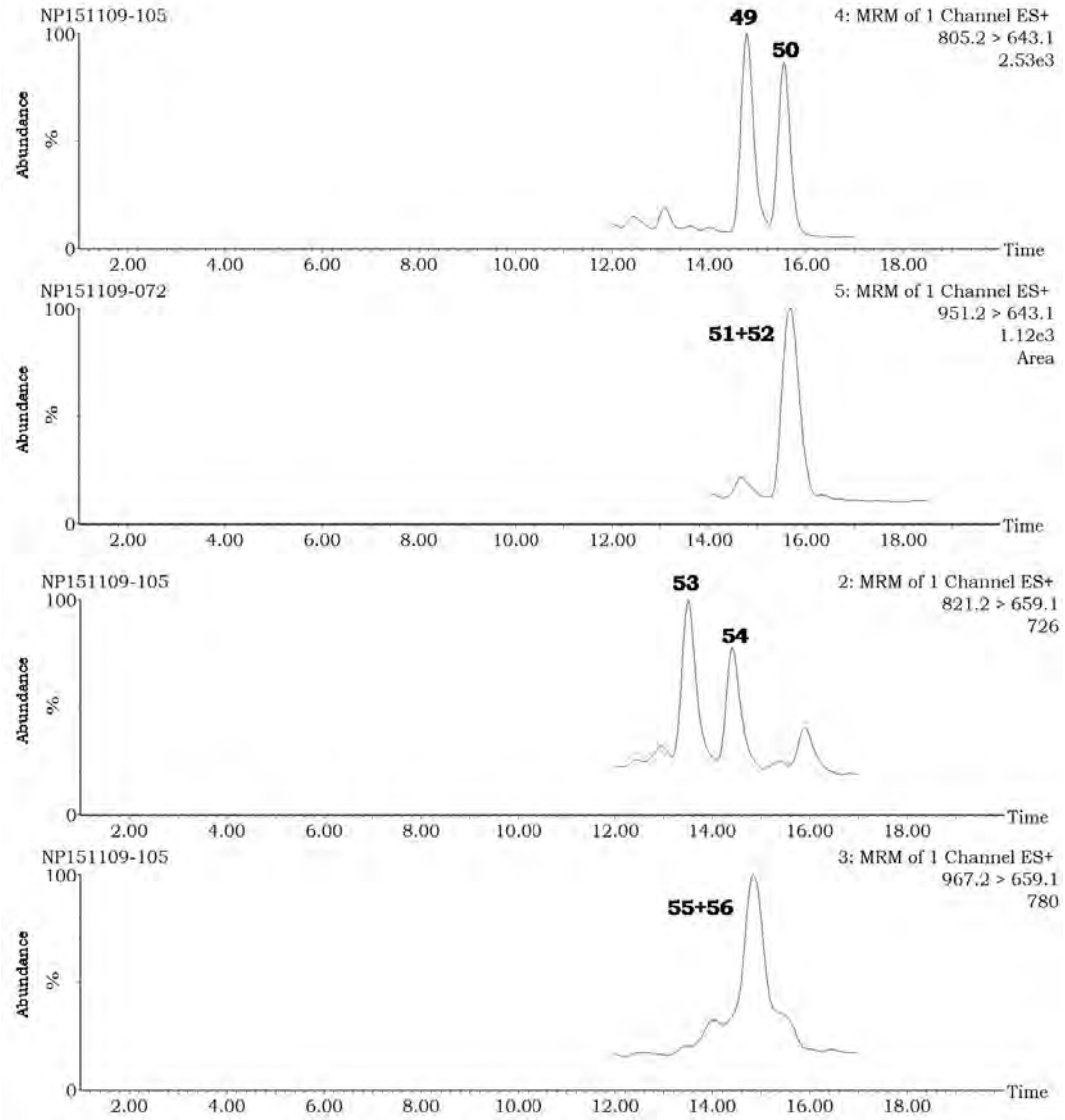



**Figure III.9.** MRM chromatograms achieved of anthocyanin derivatives with vinylflavanols.

The anthocyanin derivatives quantification was carried out by external calibration method. The pattern used was malvidin-3-O-glucoside, so all concentrations are expressed as mg/L equivalents of malvidin-3-O-glucoside.

The stock solution and the intermediate and standard solutions, all were prepared using methanol with hydrochloric acid to 0.1% (v/v) as solvent to ensure stability of the pattern, as the same way as anthocyanin quantification. The standard solutions were not prepared with more than 20% of methanol in order to not distort chromatographic peak Standard solutions were injected in the range 0.001-100 mg/L.

Mass spectrometry is a technique that has some instability and is necessary to inject the standard solutions of Mv-3-O-glucoside periodically. In this work, injections of standards solutions were inserted every 12 samples of wine.

Calibration lines were calculated for different concentration ranges: 0.001-0.01 μg/mL, 0.01-0.1 μg/mL, 0.1-1 μg/mL, 1-10 μg/mL and 10-100 μg/mL (Table III.7).

**Table III.7.** Linear model for the five different ranges of Mv-3-glc calibration.

| Anthocyanin | Linear Range | Slope | Intercept | $R^2$ |
|---|---|---|---|---|
| **Mv-3-glc** | 0.001-0.01 | (745±18) $10^2$ | 90±10 | 0.9988 |
| | 0.01-0.1 | (60820±19) $10^2$ | 199.±29 | 0.9980 |
| | 0.1-1.0 | (505±11) $10^2$ | (202±63) 10 | 0.9990 |
| | 1.0-10.0 | (3037±54) 10 | (256±31) $10^2$ | 0.9993 |
| | 10.0-100.0 | (818±32) 10 | (27±18) $10^3$ | 0.9969 |

## III.6. IDENTIFICATION OF TANNINS BY HPLC-DAD-ESI(-)-CID-MS/MS

As we say before, it is necessary a first step before carrying, so solid phase extraction (SPE) was performed to simplify the sample matrix and to preconcentrate analytes before the identification by HPLC-DAD-ESI(-)-CID-MS/MS. This pretreatment was optimized and validated by our group previously[263].

This methodology begins with 100 millilitres of red wine, which were de-alcoholized in a Büchi (Büchi Labortechnik AG, Flawil, Switzerland) R200 rotary evaporator at 25°C during 30 min. Then, n order to preconcentrate the wine sample, an SPE procedure was adapted from the literature[264]. A empty commercial cartridges (IST, Hengoed Mid, Glam, UK, 150 mL capacity) were filled with 25 g of C18-modified silica (IST, particle size 60 μm). Both top and bottom of solid phase were covered with 20 μm polyethylene frits (IST).

[263] Sánchez-Fernández, C.; *Búsqueda de marcadores de tipo tanino en vinos tintos de Rioja: Estudio cualitativo y cuantitativo por HPLC-MS/MS*, PhD Thesis, University of Basque Country, Spain, **2012**.

[264] Sun, B.; Leandro, M. C.; de Freitas, V.; Spranger, M. I.; *Fractionation of red wine polyphenols by solid-phase extraction and liquid chromatography*, J. Chromatogr. A **2006**, 1128, 27-38.

The extraction process consists of two stages:

**First stage of SPE**

The cartridge was activated with 2 x 50 mL of methanol, washed with 2 x 50 mL of ultrapurified water and preconditioned with 2 x 50 mL of phosphate buffer (0.1 mol/L, pH = 6.5). Then, 50 millilitres of de-alcoholized wine were loaded in cartridges.

Elution began with 125 mL of 0.1 mol/L phosphate buffer (pH = 6.5) to elute phenolic acids, followed by 50 mL of ultrapurified water for the elution of compounds present in wine that are soluble in water. Then the elution of monomers and oligomers of tannins was carried out by 125 mL of ethyl acetate and, finally, the polimers and anthocyanins which still retained in cartridge were eluted with 125 mL of MeOH:HCl (99:9:0.1, v/v).

The fraction of monomers and oligomers were evaporated to dryness in the rotary evaporator, redissolved in 50 mL of ultrapurified water and was reserved for the second phase of the extraction process

**Second stage of SPE**

The cartridge was activated as the same way as in the first stage, but without the tampon solution. Then, 50 millilitres of fraction of monomers and oligomers were loaded in the cartridge and air was passed through the cartridge until it was completely dry. The monomers elute with 75 mL of diethyl ether and the oligomers with 75 mL MeOH.

The fractions of monomers, oligomers and polimers were concentrated to dryness under a stream of nitrogen in a Zimark TurboVap-LV evaporator (Hopkinton, Massachusetts, USA) at 25 °C, redissolved in 500 µL of the initial HPLC mobile phase solution ($H_2O$:HAc 99:1, v/v) for monomers and oligomers and in 1mL for the fraction of polimers. All fractions were filtered through a 0.45 mm PTFE membrane before analysis by HPLC-DAD-ESI(-)-CID-MS/MS.

The identification of the compounds was carried out by HPLC-DAD-ESI(-)-CID-MS/MS using the same equipment that was used for the anthocyanin derivatives, so it was explained in point III.4. IDENTIFICATION OF NON-COLOURED AND COULORED ANTHOCYANIN DERIVATIVES BY HPLC-DAD-ESI(+)-MS/MS".

The column used was a Phenomenex Luna C18 (2) (Torrance, USA) (150 x 4.6 mm) and the particle size is 3 µm, with a precolumn of Waters Nova-Pack C18 (Barcelona, Spain) (10 x 3.9 mm) and the particle size is 4 µm.

The solvents used in the chromatographic separation for the identification of tannins were $H_2O$:HAc (99:1 v/v) as mobile phase A and MeOH:HAc (99:1 v/v) as mobile phase B. The choice of acetic acid as an additive, is because it provides an acid pH which improves the separation of tannins from the anthocyanins present in some fractions[,266,267]. The elution gradient used was: 0-10 min, isocratic, 0% B; 1-60 min, lineal gradient, 0-15% B; 60-80 min, lineal gradient, 15-25% B; 80-100 min, lineal gradient, 25-50% B; 100-110 min, lineal gradient, 50-75% B, 110-120 min, lineal gradient, 75-95% B; 120-130 min, lineal gradient, 95-100% B; followed by a column cleaning and reconditioning. The column temperature was 30 C and the automatic injector was thermostated at 4 ºC. The injection volume was 50 µL and the flow was 0.8 mL/min. The triple quadrupole is couple to the output of the diode array detector by a *T* union which divides the flow. This union allows the entry of 0.25 mL into the spectrometer, which avoids the ionization of analytes in the electrospray source.

The parameters of the electrospray interface were as follows: capillar voltage was 2.6 kV in negative mode, cone voltage was 3 V and RF lens voltage was 0 V. The temperature of the source was 120º C. Nitrogen was used as desolvation gas and its flow and temperature was 450 L/h and 300º C, respectively.

The acquisition in MS scan was performed in centroid mode in a range from 50 to 2000 u in negative mode, collecting the mass spectra at different voltages (10, 20, 30, 40 and 60 V). The scan time was 2 seconds with an interscan time of 0.1 seconds.

The acquisition in MS/MS ($MS^2$) scan was performed in product ions scan mode, using the optimum cone voltage, collecting the mass spectra at different collision energies (10, 20, 30, 40, 50 and 60 eV).

MS spectra were used for identification of desprotonated molecule $[M-H]^-$ for being used as precursor ion in MS/MS product ions scans. Study of fragmentations on these product ions spectra was used for structural elucidation.

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[266]Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Beecher, G.; Holden, J.; Haytowitz, D.; Prior, R. L.; *Screening of Food containing Proanthocyanidins and their Structural Characterization Using LC-MS/MS and Thiolytic Degradation*, J. Agric. Food Chem. **2003**, 51, 7513-7521.

[267]Monagas, M.; Bartolomé, B.; Gómez-Cordovés, C.; *Effect of the (Graciano vs Cabernet Sauvignon) on blends of Tempranillo wine during ageing in the bottle. I. Anthocyanins, pyranoanthocyanins and non-anthocyanin phenolics*, LTW **2006**, 39, 1133-1142.

# III.7. QUANTIFICATION OF TANNINS BY HPLC-DAD-ESI(-)-CID-MS/MS

Firstly, before carrying out the tannins assay, a solid phase extraction (SPE) was performed to simplify the sample matrix and avoid the ionic saturation during the subsequent sample analysis by HPLC-DAD-ESI(-)-CID-MS/MS. This pretreatment was optimized and validated by our group previously[269]. SPE conditions are detailed in Figure III.3.

The column used was a Phenomenex Onyx Monolithic C18 (100x3.0 mm), which allows quicker separations[270]. The mobile phases used were $H_2O$:HAc (99:1, v/v) (phase A) and MeOH:HAc (99:1, v/v) (phase B. The gradient elution used was: 0-1.03 min, isocratic 19.3 % B; 1.03-7.47 min lineal gradient, 19.3-20 % B; 7.47-11.25 min, lineal gradient, 20-25 % B; 11.25-16.03 min, lineal gradient, 25-45% B; 16.03-19.92 min, lineal gradient, 45-75 % B; 19.92-24.70 min, lineal gradient 75-100 % B; 24.70-28.00 min, isocratic 100% B, followed by column washing and reconditioning. The column temperature was 30 °C and the autosampler was thermostated at 4 °C. The volume injection was 50 µL, and the flow rate was 0.3 mL/min. All the effluent from the column is introduced into the ESI interface, without flow split.

For MRM experiments, the precursor and product ions selected for the transition were those with high intensities in the $MS^1$ experiments in scan mode and in the $MS^2$ experiments in product ion scan mode previously performed. Table III.8 collect the retention time, optimum transitions, cone voltages and collision energies for each tannin studied and Figures III.10 to III.17 show an example of the MRM chromatograms achieved.

[269]Sánchez-Fernández, C.; *Búsqueda de marcadores de tipo tanino en vinos tintos de Rioja: Estudio cualitativo y cuantitativo por HPLC-MS/MS*, PhD Thesis, University of Basque Country, Spain, **2012**.

[270]Rostagno, M.; Palma, M.; García-Barroso, C.; *Fast analysis of soy isoflavones by high-performance liquid chromatography with monolithic columns*, Anal. Chim. Acta **2007**, 582, 243-249.

**Table III.8.** Tannins and their corresponding retention time, mass transitions, cone voltages (CV) and collision energies (CE).

| | | Compound | $t_R$ (min) | Transition | CV (V) | CE (eV) |
|---|---|---|---|---|---|---|
| *Monomeric tannins* | **57** | Catechin | 12.1 | 289→137 | 25 | 25 |
| | **58** | Epicatechin | 16.1 | 289→137 | 25 | 25 |
| | **59** | Gallocatechin | 7.5 | 305→137 | 25 | 25 |
| | **60** | Epigallocatechin | 12.5 | 305→137 | 25 | 25 |
| *Procyainidins homodimers B* | **61** | PCB1 | 11.6 | 577→289 | 25 | 25 |
| | **62** | PCB2 | 14.0 | 577→289 | 25 | 25 |
| | **63** | $((epi)cat)_2$ 1 | 10.8 | 577→289 | 25 | 25 |
| *Prodelphinidins homodimers B* | **64** | $((epi)gallocat)_2$ 1 | 4.5 | 609→305 | 25 | 25 |
| | **65** | $((epi)gallocat)_2$ 2 | 7.4 | 609→305 | 25 | 25 |
| | **66** | $((epi)gallocat)_2$ 3 | 11.6 | 609→305 | 25 | 25 |
| *Mixed dimers B* | **67** | ((epi)cat-(epi)gallocat) 1 | 8.8 | 593→305 | 35 | 25 |
| | **68** | ((epi)cat-(epi)gallocat) 2 | 11.8 | 593→305 | 35 | 25 |
| | **69** | ((epi)gallocat-(epi)cat) 1 | 8.0 | 593→289 | 35 | 25 |
| | **70** | ((epi)gallocat-(epi)cat) 2 | 10.7 | 593→289 | 35 | 25 |
| *Procyanidins homotrimers B* | **71** | PCC1 | 16.7 | 865→577 | 25 | 25 |
| | **72** | $((epi)cat)_3$ 1 | 7.7 | 865→577 | 25 | 25 |
| | **73** | $((epi)cat)_3$ 2 | 11.9 | 865→577 | 25 | 25 |
| *Procyanidins homodimers A* | **74** | $((epi)cat)_2$A 1 | 11 | 575→285 | 25 | 25 |
| | **75** | $((epi)cat)_2$A 2 | 24.2 | 575→285 | 25 | 25 |
| *Mixed dimers A* | **76** | ((epi)cat-(epi)gallocat)A 1 | 11.6 | 591→303 | 55 | 25 |
| | **77** | ((epi)cat-(epi)gallocat)A 2 | 13.2 | 591→303 | 55 | 25 |
| *Mixed trimers with one A bond and one B bond* | **78** | (epi)cat-((epi)cat-(epi)gallocat) A 1 | 11.1 | 879→591 | 35 | 25 |
| | **79** | (epi)cat-((epi)cat-(epi)gallocat) A 2 | 12.9 | 879→591 | 35 | 25 |
| *p-Vinyl tannins* | **80** | p-vinyl(epi)cat 1 | 17.7 | 315→163 | 55 | 25 |
| | **81** | p-vinyl(epi)cat 2 | 21.6 | 315→163 | 55 | 25 |
| | **82** | p-vinyl(epi)cat 3 | 22.9 | 315→163 | 55 | 25 |
| *Tannins with furfuryl bridge* | **83** | (epi)cat-furfuryl-(epi)cat 1 | 5.7 | 657→369 | 55 | 25 |
| | **84** | (epi)cat-furfuryl-(epi)cat 2 | 10.6 | 657→369 | 55 | 25 |
| *O-glycosylated tannis* | **85** | (epi)cat-glycoside 1 | 11.6 | 451→289 | 25 | 25 |
| | **86** | (epi)cat-glycoside 2 | 13.6 | 451→289 | 25 | 25 |

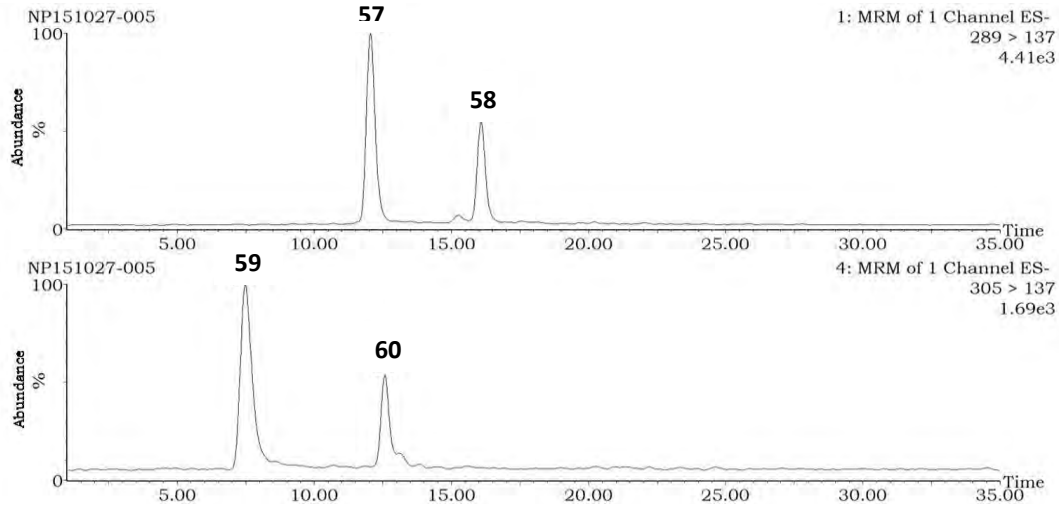



**Figure III.10.** MRM chromatograms achieved for the 4 monomeric tannins.

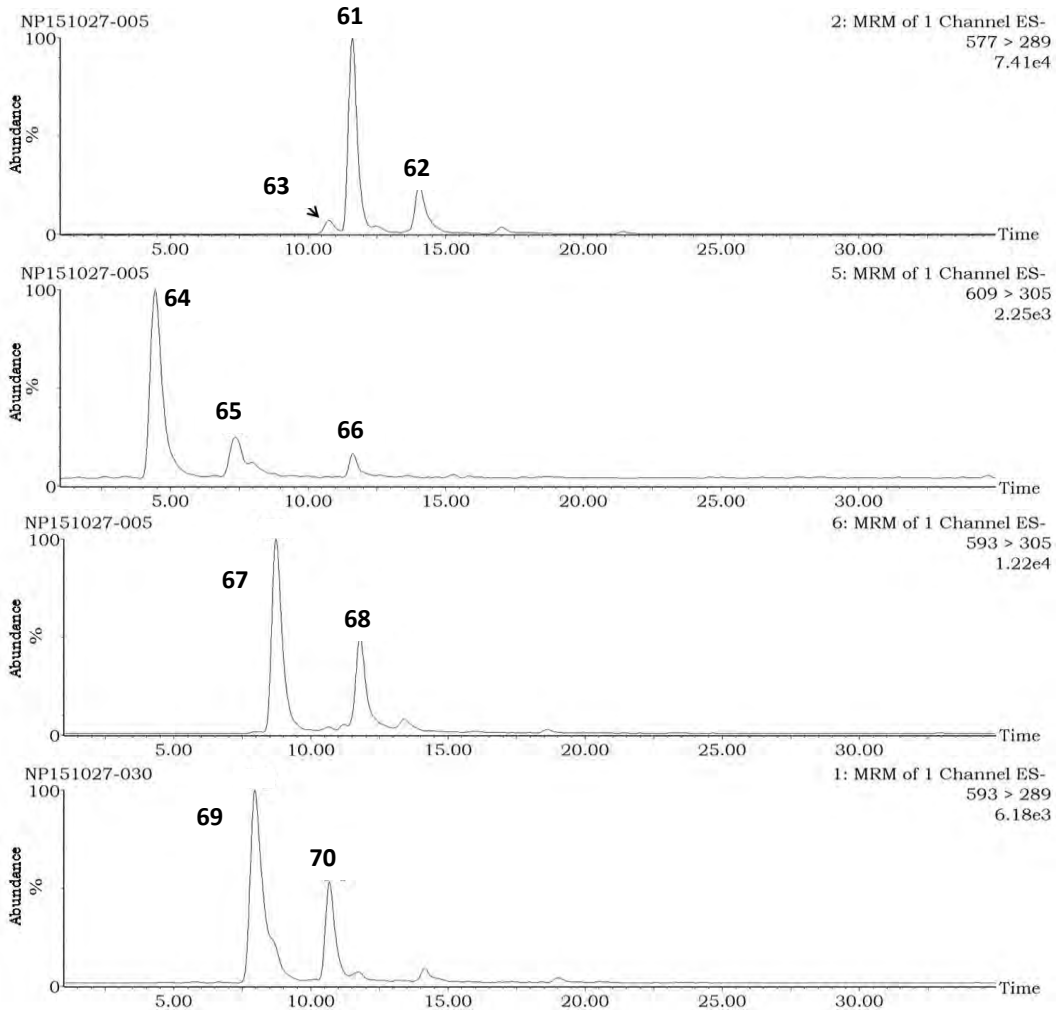



**Figure III.11.** MRM chromatograms achieved for the procyanidin dimers B (Procyanidins B), prodelphinidin dimers B (Prodelphinidins B) and mixed dimmers with B bond.

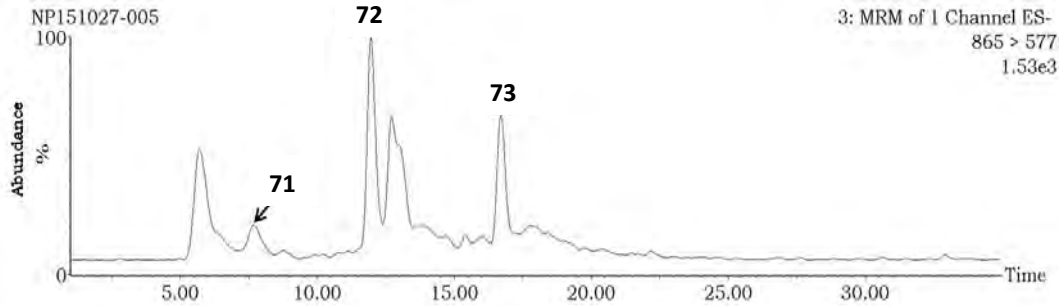



**Figure III.12.** MRM chromatograms achieved for the procyanidin trimers with B bond (Procyanidins C).

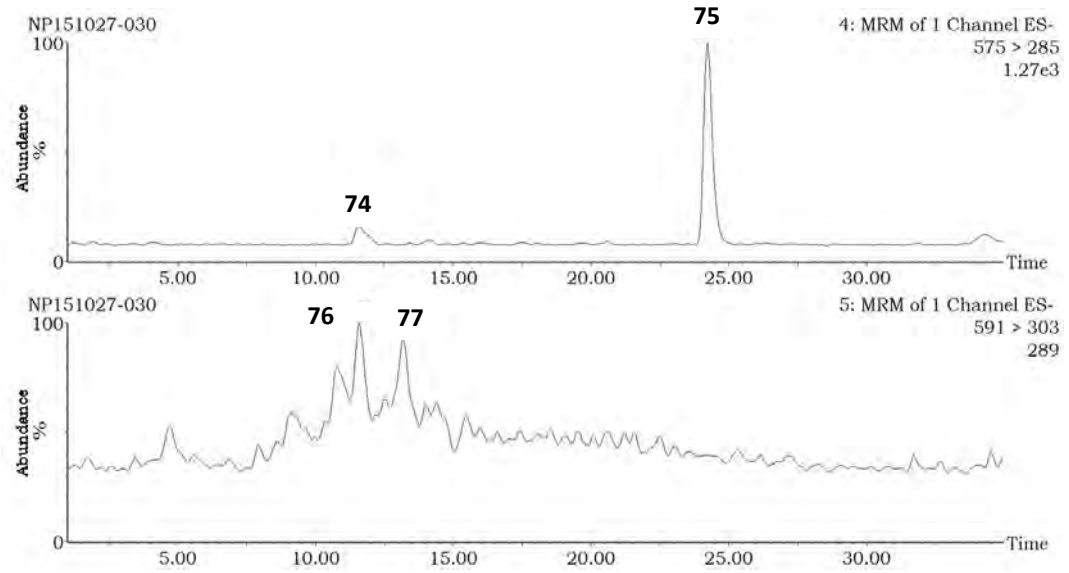



**Figure III.13.** MRM chromatograms achieved for the procyanidin dimers A (Procyanidins A) and mixed dimmers with A bond.

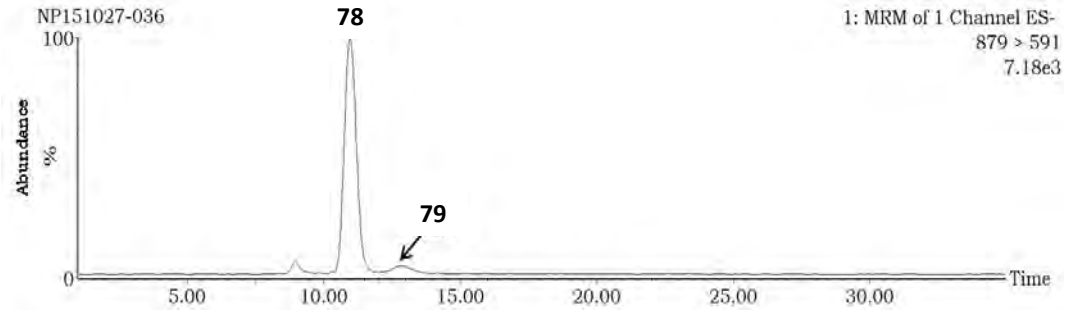



**Figure III.14.** MRM chromatograms achieved for the mixed trimers with a A bond and a B bond.

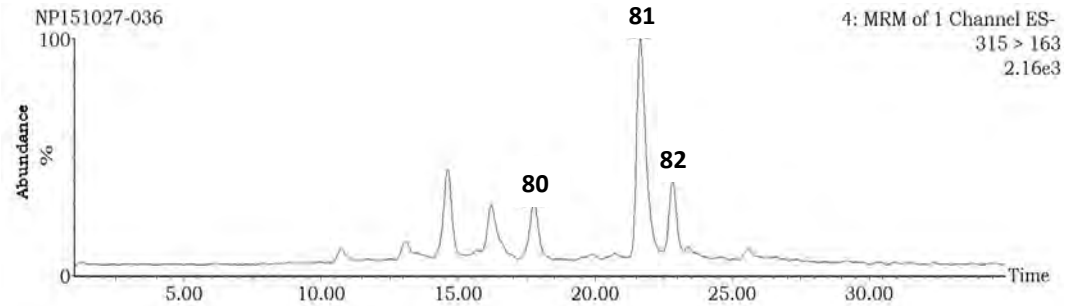



**Figure III.15.** MRM chromatograms achieved for (epi)catechin vinyl-flavan-3-ols compounds.

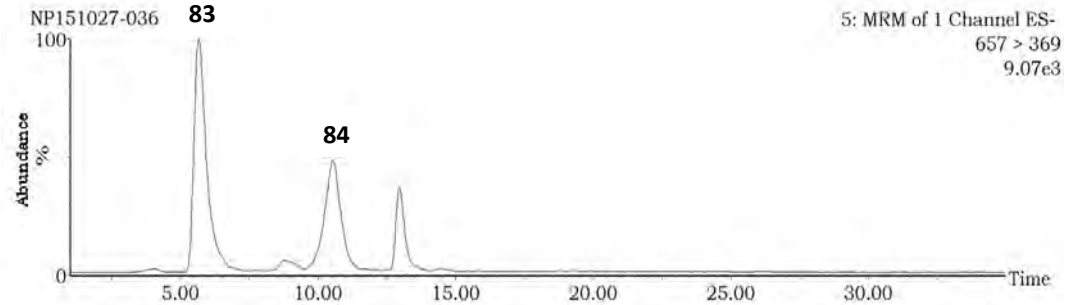



**Figure III.16.** MRM chromatograms achieved for tannins with furfuryl bridge.

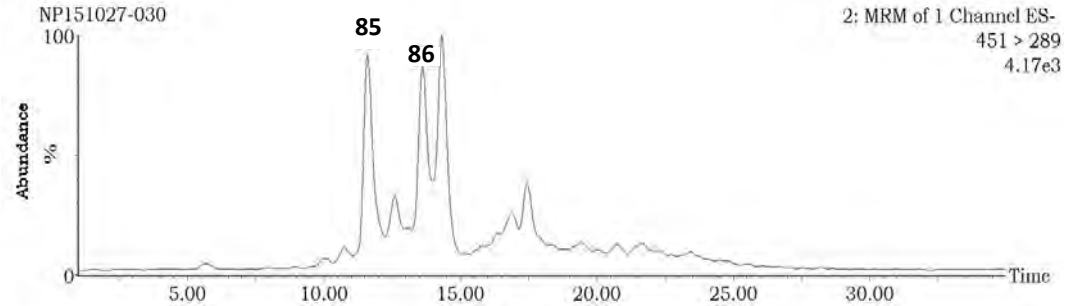



**Figure III.17.** MRM chromatograms achieved for O-glycosylated tannins.

The tannins quantification was carried out by external calibration method. The pattern used was catechin, so all concentrations are expressed as mg/L equivalents of catechin.

The stock solution was prepared at concentration close to 1000 mg/L in $H_2O$:MeOH (80:20 v/v), and stored at 4 °C protected from the light.

Corresponding injections were interspersed at 15 concentration levels between 0.01 and 150 mg/L of standard solutions of catechin each 12 samples analysis. For this reason it was necessary that the chromatographic method of patterns injection was as quick as possible, but without distorting the measure. This is why it is injected into an isocratic method at 19.3 % B (0-7 min). This is the catechin eluting percentage in the longer (38 minutes) elution method that is used for the samples separation.

Calibration was divided in four concentration ranges: 0.01-0.1 mg/L, 0.1-1.0 mg/L, 1.0-10 mg/L and 10-150 mg/L. When the curve of the full calibration is studied, there is not observed linearity due to the drop saturation process. The first step in the electrospray ionization is the formation of fine beads with an excess of negative ions (or positive, when working in positive ionization mode, ESI+) because of the voltage applied in the capillary of the probe. When the surface of the drop is fully occupied with charged molecules of the analyte, there is no gain in response with increasing concentration, because some of the analyte ions can not reach the surface

and do not pass to gas phase. At this time the drop saturation is produced. This trend is obviously more pronounced in higher concentration ranges.

If we divide the full calibration curve in the four ranges described above, it can be observed a better fit for what would be the equation of a straight line (Table III.9).

**Table III.9.** Linear model for the four different ranges of catechin calibration.

| Tannin | Linear Range | Slope | Intercept | $R^2$ |
|---|---|---|---|---|
| Catechin | 0.01-0.1 | (61.9±2.9).10 | 4.71±1.66 | 0.9958 |
| | 0.1-1.0 | (59.8±3.8).10 | 14.84±4.88 | 0.9922 |
| | 1.0-10.0 | (72.1±1.7).10 | (23.3±9.6).10 | 0.9989 |
| | 10.0-150.0 | 199.36±9.47 | (65.1±8.1).$10^2$ | 0.9933 |

## III.8. COLOUR MEASUREMENTS

The chromatic properties of different red wine samples were carried out in order to characterize the colour of the wine.

### III.8.1 STANDARD PARAMETERS

The standard parameters were determined according to the methodology described by *Glories*[271,272].

The absorbances at 420, 520 and 620 nm of each sample were measured in a 1 mm cuvette, using an UV/Vis spectrophotometer from Bioware (Edmonton, Canada). The results were multiplied by 10 to refer to a 10 mm cuvette (standard cuvette). The colouring intensity indicates how much color the wine has and was calculated as follow:

$$IC = A_{420}+A_{520}+A_{620}$$

The colour tonality indicates the relative importance of yellow colour over the red one. It was expressed in percent and was calculated as follows:

$$\text{Tonalidad (T)} = (A_{420}/A_{520}) \times 100$$

[271]Glories, Y.; *The color of red wines. Part 1. Anthocyanin and tannin equilibriums*, Connaissance Vigne Vin **1984**, 18, 195-217.

[272]Glories, Y.; *The color of red wines. Part 2. Measurement, origin and interpretation*, Connaissance Vigne Vin **1984**, 18, 253-271.

### III.8.2 CIELAB SPACE

As mentioned in Chapter 1, in 1976 the *Commission Internationale de l'Éclairage* (CIE) established some international standards for the correct definition of colour. The values X, Y and Z represent the three base colours (X: virtual red colour, Y: virtual green colour and Z: virtual blue colour).

The transmittance at 450, 520, 570 and 630 nm of each sample were measured in a 2 mm cuvette using an UV/Vis spectrophotometer from Bioware (Edmonton, Canada). Then, the X, Y and Z values were calculated as follows:

$$X = 19.717\ T_{450} + 1.884\ T_{520} + 42.539\ T_{570} + 32.474\ T_{630} - 1.841$$
$$Y = 7.950\ T_{450} + 34.764\ T_{520} + 42.736\ T_{570} + 15.759\ T_{630} - 1.180$$
$$Z = 103.518\ T_{450} + 4.190\ T_{520} + 0.251\ T_{570} - 1.831\ T_{630} + 0.818$$

The CIE defined in 1976 the CIELAB space. It can be possible to define a colour within the CIELAB space by using the parameters L*, a* and b*. The L* parameter goes from while to black, parameter a* from green to red and b* parameter from yellow to blue.

The parameters L*, a* and b* is calculated with X, Y and Z values and according to the following equations:

$$L^* = 116\ (Y/100)^{1/3} - 16$$
$$a^* = 500\ [(X/94.825)^{1/3} - (Y/100)^{1/3}]$$
$$b^* = 200\ [(Y/100)^{1/3} - (Z/107.383)^{1/3}]$$

# CHAPTER IV

## IDENTIFICATION OF NON COLOURED AND COLOURED ANTHOCYANIN DERIVATIVES AND TANNINS

# Chapter IV

# IDENTIFICATION OF NON-COLOURED AND COLOURED ANTHOCYANIN DERIVATIVES AND TANNINS

In this chapter the identification of several anthocyanin derivatives and tannins in red wines that had been previously identified by our group [273, 274] will be summarized. Also identification of eighteen new non-coloured anthocyanin derivatives identified in the present work will be detailed. The different characteristics of the fragmentation patterns observed for the different families will also be discussed.

## IV.1. IDENTIFICATION OF NON-COLOURED AND COLOURED ANTHOCYANIN DERIVATIVES BY HPLC-DAD-ESI(+)-CID-MS/MS

As mentioned anthocyanins monoglucosides (acylated or non-acylated) are the precursors of different families of anthocyanin-derived pigments, which are formed during wine making and aging.

[273] Sánchez-Ilárduya. M. B.; *Pigmentos derivados antociánicos de los vinos tintos de Rioja: Estudio analítico, influencia en el color y evolución durante la crianza. PhD* Thesis, Universidad del País Vasco, Spain, **2010**.

[274] Sánchez-Fernández, C.; *Búsqueda de marcadores de tipo tanino en vinos tintos de Rioja: Estudio cualitativo y cuantitativo por HPLC-MS/MS. PhD* Thesis, Universidad del País Vasco, Spain, **2012**.

## IV.1.1. Direct condensation with flavanols

70 anthocyanin derivatives formed by direct condensation with flavanols have been identified. These condensations can be flavanol-anthocyanin or anthocyanin-flavanol type, depending on the position of the flavanol in the compound structure.

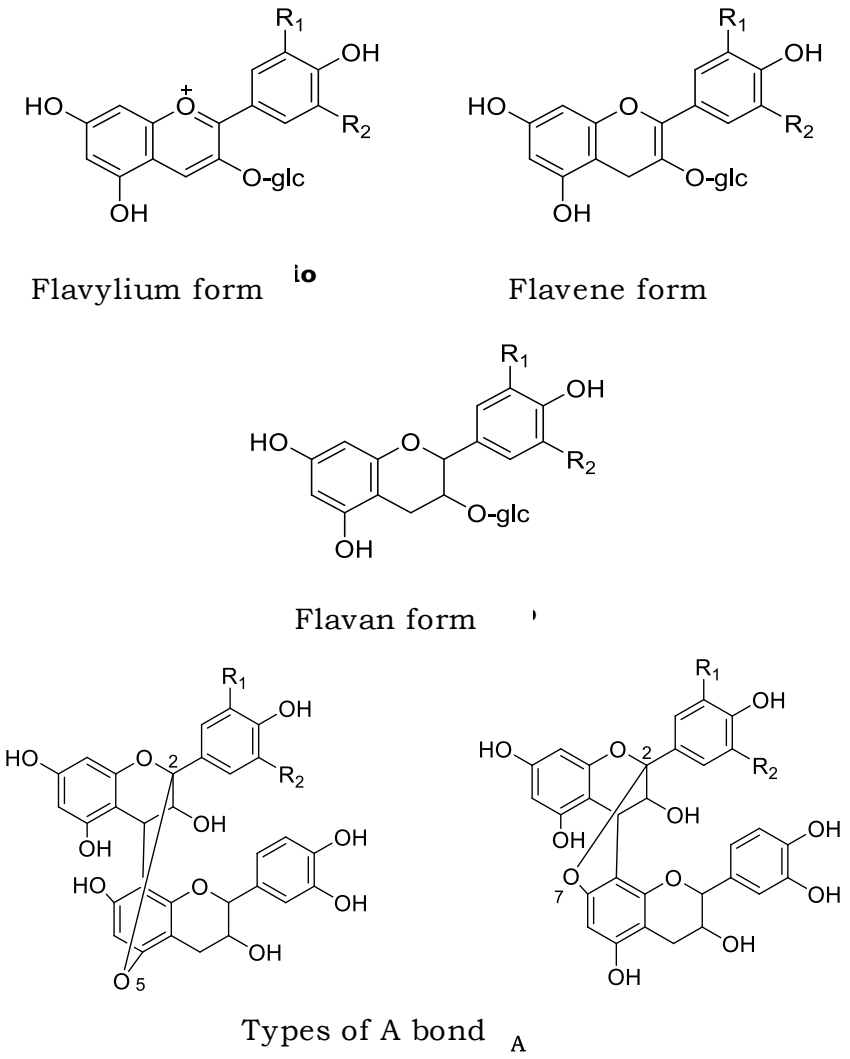
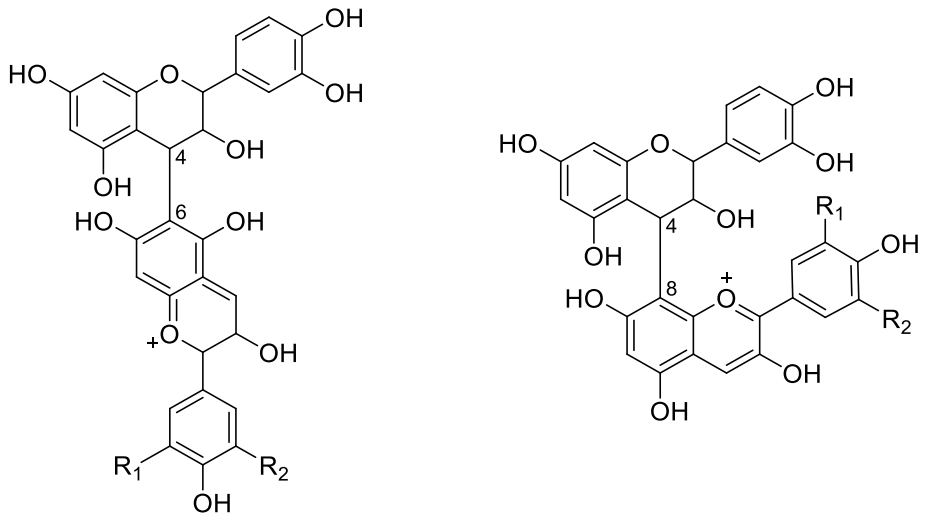



**Figure IV.1.** Different forms of anthocyanins and bond types that can take place in direct condensation with flavanols.




Types of B bond

**Figure IV.1.** Cont.

In addition, anthocyanin can appear at different forms: as flavylium form or colourless forms as flavene and flavan forms, and both units (anthocyanin and flavanol units) can be bonded by an A or B bond. These structures are shown in Figure IV.1.

**a) Direct condensation with flavanols. Flavylium form**

Thirty derivatives of this type were identified by our group [275]. In these compounds the flavanol and the anthocyanin, which is as flavylium form, are bonded by a B bond (Table IV.1.).

[275] Sánchez-Ilárduya. M. B.; *Pigmentos derivados antociánicos de los vinos tintos de Rioja: Estudio de analítico, influencia en el color y evolución durante la crianza.* PhD Thesis, Universidad del País Vasco, Spain, **2010**.

**Table IV.1.** Retention time and molecular ion achieved by HPLC-DAD-ESI(+)-CID-MS/MS of direct condensation products (flavylium form). The numbers written in bold correspond to the compounds chosen as markers.

| Number | $t_R$ (min) | Compound | $[M]^+$ (m/z) |
|---|---|---|---|
| *1* | 4.95 | Catechin-Dp-3-glc | 753.1 |
| *2* | 6.61 | Epicatechin-Dp-3-glc | 753.1 |
| *3* | 6.17 | Catechin-Cy-3-glc | 737.2 |
| *4* | 6.90 | Catechin-Pt-3-glc | 767.1 |
| *5* | 10.80 | Epicatechin-Pt-3-glc | 767.1 |
| *6* | 9.42 | Catechin-Pn-3-glc | 751.2 |
| *7* | 13.25 | Pn-3-glc-catechin | 751.2 |
| *8* | 3.03 | (Epi)catechin-Mv-3-glc | 780.9 |
| **14** | 10.37 | Catechin-Mv-3-glc | 780.9 |
| **15** | 20.63 | Epicatechin-Mv-3-glc | 780.9 |
| *9* | 13.28 | Mv-3-glc-catechin | 780.9 |
| *10* | 25.93 | Mv-3-glc-epicatechin | 780.9 |
| *11* | 40.35 | (Epi)catechin-Mv-3-(6-Ac)-glc | 823.0 |
| *12* | 50.87 | (Epi)Catechin-Dp-3-(6-p-coum)-glc | 899.1 |
| *13* | 54.15 | (Epi)Catechin-Cy-3-(6-p-coum)-glc | 883.1 |
| *14* | 61.35 | (Epi)Catechin-Pt-3-(6-p-coum)-glc | 913.0 |
| *15* | 64.7 | (Epi)Catechin-Pn-3-(6-p-coum)-glc | 897.0 |
| **16** | 67.25 | Catechin-Mv-3-(6-p-coum)-glc cis | 926.9 |
| *16* | 69.82 | Catechin-Mv-3-(6-p-coum)-glc trans | 926.9 |
| **17** | 71.53 | Epicatechin-Mv-3-(6-p-coum)-glc | 926.9 |
| *17* | 3.22 | (Epi)gallocatechin-Dp-3-glc | 769.1 |
| *18* | 3.50 | (Epi)gallocatechin-Cy-3-glc | 753.1 |
| *19* | 3.77 | (Epi)gallocatechin-Pt-3-glc | 783.0 |
| *20* | 4.85 | (Epi)gallocatechin-Pn-3-glc | 767.2 |
| **18** | 4.85 | (Epi)gallocatechin-Mv-3-glc | 796.9 |
| **19** | 46.07 | (Epi)gallocatechin-Mv-3-(6-p-coum)-glc | 943.1 |
| *21* | 77.25 | (Epi)catechin-(epi)catechin-Mv-3-(6-p-coum)-glc | 1215.0 |
| *22* | 11.43 | (Epi)catechin-(epi)gallocatechin-Pn-3-glc | 1055.1 |
| **20** | 17.9 | (Epi)catechin-(epi)catechin-Mv-3-glc* | 1069.0 |
| **21** | 11.43 | (Epi)catechin-(epi)gallocatechin-Mv-3-glc* | 1085.0 |

*: For these compounds the optimum transitions, cone voltage and collision energies were optimized in this work.

On the one hand, the mass spectra of compounds *1-20* and makers **14**-**19** show the molecular ion $[M^+]$ and some intense fragments. The fragmentation pattern for

these derivatives is characterised by the loss of glucose (-162 u in the case of glucosides and -308 u in the case of p-coumaroyl-glucosides) from the molecular ion $[M]^+$, giving rise to the aglycone ion $[M\text{-glc}]^+$. In most cases an intense fragment at -152 u for (epi)catechin or at -168 u for (epi)gallocatechin is observed, corresponding to a retro-Diels-Alder fragmentation that implies a 1/3 breaking of flavanol C ring. The rupture between C2 and C4 of the flavanol C-ring, which means the loss of 246 u for (epi)catechin and 262 u for (epi)galocatechin, and the loss of phloroglucinol (flavanol A-ring) generated by the rupture between C1 and C4 of flavanol C-ring, which means the loss of 126 u, involves that flavanol is the upper unit. Some authors said that this loss does not occur in the ring A of the anthocyanin so this fragmentation is essential to elucidate the position of flavanol in the pigment structure[276,277]. Moreover, loss of entire flavanol unit is observed at -288 u for (epi)catechin or -304 u for (epi)gallocatechin, usually together with sugar loss. The mass spectrum can be seen in Figure IV.2 using Catechin-Mv-3-glc as an example.

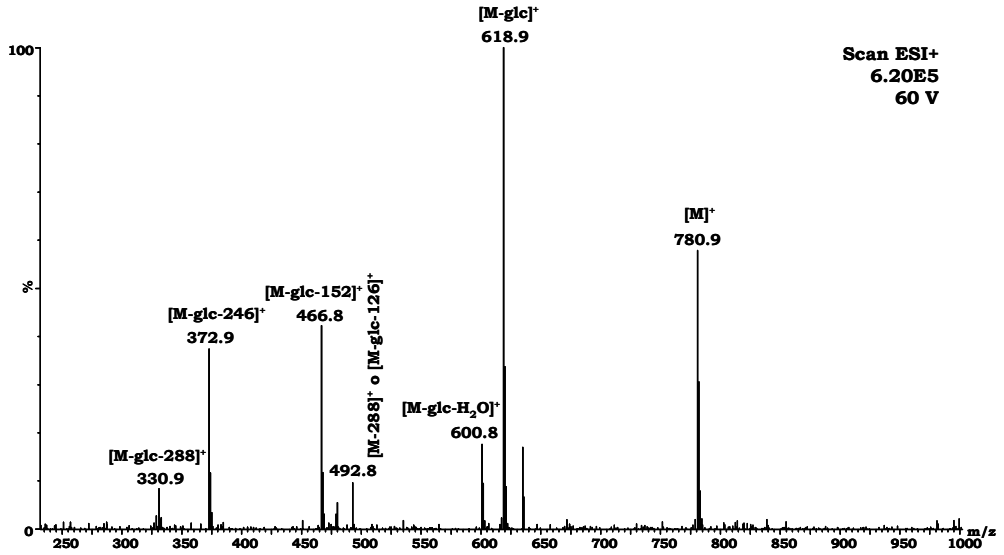



**Figure IV.2.** ESI(+)-MS spectrum of catechin-Mv-3-glc (**14**), obtained at a cone voltage of 60V.

On the other hand, the other four compounds (*21*, *22*, **20**, **21**) have two flavanol units in their structure, two (epi)catechin units in compounds *21* and **20** and an (epi)catechin and an (epi)gallocatechin unit in compounds *22* and **21**. The two more

[276] Salas, E.; Fulcrand, H.; Meudec, E.; Cheynier, V.; *Reactions of anthocyanins and tannins in model solutions,* J. Agric. Food Chem. **2003**, 51, 7951-7961.

[277] Friedrich, W.; Eberhardt, A.; Galensa, R.; *Investigation of proanthocyanidins by HPLC with electrospray ionization mass spectrometry,* Eur. Food Res. Technol. **2000**, 211, 56-64.

concentrated (**20** and **21**) were selected to be included in the list of anthocyanic derivative markers to be quantified by HPLC-DAD-ESI(+)-MS/MS.

The mass spectra of these compounds show the molecular ion $[M]^+$ at m/z 1069.0 and 1085.0 and the aglycone ion at m/z 906.9 and 922.9. The difference between the value of molecular ion (16 u) indicates that compound *22* and **21** has a (epi)gallocatechin as a second flavanol unit.

The fragmentation pattern for these derivatives is characterised by the loss of a flavanol unit (-288 u) from the molecular ion $[M]^+$, observed at m/z 780.9 for compound *21* and **20** and at 797.0 for compound *22* and **21**, followed by the loss of glucose at m/z 618.8 and 634.9, respectively. In the mass spectrum of compound **20** can also be seen a fragmentation ion at m/z 373.0, which involves a loss of 246 u from the ion at m/z 618.8, so this fragment indicates that at least one unit of epicatechin is the upper unit. The mass spectrum is shown in Figure IV.3 using (epi)catechin-(epi)catechin-Mv-3-glc (**20**) as an example.

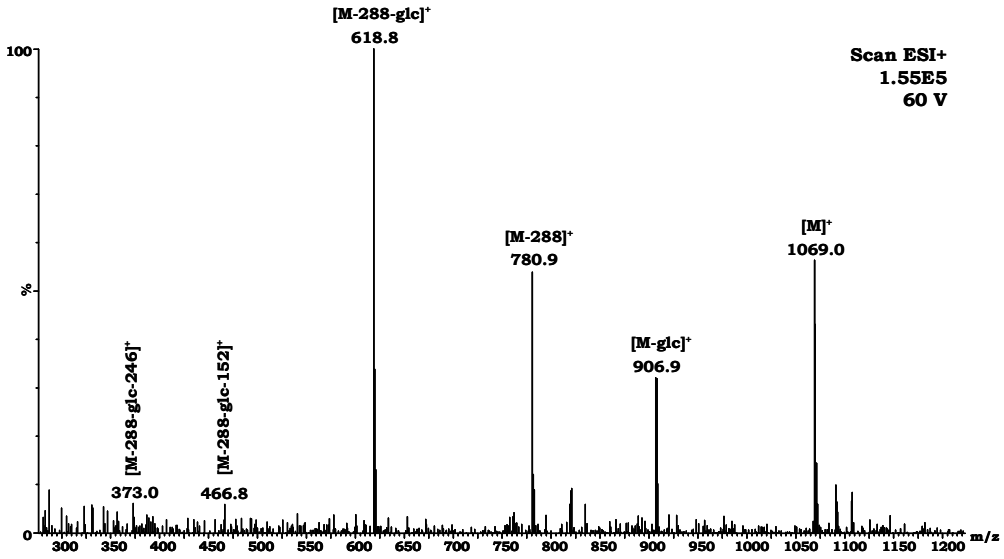



**Figure IV.3.** ESI(+)-MS spectrum of (epi)catechin-(epi)catechin-Mv-3-glc (**20**), obtained in scan mode at 60V.

In addition the optimum transitions, cone voltages and collision energies for compounds **20** and **21** were optimized during this work. So the highest intensity fragment ion, which corresponds to the $[M\text{-}288\text{-}glc]^+$ ion at m/z 618.8 for the compound **20** and 634.9 for the compound **21**, were chosen as an optimum transition for MRM experiment. Results are shown in Figure IV.4 and optimum transition, cone voltage and collision energy for these derivatives are reported in Table IV.2.

**Table IV.2.** Optimum transitions, cone voltages and collision energies for new direct condensation products (flavylium form).

| Compound | | Transition | CV (V) | CE (eV) |
|---|---|---|---|---|
| **20** | (Epi)Cat-(Epi)Cat-Mv-3-glc | 1069.0→618.8 | 50 | 30 |
| **21** | (Epi)Cat-(Epi)Galocat-Mv-3-glc | 1085.0→634.9 | 50 | 30 |

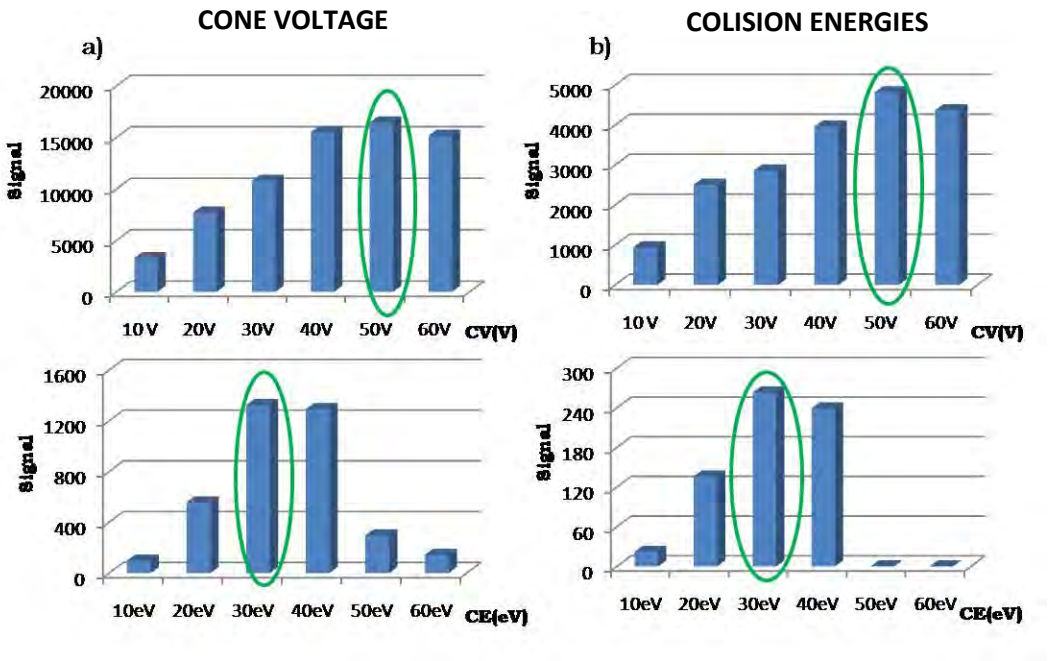



**Figure IV.4.** Variations of the chromatogram area for the new compounds, which were identified as a) (epi)cat-(epi)cat-Mv-3-glc and; b) (epi)cat-(epi)gallocat-Mv-3-glc, depending on cone voltages and collision energy values.

### b) Direct condensation with flavanols. A bond type and flavene and flavan forms.

Forty anthocyanin derivatives, which have flavene form or A bond type, were identified as uncoloured direct condensation derivatives (Tables IV.3 and IV.4).

**Table IV.3.** Retention time and molecular ion achieved by HPLC-DAD-ESI(+)-CID-MS/MS of direct condensation products (flavene form and with A bond type). The numbers written in bold correspond to the compounds chosen as markers

| | Number | $t_R$ (min) | Compound | $[M+H]^+$ (m/z) |
|---|---|---|---|---|
| *Direct condensation Anthocyanin-Flavanol. Flavene form (type 1)* | *23* | 11.73 | Dp-3-glc-catechin | 754.9 |
| | *24* | 12.88 | Dp-3-glc-epicatechin | 754.9 |
| | *25* | 20.13 | Pt-3-glc-catechin | 769.1 |
| | *26* | 21.50 | Pt-3-glc-epicatechin | 769.1 |
| | *27* | 30.86 | Pn-3-glc-catechin | 752.9 |
| | *28* | 32.68 | Pn-3-glc-epicatechin | 752.9 |
| | **22** | 34.23 | Mv-3-glc-catechin* | 783.2 |
| | **23** | 36.20 | Mv-3-glc-epicatechin* | 783.2 |
| *Direct condensation Flavanol-Anthocyanin. Flavene form (type 2)* | *29* | 16.10 | Catechin-Pt-3-glc | 769.1 |
| | *30* | 16.85 | Epicatechin-Pt-3-glc | 769.1 |
| | *31* | 26.03 | (Epi)catechin-Pn-3-glc | 752.9 |
| | *32* | 25.27 | (Epi)catechin-Mv-3-glc | 783.1 |
| | **24** | 27.55 | Catechin-Mv-3-glc* | 783.2 |
| | **25** | 28.50 | Epicatechin-Mv-3-glc* | 783.2 |
| *Direct condensation Anthocyanin-Flavanol. A-type biciclyc bond (type 3)* | *33* | 29.07 | Dp-3-glc-A-(epi)catechin | 755.1 |
| | *34* | 37.20 | Pt-3-glc-A-cetechin | 769.2 |
| | *35* | 42.55 | Pt-3-glc-A-epicatechin | 769.2 |
| | *36* | 55.42 | Pn-3-glc-A-(epi)catechin | 753.1 |
| | *37* | 23.77 | Mv-3-glc-A-(epi)gallocatechin | 798.9 |
| | *38* | 39.85 | Mv-3-glc-A-(epi)gallocatechin | 798.9 |
| | **26** | 57.38 | Mv-3-glc-A-catechin* | 783.1 |
| | **27** | 60.90 | Mv-3-glc-A-epicatechin* | 783.1 |

*: For these compounds the optimum transitions, cone voltage and collision energies were optimized in this work.

In before compounds, where the anthocyanin is as flavylium form, the molecular ion $[M]^+$ could be detected. In contrast, all these compounds are not charged and they are ionized forming the protonated molecule $[M+H]^+$ (also known as quasi molecular ion), which could be observed at a m/z ratio one unit greater than molecular ion for the equivalent flavilium form. They also present adducts with $Na^+$ and $K^+$ in their mass spectra, that could be seen at 22 and 38 units above than $[M+H]^+$.

The fragmentation pattern for these compounds is characterized by the loss of glucose (-162 u) from the protonated molecule $[M+H]^+$, giving rise to the aglycone ion $[M+H\text{-glc}]^+$. Retro Diels-Alder (RDA) fragmentations of flavanol C-ring from the protonated molecule and the aglycone ion are also observed, involving a loss of 152 u if the flavanol is (epi)catechin and 168 u if it is (epi)gallocatechin.

In derivatives where the anthocyanin is as flavylium form, the fragment ion generated by the rupture 1/4 of the flavanol C-ring (126 u) indicated that flavanol is the upper unit. For these compounds, Remy-Tanneau et al (2003) explain the loss of

126 u as the loss of the anthocyanin A-ring. This loss may also occur from the retro Diels-Alder reaction product[278].

Some differences were observed in relative ion intensities in the mass spectra of the compounds in Table IV.3, so a classification of these derivatives into three different groups was done.

Type 1 and 2 compounds, with shorter and closed retention times, could be considered as flavene derivatives. At a previous research which has been done in our group showed that F–A coloured pigments elute before than the corresponding coloured A–F derivatives[279]. Therefore type 1 compounds, that have longer retention times, could be assigned as A–F flavene structure while type 2 would be identified as F–A flavene structure. Taking this assignation into account, type 3 derivatives, those showing longer retention times, could be interpreted as A-type bicyclic derivatives.

Mass spectra of type 1 derivatives show high intensity fragment ions corresponding to the RDA fragmentation of the protonated molecule, of the aglycone ion and the product resulting from the cleavage of anthocyanin C-ring after the RDA fragmentation. An example of type 1 derivatives fragmentation can be observed in Figure IV.5, which shows the ESI(+)-MS spectra of the marker **22**, Mv-3-glc-catechin.

In conclusion, type 1 derivatives suffer more easily retro Diels Alder fragmentations.

In the mass spectrum of compounds *29-32* and **24**-**25**, the most intense ions observed are those corresponding to the loss of glucose from the protonated molecule, the retro Diels-Alder fragmentation from the aglycone and the anthocyanidin ion, after losing the glucose and flavanol unit. Then, type 2 compounds presents more willing to the loss of glucose. An example of the fragmentation for these compounds can be seen in the ESI(+)-MS spectrum of marker **24**, catechin-Mv-3-glc (Figure IV.6).

[278] Remy-Tanneau, S.; Le Guernevé, C.; Meudec, E.; Cheynier, V.; *Characterization of a colorless anthocyanin-flavan-3-ol dimer containing both carbon-carbon and ether interflavanoid linkages by NMR and mass spectrometry*, J. Agric. Food Chem. **2003**, 51, 3592-3597.

[279] Sánchez-Ilárduya, M. B.; Sánchez-Fernández, C.; Garmón-Lobato, S.; Abad-García, B.; Berrueta, L.A.; Gallo, B.; Vicente, F.; *Detection of non-coloured anthocyanin-flavanol derivatives in Rioja aged red wines by liquid chromatography-mass spectrometry*, Talanta **2014**, 121, 81-88.

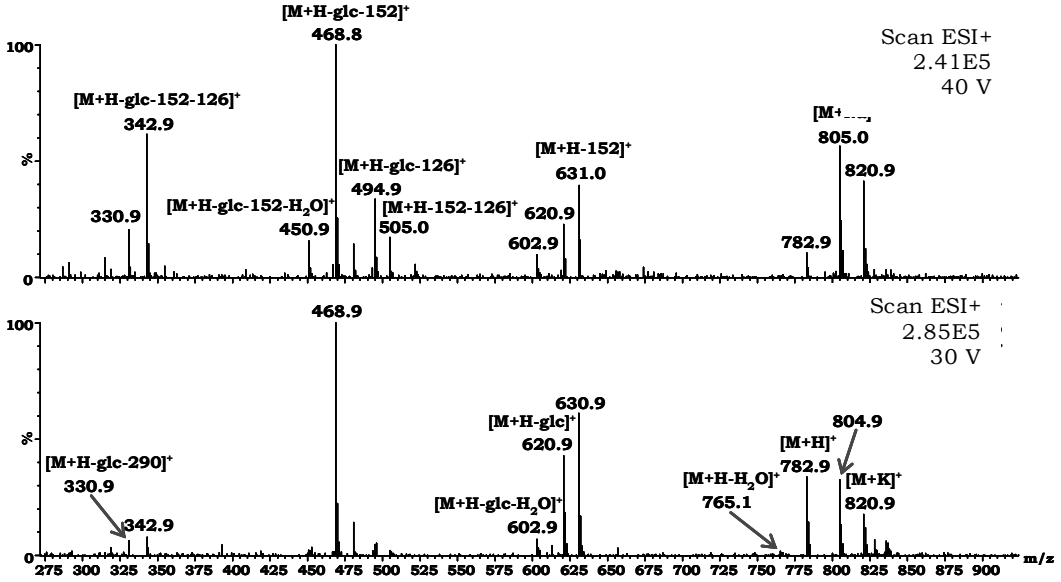



**Figure IV.5.** ESI(+)-MS spectra of A-F type 1 compounds, obtained at cone voltages of 30V and 40V. Example: Mv-3-glc-catechin as flavene form (**22**).

Finally, the retro Diels-Alder fragmentations and the loss of glucose from the protonated molecule occur with similar intensities in the mass spectrum of type 3 derivatives (*33-38* and **26**-**27**). The mass spectrum of marker **26** can be seen in Figure IV.7, as an example of these compounds.

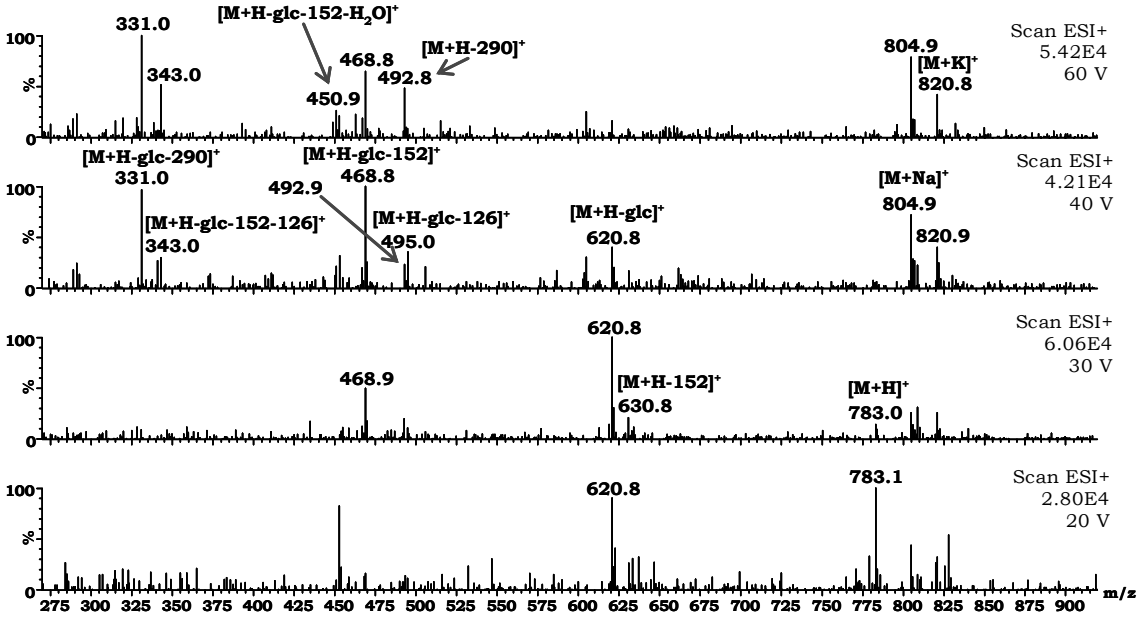



**Figure IV.6.** ESI(+)-MS spectra of A-F type 2 compounds, obtained in scan mode at 20V, 30V, 40V and 50V. Example: catechin-Mv-3-glc as flavene form (**24**).

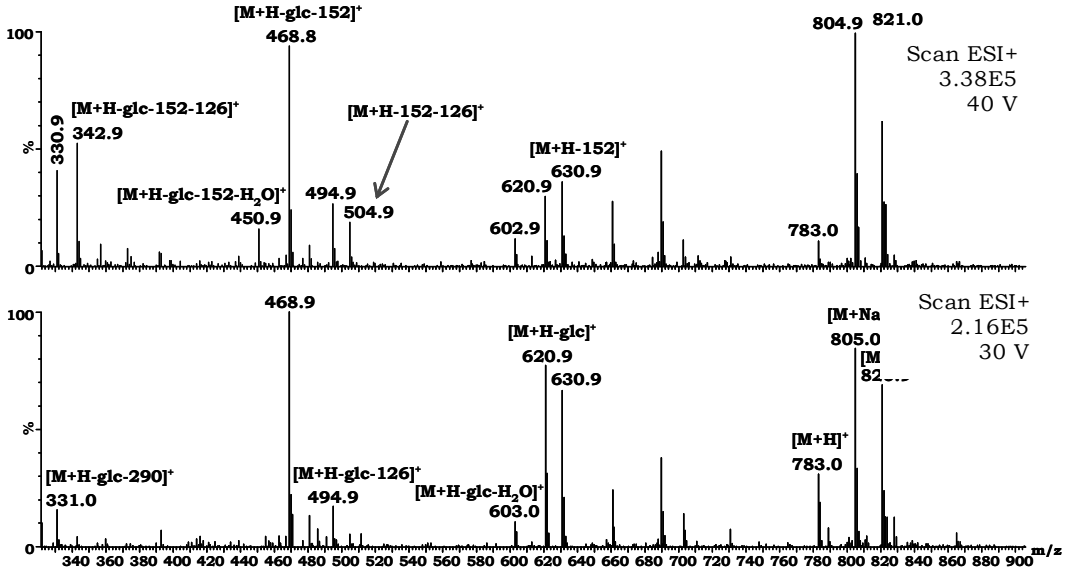



**Figure IV.7.** ESI(+)-MS spectra of F-A type 3 compounds, obtained in scan mode at 30V and 40V. Example: Mv-3-glc-A-catechin (**26**) with A-type biciclyc bond.

A set of experiments were done for optimizing cone voltage (10, 20, 30, 40, 50 and 60 V) and collision energy (10, 20, 30, 40, 50 and 50 eV) for compounds **22**-**27**. Results are shown in Figure IV.8, which shows a graphic example for compound **22**, and optimum transition, cone voltage and collision energy for these derivatives are reported in Table IV.5.

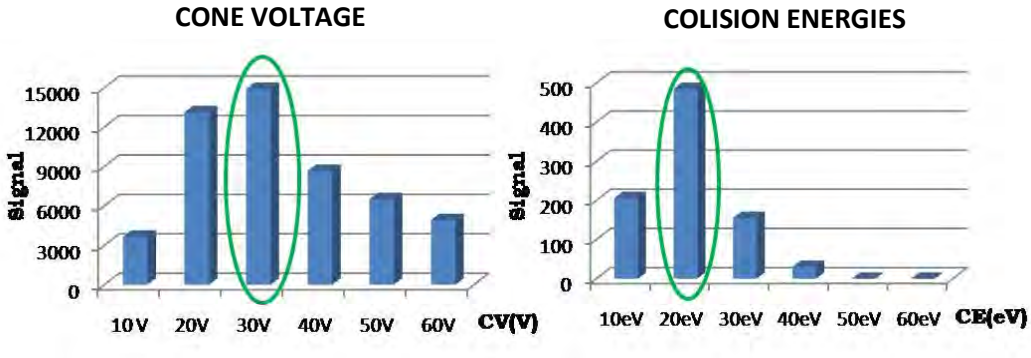



**Figure IV.8.** Variations of the chromatogram area for compounds 37, 38, 43, 44, 51 and 52 depending on cone voltages and collision energy values. Example: Mv-3-glc-catechin (37).

It have also been identified eighteen new colorless compounds formed with an anthocyanin and two flavanols units, in which can be two units of (epi)catechin or a unit of (epi)catechin and other of (epi)gallocatechin (Table IV.4) and they also have an A bond.

**Table IV.4.** Retention time and molecular ion achieved by HPLC-DAD-ESI(+)-CID-MS/MS of direct condensation products with two flavanols. The numbers written in bold correspond to the compounds chosen as markers.

| | Number | $t_R$ (min) | Compound | $[M+H]^+$ (m/z) |
|---|---|---|---|---|
| *Flavanol-Anthocyanin-A-Flavanol* | *39* | 5.97 | (Epi)catechin-Dp-3-glc-A-(epi)catechin | 1043.1 |
| | *40* | 7.13 | (Epi)catechin-Pt-3-glc-A-(epi)catechin | 1057.2 |
| | *41* | 12.47 | (Epi)catechin-Pt-3-glc-A-(epi)catechin | 1057.2 |
| | *42* | 7.77 | (Epi)catechin-Pn-3-glc-A-(epi)catechin | 1040.9 |
| | *43* | 13.40 | (Epi)catechin-Pn-3-glc-A-(epi)catechin | 1040.9 |
| | **28** | 7.95 | (Epi)catechin-Mv-3-glc-A-(epi)catechin 1* | 1071.3 |
| | **29** | 13.42 | (Epi)catechin-Mv-3-glc-A-(epi)catechin 2* | 1071.3 |
| | **30** | 6.50 | (Epi)catechin-Mv-3-glc-A-(epi)gallocatechin* | 1087.3 |
| | **31** | 7.80 | (Epi)gallocatechin-Mv-3-glc-A-(epi)catechin* | 1087.3 |
| *Anthocyanin-Flavanol-A-Flavanol* | *44* | 14.65 | Pt-3-glc-(epi)catechin-A-(epi)catechin 1 | 1057.2 |
| | *45* | 23.10 | Pt-3-glc-(epi)catechin-A-(epi)catechin 2 | 1057.2 |
| | *46* | 23.77 | Pn-3-glc-(epi)catechin-A-(epi)catechin 1 | 1040.9 |
| | *47* | 31.50 | Pn-3-glc-(epi)catechin-A-(epi)catechin 2 | 1040.9 |
| | *48* | 73.08 | Mv-3-(6-p-coum)-glc-(epi)catechin-A-(epi)catechin 1 | 1217.3 |
| | *49* | 85.02 | Mv-3-(6-p-coum)-glc-(epi)catechin-A-(epi)catechin 2 | 1217.3 |
| | *50* | 19.03 | Mv-3-glc-(epi)gallocatechin-A-(epi)catechin | 1087.3 |
| | **32** | 15.22 | Mv-3-glc-(epi)catechin-A-(epi)catechin* | 1071.3 |
| *Flavanol-A-Flavanol-Anthocyanin* | **33** | 14.30 | (Epi)catechin-A-(epi)catechin-Mv-3-glc* | 1071.3 |

*: For these compounds the optimum transitions, cone voltage and collision energies were optimized in this work.

In these trimers it can be distinguished three different types of compounds. The first type of trimers (*39*-*43* and **28**-**31**) shows a similar fragmentation pattern to the dimmers previously described, once they lost a unit of (epi)catechin, so the resulting fragment has an anthocyanin-A-flavanol structure when the upper or lower unit is lost. This is why a flavanol-anthocyanin-A-flavanol structure is proposed for these compounds.

On one hand, the fragmentation pattern for these compounds is characterized by the loss of a unit of (epi)catechin (-288 u) from the protonated molecule $[M+H]^+$. The Retro Diels-Alder (RDA) fragmentations of flavanol C-ring from the quasi-molecular ion, which involves a loss of -152 u, the dimer generated by the loss of a unit of (epi)epicatechin and its corresponding aglycone are also observed at m/z 919, 631 and 469, respectively.

As in the description of dimmers, it is also observed fragment ions at m/z 495, due to the loss of a unit of flavanol, glucose and the phloroglucinol ring of the anthocyanin, and its corresponding fragmentation retro Diels-Alder (m/z 343).

Compounds *39-43* could be the same derivative as compounds **28** and **29**, but with the aglycones delphinidin, petunidin and peonidin.

On the other hand, compounds **30** and **31** are formed by a unit of (epi)catechin and other unit of (epi)gallocatechin, so their quasi-molecular ion $[M+H]^+$ is detected at m/z 1087.3.

The mass spectrum of compound **30** is characterized by the loss of glucose and a (epi)catechin unit from the protonated molecule $[M+H]^+$, giving rise the compounds observed at m/z 925 (-162 u) and 799 (-288 u), respectively. The Retro Diels-Alder (RDA) fragmentations of flavanol C-ring from the dimer generated by the loss of a flavanol unit (m/z 799), involving a loss of 168 u, and its corresponding aglycone (m/z 637) are also observed at m/z 631 and 469, respectively. In addition, the fragment ions at m/z 799, 637 and 511, could indicate that (epi)gallocatechin unit is bound to the anthocyanin, so the proposal structure for compound **30** is (epi)catechin-Mv-3-glc-A-(epi)gallocatechin.

In contrast, the mass spectrum for compound **31** shows a fragment ion at m/z 783 from the protonated molecule, involving a loss of 304 u, which means the loss of the (epi)gallocatechin unit, giving rise to a compound with the same structure as compound **26** or **27**. Moreover the fragments ions at m/z 783, 621 and 495 could indicate that the (epi)catechin is bound to the anthocyanin, so the proposal structure for compound **31** is (epi)gallocatechin-Mv-3-glc-A-(epi)catechin.

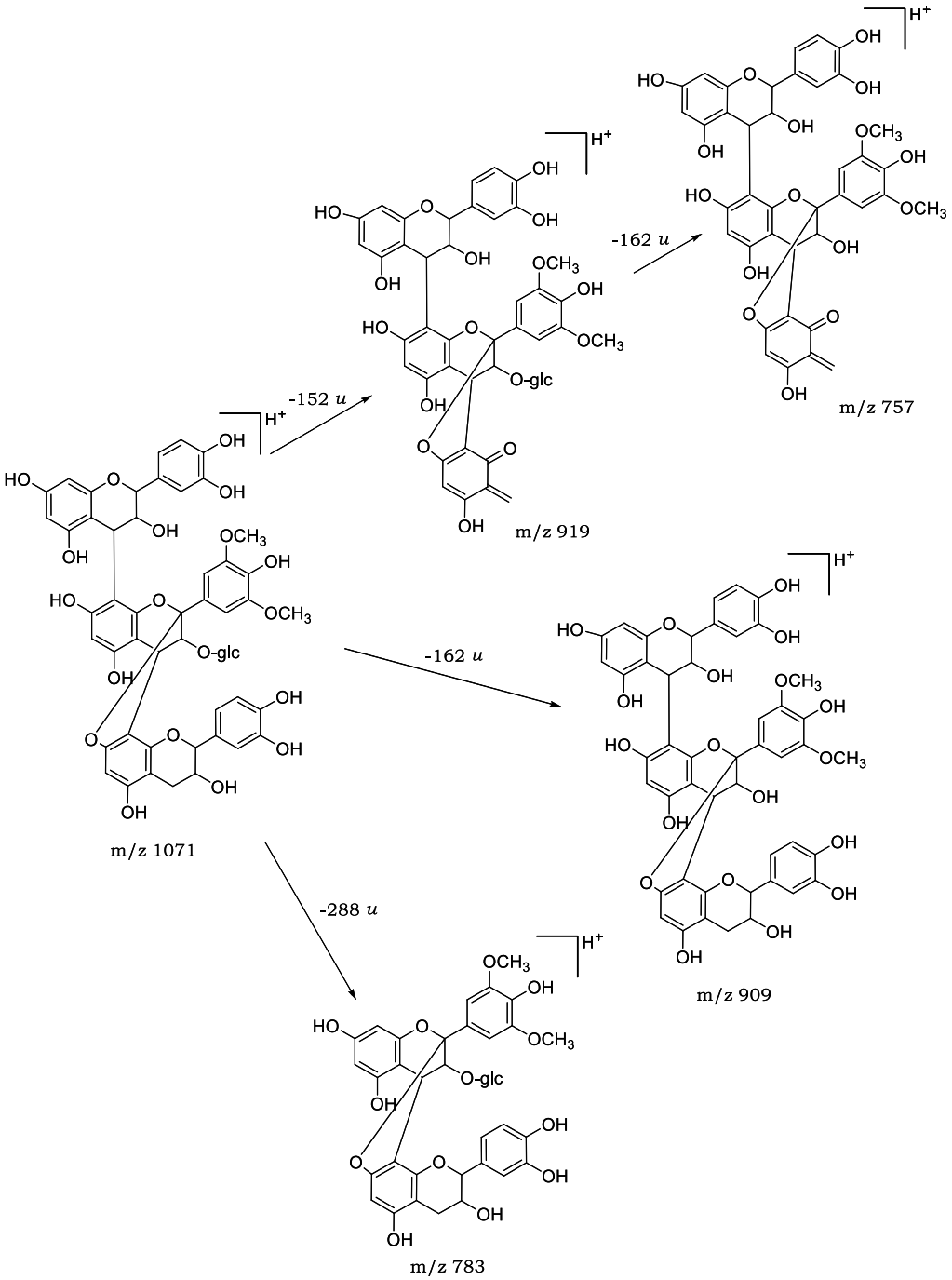



**Figure IV.9.** Fragmentation pattern of compounds formed by direct condensation flavanol-anthocyanin-A-flavanol. Example: (epi)catechin-Mv-3-glc-A-(epi)catechin (**28**).

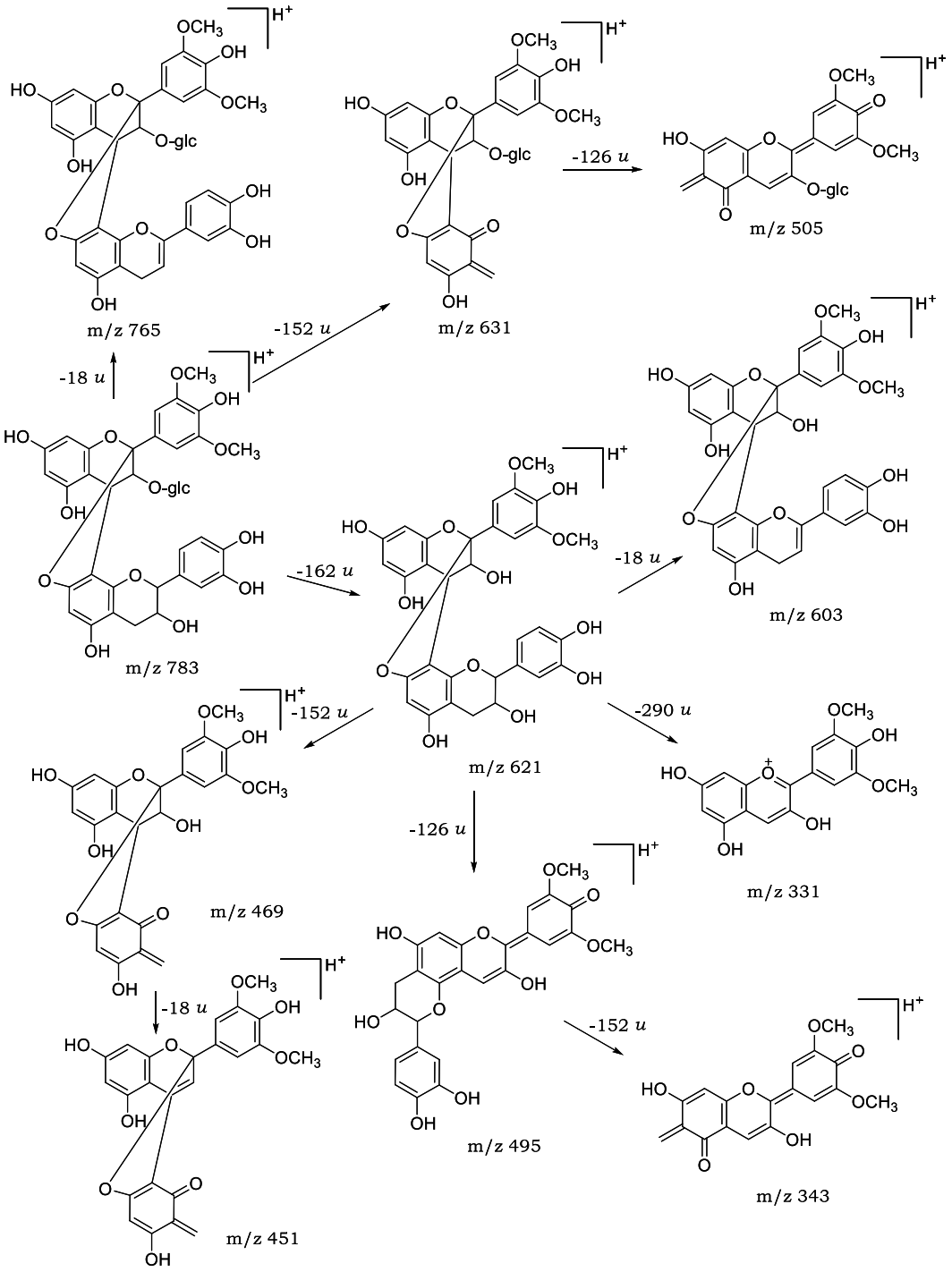



**Figure IV.9.** Cont.

In conclusion, trimers may have an A biciclyc bond, so they could only lose one flavanol unit. However, we can not rule out another structure in which the anthocyanin is as flavene form and it is necessary complementary techniques to support the proposal structure.

The fragmentation patter of compounds *39-43* and **28**-**31** is in Figure IV.9 and the mass spectra for these compounds can be seen from Figure IV.10 to IV.11, using the compound **28** and **30** as an example.

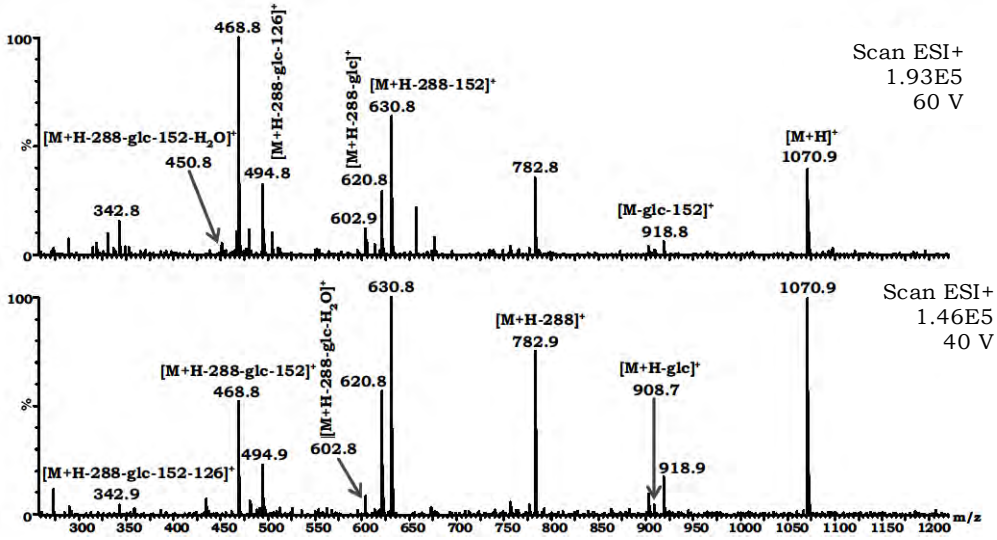



**Figure IV.10.** ESI(+)-MS spectra of (epi)catechin-Mv-3-glc-A-(epi)catechin (**28**), obtained at a cone voltage of 40V and 60V.

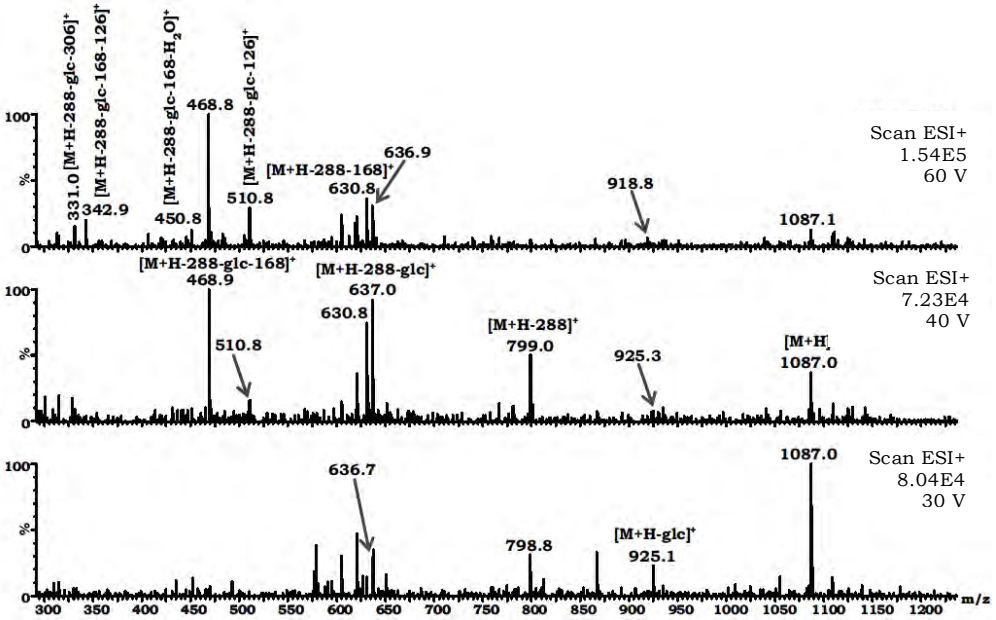



**Figure IV.11.** ESI(+)-MS spectra of (epi)gallocatechin-Mv-3-glc-A-(epi)catechin (**30**), obtained at a cone voltage of 30V, 40V and 60V.

The last colorless trimers (compounds *44-50* and **32**-**33**) seem to recover the flavylium form when a flavanol unit is lost, that could come from the rupture of A bond. For this type of compound a structure in which anthocyanin is in the flavan form and flavanols units are linked by an A bond is proposed.

The most intense ions observed are those corresponding to the loss of glucose from the protonated molecule and the loss of a flavanol unit and its loss of glucose. The retro Diels-Alder fragmentation from the aglycone and the fragmentation ion which is generated by the loss of the other flavanol unit, are also observed in the mass spectrum. Other observed ions are those which corresponding to the loss of water from the dimer in flavilium form, the Retro Diels-Alder fragmentation from the aglycone form and the ion generate by the loss of second flavanol unit.

In addition, fragment ions at m/z 739, 577 and 457, which correspond to the loss of 42 u from dimer at m/z 781 and the loss of 42 u (rupture between C2 and C4 of flavanol) and 162 u (rupture between C1 and C4 of flavanol) from fragment ion at m/z 619, are observed in the mass spectrum of compounds *44-50* and **32**. These losses indicate that flavanol is the bottom unit when the first flavanol is lost, so the structure for these compounds could be anthocyanin-flavanol-A-flavanol. Then the structure for compound **33** might be flavanol-A-flavanol-anthocyanin because fragment ions at 739, 577 and 457 are not observed in its spectrum.

The quasi-molecular ion of compounds *48-49* is observed at m/z 1217, which corresponds to p-coumaroylated derivatives. The structure for these compounds could be Mv-3-(6-p-coum)-glc-(epicatechin)-A-(epi)catechin because the fragment ion at m/z 577 and 457 are also observed in their mass sprectum.

Compound *50* could be Mv-3-glc-(epi)gallocatechin-A-(epi)catechin because the quasimolecular ion is observed at m/z 1087, which indicates that the compound contains an (epi)gallocatechin unit. In addition, the fragment ion at m/z 797, 635, 617 and 593 indicate that the (epi)gallocatechin unit is bonded to the anthocyanin when a flavanol unit is lost.

The fragmentation patters of compounds *44-50* and **32** and of compound **33** are in Figure IV.12 and IV.13. The mass spectra for both types of compounds can be seen in Figure IV.14 and IV.15, using compounds **32** and **33** as example.

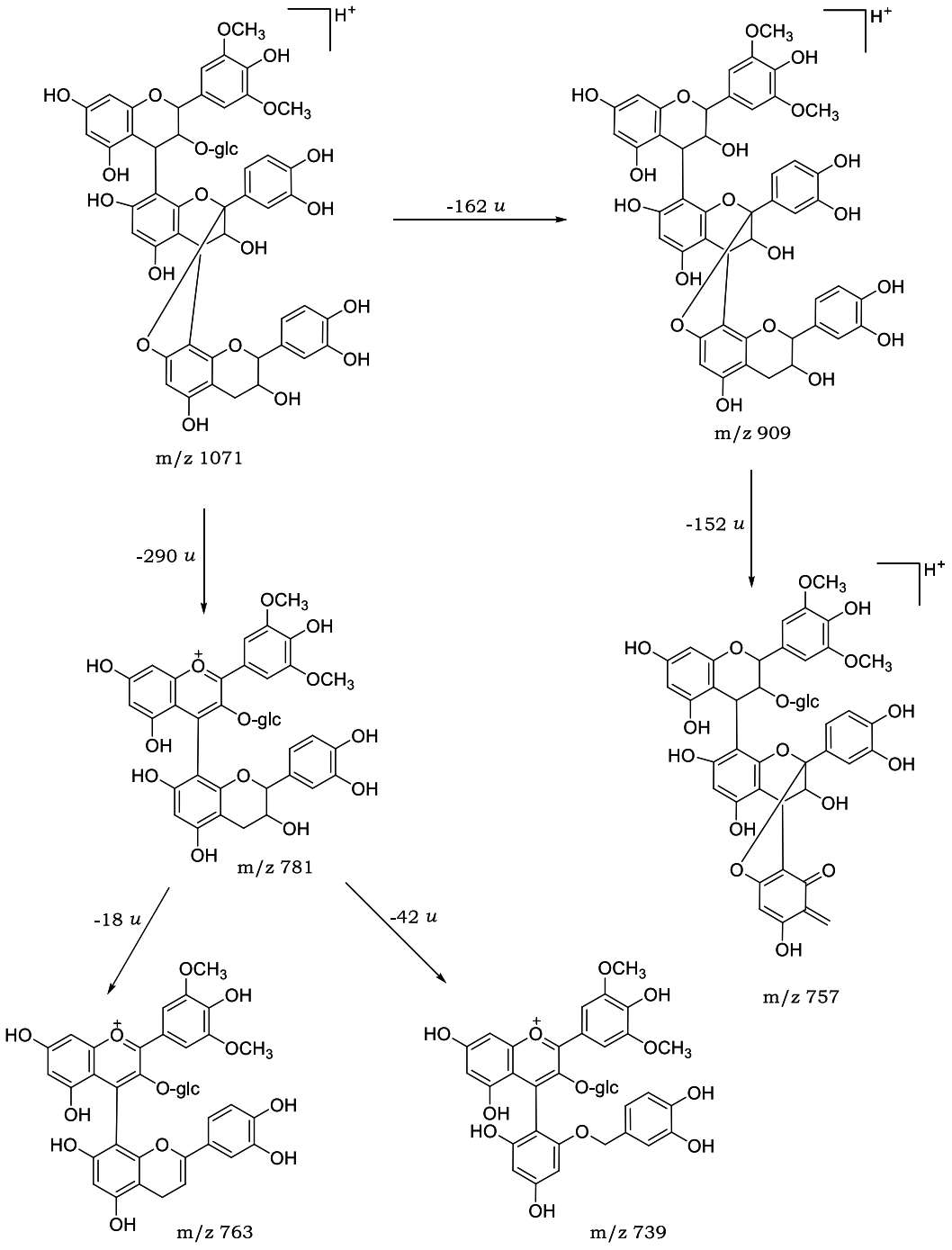



**Figure IV.12.** Fragmentation pattern of compounds formed by direct condensation anthocyanin-flavanol-A-flavanol. Example: Mv-3-glc-(epi)catechin-A-(epi)catechin (**32**).

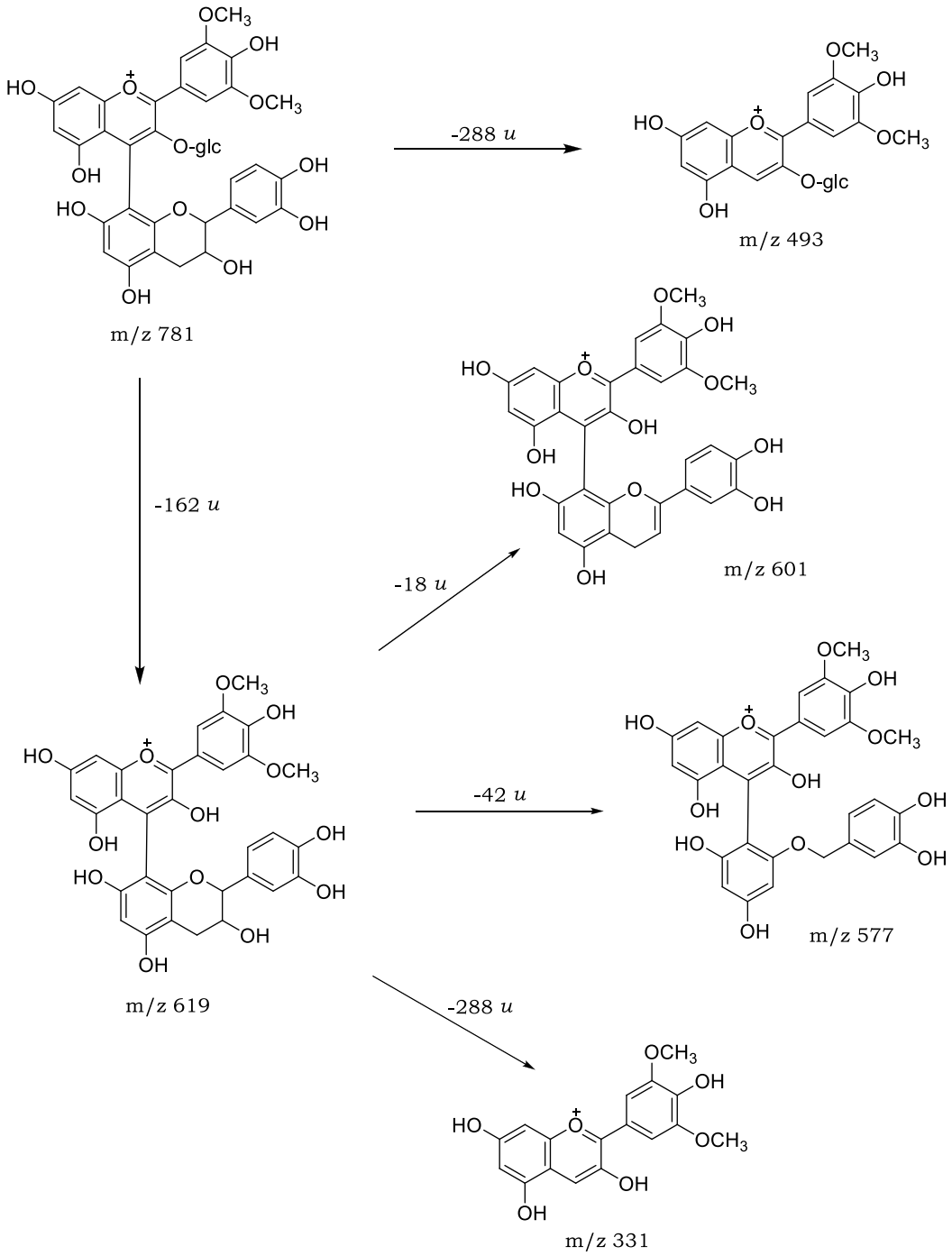



**Figure IV.12.** Cont.

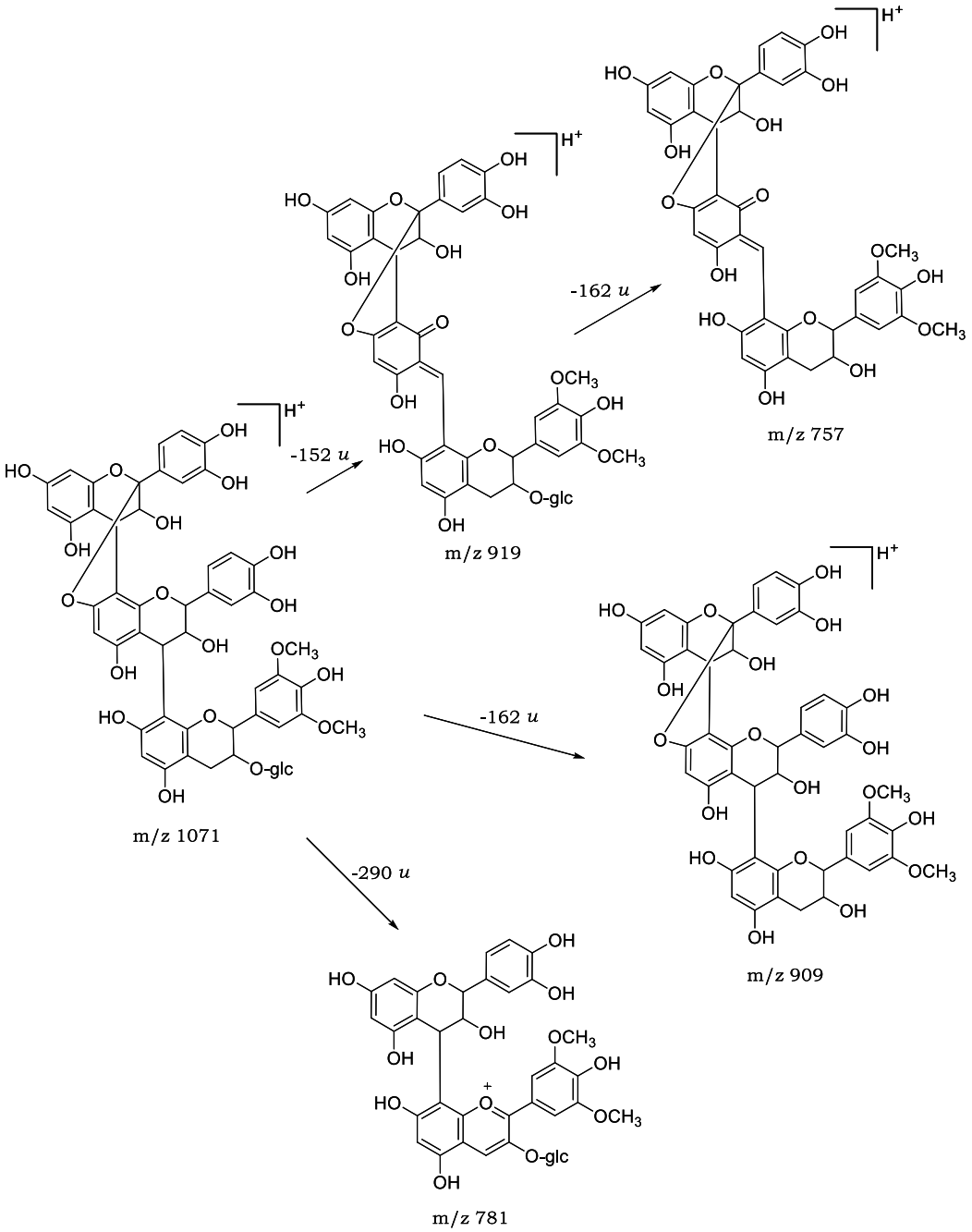



**Figure IV.13.** Fragmentation pattern of compounds formed by direct condensation flavanol-A-flavanol-anthocyanin. Example: (epi)catechin-A-(epi)catechin-Mv-3-glc (**33**).

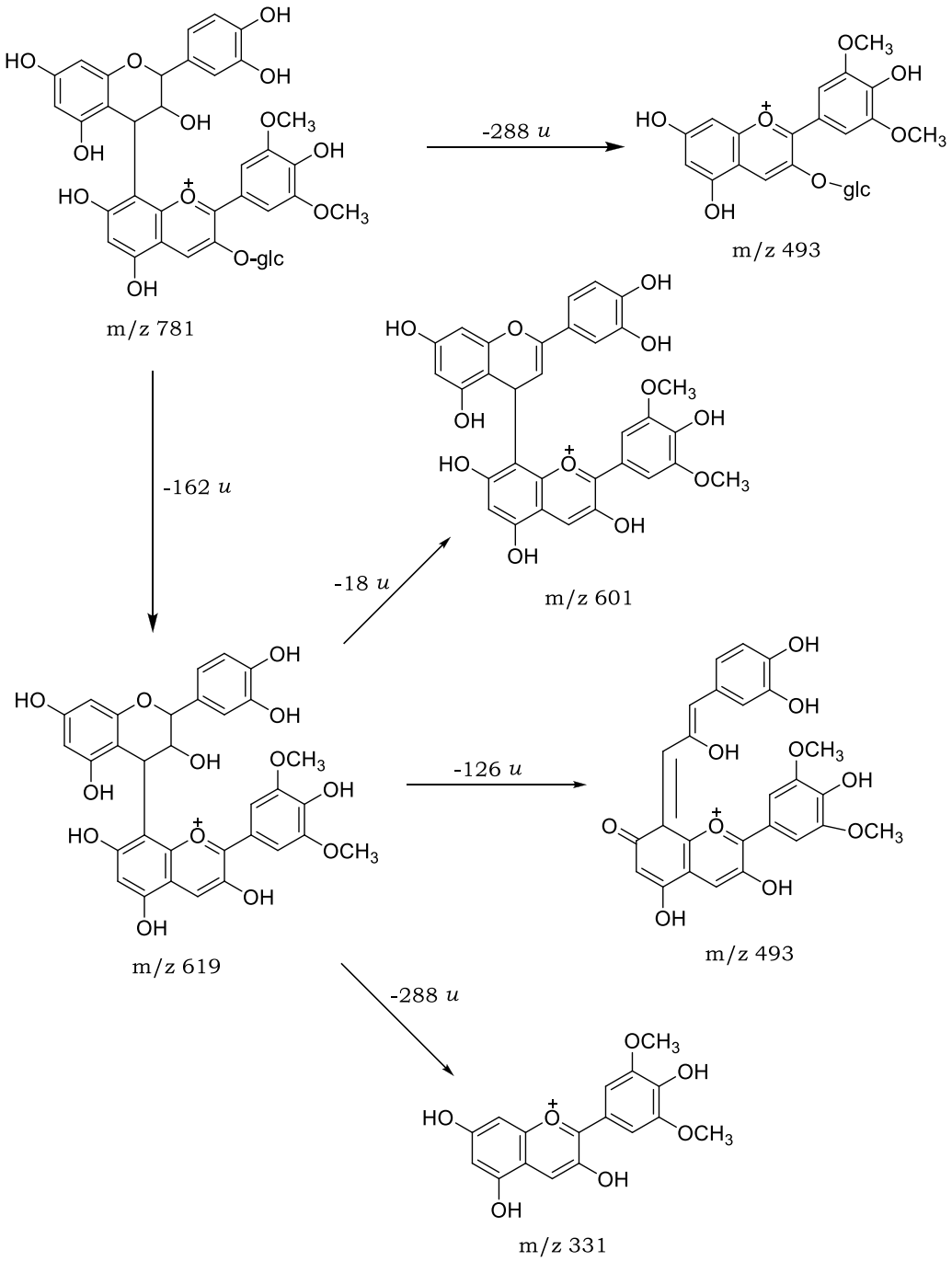



**Figure IV.13.** Cont.

A set of experiments were done for optimizing cone voltage (10, 20, 30, 40, 50 and 60 V) and collision energy (10, 20, 30, 40, 50 and 50 eV) for the compounds from **28**-**33**. In Figure IV.16 could be seen the graphics for the optimum cone voltage and collision energy of each compound.

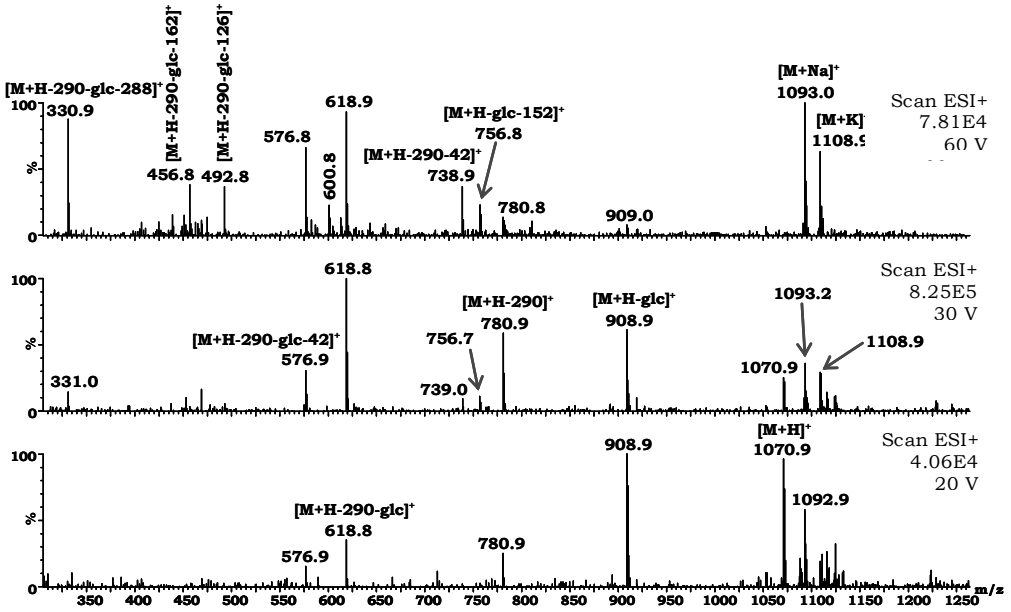



**Figure IV.14.** ESI(+)-MS spectra of Mv-3-glc-(epi)catechin-A-(epi)catechin (**32**), obtained at a cone voltage of 20V, 30V and 60V.

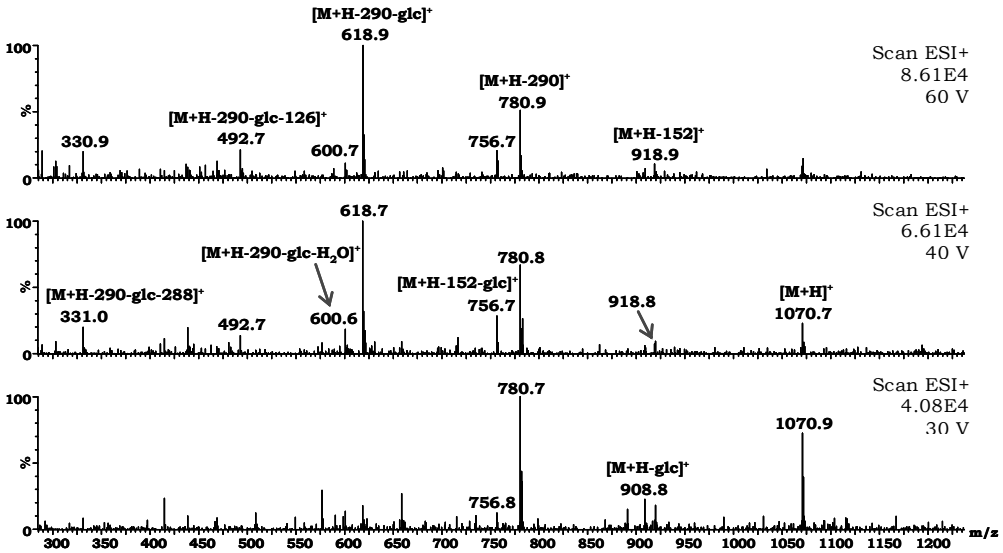



**Figure IV.15.** ESI(+)-MS spectra of (epi)catechin-A-(epi)catechin-Mv-3-glc (**33**), obtained at a cone voltage of 30V, 40V and 60V.

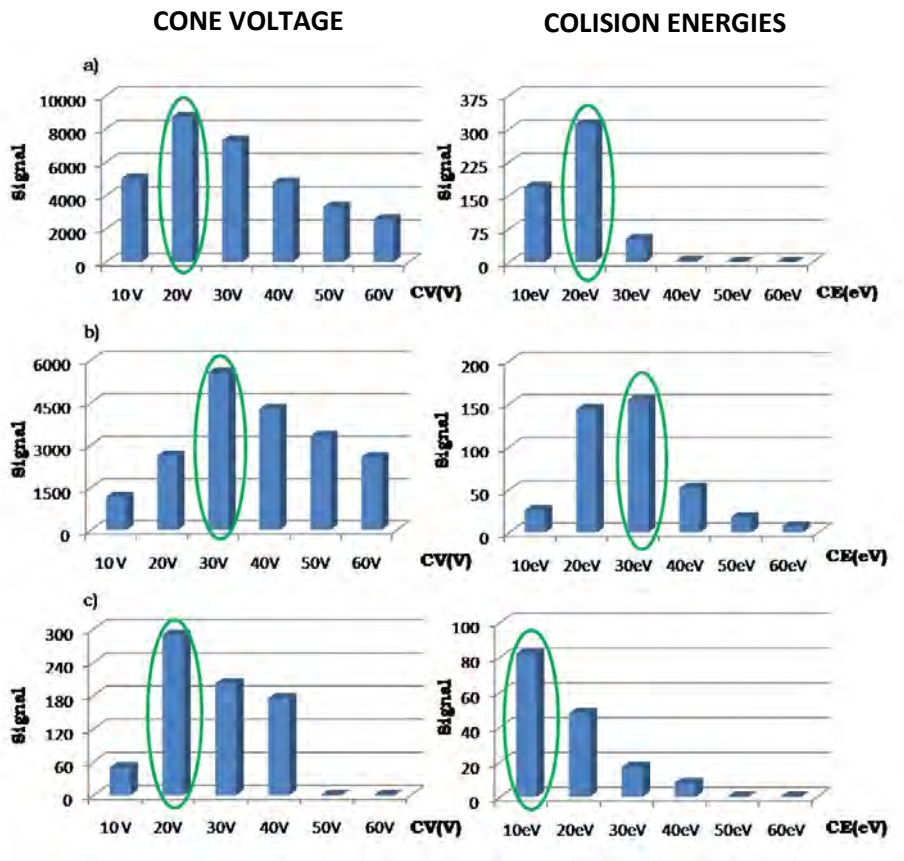



**Figure IV.16.** Variations of the chromatogram area for the new compounds, which were identified a a) (epi)cat-Mv-3-glc-A-(epi)cat; b) (epi)cat-Mv-3-glc-A-(epi)gallocat; c) (epi)cat-A-(epi)cat-Mv-3-glc, depending on cone voltages and collision energy values.

All these derivatives are reported in Table IV.5, in which optimum transitions, cone voltages and collision energies are collected.

**Table IV.5.** Optimum transitions, cone voltages and collision energies of direct condensation products.

| | | Compound | Transition | CV (V) | CE (eV) |
|---|---|---|---|---|---|
| *Direct condensation Anthocyanin-Flavanol. Flavene form (type 1)* | **22** | Mv-3-glc-Catechin | 783.2→469.1 | 30 | 20 |
| | **23** | Mv-3-glc-Epicatechin | | | |
| *Direct condensation Flavanol-Anthocyanin. Flavene form (type 2)* | **24** | Catechin-Mv-3-glc | 783.2→469.1 | 30 | 20 |
| | **25** | Epicatechin-Mv-3-glc | | | |

**Table IV.5.** Cont.

| | Compound | | Transition | CV (V) | CE (eV) |
|---|---|---|---|---|---|
| *Direct condensation Anthocyanin-Flavanol. A-type biciclyc bond (type 3)* | **26** | Mv-3-glc-A-Catechin | 783.2→469.1 | 30 | 20 |
| | **27** | Mv-3-glc-A-Epicatechin | | | |
| *Flavanol-Anthocyanin-A-Flavanol* | **28** | (Epi)Catechin-Mv-3-glc-A-(Epi)Catechin 1 | 1071.3→783.2 | 20 | 20 |
| | **29** | (Epi)Catechin-Mv-3-glc-A-(Epi)Catechin 2 | 1071.3→783.2 | 20 | 20 |
| | **30** | (Epi)Gallocatechin-Mv-3-glc-A-(Epi)Catechin 1 | 1087.3→631.2 | 30 | 30 |
| | **31** | (Epi)Gallocatechin-Mv-3-glc-A-(Epi)Catechin 2 | | | |
| *Anthocyanin-Flavanol-A-Flavanol* | **32** | Mv-3-glc-(Epi)Catechin-A-(Epi)Catechin | 1071.3→781.0 | 20 | 10 |
| *Flavanol-A-Flavanol-Anthocyanin* | **33** | (Epi)Catechin-A-(Epi)Catechin-Mv-3-glc | 1071.3→781.2.2 | 20 | 10 |

### c) Condensation with flavanols mediated by acetaldehyde

This type of condensation makes an ethylidene bridge in the structure of the compound (Figure IV.17). The reaction between the flavanol and anthocyanin may take place by the carbons C6 or C8, but is more likely to occur by the C8 position because the negative charge is better stabilized[280]. Furthermore, the ethylidene bridge has an asymmetric carbon atom and can generate diastereomers[281].

A total of six compounds of direct condensation mediated by acetaldehyde were identified by our group (Table IV.6)

[280]De Freitas, V.; Mateus, N. *Chemical transformations of anthocyanins yielding a variety of colours (Review).* Environ. Chem. Lett. **2006**, 4, 175-183.

[281]Atanasova, V.; Fulcrand, H.; Cheynier, V.; Moutonet, M. *Effect of oxygenation on polyphenol changes occurring in the course of wine-making*. Anal. Chim. Acta **2002**, 458, 15-27.

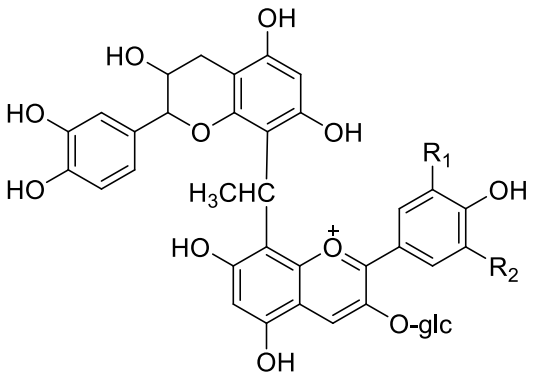



**Figure IV.17.** Chemical structure of anthocyanin derivatives formed by condensation with flavanols mediated by acetaldehyde.

The mass spectrum of this compound shows the molecular ion $[M]^+$.The most important fragment ion observed in the mass spectrum of this type of compounds was the loss of flavanol unit due to the rupture of C-C bond, which link ethyl and flavanol together. This fragment correspond to a loss of 290 u in the case of the (epi)catechin and 306 u in the case of the (epi)gallocatechin. The loss of glucose (-162 u), or p-coumaroylglucose (-308 u), is also observed in the mass spectrum. The mass spectrum pattern for these derivatives will be explained using Mv-3-glc-8-ethyl-catechin 1 (**34**) as an example in Figure IV.18.

**Table IV.6.** Retention time and molecular ion achieved by HPLC-DAD-ESI(+)-CID-MS/MS of direct condensation products mediated by acetaldehyde. The numbers written in bold correspond to the compounds chosen as markers.

| Number | $t_R$ (min) | Compound | $[M]^+$ (m/z) |
|---|---|---|---|
| *51* | 52.85 | Pt-3-glc-8-ethyl-(epi)catechin | 795.1 |
| **34** | 60.17 | Mv-3-glc-8-ethyl-catechin 1 | 808.9 |
| **35** | 65.41 | Mv-3-glc-8-ethyl-catechin 2 | 808.9 |
| **36** | 68.57 | Mv-3-glc-8-ethyl-epicatechin | 808.9 |
| **37** | 89.88 | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin1 | 955.0 |
| **38** | 93.95 | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin 2 | 955.0 |
| *52* | 61.33 | Mv-3-glc-8-ethyl-(epi)gallocatechin | 824.9 |

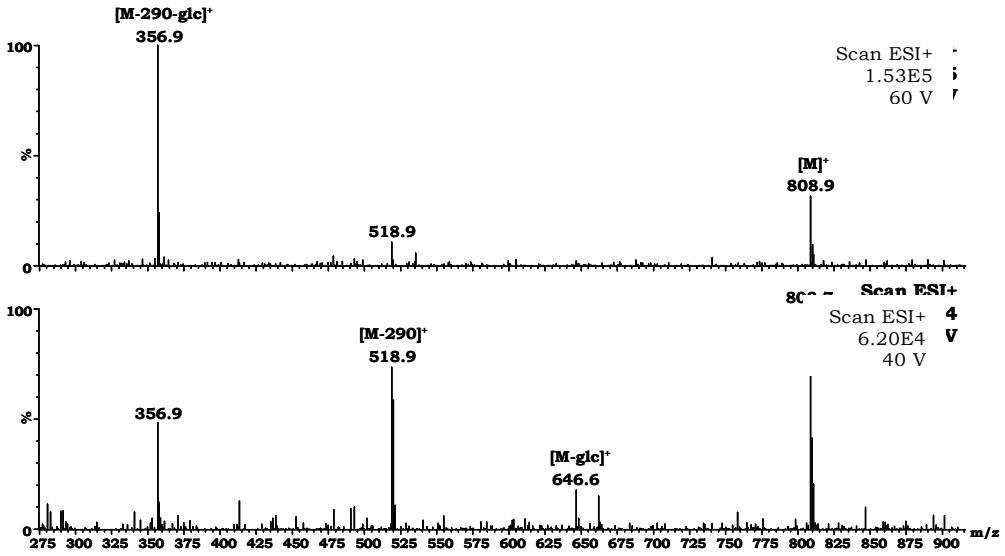
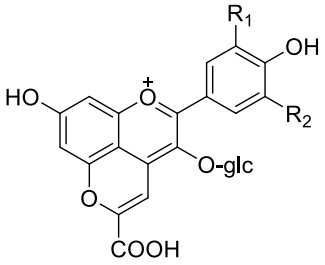



**Figure IV.18.** ESI(+)-MS spectra of ethylidene-bridge anthocyanin-flavanol condensation derivatives, obtained at a cone voltage of 40 and 60V. Example: Mv-3-glc-8-ethyl-catechin 1 (**34**).

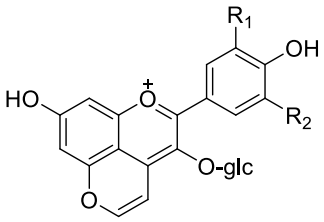
## IV.1.2. Pyranoanthocyanins

The other major anthocyanin pigments derived group is formed by a nucleophilic cycloaddition reaction of some compounds present in the wine to the anthocyanin in the flavylium cation form, leading to the formation of a new pyranic ring in the structure between the carbon C4 and hydroxyl group at the position 5 of anthocyanin. Due to the formation of this new pyranic ring these compounds are called pyranoanthocyanins.

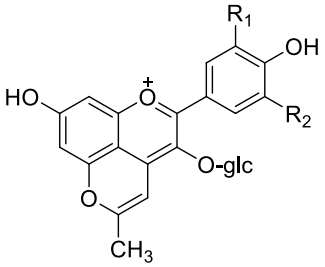
Among the molecules which may undergo this type of reaction, pyruvic acid, acetaldehyde, acetoacetic acid, hydroxycinnamic acids and vinylflavanols are included (Figure IV.19). These compounds show keto-enol tautomerism and may react in the enol form with anthocyanins.



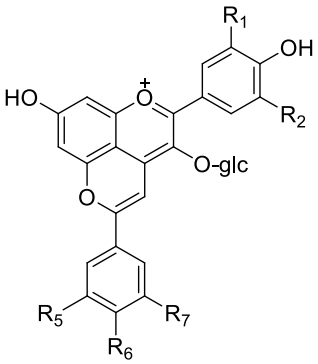

(A)




(B)

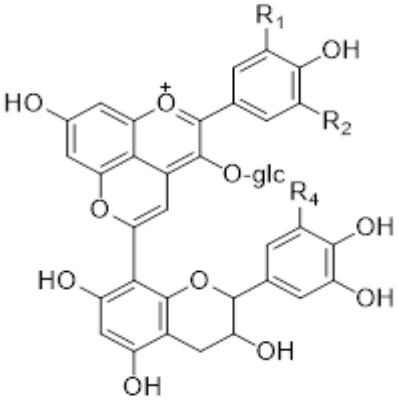



(C)




(D)

| Derivatives | R5 | R6 | R7 |
|---|---|---|---|
| Anthocyanin-vinylphenol | -H | -OH | -H |
| Anthocyanin-vinylcatechol | -H | -OH | -OH |
| Anthocyanin-vinylguaicol | -H | -OH | $-OCH_3$ |
| Anthocyanin-vinylsyringol | $-OCH_3$ | -OH | $-OCH_3$ |




(E)

| Flavanol | R4 |
|---|---|
| (epi)catechin | -H |
| (epi)gallocatechin | -OH |

**Figure IV.19.** Chemical structure of anthocyanin derivatives with: (A) pyruvic acid, (B) acetaldehyde, (C) acetoacetic acid, (D) hydroxycinnamic acids and (E) vinylflavanols.

### a) Derivatives with pyruvic acid, acetaldehyde and acetoacetic acid

Twenty three compounds were identified and some of them were analysed during this investigation. Among them, fourteen are anthocyanin derivatives with pyruvic acid (called Vitisins A), four are anthocyanin derivatives with acetaldehyde (called Vitisins B) and five are derivatives with acetoacetic acid (Table IV.7).

**Table IV.7.** Retention time and molecular ion achieved by HPLC-DAD-ESI(+)-CID-MS/MS of Vitisins A, B and derivatives with acetoacetic acid. The numbers written in bold correspond to the compounds chosen as markers.

| | Number | $t_R$ (min) | Compound | $[M]^+$ (m/z) |
|---|---|---|---|---|
| *Vitisins A* | *53* | 10.62 | Dp-3-glc-pyruvic | 533.2 |
| | *54* | 18.07 | Cy-3-glc-pyruvic | 516.9 |
| | *55* | 21.40 | Pt-3-glc-pyruvic | 547.1 |
| | *56* | 31.33 | Pn-3-glc-pyruvic | 531.1 |
| | **39** | 36.20 | Mv-3-glc-pyruvic | 561.0 |
| | *57* | 42.12 | Pt-3-(6-Ac)-glc-pyruvic | 589.3 |
| | *58* | 48.25 | Mv-3-(6-Ac)-glc-pyruvic | 603.1 |
| | *59* | 41.68 | Dp-3-(6-p-coum)-glc-pyruvic | 679.0 |
| | *60* | 57.90 | Cy-3-(6-p-coum)-glc-pyruvic | 663.1 |
| | *61* | 60.05 | Pt-3-(6-p-coum)-glc-pyruvic | 692.9 |
| | *62* | 72.27 | Pn-3-(6-p-coum)-glc-pyruvic | 677.1 |
| | **40** | 69.65 | Mv-3-(6-p-coum)-glc-pyruvic cis | 707.2 |
| | *63* | 73.35 | Mv-3-(6-p-coum)-glc-pyruvic trans | 707.2 |
| | *64* | 63.08 | Mv-3-(6-caff)-glc-pyruvic | 723.1 |
| *Vitisins B* | *65* | 38.50 | Pn-3-glc-acetaldehyde | 487.0 |
| | **41** | 44.38 | Mv-3-glc-acetaldehyde | 517.1 |
| | *66* | 82.97 | Pn-3-(6-p-coum)-glc-acetaldehyde | 632.9 |
| | **42** | 83.70 | Mv-3-(6-p-coum)-glc-acetaldehyde | 663.1 |
| *Derivative with acetoacetic acid* | *67* | 41.80 | Pt-3-glc-vinylmethyl | 517.1 |
| | *68* | 54.70 | Pn-3-glc-vinylmethyl | 501.2 |
| | **43** | 59.22 | Mv-3-glc-vinylmethyl | 530.9 |
| | *69* | 91.30 | Pn-3-(6-p-coum)-glc-vinylmethyl | 647.1 |
| | *70* | 92.20 | Mv-3-(6-p-coum)-glc-vinylmethyl | 677.2 |

The molecular ion $[M]^+$ was observed in the mass spectrum for each compounds. The great stability of this new pyranic ring made that only glucose or glucose acylated losses are observed in the mass spectra of these derivatives, so the fragment ion corresponded to aglycone is the only fragment that can be seen in the mass spectrum. The mass spectrum for each compounds could be seen from Figure IV.20 to IV.22, using Mv-3-glc-pyruvic (**39**),Mv-3-glc-acetaldehyde (**41**) and Mv-3-glc-vinylmethyl (**43**) as examples.

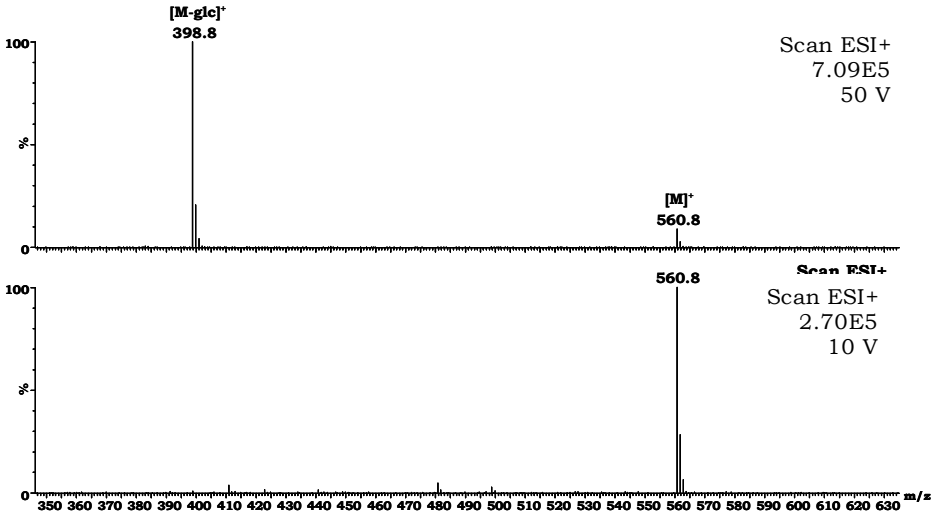



**Figure IV.20.** ESI(+)-MS spectra of Mv-3-glc-pyruvic (**39**), obtained at a cone voltage of 10V and 50V.

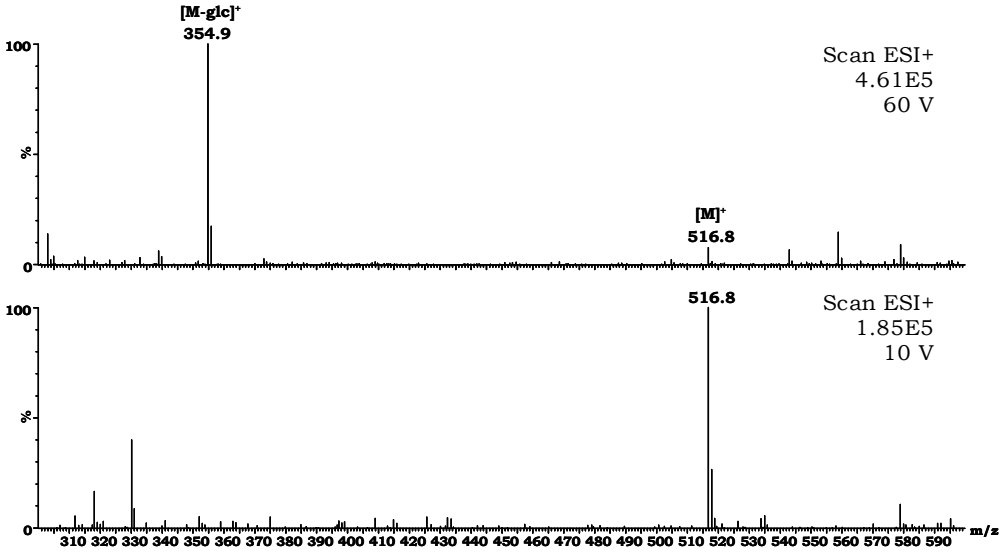



**Figure IV.21.** ESI(+)-MS spectra of Mv-3-glc-acetaldehyde (**41**), obtained at a cone voltage of 10V and 60V.

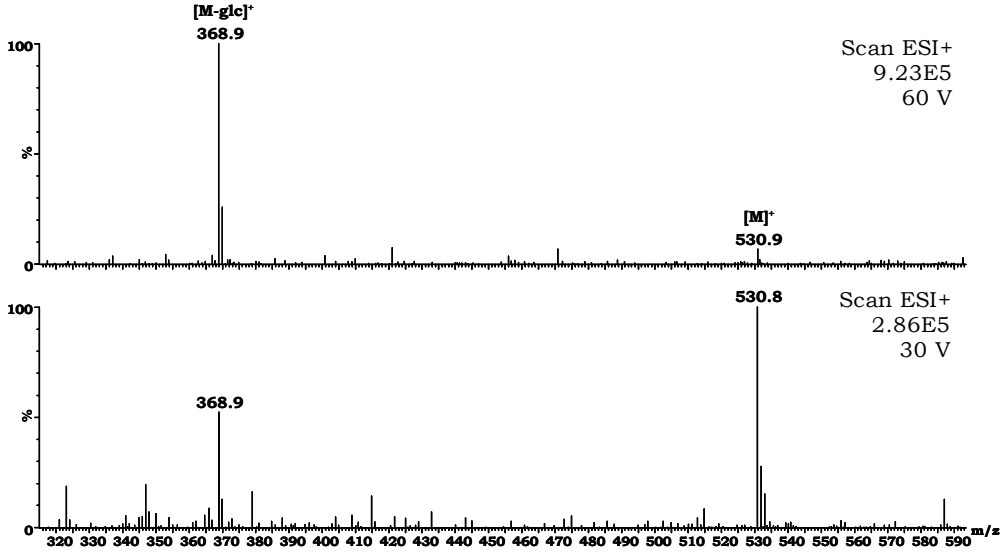



**Figure IV.22.** ESI(+)-MS spectra of Mv-3-glc-vinylmetyl (**43**), obtained at a cone voltage of 30V and 60V.

### b) Derivatives with hydroxycinnamic acids

Twenty seven derivatives with hydroxycinnamic acids are shown in the Table IV.8.

The mass spectrum observed in this type of anthocyanin derivatives are similar of those formed with pyruvic acid, acetaldehyde and acetoacetic acid. The molecular ion $[M]^+$ was observed in the spectrum for each compounds and only glucose or glucose acylated losses (-162 u) are observed, so the fragment ion corresponded to aglycone is the only fragment that can be seen in the mass spectrum. The mass spectrum could be seen from Figure IV.23 to IV.25, using Mv-3-glc-4-vinylphenol (**44**), Mv-3-glc-4-vinylcatechol (**46**) and Mv-3-glc-4-vinylguaiacol (**47**) as examples.

**Table IV.8.** Retention time and molecular ion achieved by HPLC-DAD-ESI(+)-CID-MS/MS of derivatives with hydroxycinnamic acid. The numbers written in bold correspond to the compounds chosen as markers.

| Number | $t_R$ (min) | Compound | $[M]^+$ (m/z) |
|---|---|---|---|
| *71* | 78.28 | Dp-3-glc-4-vinylphenol | 581.1 |
| *72* | 86.90 | Cy-3-glc-4-vinylphenol | 565.2 |
| *73* | 89.78 | Pt-3-glc-4-vinylphenol | 595.2 |
| *74* | 97.90 | Pn-3-glc-4-vinylphenol | 579.1 |
| **44** | 99.48 | Mv-3-glc-4-vinylphenol | 608.9 |
| *75* | 106.53 | Pn-3-(6-Ac)-glc-4-vinylphenol | 621.1 |
| *76* | 107.32 | Mv-3-(6-Ac)-glc-4-vinylphenol | 651.1 |
| *77* | 97.95 | Dp-3-(6-p-coum)-glc-4-vinylphenol | 727.3 |
| *78* | 105.67 | Cy-3-(6-p-coum)-glc-4-vinylphenol | 711.3 |
| *79* | 107.72 | Pt-3-(6-p-coum)-glc-4-vinylphenol | 741.2 |
| *80* | 114.02 | Pn-3-(6-p-coum)-glc-4-vinylphenol | 725.1 |
| **45** | 114.73 | Mv-3-(6-p-coum)-glc-4-vinylphenol | 755.0 |
| *81* | 70.38 | Dp-3-glc-4-vinylcatechol | 597.2 |
| *82* | 79.50 | Cy-3-glc-4-vinylcatechol | 580.9 |
| *83* | 82.57 | Pt-3-glc-4-vinylcatechol | 611 |
| *84* | 90.78 | Pn-3-glc-4-vinylcatechol | 595.1 |
| **46** | 92.57 | Mv-3-glc-vinylcatechol | 625.1 |
| *85* | 100.03 | Mv-3-(6-Ac)-glc-4-vinylcatechol | 667 |
| *86* | 89.73 | Dp-3-(6-p-coum)-glc-4-vinylcatechol | 743 |
| *87* | 97.37 | Cy-3-(6-p-coum)-glc-4-vinylcatechol | 727.1 |
| *88* | 99.48 | Pt-3-(6-p-coum)-glc-4-vinylcatechol | 757.3 |
| *89* | 108.32 | Pn-3-(6-p-coum)-glc-4-vinylcatechol | 741.2 |
| *90* | 109.23 | Mv-3-(6-p-coum)-glc-4-vinylcatechol | 771 |
| *91* | 81.48 | Dp-3-glc-4-vinylguaiacol | 610.9 |
| *92* | 100.63 | Pn-3-glc-4-vinylguaiacol | 609.1 |
| **47** | 102.25 | Mv-3-glc-vinylguaiacol | 639.1 |
| **48** | 115.58 | Mv-3-(6-p-coum)-glc-4-vinylguaiacol | 784.9 |
| *93* | 104.12 | Mv-3-glc-vinylsyringol | 669.2 |

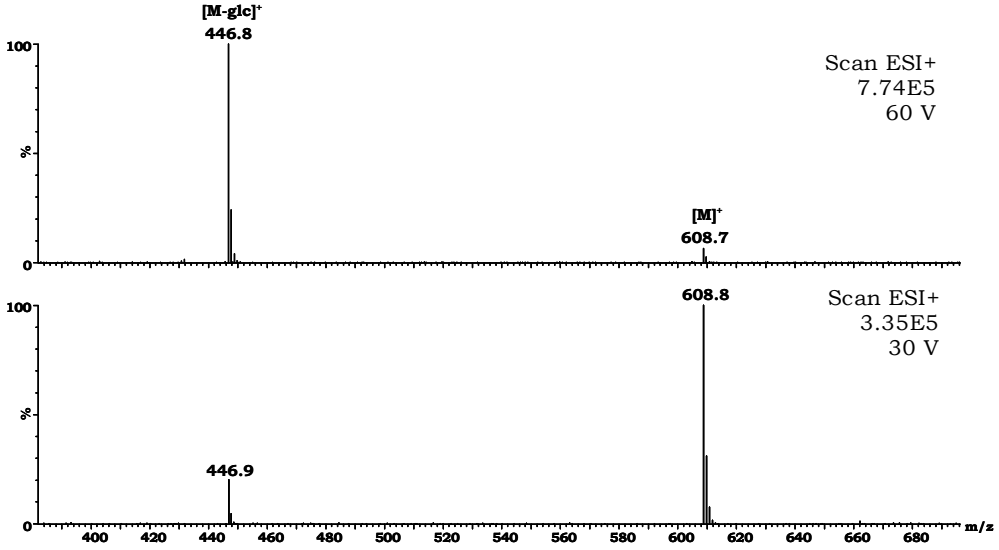



**Figure IV.23.** ESI(+)-MS spectra of Mv-3-glc-4-vinylphenol (**44**), obtained at a cone voltage of 30V and 60V.

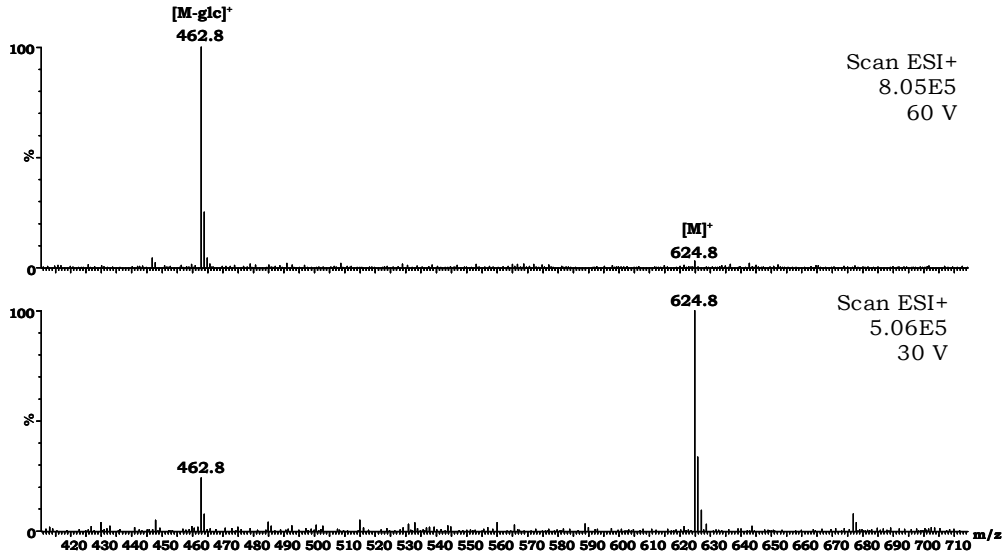



**Figure IV.24.** ESI(+)-MS spectra of Mv-3-glc-4-vinylcatecol (**46**), obtained at a cone voltage of 30V and 60V.

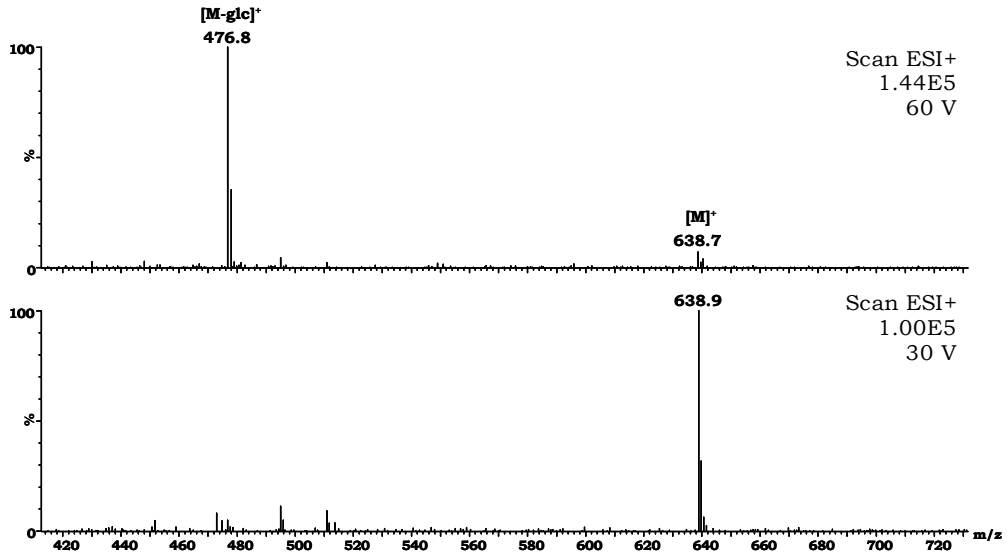



**Figure IV.25.** ESI(+)-MS spectra of Mv-3-glc-4-vinylguaiacol (**47**), obtained at a cone voltage of 30V and 60V.

### c) Derivatives with vinylflavanols

Twenty one derivatives with vinylflavanols were identified (Table IV.9), of which seventeen contain an (epi)catechin unit (compounds from *94* to *106* and from **49** to **52**) and an (epi)gallocatechin unit for the other four compounds (from **53** to **56**).

As in previous pyranoanthocyanins, the stability provided by the new pyranic ring makes that fragmentations of aglycone are not observed in the mass spectrum. However, for these compounds, it is also observed the ion of retro Diels-Alder fragmentation of flavanol, besides fragment ion corresponding to the loss of glucose. The mass spectrum could be seen in Figure IV.26 using Mv-3-glc-4-vinylepicatechin (**50**) as an example.

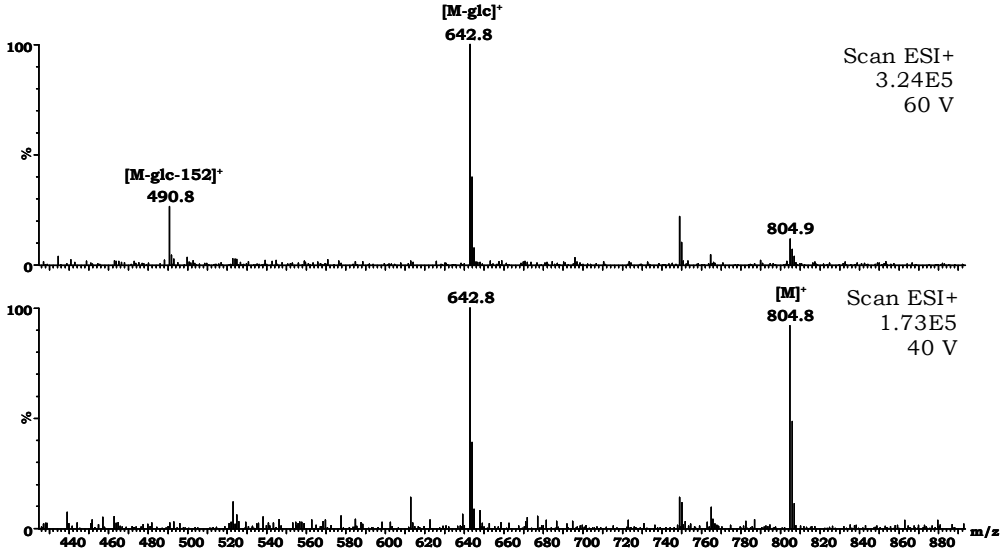



**Figure IV.26.** ESI(+)-MS spectra of vinylflavanols derivatives obtained in scan mode at 40V and 60 V. Example: Mv-3-glc-4-vinyl(epi)catechin (**50**).

**Table IV.9.** Retention time and molecular ion achieved by HPLC-DAD-ESI(+)-CID-MS/MS of derivatives with vinylflavanols. The numbers written in bold correspond to the compounds chosen as markers.

| Number | $t_R$ (min) | Compound | $[M]^+$ (m/z) |
|---|---|---|---|
| *94* | 67.60 | Dp-3-glc-4-vinylcatechin | 777.1 |
| *95* | 79.35 | Dp-3-glc-4-vinylepicatechin | 777.1 |
| *96* | 77.27 | Pt-3-glc-4-vinylcatechin | 791 |
| *97* | 87.32 | Pt-3-glc-4-vinylepicatechin | 791 |
| *98* | 83.83 | Pn-3-glc-4-vinylcatechin | 774.9 |
| *99* | 93.82 | Pn-3-glc-4-vinylepicatechin | 774.9 |
| *100* | 78.60 | Dp-3-(6-p-coum)-glc-4-vinyl(epi)catechin | 923.1 |
| *101* | 87.70 | Pt-3-(6-p-coum)-glc-4-vinylcatechin | 937.3 |
| *102* | 90.82 | Pt-3-(6-p-coum)-glc-4-vinylepicatechin | 937.3 |
| *103* | 95.45 | Pn-3-(6-p-coum)-glc-4-vinylcatechin | 920.9 |
| *104* | 98.18 | Pn-3-(6-p-coum)-glc-4-vinylepicatechin | 920.9 |
| *105* | 64.25 | Mv-3-glc-4-vinyl(epi)catechin-(epi)catechin 1 | 1093.1 |
| *106* | 65.73 | Mv-3-glc-4-vinyl(epi)catechin-(epi)catechin 2 | 1093.1 |

**Table IV.9.** Cont.

| Number | $t_R$ (min) | Compound | $[M]^+$ (m/z) |
|---|---|---|---|
| **49** | 86.02 | Mv-3-glc-4-vinylcatechin* | 805.2 |
| **50** | 95.00 | Mv-3-glc-4-vinylepicatechin* | 805.2 |
| **51** | 97.28 | Mv-3-(6-p-coum)-glc-4-vinylcatechin* | 951.2 |
| **52** | 100.18 | Mv-3-(6-p-coum)-glc-4-vinylepicatechin* | 951.2 |
| **53** | 73.00 | Mv-3-glc-4-vinylgallocatechin* | 821.2 |
| **54** | 81.83 | Mv-3-glc-4-vinylepigallocatechin* | 821.2 |
| **55** | 87.32 | Mv-3-(6-p-coum)-glc-4-vinyl(epi)gallocatechin 1* | 967.2 |
| **56** | 91.85 | Mv-3-(6-p-coum)-glc-4-vinyl(epi)gallocatechin 2* | 967.2 |

The highest intensity fragment ions for the compounds **49**-**52** and **53**-**56**, correspond to the aglycone ion at m/z 643.1 and 659.1, respectively. These fragment ions were chosen as an optimum transition for MRM experiment. Then a set of experiments were done for optimizing cone voltage (10, 20, 30, 40, 50 and 60 V) and collision energy (10, 20, 30, 40, 50 and 50 eV). In Figure IV.27 and IV.28 could be seen the graphics for the optimum cone voltage and collision energy of each compound.

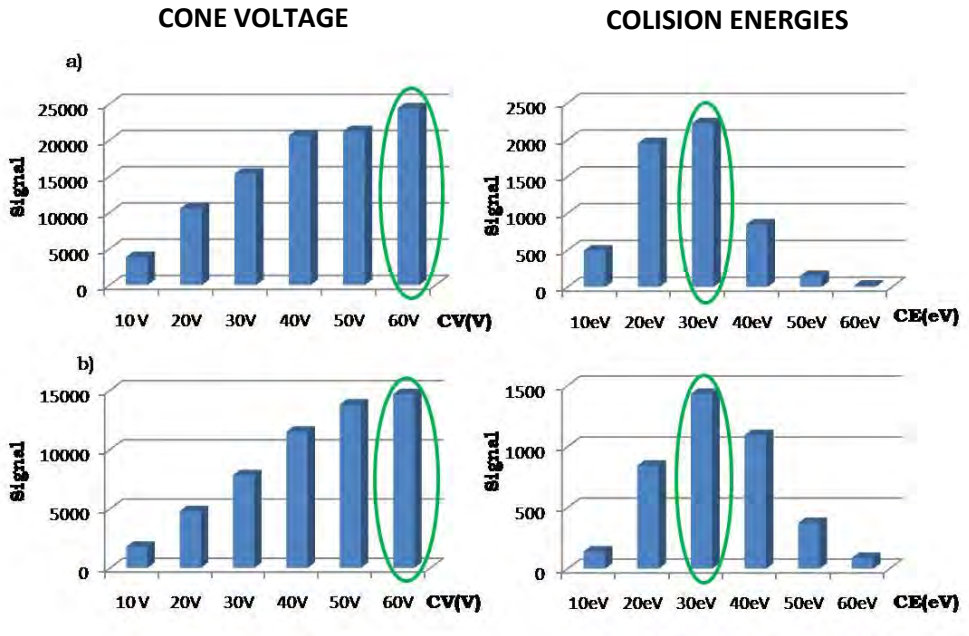



**Figure IV.27.** Variations of the chromatogram area for the new compounds, which were identified as a) Mv-3-glc-4-vinylcatechin; b) Mv-3-(6-p-coum)-glc-4-vinylcatechin, depending on cone voltages and collision energy values.

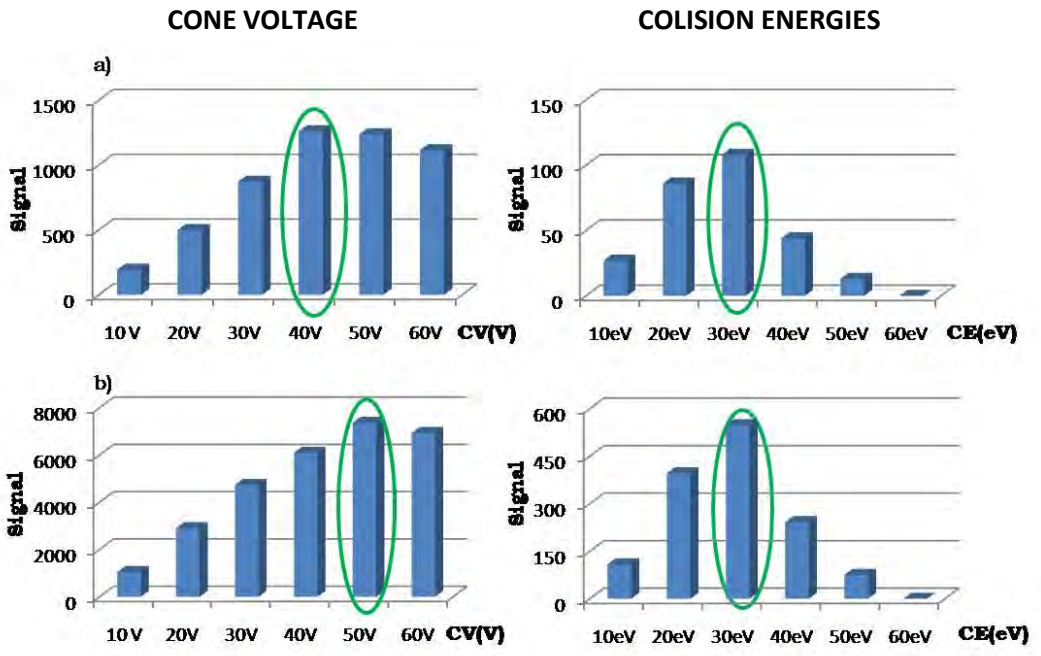



**Figure IV.28.** Variations of the chromatogram area for the new compounds, which were identified as a) Mv-3-glc-4-vinylgallocatechin ; b) Mv-3-(6-p-coum)-glc-4-vinyl(epi)gallocatechin, depending on cone voltages and collision energy values.

All these derivatives are reported in Table IV.10, in which retention times, optimum transitions, cone voltages and collision energies are collected.

**Table IV.10.** Optimum transitions, cone voltages and collision energies of derivatives formed by anthocyanin and vinylflavanols.

| Compound | | Transition | CV (V) | CE (eV) |
|---|---|---|---|---|
| **49** | Mv-3-glc-4-vinylcatechin | 805.2→643.1 | 60 | 30 |
| **50** | Mv-3-glc-4-vinylepicatechin | 805.2→643.1 | 60 | 30 |
| **51** | Mv-3-(6-*p*-coum)-glc-4-vinylcatechin | 951.2→643.1 | 60 | 30 |
| **52** | Mv-3-(6-*p*-coum)-glc-4-vinylepicatechin | | | |
| **53** | Mv-3-glc-4-vinylgallocatechin | 821.2→659.1 | 40 | 30 |
| **54** | Mv-3-glc-4-vinylepigallocatechin | 821.2→659.1 | 40 | 30 |
| **55** | Mv-3-(6-*p*-coum)-glc-4-vinyl(Epi)gallocatechin 1 | 967.2→659.1 | 50 | 30 |
| **56** | Mv-3-(6-*p*-coum)-glc-4-vinyl(Epi)gallocatechin 2 | | | |

## IV.2. IDENTIFICATION OF TANNINS BY HPLC-DAD-ESI(-)-CID-MS/MS

The identification of tannins, previously performed in our group, was carried out by HPLC-DAD-ESI(-)-CID-MS/MS using the same equipment that was used for the anthocyanin derivatives that was explained in the before chapter.

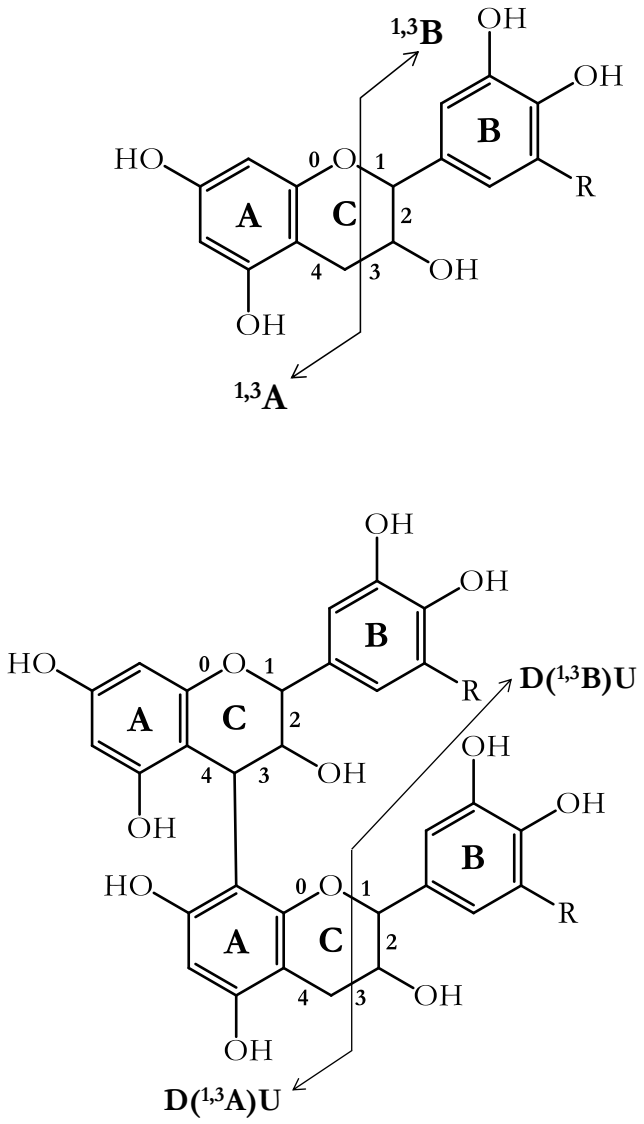



**Figure IV.29.** Nomenclature used to describe the fragment ions detected in MS.

The fragmentation pattern for tannins is characterised by the rupture of C-ring bonds at positions 1/3, 0/2, 0/3, 0/4, 1/4 or 1/2. The most used nomenclature for

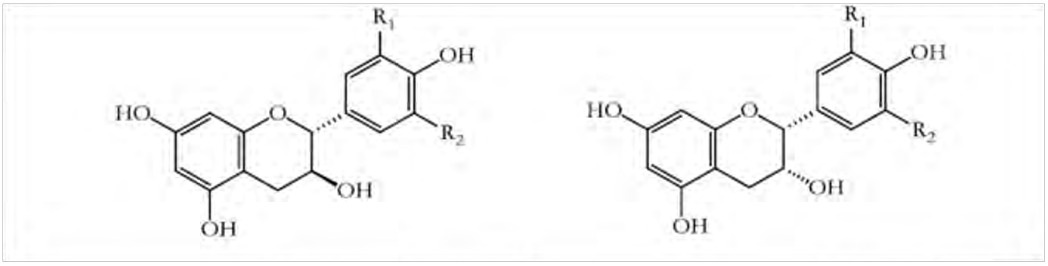
the study of the fragmentations is the one proposed by Ma et all[283] (Figure IV.29). As the major fragments are derived from the rupture of C-ring, the terms $^{i,j}A^{+/-}$ and $^{i,j}B^{+/-}$ are used to refer what ring, A or B respectively, is intact and the superscripts *i* and *j* indicate the C-ring bonds that have been broken.

When the compound has more than one flavan-3-ol unit, this nomenclature is not appropriate because there are two C-rings where the fragmentation may happen. So in this case, the two subunits can be differentiate as *U* (up) and *D* (down) and the fragmentations in each subunit are indicated in brackets. For example, the protonated or deprotonated molecule will be $UD^{+/-}$ or ion fragmentation from Retro Diels Alder (RDA) losing the B-ring by the rupture of C-ring bond at position 1/3 of the U subunit will be $U(^{1,3}A)D^{+/-}$ (Figure IV.23). In addition, when the number of flavan-3-ols units increases the letter *M* will be added, for example the protonated or deprotonated molecule of a trimer will be $UMD^{+/-}$ or for a tetramer $UM1M2D^{+/-}$.

## IV.2.1. Monomeric tannins

The four main monomers in wine, which are also present in the grape, are catechin, epicatechin, gallocatechin and epigallocatechin (Table IV.11).

The difference between (epi)catechin and (epi)gallocatechin is that the second one has one more OH in the B-ring (Figure IV.30), so the observed masses for the fragments of (epi)gallocatechin have 16 units more than those ones corresponding to (epi)catechin.




| | R1 | R2 | | R1 | R2 |
|---|---|---|---|---|---|
| (+)-Catechin | H | OH | (-)-Epicatechin | H | OH |
| (+)-Gallocatechin | OH | OH | (-)-Epigallocatechin | OH | OH |
| (+)-Afzelechin | H | H | (-)-Epiafzelechin | H | H |

**Figure IV.30.** Chemical structure of monomeric tannins.

[283]Ma, Y.; Li, Q.; Van den Heuvel, H.; Claeys, M.; *Characterisation of flavone and flavonol aglycones by collision-induced dissociation tandem mass spectrometry*, Rapid Commun. Mass Spectrom. **1997**, 11, 1357-1364.

**Table IV.11.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of monomeric tannins. The numbers written in bold correspond to the compounds chosen as markers.

| Number | $t_R$ (min) | Compound | $[M-H]^-$ (m/z) |
|---|---|---|---|
| **59** | 24.62 | Gallocatechin | 305.2 |
| **57** | 46.54 | Catechin | 289.2 |
| **60** | 52.21 | Epigallocatechin | 305.2 |
| **58** | 68.21 | Epicatechin | 289.2 |

The mass spectra of monomeric tannins show the molecular ion $[M-H]^-$ at m/z 289.2 and 305.2 for (epi)catechin and (epi)gallocatechin, respectively. One of the most intense fragments is $^{1,3}A$ at m/z 137. However, the fragment corresponding to the rupture $^{1,3}A$-CO at m/z 109 is more intense than fragment at 137. This is because the loss of CO is the most probably fragmentation in negative mode (ESI-). The fragment $^{1,3}B^-$ and its decarboxilation and the fragment $^{1,4}A^-$+2H are also intense fragment in the product ion spectrum.

An example of the mass spectrum in product ion scan mode for monomeric tannins can be seen in Figure IV.31.

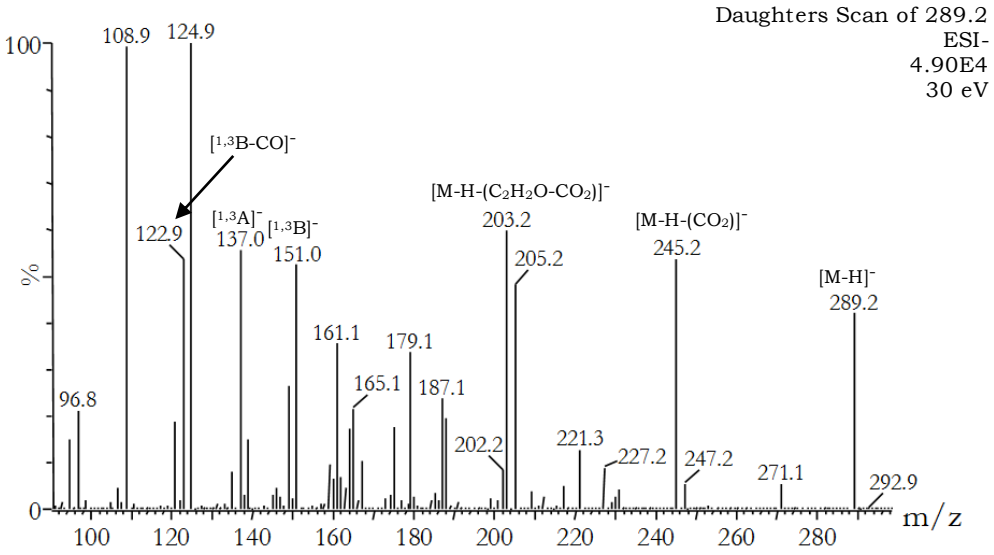



**Figure IV.31.** ESI(-)-$MS^2$ spectrum of monomeric tannins, obtained at a collision energy of 30 eV. Example: Catechin (**57**).

## IV.2.2. Procyanidins homodimers B

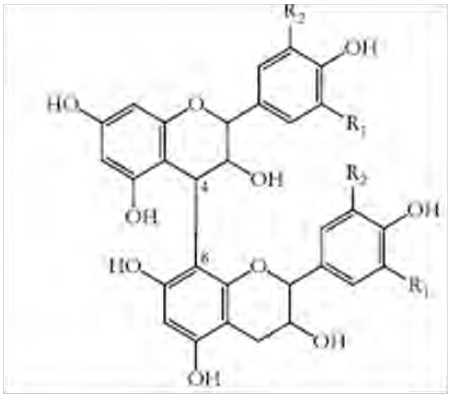
These compounds, which are formed by two units of flavan-3-ol (catechin and/or epicatechin) with an interflavonoid bond, have been detected in the grape and in young and aged wines (Figure IV.32).

Nine different procyanidins B, which are shown in Table IV.12, were identified.

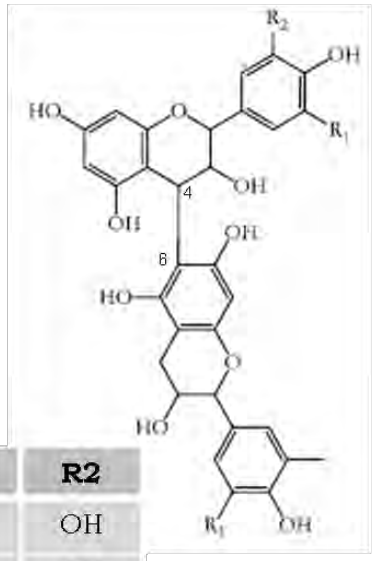
**Table IV.12.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of procyanidins homodimers B. The numbers written in bold correspond to the compounds chosen as markers.

| Number | $t_R$ (min) | Compound | $[UD]^-$ (m/z) |
|---|---|---|---|
| *107* | 41.09 | $((epi)cat)_2$ | 576.9 |
| **63** | 42.56 | $((epi)cat)_2$ | 577.0 |
| **61** | 44.92 | PCB1 | 576.9 |
| *108* | 52.17 | $((epi)cat)_2$ | 576.8 |
| *109* | 58.43 | $((epi)cat)_2$ | 576.9 |
| **62** | 60.40 | PCB2 | 577.0 |
| *110* | 73.86 | $((epi)cat)_2$ | 576.8 |
| *111* | 83.54 | $((epi)cat)_2$ | 577.1 |
| *112* | 89.60 | $((epi)cat)_2$ | 577.1 |

**B1-B4 Type**



**B5-B8 Type**



| | R1 | R2 |
|---|---|---|
| Procyanidin B1-B4 | H | OH |
| Procyanidin B5-B8 | H | OH |
| Prodelphinidin B1-B4 | OH | H |
| Prodelphinidin B5-B8 | OH | H |
| Propelargonidin B1-B4 | H | H |
| Propelargonidin B5-B8 | H | H |

**Figure IV.32.** Chemical structure of procyanidins homodimers B.

Several works on structural elucidation by $MS^2$ have been done, being the rupture of the interflavanic bond and the Retro Diels Alder fragmentation of catechin unit the most intense fragments.

The mass spectra of these compounds show the molecular ion $[UD]^-$ at m/z 576.9. The ions at m/z 289 and 287 are from the rupture of the interflavanic bond ($[D]^-$ and $[U]^-$, respectively). In this rupture the electrons of the bond remain in the

subunit D, so this subunit is the same as the corresponding monomer, whereas the subunit U has two hydrogens less than the monomer[284,285].

The fragment $[U(^{1,3}A)D]^-$ and its loss of $H_2O$ at m/z 424.9 and 406.9, respectively, are also intense fragment in the product ion spectrum. The proposed mechanism for the fragment $[U(^{1,3}A)D]^-$ is a Retro Diels-Alder fragmentation, which occurs in the subunit U because the ion fragment formed by this rupture has a greater conjugation than the one which is formed by the RDA fragmentation in the subunit D[284].

An example of the mass spectrum in product ion scan mode for procyanidin homodimers B can be seen in Figure IV.33.

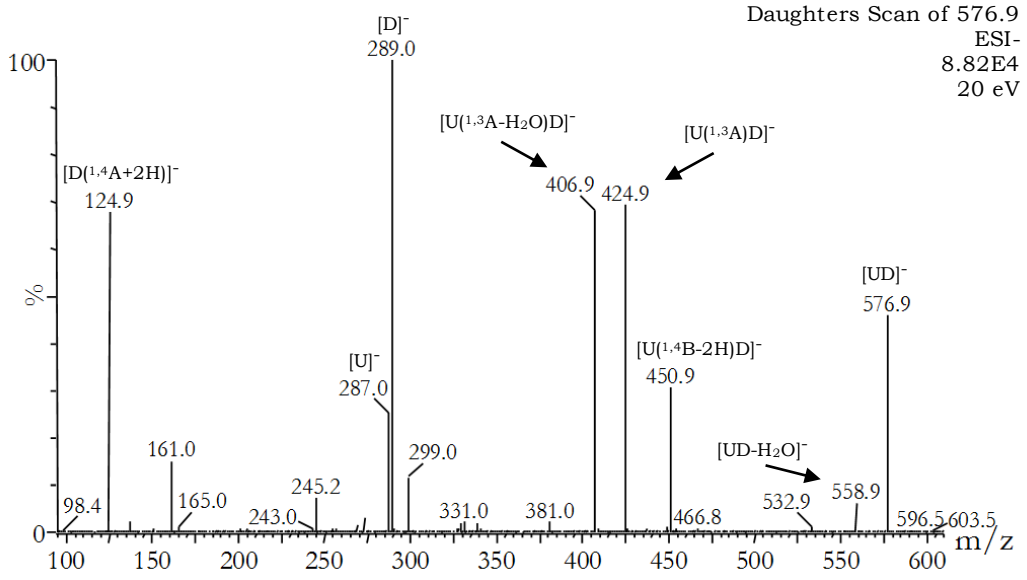



**Figure IV.33.** ESI(-)-$MS^2$ spectrum of procyanidins homodimers B, obtained at a collision energy of 20 eV. Example: PCB1 (**61**).

## IV.2.3. Prodelphinidins homodimers B

These compounds are formed by two units of gallocatechin and/or epigalocatechin with an interflavonoid bond. These compounds are more polar than procyanidins B because they present an additional OH.

[284] Gu, L.; Kelm, M.A.; Hammerstone, J. F.; Zhang, Z.; Beecher, G.; Holden, J.; Haytowitz, D.; Prior, R. L.; *Liquid chromatographic/electrospray ionization massspectrometric studies of proanthocyanidins in foods*, J. Mass spectrum. **2003**, 38, 1272-1280.

[285] Fraser, K.; Harrison, S. J.; Lane, G. A.; Otter, D. E.; Hemar, Y.; Quek, S. Y.; Rasmussen, S.; HPLC-*MS/MS profiling of proanthocyanidins in teas: A comparative study*, J. Food Compos. Anal. **2012**, 26, 43-51.

Three different prodelphinidins homodimers B were identified and analysed during this investigation (Table IV.13).

**Table IV.13.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of prodelphinidins homodimers B. The numbers written in bold correspond to the compounds chosen as markers.

| Number | $t_R$ (min) | Compound | $[UD]^-$ (m/z) |
|---|---|---|---|
| **64** | 11.90 | ((epi)gallocatechin)$_2$ 1 | 608.9 |
| **65** | 28.45 | ((epi)gallocatechin)$_2$ 2 | 608.9 |
| **66** | 42.89 | ((epi)gallocatechin)$_2$ 3 | 608.7 |

In the mass spectra of these compounds the similarity of the prodelphinidins and procyanidins is observed. The most intense fragment is the rupture of the interflavanic bond $[D]^-$ at m/z 305. The fragment corresponding to the rupture of Retro Diels Alder and its loss of $H_2O$ at m/z 440.9 and 422.9, respectively, are also intense fragment in the product ion spectrum.

The mass spectrum in product ion scan mode for procyanidins B can be seen in Figure IV.34, using ((epi)gallocatechin)$_2$ 1 as example.

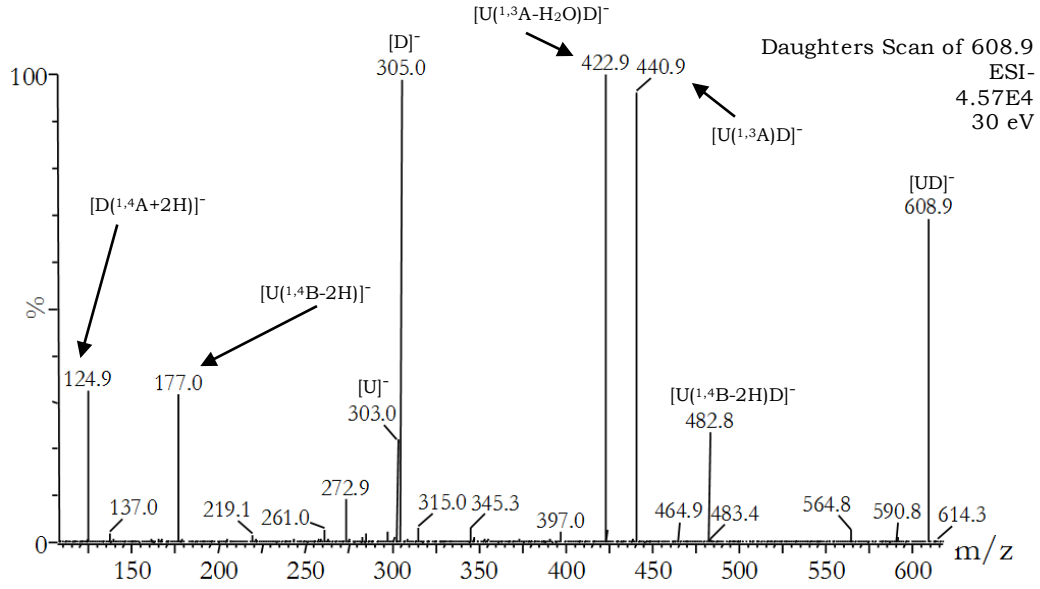



**Figure IV.34.** ESI(-)-MS$^2$ spectrum of prodelphinidins homodimers B, obtained at a collision energy of 30 eV. Example: ((epi)gallocatechin)$_2$ 1 (**64**).

## IV.2.4. Mixed dimers B

The mixed dimmers are formed by two monomers of flavan-3-ol which have different molecular weights, so the interflavonoid bond occurs between different flavan-3-ols, for example, (epi)catechin and (epi)gallocatechin (Figure IV.35).

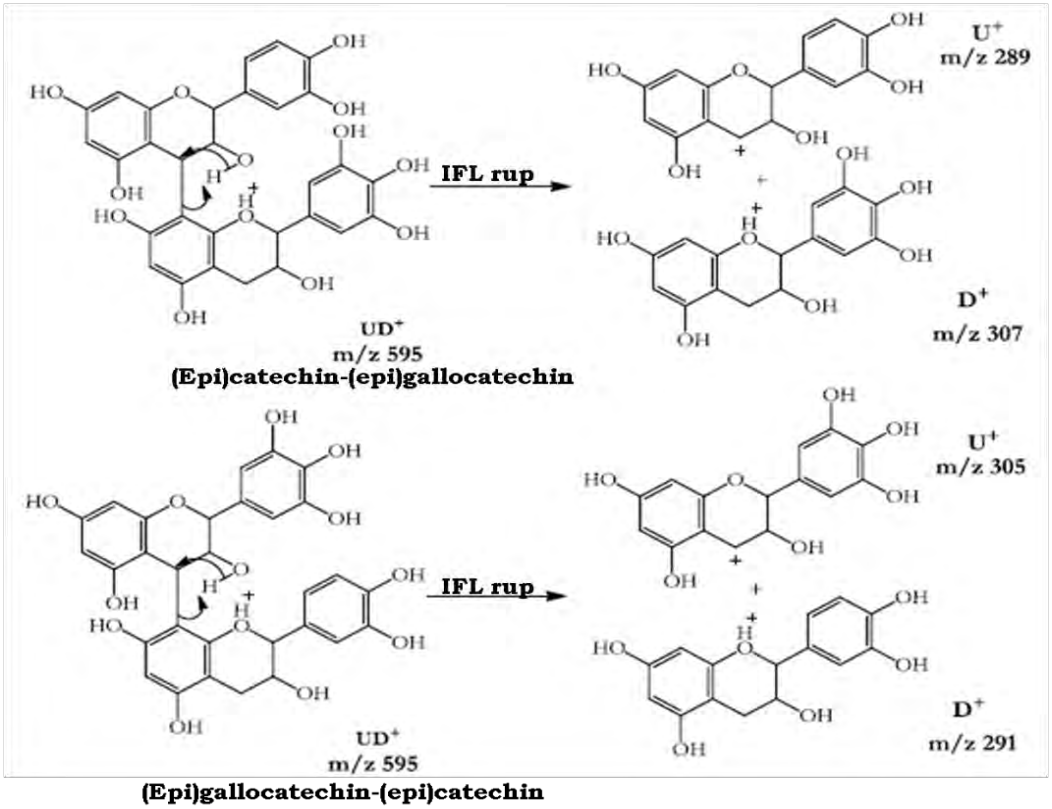



**Figure IV.35.** Generated fragment ions in the mixed dimers interflavonoid bond rupture.

Twelve different mixed dimers B have been detected (Table IV.14 and IV.15). Eight of them have the (epi)catechin monomer as the top unit (procyanidins heterodimers B), whereas in the other four compounds the (epi)gallocatechin monomer is the upper unit (prodelphinidins heterodimers B).

**Table IV.14.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of procyanidin heterodimers B. The numbers written in bold correspond to the compounds chosen as markers.

| Number | $t_R$ (min) | Compound | $[UD]^-$ (m/z) |
|---|---|---|---|
| *113* | 5.45 | (epi)catechin-(epi)gallocatechin | 592.9 |
| *114* | 5.91 | (epi)catechin-(epi)gallocatechin | 593.0 |
| *115* | 6.48 | (epi)catechin-(epi)gallocatechin | 592.9 |
| *116* | 16.90 | (epi)catechin-(epi)gallocatechin | 592.8 |
| **67** | 30.44 | (epi)catechin-(epi)gallocatechin | 592.8 |
| **68** | 47.55 | (epi)catechin-(epi)gallocatechin | 592.9 |
| *117* | 56.47 | (epi)catechin-(epi)gallocatechin | 592.9 |
| *118* | 72.03 | (epi)catechin-(epi)gallocatechin | 592.8 |

**Table IV.15.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of prodelphinidin heterodimers B. The numbers written in bold correspond to the compounds chosen as markers.

| Number | $t_R$ (min) | Compound | $[UD]^-$ (m/z) |
|---|---|---|---|
| **69** | 26.17 | (epi)gallocatechin-(epi)catechin | 592.9 |
| *119* | 28.80 | (epi)gallocatechin-(epi)catechin | 592.9 |
| **70** | 38.94 | (epi)gallocatechin-(epi)catechin | 592.9 |
| *120* | 61.20 | (epi)gallocatechin-(epi)catechin | 592.8 |

The mass spectra of these compounds show the molecular ion $[UD]^-$ at m/z 592.8. As it could be seen before (*IV.2.2. Procyanidins B*), when the interflavanic bond is broken, the electrons remain in the subunit D, so in these compounds, in which the two subunits have different mass, is possible to know which monomer is the top unit and which one is lower. If fragment ions at m/z 305.0 ($[D]^-$) and 287.0 ($[U]^-$) are more intense than ones at m/z 303.0 ($[D]^-$) and 289.0 ($[U]^-$), means that the upper unit is the (epi)catechin, whereas if the second pair of ions are more intense than the first ones, then the (epi)gallocatechin is the upper unit.

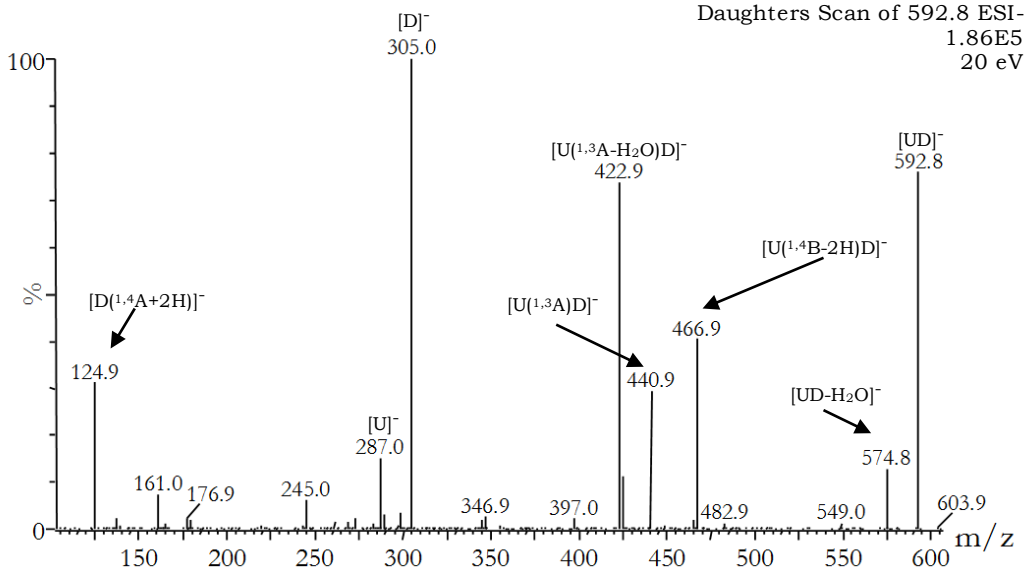



**Figure IV.36.** ESI(-)-$MS^2$ spectrum of mixed dimmers B, obtained at a collision energy of 20 eV. Example: (epi)catechin-(epi)gallocatechin (**67**).

The fragment ions of the RDA rupture ($[UD(^{1,3}A)]^-$, $[U(^{1,3}A)D]^-$, $[U(^{1,3}A\text{-}H_2O)D]^-$) also help in structural elucidation. This fragmentation occurs mainly in the subunit U, because the ion fragment formed by this rupture has a greater conjugation than the one which is formed by the RDA fragmentation in the subunit

$D^{288}$. If the mass loss from $[UD]^-$ is 152 and 170 u, the (epi)catechin will be the upper unit, whereas if the (epi)gallocatechin is the upper unit, the loss will be 168 and 186 u.

The mass spectrum in product ion scan mode for mixed dimers B can be seen in Figures IV.36 and IV.37.

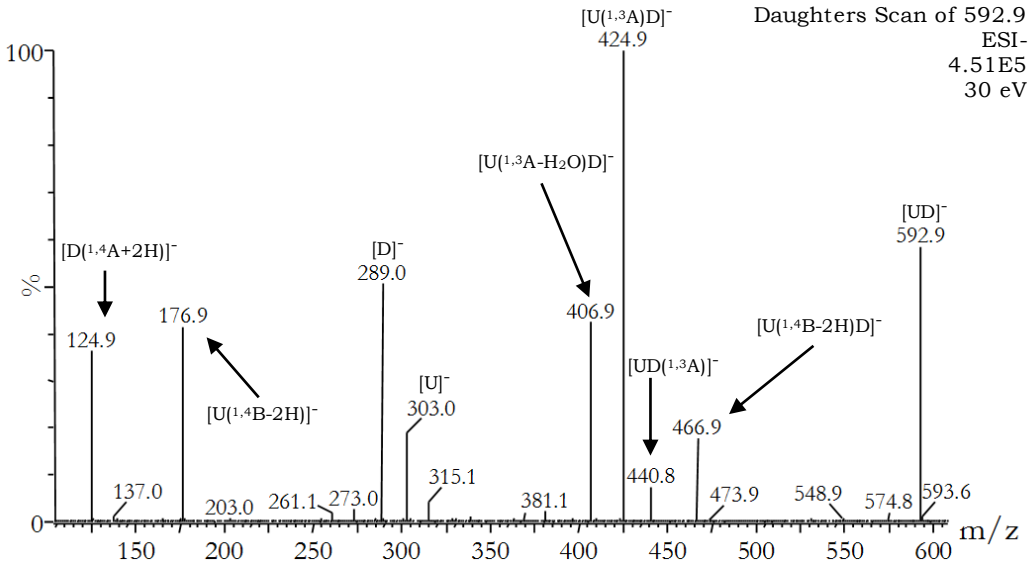



**Figure IV.37.** ESI(-)-$MS^2$ spectrum of mixed dimmers B, obtained at a collision energy of 30 eV. Example: (epi)gallocatechin-(epi)catechin (**69**).

## IV.2.5. Procyanidins homotrimers B

These compounds are formed by three units of catechin and/or epicatechin, with an interflavonoid bond between them.

Eleven different procyanidins homotrimers B were identified and three of them (**71**, **72** and **73**) were chosen as markers (Table IV.16).

[288] Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Zhang, Z.; Beecher, G.; Holden, J.; Haytowitz, D.; Prior, R. L.; *Liquid chromatographic/electrospray ionization massspectrometric studies of proanthocyanidins in foods*, J. Mass spectrum. **2003**, 38, 1272-1280.

**Table IV.16.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of procyanidin homotrimers B. The numbers written in bold correspond to the compounds chosen as markers.

| Number | $t_R$ (min) | Compound | $[UMD]^-$ (m/z) |
|---|---|---|---|
| *121* | 20.8 | ((epi)catechin)$_3$ | 864.9 |
| *122* | 48.57 | ((epi)catechin)$_3$ | 865.1 |
| **72** | 54.60 | ((epi)catechin)$_3$ | 865.0 |
| *123* | 56.74 | ((epi)catechin)$_3$ | 864.9 |
| *124* | 57.22 | ((epi)catechin)$_3$ | 864.9 |
| **73** | 68.50 | ((epi)catechin)$_3$ | 864.9 |
| *125* | 69.98 | ((epi)catechin)$_3$ | 865.0 |
| **71** | 72.84 | PCC1 | 864.9 |
| *126* | 74.20 | ((epi)catechin)$_3$ | 865.1 |
| *127* | 76.51 | ((epi)catechin)$_3$ | 864.9 |
| *128* | 88.69 | ((epi)catechin)$_3$ | 865.1 |

The fragmentations observed in these compounds are similar to dimmers B (procyanidin homodimers B, prodelphinidin homodimers B and mixed dimers B). The molecular ion $[UMD]^-$ was observed in the spectrum at m/z 865.0. In addition, some fragment ions with higher intensities are those in which RDA fragmentation occurs ($[U(^{1,3}A\text{-}H_2O)MD]^-$ at m/z 694.9, $[U(^{1,3}A)MD(^{1,3}A\text{-}H_2O)]^-$at m/z 542.8 and $[M(^{1,3}A\text{-}H_2O)D]^-$ at m/z 406.9), so this type of fragmentation seems to be highly favoured.

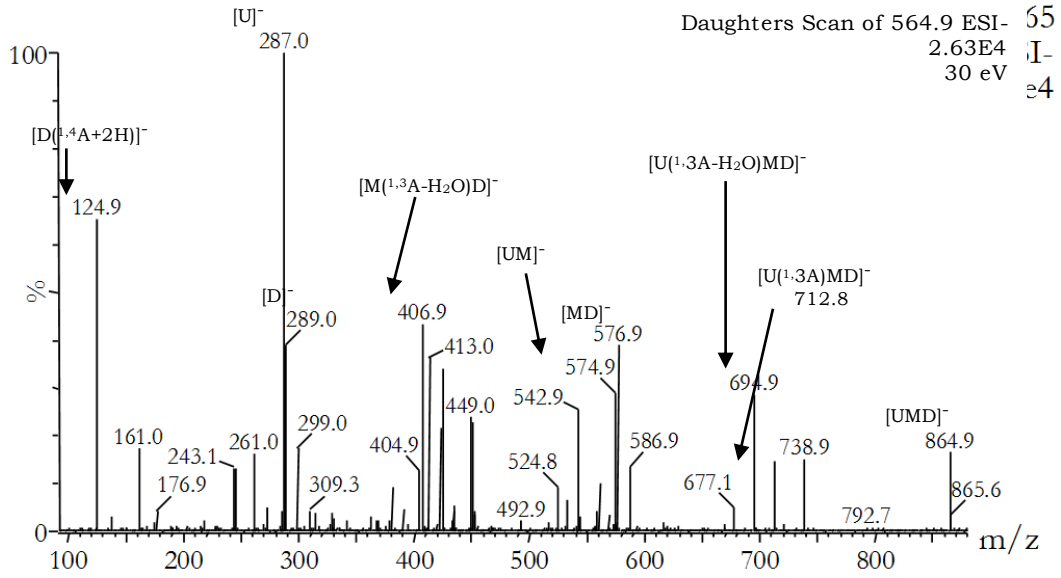



**Figure IV.38.** ESI(-)-$MS^2$ spectrum of procyanidins dimers B, obtained at a collision energy of 30 eV. Example: PCC1(**71**).

Fragment ions in which the rupture of interflavonoid bond occurs, have also higher intensities, giving the fragment ions $[MD]^-$ at m/z 576.9, $[UM]^-$ at m/z 574.9,

$[D]^-$ at m/z 289.0 and $[U]^-$ at m/z 287.0[291,292]. These fragmentations will help to elucidate which flaval-3-ol is the upper unit, the middle one and which one is at the bottom in mixed trimers. The mass spectrum in product ion scan mode for procyanidins homotrimers B can be seen in Figure IV.38.

## IV.2.6. Mixed trimers

The mixed trimers are formed by three monomers of flavan-3-ol which have different molecular weights, so they can be formed by two units of (epi)catechin and one (epi)gallocatechin or two units of (epi)gallocatechin and one (epi)catechin.

### a)Trimers with two units of (epi)catechin and one (epi)gallocatechin

There are three types of these trimers which depend on the positions of the flavan-3-ols: $((epi)catechin)_2$-(epi)gallocatechin, (epi)gallocatechin-$((epi)catechin)_2$ and (epi)catechin-(epi)gallocatechin-(epi)catechin (Table IV.17).

**Table IV.17.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of procyanidin heterotrimers with two units of (epi)catechin.

| Number | $t_R$ (min) | Compound | $[UMD]^-$ (m/z) |
|---|---|---|---|
| *129* | 14.03 | $((epi)catechin)_2$-(epi)gallocatechin | 880.8 |
| *130* | 38.90 | $((epi)catechin)_2$-(epi)gallocatechin | 880.7 |
| *131* | 40.27 | $((epi)catechin)_2$-(epi)gallocatechin | 880.9 |
| *132* | 53.61 | $((epi)catechin)_2$-(epi)gallocatechin | 880.9 |
| *133* | 61.20 | $((epi)catechin)_2$-(epi)gallocatechin | 881.1 |
| *134* | 11.75 | (epi)gallocatehin-$((epi)catechin)_2$ | 880.9 |
| *135* | 55.53 | (epi)gallocatehin-$((epi)catechin)_2$ | 880.8 |
| *136* | 20.33 | (epi)catechin-(epi)gallocatechin-(epi)catechin | 881.1 |
| *137* | 33.75 | (epi)catechin-(epi)gallocatechin-(epi)catechin | 881.1 |
| *138* | 46.59 | (epi)catechin-(epi)gallocatechin-(epi)catechin | 881.1 |
| *139* | 56.94 | (epi)catechin-(epi)gallocatechin-(epi)catechin | 880.8 |
| *140* | 63.26 | (epi)catechin-(epi)gallocatechin-(epi)catechin | 880.9 |

**$((epi)catechin)_2$-(epi)gallocatechin trimers**

Five trimmers have been detected (from *129* to *133*) as it can be seen in Table IV.17.

The mass spectrum of these compounds is characterized by fragments ions at m/z 592.9 and 574.8 which are the products of the rupture of the interflavanic bond.

[291] Li, H. J.; Deinzer, M. L.; *Tandem Mass Spectrometry for Sequencing Proanthocyanidins*, Anal. Chem. **2007**, 19, 1739-1748.

[292] Pati, S.; Losito, I.; Gambacorta, G.; La Ntte, E.; Palmisano, F.; Zambonin, P.G.; *Simultaneous separation and identification of oligomeric procyanidins and anthocyanin-derived pigments in raw red wine by HPLC-UV-ESI-$MS^n$*, J. Mass. Spectrom. **2006**, 41, 861-871.

The first one is $[MD]^-$ fragment in which the subunit U ((epi)catechin) is lost and the second one corresponds to the $[UM]^-$ fragment with the loss of the subunit D ((epi)gallocatechin). Then the subunit U and D are also detected at m/z 287.0 and 304.9, respectively.

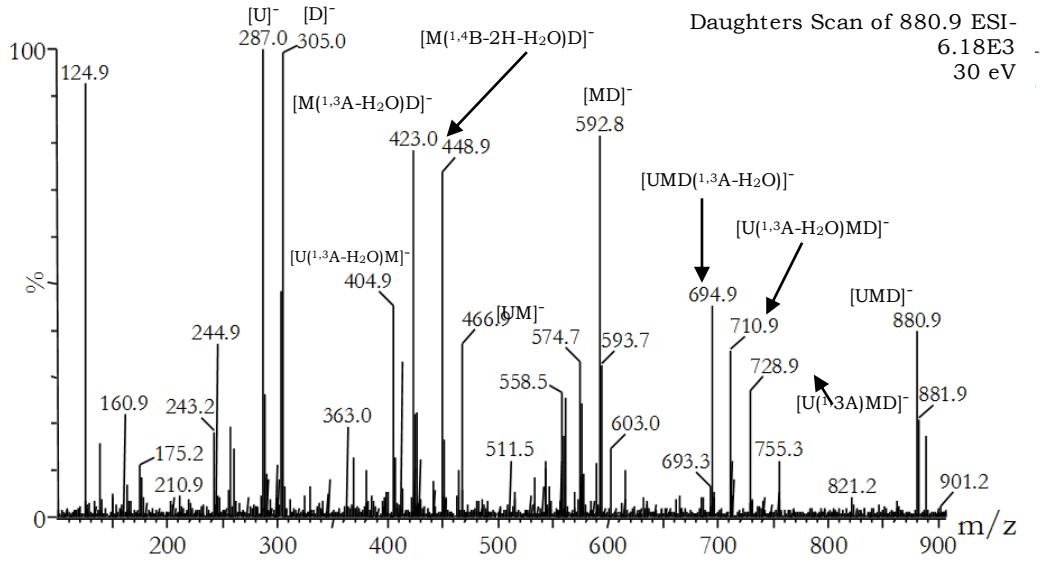



**Figure IV.39.** ESI(-)-$MS^2$ spectrum of procyanidins heterotrimers, obtained at a collision energy of 30 eV. Example: ((epi)catechin)$_2$-(epi)gallocatechin (*131*).

The remaining ions detected are the usual ones which were identified in the other compounds. The product ions of the RDA fragmentation are observed at m/z 729.1, 711.0, 560.9, 440.9, 423.0, 413.1 and 405.3.

The mass spectrum of these compounds is shown in Figure IV.39, using the compound *131* as example.

**(epi)gallocatechin-((epi)catechin)$_2$ trimers**

Two compounds of this family have been detected.

As in the previous compounds, the product ions $[UM]^-$, $[MD]^-$ are observed at 590.8 and 576.9, respectively. These fragments help with the elucidation of the position of the subunits. In addition, the fragments ion corresponding to the subunit $[U]^-$, $[M]^-$, $[M-2H]^-$ and $[D]^-$ are also observed. Although the fragment ions are the same as previous compounds, the relative abundances are different.

An example of the mass spectrum of these compounds is shown in Figure IV.40.

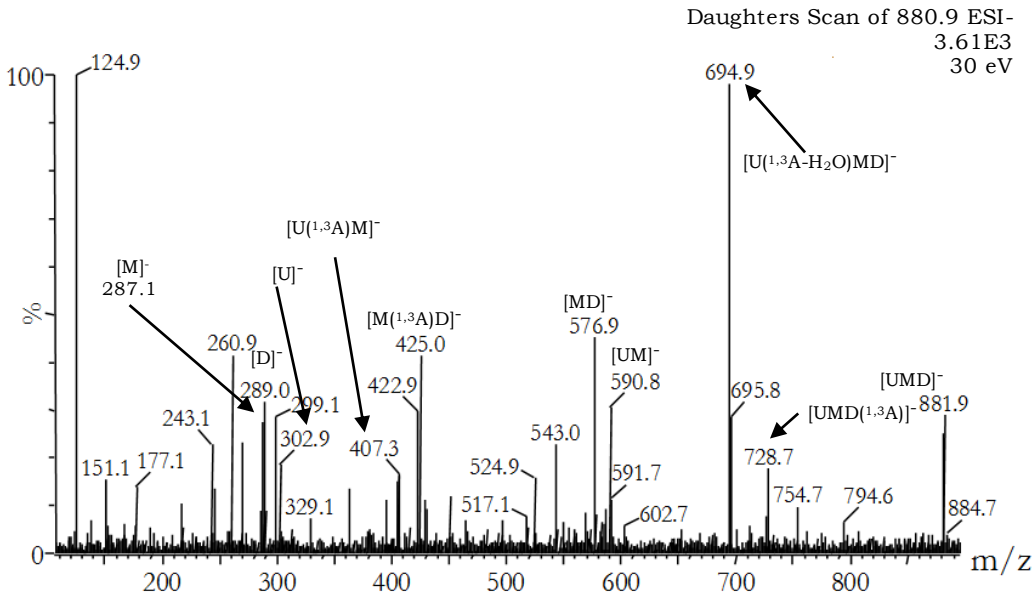



**Figure IV.40.** ESI(-)-MS² spectrum of procyanidins heterodimers, obtained at a collision energy of 30 eV. Example: (epi)gallocatechin-((epi)catechin)₂ (*134*).

**(epi)catechin-(epi)gallocatechin-(epi)catechin trimers**

Five trimers of this type were identified by our group.

The mass spectra of these compounds show the fragment ions $[MD]^-$ at m/z 593.2, $[UM]^-$ at 591.0 and $[M]^-$ at 302.7, which allow to classify these trimers as (epi)catechin-(epi)gallocat-(epi)catechin. The RDA fragmentation is also observed in the mass spectra. An example for these compounds is shown in Figure IV.41.

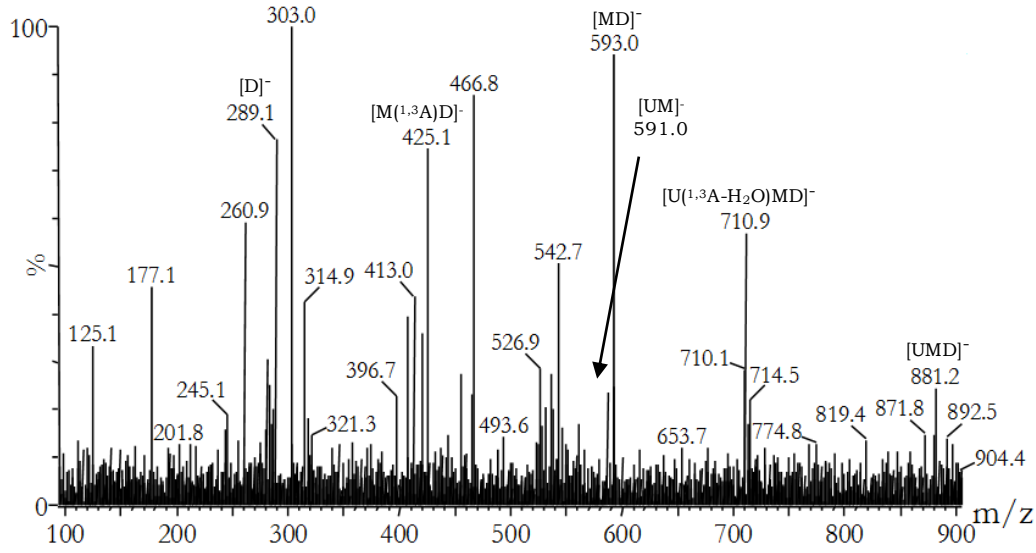



**Figure IV.41.** Mass spectrum ESI(-) of the product ions of procyanidins heterotrimers obtained in scan mode at 30 eV. Example: (epi)catechin-(epi)gallocatechin-(epi)catechin (*137*).

### b) Trimers with two units of (epi)gallocatechin and one (epi) catechin

Only two trimers with this structure (two (epi)gallocatechin and one (epi)catechin unit) have been detected (Table IV.18).

**Table IV.18.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of prodelphinidin heterotrimers with two units of (epi)gallocatechin.

| Number | $t_R$ (min) | Compound | $[UMD]^-$ (m/z) |
|---|---|---|---|
| *141* | 26.41 | ((epi)gallocatechin)$_2$-(epi)catechin | 897.2 |
| *142* | 30.86 | ((epi)gallocatechin-(epi)catechin-(epi)gallocatechin | 896.9 |

As in the previously mixed trimers, the fragments ion $[UM]^-$ and $[MD]^-$ are observed in the mass spectrum, so the two compounds can be distinguished.

On the one hand, the mass spectrum of compound *141* shows the RDA fragmentation (m/z 711) of the upper unit of (epi)gallocat, followed by a loss of $H_2O$, as the base peak. The same fragmentation on the lower unit is also intense.

On the other hand, the mass spectrum of compound *142* shows an unusual fragmentation that calls $[U(^{1,2}A\text{-}2H_2O)MD]^-$ at 723.2. In addition, the RDA fragmentation at 728.9 and 710.7 are also intense peaks.

The mass spectrum of these compounds could be seen in Figure IV.42 and IV.43.

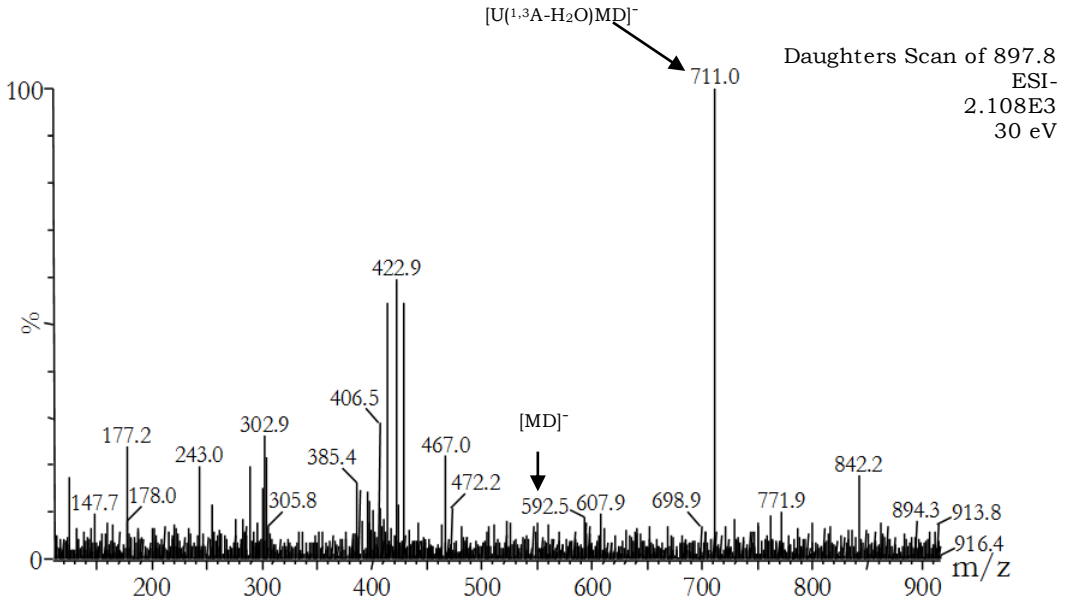


**Figure IV.42.** ESI(-)-MS² spectrum of compound *141*, obtained at a collision energy of 30 eV.

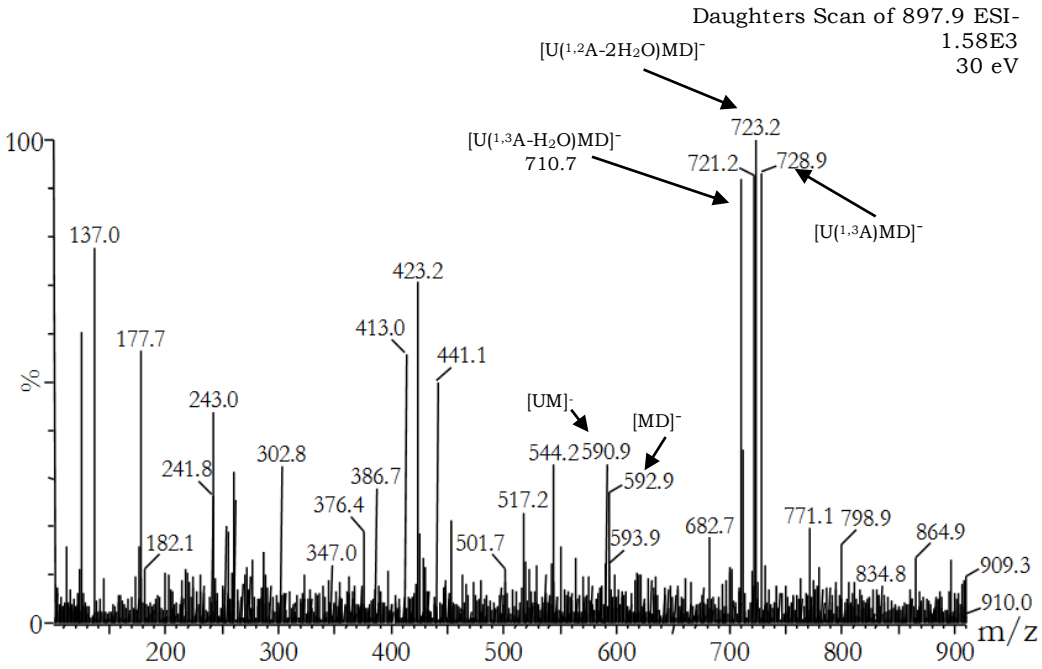



**Figure IV.43.** ESI(-)-MS² spectrum of compound *142*, obtained at a collision energy of 30 eV.

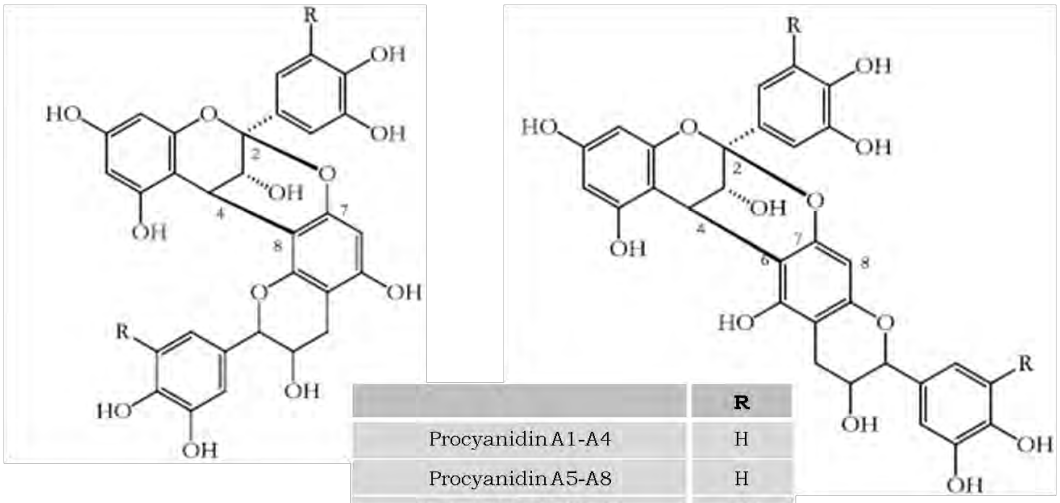
## IV.2.7. Procyanidins dimers A

These compounds, which are formed by two units of flavan-3-ol (catechin and/or epicatechin), have two different bonds, one A bond, which is an ether bond between carbons 2 and 7, and one B bond. This new A bond causes that compounds have 2 units less than its homologue with B bond (Figure IV.44).

Five different procyanidins A have been identified (Table IV.19).

**Table IV.19.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of procyanidins dimers A. The numbers written in bold correspond to the compounds chosen as markers.

| Number | $t_R$ (min) | Compound | $[UD]^-$ (m/z) |
|---|---|---|---|
| *143* | 15.20 | ((epi)catechin)₂ A | 574.7 |
| *144* | 20.64 | ((epi)catechin)₂ A | 574.7 |
| **74** | 55.99 | ((epi)catechin)₂ A | 574.9 |
| *145* | 76.52 | PCA | 575.0 |
| **75** | 85.01 | ((epi)catechin)₂ A | 574.7 |



| | R |
|---|---|
| Procyanidin A1-A4 | H |
| Procyanidin A5-A8 | H |
| Prodelphinidin A1-A4 | OH |
| Prodelphinidin A5-A8 | OH |

**Figure IV.44.** Chemical structure of procyanidins dimers A.

The ion at m/z 423.1, which has high intensity in the spectrum, is the result of the RDA rupture of C1-C3 bond in the C-ring of the subunit D. The rupture between C1-C2 bond in the C-ring is also observed which is followed by the loss of water molecule and a carbonyl group and keeping up the A-ring in the structure. This fragment corresponds to the ion at m/z 407.1.

Another fragmentation that could be observed in these compounds is the rupture between C1-C4 bond of the C-ring, in which the B-ring is conserved in the structure. In this case, it would be seen two ions, one of them at m/z 449.2 when the fragmentation occurs in U subunit and other at m/z 163.0 if the rupture is in the D subunit.

The rupture of the interflavonoid bonds is difficult and there is not enough studies about this in the bibliography. Some authors said that the fragment ions at m/z 285.1 and 289.1 in $MS^2$ spectra is clear[293,294]. The ion at 289.1 is the lower unit $[D]^-$ while the fragment at 285.1 is the upper one $[U]^-$. So this fragmentation is essential to elucidate which flavan-3-ol is the upper unit and which one is at the bottom as it could be seen in mixed dimers B. The mass spectrum can be seen in Figure IV.45, using ((epi)catechin)$_2$ A (**74**) as an example.

[293] Sarnoski, P. J.; Johnson, J. V.; Reed, K. A.; Tanko, J. M.; O´Keefe, S. F.; *Separation and characterization of proanthocyanidins in Virginia type peanut skins by LC-MS$^n$*, Food Chem. **2012**, 131, 927-939.

[294] Lu, W. H.; Huang, W. T.; Kumaran, A.; Ho, C. T.; Hwang, L. S.; *Transformation of Proanthocyanidin A2 to Its Isomers under Different Physiological pH Conditions and Common Cell Culture Medium*, J. Agric. Food Chem. **2011**, 59, 6214-6220.

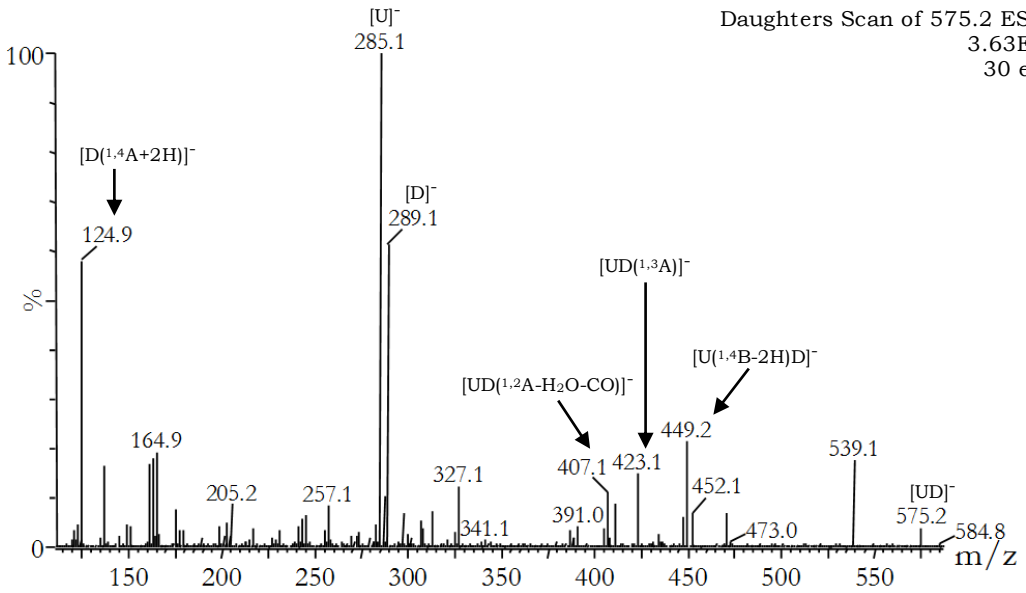



**Figure IV.45.** ESI(-)-MS² spectrum of procyanidins dimers A, obtained at a collision energy of 30 eV. Example: ((epi)catechin)₂ A (**74**).

## IV.2.8. Prodelphinidin dimers A

These compounds, which are formed by two units of flavan-3-ol (gallocatechin and/or epigallocatechin), have two different bonds, one A bond, which is an ether bond between carbons 2 and 7, and one B bond. This new A bond causes that compounds have 2 units less than its homologue with B bond.

Four different procyanidins dimers A have been identified by our group (Table IV.20).

**Table IV.20.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of prodelphinidins dimers A.

| Number | $t_R$ (min) | Compound | [UD]⁻ (m/z) |
|---|---|---|---|
| *146* | 51.03 | ((epi)gallocatechin)₂ A | 606.6 |
| *147* | 53.30 | ((epi)gallocatechin)₂ A | 606.9 |
| *148* | 55.28 | ((epi)gallocatechin)₂ A | 606.9 |
| *149* | 69.71 | ((epi)gallocatechin)₂ A | 606.9 |

In the mass spectra of these compounds the similarity of the prodelphinidins and procyanidins is observed. The most intense fragment is the rupture of the interflavanic bond [U]- at m/z 301.1, although the other fragment ion of the rupture of interflavanic bond [D-] is also observed at 305. The fragment corresponding to the

rupture of Retro Diels Alder at m/z 439.1 and 137.0, respectively, are also intense fragment in the product ion spectrum.

The mass spectrum in product ion scan mode for prodelphinidins dimers A can be seen in Figure IV.46.

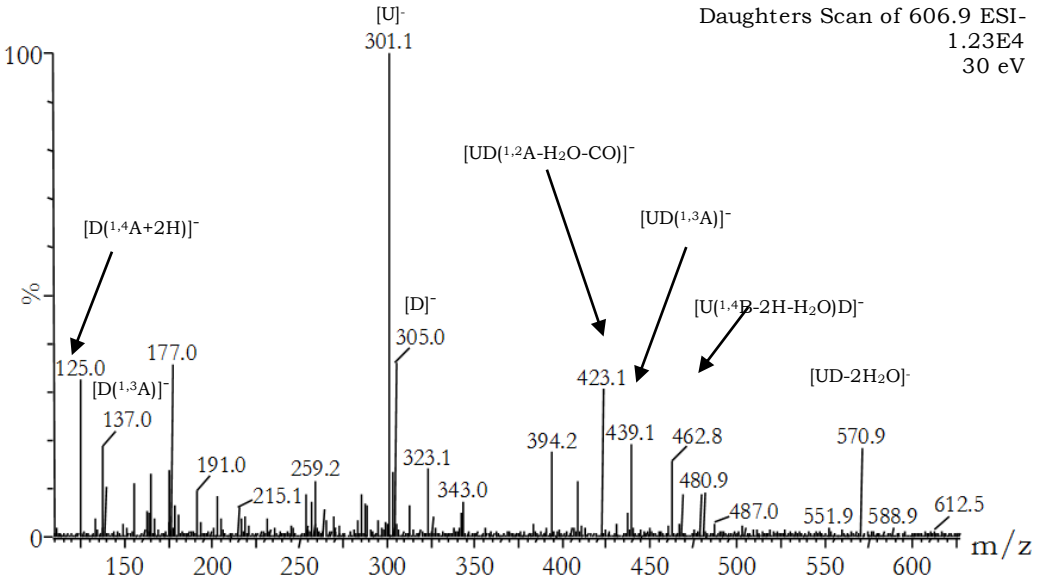



**Figure IV.46.** ESI(-)-MS$^2$ spectrum of prodelphinidins dimers A, obtained at a collision energy of 30 eV. Example: ((epi)gallocatechin)$_2$ A (*146*).

## IV.2.9. Mixed dimers A

As mixed dimers B, these compounds have two flavan-3-ols which have different molecular weights, so the A and B bonds occur between (epi)catechin and (epi)gallocatechin.

Two different mixed dimers A were identified and both have the (epi)gallocatechin as the top unit (Table IV.21).

**Table IV.21.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of mixed dimers A. The numbers written in bold correspond to the compounds chosen as markers.

| Number | $t_R$ (min) | Compound | [UD]$^-$ (m/z) |
|---|---|---|---|
| **76** | 67.13 | ((epi)gallocatechin-(epi)catechin)A | 591.2 |
| **77** | 72.88 | ((epi)gallocatechin-(epi)catechin)A | 591.0 |

The mass spectra of these compounds show the molecular ion [UD]$^-$ at m/z 590.9 and their fragmentation pattern is the same as procyanidins A.

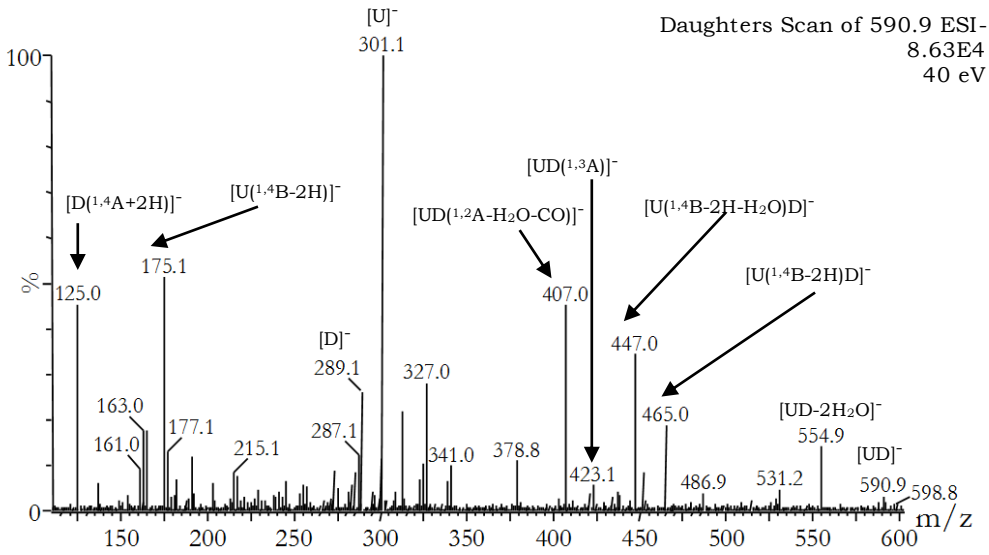
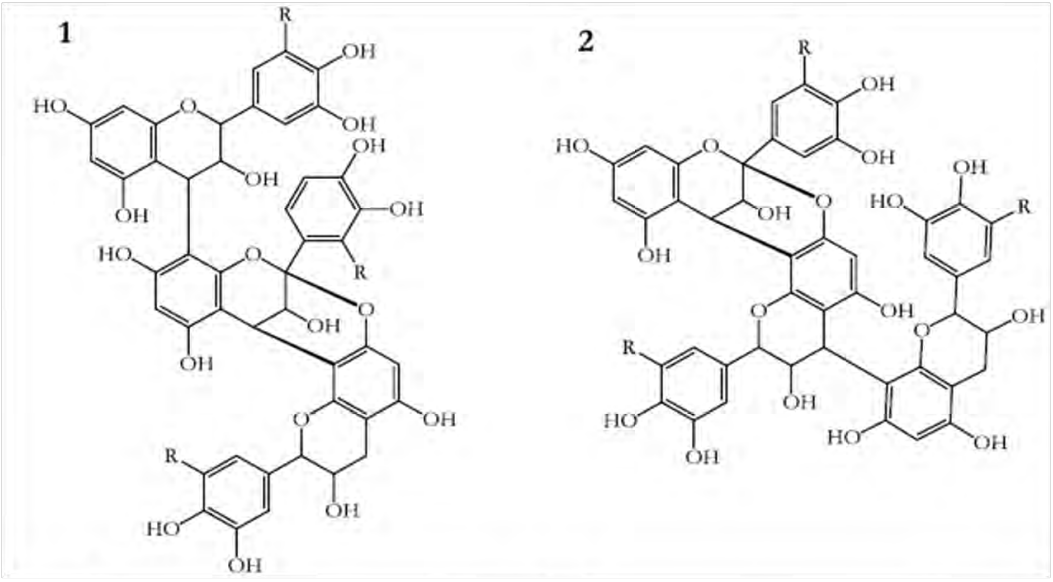



**Figure IV.47.** ESI(-)-MS² spectrum of mixed dimers A, obtained at a collision energy of 40 eV. Example: ((epi)gallocatechin-(epi)catechin) A 1 (**77**).

The rupture of the interflavonoid bond helps in the differentiation of which monomer is the upper unit and which one is at the bottom. If fragment ions at m/z 289.0 ($[D]^-$) and 301.0 ($[U]^-$) are more intense than ones at m/z 303.0 ($[D]^-$) and 285.0 ($[U]^-$), means that the upper unit is the (epi)gallocatechin, whereas if the second pair of ions are more intense than the first ones, then the (epi)catechin is the upper unit. The mass spectrum can be seen in Figure IV.47.

## IV.2.10. Mixed trimers with one A bond and one B bond

These compounds are formed by three monomers of flavan-3-ol which can be formed by two (epi)catechin units and one (epi)gallocatechin unit or one (epi)catechin unit and two (epi)gallocatechin units (Figure IV.48).




| 1 | R |
|---|---|
| (Epi)cat-((epi)cat)$_2$A | H |
| (Epi)gallocat-((epi)gallocat)$_2$A | OH |

| 2 | R |
|---|---|
| ((Epi)cat)$_2$A-(epi)cat | H |
| ((Epi)gallocat)$_2$A-(epi)gallocat | OH |

**Figure IV.48.** Chemical structure of mixed trimmers with A and B bond.

### a) Trimers formed by two units of (epi)catechin and one (epi)gallocatechin

There are six types of mixed trimers formed by two units of (epi)catechin and one of (epi)gallocatechin, which are differentiated by the distribution of the subunits and the position of the A bond.

1. ((epi)catechin)$_2$A-(epi)gallocatechin *(C-C)A-GC*
2. (epi)catechin)-((epi)catechin-(epi)gallocatechin)A *C-(C-GC)A*
3. (epi)gallocatechin-((epi)catechin)$_2$A *GC-(C-C)A*
4. ((epi)gallocatechin)-(epi)catechin)A-(epi)catechin)A (GC-C)A-C)
5. ((epi)catechin-(epi)gallocatechin)A-(epi)catechin *(C-GC)A-C*
6. (epi)catechin-((epi)gallocatechin-(epi)catechin)A *C-(GC-C)A*

These six types of mixed trimers can be differentiated by studying the fragment ions of the rupture of the interflavanoid bond (A or B bond).

A total of 13 tannins of this type have been detected (Table IV.22). One of them with the second structure C-(C-GC)A, six have the structure (GC-C)A-C and another six compounds with the structure C-(GC-C)A.

**Table IV.22.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of mixed trimers A with two units of (epi)catechin. The numbers written in bold correspond to the compounds chosen as markers.

| | **Number** | **$t_R$ (min)** | **Compound** | **[UMD]$^-$ (m/z)** |
|---|---|---|---|---|
| *C-(C-GC)A* | *150* | 37.75 | (epi)catechin-((epi)catechin-(epi)gallocatechin) A | 878.8 |
| *(GC-C)A-C* | **78** | 42.57 | ((epi)gallocatechin-(epi)catechin)A-(epi)catechin | 878.9 |
| | *151* | 46.25 | ((epi)gallocatechin-(epi)catechin)A-(epi)catechin | 878.9 |
| | *152* | 52.68 | ((epi)gallocatechin-(epi)catechin)A-(epi)catechin | 878.80 |
| | *153* | 55.23 | ((epi)gallocatechin-(epi)catechin)A-(epi)catechin | 878.9 |
| | *154* | 58.43 | ((epi)gallocatechin-(epi)catechin)A-(epi)catechin | 878.8 |
| | *155* | 69.23 | ((epi)gallocatechin-(epi)catechin)A-(epi)catechin | 879.0 |
| *C-(GC-C)A* | **79** | 48.53 | (epi)catechin-((epi)gallocatechin-(epi)catechin)A | 878.9 |
| | *156* | 64.26 | (epi)catechin-((epi)gallocatechin-(epi)catechin)A | 878.8 |
| | *157* | 71.10 | (epi)catechin-((epi)gallocatechin-(epi)catechin)A | 878.9 |
| | *158* | 75.40 | (epi)catechin-((epi)gallocatechin-(epi)catechin)A | 878.8 |
| | *159* | 76.87 | (epi)catechin-((epi)gallocatechin-(epi)catechin)A | 879.0 |
| | *160* | 83.93 | (epi)catechin-((epi)gallocatechin-(epi)catechin)A | 878.9 |

The mass spectra of the tannins with C-(C-GC)A structure shows the three fragment ions of the rupture of interflavanoid bond, [MD]-, [UM+2H]- and [UM]- at m/z 590.8, 574.7 and 572.7, respectively. The fragmentations of RDA are also identified, being the ion [M($^{1,3}$A)D] the base peak at m/z 439.2. The mass spectrum for these compounds can be seen in Figure IV.49.

The mass sprectra of mixed trimers *151-154* and **78**-**79**, which have the structure (GC-C)A-C, shows the fragments ions [UM]$^-$, [MD]$^-$ and [MD-2H]$^-$ at m/z 589.0, 576.8 and 574.8, respectively. Other fragment ions detected are those from the ruptures $^{1,3}$A, $^{1,2}$A and $^{0,4}$B, following by the loss of water and CO. An example of mass spectrum can be observed in Figure IV.50.

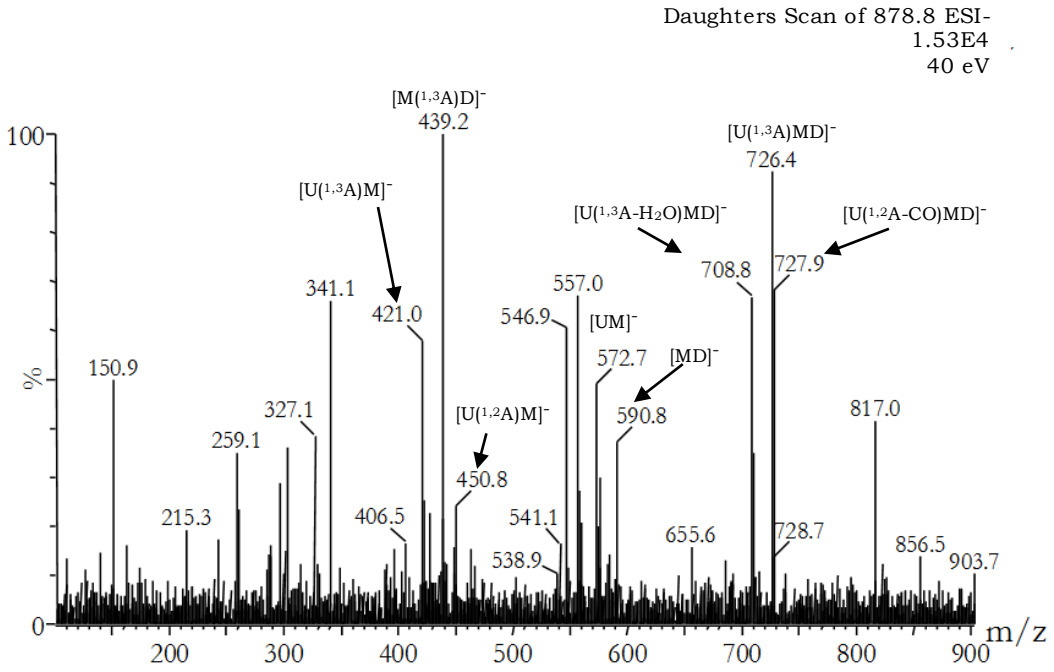



**Figure IV.49.** ESI(-)-MS² spectrum of mixed trimers with a structure C-(C-GC)A (*150*), obtained at a collision energy of 40 eV.

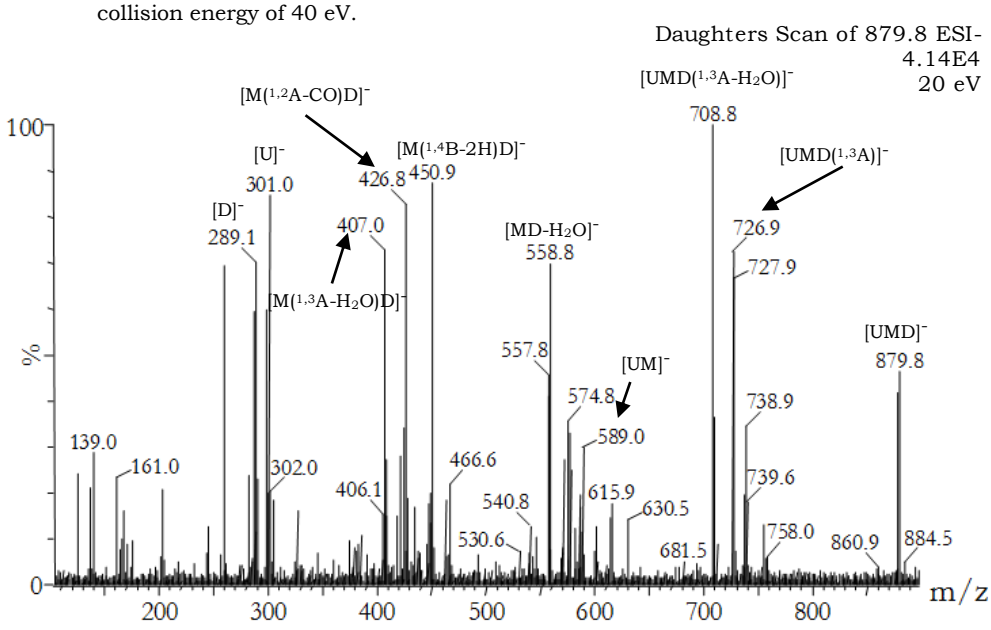



**Figure IV.50.** ESI(-)-MS² spectrum of mixed trimers with a structure (GC-C)A-C (**78**), obtained at a collision energy of 20 eV.

Finally, for the last six mixed trimers with the structure C-(GC-C)A, the fragment ions $[MD]^-$ or $[UM+2H]^-$ at m/z 590.9, $[UM]^-$ at 588.8, $[M]^-$ at 301.0, $[D]^-$ at 289.1 and $[U]^-$ at 287.1 are detected in the mass spectrum (Figure IV.51). The fragment ion $[MD]^-$ is the base peak for these trimers, except for the compounds *155* and *158*, in which the fragment ion $[U(^{1,3}A\text{-}H_2O)MD]^-$ is the most intense one.

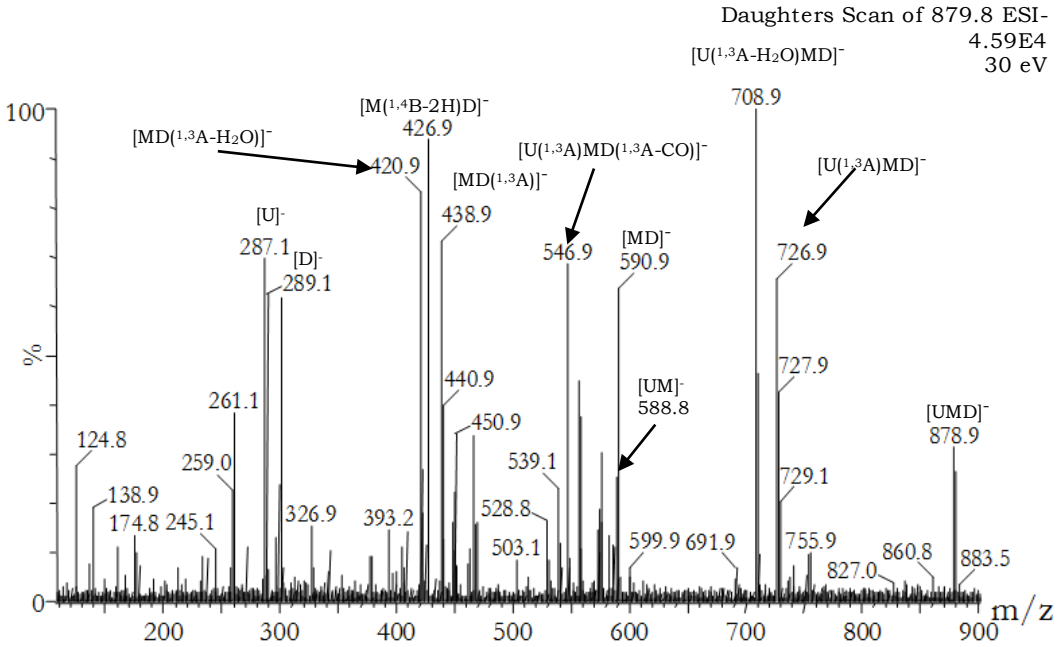



**Figure IV.51.** ESI(-)-MS² spectrum of mixed trimers with a structure C-(GC-C)A (79), obtained at a collision energy of 30 eV.

### b) Trimers formed by two units of (epi)gallocatechin and one (epi)catechin

As in the previous compounds, there are six types of mixed trimers which are differentiated by the distribution of the subunits and the position of the A bond (Table IV.23).

**Table IV.23.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of mixed trimers A with two units of (epi)gallocatechin. The numbers written in bold correspond to the compounds chosen as markers.

| Number | $t_R$ (min) | Compound | [UMD]⁻ (m/z) |
|---|---|---|---|
| *161* | 35.01 | ((epi)catechin-(epi)gallocatechin)A-(epi)gallocatechin | 894.9 |
| *162* | 44.29 | (epi)catechin-((epi)gallocatechin-(epi)gallocatechin)A | 894.8 |
| *163* | 68.94 | (epi)gallocatechin-((epi)catechin-(epi)gallocatechin)A | 894.8 |

3 tannins of this type have been detected, one of them with the structure (C-GC)A-GC, another one have the structure C-(GC-GC)A and the last compound with the structure GC-(C-GC)A.

The fragment corresponding to the rupture of B bond, which is easier to break than A bond, is detected with high intensity at m/z 588.8 for compound *161*, at 590.9 for compound *162* and at 606.8 for the last mixed trimer (*163*). Other fragment ions

that are also observed in these trimers are those which correspond to the RDA, $^{1,2}$A and $^{1,4}$B rupture. An example of mass spectrum can be observed in Figure IV.52.

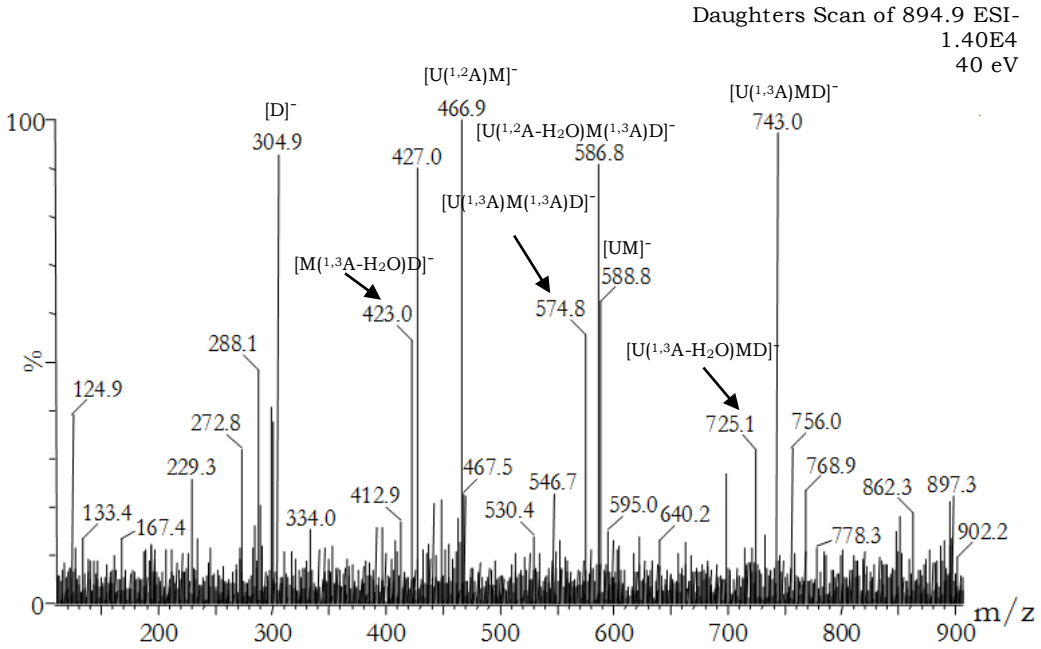



**Figure IV.52.** ESI(-)-MS$^2$ spectrum of mixed trimmers, with two units of (epi)gallocatechin and a (epi)catechin (*162*), obtained at a collision energy of 40 eV.

## IV.2.11. Ethylidene bridged tannins

As explained in the introduction, these tannins formation occurs by a nucleophilic addition acid-catalyzed, forming an ethylidene bridge between two or more flavan-3-ols that take part in it. If the reaction only occur with the catechin diasteromer, four different isomers could be formed, according to the C6 or C8 involved positions of the A ring: cat-6-et-6-cat, cat-8-et-8-cat and cat-6-et-8-cat R and S, these latters due to the asymmetric carbon of the ethylidene bridge[295,296,297]. From the epicatechin isomer, they may form other four and another four if the two diasteromers enter into reaction. In Figure IV.53, structures for these compounds are shown.

[295] Flamini, R.; *Mass Spectrometry in Grape and Wine Chemistry. Part I,* Polyphenols, Mass Spectrom. Reviews **2003**, 22, 218-250.

[296] Fulcrand, H.; Doco, T.; Es-Safi, N. E.; Cheynier, V.; Moutounet, M.; *Study of the acetaldehyde induced polymerization of flavan-3-ols by liquid chromatography-ion spray mass spectrometry*, J. Chrom. A. **1996**, 752, 85-91.

[297] Drinkine, J.; Lopes, P.; Kennedy, J. A.; Teissedre, P. L.; Saucier, C.; *Ethylidene-Bridged Flavan-3-ols in Red Wine and Correlation with Wine Age*, J. Agric. Food Chem. **2007**, 55, 6292-6299.

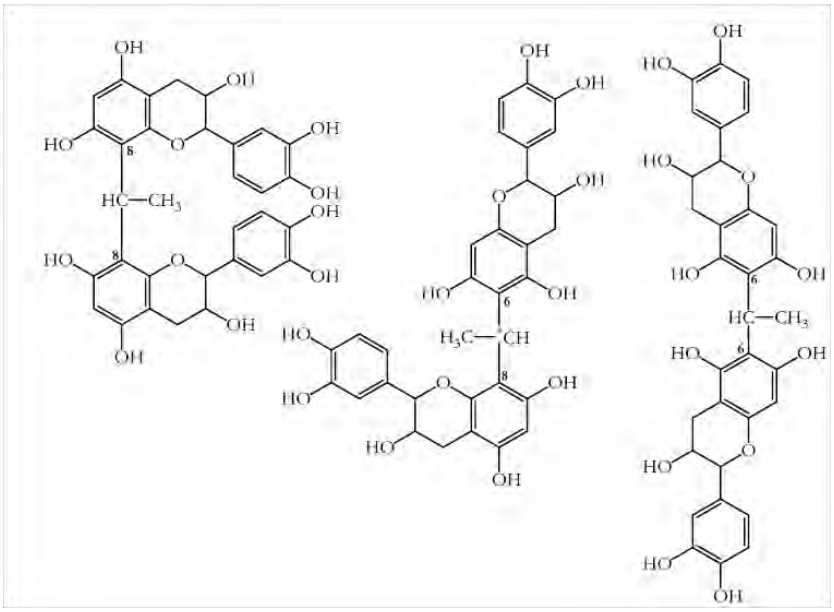


**Figure IV.53.** Epicatechin dimers formed by indirect condensation mediated by acetaldehyde.(*: asymmetric carbon of the ethylidene bridge).

Nine tannins of this type were detected, consisting of (epi)catechin monomers and there was not detected any which contain (epi)gallocatechin (Table IV.24).

**Table IV.24.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of tannins mediated by acetaldehyde. The numbers written in bold correspond to the compounds chosen as markers.

| **Number** | **$t_R$ (min)** | **Compound** | **[C-et-C]$^-$ (m/z)** |
|---|---|---|---|
| *164* | 57.65 | (epi)catechin-ethyl-(epi)catechin | 605.0 |
| *165* | 64.99 | (epi)catechin-ethyl-(epi)catechin | 605.2 |
| *166* | 78.10 | (epi)catechin-ethyl-(epi)catechin | 605.0 |
| *167* | 83.54 | (epi)catechin-ethyl-(epi)catechin | 605.0 |
| *168* | 84.47 | (epi)catechin-ethyl-(epi)catechin | 605.0 |
| *169* | 90.32 | (epi)catechin-ethyl-(epi)catechin | 605.0 |
| *170* | 96.06 | (epi)catechin-ethyl-(epi)catechin | 605.1 |
| *171* | 97.93 | (epi)catechin-ethyl-(epi)catechin | 605.2 |
| *172* | 99.03 | (epi)catechin-ethyl-(epi)catechin | 605.0 |

In contrast with the fragmentation studies of the other compounds, it is not possible to differentiate in which subunit of flaval-3-ol has occurred the rupture because the product ions are symmetrical when the ethylidene bridge is between positions C6-C6 or C8-C8. For this reason, the nomenclature *C-et-C* indicates the

tannin (epi)catechin-ethyl-(epi)catechin, *νC* is used for the product ion of the loss of a (epi)catechin unit and *C* for the complementary ion of (epi)catechin.

Most of the tannins mediated by acetaldehyde present the fragmentation ion of the rupture of ethyl bridge, which has the vinyl group (*[νC]-*), as the most intensive fragment at m/z 314.9. In addition, the complementary ion (*[C]-*) at m/z 289.0 is also detected which high intensity.

Other fragmentations which were observed in these tannins, were the RDA rupture that may occur in one subunit (m/z 452.9) and the loss of B-ring (fragmentation has already described for dimers), which is only detected in two tannins mediated by acetaldehyde (*166* and *172*). An example of mass spectrum can be observed in Figure IV.54

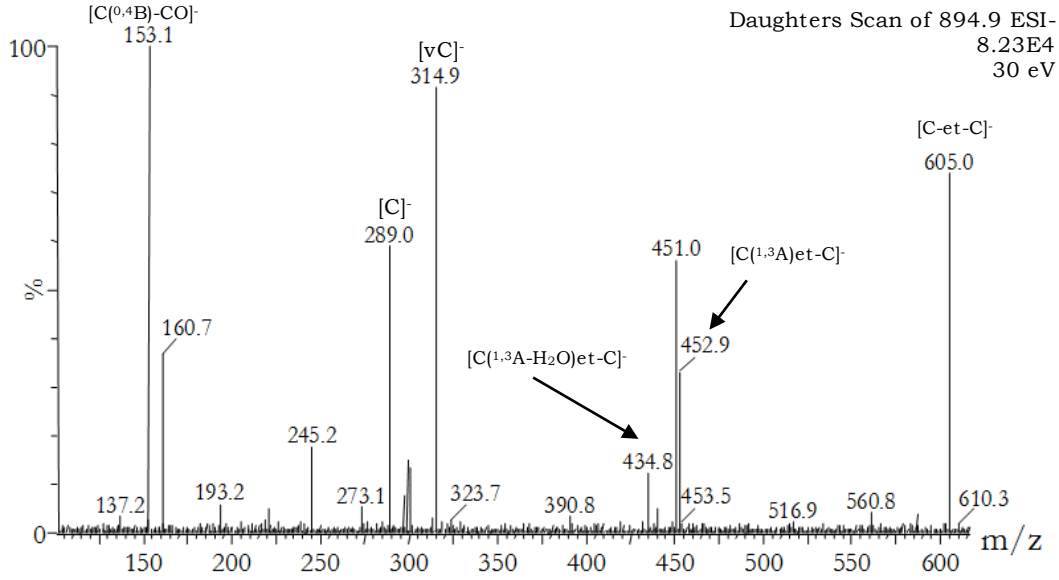
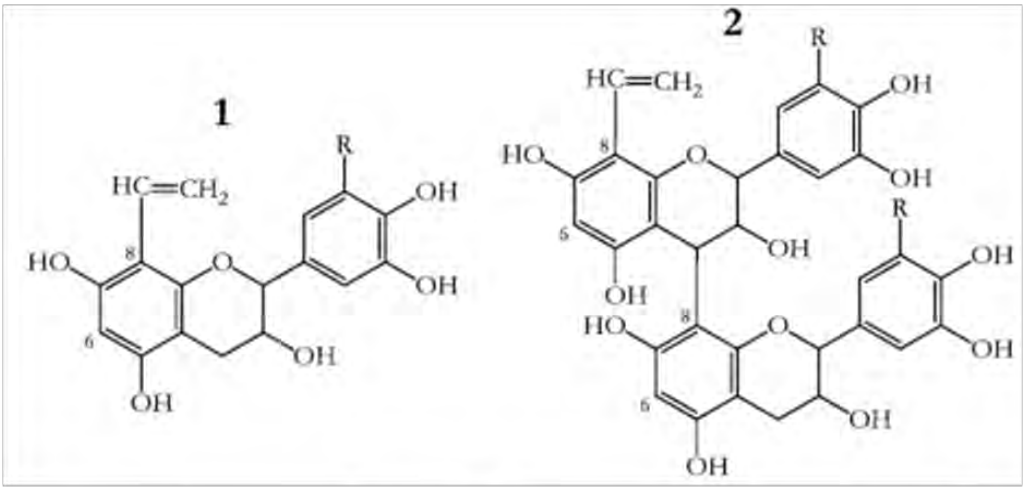



**Figure IV.54.** ESI(-)-MS² spectrum of tannins mediated by acetaldehyde (*164*), obtained at a collision energy of 30 eV.

## IV.2.12. p-Vinyl tannins

The vinyl-(epi)catechins and vinyl-(epi)gallocatechins are products of the depolymerisation of ethylidene-bridged tannins or can also be formed by the dehydration of the acetaldehyde in the flavan-3-ol condensation[298]. Furthermore, it is also possible to form vinyl-procyanidins and vinyl-prodelphinidins, as well as mixed dimers. The general structure for these tannins is shown in Figure IV.55.

[298] Drinkine, J.; Lopes, P.; Kennedy, J. A.; Teissedre, P. L.; Saucier, C.; *Ethylidene-Bridged Flavan-3-ols in Red Wine and Correlation with Wine Age*, J. Agric. Food Chem. **2007**, 55, 6292-6299.




| 1 | R |
|---|---|
| Vinyl-(epi)catechin | H |
| Vinyl-(epi)gallocatechin | OH |

| 2 | R |
|---|---|
| Vinyl-procyanidin | H |
| Vinyl-prodelphinidin | OH |

**Figure IV.55.** Vinyl-flavan-3-ols and vinyl-dimers chemical structures.

### a) Vinyl flavan-3-ols

Four vinyl flavan-3-ols which only have (epi)catechin units, have been detected (Table IV.25).

**Table IV.25.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of vinyl flavan-3-ols. The numbers written in bold correspond to the compounds chosen as markers.

| Number | $t_R$ (min) | Compound | [v-C]$^-$ (m/z) |
|---|---|---|---|
| **80** | 87.31 | Vinyl-(epi)catechin | 315.0 |
| **81** | 92.54 | Vinyl-(epi)catechin | 315.1 |
| **82** | 95.95 | Vinyl-(epi)catechin | 315.0 |
| *173* | 105.90 | Vinyl-(epi)catechin | 315.0 |

The mass spectrum of these compound shows the fragment ion of the RDA rupture at m/z 163.1 (when the rupture occurs in ring B) and at 151.0 (when the rupture is in ring A). This rupture appears to be highly favoured because the fragment ions have high intensities. What is more, the base peak corresponds to the fragment ion at m/z 151.0.

The remaining fragments observed are those that have usually been detected in the other tannins.

The mass spectrum for these tannins is shown in Figure IV.56.

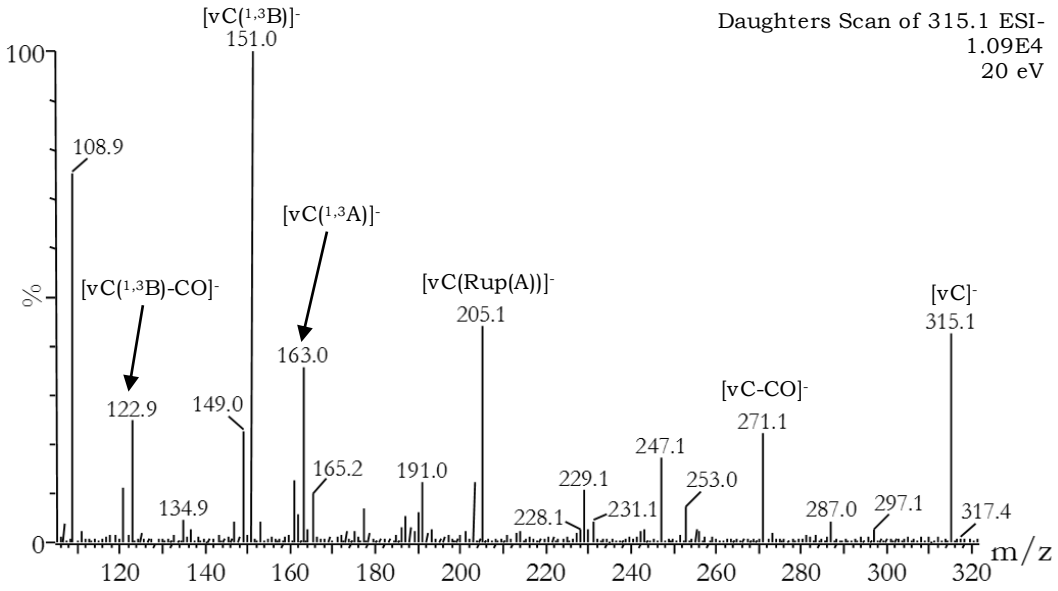



**Figure IV.56.** ESI(-)-MS² spectrum of vinyl flan-3-ols (**80**), obtained at a collision energy of 20 eV.

### b) Vinyl-procyanidines

Vinyl-procyanidines may have multiple structures because of the different positions of the interflavanic bonds (4-8, 4-6, 6´-8 and 6´-6) and the vinyl group which can be bond to the upper or lower unit of (epi)catechin as well as position 8 or 6 of that unit. With the information from MS/MS is possible to differentiate if the vinyl group is bonded to the upper or lower subunit. However, it can not be distinguished if it is bonded to the position 8 or 6 of the (epi)catechin unit.

Three tannins of this type have been found (Table IV.26) in which the vinyl group is the upper subunit (vinyl-(epi)catechin-(epi)catechin).

As shown in Figure IV.57, the product ions at m/z 313.1 and 289.1 are those that correspond to the rupture of interflavanic bond, which demonstrate that the vinyl group is the upper subunit.

**Table IV.26.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of vinyl procyanidines.

| Number | $t_R$ (min) | Compound | [v-UD]- (m/z) |
|---|---|---|---|
| *174* | 73.89 | Vinyl-(epi)catechin-(epi)catechin | 603.0 |
| *175* | 76.52 | Vinyl-(epi)catechin-(epi)catechin | 602.8 |
| *176* | 86.71 | Vinyl-(epi)catechin-(epi)catechin | 603.0 |

The fragmentation ions of RDA ruptures are also observed in the mass spectra at m/z 451.0, 299.0 and 151.1 and the remaining fragments observed are those that have usually been detected in the other tannins.

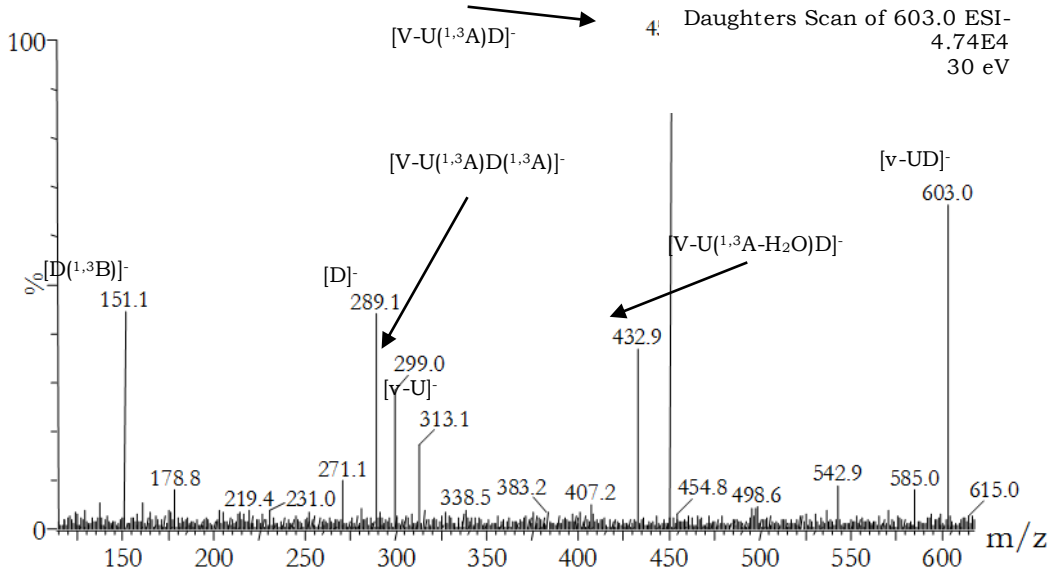



**Figure IV.57.** ESI(-)-MS$^2$ spectrum of vinyl-procyanidines (*174*), obtained at a collision energy of 30 eV.

## IV.2.13. Tannins formed by condensation mediated by furanic aldehydes

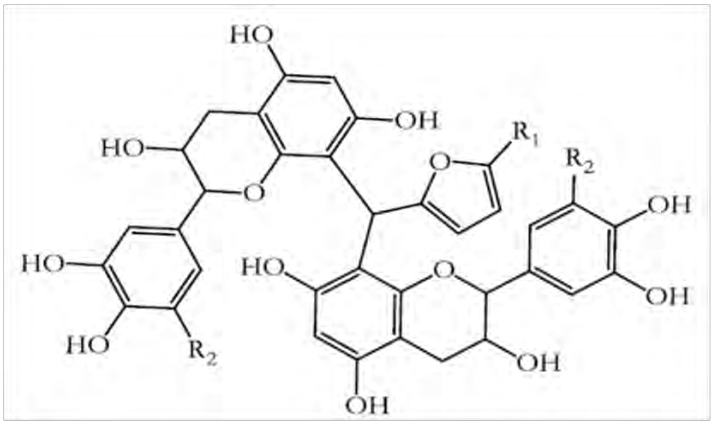
As in the ethylidene-bridged tannins, these tannins formation occurs by nucleophilic addition acid-catalyzed between the furan aldehydes and flavan-3-ols, forming a furfuryl bridge or hydroxymethylfurfuryl (HMF) bridge[299] between the two or more flavan-3-ols that take part. Such compounds have already been detected in wine and it is believed that they come from the oak wood[300,301]. If the reaction would only occur with a single type of diastereoisomer, for example the catechin, four different isomers may form: cat-6-fur-6-cat, cat-8-fur-8-cat and cat-6-fur-8-cat R and S, due to the asymmetric carbon of the furfuryl bridge in the latter case. Figure IV.58 shows the

[299]Nonier Bourden, M. F.; Vivas, N.; Absalon, C.; Vitry, C.; Fouquet, E.; Vivas de Gaulejac, N.; *Structural diversity of nucleophilic adducts from flavanols and oak wood aldehydes*, Food Chem. **2008**, 107, 1494-1505.

[300]Nonier, M. F.; Pianet, I.; Laguerre, M.; Vivas, N.; Vivas de Gaulejac, N.; *Condensation products derived from flavan-3-ols oak Wood aldehydes reaction. 1. Structural investigation*, Anal. Chem. Acta. **2006**, 76-83.

[301]Nonier, M. F.; Vivas, N.; Vivas de Gaulejac, N.; Absalon, C.; Vitry, C.; *Study by LC/ESI/MSn and ESI/HR/MS of $SO_2$ interactions in flavanols–aldehydes nucleophilic reactions*, Food Chem. **2010**, 122, 488-494.

general structure of these compounds. With the information from MS/MS is not possible to distinguish between the different bond positions from the bridge to flavan-3-ol units.



| | R1 | R2 |
|---|---|---|
| (epi)cat-furfuryl-(epi)cat | H | H |
| (epi)gallocat-furfuryl-(epi)gallocat | H | OH |
| (epi)cat-Hydroxymethylfurfuryl-(epi)cat | $CH_2OH$ | H |
| (epi)gallocat-Hydroxymethylfurfuryl-(epi)gallocat | $CH_2OH$ | OH |

**Figure IV.58.** Chemical structures for tannins with furfuryl or hydroxyfurfuryl bridge (with the bridge in positions 8,8).

### a) Procyanidins dimers with furfuryl bridge

Four tannins with the structure (epi)catechin-furfuryl-(epi)catechin have been found (Table IV.27).

As with ethylene tannins, it is not possible to differentiate in which subunit of flaval-3-ol has occurred the rupture because the product ions are symmetrical when the furfuryl bridge is between positions C6-C6 or C8-C8. For this reason, the nomenclature *C-f-C* indicates the tannin (epi)catechin-furfuryl-(epi)catechin, [*f-C*] is used for the product ion of the loss of a (epi)catechin unit and *C* for the complementary ion of (epi)catechin.

**Table IV.27.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of procyanidins dimers with furfuryl bridge. The numbers written in bold correspond to the compounds chosen as markers.

| Number | $t_R$ (min) | Compound | [C-f-C]$^-$ (m/z) |
|---|---|---|---|
| **83** | 31.10 | (epi)catechin-furfuryl-(epi)catechin | 656.9 |
| **84** | 45.22 | (epi)catechin-furfuryl-(epi)catechin | 656.9 |
| *177* | 51.49 | (epi)catechin-furfuryl-(epi)catechin | 656.8 |
| *178* | 65.40 | (epi)catechin-furfuryl-(epi)catechin | 656.8 |

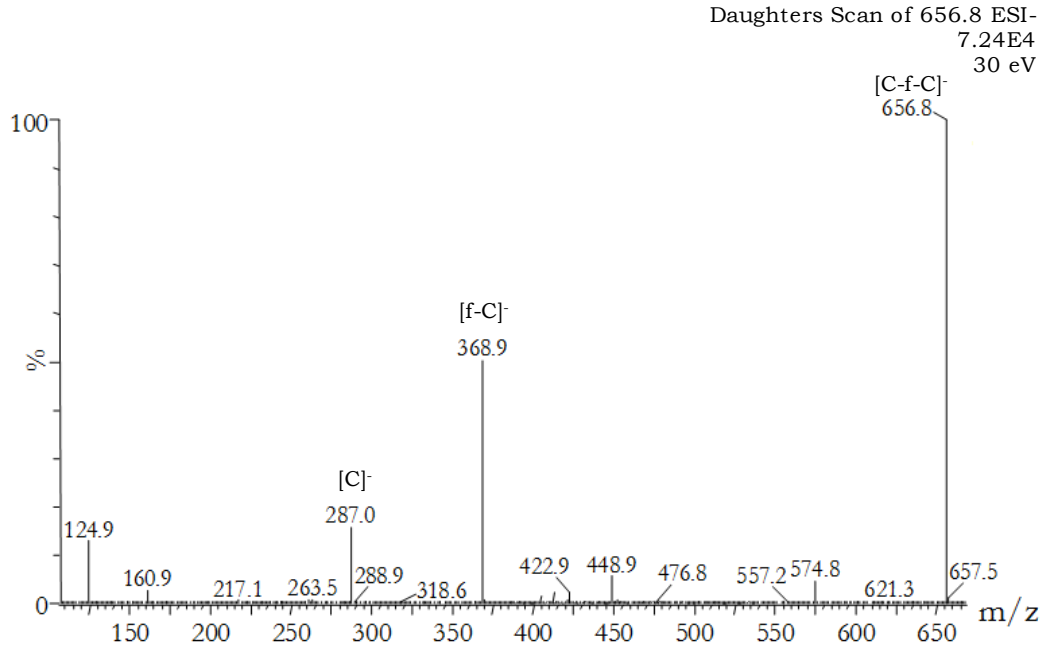



**Figure IV.59.** ESI(-)-MS² spectrum of procyanidins dimers with furfuryl bridge (**83**), obtained at a collision energy of 30 eV.

The fragments ions observed in the mass spectra (Figure IV.59) for these tannins are the most important ones, which are the rupture of furfuryl bridge (m/z 369.0) and the detection of a (epi)catechin unit (m/z 287.0).

### b) Prodelphinidins dimers with furfuryl bridge

One tannin with the structure (epi)gallocatechin-furfuryl-(epi)galocatechin has been found (Table IV.28).

**Table IV.28.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of prodelphinidins dimers with furfuryl bridge.

| Number | $t_R$ (min) | Compound | [GC-f-GC]- (m/z) |
|---|---|---|---|
| *179* | 18.76 | (epi)gallocatechin-furfuryl-(epi)gallocatechin | 688.8 |

The mass spectrum of this compound is similar as procyanidins dimers with furfuryl bridge (Figure IV.60). The most intensive ions are those which came from the rupture of furfuryl bridge at m/z 384.9 and the detection of (epi)gallocatechin unit at m/z 302.9.

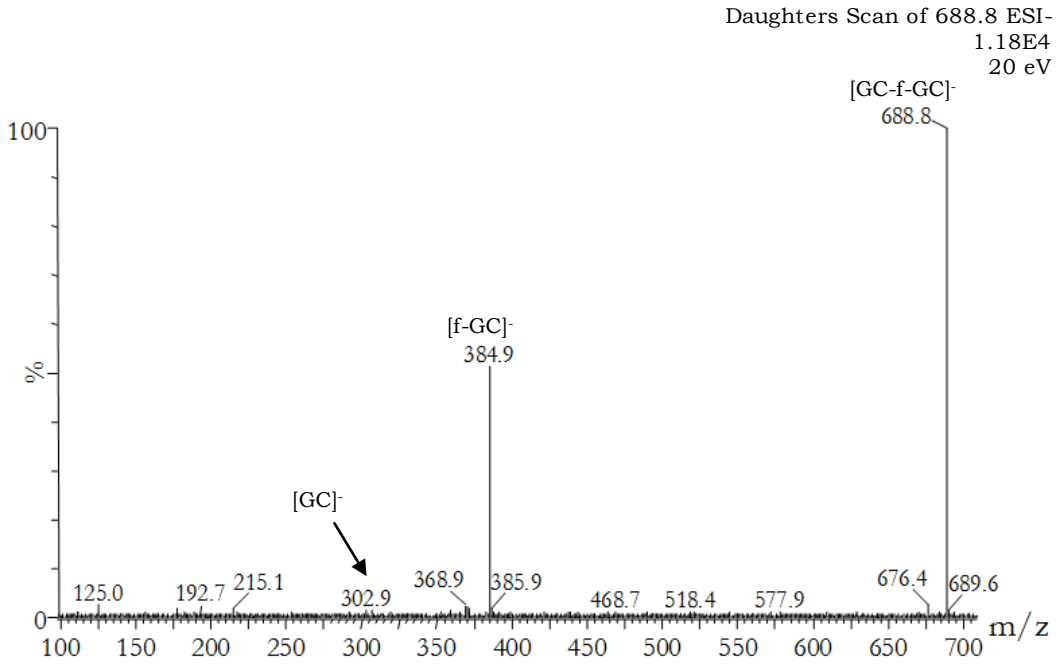



**Figure IV.60.** ESI(-)-MS$^2$ spectrum of prodelphinidins dimers with furfuryl bridge (*179*), obtained at a collision energy of 20 eV.

## IV.2.14. O-glycosylated tannins

There is few studies about the detection of O-glycosylated flavan-3-ols in wine, but this type of tannins exist in the nature, in the wood of some trees[302] and in some plants[303,304,305] and even in the cocoa liquor[306].

Two compounds were detected whose m/z ratio is 451.2 (Table IV.29). This mass agrees with an (epi)catechin attached to an hexose such as glucose, galactose or mannose, sugars that are present in the wine[312,313] as free form and bound to other compounds forming glycosides, such as anthocyanins. The differentiation between hexoses and determining the bond position between the hexose and the flavan-3-ol

[302]Bekker, M.; Bekker, R.; Brandt, V. E.; *Two flavonoid glicosides and a miscellaneous flavan from the bark of Guibourtia colesperma*. Phytochem. **2006**, 67, 818-823.

[303]Lokvan, J.; Coley, P. D.; Kursar, T. A.; *Cinnamoyl glucosides of catechin and dimeric procyanidins fromyoung leaves of Inga umbellifera (Fabaceae)*, Phytochem. **2004**, 65, 351-358.

[304]Karioti, A.; Bilia, A. R.; Gabbiani, Messori, L.; Skaltsa, H. *Proanthocyanidin glycosides from the leaves of Quercus ilex L. (Fagaceae)*. Tetrahedron Let. **2009**, 50, 1771-1776.

[305]Friedrich, W.; Galensa, R. *Identification of a new flavanol glucoside from barley (Hordeum vulgare L.) and malt*. Eur. Food Res. Technol. **2002**, 214, 388-393.

[306]Hatano, T.; Miyatake, H.; Natsume, M.; Osakabe, N.; Takizawa, T.; Ito, H.; Yoshida, T.; *Proanthocyanidins glycosides and related polyphenols from cacao liquor and their antioxidant effects*, Phytochem. **2002**, 59, 749-758.

[312]Vidal, S.; Williams, P.; Doco, T.; Moutounet, M.; Pellerin, P.; *The polysaccharides of red wine: total fractionation and characterization*, Carbohyd. Polym. 54 **2003**, 439-447.

[313]Riou, V.; Vernhet, A.; Doco, T.; Moutounet, M.; *Aggregation of grape seed tannins in model wine-effect of wine polysaccharides*, Food Hydrocolloids. **2002**, 16, 17-23.

require the use of NMR. In literature, these tannins have been found together through an ether link at positions 7 and 3, as shown in Figure V.61.

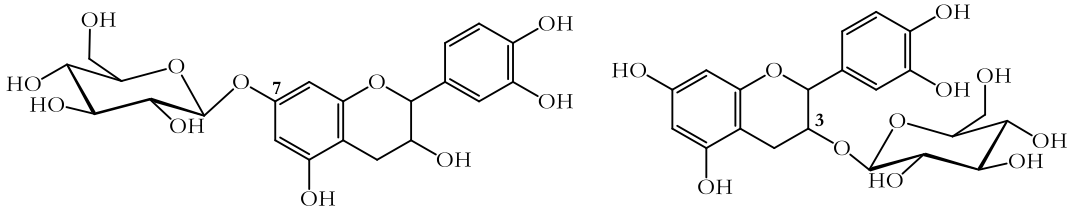



**Figure IV.61.** Structure for (epi)catechin-7-O-glycoside and (epi)catechin-3-O-glycoside compounds.

**Table IV.29.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of O-glycosilated tannins. The numbers written in bold correspond to the compounds chosen as markers.

| Number | $t_R$ (min) | Compound | [C-glc]$^-$ (m/z) |
|---|---|---|---|
| **85** | 35.40 | (epi)catechin-3-O-glycoside | 451.2 |
| **86** | 55.27 | (epi)catechin-3-O-glycoside | 451.3 |

The ion fragment which corresponds to the loss of glucose is observed at m/z 289.2. Furthermore, this ion is the most intensive peak in the mass spectrum (Figure IV.62). The other fragments are those that have usually been detected with other flavan-3-ols being the RDA rupture the most characteristic ones.

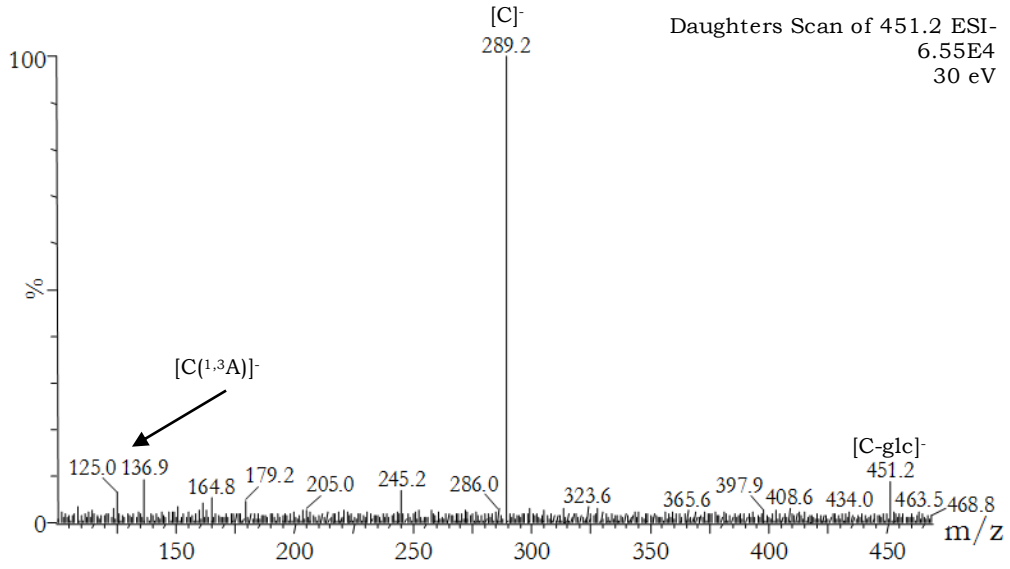



**Figure IV.62.** ESI(-)-MS$^2$ spectrum of O-glycosilated tannins (**85**), obtained at a collision energy of 30 eV.

## IV.2.15. Galloylated tannins

The nomenclature that is used for these compounds differs from the other because the ether bond, which is between the flavan-3-ol and the galoyl group, can be broken in two different parts. The general nomenclature for these compounds will be U-O-gal, being U the flavan-3-ol unit, gal is the galoyl group and O is the ether bond. Therefore, the two possible ruptures will be called as U-O when the galoyl group is lost and the flavan-3-ol unit is detected without the loss of OH group, or U when the loss of OH group occurs.

### a) Galloylated monomer.

Only epicatechin-gallato compound was found in red wine by our group (Table IV.30).

**Table IV.30.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of galloylated monomer.

| Number | $t_R$ (min) | Compound | [U-O-gal]$^-$ (m/z) |
|---|---|---|---|
| *180* | 79.93 | (epi)catechin-gallato | 441.0 |

The fragment ions detected in the CID-MS/MS experiments for this compound are those that correspond to the rupture of ether bond: the ion at m/z 289.1 is [U-O]$^-$ and the ones at 271.1 is [U]$^-$.

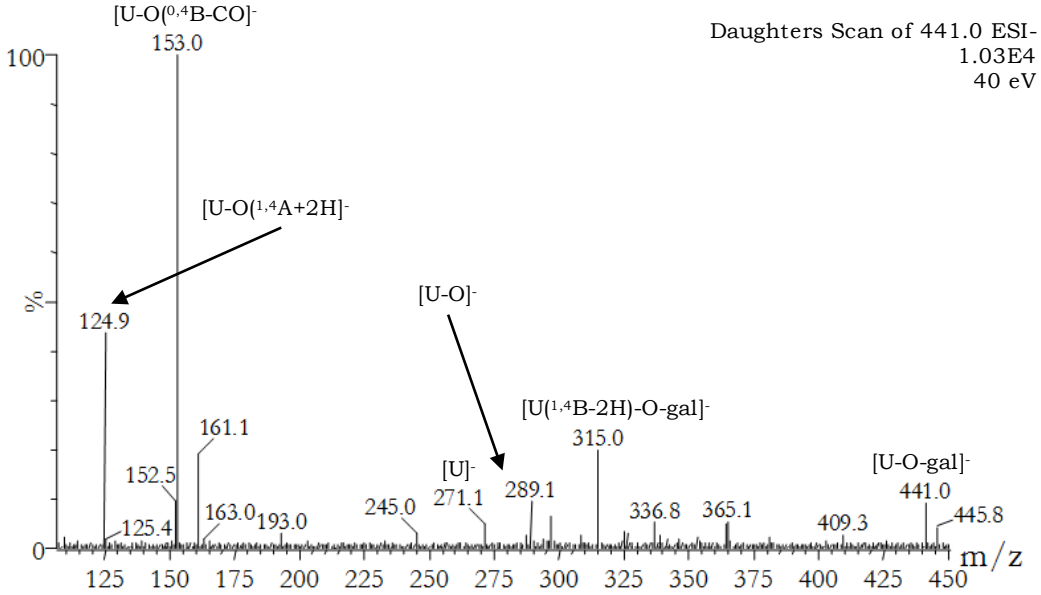



**Figure IV.63.** ESI(-)-MS$^2$ spectrum of (epi)catechin-gallato (*180*), obtained at a collision energy of 40 eV.

As in before compounds (O-glycosylated tannins), the fragment ions detected from the loss of the galloyl group are also those which have already found for the (epi)catechin monomer.

The mass spectra for these tannins is shown in Figure IV.63.

### b) Galloylated dimers

Flavan-3-ols dimers may appear galloylated in one subunit or in both ones. Two compounds of this type have been found which have two (epi)catechin units bond to a single galloyl group (Table IV.31).

**Table IV.31.** Retention time and molecular ion achieved by HPLC-DAD-ESI(-)-CID-MS/MS of galloylated dimers.

| Number | $t_R$ (min) | Compound | [gal-O-UD]$^-$ (m/z) |
|---|---|---|---|
| *181* | 64.59 | Gallato-O-(epi)catechin-(epi)catechin | 729.0 |
| *182* | 71.90 | Gallato-O-(epi)catechin-(epi)catechin | 728.8 |

As in previous cases, in these tannins it can be known which (epi)catechin unit is attached to the galoyl group by looking at the fragmentation of the interflavanic bond after the rupture of ether bond.

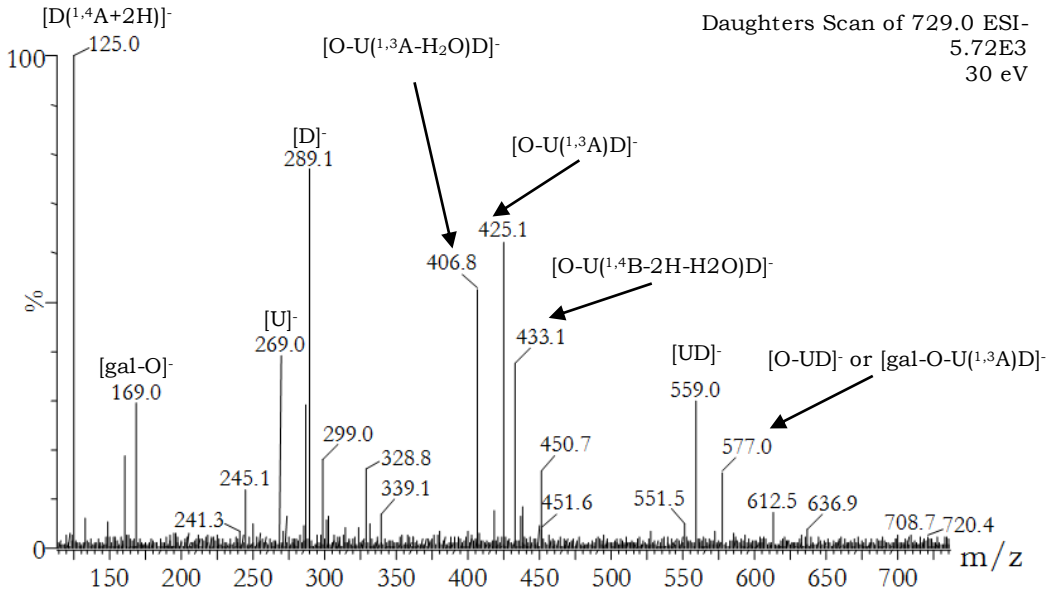



**Figure IV.64.** ESI(-)-MS$^2$ spectrum of galloylated dimers (*182*), obtained at a collision energy of 30 eV.

The fragment ions identified as the product ion of the rupture of the interflavanic bond and the ether bond of the galoyl group are those with m/z 577.0 ($[O\text{-}UD]^-$), 559.0 ($[UD]^-$), 289.1 ($[D]^-$), 287.0 ($[U\text{-}O]^-$) and 269.0 ($[U]^-$). If fragment ions at m/z 269.0 ([U]-) is detected, means that the galoyl group is bonded to the upper unit, whereas if the fragment ion at m/z 289.1 appears, then the galoyl group is in the lower unit. The rest fragment are the usually ones.

The mass spectra for these tannins is shown in Figure IV.64, using gallato-O-(epi)catechin-(epi)catechin (*181*) as an exmple.

# CHAPTER V

## EVOLUTION OF ANTHOCYANIN DERIVATIVES AND TANNINS DURING DIFFERENT STAGES OF WINEMAKING



# Chapter V

# EVOLUTION OF ANTHOCYANIN DERIVATIVES AND TANNINS DURING DIFFERENT STAGES OF WINEMAKING

Evolutionary profiles during different stages of winemaking for several selected anthocyanin derivatives and tannins will be presented in this chapter. To summarize as much as possible, the profiles of some anthocyanin derivatives and some tannins have been selected and presented in order to explain the global evolution. All markers concentrations were collected inside the Annex I as focused in the profile of each compound.

## V.1. PROFILES DURING THE FERMENTATION STAGE

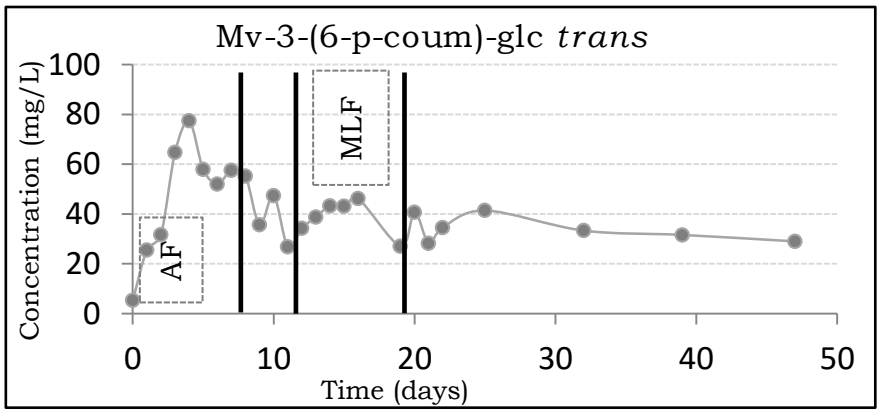
### V.1.1. Anthocyanins derivatives

As stated, since the end of alcoholic fermentation, the concentration of anthocyanins starts to decline in the first steps of winemaking due to condensation reactions, polymerization, oxidation and precipitation. Some of these reactions involve the degradation of anthocyanins but other leading to the formation of pigments that provide different hues to wine.

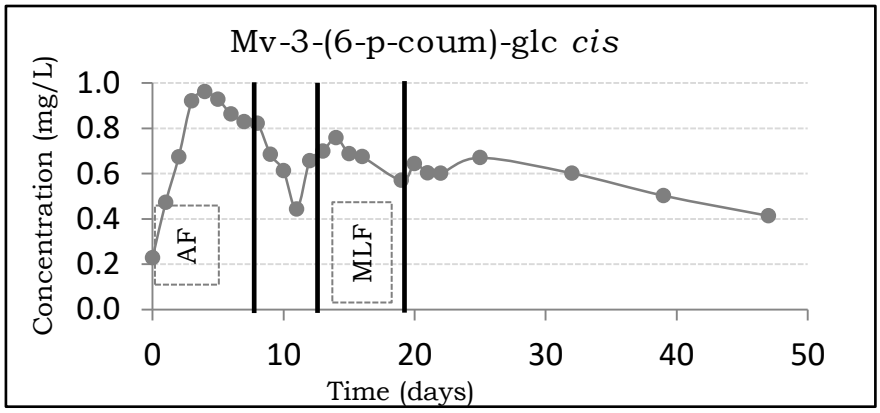
## a) Anthocyanins profiles

Mv-3-(6-*p*-coum)-glc *trans* and his *cis* isomer were selected as markers for the anthocyanins group. Mv-3-glc was not chosen as a marker because his concentration was usually greater than the calibration range used, which reach 100 mg/L.

Profiles achieved for Mv-3-(6-*p*-coum)-glc *trans* and its *cis* isomer along the fermentation are shown in Figures V.1 (a-b), respectively. Profile pattern for both is the same, with a high increasing curve in the beginning of maceration and alcoholic fermentation (AF) which decrease from the 4th or 5th day of AF to its end and followed by a more stable period during the malolactic fermentation (MLF) until its end.




**(a)**




**(b)**

**Figure V.1.** Extraction and evolution profiles for **(a)** Mv-3-(6-*p*-coum)-glc *trans*; **(b)** Mv-3-(6-*p*-coum)-glc cis during fermentations.

## b) Profiles of anthocyanin derivatives by direct condensation with flavanols

Condensation of anthocyanins in flavylium form with flavanols

As an example of the formation and evolution profiles of this type of compounds during the fermentation stage, Figure V.2 shows the profile for the Catechin-Mv-3-glc along the wine fermentation.

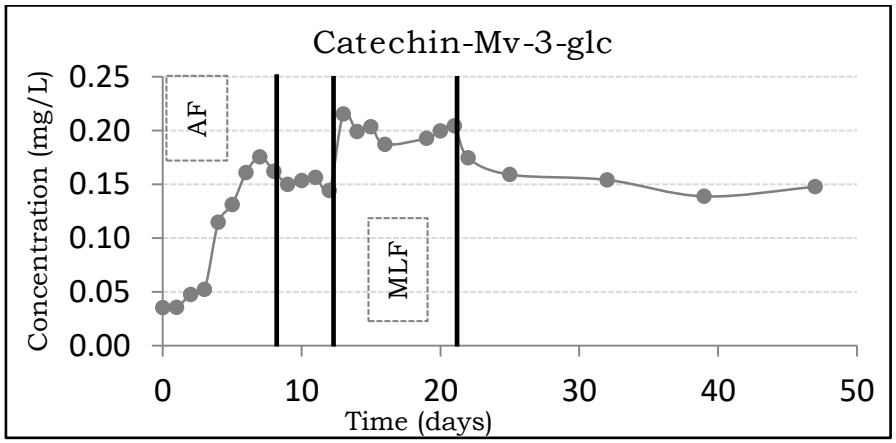



**Figure V.2.** Formation and evolution profile for Catechin-Mv-3-glc (**14**) during fermentations.

In this case, as it happened in anthocyanins profiles, there is an increase of the concentration during the first middle of alcoholic fermentation, decreasing before its end and a more stable period during the malolactic fermentation. However, increase of condensation derivatives is delayed against that of anthocyanins, obviously as a consequence of the fact that condensation derivatives need that anthocyanins and flavan-3-ols (or better tannin dimers B) had been first extracted from grape to must, before to react each between to form condensation derivatives. So, as shown in Figures, maximum concentration of anthocyanins are reached at day 4 of maceration, whereas maximum level of condensation derivatives area reached at day 7 of maceration-AF.

The same profile is observed for the other five markers (**15**-**19**) of anthocyanin derivatives by direct condensation with flavanols in its flavylium form.

Figure V.3 collects the relative evolution profile of the three greatest compounds of this type. The relative evolution profile refers to the percentage of concentration of each individual marker against to sum of concentrations of all anthocyanin derivatives markers for each anthocyanin (**14**-**15**, **18**, **34**-**36**, **39**, **41**, **43**-**44**, **46**-**47** for derivatives

with Mv-3-glc and **16**-**17**, **19**, **37**-**38**, **40**, **42**, **45**, **48** for those with Mv-3-(6-p-coum)-glc; see Table III.6 for names). Showing the graphic, Catechin-Mv-3-glc is the major one, with levels three times greater than the other non-acetylated markers in the beginning of the AF. However the contribution of this compound decreases during AF and remain stables before the end of the fermentation.

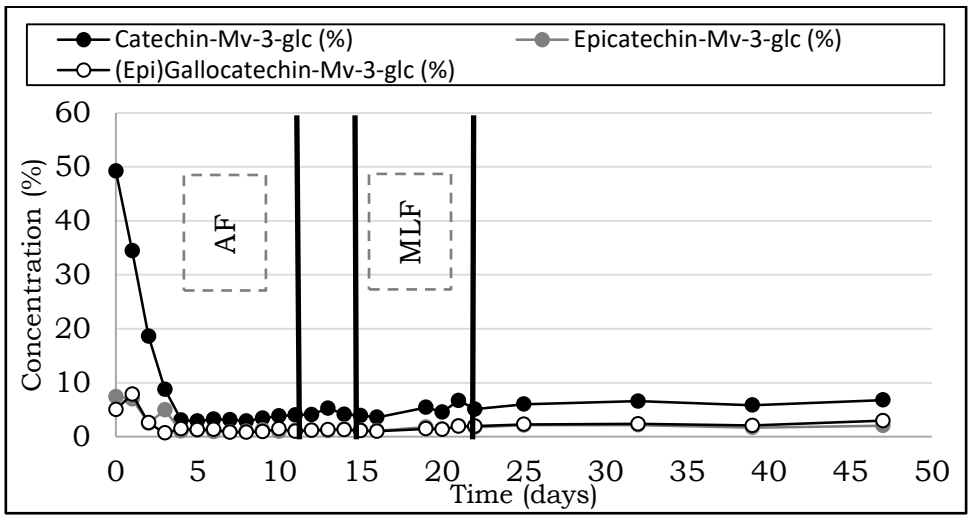
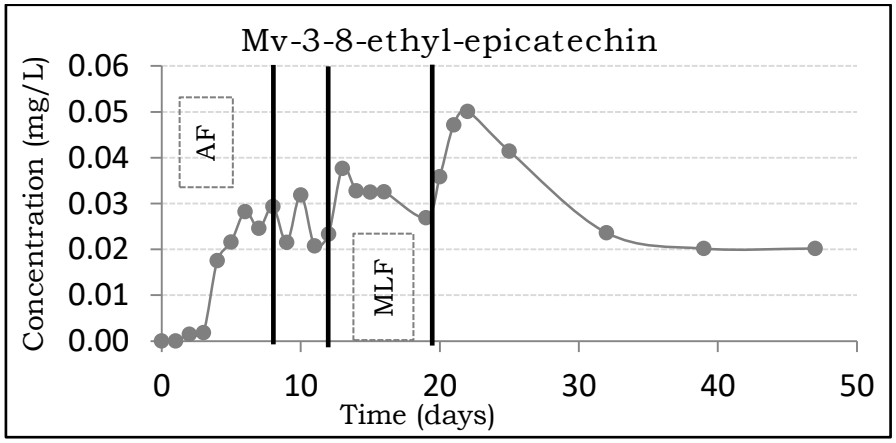


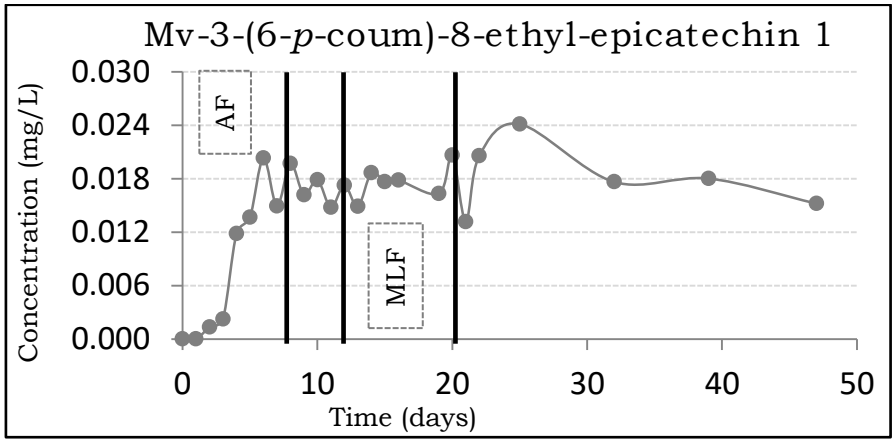

**Figure V.3.** Relative evolution profile for Catechin-Mv-3-glc (**14**), Epicatechin-Mv-3-glc (**15**) and (Epi)gallocatechin-Mv-3-glc (**18**) during fermentations.

Ethylidene-Bridged Anthocyanin-Flavanol condensation derivatives

Figure V.4 shows a representative fermentation profile for condensation derivatives with ethylidene bridge. The formation of these compounds is similar compared with direct condensation ones, but increases along MLF are higher with also higher decreases after the end of MLF (Figure V.4 a). On the other hand, after the initial grown, those markers that are acylated with *p*-coumaric acid have a more stable concentration (Figure V.4 b).




**(a)**




**(b)**

**Figure V.4.** Formation and evolution profile for **(a)** Mv-3-8-ethyl-catechin (**34**); **(b)** Mv-3-(6-p-coum)-8-ethyl-epicatechin 1 (**37**) during fermentation phase.

## c) Pyranoanthocyanins profiles

Derivatives with pyruvic acid, acetaldehyde and acetoacetic acid

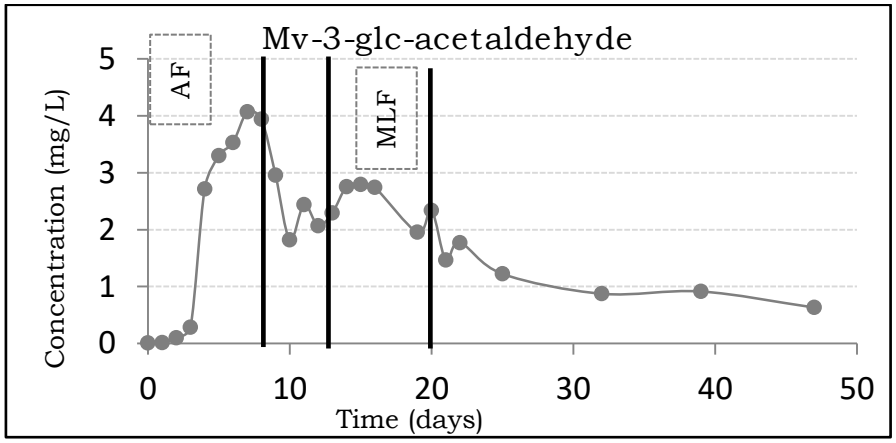
Pyruvic acid is the most important metabolic intermediate, derived from the metabolism of yeast during fermentation and by the activity of the lactic bacteria during the malolactic fermentation[309].

Figure V.5 (a-b) shows the formation and evolution profiles of Mv-3-glc-acetaldehyde (vitisin B) and Mv-3-glc-pyruvic (vitisin A), respectively during the fermentation stage.

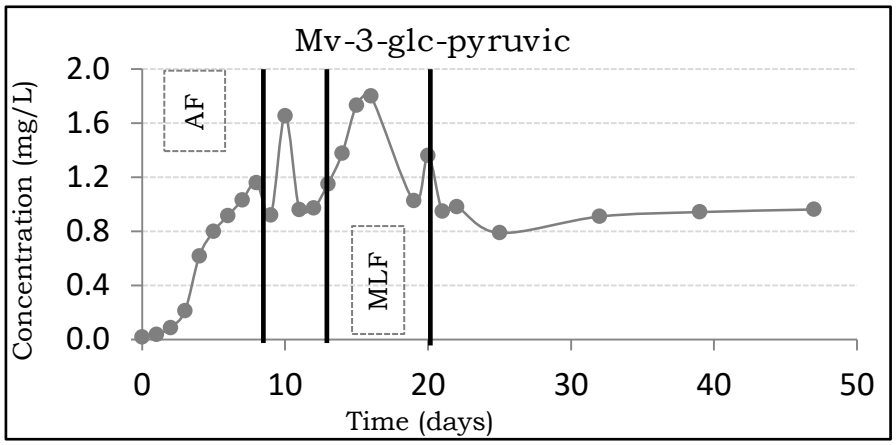
[309]Morata, A.; Calderón, F.; González, M. C.; Gómez-Cordovés, M. C.; Suárez, J. A.; *Formation of highly stable pyranoanthocyanins (vitisins A and B) in red wines by the addition of pyruvic acid and acetaldehyde*, Food Chem. **2006**, 100, 1144-1152.

The formation of vitisin B during the initial steps of maceration and alcoholic fermentation (Figure V.5 (a)) is parallel, but delayed as mentioned, to the extraction of its anthocyanin precursor. After that a decrease is observed in the last days of AF, a new increase when a vat transfer was made after the end of AF and before the start of MLF and a new decrease along MLF to finish with a stabilization period after the end of this fermentation. The initial period of AF supposes a great start of yeasts metabolisms with high production of acetaldehyde, whereas vat transfer implies a great oxygenation of wine. Both pulses at the start of AF and after the vat transfer at the end of AF are also observed for the respective derivatives with pyruvic acid (vitisin A) (Figure V.5 (b)) and acetoacetic acid, but in these cases the culming point of concentration appears in the start of MLF, instead of in the middle of AF.

However, a clear difference is observed. Formation of vitisin A along AF is slower and less intense than vitisin B, but decrease of vitisin A is also slower (-30%) than vitisin B (near -50%). On the other hand, during the second pulse (vat transfer and MLF) behavior seems to be the opposite, bigger increases of vitisin A (+95% vs +45%) but similar decreases. Also the 3 weeks period after the end of MLF supposes a decrease for vitisin B (-50%), but a stable period for vitisin A (+20%). As a result the concentration drop from the maximum level to that found after one or two weeks of the end of MLF is considerably higher for vitisin B (-78% vs -48%). So, although levels of vitisin B are higher than those of vitisin A all along fermentations, one or two weeks after the end of MLF levels of both are similar. This suggests a more unstable behavior of vitisin B along fermentations.




**(a)**




**(b)**

**Figure V.5.** Formation and evolution profile for **(a)** Mv-3-glc-acetaldehyde (**39**); **(b)** Mv-3-glc-pyruvic (**41**) during fermentation phase.

Regarding the relative evolution profile of vitisin B and vitisin A along fermentations, the same conclusion is attained. The contribution of vitisin B during AF is nearly four times that of vitisin A, but along MLF decrease to nearly two times and at the end of MLF both are nearly equal. This shows that vitisins B are formed perhaps quicker than vitisins A, but also more rapidly destroyed, being more unstable as referred in previously studies[310].

[310]Sánchez-Ilárduya. M.B.;*Pigmentos derivados antociánicos de los vinos tintos de la Rioja: Estudio de analítico, influencia en el color y evolución durante la crianza. PhD* Thesis, Universidad del País Vasco, Spain, **2010**.

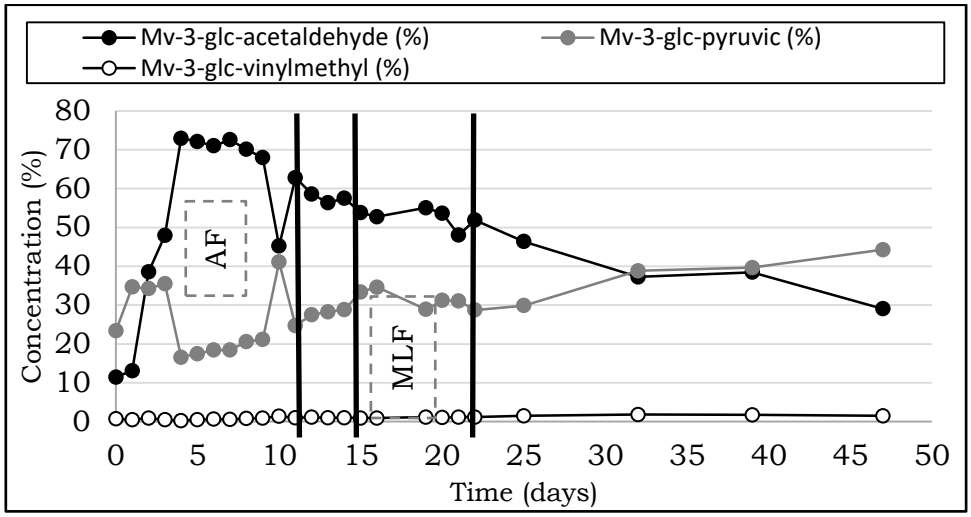
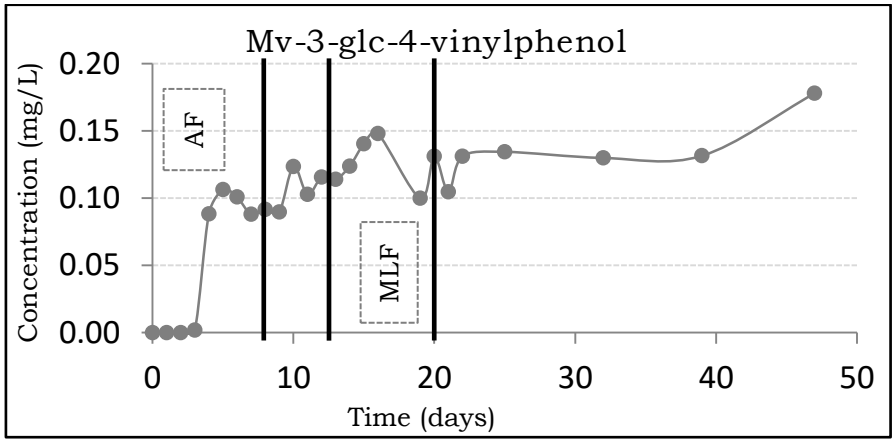



**Figure V.6.** Relative evolution profile for Mv-3-glc-acetaldehyde (**39**), Mv-3-glc-pyruvic (**41**) and Mv-3-glc-vinylmethyl (**43**) during fermentations.

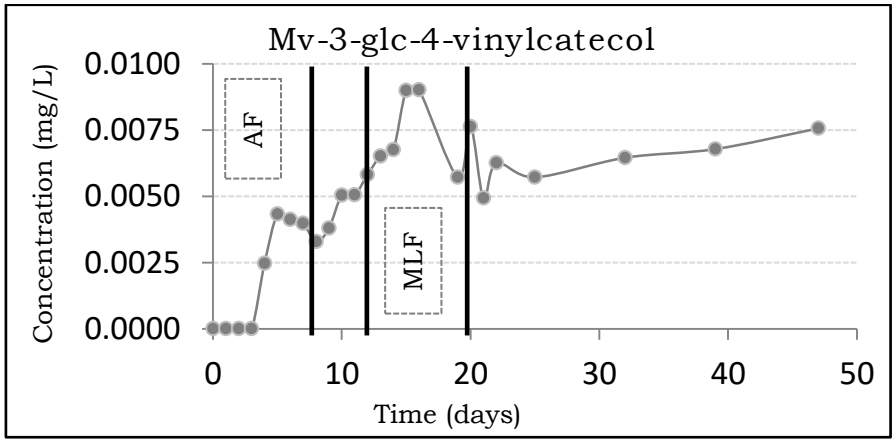
Derivatives with hydroxycinnamic acids

The profiles observed in this type of pyranoanthocyanins are similar to those formed with pyruvic acid, acetaldehyde and acetoacetic acid. The high point of concentration is observed in the MLF. The existing difference between them is that when the malolactic fermentation is ended, the concentration becomes stable but with a slight increase, as it can be seen in Figure V.7.

The relative evolution profile for Mv-3-glc-4-vynilphenol (**44**), Mv-3-glc-4-vinylcatecol (**46**) and Mv-3-glc-vinylguaiacol (**47**) (Figure V.8) shows that contribution of these compounds increase during fermentations and tend to remain stables after the end of the MLF.




**(a)**




**(b)**

**Figure V.7.** Formation and evolution profiles for **(a)** Mv-3-glc-4-vinylphenol (**44**); **(b)** Mv-3-glc-4-vinylcatecol (**46**) in fermentations.

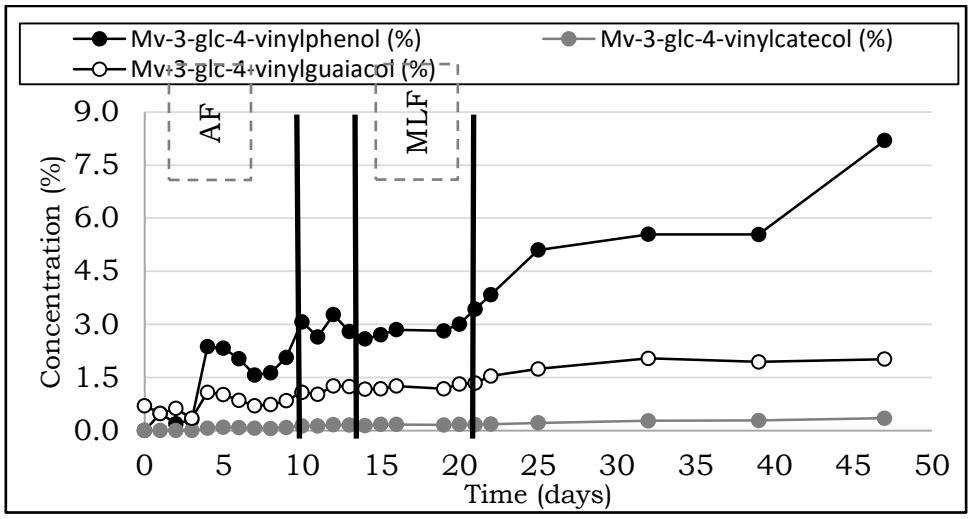


**Figure V.8.** Relative evolution profile for Mv-3-glc-vinylphenol (**44**), Mv-3-glc-4-vinylcatecol (**46**) and Mv-3-glc-4-vinylguaiacol (**47**) in the fermentation stage.

## V.1.2. Tannins

### a) Flavan-3-ols monomers profiles

Evolution profile achieved for the major monomer, catechin, during the fermentation is shown in Figure V.9. All other monomers (epicatechin, gallocatechin and epigallocatechin) have the same profile in the fermentation stage. Concentrations of monomers increases in the first days of maceration and AF, to be quite stable from the middle to the end of AF and in the short period until the start of malolactic fermentation, and showing a significant increase from the middle to the end of this fermentation.

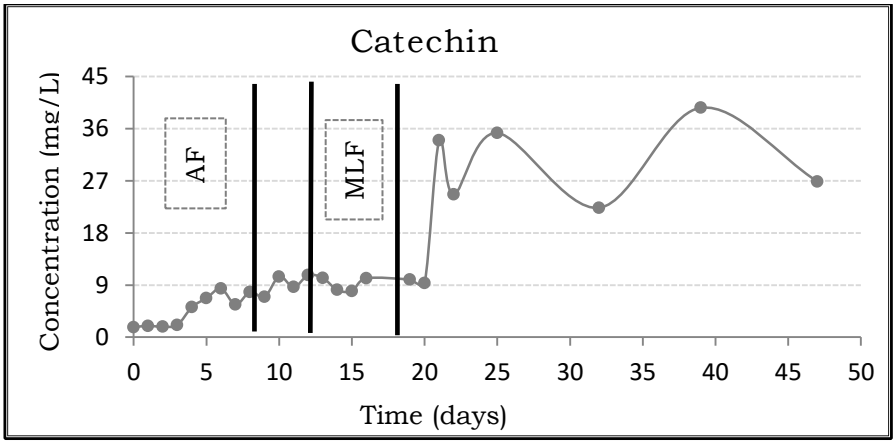



**Figure V.9.** Formation and evolution profile representative for the tannin monomers in fermentations. Ex: Catechin (**57**)

### b) Profiles of dimers with B bond

<u>Procyanidin dimers with B bond</u>

The profile achieved for the procyanidin dimers is shown in Figure V.10, using the PCB1 dimer profile as representative of the 3 markers of this type (PCB1 (**61**), PCB2 (**62**) and ((epi)cat)$_2$ 1 (**63**)) because the results obtained were very similar.

Their concentration increase during the AF and in the short period until the start of MLF. The biggest concentration is reached at the beginning of MLF. Then their levels decrease during MLF and remains stable before the end of fermentation.

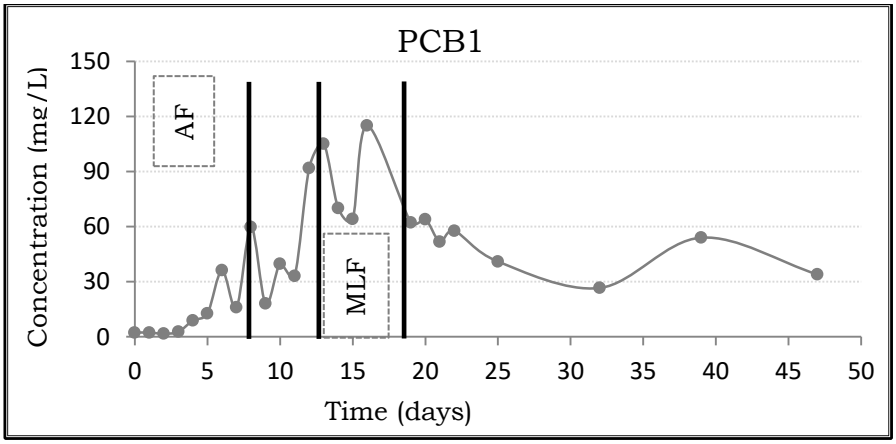



**Figure V.10.** PCB1 (**61**) profiles obtained in fermentations.

However, in the case of PCB2 and $((epi)cat)_2$ 1 (Figure V.11) the biggest concentration is reached at the end of the MLF, after that stage, the concentration remains stable.

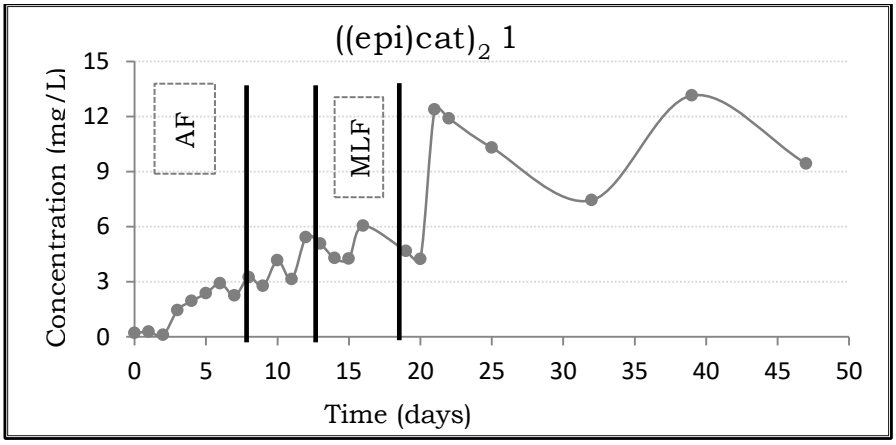



**Figure V.11.** $((epi)cat)_2$ 1 (**63**) profile achieve for fermentations.

## Prodelphinidin dimers with B bond

The profiles achieved for prodelphinidin dimers resemble to those obtained for procyanidin dimers but with a more stable behaviour along MLF. So, their concentration rise during the AF and then after the begining of the MLF the concentration values become stables. As an example, Figure V.12 describe the profile for the $((epi)gallocat)_2$ 1 (**64**).

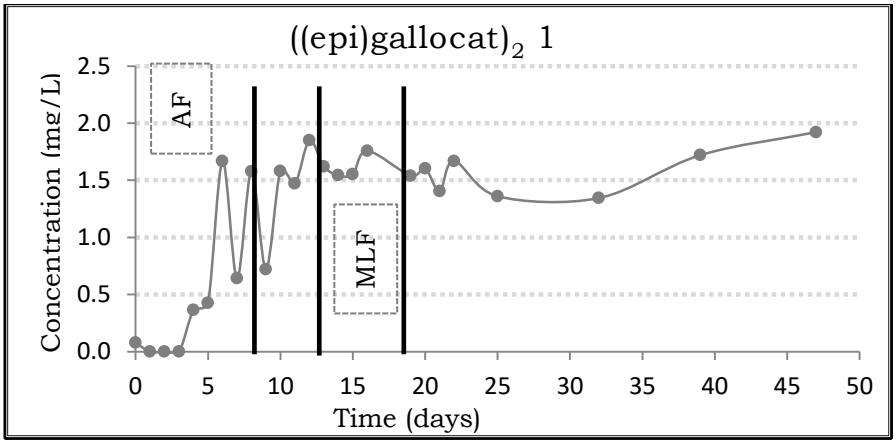
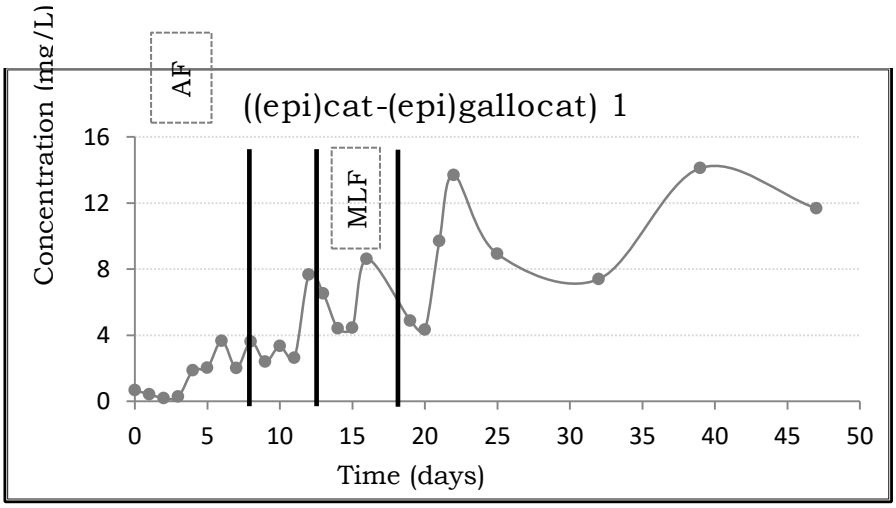
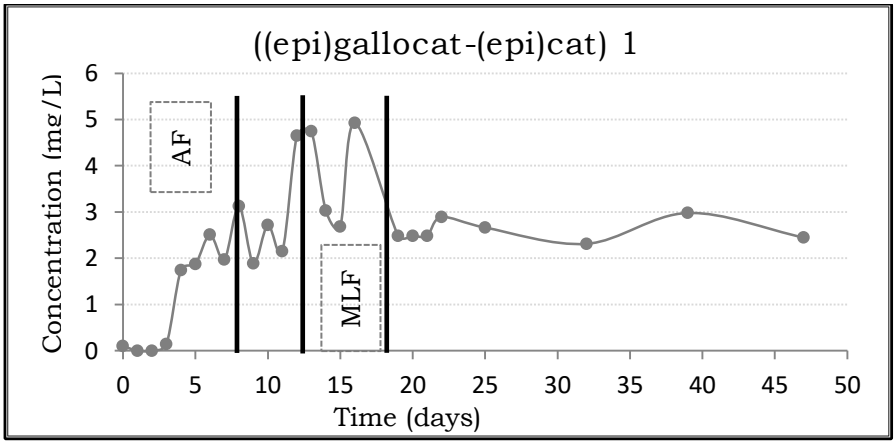



**Figure V.12.** ((epi)gallocat)$_2$ 1 (**64**) formation and evolution profile achieved in fermentations.

## Mixed dimers with B bond




**(a)**



**(b)**

**Figure V.13.** Formation and evolution profile achieved for **(a)** (epi)cat-(epi)gallocat) 1 (**67**) and **(b)** (epi)gallocat-(epi)cat 1 (**69**) in fermentation.

As happened before with the profiles of prodelphinidin dimers, the mixed dimers profiles are analogous to the procyanidin dimers. The concentration increases until the FML and then, become stable for all the mixed dimers markers. However, whereas for mixed dimers with an (epi)catechin as upper unit continues increasing their levels along MLF, mixed dimers with an (epi)gallocatechin as upper unit levels seems to decrease along MLF.

As an example of it, the profiles carried out for the (epi)cat-(epi)gallocat 1 (**67**) and the (epi)gallocat-(epi)cat 1 (**69**) mixed dimers are shown in Figure V.13.

## c) Profiles of trimers with B bond (Procyanidin homotrimers B)

The same above general profile is obtained for the procyanidin trimers: the concentration rises until the MLF and then remains stable (Figure V.14).

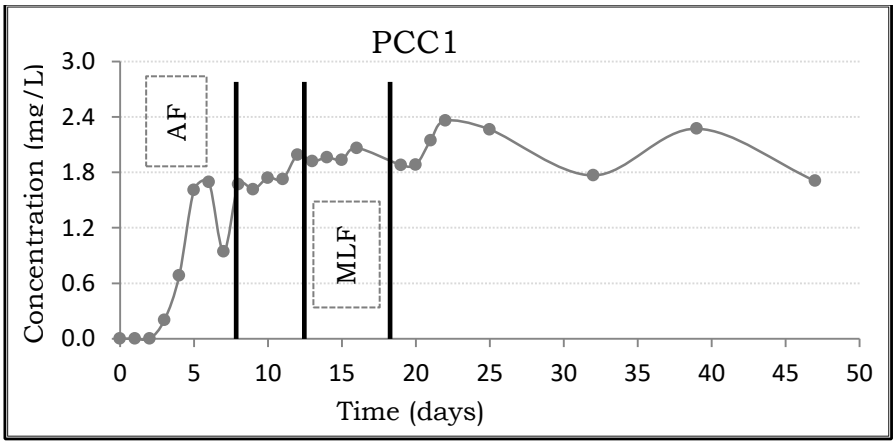



**Figure V.14.** Profile for PCC1 (**71**) achieved during fermentations.

In the case of the profile obtained for the trimer ((epi)cat)$_3$ 2 (**73**) (Figure V.15), its concentration reached until the MLF is finished and, as in the rest of described compounds, remains stable after that.

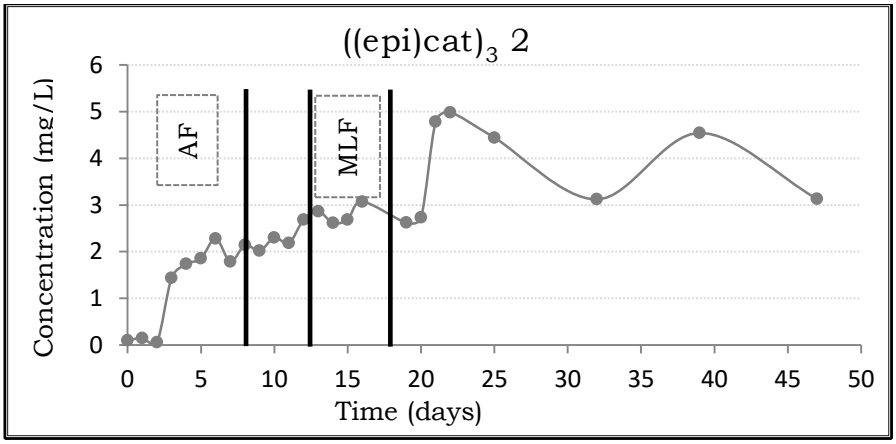



**Figure V.15.** Profile for ((epi)cat)$_3$ 2 (**73**) achieved during fermentations.

### d) Profiles of dimers with A bond

Procyanidin dimers with A bond

From the three markers of this type only two were detected along fermentations and just in 3 or 4 moments along fermentations, so there is not enough data to construct the formation and evolution profile. So, Figure V.16 shows the profile for ((epi)cat)$_2$ A 1 obtained in the fermentations of another wine, confirming the same general tendency during fermentations.

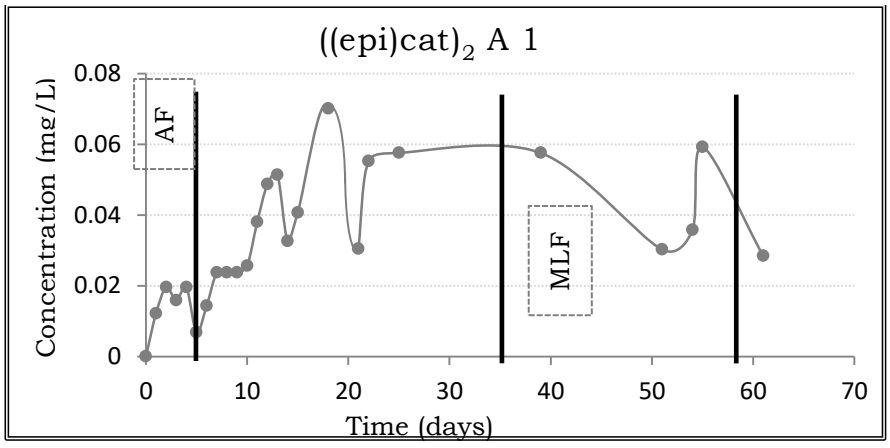



**Figure V.16.** Profile for ((epi)cat)$_2$ A 1 (**75**) achieved during fermentations.

Their levels increasealong AF and the biggest concentration is reached before the start of MLF. Then their levels decrease along MLF.

## Prodelphinidin dimers with A bond

The same general tendency is shown for ((epi)gallocat)$_2$ A (*146*), formation and increases along AF, with maximum concentration at the start of MLF, slight decrease along MLF and stable behaviour after the end of MLF, as it can be observed in Figure V.17.

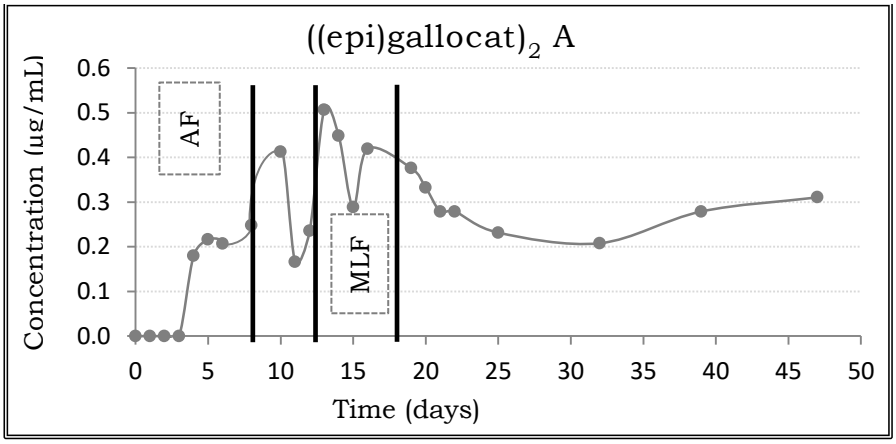



**Figure V.17.** Profile for ((epi)gallocat)$_2$ A (*146*) achieved during fermentations.

## Mixed dimers with A bond

As it was observed in Figure V.18, these compounds reach their top of concentration levels at the end of AF. Then a light reduction can be observed during the MLF and, finally, it remains stable. Any mixed A dimer with (epi)gallocatechin as upper unit were selected as marker due to their lower concentrations, suggesting a more unstable behaviour of their corresponding precursors (mixed B dimers with (epi)gallocatechin as upper unit), as found for these against their homologues with (epi)catechin as upper unit along AF (lower levels) and MLF (decreasing tendency), as mentioned in b) item.

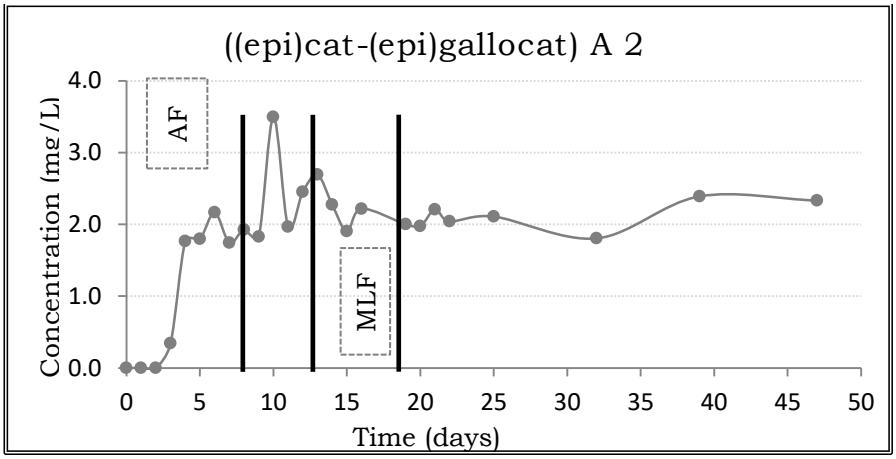



**Figure V.18.** Profile for ((epi)cat-(epi)gallocat) A 2 (**78**) achieved during fermentations.

Another interesting fact emerges when considering levels of the different types of B and A homo- and hetero-dimers. Whereas ratio of A vs B dimers for procyanidins is very low, the corresponding ratios for prodelphinidins or mixed dimers are higher. This suggests a favoured formation of additional A bond when an (epi)gallocatechin unit is implied. In fact, mixed trimers with one A bond (and other B bond) had higher levels when A bond implies one or two (epi)gallocatechin units.

## e) Profiles of mixed trimers with one A bond and one B bond

The same profile described for the other markers is also repeated for the mixed A trimers. As an example, for (epi)cat-((epi)gallocat)$_2$ A 1 (**81**) (Figure V.19) the maximum level of concentration is reached just before the beginning of the MLF. After that point the concentration values decrease slightly and become stables after its end.

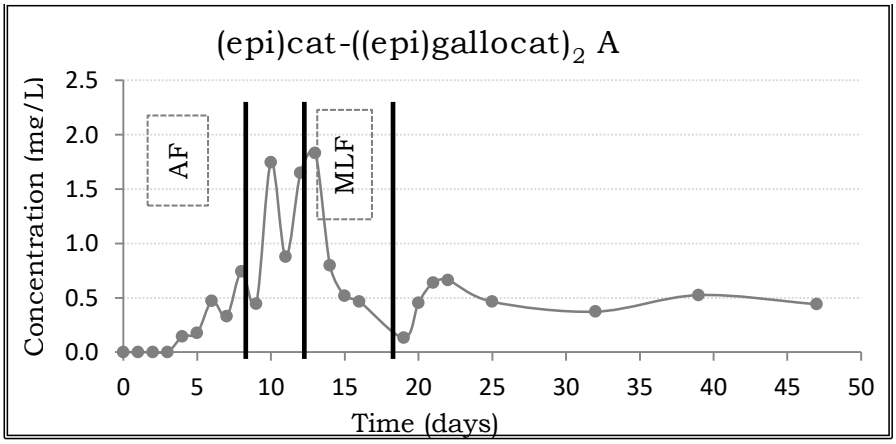



**Figure V.19.** Profile for (epi)cat-((epi)gallocat)$_2$ A (**81**) achieved during fermentations.

## f) Profiles of tannins mediated by acetaldehyde

For this type of markers, the formation and evolution profile is not clear. For the studied wine fermentation (Figure V.20) is similar to those of other tannins with even an increasing tendency after the end of MLF, but for other wine fermentations this increasing tendency was not confirmed. Any case, for all levels were very low.

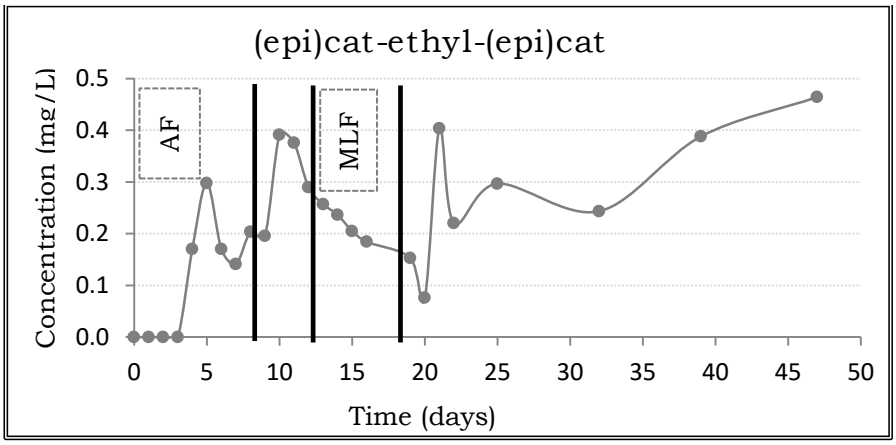



**Figure V.20.** Formation and evolution profile achieved for (epi)cat-ethyl-(epi)cat (*163*) achieved during fermentations.

## g) Profiles of p-vinyl tannins

Contrary to ethylydene bridged tannins, vinyl-tannins presented very high levels (Figure V.21), suggesting that depolymerisation of the first to produce vinyl-tannins is favoured. Profiles for the last followed the general behaviour, reaching maximum concentrations at the start of MLF, decreasing tendency along MLF and a stable period at its end.

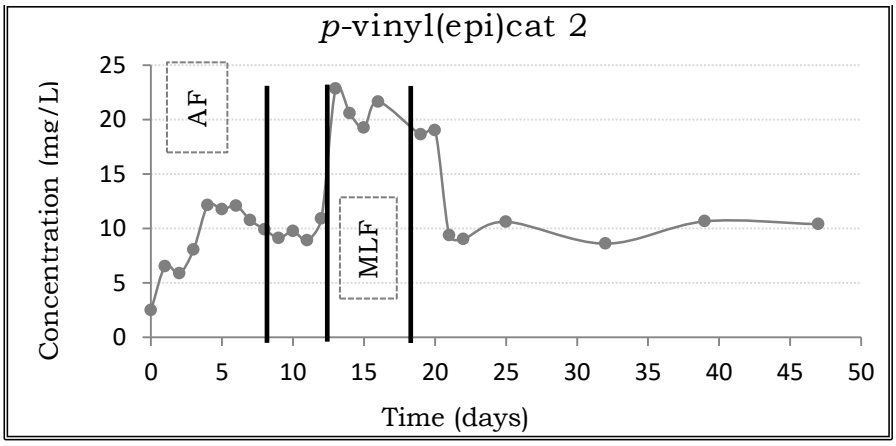



**Figure V.21.** Formation and evolution profile achieved for p-vinyl(epi)catechin 2 (**82**) achieved during fermentations.

### h) Profiles of tannins with furfuryl bridge

The formation and evolution profile for (epi)cat-furfuryl-(epi)cat 2 (**85**) compound (Figure V.22) reaches the highest point of concentration before the beginning of MLF and when the fermentation process is finished the concentration of the compound remains stable.

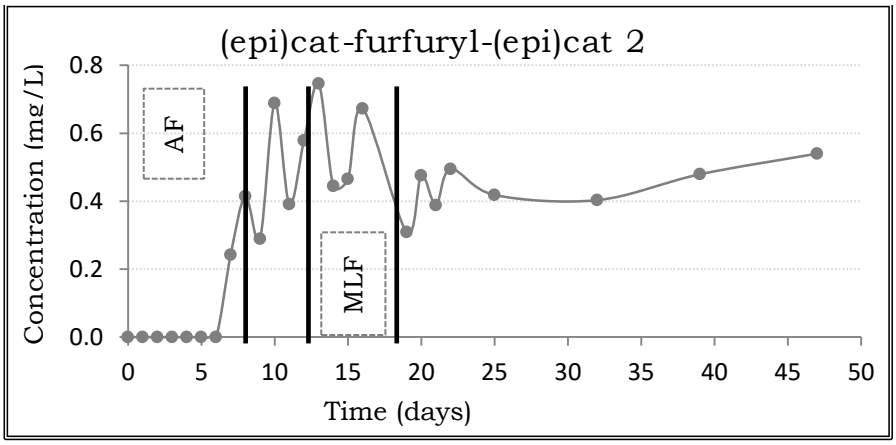



**Figure V.22.** Formation and evolution profile achieved for (epi)cat-furfuryl-(epi)cat 2 (**85**) achieved during fermentations.

### i) O-Glycosylated flavan-3-ols profiles

Profile observed for (epi)cat-glycoside 1 (**86**) along fermentations (Figure V.23) showed the general behaviour: the compound concentration increases until the beginning of the MLF and after the end of this stage the concentration remains stable.

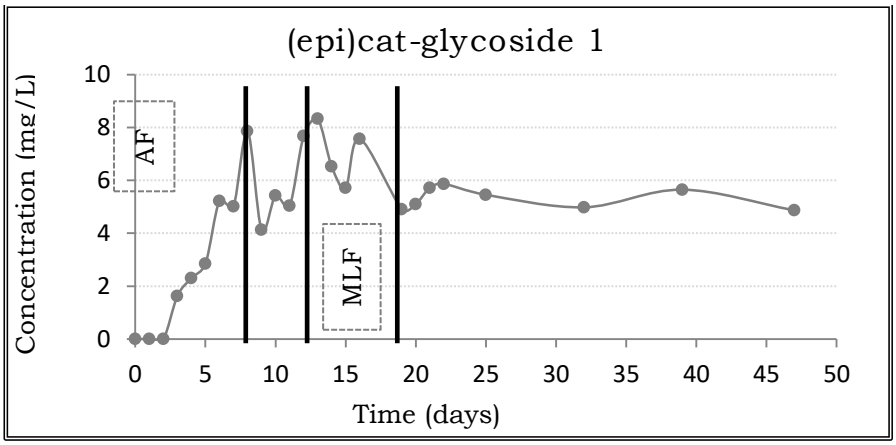



**Figure V.23.** Formation and evolution profile achieved for (epi)cat-glycoside 1 (**86**) achieved during fermentations.

## j) Galloylated tannins profiles

For these compounds the achieved profile (Figure V.24) is similar to those that were explained before. The concentration rises until the step of MLF and becomes stable after it.

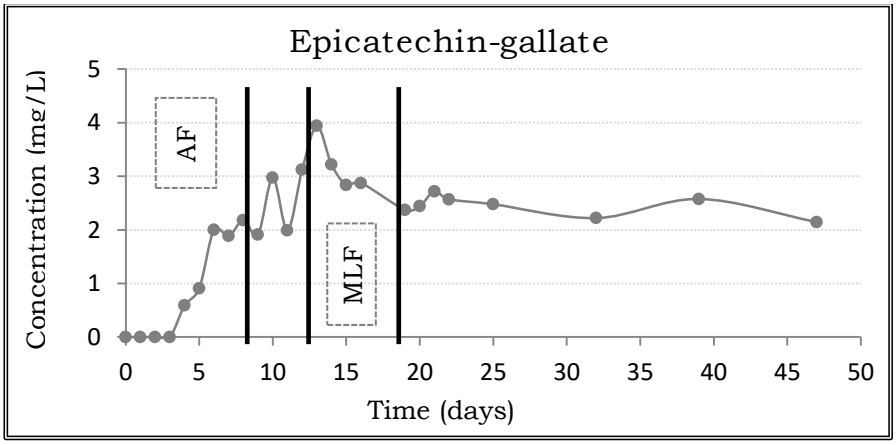



**Figure V.24.** Formation and evolution profile achieved for epicatechin-gallate (*179*) achieved during fermentations.

## k) Contribution of each tannin class to total tannins

The different participation of each tannin group in total tannins quantified was examined. The relative evolution profile refers to the percentage of concentration of each individual marker against to sum of concentrations of all taninns markers with an (epi)catechin unit in their structure (**57**-**58**, **61**-**63**, **67**-**86**; see Table III.8 for names). Figure V.25 shows the participation of monomers, dimers, trimers and mixed dimers with B bond type.

As it can be observed, monomers percentage decreases during the AF and increases slightly in the MLF, after that remains stable. In contrast, the dimers percentage increases until the beginning of the MLF, when starts to slightly decrease and, as it happened with the monomers, remains stable after the end of MLF. On the other hand, the contribution of trimers and mixed dimers with B bond are low and nearly constant during all the fermentation process, being more important mixed dimers than trimers. It seems that during AF monomers decreases because participate in the formation of dimers, and in a lesser extension mixed dimers and trimers, which increases; and during MLF B dimers slightly decrease releasing monomers, which slightly increases during this second fermentation.

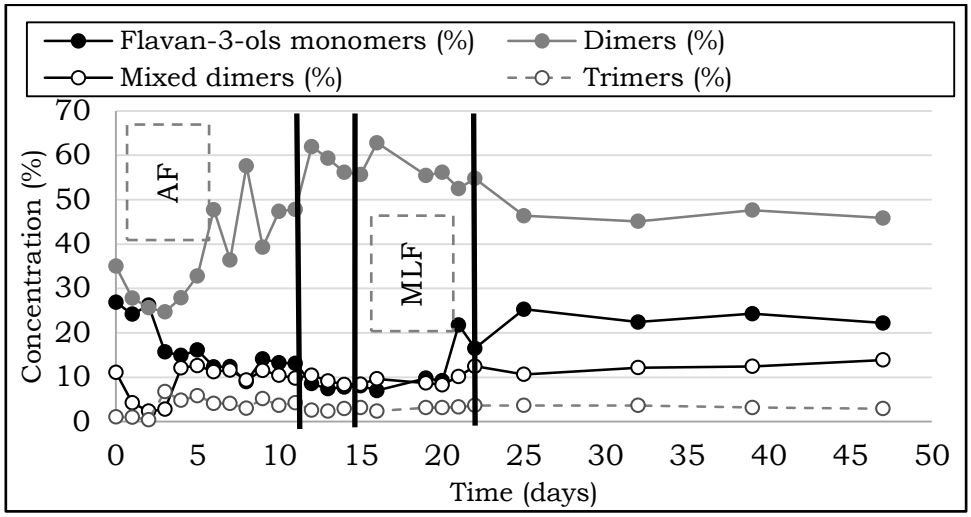



**Figure V.25.** Relative evolution profile for monomers, dimers, trimers and mixed dimers with B bond.

Figure V.26 shows the relative contribution referring to the tannins with A bond. The percentage of these compounds are insignificant comparing with those tannins markers with B bond type.

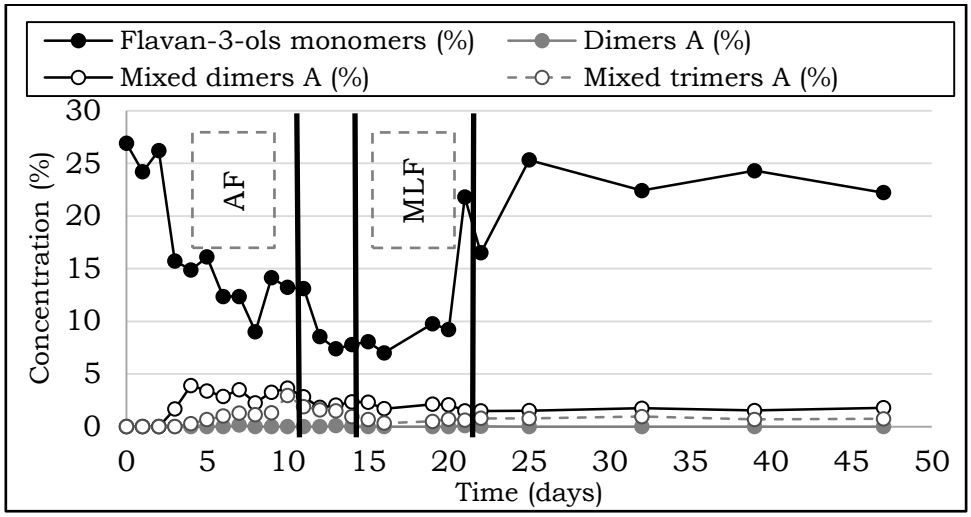



**Figure V.26.** Relative evolution profile for monomers, dimers, mixed trimers and mixed dimers with A bond.

# V.2. PROFILES DURING THE AGEING IN OAK BARRELS

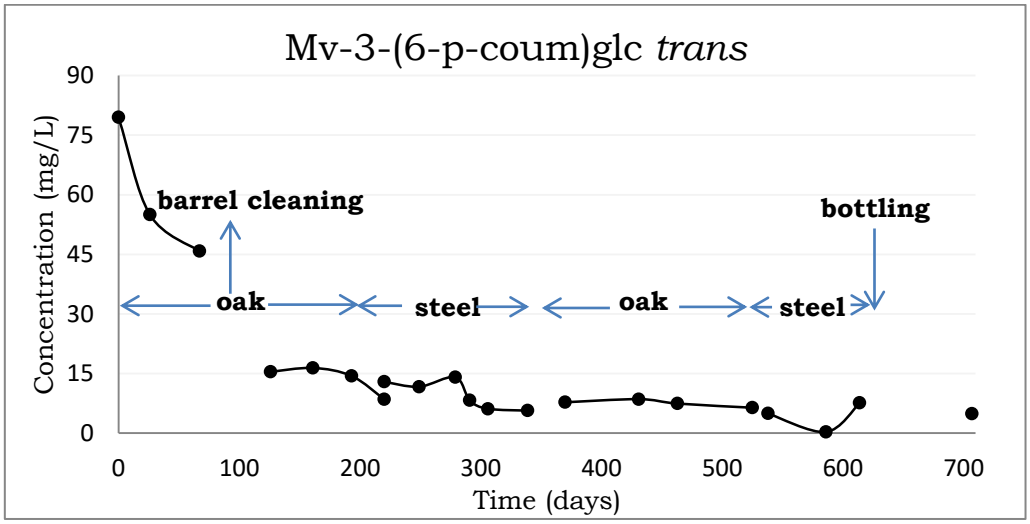
## V.2.1. Anthocyanins derivatives

Evolution profiles of some major anthocyanins derivatives along the ageing in oak barrels will be presented belong (individual concentration data for all sampling times and derivatives are collected in Annex I).

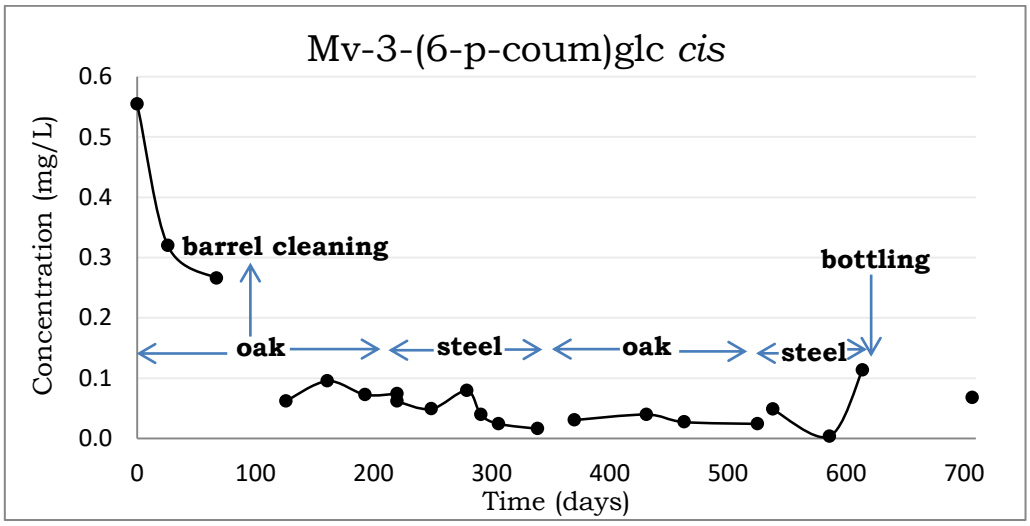
### a) Anthocyanins profiles

The profiles for the anthocyanins Mv-3-(6-*p*-coum)-glc *trans* and his *cis* isomer are represented in Figure V.27 (a) and (b). As it can be seen in these cases, big changes in profiles happen when processes such as vat transfers are made to wine more than along quiet periods in barrel or in stainless steel deposit. This is a general behavior for most instances and anthocyanins derivatives profiles. However, when interpreting results an important fact should be taken into account: transfers from barrel to steel vat also suppose the mixture of wine from de studied barrel with wine of other barrels from the same lot but perhaps somewhat different in phenolic contents. For this reason changes in those barrel to steel vat transfers do not correspond exactly to the studied wine. Any case, as the wine belongs to the same lot some probable conclusion could be attained. For steel vat to barrel transfers this problem does not exists. Also the cleaning barrel process, that supposes wine transfer from one barrel to another cleaned barrel, does not suffer from this problem.

As an example, when the cleaning of the barrel is carried out losses of 66 and 77% are experienced for *trans* and *cis* Mv-3-(6-*p*-coum)-glc isomers, respectively. This great decrease during the cleaning barrel process is also observed for direct (-40% to -65%) and ethylidene-bridged (losses of 18-66%) A-$F^+$ condensation derivatives and for pyranoanthocyanin derivatives with vinylphenols (-46%), but not for acetaldehyde (+59%), pyruvic acid (+75%) or acetoacetic acid (+54%) derivatives. All wine transfers (barrel cleaning, barrel to steel or steel to barrel) suppose a great oxygenation of wine.




**(a)**




**(b)**

**Figure V.27.** Evolution profiles for (a) Mv-3-(6-p-coum)-glc trans; (b) Mv-3-(6-p-coum)-glc cis during the ageing phase in oak barrel of D.O.Ca. Rioja alavesa wine qualified as "*Reserva*".

### b) Profiles of anthocyanin derivatives (direct condensation with flavanols, ethylidene-bridged anthocyanin-flavanol derivatives and pyranoanthocyanins)

The profile of Catechin-Mv-3-glc (**14**), the one with the greatest concentration of this type of anthocyanin derivatives pigments, is shown in Figure V.28.

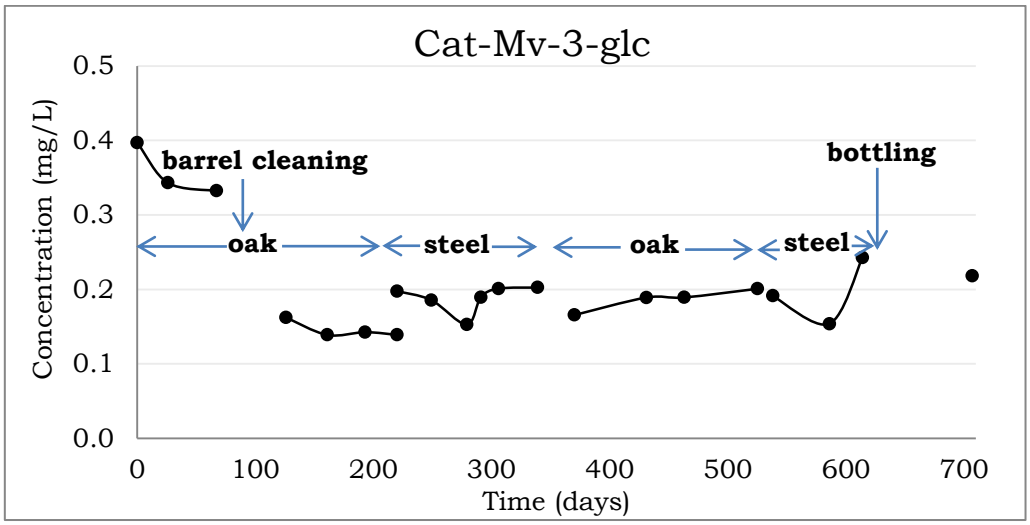



**Figure V.28.** Evolution profiles for Catechin-Mv-3-glc (**14**) during the ageing phase in oak barrel of D.O.Ca. Rioja alavesa wine qualified as "*Reserva*".

On the one hand, the period in barrel after the oak barrel cleaning supposes a slight decrease for this derivative (-14%). A similar behaviour show ethylidene-bridged derivatives (-35%) and pyranoanthocyanins (from -8% to -34%). On the other hand, the second barrel period supposes a more stable period with nearly no losses for pyranoanthocyanins derivatives with pyruvic (+0%) and acetoacetic (+0%) acids, slight losses for ethylidene-bridged derivatives (-6% to -12%) and for pyranoanthocyanins derivatives with acetaldehyde (-16%), and increases for pyranoanthocyanins derivatives with vinylphenols (+2% to +17%) and for direct condensation derivatives (+21%).

Periods within stainless steel deposit in the case of A-$F^+$ direct condensation derivatives suppose two opposite behaviors: the first steel period, with a slight decrease (+2% to -10%), and the second, with an increasing tendency (+27% to +35%). The cause of the great changes in the last period within deposit before bottling is unknown, but probably is due to mixtures of wines made at winery before bottling, so

no significant consequences should be taken from the second steel period (the same applies for all derivatives).

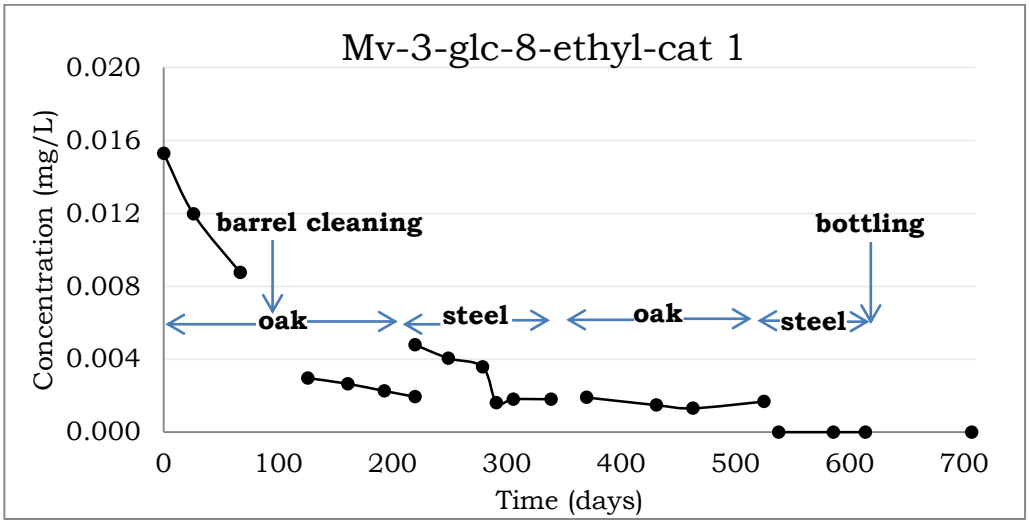
Comparing the levels between the start of the first barrel period and the end of the second barrel period, Catechin-Mv-3-glc (**14**) has suffered an increase of 24% (-49% from the start of ageing, t=0 days), whereas ethylidene-bridged derivatives have losses of 44-46% (-85 to -89%, from t=0 days), showing a more unstable behavior as previously reported[311,312]. Concerning pyranoanthocyanins differences in the levels between the start of the first and the end of second barrel periods are +9% (+38%, from t=0 days), +9% (+21%, from t=0 days), -34% (-77%, from t=0 days) and -41% (-29%, from t=0 days) for derivatives with pyruvic acid, acetoacetic acid, vinylphenol and acetaldehyde, respectively. This order could be stated as the stability order of pyranoanthocyanins, being more stable the derivatives with pyruvic and acetoacetic acids than those with vinylphenols and acetaldehyde.

Evolution profiles of anthocyanin derivatives pigments formed by anthocyanin-flavanol condensation mediated by acetaldehyde (Figure V.29 a-b) shows changes during barrel cleaning and first transfer from barrel to deposit similar to those of direct condensation derivatives, but in the period inside the deposit ethylidene-bridged derivatives highly decrease (-62%), contrary to them (+2% to -10%); showing the more unstable behavior above mentioned, also within steel vat periods.

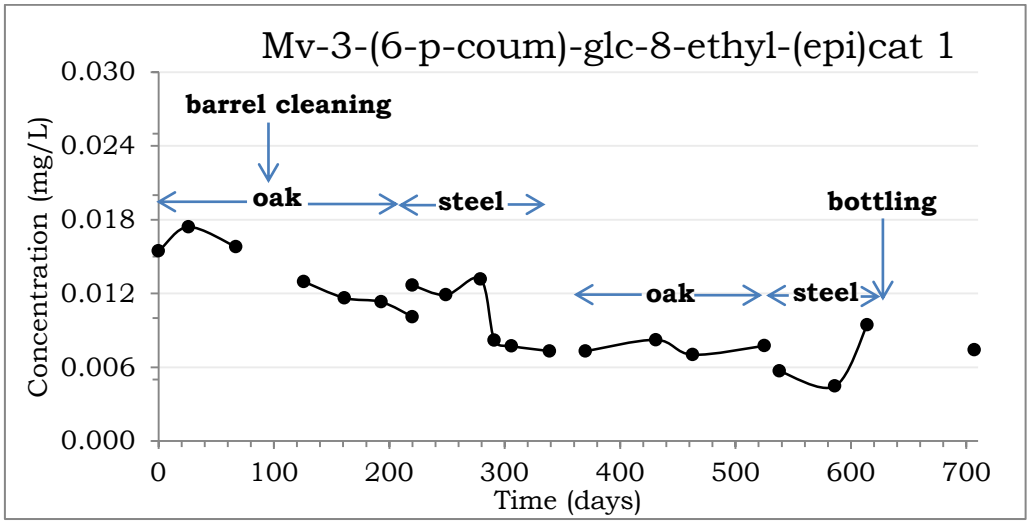
Levels of ethylidene-bridged derivatives during the second barrel period are very low, twenty times lower than those of direct condensation derivatives, and sligthly decrease (-6% to -12%) during this period, whereas amounts of direct condensation derivatives suffer a significant increase (+6% to +21%), confirming unstability of ethylidine-bridged derivatives against direct condensation ones even when ageing process is advanced.

[311]Cano-López, M.; Pardo-Mínguez, F.; Schmauch, G.; Saucier, C.; Teissedre, P. L.; López-Roca, J. M.; Gómez-Plaza, E.: *Effect of micro-oxygenation on color and anthocyanin-related compounds of wines with different phenolic contents*, J. Agric. Food Chem. **2008**, 56, 5932-5941.

[312]Escribano-Bailón, T.; Álvarez-García, M.; Rivas-Gonzalo, J. C.; Heredia, F. J.; Santos-Buelga, C.; *Color and stability of pigments derived from the acetaldehyde-mediated condensation between Malvidin-3-O-glucoside and (+)-Catechin*, J. Agric. Food Chem. **2001**, 49, 1213-1217.




**(a)**




**(b)**

**Figure V.29.** Formation and evolution profile for **(a)** Mv-3-ethyl-epicatechin 1 (**34**); **(b)** Mv-3-(6-p-coum)-ethyl-epicatechin 1 (**37**) during the ageing of a *Reserva* wine.

Mv-3-glc-acetaldehyde (**39**), Mv-3-pyruvic (**41**) and Mv-3-glc-vinylmethyl (**43**) profiles (Figures IV.30 to IV.32) reveals the mentioned greater stability of pyranoanthocyanins derivatives with pyruvic and acetoacetic acids against those of derivatives with acetaldehyde and vinylphenol.

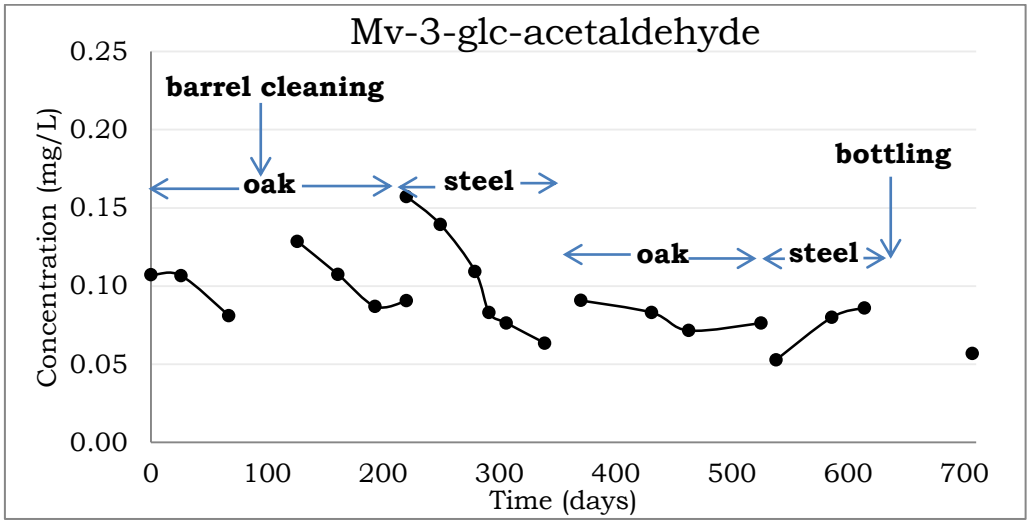



**Figure V.30.** Evolution profile for Mv-3-glc-acetaldehyde (**39**) during the ageing in a *Reserva* wine.

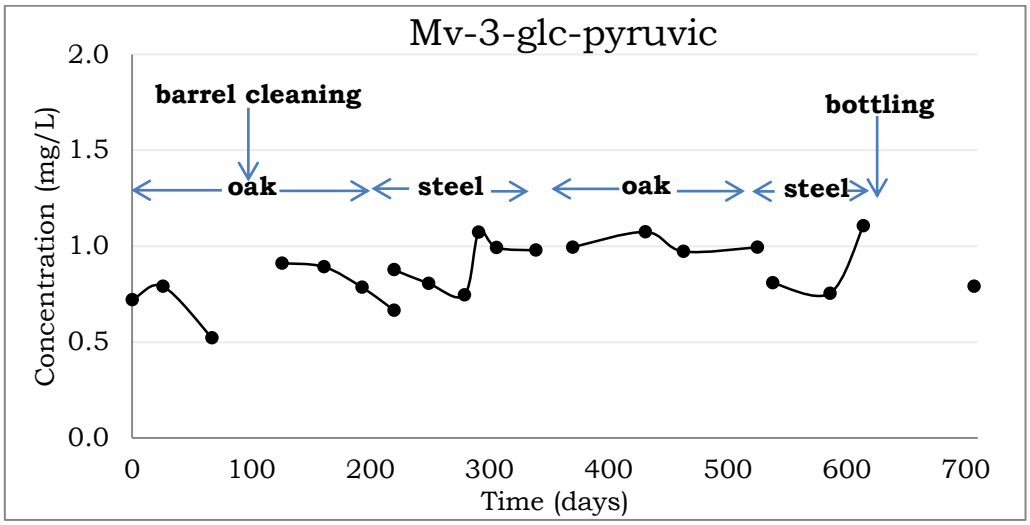



**Figure V.31.** Evolution profile for Mv-3-glc-pyruvic (**41**) during the ageing in barrel of a *Reserva* wine.

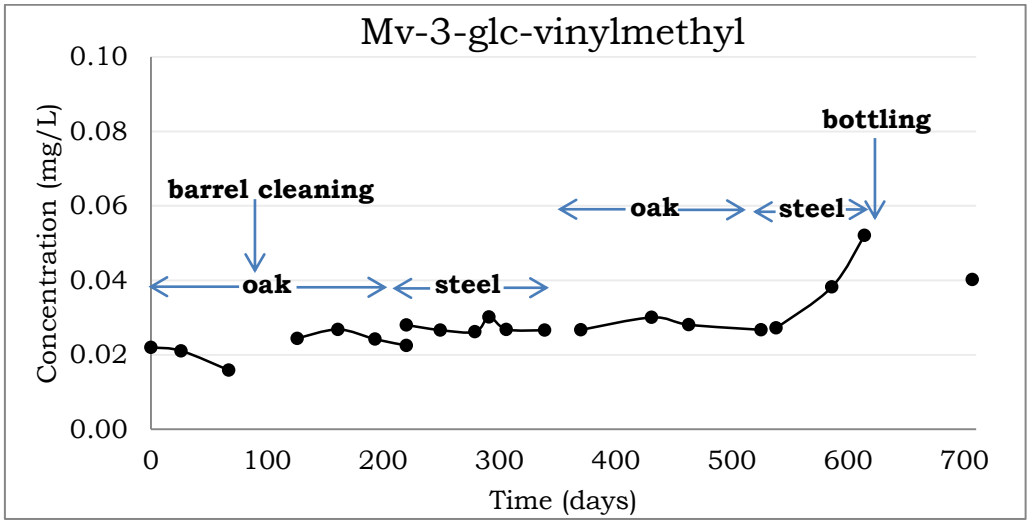



**Figure V.32.** Evolution profile for Mv-3-glc-vinylmethyl (**43**) during the ageing in barrel of a *Reserva* wine.

Although for condensation derivatives the action of barrel cleaning was deleterious concerning their concentration levels, this is not the case for the pyranoanthocyanins derivatives from the pyruvic acid, acetaldehyde and acetoacetic acid, as previously mentioned.

Relative evolution profile for Mv-3-glc-acetaldehyde (**39**) and Mv-3-glc-pyruvic (**41**) (Figure IV.33) shows that the last is the one with highest levels of concentration of this type of anthocyanin derivative pigments and also the increased contribution of pyranoanthocyanins in the total anthocyanic derivatives along ageing.

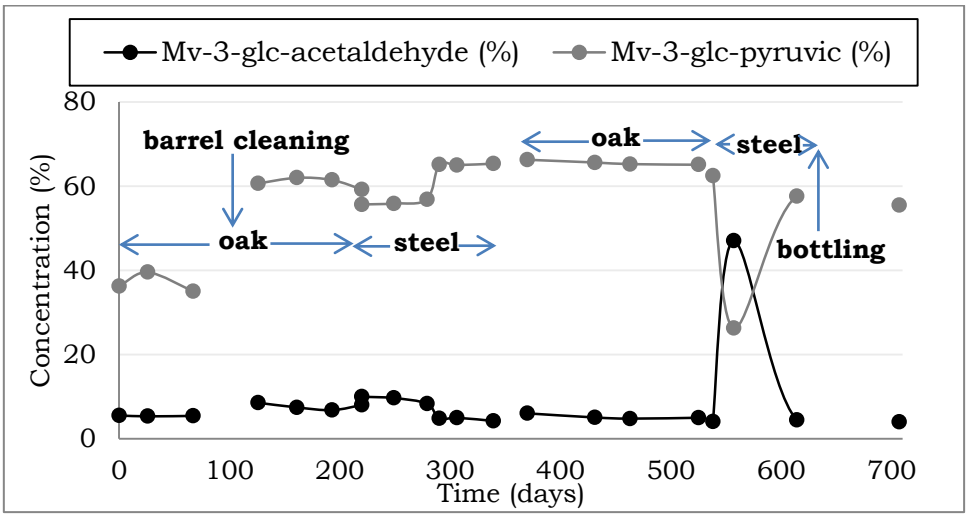



**Figure V.33.** Relative evolution profile for Mv-3-glc-acetaldehyde and Mv-3-glc-pyruvic during the ageing of a *Reserva* wine.

Mv-3-glc-4-vinylphenol (**44**) profile (Figure IV.34) shows that in this case, the barrel cleaning greatly decrease the concentration of this marker.

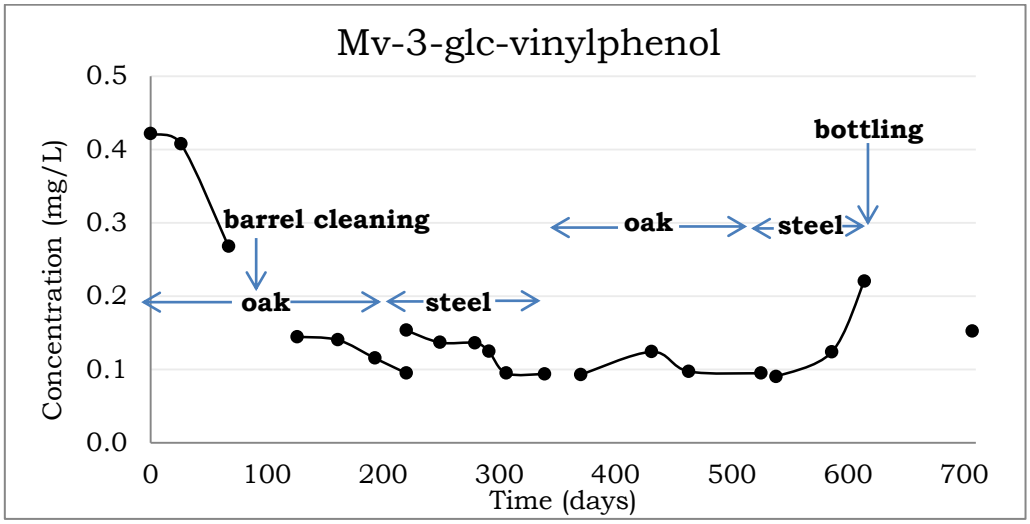



**Figure V.34.** Evolution profile for Mv-3-glc-4-vinylphenol (**44**) during the ageing of a *Reserva* wine.

Relative evolution profile for Mv-3-glc-4-vinylphenol (**44**), Mv-3-glc-4-vinylcatecol (**46**) and Mv-3-glc-vinylguaiacol (**47**) (Figure IV.35) shows a good stability of these pyranoanthocyanins after the initial losses.

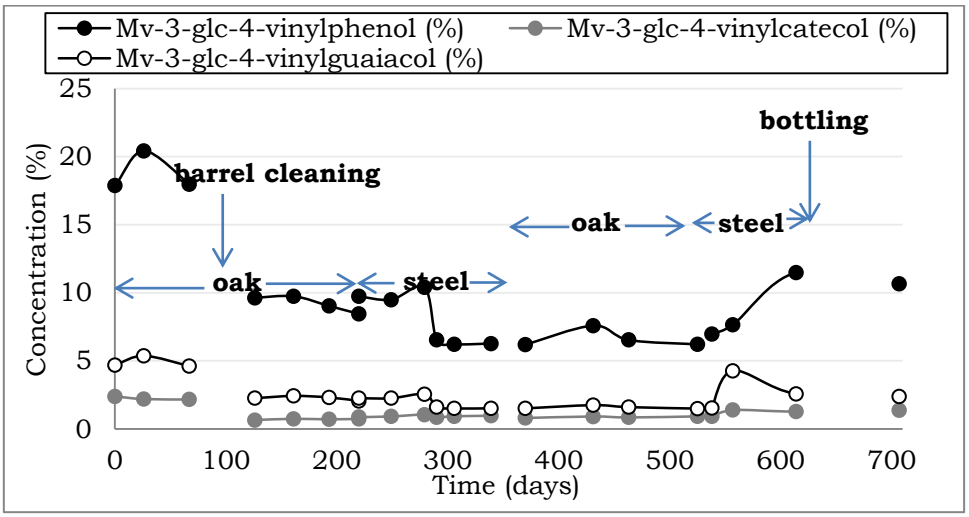



**Figure V.35.** Relative evolution profile for Mv-3-glc-vinylphenol (**44**), Mv-3-glc-4-vinylcatecol (**46**) and Mv-3-glc-4-vinylguaiacol (**47**) during the ageing of a *Reserva* wine.

## V.2.2. Tannins

### a) Flavan-3-ols monomers profiles

As has been mentioned before, the four most abundant monomers in wine that are also present in the grapes are catechin, epicatechin, gallocatechin and epigallocatechin.

Figure V.36 shows the profile achieved for the main monomer, the catechin (**57**), in oak barrel of a D.O.Ca. Rioja alavesa wine qualified as "*Reserva*". All the other monomers (epicatechin, gallocatechin and epigallocatechin) describe the same profile.

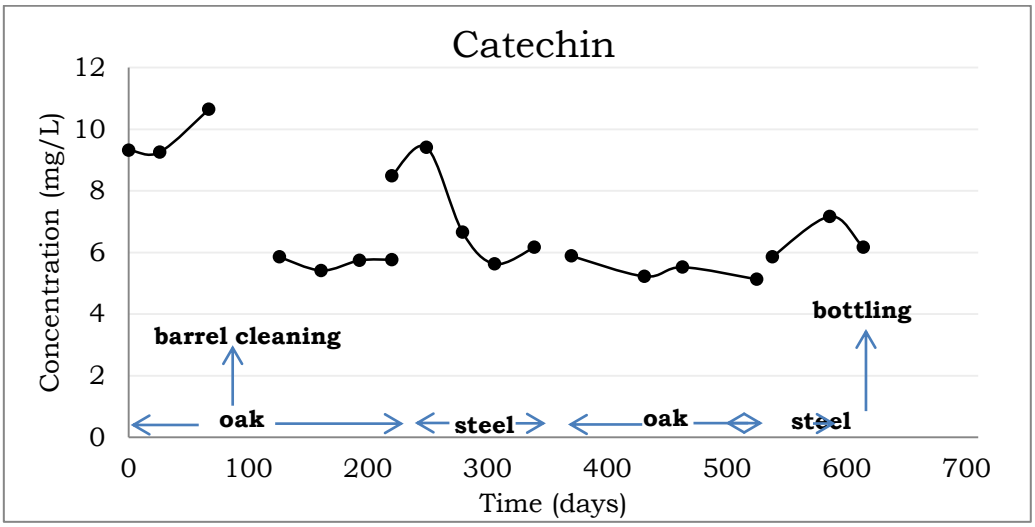



**Figure V.36.** Evolution profile representative for the flavan-3-ols monomers in a *Reserva* wine.

As it happens with the profiles described before for the anthocyanins derivatives, great concentration changes were observed when the wine is transferred from the deposit to the oak barrel or viceversa. Also, the greatest change happens when the oak barrel is cleaned. However, this type of compounds remains quite stable after the oak barrel cleaning.

### b) Profiles of dimers with B bond

Looking through the ageing evolution profiles achieved for the 3 procyanidin dimers with B bond, the 3 prodelphinidin dimers with B bond and the 4 mixed dimers with B bond, it is remarkable the similarity between them. As an example of the profile, Figure V.37 represents the profile obtained for the PCB1 (**61**).

The profile achieved during the ageing of a Reserva wine describes a decrease of concentration when the barrel is cleaned and a progressive decrease of concentration during the first barrel period, deposit stay and first part of the second barrel period which reverses with an increasing in the second part. The final stay in stainless steel deposit before bottling supposes great changes due to mixtures of wines made at winery before bottling.

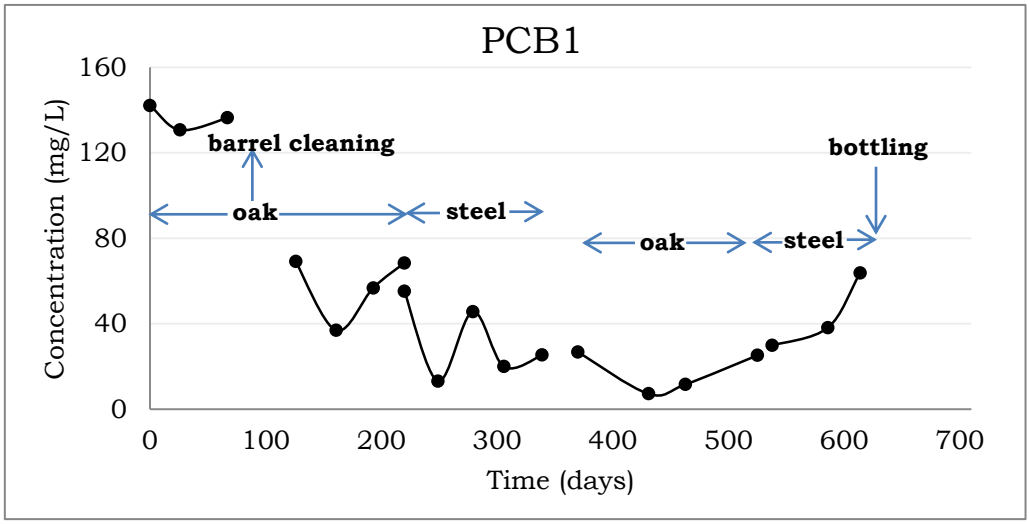



**Figure V.37.** Evolution profile representative for the tannin dimers with B bond achieved in a *Reserva* wine.

### c) Profiles of trimers with B bond

Figure V.38 shows the profiles obtained for PCC1 (**71**) as a representation of the rimers with B bond evolution during the ageing of this *Reserva* wine.

Resembling to the profiles achieved in tannin dimers, the concentration of PCC1 (**71**) in wine tends to decrease during the ageing in oak barrels until it was transferred to the final stainless steel deposit for its bottling step.

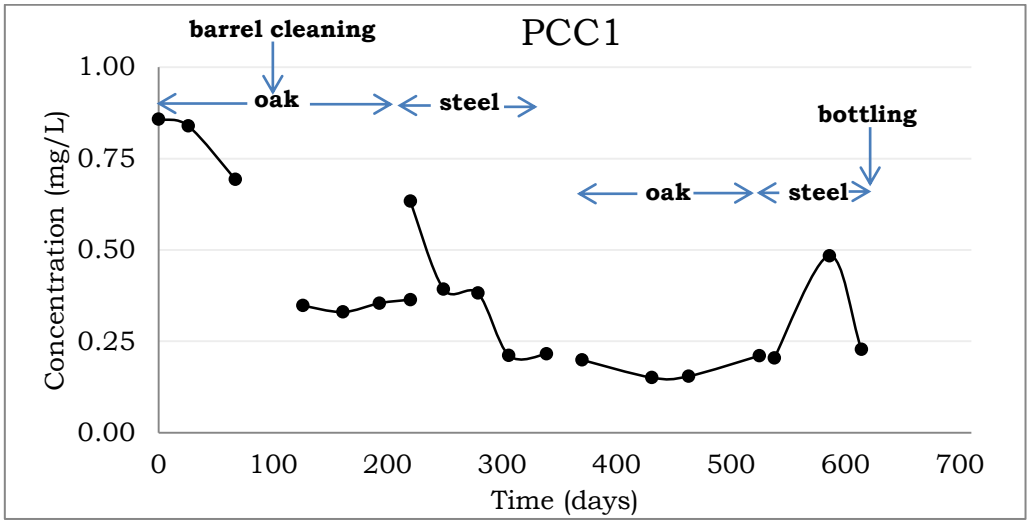



**Figure V.38.** Evolution profile representative for the tannin trimers with B bond achieved in a *Reserva* wine.

### d) Profiles of dimers with A bond

Procyanidin and prodelphinidin dimers with A bond

As it happened in fermentation stage, from the three markers of this type only two were detected and just in 3 or 4 moments along ageing, so there is not enough data to construct the formation and evolution profile. So, Figure V.39 shows the profile for ((epi)cat)$_2$ A 1 obtained in the ageing of another wine (*Reserva* wine from 2010).

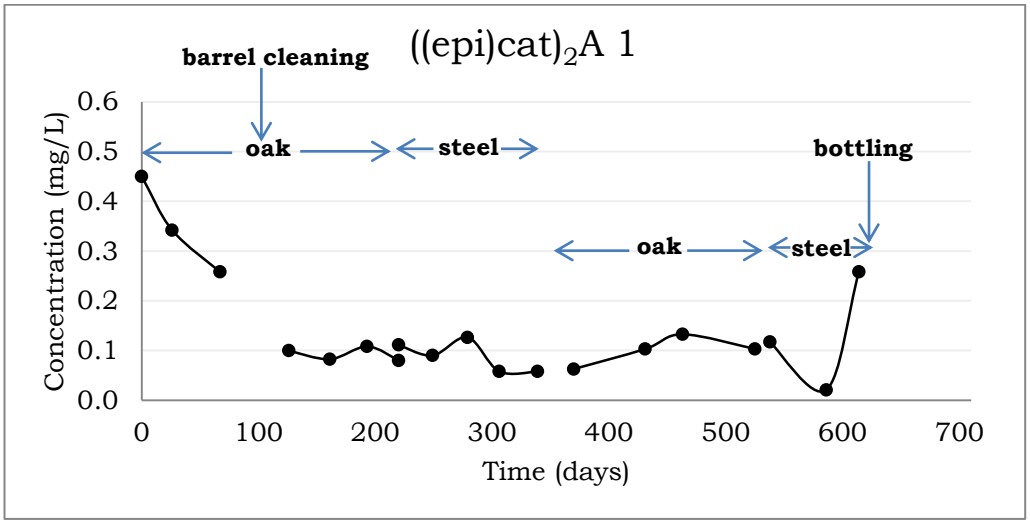



**Figure V.39.** Evolution profile representative for procyanidin and prodelphinidin dimers with A bond achieved in *Reserva* wine. Ex: ((epi)cat)2 A 1 (**75)**

Procyanidin and prodelphinidin dimers with A bond presented the same profiles during ageing in oak barrel, being greater the concentrations of the prodelphinidin dimers. Their profiles show a continuous decrease during all the ageing process until this type of dimers disappear from the wine matrix.

**Figure V.27.**

Mixed dimers with A bond

Despite changes during corrections and transfers, *Reserva* wine mixed dimers with A bond remain stable during the ageing process (Figure V.40).

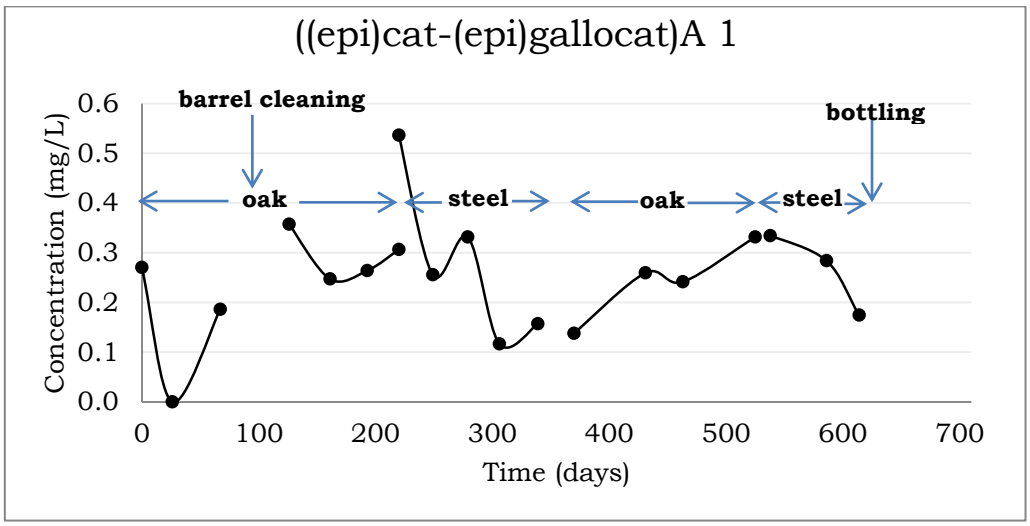
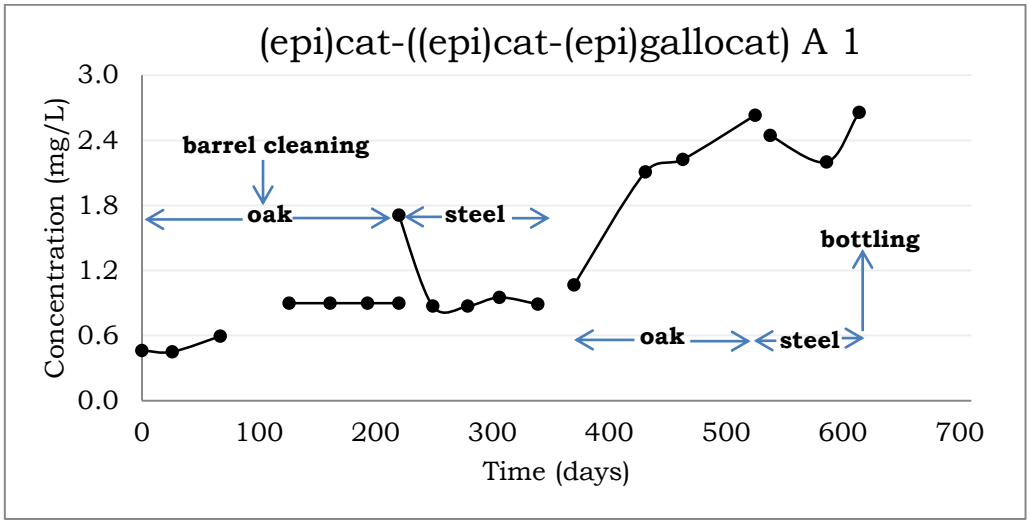


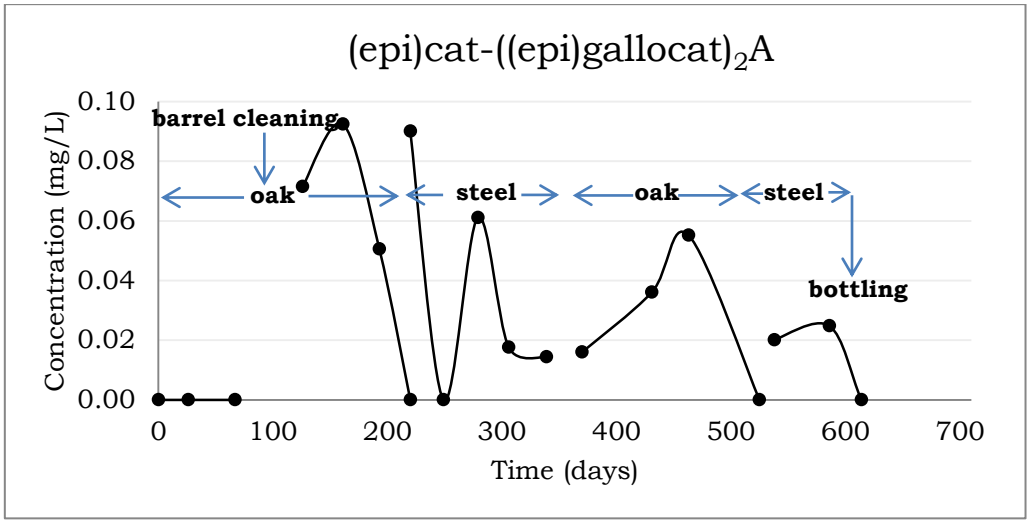

**Figure V.40.** Evolution profile representative for the mixed dimers with A bond achieved for *Reserva* wine. Ex: ((epi)cat-(epi)gallocat) A 1 (**77**)

## e) Profiles of trimers with A bond

Profile of (epi)cat-(epi)cat-(epi)gallocat) A 1 (**79**) (Figure V.41 (a)) shows an increasing of this compound during the ageing in oak barrel. Evolution of (epi)cat-((epi)gallocat)$_2$ A (Figure V.41 (b)) also supposes increases during the ageing of a *Reserva* wine.




**(a)**




**(b)**

**Figure V.41.** Evolution profile representative for the mixed trimers with A bond achieved in a *Reserva* wine: (a) (epi)cat-((epi)cat-(epi)gallocat) A 1 (**79**); (b) (epi)cat-((epi)gallocat)2 A (**81**).

## f) Profiles of tannins mediated by acetaldehyde

The profile achieved for (epi)gallocat-ethyl-(epi)gallocat (Figure V.42) is dissimilar to those profiles that were described before. Very low concentration are observed, near LOQ, being the cleaning of the barrel and the transfers to the stainless steel deposit the steps that benefit the grown of the compound, whereas the corrections and the period of time that the wine passed inside the oak barrel favour the decrease of the tannin marker.

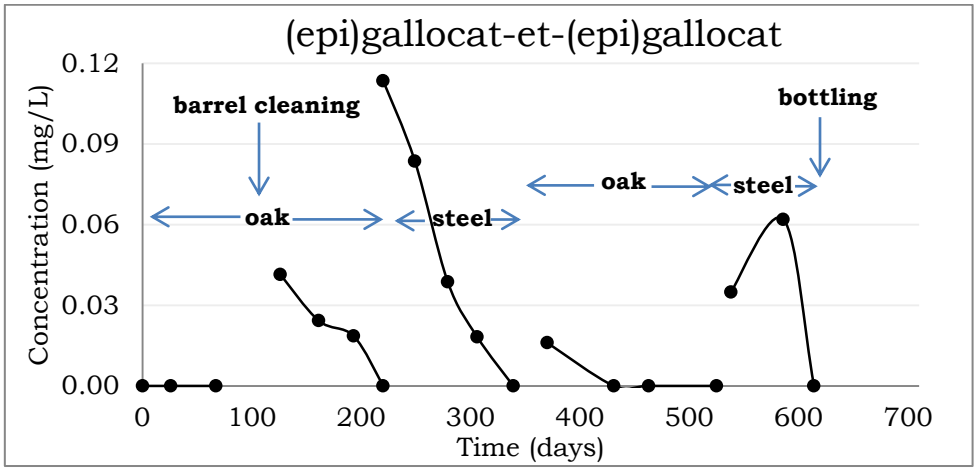



**Figure V.42.** Evolution profile for the tannin mediated by acetaldehyde achieved in *Reserva* wine ageing in oak barrel.

## g) Profiles of p-vinyl tannins

Profile for *p*-vinyl(epi)cat 2 (**82**) (Figure V.43) shows a decreasing tendency to reach low level around a few mg/L.

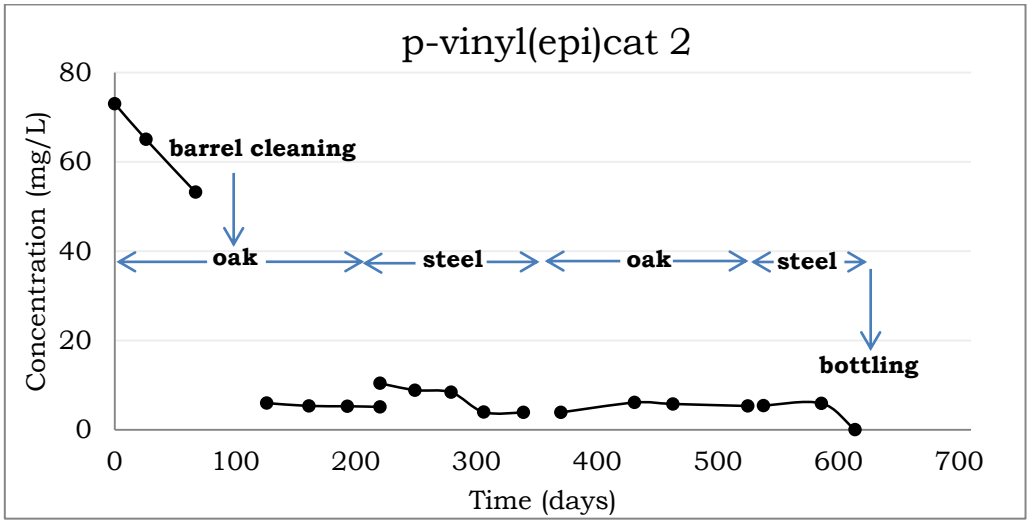



**Figure V.43.** Evolution profile for p-vinyl(epi)cat 2 (**82**) tannin achieved in *Reserva* wine.

## h) Profiles of tannins with furfuryl bridge

Profile (Figure V.44) shows high initial levels of (epi)cat-furfuryl-(epi)cat 1 (**84**) which decrease nearly to zero after the barrel cleaning.

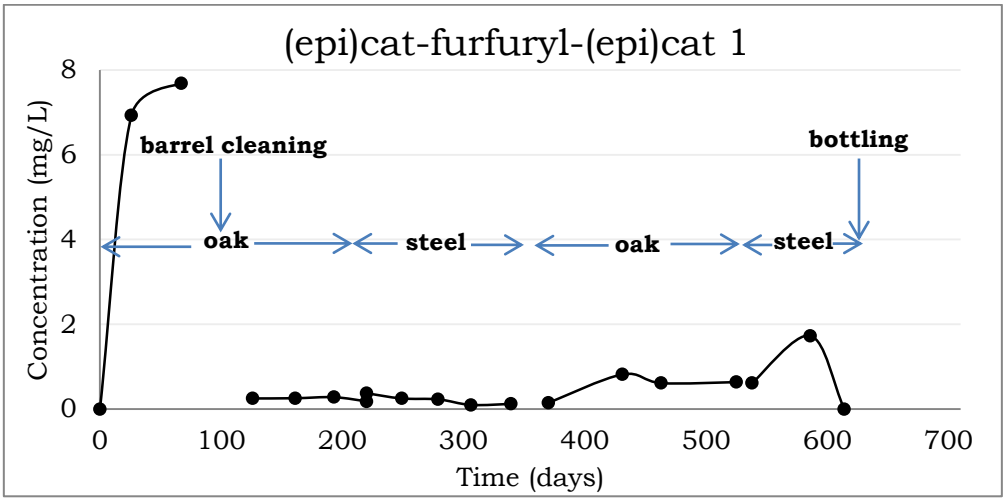



**Figure V.44.** Evolution profile for (epi)cat-furfuryl-(epi)cat 1 (**84**) tannin achieved in *Reserva* wine.

### i) O-Glycosylated flavan-3-ols profiles

Profiles achieved for the both flavan-3-ols O-glycosylated are very similar (Figure V.45). Both compounds suffered slight oscillations, with a general tendency to decrease along ageing.

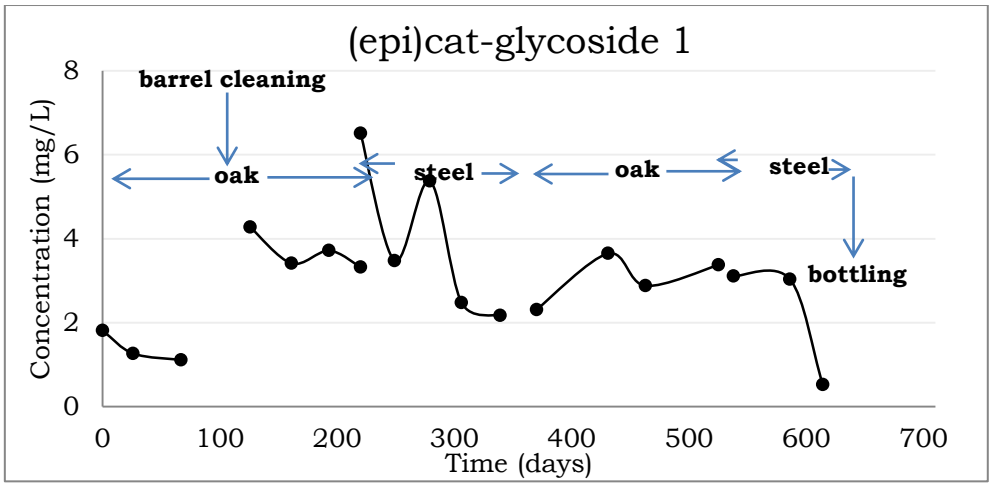



**Figure V.45.** Evolution profiles for (epi)cat-glycoside 1 (**86**) achieved in *Reserva* wine.

### j) Galloylated tannins profiles

Very low concentrations of these type of tannins were observed in all cases. As an example, Figure V.46 shows the evolution profile of Epicatechin-gallate.

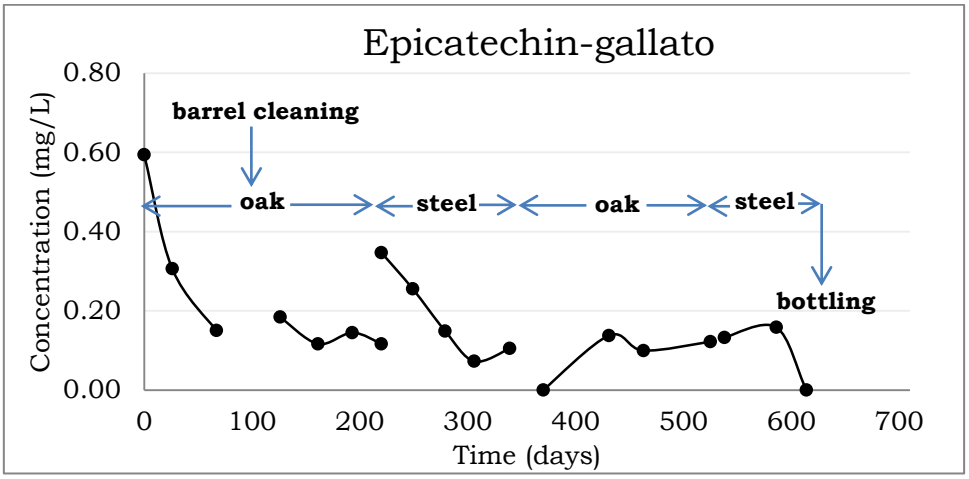



**Figure V.46.** Representative evolution profile for Epicat-gallate (*180*) achieved in *Reserva* wine.

## k) Contribution of each tannin class to total tannins

Figure V.47 shows the participation in relation to total analyzed tannins of monomers, dimers, trimers and mixed dimers with B bond type of the *Reserva* wine.

The period in barrel after the oak barrel cleaning supposes a slight decrease for monomers (-3%), dimers (-16% B bond and -15% A bond) and trimers (-2% B bond, -5% A bond).

The period within oak barrels in the case of monomers, dimers (both bond types), and trimers have a decrease tendency. The transfer to the stainless steel deposit also causes an increase in the cases of dimers with B bond (+30% in the first transfer and +68% in the second one), trimers with B bond (+57% and +11%, respectively in each transfer and trimers with A bond (+94% and +15%). While monomers and dimers A have a split in two behaviour: in the first transfer they increase (+68% and +39%, respectively), whereas in the second transfer a slight decrease is produced (-2% and -4%, respectively).

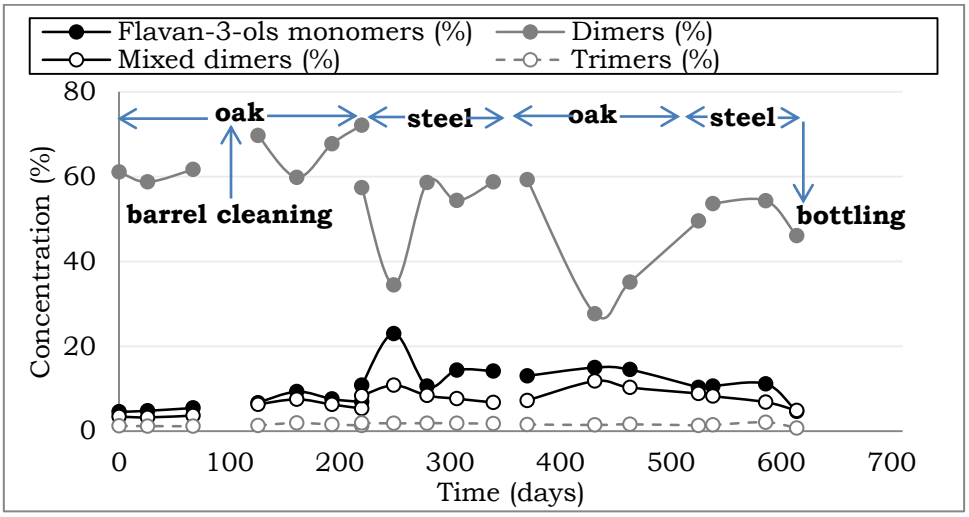



**Figure V.47.** Relative evolution profile for *Reserva* wine monomers, dimers, trimers and mixed dimers with B bond.

Figure V.48 shows the contributions of tannins with A bond. Some major contribution of mixed trimers with A bond can be observed along ageing process, that is also produced for mixed dimers with B bond. Both facts seem to point out an increasing relevance of more oxygenated tannins.

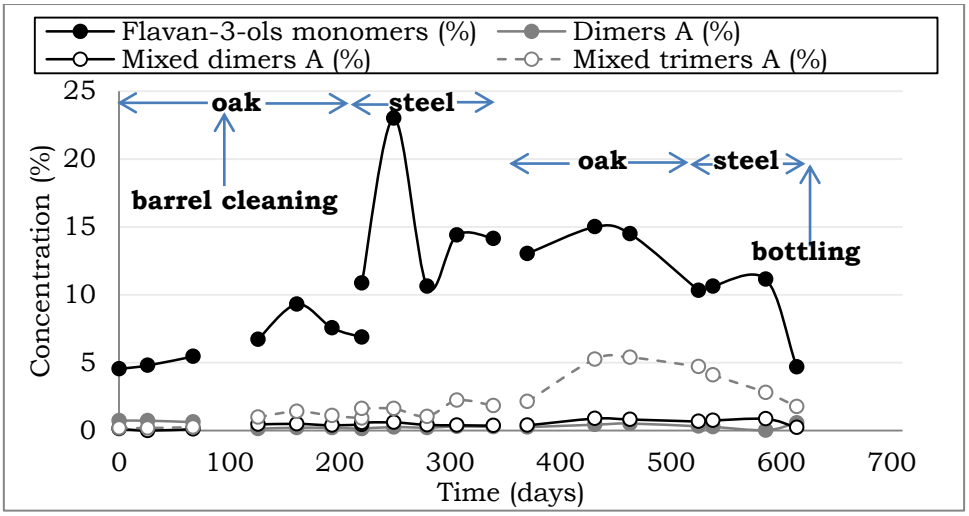



**Figure V.48.** Relative evolution profile for *Reserva* wine monomers, dimers, mixed trimers and mixed dimers with A bond.

# CHAPTER VI

## ANTHOCYANIN DERIVATIVES AND TANNINS: A COMPARISON BETWEEN RIOJA AND BORDEAUX WINES

# Chapter VI

# ANTHOCYANIN DERIVATIVES AND TANNINS: A COMPARISON BETWEEN RIOJA AND BORDEAUX WINES

In this chapter a comparison of anthocyanin derivatives and tanninsbetween Rioja and Bordeaux wines will be presented.

Although a total of 254 compounds were identified, only compounds which were in higher concentration were chosen to be quantified. So 13 free anthocyanins, 21 anthocyanin derivatives (11 flavanol-anthocyanin condensation pigments and 10 pyranoanthocyanin pigments) and 37 tannins were quantified during this research. The individual results were collected inside the Annex II.

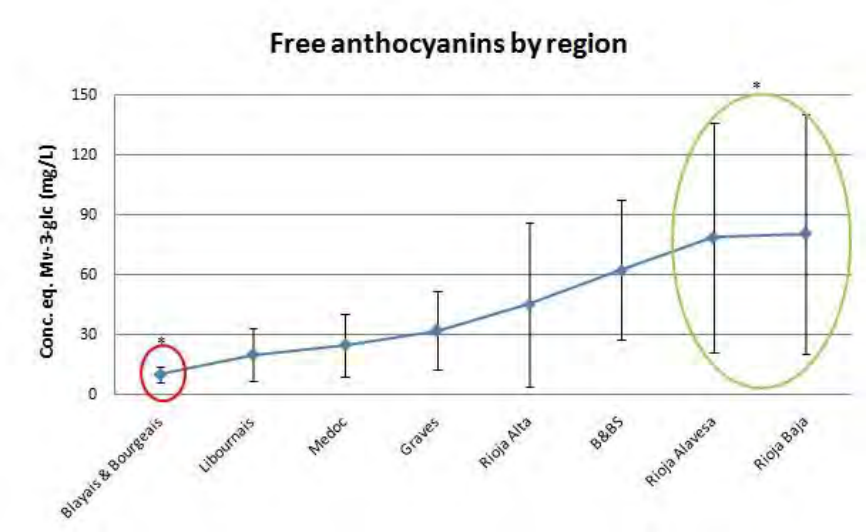
## VI.1. FREE ANTHOCYANINS

As mentioned in the third chapter, 13 anthocyanins (5 non acylated anthocyanins and 8 acylated ones) were analyzed in red wine samples from Rioja (96 wines) and Bordeaux (94 wines). The 13 anthocyanins are listed in Table VI.1.

**Table VI.1.** List of the 13 anthocyanins quantified in this research.

| **Free anthocyanins** | |
|---|---|
| Dp-3-glc | Pn-3-(6-Ac)-glc |
| Cy-3-glc | Mv-3-(6-Ac)-glc |
| Pt-3-glc | Mv-3-(6-caff)-glc |
| Pn-3-glc | Pt-3-(6-p-coum)-glc |
| Mv-3-glc | Pn-3-(6-p-coum)-glc |
| Dp-3-(6-Ac)-glc | Mv-3-(6-p-coum)-glc |
| Pt-3-(6-Ac)-glc | |

As mentioned in the first chapter, *Tempranillo* is the most used variety in Rioja wines, while *Cabernet Sauvignon* and *Merlot* are in Bordeaux ones. *Garnacha* or *Graciano* varieties are also used in Rioja wines, but usually they are not employed as the main variety.

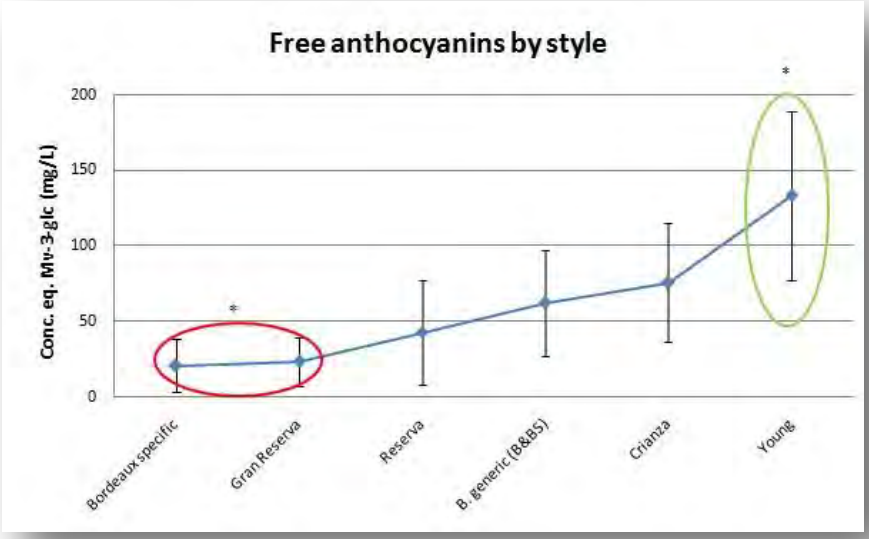



*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.1.** Mean± standard deviation concentrations for total free anthocyanins by region.

In general, Rioja wines show higher levels of total free anthocyanins (obtained as sum of the 13 individual anthocyanins) than those ones which come from the different regions of Bordeaux and their levels decrease when the time of ageing is higher, i.e. young wines are those with highest concentrations of free anthocyanins, followed in this order by *Crianza* ones, and then *Reserva* and *Gran Reserva* wines. This observation can also be seen in generic denominations of Bordeaux wines (Bordeaux and Bordeaux Supérieur (B&BS)), which have less ageing time in barrel, than the ones

with a specific denomination of Bordeaux. The graphics are show in Figures VI.1 and VI.2 and the mean, standard deviation, minimum and maximum values for total free anthocyanins, which are in equivalents of Mv-3-glc, can be seen in Tables VI.2 and VI.3.




*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.2.** Mean ± standard deviation concentrations for total free anthocyanins by winestyle.

**Table VI.2.** Total free anthocyanins concentration by region: mean in equivalents of Mv-3-glc (mg/L), standard deviation and minimum and maximum values for each region.

| | **Total anthocyanins(mg/L)** | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Blayais & Bourgeais | 10.25 | 4.23 | 4.85 | 15.20 |
| Libournais | 19.98 | 13.12 | 1.34 | 50.85 |
| Médoc | 24.99 | 15.78 | 2.63 | 69.46 |
| Graves | 32.25 | 19.70 | 4.00 | 85.77 |
| Rioja Alta | 45.29 | 41.20 | 2.72 | 203.23 |
| B&BS | 62.58 | 34.88 | 2.90 | 111.83 |
| Rioja Alavesa | 78.73 | 57.12 | 1.77 | 319.96 |
| Rioja Baja | 80.53 | 60.18 | 5.33 | 209.14 |

**Table VI.3.** Total free anthoctyanins concentration by wine style: mean in equivalents of Mv-3-glc (mg/L), standard deviation and minimum and maximum values for young, *Crianza*, *Reserva* and *Gran Reserva* wines and specific and generic denominations of Bordeaux ones.

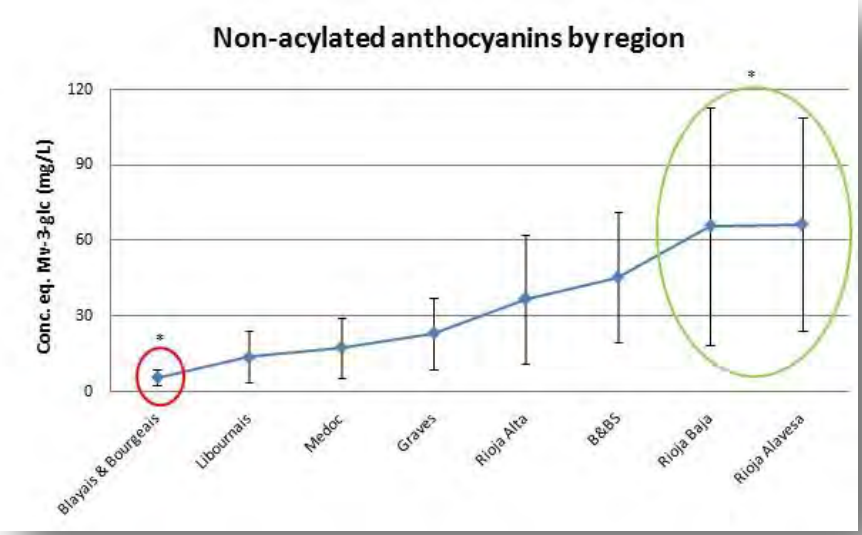
| | **Total anthocyanins(mg/L)** | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Bordeaux specific | 20.77 | 17.53 | 1.77 | 55.11 |
| *Gran Reserva* | 23.57 | 15.90 | 1.34 | 85.77 |
| *Reserva* | 42.80 | 34.29 | 2.60 | 144.39 |
| B. generic (B&BS) | 62.58 | 34.88 | 2.90 | 111.83 |
| *Crianza* | 75.80 | 39.03 | 17.61 | 189.64 |
| Young | 133.37 | 55.92 | 11.68 | 319.96 |

Within free anthocyanins, Rioja wines have higher concentration of non-acylated and coumaroylated anthocyanins than Bordeaux ones. Thus, the levels of generic wines of Bordeaux (B&BS) are lower than Young wines of Rioja, even they are somewhat lower than *Crianza* ones. In contrast, the differences between *Reserva* and *Gran Reserva* wines and specific wines of Bordeaux are not so pronounced (Figures VI.3 to VI.6, Tables VI.4 to VI.7).On the other hand, Bordeaux wines have higher levels of acetylated anthocyanins than Rioja wines (Figures VI.7 and VI.8, Tables VI.8 and VI.9).

These results are according with other authors, as a consequence of the different grape varieties used in each region. In one hand, in *Cabernet Sauvignon* and *Merlot*, the main cultivars at Bordeaux, acetylated represent 20-30% of total anthocyanin content, whereas in *Tempranillo* represents only 2-6%. In the other hand, the coumaroylated represents 9% of total anthocyanin content in *Cabernet Sauvignon* and *Merlot*, while in *Tempranillo* and *Graciano* represents 38% and 17%, respectively[440,441].

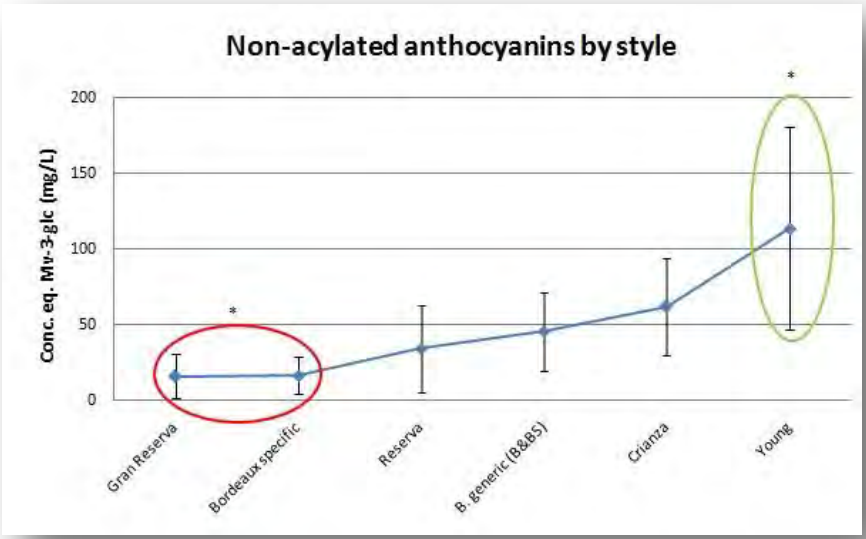
[440]Ryan, J. M.; Revilla, E.; *Anthocyanin composition of Cabernet Sauvignon and Tempranillo grapes at different stages of Ripening*, J. Agric. Food Chem. **2003**, 51, 3372-3378.

[441]Núñez, V.; Monagas, M.; Gomez-Cordovés, M. C.; Bartolomé, B.; *Vitis vinifera L. cv. Graciano grapes characterized by its anthocyanin profile,* Postharvest Biol. Technol. **2004**, 31, 69-79.




*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.3.** Mean ± standard deviation concentrations for non-acylated anthocyanins by region.




*: significant groups (α=0.05, by Scheffé test)

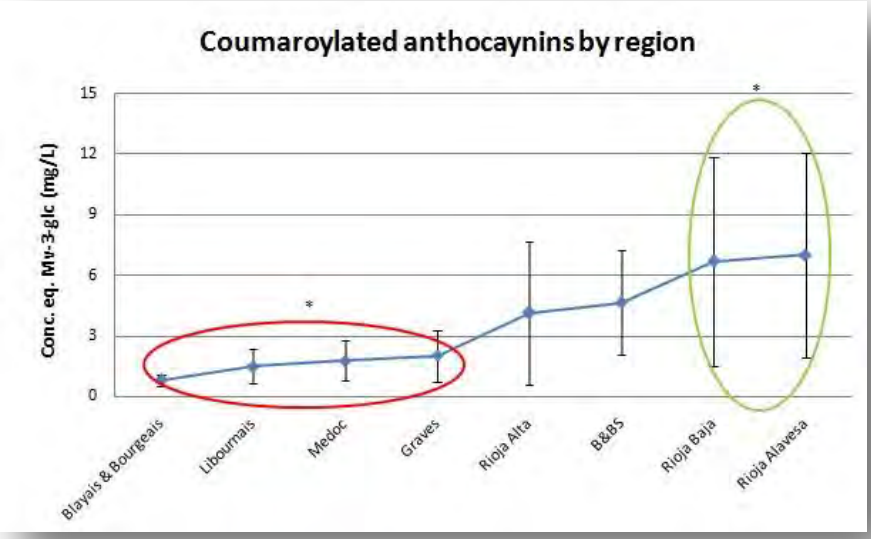
**Figure.VI.4.** Mean ± standard deviation concentrations for non-acylated anthocyanins by wine style.

**Table VI.4.** Total non-acylated anthocyanins concentration by region: mean in equivalents of Mv-3-glc (mg/L), standard deviation and minimum and maximum values for each region.

| | Non-acylated anthocyanins (mg/L) | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Blayais & Bourgeais | 5.645 | 3.26 | 1.73 | 10.28 |
| Libournais | 13.97 | 10.08 | 0.33 | 36.61 |
| Médoc | 17.48 | 12.14 | 0.82 | 52.57 |
| Graves | 23.10 | 14.13 | 2.06 | 61.07 |
| Rioja Alta | 36.80 | 25.48 | 1.04 | 175.94 |
| B&BS | 45.34 | 25.87 | 0.90 | 86.49 |
| Rioja Baja | 65.90 | 47.11 | 0.71 | 279.49 |
| Rioja Alavesa | 66.51 | 42.43 | 2.02 | 187.97 |

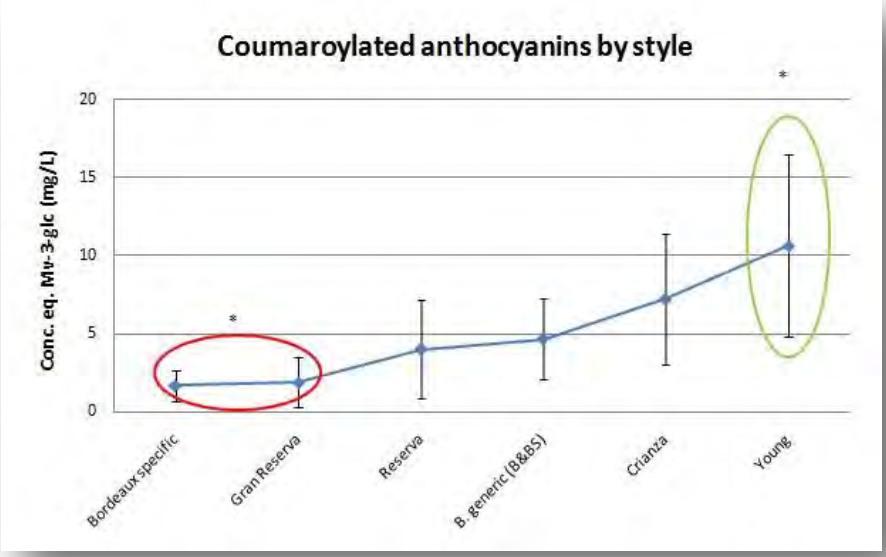
**Table VI.5.** Total non-acylated anthocyanins concentration by wine style: mean in equivalents of Mv-3-glc (mg/L), standard deviation and minimum and maximum values for young, *Crianza*, *Reserva* and *Gran Reserva* wines and specific and generic denominations of Bordeaux ones.

| | Non-acylated anthocyanins (mg/L) | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| *Gran Reserva* | 15.76 | 14.59 | 0.701 | 44.39 |
| Bordeaux specific | 16.48 | 12.04 | 0.33 | 61.07 |
| *Reserva* | 34.16 | 28.75 | 1.09 | 118.77 |
| B. generic (B&BS) | 45.34 | 25.87 | 0.90 | 86.49 |
| *Crianza* | 61.91 | 32.10 | 13.56 | 154.24 |
| Young | 113.78 | 67.06 | 8.15 | 279.49 |




*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.5.** Mean ± standard deviation concentrations for coumaroylated anthocyanins by region.




*: significant groups (α=0.05, by Scheffé test)

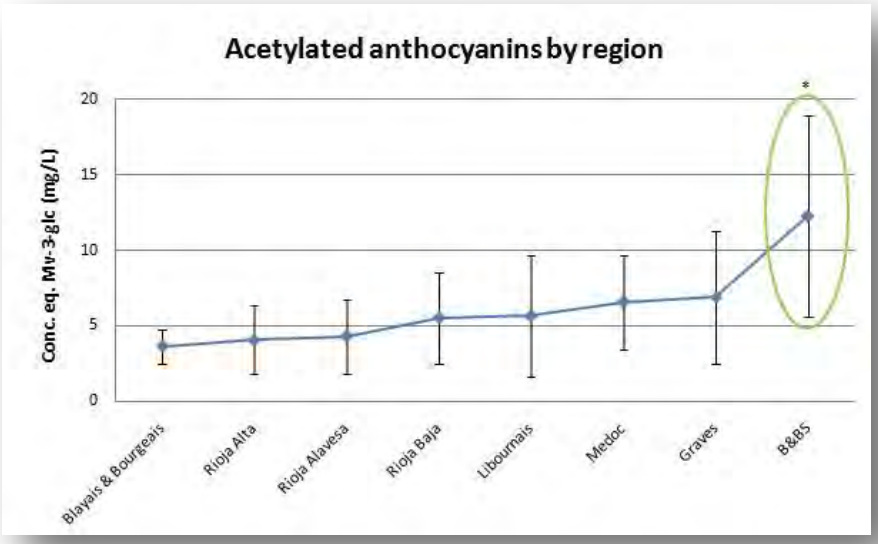
**Figure.VI.6.** Mean ± standard deviation concentrations for coumaroylated anthocyanins by wine style.

**Table VI.6.** Total coumaroylated anthocyanins concentration by region: mean in equivalents of Mv-3-glc (mg/L), standard deviation and minimum and maximum values for each region.

| | Coumaroylated anthocyanins (mg/L) | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Blayais & Bourgeais | 0.81 | 0.30 | 0.48 | 1.16 |
| Libournais | 1.50 | 0.85 | 0.35 | 3.20 |
| Médoc | 1.78 | 0.99 | 0.51 | 4.93 |
| Graves | 2.04 | 1.27 | 0.50 | 5.59 |
| Rioja Alta | 4.15 | 3.56 | 0.54 | 15.26 |
| B&BS | 4.66 | 2.58 | 0.65 | 8.44 |
| Rioja Baja | 6.70 | 5.19 | 0.38 | 23.42 |
| Rioja Alavesa | 7.01 | 5.08 | 0.47 | 20.75 |

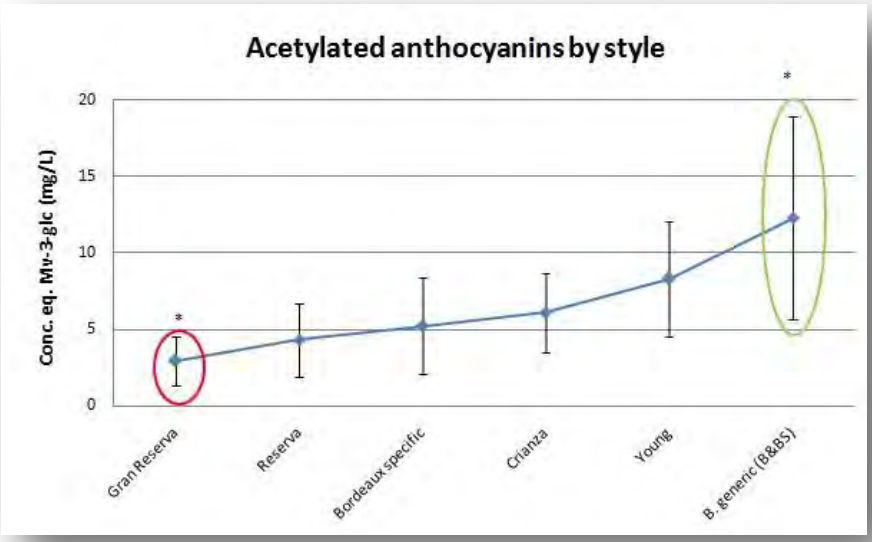
**Table VI.7.** Total coumaroylated anthocyanins concentration by wine style: mean in equivalents of Mv-3-glc (mg/L), standard deviation and minimum and maximum values for young, *Crianza*, *Reserva* and *Gran Reserva* wines and specific and generic denominations of Bordeaux ones.

| | Coumaroylated anthocyanins (mg/L) | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Bordeaux specific | 1.67 | 1.00 | 0.35 | 5.59 |
| *Gran Reserva* | 1.87 | 1.61 | 0.39 | 5.34 |
| *Reserva* | 4.01 | 3.15 | 0.38 | 13.86 |
| B. generic (B&BS) | 4.66 | 2.58 | 0.65 | 8.44 |
| *Crianza* | 7.26 | 4.20 | 1.74 | 20.75 |
| Young | 10.66 | 5.82 | 1.25 | 23.42 |




*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.7.** Mean ± standard deviation concentrations for acetylated anthocyanins by region.




*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.8.** Mean ± standard deviation concentrations for acetylated anthocyanins by wine style.

**Table VI.8.** Total acetylated anthoctyanins concentration by region: mean in equivalents of Mv-3-glc (mg/L), standard deviation and minimum and maximum values for each region.

| | Acetylated anthocyanins (mg/L) | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Blayais & Bourgeais | 3.63 | 1.13 | 2.49 | 4.82 |
| Rioja Alta | 4.05 | 2.27 | 0.68 | 11.02 |
| Rioja Alavesa | 4.32 | 2.45 | 0.55 | 15.52 |
| Rioja Baja | 5.52 | 3.00 | 2.21 | 15.02 |
| Libournais | 5.66 | 3.99 | 0.36 | 10.79 |
| Médoc | 6.59 | 3.11 | 0.91 | 11.55 |
| Graves | 6.93 | 4.40 | 1.25 | 18.95 |
| B&BS | 12.31 | 6.66 | 1.17 | 20.43 |

**Table VI.9.** Total acetylated anthocyanins concentration bywine style: mean in equivalents of Mv-3-glc (mg/L), standard deviation and minimum and maximum values for young, Crianza, Reserva and Gran Reserva wines and specific and generic denomination of Bordeaux ones.

| | Acetylated anthocyanins (mg/L) | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| *Gran Reserva* | 2.95 | 1.58 | 0.55 | 6.12 |
| *Reserva* | 4.33 | 2.40 | 1.06 | 10.94 |
| Bordeaux specific | 5.23 | 3.13 | 0.36 | 18.95 |
| *Crianza* | 6.13 | 2.59 | 2.17 | 13.36 |
| Young | 8.33 | 3.75 | 2.14 | 15.52 |
| B. generic (B&BS) | 12.31 | 6.66 | 1.17 | 20.43 |

## VI.2. ANTHOCYANIN DERIVATIVES

As explained in the first chapter, free anthocyanins (non-acylated, coumaroylated and acetylated anthocyanins), which are in high concentration in grape must, can react during maturation and ageing with other compounds that are in wine matrix, undergoing new anthocyanin derivatives that change the initial colour of must and young wine. These anthocyanin derivatives could be grouped as tannin-anthocyanin derivatives, which are formed by direct condensation or through a molecule of acetaldehyde (which generates an ethylidene bridge between both parts) and different pyranoanthocyanin classes, which are formed when the free anthocyanin react with pyruvic acid (Vitisins A), acetaldehyde (Vitisins B), acetoacetic acid, vinylphenols or vinylflavanols. The tannin-anthocyanin derivatives give violet hues to wine, while the different pyranoanthocyanin give orange colours.

## VI.2.1. Tannin-anthocyanin derivatives

Their UV-vis absorption spectra are similar to that of anthocyanins, indicating that the chromophore group is still present, showing an additional shoulder at 450 nm, more pronounced in the case of pigments formed by condensation mediated by acetaldehyde[442] and bathochromic shifts in the wavelength of maximum absorption, giving to the wine a purplish tinge.

The decrease of astringency during ageing is considered the result of anthocyanin-flavanol condensation, both, ethylidene-bridged and direct condensation[443],as well as a wine colour stabilization and decreased bitterness, accompanied by an increase in feelings of complexity and volume in the mouth[444].As mentioned, two mechanisms have been described for the formation of pigments by direct anthocyanin-flavanol condensation leading to F-$A^+$ or $A^+$-F depending on the relative position of the flavanol and anthocyanin in the compound.

11 tannin-anthocyanin derivatives (6 are formed by direct condensation and 5 mediated by acetaldehyde) were quantified in red wine samples from Rioja and Bordeaux. The 11 tannin-anthocyanin derivatives were listed in Table VI.10.

**Table VI.10.** List of the 11 tannin-anthocyanin derivatives quantified in this research.

| **Tannin-anthocyanin derivative** | |
|---|---|
| Catechin-Mv-3-glc | Mv-3-(6-p-coum)-glc-8-ethyl-catechin 1 |
| Epicatechin-Mv-3-glc | Mv-3-(6-p-coum)-glc-8-ethyl-catechin 2 |
| Catechin-Mv-3-(6-p-coum)-glc | Mv-3-(6-p-coum)-glc-8-ethyl-epicatechin |
| Epicatechin-Mv-3-(6-p-coum)-glc | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin 1 |
| (Epi)gallocatechin-Mv-3-glc | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin 2 |
| (Epi)gallocatechin-Mv-3-(6-p-coum)-glc | |

On the one hand, no significant differences in the total concentration of tannin-anthocyanin derivatives can be appreciated when comparing the Rioja and Bordeaux wines. Although it can be seen that levels decrease when the time of ageing is higher, i.e. young wines are those with highest concentrations of tannin-anthocyanin derivatives, followed by *Crianza* ones, *Reserva* and *Gran Reserva*.

[442]Fulcrand, H.; Cameira dos Santos, P. J.; Sarni-Manchado, P.; Cheynier, V.; Favre-Bonvin, J.; *Structure of new anthocyanin-derived pigments*, J. Chem. Soc. **1996**, 1, 735-739.

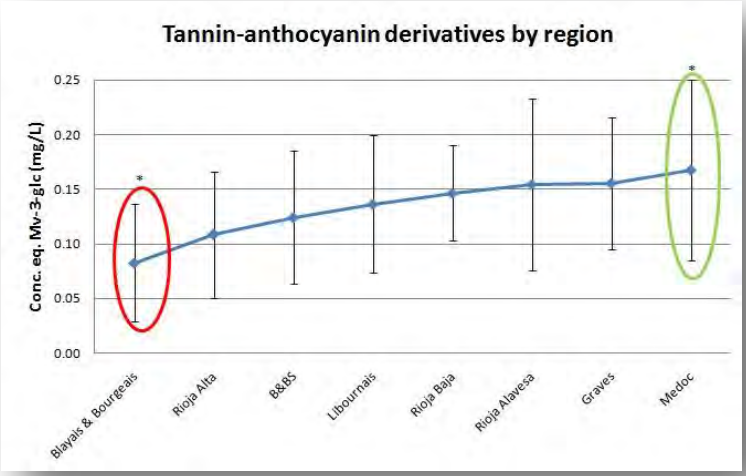
[443]Es-Safi, N. E.; Fulcrand, H.; Cheynier, V.; Moutounet, M.; *Studies on the acetaldehyde-induced condensation of (-)-epicatechin and malvidin-3-O-glucoside in a model solution system*,J. Agric. Food Chem. **1999**, 47, 2096-2102.

[444]Hidalgo Togores, J.;*Crianza de vinos*. En Tratado de enología. Tomo II. Ediciones Mundi-Prensa, Madrid, **2002**, pp. 857-944.

On the other hand, the levels of *Gran Reserva* and *Reserva* wines are lower that the specific wines of Bordeaux. In contrast the differences between specific and generic wines of Bordeaux and young and *Crianza* ones from Rioja are not so pronounced.

The graphics are show in Figures VI.9 to VI.10 and the results, which are in equivalents of Mv-3-glc, can be seen in Tables VI.11 to VI.12.

The same conclusions will be found if both types of tannin-anthocyanin derivatives, those by direct condensation and those with ethylidene bridge, are examined separately.

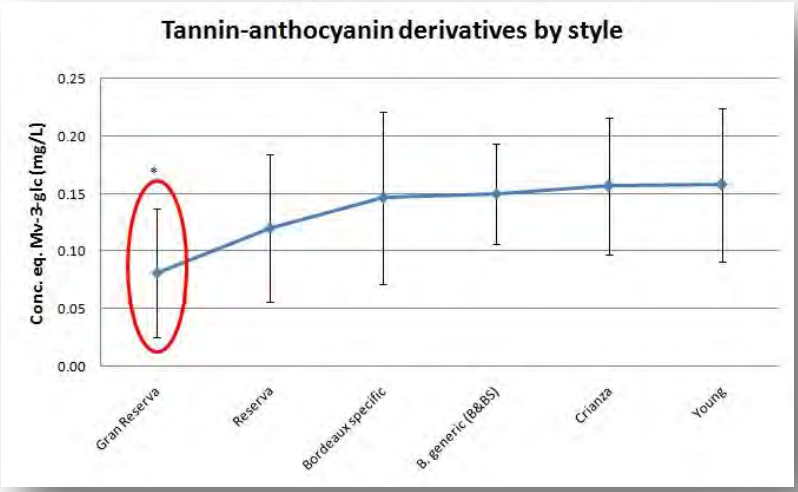



*: significant group (α=0.05, by HSD of Tukey test)

**Figure.VI.9.** Mean ± standard deviation concentrations for total tannin-anthocyanin derivative pigments by region.

**Table VI.11.** Concentration of total tannin-anthocyanin derivative pigments by region: mean in equivalents of Mv-3-glc (mg/L), standard deviation and minimum and maximum values for each region.

| | **Tannin-anthocynin derivatives (mg/L)** | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Blayais & Bourgeais | 0.083 | 0.074 | 0.038 | 0.213 |
| Rioja Alta | 0.108 | 0.058 | 0.011 | 0.264 |
| B&BS | 0.124 | 0.061 | 0.015 | 0.194 |
| Libournais | 0.137 | 0.063 | 0.024 | 0.269 |
| Rioja Baja | 0.147 | 0.044 | 0.015 | 0.235 |
| Rioja Alavesa | 0.154 | 0.078 | 0.011 | 0.308 |
| Graves | 0.155 | 0.061 | 0.062 | 0.264 |
| Médoc | 0.168 | 0.082 | 0.016 | 0.378 |




*: significant group (α=0.05, by HSD of Tukey test)

**Figure.VI.10.** Mean ± standard deviation concentrations for total tannin-anthocyanin derivative pigments by wine style.

**Table VI.12.** Concentration of total tannin-anthocyanin derivative pigments by wine style: mean in equivalents of Mv-3-glc (mg/L), standard deviation and minimum and maximum values for young, *Crianza*, *Reserva* and *Gran Reserva* wines and specific and generic denomination of Bordeaux ones.

| | **Tannin-anthocynin derivatives (mg/L)** | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| *Gran Reserva* | 0.081 | 0.056 | 0.011 | 0.182 |
| *Reserva* | 0.120 | 0.064 | 0.015 | 0.214 |
| Bordeaux specific | 0.147 | 0.075 | 0.016 | 0.378 |
| B. generic (B&BS) | 0.150 | 0.044 | 0.015 | 0.194 |
| *Crianza* | 0.157 | 0.059 | 0.068 | 0.265 |
| Young | 0.158 | 0.066 | 0.060 | 0.308 |

## VI.2.2. Pyranoanthocyanins

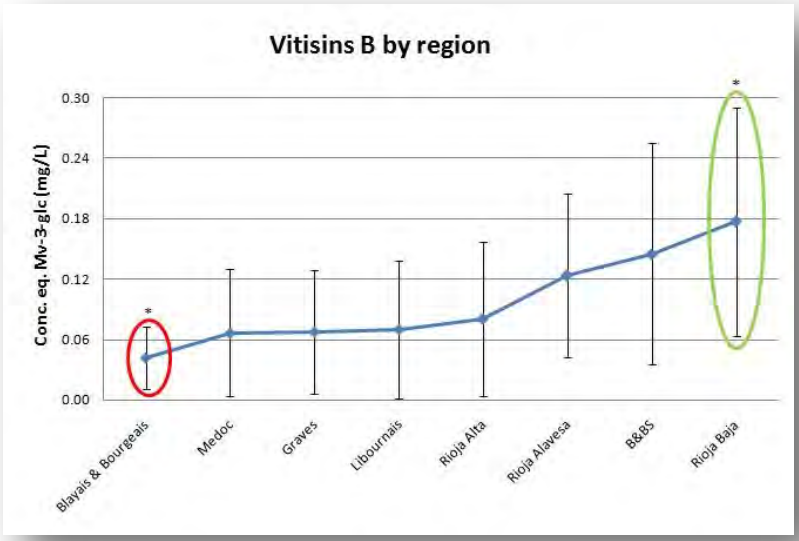
Such compounds present hypsochromic shifts in wavelength of maximum absorption of the UV-vis spectrum, giving the wine a hue tile. One possible explanation for this hypsochromic effect observed might be the charge of C ring, which may be delocalized by resonance and being partially located in the oxygen atom of the

new D ring formed. A small band in the region of 370 nm, characteristic of 4-replaced anthocyanins, is also seen[445].

10 pyranoanthocyanins: 2 derivatives with pyruvic acid (Vitisins A), 2 derivatives with acetaldehyde (Vitisins B), 1 derivative with acetoacetic acid and 5 derivatives with hydroxycinnamic acid were analyzed in red wine samples from Rioja and Bordeaux. The 10 pyranoanthocyanins are listed in Table VI.13.

**Table VI.13.** List of the 10 pyranoanthocyan in pigments quantified in this research.

| **Pyranoanthocyanins** | |
|---|---|
| Mv-3-glc-pyruvic | Mv-3-glc-4-vinylphenol |
| Mv-3-(6-p-coum)-glc-pyruvic | Mv-3-(6-p-coum)-glc-4-vinylphenol |
| Mv-3-glc-acetaldehyde | Mv-3-glc-4-vinylcatecol |
| Mv-3-(6-p-coum)-glc-acetaldehyde | Mv-3-glc-4-vinylguaiacol |
| Mv-3-glc-vinylmetyl | Mv-3-(6-p-coum)-glc-4-vinylguaiacol |



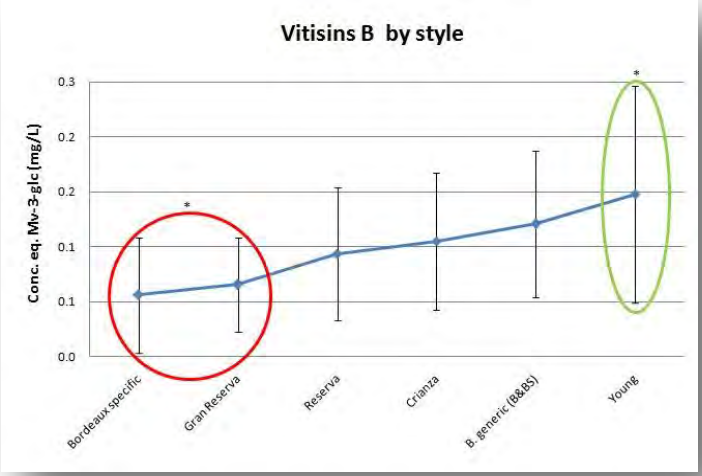

*: significant group (α=0.05, by Scheffé test)

**Figure.VI.11.** Mean ± standard deviation concentrations for Vitisins B by region.

In one hand, Rioja wines have higher concentration of anthocyanins derivatives with acetaldehyde (Vitisins B) than Bordeaux ones (Figure VI.11, Table VI.14). This observation is also present in wines of both region with less ageing, such as Rioja young wines and generic denominations of Bordeaux (B&BS), when compared with

[445]Fulcrand, H.; Benabdeljalil, C.; Rigaud, J.; Cheynier, V.; Moutounet, M.*; A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins*, Phytochem.**1998**, 47, 1401-1407.

more aged wines such as *Crianza*, *Reserva* and *Gran Reserva* in Rioja and specific denominations in Bordeaux (Figure VI.12, Table VI.15).This kind of derivatives appears quickly during the first steps of alcoholic fermentation, but they show poor stability in the rest of maturation steps and ageing[446].




*: significant group (α=0.05, by Scheffé test)

**Figure.VI.12.** Mean ± standard deviation concentrations for Vitisins B by wine style.

**Table VI.14.** Concentration of total Vitisins B by region: mean in equivalents of Mv-3-glc (mg/L), standard deviation and minimum and maximum values for each region.

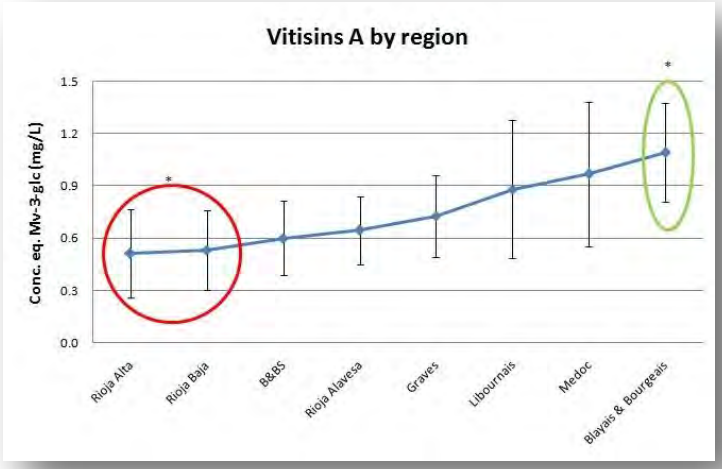
| | Vitisins B (mg/L) | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Blayais & Bourgeais | 0.042 | 0.031 | 0.007 | 0.091 |
| Médoc | 0.066 | 0.063 | 0.004 | 0.317 |
| Graves | 0.068 | 0.061 | 0.007 | 0.264 |
| Libournais | 0.070 | 0.077 | 0.010 | 0.246 |
| Rioja Alta | 0.080 | 0.077 | 0.003 | 0.333 |
| Rioja Alavesa | 0.123 | 0.082 | 0.010 | 0.467 |
| B&BS | 0.145 | 0.110 | 0.004 | 0.685 |
| Rioja Baja | 0.177 | 0.114 | 0.001 | 0.820 |

[446] Rasines-Perea, Z.; *Study of the evolution of anthocyanins, tannins and derivatives during making and ageing of red wine from Rioja by FT-IR and HPLC-MS/MS*, PhD thesis, Universidad del Pais Vasco/EuskalHerrikoUnibertsitatea, Spain, **2014**.

**Table VI.15.** Concentration of total Vitisins B by wine style: mean in equivalents of Mv-3-glc (mg/L), standard deviation and minimum and maximum values for young, *Crianza*, *Reserva* and *Gran Reserva* wines and specific and generic denomination of Bordeaux ones.

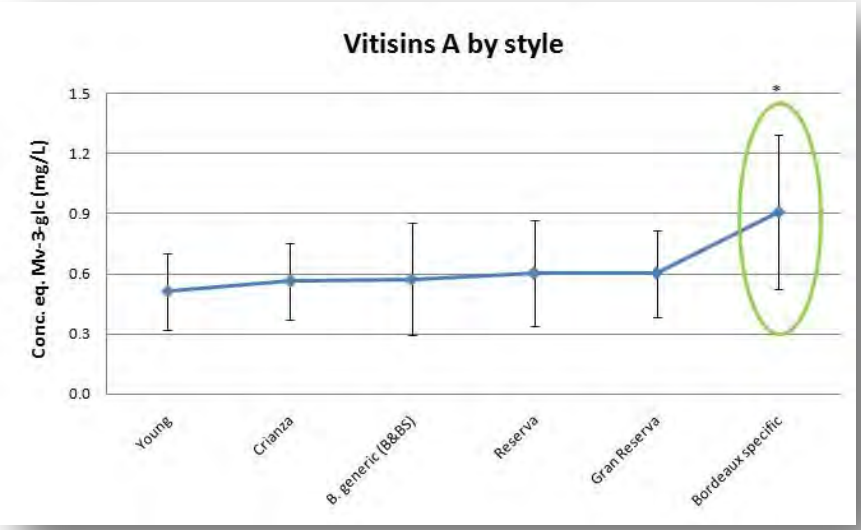
| | Vitisins B (mg/L) | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Bordeaux specific | 0.067 | 0.063 | 0.004 | 0.317 |
| *Gran Reserva* | 0.079 | 0.052 | 0.001 | 0.456 |
| *Reserva* | 0.112 | 0.073 | 0.034 | 0.522 |
| *Crianza* | 0.126 | 0.075 | 0.007 | 0.820 |
| B. generic (B&BS) | 0.145 | 0.080 | 0.004 | 0.685 |
| Young | 0.177 | 0.118 | 0.026 | 0.467 |

In the other hand, Rioja wines present lower levels of anthocyanins derivatives with pyruvic acid (Vitisins A) (Figure VI. 13, Table VI.16) than Bordeaux wines. This observation is also present in wines of both region with less ageing, such as young and *Crianza* wines of Rioja and generic denominations of Bordeaux (B&BS), when compared with more aged wines such as *Reserva* and *Gran Reserva* in Rioja and specific denominations in Bordeaux (Figure VI.14, Table VI.17).These compounds have a higher stability during maturation and ageing than Vitisins B, so the differences found in the diverse styles are according with our previous studies.




*: significant group (α=0.05, by Scheffé test)

**Figure.VI.13.** Mean ± standard deviation concentrations for Vitisins A by region.




*: significant group (α=0.05, by Scheffé test)

**Figure.VI.14.** Mean ± standard deviation concentrations for Vitisins A by wine style.

**Table VI.16.** Concentration of total Vitisins A by region: mean in equivalents of Mv-3-glc (mg/L), standard deviation and minimum and maximum values for each region.

| | Vitisins A (mg/L) | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Rioja Alta | 0.510 | 0.254 | 0.128 | 1.188 |
| Rioja Baja | 0.532 | 0.231 | 0.159 | 1.197 |
| B&BS | 0.601 | 0.216 | 0.168 | 0.991 |
| Rioja Alavesa | 0.646 | 0.195 | 0.334 | 1.201 |
| Graves | 0.727 | 0.233 | 0.400 | 1.081 |
| Libournais | 0.881 | 0.396 | 0.334 | 1.822 |
| Médoc | 0.969 | 0.415 | 0.301 | 1.895 |
| Blayais & Bourgeais | 1.092 | 0.283 | 0.721 | 1.471 |

**Table VI.17.** Concentration of total Vitisins A by wine style: mean in equivalents of Mv-3-glc (mg/L), standard deviation and minimum and maximum values for Young, *Crianza*, *Reserva* and *Gran Reserva* wines and specific and generic denomination of Bordeaux ones.

| | Vitisins A (mg/L) | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Young | 0.513 | 0.191 | 0.198 | 0.902 |
| *Crianza* | 0.562 | 0.191 | 0.325 | 0.963 |
| B. generic (B&BS) | 0.574 | 0.280 | 0.168 | 0.991 |
| *Reserva* | 0.601 | 0.264 | 0.128 | 1.188 |
| *Gran Reserva* | 0.601 | 0.216 | 0.298 | 1.201 |
| Bordeaux specific | 0.908 | 0.385 | 0.301 | 1.895 |

In contrast, no significant differences can be seen in derivatives with acetoacetic and hydroxycinnamic acids, although Rioja wines tend to present lower levels of derivatives with hydroxycinnamic acids than Bordeaux wines (see Figures VI.15 to VI.18, Tables VI.18 to VI.21).

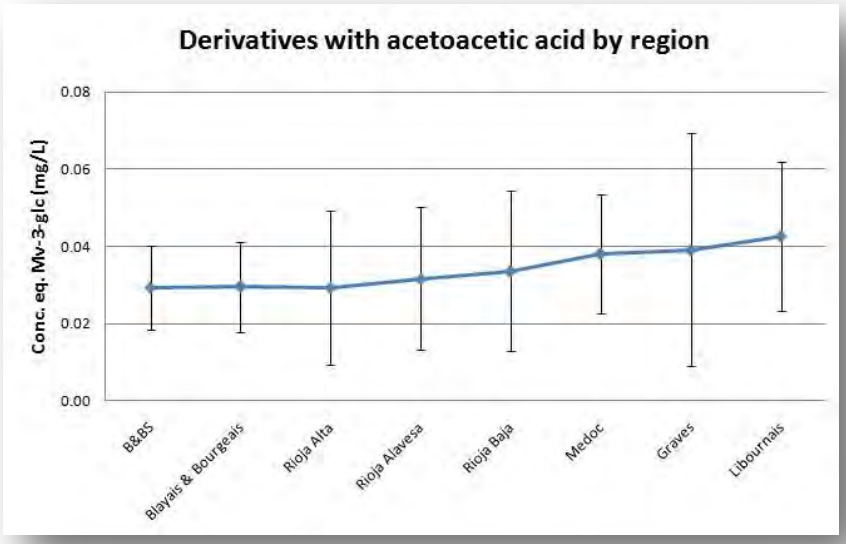



**Figure.VI.15.** Mean ± standard deviation concentrations for anthocyanin-derivatives with acetoacetic acid by region.

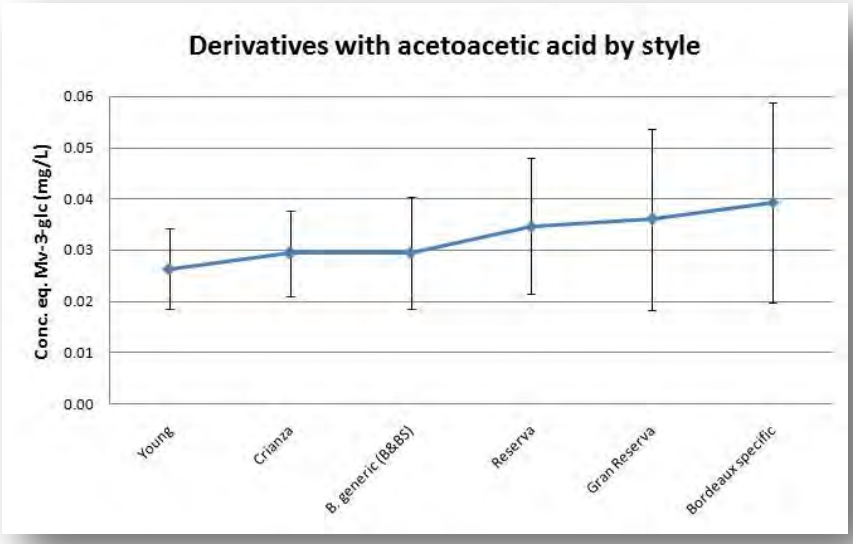



**Figure.VI.16.** Mean ± standard deviation concentrations for anthocyanin-derivatives with acetoacetic acid by wine style.

**Table VI.18.** Concentration of total anthocyanin derivatives with acetoacetic acid by region: mean in equivalents of Mv-3-glc (mg/L), standard deviation and minimum and maximum values for each region.

| | **Derivatives with acetoacetic acid (mg/L)** | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| B&BS | 0.029 | 0.011 | 0.009 | 0.044 |
| Blayais & Bourgeais | 0.030 | 0.012 | 0.016 | 0.046 |
| Rioja Alta | 0.029 | 0.020 | 0.008 | 0.095 |
| Rioja Alavesa | 0.032 | 0.018 | 0.008 | 0.083 |
| Rioja Baja | 0.034 | 0.021 | 0.005 | 0.116 |
| Médoc | 0.028 | 0.015 | 0.010 | 0.074 |
| Graves | 0.039 | 0.030 | 0.009 | 0.099 |
| Libournais | 0.043 | 0.019 | 0.013 | 0.091 |

**Table VI.19.** Concentration of total anthocyanin derivatives with acetoacetic acid by wine style: mean in equivalents of Mv-3-glc (mg/L), standard deviation and minimum and maximum values for young, *Crianza*, *Reserva* and *Gran Reserva* wines and specific and generic denomination of Bordeaux ones.

| | **Derivatives with acetoacetic acid (mg/L)** | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Young | 0.026 | 0.008 | 0.008 | 0.088 |
| *Crianza* | 0.029 | 0.008 | 0.011 | 0.025 |
| B. generic (B&BS) | 0.029 | 0.0011 | 0.009 | 0.044 |
| *Reserva* | 0.035 | 0.013 | 0.010 | 0.116 |
| *Gran Reserva* | 0.036 | 0.018 | 0.005 | 0.095 |
| Bordeaux specific | 0.039 | 0.020 | 0.009 | 0.099 |

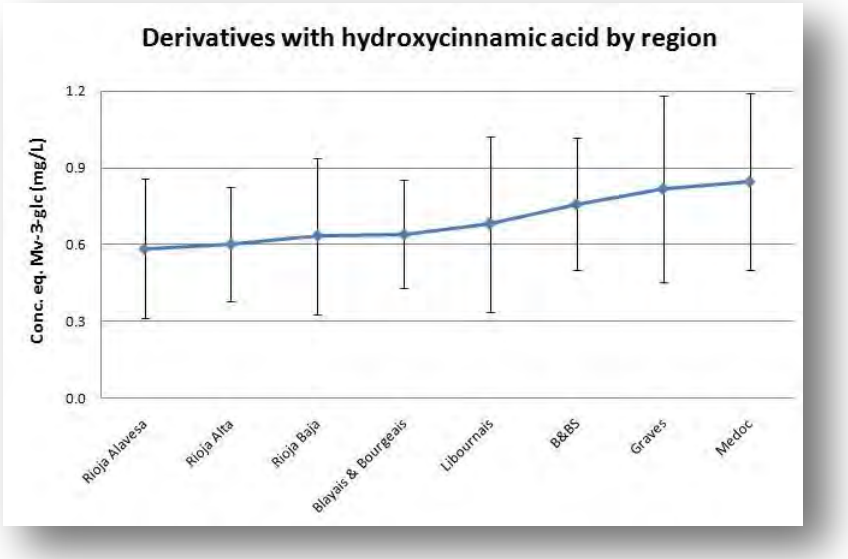



**Figure.VI.17.** Mean ± standard deviation concentrations for anthocyanin derivatives with hydroxycinnamic acids by region.

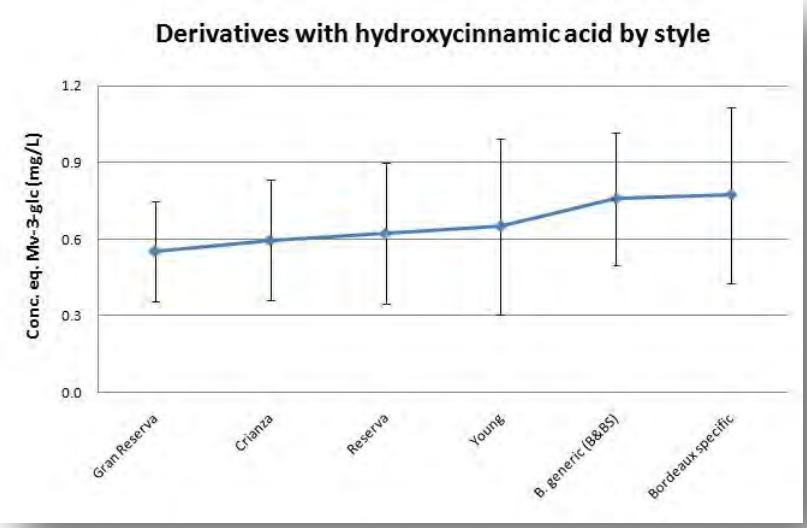



**Figure.VI.18.** Mean ± standard deviation concentrations for anthocyanin derivatives with hydroxycinnamic acids by wine style.

**Table VI.20.** Concentration of total anthocyanin derivatives with hydroxycinnamic acids by region: mean in equivalents of Mv-3-glc (mg/L), standard deviation and minimum and maximum values for each region.

| | **Derivatives with hydroxycinnamic acids (mg/L)** | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Rioja Alavesa | 0.584 | 0.272 | 0.115 | 1.599 |
| Rioja Alta | 0.601 | 0.223 | 0.184 | 1.110 |
| Rioja Baja | 0.633 | 0.307 | 0.249 | 1.525 |
| Blayais & Bourgeais | 0.642 | 0.211 | 0.363 | 0.898 |
| Libournais | 0.680 | 0.343 | 0.215 | 1.368 |
| B&BS | 0.758 | 0.259 | 0.361 | 1.228 |
| Graves | 0.818 | 0.364 | 0.333 | 1.588 |
| Médoc | 0.847 | 0.344 | 0.247 | 1.770 |

**Table VI.21.** Concentration of total anthocyanin derivatives with hydroxycinnamic acids bywinestyle: mean in equivalents of Mv-3-glc (mg/L), standard deviation and minimum and maximum values for young, *Crianza*, *Reserva* and *Gran Reserva* wines and specific and generic denomination of Bordeaux ones.

| | **Derivatives with hydroxycinnamic acids (mg/L)** | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| *Gran Reserva* | 0.554 | 0.195 | 0.282 | 1.107 |
| *Reserva* | 0.596 | 0.237 | 0.152 | 1.042 |
| *Crianza* | 0.623 | 0.278 | 0.180 | 1.262 |
| Young | 0.649 | 0.345 | 0.115 | 1.599 |
| B. generic (B&BS) | 0.758 | 0.259 | 0.361 | 1.228 |
| Bordeaux specific | 0.774 | 0.345 | 0.215 | 1.770 |

The differences found in anthocyanin derivatives must be due to the variety of grape used (mainly Tempranillo in Rioja and Cabernet Sauvignon and Merlot in Bordeaux), the weather conditions and the ground where grapes grown and the little differences in the techniques used in the winemaking process.

## VI.3. COLOUR

Colour evolution during vinification and wine ageing has been attributed to changes in the concentration of free anthocyanins and anthocyanin derivatives[447]. The parameter b* is a color parameter indicating the strength of two opposite colours, blue and yellow, so a higher b*value means a higher proportion of yellow.

Bordeaux wines present higher levels of b* parameter (Figure VI.19, Table V.22) than Rioja ones, so in spite of their lower levels of Vitisins B, the higher levels of Vitisins A and anthocyanin derivatives with hydroxycinamic acid overcome that difference. Moreover, Figure VI. 20 shows that b* parameter is higher when the wine has a longer ageing time. These results are in accordance with higher levels of the different pyranoanthocyanin classes in wines with more ageing.

[447] Sánchez-Ilárduya, M.B.;*Pigmentos derivados antociánicos de los vinos tintos de la Rioja: Estudio de analítico, influencia en el color y evolución durante la crianza,*.Tesis Doctoral, Universidad del País Vasco, España, **2010**.

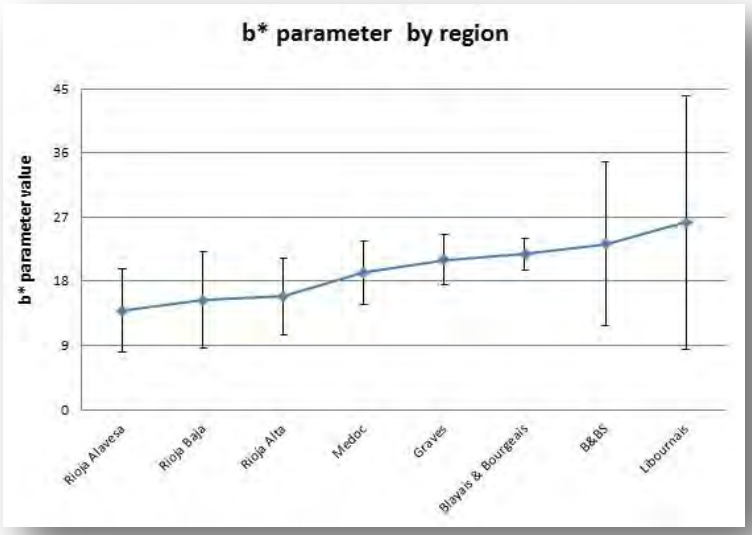



**Figure.VI.19.** Mean ± standard deviation values for b* parameter by region.

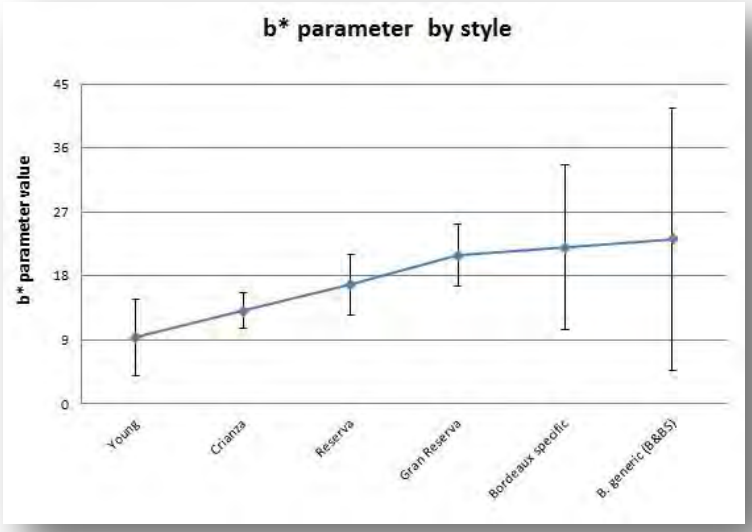



**Figure.VI.20.** Mean ± standard deviation values for b* parameter by style.

**Table VI.22.** Values of b* parameter by region: mean, standard deviation and minimum and maximum values for each region.

| | b* parameter | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Rioja Alavesa | 13.882 | 5.801 | 3.594 | 26.645 |
| Rioja Baja | 15.334 | 6.721 | 2.554 | 29.047 |
| Rioja Alta | 15.920 | 5.385 | 3.268 | 26.340 |
| Médoc | 19.229 | 4.462 | 10.388 | 30.038 |
| Graves | 21.021 | 3.468 | 16.195 | 25.724 |
| Blayais & Bourgeais | 21.812 | 2.235 | 19.095 | 24.273 |
| B&BS | 23.234 | 11.430 | 7.297 | 125.071 |
| Libournais | 26.281 | 18.809 | 16.469 | 118.089 |

**Table VI.23.** Values of b* parameter by wine style: mean, standard deviation and minimum and maximum values for young, *Crianza*, *Reserva* and *Gran Reserva* wines and specific and generic denomination of Bordeaux ones.

| | b* parameter | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Young | 9.373 | 5.366 | 2.554 | 20.124 |
| *Crianza* | 13.113 | 2.470 | 7.874 | 17.414 |
| *Reserva* | 16.802 | 4.247 | 10.333 | 25.134 |
| *Gran Reserva* | 20.893 | 4.368 | 13.558 | 29.047 |
| Bordeaux specific | 22.027 | 8.642 | 10.388 | 118.089 |
| B. generic (B&BS) | 23.234 | 11.430 | 7.297 | 125.071 |

## VI.4. TANNINS

37 tannins, previously identified, were quantified during in this study.

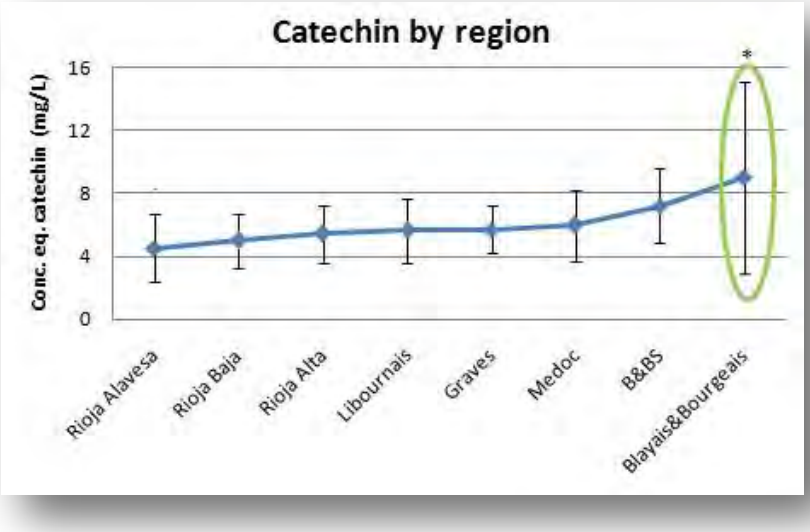
### VI.4.1. Monomeric flavan-3-ols

As mentioned in the introduction of this work, the flavan-3-ols monomers are the units constituent of the condensed tannins or proanthocyanidins, being generally their precursors. The four most abundant monomers in wine, that are also present in the grapes, are catechin, epicatechin, gallocatechin and epigallocatechin.

(+)-Catechin (Figure VI.21, Table VI.24) present higher levels than (-)-epicatechin (Figure VI.22, Table VI.25) in wines of both regions, in accordance with Monagas et

al[448], working with *Tempranillo*, *Graciano* and *Cabernet Sauvignon* red wines, and Chira et al[449], in *Tempranillo* wines.

In general, Bordeaux wines have higher levels of total monomeric tannins (obtained as sum of the four individual monomeric tannins) than those ones which come from the different regions of Rioja. Their levels decrease when the time of ageing is higher, i.e. young wines are those with highest concentrations of monomeric tannins, followed by *Crianza* ones, *Reserva* and *Gran Reserva*. This observation can be also seen in generic denominations of Bordeaux wines (Bordeaux and Bordeaux Supérieur (B&BS)), which have less ageing time in barrel, than the ones with a specific denomination of Bordeaux. The graphics are shown in Figures VI.23 and VI.24 and the mean, standard deviation, minimum and maximum values for total monomeric flavan-3-ols, which are in equivalents of catechin, can be seen in Tables VI.26 and VI.27.

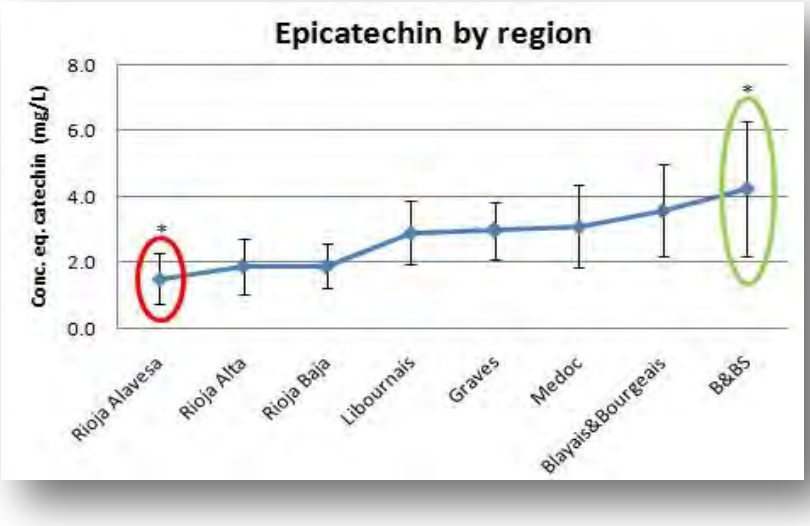



*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.21.** Mean ± standard deviation concentrations for catechin by region.

[448]Monagas, M.; Gómez-Cordovés, C.; Bartolome, B.; Laureano, O.; Ricardo da Silva, J.; *Monomeric, oligomeric and polymeric flavan-3-ol composition of wines and grapes from Vitis vinifera L. cv. Graciano, Tempranillo and Cabernet sauvignon*, J. Agric. Food Chem.**2003**, 51, 6475-6481.

[449]Chira, K.; Pacella, N.; Jourdes, M.; Teissedre, P. L.; *Chemical and sensory evaluation of Bordeaux wines (Cabernet sauvignon and Merlot) and correlation with wine age*, Food Chem. **2011**, 126, 1971–1977.




*: significant groups (α=0.05, by Scheffé test)

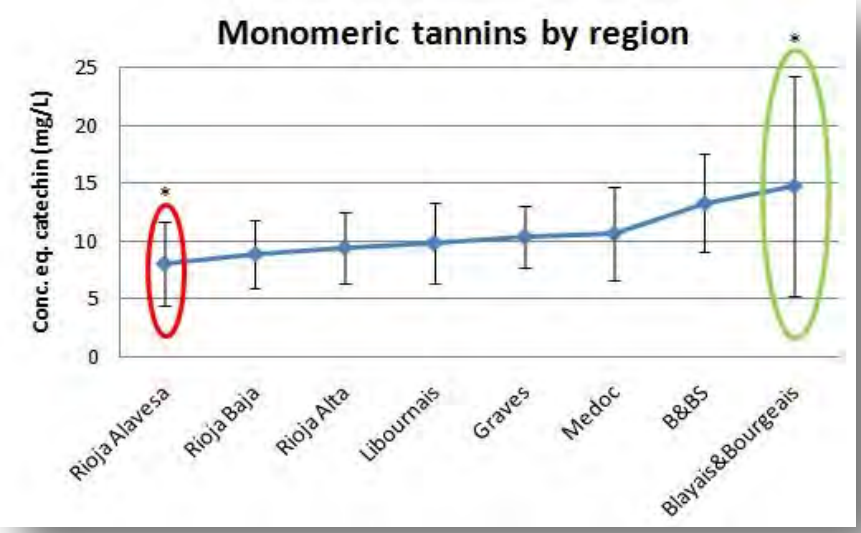
**Figure.VI.22.** Mean ± standard deviation concentrations for epicatechin by region.

**Table VI.24.** Catechin concentration by region: mean in equivalents of Catechin (mg/L), standard deviation and minimum and maximum values for each region.

| | Catechin (mg/L) | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Rioja Alavesa | 4.55 | 2.16 | 1.14 | 9.46 |
| Rioja Baja | 4.99 | 1.72 | 2.42 | 9.91 |
| Rioja Alta | 5.42 | 1.87 | 2.44 | 9.02 |
| Libournais | 5.65 | 2.04 | 1.56 | 9.35 |
| Graves | 5.71 | 1.48 | 1.98 | 7.89 |
| Médoc | 5.96 | 2.27 | 2.45 | 11.99 |
| B&BS | 7.23 | 2.39 | 1.69 | 10.02 |
| Blayais & Bourgeais | 9.04 | 6.08 | 4.33 | 19.39 |

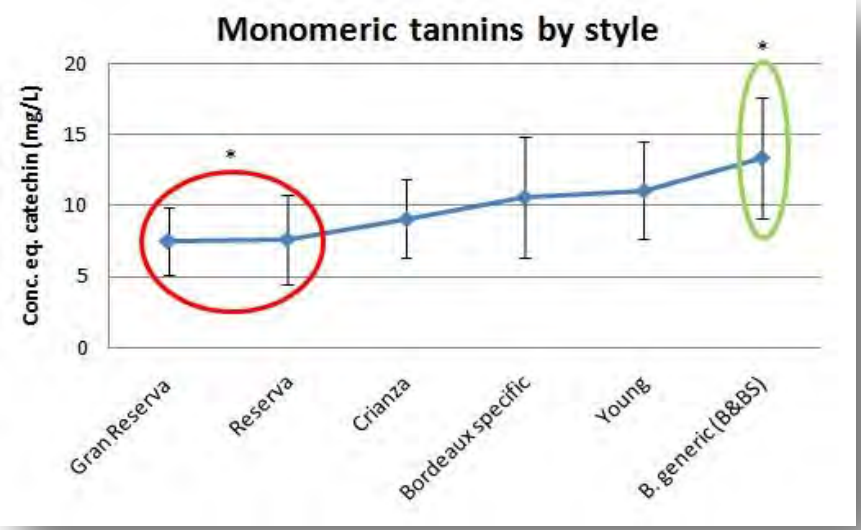
**Table VI.25.** Epicatechin concentrations by region: mean in equivalents of Catechin (mg/L), standard deviation and minimum and maximum values for each region.

| | Epicatechin (mg/L) | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Rioja Alavesa | 1.52 | 0.75 | 0.49 | 3.80 |
| Rioja Alta | 1.89 | 0.84 | 0.79 | 4.60 |
| Rioja Baja | 1.90 | 0.69 | 0.93 | 3.97 |
| Libournais | 2.91 | 0.97 | 1.00 | 4.96 |
| Graves | 2.97 | 0.87 | 0.91 | 4.40 |
| Médoc | 3.10 | 1.25 | 0.95 | 6.45 |
| Blayais&Bourgeais | 3.59 | 1.40 | 1.05 | 6.37 |
| B&BS | 4.25 | 2.06 | 1.91 | 6.93 |




*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.23.** Mean ± standard deviation concentrations for total monomeric flavan-3-ols by region.




*: significant groups (α=0.05, by HSD of Tukey test)

**Figure.VI.24.** Mean ± standard deviation concentrations for total monomeric flavan3-ols by wine style.

**Table VI.26.** Total monomeric flavan-3-ols concentration by region: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for each region.

| | Monomeric flavan-3-ols (mg/L) | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Rioja Alavesa | 8.07 | 3.63 | 2.43 | 16.43 |
| Rioja Baja | 8.88 | 2.92 | 4.06 | 17.60 |
| Rioja Alta | 9.48 | 3.10 | 3.94 | 15.96 |
| Libournais | 9.90 | 3.52 | 3.04 | 16.67 |
| Graves | 10.37 | 2.69 | 3.67 | 14.44 |
| Médoc | 10.67 | 4.05 | 4.11 | 21.03 |
| B&BS | 13.35 | 4.22 | 3.27 | 18.44 |
| Blayais & Bourgeais | 14.77 | 9.50 | 7.93 | 30.83 |

**Table VI.27.** Total monomeric tannins concentration by wine style: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for young, *Crianza*, *Reserva* and *Gran Reserva* wines and specific and generic denominations of Bordeaux ones.

| | Monomeric flavan-3-ols (mg/L) | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| *Gran Reserva* | 7.51 | 2.39 | 3.33 | 12.59 |
| *Reserva* | 7.59 | 3.13 | 2.43 | 15.96 |
| *Crianza* | 9.08 | 2.74 | 3.50 | 14.43 |
| Bordeaux specific | 10.62 | 4.25 | 3.04 | 30.83 |
| Young | 11.07 | 3.45 | 3.84 | 17.60 |
| B. generic (B&BS) | 13.35 | 4.22 | 3.27 | 18.44 |

## VI.4.2. Dimers with B bond

### a) Procyanidin homodimers B

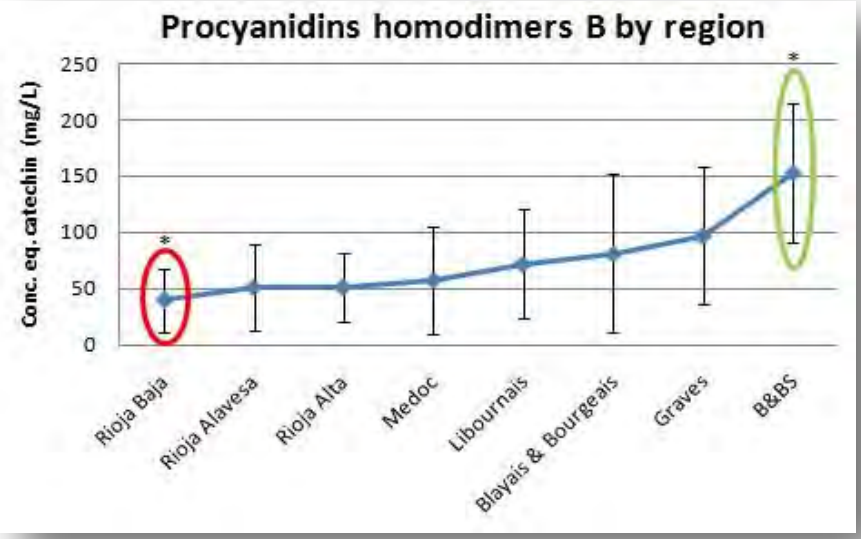
The different procyanidin homodimers that were chosen as markers can be seen in Table VI.28.

**Table VI.28.** List of the 3 procyanidin homodimers B quantified in this research.

| **Procyanidin homodimers B** | | |
|---|---|---|
| Procyanidin B1 | Procyanidin B2 | ((epi)cat)$_2$ 1 |

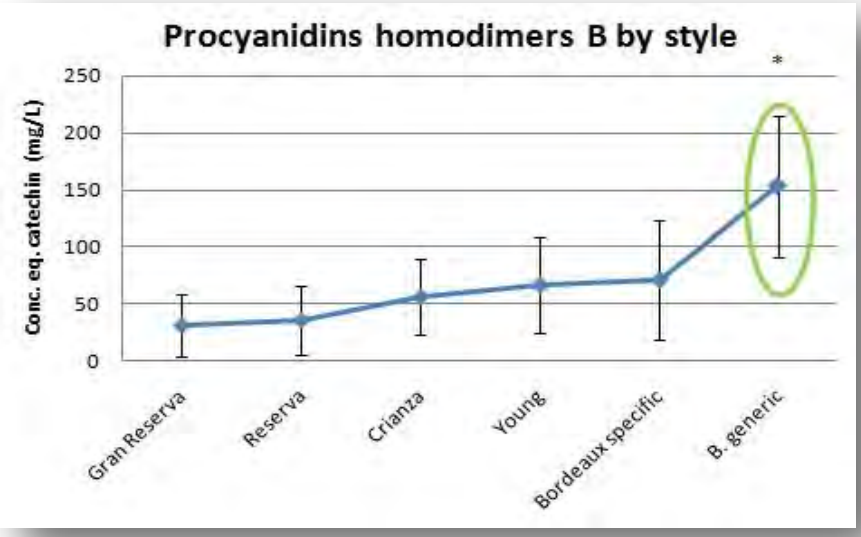
In general, Bordeaux wines have higher levels of total procyanidins homodimers B (obtained as sum of the three procyanidin homodimers B) than those ones which come from the different regions of Rioja. Their levels also decrease, as for monomers, when the time of ageing is higher, i.e. young wines are those with highest

concentrations of procyanidin homodimers B, followed in this order by *Crianza* ones, *Reserva* and *Gran Reserva*. This observation can be also seen in generic denominations of Bordeaux wines (Bordeaux and Bordeaux Supérieur (B&BS)), which have less ageing time in barrel, than the ones with a specific denomination of Bordeaux (Figures VI.25 to VI.26, Tables VI.29 and VI.30).




*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.25.** Mean ± standard deviation concentrations for procyanidin homodimers B by region.




*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.26.** Mean ± standard deviation concentrations for procyanidin homodimers B by wine style.

**Table VI.29.** Total procyanidins homodimers B concentrations by region: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for each region.

| | **Procyanidin homodimers B (mg/L)** | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Rioja Baja | 39.48 | 28.15 | 2.89 | 173.44 |
| Rioja Alavesa | 50.82 | 39.00 | 3.88 | 137.94 |
| Rioja Alta | 51.45 | 30.35 | 2.92 | 117.00 |
| Médoc | 57.57 | 47.39 | 8.02 | 209.28 |
| Libournais | 71.91 | 48.37 | 3.66 | 205.25 |
| Blayais & Bourgeais | 81.33 | 70.83 | 18.25 | 169.29 |
| Graves | 97.03 | 61.08 | 19.67 | 191.48 |
| B&BS | 153.01 | 62.34 | 8.61 | 201.88 |

**Table VI.30.** Total procyanidin homodimers B concentrations by wine style: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for young, *Crianza*, *Reserva* and *Gran Reserva* wines and specific and generic denominations of Bordeaux ones.

| | **Procyanidin homodimers B (mg/L)** | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| *Gran Reserva* | 31.20 | 27.11 | 2.92 | 91.07 |
| *Reserva* | 35.72 | 30.24 | 2.89 | 110.07 |
| *Crianza* | 56.21 | 33.16 | 14.26 | 137.94 |
| Young | 65.87 | 42.16 | 7.44 | 173.44 |
| Bordeaux specific | 70.15 | 52.45 | 3.66 | 209.28 |
| B. generic | 153.01 | 62.34 | 8.61 | 201.88 |

Within procyanidin homodimers B, the procyanidin B1 (PCB1) presents higher concentration than procyanidin B2 (PCB2) in wines of both regions (Figures VI.27 and VI.28, Tables VI.31 and VI.32). The first one is the major dimer in grape skin and the second one is the main one in seeds[450,451,452].In addition it is easier to extract skins

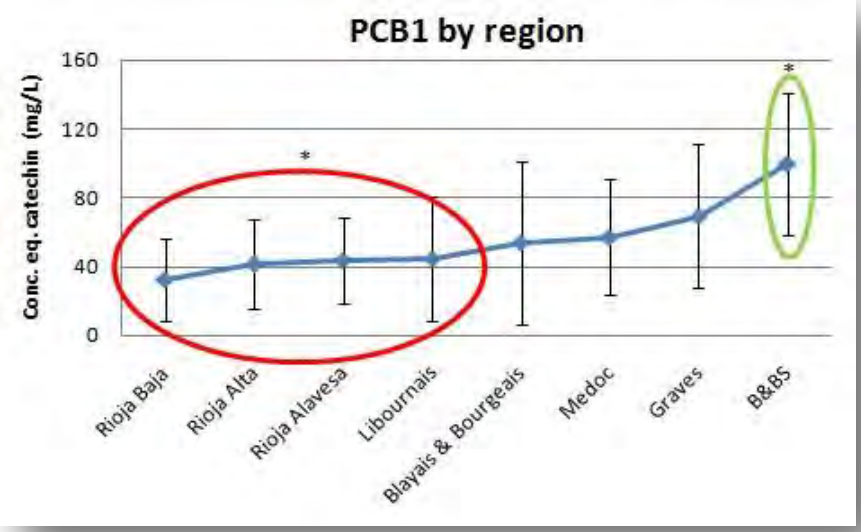
[450]Monagas, M.; Gómez-Cordovés, C.; Bartolome, B.; Laureano, O.; Ricardo da Silva, J.; *Monomeric, oligomeric and polymeric flavan-3-ol composition of wines and grapes from Vitis vinifera L. cv. Graciano, Tempranillo and Cabernet sauvignon*, J. Agric. Food Chem. **2003**, 51, 6475-6481.

[451]De Freitas, V. A. P.; Glories, Y.; Monique, A.; *Developmental Changes of Procyanidins in Grapes of Red Vitis vinifera Varieties and Their Composition in Respective Wines*, Am. J. Enol. Vitic.,**2000**, 35, 397-403.

[452]Gonzalez-Manzano, S.; Santos-Buelga, C.; Perez-Alonso, J. J.; Rivas-Gonzalo, J. C.; Escribano-Bailón, M. T.; *Characterization of the mean degree of polymerization of proanthocyanidins in red*

compounds than seed ones during maceration, so is easier to extract PCB1 than PCB2. So, lower ratios of PCB1/PCB2 could indicate longer maceration times. Figure VI.29 and Table VI.33 shows that Bordeaux wines present lower ratios of PCB1/PCB2 than Rioja ones, suggesting longer maceration times. However, other variables should be taken into account due to their influence in tannin wine content, such as grape variety, climatic conditions where grapes grow, cultural practices and other vinification conditions[453].

In addition, Figure VI.30 and Table VI.34 shows that time of maceration increases when wines with longer aging is made, i.e. young wines are those with highest PCB1/PCB2 ratio, followed by *Crianza* ones, *Reserva* and *Gran Reserva*.

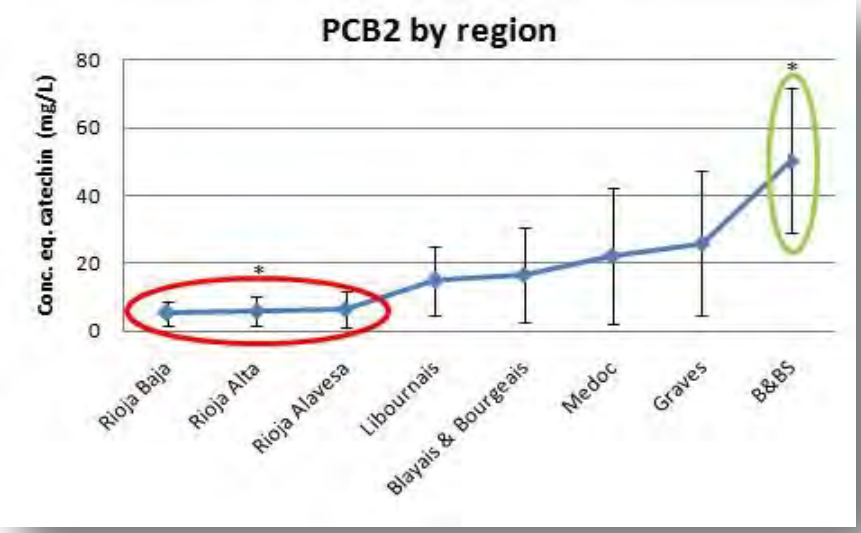



*: significant groups (α=0.05, by HSD of Tukey test)

**Figure.VI.27.** Mean ± standard deviation concentrations for procyanidin B1 by region.

*wines using Liquid Chromatography-Mass Spectrometry (LC-MS)*, J. Agric. Food Chem. **2006**, 54, 4326-4332.

[453] Sacchi, K. L.; Bisson, L. F.; Adams, D. O.; *A Review of the Effect of Winemaking Techniques on Phenolic Extraction in Red Wines*, Am. J. Enol. Vitic.**2005**, 56, 197-206.




*: significant groups (α=0.05, by Scheffé test)

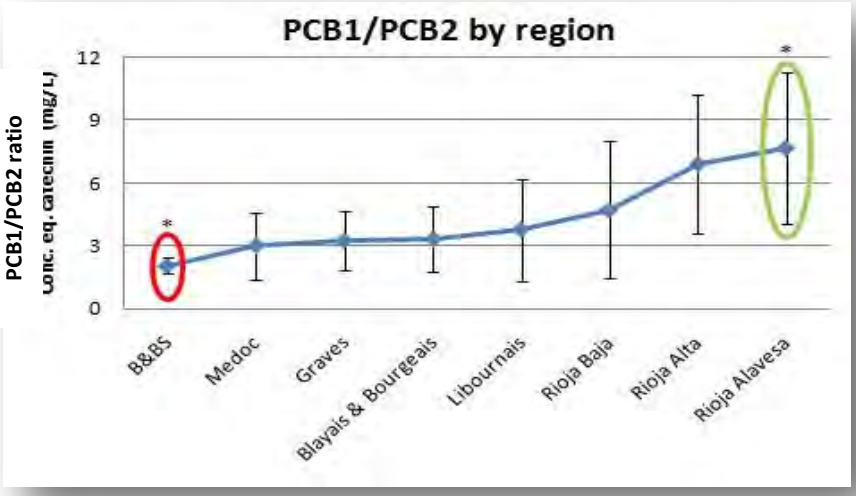
**Figure.VI.28.** Mean ± standard deviation concentrations for procyanidin B2 by region.

**Table VI.31.** Procyanidin B1 concentrations by region: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for each region.

| | **Procyanidin B1 (mg/L)** | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Rioja Baja | 32.34 | 33.57 | 1.70 | 146.00 |
| Rioja Alta | 41.36 | 26.26 | 2.01 | 107.11 |
| Rioja Alavesa | 43.55 | 35.29 | 2.47 | 122.88 |
| Libournais | 44.55 | 35.78 | 1.84 | 146.29 |
| Blayais & Bourgeais | 53.94 | 47.05 | 11.77 | 111.23 |
| Médoc | 57.35 | 33.66 | 5.02 | 118.51 |
| Graves | 69.41 | 41.98 | 11.28 | 128.68 |
| B&BS | 99.73 | 41.16 | 5.99 | 133.89 |

**Table VI.32.** Procyanidin B2 concentrations by region: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for each region.

| | Procyanidin B2 (mg/L) | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Rioja Baja | 5.29 | 3.59 | 1.18 | 16.83 |
| Rioja Alta | 6.14 | 4.37 | 1.08 | 23.92 |
| Rioja Alavesa | 6.67 | 5.36 | 0.82 | 30.92 |
| Libournais | 14.89 | 10.18 | 2.68 | 84.87 |
| Blayais & Bourgeais | 16.58 | 13.98 | 1.70 | 55.75 |
| Médoc | 22.18 | 20.16 | 5.34 | 60.33 |
| Graves | 25.93 | 21.20 | 4.60 | 68.60 |
| B&BS | 50.29 | 21.47 | 2.36 | 68.72 |

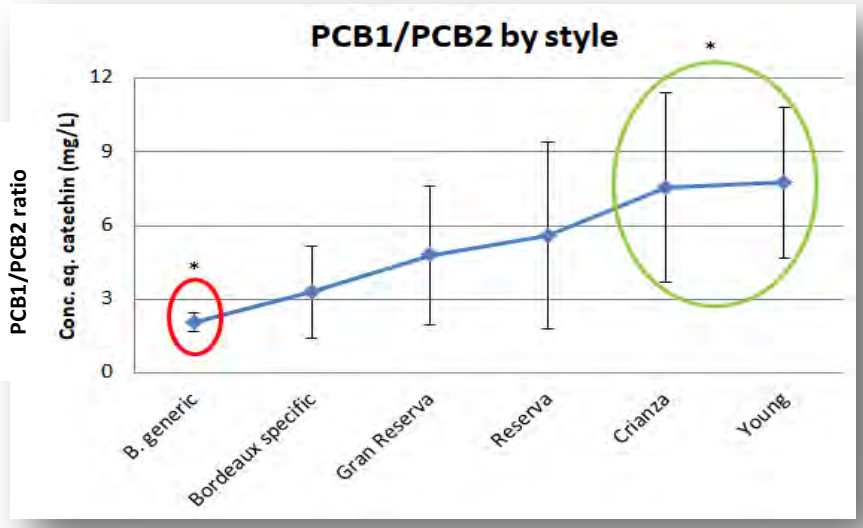



*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.29.** Mean ± standard deviation values for ratio PCB1/PCB2 by region.

**Table VI.33.** PCB1/PCB2 ratios by region: mean, standard deviation and minimum and maximum values for each region.

| | PCB1/PCB2 ratio | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| B&BS | 2.06 | 0.37 | 1.44 | 2.62 |
| Médoc | 2.99 | 1.57 | 0.69 | 7.84 |
| Graves | 3.23 | 1.42 | 1.46 | 5.85 |
| Blayais & Bourgeais | 3.31 | 1.55 | 1.72 | 5.56 |
| Libournais | 3.75 | 2.43 | 1.08 | 10.05 |
| Rioja Baja | 4.73 | 3.27 | 0.54 | 16.08 |
| Rioja Alta | 6.87 | 3.31 | 2.20 | 14.29 |
| Rioja Alavesa | 7.64 | 3.62 | 1.88 | 15.11 |




*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.30.** Mean ± standard deviation values for ratio PCB1/PCB2 by style.

**Table VI.34.** PCB1/PCB2 ratios by style: mean, standard deviation and minimum and maximum values for each region.

| | PCB1/PCB2 ratio | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| B. generic | 2.06 | 0.37 | 1.44 | 2.62 |
| Bordeaux specific | 3.30 | 1.88 | 0.69 | 10.05 |
| *Gran Reserva* | 4.79 | 2.83 | 1.88 | 10.58 |
| *Reserva* | 5.59 | 3.78 | 0.54 | 14.46 |
| *Crianza* | 7.54 | 3.85 | 2.05 | 16.08 |
| Young | 7.74 | 3.08 | 2.20 | 15.11 |

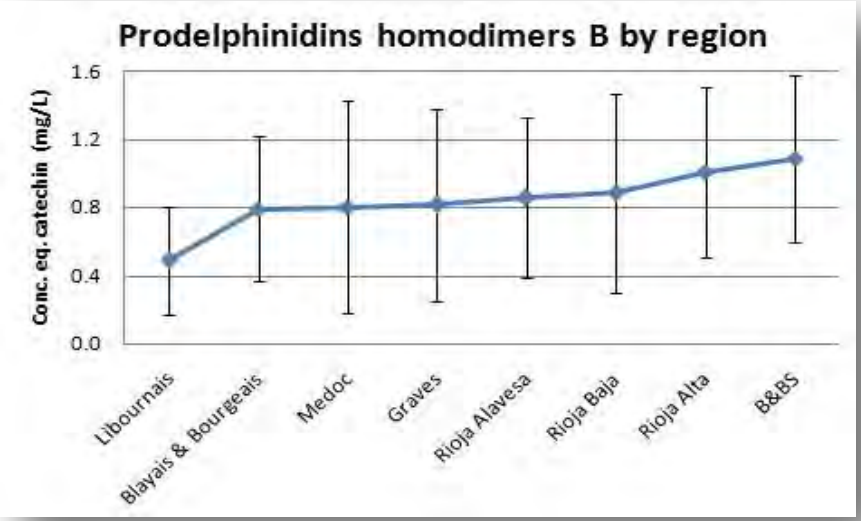
## b) Prodelphinidin homodimers B

The (epi)gallocatechin dimers are more polar than those of (epi)catechin, as they have an additional OH group. Three prodelphinidin homodimers B were quantified in this study (Table VI.35).

**Table VI.35.** List of the3 prodelphinidin homodimers B quantified in this research.

| **Prodelphinidin homodimers B** | | |
|---|---|---|
| ((epi)gallocat)$_2$ 1 | ((epi)gallocat)$_2$ 2 | ((epi)gallocat)$_2$ 3 |

On the one hand, any clear difference appears between wines of both regions for total prodelphinidin homodimers B. So, the order from higher level is generic Bordeaux appellations, Rioja regions and specific Bordeaux appellations. On the other hand the general decrease of all tannins classes along ageing is also observed (Figures VI.31 and VI.32, Tables VI.36 and VI.37).

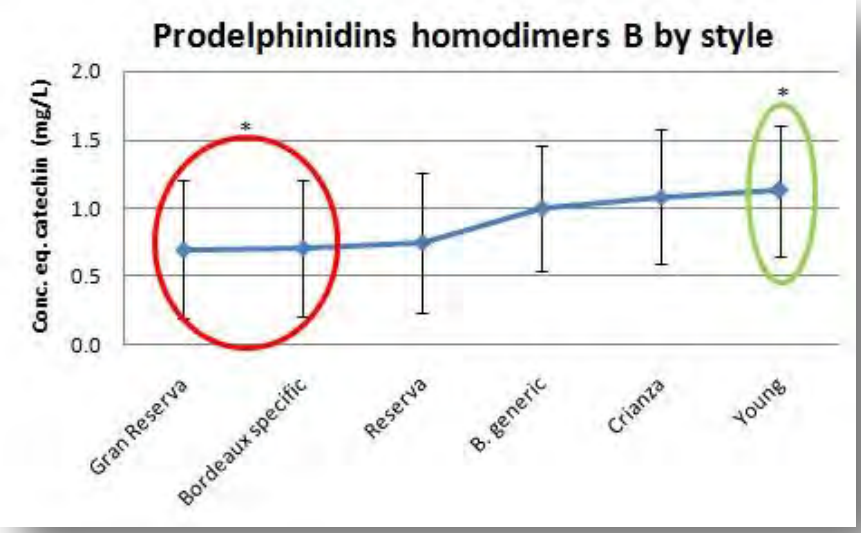



*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.31.** Mean ± standard deviation concentrations for prodelphinidins homodimers B by region.

**Table VI.36.** Total prodelphinidin homodimers B concentrations by region: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for each region.

| | **Prodelphinidin homodimers B(mg/L)** | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Libournais | 0.49 | 0.31 | 0.06 | 1.39 |
| Blayais & Bourgeais | 0.79 | 0.43 | 0.11 | 1.73 |
| Médoc | 0.80 | 0.62 | 0.34 | 1.86 |
| Graves | 0.81 | 0.57 | 0.14 | 2.54 |
| Rioja Alavesa | 0.85 | 0.47 | 0.30 | 1.88 |
| Rioja Baja | 0.88 | 0.59 | 0.10 | 2.11 |
| Rioja Alta | 1.01 | 0.50 | 0.11 | 1.94 |
| B&BS | 1.08 | 0.49 | 0.32 | 2.20 |




*: significant groups (α=0.05, by HSD of Tukey test)

**Figure.VI.32.** Mean ± standard deviation concentrations for prodelphinidin homodimers B by wine style.

**Table VI.37.** Total prodelphinidin homodimers B concentrations by wine style: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for young, *Crianza*, *Reserva* and *Gran Reserva* wines and specific and generic denominations of Bordeaux ones.

| | **Prodelphinidin homodimers B(mg/L)** | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| *Gran Reserva* | 0.70 | 0.50 | 0.11 | 1.94 |
| Bordeaux specific | 0.71 | 0.50 | 0.06 | 2.54 |
| *Reserva* | 0.75 | 0.51 | 0.10 | 2.11 |
| B. generic | 1.00 | 0.45 | 0.38 | 2.11 |
| *Crianza* | 1.08 | 0.49 | 0.32 | 2.20 |
| Young | 1.13 | 0.48 | 0.32 | 1.94 |

## c) Mixed dimers with B bond

Mixed dimers or heterodimers are those which are formed by two monomers of flavan-3-ol of different molecular weight. Two different isomers appears as a consequence of which one is upper (U) and down (D) monomer and thus it is possible to differentiate between the heterodimers (epi)catechin-(epi)gallocatechin or (epi)gallocatechin-(epi)catechin, the firsts being procyanidins and the seconds prodelphinidins.

## Procyanidin heterodimers B

Two procyanidin heterodimers were selected as markers (Table VI.38).

**Table VI.38.** List of the2 procyanidins heterodimers B analyzed in this research.

| Procyanidin heterodimers B | |
|---|---|
| ((epi)cat-(epi)gallocat) 1 | ((epi)cat-(epi)gallocat) 2 |

No significant differences in procyanidin heterodimers B between wines of both regions can be observed. Decreasing effect due to ageing time was again observed. The graphics are shown in Figures VI.33 to VI.34 and the mean, standard deviation, minimum and maximum values for procyanidin heterodimers B, which are in equivalents of catechin, can be seen in Tables VI.39 and VI.40.

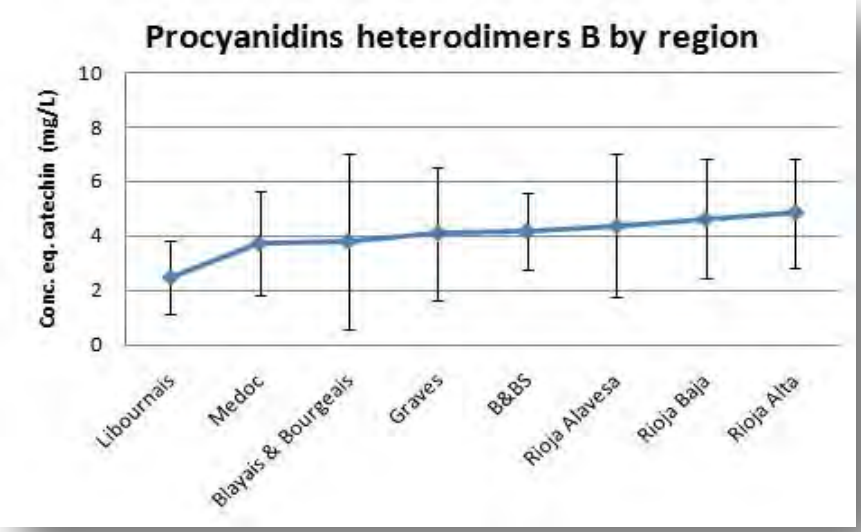



**Figure.VI.33.** Mean ± standard deviation concentrations for procyanidin heterodimers B by region.

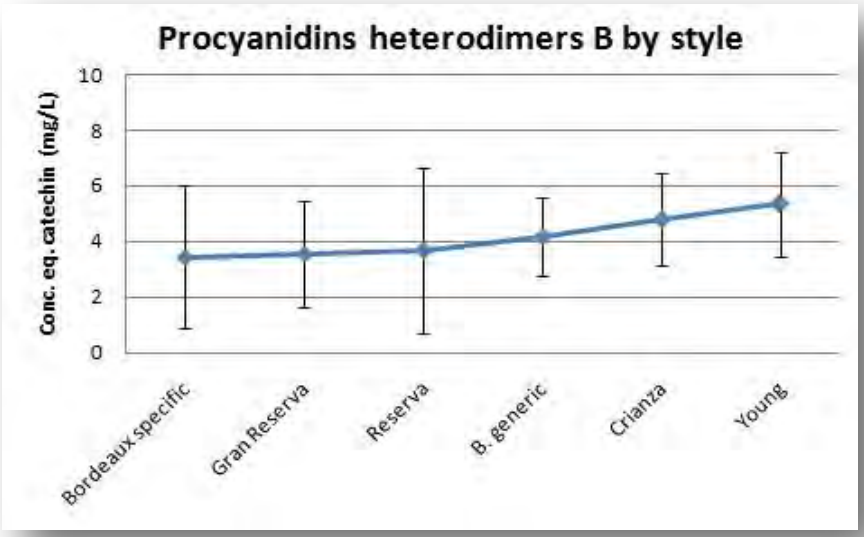



**Figure.VI.34.** Mean ± standard deviation concentrations for procyanidin heterodimers B by wine style.

**Table VI.39.** Total procyanidin heterodimers B concentrations by region: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for each region.

| | **Procyanidin heterodimers B(mg/L)** | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Libournais | 2.47 | 1.36 | 0.36 | 5.17 |
| Médoc | 3.74 | 1.91 | 0.87 | 16.32 |
| Blayais & Bourgeais | 3.80 | 3.23 | 1.90 | 7.93 |
| Graves | 4.09 | 2.46 | 1.44 | 9.92 |
| B&BS | 4.17 | 1.42 | 1.25 | 5.97 |
| Rioja Alavesa | 4.38 | 2.65 | 0.49 | 13.91 |
| Rioja Baja | 4.63 | 2.19 | 0.39 | 8.14 |
| Rioja Alta | 4.83 | 1.98 | 0.49 | 7.97 |

**Table VI.40.** Total procyanidin heterodimers B concentrations by wine style: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for young, *Crianza*, *Reserva* and *Gran Reserva* wines and specific and generic denominations of Bordeaux ones.

| | **Procyanidin heterodimers B(mg/L)** | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Bordeaux specific | 3.43 | 2.57 | 0.36 | 16.32 |
| *Gran Reserva* | 3.54 | 1.89 | 0.39 | 6.89 |
| *Reserva* | 3.65 | 2.99 | 0.49 | 13.91 |
| B. generic | 4.17 | 1.42 | 1.25 | 5.97 |
| *Crianza* | 4.79 | 1.68 | 1.84 | 7.45 |
| Young | 5.35 | 1.89 | 1.38 | 8.14 |

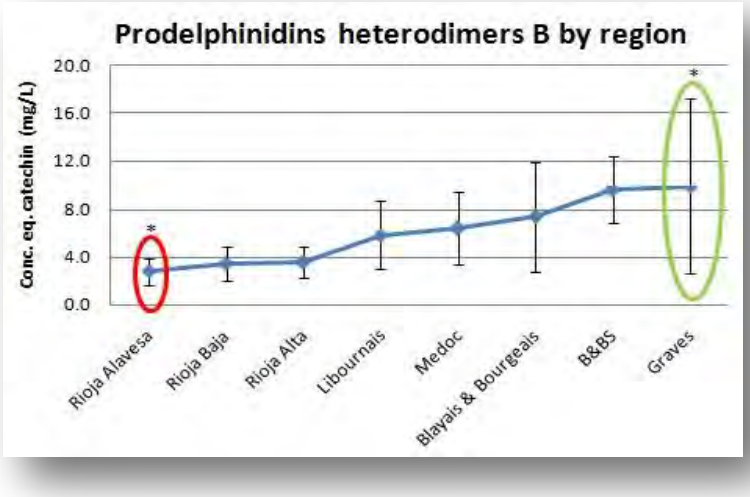
## Prodelphinidin heterodimers B

Two prodelphinidin heterodimers were selected as markers (Table VI.41).

**Table VI.41.** List of the 2 prodelphinidin heterodimers B analyzed in this research.

| **Prodelphinidin heterodimers B** | |
|---|---|
| ((epi)gallocat-(epi)cat) 1 | ((epi)gallocat-(epi)cat) 2 |

In this case, Bordeaux wines have higher levels of prodelphinidin heterodimers B (obtained as sum of the two individual prodelphinidins heterodimers B) than those ones which come from the different regions of Rioja (Figure VI.35, Table VI. 42). Decreasing along ageing is again observed (Figure VI.36 and Table VI.43).

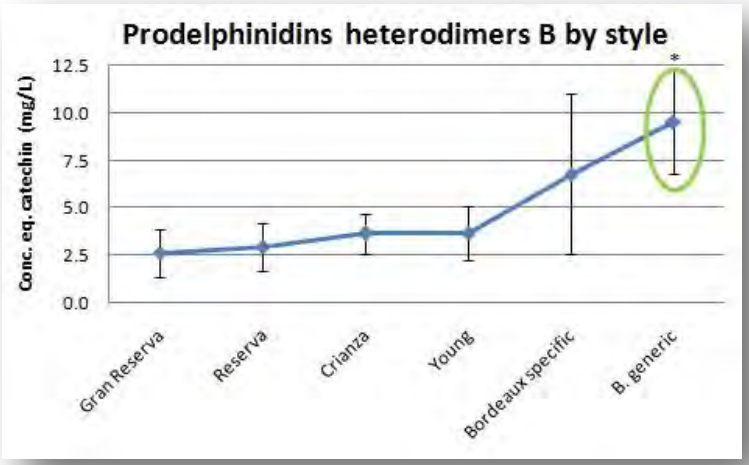



*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.35.** Mean± standard deviation concentrations for prodelphinidin heterodimers B by region.

**Table VI.42.** Total prodelphinidin heterodimers B concentrations by region: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for each region.

| | **Prodelphinidin heterodimers B(mg/L)** | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Rioja Alavesa | 2.78 | 1.10 | 0.65 | 5.01 |
| Rioja Baja | 3.41 | 1.44 | 1.38 | 6.06 |
| Rioja Alta | 3.54 | 1.29 | 0.44 | 5.78 |
| Libournais | 5.82 | 2.87 | 0.84 | 15.18 |
| Médoc | 6.35 | 3.05 | 2.37 | 14.25 |
| Blayais & Bourgeais | 7.34 | 4.59 | 2.83 | 13.83 |
| B&BS | 9.56 | 2.78 | 4.43 | 15.45 |
| Graves | 9.91 | 7.30 | 3.18 | 28.79 |




*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.36.** Mean ± standard deviation concentrations for prodelphinidin heterodimers B by wine style.

Total prodelphinidin heterodimers B concentrations by wine style: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for young, *Crianza*, *Reserva* and *Gran Reserva* wines and specific and generic denominations of Bordeaux ones.

| | **Prodelphinidin heterodimers B (mg/L)** | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| *Gran Reserva* | 2.64 | 1.24 | 0.44 | 5.11 |
| *Reserva* | 2.98 | 1.25 | 1.03 | 5.78 |
| *Crianza* | 3.65 | 1.06 | 1.95 | 5.84 |
| Young | 3.71 | 1.42 | 0.87 | 6.06 |
| Bordeaux specific | 6.80 | 4.22 | 0.84 | 28.79 |
| B. generic | 9.56 | 2.78 | 4.43 | 15.45 |

## VI.4.3. Procyanidin homotrimers B

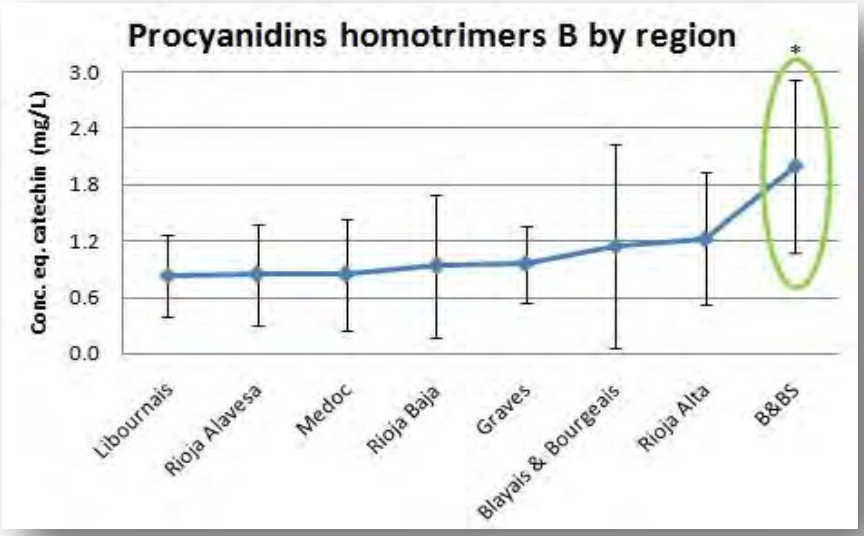
Trimers of (epi)catechin are called procyanidins C and in this work as procyanidins homotrimers B, being the most common the procyanidin C1 (PCC1), which is composed of three epicatechin units linked by B bonds between carbons 4 and 8.

Three procyanidin homotrimers B were selected as markers (Table VI.44).

**Table VI.43.** List of the 3 procyanidin homotrimers Bquantified in this research.

| **Procyanidin homotrimers B** | | |
|---|---|---|
| PCC1 | $((epi)cat)_3$ 1 | $((epi)cat)_3$ 2 |

On the one hand, no significant differences in the levels of total procyanidin homotrimers B (obtained as sum of the three individual procyanidins homotrimers B) can be appreciated when comparing Rioja and Bordeaux wines, except in the case of generic Bordeaux wines, with a significant higher level. Again decreasing with ageing time is observed for both regions (Figures VI.37 and VI.38, Tables VI.45 and VI.46).

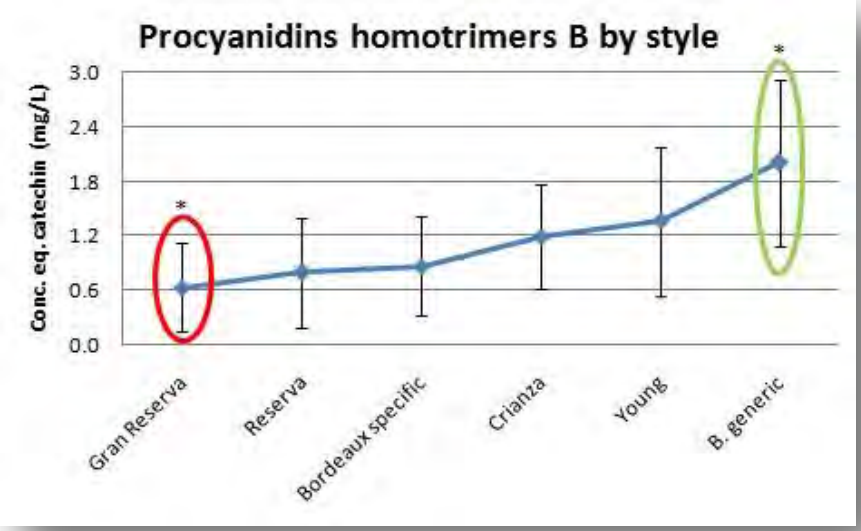



*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.37.** Mean ± standard deviation concentrations for procyanidin homotrimers B by region.

**Table VI.44.** Total procyanidin homotrimers B concentrations by region: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for each region.

| | **Procyanidin homotrimer B (mg/L)** | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Libournais | 0.83 | 0.43 | 0.08 | 1.63 |
| Rioja Alavesa | 0.84 | 0.54 | 0.07 | 1.90 |
| Médoc | 0.84 | 0.59 | 0.18 | 3.15 |
| Rioja Baja | 0.93 | 0.76 | 0.08 | 3.32 |
| Graves | 0.96 | 0.41 | 0.39 | 1.71 |
| Blayais & Bourgeais | 1.15 | 1.09 | 0.42 | 2.97 |
| Rioja Alta | 1.22 | 0.70 | 0.06 | 2.65 |
| B&BS | 2.01 | 0.92 | 0.26 | 3.14 |




*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.38.** Mean ± standard deviation concentrations for procyanidin homotrimers B by wine style.

**Table VI.45.** Total procyanidin homotrimers B concentrations by wine style: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for young, *Crianza*, *Reserva* and *Gran Reserva* wines and specific and generic denominations of Bordeaux ones.

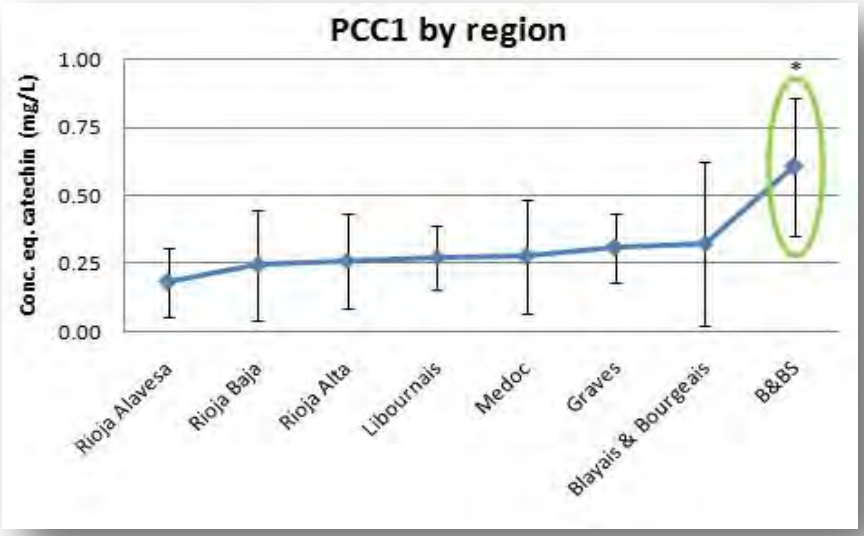
| | **Procyanidin homotrimer B (mg/L)** | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| *Gran Reserva* | 0.64 | 0.48 | 0.06 | 1.69 |
| *Reserva* | 0.80 | 0.61 | 0.08 | 2.65 |
| Bordeaux specific | 0.87 | 0.55 | 0.08 | 3.15 |
| *Crianza* | 1.20 | 0.57 | 0.39 | 2.00 |
| Young | 1.37 | 0.82 | 0.16 | 3.32 |
| B. generic | 2.01 | 0.92 | 0.26 | 3.14 |

Within procyanidin homotrimers B, the procyanidin C1 (PCC1) presents lower concentrations than $((epi)cat)_3$ 2 in wines of both regions (Figures VI.39 and VI.40, Tables VI.47 and VI.48). The first one is the major trimer in grape seeds and the second one is the main one in skin[454,455,456,457,458]. So the second is easier to extract

[454]Monagas, M.; Gómez-Cordovés, C.; Bartolome, B.; Laureano, O.; Ricardo da Silva, J.; *Monomeric, oligomeric and polymeric flavan-3-ol composition of wines and grapes from Vitis vinifera L. cv. Graciano, Tempranillo and Cabernet sauvignon*, J. Agric. Food Chem.**2003**, 51, 6475-6481.

[455]Ricardo da Silva, J. M.; Rosec, J. P.; Bourzeix, M.; Mourgues, J.; Moutounet, M.;*Dimer and trimer procyanidins in Carignan and Mourvedre grapes and red wines*, Vitis, **1992**, 31, 55-63.

along maceration and lower values of ratio ((epi)cat)$_3$ 2/PCC1 could suggest longer maceration times (Figure IV.41, Table IV.49), so Bordeaux wines have longer maceration times than Rioja wines. In addition, as in the case of PCB1/PCB2 ratio, Figure VI.42 and Table IV.50 shows that time of maceration increases when wines with longer aging is made.



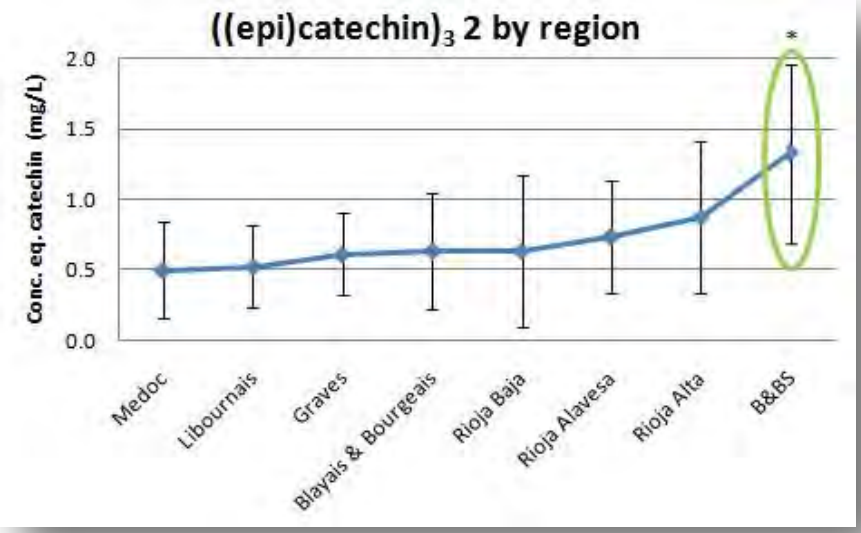

*: significant groups (α=0.05, by HSD of Tukey test)

**Figure.VI.39.** Mean ± standard deviation concentrations for PCC1 by region.

[456]De Freitas, V. A. P.; Glories, Y.; Monique, A.; *Developmental Changes of Procyanidins in Grapes of Red Vitis vinifera Varieties and Their Composition in Respective Wines*, Am. J. Enol. Vitic.**2000**, 35, 397-403.

[457]Fuleki, T.; Ricardo da Silva, J. M.; *Catechin and Procyanidin Composition of Seeds from Grape Cultivars Grown in Ontario*, J. Agric. FoodChem.**1997**, 45, 1156-1160.

[458]Gonzalez-Manzano, S.; Santos-Buelga C.; Perez-Alonso, J. J.; Rivas-Gonzalo, J. C.; Escribano-Bailón, M. T.; *Characterization of the mean degree of polymerization of proanthocyanidins in red wines using Liquid Chromatography-Mass Spectrometry (LC-MS)*, J. Agric. Food Chem. **2006**, 54, 4326-4332.




*: significant groups (α=0.05, by Scheffé test)

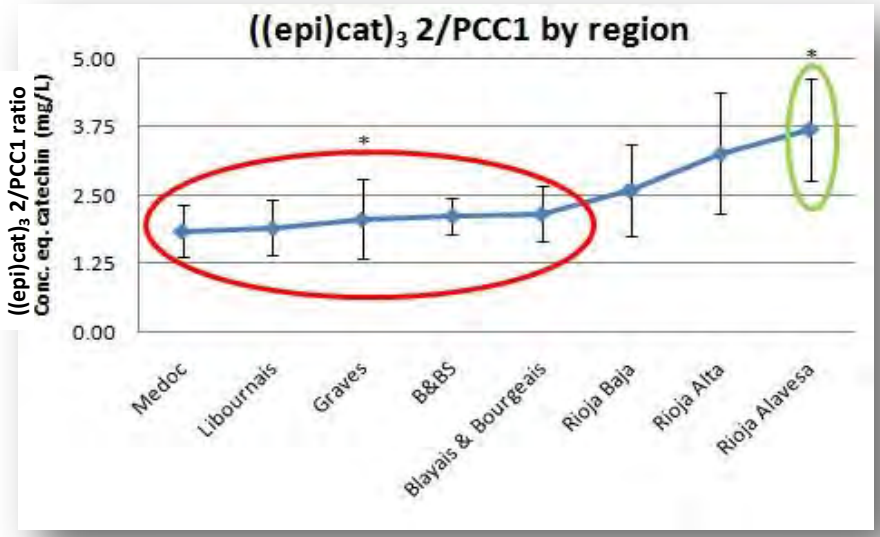
**Figure.VI.40.** Mean ± standard deviation concentrations for ((epi)cat)$_3$ 2 by region.

**Table VI.46.** Procyanidin C1 concentrations by region: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for each region.

| | **Procyanidin C1 (mg/L)** | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Rioja Alavesa | 0.18 | 0.13 | 0.02 | 0.49 |
| Rioja Baja | 0.25 | 0.22 | 0.03 | 1.09 |
| Rioja Alta | 0.26 | 0.18 | 0.03 | 0.80 |
| Libournais | 0.27 | 0.12 | 0.03 | 0.49 |
| Médoc | 0.28 | 0.21 | 0.06 | 1.24 |
| Graves | 0.31 | 0.13 | 0.09 | 0.56 |
| Blayais & Bourgeais | 0.32 | 0.30 | 0.14 | 0.84 |
| B&BS | 0.61 | 0.25 | 0.08 | 0.89 |

**Table VI.47.** ((epi)cat)$_3$ 2 concentrations by region: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for each region.

| | **((epi)cat)$_3$ 2 (mg/L)** | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Médoc | 0.50 | 0.35 | 0.11 | 1.76 |
| Libournais | 0.53 | 0.29 | 0.04 | 1.10 |
| Graves | 0.62 | 0.29 | 0.29 | 1.09 |
| Blayais & Bourgeais | 0.63 | 0.41 | 0.26 | 1.88 |
| Rioja Baja | 0.64 | 0.54 | 0.04 | 2.23 |
| Rioja Alavesa | 0.74 | 0.40 | 0.05 | 1.42 |
| Rioja Alta | 0.88 | 0.54 | 0.03 | 2.06 |
| B&BS | 1.33 | 0.63 | 0.17 | 1.98 |

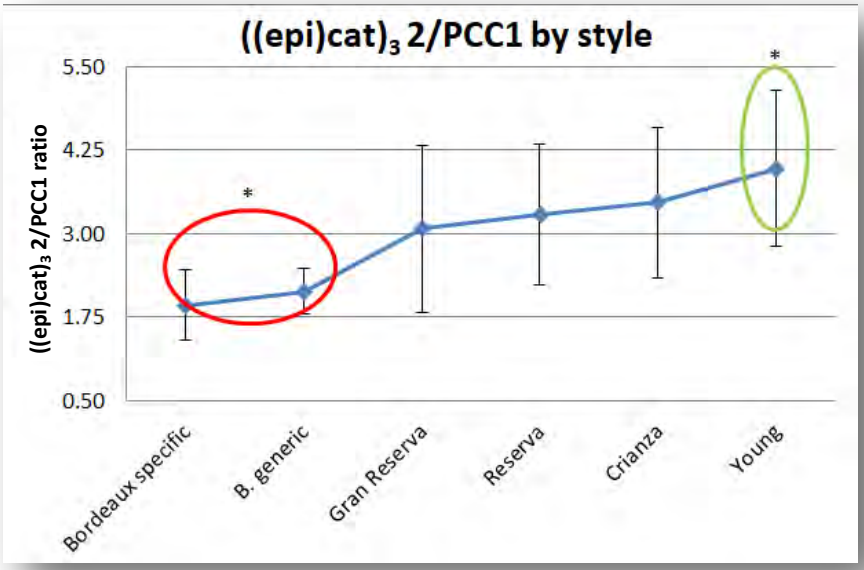



*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.41.** Mean± standard deviation concentrations for values of $((epi)cat)_3$ 2/PCC1 ratio by region.

**Table VI.48.** Values of $((epi)cat)_3$ 2/PCC1 ratio by region: mean, standard deviation and minimum and maximum values for each region.

| | **$((epi)cat)_3$ 2/PCC1 ratio** | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Médoc | 1.86 | 0.46 | 1.02 | 2.95 |
| Libournais | 1.92 | 0.51 | 0.91 | 2.85 |
| Graves | 2.07 | 0.72 | 1.20 | 3.32 |
| B&BS | 2.13 | 0.34 | 1.42 | 2.51 |
| Blayais & Bourgeais | 2.17 | 0.50 | 1.68 | 3.00 |
| Rioja Baja | 2.60 | 0.85 | 1.15 | 3.94 |
| Rioja Alta | 3.28 | 1.10 | 1.16 | 6.08 |
| Rioja Alavesa | 3.71 | 0.94 | 2.29 | 5.63 |




*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.42.** Mean ± standard deviation concentrations for values of ((epi)cat)$_3$ 2/PCC1 ratio by style.

**Table VI.49.** Values of ((epi)cat)$_3$ 2/PCC1 ratio by style: mean, standard deviation and minimum and maximum values for each region.

| | ((epi)cat)$_3$ 2/PCC1 ratio | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Bordeaux specific | 1.93 | 0.53 | 0.91 | 3.32 |
| B. generic | 2.13 | 0.34 | 1.42 | 2.51 |
| *Gran Reserva* | 3.07 | 1.25 | 1.16 | 5.63 |
| *Reserva* | 3.28 | 1.05 | 1.15 | 5.45 |
| *Crianza* | 3.46 | 1.13 | 1.39 | 6.08 |
| Young | 3.97 | 1.17 | 1.46 | 5.02 |

## VI.4.4. Dimers with A bond

Besides the link-type B, these tannins have other ether-type bond between carbons 2 and 7 and, as in previous cases, can be formed by two identical monomers (procyanidins and prodelphinidins) or be mixed.

### a) Procyanidin homodimers A

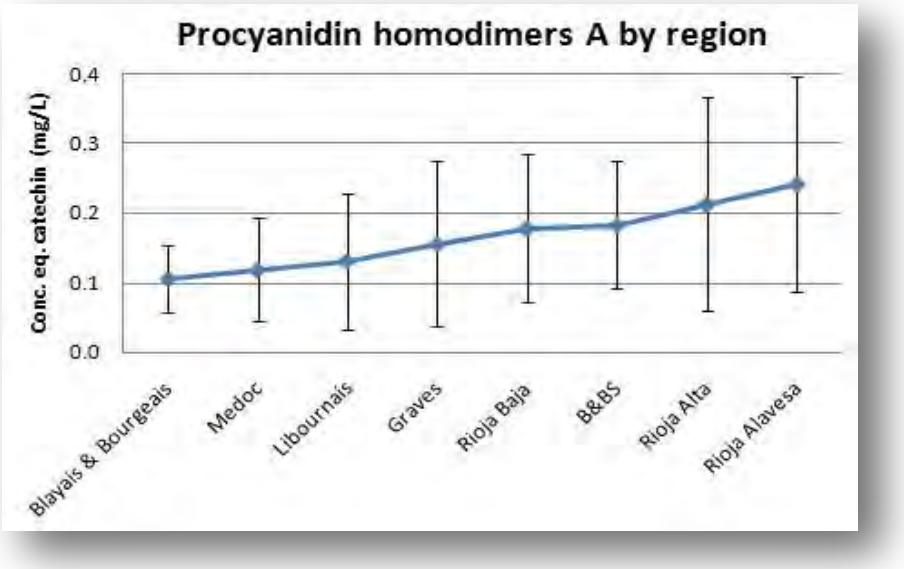
Two procyanidin homodimers A were selected as markers (Table VI.51).

**Table VI.50.** List of the procyanidin homodimers A quantified in this research.

| Procyanidin homodimers A | |
|---|---|
| ((epi)cat)$_2$ A 1 | ((epi)cat)$_2$ A 2 |

On the one hand, levels of procyanidin homodimers A (obtained as sum of the two individual procyanidin homodimers A) seems to be slightly higher for Rioja wines. Again levels decrease when the time of ageing is higher.

The graphics are show in Figures VI.43 and VI.44 and the results, which are in equivalents of catechin, can be seen in Table VI.52 to VI.53.

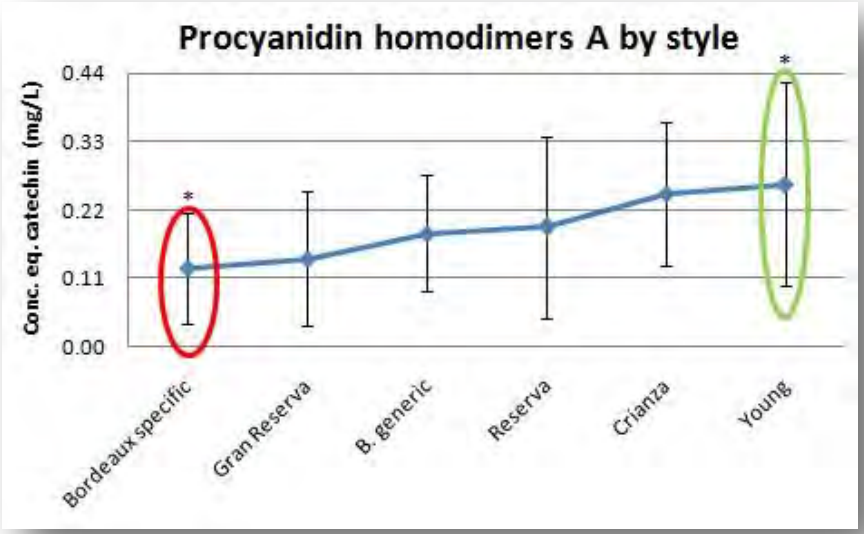



*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.43.** Mean ± standard deviation concentrations for procyanidin homodimers A by region.

**Table VI.51.** Total procyanidin homodimers A concentrations by region: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for each region.

| | Procyanidin homodimers A (mg/L) | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Blayais & Bourgeais | 0.11 | 0.05 | 0.06 | 0.18 |
| Médoc | 0.12 | 0.07 | 0.02 | 0.35 |
| Libournais | 0.13 | 0.10 | 0.02 | 0.35 |
| Graves | 0.16 | 0.12 | 0.03 | 0.38 |
| Rioja Baja | 0.18 | 0.11 | 0.05 | 0.54 |
| B&BS | 0.18 | 0.09 | 0.06 | 0.34 |
| Rioja Alta | 0.21 | 0.15 | 0.01 | 0.55 |
| Rioja Alavesa | 0.24 | 0.15 | 0.05 | 0.55 |




*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.44.** Mean ± standard deviation concentrations for procyanidin homodimers A by wine style.

**Table VI.52.** Total procyanidin homodimers A concentrations by wine style: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for young, *Crianza*, *Reserva* and *Gran Reserva* wines and specific and generic denominations of Bordeaux ones.

| | **Procyanidin homodimers A (mg/L)** | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Bordeaux specific | 0.13 | 0.09 | 0.02 | 0.38 |
| *Gran Reserva* | 0.14 | 0.11 | 0.01 | 0.44 |
| B. generic | 0.18 | 0.09 | 0.06 | 0.34 |
| *Reserva* | 0.19 | 0.15 | 0.01 | 0.55 |
| *Crianza* | 0.25 | 0.11 | 0.05 | 0.50 |
| Young | 0.26 | 0.16 | 0.05 | 0.55 |

## b) Procyanidin heterodimers A

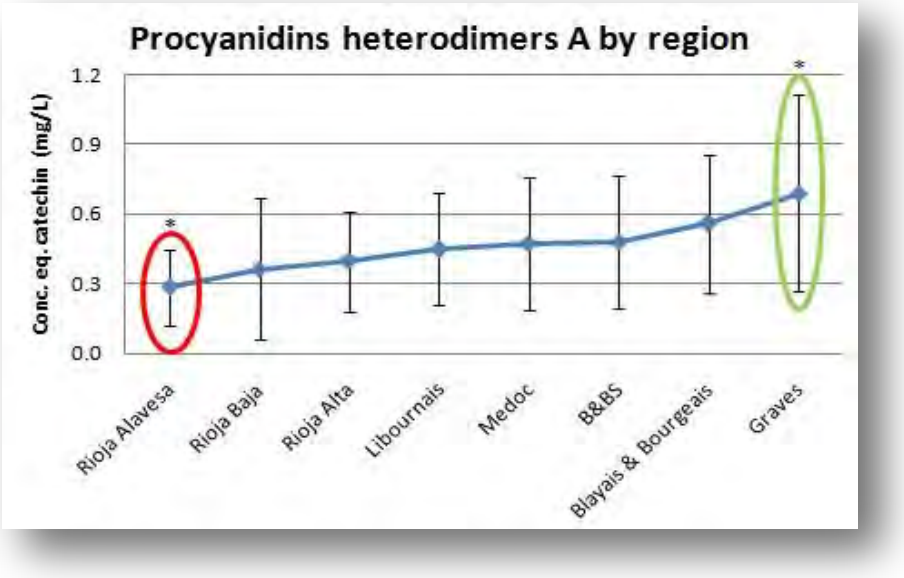
Six tannins from this type of dimers were detected by our group, but only those that present the ((epi)cat-(epi)gallocat) A structure were chosen as markers, while the rest having the (epi)gallocatechin as upper unit are minor (almost in Rioja wines).

The two procyanidins heterodimers A which were selected as markers are shown in Table VI.54.

**Table VI.53.** List of the2 procyanidin heterodimers A quantified in this research.

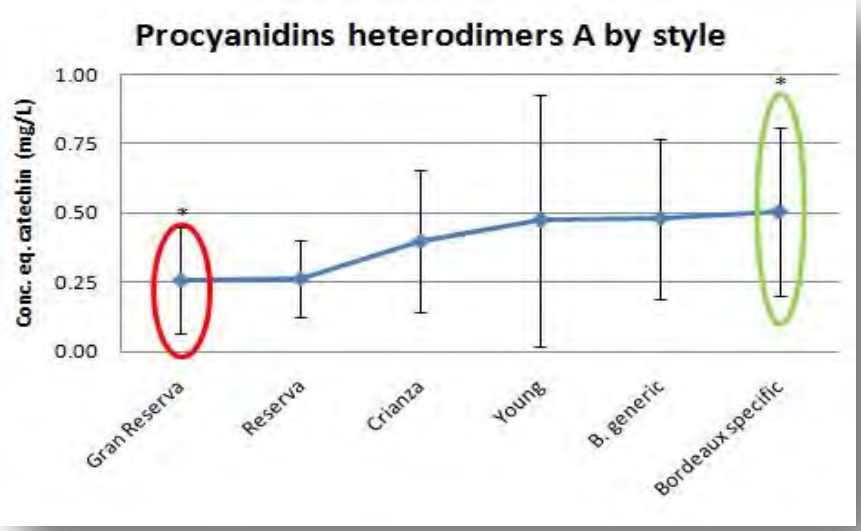
| Procyanidin heterodimers A | |
|---|---|
| ((epi)cat-(epi)gallocat) A 1 | ((epi)cat-(epi)gallocat) A 2 |

Bordeaux wines have higher levels of procyanidin heterodimers A (obtained as sum of the two individual procyanidins heterodimers A) than those ones which come from the different regions of Rioja and their levels decrease when the time of ageing is higher, i.e. young wines are those with highest concentrations of procyanidins heterodimers A, followed by *Crianza* ones, *Reserva* and *Gran Reserva*. In contrast the differences between specific and generic wines of Bordeaux are not so pronounced (Figures VI.45 and VI.46, Tables VI.55 and VI.56).




*: significant groups (α=0.05, by HSD ofTukey test)

**Figure.VI.45.** Mean ± standard deviation concentrations for procyanidin heterodimers A by region.




*: significant groups (α=0.05, by HSD of Tukey test)

**Figure.VI.46.** Mean ± standard deviation concentrations for procyanidin heterodimers A by wine style.

**Table VI.54.** Total procyanidin heterodimers A concentrations by region: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for each region.

| | **Procyanidin heterodimers A (mg/L)** | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Rioja Alavesa | 0.28 | 0.16 | 0.05 | 0.83 |
| Rioja Baja | 0.36 | 0.30 | 0.06 | 2.30 |
| Rioja Alta | 0.40 | 0.22 | 0.07 | 1.23 |
| Libournais | 0.45 | 0.24 | 0.12 | 1.24 |
| Médoc | 0.47 | 0.29 | 0.11 | 1.55 |
| B&BS | 0.48 | 0.29 | 0.18 | 1.37 |
| Blayais & Bourgeais | 0.56 | 0.30 | 0.27 | 0.90 |
| Graves | 0.69 | 0.42 | 0.32 | 1.80 |

**Table VI.55.** Total procyanidin heterodimers A concentrations by wine style: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for young, *Crianza*, *Reserva* and *Gran Reserva* wines and specific and generic denominations of Bordeaux ones.

| | **Procyanidin heterodimers A (mg/L)** | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| *Gran Reserva* | 0.26 | 0.19 | 0.05 | 0.83 |
| *Reserva* | 0.27 | 0.14 | 0.06 | 0.62 |
| *Crianza* | 0.40 | 0.26 | 0.13 | 1.07 |
| Young | 0.47 | 0.46 | 0.09 | 2.30 |
| B. generic | 0.48 | 0.29 | 0.18 | 1.37 |
| Bordeaux specific | 0.50 | 0.31 | 0.11 | 1.80 |

## VI.4.5. Mixed trimers with A bond

The so called A type trimers are those which are formed by three monomers that are joined by one A bond and one type B or by two A type bonds. These kind of trimers have been detected in fruits with high polyphenol contents[459,460,461].

The two mixed trimers with A bond which were selected as markers are shown in Table VI.57.

**Table VI.56.** List of the3 mixed trimers with A bond quantified in this research.

| Mixed trimers with A and B bond | |
|---|---|
| (epi)cat-((epi)cat-(epi)gallocat)A 1 | (epi)cat-((epi)cat-(epi)gallocat)A 2 |

No significant differences in mixed trimers with A and B bond can be seen between both regions. In contrast with general behavior for other tannins their levels increase when the time of ageing is higher, i.e. *Gran Reserva* wines are those with highest concentrations of mixed trimers with A bond, followed by *Reserva* ones, *Crianza* and young. This observation can be also seen in generic denominations of Bordeaux wines (Bordeaux and Bordeaux Supérieur (B&BS)), which have less ageing time in barrel, than the ones with a specific denomination of Bordeaux. So it seems that these kinds of compounds are formed and increase their concentrations as ageing progress.

The graphics are shown in Figures VI.47 and VI.48 and the mean, standard deviation, minimum and maximum values for total procyanidin heterodimers A, which are in equivalents of catechin, can be seen in Tables VI.58 and VI.59.

[459]Flamini, R.;*Mass Spectrometry in Grape and Wine Chemistry Part I, Polyphenols Mass Spectrom. Reviews* **2003**, 22, 218-250.

[460]Cheynier, V.; Doco, T.; Fulcrand, H.; Guyot, S.; Le Roux, E.; Souquet, J.M.; Rigaud, J.; Moutounet, M.; *ESI-MS analysis of polyphenolic oligómeros and polymers*, Anal. Mag. **1997**, 25(8), 32-37.

[461]Monagas, M.; Quintanilla-López, J. E.; Gómez-Cordovés, C.; Bartolomé, B.; Lebrón-Aguilar, R.; *MALDI-TOF MS analysis of plant proanthocyanidins*, J. Pharm. Biom. Anal. **2010**, 51, 358-372.

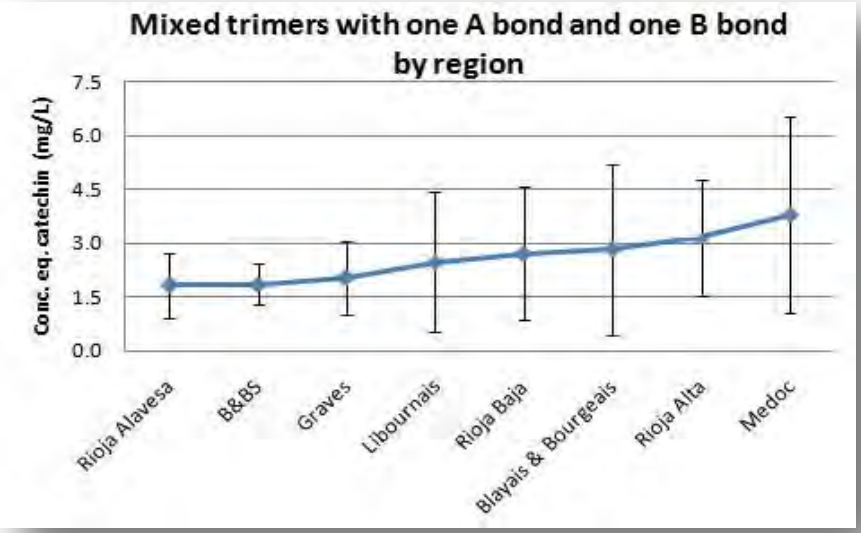



**Figure.VI.47.** Mean ± standard deviation concentrations for mixed trimers with one A bond and one B bond by region.

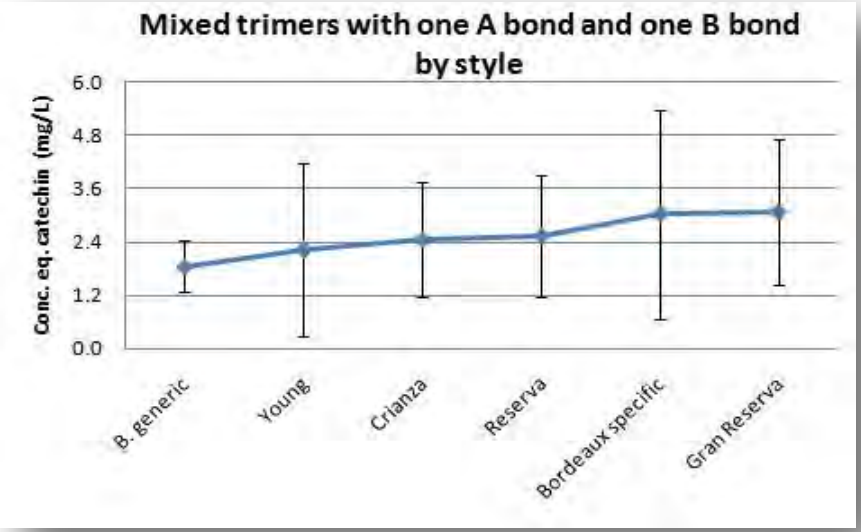



**Figure.VI.48.** Mean ± standard deviation concentrations for mixed trimers with one A bond and one B bond by style.

**Table VI.57.** Total mixed trimers with one A bond and one B bond concentrations by region: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for each region.

| | **Mixed trimers with one A bond and B bond (mg/L)** | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Rioja Alavesa | 1.82 | 0.90 | 0.12 | 3.81 |
| B&BS | 1.84 | 0.57 | 0.93 | 2.79 |
| Graves | 2.04 | 1.02 | 0.01 | 4.20 |
| Libournais | 2.47 | 1.96 | 0.23 | 10.34 |
| Rioja Baja | 2.71 | 1.84 | 0.33 | 6.86 |
| Blayais & Bourgeais | 2.83 | 2.37 | 1.05 | 6.98 |
| Rioja Alta | 3.17 | 1.62 | 0.67 | 6.40 |
| Médoc | 3.81 | 2.74 | 0.97 | 11.96 |

**Table VI.58.** Total mixed trimers with one A bond and one B bond concentrations by wine style: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for young, Crianza, Reserva and Gran Reserva wines and specific and generic denominations of Bordeaux ones.

| | **Mixed trimers with one A bond and B bond (mg/L)** | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| B. generic | 1.84 | 0.57 | 0.93 | 2.79 |
| Young | 2.20 | 1.95 | 0.12 | 6.86 |
| *Crianza* | 2.46 | 1.30 | 0.40 | 5.76 |
| *Reserva* | 2.53 | 1.37 | 0.67 | 6.11 |
| Bordeaux specific | 3.02 | 2.35 | 0.01 | 11.96 |
| *Gran Reserva* | 3.07 | 1.65 | 0.86 | 6.64 |

## VI.4.6. p-Vinyl tannins

The vinyl tannins are products of the depolymerization of ethylidene-bridged tannins or can also be formed by the dehydration of the acetaldehyde in the flavan-3-ol condensation[462]. Furthermore, it is also possible to form vinyl-procyanidins and vinyl-prodelphinidins, as well as vinyl-mixed dimers.

[462]Drinkine, J.; Lopes, P.; Kennedy, J. A.; Teissedre, P. L.; Saucier, C.; *Ethylidene-Bridged Flavan-3-ols in Red Wine and Correlation with Wine Age*, J. Agric. Food Chem. **2007**, 55, 6292-6299.

The three p-vinyl tannins which were selected as markers are shown in Table VI.60.

**Table VI.59.** List of the 3 vinyl tanninsquantified in this research.

| **p-Vinyl tannins** | | |
|---|---|---|
| p-vinyl(epi)cat 1 | p-vinyl(epi)cat 2 | p-vinyl(epi)cat 3 |

Rioja wines have higher levels of p-vinyl tannins (obtained as sum of the three individual p-vinyl tannins) than those ones which come from the different regions of Bordeaux and their levels decrease in the case of Rioja wines (not for Bordeaux ones) when the time of ageing is higher (Figures VI.49 and VI.50, Tables VI.61 and VI.62).

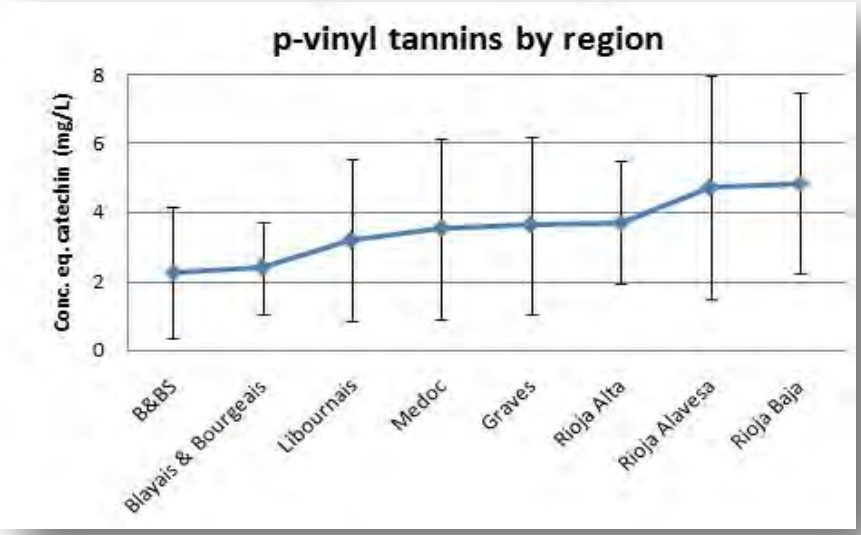
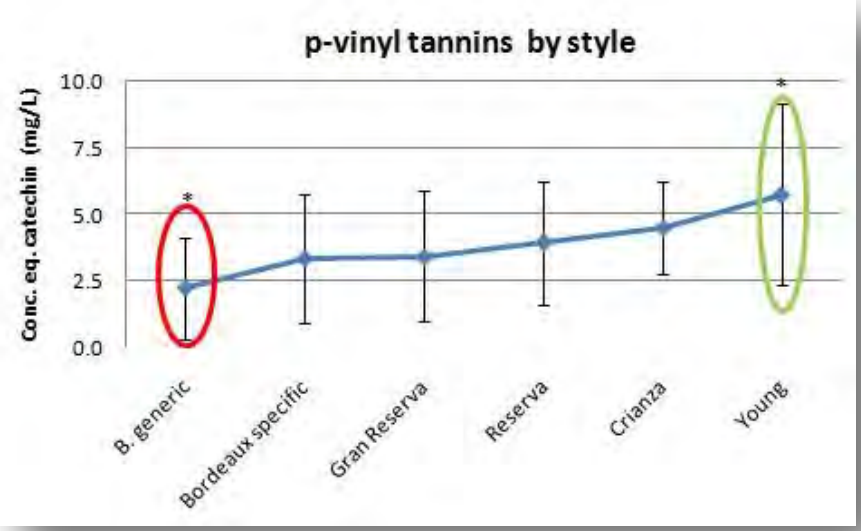



**Figure.VI.49.** Mean ± standard deviation concentrations for p-vinyl tannins by region.




*: significant groups (α=0.05, by HSD of Tukey test)

**Figure.VI.50.** Mean ± standard deviation concentrations for p-vinyl tannins by wine style.

**Table VI.60.** Total p-vinyl tannins concentrations by region: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for each region.

| | **p-Vinyl tannins (mg/L)** | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| B&BS | 2.26 | 1.90 | 0.88 | 8.44 |
| Blayais & Bourgeais | 2.38 | 1.33 | 0.86 | 3.72 |
| Libournais | 3.18 | 2.34 | 0.38 | 9.15 |
| Médoc | 3.53 | 2.61 | 0.44 | 11.19 |
| Graves | 3.63 | 2.56 | 0.01 | 9.09 |
| Rioja Alta | 3.70 | 1.78 | 0.28 | 6.86 |
| Rioja Alavesa | 4.72 | 3.24 | 0.99 | 17.91 |
| Rioja Baja | 4.85 | 2.64 | 0.48 | 9.84 |

**Table VI.61.** Total p-vinyl tannins concentrations by wine style: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for young, *Crianza*, *Reserva* and *Gran Reserva* wines and specific and generic denominations of Bordeaux ones.

| | **p-Vinyl tannins (mg/L)** | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| B. generic | 2.26 | 1.90 | 0.88 | 8.44 |
| Bordeaux specific | 3.36 | 2.43 | 0.01 | 11.19 |
| *Gran Reserva* | 3.46 | 2.45 | 0.28 | 7.93 |
| *Reserva* | 3.96 | 2.33 | 0.58 | 9.97 |
| *Crianza* | 4.52 | 1.72 | 1.36 | 8.57 |
| Young | 5.76 | 3.39 | 0.77 | 17.91 |

## VI.4.7. Tannins formed by condensation mediated by furfuryl

The formation of these tannins occurs by acid-catalyzed nucleophilic addition between the furan aldehydes and flavan-3-ols, forming a furfuryl or hydroxymethylfurfuryl (HMF) bridge[463] between the two or more flavan-3-ols that take part. Such compounds have already been detected in wine and it is believed that they come from the oak wood[464,465]. If the reaction would only occur with a single type of diastereoisomer, for example the catechin, four different isomers may form: cat-6-fur-6-cat, cat-8-fur-8-cat andcat-6-fur-8-cat R and S, due to the asymmetric carbon of the furfuryl bridge in the latter case.

Two tannins with furfuryl bridge were chosen as markers (Table VI.63).

**Table VI.62.** List of the 2 tannins with furfuryl bridge quantified in this research.

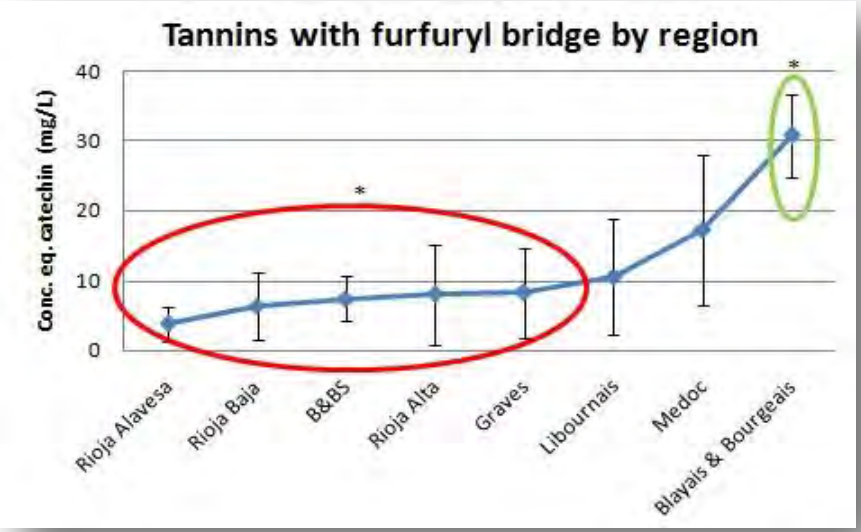
| **Tannins with furfuryl bridge** | |
|---|---|
| (epi)cat-furfuryl-(epi)cat 1 | (epi)cat-furfuryl-(epi)cat 2 |

[463]Nonier Bourden, M. F.; Vivas, N.; Absalon, C.; Vitry, C.; Fouquet, E.; Vivas de Gaulejac, N.; *Structural diversity of nucleophilic adducts from flavanols and oak wood aldehydes*, Food Chem. **2008**, 107, 1494-1505.

[464]Nonier, M-F.; Pianet, I.; Laguerre, M.; Vivas, N.; Vivas de Gaulejac, N.; *Condensation products derived from flavan-3-ols oak Wood aldehydes reaction. 1. Structural investigation,* Anal. Chem. Acta. **2006**, 76-83.

[465]Nonier, M.F.; Vivas, N.; Vivas de Gaulejac, N.; Absalon, C.; Vitry, C.; *Study by LC/ESI/MSn and ESI/HR/MS of SO2 interactions in flavanols–aldehydes nucleophilic reactions*, Food Chem. **2010**, 122,488-494.

High levels of these kind of tannins have been found in wines of both regions, being, in general, higher for Bordeaux ones. Levels of tannins with furfuryl bridge increase when the time of ageing is higher, showing their formation and accumulation along ageing. The graphics are shown in Figures VI.51 and VI.52 and the mean, standard deviation, minimum and maximum values for total tannins with furfuryl bridge, which are in equivalents of catechin, can be seen in Tables VI.64 and VI.65.




*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.51.** Mean ± standard deviation concentrations for tannins with furfuryl bridge by region.

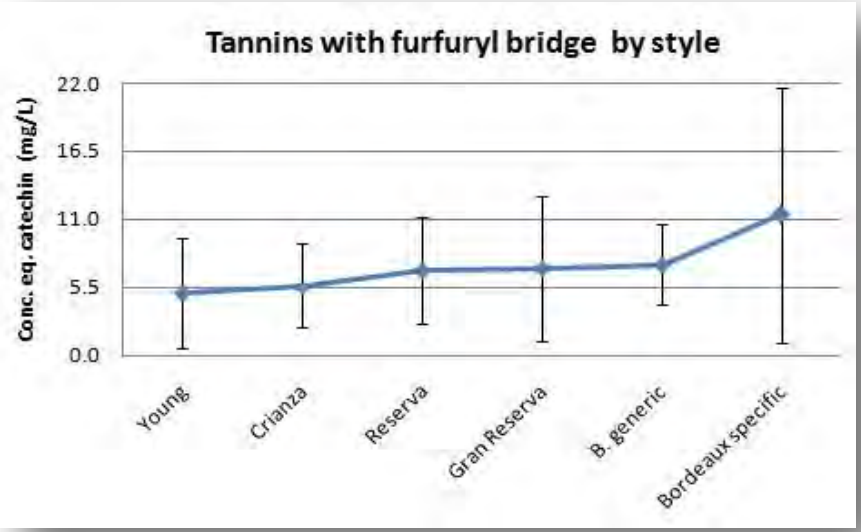


**Figure.VI.52.** Mean ± standard deviation concentrations for tannins with furfuryl bridge by wine style.

**Table VI.63.** Total tannins with furfuryl bridge concentrations by region: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for each region.

| | **Tannins with furfuryl bridge (mg/L)** | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Rioja Alavesa | 3.80 | 2.49 | 0.16 | 10.69 |
| Rioja Baja | 6.32 | 4.82 | 0.26 | 16.89 |
| B&BS | 7.34 | 3.21 | 1.42 | 13.12 |
| Rioja Alta | 8.00 | 7.21 | 0.01 | 30.02 |
| Graves | 8.26 | 6.41 | 0.96 | 31.92 |
| Libournais | 10.46 | 8.25 | 1.10 | 77.08 |
| Médoc | 17.21 | 10.84 | 2.30 | 144.55 |
| Blayais & Bourgeais | 30.76 | 5.99 | 2.87 | 30.85 |

**Table VI.64.** Total tannins with furfuryl bridge concentrations by wine style: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for young, *Crianza*, *Reserva* and *Gran Reserva* wines and specific and generic denominations of Bordeaux ones.

| | **Tannins with furfuryl bridge (mg/L)** | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Young | 5.01 | 4.42 | 0.16 | 18.71 |
| *Crianza* | 5.63 | 3.41 | 0.26 | 13.25 |
| *Reserva* | 6.88 | 4.29 | 1.59 | 16.41 |
| *Gran Reserva* | 6.99 | 5.83 | 1.09 | 31.92 |
| B. generic | 7.34 | 3.21 | 1.42 | 13.12 |
| Bordeaux specific | 11.34 | 10.35 | 1.10 | 77.08 |

## VI.4.8. O-glycosylated flavan-3-ols

There is few studies about the detection of O-glycosylated flavan-3-ols in wine, but this type of tannins exist in the nature, in the wood of some trees[466] and in some plants[467,468,469] and even in the cocoa liquor[470].

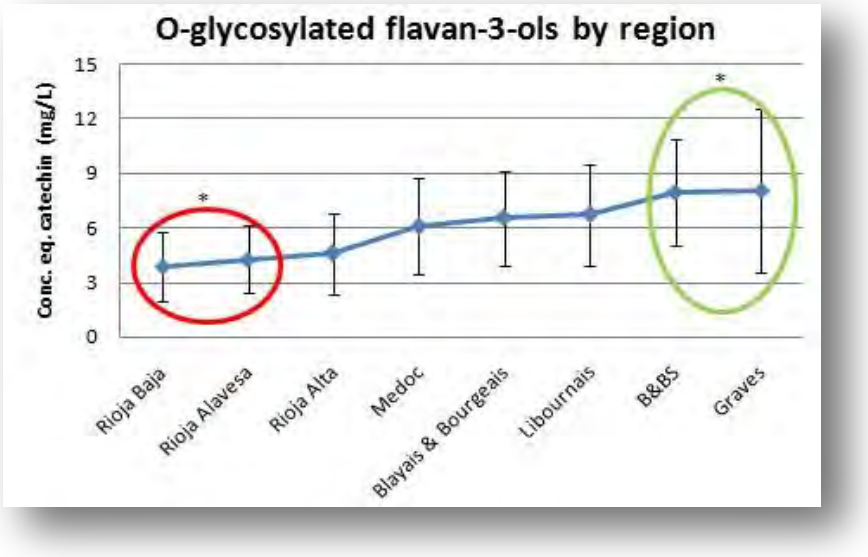
[466]Bekker, M.; Bekker, R.; Brandt, V. E.; *Two flavonoid glicosides and a miscellaneous flavan from the bark of Guibourtia colesperma*. Phytochem. **2006**, 67, 818-823.

The two O-glycosylated flavan-3-ols which were selected as markers are shown in Table VI.66.

**Table VI.65.** List of the O-glycosylated flavan-3-ols analyzed in this research.

| O-Glycosylated flavan-3-ols | |
|---|---|
| (epi)cat-glycoside 1 | (epi)cat-glycoside 2 |

Bordeaux wines have higher levels of O-glycosylated flavan-3-ols (obtained as sum of the two individual O-glycosylated flavan-3-ols) than those ones which come from the different regions of Rioja and their levels decrease when the time of ageing is higher (Figures VI.53 and VI.54, Tables VI.67 and VI.68).




*: significant groups (α=0.05, by Scheffé test)

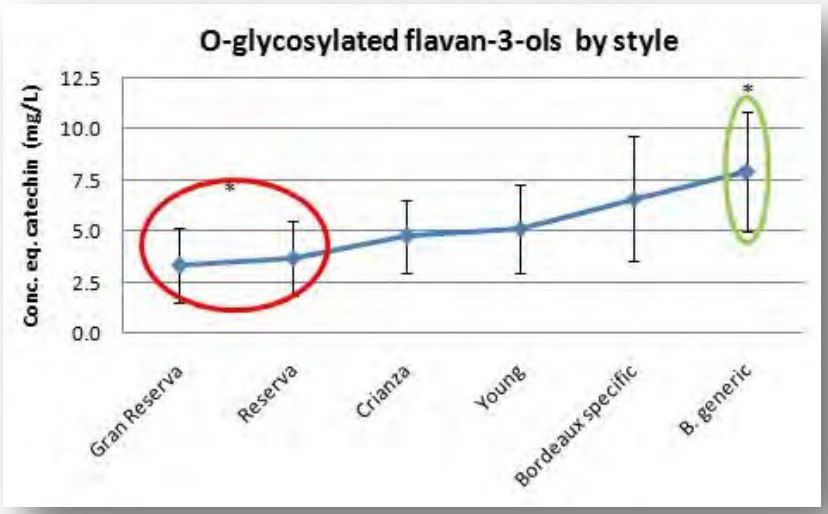
**Figure.VI.53.** Mean ± standard deviation concentrations for O-glycosylated flavan-3-ols by region.

[467]Lokvan, J.; Coley, P. D.; Kursar, T. A.; *Cinnamoyl glucosides of catechin and dimeric procyanidins fromyoung leaves of Inga umbellifera (Fabaceae)*, Phytochem. **2004**, 65, 351-358.

[468]Karioti, A.; Bilia, A. R.; Gabbiani, Messori, L.; Skaltsa, H. *Proanthocyanidin glycosides from the leaves of Quercus ilex L. (Fagaceae)*. Tetrahedron Let. **2009**, 50, 1771-1776.

[469]Friedrich, W.; Galensa, R. *Identification of a new flavanol glucoside from barley (Hordeum vulgare L.) and malt*. Eur. Food Res. Technol. **2002**, 214, 388-393.

[470]Hatano, T.; Miyatake, H.; Natsume, M.; Osakabe, N.; Takizawa, T.; Ito, H.; Yoshida, T.; *Proanthocyanidins glycosides and related polyphenols from cacao liquor and their antioxidant effects*, Phytochem. **2002**, 59, 749-758.




*: significant groups (α=0.05, by Scheffé test)

**Figure.VI.54.** Mean ± standard deviation concentrations for O-glycosylated flavan-3-ols by wine style.

**Table VI.66.** Total O-glycosylated flavan-3-ols concentrations by region: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for each region.

| | **O-Glycosylated flavan-3-ols (mg/L)** | | | |
|---|---|---|---|---|
| **Region** | **Mean** | **Standard deviation** | **Min** | **Max** |
| Rioja Baja | 3.86 | 1.88 | 0.93 | 9.68 |
| Rioja Alavesa | 4.27 | 1.90 | 1.24 | 7.97 |
| Rioja Alta | 4.58 | 2.24 | 0.71 | 9.57 |
| Médoc | 6.06 | 2.63 | 1.98 | 13.43 |
| Blayais & Bourgeais | 6.51 | 2.56 | 3.56 | 9.23 |
| Libournais | 6.69 | 2.78 | 0.90 | 14.15 |
| B&BS | 7.94 | 2.91 | 3.86 | 16.11 |
| Graves | 8.04 | 4.50 | 3.01 | 17.70 |

**Table VI.67.** Total O-glycosylated flavan-3-ols concentrations by wine style: mean in equivalents of catechin (mg/L), standard deviation and minimum and maximum values for young, *Crianza*, *Reserva* and *Gran Reserva* wines and specific and generic denominations of Bordeaux ones.

| | **O-Glycosylated flavan-3-ols (mg/L)** | | | |
|---|---|---|---|---|
| **Style** | **Mean** | **Standard deviation** | **Min** | **Max** |
| *Gran Reserva* | 3.34 | 1.84 | 0.71 | 7.02 |
| *Reserva* | 3.73 | 1.83 | 1.24 | 9.57 |
| *Crianza* | 4.77 | 1.79 | 2.02 | 7.97 |
| Young | 5.13 | 2.13 | 1.30 | 9.68 |
| Bordeaux specific | 6.62 | 3.06 | 0.90 | 17.70 |
| B. generic | 7.94 | 2.91 | 3.86 | 16.11 |

## VI.5. Effect of mixture of grape varieties in Rioja wines

Although the predominant variety used in the production of red wines in Rioja is *Tempranillo* and many of the wines are monovarietal wines (100% *Tempranillo*), it is also common in many wines to mix this predominant variety with another such as *Graciano*, *Mazuelo* or *Garnacha*. This mixture can affect to the levels of anthocyanins and tannins in wines.

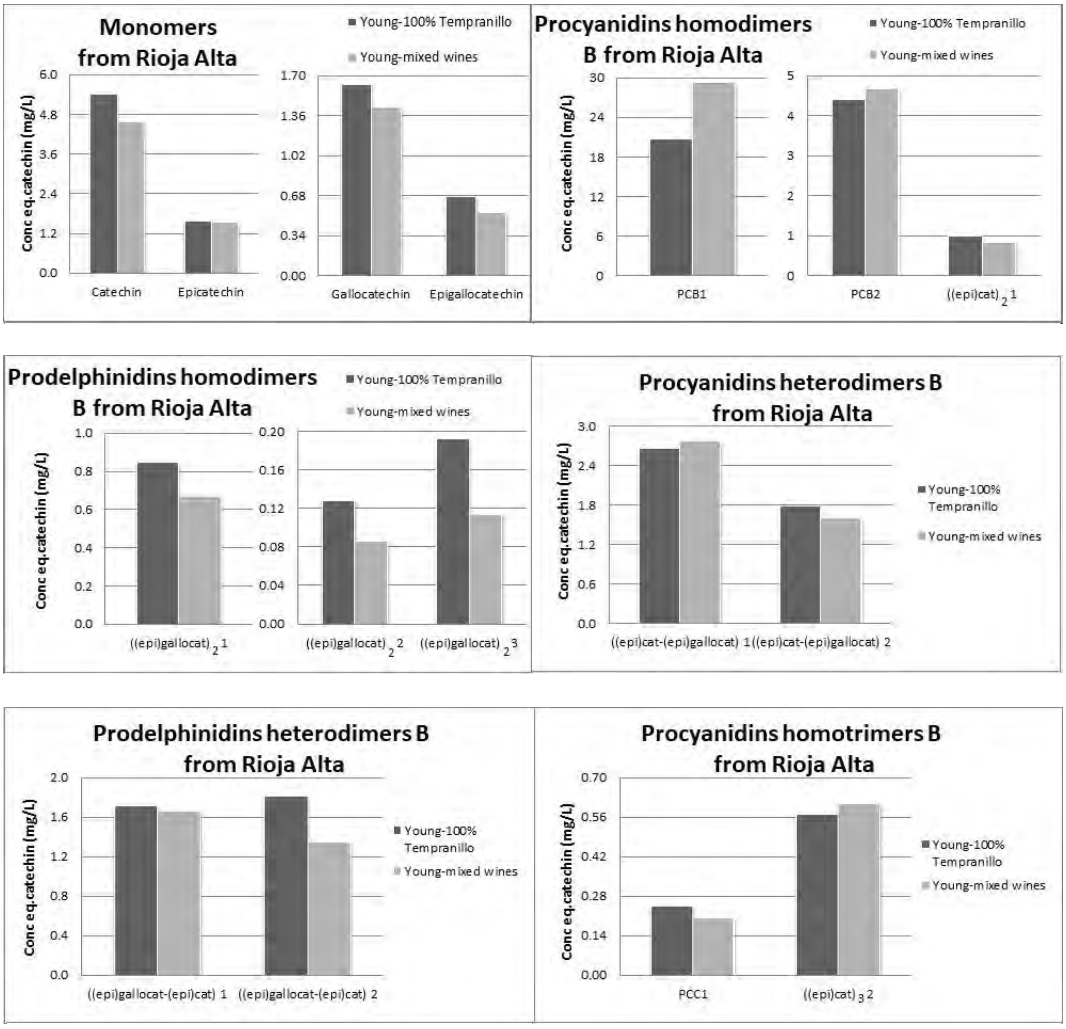



**Figure.VI.55.** Meanconcentrations of different tannins for*Tempranillo* and mixed young wines from Rioja Alta.

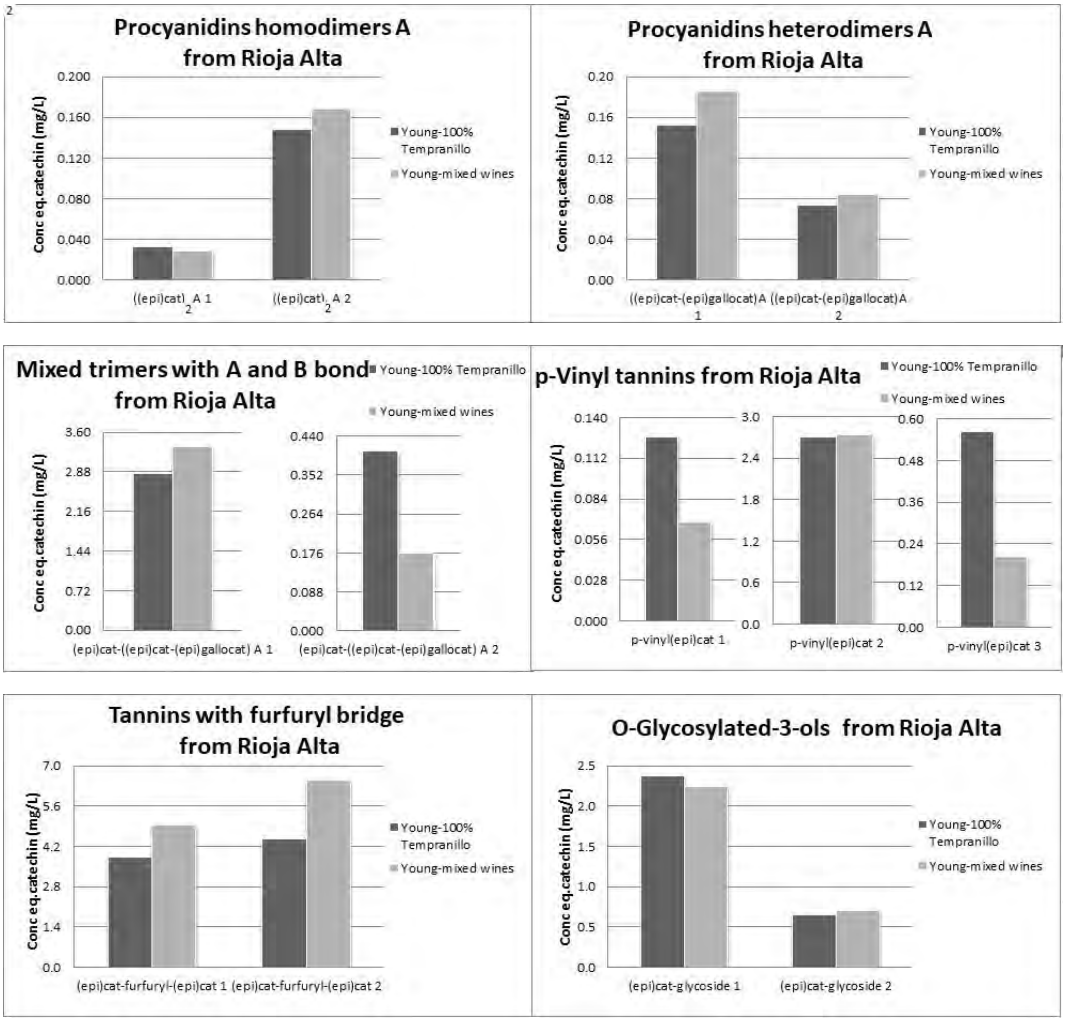



**Figure.VI.55.** Cont.

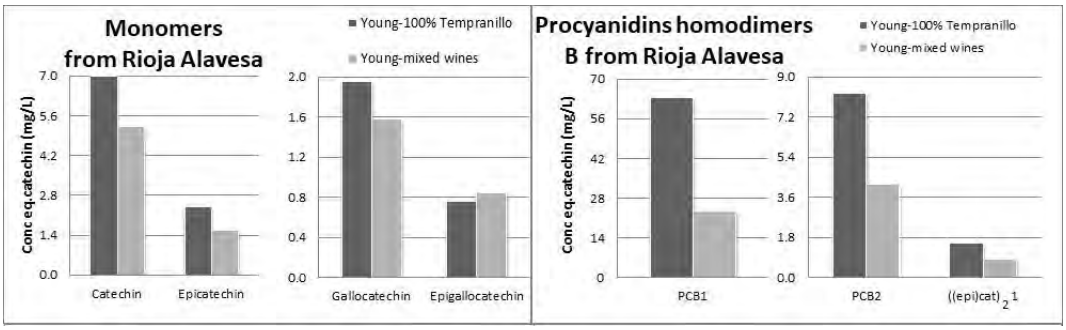



**Figure.VI.56.** Mean concentrations of different tannins for *Tempranillo* and mixed young wines from Rioja Alavesa.

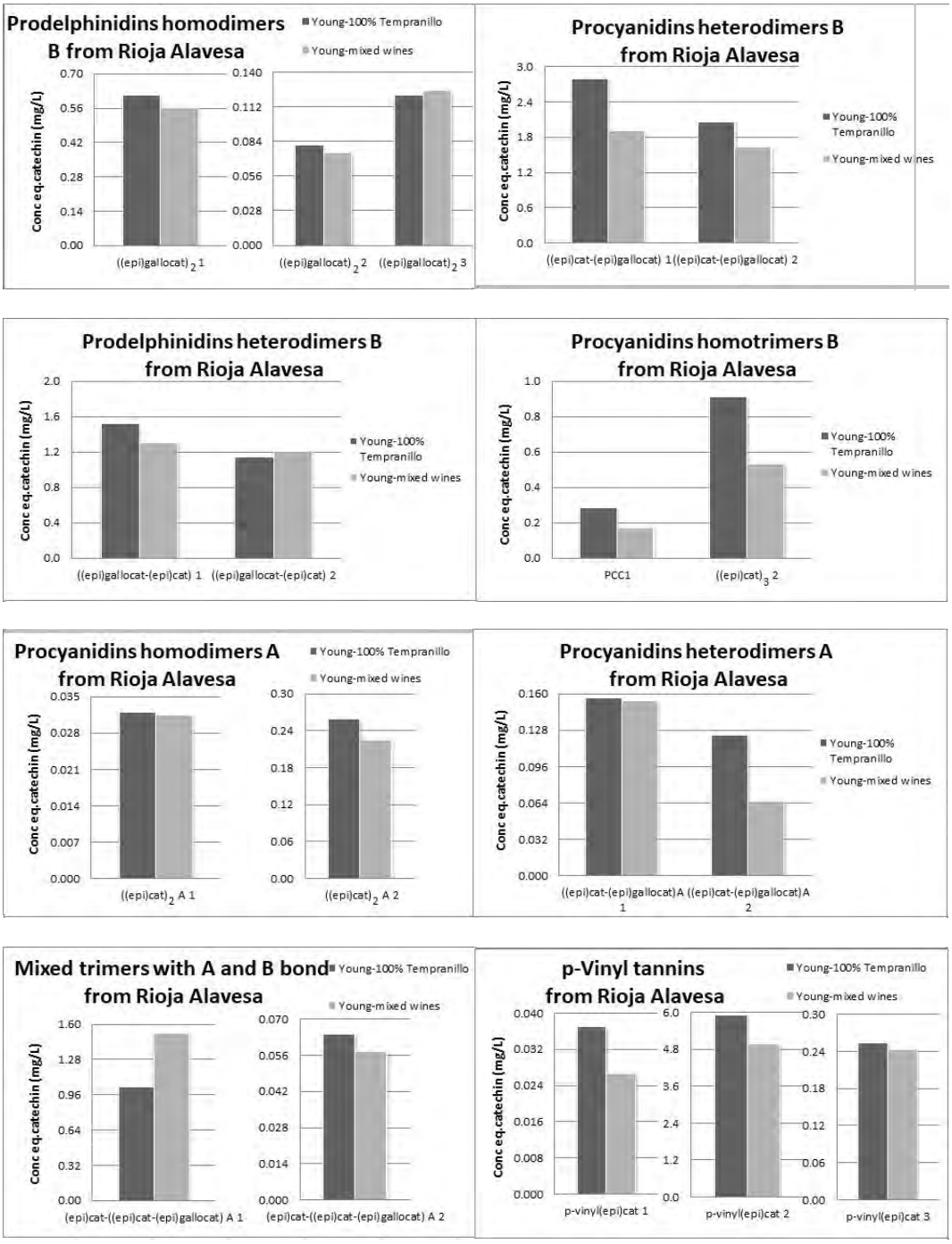



**Figure.VI.56.** Cont.

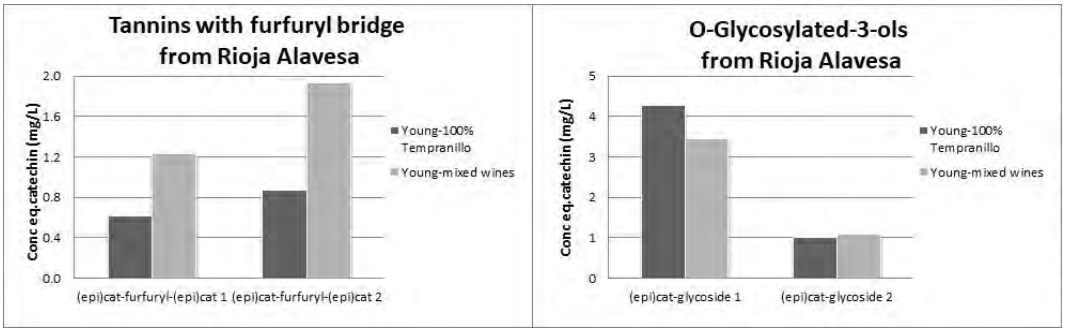



**Figure.VI.56.** Cont.

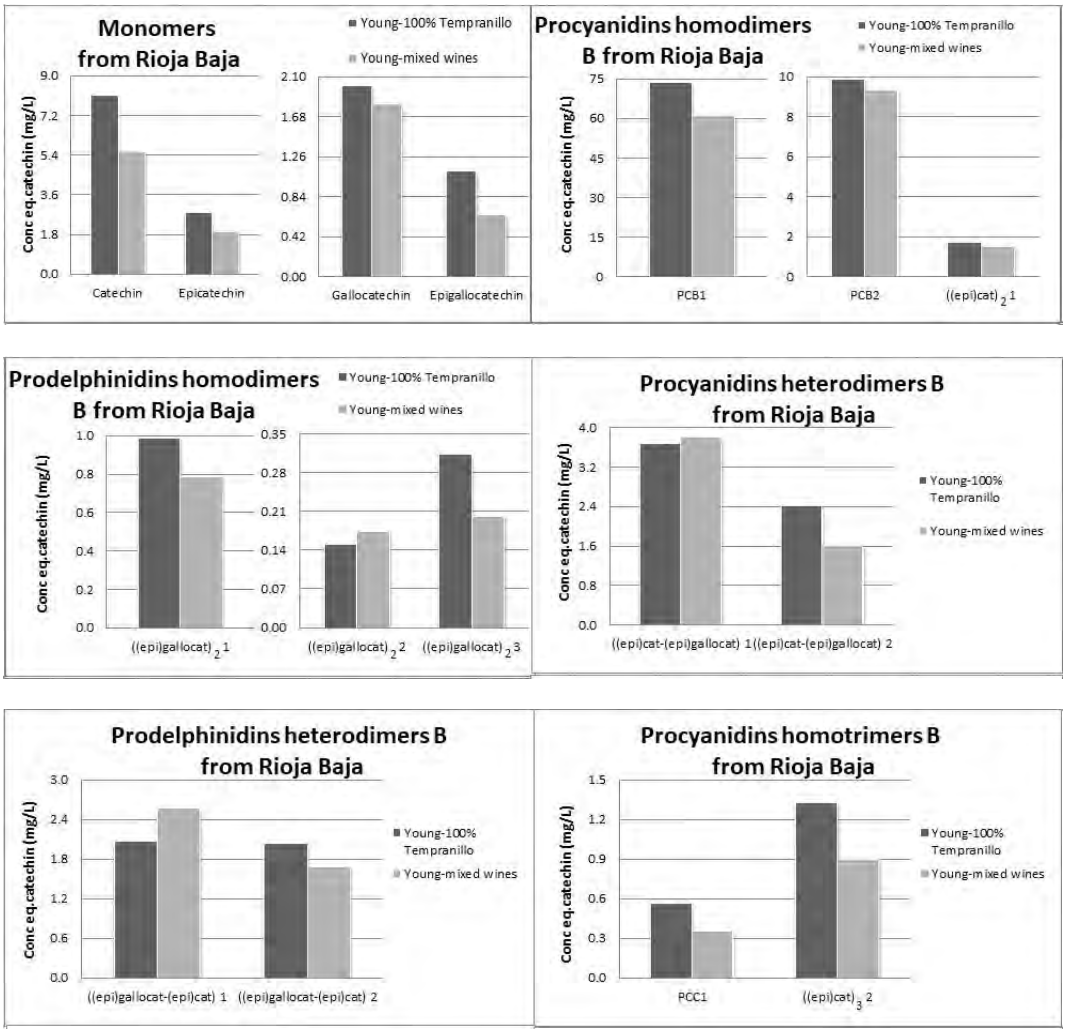



**Figure.VI.57.** Mean concentrations of different tannins for *Tempranillo* and mixed young wines from Rioja Baja.

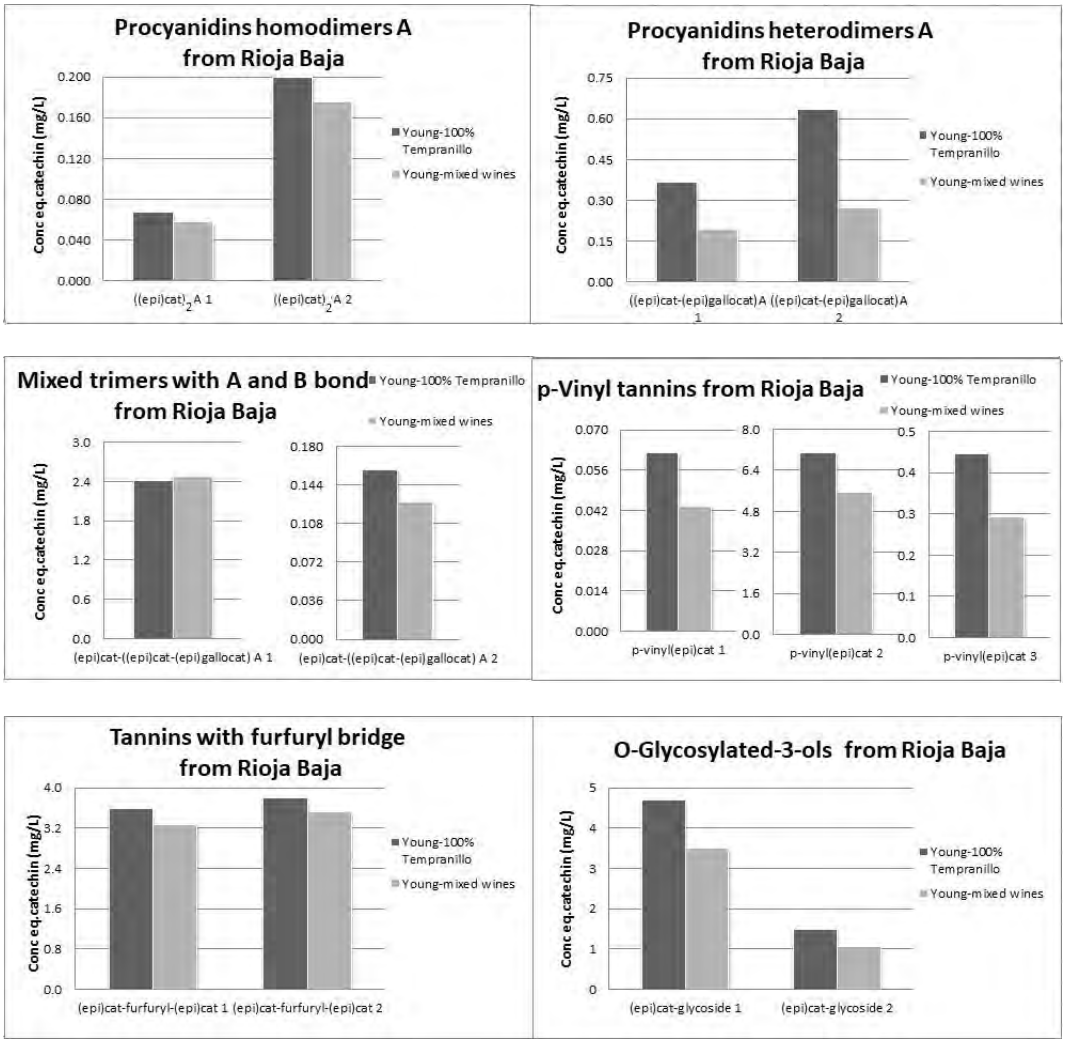



**Figure.VI.57.** Cont.

On one hand, Figures VI.55 to 57 (for wines of Rioja Alta, Alavesa and Baja, respectively) shows that almost all levels of tannins are lower in young wines which are made by mixing varieties of grapes (*Tempranillo-Graciano* wines and *Tempranillo-Garnacha* wines, with 5-40% of the secondary variety) than wines 100% *Tempranillo* (*Tempranillo* wines). This difference could indicate an increased reactivity of tannins along firsts winemaking steps in mixed wines, being less pronounced in wines from Rioja Baja, in which *Garnacha* is more used. This low reactivity of tannins in *Tempranillo* monovarietal young wines has already been mentioned in the bibliography for young red wines of Cabernet Sauvignon, Graciano and Tempranillo varieties during bottle aging[502] being associated with its particular tannin-anthocyanin ratio[503,504] and

[502]Monagas, M.; Bartolomé, B.; Goméz-Cordovés, C.; *Evolution of polyphenols in red wines from Vitis vinifera L. during aging in the bottle*, Eur. Food Res. Technol. **2005**, 220, 331-340.

a high pH[505]. Figure VI.58 shows that mixed young wines have lower pH than *Tempranillo* monovarietal wines for Rioja Alta y Alavesa wines, but for Rioja Baja the difference does not exist.

In addition, this higher reactivity of tannins in mixed wines also occurs in free anthocyanins compounds, so these wines should have lower levels of than 100% Tempranillo wines. This fact is confirmed in Figures VI. 59 to VI.61, being less pronounced in wines from Rioja Baja.

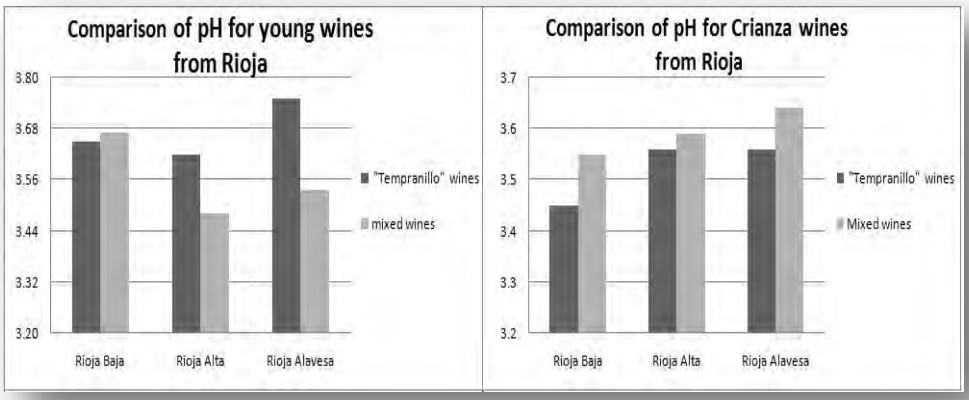
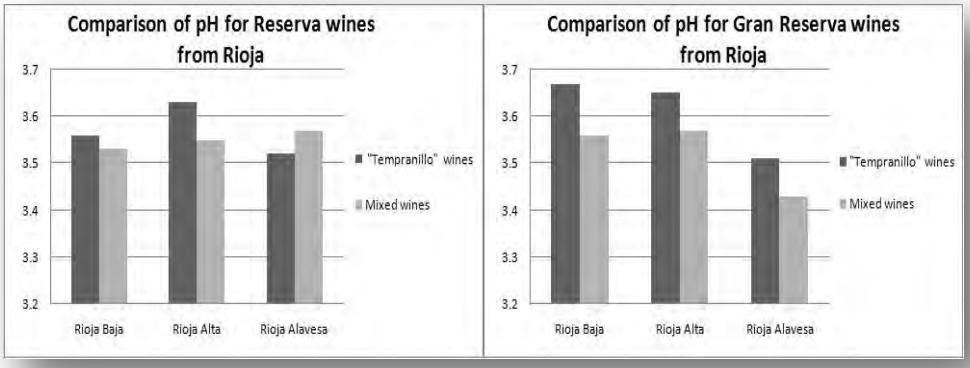



**Figure.VI.58.**




[503]Monagas, M.; Martín-Álvarez, P. J.; Goméz-Cordovés, C.; Bartolomé, B.; *Effect of the modifieer (Graciano and Cabernet sauvignon) on blends of Tempranillo wine during ageing in the botel*, LWT Food Sci. Technol. **2007**, 40, 107-115.

[504]Monagas, M.; Bartolomé, B.; Goméz-Cordovés,C.; *Effect of the modifieer (Graciano and Cabernet sauvignon) on blends of Tempranillo wine during ageing in the botel. I Anthocyanins, pyranoanthocyanins and non-anthocyanin phenolics*, LWT Food Sci. Technol. **2006**, 39, 1133-1142.

[505]Monagas, M.; Goméz-Cordovés`, C.; Bartolomé, B.; *Evaluation of different Saccharomyces cerevisiae strains for red winemaking. Influence on the anthocyani, pyranoanthocyanin and non-anthocyanin phenolic content and colour characteristics of wines*, Food Chem. **2007**, 104, 814-823.

**Figure.VI.58.** Values of pH for Tempranillo and mixed wines from Rioja.

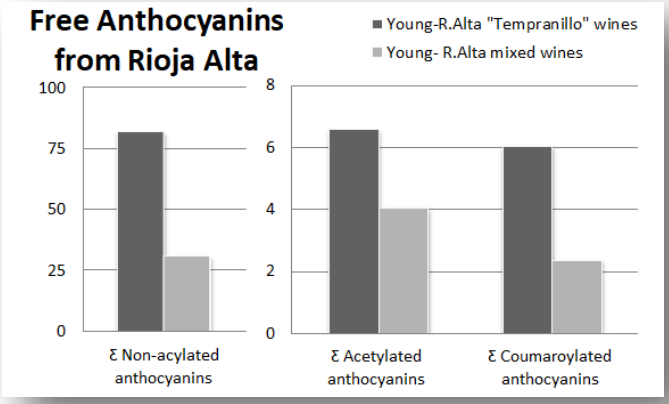



**Figure.VI.59.** Mean concentrations of free anthocyanins for *Tempranillo* and mixed young wines from Rioja Alta.

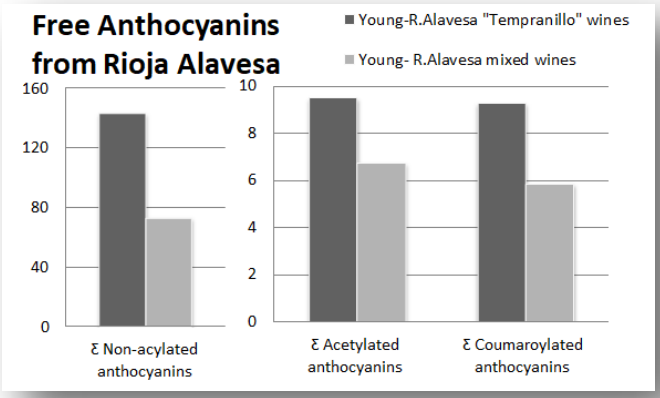



**Figure.VI.60.** Mean concentrations of free anthocyanins for *Tempranillo* and mixed young wines from Rioja Alavesa.

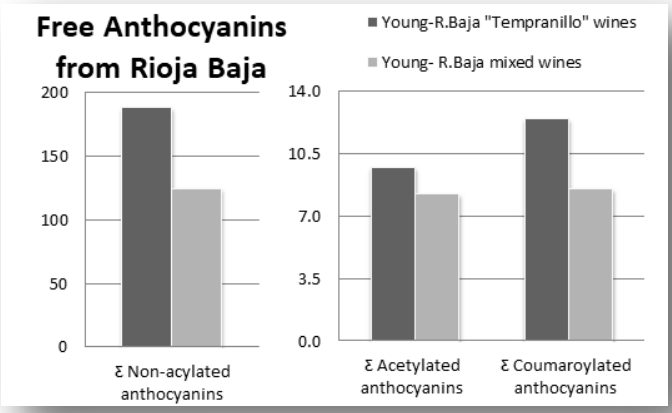


**Figure.VI.61.** Mean concentrations of free anthocyanins for *Tempranillo* and mixed young wines from Rioja Baja.

On the other hand, anthocyanin derivatives, which are the reaction products of free anthocyanins and tannins, should have higher level in mixed wines. However, this is not supported by the data of this research (Figures VI. 62 to VI.64), probably due to the fact that wine samples in this work are final bottled wines, not wine samples along or just at the end of fermentations and aging, and time passed after have also influence.

In the case of aged wines (Figures can be seen on the Annex II) the lower levels of tannins in mixed wines is also observed in most cases (*Crianza* wines from Rioja Alavesa and *Reserva* and *Gran reserva* wines from Rioja Alta and Alavesa). However, when ageing advance other factors than initial tannin reactivity in must could have effect on the different compounds present in wines.

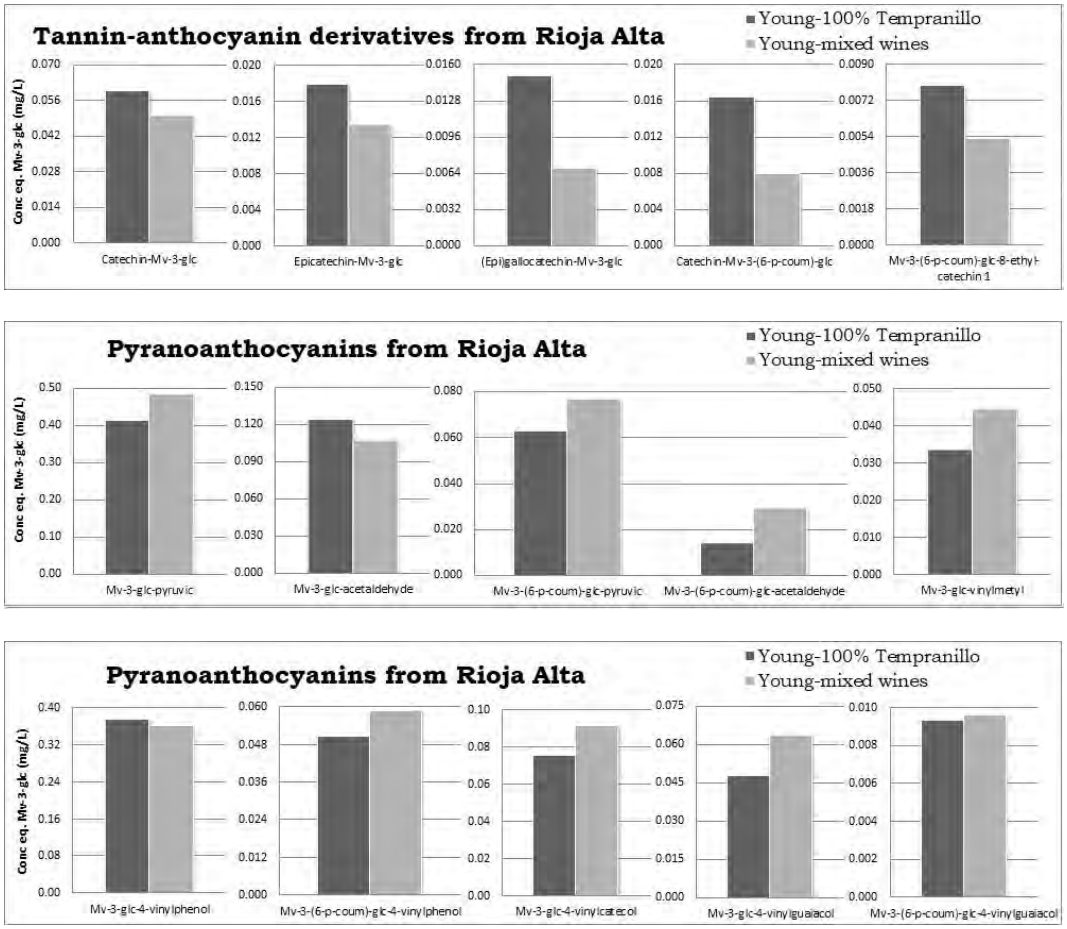



**Figure.VI.62.** Mean concentrations of anthocyanin derivatives for *Tempranillo* and mixed young wines from Rioja Alta.

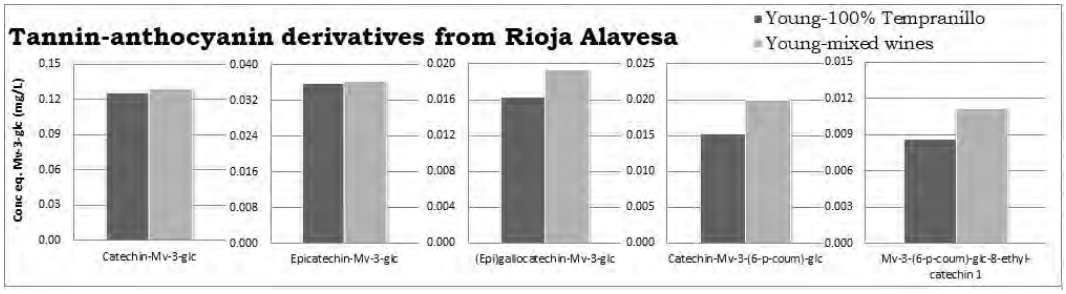



**Figure.VI.63.**

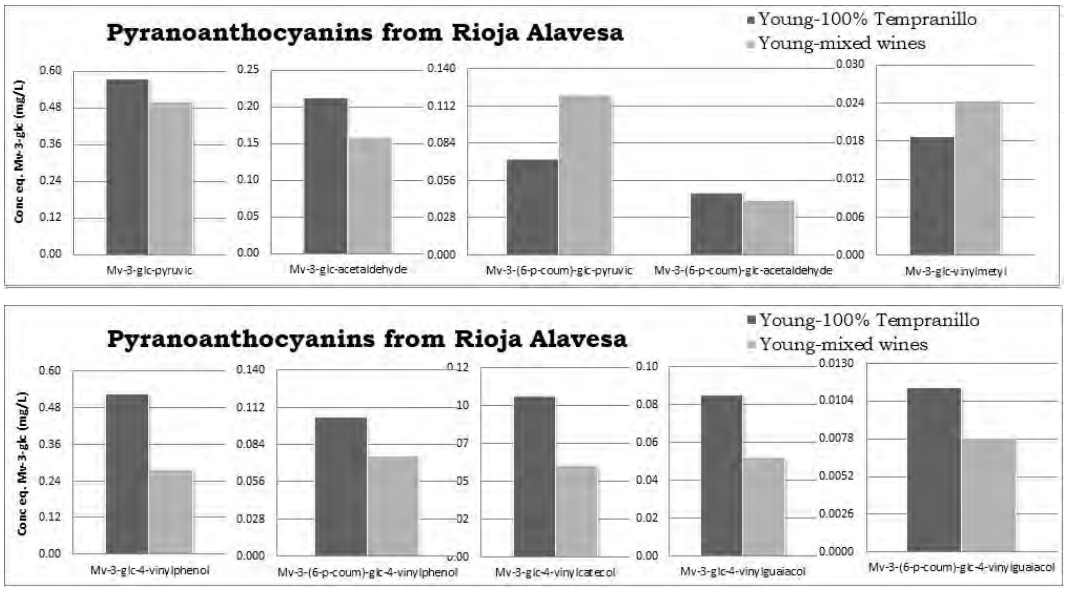



**Figure.VI.64.** Meanconcentrations of anthocyanin derivatives for*Tempranillo* and mixed young wines from Rioja Alavesa.

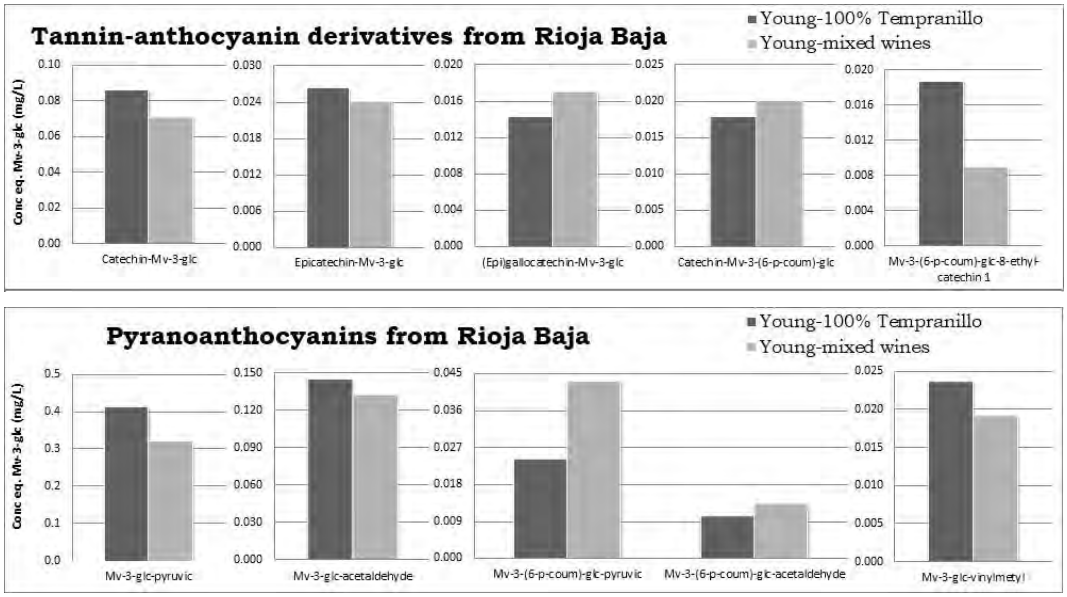


**Figure.VI.64.** Mean concentrations of anthocyanin derivatives for *Tempranillo* and mixed young wines from Rioja Baja.

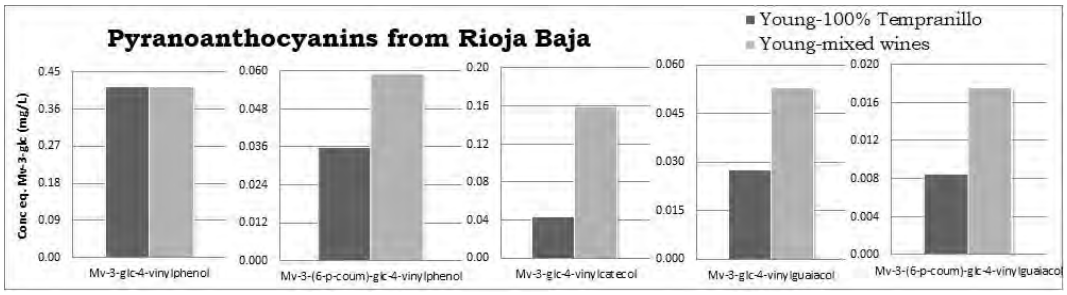



**Figure.VI.64.** Cont.

## VI.6. Differences in anthocyanin, anthocyanin derivatives and tannin composition between Rioja red wines aged in American and French oak barrels

Aging wine in oak barrels is a traditional, common practice in winemaking that enables obtaining high quality wines. During this aging, wood releases different compounds to wines and allows small amounts of oxygen to pass through the pores[506]. These facts enhance the occurrence of different phenomena and reactions that increase wine stability and improve wine sensorial characteristics.

It is well-known that the characteristics of the final wines aged in wood barrels are profoundly influence by the geographical origin of wood used[507,508,509], but others factors such as toast, pore size, contact surface between wine and wood or aging time have to be taken into account.

The pore size depends mainly on the geographical origin and this factor is different for American and French oaks. In order to study the differences on anthocyanin, anthocyanin derivatives and tannin composition of Rioja wines aged in different types of oak barrels, samples with different winemaking conditions

[506] Ortega-Heras, M.; Pérez-Magariño, S.; Cano-Mozo, E.; Gonzáliez-San José, M. L.; *Differences in the phenolic composition and sensory profile between red wines aged in oak barrels and wines aged with oak chips*, LWT Food Sci. Technol. **2010**, 43, 1533-1541.

[507] Frangipane, M. T.; De Santis, D.; Ceccarelli, A.; *Influence of oak woods of different geographical origins on quality of wines aged in barriques usin oak chips*, Food Chem. **2007**, 103, 46-54.

[508] Ortega-Heras, M.; Gonzaléz.Huerta, C.; Gonzaléz-SanJosé, M. L.; *Discussion about the influence of aging process, kind of wine and oenological parameters on the levels of wood volatile compounds present in red wines*, Food Chem **2007**, 103, 1434-1448.

[509] Del Alamo-Sanza, M.; Nevares-Domínguez, I.; *Wine aging in botlle from artificial systems and oak woods: anthocyanin composition*, Anal. Chim. Acta **2006**, 563, 255-263.

concerning grape cultivar (wines made by *Tempranillo* grapes and by *Tempranillo-Graciano* grapes with 10% of the secondary variety), oak origin (French and American oak barrels) and barrel ageing period (*Crianza* and *Reserva* wines) were sampled

It is known that French oak barrels have higher pore size than American ones, so greater amounts of oxygen pass through the pores. Due to this fact the reactivity of wines which are aged in French oak barrels will be higher than those which are aged in American ones. This is supported by the ratios of French oak barrels between American ones which will be seen in bellow tables (the individual results were collected inside the Annex II).

On one hand, tannins ratios (Table VI. 69) show that almost all levels of tannins are lower in *Crianza* wines which are aged in French oak barrels than those which are aged in American ones. This difference could indicate an increased reactivity of tannins along aging in French oak barrels. In the case of *Crianza* mixed wines (which are made by mixing varieties of grapes) the lower levels of tannins are also observed.

**Table VI.68.** French oak barrels/American oak barrels ratio of the different families of tannins for 100% *Tempranillo* wines and mixed wines from Rioja Alavesa and Alta.

| | **Ratio (French oak/American oak)** | | | |
|---|---|---|---|---|
| **Tannins** | ***Crianza* 100% *Tempranillo*** | ***Reserva* 100% *Tempranillo*** | ***Crianza* mixed wines** | ***Reserva* mixed wines** |
| **Flavan-3-ols** | 0.99 | 1.04 | 0.92 | 0.56 |
| **Procyanidins homodimers B** | 0.44 | 0.99 | 0.86 | 0.28 |
| **Prodelphinidins homodimers B** | 0.64 | 0.99 | 0.79 | 0.28 |
| **Procyanidins heterodimers B** | 0.75 | 1.25 | 0.84 | 0.31 |
| **Prodelphinidins heterodimers B** | 0.71 | 1.07 | 0.83 | 0.33 |
| **Procyanidins homotrimers B** | 0.65 | 1.01 | 0.70 | 0.53 |
| **Procyanidins homodimers A** | 0.40 | 0.34 | 1.14 | 0.40 |
| **Procyanidins heterodimers A** | 0.41 | 0.98 | 1.21 | 0.87 |
| **Mixed trimers with A bond** | 0.42 | 1.29 | 1.74 | 0.33 |
| **p-Vinyl tannins** | 0.50 | 0.24 | 0.75 | 0.48 |
| **Tannins with furfuryl bridge** | 0.11 | 3.68 | 0.63 | 0.15 |
| **O-glycosylated flavan-3-ols** | 0.56 | 0.94 | 0.61 | 0.38 |

**Table VI.69.** French oak barrels/American oak barrels ratio of the different families of anthocyanins for 100% *Tempranillo* wines and mixed wines from Rioja Alavesa and Alta.

| | **Ratio (French oak/American oak)** | | | |
|---|---|---|---|---|
| **Anthocyanins** | ***Crianza* 100% *Tempranillo*** | ***Reserva* 100% *Tempranillo*** | ***Crianza* mixed wines** | ***Reserva* mixed wines** |
| **Non acylated anthocyanins** | 0.40 | 0.21 | 0.39 | 0.49 |
| **Acetylated anthocyanins** | 0.66 | 0.53 | 0.47 | 0.44 |
| **Coumaroylated anthocyanins** | 1.41 | 0.21 | 0.34 | 0.61 |
| **Total free anthocyanins** | 0.45 | 0.25 | 0.39 | 0.48 |

In addition, this higher reactivity of tannins in French oak barrels also occurs in free anthocyanins compounds, so these wines should have lower levels of than wines aged in American oak barrels. This fact is confirmed in Table VI. 70, in which ratios show lower level of anthocyanins in wines aged with French oak barrels.

On the other hand, anthocyanin derivatives, which are the reaction products of free anthocyanins and tannins, have higher ratios which indicate higher levels of anthocyanin derivatives in wines aged in French oak barrels. These results can be seen in wines made by 100% *Tempranillo* grapes and by mixing *Tempranillo* and *Graciano* grapes. The ratios of anthocyanin derivatives between French and American.oak are showed in Table VI. 71. These differences are less pronounced in wines from Rioja Baja (the ratios between French and American oak of wines from Rioja Baja could be seen in Annex II)

In conclusion these results show that aging in French oak barrels increases the reactivity of anthocyanins and tannins in 100% *Tempranillo* wines and wines which are made by mixing varieties of grapes (*Tempranillo-Graciano* wines with 10% of the secondary variety). This higher reactivity is due to the fact that French oak has bigger pores than American one, so greater amounts of oxygen, which causes the reactions of the different compounds presents in wine during the aging, are allowed to pass through the pores.

In the case of wines with larger periods in barrels such as *Reserva* wines this conclusion is not observed. However, *Reserva* wines have an additional aging period in bottle that could have effect on anthocyanin, anthocyanin derivatives and tannin levels.

**Table VI.70.** French oak barrels/American oak barrels ratio of the different families of anthocyanin derivatives for 100% *Tempranillo* wines and mixed wines from Rioja Alavesa and Alta.

| | **Ratio (French oak/American oak)** | | | |
|---|---|---|---|---|
| **Anthocyanin derivatives** | ***Crianza* 100% *Tempranillo*** | ***Reserva* 100% *Tempranillo*** | ***Crianza* mixed wines** | ***Reserva* mixed wines** |
| **Vititisins A** | 2.34 | 0.70 | 1.13 | 0.37 |
| **Vitisins B** | 0.64 | 0.13 | 0.98 | 0.63 |
| **Derivatives with acetoacetic acid** | 2.68 | 0.59 | 1.23 | 0.98 |
| **Derivatives with hydroxicinamic acid** | 1.79 | 0.46 | 1.15 | 1.18 |
| **Derivatives with vinylflavanols** | 3.88 | 0.44 | 1.42 | 1.02 |
| **Flavanol-Anthocyanin-A-Flavanol** | 7.11 | 0.80 | 0.81 | 0.29 |
| **Flavanol-A-Flavanol-Anthocyanin** | 1.63 | <0.001 | <0.001 | <0.001 |
| **Anthocyanin-Flavanol-A-Flavanol** | n.d. | n.d. | n.d. | n.d. |
| **Anthocyanin-Flavanol. Flavene form** | n.d. | n.d. | n.d. | n.d. |
| **Flavanol-Anthocyanin. Flavene form** | 0.64 | 0.35 | 2.63 | 0.84 |
| **Anthocyanin-A-Flavavanol** | n.d. | n.d. | n.d. | n.d. |
| **Anthocyanin-Flavanol. Flavylium form** | 0.55 | 0.66 | 0.41 | 0.43 |
| **Total Tanin-Anthocyanin** | 0.68 | 0.50 | 0.79 | 0.51 |
| **Total Pyranoanthocyanins** | 1.88 | 0.56 | 1.13 | 0.60 |

# CAPÍTULO VII

## COMPARACIÓN MEDIANTE ANÁLISIS DE COMPONENTES PRINCIPALES DE LA COMPOSICIÓN ANTOCIÁNICA Y TÁNICA DE VINOS TINTOS DE RIOJA Y BURDEOS

# Capítulo VII

## COMPARACIÓN MEDIANTE ANÁLISIS DE COMPONENTES PRINCIPALES DE LA COMPOSICIÓN ANTOCIÁNICA Y TÁNICA DE VINOS TINTOS DE RIOJA Y BURDEOS

El gran volumen de información obtenida en este trabajo en relación a la composición de antocianos, derivados antociánicos y taninos de los vinos de Rioja y Burdeos ha permitido realizar un estudio de PCA para la diferenciación de los vinos procedentes de diferente región o con diferente tipo de crianza.

**Tabla VII.1.** Valores medios y desviación estándar (X ± DS), mínimos y máximos (conc. Mínima – conc. Máxima) de las concentraciones en mg/L de las antocianinas libres para cada región.

| Antocianinas libres | Rioja | Burdeos |
|---|---|---|
| Dp-3-glc | 7.17 ± 6.89 (0.06 - 37.67) | 2.57 ± 2.17 (0.04 - 12.29) |
| Pt-3-glc | 9.11 ± 8.86 (0.08 - 44.66) | 2.90 ± 2.70 (0.04 - 14.74) |
| Pn-3-glc | 3.03 ± 2.43 (0.03 - 17.10) | 1.43 ± 0.56 (0.05 - 9.40) |
| Mv-3-glc | 36.44 ± 35.40 (0.53 - 176.04) | 13.32 ± 11.30 (0.55 - 47.94) |
| Dp-3-(6-Ac)-glc | 1.66 ± 0.63 (0.32 - 3.12) | 1.89 ± 0.77 (0.31 - 4.50) |
| Mv-3-(6-Ac)-glc | 3.47 ± 2.92 (0.19 - 12.44) | 3.95 ± 3.80 (0.24 - 16.02) |
| Mv-3-(6-p-coum)-glc | 4.16 ± 3.81 (0.07 - 16.98) | 1.39 ± 1.07 (0.10 - 6.01) |

Este estudio ha partido de los contenidos de 51 compuestos fenólicos (7 antocianinas, 15 derivados antociánicos y 29 taninos) cuantificados en las 190 muestras de vinos. Entre las 190 muestras, 96 fueron de vinos tintos procedentes de

Rioja (de las tres diferentes zonas de Rioja: 32 vinos de Rioja *Alavesa*, 32 vinos de Rioja *Alta* y 32 vinos de Rioja *Baja*) y las otras 94 fueron de Burdeos (pertenecientes a las diferentes zonas vinícolas de Burdeos: 36 vinos de *Médoc*, 13 vinos de *Graves*, 5 vinos de *Blayais & Bourgeais*, 27 vinos de *Libournais* y 13 vinos de denominaciones genéricas de Burdeos, *Bordeaux & Bordeaux Supérieur*). Dichas muestras son vinos de cosechas comprendidas entre 1994 y 2013.

**Tabla VII.2.** Valores medios y desviación estándar (X ± DS), mínimos y máximos (conc. Mínima – conc. máxima) de las concentraciones en mg/L de los derivados antociánicos para cada región.

| **Derivados Antociánicos** | **Rioja** | **Burdeos** |
|---|---|---|
| Mv-3-glc-acetaldehído | 0.11 ± 0.07 (0.00 - 0.70) | 0.07 ± 0.05 (0.00 - 0.67) |
| Mv-3-glc-vinilmetil | 0.03 ± 0.02 (0.00 - 0.12) | 0.04 ± 0.02 (0.01 - 010) |
| Mv-3-glc-pirúvico | 0.48 ± 0.20 (0.09 - 1.09) | 0.74 ± 0.32 (0.14 - 1.60) |
| Mv-3-glc-4-vinilfenol | 0.37 ± 0.16 (0.07 - 0.91) | 0.47 ± 0.23 (0.12 - 1.08) |
| Mv-3-glc-4-vinilcatecol | 0.11 ± 0.09 (0.02 - 0.61) | 0.17 ± 0.10 (0.04 - 0.50) |
| Mv-3-glc-4-vinilguaiacol | 0.05 ± 0.04 (0.00 - 0.20) | 0.05 ± 0.02 (0.02 - 0.12) |
| Mv-3-(6-p-coum)-glc-acetaldehído | 0.02 ± 0.01 (0.00 - 0.12) | 0.01 ± 0.01 (0.00 - 0.06) |
| Mv-3-(6-p-coum)-glc-pirúvico | 0.08 ± 0.04 (0.00 - 0.32) | 0.13 ± 0.08 (0.02 - 0.35) |
| Mv-3-(6-p-coum)-glc-4-vinilfenol | 0.06 ± 0.04 (0.00 - 0.21) | 0.07 ± 0.04 ( 0.01 - 0.23) |
| Catequin-Mv-3-glc | 0.07 ± 0.04 (0.00 - 0.18) | 0.08 ± 0.04 (0.01 - 0.21) |
| Epicatequin-Mv-3-glc | 0.02 ± 0.01 (0.00 - 0.05) | 0.04 ± 0.02 (0.00 - 0.09) |
| Mv-3-(6-p-coum)-glc-4-vinilguaiacol | 0.01 ± 0.01 (0.00 - 0.04) | 0.01 ± 0.00 (0.00 - 0.02) |
| (Epi)Galocatequin-Mv-3-glc | 0.01 ± 0.01 (0.00 - 0.03) | 0.01 ± 0.01 (0.00 - 0.04) |
| Catequin-Mv-3-(6-p-coum)-glc | 0.01 ± 0.01 (0.00 - 0.03) | 0.01 ± 0.01 (0.00 - 0.05) |
| Mv-3-(6-p-coum)-glc-8-etil-(epi)catequina 1 | 0.01 ± 0.01 (0.00 - 0.04) | 0.01 ± 0.01 (0.00 - 0.04) |

En las tablas VII.1 a VII.3 se resumen algunas características estadísticas de las concentraciones encontradas para cada una de las familias estudiadas en los vinos tintos analizados. Para los posteriores tratamientos quimiométricos los valores de concentración de los compuestos que se encontraban por debajo del límite de detección se toman como la mitad de dicho valor. Con esto se evita que los tratamientos quimiométricos magnifiquen la diferencia entre una concentración muy pequeña y una concentración nula, pudiendo direccionarse los tratamientos hacia estas diferencias y no hacia otras más significantes.

**Tabla VII.3.** Valores medios y desviación estándar (X ± DS), mínimos y máximos (conc. Mínima – conc. Máxima) de las concentraciones en mg/L de los taninos para cada región.

| Taninos | Rioja | Burdeos |
|---|---|---|
| Catequina | 4.99 ± 1.94 (1.14 - 9.91) | 6.20 ± 2.54 (1.56 - 19.39) |
| Epicatequina | 1.77 ± 0.77 (0.49 - 4.60) | 3.24 ± 1.26 (0.91 - 6.93) |
| Galocatequina | 1.40 ± 0.51 (0.40 - 3.06) | 1.17 ± 0.53 (0.02 - 3.16) |
| Epigalocatequina | 0.65 ± 0.32 (0.18 - 1.81) | 0.44 ± 0.24 (0.09 - 1.35) |
| PCB1 | 40.15 ± 32.10 (1.70 - 146.0) | 58.38 ± 41.03 (3.95 - 146.29) |
| PCB2 | 6.03 ± 4.49 (0.82 - 30.92) | 22.40 ± 19.64 (2.36 - 84.87) |
| $((epi)cat)_2$ 1 | 1.07 ± 0.69 (0.09 - 3.52) | 1.66 ± 1.16 (0.26 - 5.90) |
| $((epi)galocat)_2$ 1 | 0.64 ± 0.36 (0.07 - 1.74) | 0.57 ± 0.39 (0.05 - 1.87) |
| $((epi)galocat)_2$ 2 | 0.10 ± 0.08 (0.00 - 0.30) | 0.07 ± 0.06 (0.00 - 0.33) |
| $((epi)galocat)_2$ 3 | 0.16 ± 0.10 (0.01 - 0.43) | 0.14 ± 0.09 (0.01 - 0.45) |
| ((epi)cat-(epi)galocat) 1 | 2.58 ± 1.34 (0.26 - 6.01) | 2.01 ± 1.36 (0.39 - 7.50) |
| ((epi)cat-(epi)galocat) 2 | 1.76 ± 1.18 (0.14 - 9.71) | 1.55 ± 1.46 (0.21 - 11.96) |
| ((epi)galocat-(epi)cat) 1 | 1.75 ± 0.74 (0.23 - 3.92) | 3.11 ± 1.56 (0.88 - 9.46) |
| ((epi)galocat-(epi)cat) 2 | 1.50 ± 0.68 (0.21 - 3.39) | 4.14 ± 2.66 (0.56 - 19.33) |
| PCC1 | 0.23 ± 0.18 (0.02 - 1.09) | 0.33 ± 0.22 (0.06 - 1.24) |
| $((epi)cat)_3$ 2 | 0.71 ± 0.50 (0.03 - 2.23) | 0.66 ± 0.48 (0.05 - 1.97) |
| $((epi)cat)_2$A 1 | 0.04 ± 0.03 (0.00 - 0.14) | 0.04 ± 0.02 (0.00 - 0.09) |
| $((epi)cat)_2$A 2 | 0.17 ± 0.13 (0.00 - 0.50) | 0.09 ± 0.08 (0.01 - 0.29) |

**Tabla VII.3.** Continuación.

| Taninos | Rioja | Burdeos |
|---|---|---|
| ((epi)cat-(epi)galocat)A 1 | 0.20 ± 0.13 (0.02 - 0.67) | 0.23 ± 0.12 (0.03 - 0.61) |
| ((epi)cat-(epi)galocat)A 2 | 0.15 ± 0.11 (0.01 - 1.69) | 0.27 ± 0.25 (0.04 - 1.50) |
| (epi)cat-((epi)cat-(epi)galocat) A 1 | 2.40 ± 4.51 (0.09 - 6.70) | 2.58 ± 1.98 (0.00 - 10.47) |
| (epi)cat-((epi)cat-(epi)galocat) A 2 | 0.17 ± 0.14 (0.01 - 0.90) | 0.30 ± 0.26 (0.00 - 1.49) |
| p-vinil(epi)cat 1 | 0.06 ± 0.04 (0.00 - 0.18) | 0.05 ± 0.04 (0.00 - 0.24) |
| p-vinil(epi)cat 2 | 3.99 ± 2.63 (0.14 - 17.76) | 2.67 ± 2.31 (0.00 - 10.41) |
| p-vinil(epi)cat 3 | 0.38 ± 0.24 (0.04 - 1.82) | 0.55 ± 0.47 (0.00 - 2.98) |
| (epi)cat-furfuril-(epi)cat 1 | 2.65 ± 2.27 ( 0.00 - 11.38) | 3.74 ± 3.64 (0.00 - 47.57) |
| (epi)cat-furfuril-(epi)cat 2 | 3.55 ± 3.09 (0.07 - 20.54) | 9.73 ± 6.59 (0.00 - 96.98) |
| (epi)cat-glucosa 1 | 3.16 ± 1.59 (0.40 - 7.43) | 4.69 ± 2.19 (1.04 - 13.36) |
| (epi)cat-glucosa 2 | 1.08 ± 0.53 (0.25 - 3.12) | 2.18 ± 0.96 (0.54 - 5.85) |

## VII.1. ANÁLISIS UNIVARIANTE

Para visualizar las diferencias entre los vinos tintos, en las Figuras VII.1 a VII.16 se representaron los gráficos de Box-Whiskers para cada una de las variables (cada concentración de un compuesto). Este tipo de gráfico divide los datos en cuatro áreas de igual frecuencia o cuartiles. La caja engloba el 50% de los datos centrales entre el primer y el tercer cuartil. La línea horizontal que aparece dentro de la caja representa la mediana y las líneas verticales son llamadas "bigotes", las cuales se extienden desde cada uno de los bordes de la caja. Los extremos de estos bigotes indican el recorrido de los datos de la siguiente manera: la barra inferior se dibuja desde el borde inferior de la caja (o primer cuartil) hasta el menor valor en una distancia no superior a 1.5 veces el rango intercuartílico (es el rango que comprende el 50% de los datos centrales), y la otra barra, desde la parte superior de la caja (o tercer cuartil) hasta el mayor valor en una distancia menor a 1,5 veces el rango intercuartílico. Las valores que caen fuera de los bigotes pueden ser de dos clases: los valores que caen hasta tres veces el rango intercuartílico son los valores atípicos "moderados" que aparecen en el

grafico representados con un circulo y los valores que se encuentren más alejados de 3 veces el rango intercuartílico son los valores atípicos "extremos" y se representan con un asterisco en el gráfico.

En estos gráficos se observa que para algunas variables las cajas que representan los valores de las diferentes variables para cada región, no se solapan o lo hacen mínimamente y, por ello, son variables más discriminantes para la diferenciación de los vinos de Rioja y Burdeos. Esas variables son: Pt-3-glc, Mv-3-(6-p-coum)-glc, Epicatequina, PCB2, ((epi)galocat-(epi)cat) 2, ((epi)cat-(epi)galocat)A 2 y (epi)cat-glucosa 2. Sin embargo, ninguna variable por si sóla es completamente discriminante; por lo que se deben utilizar tratamientos multivariantes para intentar conseguir la discriminación.

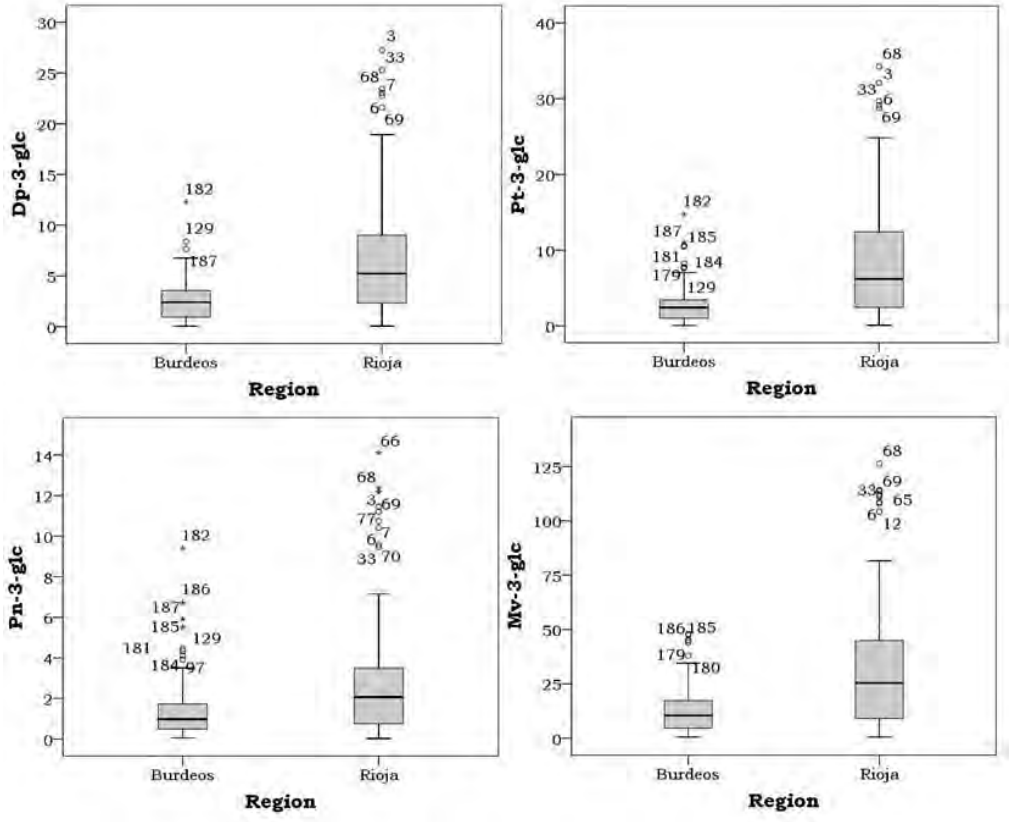



**Figura.VII.1.** Gráficos Box-Whiskers para las variables **antocianinas no aciladas** en muestras de Rioja y Burdeos.

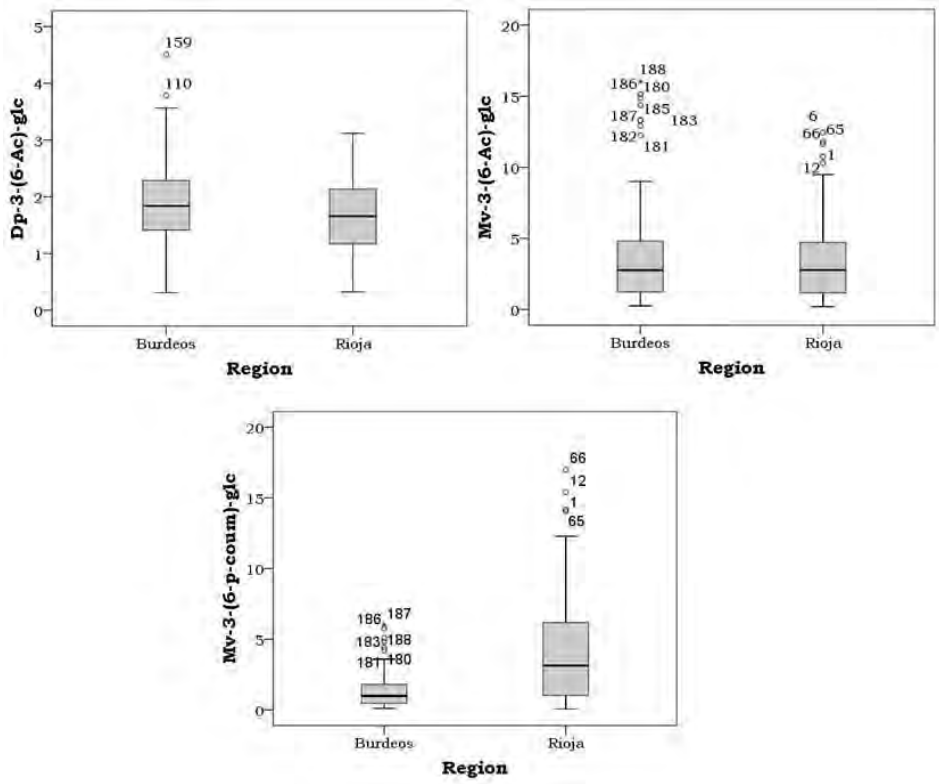



**Figura.VII.2.** Gráficos Box-Whiskers para las variables **antocianinas acetiladas** y **coumaroiladas** en muestras de Rioja y Burdeos.

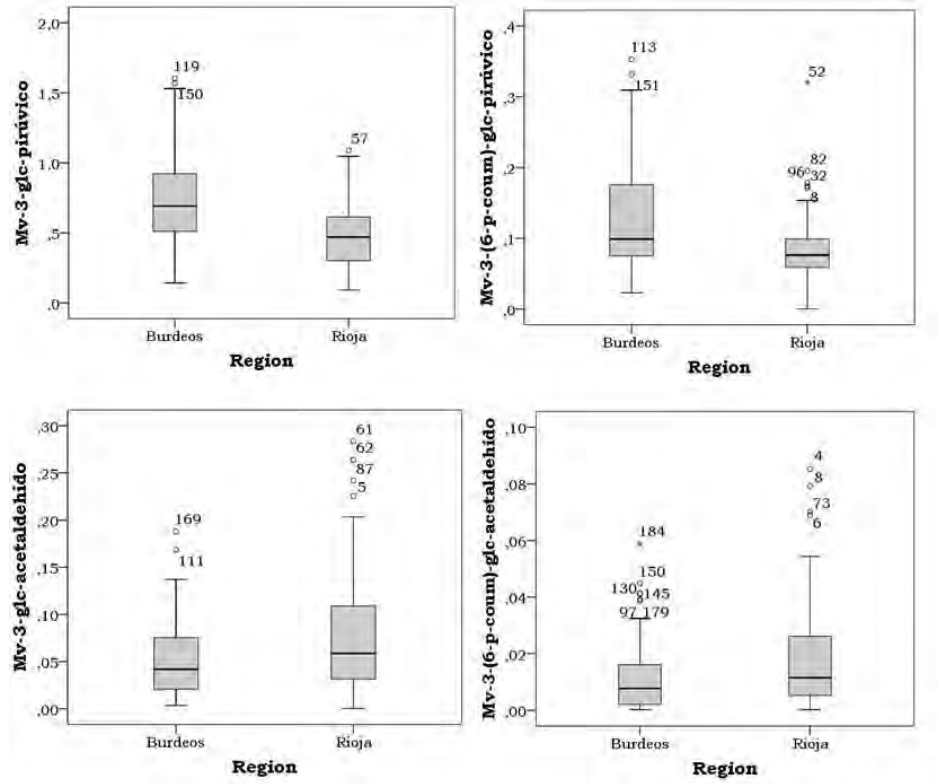



**Figura.VII.3.** Gráficos Box-Whiskers para las variables **Vitisinas A** y **Vitisinas B** en muestras de Rioja y Burdeos.

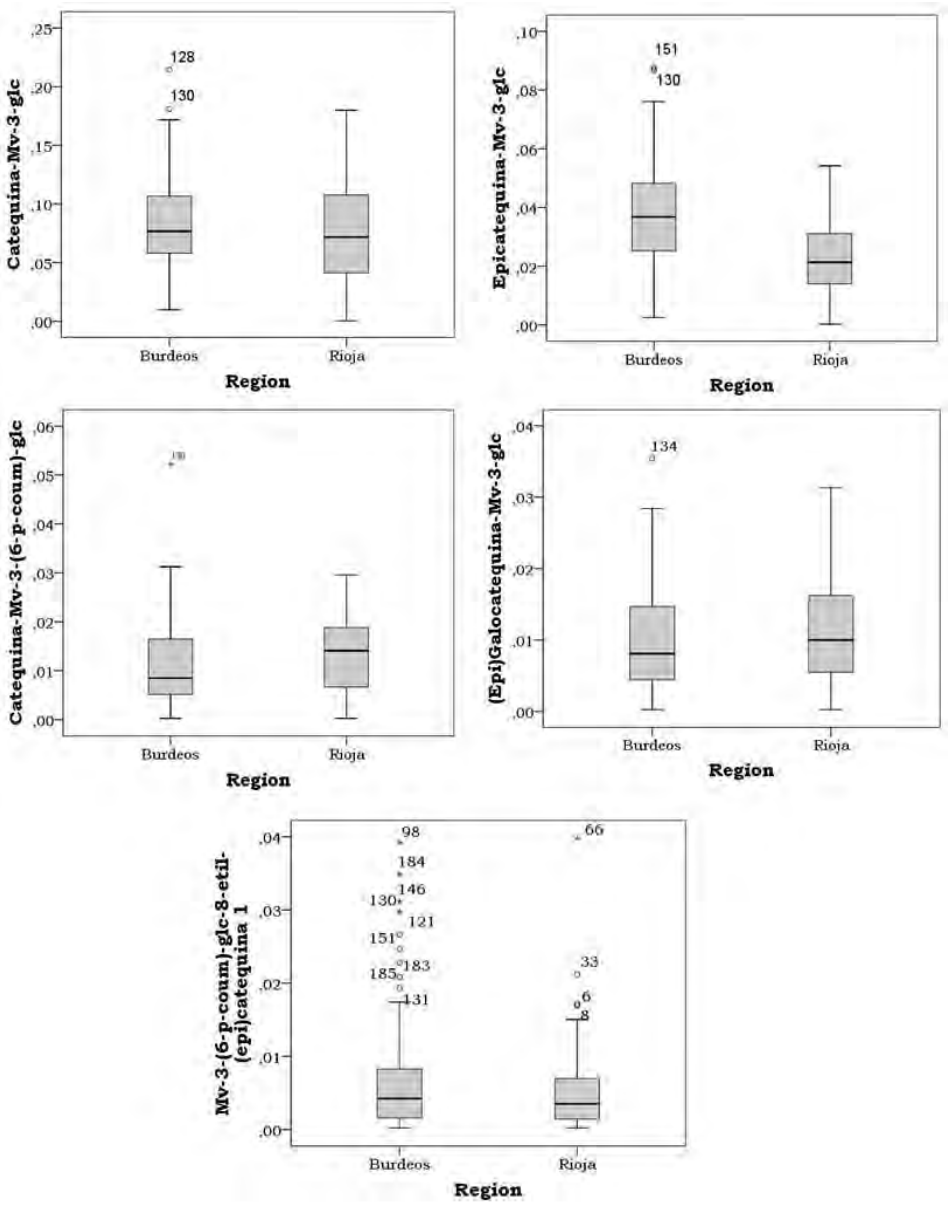



**Figura.VII.4.** Gráficos Box-Whiskers para las variables **derivados antocianina-tanino** en muestras de Rioja y Burdeos.

**Figura.VII.5.**

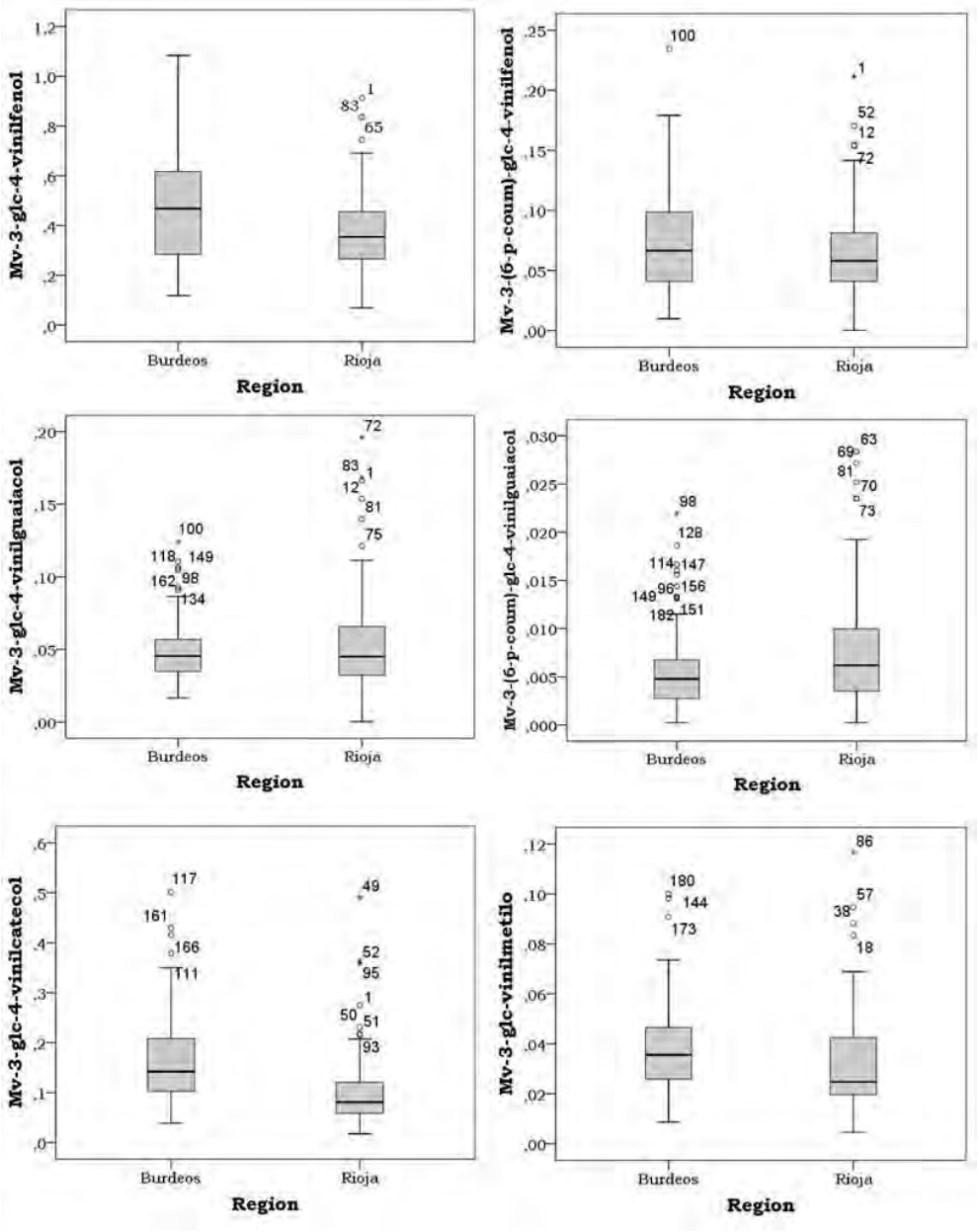



**Figura.VII.6.** Gráficos Box-Whiskers para las variables **derivados antociánicos con ácido hidroxicinámico** y á**cido acetoacético** en muestras de Rioja y Burdeos.

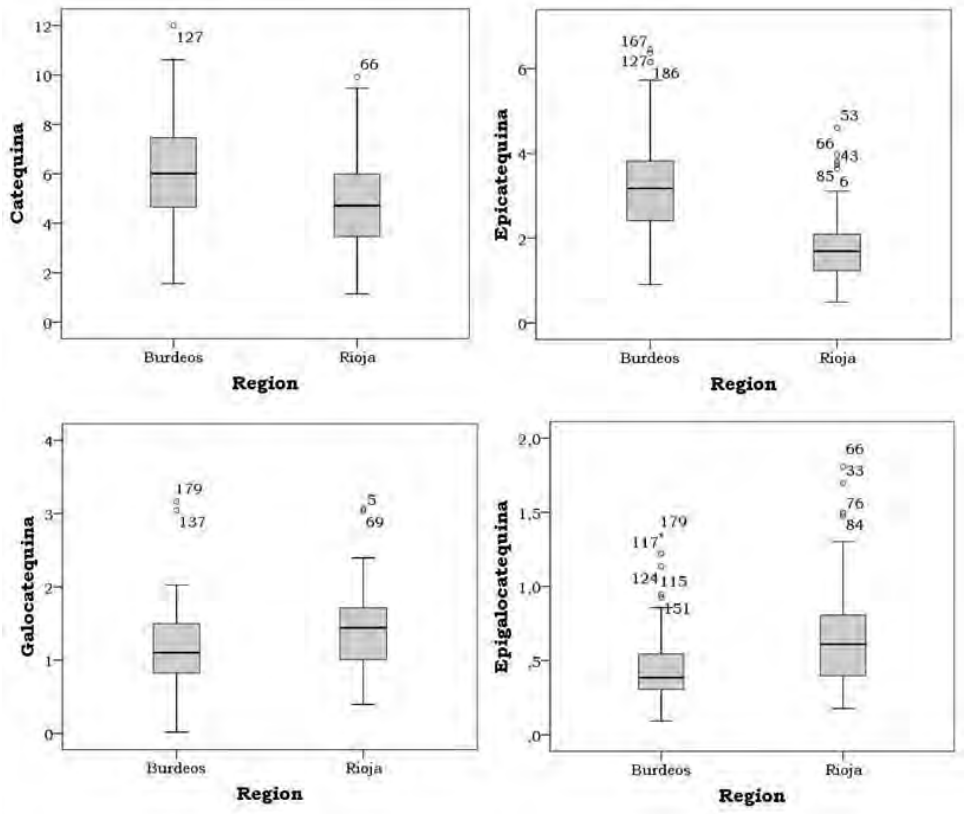



**Figura.VII.7.** Gráficos Box-Whiskers para las variables **taninos monoméricos** en muestras de Rioja y Burdeos.

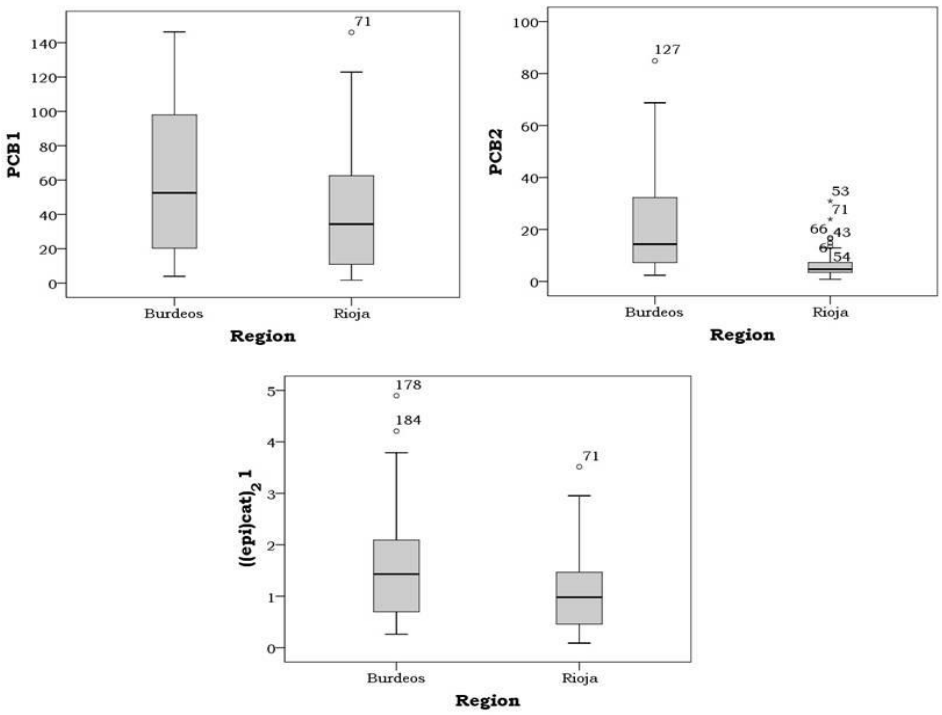



**Figura.VII.8.** Gráficos Box-Whiskers para las variables **procianidinas homodímeras B** en muestras de Rioja y Burdeos.

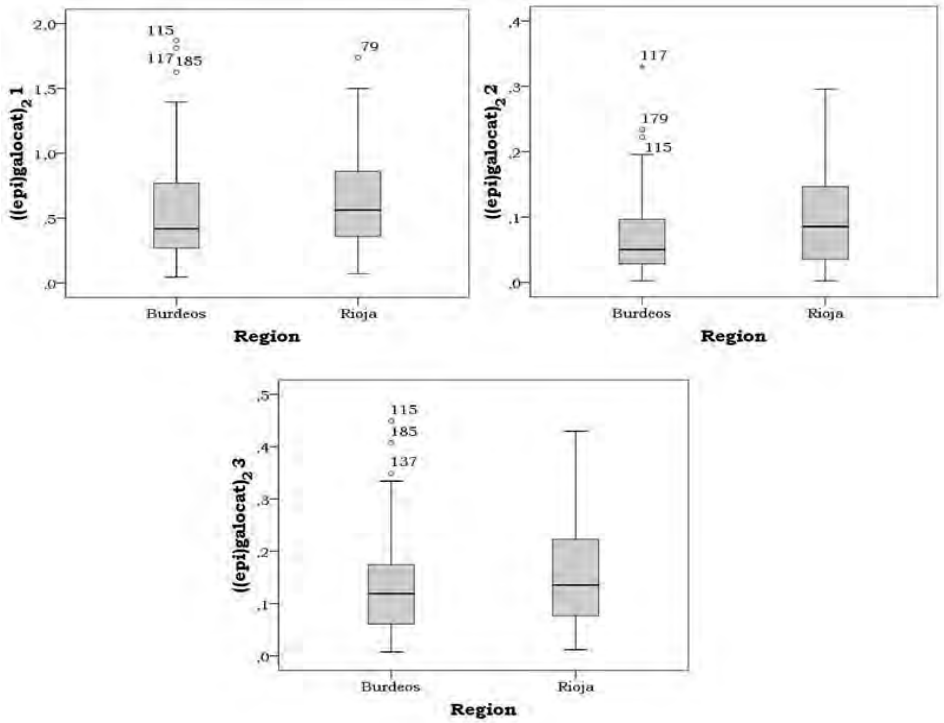



**Figura.VII.9.** Gráficos Box-Whiskers para las variables **prodelfinidinas homodímeras B** en muestras de Rioja y Burdeos.

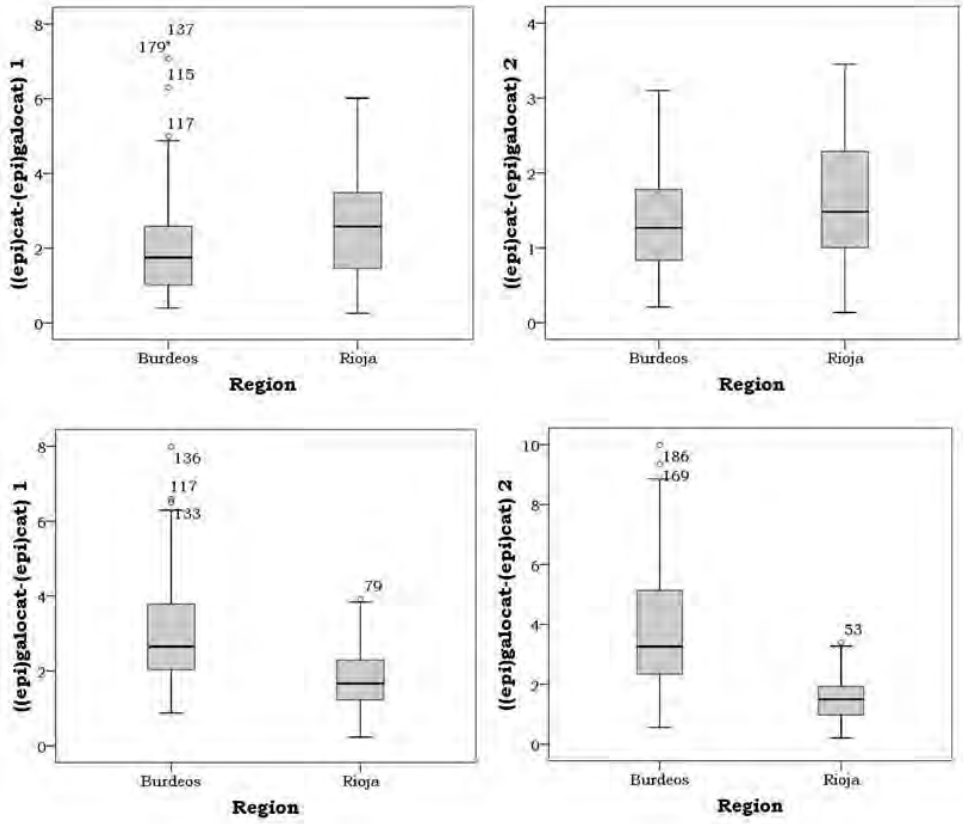



**Figura.VII.10.** Gráficos Box-Whiskers para las variables **procianidinas** y **prodelfinidinas heterodímeras B** en muestras de Rioja y Burdeos.

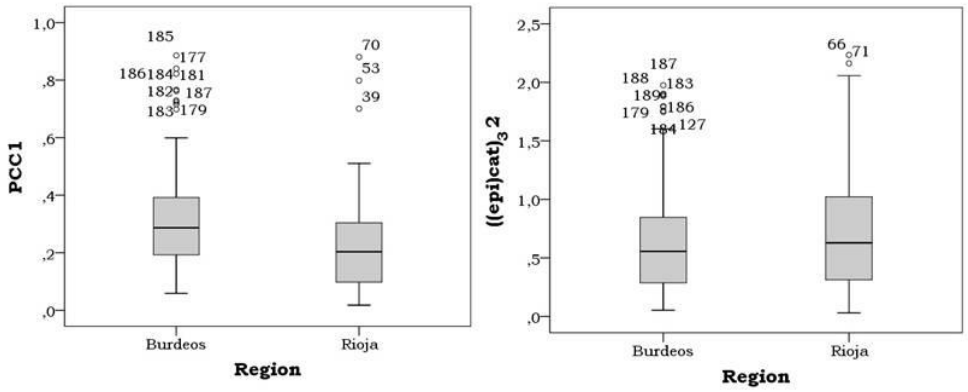



**Figura.VII.11.** Gráficos Box-Whiskers para las variables **procianidinas heterodímeras B** en muestras de Rioja y Burdeos.

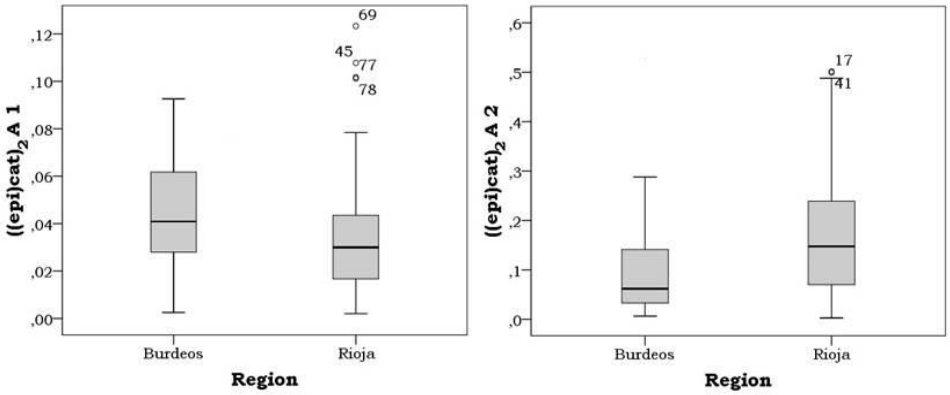



**Figura.VII.12.** Gráficos Box-Whiskers para las variables **procianidinas homodímeras A** en muestras de Rioja y Burdeos.

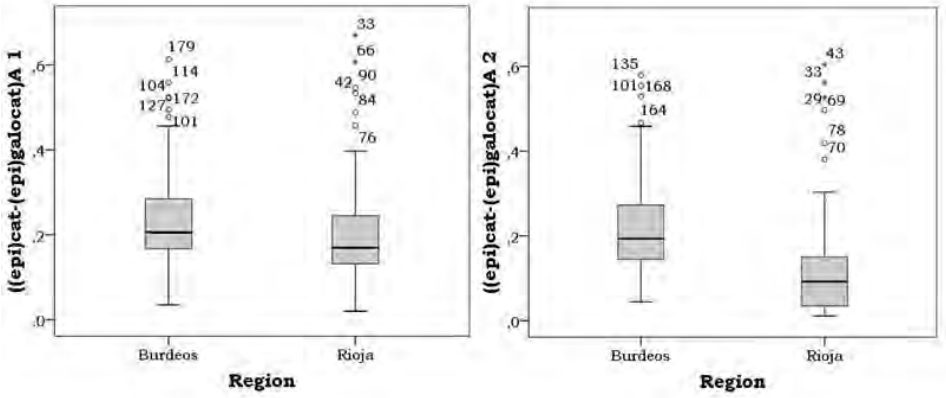



**Figura.VII.13.** Gráficos Box-Whiskers para las variables **procianidinas heterodímeras A** en muestras de Rioja y Burdeos.

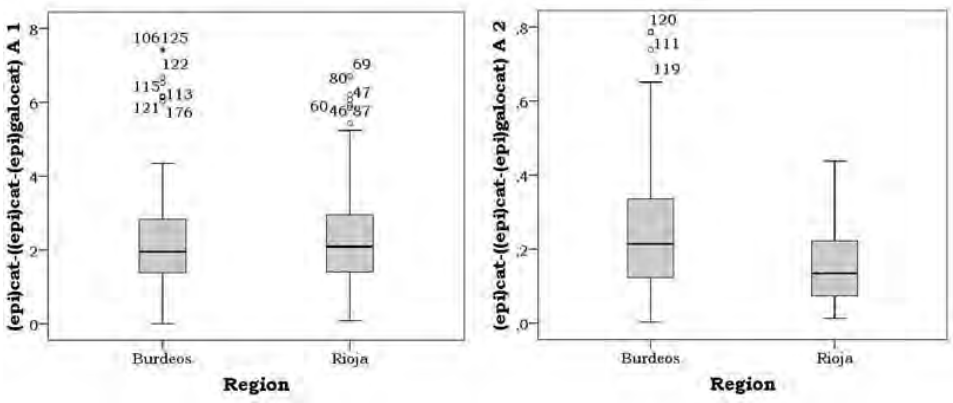



**Figura.VII.14.** Gráficos Box-Whiskers para las variables **trímeros mixtos con enlace A** en muestras de Rioja y Burdeos.

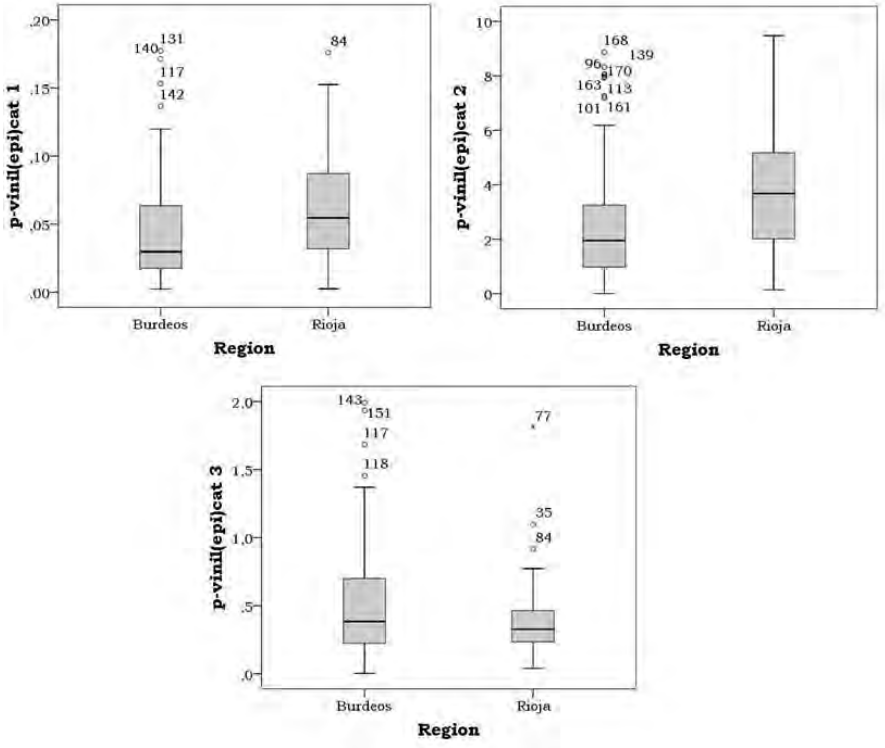



**Figura.VII.15.** Gráficos Box-Whiskers para las variables **taninos p-vinílicos** en muestras de Rioja y Burdeos.

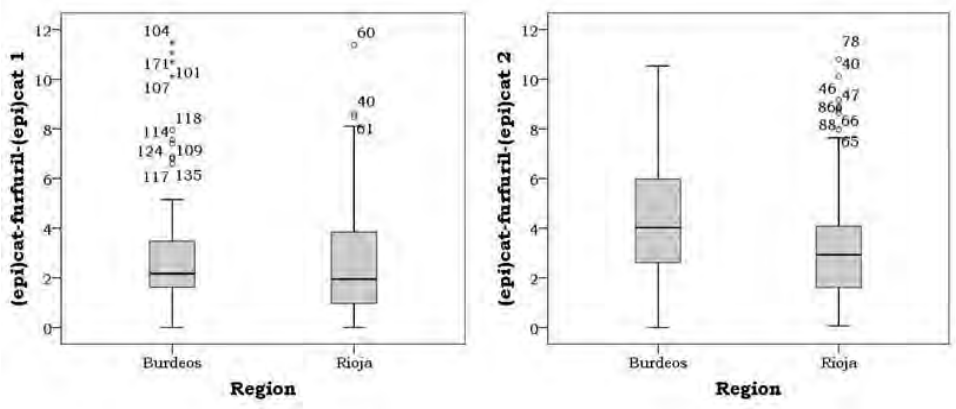



**Figura.VII.16.** Gráficos Box-Whiskers para las variables **taninos con enlace de furfuril** en muestras de Rioja y Burdeos.

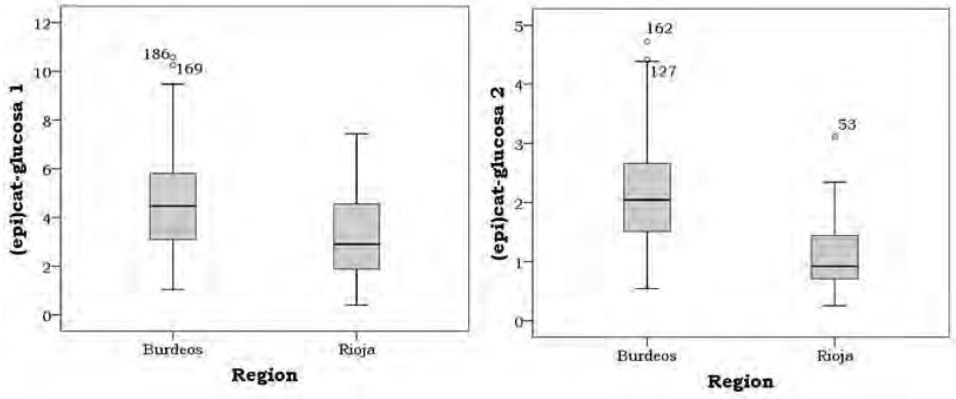



**Figura.VII.17.** Gráficos Box-Whiskers para las variables **taninos O-glicosilados** en muestras de Rioja y Burdeos.

## VII.2. TRATAMIENTO MULTIVARIANTE MEDIANTE ANÁLISIS DE COMPONENTES PRINCIPALES

Para la diferenciación de los vinos de Rioja y Burdeos en base a los polifenoles analizados es necesario recurrir al análisis estadístico multivariante.

Para llevar a cabo el análisis multivariante, la información química sobre las diferentes muestras se recoge en una matriz. Esta matriz contiene información sobre las muestras, pero también contiene información redundante y ruido. Cuando la matriz representa diferentes vinos, ésta puede contener información para diferenciar entre vinos elaborados a partir de variedades de uva diferentes e incluso diferenciar la región donde la uva ha sido cultivada o vinos con diferente tiempo de crianza.

El objetivo de este estudio es, por tanto, diferenciar las muestras de vinos pertenecientes a diferentes regiones de Rioja y Burdeos y con diferentes tiempos de crianza.

### VII.2.1. Análisis de Componentes Principales según la región: Rioja y Burdeos

La técnica de Análisis de Componentes Principales (PCA) se aplicó a la matriz completa de datos con el fin de reducir la dimensionalidad de dicha matriz con la pérdida de la mínima cantidad de información posible. Además, el autoescalado de los datos hace que el tratamiento de PCA considere a todas las variables por igual y no tenga en cuenta la diferente magnitud de sus valores.

El número de Componentes Principales (PCs) significantes indica el número de componentes fundamentales que explican la máxima variabilidad en la matriz de datos. Existen diferentes criterios para determinar el número de PCs significantes. Uno de ellos consiste en construir el gráfico de sedimentación mostrando el autovalor de cada PC (Figura VII.17). Sin embargo, el más utilizado consiste en considerar los PCs con autovalor mayor que 1; los cuales explican más varianza que el promedio.

En la matriz de datos, de los 51 posibles PCs, 11 tienen autovalores mayores que 1 acumulando el 82% de la varianza total del sistema. Esto supone que al reducir el número de variables de 51 a 11, se explica el 82% de la variabilidad total de la matriz de datos original (Tabla VII.4).

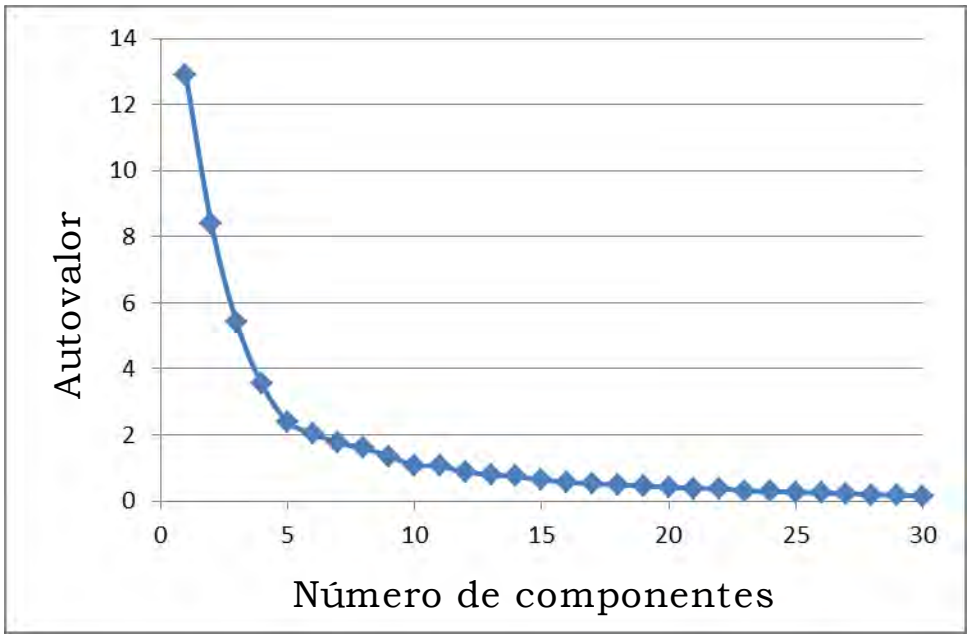



**Figura.VII.18.** Gráfico de sedimentación.

**Tabla VII.4.** Autovalores, varianza explicada y acumulada para los PCs con autovalor > 1.

| Componentes | Autovalor | %Varianza explicada | %Varianza acumulada |
|---|---|---|---|
| 1 | 12.9 | 25.3 | 25.3 |
| 2 | 8.4 | 16.5 | 41.8 |
| 3 | 5.4 | 10.6 | 52.4 |
| 4 | 3.5 | 6.9 | 59.3 |
| 5 | 2.4 | 4.7 | 64.1 |
| 6 | 2.0 | 4.0 | 68.1 |
| 7 | 1.8 | 3.5 | 71.5 |
| 8 | 1.6 | 3.1 | 74.7 |
| 9 | 1.4 | 2.7 | 77.3 |
| 10 | 1.1 | 2.1 | 79.4 |
| 11 | 1.1 | 2.1 | 81.5 |

La Tabla VII.5 recoge los "loadings" (coeficientes de las combinaciones lineales sobre las variables originales que definen las variables nuevas) para poder calcular a partir de ellos los "scores", las nuevas coordenadas de los objetos (cada una de las 189 muestras) en el espacio definido por los componentes principales. Estos loadings nos indican la contribución de cada variable original a cada uno de los PCs (nuevas variables). Así, las variables que contribuyen mayoritariamente al primer componente principal (PC1), el cual explica el 25.3% de la varianza total del sistema, son los taninos catequina, galocatequina, PCB1, $((epi)cat)_2$ 1, $((epi)galocat)_2$ 1, $((epi)galocat)_2$ 3,((epi)cat-(epi)galocat) 1, ((epi)galocat-(epi)cat) 1, PCC1, $((epi)cat)_3$ 2, ((epi)cat-(epi)galocat)A 1 y (epi)cat-glucosa 1. El segundo componente principal (PC2), que acumula un 16.5% del total de la varianza, está correlacionado positivamente con las antocianinas libres Delfinidina-3-O-glucósido, Petunidina-3-O-glucósido, Peonidina-3-O-glucósido, Malvidina-3-O-glucósido, Malvidin-3-O-(6-acetil)-glucósido y Malvidin-3-O-(6-p-coumaroil)-glucósido y los derivados antociánicos Malvidin-3-(6-p-coumaroil)-glc-4-vinilguaicol, (Epi)galocatequina-Mv-3-glucósido y Catequina-Malvidin-3-(6p-coum)-glucósido. En el tercer componente principal (PC3: 10.6% de la varianza total) las variables dominantes son la antocianina libre Delfinidina-3-O-(6-acetil)-glucósido y los derivados antociánicos Malvidin-3-glc-pirúvico, Malvidin-3-(6-p-coum)-glc-pirúvico, Malvidin-3-glc-vinilmetil, Malvidin-3-glc-4-vinilfenol, Malvidin-3-(6-p-coum)-glc-4-vinilfenol y Epicatequin-Malvidin-3-glucósido.

**Tabla VII.5.** Loadings o coeficientes de las combinaciones lineales sobre las variables originales que definen las variables nuevas.

| Variable | PC1 | PC2 | PC3 |
|---|---|---|---|
| **Dp-3-glc** | 0.127 | **0.264** | -0.098 |
| **Pt-3-glc** | 0.122 | **0.272** | -0.102 |
| **Pn-3-glc** | 0.134 | **0.236** | -0.072 |
| **Mv-3-glc** | 0.116 | **0.277** | -0.082 |
| **Dp-3-(6-Ac)-glc** | 0.010 | 0.030 | **0.214** |
| **Mv-3-(6-Ac)-glc** | 0.136 | **0.203** | 0.115 |
| **Mv-3-(6-p-coum)-glc** | 0.104 | **0.282** | -0.086 |
| **Cat-Mv-3-glc** | 0.030 | 0.154 | 0.188 |
| **Cat-Mv-3-(6-p-coum)-glc** | 0.076 | **0.229** | 0.127 |
| **Epicat-Mv-3-glc** | 0.054 | 0.111 | **0.322** |
| **(Epi)Galocat-Mv-3-glc** | 0.039 | **0.225** | 0.086 |
| **Mv-3-(6-p-coum)-glc-8-etil-(epi)cat 1** | 0.085 | 0.161 | 0.177 |
| **Mv-3-glc-pirúvico** | -0.016 | 0.015 | **0.322** |
| **Mv-3-(6-p-coum)-glc-pirúvico** | -0.047 | -0.043 | **0.284** |
| **Mv-3-glc-acetaldehido** | 0.025 | 0.177 | 0.055 |
| **Mv-3-(6-p-coum)-glc-acetaldehido** | 0.014 | 0.174 | 0.069 |
| **Mv-3-glc-vinilmetil** | -0.057 | 0.046 | **0.203** |
| **Mv-3-glc-4-vinilfenol** | -0.012 | 0.073 | **0.227** |
| **Mv-3-(6-p-coum)-glc-4-vinilfenol** | -0.009 | 0.107 | **0.254** |
| **Mv-3-glc-4-vinilcatecol** | -0.054 | -0.096 | 0.160 |
| **Mv-3-glc-4-vinilguaiacol** | -0.044 | 0.128 | 0.160 |
| **Mv-3-(6-p-coum)-glc-4-vinilguaicol** | -0.004 | **0.216** | 0.104 |
| **Catequina** | **0.210** | -0.089 | 0.053 |
| **Epicatequina** | 0.178 | -0.122 | 0.186 |
| **Galocatequina** | **0.219** | 0.027 | -0.114 |
| **Epigalocatequina** | 0.168 | 0.083 | -0.124 |
| **PCB1** | **0.232** | -0.031 | 0.063 |
| **PCB2** | 0.179 | -0.112 | 0.155 |
| **((epi)cat)$_2$ 1** | **0.245** | -0.086 | 0.056 |
| **((epi)galocat)$_2$ 1** | **0.198** | 0.028 | -0.075 |
| **((epi)galocat)$_2$ 2** | 0.151 | 0.018 | -0.148 |
| **((epi)galocat)$_2$ 3** | **0.231** | 0.028 | -0.093 |

**Tabla VII.5.** Continuación.

| Variable | PC1 | PC2 | PC3 |
|---|---|---|---|
| **((epi)cat-(epi)galocat) 1** | **0.219** | 0.013 | -0.122 |
| **((epi)cat-(epi)galocat) 2** | 0.174 | -0.045 | -0.068 |
| **((epi)galocat)-(epi)cat) 1** | **0.203** | -0.115 | 0.100 |
| **((epi)galocat)-(epi)cat) 2** | 0.173 | -0.155 | 0.157 |
| **PCC1** | **0.235** | -0.043 | 0.045 |
| **$((epi)cat)_3$ 2** | **0.241** | 0.021 | -0.043 |
| **$((epi)cat)_2$A 1** | 0.130 | -0.037 | -0.023 |
| **$((epi)cat)_2$A 2** | 0.041 | 0.146 | -0.089 |
| **((epi)cat-(epi)galocat)A 1** | **0.209** | -0.045 | -0.004 |
| **((epi)cat-(epi)galocat)A 2** | 0.112 | -0.034 | 0.041 |
| **(epi)cat-((epi)cat-(epi)galocat)A 1** | 0.107 | -0.155 | -0.093 |
| **(epi)cat-((epi)cat-(epi)galocat)A 2** | 0.112 | -0.187 | 0.004 |
| **p-vinil(epi)cat 1** | 0.030 | -0.024 | -0.101 |
| **p-vinil(epi)cat 2** | 0.060 | 0.101 | -0.121 |
| **p-vinil(epi)cat 3** | 0.022 | -0.004 | 0.120 |
| **(epi)cat-furfuril-(epi)cat 1** | 0.127 | -0.165 | -0.039 |
| **(epi)cat-furfuril-(epi)cat 2** | 0.124 | -0.190 | 0.005 |
| **(epi)cat-glucosa 1** | **0.203** | -0.067 | 0.119 |
| **(epi)cat-glucosa 2** | 0.160 | -0.153 | 0.175 |

Si se representan los scores de las muestras en el espacio definido por el primer y tercer componente principal y por el PC2 y PC3 (Figuras VII.18 y VII.19), se observan dos agrupamientos.

En el primer gráfico (Figura IX.18) los grupos no se encuentran tan separados como en el segundo (Figura IX.19). En este último se ven los dos grupos algo más definidos, aunque no exista una separación completa entre ambos. Se puede observar que mientras que los vinos de Burdeos tienden a posicionarse hacia el cuadrante superior izquierdo (es decir, valores negativos del componente principal, PC2, y positivos del PC3), los vinos de Rioja tienden a situarse hacia el cuadrante inferior derecho (es decir, valores positivos del PC2 y negativos del PC3).

Las variables con mayor peso en el PC2 son las antocianinas no aciladas y coumaroiladas y 3 derivados antociánicos (dos compuestos tanino-antocianina, Catequina-Malvidina-3-(6-p-coum)-glc y (Epi)galocat-Malvidina-3-glc, y una piranoantocianina, la Malvidina-3-(6-p-coum)glc-4-vinilguaicol).

Como ya se vio en el capítulo 6, los vinos de Rioja presentan mayores concentraciones de dichos compuestos, lo que explica que estos vinos estén localizados en la parte derecha del PC2, mientras que los vinos de Burdeos se sitúan en valores negativos del PC2.

Las variables de mayor influencia en el PC3 son la delfinidina-3-(6-Ac)-glc, las vitisinas A, un derivado antociánico con ácido acetoacético (Mv-3-glc-vinilmetil) y dos derivados antociánicos con ácidos hidroxicinámicos (Mv-3-glc-4-vinilfenol, Mv-3-(6-p-coum)-glc-4-vinilfenol). En el capítulo 6 se vio que los vinos de Burdeos presentan mayores concentraciones de estos compuestos que los vinos de Rioja. Por ello los vinos de Burdeos se sitúan en la parte superior del PC3, es decir presentan valores positivos en el PC3, mientras que los vinos de Rioja se sitúan en la parte negativa del PC3.

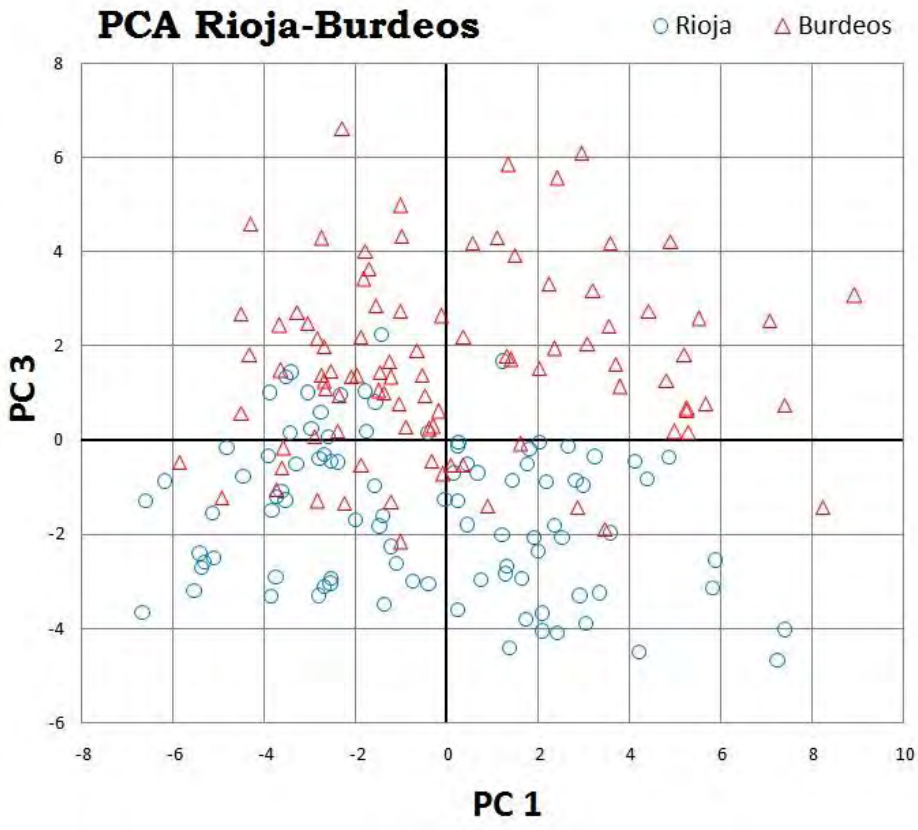



**Figura.VII.19.** Representación de las muestras de vinos tintos en el espacio definido por el primer y tercer componente principal.

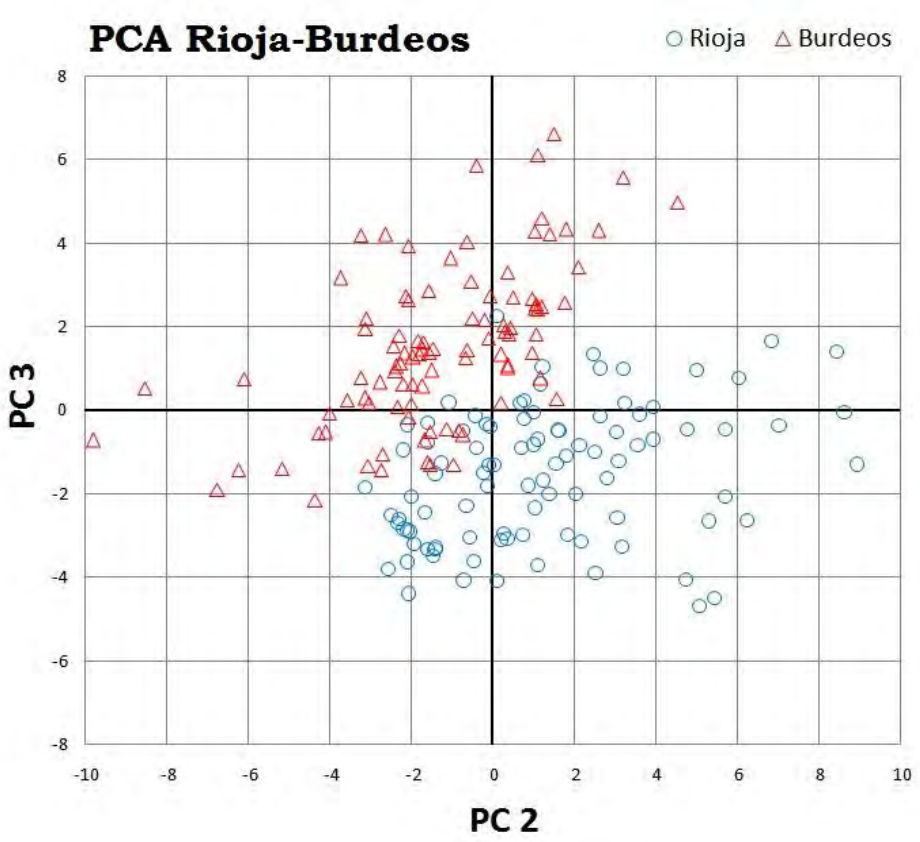



**Figura.VII.20.** Representación de las muestras de vinos tintos en el espacio definido por el PC2 y PC3.

### VII.2.2. Análisis de Componentes Principales según la subzona de Rioja: Rioja Alavesa, Rioja Alta y Rioja Baja

En este subapartado se estudia la distribución de las tres regiones diferentes de Rioja, Rioja Alavesa, Rioja Alta y Rioja Baja.

Si se representan los scores de las muestras en el espacio definido por el segundo y tercer componente principal (Figuras VII.20) en función de las tres subzonas diferentes de Rioja, se puede ver como no existe separación física entre las mismas. Las subzonas de Rioja Alavesa y Rioja Baja se superponen, mientras que la Rioja Alta parece que tiene mayor tendencia a posicionarse hacia la izquierda del PC2. Las variables con mayor peso en dicho PC son las antocianinas no aciladas y coumaroiladas y como ya se vio en el capítulo 6 (Figura VI.3 y VI.5) los valores de dichos compuestos son menores en la Rioja Alta, mientras que para la Rioja Alavesa y la Rioja Baja los valores son mayores y muy similares. Por esta razón la región de la Rioja Alta se sitúa más hacia la izquierda del gráfico.

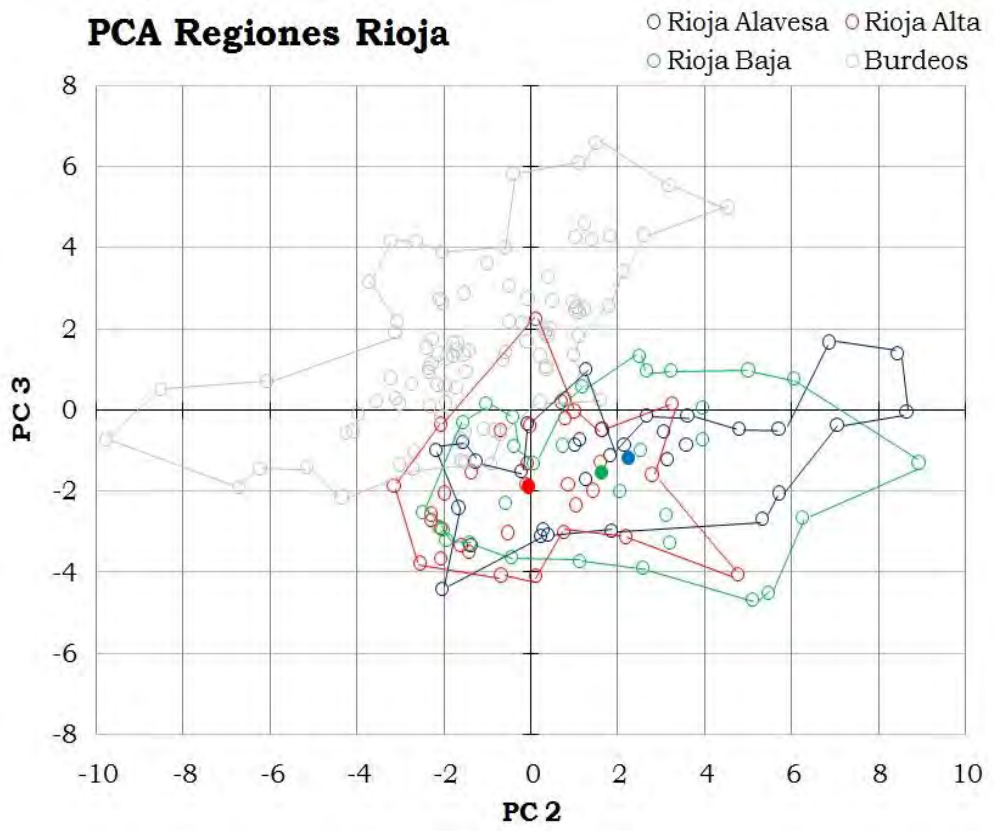



**Figura.VII.21.** Representación de tres subzonas de Rioja en el espacio definido por el PC2 y PC3.

### VII.2.3. Análisis de Componentes Principales según el estilo de vino de Rioja: Joven, *Crianza*, *Reserva* y *Gran Reserva*

Como en el caso anterior, en el que se observó la distribución de las muestras en función de las regiones de Rioja, se estudia la posición de los diferentes tipos de vino de Rioja: Joven, *Crianza*, *Reserva* y *Gran Reserva* (Figura VII.21).

Al representar los scores de las muestras en el espacio definido por el PC2 y el PC3, se puede observar que, al igual que ocurre en el caso anterior, parece que la tendencia de la separación tiene lugar a lo largo del PC2, es decir que las posibles diferencias se encuentran en las concentraciones de las antocianinas no aciladas y coumaroiladas y de tres derivados antociánicos (dos compuestos tanino-antocianina, Catequina-Malvidina-3-(6-p-coum)-glc y (Epi)galocat-Malvidina-3-glc, y una piranoantocianina, la Malvidina-3-(6-p-coum)glc-4-vinilguaicol).

En la Figura VII.21 se ha representado tanto el conjunto de muestras pertenecientes a cada tipo de vino, uniendo con una línea las muestras que se

encuentran en el contorno exterior de cada grupo, así como el punto centroide de cada grupo. Como se puede ver en dicha figura tanto el conjunto de muestras de cada grupo como su punto centroide avanzan hacia la parte negativa del PC2 a medida que vamos del grupo con menos crianza (Joven) al grupo con mayor tiempo de crianza (*Gran Reserva*). Esto implica que las antocianinas no aciladas y coumaroiladas y los tres derivados antocianicos mencionados anteriormente (Catequina-Malvidina-3-(6-p-coum)-glc, (Epi)galocat-Malvidina-3-glc y Malvidina-3-(6-p-coum)glc-4-vinilguaicol) disminuyen su concentración a medida que aumenta el tiempo de envejecimiento en barrica y en botella. Este hecho ya se pudo ver en los gráficos del capítulo 6 (Figura VI.4, VI.6, VI.10 y VI.18).

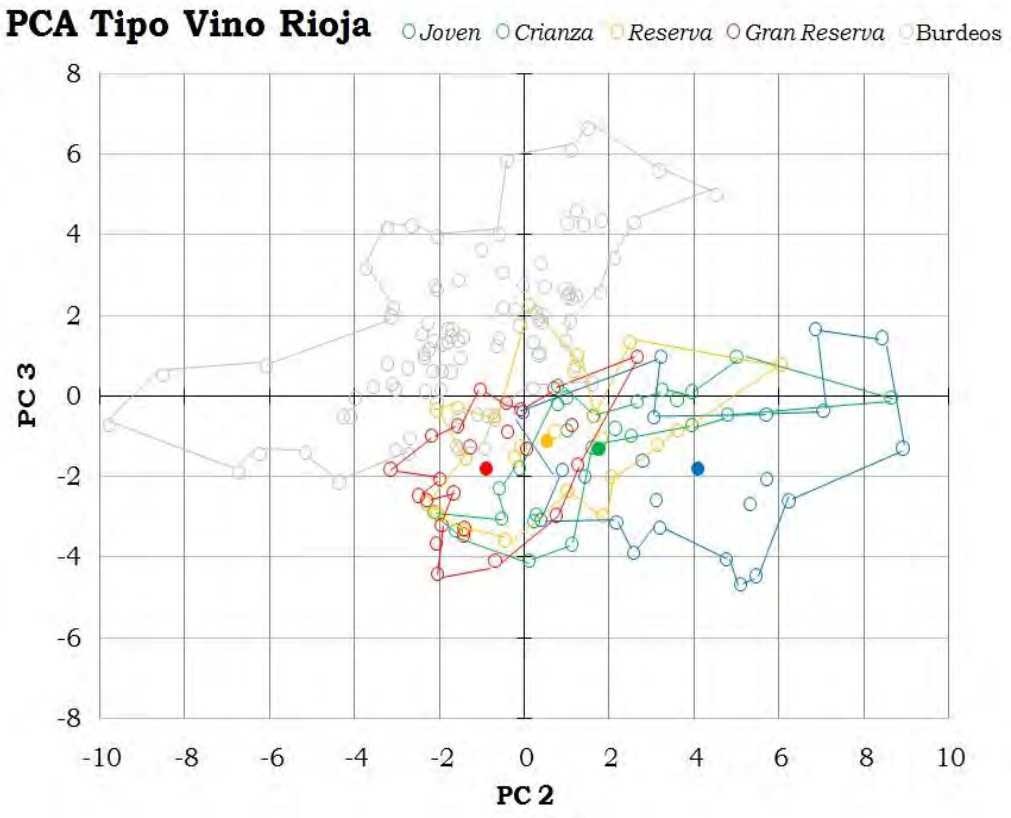



**Figura.VII.22.** Representación de los tipos de vino de Rioja en el espacio definido por el PC2 y PC3. Centroide de cada grupo representado por un punto relleno del color correspondiente a cada grupo.

### VII.2.4. Análisis de Componentes Principales según la puntuación en la guía Peñín

Existe en nuestro país una guía en la que se evalúa organolépticamente los distintos vinos de cada región vitivinícola de España y que recibe el nombre de *Guia*

*Peñín*. Dicha evaluación organoléptica, dada en una escala con máximo en 10, es realizada por un panel de catadores expertos.

En la Figura VII.22 se representan los puntos centroides de cada grupo de vinos de Rioja, agrupandolos según su puntuación en la *Guía Peñín* en función de 5 intervalos diferentes de calificaciones (8.2 a <8.4, 8.4 a <8.6, 8.6 a <8.8, 8.8 a <9.0 y 9.0 a 9.2).

Como se puede observar en el gráfico los puntos centroides para los diferentes intervalos de puntuación tienen tendencia a avanzar hacia el lado negativo del PC3 y positivo del PC2 a medida que crece la puntuación de la *Guía Peñín*. Si lo comparamos con la tendencia que se ha encontrado en el apartado anterior con los diferentes tipos de vino de Rioja, se ve que hay una relación entre la calificación y el tipo de vino. Se puede apreciar que el valor de la calificación del vino aumenta al aumentar el tiempo de envejecimiento, es decir, que el envejecimiento en barrica y botella parece mejorar la valoración organoléptica de los vinos por el panel de catadores expertos.

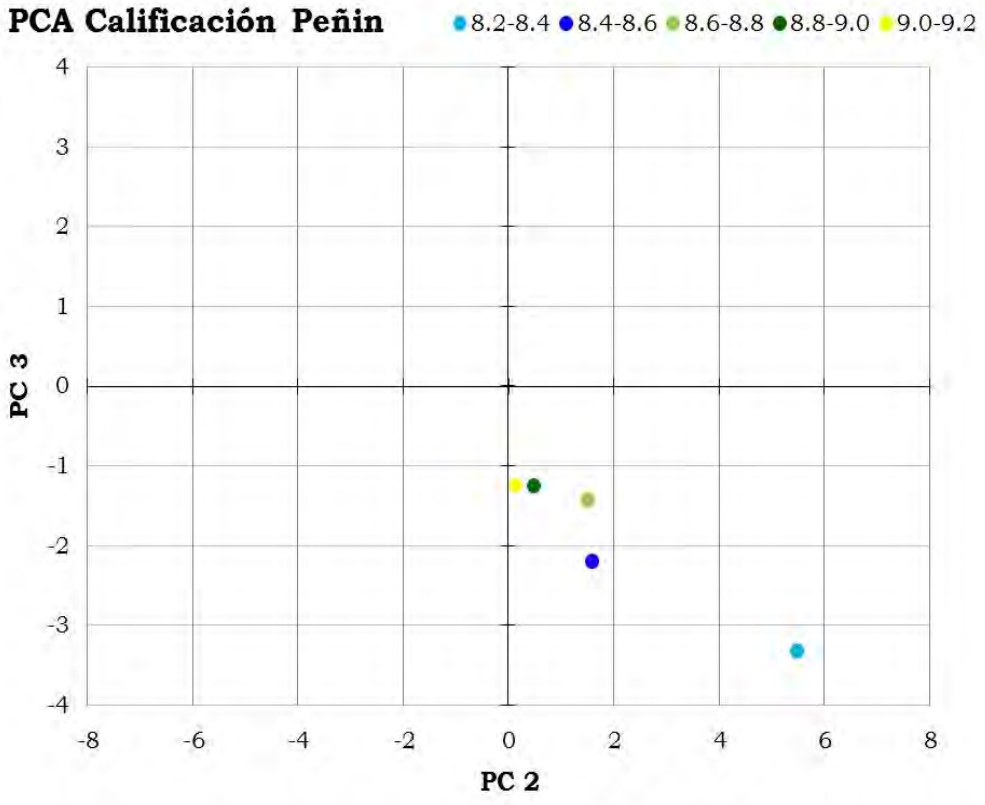



**Figura.VII.23.** Representación de las calificaciones de la guía Peñin en el espacio definido por el PC2 y PC3.

# CHAPTER VIII

## CONCLUSIONS

# Chapter VIII

## CONCLUSIONS

The main conclusions that have been reached, following the development of this work, are reflected below:

IDENTIFICATION OF NEW ANTHOCYANIN DERIVATIVES

1) 18 anthocyanin derivatives, which are formed by direct condensation of anthocyanin with two units of flavanols, have been identified during this work.
2) Cone voltages and collision energies of 14 compounds formed by direct condensation of anthocyanin with flavanols, some of them coloured and some non coloured, and 8 derivatives with vinylflavanols have also been optimized.
3) Although some derivatives are not colored they have an indirect influence on the color, because their formation supposes a reduction of the contents of the anthocyanins from which they come, and therefore of the color of the wine.

EVOLUTION OF ANTHOCYANIN DERIVATIVES AND TANNINS DURING DIFFERENTS STAGES OF WINEMAKING

4) Extraction of anthocyanins from grape occurs mainly in the first 4 or 5 days of maceration-alcoholic fermentation, with high increases of their contents in must. After that a progressive and continuous decreasing of anthocyanin levels along fermentations and ageing is produced.

5) Formation of anthocyanin derivatives starts quickly and in parallel as anthocyanins are being extracted from grape. Thus, maximum concentrations of anthocyanin derivatives are reached just a few days (2-3 days) after highest levels of anthocyanins have been produced.
6) Catechin-Mv-3-glc is the major anthocyanin derivative formed by direct condensation between anthocyanins and flavanols. Direct condensation derivatives represent about 10% of total anthocyanin derivatives at the end of fermentations. Condensation derivatives by ethylidene bridge are minor derivatives.
7) Within pyranoanthocyanins, vitisin A and B are the major ones, being each one around 40% of total anthocyanin derivatives, at the end of fermentations. The remaining 10% of total anthocyanin derivatives are pyranoanthocyanins with vinylphenols.
8) Vitisin B has a more unstable behavior than vitisin A along fermentations, with a quicker and stronger formation along AF but also a more intense degradation. So, although levels of vitisin B are higher than those of vitisin A all along fermentations, one or two weeks after the end of MLF levels of both are similar.
9) The more unstable behavior of ethylidene-bridged anthocyanin derivatives against direct condensation products observed along fermentations is confirmed along the wine ageing in both barrel and steel vat periods.
10) A stability order of the different classes of pyranoanthocyanins along barrel ageing has been stated, being more stable the derivatives with pyruvic (vitisins A) and acetoacetic acids than those with vinylphenols and acetaldehyde (vitisins B).
11) Extraction of tannins from grape occurs slower than that of anthocyanins and their contents in must increases all along maceration-alcoholic fermentation.
12) Major tannins are procyanidin dimers with B bond (45% of total tannins after fermentations), followed by monomers (near 25%) and mixed dimers with B bond (more than 10%).
13) Within dimers with B bond, mixed ones have higher levels than those of prodelphinidins. The same happens for dimers with A bond.
14) Higher levels of prodelphinidin and mixed dimers with A bond against those of procyanidin dimers with A bond suggests a favoured formation of additional A bond when an (epi)gallocatechin unit is implied.

15) Contrary to ethylydene bridged tannins, vinyl-tannins presented very high levels, suggesting that depolymerisation of the first to produce the last is favoured.
16) Tannins suffer a general continuous decreasing all along ageing. But some major contribution of mixed trimers with A bond can be observed along ageing process, that is also produced for mixed dimers with B bond. Both facts seem to point out an increasing relevance of more oxygenated tannins as ageing advance.

ANTHOCYANINS AND TANNINS IN RIOJA AND BORDEAUX WINES

17) Some significant differences between red wines from Rioja and those from Bordeaux have been found, as consequence of the different grape varieties used in each region. However, major differences are due to the different wine styles within each region, mainly related with extension of time ageing.
18) Thus, total free anthocyanin content is more related with ageing time of wine than production region, but some differences within each kind of free anthocyanins can be observed.
19) Bordeaux wines have higher levels of acetylated anthocyanins, while Rioja wines present higher levels of non-acylated and coumaroylated anthocyanins as a consequence of the different grape varieties used, mainly *Tempranillo* in Rioja and *Cabernet Sauvignon* and *Merlot* in Bordeaux.
20) Bordeaux specific wines seem to preserve higher levels of tannin-anthocyanin condensation products than *Reserva* and *Gran Reserva* Rioja wines, but no data on precise time ageing for Bordeaux wines was available to compare.
21) Concerning pyranoanthocyanins, Bordeaux wines have higher levels of vitisins A and derivatives with vinylphenols, while Rioja wines present higher levels of vitisins B.
22) Bordeaux wines present higher levels of b* parameter, proportion of yellow, than Rioja wines, so in spite of their lower levels of Vitisins B, the higher levels of Vitisins A and pyranoanthocyanin derivatives with vinylphenols overcome that difference. For both this colour parameter increases as time ageing increases, in accordance with higher levels of the different pyranoanthocyanin classes in wines with more ageing.
23) In general, Bordeaux wines have higher levels of tannins than Rioja wines. In relation to time ageing, a general decrease of all tannin classes is

observed for all wines along ageing, with the exception of mixed trimers with A and B bond which slightly increase and tannins with furfuryl bridge which show high increases along ageing, as a consequence of wood origin of furfurals.

24) Catechin is the major flavan-3-ol monomer in wines of both regions. However, the major tannins class in red wines of both regions are homodimers B, with procyanidin B1 as the major one in both cases.
25) Bordeaux wines present lower ratios of PCB1/PCB2 and ((epi)cat)$_3$ 2/PCC1, both facts could indicate longer maceration times.
26) No significant differences were found between wines of both regions in some tannin classes, such as prodelphinidin homodimers B, procyanidin heterodimers B, procyanidin homotrimers B and mixed trimers with A and B bond.
27) However, Rioja wines showed lower levels of monomers, procyanidins homodimers B, prodelphinidin heterodimers B, procyanidin heterodimers A, tannins with furfuryl bridge and O-glycosylated flavan-3-ols; but slightly higher levels of procyanidin homodimers A, and higher concentrations of p-vinyl tannins
28) Within Rioja wines, levels of free anthocyanins and tannins are lower in young wines which are made by mixing varieties of grapes than those in wines 100% *Tempranillo*. This difference indicates an increased reactivity of both compounds along firsts winemaking steps in mixed wines, which can be associated to the lower pH of mixed young wines found in wines of the present work. As a consequence anthocyanin derivatives should have higher levels in mixed young wines. However, results in this study do not comply this; probably due to the fact that wine samples in this work are final bottled wines, not wine samples along or just at the end of fermentations, and time passed after have also influence.

# CAPÍTULO IX

## BIBLIOGRAFÍA

# BIBLIOGRAFÍA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# ANNEXES

# ANNEX I

Tabla Anexo I.1 Anthocyanin derivatives concentrations of the samples analysed during the fermentation process of the 2012 wine.

| Time (days) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 19 | 20 | 21 | 22 | 25 | 32 | 39 | 47 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mv-3-glc-acetaldehyde | 0.0082 | 0.0135 | 0.0980 | 0.2843 | 2.7168 | 3.2986 | 3.5310 | 4.0675 | 3.9403 | 2.9549 | 1.8181 | 2.4379 | 2.0705 | 2.2918 | 2.7524 | 2.7926 | 2.7403 | 1.9542 | 2.3360 | 1.4659 | 1.7722 | 1.2220 | 0.8734 | 0.9132 | 0.6313 |
| Mv-3-glc-vinylmetyl | <0.001 | <0.001 | 0.0023 | 0.0028 | 0.0098 | 0.0224 | 0.0314 | 0.0326 | 0.0444 | 0.0389 | 0.0539 | 0.0389 | 0.0394 | 0.0408 | 0.0474 | 0.0477 | 0.0467 | 0.0408 | 0.0454 | 0.0349 | 0.0397 | 0.0391 | 0.0425 | 0.0415 | 0.0318 |
| Mv-3-glc-pyruvic | 0.0167 | 0.0357 | 0.0871 | 0.2108 | 0.6166 | 0.7994 | 0.9150 | 1.0310 | 1.1581 | 0.9189 | 1.6523 | 0.9592 | 0.9713 | 1.1500 | 1.3772 | 1.7322 | 1.7988 | 1.0260 | 1.3602 | 0.9475 | 0.9817 | 0.7883 | 0.9095 | 0.9423 | 0.9617 |
| Mv-3-glc-4-vinylphenol | nd | <0.001 | <0.001 | 0.0018 | 0.0882 | 0.1065 | 0.1008 | 0.0880 | 0.0915 | 0.0897 | 0.1236 | 0.1027 | 0.1157 | 0.1140 | 0.1238 | 0.1404 | 0.1480 | 0.1000 | 0.1311 | 0.1047 | 0.1311 | 0.1345 | 0.1299 | 0.1316 | 0.1780 |
| Mv-3-glc-4-vinylcatechol | nd | nd | nd | nd | 0.0025 | 0.0043 | 0.0041 | 0.0040 | 0.0033 | 0.0038 | 0.0050 | 0.0051 | 0.0058 | 0.0065 | 0.0068 | 0.0090 | 0.0090 | 0.0057 | 0.0076 | 0.0049 | 0.0063 | 0.0057 | 0.0065 | 0.0068 | 0.0076 |
| Mv-3-glc-4-vinylguaiacol | <0.001 | <0.001 | 0.0016 | 0.0021 | 0.0402 | 0.0465 | 0.0424 | 0.0394 | 0.0412 | 0.0365 | 0.0435 | 0.0397 | 0.0446 | 0.0506 | 0.0559 | 0.0612 | 0.0653 | 0.0420 | 0.0573 | 0.0410 | 0.0527 | 0.0460 | 0.0478 | 0.0461 | 0.0439 |
| Mv-3-(6-p-coum)-glc-acetaldehyde | <0.001 | 0.0033 | 0.0304 | 0.0870 | 0.7783 | 0.6817 | 0.6687 | 0.4872 | 0.4938 | 0.3483 | 0.2402 | 0.2439 | 0.2348 | 0.2476 | 0.2854 | 0.3151 | 0.3431 | 0.1997 | 0.2990 | 0.1813 | 0.2246 | 0.2053 | 0.1479 | 0.1405 | 0.0794 |
| Mv-3-(6-p-coum)-glc-pyruvic | 0.0036 | 0.0071 | 0.0175 | 0.0393 | 0.1215 | 0.1194 | 0.1330 | 0.1066 | 0.1111 | 0.0930 | 0.1094 | 0.0833 | 0.0885 | 0.0896 | 0.0955 | 0.1085 | 0.1265 | 0.0808 | 0.1060 | 0.0917 | 0.0588 | 0.1331 | 0.1031 | 0.1097 | 0.0907 |
| Mv-3-(6-p-coum)-glc-4-vinylphenol | nd | nd | <0.001 | <0.001 | 0.0418 | 0.0320 | 0.0269 | 0.0223 | 0.0233 | 0.0191 | 0.0259 | 0.0224 | 0.0211 | 0.0226 | 0.0263 | 0.0330 | 0.0361 | 0.0162 | 0.0305 | 0.0175 | 0.0239 | 0.0296 | 0.0255 | 0.0307 | 0.0345 |
| Catechin-Mv-3-glc | 0.0350 | 0.0355 | 0.0474 | 0.0522 | 0.1145 | 0.1310 | 0.1608 | 0.1754 | 0.1621 | 0.1499 | 0.1533 | 0.1563 | 0.1442 | 0.2151 | 0.1990 | 0.2033 | 0.1870 | 0.1928 | 0.1994 | 0.2044 | 0.1745 | 0.1589 | 0.1540 | 0.1387 | 0.1476 |
| Epicatechin-Mv-3-glc | 0.0053 | 0.0072 | 0.0063 | 0.0294 | 0.0382 | 0.0525 | 0.0478 | 0.0496 | 0.0560 | 0.0535 | 0.0404 | 0.0462 | 0.0404 | 0.0463 | 0.0675 | 0.0612 | 0.0568 | 0.0630 | 0.0627 | 0.0613 | 0.0596 | 0.0561 | 0.0511 | 0.0405 | 0.0447 |
| Mv-3-(6-p-coum)-glc-4-vinylguaiacol | nd | nd | <0.001 | <0.001 | 0.0064 | 0.0095 | 0.0081 | 0.0068 | 0.0077 | 0.0051 | 0.0060 | 0.0052 | 0.0044 | 0.0062 | 0.0070 | 0.0086 | 0.0110 | 0.0033 | 0.0082 | 0.0047 | 0.0067 | 0.0074 | 0.0058 | 0.0070 | 0.0054 |
| (Epi)Gallocatechin-Mv-3-glc | 0.0036 | 0.0081 | 0.0066 | 0.0040 | 0.0538 | 0.0598 | 0.0666 | 0.0464 | 0.0463 | 0.0416 | 0.0585 | 0.0384 | 0.0416 | 0.0535 | 0.0621 | 0.0575 | 0.0522 | 0.0513 | 0.0601 | 0.0592 | 0.0667 | 0.0605 | 0.0558 | 0.0492 | 0.0644 |
| Mv-3-glc-8-ethyl-catechin 1 | <0.001 | <0.001 | <0.001 | 0.0012 | 0.0116 | 0.0127 | 0.0194 | 0.0172 | 0.0215 | 0.0167 | 0.0165 | 0.0141 | 0.0142 | 0.0258 | 0.0246 | 0.0202 | 0.0245 | 0.0173 | 0.0279 | 0.0336 | 0.0339 | 0.0381 | 0.0213 | 0.0184 | 0.0177 |
| Mv-3-glc-8-ethyl-catechin 2 | <0.001 | <0.001 | 0.0027 | 0.0028 | 0.0143 | 0.0178 | 0.0246 | 0.0273 | 0.0284 | 0.0214 | 0.0231 | 0.0219 | 0.0214 | 0.0363 | 0.0343 | 0.0330 | 0.0349 | 0.0293 | 0.0347 | 0.0449 | 0.0473 | 0.0460 | 0.0283 | 0.0274 | 0.0240 |
| Mv-3-glc-8-ethyl-epicatechin | <0.001 | <0.001 | 0.0015 | 0.0018 | 0.0175 | 0.0216 | 0.0282 | 0.0246 | 0.0293 | 0.0215 | 0.0319 | 0.0207 | 0.0233 | 0.0376 | 0.0328 | 0.0325 | 0.0326 | 0.0269 | 0.0358 | 0.0472 | 0.0501 | 0.0414 | 0.0236 | 0.0202 | 0.0202 |
| Catechin-Mv-3-(6-p-coum)- | 0.0034 | 0.0055 | 0.0075 | 0.0102 | 0.0275 | 0.0258 | 0.0271 | 0.0200 | 0.0236 | 0.0232 | 0.0202 | 0.0167 | 0.0204 | 0.0236 | 0.0262 | 0.0236 | 0.0226 | 0.0267 | 0.0314 | 0.0269 | 0.0333 | 0.0322 | 0.0295 | 0.0254 | 0.0244 |
| Epicatechin-Mv-3-(6-p- | <0.001 | 0.0012 | 0.0036 | 0.0042 | 0.0045 | 0.0043 | 0.0034 | 0.0029 | 0.0032 | 0.0023 | <0.001 | 0.0012 | <0.001 | 0.0016 | 0.0015 | 0.0014 | 0.0011 | <0.001 | 0.0012 | <0.001 | 0.0014 | 0.0009 | <0.001 | <0.001 | <0.001 |
| (Epi)gallocatechin-Mv-3-(6- | 0.0014 | 0.0042 | 0.0054 | 0.0055 | 0.0231 | 0.0198 | 0.0216 | 0.0100 | 0.0130 | 0.0121 | 0.0127 | 0.0096 | 0.0097 | 0.0130 | 0.0129 | 0.0115 | 0.0108 | 0.0119 | 0.0134 | 0.0116 | 0.0134 | 0.0142 | 0.0111 | 0.0125 | 0.0114 |
| Mv-3-(6-p-coum)-glc-8-ethyl-catechin 1 | <0.001 | <0.001 | 0.0013 | 0.0022 | 0.0118 | 0.0137 | 0.0203 | 0.0149 | 0.0197 | 0.0162 | 0.0179 | 0.0148 | 0.0173 | 0.0149 | 0.0187 | 0.0177 | 0.0178 | 0.0163 | 0.0206 | 0.0132 | 0.0206 | 0.0241 | 0.0176 | 0.0180 | 0.0152 |
| Mv-3-(6-p-coum)-glc-8-ethyl-catechin 2 | nd | nd | <0.001 | <0.001 | 0.0057 | 0.0053 | 0.0064 | 0.0067 | 0.0086 | 0.0056 | 0.0079 | 0.0059 | 0.0066 | 0.0057 | 0.0075 | 0.0073 | 0.0059 | 0.0066 | 0.0075 | 0.0048 | 0.0056 | 0.0073 | 0.0062 | 0.0057 | 0.0044 |

Tabla Anexo I.2 Tannins concentrations of the samples analysed during the fermentation process of the 2012 wine.

| Time (days) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 19 | 20 | 21 | 22 | 25 | 32 | 39 | 47 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Catechin | 1.7328 | 1.9572 | 1.8070 | 2.1161 | 5.1911 | 6.7496 | 8.3885 | 5.6437 | 7.8126 | 6.9582 | 10.4636 | 8.6620 | 10.6956 | 10.2271 | 8.1671 | 7.9642 | 10.1606 | 9.9438 | 9.3615 | 33.9927 | 24.6592 | 35.2561 | 22.3534 | 39.6149 | 26.8859 |
| Epicatechin | 0.8589 | 1.7353 | 1.5870 | 1.7123 | 2.2998 | 2.8241 | 3.5531 | 2.6558 | 3.5495 | 2.9978 | 4.2819 | 3.3881 | 4.9850 | 4.9346 | 3.9422 | 3.7429 | 5.0649 | 4.0760 | 4.0226 | 11.8078 | 8.5387 | 11.4209 | 7.6108 | 12.5935 | 9.8889 |
| Gallocatechin | 0.3065 | 0.2755 | 0.1346 | 1.4176 | 1.6798 | 1.7286 | 2.0223 | 0.8364 | 1.6954 | 1.6036 | 2.1141 | 1.6944 | 2.2785 | 2.0580 | 1.8053 | 1.8455 | 2.5380 | 2.0003 | 1.9705 | 3.1652 | 2.4821 | 2.8414 | 1.5740 | 3.9903 | 2.8493 |
| Epigallocatechin | 0.3065 | 0.3664 | nd | nd | 1.7649 | 1.8195 | 2.2328 | 1.5774 | 1.6692 | 1.7025 | 2.2503 | 1.6611 | 2.1504 | 1.9321 | 1.6575 | 1.8000 | 1.9495 | 1.8962 | 1.7082 | 1.9452 | 1.4911 | 2.4900 | 1.7438 | 3.2323 | 3.6271 |
| PCB1 | 2.3437 | 2.3055 | 1.7066 | 2.7590 | 8.9176 | 12.7284 | 36.1880 | 15.9796 | 59.7428 | 18.1524 | 39.7551 | 33.0888 | 91.7494 | 105.0995 | 70.0592 | 64.1304 | 114.9502 | 62.2478 | 64.0332 | 51.7604 | 57.6040 | 40.9927 | 26.6029 | 54.0045 | 33.9567 |
| PCB2 | 0.8134 | 1.6721 | 1.5181 | 1.8022 | 3.1752 | 4.4008 | 7.0586 | 6.2540 | 9.6364 | 6.7655 | 8.9293 | 7.7077 | 16.6121 | 11.7413 | 12.8405 | 12.5039 | 15.4607 | 12.6193 | 13.3826 | 46.2361 | 40.7362 | 34.2059 | 26.2621 | 35.1614 | 32.5635 |
| $(epi)cat)_2$ 1 | 0.2155 | 0.2709 | 0.0974 | 1.4530 | 1.9524 | 2.3744 | 2.9130 | 2.2442 | 3.2469 | 2.7698 | 4.1609 | 3.1420 | 5.4228 | 5.0658 | 4.2884 | 4.2561 | 6.0407 | 4.6627 | 4.2272 | 12.3684 | 11.8867 | 10.3035 | 7.4450 | 13.1423 | 9.4270 |
| $(epi)gallocat)_2$ 1 | 0.0784 | nd | nd | nd | 0.3664 | 0.4255 | 1.6673 | 0.6430 | 1.5784 | 0.7212 | 1.5825 | 1.4705 | 1.8488 | 1.6217 | 1.5438 | 1.5543 | 1.7581 | 1.5395 | 1.6033 | 1.4043 | 1.6688 | 1.3608 | 1.3450 | 1.7201 | 1.9215 |
| $(epi)gallocat)_2$ 2 | nd | nd | nd | nd | 0.1164 | 0.1755 | 0.3482 | 0.0625 | 0.3099 | 0.2235 | 0.4909 | 0.2030 | 0.6225 | 0.2527 | 0.4363 | 0.4139 | 0.8616 | 0.5213 | 0.4900 | 0.1800 | 0.4666 | 0.3693 | 0.3693 | 0.6072 | 0.6830 |
| $(epi)gallocat)_2$ 3 | nd | nd | nd | nd | 0.2118 | 0.1891 | 0.4618 | 0.3222 | 0.4127 | 0.2893 | 0.7788 | 0.3963 | 0.6801 | 1.4949 | 0.9869 | 0.8750 | 1.4722 | 0.7317 | 0.9422 | 0.9155 | 0.9858 | 0.7046 | 0.6181 | 0.9101 | 0.8344 |
| PCC1 | nd | nd | nd | 0.2028 | 0.6845 | 1.6099 | 1.6951 | 0.9433 | 1.6722 | 1.6147 | 1.7418 | 1.7267 | 1.9900 | 1.9198 | 1.9627 | 1.9347 | 2.0641 | 1.8796 | 1.8814 | 2.1465 | 2.3597 | 2.2650 | 1.7675 | 2.2729 | 1.7083 |
| $(epi)cat)_3$ 1 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| $(epi)cat)_3$ 2 | 0.1002 | 0.1482 | 0.0575 | 1.4396 | 1.7353 | 1.8520 | 2.2835 | 1.7882 | 2.1403 | 2.0172 | 2.3017 | 2.1857 | 2.6881 | 2.8659 | 2.6149 | 2.6875 | 3.0705 | 2.6202 | 2.7312 | 4.7879 | 4.9853 | 4.4444 | 3.1217 | 4.5431 | 3.1296 |
| (epi)cat-(epi)gallocat) 1 | 0.6639 | 0.4209 | 0.1800 | 0.2755 | 1.8616 | 2.0232 | 3.6564 | 2.0122 | 3.6121 | 2.3955 | 3.3488 | 2.6296 | 7.6593 | 6.5128 | 4.3995 | 4.4528 | 8.6113 | 4.8813 | 4.3260 | 9.7034 | 13.6871 | 8.9177 | 7.3976 | 14.1175 | 11.6735 |
| (epi)cat-(epi)gallocat) 2 | 0.2350 | 0.2300 | 0.1255 | 0.2164 | 1.8309 | 1.9917 | 2.7274 | 2.0263 | 2.7284 | 2.1433 | 2.8858 | 2.2765 | 3.5677 | 3.6633 | 2.7644 | 2.6613 | 3.7140 | 2.7837 | 2.8781 | 6.3632 | 6.0197 | 5.5183 | 4.3299 | 6.7817 | 6.4580 |
| (epi)gallocat)-(epi)cat) 1 | 0.1002 | nd | nd | 0.1437 | 1.7458 | 1.8740 | 2.5083 | 1.9739 | 3.1249 | 1.8911 | 2.7203 | 2.1504 | 4.6481 | 4.7466 | 3.0311 | 2.6849 | 4.3241 | 2.4820 | 2.4838 | 2.4867 | 2.8896 | 2.6639 | 2.3114 | 2.9786 | 2.4463 |
| (epi)gallocat)-(epi)cat) 2 | 0.0711 | nd | nd | 0.0376 | 0.6618 | 1.6185 | 1.9821 | 1.8014 | 2.3431 | 1.6924 | 2.6739 | 1.9517 | 3.3054 | 3.7918 | 2.7819 | 2.4383 | 3.6292 | 2.3019 | 2.3448 | 2.7970 | 2.5308 | 2.4831 | 2.1837 | 2.8788 | 2.3878 |
| (epi)cat-glycoside 1 | nd | nd | nd | 1.6224 | 2.3074 | 2.8461 | 5.2236 | 5.0193 | 7.8570 | 4.1346 | 5.4208 | 5.0375 | 7.6734 | 8.3271 | 6.5312 | 5.7215 | 7.5760 | 4.9049 | 5.1042 | 5.7185 | 5.8677 | 5.4559 | 4.9811 | 5.6474 | 4.8660 |
| (epi)cat-glycoside 2 | nd | nd | nd | 2.0902 | 1.9582 | 1.7802 | 2.1103 | 2.6669 | 3.1743 | 2.3189 | 2.3824 | 2.4903 | 3.7261 | 4.2430 | 3.4561 | 3.2165 | 4.3138 | 3.1343 | 2.9454 | 3.3590 | 3.0137 | 2.8968 | 2.5515 | 2.7655 | 2.6423 |
| PCA 2 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| $(epi)cat)_2A$ 1 | nd | nd | nd | nd | nd | nd | nd | 0.0571 | nd | nd | nd | nd | nd | 0.1319 | nd | nd | nd | nd | nd | 0.0665 | 0.0611 | nd | nd | nd | nd |
| $(epi)cat)_2A$ 2 | nd | nd | nd | nd | nd | nd | nd | 0.0462 | nd | nd | nd | nd | nd | 0.0566 | nd | nd | nd | nd | nd | 0.1527 | nd | nd | nd | nd | nd |
| (epi)cat-(epi)gallocat)A 1 | nd | nd | nd | 0.0642 | 0.2028 | 0.2209 | 0.5891 | 0.6143 | 0.9186 | 0.4744 | 0.5772 | 0.6348 | 0.8816 | 1.4748 | 1.3848 | 1.4429 | 1.4590 | 1.0582 | 1.0437 | 0.9384 | 0.9423 | 0.7015 | 0.5238 | 0.9068 | 0.6462 |
| (epi)cat-(epi)gallocat)A 2 | nd | nd | nd | 0.3437 | 1.7659 | 1.7955 | 2.1668 | 1.7428 | 1.9285 | 1.8266 | 3.4961 | 1.9658 | 2.4520 | 2.6919 | 2.2731 | 1.9032 | 2.2180 | 2.0003 | 1.9740 | 2.2089 | 2.0425 | 2.1091 | 1.8042 | 2.3896 | 2.3303 |
| $(epi)gallocat)_2A$ | nd | nd | nd | nd | 0.1800 | 0.2164 | 0.2073 | nd | 0.2482 | nd | 0.4127 | 0.1659 | 0.2359 | 0.5066 | 0.4485 | 0.2889 | 0.4195 | 0.3760 | 0.3324 | 0.2790 | 0.2790 | 0.2317 | 0.2080 | 0.2790 | 0.3106 |
| (epi)cat-((epi)cat- | nd | nd | nd | nd | nd | 0.1188 | 0.2531 | 0.3285 | 0.3676 | 0.2581 | 0.6023 | 0.5162 | 0.5866 | 0.5794 | 0.3195 | 0.3091 | 0.2571 | 0.2467 | 0.3507 | 0.3195 | 0.4336 | 0.5021 | 0.4032 | 0.4488 | 0.3727 |
| (epi)cat-((epi)cat-(epi)gallocat) A 2 | nd | nd | nd | nd | nd | 0.0990 | 0.2403 | 0.1955 | 0.3285 | 0.2268 | 0.9386 | 0.3363 | 0.6883 | 0.6834 | 0.3715 | 0.1635 | <0.001 | 0.3403 | 0.2259 | 0.3499 | 0.4793 | 0.4488 | 0.5097 | 0.4869 | 0.4032 |
| (epi)cat-((epi)galocat)$_2$A | nd | nd | nd | nd | 0.1443 | 0.1763 | 0.4707 | 0.3285 | 0.7431 | 0.4458 | 1.7452 | 0.8760 | 1.6480 | 1.8324 | 0.7978 | 0.5171 | 0.4651 | 0.1323 | 0.4547 | 0.6390 | 0.6619 | 0.4640 | 0.3727 | 0.5249 | 0.4412 |
| (epi)cat-ethyl-(epi)cat | nd | nd | nd | nd | 0.1699 | 0.2979 | 0.1699 | 0.1408 | 0.2034 | 0.1955 | 0.3911 | 0.3754 | 0.2894 | 0.2571 | 0.2363 | 0.2051 | 0.1843 | 0.1531 | 0.0761 | 0.4032 | 0.2205 | 0.2966 | 0.2434 | 0.3879 | 0.4640 |
| p-vinyl(epi)cat 1 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| p-vinyl(epi)cat 2 | 2.5003 | 6.5227 | 5.8657 | 8.0545 | 12.1238 | 11.7485 | 12.0764 | 10.7505 | 9.9091 | 9.1242 | 9.7495 | 8.8926 | 10.9173 | 22.8266 | 20.5653 | 19.2453 | 21.6386 | 18.6236 | 19.0265 | 9.3710 | 9.0368 | 10.6190 | 8.6103 | 10.6560 | 10.3958 |
| p-vinyl(epi)cat 3 | nd | nd | nd | nd | 1.7838 | 0.5858 | 0.6114 | 1.6876 | 0.6414 | 1.7092 | 2.2661 | 1.6552 | 0.8213 | 0.8810 | 1.9679 | 2.5688 | 0.6418 | 1.9401 | 1.8463 | 2.0188 | 2.0320 | 2.0359 | 1.9488 | 2.0227 | 2.0835 |
| (epi)cat-furfuryl-(epi)cat 1 | nd | nd | nd | nd | nd | nd | nd | 0.1955 | 0.2112 | 0.2112 | 0.4380 | 0.2190 | 0.4067 | 0.4131 | 0.3091 | 0.2987 | 0.3299 | 0.2571 | 0.2571 | 0.2129 | 0.2510 | 0.2434 | 0.1825 | 0.3956 | 0.3347 |
| (epi)cat-furfuryl-(epi)cat 2 | nd | nd | nd | nd | nd | nd | nd | 0.2425 | 0.4145 | 0.2894 | 0.6883 | 0.3911 | 0.5788 | 0.7458 | 0.4443 | 0.4651 | 0.6730 | 0.3091 | 0.4755 | 0.3879 | 0.4945 | 0.4184 | 0.4032 | 0.4793 | 0.5401 |

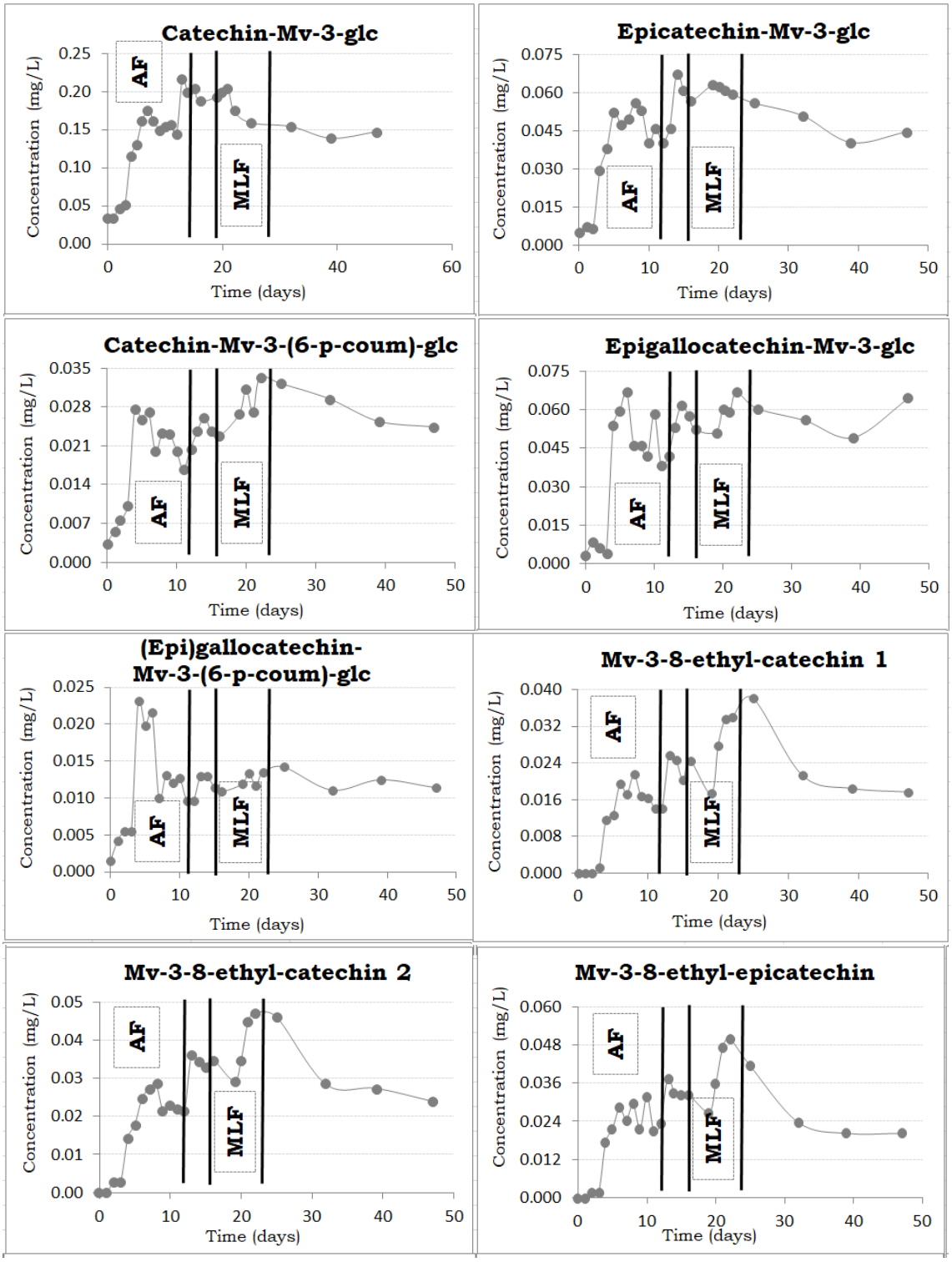
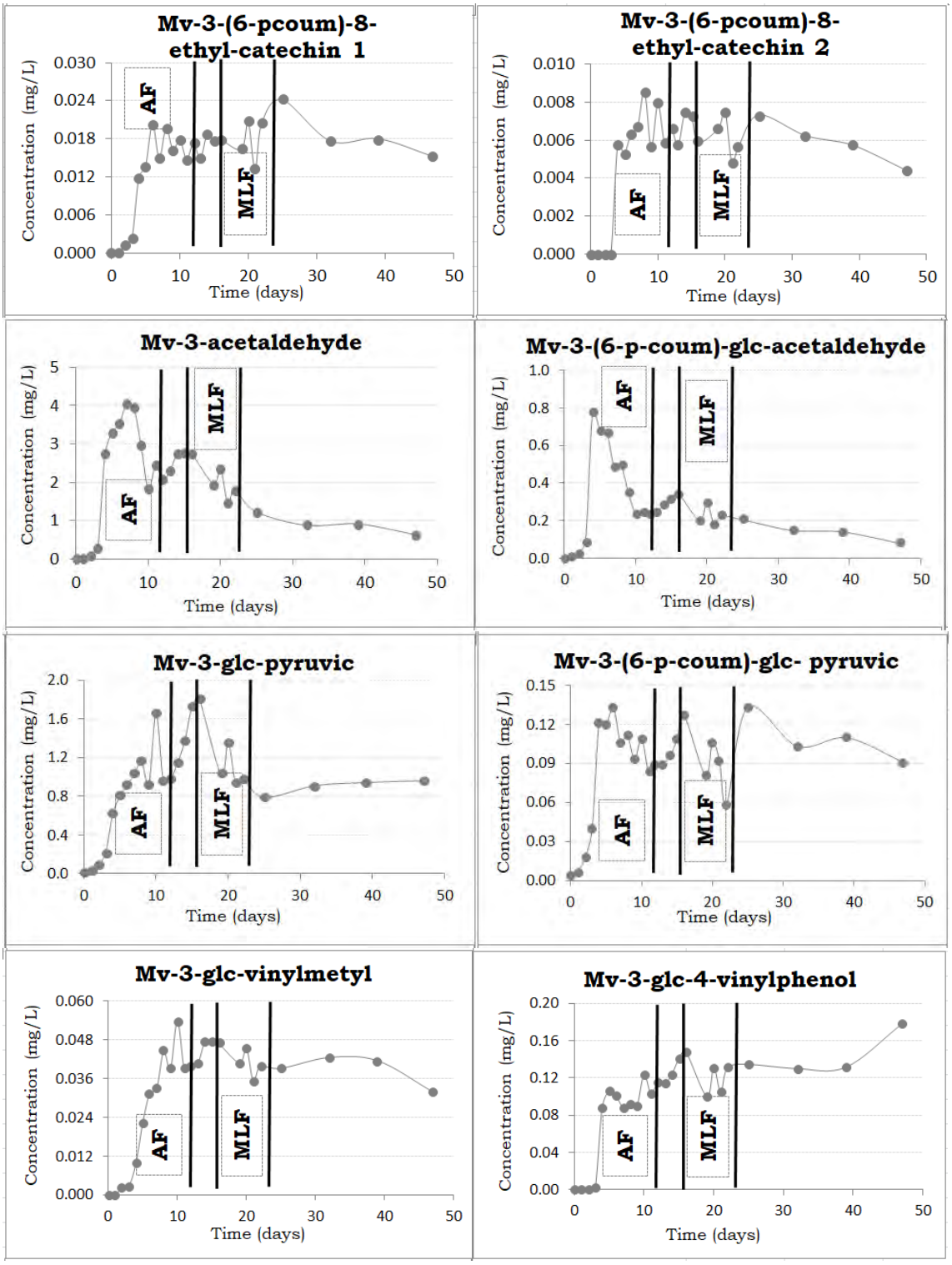
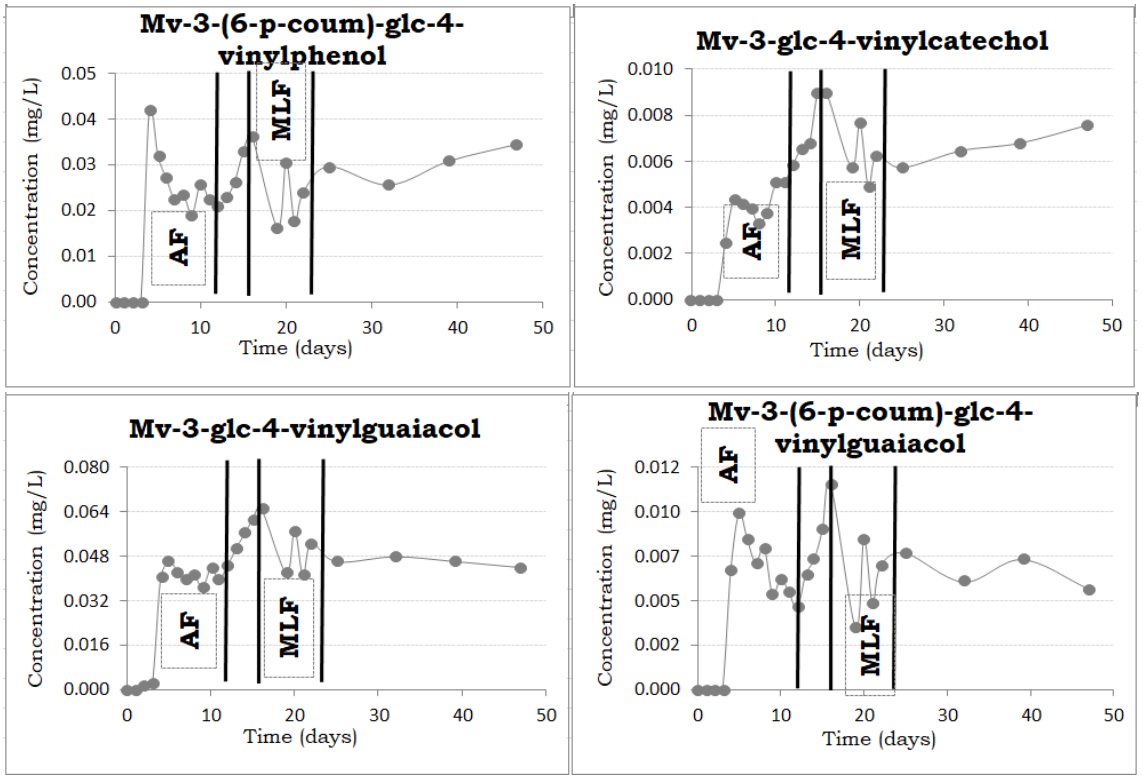



Figure Annex I.1 Formation and evolution profiles for the anthocyanin derivatives in the fermentation studied.




Continuation of Figure Annex I.1.




Continuation of Figure Annex I.1.

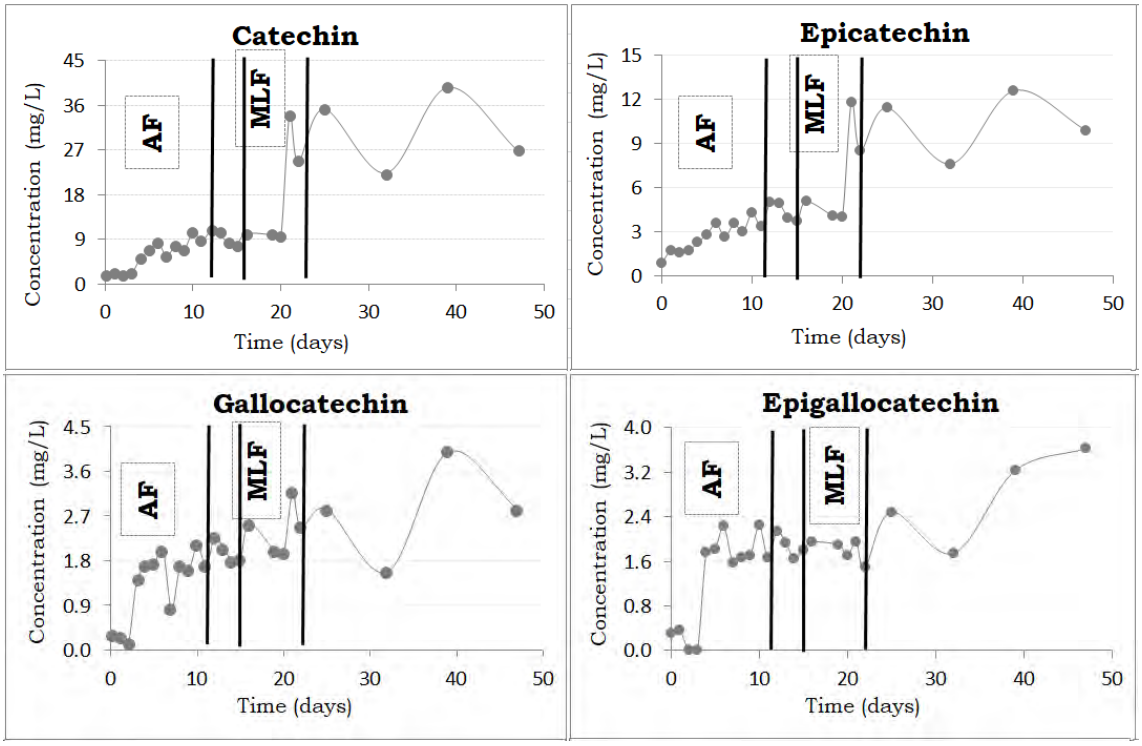
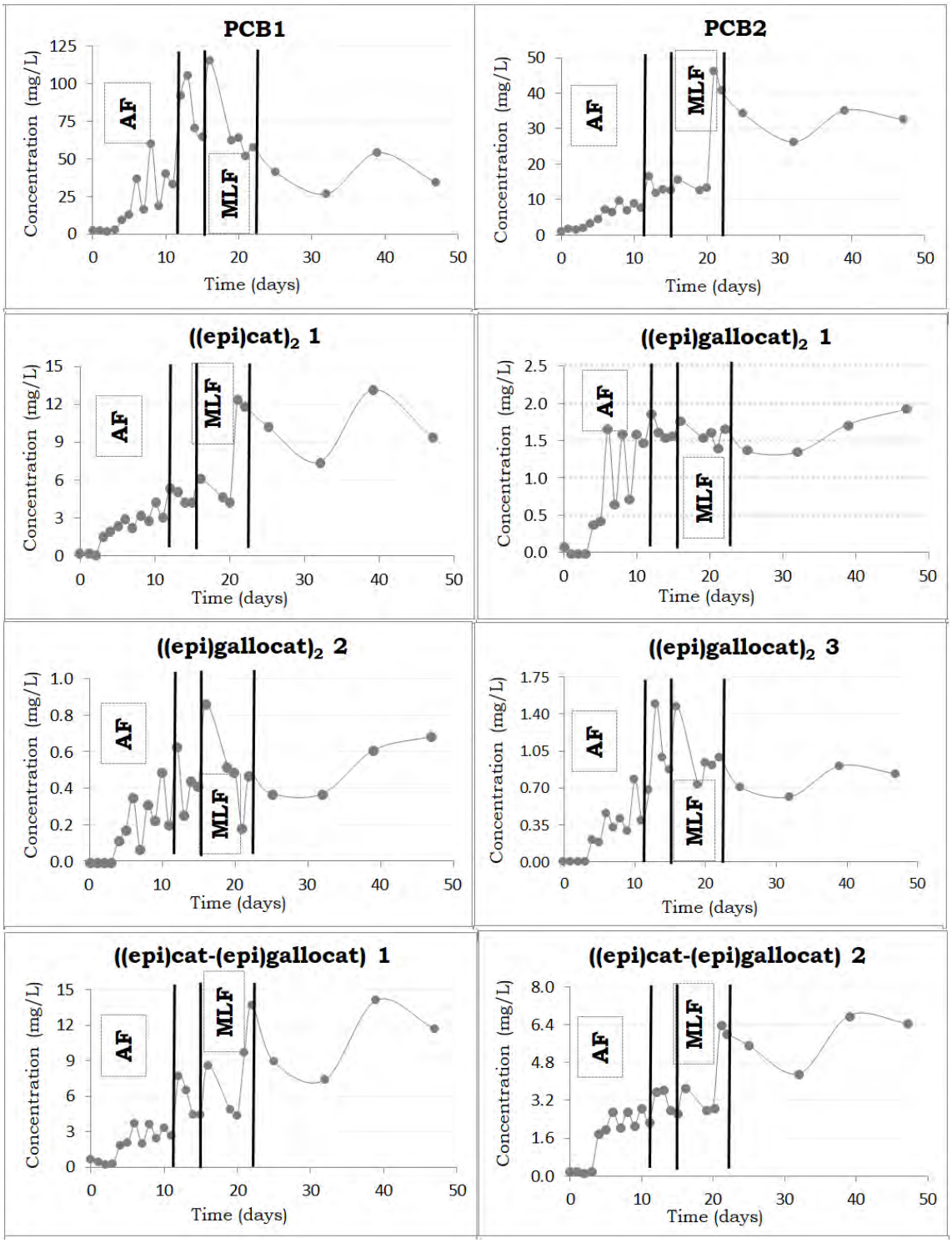
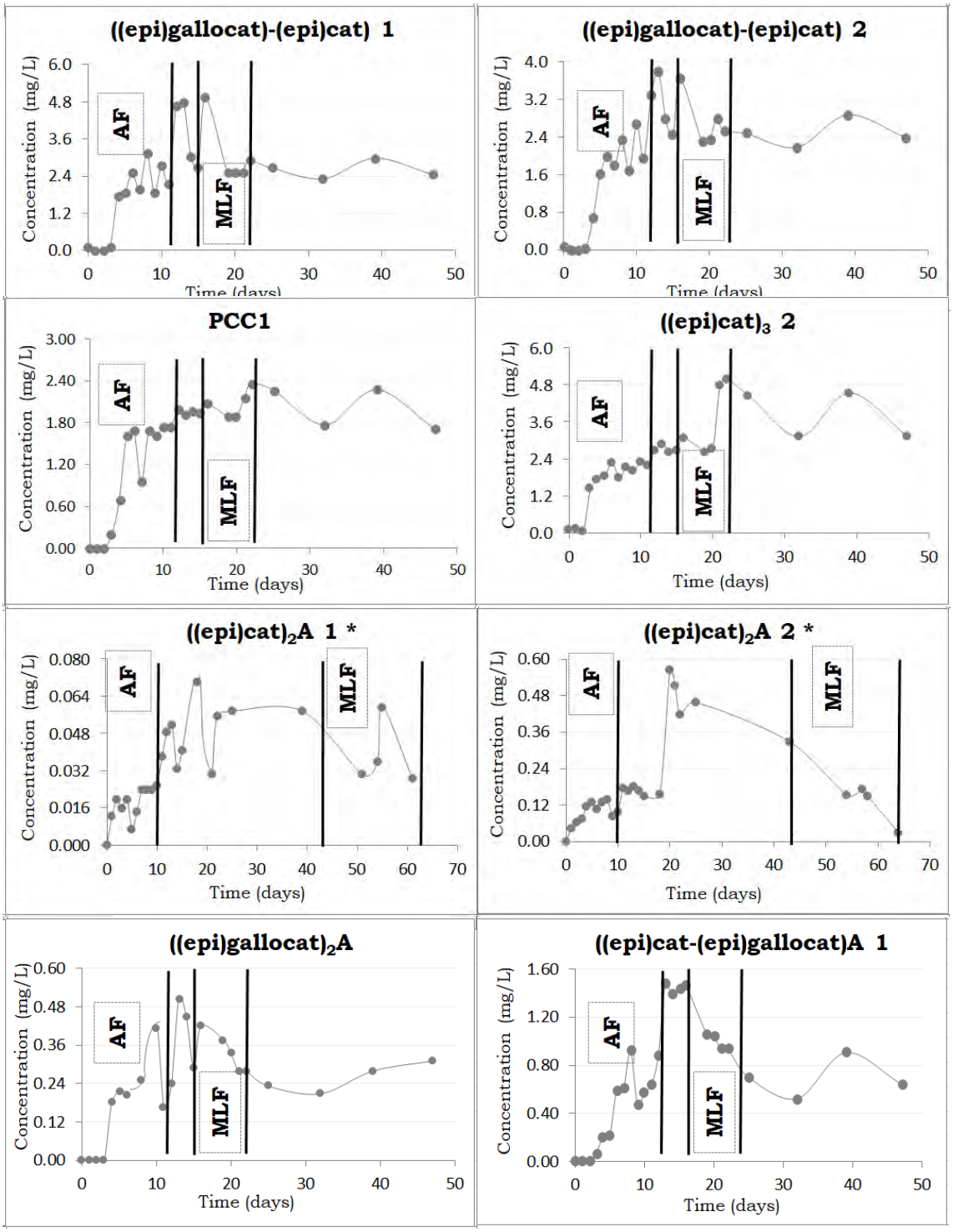


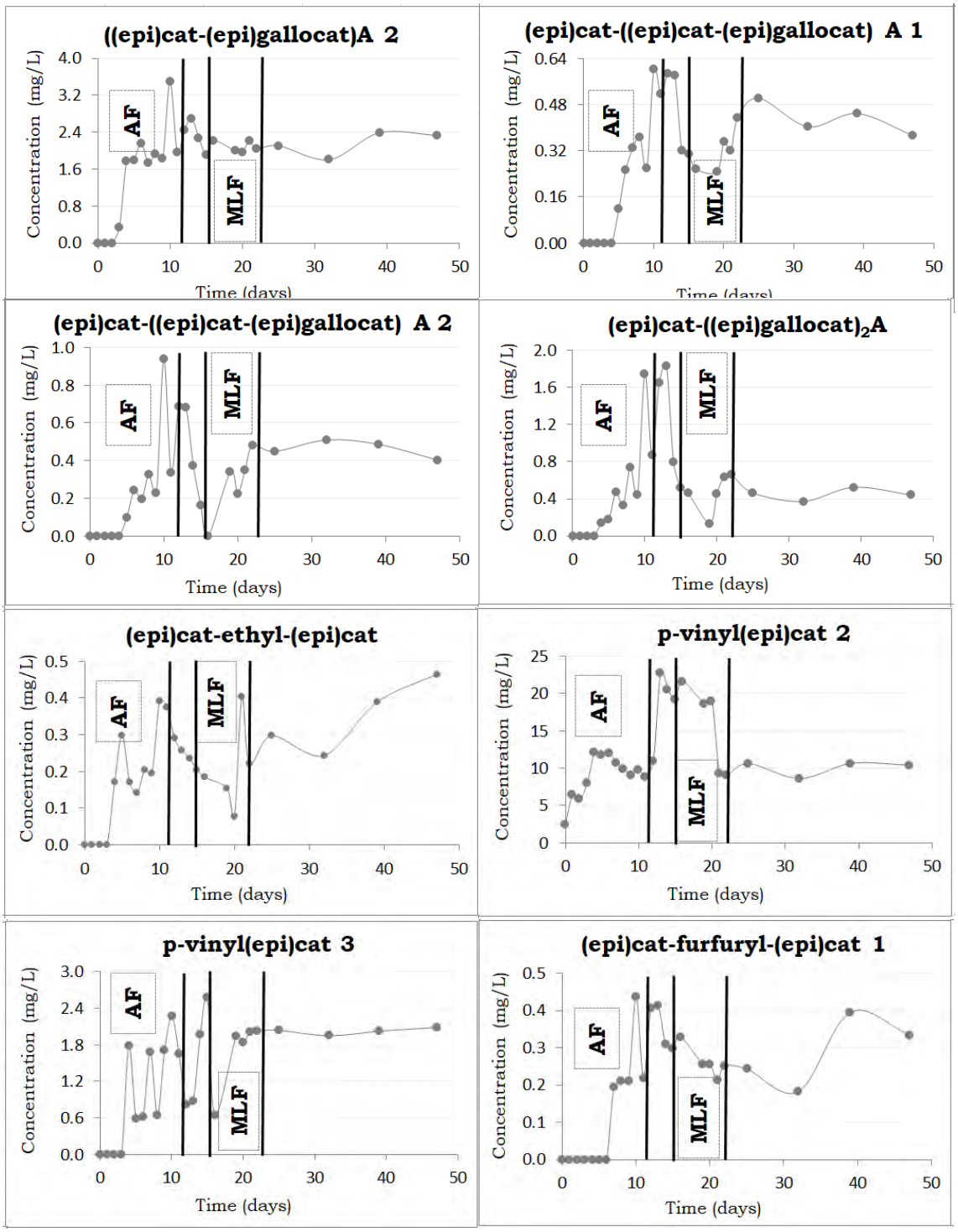

Figure Annex I.2 Formation and evolution profiles for the tannins in the fermentation studied.
*Formation and evolution profiles for these tannins in a fermentation of 2011.



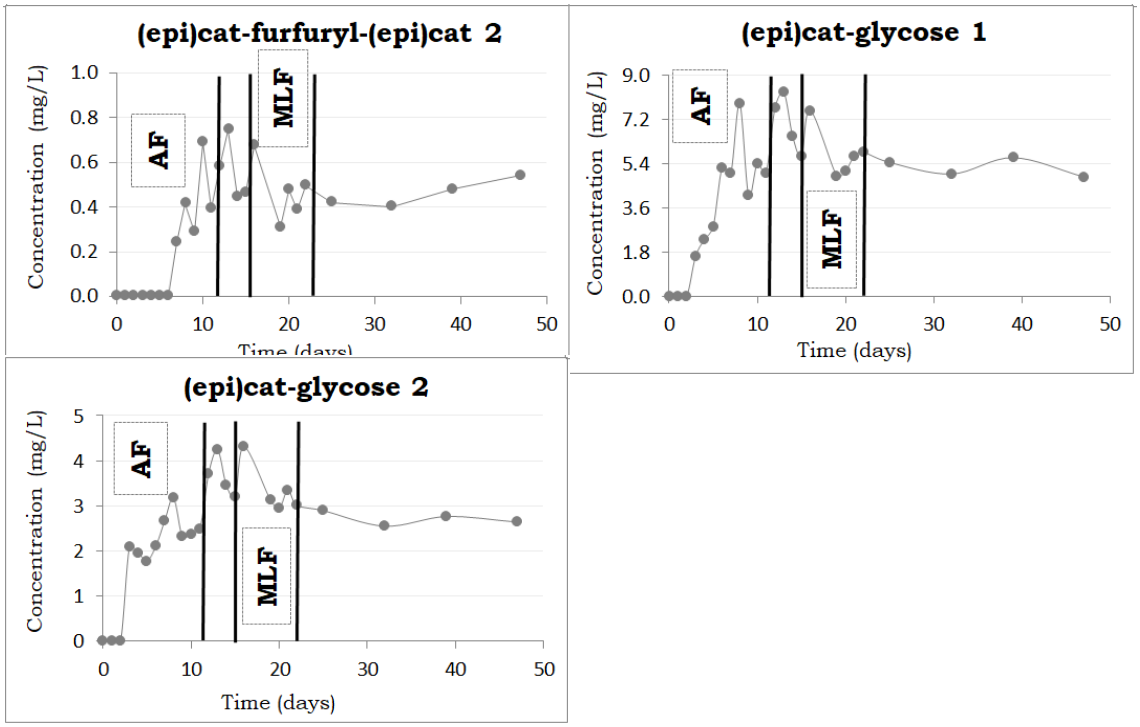

Continuation of Figure Annex I.2.
*Formation and evolution profiles for these tannins in a fermentation of 2011.




Continuation of Figure Annex I.2.
*Formation and evolution profiles for these tannins in a fermentation of 2011.




Continuation of Figure Annex I.2.
*Formation and evolution profiles for these tannins in a fermentation of 2011.




Continuation of Figure Annex I.2.
*Formation and evolution profiles for these tannins in a fermentation of 2011.

Tabla Anexo I.3 Anthocyanin derivatives concentrations of the samples analysed during the ageing inside oak barrel for *Reserva* wine of 2012.

| Time (days) | 0 | 26 | 67 | 126 | 161 | 193 | 220 | 220 | 249 | 279 | 291 | 306 | 339 | 370 | 431 | 463 | 525 | 538 | 586 | 614 | 707 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mv-3-glc-acetaldehyde | 0.1072 | 0.1066 | 0.0811 | 0.1286 | 0.1074 | 0.0870 | 0.0906 | 0.1572 | 0.1394 | 0.1093 | 0.0830 | 0.0763 | 0.0635 | 0.0907 | 0.0830 | 0.0715 | 0.0763 | 0.0528 | 0.0800 | 0.0859 | 0.0569 |
| Mv-3-glc-vinylmetyl | 0.0220 | 0.0211 | 0.0159 | 0.0244 | 0.0268 | 0.0242 | 0.0225 | 0.0280 | 0.0266 | 0.0262 | 0.0301 | 0.0268 | 0.0266 | 0.0267 | 0.0300 | 0.0281 | 0.0267 | 0.0272 | 0.0382 | 0.0521 | 0.0402 |
| Mv-3-glc-pyruvic | 0.7213 | 0.7898 | 0.5217 | 0.9109 | 0.8923 | 0.7856 | 0.6659 | 0.8768 | 0.8055 | 0.7456 | 1.0719 | 0.9923 | 0.9785 | 0.9947 | 1.0742 | 0.9723 | 0.9943 | 0.8085 | 0.7547 | 1.1059 | 0.7911 |
| Mv-3-glc-4-vinylphenol | 0.4215 | 0.4078 | 0.2677 | 0.1446 | 0.1403 | 0.1155 | 0.0951 | 0.1535 | 0.1368 | 0.1363 | 0.1247 | 0.0949 | 0.0940 | 0.0928 | 0.1242 | 0.0974 | 0.0950 | 0.0902 | 0.1237 | 0.2203 | 0.1522 |
| Mv-3-glc-4-vinylcatechol | 0.0267 | 0.0439 | 0.0323 | 0.0099 | 0.0108 | 0.0090 | 0.0084 | 0.0137 | 0.0133 | 0.0139 | 0.0152 | 0.0143 | 0.0145 | 0.0121 | 0.0150 | 0.0126 | 0.0141 | 0.0120 | 0.0274 | 0.0245 | 0.0197 |
| Mv-3-glc-4-vinylguaiacol | 0.1051 | 0.1073 | 0.0688 | 0.0339 | 0.0350 | 0.0296 | 0.0231 | 0.0356 | 0.0325 | 0.0335 | 0.0287 | 0.0230 | 0.0228 | 0.0227 | 0.0286 | 0.0239 | 0.0229 | 0.0199 | 0.0351 | 0.0495 | 0.0341 |
| Mv-3-(6-p-coum)-glc-acetaldehyde | 0.1215 | 0.0924 | 0.0934 | 0.0435 | 0.0394 | 0.0327 | 0.0260 | 0.0389 | 0.0281 | 0.0326 | 0.0176 | 0.0159 | 0.0137 | 0.0176 | 0.0174 | 0.0155 | 0.0157 | 0.0101 | 0.0033 | 0.0215 | 0.0078 |
| Mv-3-(6-p-coum)-glc- pyruvic | 0.2557 | 0.2127 | 0.2565 | 0.1137 | 0.1201 | 0.0991 | 0.0918 | 0.1410 | 0.1034 | 0.1551 | 0.1195 | 0.0966 | 0.0993 | 0.0885 | 0.1190 | 0.0969 | 0.0967 | 0.0798 | 0.0318 | 0.1732 | 0.0904 |
| Mv-3-(6-p-coum)-glc-4-vinylphenol | 0.1206 | 0.0936 | 0.1099 | 0.0657 | 0.0673 | 0.0534 | 0.0444 | 0.0495 | 0.0414 | 0.0526 | 0.0317 | 0.0268 | 0.0275 | 0.0294 | 0.0316 | 0.0266 | 0.0267 | 0.0195 | 0.0146 | 0.0591 | 0.0332 |
| Catechin-Mv-3-glc | 0.3969 | 0.3431 | 0.3324 | 0.1624 | 0.1389 | 0.1426 | 0.1389 | 0.1976 | 0.1856 | 0.1527 | 0.1893 | 0.2011 | 0.2024 | 0.1655 | 0.1890 | 0.1892 | 0.2008 | 0.1914 | 0.1538 | 0.2424 | 0.2180 |
| Epicatechin-Mv-3-glc | 0.0741 | 0.0809 | 0.0841 | 0.0425 | 0.0433 | 0.0440 | 0.0418 | 0.0480 | 0.0445 | 0.0434 | 0.0451 | 0.0450 | 0.0432 | 0.0424 | 0.0450 | 0.0448 | 0.0449 | 0.0525 | 0.0441 | 0.0812 | 0.0650 |
| Mv-3-(6-p-coum)-glc-4-vinylguaiacol | 0.0195 | 0.0157 | 0.0199 | 0.0097 | 0.0100 | 0.0096 | 0.0069 | 0.0099 | 0.0078 | 0.0109 | 0.0056 | 0.0042 | 0.0048 | 0.0044 | 0.0052 | 0.0043 | 0.0039 | 0.0030 | 0.0013 | 0.0079 | 0.0043 |
| (Epi)Gallocatechin-Mv-3-glc | 0.0511 | 0.0555 | 0.0555 | 0.0329 | 0.0338 | 0.0318 | 0.0308 | 0.0477 | 0.0413 | 0.0386 | 0.0420 | 0.0458 | 0.0455 | 0.0461 | 0.0420 | 0.0446 | 0.0458 | 0.0366 | 0.0329 | 0.0529 | 0.0463 |
| Mv-3-glc-8-ethyl-catechin 1 | 0.0153 | 0.0120 | 0.0088 | 0.0030 | 0.0027 | 0.0023 | 0.0019 | 0.0048 | 0.0041 | 0.0036 | 0.0016 | 0.0018 | 0.0018 | 0.0019 | 0.0015 | 0.0013 | 0.0017 | <0.001 | <0.001 | <0.001 | <0.001 |
| Mv-3-glc-8-ethyl-catechin 2 | 0.0282 | 0.0246 | 0.0190 | 0.0076 | 0.0067 | 0.0058 | 0.0048 | 0.0103 | 0.0093 | 0.0072 | 0.0036 | 0.0042 | 0.0039 | 0.0044 | 0.0034 | 0.0037 | 0.0041 | 0.0022 | 0.0016 | 0.0034 | 0.0020 |
| Mv-3-glc-8-ethyl-epicatechin | 0.0051 | 0.0041 | 0.0024 | 0.0013 | 0.0013 | <0.001 | <0.001 | 0.0018 | 0.0018 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.0046 | <0.001 | <0.001 |
| Catechin-Mv-3-(6-p-coum)-glc | 0.0372 | 0.0417 | 0.0485 | 0.0177 | 0.0200 | 0.0169 | 0.0150 | 0.0189 | 0.0174 | 0.0179 | 0.0268 | 0.0214 | 0.0196 | 0.0245 | 0.0268 | 0.0264 | 0.0213 | 0.0243 | 0.0017 | 0.0404 | 0.0339 |
| Epicatechin-Mv-3-(6-p-coum)-glc | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | nd | <0.001 | <0.001 | nd | nd | nd | nd | nd | nd | nd | nd | <0.001 | <0.001 | <0.001 |
| (Epi)gallocatechin-Mv-3-(6-p-coum)-glc | 0.0143 | 0.0159 | 0.0175 | 0.0062 | 0.0071 | 0.0073 | 0.0063 | 0.0071 | 0.0064 | 0.0083 | 0.0108 | 0.0071 | 0.0067 | 0.0095 | 0.0107 | 0.0092 | 0.0070 | 0.0089 | <0.001 | 0.0104 | 0.0093 |
| Mv-3-(6-p-coum)-glc-8-ethyl-catechin 1 | 0.0155 | 0.0174 | 0.0158 | 0.0130 | 0.0116 | 0.0113 | 0.0101 | 0.0127 | 0.0119 | 0.0132 | 0.0082 | 0.0077 | 0.0073 | 0.0073 | 0.0082 | 0.0070 | 0.0077 | 0.0057 | 0.0045 | 0.0094 | 0.0074 |
| Mv-3-(6-p-coum)-glc-8-ethyl-catechin 2 | <0.001 | <0.001 | <0.001 | 0.0035 | 0.0028 | 0.0028 | 0.0018 | 0.0025 | 0.0029 | 0.0033 | 0.0014 | <0.001 | <0.001 | <0.001 | 0.0015 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

Tabla Anexo I.4 Tannins concentrations of the samples analysed during the ageing inside oak barrel for *Reserva* wine of 2012.

| Time (Days) | 0 | 26 | 67 | 126 | 161 | 193 | 220 | 220 | 249 | 279 | 306 | 339 | 370 | 431 | 463 | 525 | 538 | 586 | 614 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Catechin | 9.3213 | 9.2597 | 10.6484 | 5.8543 | 5.4145 | 5.7436 | 5.7639 | 8.4878 | 9.4126 | 6.6585 | 5.6266 | 6.1694 | 5.8864 | 5.2221 | 5.5204 | 5.1269 | 5.8590 | 7.1687 | 6.1699 |
| Epicatechin | 2.7961 | 2.5144 | 2.3449 | 1.7292 | 1.5352 | 1.5809 | 1.5931 | 4.2420 | 4.1680 | 3.4138 | 1.3132 | 1.3364 | 1.2784 | 1.2819 | 1.3546 | 1.2957 | 1.3624 | 2.4497 | 1.2270 |
| Gallocatechin | 4.0879 | 3.7952 | 4.0615 | 1.6967 | 1.4814 | 1.5870 | 1.4783 | 2.4628 | 2.3100 | 1.8888 | 1.7409 | 1.8864 | 1.6561 | 1.8344 | 1.8904 | 1.6863 | 1.6794 | 1.7841 | 1.6715 |
| Epigallocatechin | 1.2534 | 1.1301 | 1.7045 | 1.1737 | 0.8791 | 1.1990 | 1.2204 | 1.7360 | 1.7921 | 1.5451 | 0.8882 | 0.8824 | 0.7994 | 0.8276 | 0.8301 | 0.8364 | 0.9226 | 1.5645 | 0.6787 |
| PCB1 | 142.1549 | 130.6853 | 136.3735 | 69.1834 | 36.8577 | 56.7861 | 68.4407 | 55.2436 | 13.1296 | 45.6296 | 19.9239 | 25.3333 | 26.8098 | 7.1872 | 11.6040 | 25.1693 | 29.9294 | 38.1283 | 63.7303 |
| PCB2 | 17.2730 | 10.4670 | 7.9855 | 8.1331 | 6.5875 | 7.3847 | 7.2293 | 10.0284 | 5.8578 | 8.1024 | 5.6212 | 5.2819 | 5.1007 | 4.2672 | 4.3938 | 5.0189 | 5.7304 | 7.0612 | 8.0823 |
| ((epi)cat)$_1$1 | 3.6478 | 3.0734 | 2.5364 | 1.3636 | 1.2183 | 1.4641 | 1.4580 | 1.9938 | 1.3625 | 1.8088 | 0.6643 | 0.5427 | 0.6672 | 0.5428 | 0.6690 | 0.6490 | 0.7714 | 1.7026 | 1.0905 |
| ((epi)gallocat)$_1$1 | 1.0124 | 0.8914 | 0.8664 | 0.1868 | 0.1547 | 0.1820 | 0.1949 | 0.2913 | 0.1242 | 0.3269 | 0.0958 | 0.0898 | 0.1199 | 0.1181 | 0.1118 | 0.1306 | 0.1743 | 0.2071 | 0.8685 |
| ((epi)gallocat)$_1$2 | 0.2866 | 0.2720 | 0.2637 | 0.1096 | 0.0905 | 0.0967 | 0.0999 | 0.2119 | 0.1214 | 0.1461 | 0.0460 | 0.0549 | 0.0689 | 0.0687 | 0.0740 | 0.0780 | 0.0833 | 0.1270 | 0.1802 |
| ((epi)gallocat)$_1$3 | 0.2970 | 0.2157 | 0.2199 | 0.1514 | 0.1241 | 0.1611 | 0.1530 | 0.3132 | 0.1708 | 0.2530 | 0.0888 | 0.1006 | 0.0709 | 0.0780 | 0.0806 | 0.0873 | 0.1031 | 0.2396 | 0.1198 |
| PCC1 | 0.8581 | 0.8393 | 0.6933 | 0.3478 | 0.3301 | 0.3543 | 0.3639 | 0.6338 | 0.3927 | 0.3817 | 0.2116 | 0.2154 | 0.1990 | 0.1505 | 0.1543 | 0.2105 | 0.2043 | 0.4841 | 0.2282 |
| ((epi)cat)$_1$1 | nd | nd | nd | nd | nd | nd | nd | 0.0193 | 0.0133 | 0.0253 | nd | nd | 0.0081 | nd | 0.0167 | 0.0140 | 0.0273 | nd | 0.1302 |
| ((epi)cat)$_1$2 | 2.4242 | 2.0853 | 2.0324 | 1.1848 | 1.1158 | 1.1625 | 1.0853 | 1.6251 | 0.6694 | 1.3792 | 0.6855 | 0.7135 | 0.6575 | 0.4803 | 0.5928 | 0.6215 | 0.7514 | 1.3370 | 0.8289 |
| ((epi)cat-(epi)gallocat) 1 | 3.1218 | 2.8709 | 3.2891 | 2.3364 | 1.7332 | 1.8348 | 1.6601 | 2.8554 | 1.6823 | 2.2885 | 1.1016 | 1.0614 | 1.1971 | 1.5302 | 1.5027 | 1.7746 | 1.6078 | 1.5985 | 2.6575 |
| ((epi)cat-(epi)gallocat) 2 | 1.4646 | 1.0377 | 1.0949 | 1.8866 | 1.5728 | 1.7220 | 1.6195 | 2.8160 | 2.0045 | 2.3494 | 0.9855 | 0.9882 | 1.0311 | 1.5695 | 1.3212 | 1.4841 | 1.5852 | 1.5577 | 0.7788 |
| ((epi)gallocat)-(epi)cat) 1 | 3.5466 | 3.2274 | 3.4915 | 1.6042 | 1.1777 | 1.3189 | 1.2336 | 2.2683 | 1.3673 | 1.7527 | 0.9500 | 0.8322 | 1.0427 | 1.0405 | 1.1151 | 1.2005 | 1.2182 | 1.4366 | 3.4783 |
| ((epi)gallocat)-(epi)cat) 2 | 1.0082 | 0.8143 | 0.8518 | 1.3016 | 1.1188 | 1.2082 | 1.1848 | 1.8458 | 1.3315 | 1.5666 | 0.6517 | 0.7048 | 0.7087 | 0.9750 | 0.9400 | 1.0719 | 1.1769 | 1.3076 | 0.7642 |
| (epi)cat-glycoside 1 | 1.8080 | 1.2644 | 1.1125 | 4.2751 | 3.4129 | 3.7166 | 3.3185 | 6.5093 | 3.4747 | 5.3757 | 2.4748 | 2.1721 | 2.3105 | 3.6460 | 2.8786 | 3.3742 | 3.1053 | 3.0326 | 0.5202 |
| (epi)cat-glycoside 2 | 0.6453 | 0.3784 | 0.3805 | 1.9221 | 1.6784 | 1.7637 | 1.6469 | 2.0821 | 1.5785 | 1.6573 | 1.0819 | 0.9980 | 1.0998 | 1.7079 | 1.4291 | 1.5342 | 1.4772 | 1.9437 | 0.2366 |
| PCA 2 | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.0418 | nd | <LOQ | nd | nd | 0.0201 | nd | nd | nd | nd |
| ((epi)cat)$_1$A 1 | 0.4501 | 0.3422 | 0.2583 | 0.0997 | 0.0827 | 0.1082 | 0.0798 | 0.1113 | 0.0900 | 0.1265 | 0.0581 | 0.0581 | 0.0629 | 0.1030 | 0.1326 | 0.1030 | 0.1171 | 0.0207 | 0.2583 |
| ((epi)cat)$_1$A 2 | 1.4938 | 1.4473 | 1.2147 | 0.0571 | 0.0742 | 0.0827 | 0.0600 | 0.0611 | 0.0611 | 0.0322 | 0.0888 | 0.0807 | 0.0759 | 0.0839 | 0.0935 | 0.0903 | 0.0743 | nd | 0.6179 |
| ((epi)cat-(epi)gallocat)A 1 | 0.2703 | nd | 0.1864 | 0.3577 | 0.2471 | 0.2641 | 0.3067 | 0.5365 | 0.2556 | 0.3315 | 0.1167 | 0.1574 | 0.1377 | 0.2594 | 0.2413 | 0.3319 | 0.3344 | 0.2841 | 0.1744 |
| ((epi)cat-(epi)gallocat)A 2 | 0.0495 | nd | 0.0495 | 0.1507 | 0.1224 | 0.1082 | 0.1706 | 0.1493 | 0.0997 | 0.0611 | 0.0710 | 0.0338 | 0.0710 | 0.1222 | 0.1455 | 0.0807 | 0.1662 | 0.4562 | 0.1864 |
| ((epi)gallocat)$_1$A | 0.2104 | 0.2104 | 0.0666 | nd | nd | nd | nd | nd | nd | nd | 0.0079 | 0.0144 | nd | nd | nd | nd | 0.0265 | nd | 0.0214 |
| (epi)cat-((epi)cat- | 0.4621 | 0.4501 | 0.5939 | 0.8983 | 0.8983 | 0.8983 | 0.8983 | 1.7092 | 0.8706 | 0.8706 | 0.9509 | 0.8877 | 1.0662 | 2.1077 | 2.2229 | 2.6318 | 2.4447 | 2.1990 | 2.6568 |
| (epi)cat-((epi)cat- | 0.0306 | nd | nd | 0.1422 | 0.0610 | 0.1053 | 0.0785 | 0.0997 | 0.0804 | 0.0515 | 0.1040 | 0.0710 | 0.0856 | 0.1352 | 0.2775 | 0.3008 | 0.3086 | 0.1909 | 0.1385 |
| (epi)cat-((epi)gallocat)$_1$A | nd | nd | nd | 0.0715 | 0.0924 | 0.0506 | nd | 0.0900 | nd | 0.0611 | 0.0176 | 0.0144 | 0.0160 | 0.0361 | 0.0552 | nd | 0.0201 | 0.0248 | nd |
| (epi)cat-ethyl-(epi)cat | nd | nd | nd | 0.0415 | 0.0243 | 0.0186 | nd | 0.1135 | 0.0836 | 0.0388 | 0.0182 | nd | 0.0161 | nd | nd | nd | 0.0349 | 0.0619 | nd |
| p-vinyl(epi)cat 1 | 1.1807 | nd | 0.9453 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.1715 | nd | nd | 1.6138 | nd |
| p-vinyl(epi)cat 2 | 73.0302 | 65.0228 | 53.2224 | 5.9863 | 5.3443 | 5.2758 | 5.1234 | 10.4034 | 8.8833 | 8.3838 | 3.9545 | 3.8843 | 3.8892 | 6.0933 | 5.7846 | 5.3269 | 5.4041 | 5.9336 | nd |
| p-vinyl(epi)cat 3 | nd | 1.1807 | nd | 1.8243 | 1.9425 | 1.8534 | 1.6908 | 1.5109 | 1.5326 | 1.4023 | 0.5373 | 0.5352 | 0.5020 | 1.4209 | 1.4999 | 1.4317 | 0.9750 | 1.9575 | 20.7712 |
| (epi)cat-furfuryl-(epi)cat 1 | nd | 6.9304 | 7.6803 | 0.2521 | 0.2571 | 0.2822 | 0.1820 | 0.3752 | 0.2520 | 0.2315 | 0.0991 | 0.1240 | 0.1489 | 0.8198 | 0.6140 | 0.6373 | 0.6140 | 1.7302 | nd |
| (epi)cat-furfuryl-(epi)cat 2 | nd | 1.6806 | 1.1807 | 1.9870 | 1.7987 | 1.8569 | 1.7627 | 1.4729 | 0.4574 | 0.7962 | 0.9547 | 0.8613 | 0.9485 | 2.5787 | 2.6882 | 2.8085 | 2.6487 | 2.7056 | 43.5232 |

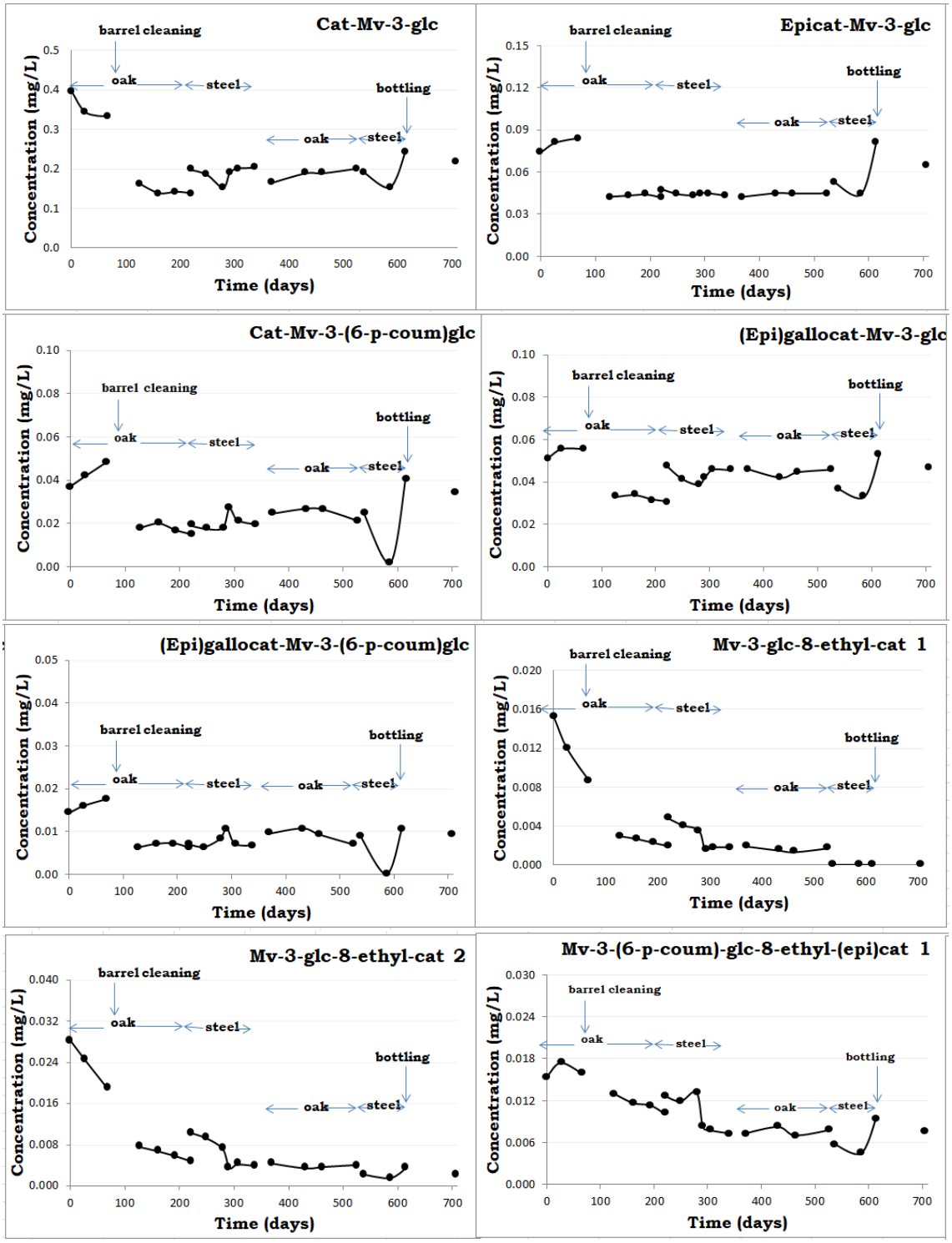
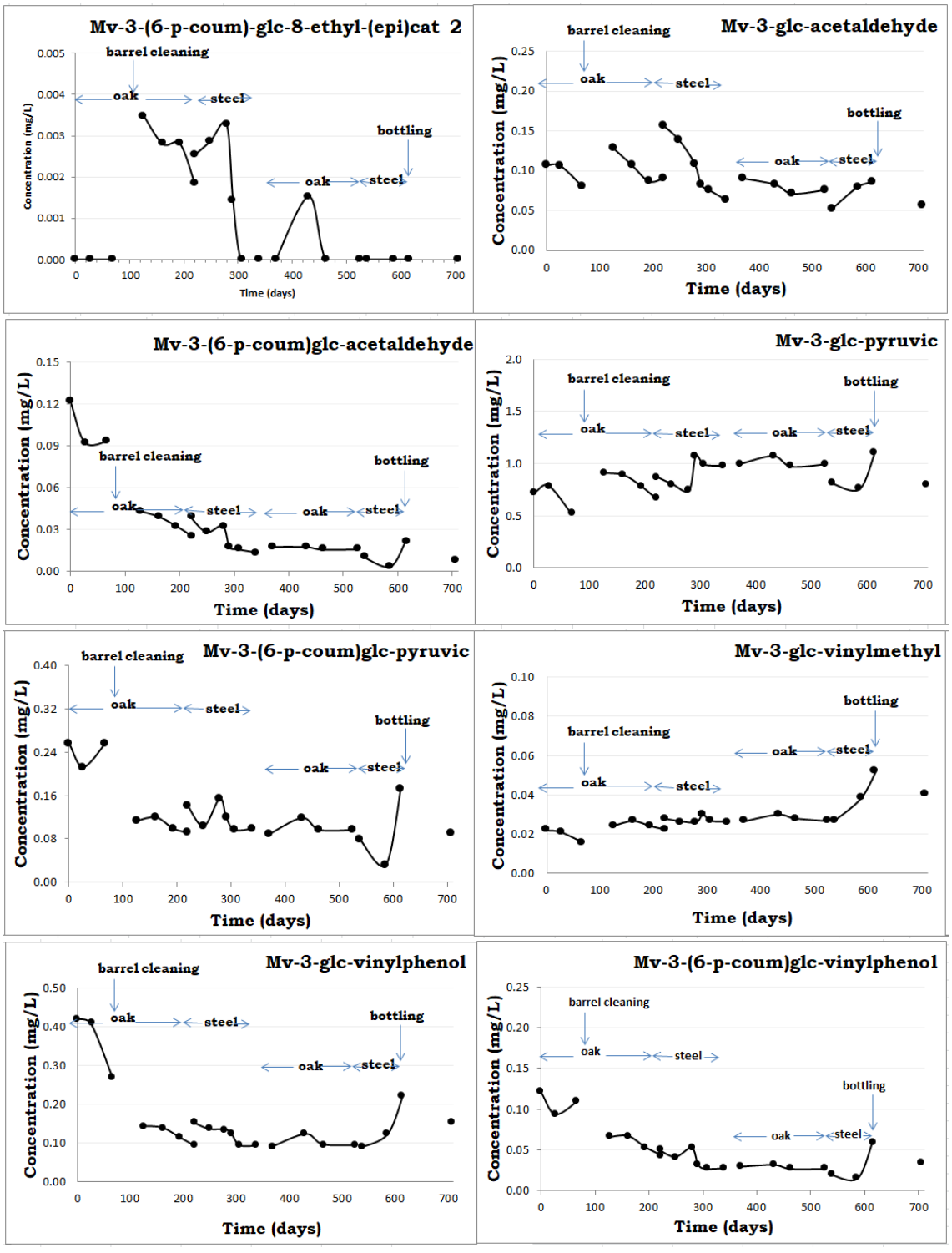
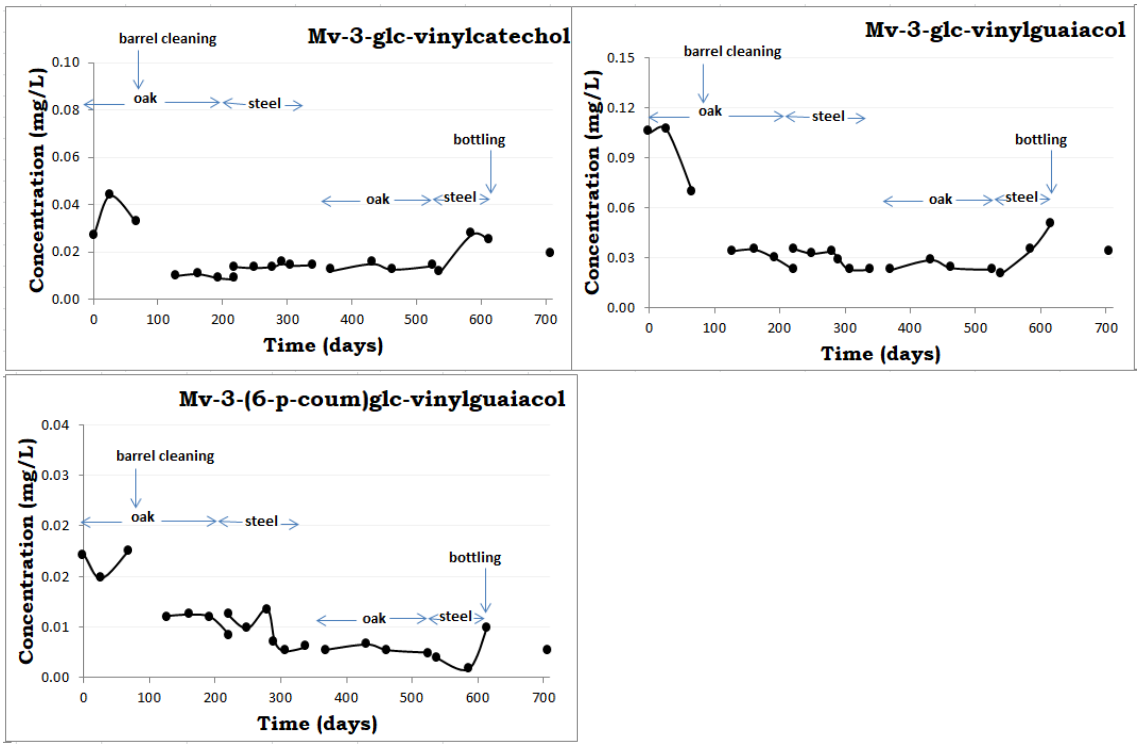



Figure Annex I.3 Evolution profiles for the anthocyanin derivatives in a wine of 2012 qualified as *Reserva*.




Continuation of Figure Annex I.3.




Continuation of Figure Annex I.3.

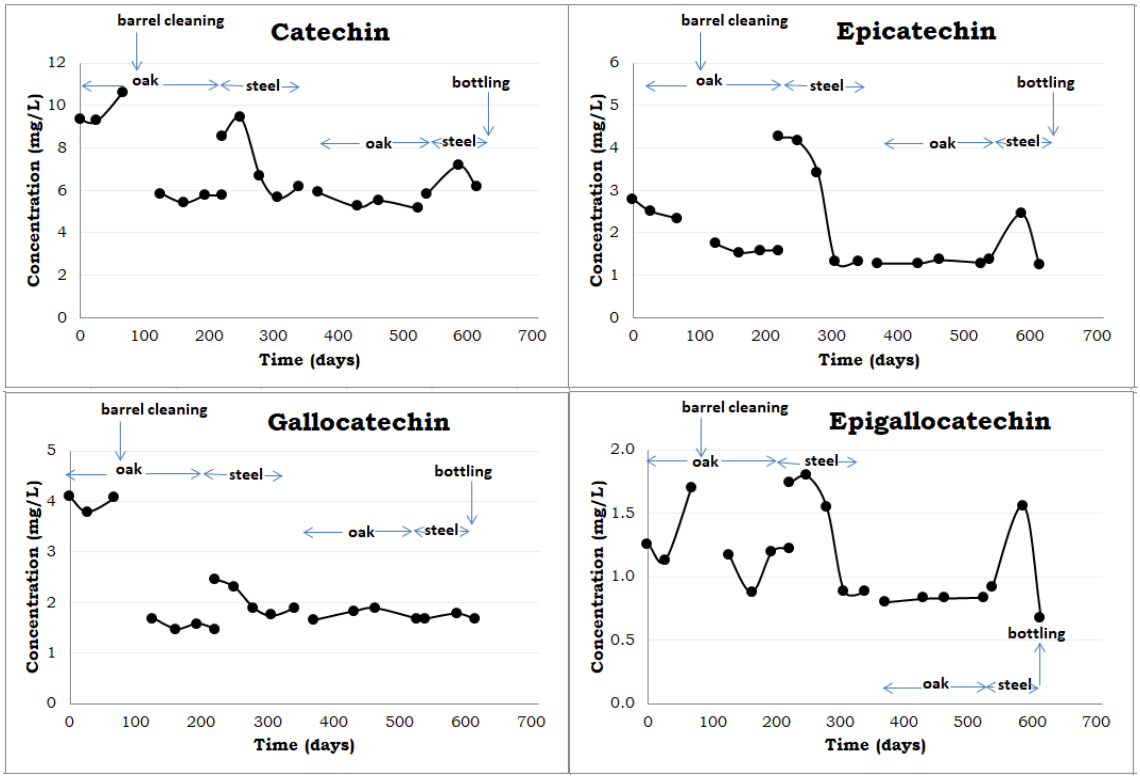
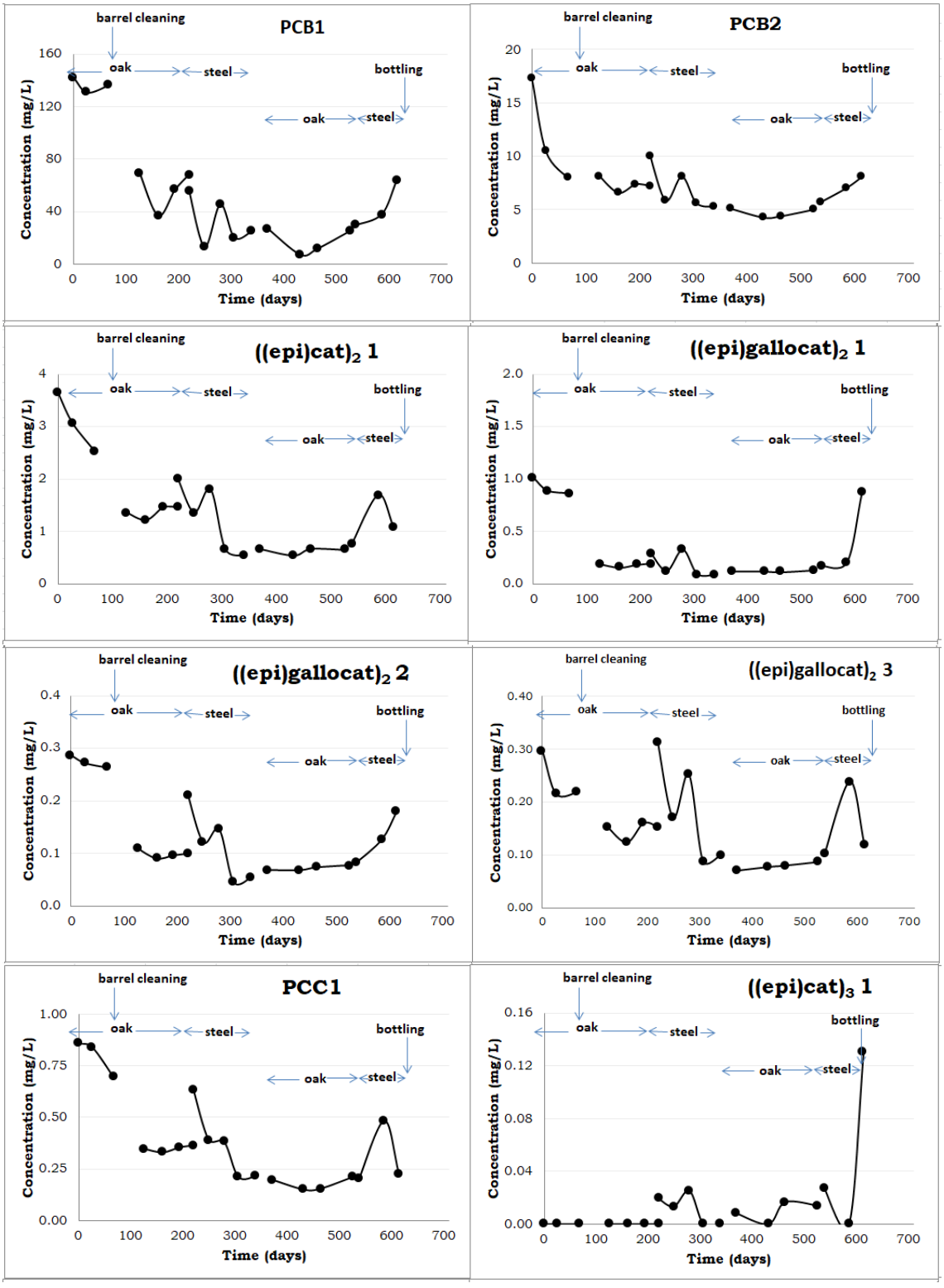
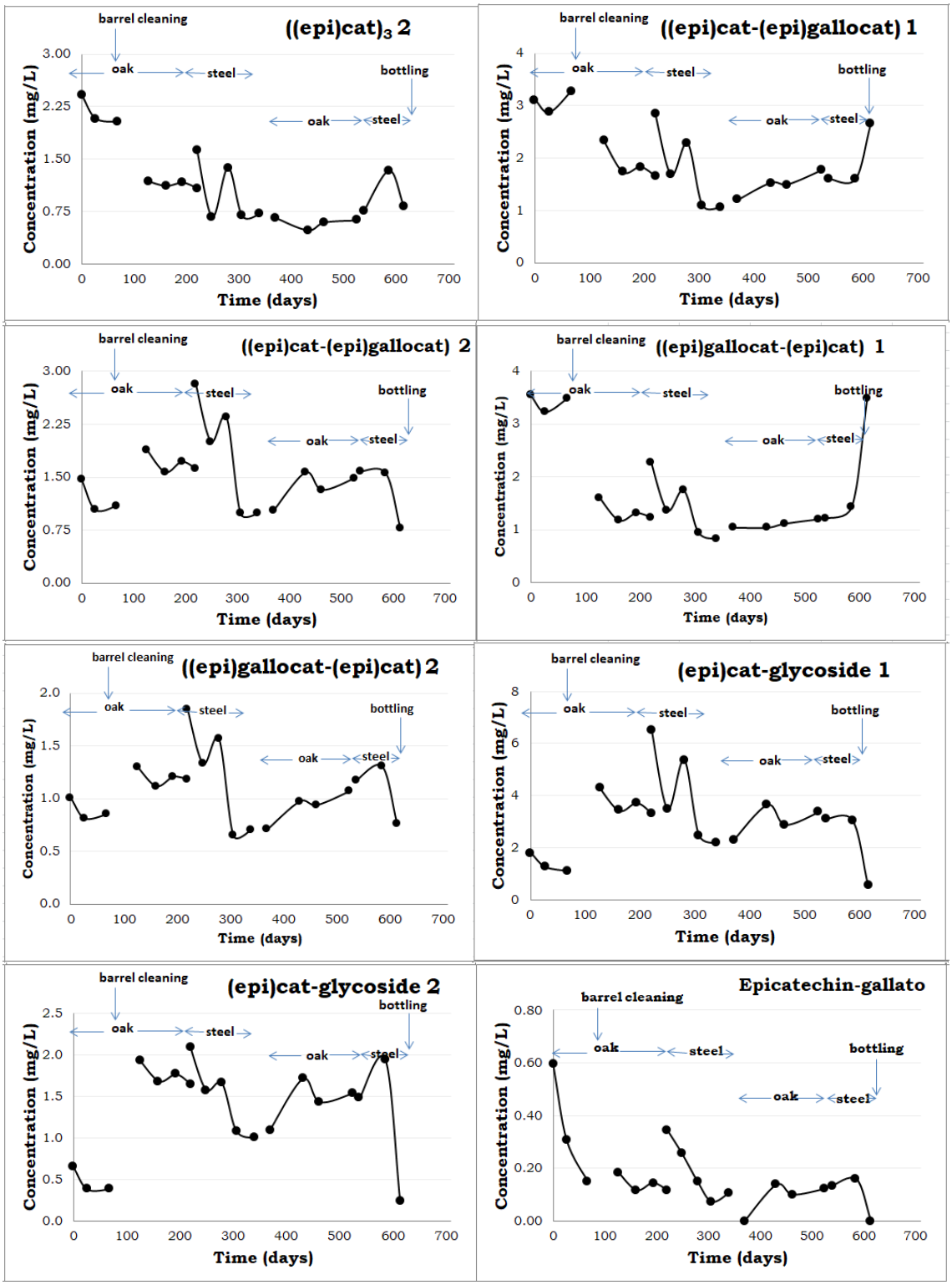


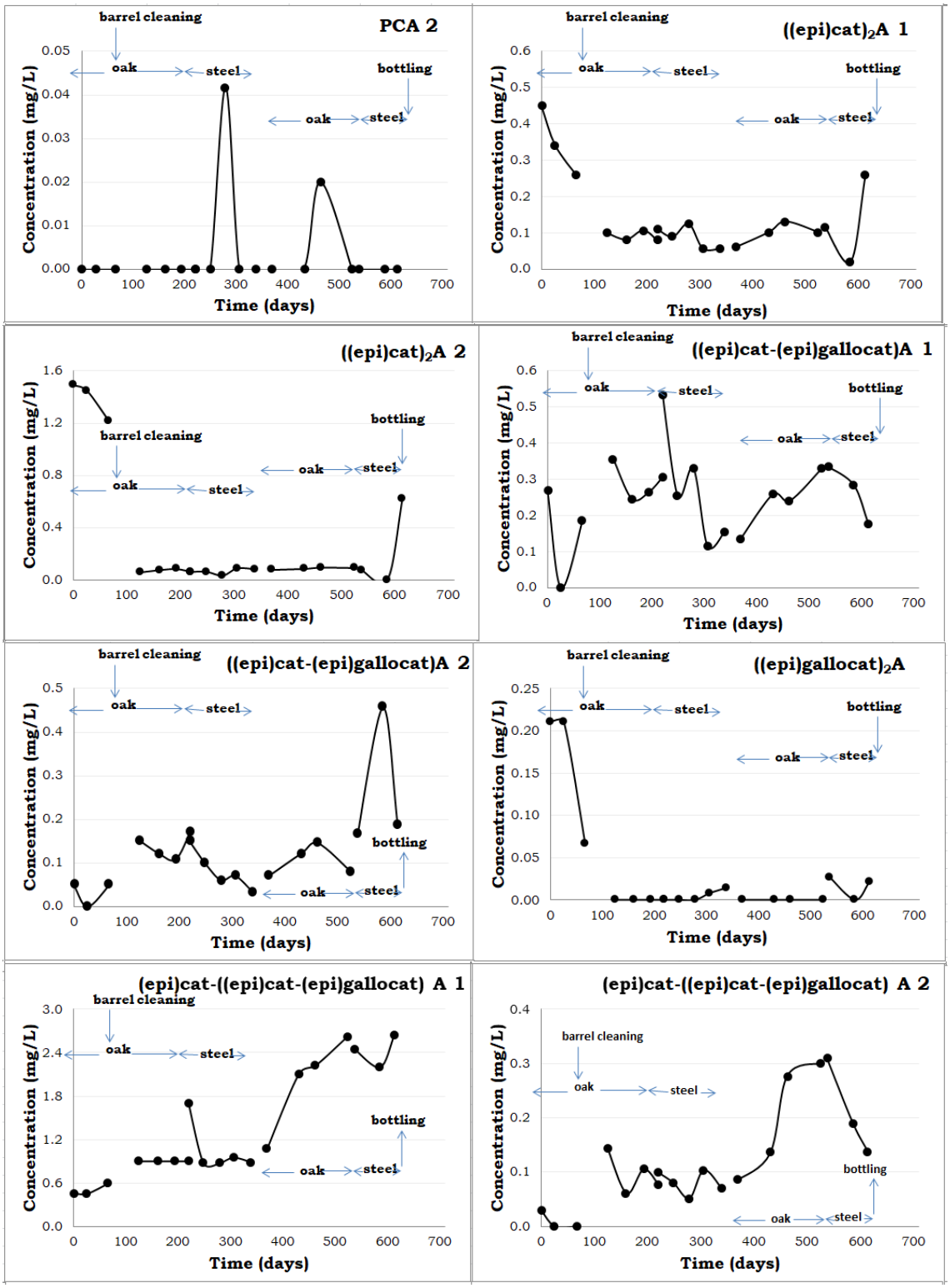

Figure Annex I.4 Evolution profiles for the tannins in a wine of 2012 qualified as *Reserva*.



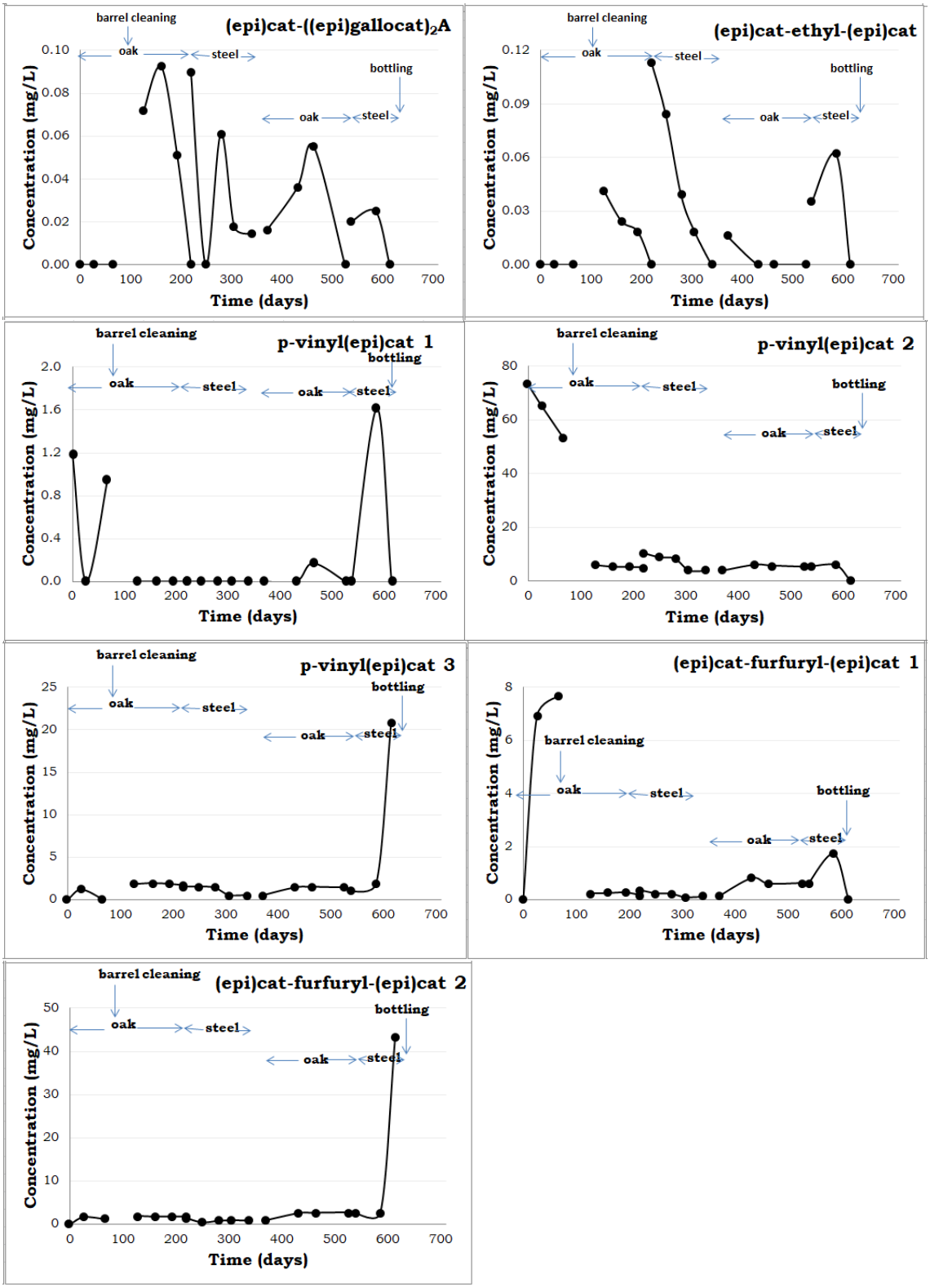

Continuation of Figure Annex I.4.




Continuation of Figure Annex I.4.




Continuation of Figure Annex I.4.




Continuation of Figure Annex I.4.

# ANNEX II

Table Annex II.1 Anthocyanins concentrations of the samples from Rioja Alavesa.

| Sample | Dp-3-glc | Cy-3-glc | Pt-3-glc | Pn-3-glc | Mv-3-glc | Dp-3-(6-Ac)-glc | Pt-3-(6-Ac)-glc | Pn-3-(6-Ac)-glc | Mv-3-(6-Ac)-glc | Mv-3-(6-caff)-glc | Pt-3-(6-p-coum)-glc | Pn-3-(6-p-coum)-glc | Mv-3-(6-p-coum)-glc |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **RV-J-01** | 13.12 | 0.50 | 23.64 | 3.67 | 113.55 | 1.67 | 0.30 | 0.45 | 10.29 | 0.97 | 2.88 | 2.14 | 14.05 |
| **RV-J-02** | 3.75 | 0.27 | 6.64 | 1.93 | 29.77 | 1.54 | 0.12 | 0.08 | 3.16 | 0.28 | 0.98 | 0.50 | 3.69 |
| **RV-J-03** | 27.26 | 3.29 | 32.08 | 11.20 | 114.14 | 1.41 | 0.12 | 0.38 | 6.00 | 0.71 | 1.91 | 1.53 | 9.09 |
| **RV-J-04** | 13.26 | 0.84 | 17.40 | 4.48 | 70.33 | 1.56 | 0.09 | 0.25 | 6.30 | 0.51 | 1.41 | 1.10 | 6.63 |
| **RV-J-05** | 12.85 | 0.64 | 17.15 | 3.59 | 73.24 | 1.82 | 0.08 | 0.22 | 6.96 | 0.41 | 1.76 | 0.69 | 7.64 |
| **RV-J-06** | 22.73 | 2.30 | 28.72 | 10.41 | 111.33 | 1.45 | 0.13 | 1.00 | 12.44 | 0.60 | 2.10 | 1.57 | 9.72 |
| **RV-J-07** | 23.01 | 3.53 | 23.62 | 10.74 | 78.93 | 0.67 | 0.12 | 0.37 | 6.46 | 0.64 | 1.88 | 1.29 | 8.55 |
| **RV-J-08** | 14.58 | 0.75 | 17.56 | 4.19 | 67.88 | 1.91 | 0.09 | 0.26 | 6.85 | 0.40 | 1.83 | 0.76 | 8.03 |
| **RV-C-09** | 7.09 | 0.51 | 8.87 | 1.78 | 36.32 | 1.70 | 0.16 | <0.005 | 3.75 | 0.51 | 1.34 | 0.97 | 4.40 |
| **RV-C-10** | 8.57 | 0.76 | 12.53 | 3.48 | 45.68 | 2.32 | 0.31 | 0.15 | 4.76 | 0.60 | 1.84 | 1.25 | 6.40 |
| **RV-C-11** | 10.48 | 0.82 | 15.98 | 4.66 | 67.03 | 2.19 | 0.41 | 0.28 | 6.77 | 0.72 | 2.18 | 1.43 | 8.00 |
| **RV-C-12** | 16.05 | 0.77 | 24.84 | 4.50 | 108.08 | 1.73 | 0.42 | 0.46 | 10.75 | 1.29 | 3.35 | 1.99 | 15.40 |
| **RV-C-13** | 8.90 | 0.71 | 13.91 | 3.70 | 55.24 | 1.79 | 0.26 | 0.33 | 6.22 | 0.57 | 1.83 | 1.34 | 7.19 |
| **RV-C-14** | 4.42 | 0.38 | 6.62 | 2.18 | 29.25 | 1.98 | 0.14 | 0.10 | 2.81 | 0.29 | 0.82 | 0.51 | 3.13 |
| **RV-C-15** | 9.95 | 0.95 | 12.29 | 3.25 | 41.94 | 2.45 | 0.35 | 0.17 | 4.50 | 0.49 | 1.49 | 0.84 | 4.86 |
| **RV-C-16** | 7.72 | 0.59 | 8.95 | 2.18 | 32.27 | 1.33 | 0.07 | 0.14 | 2.99 | 0.35 | 0.78 | 0.74 | 3.37 |
| **RV-R-17** | 6.44 | 0.60 | 6.68 | 2.32 | 25.92 | 2.56 | 0.16 | 0.08 | 2.46 | 0.40 | 0.78 | 0.68 | 2.67 |
| **RV-R-18** | 4.62 | 0.35 | 5.98 | 2.04 | 22.99 | 2.98 | 0.14 | 0.08 | 2.78 | 0.36 | 1.35 | 0.62 | 4.10 |
| **RV-R-19** | 4.98 | 0.41 | 4.96 | 1.77 | 20.63 | 2.55 | 0.17 | 0.03 | 2.36 | 0.32 | 0.86 | 0.50 | 3.02 |
| **RV-R-20** | 8.66 | 0.64 | 12.08 | 2.85 | 42.14 | 2.00 | 0.27 | 0.16 | 3.86 | 0.55 | 1.70 | 0.97 | 5.61 |
| **RV-R-21** | 3.93 | 0.37 | 3.78 | 1.47 | 13.47 | 1.70 | 0.07 | 0.06 | 1.77 | 0.31 | 0.50 | 0.57 | 1.94 |
| **RV-R-22** | 1.65 | 0.16 | 1.85 | 0.63 | 6.94 | 1.88 | 0.04 | 0.02 | 0.83 | 0.16 | 0.25 | 0.31 | 0.95 |
| **RV-R-23** | 13.33 | 1.33 | 13.98 | 3.79 | 45.72 | 2.46 | 0.43 | 0.20 | 4.78 | 0.55 | 1.67 | 0.98 | 5.24 |
| **RV-R-24** | 9.11 | 0.57 | 11.25 | 2.61 | 43.93 | 2.20 | 0.09 | 0.20 | 4.11 | 0.31 | 1.00 | 0.52 | 4.26 |
| **RV-GR-25** | 5.99 | 0.53 | 5.83 | 2.52 | 26.21 | 2.23 | 0.18 | 0.13 | 3.58 | 0.33 | 1.28 | 0.61 | 3.45 |
| **RV-GR-26** | 2.89 | 0.13 | 2.74 | 1.28 | 8.66 | 2.91 | 0.03 | 0.03 | 1.48 | 0.15 | 0.38 | 0.31 | 1.49 |
| **RV-GR-27** | 0.40 | 0.06 | 0.41 | 0.16 | 2.10 | 2.43 | 0.02 | 0.05 | 0.87 | 0.06 | 0.14 | 0.21 | 0.32 |
| **RV-GR-28** | 0.17 | 0.03 | 0.23 | 0.08 | 1.51 | 1.66 | 0.04 | 0.03 | 0.48 | 0.23 | 0.14 | 0.56 | 0.17 |
| **RV-GR-29** | 1.98 | 0.23 | 2.23 | 0.55 | 5.82 | 2.15 | 0.07 | 0.05 | 0.92 | 0.20 | 0.21 | 0.42 | 0.69 |
| **RV-GR-30** | 6.91 | 0.74 | 7.26 | 2.71 | 26.63 | 2.83 | 0.24 | 0.03 | 2.46 | 0.30 | 0.79 | 0.24 | 3.14 |
| **RV-GR-31** | 1.16 | 0.19 | 1.19 | 0.55 | 5.82 | 2.52 | 0.01 | 0.05 | 1.09 | 0.15 | 0.19 | 0.16 | 0.57 |
| **RV-GR-32** | 0.22 | 0.04 | 0.33 | 0.12 | 1.70 | 1.90 | 0.03 | 0.05 | 0.49 | 0.07 | 0.16 | 0.12 | 0.19 |

Table Annex II.2 Anthocyanins concentrations of the samples from Rioja Alta.

| Sample | Dp-3-glc | Cy-3-glc | Pt-3-glc | Pn-3-glc | Mv-3-glc | Dp-3-(6-Ac)-glc | Pt-3-(6-Ac)-glc | Pn-3-(6-Ac)-glc | Mv-3-(6-Ac)-glc | Mv-3-(6-caff)-glc | Pt-3-(6-p-coum)-glc | Pn-3-(6-p-coum)-glc | Mv-3-(6-p-coum)-glc |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **RA-J-33** | 25.27 | 2.93 | 29.68 | 9.47 | 108.59 | 1.55 | 0.71 | 0.45 | 8.30 | 1.01 | 2.69 | 1.89 | 10.67 |
| **RA-J-34** | 7.85 | 0.48 | 10.86 | 2.77 | 41.71 | 1.50 | 0.05 | 0.15 | 4.01 | 0.42 | 1.10 | 0.71 | 4.80 |
| **RA-J-35** | 9.01 | 0.75 | 10.89 | 2.71 | 39.61 | 1.17 | 0.05 | 0.19 | 3.52 | 0.42 | 1.06 | 0.82 | 4.61 |
| **RA-J-36** | 8.45 | 1.01 | 7.89 | 3.21 | 25.28 | 2.19 | 0.03 | 0.04 | 1.92 | 0.26 | 0.58 | 0.46 | 2.13 |
| **RA-J-37** | 8.42 | 0.52 | 9.69 | 2.11 | 4.00 | 1.30 | 0.08 | 0.14 | 3.37 | 0.30 | 0.81 | 0.43 | 3.39 |
| **RA-J-38** | 2.22 | 0.35 | 2.12 | 1.86 | 15.35 | 2.90 | 0.01 | 0.06 | 2.43 | 0.21 | 0.54 | 0.34 | 1.73 |
| **RA-J-39** | 10.55 | 0.55 | 16.76 | 4.83 | 71.15 | 1.23 | 0.06 | 0.38 | 7.55 | 0.59 | 2.03 | 1.19 | 9.22 |
| **RA-J-40** | 1.00 | 0.08 | 1.20 | 0.38 | 5.50 | 1.29 | 0.01 | 0.03 | 0.80 | 0.13 | 0.18 | 0.32 | 0.75 |
| **RA-C-41** | 9.01 | 0.75 | 14.19 | 3.91 | 53.30 | 1.94 | 0.27 | 0.17 | 5.02 | 0.64 | 1.93 | 1.31 | 7.11 |
| **RA-C-42** | 5.60 | 0.59 | 5.92 | 2.02 | 25.00 | 1.84 | 0.19 | 0.01 | 2.41 | 0.38 | 0.68 | 0.79 | 2.59 |
| **RA-C-43** | 6.74 | 0.33 | 10.40 | 2.16 | 37.67 | 1.27 | 0.05 | 0.14 | 3.94 | 0.39 | 1.00 | 0.70 | 4.17 |
| **RA-C-44** | 7.53 | 0.53 | 8.35 | 2.15 | 31.34 | 0.96 | 0.04 | 0.11 | 2.95 | 0.32 | 0.81 | 0.55 | 3.49 |
| **RA-C-45** | 5.04 | 0.32 | 5.41 | 1.26 | 19.69 | 0.96 | 0.03 | 0.07 | 1.71 | 0.22 | 0.55 | 0.38 | 2.37 |
| **RA-C-46** | 3.40 | 0.23 | 3.75 | 1.45 | 17.64 | 1.36 | 0.07 | 0.09 | 1.76 | 0.21 | 0.49 | 0.41 | 1.90 |
| **RA-C-47** | 5.53 | 0.24 | 10.13 | 2.19 | 41.46 | 1.20 | 0.06 | 0.18 | 4.66 | 0.35 | 1.25 | 0.65 | 5.96 |
| **RA-C-48** | 4.41 | 0.20 | 7.87 | 2.33 | 36.58 | 1.42 | 0.07 | 0.18 | 3.66 | 0.35 | 1.06 | 0.79 | 5.52 |
| **RA-R-49** | 0.65 | 0.07 | 0.86 | 0.19 | 2.85 | 0.85 | 0.04 | 0.02 | 0.41 | 0.33 | 0.08 | 0.91 | 0.26 |
| **RA-R-50** | 0.32 | 0.03 | 0.48 | 0.08 | 2.21 | 0.91 | 0.03 | 0.02 | 0.39 | 0.21 | 0.09 | 0.53 | 0.21 |
| **RA-R-51** | 2.39 | 0.29 | 2.46 | 0.94 | 8.24 | 2.21 | 0.02 | 0.03 | 1.23 | 0.31 | 0.32 | 0.62 | 0.93 |
| **RA-R-52** | 2.65 | 0.18 | 2.94 | 0.70 | 11.83 | 2.15 | 0.02 | 0.03 | 1.40 | 0.13 | 0.34 | 0.25 | 1.32 |
| **RA-R-53** | 5.10 | 0.42 | 4.96 | 1.40 | 16.61 | 2.18 | 0.03 | 0.04 | 1.95 | 0.26 | 0.51 | 0.40 | 1.89 |
| **RA-R-54** | 4.89 | 0.39 | 5.14 | 1.28 | 18.40 | 1.09 | 0.04 | 0.02 | 1.98 | 0.22 | 0.57 | 0.32 | 2.22 |
| **RA-R-55** | 0.61 | 0.06 | 0.75 | 0.20 | 2.73 | 1.06 | 0.02 | 0.02 | 0.34 | 0.12 | 0.08 | 0.26 | 0.30 |
| **RA-R-56** | 5.23 | 0.24 | 6.01 | 2.04 | 29.25 | 1.04 | 0.03 | 0.14 | 3.01 | 0.33 | 1.05 | 0.68 | 4.35 |
| **RA-GR-57** | 4.56 | 0.49 | 4.53 | 2.07 | 19.32 | 2.53 | 0.11 | 0.03 | 1.99 | 0.28 | 0.71 | 0.44 | 2.46 |
| **RA-GR-58** | 0.11 | 0.02 | 0.15 | 0.05 | 1.01 | 0.45 | 0.02 | 0.01 | 0.20 | 0.16 | 0.05 | 0.42 | 0.07 |
| **RA-GR-59** | 0.07 | 0.02 | 0.12 | 0.04 | 0.78 | 0.88 | 0.02 | 0.02 | 0.33 | 0.16 | 0.10 | 0.52 | 0.12 |
| **RA-GR-60** | 0.46 | 0.08 | 0.52 | 0.13 | 1.94 | 1.56 | 0.05 | 0.06 | 0.49 | 0.15 | 0.11 | 0.39 | 0.16 |
| **RA-GR-61** | 2.46 | 0.26 | 2.36 | 0.68 | 7.84 | 2.37 | 0.03 | 0.05 | 0.73 | 0.12 | 0.17 | 0.20 | 0.49 |
| **RA-GR-62** | 4.66 | 0.43 | 4.46 | 1.27 | 14.74 | 1.18 | 0.03 | 0.02 | 1.51 | 0.22 | 0.40 | 0.32 | 1.51 |
| **RA-GR-63** | 2.14 | 0.18 | 2.39 | 0.68 | 9.43 | 0.98 | 0.04 | 0.02 | 0.89 | 0.15 | 0.25 | 0.30 | 1.03 |
| **RA-GR-64** | 4.67 | 0.22 | 5.34 | 1.65 | 25.38 | 1.06 | 0.08 | 0.10 | 2.76 | 0.30 | 0.89 | 0.57 | 3.59 |

Table Annex II.3 Anthocyanins concentrations of the samples from Rioja Baja.

| Sample | Dp-3-glc | Cy-3-glc | Pt-3-glc | Pn-3-glc | Mv-3-glc | Dp-3-(6-Ac)-glc | Pt-3-(6-Ac)-glc | Pn-3-(6-Ac)-glc | Mv-3-(6-Ac)-glc | Mv-3-(6-caff)-glc | Pt-3-(6-p-coum)-glc | Pn-3-(6-p-coum)-glc | Mv-3-(6-p-coum)-glc |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **RB-J-65** | 10.34 | 0.61 | 17.85 | 7.14 | 104.26 | 1.34 | 0.33 | 1.63 | 11.79 | 0.84 | 2.90 | 2.76 | 14.18 |
| **RB-J-66** | 37.67 | 4.02 | 44.66 | 17.10 | 176.04 | 2.30 | 0.77 | 0.82 | 11.63 | 1.53 | 3.75 | 2.69 | 16.98 |
| **RB-J-67** | 13.44 | 2.17 | 17.97 | 14.11 | 74.98 | 0.99 | 0.13 | 1.14 | 7.53 | 0.59 | 1.31 | 1.80 | 6.61 |
| **RB-J-68** | 12.63 | 0.92 | 18.61 | 6.43 | 77.63 | 1.11 | 0.25 | 0.30 | 5.53 | 0.53 | 1.76 | 1.19 | 9.01 |
| **RB-J-69** | 23.43 | 2.30 | 34.22 | 12.35 | 126.24 | 1.53 | 0.11 | 0.59 | 9.48 | 1.02 | 2.90 | 2.03 | 12.28 |
| **RB-J-70** | 21.59 | 2.27 | 29.11 | 12.16 | 112.22 | 0.92 | 0.10 | 0.54 | 7.79 | 0.91 | 2.47 | 1.85 | 11.46 |
| **RB-J-71** | 18.91 | 2.55 | 20.74 | 9.59 | 79.30 | 1.31 | 0.11 | 0.31 | 4.21 | 0.57 | 1.39 | 1.10 | 6.77 |
| **RB-J-72** | 5.16 | 0.32 | 6.61 | 1.81 | 25.41 | 0.63 | 0.04 | 0.14 | 2.50 | 0.35 | 0.54 | 1.16 | 2.85 |
| **RB-C-73** | 5.70 | 0.49 | 7.29 | 3.41 | 39.44 | 1.98 | 0.20 | 0.23 | 3.62 | 0.35 | 1.29 | 1.30 | 4.38 |
| **RB-C-74** | 5.43 | 0.72 | 5.78 | 2.30 | 20.20 | 2.59 | 0.09 | 0.06 | 2.33 | 0.39 | 0.69 | 0.63 | 2.58 |
| **RB-C-75** | 10.88 | 0.77 | 15.38 | 4.49 | 66.94 | 2.12 | 0.28 | 0.25 | 6.67 | 2.11 | 2.11 | 1.45 | 8.03 |
| **RB-C-76** | 7.94 | 0.70 | 9.74 | 3.51 | 44.01 | 1.95 | 0.25 | 0.17 | 4.05 | 0.49 | 1.45 | 0.96 | 5.42 |
| **RB-C-77** | 3.20 | 0.33 | 3.97 | 1.26 | 15.64 | 1.66 | 0.05 | 0.12 | 1.72 | 0.19 | 0.25 | 0.26 | 1.61 |
| **RB-C-78** | 12.34 | 1.14 | 17.15 | 11.45 | 78.21 | 1.88 | 0.11 | 0.82 | 6.61 | 0.51 | 1.58 | 1.82 | 8.68 |
| **RB-C-79** | 7.99 | 0.56 | 8.33 | 2.33 | 32.35 | 1.16 | 0.12 | 0.13 | 3.14 | 0.34 | 1.03 | 0.47 | 4.52 |
| **RB-C-80** | 1.31 | 0.12 | 1.81 | 0.69 | 9.63 | 1.22 | 0.03 | 0.02 | 0.90 | 0.14 | 0.28 | 0.45 | 1.01 |
| **RB-R-81** | 3.54 | 0.27 | 5.09 | 1.86 | 28.92 | 2.08 | 0.07 | 0.10 | 2.76 | 0.30 | 0.69 | 0.80 | 2.82 |
| **RB-R-82** | 6.56 | 0.71 | 6.19 | 2.36 | 21.54 | 3.12 | 0.10 | 0.07 | 2.77 | 0.39 | 0.81 | 0.69 | 3.06 |
| **RB-R-83** | 13.64 | 0.79 | 18.05 | 4.71 | 81.58 | 1.99 | 0.35 | 0.30 | 8.30 | 0.81 | 2.54 | 1.68 | 9.64 |
| **RB-R-84** | 8.56 | 0.72 | 10.26 | 3.42 | 44.29 | 2.04 | 0.23 | 0.16 | 4.19 | 0.46 | 1.40 | 0.87 | 4.81 |
| **RB-R-85** | 0.97 | 0.13 | 1.19 | 0.82 | 5.83 | 1.47 | 0.11 | 0.08 | 0.55 | 0.12 | 0.17 | 0.29 | 0.64 |
| **RB-R-86** | 0.11 | 0.05 | 0.12 | 0.06 | 0.76 | 0.77 | 0.03 | 0.02 | 0.24 | 0.07 | 0.12 | 0.14 | 0.12 |
| **RB-R-87** | 5.67 | 0.43 | 6.17 | 1.46 | 21.96 | 1.42 | 0.05 | 0.03 | 2.50 | 0.31 | 0.76 | 0.45 | 2.98 |
| **RB-R-88** | 0.73 | 0.07 | 1.02 | 0.44 | 5.66 | 1.01 | 0.03 | 0.02 | 0.60 | 0.15 | 0.15 | 0.51 | 0.62 |
| **RB-GR-89** | 3.96 | 0.33 | 5.57 | 2.61 | 31.91 | 1.95 | 0.11 | 0.18 | 3.07 | 0.31 | 0.79 | 0.92 | 3.38 |
| **RB-GR-90** | 3.13 | 0.31 | 3.40 | 1.52 | 14.92 | 1.96 | 0.04 | 0.02 | 1.71 | 0.25 | 0.45 | 0.37 | 2.07 |
| **RB-GR-91** | 0.06 | 0.01 | 0.08 | 0.03 | 0.53 | 0.32 | 0.02 | 0.01 | 0.19 | 0.12 | 0.03 | 0.29 | 0.07 |
| **RB-GR-92** | 0.61 | 0.10 | 0.79 | 0.23 | 3.53 | 0.62 | 0.01 | 0.01 | 0.34 | 0.12 | 0.10 | 0.20 | 0.25 |
| **RB-GR-93** | 2.16 | 0.20 | 2.48 | 0.61 | 11.16 | 2.20 | 0.02 | 0.01 | 1.58 | 0.13 | 0.25 | 0.24 | 1.29 |
| **RB-GR-94** | 0.36 | 0.04 | 0.48 | 0.20 | 2.42 | 0.67 | 0.02 | 0.03 | 0.28 | 0.12 | 0.08 | 0.33 | 0.20 |
| **RB-GR-95** | 2.63 | 0.21 | 3.13 | 0.95 | 14.21 | 0.99 | 0.02 | 0.02 | 0.97 | 0.14 | 0.26 | 0.27 | 1.00 |
| **RB-GR-96** | 0.86 | 0.11 | 1.04 | 0.37 | 5.06 | 1.11 | 0.05 | 0.03 | 0.58 | 0.17 | 0.12 | 0.44 | 0.36 |

Table Annex II.4 Anthocyanins concentrations of the samples from Médoc.

| Sample | Dp-3-glc | Cy-3-glc | Pt-3-glc | Pn-3-glc | Mv-3-glc | Dp-3-(6-Ac)-glc | Pt-3-(6-Ac)-glc | Pn-3-(6-Ac)-glc | Mv-3-(6-Ac)-glc | Mv-3-(6-caff)-glc | Pt-3-(6-p-coum)-glc | Pn-3-(6-p-coum)-glc | Mv-3-(6-p-coum)-glc |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BO-M-01 | 2.13 | 0.43 | 2.43 | 0.98 | 11.72 | 2.19 | 0.02 | 0.25 | 3.38 | 0.22 | 0.22 | 0.32 | 1.29 |
| BO-M-02 | 6.37 | 1.61 | 5.84 | 4.11 | 23.82 | 2.60 | 0.09 | 0.54 | 4.53 | 0.31 | 0.43 | 0.62 | 2.55 |
| BO-M-03 | 1.32 | 0.27 | 1.77 | 0.65 | 13.28 | 3.25 | 0.02 | 0.26 | 4.66 | 0.19 | 0.22 | 0.52 | 1.34 |
| BO-M-04 | 1.76 | 0.44 | 2.60 | 1.21 | 20.33 | 3.11 | <0.01 | 0.37 | 7.63 | 0.15 | 0.16 | 0.43 | 1.75 |
| BO-M-05 | 2.74 | 0.38 | 2.84 | 0.92 | 13.98 | 2.44 | 0.03 | 0.30 | 4.27 | 0.19 | 0.18 | 0.43 | 1.31 |
| BO-M-06 | 6.76 | 1.28 | 6.09 | 2.72 | 24.27 | 1.77 | 0.03 | 0.64 | 7.66 | 0.31 | 0.37 | 0.50 | 2.38 |
| BO-M-07 | 1.00 | 0.30 | 1.00 | 0.63 | 4.78 | 1.32 | 0.01 | 0.06 | 0.85 | 0.18 | 0.12 | 0.25 | 0.40 |
| BO-M-08 | 0.25 | 0.13 | 0.40 | 0.20 | 2.36 | 1.78 | 0.03 | 0.06 | 0.68 | 0.15 | 0.10 | 0.24 | 0.22 |
| BO-M-09 | 1.09 | 0.25 | 1.14 | 0.50 | 5.17 | 1.07 | <0.01 | 0.10 | 0.89 | 0.14 | 0.11 | 0.32 | 0.39 |
| BO-M-10 | 1.00 | 0.21 | 0.84 | 0.45 | 9.68 | 0.84 | 0.02 | 0.17 | 2.68 | 0.15 | 0.17 | 0.39 | 0.72 |
| BO-M-11 | 2.56 | 0.45 | 2.35 | 1.09 | 10.18 | 2.14 | 0.01 | 0.25 | 2.49 | 0.23 | 0.19 | 0.50 | 0.90 |
| BO-M-12 | 1.74 | 0.28 | 1.81 | 0.65 | 10.47 | 1.46 | 0.01 | 0.17 | 2.86 | 0.15 | 0.14 | 0.34 | 0.92 |
| BO-M-13A | 2.20 | 0.35 | 2.37 | 0.80 | 13.14 | 2.86 | 0.04 | 0.24 | 2.77 | 0.24 | 0.38 | 0.51 | 1.18 |
| BO-M-14A | 0.25 | 0.06 | 0.29 | 0.16 | 2.32 | 1.83 | 0.01 | 0.06 | 0.67 | 0.16 | 0.09 | 0.50 | 0.18 |
| BO-M-15A | 2.07 | 0.35 | 1.77 | 0.92 | 18.38 | 3.78 | 0.01 | 0.36 | 7.15 | 0.15 | 0.18 | 0.39 | 1.62 |
| BO-M-16A | 2.53 | 0.63 | 2.48 | 1.13 | 10.46 | 1.66 | 0.23 | 0.27 | 3.18 | 0.21 | 0.24 | 0.54 | 0.84 |
| BO-M-17A | 0.58 | 0.18 | 0.60 | 0.27 | 2.39 | 1.27 | <0.01 | 0.05 | 0.51 | 0.19 | 0.10 | 0.36 | 0.23 |
| BO-M-18A | 3.08 | 0.44 | 3.38 | 1.18 | 18.42 | 1.74 | 0.02 | 0.44 | 6.07 | 0.23 | 0.28 | 0.44 | 2.08 |
| BO-M-19A | 5.46 | 0.89 | 4.77 | 2.59 | 19.06 | 1.44 | 0.03 | 0.39 | 4.40 | 0.26 | 0.33 | 0.42 | 1.99 |
| BO-M-20A | 1.64 | 0.30 | 2.07 | 0.64 | 12.12 | 2.60 | 0.03 | 0.24 | 3.93 | 0.19 | 0.21 | 0.33 | 1.25 |
| BO-M-21A | 3.57 | 0.69 | 3.41 | 1.64 | 13.98 | 2.29 | 0.03 | 0.24 | 2.68 | 0.19 | 0.29 | 0.39 | 1.74 |
| BO-M-22B | 0.82 | 0.21 | 0.90 | 0.42 | 4.48 | 1.66 | 0.01 | 0.10 | 1.26 | 0.20 | 0.11 | 0.45 | 0.40 |
| BO-M-23B | 1.51 | 0.31 | 1.99 | 0.69 | 15.56 | 2.65 | 0.01 | 0.30 | 5.72 | 0.18 | 0.14 | 0.48 | 1.20 |
| BO-M-24B | 3.03 | 0.46 | 3.20 | 1.13 | 18.83 | 2.42 | 0.03 | 0.34 | 5.89 | 0.15 | 0.19 | 0.35 | 1.45 |
| BO-M-25B | 0.29 | 0.10 | 0.30 | 0.22 | 1.58 | 0.87 | 0.02 | 0.07 | 0.29 | 0.15 | 0.09 | 0.27 | 0.16 |
| BO-M-26B | 0.65 | 0.18 | 0.89 | 0.43 | 6.29 | 2.28 | 0.01 | 0.07 | 1.75 | 0.19 | 0.11 | 0.59 | 0.54 |
| BO-M-27B | 0.05 | 0.02 | 0.08 | 0.05 | 0.61 | 0.57 | 0.05 | 0.05 | 0.24 | 0.18 | 0.07 | 0.55 | 0.10 |
| BO-M-28B | 0.12 | 0.03 | 0.20 | 0.12 | 1.77 | 1.55 | 0.01 | 0.10 | 0.60 | 0.17 | 0.08 | 0.43 | 0.19 |
| BO-M-29B | 1.48 | 0.27 | 1.45 | 0.58 | 8.30 | 2.23 | 0.01 | 0.14 | 2.16 | 0.25 | 0.12 | 0.62 | 0.59 |
| BO-M-30B | 1.25 | 0.30 | 1.71 | 0.60 | 9.17 | 2.20 | 0.03 | 0.16 | 2.50 | 0.14 | 0.12 | 0.27 | 0.68 |
| BO-M-31B | 2.39 | 0.43 | 2.14 | 1.40 | 8.85 | 0.71 | 0.01 | 0.33 | 2.30 | 0.30 | 0.17 | 0.70 | 0.74 |
| BO-M-32B | 3.60 | 0.53 | 3.92 | 1.63 | 19.13 | 1.56 | 0.04 | 0.50 | 6.52 | 0.26 | 0.32 | 0.52 | 2.49 |
| BO-M-33B | 0.32 | 0.12 | 0.32 | 0.16 | 1.95 | 1.39 | 0.04 | 0.03 | 0.53 | 0.19 | 0.08 | 0.60 | 0.15 |
| BO-M-34B | 8.39 | 1.15 | 7.73 | 4.33 | 30.97 | 1.50 | 0.07 | 0.98 | 8.99 | 0.41 | 0.62 | 0.74 | 3.57 |
| BO-M-35B | 3.31 | 0.46 | 3.74 | 1.34 | 17.61 | 1.97 | 0.04 | 0.32 | 4.46 | 0.20 | 0.33 | 0.36 | 1.89 |
| BO-M-36B | 2.09 | 0.39 | 2.42 | 0.98 | 10.79 | 2.16 | 0.02 | 0.20 | 2.29 | 0.19 | 0.25 | 0.35 | 1.40 |

Table Annex II.5 Anthocyanins concentrations of the samples from Graves.

| Sample | Dp-3-glc | Cy-3-glc | Pt-3-glc | Pn-3-glc | Mv-3-glc | Dp-3-(6-Ac)-glc | Pt-3-(6-Ac)-glc | Pn-3-(6-Ac)-glc | Mv-3-(6-Ac)-glc | Mv-3-(6-caff)-glc | Pt-3-(6-p-coum)-glc | Pn-3-(6-p-coum)-glc | Mv-3-(6-p-coum)-glc |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BO-G-37** | 2.85 | 0.48 | 3.22 | 1.06 | 16.65 | 1.37 | 0.07 | 0.36 | 6.36 | 0.17 | 0.22 | 0.34 | 1.47 |
| **BO-G-38** | 3.10 | 0.62 | 2.95 | 1.30 | 15.73 | 1.83 | 0.03 | 0.27 | 4.37 | 0.20 | 0.20 | 0.44 | 1.31 |
| **BO-G-39** | 3.22 | 0.50 | 3.35 | 1.17 | 16.07 | 2.22 | 0.03 | 0.35 | 4.70 | 0.17 | 0.30 | 0.38 | 1.59 |
| **BO-G-40** | 2.41 | 0.39 | 2.17 | 0.83 | 8.95 | 1.21 | <0.01 | 0.22 | 2.42 | 0.18 | 0.12 | 0.46 | 0.52 |
| **BO-G-41** | 2.42 | 0.48 | 2.41 | 0.97 | 10.68 | 2.57 | 0.02 | 0.19 | 2.72 | 0.16 | 0.23 | 0.26 | 0.90 |
| **BO-G-42** | 2.54 | 0.46 | 2.59 | 1.25 | 10.36 | 2.27 | <0.01 | 0.20 | 2.83 | 0.18 | 0.18 | 0.30 | 0.97 |
| **BO-G-43** | 0.16 | 0.10 | 0.29 | 0.14 | 1.37 | 0.71 | 0.08 | 0.05 | 0.41 | 0.19 | 0.09 | 0.22 | 0.19 |
| **BO-G-44B** | 2.52 | 0.40 | 2.71 | 0.82 | 16.74 | 2.41 | 0.02 | 0.28 | 5.32 | 0.13 | 0.24 | 0.34 | 1.45 |
| **BO-G-45B** | 3.54 | 0.59 | 3.11 | 1.43 | 11.98 | 1.48 | 0.05 | 0.36 | 3.41 | 0.23 | 0.21 | 0.36 | 0.92 |
| **BO-G-46B** | 0.95 | 0.22 | 1.28 | 0.50 | 5.74 | 0.94 | 0.02 | 0.14 | 1.31 | 0.19 | 0.12 | 0.45 | 0.47 |
| **BO-G-47B** | 2.83 | 0.42 | 4.01 | 1.40 | 24.31 | 1.88 | 0.04 | 0.94 | 7.90 | 0.25 | 0.32 | 0.60 | 2.14 |
| **BO-G-48B** | 3.80 | 0.49 | 3.98 | 1.73 | 20.45 | 1.87 | 0.02 | 0.25 | 4.62 | 0.15 | 0.33 | 0.29 | 2.02 |
| **BO-G-87** | 5.30 | 0.54 | 8.22 | 3.10 | 43.91 | 2.96 | 0.07 | 1.05 | 14.88 | 0.16 | 0.45 | 0.73 | 4.41 |



Table Annex II.6 Anthocyanins concentrations of the samples from Blayais & Bourgeais.

| Sample | Dp-3-glc | Cy-3-glc | Pt-3-glc | Pn-3-glc | Mv-3-glc | Dp-3-(6-Ac)-glc | Pt-3-(6-Ac)-glc | Pn-3-(6-Ac)-glc | Mv-3-(6-Ac)-glc | Mv-3-(6-caff)-glc | Pt-3-(6-p-coum)-glc | Pn-3-(6-p-coum)-glc | Mv-3-(6-p-coum)-glc |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BO-BB-74** | 1.19 | 0.29 | 1.56 | 0.70 | 6.55 | 2.20 | 0.02 | 0.09 | 1.29 | 0.15 | 0.24 | 0.13 | 0.78 |
| **BO-BB-82B** | 0.81 | 0.27 | 1.08 | 0.53 | 4.57 | 3.30 | 0.04 | 0.15 | 1.22 | 0.23 | 0.26 | 0.23 | 0.60 |
| **BO-BB-84** | 0.12 | 0.05 | 0.20 | 0.11 | 1.25 | 1.77 | 0.01 | 0.10 | 0.60 | 0.15 | 0.09 | 0.22 | 0.18 |
| **BO-BB-85** | 0.48 | 0.15 | 0.59 | 0.32 | 3.37 | 3.56 | <0.01 | 0.14 | 1.11 | 0.14 | 0.13 | 0.22 | 0.35 |
| **BO-BB-86** | 0.42 | 0.15 | 0.55 | 0.30 | 2.64 | 1.86 | 0.02 | 0.07 | 0.56 | 0.14 | 0.13 | 0.21 | 0.31 |

Table Annex II.8 Anthocyanins concentrations of the samples from Libournais.

| Sample | Dp-3-glc | Cy-3-glc | Pt-3-glc | Pn-3-glc | Mv-3-glc | Dp-3-(6-Ac)-glc | Pt-3-(6-Ac)-glc | Pn-3-(6-Ac)-glc | Mv-3-(6-Ac)-glc | Mv-3-(6-caff)-glc | Pt-3-(6-p-coum)-glc | Pn-3-(6-p-coum)-glc | Mv-3-(6-p-coum)-glc |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BO-L-49 | 0.22 | 0.11 | 0.37 | 0.23 | 1.88 | 1.32 | 0.04 | 0.08 | 0.54 | 0.21 | 0.14 | 0.31 | 0.32 |
| BO-L-50 | 3.57 | 0.92 | 3.16 | 1.84 | 10.06 | 1.88 | 0.10 | 0.20 | 1.62 | 0.21 | 0.31 | 0.27 | 0.98 |
| BO-L-51 | 3.78 | 0.33 | 1.51 | 0.88 | 9.80 | 3.39 | 0.01 | 0.17 | 2.56 | 0.13 | 0.21 | 0.27 | 0.90 |
| BO-L-52 | 0.66 | 0.20 | 0.62 | 0.46 | 3.89 | 1.42 | <0.01 | 0.05 | 0.72 | 0.11 | 0.11 | 0.14 | 0.38 |
| BO-L-53 | 2.96 | 0.45 | 3.41 | 1.41 | 0.55 | 0.87 | 0.11 | 0.35 | 4.05 | 0.18 | 0.26 | 0.41 | 1.95 |
| BO-L-54 | 0.02 | <0.01 | 0.04 | 0.03 | 0.24 | 0.17 | 0.03 | 0.04 | 0.12 | 0.13 | 0.02 | 0.43 | 0.07 |
| BO-L-55 | 0.32 | 0.15 | 0.53 | 0.26 | 2.97 | 2.77 | 0.01 | 0.11 | 1.22 | 0.18 | 0.13 | 0.17 | 0.37 |
| BO-L-56 | 4.92 | 0.82 | 5.25 | 2.32 | 23.30 | 2.76 | 0.06 | 0.68 | 7.29 | 0.26 | 0.35 | 0.50 | 2.35 |
| BO-L-57B | 2.88 | 0.58 | 2.54 | 2.04 | 9.48 | 0.80 | 0.02 | 0.39 | 2.00 | 0.29 | 0.21 | 0.55 | 0.96 |
| BO-L-58B | 3.11 | 0.57 | 3.30 | 1.61 | 13.74 | 1.41 | 0.03 | 0.36 | 3.35 | 0.27 | 0.29 | 0.51 | 1.70 |
| BO-L-59 | 3.96 | 0.75 | 4.00 | 1.83 | 14.40 | 1.97 | 0.07 | 0.41 | 3.54 | 0.38 | 0.43 | 0.47 | 1.80 |
| BO-L-60 | 2.22 | 0.46 | 2.44 | 0.98 | 9.86 | 2.07 | 0.02 | 0.17 | 1.93 | 0.21 | 0.23 | 0.37 | 1.12 |
| BO-L-61 | 1.12 | 0.45 | 1.10 | 0.73 | 3.47 | 0.84 | 0.06 | 0.04 | 0.38 | 0.16 | 0.21 | 0.19 | 0.46 |
| BO-L-62 | 0.14 | 0.03 | 0.07 | 0.09 | 0.70 | 0.97 | 0.01 | 0.06 | 0.31 | 0.04 | 0.20 | 0.12 | 0.29 |
| BO-L-63A | 4.32 | 1.09 | 4.25 | 2.58 | 16.05 | 2.00 | 0.03 | 0.60 | 4.91 | 0.30 | 0.28 | 0.53 | 1.63 |
| BO-L-64A | 0.93 | 0.22 | 1.48 | 0.62 | 8.96 | 1.75 | 0.04 | 0.23 | 2.32 | 0.15 | 0.16 | 0.31 | 0.78 |
| BO-L-65A | 3.51 | 0.63 | 3.62 | 1.72 | 14.90 | 4.50 | 0.01 | 0.31 | 3.90 | 0.15 | 0.25 | 0.33 | 1.55 |
| BO-L-66A | 3.93 | 0.89 | 3.21 | 2.19 | 13.14 | 2.84 | 0.01 | 0.30 | 3.08 | 0.22 | 0.31 | 0.41 | 1.59 |
| BO-L-67B | 1.31 | 0.23 | 2.13 | 0.80 | 12.63 | 0.94 | <0.01 | 0.35 | 3.76 | 0.19 | 0.12 | 0.67 | 0.87 |
| BO-L-68B | 0.04 | 0.04 | 0.04 | 0.07 | 0.63 | 1.27 | 0.02 | 0.09 | 0.42 | 0.05 | 0.07 | 0.15 | 0.13 |
| BO-L-73 | 2.51 | 0.54 | 2.46 | 1.34 | 9.79 | 1.84 | 0.14 | 0.32 | 2.35 | 0.12 | 0.07 | 0.30 | 0.76 |
| BO-L-75 | 5.52 | 1.11 | 4.97 | 2.03 | 17.29 | 2.17 | 0.03 | 0.57 | 4.20 | 0.33 | 0.47 | 0.49 | 2.07 |
| BO-L-76 | 0.62 | 0.16 | 0.56 | 0.31 | 4.45 | 2.77 | 0.01 | 0.08 | 1.42 | 0.11 | 0.12 | 0.23 | 0.39 |
| BO-L-77 | 0.10 | 0.06 | 0.13 | 0.11 | 0.73 | 0.73 | 0.01 | 0.08 | 0.28 | 0.16 | 0.10 | 0.26 | 0.17 |
| BO-L-79 | 1.78 | 0.54 | 1.93 | 1.36 | 7.96 | 1.64 | 0.07 | 0.03 | 1.09 | 0.22 | 0.29 | 0.38 | 0.99 |
| BO-L-80 | 1.67 | 0.33 | 1.94 | 0.76 | 8.40 | 1.97 | 0.03 | 0.18 | 1.84 | 0.17 | 0.19 | 0.28 | 0.72 |
| BO-L-81 | 0.78 | 0.20 | 0.99 | 0.45 | 3.91 | 2.12 | <0.01 | 0.11 | 0.94 | 0.14 | 0.10 | 0.29 | 0.36 |

Table Annex II.7 Anthocyanins concentrations of the samples from Bordeaux & Bordeaux Superieur.

| Sample | Dp-3-glc | Cy-3-glc | Pt-3-glc | Pn-3-glc | Mv-3-glc | Dp-3-(6-Ac)-glc | Pt-3-(6-Ac)-glc | Pn-3-(6-Ac)-glc | Mv-3-(6-Ac)-glc | Mv-3-(6-caff)-glc | Pt-3-(6-p-coum)-glc | Pn-3-(6-p-coum)-glc | Mv-3-(6-p-coum)-glc |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BO-B-69 | 3.13 | 0.57 | 3.22 | 1.44 | 12.85 | 2.29 | 0.15 | 0.28 | 2.76 | 0.09 | 0.07 | 0.38 | 1.21 |
| BO-B-70 | 1.35 | 0.37 | 1.77 | 0.70 | 6.79 | 0.31 | 0.02 | 0.21 | 1.30 | 0.18 | 0.17 | 0.40 | 0.61 |
| BO-BS-71 | 0.09 | 0.05 | 0.08 | 0.10 | 0.59 | 0.82 | 0.02 | 0.07 | 0.27 | 0.17 | 0.16 | 0.26 | 0.24 |
| BO-BS-72 | 4.09 | 0.65 | 4.79 | 2.25 | 24.87 | 1.63 | 0.05 | 0.69 | 7.89 | 0.26 | 0.30 | 1.00 | 2.44 |
| BO-B-88 | 3.86 | 0.39 | 7.02 | 3.50 | 38.02 | 1.65 | 0.06 | 1.34 | 12.24 | 0.24 | 0.57 | 0.99 | 4.20 |
| BO-B-89 | 5.16 | 0.89 | 7.61 | 3.93 | 33.50 | 1.42 | 0.06 | 1.36 | 12.92 | 0.22 | 0.58 | 0.91 | 3.59 |
| BO-B-90 | 12.29 | 2.55 | 14.74 | 9.40 | 47.52 | 1.43 | 0.11 | 2.38 | 13.30 | 0.76 | 1.06 | 1.45 | 4.84 |
| BO-B-91 | 5.00 | 0.82 | 5.72 | 2.47 | 24.76 | 1.87 | 0.15 | 0.95 | 7.92 | 0.17 | 0.49 | 0.67 | 2.38 |
| BO-B-92 | 5.72 | 1.05 | 7.75 | 4.46 | 34.53 | 2.44 | 0.12 | 1.87 | 13.32 | 0.18 | 0.53 | 0.88 | 3.37 |
| BO-B-93 | 6.62 | 0.84 | 10.59 | 5.53 | 44.90 | 2.28 | 0.07 | 1.77 | 15.13 | 0.33 | 0.81 | 1.09 | 5.08 |
| BO-B-94 | 6.36 | 1.04 | 10.48 | 6.72 | 45.03 | 1.25 | 0.07 | 1.95 | 14.38 | 0.42 | 0.95 | 1.49 | 6.01 |
| BO-B-95 | 7.65 | 1.12 | 11.01 | 5.91 | 47.94 | 2.39 | 0.07 | 1.95 | 16.02 | 0.38 | 0.95 | 1.22 | 5.78 |
| BO-B-96 | 2.44 | 0.35 | 3.41 | 2.25 | 16.79 | 1.65 | 0.02 | 0.52 | 4.81 | 0.19 | 0.39 | 0.66 | 2.45 |

Table Annex II.9 Anthocyanin derivatives concentrations of the samples from Rioja Alavesa.

| Sample | Mv-3-(6-p-coum)-glc cis | Mv-3-(6-p-coum)-glc trans | Mv-3-glc-acetaldehyde | Mv-3-(6-p-coum)-glc-acetaldehyde | Mv-3-glc-pyruvic | Mv-3-(6-p-coum)-glc- pyruvic | Mv-3-glc-vinylmetyl | Mv-3-glc-4-vinylphenol | Mv-3-glc-4-vinylcatechol | Mv-3-glc-4-vinylguaiacol | Mv-3-(6-p-coum)-glc-4-vinylphenol | Mv-3-(6-p-coum)-glc-4-vinylguaiacol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **RV-J-01** | 0.1662 | 7.9473 | 0.1038 | 0.0214 | 0.4012 | 0.0698 | 0.0290 | 0.9121 | 0.2744 | 0.1662 | 0.2114 | 0.0356 |
| **RV-J-02** | 0.0321 | 2.1212 | 0.0337 | 0.0048 | 0.2671 | 0.0673 | 0.0093 | 0.0693 | 0.0320 | 0.0040 | 0.0093 | <0.001 |
| **RV-J-03** | 0.1586 | 8.5657 | 0.1386 | 0.0136 | 0.5521 | 0.0615 | 0.0081 | 0.3468 | 0.0231 | 0.0422 | 0.0666 | 0.0044 |
| **RV-J-04** | 0.0450 | 2.8592 | 0.1194 | 0.0852 | 0.5434 | 0.0522 | 0.0265 | 0.5248 | 0.1884 | 0.0664 | 0.0815 | 0.0047 |
| **RV-J-05** | 0.1119 | 5.1871 | 0.2256 | 0.0411 | 0.6351 | 0.1137 | 0.0246 | 0.5651 | 0.0570 | 0.1113 | 0.1138 | 0.0138 |
| **RV-J-06** | 0.2075 | 7.9206 | 0.3971 | 0.0702 | 0.6119 | 0.0801 | 0.0146 | 0.3944 | 0.0340 | 0.0547 | 0.0681 | 0.0041 |
| **RV-J-07** | 0.1653 | 7.0392 | 0.2929 | 0.0543 | 0.6933 | 0.0583 | 0.0089 | 0.4178 | 0.0381 | 0.0712 | 0.0875 | 0.0058 |
| **RV-J-08** | 0.1599 | 6.3388 | 0.2838 | 0.0793 | 0.7284 | 0.1736 | 0.0392 | 0.4866 | 0.0853 | 0.1006 | 0.1416 | 0.0155 |
| **RV-C-09** | 0.0366 | 2.0773 | 0.0310 | 0.0064 | 0.4317 | 0.0776 | 0.0229 | 0.3260 | 0.1508 | 0.0249 | 0.0516 | 0.0021 |
| **RV-C-10** | 0.0551 | 3.7418 | 0.0557 | 0.0069 | 0.8641 | 0.0897 | 0.0446 | 0.4490 | 0.0604 | 0.0442 | 0.0698 | 0.0088 |
| **RV-C-11** | 0.0838 | 5.1042 | 0.0643 | 0.0170 | 0.5313 | 0.1005 | 0.0290 | 0.3297 | 0.0636 | 0.0562 | 0.0920 | 0.0134 |
| **RV-C-12** | 0.1373 | 8.6564 | 0.1085 | 0.0288 | 0.3843 | 0.0779 | 0.0213 | 0.5292 | 0.0893 | 0.1536 | 0.1545 | 0.0383 |
| **RV-C-13** | 0.0873 | 4.3610 | 0.0573 | 0.0085 | 0.4790 | 0.0708 | 0.0521 | 0.3569 | 0.0746 | 0.0529 | 0.0639 | 0.0064 |
| **RV-C-14** | 0.0164 | 1.7235 | 0.0298 | 0.0045 | 0.4769 | 0.0873 | 0.0111 | 0.0985 | 0.0295 | 0.0058 | 0.0175 | n.d. |
| **RV-C-15** | 0.0630 | 3.2200 | 0.2034 | 0.0440 | 0.6420 | 0.1081 | 0.0442 | 0.4412 | 0.0920 | 0.0426 | 0.0961 | 0.0056 |
| **RV-C-16** | 0.0237 | 1.9947 | 0.0719 | 0.0150 | 0.3625 | 0.0507 | 0.0152 | 0.4711 | 0.1442 | 0.0514 | 0.0812 | 0.0095 |
| **RV-R-17** | 0.0147 | 0.8824 | 0.0852 | 0.0133 | 0.6151 | 0.0864 | 0.0317 | 0.1889 | 0.1115 | 0.0437 | 0.0305 | 0.0042 |
| **RV-R-18** | 0.0247 | 2.0821 | 0.0479 | 0.0070 | 1.0473 | 0.1536 | 0.0834 | 0.4541 | 0.0530 | 0.0565 | 0.0612 | 0.0093 |
| **RV-R-19** | 0.0155 | 1.0704 | 0.0471 | 0.0095 | 0.6053 | 0.1133 | 0.0470 | 0.4049 | 0.0526 | 0.0592 | 0.0688 | 0.0093 |
| **RV-R-20** | 0.0243 | 2.2486 | 0.0659 | 0.0149 | 0.4238 | 0.0739 | 0.0203 | 0.3110 | 0.0680 | 0.0823 | 0.0619 | 0.0137 |
| **RV-R-21** | 0.0103 | 0.6561 | 0.0175 | 0.0037 | 0.3025 | 0.0716 | 0.0243 | 0.3175 | 0.1039 | 0.0573 | 0.0646 | 0.0070 |
| **RV-R-22** | 0.0030 | 0.4934 | 0.0119 | 0.0023 | 0.4279 | 0.0832 | 0.0101 | 0.0884 | 0.0723 | 0.0060 | 0.0130 | <0.001 |
| **RV-R-23** | 0.0482 | 2.7395 | 0.1946 | 0.0381 | 0.5861 | 0.0925 | 0.0532 | 0.3203 | 0.0942 | 0.0456 | 0.0670 | 0.0056 |
| **RV-R-24** | 0.0430 | 2.6260 | 0.1955 | 0.0319 | 0.6206 | 0.0715 | 0.0327 | 0.2047 | 0.0245 | 0.0285 | 0.0337 | 0.0035 |
| **RV-GR-25** | n.d. | 0.0010 | 0.0504 | 0.0024 | 0.4652 | 0.0519 | 0.0435 | 0.2379 | 0.0367 | n.d. | n.d. | 0.0073 |
| **RV-GR-26** | 0.0023 | 0.6237 | 0.0370 | 0.0059 | 0.8492 | 0.1304 | 0.0556 | 0.4245 | 0.0579 | 0.0541 | 0.0540 | 0.0085 |
| **RV-GR-27** | <0.001 | 0.0599 | 0.0219 | 0.0045 | 0.5080 | 0.1061 | 0.0331 | 0.4562 | 0.0555 | 0.0369 | 0.0650 | 0.0034 |
| **RV-GR-28** | <0.001 | 0.0930 | 0.0173 | 0.0033 | 0.3583 | 0.0946 | 0.0212 | 0.3906 | 0.1720 | 0.0168 | 0.0573 | 0.0020 |
| **RV-GR-29** | <0.001 | 0.0030 | 0.0236 | <0.001 | 0.4008 | <0.001 | 0.0214 | 0.4292 | 0.1039 | <0.001 | <0.001 | 0.0040 |
| **RV-GR-30** | 0.0180 | 0.9991 | 0.1207 | 0.0171 | 0.6840 | 0.0832 | 0.0170 | 0.4359 | 0.0412 | 0.0386 | 0.0538 | 0.0025 |
| **RV-GR-31** | 0.0019 | 0.3177 | 0.0682 | 0.0108 | 0.7255 | 0.1027 | 0.0595 | 0.2488 | 0.0606 | 0.0249 | 0.0306 | <0.001 |
| **RV-GR-32** | <0.001 | 0.1566 | 0.0475 | 0.0051 | 0.6306 | 0.1713 | 0.0613 | 0.3578 | 0.0967 | 0.0685 | 0.0608 | 0.0051 |

n.d. not detected

Continuation of Table Annex II. 9

| Sample | Catechin-Mv-3-glc | Catechin-Mv-3-(6-p-coum)-glc | Epicatechin-Mv-3-glc | Epicatechin-Mv-3-(6-p-coum)-glc | (Epi)Gallocatechin-Mv-3-glc | (Epi)Gallocatechin-Mv-3-(6-p-coum)-glc | Mv-3-glc-8-ethyl-catechin 1 | Mv-3-glc-8-ethyl-catechin 2 | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin 1 | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin 2 | Mv-3-glc-8-ethyl-epicatechin |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **RV-J-01** | 0.1078 | 0.0220 | 0.0327 | n.d. | 0.0252 | 0.0052 | <0.001 | 0.0006 | 0.0057 | <0.001 | <0.001 |
| **RV-J-02** | 0.0770 | 0.0141 | 0.0191 | n.d. | 0.0127 | 0.0019 | <0.001 | <0.001 | 0.0050 | <0.001 | <0.001 |
| **RV-J-03** | 0.0851 | 0.0077 | 0.0273 | n.d. | 0.0086 | 0.0019 | 0.0030 | 0.0059 | 0.0083 | 0.0018 | <0.001 |
| **RV-J-04** | 0.1591 | 0.0065 | 0.0235 | n.d. | 0.0093 | 0.0012 | <0.001 | 0.0013 | 0.0032 | <0.001 | <0.001 |
| **RV-J-05** | 0.1488 | 0.0227 | 0.0498 | n.d. | 0.0249 | 0.0046 | <0.001 | <0.001 | 0.0080 | <0.001 | <0.001 |
| **RV-J-06** | 0.1457 | 0.0183 | 0.0542 | n.d. | 0.0174 | 0.0030 | 0.0040 | 0.0071 | 0.0170 | 0.0012 | <0.001 |
| **RV-J-07** | 0.1068 | 0.0141 | 0.0274 | n.d. | 0.0120 | 0.0036 | 0.0016 | 0.0048 | 0.0093 | <0.001 | <0.001 |
| **RV-J-08** | 0.1800 | 0.0259 | 0.0535 | n.d. | 0.0260 | 0.0055 | <0.001 | 0.0025 | 0.0172 | <0.001 | <0.001 |
| **RV-C-09** | 0.1615 | 0.0186 | 0.0312 | <0.001 | 0.0217 | 0.0054 | <0.001 | <0.001 | 0.0022 | <0.001 | <0.001 |
| **RV-C-10** | 0.1249 | 0.0246 | 0.0426 | n.d. | 0.0184 | 0.0062 | <0.001 | <0.001 | 0.0051 | <0.001 | <0.001 |
| **RV-C-11** | 0.1019 | 0.0248 | 0.0336 | n.d. | 0.0187 | 0.0059 | <0.001 | 0.0006 | 0.0103 | <0.001 | <0.001 |
| **RV-C-12** | 0.1133 | 0.0284 | 0.0345 | n.d. | 0.0313 | 0.0115 | <0.001 | <0.001 | 0.0052 | <0.001 | <0.001 |
| **RV-C-13** | 0.1215 | 0.0225 | 0.0341 | n.d. | 0.0214 | 0.0073 | <0.001 | 0.0008 | 0.0051 | <0.001 | <0.001 |
| **RV-C-14** | 0.0596 | 0.0101 | 0.0187 | n.d. | 0.0086 | 0.0013 | <0.001 | 0.0013 | n.d. | n.d. | <0.001 |
| **RV-C-15** | 0.1126 | 0.0214 | 0.0420 | n.d. | 0.0234 | 0.0066 | 0.0007 | 0.0034 | 0.0150 | <0.001 | <0.001 |
| **RV-C-16** | 0.0407 | 0.0097 | 0.0111 | n.d. | 0.0056 | 0.0014 | <0.001 | <0.001 | 0.0028 | <0.001 | <0.001 |
| **RV-R-17** | 0.1250 | 0.0133 | 0.0285 | n.d. | 0.0160 | 0.0021 | <0.001 | <0.001 | 0.0053 | <0.001 | <0.001 |
| **RV-R-18** | 0.1129 | 0.0232 | 0.0339 | n.d. | 0.0163 | 0.0047 | <0.001 | <0.001 | 0.0033 | <0.001 | <0.001 |
| **RV-R-19** | 0.0958 | 0.0188 | 0.0323 | n.d. | 0.0113 | 0.0019 | <0.001 | <0.001 | 0.0027 | <0.001 | <0.001 |
| **RV-R-20** | 0.1099 | 0.0148 | 0.0269 | n.d. | 0.0161 | 0.0024 | <0.001 | <0.001 | 0.0040 | <0.001 | <0.001 |
| **RV-R-21** | 0.0656 | 0.0094 | 0.0211 | n.d. | 0.0072 | 0.0013 | <0.001 | <0.001 | 0.0012 | <0.001 | <0.001 |
| **RV-R-22** | 0.0491 | 0.0059 | 0.0096 | n.d. | 0.0066 | <0.001 | <0.001 | <0.001 | 0.0014 | <0.001 | <0.001 |
| **RV-R-23** | 0.1032 | 0.0195 | 0.0405 | <0.001 | 0.0213 | 0.0053 | 0.0008 | 0.0029 | 0.0149 | <0.001 | <0.001 |
| **RV-R-24** | 0.0437 | 0.0102 | 0.0159 | n.d. | 0.0112 | 0.0032 | <0.001 | <0.001 | 0.0067 | <0.001 | <0.001 |
| **RV-GR-25** | 0.0907 | 0.0200 | 0.0329 | n.d. | 0.0103 | 0.0060 | <0.001 | <0.001 | 0.0071 | <0.001 | <0.001 |
| **RV-GR-26** | 0.0640 | 0.0079 | 0.0201 | n.d. | 0.0094 | 0.0012 | <0.001 | <0.001 | 0.0025 | <0.001 | <0.001 |
| **RV-GR-27** | 0.0158 | 0.0010 | 0.0037 | n.d. | 0.0009 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **RV-GR-28** | 0.0115 | 0.0007 | 0.0021 | n.d. | 0.0009 | <0.001 | <0.001 | <0.001 | 0.0011 | <0.001 | <0.001 |
| **RV-GR-29** | n.d. | 0.0029 | n.d. | <0.001 | 0.0068 | <0.001 | <0.001 | <0.001 | 0.0008 | <0.001 | <0.001 |
| **RV-GR-30** | 0.1123 | 0.0134 | 0.0320 | n.d. | 0.0152 | 0.0030 | <0.001 | 0.0010 | 0.0065 | <0.001 | <0.001 |
| **RV-GR-31** | 0.0351 | 0.0026 | 0.0112 | n.d. | 0.0022 | <0.001 | <0.001 | <0.001 | 0.0059 | <0.001 | <0.001 |
| **RV-GR-32** | 0.0250 | <0.001 | 0.0052 | n.d. | 0.0006 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

n.d.: not detected.

Table Annex II.10 Anthocyanin derivatives concentrations of the samples from Rioja Alta.

| Sample | Mv-3-(6-p-coum)-glc cis | Mv-3-(6-p-coum)-glc trans | Mv-3-glc-acetaldehyde | Mv-3-(6-p-coum)-glc-acetaldehyde | Mv-3-glc-pyruvic | Mv-3-(6-p-coum)-glc- pyruvic | Mv-3-glc-vinylmetyl | Mv-3-glc-4-vinylphenol | Mv-3-glc-4-vinylcatechol | Mv-3-glc-4-vinylguaiacol | Mv-3-(6-p-coum)-glc-4-vinylphenol | Mv-3-(6-p-coum)-glc-4-vinylguaiacol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RA-J-33 | n.d. | n.d. | 0.3332 | n.d. | 0.3555 | n.d. | 0.0307 | 0.3510 | 0.0664 | n.d. | n.d. | 0.0057 |
| RA-J-34 | 0.0367 | 2.4532 | 0.0383 | 0.0079 | 0.4357 | 0.0598 | 0.0234 | 0.2642 | 0.0974 | 0.0482 | 0.0425 | 0.0074 |
| RA-J-35 | 0.0345 | 2.2890 | 0.1848 | 0.0334 | 0.3663 | 0.0554 | 0.0268 | 0.4425 | 0.1552 | 0.1070 | 0.0830 | 0.0184 |
| RA-J-36 | 0.0064 | 0.9095 | 0.0676 | 0.0097 | 0.5693 | 0.1201 | 0.0689 | 0.5273 | 0.0990 | 0.1074 | 0.1083 | 0.0145 |
| RA-J-37 | 0.0699 | 2.9642 | 0.1054 | 0.0246 | 0.3555 | 0.0585 | 0.0249 | 0.3071 | 0.0562 | 0.0420 | 0.0612 | 0.0065 |
| RA-J-38 | 0.0124 | 0.6456 | 0.1312 | 0.0277 | 0.7853 | 0.1119 | 0.0882 | 0.2801 | 0.0431 | 0.0414 | 0.0421 | 0.0068 |
| RA-J-39 | 0.0099 | 0.5672 | 0.0773 | 0.0273 | 0.3522 | 0.0749 | 0.0217 | 0.4334 | 0.0607 | 0.0398 | 0.0419 | 0.0127 |
| RA-J-40 | 0.0104 | 0.5642 | 0.0035 | 0.0262 | 0.3019 | 0.0619 | 0.0190 | 0.3604 | 0.0773 | 0.0423 | 0.0516 | 0.0037 |
| RA-C-41 | 0.0601 | 4.6369 | 0.0677 | 0.0071 | 0.7158 | 0.0925 | 0.0399 | 0.6554 | 0.0808 | 0.0653 | 0.0904 | 0.0116 |
| RA-C-42 | 0.0252 | 1.4433 | 0.1342 | 0.0323 | 0.4818 | 0.0904 | 0.0288 | 0.5402 | 0.2072 | 0.0737 | 0.1004 | 0.0107 |
| RA-C-43 | 0.0465 | 2.7818 | 0.0578 | 0.0136 | 0.5000 | 0.0702 | 0.0295 | 0.4388 | 0.1774 | 0.0589 | 0.0659 | 0.0087 |
| RA-C-44 | 0.0365 | 2.1088 | 0.0373 | 0.0074 | 0.2957 | 0.0473 | 0.0166 | 0.2940 | 0.0932 | 0.0308 | 0.0445 | 0.0035 |
| RA-C-45 | 0.0289 | 1.0401 | 0.0328 | 0.0064 | 0.2911 | 0.0472 | 0.0209 | 0.3842 | 0.0774 | 0.0318 | 0.0610 | 0.0031 |
| RA-C-46 | 0.0631 | 3.3517 | 0.0321 | 0.0096 | 0.3147 | 0.0729 | 0.0163 | 0.2654 | 0.0656 | 0.0381 | 0.0483 | 0.0042 |
| RA-C-47 | 0.0629 | 3.9332 | 0.0357 | 0.0131 | 0.2865 | 0.0677 | 0.0114 | 0.1077 | 0.0334 | 0.0108 | 0.0213 | 0.0109 |
| RA-C-48 | 0.0569 | 3.4303 | 0.0433 | 0.0151 | 0.5512 | 0.0837 | 0.0291 | 0.4824 | 0.1085 | 0.0943 | 0.0783 | 0.0127 |
| RA-R-49 | 0.0019 | 0.1852 | 0.0158 | 0.0033 | 0.2222 | 0.0755 | 0.0240 | 0.4922 | 0.4906 | 0.0541 | 0.0684 | 0.0049 |
| RA-R-50 | <0.001 | 0.2219 | 0.0066 | <0.001 | 0.2700 | 0.0723 | 0.0120 | 0.3020 | 0.2309 | 0.0170 | 0.0213 | 0.0018 |
| RA-R-51 | 0.0053 | 0.5335 | 0.0458 | 0.0110 | 0.5333 | 0.1193 | 0.0282 | 0.4322 | 0.2176 | 0.0512 | 0.0973 | 0.0100 |
| RA-R-52 | 0.0181 | 1.4958 | 0.0540 | 0.0168 | 0.8105 | 0.3202 | 0.0291 | 0.5049 | 0.3584 | 0.0674 | 0.1701 | 0.0094 |
| RA-R-53 | 0.0083 | 0.7026 | 0.0188 | 0.0047 | 0.5876 | 0.0894 | 0.0236 | 0.3148 | 0.0667 | 0.0287 | 0.0488 | 0.0033 |
| RA-R-54 | 0.0189 | 1.0868 | 0.0645 | 0.0214 | 0.3145 | 0.0673 | 0.0162 | 0.4068 | 0.0497 | 0.0445 | 0.0729 | 0.0066 |
| RA-R-55 | <0.001 | 0.1409 | 0.0228 | 0.0034 | 0.2758 | 0.0501 | 0.0258 | 0.2977 | 0.0942 | 0.0309 | 0.0353 | 0.0018 |
| RA-R-56 | 0.0403 | 2.6310 | 0.0185 | 0.0081 | 0.2973 | 0.0673 | 0.0238 | 0.4635 | 0.0751 | 0.0898 | 0.0977 | 0.0152 |
| RA-GR-57 | 0.0082 | 1.1095 | 0.0501 | 0.0043 | 1.0891 | 0.0988 | 0.0947 | 0.5232 | 0.0695 | 0.0536 | 0.0453 | 0.0060 |
| RA-GR-58 | <0.001 | 0.0541 | 0.0025 | <0.001 | 0.0922 | 0.0353 | 0.0080 | 0.1844 | 0.1348 | 0.0179 | 0.0263 | 0.0008 |
| RA-GR-59 | <0.001 | 0.0468 | 0.0070 | <0.001 | 0.2024 | 0.0547 | 0.0169 | 0.2700 | 0.1956 | 0.0366 | 0.0609 | 0.0037 |
| RA-GR-60 | <0.001 | 0.2985 | 0.0637 | 0.0011 | 0.6585 | 0.1287 | 0.0348 | 0.1749 | 0.1128 | 0.0527 | 0.0816 | 0.0035 |
| RA-GR-61 | <0.001 | 0.3022 | 0.0637 | 0.0066 | 0.6780 | 0.1150 | 0.0478 | 0.1955 | 0.1075 | 0.0326 | 0.0263 | <0.001 |
| RA-GR-62 | 0.0126 | 0.5283 | 0.0225 | 0.0064 | 0.2345 | 0.0529 | 0.0200 | 0.2384 | 0.0579 | 0.0373 | 0.0460 | 0.0042 |
| RA-GR-63 | 0.0085 | 0.4955 | 0.0253 | 0.0053 | 0.2229 | 0.0425 | 0.0212 | 0.3274 | 0.0771 | 0.0274 | 0.0452 | 0.0032 |
| RA-GR-64 | 0.0326 | 2.1482 | 0.0184 | 0.0098 | 0.2926 | 0.0769 | 0.0179 | 0.3340 | 0.0716 | 0.0589 | 0.0756 | 0.0103 |

n.d.: not detected.

Continuation of Table Annex II. 10

| Sample | Catechin-Mv-3-glc | Catechin-Mv-3-(6-p-coum)-glc | Epicatechin-Mv-3-glc | Epicatechin-Mv-3-(6-p-coum)-glc | (Epi)Gallocatechin-Mv-3-glc | (Epi)Gallocatechin-Mv-3-(6-p-coum)-glc | Mv-3-glc-8-ethyl-catechin 1 | Mv-3-glc-8-ethyl-catechin 2 | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin 1 | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin 2 | Mv-3-glc-8-ethyl-epicatechin |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **RA-J-33** | n.d. | 0.0153 | n.d. | n.d. | 0.0196 | 0.0030 | 0.0091 | 0.0174 | 0.0212 | 0.0037 | 0.0002 |
| **RA-J-34** | 0.0738 | 0.0187 | 0.0272 | n.d. | 0.0132 | 0.0055 | <0.001 | <0.001 | 0.0022 | <0.001 | <0.001 |
| **RA-J-35** | 0.0705 | 0.0127 | 0.0160 | n.d | 0.0149 | 0.0041 | <0.001 | <0.001 | 0.0026 | <0.001 | <0.001 |
| **RA-J-36** | 0.1136 | 0.0084 | 0.0216 | n.d. | 0.0137 | <0.001 | <0.001 | <0.001 | 0.0028 | <0.001 | <0.001 |
| **RA-J-37** | 0.0661 | 0.0183 | 0.0274 | n.d | 0.0170 | 0.0048 | <0.001 | <0.001 | 0.0089 | <0.001 | <0.001 |
| **RA-J-38** | 0.0412 | 0.0060 | 0.0170 | n.d. | 0.0035 | <0.001 | <0.001 | <0.001 | 0.0070 | <0.001 | <0.001 |
| **RA-J-39** | 0.0460 | 0.0217 | 0.0130 | n.d. | 0.0115 | 0.0063 | <0.001 | <0.001 | 0.0046 | <0.001 | <0.001 |
| **RA-J-40** | 0.0390 | 0.0051 | 0.0075 | n.d. | 0.0022 | <0.001 | <0.001 | <0.001 | 0.0065 | <0.001 | <0.001 |
| **RA-C-41** | 0.1634 | 0.0261 | 0.0402 | n.d. | 0.0218 | 0.0065 | <0.001 | <0.001 | 0.0065 | <0.001 | <0.001 |
| **RA-C-42** | 0.1100 | 0.0124 | 0.0301 | n.d. | 0.0156 | 0.0016 | <0.001 | 0.0022 | 0.0100 | <0.001 | <0.001 |
| **RA-C-43** | 0.0783 | 0.0212 | 0.0289 | n.d. | 0.0176 | 0.0060 | <0.001 | <0.001 | 0.0029 | <0.001 | <0.001 |
| **RA-C-44** | 0.0728 | 0.0200 | 0.0284 | n.d. | 0.0146 | 0.0052 | <0.001 | <0.001 | 0.0022 | <0.001 | <0.001 |
| **RA-C-45** | 0.0503 | 0.0142 | 0.0160 | n.d | 0.0073 | 0.0024 | <0.001 | <0.001 | 0.0015 | <0.001 | <0.001 |
| **RA-C-46** | 0.0515 | 0.0127 | 0.0192 | n.d. | 0.0068 | 0.0051 | <0.001 | <0.001 | 0.0019 | <0.001 | <0.001 |
| **RA-C-47** | 0.0505 | 0.0214 | 0.0151 | n.d. | 0.0059 | 0.0058 | <0.001 | <0.001 | 0.0019 | <0.001 | <0.001 |
| **RA-C-48** | 0.0918 | 0.0183 | 0.0221 | n.d. | 0.0097 | 0.0046 | <0.001 | <0.001 | 0.0020 | <0.001 | <0.001 |
| **RA-R-49** | 0.0468 | 0.0034 | 0.0142 | <0.001 | 0.0057 | 0.0006 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **RA-R-50** | 0.0206 | 0.0016 | 0.0086 | n.d. | 0.0019 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **RA-R-51** | 0.0353 | 0.0068 | 0.0121 | n.d. | 0.0046 | 0.0009 | <0.001 | <0.001 | 0.0067 | <0.001 | <0.001 |
| **RA-R-52** | 0.1366 | 0.0201 | 0.0442 | n.d | 0.0072 | 0.0025 | <0.001 | <0.001 | 0.0035 | <0.001 | <0.001 |
| **RA-R-53** | 0.0559 | 0.0124 | 0.0190 | n.d. | 0.0065 | 0.0022 | <0.001 | <0.001 | 0.0007 | <0.001 | <0.001 |
| **RA-R-54** | 0.0853 | 0.0164 | 0.0198 | n.d. | 0.0085 | 0.0039 | <0.001 | <0.001 | 0.0035 | <0.001 | <0.001 |
| **RA-R-55** | 0.0226 | 0.0028 | 0.0107 | n.d | 0.0011 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **RA-R-56** | 0.0684 | 0.0189 | 0.0288 | n.d. | 0.0101 | 0.0068 | <0.001 | <0.001 | 0.0008 | <0.001 | <0.001 |
| **RA-GR-57** | 0.1127 | 0.0127 | 0.0273 | n.d. | 0.0126 | 0.0024 | <0.001 | <0.001 | 0.0024 | <0.001 | <0.001 |
| **RA-GR-58** | 0.0066 | <0.001 | 0.0017 | n.d. | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **RA-GR-59** | 0.0090 | <0.001 | <0.001 | n.d. | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | n.d. |
| **RA-GR-60** | 0.0354 | 0.0015 | 0.0063 | n.d. | 0.0015 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **RA-GR-61** | 0.0933 | 0.0044 | 0.0195 | n.d. | 0.0054 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **RA-GR-62** | 0.0490 | 0.0107 | 0.0193 | n.d. | 0.0057 | 0.0012 | <0.001 | <0.001 | 0.0019 | <0.001 | <0.001 |
| **RA-GR-63** | 0.0269 | 0.0102 | 0.0140 | n.d. | 0.0039 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **RA-GR-64** | 0.0657 | 0.0182 | 0.0222 | n.d. | 0.0087 | 0.0064 | <0.001 | <0.001 | 0.0000 | <0.001 | <0.001 |

n.d.: not detected.

Table Annex II.11 Anthocyanin derivatives concentrations of the samples from Rioja Baja.

| Sample | Mv-3-(6-p-coum)-glc cis | Mv-3-(6-p-coum)-glc trans | Mv-3-glc-acetaldehyde | Mv-3-(6-p-coum)-glc-acetaldehyde | Mv-3-glc-pyruvic | Mv-3-(6-p-coum)-glc- pyruvic | Mv-3-glc-vinylmetyl | Mv-3-glc-4-vinylphenol | Mv-3-glc-4-vinylcatechol | Mv-3-glc-4-vinylguaiacol | Mv-3-(6-p-coum)-glc-4-vinylphenol | Mv-3-(6-p-coum)-glc-4-vinylguaiacol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RB-J-65 | n.d. | n.d. | 0.2833 | n.d. | 0.1980 | n.d. | 0.0215 | 0.7442 | 0.0802 | n.d. | n.d. | 0.0284 |
| RB-J-66 | <0.001 | <0.001 | 0.2639 | n.d. | 0.6152 | n.d. | 0.0279 | 0.5848 | 0.0204 | <0.001 | <0.001 | 0.0155 |
| RB-J-67 | 0.1283 | 5.7279 | 0.1404 | 0.0316 | 0.2677 | 0.0424 | 0.0188 | 0.3570 | 0.0584 | 0.0340 | 0.0746 | 0.0070 |
| RB-J-68 | 0.0837 | 4.8688 | 0.0810 | 0.0162 | 0.3408 | 0.0463 | 0.0226 | 0.2585 | 0.0712 | 0.0403 | 0.0491 | 0.0054 |
| RB-J-69 | 0.1240 | 7.0270 | 0.1236 | 0.0194 | 0.4859 | 0.0423 | 0.0218 | 0.2181 | 0.0277 | 0.0208 | 0.0403 | 0.0017 |
| RB-J-70 | 0.1226 | 6.3849 | 0.0903 | 0.0140 | 0.2822 | 0.0254 | 0.0207 | 0.3999 | 0.0373 | 0.0417 | 0.0587 | 0.0044 |
| RB-J-71 | 0.0742 | 4.1674 | 0.0922 | 0.0107 | 0.3680 | 0.0295 | 0.0168 | 0.2120 | 0.0174 | 0.0129 | 0.0264 | 0.0272 |
| RB-J-72 | 0.0154 | 2.5100 | 0.0218 | 0.0038 | 0.2891 | 0.1015 | 0.0168 | 0.5422 | 0.6097 | 0.1960 | 0.1535 | 0.0235 |
| RB-C-73 | 0.0318 | 1.7300 | 0.4533 | 0.0690 | 0.3579 | 0.0687 | 0.0489 | 0.4009 | 0.1044 | 0.1050 | 0.0878 | 0.0188 |
| RB-C-74 | 0.0196 | 1.1146 | 0.1751 | 0.0337 | 0.8478 | 0.1150 | 0.0442 | 0.3159 | 0.1242 | 0.0493 | 0.0408 | 0.0056 |
| RB-C-75 | 0.1148 | 7.1669 | 0.0689 | 0.0278 | 0.7027 | 0.0993 | 0.0515 | 0.6915 | 0.1030 | 0.1212 | 0.1031 | 0.0235 |
| RB-C-76 | 0.0535 | 2.8655 | 0.0968 | 0.0202 | 0.4982 | 0.0831 | 0.0458 | 0.3331 | 0.0870 | 0.0847 | 0.0522 | 0.0098 |
| RB-C-77 | 0.0126 | 0.9236 | 0.1143 | 0.0261 | 0.4469 | 0.0868 | 0.0172 | 0.1896 | 0.0320 | 0.0348 | 0.0350 | 0.0046 |
| RB-C-78 | 0.0956 | 9.3920 | 0.1835 | 0.0390 | 0.6129 | 0.0911 | 0.0199 | 0.3462 | 0.0682 | 0.0484 | 0.0827 | 0.0070 |
| RB-C-79 | 0.0516 | 3.7315 | 0.0348 | 0.0104 | 0.2652 | 0.0593 | 0.0228 | 0.1328 | 0.0282 | 0.0383 | 0.0377 | 0.0117 |
| RB-C-80 | 0.0102 | 0.4262 | 0.0331 | 0.0086 | 0.2633 | 0.0650 | 0.0231 | 0.2664 | 0.1231 | 0.0646 | 0.0472 | 0.0100 |
| RB-R-81 | 0.0168 | 1.1533 | 0.3705 | 0.0451 | 0.5735 | 0.0827 | 0.0479 | 0.6205 | 0.1768 | 0.1398 | 0.0698 | 0.0192 |
| RB-R-82 | 0.0199 | 2.3394 | 0.1300 | 0.0330 | 1.0013 | 0.1955 | 0.0407 | 0.3133 | 0.1043 | 0.0500 | 0.0518 | 0.0082 |
| RB-R-83 | 0.0864 | 5.1164 | 0.1763 | 0.0232 | 0.5738 | 0.0627 | 0.0426 | 0.8364 | 0.1265 | 0.1684 | 0.1054 | 0.0252 |
| RB-R-84 | 0.0384 | 2.7604 | 0.1093 | 0.0198 | 0.5693 | 0.0858 | 0.0423 | 0.3315 | 0.0781 | 0.0862 | 0.0497 | n.d. |
| RB-R-85 | 0.0018 | 0.3059 | 0.0596 | 0.0115 | 0.4474 | 0.0999 | 0.0372 | 0.5352 | 0.1217 | 0.0718 | 0.0926 | 0.0043 |
| RB-R-86 | <0.001 | 0.1931 | 0.7008 | 0.1190 | 0.3566 | 0.0848 | 0.1164 | 0.1689 | 0.1188 | 0.0225 | 0.0250 | <0.001 |
| RB-R-87 | 0.0345 | 1.9321 | 0.0521 | 0.0181 | 0.3694 | 0.0730 | 0.0217 | 0.1537 | 0.0705 | 0.0318 | 0.0367 | 0.0068 |
| RB-R-88 | 0.0071 | 0.2564 | 0.0399 | 0.0111 | 0.2397 | 0.0655 | 0.0216 | 0.3182 | 0.1605 | 0.0486 | 0.0526 | 0.0077 |
| RB-GR-89 | 0.0288 | 1.6334 | 0.4126 | 0.0433 | 0.6023 | 0.0775 | 0.0585 | 0.6234 | 0.1937 | 0.0954 | 0.0645 | 0.0130 |
| RB-GR-90 | 0.0234 | 1.5936 | 0.0812 | 0.0344 | 0.5088 | 0.1374 | 0.0432 | 0.2474 | 0.0699 | 0.0579 | 0.0569 | 0.0092 |
| RB-GR-91 | <0.001 | 0.0539 | <0.001 | <0.001 | 0.1162 | 0.0430 | 0.0046 | 0.4265 | 0.1483 | 0.0396 | 0.0525 | 0.0015 |
| RB-GR-92 | <0.001 | 0.1817 | 0.0051 | <0.001 | 0.2065 | 0.0486 | 0.0131 | 0.1916 | 0.1195 | 0.0404 | 0.0213 | 0.0018 |
| RB-GR-93 | 0.0083 | 0.6274 | 0.0654 | 0.0127 | 0.6725 | 0.1330 | 0.0592 | 0.4479 | 0.1064 | 0.0647 | 0.0816 | 0.0041 |
| RB-GR-94 | <0.001 | 0.2095 | 0.0079 | <0.001 | 0.2780 | 0.0681 | 0.0194 | 0.2987 | 0.2152 | 0.0358 | 0.0426 | 0.0014 |
| RB-GR-95 | 0.0144 | 0.6539 | 0.2418 | 0.0453 | 0.6733 | 0.0941 | 0.0396 | 0.1925 | 0.1124 | 0.0372 | 0.0409 | 0.0018 |
| RB-GR-96 | 0.0032 | 0.4014 | 0.1927 | 0.0155 | 0.5015 | 0.1786 | 0.0283 | 0.5456 | 0.3635 | 0.0859 | 0.1054 | 0.0064 |

n.d.: not detected.

Continuation of Table Annex II. 11

| Sample | Catechin-Mv-3-glc | Catechin-Mv-3-(6-p-coum)-glc | Epicatechin-Mv-3-glc | Epicatechin-Mv-3-(6-p-coum)-glc | (Epi)Gallocatechin-Mv-3-glc | (Epi)Gallocatechin-Mv-3-(6-p-coum)-glc | Mv-3-glc-8-ethyl-catechin 1 | Mv-3-glc-8-ethyl-catechin 2 | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin 1 | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin 2 | Mv-3-glc-8-ethyl-epicatechin |
|---|---|---|---|---|---|---|---|---|---|---|---|
| RB-J-65 | n.d. | 0.0296 | n.d. | n.d. | 0.0240 | 0.0071 | 0.0017 | 0.0042 | 0.0150 | <0.001 | <0.001 |
| RB-J-66 | 0.1127 | 0.0203 | 0.0333 | n.d. | 0.0193 | 0.0051 | 0.0231 | 0.0302 | 0.0398 | 0.0081 | 0.0063 |
| RB-J-67 | 0.0530 | 0.0200 | 0.0308 | n.d. | 0.0088 | 0.0043 | 0.0017 | 0.0055 | 0.0112 | 0.0016 | <0.001 |
| RB-J-68 | 0.0696 | 0.0181 | 0.0231 | n.d. | 0.0115 | 0.0029 | 0.0015 | 0.0049 | 0.0050 | <0.001 | <0.001 |
| RB-J-69 | 0.1074 | 0.0176 | 0.0330 | n.d. | 0.0142 | 0.0031 | 0.0011 | 0.0048 | 0.0103 | <0.001 | <0.001 |
| RB-J-70 | 0.0761 | 0.0152 | 0.0225 | n.d. | 0.0122 | 0.0026 | 0.0006 | 0.0046 | 0.0112 | <0.001 | <0.001 |
| RB-J-71 | 0.0407 | 0.0179 | 0.0205 | n.d. | 0.0130 | 0.0043 | 0.0011 | 0.0065 | 0.0120 | 0.0015 | <0.001 |
| RB-J-72 | 0.1504 | 0.0151 | 0.0356 | n.d. | 0.0250 | 0.0040 | <0.001 | <0.001 | 0.0051 | <0.001 | <0.001 |
| RB-C-73 | 0.0808 | 0.0124 | 0.0198 | n.d. | 0.0162 | 0.0025 | 0.0013 | 0.0034 | 0.0104 | <0.001 | <0.001 |
| RB-C-74 | 0.0891 | 0.0148 | 0.0268 | n.d. | 0.0120 | 0.0018 | <0.001 | 0.0009 | 0.0045 | <0.001 | <0.001 |
| RB-C-75 | 0.0992 | 0.0218 | 0.0318 | n.d. | 0.0192 | 0.0050 | <0.001 | 0.0012 | 0.0070 | <0.001 | <0.001 |
| RB-C-76 | 0.1382 | 0.0189 | 0.0310 | n.d. | 0.0240 | 0.0049 | <0.001 | 0.0013 | 0.0073 | <0.001 | <0.001 |
| RB-C-77 | 0.0412 | 0.0092 | 0.0103 | n.d. | 0.0049 | 0.0009 | <0.001 | <0.001 | 0.0055 | <0.001 | <0.001 |
| RB-C-78 | 0.0895 | 0.0197 | 0.0246 | n.d. | 0.0087 | 0.0029 | <0.001 | <0.001 | 0.0096 | <0.001 | <0.001 |
| RB-C-79 | 0.0635 | 0.0176 | 0.0190 | n.d. | 0.0123 | 0.0045 | <0.001 | <0.001 | 0.0071 | <0.001 | <0.001 |
| RB-C-80 | 0.0372 | 0.0084 | 0.0140 | n.d. | 0.0052 | <0.001 | <0.001 | <0.001 | 0.0024 | <0.001 | <0.001 |
| RB-R-81 | 0.0914 | 0.0090 | 0.0276 | n.d. | 0.0143 | 0.0009 | 0.0020 | 0.0042 | 0.0070 | <0.001 | <0.001 |
| RB-R-82 | 0.0904 | 0.0161 | 0.0275 | n.d. | 0.0090 | 0.0028 | <0.001 | 0.0008 | 0.0046 | <0.001 | <0.001 |
| RB-R-83 | 0.1187 | 0.0177 | 0.0322 | n.d. | 0.0208 | 0.0046 | <0.001 | 0.0018 | 0.0058 | <0.001 | <0.001 |
| RB-R-84 | 0.1249 | 0.0167 | 0.0361 | n.d. | 0.0252 | 0.0044 | <0.001 | 0.0008 | n.d. | n.d. | <0.001 |
| RB-R-85 | 0.0449 | 0.0026 | 0.0144 | n.d. | 0.0017 | <0.001 | <0.001 | <0.001 | 0.0022 | <0.001 | <0.001 |
| RB-R-86 | 0.0088 | <0.001 | <0.001 | n.d. | 0.0012 | <0.001 | <0.001 | 0.0021 | 0.0035 | <0.001 | <0.001 |
| RB-R-87 | 0.0362 | 0.0151 | 0.0110 | n.d. | 0.0069 | 0.0035 | <0.001 | <0.001 | 0.0025 | <0.001 | <0.001 |
| RB-R-88 | 0.0174 | 0.0043 | 0.0068 | n.d. | 0.0013 | <0.001 | <0.001 | <0.001 | 0.0012 | <0.001 | <0.001 |
| RB-GR-89 | 0.0832 | 0.0123 | 0.0205 | n.d. | 0.0163 | 0.0024 | 0.0017 | 0.0040 | 0.0086 | 0.0009 | <0.001 |
| RB-GR-90 | 0.0739 | 0.0172 | 0.0320 | n.d. | 0.0099 | 0.0029 | <0.001 | 0.0004 | 0.0065 | <0.001 | <0.001 |
| RB-GR-91 | 0.0138 | <0.001 | <0.001 | n.d. | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | n.d. | <0.001 |
| RB-GR-92 | 0.0643 | 0.0025 | 0.0154 | n.d. | 0.0049 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| RB-GR-93 | 0.0620 | 0.0045 | 0.0147 | n.d. | 0.0040 | <0.001 | <0.001 | <0.001 | 0.0012 | <0.001 | <0.001 |
| RB-GR-94 | 0.0339 | <0.001 | 0.0021 | n.d. | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| RB-GR-95 | 0.0825 | 0.0070 | 0.0217 | n.d. | 0.0084 | <0.001 | <0.001 | <0.001 | 0.0025 | <0.001 | <0.001 |
| RB-GR-96 | 0.0593 | 0.0054 | 0.0203 | n.d. | 0.0044 | <0.001 | <0.001 | <0.001 | 0.0026 | <0.001 | <0.001 |

n.d.: not detected.

Table Annex II.12 Anthocyanin derivatives concentrations of the samples from Médoc.

| Sample | Mv-3-(6-p-coum)-glc cis | Mv-3-(6-p-coum)-glc trans | Mv-3-glc-acetaldehyde | Mv-3-(6-p-coum)-glc-acetaldehyde | Mv-3-glc-pyruvic | Mv-3-(6-p-coum)-glc- pyruvic | Mv-3-glc-vinylmetyl | Mv-3-glc-4-vinylphenol | Mv-3-glc-4-vinylcatechol | Mv-3-glc-4-vinylguaiacol | Mv-3-(6-p-coum)-glc-4-vinylphenol | Mv-3-(6-p-coum)-glc 4-vinylguaiacol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BO-M-01 | 0.0138 | 1.0837 | 0.0567 | 0.0137 | 0.9318 | 0.1384 | 0.0597 | 0.4187 | 0.1059 | 0.0549 | 0.0682 | 0.0097 |
| BO-M-02 | 0.0171 | 2.2969 | 0.2784 | 0.0387 | 1.4344 | 0.1551 | 0.0586 | 0.8748 | 0.1041 | 0.0910 | 0.1098 | 0.0160 |
| BO-M-03 | 0.0159 | 0.9285 | 0.0566 | 0.0095 | 1.0114 | 0.2722 | 0.0459 | 0.6172 | 0.2314 | 0.0575 | 0.1353 | 0.0068 |
| BO-M-04 | 0.0332 | 1.8722 | 0.0480 | 0.0076 | 1.5306 | 0.2453 | 0.0466 | 0.9816 | 0.2698 | 0.1241 | 0.2343 | 0.0220 |
| BO-M-05 | 0.0271 | 1.8134 | 0.0380 | 0.0080 | 1.5193 | 0.2189 | 0.0431 | 0.5422 | 0.3139 | 0.0587 | 0.0711 | 0.0053 |
| BO-M-06 | 0.0211 | 1.8784 | 0.0845 | 0.0096 | 1.0073 | 0.0925 | 0.0662 | 0.1628 | 0.1340 | 0.0261 | 0.0199 | 0.0013 |
| BO-M-07 | 0.0011 | 0.2880 | 0.0286 | 0.0019 | 0.7955 | 0.0911 | 0.0290 | 0.3712 | 0.1246 | 0.0306 | 0.0366 | 0.0008 |
| BO-M-08 | <0.001 | 0.1355 | 0.0153 | <0.001 | 0.6049 | 0.0976 | 0.0363 | 0.2176 | 0.1426 | 0.0274 | 0.0191 | <0.001 |
| BO-M-09 | 0.0017 | 0.2419 | 0.0086 | <0.001 | 0.3612 | 0.0625 | 0.0154 | 0.3984 | 0.1652 | 0.0442 | 0.0452 | 0.0016 |
| BO-M-10 | 0.0026 | 0.3092 | 0.1136 | 0.0080 | 0.4503 | 0.0658 | 0.0232 | 0.3775 | 0.1674 | 0.0449 | 0.0553 | 0.0022 |
| BO-M-11 | 0.0331 | 0.8675 | 0.0276 | 0.0071 | 0.9002 | 0.2425 | 0.0424 | 0.3486 | 0.3430 | 0.0382 | 0.0665 | 0.0067 |
| BO-M-12 | 0.0065 | 0.4830 | 0.0479 | 0.0087 | 0.5473 | 0.0675 | 0.0253 | 0.3854 | 0.1718 | 0.0445 | 0.0430 | 0.0027 |
| BO-M-13A | 0.0104 | 1.3352 | 0.0333 | 0.0034 | 0.9559 | 0.1639 | 0.0426 | 0.2769 | 0.1422 | 0.0308 | 0.0546 | 0.0038 |
| BO-M-14A | <0.001 | 0.0950 | 0.0124 | 0.0010 | 0.5509 | 0.1141 | 0.0285 | 0.5554 | 0.2521 | 0.0489 | 0.0563 | 0.0024 |
| BO-M-15A | 0.0131 | 1.0229 | 0.0813 | 0.0084 | 1.1570 | 0.2211 | 0.0496 | 0.6809 | 0.1599 | 0.0531 | 0.1610 | 0.0059 |
| BO-M-16A | 0.0254 | 1.0494 | 0.0639 | 0.0146 | 0.8287 | 0.1531 | 0.0454 | 0.6670 | 0.3787 | 0.0667 | 0.0957 | 0.0081 |
| BO-M-17A | 0.0196 | 0.8469 | 0.0420 | 0.0107 | 0.6915 | 0.3530 | 0.0284 | 0.5704 | 0.3251 | 0.0569 | 0.0800 | 0.0081 |
| BO-M-18A | 0.0166 | 0.8921 | 0.0328 | 0.0090 | 0.5113 | 0.0781 | 0.0428 | 0.2255 | 0.1419 | 0.0345 | 0.0295 | 0.0031 |
| BO-M-19A | 0.0140 | 0.6551 | 0.0763 | 0.0112 | 0.4441 | 0.0442 | 0.0354 | 0.1476 | 0.0599 | 0.0199 | 0.0180 | 0.0012 |
| BO-M-20A | 0.0308 | 1.7193 | 0.0475 | 0.0172 | 1.1350 | 0.3094 | 0.0515 | 0.5121 | 0.1937 | 0.0721 | 0.1097 | 0.0156 |
| BO-M-21A | 0.0102 | 0.5837 | 0.0805 | 0.0146 | 0.6694 | 0.0779 | 0.0550 | 0.4697 | 0.0855 | 0.0484 | 0.0653 | 0.0043 |
| BO-M-22B | 0.0098 | 0.4865 | 0.0114 | 0.0021 | 0.9475 | 0.3061 | 0.0393 | 1.0201 | 0.5014 | 0.1063 | 0.1348 | 0.0076 |
| BO-M-23B | 0.0263 | 1.7618 | 0.1011 | 0.0217 | 1.6015 | 0.2933 | 0.0486 | 0.6171 | 0.3499 | 0.0820 | 0.0999 | 0.0090 |
| BO-M-24B | 0.0206 | 1.3885 | 0.1685 | 0.0285 | 0.9231 | 0.1792 | 0.0340 | 0.4538 | 0.1616 | 0.0487 | 0.0819 | 0.0072 |
| BO-M-25B | 0.0161 | 1.0830 | 0.0121 | 0.0077 | 0.5188 | 0.1227 | 0.0167 | 0.8492 | 0.1768 | 0.0291 | 0.0367 | 0.0057 |
| BO-M-26B | 0.0030 | 0.4376 | 0.0215 | 0.0017 | 0.7998 | 0.1668 | 0.0266 | 0.7218 | 0.3353 | 0.0747 | 0.1166 | 0.0097 |
| BO-M-27B | <0.001 | 0.0448 | 0.0035 | <0.001 | 0.2386 | 0.0751 | 0.0134 | 0.5051 | 0.3183 | 0.0567 | 0.0683 | 0.0056 |
| BO-M-28B | <0.001 | 0.0925 | 0.0070 | <0.001 | 0.5706 | 0.1474 | 0.0354 | 0.6773 | 0.2443 | 0.0641 | 0.0844 | 0.0058 |
| BO-M-29B | 0.0041 | 0.3456 | 0.0391 | 0.0038 | 0.7291 | 0.0990 | 0.0269 | 0.6859 | 0.3259 | 0.0550 | 0.0792 | 0.0052 |
| BO-M-30B | 0.0021 | 0.4553 | 0.0524 | 0.0067 | 0.6208 | 0.0903 | 0.0309 | 0.3607 | 0.1090 | 0.0470 | 0.0524 | 0.0040 |
| BO-M-31B | 0.0045 | 0.2359 | 0.0048 | <0.001 | 0.2784 | 0.0229 | 0.0096 | 0.1924 | 0.3414 | 0.0288 | 0.0165 | 0.0009 |
| BO-M-32B | 0.0217 | 1.4884 | 0.0195 | 0.0027 | 0.6595 | 0.0781 | 0.0361 | 0.2143 | 0.1365 | 0.0303 | 0.0272 | 0.0028 |
| BO-M-33B | <0.001 | 0.0646 | 0.0150 | <0.001 | 0.4393 | 0.0689 | 0.0151 | 0.4949 | 0.3099 | 0.0376 | 0.0350 | <0.001 |
| BO-M-34B | 0.1328 | 6.4441 | 0.0705 | 0.0313 | 0.8077 | 0.1761 | 0.0388 | 0.5656 | 0.0770 | 0.0566 | 0.1314 | 0.0186 |
| BO-M-35B | 0.0351 | 3.0147 | 0.1360 | 0.0392 | 0.9715 | 0.2099 | 0.0735 | 0.2973 | 0.1436 | 0.0455 | 0.0611 | 0.0080 |
| BO-M-36B | 0.0272 | 1.9443 | 0.0773 | 0.0244 | 1.1524 | 0.2684 | 0.0601 | 0.4810 | 0.1465 | 0.0706 | 0.1153 | 0.0088 |

Continuation of Table Annex II. 12

| Sample | Catechin-Mv-3-glc | Catechin-Mv-3-(6-p-coum)-glc | Epicatechin-Mv-3-glc | Epicatechin-Mv-3-(6-p-coum)-glc | (Epi)Gallocatechin-Mv-3-glc | (Epi)Gallocatechin-Mv-3-(6-p-coum)-glc | Mv-3-glc-8-ethyl-catechin 1 | Mv-3-glc-8-ethyl-catechin 2 | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin 1 | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin 2 | Mv-3-glc-8-ethyl-epicatechin |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **BO-M-01** | 0.0966 | 0.0171 | 0.0482 | n.d. | 0.0213 | 0.0014 | <0.001 | <0.001 | 0.0055 | <0.001 | <0.001 |
| **BO-M-02** | 0.1640 | 0.0180 | 0.0598 | n.d. | 0.0284 | 0.0025 | 0.0029 | 0.0124 | 0.0391 | <0.001 | <0.001 |
| **BO-M-03** | 0.0645 | 0.0112 | 0.0383 | n.d. | 0.0062 | <0.001 | <0.001 | <0.001 | 0.0043 | <0.001 | <0.001 |
| **BO-M-04** | 0.1580 | 0.0203 | 0.0587 | n.d. | 0.0038 | 0.0026 | <0.001 | <0.001 | 0.0049 | <0.001 | <0.001 |
| **BO-M-05** | 0.1024 | 0.0123 | 0.0579 | n.d. | 0.0078 | <0.001 | <0.001 | <0.001 | 0.0040 | <0.001 | <0.001 |
| **BO-M-06** | 0.1718 | 0.0132 | 0.0402 | n.d. | 0.0268 | 0.0020 | <0.001 | <0.001 | 0.0075 | <0.001 | <0.001 |
| **BO-M-07** | 0.0873 | 0.0083 | 0.0322 | n.d. | 0.0144 | <0.001 | <0.001 | <0.001 | 0.0008 | <0.001 | <0.001 |
| **BO-M-08** | 0.0405 | <0.001 | 0.0162 | n.d. | 0.0033 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **BO-M-09** | 0.0438 | 0.0022 | 0.0180 | n.d | 0.0022 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **BO-M-10** | 0.0711 | 0.0032 | 0.0235 | n.d. | 0.0054 | <0.001 | <0.001 | <0.001 | 0.0016 | <0.001 | <0.001 |
| **BO-M-11** | 0.0980 | 0.0239 | 0.0461 | n.d. | 0.0076 | 0.0036 | <0.001 | <0.001 | 0.0057 | <0.001 | <0.001 |
| **BO-M-12** | 0.0767 | 0.0066 | 0.0252 | n.d. | 0.0160 | 0.0009 | <0.001 | <0.001 | 0.0016 | <0.001 | <0.001 |
| **BO-M-13A** | 0.1001 | 0.0107 | 0.0364 | n.d. | 0.0081 | 0.0116 | <0.001 | <0.001 | 0.0022 | <0.001 | <0.001 |
| **BO-M-14A** | 0.0369 | <0.001 | 0.0080 | n.d | 0.0045 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **BO-M-15A** | 0.0713 | 0.0069 | 0.0350 | n.d. | 0.0075 | 0.0011 | <0.001 | <0.001 | 0.0036 | <0.001 | <0.001 |
| **BO-M-16A** | 0.0865 | 0.0147 | 0.0463 | n.d. | 0.0171 | 0.0007 | <0.001 | <0.001 | 0.0129 | <0.001 | <0.001 |
| **BO-M-17A** | 0.0596 | 0.0117 | 0.0386 | n.d. | 0.0122 | 0.0016 | <0.001 | <0.001 | 0.0135 | <0.001 | <0.001 |
| **BO-M-18A** | 0.1546 | 0.0121 | 0.0417 | n.d. | 0.0146 | 0.0010 | <0.001 | <0.001 | 0.0026 | <0.001 | <0.001 |
| **BO-M-19A** | 0.1349 | 0.0076 | 0.0260 | n.d. | 0.0187 | 0.0010 | <0.001 | <0.001 | 0.0045 | <0.001 | <0.001 |
| **BO-M-20A** | 0.1085 | 0.0270 | 0.0521 | n.d. | 0.0193 | 0.0046 | <0.001 | <0.001 | 0.0082 | <0.001 | <0.001 |
| **BO-M-21A** | 0.0998 | 0.0080 | 0.0368 | n.d. | 0.0177 | <0.001 | <0.001 | <0.001 | 0.0070 | <0.001 | <0.001 |
| **BO-M-22B** | 0.0572 | 0.0101 | 0.0444 | n.d. | 0.0152 | 0.0008 | <0.001 | <0.001 | 0.0040 | <0.001 | <0.001 |
| **BO-M-23B** | 0.1086 | 0.0152 | 0.0586 | n.d. | 0.0163 | <0.001 | <0.001 | <0.001 | 0.0055 | <0.001 | <0.001 |
| **BO-M-24B** | 0.0949 | 0.0152 | 0.0343 | n.d. | 0.0199 | 0.0017 | <0.001 | 0.0013 | 0.0080 | <0.001 | <0.001 |
| **BO-M-25B** | 0.1571 | 0.0132 | 0.0441 | n.d. | 0.0118 | 0.0048 | <0.001 | <0.001 | 0.0266 | <0.001 | <0.001 |
| **BO-M-26B** | 0.0541 | 0.0064 | 0.0276 | n.d. | 0.0141 | <0.001 | <0.001 | <0.001 | 0.0008 | <0.001 | <0.001 |
| **BO-M-27B** | 0.0116 | <0.001 | 0.0025 | n.d. | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **BO-M-28B** | 0.0305 | <0.001 | 0.0108 | n.d. | 0.0027 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **BO-M-29B** | 0.0768 | 0.0066 | 0.0239 | n.d. | 0.0158 | <0.001 | <0.001 | <0.001 | 0.0022 | <0.001 | <0.001 |
| **BO-M-30B** | 0.0791 | 0.0050 | 0.0367 | n.d. | 0.0069 | <0.001 | <0.001 | <0.001 | 0.0026 | <0.001 | <0.001 |
| **BO-M-31B** | 0.0824 | 0.0027 | 0.0263 | n.d. | 0.0055 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **BO-M-32B** | 0.2144 | 0.0132 | 0.0548 | n.d. | 0.0146 | <0.001 | <0.001 | <0.001 | 0.0023 | <0.001 | <0.001 |
| **BO-M-33B** | 0.0478 | <0.001 | 0.0141 | n.d. | 0.0050 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **BO-M-34B** | 0.1811 | 0.0522 | 0.0876 | n.d. | 0.0168 | 0.0101 | <0.001 | 0.0013 | 0.0298 | 0.0007 | <0.001 |
| **BO-M-35B** | 0.1447 | 0.0305 | 0.0761 | n.d. | 0.0236 | 0.0024 | <0.001 | 0.0017 | 0.0193 | <0.001 | <0.001 |
| **BO-M-36B** | 0.1054 | 0.0169 | 0.0516 | n.d. | 0.0123 | 0.0037 | <0.001 | <0.001 | 0.0083 | <0.001 | <0.001 |

n.d.: not detected.

Table Annex II.13 Anthocyanin derivatives concentrations of the samples from Graves.

| Sample | Mv-3-(6-p-coum)-glc cis | Mv-3-(6-p-coum)-glc trans | Mv-3-glc-acetaldehyde | Mv-3-(6-p-coum)-glc-acetaldehyde | Mv-3-glc-pyruvic | Mv-3-(6-p-coum)-glc- pyruvic | Mv-3-glc-vinylmetyl | Mv-3-glc-4-vinylphenol | Mv-3-glc-4-vinylcatechol | Mv-3-glc-4-vinylguaiacol | Mv-3-(6-p-coum)-glc-4-vinylphenol | Mv-3-(6-p-coum)-glc-4-vinylguaiacol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BO-G-37 | 0.0053 | 0.6647 | 0.0293 | 0.0012 | 0.4956 | 0.0568 | 0.0158 | 0.5092 | 0.0986 | 0.0325 | 0.0457 | 0.0011 |
| BO-G-38 | 0.0160 | 0.9406 | 0.1089 | 0.0177 | 0.9837 | 0.0969 | 0.0626 | 1.0834 | 0.2998 | 0.0928 | 0.1000 | 0.0115 |
| BO-G-39 | 0.0061 | 0.5362 | 0.0414 | 0.0030 | 0.6511 | 0.0761 | 0.0214 | 0.4901 | 0.0855 | 0.0356 | 0.0611 | 0.0025 |
| BO-G-40 | <0.001 | 0.2629 | 0.0129 | <0.001 | 0.3666 | 0.0330 | 0.0087 | 0.2378 | 0.1958 | 0.0207 | 0.0188 | 0.0012 |
| BO-G-41 | <0.001 | 0.4821 | 0.0637 | 0.0067 | 0.6790 | 0.0876 | 0.0372 | 0.5479 | 0.0863 | 0.0546 | 0.0816 | 0.0056 |
| BO-G-42 | 0.0040 | 0.5614 | 0.0399 | 0.0037 | 0.7303 | 0.0946 | 0.0267 | 0.4127 | 0.1090 | 0.0474 | 0.0522 | 0.0055 |
| BO-G-43 | <0.001 | 0.1456 | 0.0091 | <0.001 | 0.3381 | 0.0757 | 0.0125 | 1.0022 | 0.1679 | 0.0641 | 0.1066 | 0.0052 |
| BO-G-44B | 0.0061 | 0.6622 | 0.0756 | 0.0069 | 0.8148 | 0.0942 | 0.0355 | 0.4228 | 0.1265 | 0.0523 | 0.0592 | 0.0038 |
| BO-G-45B | 0.0025 | 0.3289 | 0.0335 | <0.001 | 0.5458 | 0.0499 | 0.0199 | 0.1863 | 0.1053 | 0.0242 | 0.0169 | <0.001 |
| BO-G-46B | <0.001 | 0.0705 | 0.0389 | <0.001 | 0.5634 | 0.0618 | 0.0258 | 0.6593 | 0.3499 | 0.0407 | 0.0629 | 0.0051 |
| BO-G-47B | 0.0205 | 1.6294 | 0.0676 | 0.0063 | 0.8815 | 0.1202 | 0.0450 | 0.4424 | 0.2083 | 0.0691 | 0.0817 | 0.0053 |
| BO-G-48B | 0.0141 | 0.6863 | 0.2192 | 0.0266 | 0.4565 | 0.0557 | 0.0982 | 0.3099 | 0.0757 | 0.0292 | 0.0563 | 0.0033 |
| BO-G-87 | 0.0671 | 8.2686 | 0.1880 | 0.0413 | 0.9139 | 0.1225 | 0.0998 | 0.7270 | 0.1115 | 0.0495 | 0.1703 | 0.0059 |

Continuation of Table Annex II. 13.

| Sample | Catechin-Mv-3-glc | Catechin-Mv-3-(6-p-coum)-glc | Epicatechin-Mv-3-glc | Epicatechin-Mv-3-(6-p-coum)-glc | (Epi)Gallocatechin-Mv-3-glc | (Epi)Gallocatechin-Mv-3-(6-p-coum)-glc | Mv-3-glc-8-ethyl-catechin 1 | Mv-3-glc-8-ethyl-catechin 2 | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin 1 | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin 2 | Mv-3-glc-8-ethyl-epicatechin |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BO-G-37 | 0.1096 | 0.0091 | 0.0516 | n.d. | 0.0205 | <0.001 | <0.001 | <0.001 | 0.0070 | <0.001 | <0.001 |
| BO-G-38 | 0.1571 | 0.0146 | 0.0483 | n.d. | 0.0354 | 0.0010 | <0.001 | <0.001 | 0.0076 | <0.001 | <0.001 |
| BO-G-39 | 0.0984 | 0.0058 | 0.0347 | n.d. | 0.0107 | <0.001 | <0.001 | <0.001 | 0.0043 | <0.001 | <0.001 |
| BO-G-40 | 0.0715 | 0.0026 | 0.0185 | n.d. | 0.0071 | <0.001 | <0.001 | <0.001 | 0.0011 | <0.001 | <0.001 |
| BO-G-41 | 0.0819 | 0.0045 | 0.0267 | n.d. | 0.0067 | <0.001 | <0.001 | <0.001 | 0.0071 | <0.001 | <0.001 |
| BO-G-42 | 0.0900 | 0.0066 | 0.0281 | n.d. | 0.0084 | <0.001 | <0.001 | <0.001 | 0.0040 | <0.001 | <0.001 |
| BO-G-43 | 0.0386 | <0.001 | 0.0194 | n.d. | 0.0025 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| BO-G-44B | 0.1527 | 0.0073 | 0.0407 | n.d. | 0.0106 | <0.001 | <0.001 | <0.001 | 0.0034 | <0.001 | <0.001 |
| BO-G-45B | 0.1130 | 0.0030 | 0.0304 | n.d. | 0.0094 | <0.001 | <0.001 | <0.001 | 0.0024 | <0.001 | <0.001 |
| BO-G-46B | 0.0397 | 0.0066 | 0.0089 | n.d. | 0.0105 | <0.001 | <0.001 | <0.001 | 0.0065 | <0.001 | <0.001 |
| BO-G-47B | 0.1511 | 0.0085 | 0.0508 | n.d. | 0.0117 | <0.001 | <0.001 | <0.001 | 0.0076 | <0.001 | <0.001 |
| BO-G-48B | 0.0718 | 0.0060 | 0.0302 | n.d. | 0.0088 | <0.001 | <0.001 | <0.001 | 0.0042 | <0.001 | <0.001 |
| BO-G-87 | 0.0745 | 0.0175 | 0.0493 | n.d. | 0.0147 | 0.0030 | <0.001 | <0.001 | 0.0153 | <0.001 | <0.001 |

n.d.: not detected.

Table Annex II.14 Anthocyanin derivatives concentrations of the samples from Blavais & Bourgeais.

| Sample | Mv-3-(6-p-coum)-glc cis | Mv-3-(6-p-coum)-glc trans | Mv-3-glc-acetaldehyde | Mv-3-(6-p-coum)-glc-acetaldehyde | Mv-3-glc-pyruvic | Mv-3-(6-p-coum)-glc- pyruvic | Mv-3-glc-vinylmetyl | Mv-3-glc-4-vinylphenol | Mv-3-glc-4-vinylcatechol | Mv-3-glc-4-vinylguaiacol | Mv-3-(6-p-coum)-glc-4-vinylphenol | Mv-3-(6-p-coum)-glc-4-vinylguaiacol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BO-BB-74 | 0.0042 | 0.6270 | 0.0743 | 0.0161 | 1.0266 | 0.2088 | 0.0456 | 0.1827 | 0.1067 | 0.0313 | 0.0370 | 0.0056 |
| BO-BB-82B | 0.0043 | 0.3718 | 0.0391 | 0.0079 | 0.8417 | 0.2326 | 0.0209 | 0.5058 | 0.0779 | 0.0454 | 0.1046 | 0.0030 |
| BO-BB-84 | <0.001 | 0.1352 | 0.0065 | <0.001 | 0.5807 | 0.1404 | 0.0161 | 0.3313 | 0.0948 | 0.0290 | 0.0408 | 0.0016 |
| BO-BB-85 | 0.0000 | 0.1731 | 0.0352 | 0.0045 | 1.2105 | 0.2603 | 0.0353 | 0.4956 | 0.0865 | 0.0432 | 0.0829 | 0.0057 |
| BO-BB-86 | 0.0045 | 0.2561 | 0.0199 | 0.0040 | 0.7334 | 0.2241 | 0.0299 | 0.5893 | 0.1654 | 0.0537 | 0.0847 | 0.0048 |

Continuation of Table Annex II. 14.

| Sample | Catechin-Mv-3-glc | Catechin-Mv-3-(6-p-coum)-glc | Epicatechin-Mv-3-glc | Epicatechin-Mv-3-(6-p-coum)-glc | (Epi)Gallocatechin-Mv-3-glc | (Epi)Gallocatechin-Mv-3-(6-p-coum)-glc | Mv-3-glc-8-ethyl-catechin 1 | Mv-3-glc-8-ethyl-catechin 2 | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin 1 | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin 2 | Mv-3-glc-8-ethyl-epicatechin |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BO-BB-74 | 0.1134 | 0.0192 | 0.0524 | n.d. | 0.0142 | <0.001 | <0.001 | <0.001 | 0.0134 | <0.001 | <0.001 |
| BO-BB-82B | 0.0356 | 0.0036 | 0.0148 | n.d. | 0.0020 | <0.001 | <0.001 | <0.001 | 0.0058 | <0.001 | <0.001 |
| BO-BB-84 | 0.0285 | 0.0007 | 0.0062 | n.d. | 0.0027 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| BO-BB-85 | 0.0354 | 0.0052 | 0.0127 | n.d. | 0.0050 | <0.001 | <0.001 | <0.001 | 0.0016 | <0.001 | <0.001 |
| BO-BB-86 | 0.0205 | 0.0033 | 0.0100 | n.d. | 0.0010 | <0.001 | <0.001 | <0.001 | 0.0025 | <0.001 | <0.001 |

n.d.: not detected.

Table Annex II.15 Anthocyanin derivatives concentrations of the samples from Libournais.

| Sample | Mv-3-(6-p-coum)-glc cis | Mv-3-(6-p-coum)-glc trans | Mv-3-glc-acetaldehyde | Mv-3-(6-p-coum)-glc-acetaldehyde | Mv-3-glc-pyruvic | Mv-3-(6-p-coum)-glc- pyruvic | Mv-3-glc-vinylmetyl | Mv-3-glc-4-vinylphenol | Mv-3-glc-4-vinylcatechol | Mv-3-glc-4-vinylguaiacol | Mv-3-(6-p-coum)-glc-4-vinylphenol | Mv-3-(6-p-coum)-glc-4-vinylguaiacol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BO-L-49** | <0.001 | 0.3293 | 0.0476 | 0.0106 | 0.7213 | 0.1888 | 0.0563 | 0.3858 | 0.2773 | 0.0340 | 0.0645 | 0.0047 |
| **BO-L-50** | 0.0124 | 1.0357 | 0.1364 | 0.0412 | 0.8300 | 0.1819 | 0.0502 | 0.1478 | 0.0712 | 0.0305 | 0.0392 | 0.0043 |
| **BO-L-51** | 0.0023 | 0.3333 | 0.0583 | 0.0046 | 0.8008 | 0.1428 | 0.0497 | 0.2438 | 0.0753 | 0.0283 | 0.0339 | 0.0020 |
| **BO-L-52** | <0.001 | 0.2415 | 0.0119 | 0.0012 | 0.4957 | 0.0623 | 0.0328 | 0.1178 | 0.0678 | 0.0182 | 0.0098 | 0.0017 |
| **BO-L-53** | 0.0310 | 1.4558 | 0.0489 | 0.0061 | 0.4781 | 0.0565 | 0.0648 | 0.6621 | 0.1715 | 0.1047 | 0.0765 | 0.0167 |
| **BO-L-54** | <0.001 | 0.4134 | 0.0153 | 0.0010 | 0.4017 | 0.0536 | 0.0130 | 0.7610 | 0.4652 | 0.0421 | 0.0464 | 0.0022 |
| **BO-L-55** | 0.0027 | 0.3794 | 0.0294 | 0.0072 | 1.5668 | 0.0810 | 0.0543 | 0.1485 | 0.0984 | 0.0383 | 0.0688 | 0.0024 |
| **BO-L-56** | 0.0619 | 3.2794 | 0.1372 | 0.0448 | 1.4905 | 0.3316 | 0.0626 | 0.3206 | 0.1531 | 0.0791 | 0.1034 | 0.0133 |
| **BO-L-57B** | 0.0049 | 0.5562 | 0.0068 | <0.001 | 0.4873 | 0.0448 | 0.0270 | 0.3162 | 0.2610 | 0.0484 | 0.0302 | 0.0017 |
| **BO-L-58B** | 0.0160 | 0.7693 | 0.0413 | 0.0055 | 0.4433 | 0.0856 | 0.0350 | 0.6043 | 0.1600 | 0.0652 | 0.0986 | 0.0132 |
| **BO-L-59** | 0.0080 | 0.5529 | 0.0279 | 0.0270 | 0.9420 | 0.0929 | 0.0395 | 0.2439 | 0.1335 | 0.0479 | 0.0745 | 0.0043 |
| **BO-L-60** | 0.0095 | 0.8690 | 0.0983 | 0.0221 | 0.9790 | 0.1755 | 0.0293 | 0.4678 | 0.1690 | 0.0505 | 0.0783 | 0.0063 |
| **BO-L-61** | 0.0016 | 0.3149 | 0.0715 | 0.0174 | 0.3888 | 0.0636 | 0.0380 | 0.2684 | 0.0923 | 0.0396 | 0.0336 | 0.0028 |
| **BO-L-62** | <0.001 | 0.1494 | 0.0181 | 0.0054 | 0.5065 | 0.1409 | 0.0721 | 0.7528 | 0.0993 | 0.0530 | 0.0952 | 0.0055 |
| **BO-L-63A** | 0.0153 | 1.0867 | 0.0558 | 0.0084 | 1.0581 | 0.1017 | 0.0408 | 0.9560 | 0.1475 | 0.0866 | 0.1094 | 0.0144 |
| **BO-L-64A** | 0.0070 | 0.6637 | 0.0982 | 0.0175 | 0.6685 | 0.1844 | 0.0323 | 0.2692 | 0.1574 | 0.0374 | 0.0542 | 0.0031 |
| **BO-L-65A** | 0.0157 | 0.6885 | 0.0753 | 0.0157 | 1.5197 | 0.2657 | 0.0733 | 0.2690 | 0.1142 | 0.0384 | 0.0482 | 0.0034 |
| **BO-L-66A** | <0.001 | 0.1083 | 0.0075 | <0.001 | 0.7682 | 0.2158 | 0.0445 | 0.1564 | 0.1114 | 0.0409 | 0.0142 | 0.0012 |
| **BO-L-67B** | 0.0081 | 0.5897 | 0.0355 | 0.0028 | 0.4229 | 0.0564 | 0.0199 | 0.7191 | 0.4295 | 0.1106 | 0.1000 | 0.0089 |
| **BO-L-68B** | 0.0035 | 0.4672 | 0.0589 | 0.0070 | 0.6993 | 0.0839 | 0.0534 | 0.3717 | 0.1026 | 0.0510 | 0.0484 | 0.0038 |
| **BO-L-73** | 0.0041 | 0.4434 | 0.0214 | 0.0011 | 0.7640 | 0.0802 | 0.0356 | 0.6171 | 0.1061 | 0.0384 | 0.0588 | 0.0028 |
| **BO-L-75** | 0.0051 | 0.6196 | 0.0418 | 0.0038 | 0.5808 | 0.0761 | 0.0173 | 0.1710 | 0.0387 | 0.0359 | 0.0252 | 0.0031 |
| **BO-L-76** | <0.001 | 0.1787 | 0.0768 | 0.0078 | 0.7035 | 0.1228 | 0.0290 | 0.2839 | 0.1041 | 0.0227 | 0.0380 | 0.0016 |
| **BO-L-77** | <0.001 | 0.0524 | 0.0069 | <0.001 | 0.2764 | 0.0572 | 0.0188 | 0.4708 | 0.1507 | 0.0459 | 0.0557 | n.d. |
| **BO-L-79** | 0.0082 | 0.5757 | 0.2362 | 0.0274 | 1.0439 | 0.1217 | 0.0908 | 0.8076 | 0.2454 | 0.0516 | 0.0769 | 0.0074 |
| **BO-L-80** | 0.0076 | 0.6237 | 0.0518 | 0.0102 | 0.9023 | 0.1399 | 0.0498 | 0.1869 | 0.1260 | 0.0294 | 0.0258 | <0.001 |
| **BO-L-81** | <0.001 | 0.2431 | 0.0086 | 0.0014 | 0.5284 | 0.1177 | 0.0211 | 0.3729 | 0.1195 | 0.0348 | 0.0626 | 0.0028 |

Continuation of Table Annex II. 15.

| Sample | Catechin-Mv-3-glc | Catechin-Mv-3-(6-p-coum)-glc | Epicatechin-Mv-3-glc | Epicatechin-Mv-3-(6-p-coum)-glc | (Epi)Gallocatechin-Mv-3-glc | (Epi)Gallocatechin-Mv-3-(6-p-coum)-glc | Mv-3-glc-8-ethyl-catechin 1 | Mv-3-glc-8-ethyl-catechin 2 | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin 1 | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin 2 | Mv-3-glc-8-ethyl-epicatechin |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **BO-L-49** | 0.0366 | 0.0070 | 0.0240 | n.d. | 0.0026 | <0.001 | <0.001 | <0.001 | 0.0123 | <0.001 | <0.001 |
| **BO-L-50** | 0.0726 | 0.0188 | 0.0544 | n.d. | 0.0064 | <0.001 | <0.001 | 0.0046 | 0.0311 | <0.001 | <0.001 |
| **BO-L-51** | 0.0506 | 0.0051 | 0.0317 | n.d. | 0.0039 | <0.001 | <0.001 | <0.001 | 0.0029 | <0.001 | <0.001 |
| **BO-L-52** | 0.0950 | 0.0053 | 0.0432 | n.d. | 0.0038 | <0.001 | <0.001 | <0.001 | 0.0010 | <0.001 | <0.001 |
| **BO-L-53** | 0.1578 | 0.0216 | 0.0513 | n.d. | 0.0240 | 0.0026 | <0.001 | <0.001 | 0.0036 | <0.001 | <0.001 |
| **BO-L-54** | 0.0561 | 0.0083 | 0.0366 | n.d. | <0.001 | <0.001 | <0.001 | <0.001 | 0.0044 | <0.001 | <0.001 |
| **BO-L-55** | 0.0641 | 0.0061 | 0.0342 | n.d. | 0.0062 | <0.001 | <0.001 | <0.001 | 0.0010 | <0.001 | <0.001 |
| **BO-L-56** | 0.1207 | 0.0251 | 0.0868 | n.d. | 0.0081 | 0.0032 | <0.001 | 0.0020 | 0.0247 | <0.001 | <0.001 |
| **BO-L-57B** | 0.1153 | 0.0101 | 0.0368 | n.d. | 0.0180 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **BO-L-58B** | 0.0965 | 0.0187 | 0.0482 | n.d. | 0.0156 | 0.0012 | <0.001 | <0.001 | 0.0071 | <0.001 | <0.001 |
| **BO-L-59** | 0.0848 | 0.0179 | 0.0400 | n.d. | 0.0157 | 0.0010 | <0.001 | <0.001 | 0.0100 | <0.001 | <0.001 |
| **BO-L-60** | 0.1122 | 0.0117 | 0.0463 | n.d. | 0.0038 | <0.001 | <0.001 | <0.001 | 0.0085 | <0.001 | <0.001 |
| **BO-L-61** | 0.0579 | 0.0067 | 0.0265 | n.d. | 0.0052 | <0.001 | <0.001 | 0.0013 | 0.0174 | <0.001 | <0.001 |
| **BO-L-62** | 0.0276 | 0.0006 | 0.0107 | n.d. | <0.001 | <0.001 | <0.001 | <0.001 | 0.0021 | <0.001 | n.d. |
| **BO-L-63A** | 0.1150 | 0.0174 | 0.0564 | n.d. | 0.0174 | 0.0008 | <0.001 | <0.001 | 0.0077 | <0.001 | <0.001 |
| **BO-L-64A** | 0.0624 | 0.0055 | 0.0340 | n.d. | 0.0023 | <0.001 | <0.001 | <0.001 | 0.0048 | <0.001 | <0.001 |
| **BO-L-65A** | 0.0702 | 0.0102 | 0.0350 | n.d. | <0.001 | <0.001 | <0.001 | <0.001 | 0.0077 | <0.001 | <0.001 |
| **BO-L-66A** | 0.0172 | <0.001 | 0.0050 | n.d. | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **BO-L-67B** | 0.0992 | 0.0076 | 0.0328 | n.d. | 0.0053 | <0.001 | <0.001 | <0.001 | 0.0024 | <0.001 | <0.001 |
| **BO-L-68B** | 0.0690 | 0.0031 | 0.0262 | n.d. | 0.0042 | <0.001 | <0.001 | <0.001 | 0.0063 | <0.001 | <0.001 |
| **BO-L-73** | 0.1017 | 0.0107 | 0.0371 | n.d. | 0.0137 | <0.001 | <0.001 | <0.001 | 0.0018 | <0.001 | <0.001 |
| **BO-L-75** | 0.0960 | 0.0077 | 0.0254 | n.d. | 0.0057 | <0.001 | <0.001 | <0.001 | 0.0042 | <0.001 | <0.001 |
| **BO-L-76** | 0.0378 | 0.0022 | 0.0219 | n.d. | 0.0024 | <0.001 | <0.001 | <0.001 | 0.0031 | <0.001 | <0.001 |
| **BO-L-77** | 0.0209 | <0.001 | 0.0059 | n.d. | <0.001 | <0.001 | <0.001 | <0.001 | n.d. | n.d. | <0.001 |
| **BO-L-79** | 0.1203 | 0.0108 | 0.0424 | n.d. | 0.0119 | <0.001 | <0.001 | <0.001 | 0.0104 | <0.001 | 0.0029 |
| **BO-L-80** | 0.0735 | 0.0140 | 0.0424 | n.d. | 0.0070 | 0.0013 | <0.001 | <0.001 | n.d. | n.d. | <0.001 |
| **BO-L-81** | 0.0543 | 0.0028 | 0.0169 | n.d. | 0.0026 | <0.001 | <0.001 | <0.001 | 0.0010 | <0.001 | <0.001 |

n.d.: not detected.

Table Annex II.16 Anthocyanin derivatives concentrations of the samples from Bordeaux & Bordeaux. Superieur.

| Sample | Mv-3-(6-p-coum)-glc cis | Mv-3-(6-p-coum)-glc trans | Mv-3-glc-acetaldehyde | Mv-3-(6-p-coum)-glc-acetaldehyde | Mv-3-glc-pyruvic | Mv-3-(6-p-coum)-glc- pyruvic | Mv-3-glc-vinylmetyl | Mv-3-glc-4-vinylphenol | Mv-3-glc-4-vinylcatechol | Mv-3-glc-4-vinylguaiacol | Mv-3-(6-p-coum)-glc-4-vinylphenol | Mv-3-(6-p-coum)-glc-4-vinylguaiacol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BO-B-69 | 0.0087 | 0.7427 | 0.0705 | 0.0120 | 0.8773 | 0.1140 | 0.0444 | 0.4684 | 0.0995 | 0.0593 | 0.0652 | 0.0091 |
| BO-B-70 | 0.0012 | 0.4493 | 0.0082 | <0.001 | 0.1431 | 0.0246 | 0.0294 | 0.2365 | 0.2102 | 0.0786 | 0.0364 | 0.0044 |
| BO-BS-71 | <0.001 | 0.0225 | 0.0037 | <0.001 | 0.2700 | 0.0367 | 0.0092 | 0.2130 | 0.1177 | 0.0167 | 0.0131 | <0.001 |
| BO-BS-72 | 0.0172 | 0.9676 | 0.0399 | 0.0032 | 0.5444 | 0.0554 | 0.0279 | 0.6464 | 0.4154 | 0.0669 | 0.0939 | 0.0048 |
| BO-B-88 | 0.0687 | 4.1099 | 0.1199 | 0.0308 | 0.5559 | 0.0743 | 0.0137 | 0.4854 | 0.0994 | 0.0282 | 0.1068 | 0.0043 |
| BO-B-89 | 0.0420 | 3.5435 | 0.0851 | 0.0274 | 0.4727 | 0.0989 | 0.0195 | 0.8333 | 0.1512 | 0.0387 | 0.1254 | 0.0067 |
| BO-B-90 | 0.0866 | 4.7184 | 0.1347 | 0.0325 | 0.4640 | 0.0455 | 0.0258 | 0.3174 | 0.0596 | 0.0364 | 0.0700 | 0.0046 |
| BO-B-91 | 0.0294 | 2.6367 | 0.6732 | 0.0115 | 0.6018 | 0.1012 | 0.0410 | 0.6925 | 0.1242 | 0.0729 | 0.1791 | 0.0131 |
| BO-B-92 | 0.0321 | 3.1990 | 0.2430 | 0.0589 | 0.7128 | 0.0989 | 0.0418 | 0.3462 | 0.0917 | 0.0404 | 0.0882 | 0.0054 |
| BO-B-93 | 0.0570 | 5.7190 | 0.0877 | 0.0193 | 0.5936 | 0.0837 | 0.0357 | 0.5276 | 0.0718 | 0.0380 | 0.1033 | 0.0066 |
| BO-B-94 | 0.0690 | 6.6353 | 0.0624 | 0.0125 | 0.3768 | 0.0508 | 0.0239 | 0.5525 | 0.1230 | 0.0494 | 0.1035 | 0.0066 |
| BO-B-95 | 0.0798 | 7.4290 | 0.0785 | 0.0241 | 0.6777 | 0.0974 | 0.0382 | 0.5188 | 0.0652 | 0.0402 | 0.1142 | 0.0068 |
| BO-B-96 | 0.0307 | 4.7338 | 0.0357 | 0.0077 | 0.5399 | 0.1053 | 0.0309 | 0.4146 | 0.1192 | 0.0406 | 0.0753 | 0.0052 |

Continuation of Table Annex II. 16.

| Sample | Catechin-Mv-3-glc | Catechin-Mv-3-(6-p-coum)-glc | Epicatechin-Mv-3-glc | Epicatechin-Mv-3-(6-p-coum)-glc | (Epi)Gallocatechin-Mv-3-glc | (Epi)Gallocatechin-Mv-3-(6-p-coum)-glc | Mv-3-glc-8-ethyl-catechin 1 | Mv-3-glc-8-ethyl-catechin 2 | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin 1 | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin 2 | Mv-3-glc-8-ethyl-epicatechin |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BO-B-69 | 0.1067 | 0.0164 | 0.0483 | n.d. | 0.0159 | 0.0007 | <0.001 | <0.001 | 0.0058 | <0.001 | <0.001 |
| BO-B-70 | 0.1056 | 0.0059 | 0.0162 | n.d. | 0.0158 | 0.0020 | <0.001 | <0.001 | 0.0014 | <0.001 | <0.001 |
| BO-BS-71 | 0.0097 | <0.001 | 0.0032 | n.d. | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| BO-BS-72 | 0.0800 | 0.0062 | 0.0459 | n.d. | 0.0124 | <0.001 | <0.001 | <0.001 | 0.0038 | <0.001 | <0.001 |
| BO-B-88 | 0.0544 | 0.0189 | 0.0526 | n.d. | 0.0056 | 0.0032 | <0.001 | <0.001 | 0.0102 | <0.001 | <0.001 |
| BO-B-89 | 0.0629 | 0.0312 | 0.0389 | n.d. | 0.0070 | 0.0028 | <0.001 | <0.001 | 0.0150 | <0.001 | <0.001 |
| BO-B-90 | 0.0584 | 0.0165 | 0.0287 | n.d. | 0.0042 | 0.0013 | <0.001 | 0.0022 | 0.0208 | 0.0022 | <0.001 |
| BO-B-91 | 0.0740 | 0.0153 | 0.0442 | n.d. | 0.0063 | 0.0028 | 0.0038 | 0.0070 | 0.0348 | 0.0047 | 0.0029 |
| BO-B-92 | 0.0714 | 0.0210 | 0.0533 | n.d. | 0.0095 | 0.0034 | <0.001 | 0.0030 | 0.0228 | 0.0010 | <0.001 |
| BO-B-93 | 0.0589 | 0.0193 | 0.0426 | n.d. | 0.0057 | 0.0022 | <0.001 | <0.001 | 0.0122 | <0.001 | <0.001 |
| BO-B-94 | 0.0583 | 0.0280 | 0.0494 | n.d. | 0.0038 | 0.0023 | <0.001 | <0.001 | 0.0162 | <0.001 | <0.001 |
| BO-B-95 | 0.0660 | 0.0259 | 0.0523 | n.d. | 0.0087 | 0.0044 | <0.001 | <0.001 | 0.0143 | <0.001 | <0.001 |
| BO-B-96 | 0.0593 | 0.0260 | 0.0428 | n.d. | 0.0046 | 0.0032 | <0.001 | <0.001 | 0.0037 | <0.001 | <0.001 |

n.d.: not detected.

Table Annex II.17 Tannins concentrations of the samples from Rioja Alavesa.

| Sample | Catechin | Epicatechin | PCB1 | PCB2 | ((epi)cat)$_2$ 1 | PCC1 | ((epi)cat)$_3$ 1 | ((epi)cat)$_3$ 2 | Gallocatechin | Epigallocatechin | ((epi)gallocat)$_2$ 1 | ((epi)gallocat)$_2$ 2 | ((epi)gallocat)$_2$ 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **RV-J-01** | 2.0942 | 0.7726 | 5.9922 | 1.1832 | 0.2670 | 0.0445 | 0.0096 | 0.1034 | 0.6557 | 0.3207 | 0.2655 | 0.0261 | 0.0298 |
| **RV-J-02** | 3.5099 | 1.2215 | 10.4810 | 2.8595 | 0.6294 | 0.1326 | n.d | 0.3645 | 1.0281 | 0.8480 | 0.3590 | 0.0100 | 0.0897 |
| **RV-J-03** | 7.0142 | 1.9656 | 77.6405 | 5.1379 | 1.6968 | 0.2257 | 0.0052 | 0.9439 | 1.9716 | 0.8627 | 0.3327 | 0.0404 | 0.0697 |
| **RV-J-04** | 7.9157 | 2.8813 | 57.7017 | 10.6650 | 2.0971 | 0.3675 | 0.0263 | 1.3236 | 1.9929 | 0.8188 | 0.4991 | 0.0556 | 0.1457 |
| **RV-J-05** | 8.1688 | 2.7082 | 62.5453 | 9.2494 | 1.5576 | 0.2986 | 0.0086 | 0.9056 | 3.0627 | 0.9084 | 1.3237 | 0.1625 | 0.1994 |
| **RV-J-06** | 9.4588 | 3.8029 | 110.5780 | 16.8313 | 2.4070 | 0.4945 | 0.0089 | 1.2701 | 2.2405 | 0.9253 | 0.5967 | 0.0911 | 0.1122 |
| **RV-J-07** | 7.1278 | 2.2724 | 64.8870 | 6.5341 | 1.1860 | 0.2816 | 0.0071 | 0.9102 | 1.7645 | 0.7225 | 0.6504 | 0.1122 | 0.1743 |
| **RV-J-08** | 6.8991 | 1.9091 | 36.1692 | 5.5149 | 1.0343 | 0.2128 | 0.0071 | 0.6973 | 2.1211 | 0.8448 | 0.7660 | 0.1407 | 0.1608 |
| **RV-C-09** | 6.0343 | 2.1070 | 99.6313 | 7.6478 | 1.3963 | 0.3095 | 0.0161 | 1.0814 | 1.6061 | 1.0535 | 0.7443 | <0.005 | 0.2736 |
| **RV-C-10** | 4.9564 | 1.4734 | 51.8712 | 4.7251 | 1.1049 | 0.1350 | n.d. | 0.7082 | 1.4755 | 0.8105 | 0.8624 | 0.0973 | 0.1684 |
| **RV-C-11** | 1.7445 | 0.7410 | 15.8306 | 2.8840 | 0.3697 | 0.1071 | n.d. | 0.2828 | 0.8010 | 0.2181 | 0.2765 | 0.0464 | 0.0887 |
| **RV-C-12** | 3.2138 | 0.8904 | 33.9890 | 3.0488 | 0.5612 | 0.0933 | n.d. | 0.4743 | 1.6191 | 0.5478 | 0.5077 | n.d. | 0.1482 |
| **RV-C-13** | 7.6448 | 2.7231 | 122.8798 | 12.8735 | 2.1868 | 0.4152 | 0.0632 | 1.4184 | 1.7538 | 1.3017 | 0.7224 | 0.0972 | 0.3674 |
| **RV-C-14** | 2.9710 | 1.1224 | 13.0169 | 2.0736 | 0.4446 | 0.0989 | n.d. | 0.3930 | 0.9559 | 0.7693 | 0.5302 | 0.0774 | 0.1050 |
| **RV-C-15** | 6.7952 | 1.8543 | 88.2072 | 6.8558 | 1.6548 | 0.2477 | 0.0396 | 1.0195 | 1.7080 | 1.1953 | 0.7204 | 0.1233 | 0.3414 |
| **RV-C-16** | 3.9236 | 1.6114 | 85.1038 | 7.1505 | 1.8885 | 0.4150 | 0.1220 | 1.3281 | 1.9638 | 0.5208 | 1.2604 | 0.2602 | 0.2121 |
| **RV-R-17** | 1.1423 | 0.4929 | 22.0913 | 2.5923 | 0.2465 | 0.1218 | 0.0096 | 0.3160 | 0.6162 | 0.1770 | 0.2038 | 0.0169 | 0.0831 |
| **RV-R-18** | 4.1901 | 1.3132 | 33.0037 | 4.1158 | 0.7488 | 0.0973 | n.d. | 0.5301 | 1.1049 | 0.4180 | 0.6745 | 0.0892 | 0.1027 |
| **RV-R-19** | 2.4336 | 0.7615 | 13.4348 | 2.7663 | 0.3429 | 0.0979 | 0.0151 | 0.3444 | 0.9527 | 0.2244 | 0.3666 | 0.0372 | 0.0758 |
| **RV-R-20** | 2.5803 | 0.6917 | 8.1446 | 1.4712 | 0.2270 | 0.0588 | 0.0258 | 0.1910 | 0.8192 | 0.2721 | 0.2412 | 0.0134 | 0.0588 |
| **RV-R-21** | 3.4634 | 1.3211 | 10.8942 | 3.0345 | 0.3068 | 0.0575 | n.d. | 0.2746 | 1.0367 | 0.3248 | 0.3120 | 0.0121 | 0.0712 |
| **RV-R-22** | 3.4645 | 1.0626 | 12.3840 | 2.7363 | 0.6864 | 0.0774 | n.d. | 0.3889 | 1.1032 | 0.7380 | 0.5465 | 0.1418 | 0.1341 |
| **RV-R-23** | 5.3561 | 1.7686 | 94.5264 | 6.5352 | 1.4642 | 0.2377 | 0.0213 | 0.9198 | 1.4332 | 0.9777 | 0.9876 | 0.1181 | 0.3036 |
| **RV-R-24** | 3.2331 | 1.2443 | 58.1219 | 5.7872 | 1.4279 | 0.2577 | 0.0334 | 1.0759 | 1.7576 | 0.6153 | 1.0205 | 0.2799 | 0.2285 |
| **RV-GR-25** | 3.0256 | 1.1106 | 42.3561 | 4.0031 | 0.5672 | 0.1107 | 0.0077 | 0.4392 | 1.0917 | 0.3128 | 0.4139 | 0.0666 | 0.1071 |
| **RV-GR-26** | 3.1799 | 1.1792 | 10.5050 | 3.0396 | 0.4586 | 0.0394 | n.d. | 0.1923 | 1.0166 | 0.3829 | 0.3998 | 0.0798 | 0.0785 |
| **RV-GR-27** | 2.0345 | 0.6518 | 2.4673 | 1.3142 | 0.1029 | 0.0176 | n.d. | 0.0547 | 0.4394 | 0.2000 | 0.0712 | 0.0176 | 0.0189 |
| **RV-GR-28** | 2.3874 | 0.8114 | 5.0199 | 1.4769 | 0.2680 | 0.0422 | n.d. | 0.1702 | 0.7611 | 0.3889 | 0.2640 | 0.0192 | 0.0591 |
| **RV-GR-29** | 5.6398 | 1.6209 | 71.7101 | 9.8582 | 0.9657 | 0.1155 | 0.0161 | 0.6506 | 1.6667 | 0.8201 | 0.7443 | 0.1639 | 0.2377 |
| **RV-GR-30** | 5.2142 | 1.4096 | 62.5048 | 6.2367 | 1.1968 | 0.2138 | 0.0789 | 0.7044 | 1.2175 | 0.6745 | 0.5748 | 0.0919 | 0.1919 |
| **RV-GR-31** | 4.2127 | 1.8379 | 34.7266 | 6.1485 | 1.3959 | 0.3053 | 0.0829 | 0.7002 | 1.3917 | 0.3788 | 0.5982 | 0.0605 | 0.1511 |
| **RV-GR-32** | 4.5051 | 1.3719 | 7.3029 | 3.0555 | 0.3327 | 0.0769 | 0.0704 | 0.2107 | 1.0077 | 0.3157 | 0.3128 | 0.0183 | 0.0118 |

n.d.: not detected.

Continuation of Table Annex II. 17.

| Sample | ((epi)cat-(epi)gallocat) 1 | ((epi)cat-(epi)gallocat) 2 | ((epi)gallocat)-(epi)cat) 1 | ((epi)gallocat)-(epi)cat) 2 | (epi)cat-glycoside 1 | (epi)cat-glycoside 2 | Catechin-gallate | Epicatechin-gallate | (Epi)catechin-gallate | PCA 2 | $((epi)cat)_2A$ 1 | $((epi)cat)_2A$ 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **RV-J-01** | 0.9132 | 0.4629 | 0.5925 | 0.2781 | 1.0484 | 0.2544 | n.d. | n.d. | n.d. | n.d. | 0.0298 | 0.4724 |
| **RV-J-02** | 1.4652 | 1.2057 | 1.0350 | 1.0034 | 2.2332 | 1.1129 | n.d. | n.d. | 0.0192 | n.d. | 0.0391 | 0.1096 |
| **RV-J-03** | 2.4762 | 0.9852 | 1.0110 | 0.6112 | 4.7304 | 0.6886 | n.d. | n.d. | n.d. | <0.005 | 0.0274 | 0.0861 |
| **RV-J-04** | 2.6029 | 3.3871 | 1.5345 | 1.7915 | 4.2471 | 1.5167 | 0.0063 | <0.005 | 0.0134 | 0.0087 | 0.0380 | 0.0533 |
| **RV-J-05** | 4.2373 | 3.1037 | 2.4694 | 2.2065 | 5.1684 | 1.5263 | n.d. | n.d. | n.d. | n.d. | 0.0183 | 0.3809 |
| **RV-J-06** | 3.9307 | 2.2388 | 1.8957 | 1.0326 | 6.0413 | 1.1961 | n.d. | n.d. | n.d. | n.d. | 0.0381 | 0.2782 |
| **RV-J-07** | 2.6609 | 2.1799 | 1.5812 | 0.9521 | 4.3494 | 0.9136 | n.d. | n.d. | 0.0199 | n.d. | 0.0400 | 0.2782 |
| **RV-J-08** | 2.3582 | 2.0639 | 1.5677 | 1.4181 | 4.6656 | 1.0481 | n.d. | n.d. | n.d. | n.d. | 0.0235 | 0.3386 |
| **RV-C-09** | 2.6448 | 2.9108 | 1.2618 | 1.4731 | 4.9084 | 1.5736 | n.d. | n.d. | n.d. | n.d. | 0.0292 | 0.1103 |
| **RV-C-10** | 3.2877 | 1.6933 | 1.9162 | 1.3541 | 3.5211 | 0.8694 | n.d. | n.d. | n.d. | n.d. | 0.0313 | 0.0758 |
| **RV-C-11** | 0.9956 | 0.8468 | 1.0860 | 0.8658 | 2.6230 | 0.6920 | n.d. | n.d. | n.d. | n.d. | 0.0280 | 0.4455 |
| **RV-C-12** | 2.7063 | 1.9214 | 1.6995 | 0.9673 | 3.9003 | 0.7400 | n.d. | n.d. | n.d. | n.d. | 0.0464 | 0.4877 |
| **RV-C-13** | 3.9731 | 3.3850 | 2.3109 | 2.1705 | 6.0816 | 1.8868 | n.d. | n.d. | n.d. | <0.005 | 0.0344 | 0.1286 |
| **RV-C-14** | 2.0330 | 1.1438 | 1.2532 | 1.1007 | 2.3041 | 0.7638 | n.d. | n.d. | 0.0131 | n.d. | 0.0346 | 0.1035 |
| **RV-C-15** | 3.4471 | 3.0496 | 1.6312 | 1.6489 | 4.7843 | 1.4465 | n.d. | n.d. | n.d. | 0.0108 | 0.0265 | 0.2377 |
| **RV-C-16** | 4.7942 | 2.6579 | 3.2295 | 1.7772 | 1.3294 | 0.6857 | n.d. | n.d. | 0.0707 | n.d. | 0.0525 | 0.1620 |
| **RV-R-17** | 0.9103 | 0.9432 | 1.0484 | 0.7868 | 3.1399 | 0.6177 | n.d. | n.d. | n.d. | n.d. | 0.0482 | 0.4993 |
| **RV-R-18** | 2.6648 | 1.3740 | 1.6577 | 1.2735 | 2.8365 | 0.7811 | n.d. | n.d. | n.d. | n.d. | 0.0273 | 0.0515 |
| **RV-R-19** | 1.1952 | 0.7994 | 1.4766 | 0.8705 | 2.3415 | 0.6035 | n.d. | n.d. | n.d. | n.d. | 0.0427 | 0.4692 |
| **RV-R-20** | 1.3279 | 0.6390 | 0.7522 | 0.4227 | 2.1407 | 0.3351 | n.d. | n.d. | n.d. | n.d. | 0.0272 | 0.1781 |
| **RV-R-21** | 1.5657 | 1.2869 | 1.5637 | 1.0174 | 2.3308 | 0.5321 | n.d. | n.d. | n.d. | n.d. | 0.0300 | 0.0506 |
| **RV-R-22** | 1.8416 | 1.2436 | 1.1981 | 1.1307 | 2.3709 | 0.8548 | n.d. | n.d. | 0.0192 | n.d. | 0.0300 | 0.0698 |
| **RV-R-23** | 4.3070 | 2.5842 | 2.5399 | 1.7730 | 5.0931 | 1.5174 | n.d. | n.d. | n.d. | 0.0082 | 0.0449 | 0.2756 |
| **RV-R-24** | 4.2235 | 2.6361 | 2.7564 | 1.5049 | 0.6895 | 0.5551 | n.d. | n.d. | n.d. | <0,005 | 0.0372 | 0.2406 |
| **RV-GR-25** | 1.9850 | 0.9495 | 1.9116 | 0.9034 | 3.5766 | 0.8452 | n.d. | n.d. | n.d. | n.d. | 0.0445 | 0.3681 |
| **RV-GR-26** | 1.8189 | 0.9928 | 1.1764 | 0.8512 | 1.7163 | 0.6325 | n.d. | n.d. | n.d. | n.d. | 0.0300 | 0.0623 |
| **RV-GR-27** | 0.3429 | 0.1433 | 0.2952 | 0.3519 | 0.5373 | 0.7175 | n.d. | n.d. | n.d. | n.d. | 0.0107 | 0.0423 |
| **RV-GR-28** | 1.0436 | 0.8847 | 0.7693 | 0.6049 | 1.1094 | 0.7448 | n.d. | n.d. | 0.0162 | n.d. | 0.0131 | 0.0560 |
| **RV-GR-29** | 4.1977 | 9.7148 | 2.2074 | 1.5086 | 4.6735 | 1.2973 | n.d. | n.d. | n.d. | n.d. | 0.0370 | 0.1469 |
| **RV-GR-30** | 2.6788 | 2.3183 | 1.9223 | 1.5233 | 4.7680 | 1.4391 | n.d. | n.d. | n.d. | n.d. | 0.0396 | 0.1364 |
| **RV-GR-31** | 1.8894 | 1.7179 | 2.1838 | 1.9501 | 2.2290 | 1.3917 | n.d. | n.d. | n.d. | n.d. | 0.0515 | 0.1031 |
| **RV-GR-32** | 1.2634 | 0.7950 | 1.3357 | 0.9935 | 3.8731 | 1.6083 | n.d. | n.d. | n.d. | n.d. | 0.0086 | 0.1739 |

n.d.: not detected.

Continuation of Table Annex II. 17.

| Sample | ((epi)cat-(epi)gallocat)A 1 | ((epi)cat-(epi)gallocat)A 2 | ((epi)gallocat)$_2$A | (epi)cat-((epi)cat-(epi)gallocat) A 1 | (epi)cat-((epi)cat-(epi)gallocat) A 2 | (epi)cat-((epi)gallocat)$_2$A | (epi)gallocat-et-(epi)gallocat | p-vinyl(epi)cat 1 | p-vinyl(epi)cat 2 | p-vinyl(epi)cat 3 | (epi)cat-furfuryl-(epi)cat 1 | (epi)cat-furfuryl-(epi)cat 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **RV-J-01** | 0.0372 | 0.0537 | n.d. | 0.0885 | 0.0307 | n.d. | n.d. | 0.0324 | 2.7406 | 0.3525 | 0.0984 | 0.0654 |
| **RV-J-02** | 0.1716 | 0.0284 | n.d. | 1.6714 | 0.0737 | n.d. | n.d. | 0.0158 | 5.1884 | 0.2780 | 1.7968 | 3.0903 |
| **RV-J-03** | 0.1154 | 0.1638 | n.d. | 0.1536 | 0.0419 | n.d. | n.d. | 0.0223 | 2.9724 | 0.2830 | 0.0648 | 0.1320 |
| **RV-J-04** | 0.1664 | 0.0673 | n.d. | 0.9514 | 0.1203 | n.d. | n.d. | 0.0876 | 2.5752 | 0.4699 | 0.7682 | 2.0781 |
| **RV-J-05** | 0.2873 | 0.2277 | n.d. | 2.4358 | 0.0893 | n.d. | n.d. | 0.0198 | 17.7620 | 0.1244 | 1.6948 | 1.7845 |
| **RV-J-06** | 0.1726 | 0.1223 | n.d. | 1.2651 | 0.0535 | n.d. | n.d. | 0.0335 | 4.9752 | 0.2155 | 0.4879 | 0.5091 |
| **RV-J-07** | 0.1592 | 0.1039 | n.d. | 1.2775 | 0.0495 | n.d. | n.d. | 0.0255 | 4.5792 | 0.0816 | 0.5980 | 0.6212 |
| **RV-J-08** | 0.1357 | 0.1021 | n.d. | 1.3669 | 0.0415 | n.d. | n.d. | 0.0375 | 4.8157 | 0.2097 | 0.6579 | 0.7738 |
| **RV-C-09** | 0.3275 | 0.1364 | n.d. | 2.2901 | 0.1354 | n.d. | n.d. | 0.0370 | 1.3672 | 0.6613 | 1.9754 | 4.3520 |
| **RV-C-10** | 0.2217 | 0.0273 | n.d. | 2.9050 | 0.1068 | n.d. | n.d. | 0.0515 | 2.0232 | 0.4797 | 2.8686 | 4.6476 |
| **RV-C-11** | 0.1738 | 0.0555 | n.d. | 0.7965 | 0.0637 | n.d. | n.d. | 0.0324 | 3.4856 | 0.3823 | 0.9868 | 1.1416 |
| **RV-C-12** | 0.2287 | 0.0286 | n.d. | 1.3808 | 0.1170 | n.d. | n.d. | 0.0468 | 6.0217 | 0.4774 | 1.0836 | 1.6453 |
| **RV-C-13** | 0.3933 | 0.1260 | n.d. | 2.8490 | 0.2003 | n.d. | n.d. | 0.0928 | 3.6774 | 0.6326 | 2.1194 | 5.4231 |
| **RV-C-14** | 0.1376 | 0.0208 | n.d. | 1.1486 | 0.0613 | n.d. | n.d. | 0.0447 | 4.8794 | 0.3931 | 0.9562 | 1.3838 |
| **RV-C-15** | 0.3434 | 0.1739 | 0.0187 | 1.7242 | 0.1387 | n.d. | n.d. | 0.0665 | 8.0564 | 0.4509 | 0.7283 | 2.0206 |
| **RV-C-16** | 0.1366 | 0.0499 | n.d. | 2.8294 | 0.2494 | n.d. | n.d. | 0.0201 | 0.9884 | 0.3514 | 4.2567 | 4.1082 |
| **RV-R-17** | 0.1975 | 0.0206 | n.d. | 1.4942 | 0.0604 | n.d. | n.d. | 0.0637 | 3.7294 | 0.1760 | 1.0711 | 1.3455 |
| **RV-R-18** | 0.2105 | 0.0286 | n.d. | 3.4111 | 0.1499 | n.d. | n.d. | 0.0650 | 2.7999 | 0.3114 | 4.6630 | 6.0271 |
| **RV-R-19** | 0.1959 | 0.0372 | n.d. | 1.9327 | 0.1281 | n.d. | n.d. | 0.0538 | 4.8899 | 0.1925 | 3.3001 | 2.9951 |
| **RV-R-20** | 0.1472 | 0.0355 | n.d. | 0.9534 | 0.0657 | n.d. | n.d. | 0.0288 | 5.5621 | 0.2705 | 0.8269 | 1.3218 |
| **RV-R-21** | 0.1459 | 0.0630 | n.d. | 1.4803 | 0.0724 | n.d. | n.d. | 0.0573 | 0.9792 | 0.2705 | 1.9601 | 1.9796 |
| **RV-R-22** | 0.1879 | 0.0637 | n.d. | 1.5500 | 0.0985 | n.d. | n.d. | 0.0344 | 3.4188 | 0.3314 | 1.6792 | 2.0621 |
| **RV-R-23** | 0.3714 | 0.1779 | n.d. | 1.4970 | 0.1582 | n.d. | n.d. | 0.1256 | 9.4776 | 0.3686 | 0.9196 | 2.7488 |
| **RV-R-24** | 0.1315 | 0.1188 | n.d. | 1.5037 | 0.1834 | n.d. | n.d. | 0.0063 | 1.8442 | 0.4768 | 0.8965 | 1.0411 |
| **RV-GR-25** | 0.1896 | 0.0335 | n.d. | 1.1992 | 0.1264 | n.d. | n.d. | n.d. | 3.9241 | 0.1314 | 2.6915 | 2.0998 |
| **RV-GR-26** | 0.1121 | 0.0111 | n.d. | 2.2377 | 0.1283 | n.d. | n.d. | 0.0906 | 1.3629 | 0.2932 | 1.9110 | 2.5013 |
| **RV-GR-27** | 0.0300 | 0.0162 | n.d. | 0.7520 | 0.0573 | n.d. | n.d. | 0.0120 | 0.8418 | 0.1362 | 0.8663 | 1.2971 |
| **RV-GR-28** | 0.0836 | 0.0346 | n.d. | 1.1330 | 0.0447 | n.d. | n.d. | 0.0530 | 4.4100 | 0.2020 | 0.3561 | 0.7301 |
| **RV-GR-29** | 0.3375 | 0.4970 | n.d. | 1.6396 | 0.1354 | n.d. | n.d. | 0.1223 | 7.6176 | 0.1945 | 0.6728 | 2.7925 |
| **RV-GR-30** | 0.3075 | 0.0867 | n.d. | 2.1900 | 0.0698 | n.d. | n.d. | 0.0468 | 5.2918 | 0.3323 | 1.5210 | 4.3181 |
| **RV-GR-31** | 0.1571 | 0.1606 | n.d. | 3.5113 | 0.2981 | n.d. | n.d. | 0.0546 | 0.3004 | 0.7725 | 2.5441 | 3.3169 |
| **RV-GR-32** | 0.0672 | 0.0965 | n.d. | 3.0053 | 0.1094 | n.d. | n.d. | 0.0314 | 6.7182 | 0.0661 | 1.9405 | 2.2954 |

n.d.: not detected.

Table Annex II.18 Tannins concentrations of the samples from Rioja Alta.

| Sample | Catechin | Epicatechin | PCB1 | PCB2 | $((epi)cat)_2$ 1 | PCC1 | $((epi)cat)_3$ 1 | $((epi)cat)_3$ 2 | Gallocatechin | Epigallocatechin | $((epi)gallocat)_2$ 1 | $((epi)gallocat)_2$ 2 | $((epi)gallocat)_2$ 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **RA-J-33** | 7.8002 | 2.5356 | 107.1148 | 7.4975 | 2.3918 | 0.5105 | n.d. | 2.0580 | 1.8669 | 1.6973 | 1.1965 | 0.1420 | 0.3256 |
| **RA-J-34** | 5.4724 | 1.7765 | 50.0620 | 6.6625 | 1.0118 | 0.1730 | 0.0490 | 0.8678 | 1.5517 | 0.3864 | 0.7748 | 0.0654 | 0.1548 |
| **RA-J-35** | 2.7782 | 1.0263 | 21.9917 | 3.0218 | 0.8259 | 0.2033 | 0.0784 | 0.6065 | 1.5011 | 0.6278 | 0.8911 | 0.1393 | 0.2045 |
| **RA-J-36** | 5.3517 | 1.4829 | 48.4937 | 4.1505 | 0.9963 | 0.1427 | 0.0607 | 0.5511 | 1.9363 | 0.8035 | 0.9453 | 0.1625 | 0.1427 |
| **RA-J-37** | 4.4535 | 1.8181 | 65.3032 | 7.2124 | 1.7376 | 0.4203 | 0.0290 | 1.2618 | 1.7200 | 0.6421 | 1.1842 | 0.1417 | 0.2223 |
| **RA-J-38** | 5.0205 | 2.0020 | 12.0815 | 5.5038 | 0.8125 | 0.2602 | 0.0613 | 0.3810 | 1.0518 | 0.4453 | 0.4422 | 0.0271 | 0.1519 |
| **RA-J-39** | 8.1927 | 2.8956 | 80.4253 | 8.9953 | 2.1528 | 0.7009 | 0.1186 | 1.7348 | 1.7251 | 1.1495 | 0.9522 | 0.2162 | 0.3374 |
| **RA-J-40** | 8.4348 | 1.6973 | 27.9962 | 4.6577 | 1.3360 | 0.2687 | 0.3627 | 0.7189 | 2.3243 | 0.9486 | 1.2053 | 0.2181 | 0.2235 |
| **RA-C-41** | 4.4068 | 1.4514 | 28.8182 | 3.6394 | 0.5484 | 0.0919 | 0.0192 | 0.4699 | 1.1470 | 0.7320 | 0.4600 | 0.0178 | 0.0717 |
| **RA-C-42** | 5.4840 | 1.6844 | 62.0024 | 4.5953 | 1.7714 | 0.2826 | n.d. | 1.7188 | 1.4977 | 1.0320 | 0.7560 | 0.0994 | 0.2320 |
| **RA-C-43** | 7.8393 | 3.6410 | 68.6705 | 14.7364 | 1.9517 | 0.4174 | 0.0089 | 1.3782 | 1.5765 | 0.7948 | 0.4229 | 0.0654 | 0.1147 |
| **RA-C-44** | 8.8393 | 3.1088 | 72.9574 | 9.5659 | 2.2228 | 0.3791 | 0.0107 | 1.5385 | 1.7484 | 0.7310 | 0.3882 | 0.0582 | 0.1293 |
| **RA-C-45** | 4.2791 | 1.6663 | 42.9076 | 5.5957 | 1.2915 | 0.3112 | 0.0874 | 0.9148 | 1.5336 | 0.7369 | 0.8354 | 0.1843 | 0.1796 |
| **RA-C-46** | 5.7982 | 1.8816 | 62.7297 | 7.4221 | 1.7723 | 0.3862 | 0.2253 | 1.3875 | 1.6340 | 0.8618 | 1.1978 | 0.1638 | 0.2958 |
| **RA-C-47** | 6.2591 | 2.1239 | 31.1925 | 5.8604 | 1.2428 | 0.2850 | 0.1964 | 0.7262 | 1.4389 | 0.8274 | 0.8329 | 0.1638 | 0.2524 |
| **RA-C-48** | 5.0260 | 2.0833 | 31.9343 | 6.4254 | 0.9443 | 0.3591 | 0.1126 | 0.8329 | 1.4407 | 0.6101 | 0.7105 | 0.0613 | 0.2100 |
| **RA-R-49** | 3.4481 | 0.8500 | 7.5378 | 2.2360 | 0.2357 | 0.0482 | n.d. | 0.1592 | 0.8674 | 0.3228 | 0.2115 | 0.0272 | 0.0482 |
| **RA-R-50** | 3.4458 | 1.0741 | 6.5174 | 2.1518 | 0.2399 | 0.0394 | n.d. | 0.1202 | 0.8175 | 0.2848 | 0.2217 | 0.0448 | 0.0434 |
| **RA-R-51** | 3.8520 | 1.1926 | 51.4051 | 3.6152 | 1.0817 | 0.1770 | n.d. | 0.6810 | 1.0068 | 0.4854 | 0.5709 | 0.0437 | 0.1249 |
| **RA-R-52** | 4.9969 | 1.3794 | 44.0778 | 5.8244 | 0.8197 | 0.1642 | 0.1256 | 0.4810 | 1.6232 | 0.4693 | 0.6923 | 0.1021 | 0.1189 |
| **RA-R-53** | 9.0228 | 4.6013 | 76.7093 | 30.9225 | 2.4410 | 0.7985 | 0.0180 | 1.8377 | 1.6559 | 0.6800 | 0.4721 | 0.0764 | 0.1931 |
| **RA-R-54** | 6.5369 | 2.8542 | 63.3450 | 13.4380 | 1.7931 | 0.2970 | 0.0180 | 0.9444 | 1.6013 | 0.4557 | 0.4794 | 0.0746 | 0.1092 |
| **RA-R-55** | 3.6381 | 1.3804 | 14.0616 | 3.5329 | 0.7334 | 0.1713 | 0.0650 | 0.4832 | 1.1835 | 0.3610 | 0.3575 | 0.0335 | 0.1179 |
| **RA-R-56** | 5.7883 | 1.8262 | 46.7575 | 6.8516 | 1.4604 | 0.2994 | 0.1645 | 1.1858 | 1.7032 | 0.8486 | 1.0881 | 0.2445 | 0.3088 |
| **RA-GR-57** | 4.6863 | 1.3980 | 35.9253 | 4.3053 | 0.8974 | 0.1014 | n.d. | 0.5554 | 1.5278 | 0.7418 | 0.7769 | 0.0906 | 0.1121 |
| **RA-GR-58** | 2.4380 | 0.7934 | 2.0053 | 0.8230 | 0.0891 | 0.0258 | n.d. | 0.0300 | 0.5154 | 0.1948 | 0.0726 | 0.0176 | 0.0203 |
| **RA-GR-59** | 2.8492 | 0.9793 | 6.5829 | 2.4094 | 0.4365 | 0.0682 | n.d. | 0.1444 | 0.7570 | 0.2966 | 0.2028 | 0.0269 | 0.0483 |
| **RA-GR-60** | 5.6942 | 1.7434 | 32.9916 | 5.5109 | 0.9084 | 0.1512 | 0.1200 | 0.5000 | 1.5142 | 0.3979 | 0.5539 | 0.0867 | 0.1200 |
| **RA-GR-61** | 7.0762 | 2.5080 | 80.0000 | 9.1626 | 1.9074 | 0.4036 | 0.0183 | 1.2272 | 2.3946 | 0.6106 | 1.4370 | 0.1909 | 0.3157 |
| **RA-GR-62** | 7.2584 | 2.2362 | 63.9186 | 7.9756 | 1.3603 | 0.2941 | 0.1650 | 1.2352 | 2.1019 | 0.9346 | 1.3054 | 0.2385 | 0.3251 |
| **RA-GR-63** | 3.1854 | 1.2742 | 31.6771 | 4.5184 | 1.2118 | 0.3617 | 0.1410 | 0.7796 | 1.5874 | 0.5253 | 0.6933 | 0.1880 | 0.1442 |
| **RA-GR-64** | 4.2142 | 1.5472 | 15.5231 | 4.5515 | 0.5881 | 0.1849 | 0.1410 | 0.4908 | 1.4451 | 0.6399 | 0.7199 | 0.1221 | 0.2210 |

n.d.: not detected.

Continuation of Table Annex II. 18.

| Sample | ((epi)cat-(epi)gallocat) 1 | ((epi)cat-(epi)gallocat) 2 | ((epi)gallocat)-(epi)cat) 1 | ((epi)gallocat)-(epi)cat) 2 | (epi)cat-glycoside 1 | (epi)cat-glycoside 2 | Catechin-gallate | Epicatechin-gallate | (Epi)catechin-gallate | PCA 2 | $((epi)cat)_2A$ 1 | $((epi)cat)_2A$ 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **RA-J-33** | 4.0758 | 3.4522 | 2.3263 | 2.1470 | 7.4320 | 2.1127 | 0.0088 | n.d. | 0.0115 | n.d. | 0.0435 | 0.0701 |
| **RA-J-34** | 4.2740 | 1.9385 | 2.3914 | 1.7815 | 4.6145 | 1.9997 | n.d. | n.d. | n.d. | 0.0053 | 0.0326 | 0.1822 |
| **RA-J-35** | 2.9928 | 1.4814 | 1.5244 | 0.9350 | 1.7539 | 0.4369 | n.d. | n.d. | n.d. | <0,005 | 0.0043 | 0.0470 |
| **RA-J-36** | 5.1153 | 2.6913 | 2.7950 | 1.7024 | 4.6353 | 0.9793 | n.d. | n.d. | n.d. | n.d. | 0.0020 | 0.1483 |
| **RA-J-37** | 3.8167 | 2.0313 | 2.4295 | 1.6000 | 2.4627 | 0.9290 | n.d. | n.d. | n.d. | n.d. | 0.0268 | 0.1760 |
| **RA-J-38** | 1.3352 | 1.0149 | 1.2199 | 1.6724 | 1.8702 | 0.7482 | n.d. | n.d. | n.d. | <0.005 | 0.0156 | 0.1912 |
| **RA-J-39** | 3.1121 | 2.8613 | 2.3468 | 2.9149 | 5.2687 | 1.5954 | n.d. | n.d. | n.d. | n.d. | 0.1385 | 0.3356 |
| **RA-J-40** | 3.6673 | 2.8838 | 2.3886 | 2.8152 | 3.4937 | 0.7768 | n.d. | n.d. | n.d. | n.d. | 0.0784 | 0.2054 |
| **RA-C-41** | 1.8702 | 1.3227 | 2.1456 | 2.7797 | 3.5727 | 0.7446 | n.d. | n.d. | n.d. | n.d. | 0.0421 | 0.5018 |
| **RA-C-42** | 3.1549 | 2.7341 | 2.0773 | 1.7456 | 5.6664 | 1.7778 | n.d. | n.d. | n.d. | 0.0062 | 0.0408 | 0.0488 |
| **RA-C-43** | 3.3947 | 2.7699 | 1.7914 | 2.0443 | 5.3683 | 1.8691 | n.d. | n.d. | n.d. | n.d. | 0.0381 | 0.1676 |
| **RA-C-44** | 3.8840 | 2.7600 | 2.0212 | 2.0294 | 5.7484 | 1.5765 | n.d. | n.d. | n.d. | n.d. | 0.0563 | 0.1730 |
| **RA-C-45** | 3.0917 | 1.5131 | 1.6699 | 1.5018 | 2.1019 | 0.7322 | n.d. | n.d. | <0,005 | n.d. | 0.0358 | 0.0762 |
| **RA-C-46** | 3.8281 | 2.5140 | 2.2171 | 2.3468 | 4.4744 | 1.2471 | n.d. | n.d. | n.d. | n.d. | 0.1349 | 0.1656 |
| **RA-C-47** | 2.5505 | 1.8655 | 2.5365 | 1.7734 | 3.9996 | 1.1871 | n.d. | n.d. | n.d. | n.d. | 0.1078 | 0.3049 |
| **RA-C-48** | 2.0108 | 2.0162 | 1.4780 | 1.6571 | 2.9642 | 0.8125 | n.d. | n.d. | n.d. | <0.005 | 0.0784 | 0.4689 |
| **RA-R-49** | 0.8165 | 0.4744 | 0.6193 | 0.5334 | 1.7294 | 0.6340 | 0.0061 | <0.005 | 0.0253 | <0.005 | 0.0157 | 0.1994 |
| **RA-R-50** | 1.0461 | 0.5245 | 0.5007 | 0.5273 | 0.9984 | 1.0573 | n.d. | n.d. | 0.0327 | n.d. | n.d. | n.d. |
| **RA-R-51** | 2.4975 | 1.2112 | 1.3970 | 1.2215 | 3.1458 | 1.1390 | n.d. | n.d. | 0.0774 | n.d. | 0.0315 | 0.0361 |
| **RA-R-52** | 2.7719 | 1.4029 | 2.0134 | 1.5745 | 3.5472 | 1.0829 | n.d. | n.d. | n.d. | n.d. | 0.0381 | 0.1057 |
| **RA-R-53** | 3.2790 | 2.5253 | 2.3931 | 3.3881 | 6.4559 | 3.1170 | n.d. | n.d. | n.d. | n.d. | 0.0545 | 0.1439 |
| **RA-R-54** | 2.9286 | 2.2311 | 1.8774 | 2.0724 | 4.0740 | 1.8939 | n.d. | n.d. | n.d. | n.d. | 0.0217 | 0.3317 |
| **RA-R-55** | 1.8591 | 0.9101 | 1.4249 | 0.8698 | 1.5216 | 0.6397 | n.d. | n.d. | n.d. | n.d. | 0.0066 | 0.0335 |
| **RA-R-56** | 3.6068 | 2.2546 | 2.1590 | 2.0690 | 3.5233 | 0.8454 | n.d. | n.d. | n.d. | n.d. | 0.0670 | 0.3324 |
| **RA-GR-57** | 3.1265 | 1.3415 | 1.5017 | 1.1442 | 1.5174 | 0.7068 | n.d. | n.d. | n.d. | n.d. | 0.0381 | 0.0461 |
| **RA-GR-58** | 0.3133 | 0.1794 | 0.2334 | 0.2103 | 0.4021 | 0.3055 | n.d. | n.d. | 0.0437 | n.d. | n.d. | n.d. |
| **RA-GR-59** | 1.1414 | 0.6592 | 0.6538 | 0.8038 | 1.3206 | 1.3406 | n.d. | n.d. | 0.1234 | n.d. | n.d. | n.d. |
| **RA-GR-60** | 2.9397 | 1.8278 | 1.5673 | 1.6252 | 3.1520 | 1.4853 | n.d. | n.d. | n.d. | n.d. | 0.0216 | 0.1062 |
| **RA-GR-61** | 5.2648 | 2.6503 | 2.4935 | 2.2065 | 4.8741 | 1.8664 | n.d. | n.d. | n.d. | n.d. | 0.0118 | 0.1653 |
| **RA-GR-62** | 5.1601 | 2.8146 | 2.5109 | 1.7448 | 3.4662 | 0.8659 | n.d. | n.d. | n.d. | n.d. | 0.0499 | 0.1764 |
| **RA-GR-63** | 2.9734 | 1.0459 | 2.3880 | 1.6417 | 1.8734 | 0.4124 | n.d. | n.d. | <0,005 | <0,005 | 0.0366 | 0.1480 |
| **RA-GR-64** | 2.5567 | 2.1579 | 1.6065 | 1.4989 | 2.3117 | 0.4610 | n.d. | n.d. | n.d. | n.d. | 0.0328 | 0.4077 |

n.d.: not detected.

Continuation of Table Annex II. 18.

| Sample | ((epi)cat-(epi)gallocat)A 1 | ((epi)cat-(epi)gallocat)A 2 | ((epi)gallocat)$_2$A | (epi)cat-((epi)cat-(epi)gallocat) A 1 | (epi)cat-((epi)cat-(epi)gallocat) A 2 | (epi)cat-((epi)gallocat)$_2$A | (epi)gallocat-et-(epi)gallocat | p-vinyl(epi)cat 1 | p-vinyl(epi)cat 2 | p-vinyl(epi)cat 3 | (epi)cat-furfuryl-(epi)cat 1 | (epi)cat-furfuryl-(epi)cat 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **RA-J-33** | 0.6639 | 0.5611 | n.d. | 0.5693 | 0.1330 | n.d. | n.d. | 0.0584 | 5.3137 | 0.7047 | 0.1631 | 0.7371 |
| **RA-J-34** | 0.1803 | 0.1019 | n.d. | 2.7311 | 0.2225 | n.d. | n.d. | 0.0645 | 2.8017 | 0.3201 | 2.7095 | 4.5062 |
| **RA-J-35** | 0.1428 | 0.0560 | n.d. | 1.7500 | 0.1251 | n.d. | n.d. | 0.0312 | 1.4000 | 1.0386 | 1.4101 | 1.4138 |
| **RA-J-36** | 0.2674 | 0.2703 | n.d. | 2.5528 | 0.0854 | n.d. | n.d. | 0.0833 | 6.3906 | 0.3803 | 4.2806 | 2.3858 |
| **RA-J-37** | 0.1808 | 0.2840 | n.d. | 1.2708 | 0.1750 | n.d. | n.d. | 0.0729 | 4.2038 | 0.6539 | 1.6722 | 1.6083 |
| **RA-J-38** | 0.1551 | 0.0970 | n.d. | 1.8128 | 0.2395 | n.d. | n.d. | 0.1525 | 3.3174 | 0.3253 | 1.4973 | 1.8010 |
| **RA-J-39** | 0.3971 | 0.3030 | n.d. | 4.4528 | 0.2884 | n.d. | n.d. | 0.1068 | 5.5330 | 0.4144 | 4.5137 | 7.6388 |
| **RA-J-40** | 0.1584 | 0.0670 | n.d. | 5.0008 | 0.8431 | n.d. | n.d. | 0.1358 | 3.4307 | 0.2683 | 8.5887 | 10.1165 |
| **RA-C-41** | 0.1642 | 0.0152 | n.d. | 4.3615 | 0.8361 | n.d. | n.d. | 0.1488 | 3.3073 | 0.5217 | 1.3292 | 2.2713 |
| **RA-C-42** | 0.5332 | 0.2302 | n.d. | 2.4536 | 0.2286 | n.d. | n.d. | 0.1244 | 3.4555 | 0.6402 | 0.8702 | 3.0586 |
| **RA-C-43** | 0.1712 | 0.6034 | n.d. | 1.6790 | 0.2331 | n.d. | n.d. | 0.0510 | 2.3536 | 0.5112 | 2.5303 | 3.7134 |
| **RA-C-44** | 0.1313 | 0.8751 | n.d. | 2.4150 | 0.1872 | n.d. | n.d. | 0.0734 | 3.0252 | 0.3887 | 2.4079 | 3.4917 |
| **RA-C-45** | 0.1571 | 0.0470 | n.d. | 2.8653 | 0.2731 | n.d. | n.d. | 0.0442 | 2.0047 | 0.3643 | 4.3301 | 4.7783 |
| **RA-C-46** | 0.3663 | 0.1819 | n.d. | 6.0687 | 0.3345 | n.d. | n.d. | 0.0683 | 4.8704 | 0.2955 | 7.1842 | 9.1614 |
| **RA-C-47** | 0.3898 | 0.1222 | n.d. | 5.9260 | 0.2405 | n.d. | n.d. | 0.1358 | 5.0008 | 0.3736 | 7.0238 | 8.8442 |
| **RA-C-48** | 0.1880 | 0.0970 | n.d. | 3.7453 | 0.2731 | n.d. | n.d. | 0.0613 | 3.4167 | 0.3179 | 3.8670 | 3.9238 |
| **RA-R-49** | 0.1525 | 0.1018 | 0.0061 | 1.8714 | 0.1707 | n.d. | n.d. | 0.0839 | 2.8504 | 0.1343 | 0.8238 | 3.2024 |
| **RA-R-50** | 0.0529 | 0.0744 | n.d. | 1.7596 | 0.0919 | n.d. | n.d. | 0.0327 | 0.4036 | 0.1404 | 2.5553 | 3.5143 |
| **RA-R-51** | 0.2368 | 0.0407 | n.d. | 1.4648 | 0.1337 | n.d. | n.d. | 0.0551 | 1.9037 | 0.3273 | 1.3099 | 2.0922 |
| **RA-R-52** | 0.2178 | 0.0327 | n.d. | 4.0429 | 0.1691 | n.d. | n.d. | 0.0555 | 3.7376 | 0.2251 | 3.8270 | 5.2076 |
| **RA-R-53** | 0.2241 | 0.1913 | n.d. | 1.8865 | 0.2641 | n.d. | n.d. | 0.0600 | 3.5857 | 0.3866 | 2.3383 | 3.3383 |
| **RA-R-54** | 0.1767 | 0.0928 | n.d. | 2.4310 | 0.2745 | n.d. | n.d. | 0.0107 | 3.8830 | 0.3596 | 3.1511 | 4.0841 |
| **RA-R-55** | 0.1251 | 0.0762 | n.d. | 2.1483 | 0.1818 | n.d. | n.d. | 0.0755 | 1.4088 | 0.2434 | 2.5303 | 2.8765 |
| **RA-R-56** | 0.2916 | 0.2508 | n.d. | 4.1188 | 0.2843 | n.d. | n.d. | 0.0841 | 2.9428 | 0.3440 | 3.8706 | 4.1093 |
| **RA-GR-57** | 0.1365 | 0.0327 | n.d. | 2.2784 | 0.1310 | n.d. | n.d. | 0.0529 | 1.9082 | 0.2722 | 3.3396 | 3.8387 |
| **RA-GR-58** | 0.0382 | 0.0341 | n.d. | 0.6541 | 0.0187 | n.d. | n.d. | 0.0271 | 0.1508 | 0.1009 | 1.0309 | 1.3300 |
| **RA-GR-59** | 0.0820 | 0.1173 | n.d. | 1.3585 | 0.1047 | n.d. | n.d. | 0.0551 | 0.3232 | 0.2800 | 1.4217 | 2.4285 |
| **RA-GR-60** | 0.2334 | 0.0313 | n.d. | 5.8524 | 0.2593 | n.d. | n.d. | 0.0623 | 1.9834 | 0.2449 | 11.3810 | 20.5364 |
| **RA-GR-61** | 0.2221 | 0.0397 | n.d. | 3.3413 | 0.0777 | n.d. | n.d. | 0.0468 | 5.8932 | 0.2439 | 8.4811 | 12.6079 |
| **RA-GR-62** | 0.2908 | 0.1486 | n.d. | 2.2957 | 0.0937 | n.d. | n.d. | 0.0976 | 6.0861 | 0.2363 | 4.5764 | 3.5501 |
| **RA-GR-63** | 0.1125 | 0.0347 | n.d. | 3.3795 | 0.2620 | n.d. | n.d. | 0.0454 | 1.0077 | 0.1984 | 3.6295 | 3.3364 |
| **RA-GR-64** | 0.2524 | 0.1723 | n.d. | 4.5135 | 0.2358 | n.d. | n.d. | 0.1063 | 4.4663 | 0.2451 | 4.1341 | 3.6436 |

n.d.: not detected.

Table Annex II.19 Tannins concentrations of the samples from Rioja Baja.

| Sample | Catechin | Epicatechin | PCB1 | PCB2 | ((epi)cat)$_2$ 1 | PCC1 | ((epi)cat)$_3$ 1 | ((epi)cat)$_3$ 2 | Gallocatechin | Epigallocatechin | ((epi)gallocat)$_2$ 1 | ((epi)gallocat)$_2$ 2 | ((epi)gallocat)$_2$ 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **RB-J-65** | 4.0930 | 1.8581 | 10.3181 | 3.9107 | 0.3545 | 0.1395 | n.d. | 0.3416 | 0.9672 | 0.7934 | 0.3673 | 0.0575 | 0.0974 |
| **RB-J-66** | 9.9124 | 3.9663 | 118.1982 | 16.3042 | 2.9520 | 1.0877 | n.d. | 2.2340 | 1.9141 | 1.8057 | 0.6598 | 0.0302 | 0.3864 |
| **RB-J-67** | 5.5858 | 1.8837 | 56.8876 | 7.6933 | 1.2718 | 0.2630 | 0.2058 | 0.8235 | 1.8227 | 0.5163 | 1.0473 | 0.1568 | 0.2712 |
| **RB-J-68** | 7.1210 | 2.3339 | 45.1822 | 6.7944 | 1.0016 | 0.3071 | 0.1650 | 0.7777 | 1.9554 | 0.7287 | 1.0326 | 0.2369 | 0.2842 |
| **RB-J-69** | 8.2594 | 2.1615 | 68.6977 | 8.1389 | 1.9020 | 0.4084 | 0.1699 | 0.9133 | 3.0374 | 0.7695 | 1.2733 | 0.2957 | 0.3692 |
| **RB-J-70** | 7.2400 | 2.0439 | 56.7868 | 6.5671 | 1.1970 | 0.2957 | 0.1069 | 0.9640 | 2.1523 | 0.7842 | 1.2663 | 0.1846 | 0.2695 |
| **RB-J-71** | 5.6741 | 2.4828 | 145.9967 | 23.9242 | 3.5155 | 0.8804 | <0,005 | 2.1621 | 2.0458 | 0.5957 | 0.7783 | 0.2241 | 0.1854 |
| **RB-J-72** | 4.1981 | 1.1961 | 22.6537 | 2.9182 | 0.4123 | 0.0893 | 0.0052 | 0.2430 | 1.1742 | 0.5716 | 0.4509 | 0.1407 | 0.0875 |
| **RB-C-73** | 4.0793 | 1.7470 | 10.8990 | 4.6066 | 0.4677 | 0.1408 | n.d. | 0.3017 | 1.2265 | 0.6312 | 0.3454 | 0.0396 | 0.0864 |
| **RB-C-74** | 4.4305 | 1.9431 | 9.4935 | 4.6226 | 0.5851 | 0.1355 | n.d. | 0.3408 | 0.9070 | 0.4852 | 0.2949 | 0.0183 | 0.0655 |
| **RB-C-75** | 3.0435 | 1.3583 | 10.4868 | 3.3364 | 0.4408 | 0.0932 | n.d. | 0.2964 | 0.7722 | 0.4550 | 0.5311 | 0.0330 | 0.0916 |
| **RB-C-76** | 5.5548 | 2.1782 | 46.9061 | 7.6327 | 1.6276 | 0.3181 | n.d. | 1.2497 | 1.5320 | 1.4741 | 0.8978 | 0.1234 | 0.4294 |
| **RB-C-77** | 3.6713 | 1.6854 | 31.0145 | 5.6713 | 1.2929 | 0.3089 | 0.1227 | 0.8116 | 1.2378 | 0.4523 | 0.7215 | 0.1511 | 0.1630 |
| **RB-C-78** | 5.5418 | 2.9567 | 35.5504 | 9.1860 | 1.1236 | 0.3791 | 0.0162 | 0.5286 | 1.3369 | 0.4484 | 0.4393 | 0.0600 | 0.1366 |
| **RB-C-79** | 2.8869 | 0.9254 | 72.9885 | 4.5402 | 1.4684 | 0.3027 | 0.1423 | 1.1922 | 1.7330 | 0.4936 | 1.7371 | 0.1144 | 0.2628 |
| **RB-C-80** | 5.9440 | 2.4358 | 38.3636 | 7.3320 | 1.2910 | 0.2940 | 0.1276 | 0.6737 | 1.6158 | 0.7786 | 0.9323 | 0.1728 | 0.2108 |
| **RB-R-81** | 4.2110 | 1.8433 | 10.1665 | 3.8373 | 0.5422 | 0.0981 | n.d. | 0.2647 | 0.8911 | 0.4661 | 0.3202 | 0.0313 | 0.0509 |
| **RB-R-82** | 3.9601 | 1.6827 | 11.0716 | 4.6804 | 0.6945 | 0.1176 | n.d. | 0.3726 | 0.8293 | 0.5200 | 0.4027 | 0.0460 | 0.0769 |
| **RB-R-83** | 3.7596 | 1.5819 | 11.0223 | 3.5643 | 0.5232 | 0.0802 | n.d. | 0.3091 | 0.9435 | 0.7484 | 0.4788 | 0.0899 | 0.0655 |
| **RB-R-84** | 4.7659 | 2.0580 | 46.4968 | 6.7429 | 1.6297 | 0.3256 | n.d. | 1.0244 | 1.5567 | 1.4934 | 1.4998 | 0.2902 | 0.3206 |
| **RB-R-85** | 8.6830 | 3.7357 | 15.2826 | 10.7076 | 0.9042 | 0.2777 | 0.0478 | 0.3188 | 1.2580 | 0.7595 | 0.1971 | 0.0838 | 0.0538 |
| **RB-R-86** | 2.4238 | 1.0276 | 1.6981 | 1.0811 | 0.1067 | 0.0318 | 0.0118 | 0.0398 | 0.3961 | 0.2152 | 0.0738 | 0.0098 | 0.0138 |
| **RB-R-87** | 3.2612 | 0.9964 | 38.6392 | 3.8702 | 1.2907 | 0.2977 | 0.2717 | 1.0383 | 1.6791 | 0.5931 | 0.8582 | 0.2260 | 0.1949 |
| **RB-R-88** | 4.7413 | 1.7916 | 2.4196 | 4.5002 | 0.8238 | 0.2144 | 0.1512 | 0.3989 | 1.2975 | 0.5616 | 0.3790 | 0.1078 | 0.1331 |
| **RB-GR-89** | 4.4200 | 1.8202 | 6.9609 | 3.4057 | 0.3234 | 0.0899 | n.d. | 0.2457 | 0.8324 | 0.4281 | 0.2663 | 0.0297 | 0.0427 |
| **RB-GR-90** | 5.4367 | 2.1599 | 52.6726 | 8.4237 | 1.7070 | 0.2851 | n.d. | 1.0522 | 1.4204 | 1.1383 | 0.9155 | 0.2092 | 0.2294 |
| **RB-GR-91** | 3.7179 | 1.1411 | 4.9356 | 1.8166 | 0.1823 | 0.0538 | 0.0118 | 0.0978 | 0.9223 | 0.3205 | 0.2234 | 0.0518 | 0.0498 |
| **RB-GR-92** | 5.1710 | 1.4870 | 9.0199 | 3.6951 | 0.4569 | 0.0798 | 0.0338 | 0.2448 | 1.1639 | 0.4421 | 0.4372 | 0.0438 | 0.0798 |
| **RB-GR-93** | 3.3412 | 1.3084 | 9.4542 | 4.6303 | 0.4356 | 0.1098 | 0.0918 | 0.1461 | 0.7661 | 0.3336 | 0.2514 | 0.0538 | 0.0498 |
| **RB-GR-94** | 4.3072 | 1.7370 | 7.1632 | 3.4873 | 0.3714 | 0.1067 | 0.0398 | 0.1281 | 0.9010 | 0.3024 | 0.1593 | 0.0258 | 0.0298 |
| **RB-GR-95** | 4.9296 | 1.7410 | 9.0396 | 3.5572 | 0.4007 | 0.0704 | 0.0313 | 0.2760 | 1.1548 | 0.3979 | 0.2533 | 0.0281 | 0.0639 |
| **RB-GR-96** | 5.4241 | 1.6333 | 18.3342 | 5.2828 | 0.7023 | 0.1793 | 0.0875 | 0.3251 | 1.2062 | 0.3771 | 0.4173 | 0.0911 | 0.0601 |

n.d.: not detected.

Continuation of Table Annex II. 19.

| Sample | ((epi)cat-(epi)gallocat) 1 | ((epi)cat-(epi)gallocat) 2 | ((epi)gallocat)-(epi)cat) 1 | ((epi)gallocat)-(epi)cat) 2 | (epi)cat-glycoside 1 | (epi)cat-glycoside 2 | Catechin-gallate | Epicatechin-gallate | (Epi)catechin-gallate | PCA 2 | $((epi)cat)_2A$ 1 | $((epi)cat)_2A$ 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **RB-J-65** | 1.3327 | 1.2879 | 0.8102 | 0.8243 | 1.9624 | 0.6763 | n.d. | n.d. | n.d. | n.d. | 0.0189 | 0.1729 |
| **RB-J-66** | 2.9059 | 3.2472 | 1.8787 | 2.3123 | 7.3365 | 2.3445 | n.d. | n.d. | n.d. | 0.0088 | 0.0648 | 0.1234 |
| **RB-J-67** | 4.5726 | 1.8379 | 2.9626 | 2.3995 | 4.6321 | 1.1863 | n.d. | n.d. | n.d. | <0,005 | 0.0602 | 0.1584 |
| **RB-J-68** | 3.5150 | 1.8379 | 1.9905 | 2.1508 | 3.3579 | 1.2947 | n.d. | n.d. | 0.0240 | 0.0032 | 0.0602 | 0.2401 |
| **RB-J-69** | 6.0146 | 2.1279 | 3.7699 | 2.0699 | 4.6306 | 1.3023 | n.d. | n.d. | n.d. | <0,005 | 0.0758 | 0.1584 |
| **RB-J-70** | 4.6061 | 2.1752 | 2.3339 | 1.6258 | 3.3975 | 0.7859 | n.d. | n.d. | n.d. | <0,005 | 0.0758 | 0.2352 |
| **RB-J-71** | 5.2268 | 1.5744 | 3.8377 | 2.2231 | 4.0421 | 1.5994 | n.d. | n.d. | n.d. | n.d. | 0.1233 | 0.1379 |
| **RB-J-72** | 1.8974 | 1.2112 | 1.4534 | 0.8750 | 2.1816 | 0.4727 | n.d. | n.d. | n.d. | n.d. | 0.0107 | 0.2480 |
| **RB-C-73** | 1.3942 | 1.0470 | 1.3727 | 1.2908 | 2.0637 | 0.8887 | n.d. | n.d. | n.d. | 0.0355 | 0.0231 | 0.1768 |
| **RB-C-74** | 1.1480 | 1.0767 | 1.5147 | 1.5220 | 2.0407 | 1.2690 | n.d. | n.d. | n.d. | 0.0086 | 0.0118 | 0.0411 |
| **RB-C-75** | 1.7562 | 1.3058 | 1.7404 | 0.9530 | 2.1825 | 0.7119 | n.d. | n.d. | n.d. | n.d. | 0.0151 | 0.1013 |
| **RB-C-76** | 2.7374 | 2.7642 | 2.1277 | 2.6504 | 4.6597 | 2.0472 | n.d. | n.d. | n.d. | 0.0062 | 0.0142 | 0.1154 |
| **RB-C-77** | 2.4365 | 1.2992 | 1.7609 | 1.4383 | 1.8520 | 0.9136 | n.d | n.d. | n.d. | n.d. | 0.0133 | 0.1156 |
| **RB-C-78** | 1.8046 | 1.8393 | 1.4608 | 1.9369 | 3.5864 | 1.5583 | n.d. | n.d. | n.d. | n.d. | 0.0290 | 0.2824 |
| **RB-C-79** | 5.3934 | 1.8288 | 3.9170 | 1.9268 | 2.6548 | 0.4860 | n.d. | n.d. | n.d. | <0,005 | 0.1017 | 0.4352 |
| **RB-C-80** | 2.6405 | 1.6222 | 2.4915 | 3.2750 | 4.0018 | 1.5333 | n.d. | n.d. | n.d. | n.d. | 0.1012 | 0.2434 |
| **RB-R-81** | 0.9117 | 0.9292 | 0.8879 | 1.0687 | 2.2423 | 1.1401 | n.d. | n.d. | n.d. | n.d. | 0.0297 | 0.0688 |
| **RB-R-82** | 1.6858 | 1.3145 | 1.6711 | 1.4811 | 2.0103 | 1.0101 | n.d. | n.d. | n.d. | 0.0053 | 0.0232 | 0.0769 |
| **RB-R-83** | 1.7688 | 1.4024 | 1.6669 | 1.0465 | 2.4817 | 0.8261 | n.d. | n.d. | n.d. | n.d. | 0.0151 | 0.0965 |
| **RB-R-84** | 3.8182 | 3.0712 | 2.2984 | 2.6815 | 4.5051 | 1.8454 | n.d. | n.d. | n.d. | n.d. | 0.0195 | 0.1420 |
| **RB-R-85** | 1.1882 | 1.0876 | 1.1476 | 1.4724 | 1.7484 | 1.1509 | n.d. | n.d. | n.d. | n.d. | 0.0378 | 0.0498 |
| **RB-R-86** | 0.2580 | 0.1363 | 1.0407 | 1.2402 | 1.6575 | 1.1180 | n.d. | n.d. | 0.0918 | n.d. | 0.0078 | 0.0678 |
| **RB-R-87** | 3.4318 | 1.3263 | 3.0161 | 1.6333 | 2.1255 | 0.4511 | n.d. | n.d. | n.d. | n.d. | 0.0480 | 0.2114 |
| **RB-R-88** | 1.4443 | 1.4218 | 1.2385 | 1.5997 | 1.7166 | 1.2332 | n.d. | n.d. | 0.0156 | n.d. | 0.0099 | 0.1692 |
| **RB-GR-89** | 0.7722 | 0.7294 | 0.8055 | 0.9958 | 1.8118 | 0.9006 | n.d. | n.d. | n.d. | 0.0102 | 0.0167 | 0.0590 |
| **RB-GR-90** | 3.0744 | 2.4712 | 2.2812 | 2.8243 | 4.7681 | 2.2512 | n.d. | n.d. | n.d. | n.d. | 0.0435 | 0.0701 |
| **RB-GR-91** | 0.7086 | 0.3303 | 0.9010 | 0.4833 | 0.6494 | 0.2810 | 0.0078 | 0.0078 | 0.0158 | n.d. | 0.0138 | 0.0698 |
| **RB-GR-92** | 1.5324 | 0.8533 | 1.1233 | 1.1282 | 1.7711 | 0.4701 | n.d. | n.d. | 0.0138 | n.d. | 0.0238 | 0.1018 |
| **RB-GR-93** | 1.1022 | 0.7447 | 1.2272 | 1.0226 | 1.2158 | 0.6378 | n.d. | n.d. | n.d. | n.d. | 0.0158 | 0.0518 |
| **RB-GR-94** | 0.7349 | 0.3665 | 1.0835 | 0.7612 | 1.1328 | 0.8319 | 0.0078 | n.d. | 0.0278 | n.d. | 0.0178 | 0.0298 |
| **RB-GR-95** | 1.5890 | 0.9283 | 1.2947 | 0.8205 | 1.8857 | 1.1013 | n.d. | n.d. | 0.0281 | n.d. | 0.0086 | 0.2192 |
| **RB-GR-96** | 1.8133 | 1.0598 | 1.0879 | 1.1002 | 2.0185 | 0.8532 | n.d. | n.d. | 0.0052 | n.d. | 0.0235 | 0.0802 |

n.d.: not detected.

Continuation of Table Annex II. 19.

| Sample | ((epi)cat-(epi)gallocat)A 1 | ((epi)cat-(epi)gallocat)A 2 | $((epi)gallocat)_2A$ | (epi)cat-((epi)cat-(epi)gallocat) A 1 | (epi)cat-((epi)cat-(epi)gallocat) A 2 | (epi)cat-$((epi)gallocat)_2A$ | (epi)gallocat-et-(epi)gallocat | p-vinyl(epi)cat 1 | p-vinyl(epi)cat 2 | p-vinyl(epi)cat 3 | (epi)cat-furfuryl-(epi)cat 1 | (epi)cat-furfuryl-(epi)cat 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **RB-J-65** | 0.0877 | 0.1472 | n.d. | 0.3616 | 0.0489 | n.d. | n.d. | 0.0204 | 3.3532 | 0.4596 | 0.2303 | 0.3276 |
| **RB-J-66** | 0.6067 | 1.6320 | n.d. | 0.1416 | 0.1889 | 0.0120 | 0.1373 | n.d. | 5.1510 | 0.5457 | n.d. | 0.3033 |
| **RB-J-67** | 0.2744 | 0.1503 | n.d. | 4.6332 | 0.1562 | n.d. | n.d. | 0.0546 | 5.4724 | 0.2488 | 0.0112 | 7.3677 |
| **RB-J-68** | 0.2303 | 0.1290 | n.d. | 5.2389 | 0.1578 | n.d. | n.d. | 0.0819 | 7.8075 | 0.4214 | 8.1062 | 8.7795 |
| **RB-J-69** | 0.3431 | 0.1568 | n.d. | 6.6397 | 0.1641 | n.d. | n.d. | 0.1015 | 9.3714 | 0.3665 | 7.2645 | 7.3079 |
| **RB-J-70** | 0.2646 | 0.0758 | n.d. | 1.8634 | 0.1249 | n.d. | n.d. | 0.1015 | 8.3288 | 0.3743 | 2.6459 | 2.3120 |
| **RB-J-71** | 0.1715 | 0.5259 | n.d. | 0.3832 | 0.2294 | n.d. | n.d. | <0,005 | 0.5186 | 0.2444 | 1.3372 | 1.6874 |
| **RB-J-72** | 0.0929 | 0.3805 | n.d. | 0.2385 | 0.0415 | n.d. | n.d. | 0.0375 | 8.4887 | 0.1536 | 0.5284 | 0.3662 |
| **RB-C-73** | 0.1369 | 0.0336 | n.d. | 0.9534 | 0.0221 | n.d. | n.d. | 0.0523 | 3.8645 | 0.3317 | 0.6704 | 0.9221 |
| **RB-C-74** | 0.1323 | 0.0316 | 0.0069 | 2.6025 | 0.1842 | n.d. | n.d. | 0.0871 | 3.7919 | 0.4361 | 1.3829 | 3.3844 |
| **RB-C-75** | 0.1111 | 0.0216 | 0.0053 | 1.4666 | 0.0699 | n.d. | n.d. | 0.0484 | 5.8916 | 0.5843 | 1.5252 | 2.0059 |
| **RB-C-76** | 0.4573 | 0.1915 | n.d. | 2.3602 | 0.2083 | n.d. | n.d. | 0.1287 | 6.8105 | 0.7305 | 1.7959 | 3.3162 |
| **RB-C-77** | 0.1677 | 0.0313 | n.d. | 2.8276 | 0.2594 | n.d. | n.d. | 0.0990 | 3.3344 | 1.8165 | 3.0484 | 3.6405 |
| **RB-C-78** | 0.1566 | 0.0837 | n.d. | 2.1525 | 0.2205 | n.d. | n.d. | 0.0152 | 3.4651 | 0.6132 | 2.6084 | 3.7169 |
| **RB-C-79** | 0.1582 | 0.0878 | n.d. | 4.1879 | 0.2637 | n.d. | n.d. | 0.0247 | 2.8737 | 0.3063 | 5.7977 | 4.0806 |
| **RB-C-80** | 0.2958 | 0.4188 | n.d. | 6.2026 | 0.4374 | n.d. | n.d. | 0.1234 | 5.2405 | 0.2973 | 5.6158 | 10.7985 |
| **RB-R-81** | 0.1323 | 0.0265 | 0.0086 | 1.3880 | 0.1043 | n.d. | n.d. | 0.0291 | 2.4954 | 0.5157 | 0.9382 | 2.0237 |
| **RB-R-82** | 0.1388 | 0.0199 | n.d. | 2.2481 | 0.1193 | n.d. | n.d. | 0.0721 | 4.8628 | 0.4176 | 2.3437 | 3.8123 |
| **RB-R-83** | 0.1534 | 0.0297 | 0.0086 | 1.4526 | 0.0721 | n.d. | n.d. | 0.0871 | 5.3153 | 0.7628 | 1.0002 | 2.0632 |
| **RB-R-84** | 0.4877 | 0.1367 | n.d. | 3.3546 | 0.2040 | n.d. | n.d. | 0.1760 | 7.3783 | 0.3175 | 2.7625 | 5.1602 |
| **RB-R-85** | 0.0678 | 0.0578 | n.d. | 2.1154 | 0.1677 | n.d. | n.d. | 0.0130 | 0.2484 | 0.3343 | 1.2571 | 2.1931 |
| **RB-R-86** | 0.0318 | 0.0278 | n.d. | 0.3885 | 0.0154 | n.d. | n.d. | 0.0273 | 5.1666 | 0.0391 | 0.1126 | 0.1486 |
| **RB-R-87** | 0.1525 | 0.1125 | n.d. | 5.4211 | 0.3339 | n.d. | n.d. | 0.0293 | 1.8629 | 0.3038 | 7.5508 | 5.6343 |
| **RB-R-88** | 0.1548 | 0.1620 | n.d. | 4.6255 | 0.2902 | n.d. | n.d. | 0.0324 | 3.3672 | 0.2210 | 4.1972 | 8.8300 |
| **RB-GR-89** | 0.0785 | 0.0265 | 0.0086 | 1.7190 | 0.0833 | n.d. | n.d. | 0.0205 | 3.7613 | 0.5902 | 0.6452 | 1.4437 |
| **RB-GR-90** | 0.5459 | 0.1047 | n.d. | 4.2189 | 0.3028 | n.d. | n.d. | 0.1051 | 5.0859 | 0.6725 | 4.2601 | 8.6374 |
| **RB-GR-91** | 0.0738 | 0.1445 | n.d. | 1.1742 | 0.0130 | n.d. | n.d. | 0.0249 | 0.1422 | 0.3142 | 0.3787 | 1.1639 |
| **RB-GR-92** | 0.0698 | 0.2530 | n.d. | 1.4229 | 0.0628 | n.d. | n.d. | 0.0676 | 6.8278 | 0.1677 | 0.6475 | 0.8811 |
| **RB-GR-93** | 0.0618 | 0.0158 | n.d. | 1.6267 | 0.0557 | n.d. | n.d. | 0.0533 | 1.3353 | 0.2314 | 1.8114 | 2.3519 |
| **RB-GR-94** | 0.0198 | 0.0478 | n.d. | 2.0670 | 0.1613 | n.d. | n.d. | 0.0699 | 0.3843 | 0.1592 | 2.7128 | 3.8042 |
| **RB-GR-95** | 0.1285 | 0.0346 | n.d. | 2.5606 | 0.0833 | n.d. | n.d. | 0.0352 | 3.5663 | 0.2148 | 5.0021 | 3.2705 |
| **RB-GR-96** | 0.1057 | 0.1057 | n.d. | 2.8768 | 0.1717 | n.d. | n.d. | 0.0215 | 1.6708 | 0.1903 | 5.1581 | 3.3549 |

n.d.: not detected.

Table Annex II.20 Tannins concentrations of the samples from Médoc.

| Sample | Catechin | Epicatechin | PCB1 | PCB2 | $[(epi)cat)_2$ 1 | PCC1 | $[(epi)cat)_3$ 1 | $[(epi)cat)_3$ 2 | Gallocatechin | Epigallocatechin | $[(epi)gallocat)_2$ 1 | $[(epi)gallocat)_2$ 2 | $[(epi)gallocat)_2$ 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BO-M-01** | 4.0182 | 1.8340 | 18.5700 | 4.3496 | 0.5077 | 0.0803 | n.d. | 0.2136 | 0.8704 | 0.2069 | 0.5161 | 0.0609 | 0.0545 |
| **BO-M-02** | 5.2089 | 2.9416 | 70.3373 | 8.9697 | 1.7093 | 0.1995 | <0.005 | 0.5556 | 1.5036 | 0.5497 | 1.1473 | 0.1777 | 0.1659 |
| **BO-M-03** | 6.0028 | 3.3432 | 61.8354 | 26.4191 | 1.0801 | 0.3121 | 0.0364 | 0.4534 | 1.0160 | 0.3009 | 0.2981 | 0.0311 | 0.1469 |
| **BO-M-04** | 2.7443 | 1.4539 | 27.9231 | 10.5486 | 0.6387 | 0.2390 | 0.0232 | 0.3664 | 0.7661 | 0.2128 | 0.3018 | 0.0121 | 0.0981 |
| **BO-M-05** | 8.9758 | 5.7266 | 53.7568 | 27.8264 | 1.9565 | 0.4557 | 0.0536 | 0.8128 | 1.4548 | 0.7997 | 1.2629 | 0.1078 | 0.0997 |
| **BO-M-06** | 5.5997 | 2.6013 | 14.6130 | 4.4576 | 0.5660 | 0.1241 | <0.005 | 0.3664 | 1.1437 | 0.5292 | 1.3301 | 0.0536 | 0.0780 |
| **BO-M-07** | 5.1301 | 2.4907 | 28.9269 | 5.3667 | 0.9811 | 0.1560 | 0.0503 | 0.3558 | 1.4641 | 0.4547 | 0.7535 | 0.0926 | 0.0561 |
| **BO-M-08** | 6.6497 | 3.0060 | 52.5673 | 15.4937 | 1.8991 | 0.3573 | 0.1096 | 0.5963 | 1.6469 | 0.6088 | 0.5540 | 0.1059 | 0.1632 |
| **BO-M-09** | 2.4529 | 1.4102 | 5.0165 | 2.7085 | 0.2928 | 0.1590 | 0.0197 | 0.1622 | 0.3008 | 0.1335 | 0.1925 | 0.0424 | 0.0348 |
| **BO-M-10** | 2.4630 | 1.5315 | 5.8888 | 3.5534 | 0.3725 | 0.1256 | 0.0171 | 0.1606 | 0.3614 | 0.1909 | 0.2642 | 0.0348 | 0.0373 |
| **BO-M-11** | 7.8293 | 4.5730 | 87.7976 | 30.4030 | 1.8434 | 0.3893 | 0.0934 | 0.6581 | 1.3381 | 0.3989 | 0.7093 | 0.1013 | 0.1557 |
| **BO-M-12** | 7.2616 | 3.4826 | 27.0405 | 8.9700 | 1.6515 | 0.3668 | 0.1203 | 0.6729 | 1.2989 | 0.6077 | 0.9392 | 0.1260 | 0.1798 |
| **BO-M-13A** | 6.7105 | 3.6381 | 100.0142 | 36.4286 | 1.8894 | 0.2995 | 0.0259 | 0.6143 | 1.2875 | 0.3555 | 0.5766 | 0.0964 | 0.2099 |
| **BO-M-14A** | 2.7170 | 0.9544 | 9.3111 | 3.9370 | 0.3415 | 0.1199 | 0.0311 | 0.1847 | 0.7641 | 0.1861 | 0.1791 | 0.0233 | 0.0886 |
| **BO-M-15A** | 7.6231 | 4.0102 | 29.1427 | 14.5713 | 1.4693 | 0.2863 | 0.0134 | 0.5429 | 0.9452 | 0.4629 | 0.4127 | 0.0204 | 0.0697 |
| **BO-M-16A** | 3.5270 | 1.9258 | 13.1812 | 5.4101 | 0.6838 | 0.1417 | 0.0259 | 0.2753 | 0.8250 | 0.3765 | 0.4204 | 0.0300 | 0.0531 |
| **BO-M-17A** | 6.0172 | 3.1046 | 57.8698 | 14.6523 | 1.3277 | 0.3253 | 0.0719 | 0.5787 | 0.9824 | 0.3017 | 0.2373 | 0.0186 | 0.1253 |
| **BO-M-18A** | 7.1568 | 3.7421 | 97.9593 | 20.3651 | 1.9196 | 0.4768 | 0.0248 | 0.9050 | 1.6551 | 0.8229 | 1.1385 | 0.0468 | 0.2229 |
| **BO-M-19A** | 10.6113 | 4.7366 | 102.9727 | 36.0642 | 3.5750 | 0.5992 | 0.4065 | 1.4309 | 1.9834 | 0.9449 | 1.8703 | 0.2223 | 0.4490 |
| **BO-M-20A** | 3.6792 | 2.4050 | 14.6208 | 8.3434 | 0.7010 | 0.1760 | 0.0280 | 0.2600 | 0.8136 | 0.3726 | 0.2524 | 0.0133 | 0.0699 |
| **BO-M-21A** | 6.8337 | 3.5390 | 83.6505 | 16.3674 | 2.0177 | 0.3300 | 0.1582 | 0.6884 | 1.6606 | 1.2216 | 1.8105 | 0.3300 | 0.3125 |
| **BO-M-22B** | 8.6941 | 5.4286 | 34.6443 | 12.8968 | 1.6880 | 0.2745 | 0.0192 | 0.4741 | 1.2911 | 0.7892 | 0.8496 | 0.1238 | 0.0744 |
| **BO-M-23B** | 4.5240 | 2.6633 | 6.1250 | 3.1399 | 0.3454 | 0.0644 | <0.005 | 0.1132 | 0.7262 | 0.3953 | 0.2718 | 0.0183 | 0.0482 |
| **BO-M-24B** | 7.7764 | 4.1143 | 42.1900 | 14.4943 | 1.3008 | 0.3980 | 0.1288 | 0.5992 | 1.3573 | 0.8570 | 0.7579 | 0.1826 | 0.1741 |
| **BO-M-25B** | 3.5126 | 1.7358 | 7.3534 | 7.2559 | 0.9760 | 0.2094 | 0.0751 | 0.3962 | 0.8278 | 0.1836 | 0.2910 | 0.0123 | 0.0845 |
| **BO-M-26B** | 6.0215 | 2.6636 | 24.4424 | 14.3205 | 1.0389 | 0.1173 | 0.1039 | 0.2421 | 1.1610 | 0.4565 | 0.5861 | 0.1173 | 0.1349 |
| **BO-M-27B** | 7.5821 | 3.4071 | 11.4864 | 6.6939 | 1.1424 | 0.2025 | 0.1628 | 0.2648 | 1.6741 | 0.6445 | 0.3328 | 0.1146 | 0.1316 |
| **BO-M-28B** | 7.0859 | 3.5063 | 10.8199 | 15.6673 | 1.3143 | 0.1134 | 0.1034 | 0.2379 | 1.4479 | 1.1344 | 0.7631 | 0.0562 | 0.1034 |
| **BO-M-29B** | 5.0668 | 2.3699 | 14.9531 | 7.8539 | 0.7861 | 0.1365 | 0.0777 | 0.2421 | 0.9525 | 0.3413 | 0.4101 | 0.0619 | 0.1205 |
| **BO-M-30B** | 7.3109 | 3.2723 | 90.1087 | 25.9984 | 1.8815 | 0.3225 | 0.0363 | 0.9447 | 1.2469 | 0.4122 | 0.1998 | 0.0562 | 0.1419 |
| **BO-M-31B** | 11.9921 | 6.4507 | 118.5136 | 84.8681 | 5.9019 | 1.2423 | 0.1497 | 1.7571 | 1.9306 | 0.6559 | 0.8089 | 0.0540 | 0.2903 |
| **BO-M-32B** | 6.0630 | 3.3893 | 9.9940 | 5.5967 | 0.5476 | 0.1957 | <0.005 | 0.3270 | 0.8785 | 0.4320 | 0.1105 | <0.005 | 0.0292 |
| **BO-M-33B** | 7.0709 | 3.5172 | 63.3692 | 9.4618 | 1.7028 | 0.3474 | 0.0656 | 0.6262 | 1.1862 | 0.4793 | 0.6387 | 0.0656 | 0.1731 |
| **BO-M-34B** | 2.5215 | 1.4249 | 33.7363 | 9.7496 | 0.5135 | 0.2903 | 0.0220 | 0.3254 | 0.0192 | 0.1455 | 0.1621 | 0.0163 | 0.0645 |
| **BO-M-35B** | 5.5737 | 3.1455 | 51.6827 | 10.0684 | 0.6376 | 0.2427 | 0.0135 | 0.4040 | 0.7637 | 0.2675 | 0.3585 | 0.0504 | 0.0787 |
| **BO-M-36B** | 4.6540 | 2.1942 | 6.5899 | 2.6826 | 0.3953 | 0.0947 | 0.0134 | 0.1983 | 0.7655 | 0.3060 | 0.3428 | 0.0279 | 0.0570 |

n.d.: not detected.

Continuation of Table Annex II. 20.

| Sample | ((epi)cat-(epi)gallocat) 1 | ((epi)cat-(epi)gallocat) 2 | ((epi)gallocat)-(epi)cat) 1 | ((epi)gallocat)-(epi)cat) 2 | (epi)cat-glycoside 1 | (epi)cat-glycoside 2 | Catechin-gallate | Epicatechin-gallate | (Epi)catechin-gallate | PCA 2 | $((epi)cat)_2A$ 1 | $((epi)cat)_2A$ 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BO-M-01** | 1.6690 | 0.5178 | 2.4483 | 2.3277 | 3.2665 | 1.3264 | n.d. | n.d. | n.d. | n.d. | 0.0383 | 0.1919 |
| **BO-M-02** | 3.1025 | 1.7082 | 3.5268 | 4.6013 | 5.2590 | 2.1240 | n.d. | n.d. | n.d. | n.d. | 0.0253 | 0.0330 |
| **BO-M-03** | 1.0435 | 1.0426 | 2.2554 | 2.6275 | 3.0545 | 1.3444 | n.d. | n.d. | n.d. | 0.0129 | 0.0494 | 0.0494 |
| **BO-M-04** | 0.9773 | 0.9057 | 2.0031 | 3.7948 | 5.3445 | 2.0722 | n.d. | n.d. | n.d. | 0.0149 | 0.0759 | 0.1395 |
| **BO-M-05** | 2.9805 | 1.6097 | 3.9854 | 7.3025 | 6.3493 | 2.9628 | n.d. | n.d. | n.d. | <0.005 | 0.0238 | 0.0482 |
| **BO-M-06** | 1.7809 | 1.0518 | 2.5629 | 2.0317 | 3.0130 | 0.8548 | n.d. | n.d. | n.d. | <0.005 | 0.0129 | 0.0617 |
| **BO-M-07** | 1.9321 | 1.1037 | 2.1623 | 2.5418 | 3.3584 | 1.2046 | n.d. | n.d. | n.d. | n.d. | 0.0195 | 0.0791 |
| **BO-M-08** | 2.4935 | 1.4656 | 3.0537 | 4.8394 | 6.0472 | 3.4272 | n.d. | n.d. | n.d. | n.d. | 0.0343 | 0.0845 |
| **BO-M-09** | 0.5987 | 0.2690 | 2.4587 | 3.1158 | 4.1730 | 2.0500 | n.d. | n.d. | n.d. | <0.005 | 0.0626 | 0.0348 |
| **BO-M-10** | 0.7198 | 0.2721 | 2.7129 | 3.4191 | 3.8871 | 2.1828 | n.d. | n.d. | n.d. | <0.005 | 0.0399 | 0.0222 |
| **BO-M-11** | 2.5455 | 1.8024 | 3.5593 | 7.2272 | 8.0327 | 3.4707 | n.d. | n.d. | n.d. | 0.0225 | 0.0645 | 0.1413 |
| **BO-M-12** | 2.2436 | 1.2970 | 2.7830 | 2.0909 | 2.2285 | 1.4761 | n.d. | n.d. | n.d. | 0.0205 | 0.0444 | 0.0253 |
| **BO-M-13A** | 2.5868 | 1.5681 | 3.6462 | 5.7263 | 5.7893 | 1.9341 | n.d. | n.d. | n.d. | 0.0103 | 0.0781 | 0.1147 |
| **BO-M-14A** | 0.5038 | 0.4562 | 1.3363 | 1.0333 | 1.5864 | 0.5413 | n.d. | n.d. | 0.0207 | <0.005 | 0.0390 | 0.2127 |
| **BO-M-15A** | 1.3248 | 1.2182 | 1.9029 | 2.9465 | 2.5259 | 1.7252 | n.d. | n.d. | n.d. | <0.005 | 0.0192 | 0.0063 |
| **BO-M-16A** | 1.4679 | 0.8327 | 1.6226 | 2.1723 | 2.3371 | 1.0655 | n.d. | n.d. | n.d. | 0.0133 | 0.0196 | 0.0699 |
| **BO-M-17A** | 1.0103 | 1.5805 | 2.0067 | 2.5321 | 4.1212 | 2.0193 | n.d. | n.d. | n.d. | <0.005 | 0.0688 | 0.0939 |
| **BO-M-18A** | 2.6938 | 2.3026 | 4.5777 | 6.2571 | 6.5502 | 2.3435 | n.d. | n.d. | n.d. | 0.0123 | 0.0656 | 0.1159 |
| **BO-M-19A** | 6.2979 | 3.1016 | 6.1245 | 6.4526 | 6.2847 | 2.6264 | n.d. | n.d. | n.d. | 0.0109 | 0.0779 | 0.0348 |
| **BO-M-20A** | 0.9758 | 2.8579 | 1.5233 | 2.5673 | 2.5912 | 1.6038 | n.d. | n.d. | n.d. | 0.0196 | 0.0363 | 0.0678 |
| **BO-M-21A** | 4.9921 | 2.0259 | 6.5829 | 7.6652 | 5.0821 | 2.2195 | n.d. | n.d. | n.d. | 0.0154 | 0.0280 | 0.0437 |
| **BO-M-22B** | 2.3845 | 1.4564 | 2.7652 | 4.7705 | 4.5566 | 2.1971 | 0.0105 | 0.0105 | 0.0250 | 0.0221 | 0.0105 | 0.0134 |
| **BO-M-23B** | 0.9336 | 0.4032 | 1.4872 | 1.4135 | 1.6540 | 0.7157 | n.d. | n.d. | n.d. | <0.005 | 0.0183 | 0.0346 |
| **BO-M-24B** | 2.5868 | 1.7779 | 3.5636 | 3.0960 | 3.7371 | 1.8703 | n.d. | n.d. | n.d. | 0.0157 | 0.0396 | 0.0253 |
| **BO-M-25B** | 0.9803 | 0.6882 | 1.9633 | 2.1204 | 3.2815 | 1.7539 | n.d. | n.d. | n.d. | n.d. | 0.0374 | 0.1536 |
| **BO-M-26B** | 1.2955 | 5.8788 | 2.4815 | 2.8260 | 3.4133 | 1.4825 | n.d. | n.d. | n.d. | n.d. | 0.0409 | 0.0146 |
| **BO-M-27B** | 1.9551 | 0.8485 | 2.1097 | 2.2436 | 1.9004 | 1.8307 | n.d. | n.d. | n.d. | 0.0205 | 0.0157 | 0.0109 |
| **BO-M-28B** | 1.9605 | 5.4406 | 1.8119 | 3.1927 | 2.6652 | 1.4738 | n.d. | n.d. | n.d. | 0.0091 | 0.0468 | 0.0845 |
| **BO-M-29B** | 1.2331 | 2.5816 | 2.0550 | 2.2141 | 2.6357 | 1.3562 | n.d. | n.d. | n.d. | 0.0251 | 0.0488 | 0.0567 |
| **BO-M-30B** | 1.5238 | 1.2663 | 2.6694 | 3.9928 | 4.2953 | 1.5902 | n.d. | n.d. | n.d. | <0.005 | 0.0407 | 0.0551 |
| **BO-M-31B** | 4.3594 | 11.9569 | 5.1627 | 7.5256 | 9.0096 | 4.4179 | n.d. | n.d. | n.d. | 0.0109 | 0.0683 | 0.0923 |
| **BO-M-32B** | 0.8548 | 1.3323 | 1.2067 | 1.6289 | 2.4700 | 1.6422 | n.d. | n.d. | n.d. | <0.005 | 0.0156 | 0.0346 |
| **BO-M-33B** | 1.8105 | 1.2053 | 3.1832 | 4.1074 | 5.0643 | 2.6761 | n.d. | n.d. | 0.0186 | 0.0091 | 0.0468 | 0.0248 |
| **BO-M-34B** | 0.7141 | 0.6231 | 2.0399 | 2.0761 | 5.9920 | 2.3135 | n.d. | n.d. | n.d. | 0.0192 | 0.0872 | 0.2675 |
| **BO-M-35B** | 1.3142 | 0.7596 | 2.3768 | 2.9782 | 6.3650 | 2.3519 | 0.0192 | 0.0163 | 0.0163 | n.d. | 0.0447 | 0.1704 |
| **BO-M-36B** | 1.2498 | 0.4110 | 1.8725 | 1.4298 | 1.4062 | 0.5738 | n.d. | n.d. | n.d. | 0.0134 | 0.0250 | 0.0570 |

n.d.: not detected.

Continuation of Table Annex II. 20.

| Sample | ((epi)cat-(epi)gallocat)A 1 | ((epi)cat-(epi)gallocat)A 2 | $((epi)gallocat)_2A$ | (epi)cat-((epi)cat-(epi)gallocat) A 1 | (epi)cat-((epi)cat-(epi)gallocat) A 2 | $(epi)cat-((epi)gallocat)_2A$ | (epi)gallocat-et-(epi)gallocat | p-vinyl(epi)cat 1 | p-vinyl(epi)cat 2 | p-vinyl(epi)cat 3 | (epi)cat-furfuryl-(epi)cat 1 | (epi)cat-furfuryl-(epi)cat 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BO-M-01** | 0.1466 | 0.0803 | n.d. | 1.7839 | 0.1568 | n.d. | n.d. | 0.0744 | 8.0610 | 0.2855 | 1.6285 | 2.8157 |
| **BO-M-02** | 0.3004 | 1.2541 | n.d. | 2.0486 | 0.2234 | n.d. | n.d. | 0.0393 | 2.2602 | 1.3639 | 1.8872 | 3.4735 |
| **BO-M-03** | 0.1330 | 0.1043 | n.d. | 2.2343 | 0.1446 | n.d. | n.d. | 0.0117 | 0.1475 | 0.2802 | 3.4088 | 5.3030 |
| **BO-M-04** | 0.1953 | 0.1290 | n.d. | 1.9751 | 0.2153 | n.d. | n.d. | 0.0460 | 0.6366 | 0.6434 | 1.4936 | 2.8777 |
| **BO-M-05** | 0.4351 | 0.2062 | n.d. | 3.6619 | 0.5646 | n.d. | n.d. | 0.0640 | 1.5505 | 0.5361 | 10.7077 | 33.6143 |
| **BO-M-06** | 0.2824 | 0.3296 | n.d. | 1.1183 | 0.0868 | n.d. | n.d. | 0.1139 | 7.1891 | 0.3716 | 2.1619 | 1.3960 |
| **BO-M-07** | 0.1234 | 0.2351 | n.d. | 2.6044 | 0.2513 | n.d. | n.d. | 0.0201 | 4.4280 | 0.6966 | 3.6462 | 5.8732 |
| **BO-M-08** | 0.5241 | 0.5231 | n.d. | 10.4651 | 1.4300 | n.d. | n.d. | 0.0758 | 6.1800 | 0.4238 | 11.4754 | 31.8384 |
| **BO-M-09** | 0.2253 | 0.2674 | n.d. | 2.2102 | 0.2535 | n.d. | n.d. | 0.0212 | 1.3437 | 0.2801 | 2.7057 | 6.2138 |
| **BO-M-10** | 0.2084 | 0.1447 | n.d. | 2.5572 | 0.3436 | n.d. | n.d. | 0.0061 | 0.4424 | 0.7566 | 2.1631 | 5.4833 |
| **BO-M-11** | 0.2773 | 0.1383 | n.d. | 7.4367 | 0.4658 | n.d. | n.d. | 0.0223 | 2.2754 | 0.2373 | 10.1102 | 45.3197 |
| **BO-M-12** | 0.1826 | 0.1515 | n.d. | 2.4564 | 0.3349 | n.d. | n.d. | 0.0593 | 1.4431 | 0.6979 | 3.6422 | 6.3705 |
| **BO-M-13A** | 0.2127 | 0.0442 | n.d. | 3.1410 | 0.0862 | n.d. | n.d. | 0.0203 | 0.8755 | 0.2920 | 5.8067 | 7.8683 |
| **BO-M-14A** | 0.0990 | 0.1016 | n.d. | 2.3859 | 0.1947 | n.d. | n.d. | 0.0518 | 4.1930 | 0.1416 | 1.1146 | 1.6954 |
| **BO-M-15A** | 0.1060 | 0.0861 | n.d. | 1.3438 | 0.2686 | n.d. | n.d. | <0.005 | 0.5166 | 0.5903 | 1.1483 | 2.4613 |
| **BO-M-16A** | 0.1264 | 0.3230 | n.d. | 1.4130 | 0.1718 | n.d. | n.d. | 0.0262 | 2.5061 | 1.2087 | 1.3680 | 2.5470 |
| **BO-M-17A** | 0.2287 | 0.3082 | n.d. | 1.5764 | 0.1138 | n.d. | n.d. | 0.0141 | 0.5683 | 0.1413 | 2.1318 | 3.3261 |
| **BO-M-18A** | 0.5590 | 0.1682 | n.d. | 3.0210 | 1.0736 | n.d. | n.d. | 0.0758 | 7.3305 | 1.0593 | 7.4127 | 30.4762 |
| **BO-M-19A** | 0.4320 | 0.2166 | n.d. | 6.5218 | 0.7857 | n.d. | n.d. | 0.0759 | 5.3209 | 0.3087 | 4.1019 | 10.5327 |
| **BO-M-20A** | 0.1932 | 0.1455 | n.d. | 1.0099 | 0.1738 | n.d. | n.d. | 0.0213 | 2.0630 | 0.7826 | 2.0311 | 3.3084 |
| **BO-M-21A** | 0.4495 | 0.1632 | n.d. | 6.1362 | 0.5098 | n.d. | n.d. | 0.1533 | 2.6278 | 1.6833 | 6.8656 | 18.1229 |
| **BO-M-22B** | 0.2692 | 0.4399 | n.d. | 3.3373 | 0.4228 | n.d. | n.d. | 0.2357 | 1.4324 | 1.4547 | 7.3512 | 17.1040 |
| **BO-M-23B** | 0.0861 | 0.0617 | n.d. | 0.3169 | 0.0487 | n.d. | n.d. | <0.005 | 4.1365 | 0.4933 | 1.6619 | 1.3936 |
| **BO-M-24B** | 0.2931 | 0.1656 | n.d. | 3.0765 | 0.3232 | n.d. | n.d. | 0.1176 | 2.4685 | 0.7857 | 1.7517 | 4.0687 |
| **BO-M-25B** | 0.1815 | 0.1643 | n.d. | 3.1357 | 0.1779 | n.d. | n.d. | 0.0650 | 1.0667 | 0.1290 | 3.6209 | 6.2666 |
| **BO-M-26B** | 0.1877 | 0.1637 | n.d. | 2.8881 | 0.2935 | n.d. | n.d. | 0.0653 | 0.1737 | 1.0005 | 2.6885 | 6.2601 |
| **BO-M-27B** | 0.3697 | 0.1685 | n.d. | 6.1588 | 0.7389 | n.d. | n.d. | 0.1093 | 0.6511 | 0.2237 | 5.1485 | 13.3189 |
| **BO-M-28B** | 0.2503 | 0.2627 | n.d. | 6.6796 | 0.7870 | n.d. | n.d. | 0.0887 | 1.3003 | 1.0402 | 7.5543 | 23.2193 |
| **BO-M-29B** | 0.1893 | 0.0987 | n.d. | 3.5936 | 0.3310 | n.d. | n.d. | 0.0223 | 0.5175 | 0.7017 | 2.3567 | 5.8262 |
| **BO-M-30B** | 0.0618 | 0.0485 | n.d. | 2.8617 | 0.5970 | n.d. | n.d. | 0.0287 | 4.1152 | 0.4053 | 1.2068 | 2.8146 |
| **BO-M-31B** | 0.4773 | 0.4575 | n.d. | 7.4050 | 1.0375 | n.d. | n.d. | 0.1009 | 5.2332 | 0.3876 | 47.5666 | 96.3845 |
| **BO-M-32B** | 0.1105 | 0.0834 | n.d. | 0.9235 | 0.1629 | n.d. | n.d. | 0.0487 | 3.2213 | 0.3127 | 1.8203 | 2.8643 |
| **BO-M-33B** | 0.3026 | 0.3648 | n.d. | 4.3456 | 0.4286 | n.d. | n.d. | 0.1061 | 0.4764 | 0.8443 | 4.2361 | 10.0202 |
| **BO-M-34B** | 0.2200 | 0.2799 | n.d. | 1.1431 | 0.2554 | n.d. | n.d. | 0.0338 | 1.8146 | 0.6278 | 0.6774 | 1.6195 |
| **BO-M-35B** | 0.2076 | 0.1642 | n.d. | 1.0227 | 0.1145 | n.d. | n.d. | 0.0305 | 1.7396 | 0.3073 | 0.8393 | 1.6795 |
| **BO-M-36B** | 0.0539 | 0.1563 | n.d. | 1.8886 | 0.2357 | n.d. | n.d. | 0.1772 | 10.4113 | 0.6041 | 1.3493 | 1.7451 |

n.d.: not detected.

Table Annex II.21 Tannins concentrations of the samples from Graves.

| Sample | Catechin | Epicatechin | PCB1 | PCB2 | ((epi)cat)$_2$ 1 | PCC1 | ((epi)cat)$_3$ 1 | ((epi)cat)$_3$ 2 | Gallocatechin | Epigallocatechin | ((epi)gallocat)$_2$ 1 | ((epi)gallocat)$_2$ 2 | ((epi)gallocat)$_2$ 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BO-G-37** | 7.4685 | 3.6978 | 109.7642 | 29.7541 | 2.2112 | 0.2871 | 0.0367 | 0.8620 | 1.8409 | 0.5445 | 1.0442 | 0.1191 | 0.2303 |
| **BO-G-38** | 4.4553 | 2.2668 | 24.2974 | 4.5992 | 0.8485 | 0.0887 | n.d. | 0.2945 | 1.1928 | 0.5477 | 1.0048 | 0.0695 | 0.1022 |
| **BO-G-39** | 5.5880 | 2.4364 | 109.4702 | 37.8997 | 2.9589 | 0.4576 | 0.0129 | 0.8886 | 1.6495 | 0.3163 | 0.4842 | 0.1330 | 0.2659 |
| **BO-G-40** | 5.8418 | 3.1188 | 128.6823 | 59.6382 | 3.1572 | 0.5636 | 0.0537 | 1.0909 | 1.7361 | 0.2966 | 0.9057 | 0.0426 | 0.2582 |
| **BO-G-41** | 7.8928 | 2.8884 | 108.0778 | 18.4855 | 2.0645 | 0.2494 | 0.0343 | 0.7818 | 3.0439 | 0.6125 | 1.3943 | 0.1377 | 0.3489 |
| **BO-G-42** | 5.9724 | 2.6829 | 88.1153 | 30.9128 | 2.0914 | 0.3978 | 0.0815 | 1.0611 | 1.4519 | 0.3734 | 0.4781 | 0.0149 | 0.1883 |
| **BO-G-43** | 5.8931 | 2.9899 | 50.7374 | 10.2453 | 1.3157 | 0.2104 | 0.0154 | 0.3956 | 0.6456 | 0.3287 | 0.2429 | 0.0238 | 0.0510 |
| **BO-G-44B** | 1.9789 | 0.9068 | 37.5922 | 12.5733 | 0.7431 | 0.3289 | 0.0103 | 0.4086 | 0.6367 | 0.1511 | 0.2337 | 0.0207 | 0.1581 |
| **BO-G-45B** | 6.5752 | 3.7096 | 17.2127 | 7.2997 | 0.8680 | 0.2062 | 0.0192 | 0.3322 | 0.8758 | 0.5082 | 0.4215 | 0.0483 | 0.0599 |
| **BO-G-46B** | 5.2244 | 3.4910 | 88.9128 | 42.5348 | 1.7954 | 0.3983 | 0.0154 | 0.5637 | 0.9502 | 0.3253 | 0.3189 | 0.0499 | 0.1707 |
| **BO-G-47B** | 6.8039 | 4.3972 | 11.2823 | 7.4059 | 0.9809 | 0.2955 | 0.0163 | 0.3559 | 0.9205 | 0.6237 | 0.2377 | 0.0134 | 0.0483 |
| **BO-G-48B** | 4.8451 | 2.2392 | 27.9111 | 7.1699 | 1.0026 | 0.1919 | 0.0462 | 0.3531 | 1.1539 | 0.3577 | 0.5575 | 0.0751 | 0.0940 |
| **BO-G-87** | 5.6322 | 3.0561 | 100.2955 | 68.5951 | 1.8398 | 0.3465 | 0.0692 | 0.6070 | 1.2943 | 0.3931 | 0.7691 | 0.1136 | 0.1671 |

n.d.: not detected.

Continuation of Table Annex II. 21.

| Sample | ((epi)cat-(epi)gallocat) 1 | ((epi)cat-(epi)gallocat) 2 | ((epi)gallocat)-(epi)cat) 1 | ((epi)gallocat)-(epi)cat) 2 | (epi)cat-glycoside 1 | (epi)cat-glycoside 2 | Catechin-gallate | Epicatechin-gallate | (Epi)catechin-gallate | PCA 2 | ((epi)cat)$_2$A 1 | ((epi)cat)$_2$A 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BO-G-37** | 4.5271 | 1.9644 | 6.5074 | 8.7651 | 7.7888 | 3.0280 | n.d. | n.d. | n.d. | n.d. | 0.0351 | 0.0803 |
| **BO-G-38** | 2.3809 | 1.5164 | 2.5951 | 2.2412 | 2.6772 | 0.9257 | n.d. | n.d. | n.d. | n.d. | 0.0118 | 0.0157 |
| **BO-G-39** | 3.0931 | 2.1456 | 5.9703 | 8.2131 | 8.4266 | 3.0525 | n.d. | n.d. | n.d. | <0.005 | 0.0781 | 0.2127 |
| **BO-G-40** | 4.8682 | 2.1663 | 9.4579 | 19.3290 | 13.3636 | 4.3363 | n.d. | n.d. | 0.0399 | 0.0121 | 0.0926 | 0.2878 |
| **BO-G-41** | 7.4991 | 2.4236 | 7.9869 | 8.8529 | 9.3926 | 2.2239 | n.d. | n.d. | n.d. | 0.0093 | 0.0621 | 0.1569 |
| **BO-G-42** | 2.2738 | 1.7707 | 3.8524 | 7.7699 | 9.4675 | 3.0996 | n.d. | n.d. | n.d. | 0.0093 | 0.0759 | 0.1150 |
| **BO-G-43** | 1.1304 | 0.9186 | 1.3447 | 1.8314 | 2.8478 | 1.4403 | 0.0133 | 0.0133 | 0.0489 | 0.0112 | 0.0363 | 0.0112 |
| **BO-G-44B** | 0.7151 | 0.7263 | 2.7149 | 4.8621 | 5.7405 | 2.2696 | n.d. | n.d. | n.d. | <0.005 | 0.0886 | 0.2491 |
| **BO-G-45B** | 1.7972 | 1.0439 | 2.3786 | 3.1090 | 3.3348 | 1.6098 | 0.0192 | 0.0105 | 0.0163 | 0.0076 | 0.0279 | 0.0425 |
| **BO-G-46B** | 1.3476 | 1.4632 | 2.7109 | 3.8882 | 5.0042 | 2.6513 | n.d. | n.d. | n.d. | <0.005 | 0.0845 | 0.0562 |
| **BO-G-47B** | 1.3487 | 1.0991 | 2.3624 | 2.8405 | 3.5429 | 1.7441 | 0.0105 | 0.0105 | 0.0425 | 0.0163 | 0.0279 | 0.0366 |
| **BO-G-48B** | 1.3337 | 0.7358 | 1.8866 | 2.4415 | 2.0399 | 0.9674 | n.d. | n.d. | n.d. | <0.005 | 0.0185 | 0.0207 |
| **BO-G-87** | 1.8161 | 1.0686 | 2.2459 | 2.6780 | 2.2200 | 1.3041 | n.d. | n.d. | n.d. | n.d. | 0.0372 | 0.0564 |

n.d.: not detected.

Continuation of Table Annex II. 21.

| Sample | ((epi)cat-(epi)gallocat)A 1 | ((epi)cat-(epi)gallocat)A 2 | ((epi)gallocat)$_2$A | (epi)cat-((epi)cat-(epi)gallocat) A 1 | (epi)cat-((epi)cat-(epi)gallocat) A 2 | (epi)cat-((epi)gallocat)$_2$A | (epi)gallocat-et-(epi)gallocat | p-vinyl(epi)cat 1 | p-vinyl(epi)cat 2 | p-vinyl(epi)cat 3 | (epi)cat-furfuryl-(epi)cat 1 | (epi)cat-furfuryl-(epi)cat 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BO-G-37** | 0.2388 | 1.5004 | n.d. | 1.7333 | 0.3370 | n.d. | n.d. | 0.0132 | 5.6172 | 0.5313 | 2.8157 | 6.2184 |
| **BO-G-38** | 0.1896 | 0.1401 | n.d. | 1.7187 | 0.0756 | n.d. | n.d. | 0.0522 | 3.0240 | 1.1470 | 1.5967 | 1.3607 |
| **BO-G-39** | 0.2603 | 0.6759 | n.d. | 2.8312 | 0.1859 | n.d. | n.d. | 0.0174 | 3.2636 | 0.2389 | 2.3087 | 6.0065 |
| **BO-G-40** | 0.3454 | 0.8411 | n.d. | 3.6850 | 0.5113 | n.d. | n.d. | 0.0362 | 3.7070 | 0.2039 | 6.5378 | 23.4211 |
| **BO-G-41** | 0.3681 | 0.3576 | n.d. | 1.5192 | 0.1265 | n.d. | n.d. | 0.0133 | 2.0025 | 0.2381 | 1.7462 | 2.6653 |
| **BO-G-42** | 0.2931 | 0.3332 | n.d. | 1.3952 | 0.2130 | n.d. | n.d. | 0.0334 | 0.6753 | 0.2973 | 1.6785 | 4.2764 |
| **BO-G-43** | 0.1550 | 0.2677 | n.d. | 1.7039 | 0.1941 | n.d. | n.d. | 0.0165 | 2.3289 | 0.4417 | 4.7220 | 6.3652 |
| **BO-G-44B** | 0.2183 | 0.1511 | n.d. | 2.3635 | 0.3480 | n.d. | n.d. | 0.0289 | 3.0087 | 0.3214 | 2.4638 | 3.4734 |
| **BO-G-45B** | 0.2246 | 0.5731 | n.d. | 1.2026 | 0.1889 | n.d. | n.d. | 0.1714 | 8.3213 | 0.5924 | 2.3927 | 4.0233 |
| **BO-G-46B** | 0.1922 | 0.2695 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| **BO-G-47B** | 0.2080 | 0.3454 | n.d. | 1.4770 | 0.3117 | n.d. | n.d. | 0.1365 | 4.3327 | 1.3333 | 2.7821 | 5.8484 |
| **BO-G-48B** | 0.1692 | 0.1555 | n.d. | 1.7250 | 0.0932 | n.d. | n.d. | 0.0176 | 0.9563 | 0.7260 | 1.3114 | 3.1157 |
| **BO-G-87** | 0.1343 | 0.2782 | n.d. | 1.2667 | 0.1049 | n.d. | n.d. | 0.0160 | 1.2210 | 0.2237 | 1.8601 | 2.5526 |

n.d.: not detected.

Table Annex II.22 Tannins concentrations of the samples from Blayais & Bourgeais.

| Sample | Catechin | Epicatechin | PCB1 | PCB2 | ((epi)cat)$_2$ 1 | PCC1 | ((epi)cat)$_3$ 1 | ((epi)cat)$_3$ 2 | Gallocatechin | Epigallocatechin | ((epi)gallocat)$_2$ 1 | ((epi)gallocat)$_2$ 2 | ((epi)gallocat)$_2$ 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BO-BB-74** | 4.3274 | 2.4566 | 20.3628 | 5.3422 | 0.5398 | 0.1421 | <0.005 | 0.2747 | 0.7654 | 0.3776 | 0.2885 | n.d. | 0.0445 |
| **BO-BB-82B** | 9.4590 | 4.2221 | 111.2297 | 32.2450 | 2.2637 | 0.3327 | 0.0373 | 0.9970 | 1.7510 | 0.5573 | 0.5685 | 0.0676 | 0.1973 |
| **BO-BB-84** | 5.7049 | 1.9106 | 39.3440 | 7.0763 | 0.6965 | 0.1429 | 0.0855 | 0.2869 | 1.0597 | 0.3509 | 0.3669 | 0.1118 | 0.0934 |
| **BO-BB-85** | 6.3201 | 2.4405 | 11.7713 | 5.8870 | 0.5941 | 0.1557 | 0.0619 | 0.2613 | 0.9701 | 0.3701 | 0.2517 | 0.0514 | 0.0961 |
| **BO-BB-86** | 19.3887 | 6.9259 | 104.0589 | 60.3342 | 4.8969 | 0.8400 | 0.2421 | 1.8835 | 3.1639 | 1.3479 | 1.3008 | 0.2336 | 0.3243 |

n.d.: not detected.

Continuation of Table Annex II. 22.

| Sample | ((epi)cat-(epi)gallocat) 1 | ((epi)cat-(epi)gallocat) 2 | ((epi)gallocat)-(epi)cat) 1 | ((epi)gallocat)-(epi)cat) 2 | (epi)cat-glycoside 1 | (epi)cat-glycoside 2 | Catechin-gallate | Epicatechin-gallate | (Epi)catechin-gallate | PCA 2 | ((epi)cat)$_2$A 1 | ((epi)cat)$_2$A 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BO-BB-74** | 1.5078 | 0.8287 | 2.2892 | 2.2530 | 3.4874 | 1.4559 | n.d. | n.d. | n.d. | n.d. | 0.0272 | 0.0330 |
| **BO-BB-82B** | 2.6970 | 1.6672 | 4.3593 | 5.9928 | 6.3380 | 2.7995 | n.d. | n.d. | n.d. | <0.005 | 0.0449 | 0.1351 |
| **BO-BB-84** | 1.6154 | 0.9989 | 2.8375 | 2.3109 | 4.2351 | 1.4398 | n.d. | n.d. | n.d. | 0.0199 | 0.0777 | 0.0304 |
| **BO-BB-85** | 0.9669 | 0.9333 | 1.6432 | 1.1872 | 2.2978 | 1.2594 | n.d. | n.d. | n.d. | 0.0120 | 0.0488 | 0.0698 |
| **BO-BB-86** | 7.0673 | 4.8610 | 6.2923 | 7.5406 | 6.2433 | 2.9828 | n.d. | n.d. | n.d. | 0.0157 | 0.0444 | 0.0205 |

n.d.: not detected.

Continuation of Table Annex II. 22.

| Sample | ((epi)cat-(epi)gallocat)A 1 | ((epi)cat-(epi)gallocat)A 2 | ((epi)gallocat)$_2$A | (epi)cat-((epi)cat-(epi)gallocat) A 1 | (epi)cat-((epi)cat-(epi)gallocat) A 2 | (epi)cat-((epi)gallocat)$_2$A | (epi)gallocat-et-(epi)gallocat | p-vinyl(epi)cat 1 | p-vinyl(epi)cat 2 | p-vinyl(epi)cat 3 | (epi)cat-furfuryl-(epi)cat 1 | (epi)cat-furfuryl-(epi)cat 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BO-BB-74** | 0.1817 | 0.1579 | n.d. | 1.5232 | 0.1376 | n.d. | n.d. | 0.0650 | 2.8160 | 0.8404 | 2.0038 | 3.3037 |
| **BO-BB-82B** | 0.2360 | 0.5541 | n.d. | 1.7844 | 0.2466 | n.d. | n.d. | 0.0629 | 3.2102 | 0.3728 | 1.5411 | 4.4571 |
| **BO-BB-84** | 0.1605 | 0.2757 | n.d. | 2.2685 | 0.1670 | n.d. | n.d. | 0.0247 | 0.3106 | 0.2636 | 2.5841 | 6.2068 |
| **BO-BB-85** | 0.1221 | 0.1429 | n.d. | 0.3511 | 0.0340 | n.d. | n.d. | 0.0223 | 0.6725 | 0.1670 | 0.6590 | 2.2081 |
| **BO-BB-86** | 0.6133 | 0.2303 | n.d. | 6.0348 | 0.3438 | n.d. | n.d. | 0.1009 | 1.6851 | 0.7038 | 33.3505 | 96.8378 |

n.d.: not detected.

Table Annex II.23 Tannins concentrations of the samples from Libournais.

| Sample | Catechin | Epicatechin | PCB1 | PCB2 | ((epi)cat)$_2$ 1 | PCC1 | ((epi)cat)$_3$ 1 | ((epi)cat)$_3$ 2 | Gallocatechin | Epigallocatechin | ((epi)gallocat)$_2$ 1 | ((epi)gallocat)$_2$ 2 | ((epi)gallocat)$_2$ 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BO-L-49** | 5.7750 | 3.2038 | 50.9709 | 8.1415 | 1.4375 | 0.1955 | 0.0291 | 0.3855 | 0.9909 | 0.3875 | 0.3301 | 0.0407 | 0.0599 |
| **BO-L-50** | 5.7044 | 3.0252 | 86.6854 | 8.6236 | 1.1400 | 0.2371 | 0.0196 | 0.5463 | 1.1018 | 0.4872 | 0.3841 | 0.0405 | 0.1013 |
| **BO-L-51** | 7.1256 | 3.7958 | 28.2745 | 12.0713 | 1.5570 | 0.2927 | 0.0486 | 0.5261 | 1.0007 | 0.3301 | 0.3495 | 0.0310 | 0.0673 |
| **BO-L-52** | 6.9307 | 3.8332 | 69.5258 | 33.7198 | 2.2892 | 0.3439 | 0.0954 | 0.8377 | 0.7207 | 0.3454 | 0.1092 | 0.0232 | 0.0537 |
| **BO-L-53** | 3.6645 | 2.2930 | 21.3497 | 7.3395 | 0.5729 | 0.2019 | 0.0286 | 0.2654 | 0.6648 | 0.2404 | 0.3222 | 0.0335 | 0.0480 |
| **BO-L-54** | 3.6211 | 2.0599 | 1.8445 | 1.7028 | 0.1121 | 0.0279 | 0.0105 | 0.0396 | 0.2824 | 0.1878 | 0.0483 | 0.0134 | 0.0134 |
| **BO-L-55** | 4.5455 | 2.6026 | 42.3578 | 15.8913 | 1.2736 | 0.2287 | 0.0123 | 0.3941 | 0.9330 | 0.2802 | 0.2738 | 0.0437 | 0.1190 |
| **BO-L-56** | 9.3522 | 4.9594 | 41.4777 | 11.1864 | 1.7500 | 0.2718 | 0.0773 | 0.5712 | 1.4283 | 0.9257 | 1.0019 | 0.1957 | 0.1957 |
| **BO-L-57B** | 7.1409 | 3.4000 | 76.3904 | 8.8226 | 1.1270 | 0.1951 | 0.0217 | 0.5559 | 1.3648 | 0.7983 | 0.6609 | 0.0426 | 0.1302 |
| **BO-L-58B** | 7.1288 | 3.6101 | 55.8294 | 17.9147 | 1.4661 | 0.2981 | 0.0672 | 0.6085 | 0.9973 | 0.3957 | 0.5253 | 0.1237 | 0.1269 |
| **BO-L-59** | 2.9511 | 1.4325 | 71.7320 | 8.7674 | 1.3025 | 0.2867 | 0.0311 | 0.6560 | 0.6818 | 0.1815 | 0.2008 | 0.0531 | 0.1190 |
| **BO-L-60** | 4.6553 | 2.4169 | 6.0910 | 3.5294 | 0.3401 | 0.1051 | <0.005 | 0.1721 | 0.6553 | 0.3375 | 0.2141 | 0.0129 | 0.0210 |
| **BO-L-61** | 3.4824 | 1.8409 | 32.7129 | 5.1125 | 0.3316 | 0.1099 | 0.0135 | 0.2324 | 0.4970 | 0.1600 | 0.2675 | 0.0192 | 0.0418 |
| **BO-L-62** | 2.5667 | 1.6962 | 28.1319 | 6.3243 | 0.3709 | 0.1373 | 0.0163 | 0.2262 | 0.4267 | 0.0929 | 0.2593 | 0.0163 | 0.0305 |
| **BO-L-63A** | 6.9262 | 3.6366 | 84.9055 | 15.5953 | 2.2220 | 0.3677 | 0.0657 | 0.9336 | 1.5121 | 0.6308 | 0.9395 | 0.0830 | 0.1698 |
| **BO-L-64A** | 3.1259 | 2.1092 | 7.1742 | 5.5003 | 0.5987 | 0.2068 | 0.0197 | 0.2196 | 0.3247 | 0.1351 | 0.1463 | 0.0146 | 0.0222 |
| **BO-L-65A** | 8.2082 | 3.9161 | 102.8257 | 46.2524 | 2.2800 | 0.4894 | 0.0329 | 1.0874 | 1.3879 | 0.5212 | 0.3986 | 0.0540 | 0.1544 |
| **BO-L-66A** | 6.4852 | 3.2396 | 105.5131 | 30.8921 | 2.6408 | 0.2986 | 0.0251 | 0.8459 | 1.5238 | 0.4451 | 0.6370 | 0.0396 | 0.1238 |
| **BO-L-67B** | 7.4242 | 4.3555 | 64.1509 | 30.3224 | 1.8245 | 0.4502 | 0.0315 | 0.7155 | 1.0297 | 0.3210 | 0.2564 | 0.0121 | 0.1255 |
| **BO-L-68B** | 3.3111 | 1.6097 | 3.9515 | 2.4301 | 0.2850 | 0.0590 | <0.005 | 0.0536 | 0.3139 | 0.1241 | 0.0455 | 0.0075 | 0.0075 |
| **BO-L-73** | 5.7849 | 2.9462 | 78.0281 | 11.7662 | 1.4235 | 0.2587 | 0.0383 | 0.5913 | 0.9406 | 0.3222 | 0.4175 | 0.0302 | 0.0706 |
| **BO-L-75** | 7.9345 | 3.8109 | 146.2914 | 55.7521 | 3.2029 | 0.4576 | 0.0729 | 1.1004 | 1.4868 | 0.3681 | 0.4086 | 0.0755 | 0.1917 |
| **BO-L-76** | 5.7341 | 2.9095 | 84.6312 | 35.7051 | 1.9612 | 0.3270 | 0.0196 | 0.5791 | 0.8289 | 0.2566 | 0.2362 | 0.0285 | 0.0929 |
| **BO-L-77** | 7.6734 | 3.5063 | 57.8157 | 27.3070 | 2.7415 | 0.3947 | 0.2901 | 0.7606 | 1.5720 | 0.5316 | 0.4196 | 0.1408 | 0.1433 |
| **BO-L-79** | 1.5589 | 0.9953 | 8.1750 | 5.2335 | 0.4434 | 0.2137 | 0.0154 | 0.2201 | 0.3769 | 0.1065 | 0.0876 | 0.0091 | 0.0594 |
| **BO-L-80** | 5.7704 | 3.2133 | 46.6758 | 14.4643 | 0.8319 | 0.2220 | 0.0135 | 0.3461 | 0.6459 | 0.3171 | 0.2572 | 0.0305 | 0.0504 |
| **BO-L-81** | 7.8339 | 3.8515 | 52.9539 | 17.1866 | 2.0924 | 0.3508 | 0.0157 | 0.7157 | 1.3355 | 0.3791 | 0.3031 | 0.0345 | 0.0709 |

Continuation of Table Annex II. 23.

| Sample | ((epi)cat-(epi)gallocat) 1 | ((epi)cat-(epi)gallocat) 2 | ((epi)gallocat)-(epi)cat) 1 | ((epi)gallocat)-(epi)cat) 2 | (epi)cat-glycoside 1 | (epi)cat-glycoside 2 | Catechin-gallate | Epicatechin-gallate | (Epi)catechin-gallate | PCA 2 | $((epi)cat)_2A$ 1 | $((epi)cat)_2A$ 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BO-L-49** | 1.7775 | 1.0107 | 2.6527 | 3.3477 | 4.4574 | 1.8863 | n.d. | n.d. | 0.0176 | n.d. | 0.0253 | 0.0214 |
| **BO-L-50** | 1.7736 | 1.1629 | 2.5094 | 3.2642 | 3.9925 | 1.7547 | n.d. | n.d. | n.d. | 0.0133 | 0.0280 | 0.0447 |
| **BO-L-51** | 1.2099 | 0.8176 | 2.3163 | 3.7768 | 3.0685 | 2.0367 | n.d. | n.d. | n.d. | 0.0052 | 0.0216 | 0.0263 |
| **BO-L-52** | 0.9040 | 0.8411 | 2.0012 | 3.7967 | 6.2739 | 3.1303 | n.d. | n.d. | n.d. | 0.0093 | 0.0815 | 0.1726 |
| **BO-L-53** | 0.9990 | 0.8236 | 2.2195 | 2.1779 | 3.1195 | 1.7189 | n.d. | n.d. | n.d. | n.d. | 0.0351 | 0.0125 |
| **BO-L-54** | 0.2587 | 0.1005 | 0.4557 | 0.3821 | 0.5371 | 0.3585 | 0.0105 | n.d. | 0.0396 | 0.0105 | n.d. | 0.0192 |
| **BO-L-55** | 0.9545 | 0.9996 | 1.9001 | 2.7615 | 3.1244 | 1.8658 | n.d. | n.d. | n.d. | <0.005 | 0.0343 | 0.1504 |
| **BO-L-56** | 2.7401 | 1.8223 | 3.7435 | 4.8871 | 5.8772 | 2.3830 | n.d. | n.d. | n.d. | 0.0105 | 0.0250 | 0.0192 |
| **BO-L-57B** | 2.4302 | 1.2755 | 1.8491 | 2.3447 | 3.8956 | 1.9409 | n.d. | n.d. | n.d. | n.d. | 0.0510 | 0.0782 |
| **BO-L-58B** | 1.3135 | 1.4841 | 3.3673 | 3.8726 | 5.5212 | 2.3273 | n.d. | n.d. | n.d. | 0.0120 | 0.0619 | 0.0488 |
| **BO-L-59** | 1.3115 | 1.3205 | 2.9150 | 2.3859 | 5.1865 | 1.9128 | n.d. | n.d. | n.d. | 0.0123 | 0.0280 | 0.2437 |
| **BO-L-60** | 0.7472 | 0.4032 | 1.3603 | 1.1568 | 1.6953 | 0.7655 | n.d. | n.d. | n.d. | <0.005 | 0.0238 | 0.0319 |
| **BO-L-61** | 0.7885 | 0.3606 | 2.4604 | 1.5651 | 4.2737 | 1.6126 | n.d. | n.d. | n.d. | 0.0163 | 0.0447 | 0.2861 |
| **BO-L-62** | 0.6335 | 0.3709 | 1.9382 | 1.4589 | 4.4817 | 1.8387 | n.d. | n.d. | n.d. | n.d. | 0.0617 | 0.1497 |
| **BO-L-63A** | 3.1836 | 1.8543 | 3.4170 | 3.9564 | 4.9542 | 2.0856 | n.d. | n.d. | n.d. | n.d. | 0.0311 | 0.0349 |
| **BO-L-64A** | 0.6043 | 0.3598 | 2.6522 | 4.9356 | 5.8238 | 3.9318 | n.d. | n.d. | n.d. | <0.005 | 0.0626 | 0.0803 |
| **BO-L-65A** | 1.9050 | 1.6240 | 3.0475 | 5.1547 | 4.7613 | 2.1697 | n.d. | n.d. | n.d. | <0.005 | 0.0363 | 0.0396 |
| **BO-L-66A** | 2.2218 | 1.5228 | 3.9059 | 5.8230 | 5.2998 | 1.8989 | n.d. | n.d. | 0.0085 | <0.005 | 0.0496 | 0.1113 |
| **BO-L-67B** | 1.0105 | 1.0715 | 1.6747 | 2.6944 | 5.8265 | 4.7261 | <0.005 | <0.005 | 0.0288 | <0.005 | 0.0510 | 0.0704 |
| **BO-L-68B** | 0.3926 | 0.2088 | 0.8785 | 0.5633 | 1.0439 | 0.6342 | n.d. | n.d. | n.d. | <0.005 | <0.005 | 0.0373 |
| **BO-L-73** | 1.7758 | 1.3778 | 2.5551 | 3.0987 | 5.4729 | 3.2263 | n.d. | n.d. | n.d. | n.d. | 0.0561 | 0.0771 |
| **BO-L-75** | 3.0179 | 2.1497 | 5.8300 | 9.3538 | 10.5676 | 3.5811 | n.d. | n.d. | n.d. | <0.005 | 0.0808 | 0.2701 |
| **BO-L-76** | 1.0854 | 0.9652 | 2.1472 | 3.2549 | 4.0306 | 2.2116 | n.d. | n.d. | n.d. | <0.005 | 0.0451 | 0.0207 |
| **BO-L-77** | 2.4907 | 1.9441 | 3.1478 | 5.1379 | 4.7890 | 4.3869 | n.d. | n.d. | n.d. | 0.0091 | 0.0343 | 0.0562 |
| **BO-L-79** | 0.4520 | 0.3726 | 1.3620 | 1.7087 | 2.1511 | 1.4325 | n.d. | n.d. | n.d. | n.d. | 0.0405 | 0.2760 |
| **BO-L-80** | 0.9125 | 0.9973 | 2.0422 | 2.5147 | 5.2888 | 2.5870 | n.d. | n.d. | n.d. | n.d. | 0.0645 | 0.0248 |
| **BO-L-81** | 1.4705 | 1.2004 | 2.7439 | 4.5409 | 4.6238 | 2.1872 | n.d. | n.d. | n.d. | <0.005 | 0.0310 | 0.0239 |

n.d.: not detected.

Continuation of Table Annex II. 23.

| Sample | ((epi)cat-(epi)gallocat)A 1 | ((epi)cat-(epi)gallocat)A 2 | ((epi)gallocat)$_2$A | (epi)cat-((epi)cat-(epi)gallocat) A 1 | (epi)cat-((epi)cat-(epi)gallocat) A 2 | (epi)cat-((epi)gallocat)$_2$A | (epi)gallocat-et-(epi)gallocat | p-vinyl(epi)cat 1 | p-vinyl(epi)cat 2 | p-vinyl(epi)cat 3 | (epi)cat-furfuryl-(epi)cat 1 | (epi)cat-furfuryl-(epi)cat 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BO-L-49** | 0.2054 | 0.4587 | n.d. | 1.2220 | 0.0885 | n.d. | n.d. | 0.0351 | 3.2858 | 0.4065 | 1.3278 | 2.8662 |
| **BO-L-50** | 0.1930 | 0.1665 | n.d. | 1.4138 | 0.0930 | n.d. | n.d. | 0.0602 | 4.1970 | 0.5310 | 1.6607 | 2.1311 |
| **BO-L-51** | 0.1014 | 0.2089 | n.d. | 2.2155 | 0.5040 | n.d. | n.d. | 0.0256 | 2.7824 | 0.3585 | 2.3658 | 7.5837 |
| **BO-L-52** | 0.2006 | 0.2058 | n.d. | 1.3531 | 0.2244 | n.d. | n.d. | 0.0133 | 1.7133 | 0.1538 | 1.5979 | 5.0068 |
| **BO-L-53** | 0.0900 | 0.2069 | n.d. | 1.5311 | 0.1592 | n.d. | n.d. | 0.0217 | 0.3511 | 0.5944 | 1.9787 | 3.3419 |
| **BO-L-54** | 0.0221 | 0.0976 | n.d. | 0.2299 | <0.005 | n.d. | n.d. | <0.005 | 0.7211 | 0.2708 | 1.2951 | 1.4260 |
| **BO-L-55** | 0.1159 | 0.1815 | n.d. | 1.1064 | 0.1749 | n.d. | n.d. | 0.0432 | 1.3872 | 0.2573 | 2.5987 | 4.1305 |
| **BO-L-56** | 0.4163 | 0.2456 | n.d. | 2.3671 | 0.3410 | n.d. | n.d. | 0.0261 | 1.3812 | 1.3907 | 2.8778 | 6.3142 |
| **BO-L-57B** | 0.2638 | 0.3784 | n.d. | 1.3348 | 0.0796 | n.d. | n.d. | 0.0310 | 0.1495 | 0.2022 | 2.0675 | 2.4652 |
| **BO-L-58B** | 0.2005 | 0.2581 | n.d. | 2.6746 | 0.2816 | n.d. | n.d. | 0.0139 | 0.6837 | 1.0454 | 0.2007 | 6.7312 |
| **BO-L-59** | 0.2352 | 0.2223 | n.d. | 2.5315 | 0.1810 | n.d. | n.d. | 0.0323 | 2.0036 | 0.6147 | 1.7656 | 5.0186 |
| **BO-L-60** | 0.0346 | 0.1931 | n.d. | 1.0721 | 0.0411 | n.d. | n.d. | 0.0868 | 2.7436 | 0.6527 | 1.5455 | 1.6866 |
| **BO-L-61** | 0.1724 | 0.1745 | n.d. | 0.2780 | 0.0803 | n.d. | n.d. | 0.0274 | 1.5534 | 0.1238 | 0.3254 | 0.7722 |
| **BO-L-62** | 0.1600 | 0.1931 | n.d. | 0.5781 | 0.0709 | n.d. | n.d. | 0.0149 | 0.6932 | 0.1945 | 0.6368 | 1.4078 |
| **BO-L-63A** | 0.3756 | 0.2133 | n.d. | 2.7855 | 0.3110 | n.d. | n.d. | 0.0415 | 3.3475 | 0.3201 | 3.8327 | 8.3143 |
| **BO-L-64A** | 0.3056 | 0.2132 | n.d. | 3.7487 | 0.6510 | n.d. | n.d. | 0.0326 | 3.2388 | 0.2981 | 3.4356 | 13.7605 |
| **BO-L-65A** | 0.1907 | 0.1442 | n.d. | 2.5524 | 0.4227 | n.d. | n.d. | 0.0343 | 1.5099 | 0.5796 | 2.0322 | 4.8587 |
| **BO-L-66A** | 0.1873 | 0.1465 | n.d. | 4.0026 | 0.6179 | n.d. | n.d. | 0.0343 | 5.2971 | 0.5552 | 3.8735 | 34.1974 |
| **BO-L-67B** | 0.2075 | 0.2547 | n.d. | 1.2538 | 0.2700 | n.d. | n.d. | 0.0165 | 0.8316 | 0.3474 | 1.5778 | 3.0607 |
| **BO-L-68B** | 0.0536 | 0.0639 | n.d. | 1.1812 | 0.1139 | n.d. | n.d. | 0.0868 | 7.2535 | 0.3758 | 1.0008 | 1.6039 |
| **BO-L-73** | 0.2019 | 0.2036 | n.d. | 2.0142 | 0.2715 | n.d. | n.d. | 0.0268 | 2.7727 | 0.3581 | 2.3682 | 4.7052 |
| **BO-L-75** | 0.2771 | 0.3614 | n.d. | 2.8216 | 0.1976 | n.d. | n.d. | 0.0404 | 8.8719 | 0.2419 | 3.2120 | 6.6228 |
| **BO-L-76** | 0.1783 | 0.3452 | n.d. | 1.3441 | 0.4680 | n.d. | n.d. | 0.0176 | 1.0019 | 0.4576 | 1.8356 | 5.0042 |
| **BO-L-77** | 0.5216 | 0.4669 | n.d. | 3.3419 | 0.3972 | n.d. | n.d. | 0.0952 | 7.3743 | 0.1897 | 11.0687 | 66.0078 |
| **BO-L-79** | 0.1284 | 0.0656 | n.d. | 1.8754 | 0.2451 | n.d. | n.d. | 0.0214 | 3.0564 | 0.2207 | 1.0942 | 3.4348 |
| **BO-L-80** | 0.2014 | 0.2034 | n.d. | 0.3935 | 0.0585 | n.d. | n.d. | 0.0243 | 0.3276 | 0.1651 | 1.6135 | 2.3220 |
| **BO-L-81** | 0.1909 | 0.1934 | n.d. | 3.7014 | 0.4753 | n.d. | n.d. | 0.0125 | 2.1564 | 0.3800 | 2.1715 | 9.1277 |

n.d.: not detected.

Table Annex II.24 Tannins concentrations of the samples from Bordeaux & Bordeaux Superieur.

| Sample | Catechin | Epicatechin | PCB1 | PCB2 | ((epi)cat)$_2$ 1 | PCC1 | ((epi)cat)$_3$ 1 | ((epi)cat)$_3$ 2 | Gallocatechin | Epigallocatechin | ((epi)gallocat)$_2$ 1 | ((epi)gallocat)$_2$ 2 | ((epi)gallocat)$_2$ 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BO-B-69** | 5.2649 | 3.1209 | 17.0296 | 8.2908 | 0.7400 | 0.1936 | 0.0173 | 0.2755 | 0.9723 | 0.3724 | 0.2236 | 0.0319 | 0.0609 |
| **BO-B-70** | 1.6918 | 1.0477 | 5.9870 | 2.3576 | 0.2610 | 0.0752 | 0.0146 | 0.1702 | 0.3359 | 0.1925 | 0.3948 | 0.0853 | 0.0575 |
| **BO-BS-71** | 9.8923 | 4.6251 | 101.0867 | 38.5224 | 2.0254 | 0.3215 | 0.1272 | 0.6274 | 1.0416 | 0.3980 | 0.2721 | 0.0373 | 0.0929 |
| **BO-BS-72** | 9.3098 | 6.3698 | 123.0651 | 68.7165 | 3.7404 | 0.5836 | 0.0163 | 0.9981 | 1.6781 | 0.7983 | 1.0854 | 0.1340 | 0.1783 |
| **BO-B-88** | 6.5482 | 4.3533 | 112.9601 | 62.1877 | 3.5551 | 0.6984 | 0.0137 | 1.1604 | 1.2457 | 0.4276 | 0.7208 | 0.0841 | 0.1533 |
| **BO-B-89** | 7.7957 | 4.2950 | 104.1835 | 60.2385 | 2.8065 | 0.5983 | 0.0073 | 1.3127 | 1.6205 | 0.5483 | 0.6518 | 0.0713 | 0.2378 |
| **BO-B-90** | 8.7700 | 4.9679 | 102.0062 | 64.1213 | 3.3790 | 0.7622 | 0.0414 | 1.8927 | 1.5266 | 0.4293 | 0.7467 | 0.1171 | 0.1912 |
| **BO-B-91** | 8.3932 | 4.9785 | 124.1576 | 57.4339 | 3.1952 | 0.7239 | 0.0134 | 1.7464 | 1.9106 | 0.5118 | 1.0023 | 0.0807 | 0.2520 |
| **BO-B-92** | 8.4762 | 4.6655 | 94.8160 | 66.0705 | 4.2086 | 0.7294 | 0.0180 | 1.6022 | 2.0234 | 0.6760 | 1.6259 | 0.1705 | 0.4069 |
| **BO-B-93** | 7.4498 | 4.3542 | 117.2939 | 50.0622 | 3.7038 | 0.7663 | 0.0059 | 1.7909 | 1.6621 | 0.4031 | 0.9280 | 0.0807 | 0.3342 |
| **BO-B-94** | 10.0161 | 6.1566 | 133.8880 | 64.2043 | 3.7897 | 0.8856 | <0.005 | 1.9750 | 1.8876 | 0.3846 | 0.8247 | 0.0807 | 0.3077 |
| **BO-B-95** | 4.6809 | 2.8218 | 126.3919 | 54.3650 | 3.7069 | 0.8220 | 0.0433 | 1.9029 | 1.4979 | 0.2653 | 0.8114 | 0.0471 | 0.3050 |
| **BO-B-96** | 5.7470 | 3.4936 | 133.6651 | 57.2473 | 3.6746 | 0.7160 | 0.6282 | 1.7955 | 1.0689 | 0.3395 | 1.0235 | 0.0321 | 0.1486 |

n.d.: not detected.

Continuation of Table Annex II. 24.

| Sample | ((epi)cat-(epi)gallocat) 1 | ((epi)cat-(epi)gallocat) 2 | ((epi)gallocat)-(epi)cat) 1 | ((epi)gallocat)-(epi)cat) 2 | (epi)cat-glycoside 1 | (epi)cat-glycoside 2 | Catechin-gallate | Epicatechin-gallate | (Epi)catechin-gallate | PCA 2 | $((epi)cat)_2A$ 1 | $((epi)cat)_2A$ 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BO-B-69** | 0.9974 | 0.8169 | 2.0240 | 2.4081 | 2.7618 | 1.0993 | n.d. | n.d. | n.d. | n.d. | 0.0351 | 0.0642 |
| **BO-B-70** | 0.8664 | 0.3789 | 4.3030 | 4.6207 | 4.6337 | 1.5416 | n.d. | n.d. | n.d. | 0.0146 | 0.0399 | 0.2323 |
| **BO-BS-71** | 1.7467 | 2.2608 | 3.0508 | 5.1277 | 6.2065 | 3.2097 | n.d. | n.d. | n.d. | <0.005 | 0.0449 | 0.0146 |
| **BO-BS-72** | 3.4665 | 1.9643 | 4.7490 | 7.1025 | 4.0306 | 2.2657 | n.d. | n.d. | n.d. | <0.005 | 0.0429 | 0.0296 |
| **BO-B-88** | 1.6184 | 1.2738 | 3.1878 | 3.1695 | 3.9321 | 2.3691 | n.d. | n.d. | 0.0578 | n.d. | 0.0649 | 0.0457 |
| **BO-B-89** | 2.2006 | 1.4726 | 3.9612 | 3.6307 | 3.7430 | 1.9100 | n.d. | n.d. | n.d. | n.d. | 0.0478 | 0.1058 |
| **BO-B-90** | 2.9243 | 1.7804 | 4.3047 | 5.1148 | 5.8871 | 2.7785 | n.d. | n.d. | n.d. | n.d. | 0.0521 | 0.0734 |
| **BO-B-91** | 2.7190 | 1.9719 | 4.1042 | 6.7963 | 5.3267 | 2.9951 | n.d. | n.d. | n.d. | n.d. | <0.005 | 0.1725 |
| **BO-B-92** | 3.6264 | 1.8387 | 5.7878 | 6.3051 | 4.8318 | 2.6780 | n.d. | n.d. | n.d. | n.d. | 0.0372 | 0.1533 |
| **BO-B-93** | 2.8202 | 1.8032 | 3.8280 | 4.8681 | 4.9310 | 2.4996 | n.d. | n.d. | n.d. | n.d. | 0.0452 | 0.1645 |
| **BO-B-94** | 3.3755 | 2.5993 | 5.4602 | 9.9870 | 10.2600 | 5.8514 | n.d. | n.d. | n.d. | n.d. | 0.0789 | 0.2584 |
| **BO-B-95** | 2.7420 | 2.2419 | 4.6457 | 6.0799 | 6.1351 | 2.7681 | n.d. | n.d. | n.d. | n.d. | 0.0452 | 0.2865 |
| **BO-B-96** | 2.7757 | 1.9044 | 3.9998 | 5.6611 | 5.6857 | 2.8494 | n.d. | n.d. | n.d. | n.d. | 0.0471 | 0.1990 |

n.d.: not detected.

Continuation of Table Annex II. 24.

| Sample | ((epi)cat-(epi)gallocat)A 1 | ((epi)cat-(epi)gallocat)A 2 | $((epi)gallocat)_2A$ | (epi)cat-((epi)cat-(epi)gallocat) A 1 | (epi)cat-((epi)cat-(epi)gallocat) A 2 | (epi)cat-$((epi)gallocat)_2A$ | (epi)gallocat-et-(epi)gallocat | p-vinyl(epi)cat 1 | p-vinyl(epi)cat 2 | p-vinyl(epi)cat 3 | (epi)cat-furfuryl-(epi)cat 1 | (epi)cat-furfuryl-(epi)cat 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BO-B-69** | 0.1272 | 0.2036 | n.d. | 1.1116 | 0.1070 | n.d. | n.d. | 0.0318 | 1.1585 | 0.5453 | 1.4543 | 2.8157 |
| **BO-B-70** | 0.2355 | 1.1372 | n.d. | 0.7695 | 0.1616 | n.d. | n.d. | 0.0136 | 8.0456 | 0.3780 | 0.4939 | 0.3215 |
| **BO-BS-71** | 0.2630 | 0.2626 | n.d. | 2.0832 | 0.3419 | n.d. | n.d. | 0.0667 | 0.8571 | 0.2157 | 3.1369 | 3.9830 |
| **BO-BS-72** | 0.1181 | 0.0602 | n.d. | 1.9503 | 0.4934 | n.d. | n.d. | 0.0213 | 1.0432 | 0.9212 | 1.8356 | 5.0042 |
| **BO-B-88** | 0.1671 | 0.2758 | n.d. | 1.0302 | 0.1246 | n.d. | n.d. | 0.0024 | 0.6979 | 0.1810 | 1.3202 | 3.2581 |
| **BO-B-89** | 0.1947 | 0.3327 | n.d. | 1.3867 | 0.1169 | n.d. | n.d. | 0.0001 | 1.5601 | 0.2568 | 1.7297 | 2.6939 |
| **BO-B-90** | 0.1939 | 0.1257 | n.d. | 1.3515 | 0.1169 | n.d. | n.d. | 0.0115 | 2.0132 | 0.0981 | 3.3362 | 4.8230 |
| **BO-B-91** | 0.2335 | 0.1032 | n.d. | 2.0247 | 0.1559 | n.d. | n.d. | 0.0114 | 1.7319 | 0.1261 | 2.2632 | 3.6111 |
| **BO-B-92** | 0.2846 | 0.1930 | n.d. | 1.5067 | 0.2430 | n.d. | n.d. | 0.0320 | 2.1405 | 0.1538 | 3.3024 | 5.3472 |
| **BO-B-93** | 0.2520 | 0.0732 | n.d. | 1.7959 | 0.1232 | n.d. | n.d. | 0.0059 | 1.6125 | 0.1559 | 2.5777 | 4.3801 |
| **BO-B-94** | 0.4562 | 0.1013 | n.d. | 1.3778 | 0.2683 | n.d. | n.d. | 0.0059 | 1.2543 | 0.0845 | 3.5102 | 6.8463 |
| **BO-B-95** | 0.3342 | 0.1069 | n.d. | 1.8072 | 0.1232 | n.d. | n.d. | 0.0151 | 1.7760 | 0.1707 | 3.5670 | 5.5926 |
| **BO-B-96** | 0.2971 | 0.0938 | n.d. | 2.5521 | 0.2421 | n.d. | n.d. | 0.0132 | 1.9224 | 0.1046 | 4.1726 | 6.2081 |

n.d.: not detected.

Table Annex II.25 pH values of the samples from Rioja Alavesa.

| Sample | pH | Sample | pH | Sample | pH | Sample | pH |
|---|---|---|---|---|---|---|---|
| RV-J-01 | 3.80 | RV-C-09 | 3.61 | RV-R-17 | 3.59 | RV-GR-25 | 3.56 |
| RV-J-02 | 3.54 | RV-C-10 | 3.48 | RV-R-18 | 3.38 | RV-GR-26 | 3.45 |
| RV-J-03 | 3.87 | RV-C-11 | 3.67 | RV-R-19 | 3.57 | RV-GR-27 | 3.33 |
| RV-J-04 | 3.81 | RV-C-12 | 3.77 | RV-R-20 | 3.68 | RV-GR-28 | 3.48 |
| RV-J-05 | 3.54 | RV-C-13 | 3.47 | RV-R-21 | 3.57 | RV-GR-29 | 3.63 |
| RV-J-06 | 3.77 | RV-C-14 | 3.53 | RV-R-22 | 3.51 | RV-GR-30 | 3.31 |
| RV-J-07 | 3.71 | RV-C-15 | 3.41 | RV-R-23 | 3.37 | RV-GR-31 | 3.60 |
| RV-J-08 | 3.53 | RV-C-16 | 3.76 | RV-R-24 | 3.73 | RV-GR-32 | 3.41 |

Table Annex II.26 pH values of the samples from Rioja Alta.

| Sample | pH | Sample | pH | Sample | pH | Sample | pH |
|---|---|---|---|---|---|---|---|
| RA-J-33 | 3.57 | RA-C-41 | 3.58 | RA-R-49 | 3.56 | RA-GR-57 | 3.61 |
| RA-J-34 | 3.63 | RA-C-42 | 3.52 | RA-R-50 | 3.62 | RA-GR-58 | 3.53 |
| RA-J-35 | 3.63 | RA-C-43 | 3.63 | RA-R-51 | 3.64 | RA-GR-59 | 3.62 |
| RA-J-36 | 3.59 | RA-C-44 | 3.70 | RA-R-52 | 3.59 | RA-GR-60 | 3.53 |
| RA-J-37 | 3.74 | RA-C-45 | 3.48 | RA-R-53 | 3.68 | RA-GR-61 | 3.72 |
| RA-J-38 | 3.33 | RA-C-46 | 3.57 | RA-R-54 | 3.59 | RA-GR-62 | 3.69 |
| RA-J-39 | 3.60 | RA-C-47 | 3.58 | RA-R-55 | 3.44 | RA-GR-63 | 3.47 |
| RA-J-40 | 3.49 | RA-C-48 | 3.60 | RA-R-56 | 3.62 | RA-GR-64 | 3.63 |

Table Annex II.27 pH values of the samples from Rioja Baja.

| Sample | pH | Sample | pH | Sample | pH | Sample | pH |
|---|---|---|---|---|---|---|---|
| RB-J-65 | 3.62 | RB-C-73 | 3.57 | RB-R-81 | 3.55 | RB-GR-89 | 3.47 |
| RB-J-66 | 3.72 | RB-C-74 | 3.44 | RB-R-82 | 3.50 | RB-GR-90 | 3.48 |
| RB-J-67 | 3.57 | RB-C-75 | 3.46 | RB-R-83 | 3.62 | RB-GR-91 | 3.60 |
| RB-J-68 | 3.59 | RB-C-76 | 3.52 | RB-R-84 | 3.58 | RB-GR-92 | 3.67 |
| RB-J-69 | 3.68 | RB-C-77 | 3.60 | RB-R-85 | 3.44 | RB-GR-93 | 3.44 |
| RB-J-70 | 3.65 | RB-C-78 | 3.59 | RB-R-86 | 3.62 | RB-GR-94 | 3.57 |
| RB-J-71 | 3.66 | RB-C-79 | 3.54 | RB-R-87 | 3.63 | RB-GR-95 | 3.67 |
| RB-J-72 | 3.83 | RB-C-80 | 3.45 | RB-R-88 | 3.38 | RB-GR-96 | 3.68 |

Table Annex II.28 pH values of the samples from Medoc.

| Sample | pH | Sample | pH | Sample | pH | Sample | pH |
|---|---|---|---|---|---|---|---|
| BO-M-01 | 3.56 | BO-M-10 | 3.69 | BO-M-19A | 3.53 | BO-M-28B | 3.59 |
| BO-M-02 | 3.40 | BO-M-11 | 3.56 | BO-M-20A | 3.65 | BO-M-29B | 3.59 |
| BO-M-03 | 3.64 | BO-M-12 | 3.65 | BO-M-21A | 3.55 | BO-M-30B | 3.69 |
| BO-M-04 | 3.84 | BO-M-13A | 3.42 | BO-M-22B | 3.59 | BO-M-31B | 3.80 |
| BO-M-05 | 3.59 | BO-M-14A | 3.68 | BO-M-23B | 3.63 | BO-M-32B | 3.58 |
| BO-M-06 | 3.51 | BO-M-15A | 3.79 | BO-M-24B | 3.66 | BO-M-33B | 3.78 |
| BO-M-07 | 3.54 | BO-M-16A | 3.52 | BO-M-25B | 3.45 | BO-M-34B | 3.56 |
| BO-M-08 | 3.86 | BO-M-17A | 3.52 | BO-M-26B | 3.62 | BO-M-35B | 3.55 |
| BO-M-09 | 3.65 | BO-M-18A | 3.68 | BO-M-27B | 3.63 | BO-M-36B | 3.59 |

Table Annex II.29 pH values of the samples from Graves.

| Sample | pH | Sample | pH | Sample | pH |
|---|---|---|---|---|---|
| **BO-G-37** | 3.77 | **BO-G-42** | 3.62 | **BO-G-47B** | 3.80 |
| **BO-G-38** | 3.45 | **BO-G-43** | 3.68 | **BO-G-48B** | 3.47 |
| **BO-G-39** | 3.56 | **BO-G-44B** | 3.60 | **BO-G-87** | 3.58 |
| **BO-G-40** | 3.67 | **BO-G-45B** | 3.67 | **-** | - |
| **BO-G-41** | 3.59 | **BO-G-46B** | 3.80 | **-** | - |

Table Annex II.30 pH values of the samples from Blayais & Bourgeais.

| Sample | pH | Sample | pH |
|---|---|---|---|
| **BO-BB-74** | 3.42 | **BO-BB-85** | 3.45 |
| **BO-BB-82B** | 3.39 | **BO-BB-86** | 3.63 |
| **BO-BB-84** | 3.49 | **-** | - |

Table Annex II.31 pH values of the samples from Libournais.

| Sample | pH | Sample | pH | Sample | pH | Sample | pH |
|---|---|---|---|---|---|---|---|
| **BO-L-49** | 3.47 | **BO-L-56** | 3.57 | **BO-L-63A** | 3.61 | **BO-L-75** | 3.62 |
| **BO-L-50** | 3.39 | **BO-L-57B** | 3.59 | **BO-L-64A** | 3.68 | **BO-L-76** | 3.69 |
| **BO-L-51** | 3.68 | **BO-L-58B** | 3.51 | **BO-L-65A** | 3.60 | **BO-L-77** | 3.75 |
| **BO-L-52** | 3.53 | **BO-L-59** | 3.50 | **BO-L-66A** | 3.52 | **BO-L-79** | 3.27 |
| **BO-L-53** | 3.56 | **BO-L-60** | 3.33 | **BO-L-67B** | 3.84 | **BO-L-80** | 3.64 |
| **BO-L-54** | 3.57 | **BO-L-61** | 3.22 | **BO-L-68B** | 3.61 | **BO-L-81** | 3.61 |
| **BO-L-55** | 3.59 | **BO-L-62** | 3.38 | **BO-L-73** | 3.64 | - | - |

Table Annex II.32 pH values of the samples from Bordeaux & Bordeaux Superieur.

| Sample | pH | Sample | pH | Sample | pH |
|---|---|---|---|---|---|
| **BO-B-69** | 3.59 | **BO-B-89** | 3.66 | **BO-B-94** | 3.68 |
| **BO-B-70** | 3.62 | **BO-B-90** | 3.51 | **BO-B-95** | 3.58 |
| **BO-BS-71** | 3.56 | **BO-B-91** | 3.61 | **BO-B-96** | 3.48 |
| **BO-BS-72** | 3.77 | **BO-B-92** | 3.74 | **-** | - |
| **BO-B-88** | 3.72 | **BO-B-93** | 3.58 | **-** | - |

Table Annex II.33 Parameters of CIELAB space of the samples from Rioja Alavesa.

| Sample | X | Y | Z | L* | a* | b* | H* | C* |
|---|---|---|---|---|---|---|---|---|
| RV-J-01 | 42.0368 | 33.0984 | 32.2870 | 64.2405 | 35.3821 | 4.3594 | 0.1226 | 35.6496 |
| RV-J-02 | 44.9299 | 37.4725 | 28.7114 | 67.6300 | 29.3247 | 15.3441 | 0.4821 | 33.0965 |
| RV-J-03 | 36.9446 | 27.3468 | 25.0690 | 59.2939 | 40.6421 | 6.6682 | 0.1626 | 41.1855 |
| RV-J-04 | 34.1025 | 25.6998 | 21.0822 | 57.7510 | 37.6772 | 10.9166 | 0.2820 | 39.2268 |
| RV-J-05 | 27.6013 | 18.6226 | 15.8431 | 50.2427 | 45.8356 | 8.5301 | 0.1840 | 46.6226 |
| RV-J-06 | 28.0109 | 19.9479 | 19.5046 | 51.7783 | 40.8476 | 3.5939 | 0.0878 | 41.0054 |
| RV-J-07 | 34.2391 | 25.1386 | 22.1315 | 57.2102 | 40.4823 | 8.0869 | 0.1972 | 41.2822 |
| RV-J-08 | 28.0293 | 18.7573 | 16.3240 | 50.4021 | 46.8524 | 7.7465 | 0.1639 | 47.4885 |
| RV-C-09 | 43.5444 | 35.6268 | 27.3958 | 66.2338 | 31.2945 | 14.9356 | 0.4453 | 34.6759 |
| RV-C-10 | 36.7432 | 27.6362 | 21.5584 | 59.5586 | 38.8364 | 13.1645 | 0.3268 | 41.0069 |
| RV-C-11 | 34.1237 | 25.4165 | 20.4950 | 57.4790 | 38.9234 | 11.5369 | 0.2882 | 40.5972 |
| RV-C-12 | 30.9464 | 22.1257 | 19.4149 | 54.1601 | 41.8292 | 7.8743 | 0.1861 | 42.5639 |
| RV-C-13 | 49.1775 | 40.5661 | 33.9065 | 69.8709 | 31.5818 | 11.8631 | 0.3593 | 33.7363 |
| RV-C-14 | 37.3592 | 28.8746 | 22.3000 | 60.6708 | 36.0679 | 13.7544 | 0.3643 | 38.6015 |
| RV-C-15 | 33.1300 | 23.5598 | 18.8239 | 55.6443 | 43.3452 | 11.5925 | 0.2613 | 44.8686 |
| RV-C-16 | 37.6494 | 29.4489 | 23.3509 | 61.1757 | 34.8385 | 12.7929 | 0.3519 | 37.1131 |
| RV-R-17 | 30.2900 | 21.5574 | 17.1813 | 53.5542 | 41.9898 | 11.3443 | 0.2639 | 43.4953 |
| RV-R-18 | 33.3037 | 23.9562 | 17.9439 | 56.0439 | 42.2370 | 14.0538 | 0.3212 | 44.5138 |
| RV-R-19 | 27.9543 | 19.4992 | 14.0970 | 51.2662 | 42.8304 | 14.3294 | 0.3229 | 45.1639 |
| RV-R-20 | 29.9905 | 22.0625 | 16.7940 | 54.0933 | 38.5355 | 13.0958 | 0.3276 | 40.7000 |
| RV-R-21 | 32.9908 | 24.7987 | 16.3253 | 56.8787 | 37.5307 | 18.9103 | 0.4667 | 42.0256 |
| RV-R-22 | 38.3076 | 30.0413 | 20.3806 | 61.6898 | 34.7516 | 19.0118 | 0.5006 | 39.6121 |
| RV-R-23 | 25.9524 | 17.1040 | 13.7009 | 48.3908 | 47.0831 | 10.3331 | 0.2160 | 48.2036 |
| RV-R-24 | 26.7662 | 18.4343 | 14.5718 | 50.0187 | 43.4246 | 11.0500 | 0.2492 | 44.8085 |
| RV-GR-25 | 25.6289 | 17.1702 | 11.0045 | 48.4737 | 45.3711 | 17.5691 | 0.3694 | 48.6540 |
| RV-GR-26 | 28.2185 | 19.7441 | 11.2947 | 51.5467 | 42.6662 | 22.0517 | 0.4770 | 48.0279 |
| RV-GR-27 | 32.7192 | 23.7372 | 12.3222 | 55.8237 | 41.1104 | 26.6455 | 0.5751 | 48.9903 |
| RV-GR-28 | 32.7496 | 24.6199 | 13.2025 | 56.7032 | 37.4280 | 25.9005 | 0.6053 | 45.5158 |
| RV-GR-29 | 32.1714 | 23.8021 | 14.0968 | 55.8892 | 38.8602 | 22.3005 | 0.5210 | 44.8043 |
| RV-GR-30 | 36.0475 | 25.6187 | 19.6082 | 57.6734 | 44.6472 | 13.5576 | 0.2948 | 46.6603 |
| RV-GR-31 | 28.2590 | 19.6117 | 11.6942 | 51.3953 | 43.4786 | 20.6903 | 0.4442 | 48.1505 |
| RV-GR-32 | 30.5590 | 22.1853 | 13.6015 | 54.2230 | 40.1156 | 20.6326 | 0.4750 | 45.1106 |

Table Annex II.34 Parameters of CIELAB space of the samples from Rioja Alta.

| Sample | X | Y | Z | L* | a* | b* | H* | C* |
|---|---|---|---|---|---|---|---|---|
| RA-J-33 | 39.3552 | 28.6570 | 28.5410 | 60.4777 | 43.3157 | 3.2678 | 0.0753 | 43.4387 |
| RA-J-34 | 38.3433 | 29.0691 | 24.1216 | 60.8426 | 38.5183 | 10.9096 | 0.2760 | 40.0335 |
| RA-J-35 | 28.1691 | 19.5259 | 15.0390 | 51.2968 | 43.5483 | 12.1668 | 0.2724 | 45.2160 |
| RA-J-36 | 11.1978 | 6.3335 | 3.1558 | 30.2387 | 46.0006 | 18.0024 | 0.3730 | 49.3978 |
| RA-J-37 | 34.3821 | 25.9018 | 22.5618 | 57.9438 | 37.8155 | 8.5909 | 0.2234 | 38.7791 |
| RA-J-38 | 20.2074 | 12.3669 | 6.7899 | 41.7933 | 49.5428 | 19.9654 | 0.3831 | 53.4145 |
| RA-J-39 | 42.3255 | 34.1051 | 29.2302 | 65.0459 | 32.7813 | 10.1173 | 0.2994 | 34.3070 |
| RA-J-40 | 35.8896 | 28.2995 | 18.4490 | 60.1584 | 33.4060 | 20.1236 | 0.5422 | 38.9990 |
| RA-C-41 | 43.6884 | 35.5830 | 28.9207 | 66.2001 | 31.8647 | 12.5663 | 0.3756 | 34.2530 |
| RA-C-42 | 39.2034 | 29.0377 | 24.7925 | 60.8149 | 41.3819 | 9.7449 | 0.2313 | 42.5138 |
| RA-C-43 | 36.1068 | 28.1404 | 24.3430 | 60.0154 | 34.7506 | 9.1126 | 0.2565 | 35.9255 |
| RA-C-44 | 43.2073 | 35.0214 | 29.5163 | 65.7654 | 32.3156 | 10.9362 | 0.3263 | 34.1160 |
| RA-C-45 | 44.2524 | 36.3970 | 27.2006 | 66.8222 | 30.8376 | 16.2521 | 0.4850 | 34.8581 |
| RA-C-46 | 41.1216 | 33.4729 | 26.4064 | 64.5420 | 31.2955 | 13.5645 | 0.4090 | 34.1087 |
| RA-C-47 | 43.7490 | 36.4172 | 28.3375 | 66.8374 | 29.2958 | 14.5393 | 0.4607 | 32.7053 |
| RA-C-48 | 39.0518 | 31.7852 | 24.5025 | 63.1650 | 30.7716 | 14.2771 | 0.4344 | 33.9224 |
| RA-R-49 | 43.2443 | 35.5280 | 22.0392 | 66.1577 | 30.7344 | 23.6780 | 0.6564 | 38.7976 |
| RA-R-50 | 43.1703 | 35.7621 | 21.4031 | 66.3377 | 29.7385 | 25.1344 | 0.7017 | 38.9374 |
| RA-R-51 | 28.3987 | 20.5432 | 13.8642 | 52.4459 | 39.4994 | 16.9260 | 0.4048 | 42.9732 |
| RA-R-52 | 28.3453 | 20.3525 | 13.2216 | 52.2334 | 40.2053 | 18.1463 | 0.4240 | 44.1107 |
| RA-R-53 | 29.0616 | 20.8017 | 14.0681 | 52.7318 | 40.8502 | 16.9259 | 0.3928 | 44.2179 |
| RA-R-54 | 27.8087 | 20.5625 | 14.4915 | 52.4673 | 37.0745 | 15.4607 | 0.3951 | 40.1691 |
| RA-R-55 | 33.3152 | 27.3163 | 19.7075 | 59.2660 | 28.3894 | 16.1122 | 0.5162 | 32.6429 |
| RA-R-56 | 35.6604 | 27.7678 | 21.4145 | 59.6783 | 34.7036 | 13.6321 | 0.3743 | 37.2851 |
| RA-GR-57 | 29.3974 | 21.0520 | 14.8931 | 53.0063 | 40.9602 | 15.4372 | 0.3604 | 43.7726 |
| RA-GR-58 | 51.0231 | 44.8387 | 28.2458 | 72.7856 | 23.9823 | 24.9332 | 0.8048 | 34.5950 |
| RA-GR-59 | 44.4778 | 37.6700 | 23.4104 | 67.7767 | 27.3808 | 24.0717 | 0.7212 | 36.4576 |
| RA-GR-60 | 26.4871 | 18.9589 | 9.3214 | 50.6390 | 39.6069 | 26.3400 | 0.5869 | 47.5657 |
| RA-GR-61 | 19.8224 | 12.8771 | 6.8617 | 42.5775 | 44.2543 | 21.0378 | 0.4438 | 49.0003 |
| RA-GR-62 | 31.4579 | 23.4540 | 16.5170 | 55.5369 | 37.7809 | 16.1805 | 0.4046 | 41.0999 |
| RA-GR-63 | 42.5169 | 34.4071 | 25.2258 | 65.2845 | 32.3281 | 16.7405 | 0.4778 | 36.4053 |
| RA-GR-64 | 33.5001 | 25.9458 | 19.3841 | 57.9855 | 34.5603 | 14.5296 | 0.3980 | 37.4903 |

Table Annex II.35 Parameters of CIELAB space of the samples from Rioja Baja.

| Sample | X | Y | Z | L* | a* | b* | H* | C* |
|---|---|---|---|---|---|---|---|---|
| RB-J-65 | 38.0277 | 28.8061 | 26.0723 | 60.6102 | 38.5029 | 7.3161 | 0.1878 | 39.1919 |
| RB-J-66 | 37.2221 | 26.1325 | 25.9238 | 58.1626 | 46.4306 | 3.3335 | 0.0717 | 46.5501 |
| RB-J-67 | 41.5043 | 31.5376 | 29.0159 | 62.9588 | 39.2897 | 6.8365 | 0.1723 | 39.8800 |
| RB-J-68 | 46.6095 | 37.7302 | 32.0106 | 67.8213 | 33.2983 | 10.9165 | 0.3168 | 35.0421 |
| RB-J-69 | 36.7799 | 28.5722 | 28.6776 | 60.4022 | 35.3215 | 2.9330 | 0.0828 | 35.4431 |
| RB-J-70 | 44.5995 | 34.3241 | 34.8780 | 65.2190 | 38.7595 | 2.5542 | 0.0658 | 38.8436 |
| RB-J-71 | 43.6430 | 34.8070 | 32.8046 | 65.5981 | 34.3255 | 5.9877 | 0.1727 | 34.8438 |
| RB-J-72 | 25.1792 | 18.6579 | 12.4767 | 50.2846 | 35.6641 | 16.6909 | 0.4377 | 39.3766 |
| RB-C-73 | 25.6115 | 17.1099 | 12.1824 | 48.3982 | 45.6237 | 14.2119 | 0.3020 | 47.7859 |
| RB-C-74 | 30.1595 | 21.8461 | 14.7182 | 53.8634 | 40.1654 | 17.3357 | 0.4075 | 43.7468 |
| RB-C-75 | 38.9852 | 29.4725 | 23.8489 | 61.1964 | 39.0450 | 11.9796 | 0.2977 | 40.8414 |
| RB-C-76 | 38.6243 | 29.0776 | 20.6457 | 60.8500 | 39.3873 | 17.0675 | 0.4089 | 42.9262 |
| RB-C-77 | 28.7904 | 21.8755 | 16.4114 | 53.8946 | 34.7858 | 13.5779 | 0.3721 | 37.3418 |
| RB-C-78 | 35.9773 | 27.1565 | 21.9700 | 59.1189 | 38.1810 | 11.6657 | 0.2965 | 39.9234 |
| RB-C-79 | 36.4464 | 27.7064 | 21.7345 | 59.6226 | 37.5765 | 12.9568 | 0.3320 | 39.7476 |
| RB-C-80 | 40.0598 | 31.9451 | 22.7945 | 63.2975 | 33.3740 | 17.4140 | 0.4809 | 37.6440 |
| RB-R-81 | 22.5666 | 15.7219 | 9.8970 | 46.6075 | 39.9900 | 17.6027 | 0.4147 | 43.6927 |
| RB-R-82 | 26.9732 | 18.4437 | 10.1897 | 50.0299 | 44.2196 | 22.6214 | 0.4729 | 49.6699 |
| RB-R-83 | 34.7793 | 25.2643 | 20.0274 | 57.3320 | 41.8199 | 12.1662 | 0.2831 | 43.5537 |
| RB-R-84 | 32.7007 | 23.9186 | 16.7587 | 56.0062 | 40.2577 | 16.4694 | 0.3883 | 43.4962 |
| RB-R-85 | 31.8737 | 22.7988 | 13.5572 | 54.8645 | 42.1979 | 21.8476 | 0.4777 | 47.5182 |
| RB-R-86 | 28.4488 | 20.6432 | 15.6396 | 52.5568 | 39.2183 | 12.9745 | 0.3195 | 41.3087 |
| RB-R-87 | 36.0968 | 27.3482 | 18.8176 | 59.2953 | 37.8212 | 17.8998 | 0.4420 | 41.8432 |
| RB-R-88 | 39.9349 | 32.3624 | 19.7822 | 63.6413 | 31.5015 | 23.5124 | 0.6412 | 39.3088 |
| RB-GR-89 | 34.2446 | 24.5511 | 16.7576 | 56.6354 | 42.9789 | 17.5568 | 0.3878 | 46.4265 |
| RB-GR-90 | 37.8143 | 28.4187 | 19.2034 | 60.2652 | 39.2990 | 18.8124 | 0.4465 | 43.5697 |
| RB-GR-91 | 39.2366 | 31.8053 | 16.6625 | 63.1816 | 31.2856 | 29.0473 | 0.7483 | 42.6911 |
| RB-GR-92 | 27.0728 | 20.0728 | 10.8455 | 51.9194 | 36.4793 | 23.9629 | 0.5812 | 43.6459 |
| RB-GR-93 | 31.0442 | 21.9263 | 12.2025 | 53.9487 | 43.1030 | 23.7285 | 0.5032 | 49.2027 |
| RB-GR-94 | 49.8488 | 43.0787 | 27.9147 | 71.6083 | 25.9124 | 23.4062 | 0.7346 | 34.9185 |
| RB-GR-95 | 34.6717 | 27.1183 | 20.3590 | 59.0836 | 33.9004 | 14.5588 | 0.4056 | 36.8945 |
| RB-GR-96 | 31.9938 | 24.4276 | 14.7935 | 56.5134 | 35.5262 | 21.7291 | 0.5489 | 41.6445 |

Table Annex II.36 Parameters of CIELAB space of the samples from Medoc.

| Sample | X | Y | Z | L* | a* | b* | H* | C* |
|---|---|---|---|---|---|---|---|---|
| BO-M-01 | 29.6651 | 20.8777 | 15.0463 | 52.8154 | 42.8072 | 14.7682 | 0.3322 | 45.2831 |
| BO-M-02 | 23.3081 | 14.5644 | 8.5499 | 45.0317 | 50.1393 | 19.1862 | 0.3655 | 53.6849 |
| BO-M-03 | 35.4298 | 25.9403 | 16.5011 | 57.9803 | 41.2433 | 20.4276 | 0.4599 | 46.0250 |
| BO-M-04 | 29.4514 | 21.5607 | 12.6776 | 53.5578 | 38.7903 | 21.8133 | 0.5123 | 44.5029 |
| BO-M-05 | 41.1922 | 33.2189 | 22.4215 | 64.3378 | 32.3921 | 19.8620 | 0.5501 | 37.9966 |
| BO-M-06 | 28.0193 | 19.4982 | 13.5776 | 51.2650 | 43.0929 | 15.5914 | 0.3472 | 45.8267 |
| BO-M-07 | 34.5100 | 26.1712 | 17.4002 | 58.1992 | 37.1563 | 18.8937 | 0.4704 | 41.6840 |
| BO-M-08 | 23.7395 | 17.4423 | 9.7932 | 48.8125 | 35.7631 | 21.7214 | 0.5458 | 41.8428 |
| BO-M-09 | 30.1467 | 23.1439 | 17.2008 | 55.2203 | 34.2684 | 14.1758 | 0.3922 | 37.0847 |
| BO-M-10 | 36.4763 | 27.4896 | 19.2160 | 59.4248 | 38.5282 | 17.3388 | 0.4229 | 42.2500 |
| BO-M-11 | 42.8072 | 34.5128 | 25.0609 | 65.3676 | 32.8388 | 17.1532 | 0.4814 | 37.0489 |
| BO-M-12 | 44.0909 | 36.2515 | 27.4187 | 66.7117 | 30.8417 | 15.7242 | 0.4715 | 34.6188 |
| BO-M-13A | 33.3876 | 24.3043 | 15.5936 | 56.3912 | 41.0364 | 19.6888 | 0.4473 | 45.5152 |
| BO-M-14A | 30.8755 | 22.2182 | 12.6192 | 54.2577 | 41.1454 | 23.1709 | 0.5129 | 47.2212 |
| BO-M-15A | 25.1039 | 17.2315 | 9.3007 | 48.5504 | 42.8180 | 22.8045 | 0.4894 | 48.5122 |
| BO-M-16A | 23.0710 | 15.0691 | 8.3344 | 45.7286 | 46.0694 | 21.1169 | 0.4298 | 50.6785 |
| BO-M-17A | 38.8823 | 30.4438 | 19.6223 | 62.0353 | 35.1018 | 21.0509 | 0.5402 | 40.9301 |
| BO-M-18A | 26.6478 | 19.8024 | 14.9784 | 51.6131 | 36.0679 | 12.8517 | 0.3423 | 38.2892 |
| BO-M-19A | 33.5315 | 24.0361 | 18.9097 | 56.1239 | 42.6951 | 12.2497 | 0.2794 | 44.4176 |
| BO-M-20A | 27.8300 | 19.3109 | 10.3155 | 51.0490 | 43.2726 | 24.0046 | 0.5065 | 49.4848 |
| BO-M-21A | 23.0313 | 14.2094 | 6.3912 | 44.5317 | 51.0496 | 26.2780 | 0.4754 | 57.4159 |
| BO-M-22B | 39.0619 | 31.4241 | 19.6049 | 62.8640 | 32.1007 | 22.5132 | 0.6116 | 39.2084 |
| BO-M-23B | 33.7034 | 25.6607 | 17.1509 | 57.7136 | 36.4458 | 18.5797 | 0.4714 | 40.9084 |
| BO-M-24B | 33.4407 | 24.4390 | 16.7640 | 56.5247 | 40.6478 | 17.3522 | 0.4035 | 44.1967 |
| BO-M-25B | 40.7086 | 33.2310 | 23.4855 | 64.3475 | 30.8623 | 18.0308 | 0.5287 | 35.7434 |
| BO-M-26B | 34.5087 | 25.9862 | 13.6327 | 58.0239 | 37.9070 | 27.1090 | 0.6208 | 46.6030 |
| BO-M-27B | 45.5215 | 38.4155 | 20.6021 | 68.3258 | 28.0293 | 30.0381 | 0.8200 | 41.0844 |
| BO-M-28B | 36.4531 | 29.0952 | 16.4017 | 60.8656 | 32.2412 | 25.6177 | 0.6714 | 41.1796 |
| BO-M-29B | 36.8365 | 28.3760 | 17.3139 | 60.2269 | 36.2642 | 22.5704 | 0.5567 | 42.7143 |
| BO-M-30B | 27.5447 | 19.5905 | 11.9630 | 51.3710 | 40.7453 | 19.9223 | 0.4548 | 45.3551 |
| BO-M-31B | 47.6779 | 41.0468 | 30.8789 | 70.2087 | 26.0000 | 16.6261 | 0.5689 | 30.8614 |
| BO-M-32B | 26.6245 | 20.8865 | 17.0391 | 52.8251 | 30.7484 | 10.3876 | 0.3258 | 32.4556 |
| BO-M-33B | 36.3183 | 28.6692 | 18.7243 | 60.4886 | 33.4174 | 20.1426 | 0.5424 | 39.0186 |
| BO-M-34B | 38.5264 | 29.2220 | 22.7449 | 60.9771 | 38.5263 | 13.4999 | 0.3370 | 40.8230 |
| BO-M-35B | 32.8327 | 23.8531 | 16.7272 | 55.9405 | 41.0121 | 16.4236 | 0.3809 | 44.1784 |
| BO-M-36B | 31.8115 | 23.2334 | 17.5585 | 55.3119 | 40.0431 | 13.5860 | 0.3271 | 42.2851 |

Table Annex II.37 Parameters of CIELAB space of the samples from Graves.

| Sample | X | Y | Z | L* | a* | b* | H* | C* |
|---|---|---|---|---|---|---|---|---|
| BO-G-37 | 20.6152 | 13.3703 | 6.3998 | 43.3160 | 44.9755 | 24.1470 | 0.4927 | 51.0478 |
| BO-G-38 | 31.1208 | 21.7040 | 15.0190 | 53.7116 | 44.4081 | 16.3762 | 0.3533 | 47.3314 |
| BO-G-39 | 22.2439 | 14.8366 | 9.0453 | 45.4096 | 43.6692 | 18.2070 | 0.3950 | 47.3128 |
| BO-G-40 | 27.6303 | 20.0887 | 11.4543 | 51.9374 | 38.6466 | 22.2825 | 0.5230 | 44.6102 |
| BO-G-41 | 19.5446 | 11.9571 | 5.2264 | 41.1479 | 49.0236 | 25.5090 | 0.4798 | 55.2632 |
| BO-G-42 | 25.7969 | 17.8323 | 9.9666 | 49.2920 | 42.5488 | 22.0200 | 0.4776 | 47.9090 |
| BO-G-43 | 29.4810 | 22.1318 | 11.6004 | 54.1666 | 36.2799 | 25.7244 | 0.6168 | 44.4744 |
| BO-G-44B | 26.9078 | 18.3788 | 12.4471 | 49.9524 | 44.2879 | 16.1954 | 0.3506 | 47.1562 |
| BO-G-45B | 24.8227 | 17.9116 | 11.3632 | 49.3887 | 38.0017 | 18.1405 | 0.4454 | 42.1094 |
| BO-G-46B | 37.7987 | 29.0978 | 17.9872 | 60.8678 | 36.6508 | 22.2822 | 0.5463 | 42.8926 |
| BO-G-47B | 25.4504 | 18.1799 | 9.5722 | 49.7136 | 39.2747 | 23.9572 | 0.5477 | 46.0049 |
| BO-G-48B | 26.9986 | 17.9008 | 10.1361 | 49.3756 | 47.1433 | 21.6534 | 0.4306 | 51.8784 |
| BO-G-87 | 29.6735 | 20.5954 | 13.9647 | 52.5038 | 44.1824 | 16.7823 | 0.3630 | 47.2623 |

Table Annex II.38 Parameters of CIELAB space of the samples from Blayais & Bourgeais.

| Sample | X | Y | Z | L* | a* | b* | H* | C* |
|---|---|---|---|---|---|---|---|---|
| BO-BB-74 | 23.6260 | 15.6303 | 8.0972 | 46.4857 | 45.2892 | 23.2394 | 0.4741 | 50.9037 |
| BO-BB-82B | 29.3228 | 19.7188 | 11.0785 | 51.5178 | 47.0897 | 22.6080 | 0.4476 | 52.2356 |
| BO-BB-84 | 29.9912 | 21.6187 | 11.7875 | 53.6201 | 40.5779 | 24.2728 | 0.5391 | 47.2835 |
| BO-BB-85 | 40.8104 | 31.8368 | 21.7583 | 63.2078 | 36.0892 | 19.0953 | 0.4867 | 40.8296 |
| BO-BB-86 | 50.4010 | 42.5559 | 29.8939 | 71.2525 | 28.9306 | 19.8447 | 0.6012 | 35.0826 |

Table Annex II.39 Parameters of CIELAB space of the samples from Libournais.

| Sample | X | Y | Z | L* | a* | b* | H* | C* |
|---|---|---|---|---|---|---|---|---|
| BO-L-49 | 30.0702 | 21.3097 | 11.9101 | 53.2868 | 42.3134 | 23.3674 | 0.5046 | 48.3369 |
| BO-L-50 | 20.7161 | 12.2827 | 5.5826 | 41.6619 | 52.5950 | 24.7726 | 0.4402 | 58.1370 |
| BO-L-51 | 21.4497 | 14.0589 | 5.8045 | 44.3173 | 44.6628 | 28.3749 | 0.5660 | 52.9140 |
| BO-L-52 | 31.8919 | 23.5245 | 14.7112 | 55.6086 | 39.0566 | 20.3609 | 0.4806 | 44.0452 |
| BO-L-53 | 46.2417 | 38.2955 | 27.4932 | 68.2378 | 30.4624 | 18.2407 | 0.5395 | 35.5061 |
| BO-L-54 | 49.1219 | 43.7099 | 23.7761 | 72.0342 | 22.1057 | 30.7888 | 0.9481 | 37.9027 |
| BO-L-55 | 33.4242 | 24.5523 | 11.4094 | 56.6366 | 40.1077 | 30.5088 | 0.6503 | 50.3926 |
| BO-L-56 | 28.5166 | 20.0697 | 11.5944 | 51.9159 | 42.2464 | 21.8603 | 0.4775 | 47.5671 |
| BO-L-57B | 44.1034 | 35.8460 | 25.7577 | 66.4021 | 32.2127 | 17.8062 | 0.5050 | 36.8065 |
| BO-L-58B | 26.5938 | 19.5726 | 13.2833 | 51.3505 | 36.9784 | 16.4694 | 0.4190 | 40.4802 |
| BO-L-59 | 31.4712 | 22.2362 | 13.7586 | 54.2767 | 43.2617 | 20.3399 | 0.4395 | 47.8047 |
| BO-L-60 | 30.7002 | 22.2675 | 14.3341 | 54.3097 | 40.2694 | 19.0100 | 0.4411 | 44.5309 |
| BO-L-61 | 34.7645 | 24.5927 | 16.9101 | 56.6764 | 44.5950 | 17.3017 | 0.3701 | 47.8337 |
| BO-L-62 | 30.7861 | 22.0376 | 11.2970 | 54.0668 | 41.6358 | 26.3904 | 0.5649 | 49.2949 |
| BO-L-63A | 27.6723 | 19.0311 | 10.7207 | 50.7235 | 44.0464 | 22.2594 | 0.4679 | 49.3515 |
| BO-L-64A | 35.4657 | 26.7299 | 17.1803 | 58.7235 | 38.1615 | 20.2590 | 0.4880 | 43.2056 |
| BO-L-65A | 21.9626 | 13.7777 | 5.8227 | 43.9125 | 48.8167 | 27.5977 | 0.5145 | 56.0776 |
| BO-L-66A | 23.9525 | 15.3365 | 7.7673 | 46.0917 | 48.4305 | 23.7234 | 0.4555 | 53.9288 |
| BO-L-67B | 40.3312 | 32.8174 | 23.7239 | 64.0128 | 31.1360 | 17.0474 | 0.5009 | 35.4974 |
| BO-L-68B | 27.3832 | 21.4706 | 11.8555 | 53.4607 | 31.0897 | 23.8143 | 0.6537 | 39.1623 |
| BO-L-73 | 29.4320 | 20.8247 | 13.3610 | 52.7571 | 42.1670 | 18.7006 | 0.4174 | 46.1278 |
| BO-L-75 | 19.3763 | 12.0786 | 5.6182 | 41.3408 | 47.3415 | 24.0607 | 0.4702 | 53.1050 |
| BO-L-76 | 18.6377 | 8.7579 | -0.3367 | 35.5139 | 68.6670 | 118.0889 | 1.0441 | 136.6022 |
| BO-L-77 | 38.5908 | 30.7014 | 16.4465 | 62.2548 | 33.2251 | 27.9156 | 0.6988 | 43.3958 |
| BO-L-79 | 27.5950 | 18.1044 | 10.8671 | 49.6224 | 48.4836 | 19.9406 | 0.3902 | 52.4241 |
| BO-L-80 | 32.4673 | 23.8434 | 13.2191 | 55.9307 | 39.7472 | 24.5270 | 0.5529 | 46.7057 |
| BO-L-81 | 27.4188 | 19.6692 | 9.8658 | 51.4611 | 39.8516 | 26.0661 | 0.5792 | 47.6193 |

Table Annex II.40 Parameters of CIELAB space of the samples from Bordeaux & Bordeaux Superieur.

| Sample | X | Y | Z | L* | a* | b* | H* | C* |
|---|---|---|---|---|---|---|---|---|
| **BO-B-69** | 30.6429 | 21.8239 | 10.4823 | 53.8397 | 42.0814 | 28.3252 | 0.5924 | 50.7263 |
| **BO-B-70** | 38.1323 | 29.1711 | 22.3613 | 60.9324 | 37.4517 | 14.0969 | 0.3600 | 40.0169 |
| **BO-BS-71** | 39.8989 | 31.4501 | 15.2404 | 62.8858 | 34.6455 | 31.6862 | 0.7408 | 46.9502 |
| **BO-BS-72** | 20.9380 | 9.8742 | -0.4664 | 37.6157 | 71.1068 | 125.0714 | 1.0538 | 143.8716 |
| **BO-B-88** | 44.2243 | 35.9888 | 30.8129 | 66.5113 | 32.0955 | 10.3455 | 0.3118 | 33.7217 |
| **BO-B-89** | 36.6574 | 28.2715 | 22.6808 | 60.1333 | 36.0754 | 12.1572 | 0.3250 | 38.0688 |
| **BO-B-90** | 42.8530 | 33.2445 | 30.3501 | 64.3584 | 37.3258 | 7.2972 | 0.1931 | 38.0324 |
| **BO-B-91** | 31.1082 | 22.2118 | 17.6866 | 54.2510 | 42.0362 | 11.4916 | 0.2669 | 43.5787 |
| **BO-B-92** | 31.0036 | 22.1997 | 18.1637 | 54.2382 | 41.7047 | 10.4925 | 0.2465 | 43.0044 |
| **BO-B-93** | 35.0918 | 26.7652 | 22.1851 | 58.7563 | 36.7493 | 10.6573 | 0.2823 | 38.2635 |
| **BO-B-94** | 43.9082 | 35.6615 | 29.0791 | 66.2605 | 32.2509 | 12.4351 | 0.3680 | 34.5652 |
| **BO-B-95** | 40.0335 | 30.9467 | 25.5221 | 62.4626 | 36.8903 | 11.3940 | 0.2996 | 38.6098 |
| **BO-B-96** | 47.5750 | 39.8623 | 29.9034 | 69.3713 | 29.3233 | 16.5873 | 0.5148 | 33.6897 |

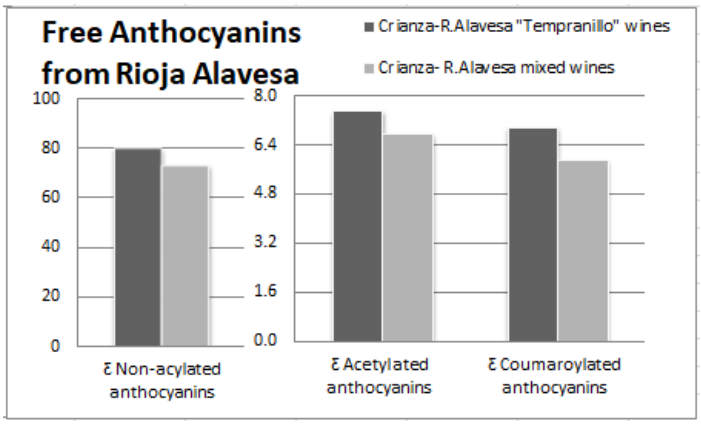



Figure Annex II.1 Mean concentrations of free anthocyanins for Tempranillo and mixed Crianza wines from Rioja Alavesa

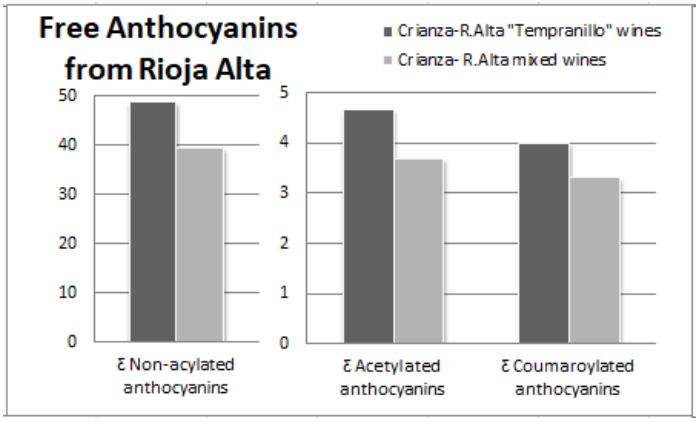



Figure Annex II.2 Mean concentrations of free anthocyanins for Tempranillo and mixed Crianza wines from Rioja Alta.

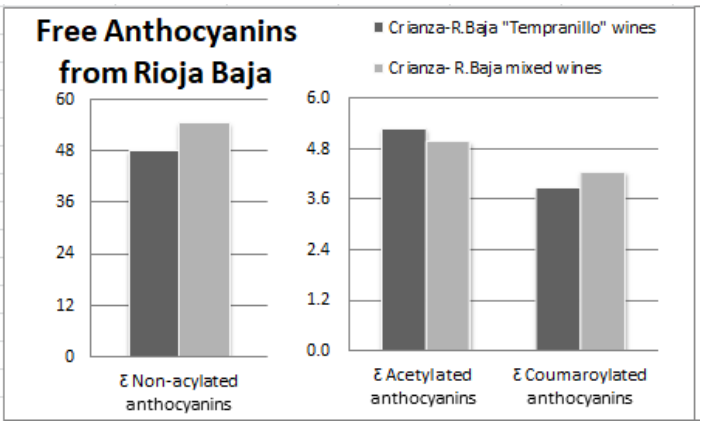



Figure Annex II.3 Mean concentrations of free anthocyanins for Tempranillo and mixed Crianza wines from Rioja Baja.

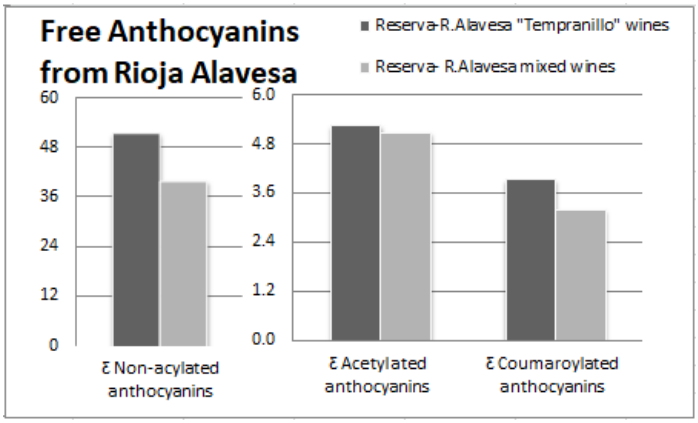



Figure Annex II.4 Mean concentrations of free anthocyanins for Tempranillo and mixed Reserva wines from Rioja Alavesa.

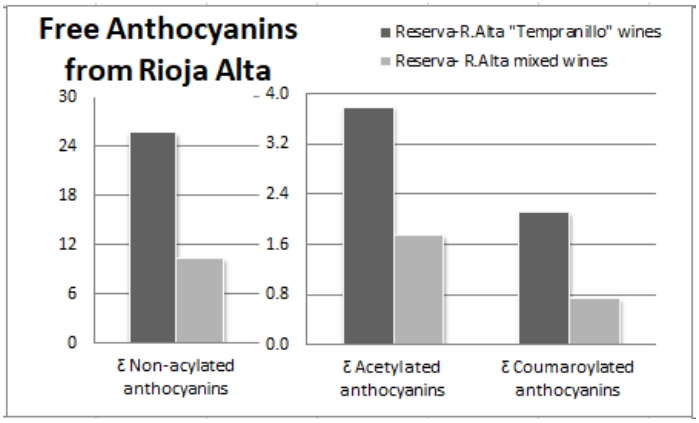



Figure Annex II.5 Mean concentrations of free anthocyanins for Tempranillo and mixed Reserva wines from Rioja Alta.

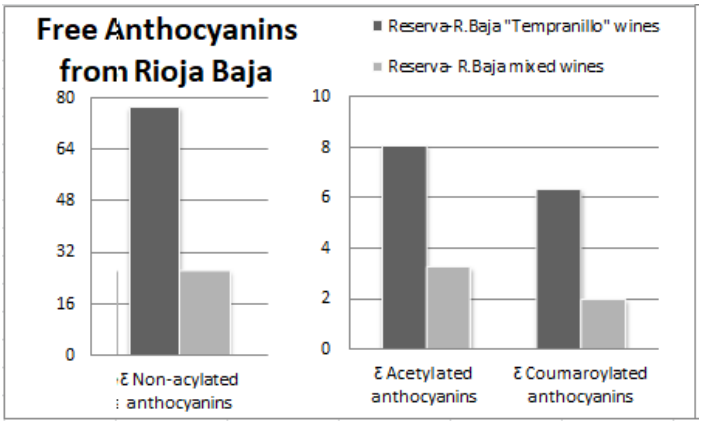



Figure Annex II.6 Mean concentrations of free anthocyanins for *Tempranillo* and mixed *Reserva* wines from Rioja Baja.

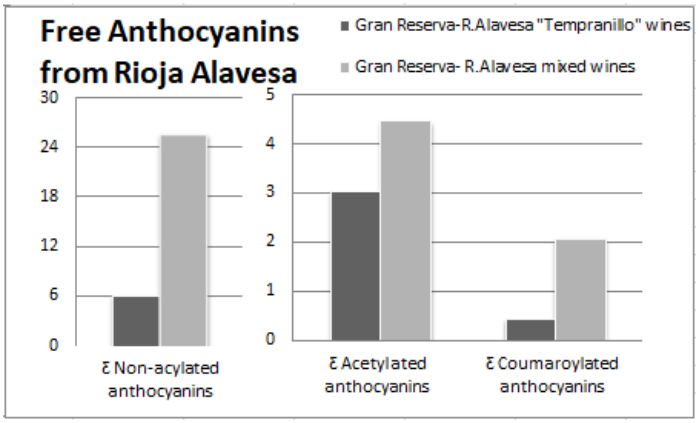



Figure Annex II.7 Mean concentrations of free anthocyanins for *Tempranillo* and mixed *Gran Reserva* wines from Rioja Alavesa.

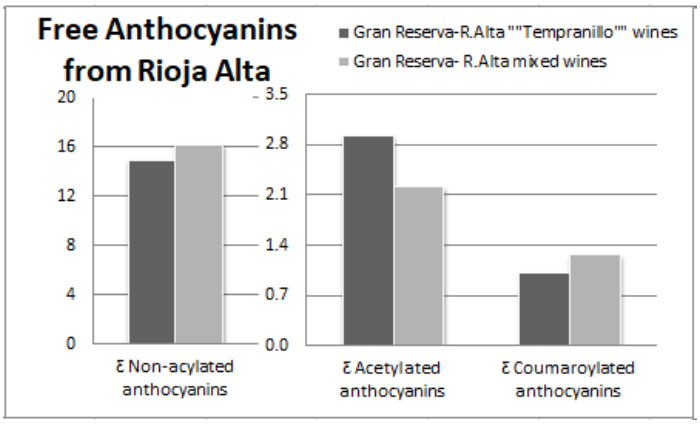



Figure Annex II.8 Mean concentrations of free anthocyanins for *Tempranillo* and mixed *Gran Reserva* wines from Rioja Alta.

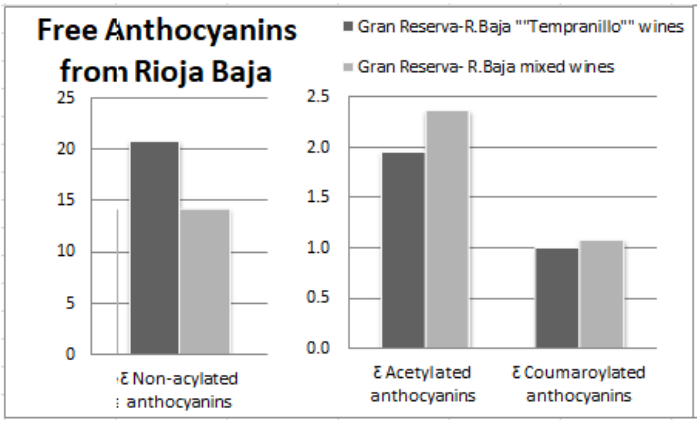



Figure Annex II.9 Mean concentrations of free anthocyanins for *Tempranillo* and mixed *Gran Reserva* wines from Rioja Baja.

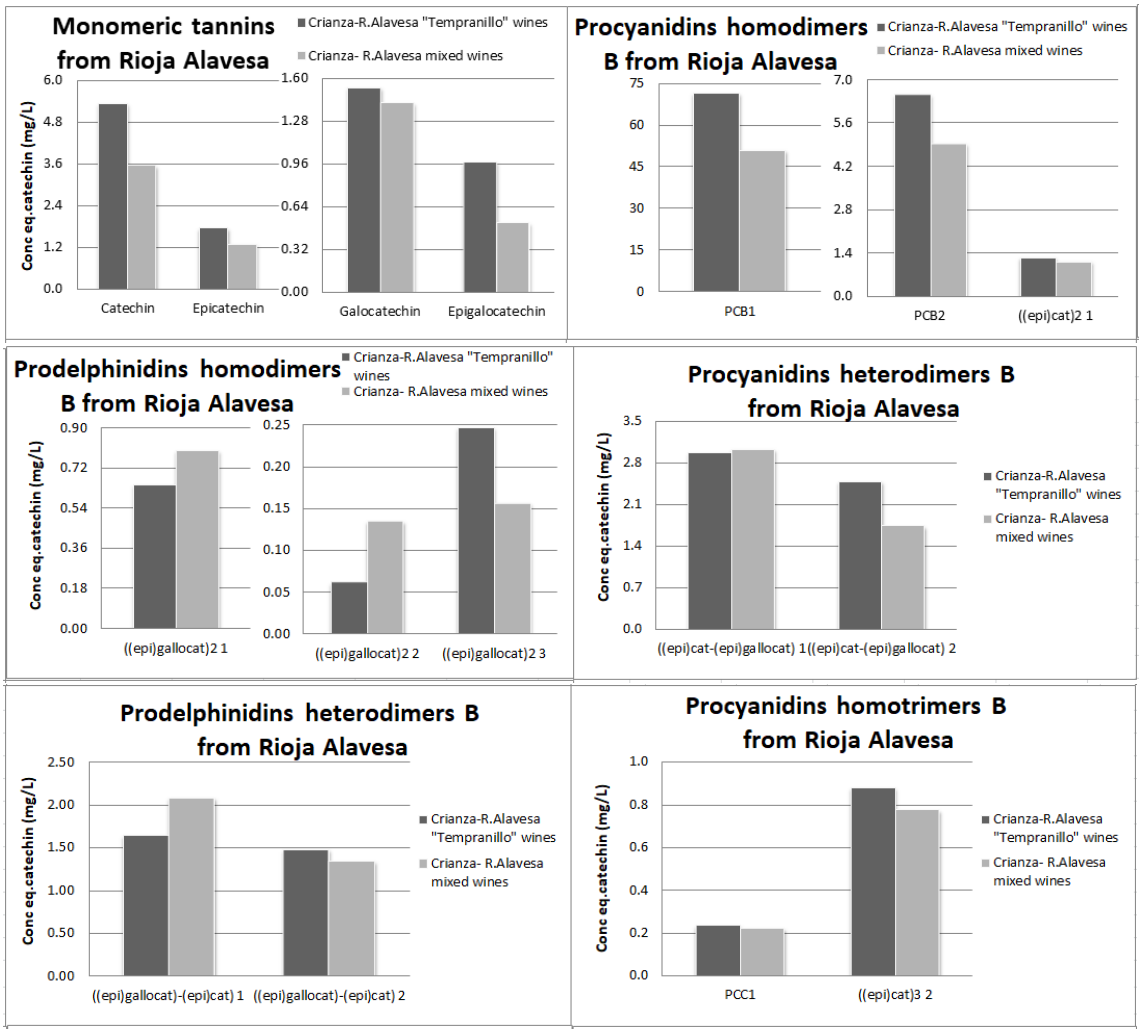



Figure Annex II.19 Mean concentrations of different tannins for *Tempranillo* and mixed *Crianza* wines from Rioja Alavesa.

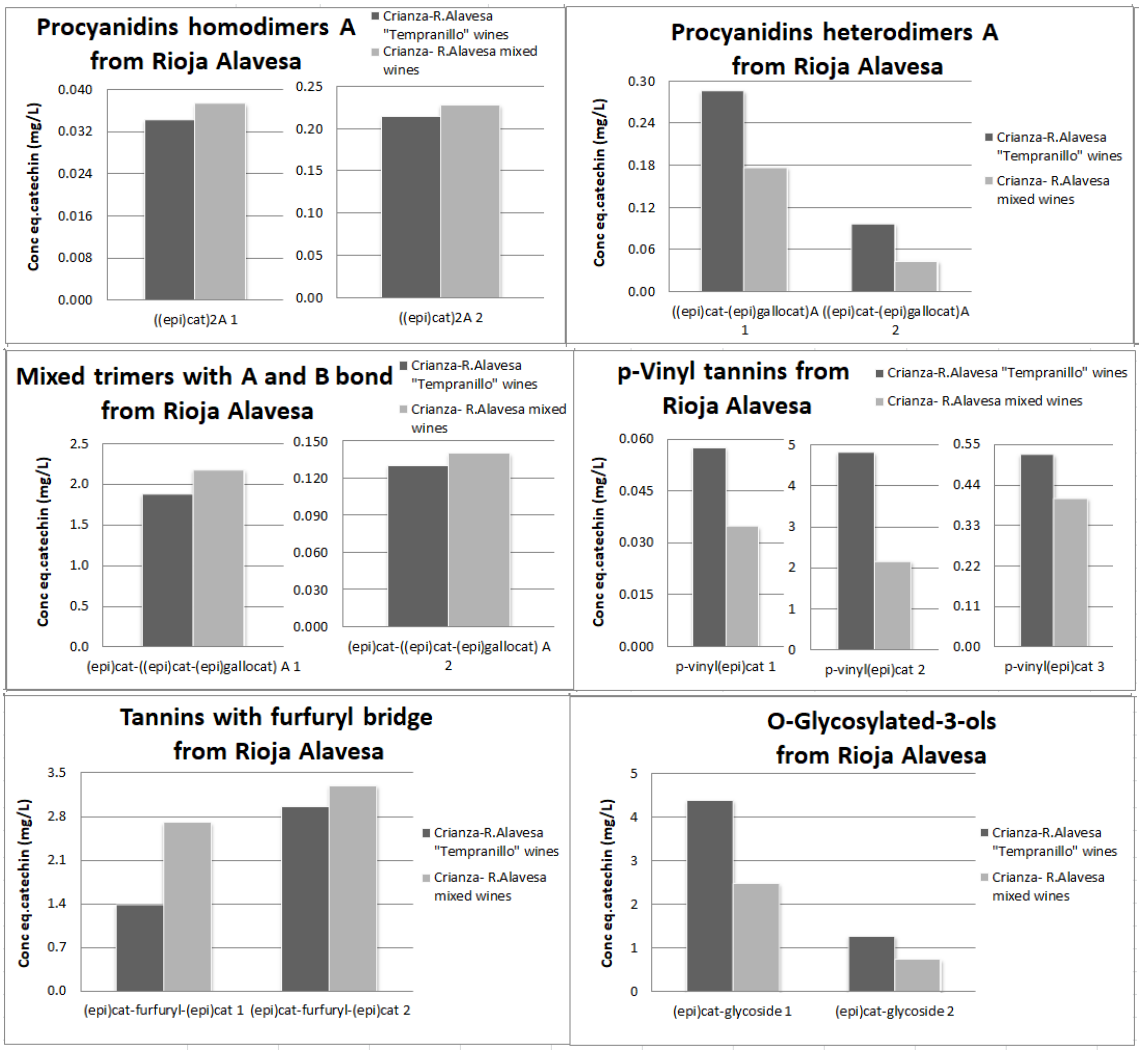



Figure Annex II.19 Cont.

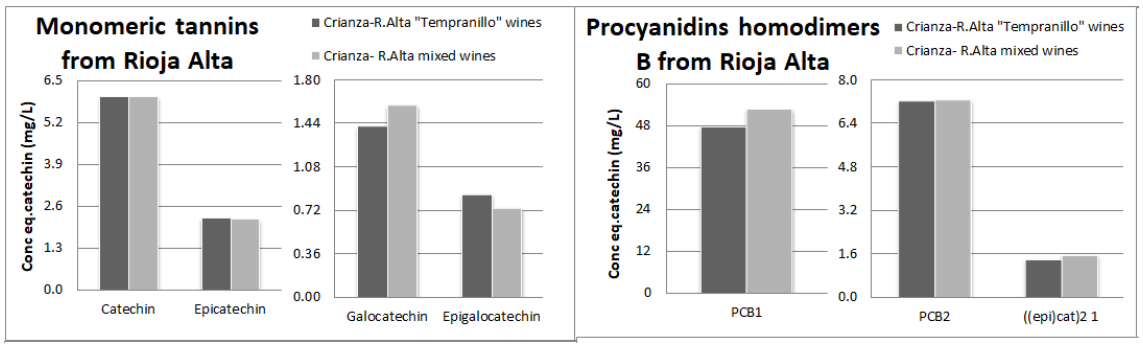



Figure Annex II.20 Mean concentrations of different tannins for *Tempranillo* and mixed *Crianza* wines from Rioja Alta.

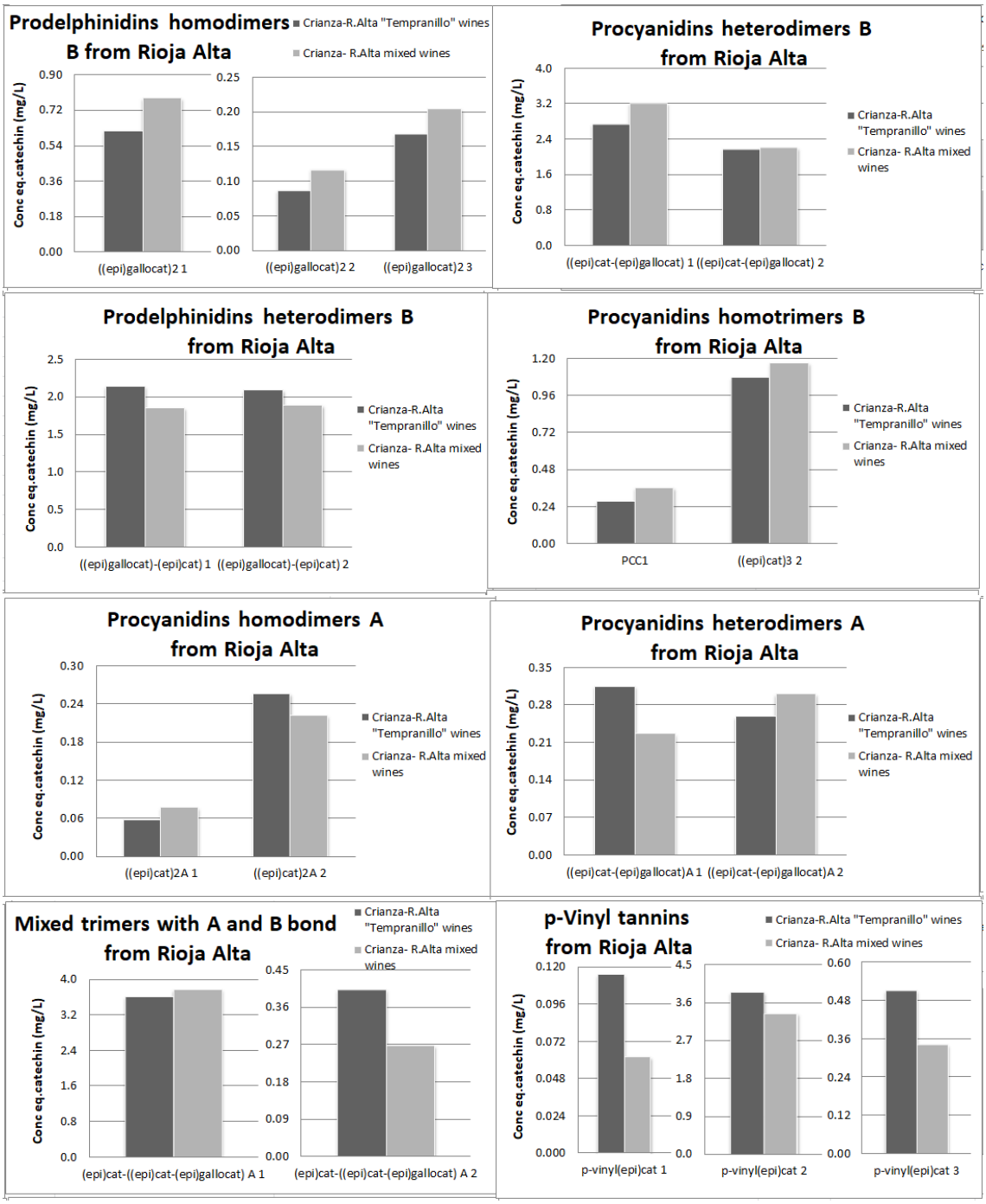



Figure Annex II.20 Cont.

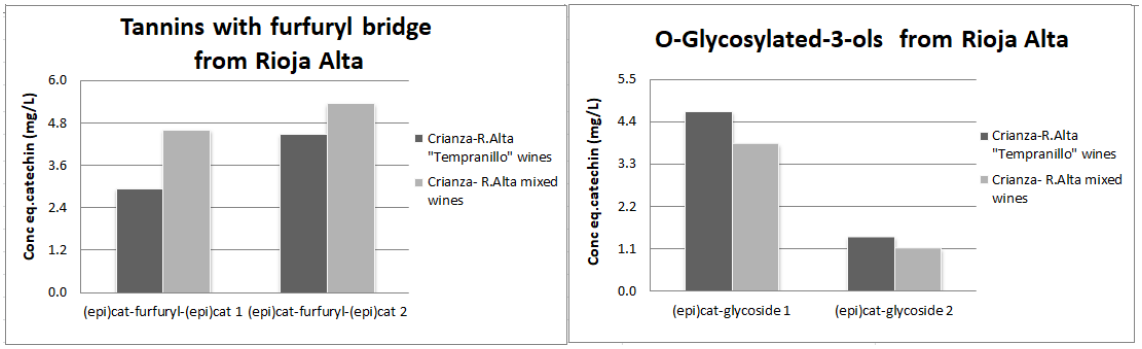



Figure Annex II.20 Cont.

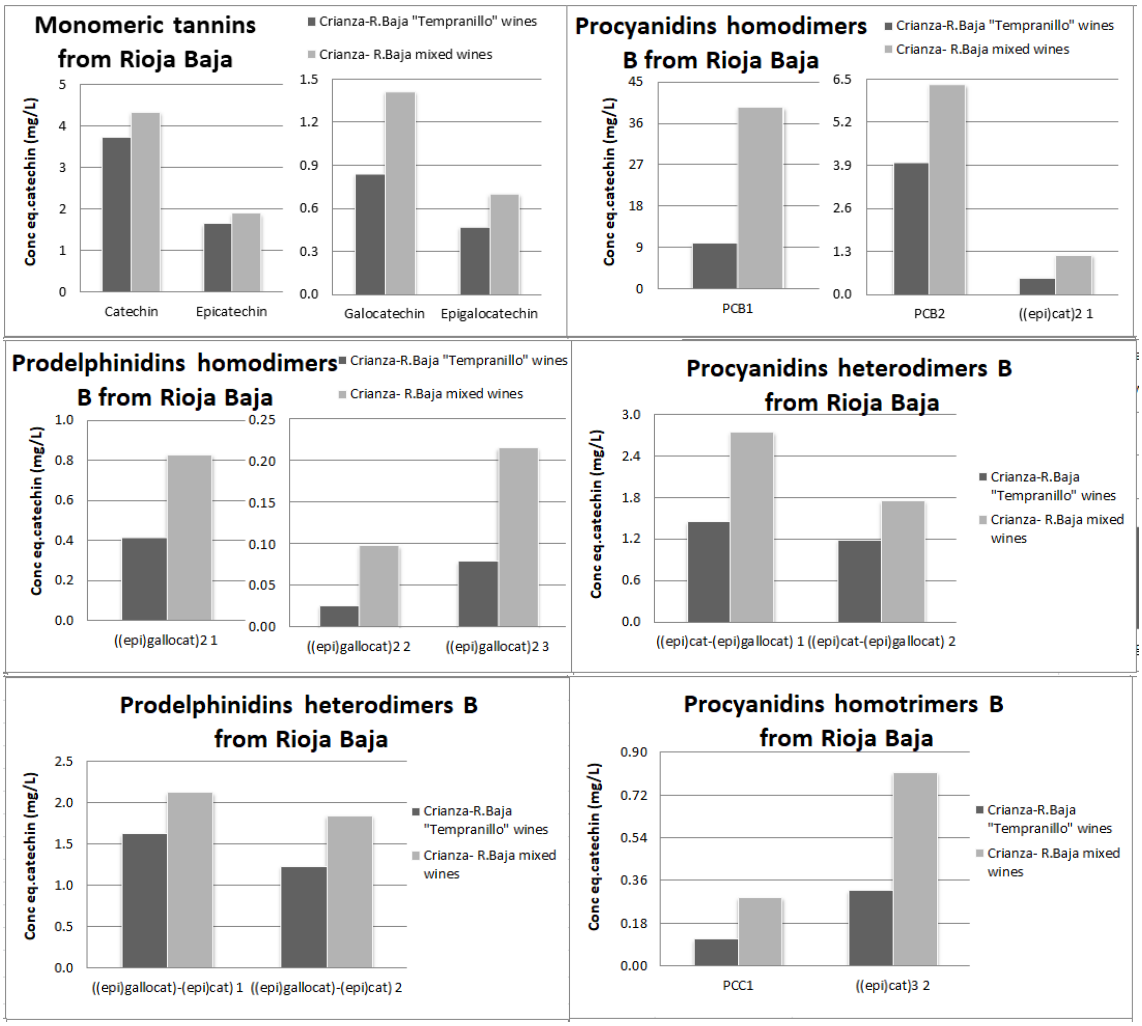



Figure Annex II.21 Mean concentrations of different tannins for *Tempranillo* and mixed *Crianza* wines from Rioja Baja.

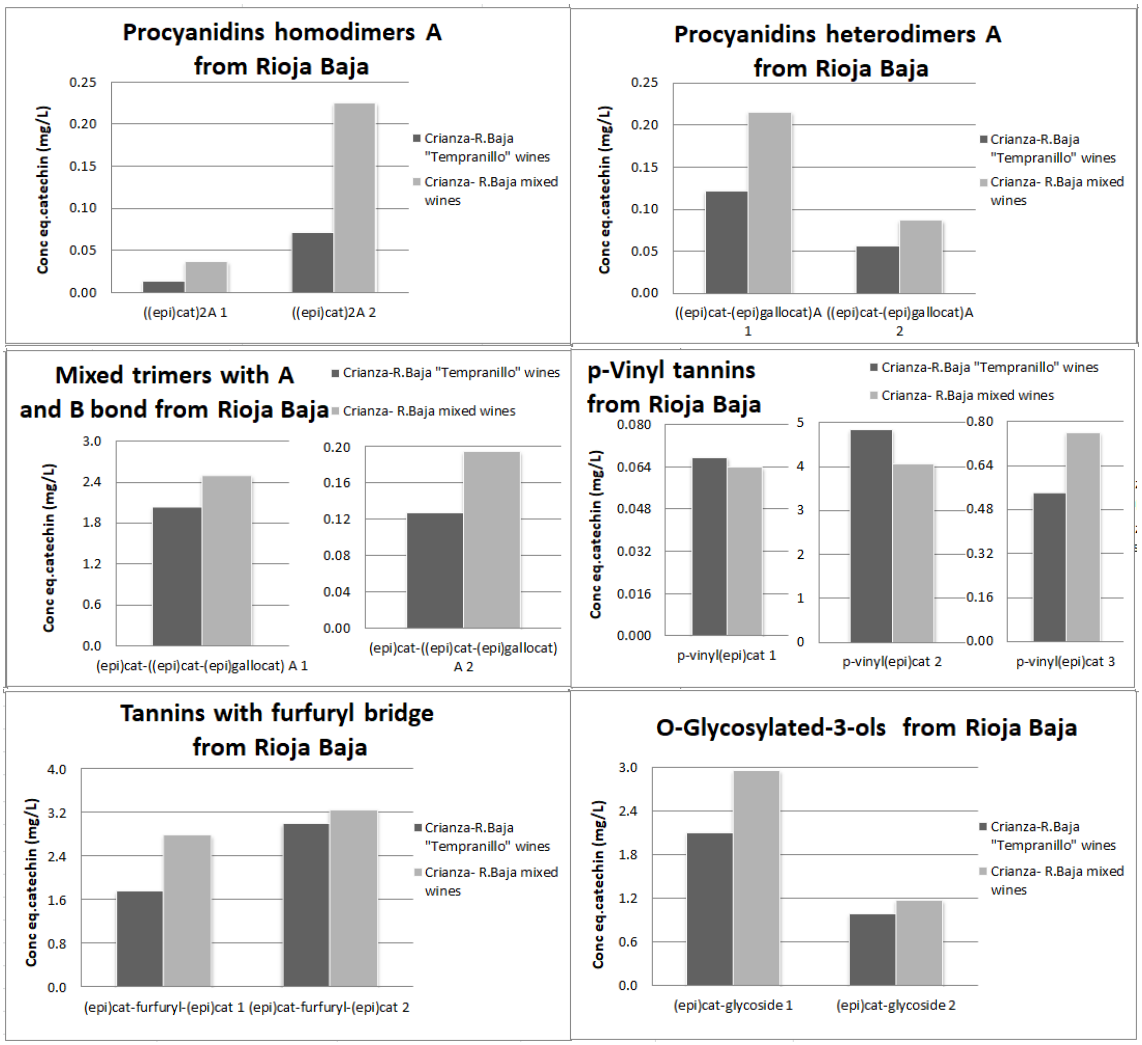



Figure Annex II.21 Cont.

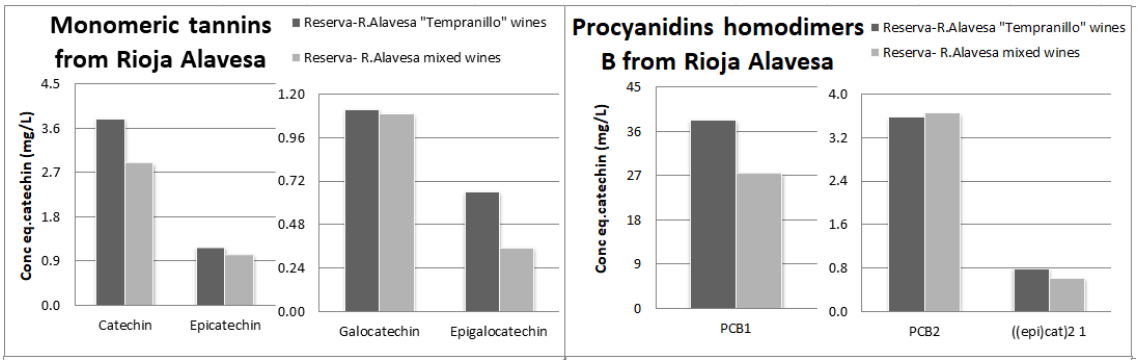



Figure Annex II.22 Mean concentrations of different tannins for *Tempranillo* and mixed *Reserva* wines from Rioja Alavesa.

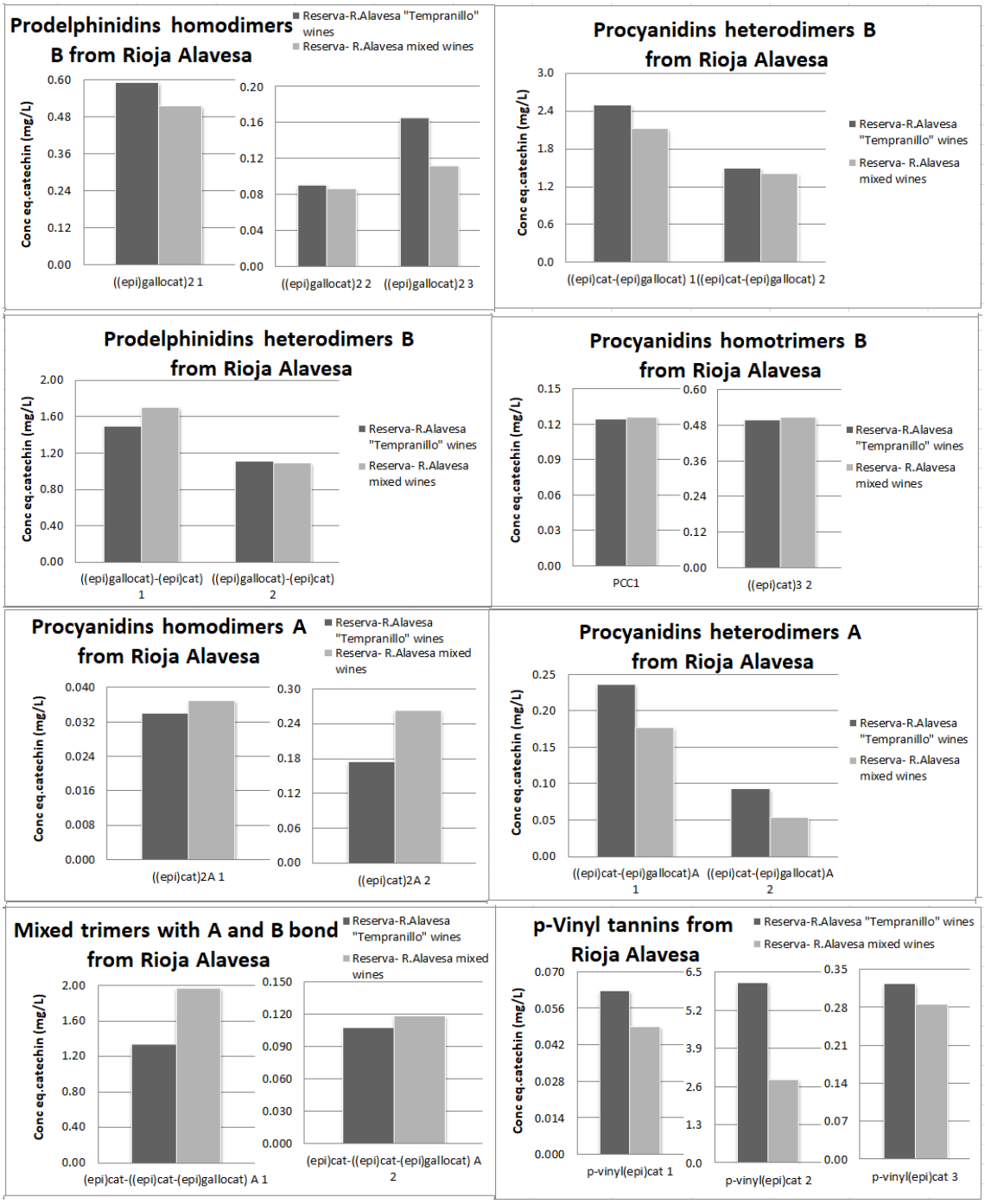



Figure Annex II.22 Cont.

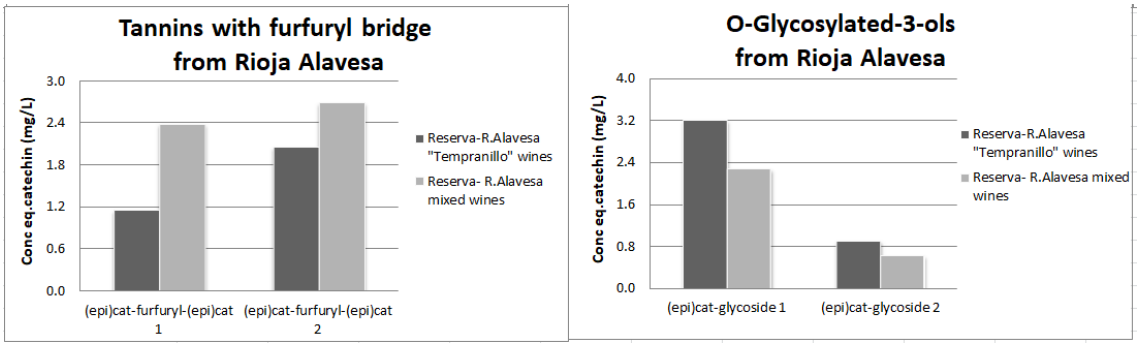



Figure Annex II.22 Cont.

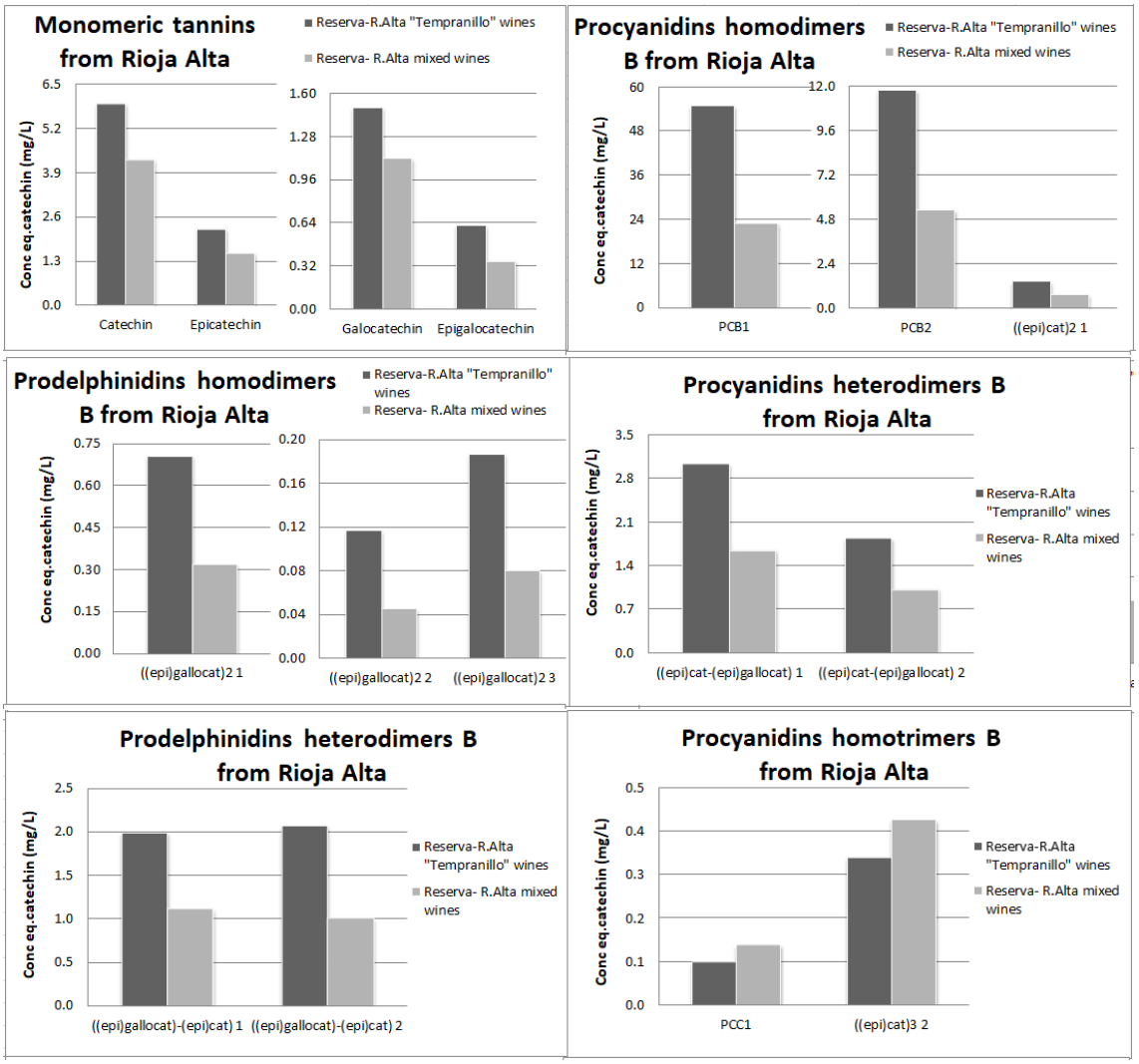



Figure Annex II.23 Mean concentrations of different tannins for *Tempranillo* and mixed *Reserva* wines from Rioja Alta.

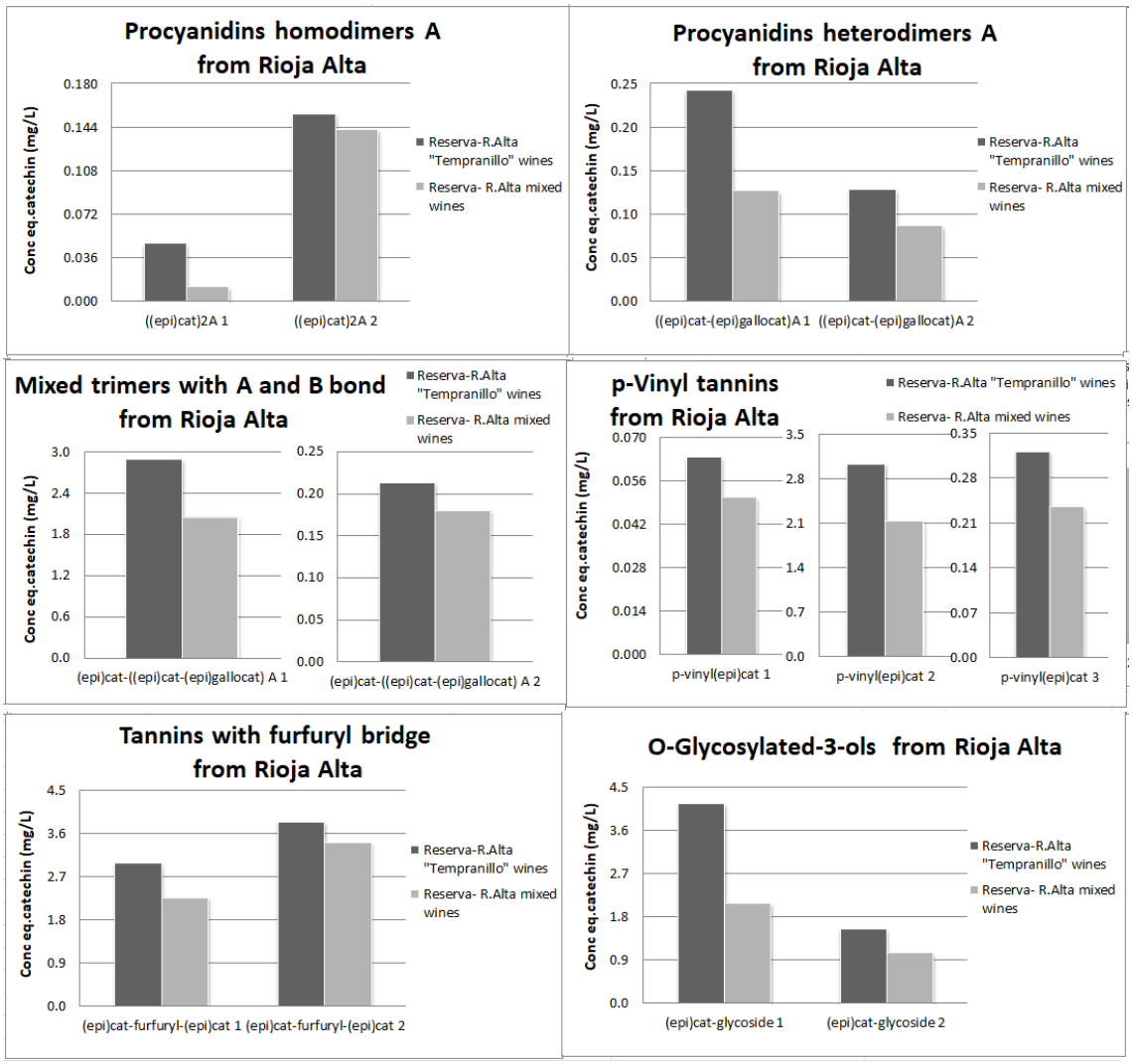



Figure Annex II.23 Cont.

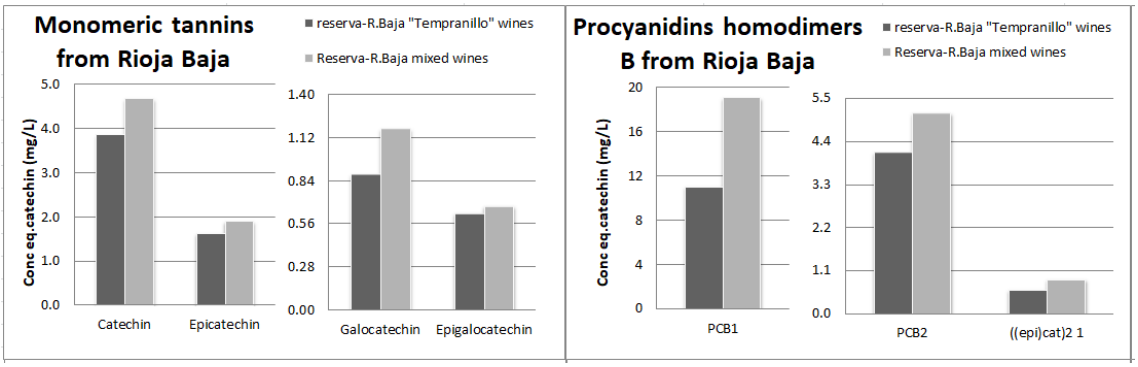



Figure Annex II.24 Mean concentrations of different tannins for *Tempranillo* and mixed *Reserva* wines from Rioja Baja.

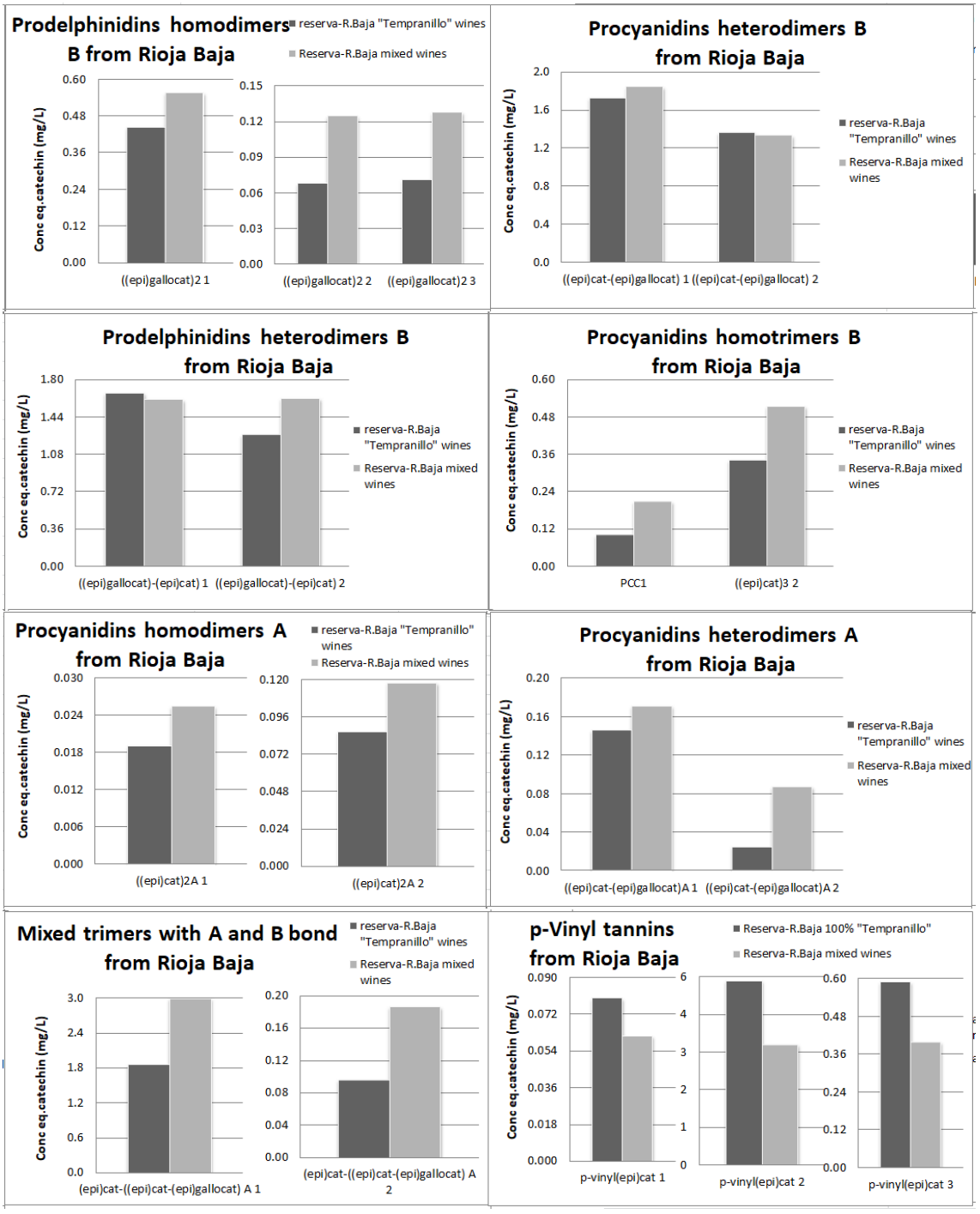



Figure Annex II.24 Cont.

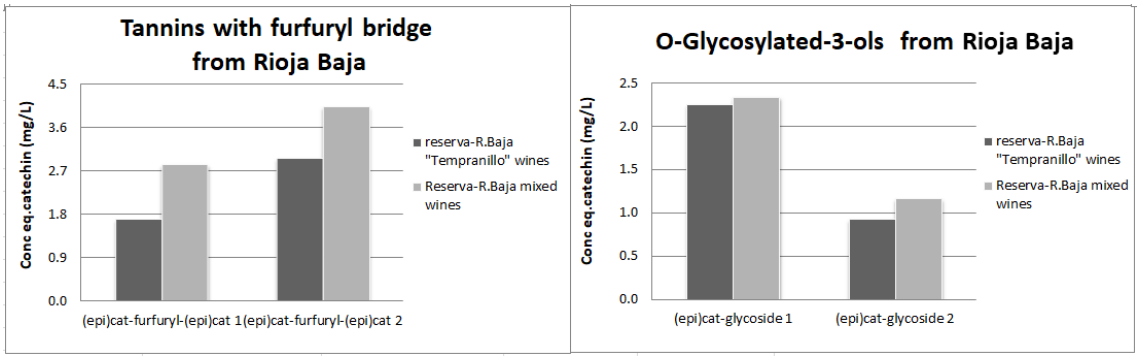



Figure Annex II.24 Cont.

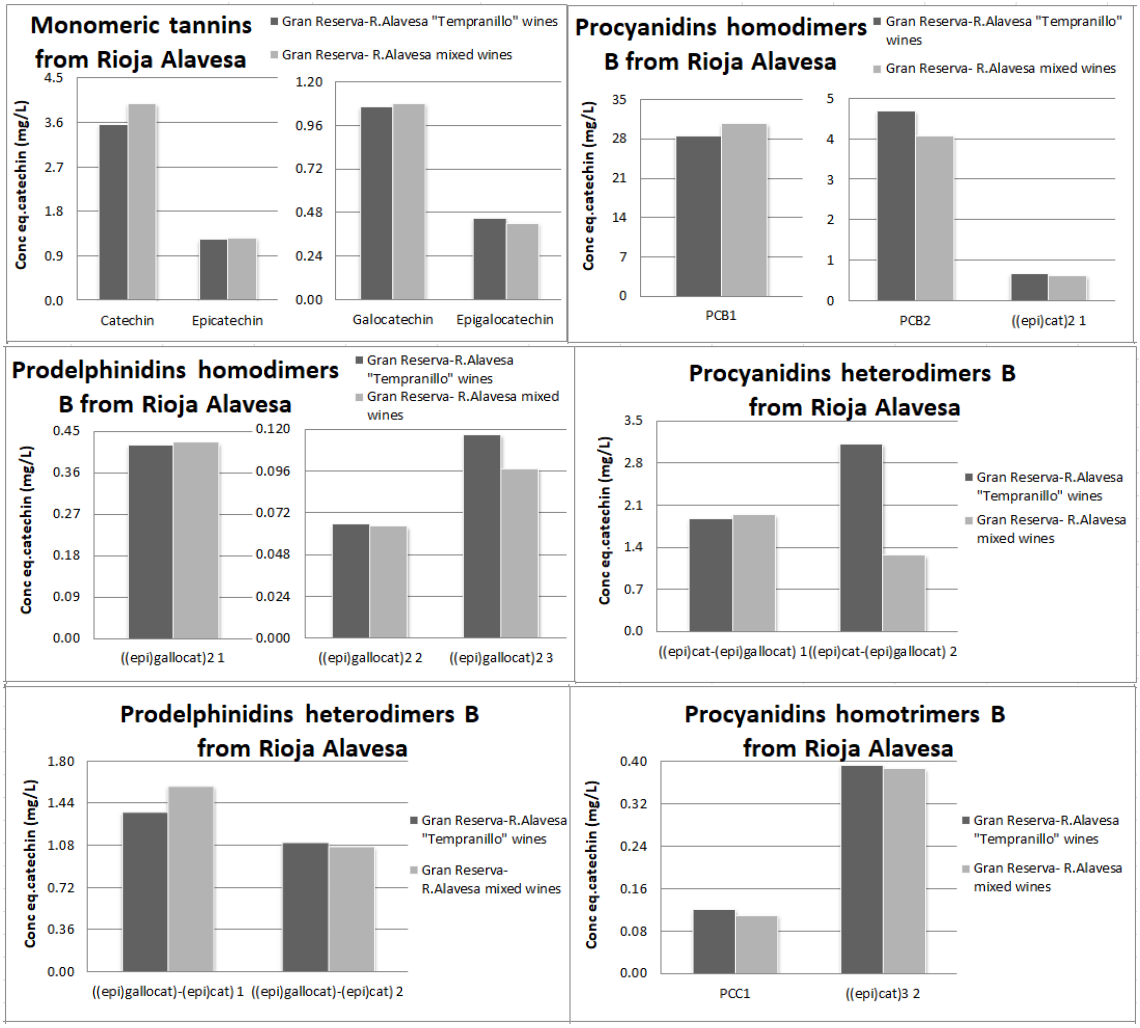



Figure Annex II.25 Mean concentrations of different tannins for *Tempranillo* and mixed *Gran Reserva* wines from Rioja Alavesa.

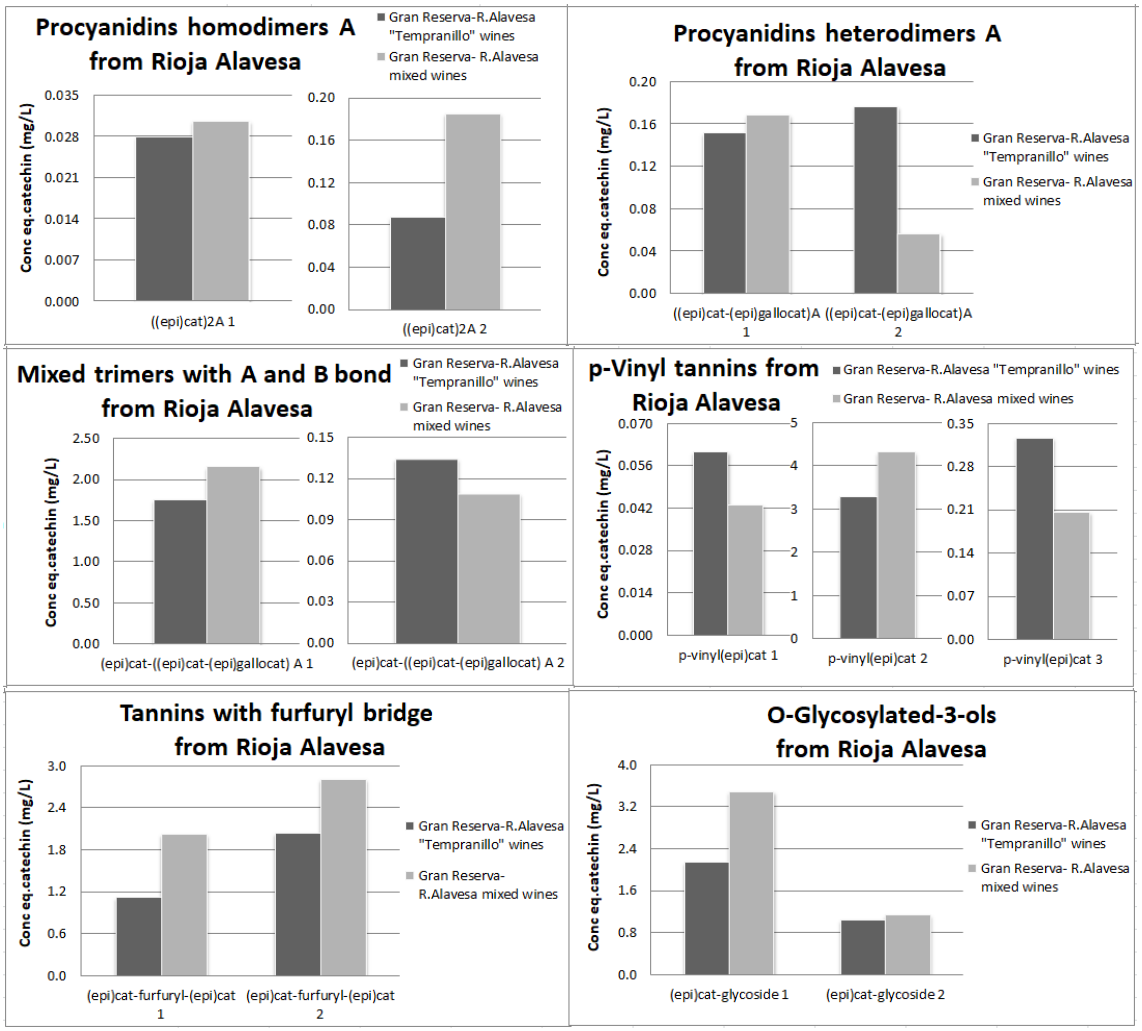



Figure Annex II.25 Cont.

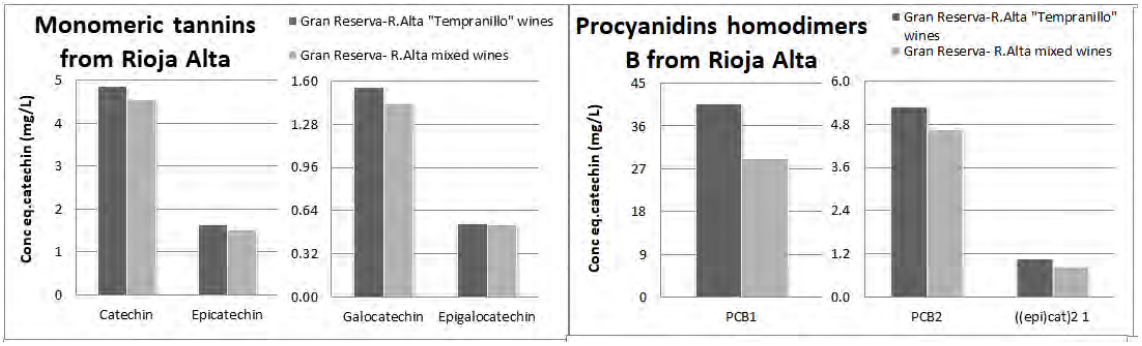



Figure Annex II.26 Mean concentrations of different tannins for *Tempranillo* and mixed *Gran Reserva* wines from Rioja Alta.

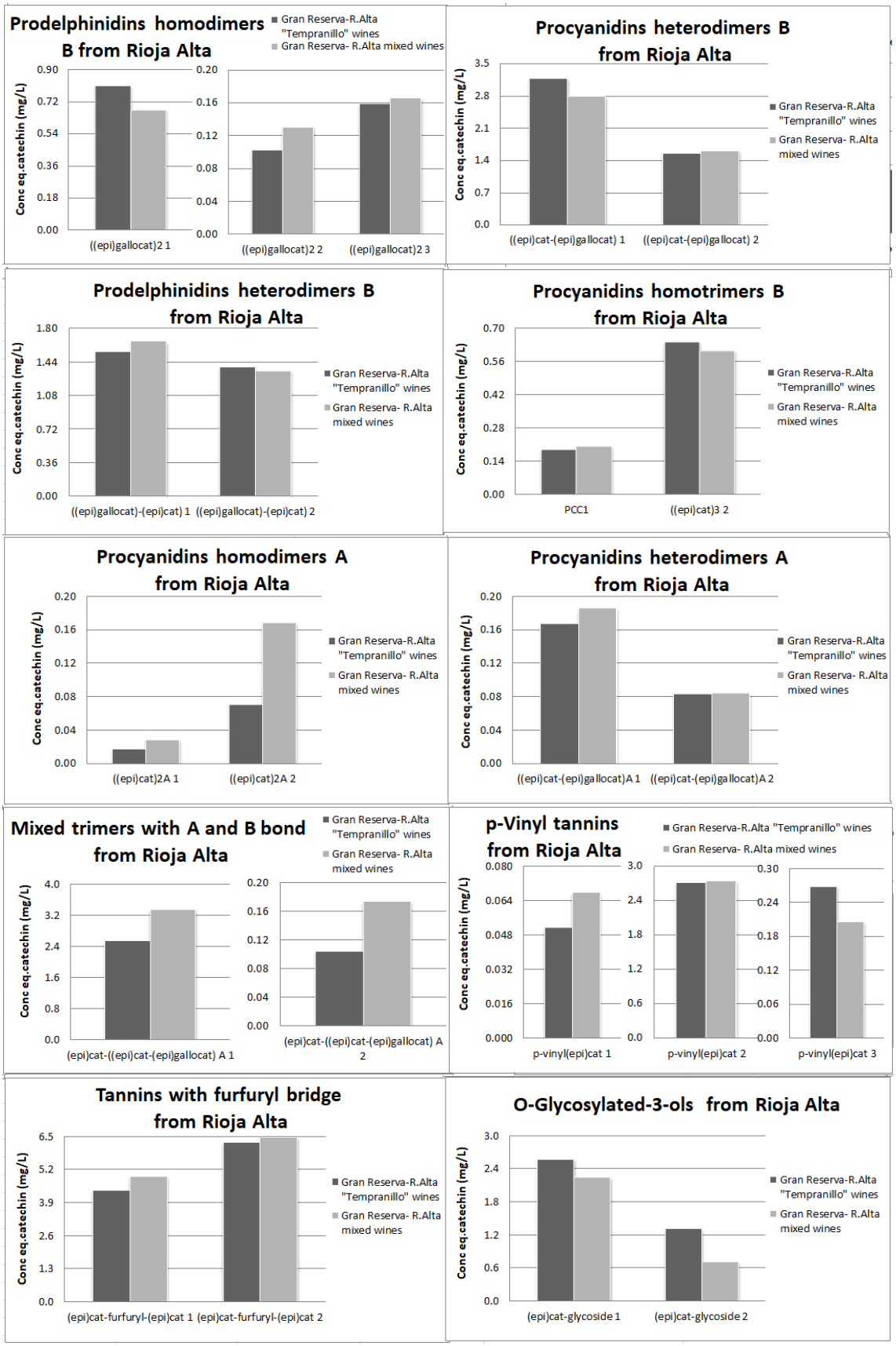



Figure Annex II.26 Cont.

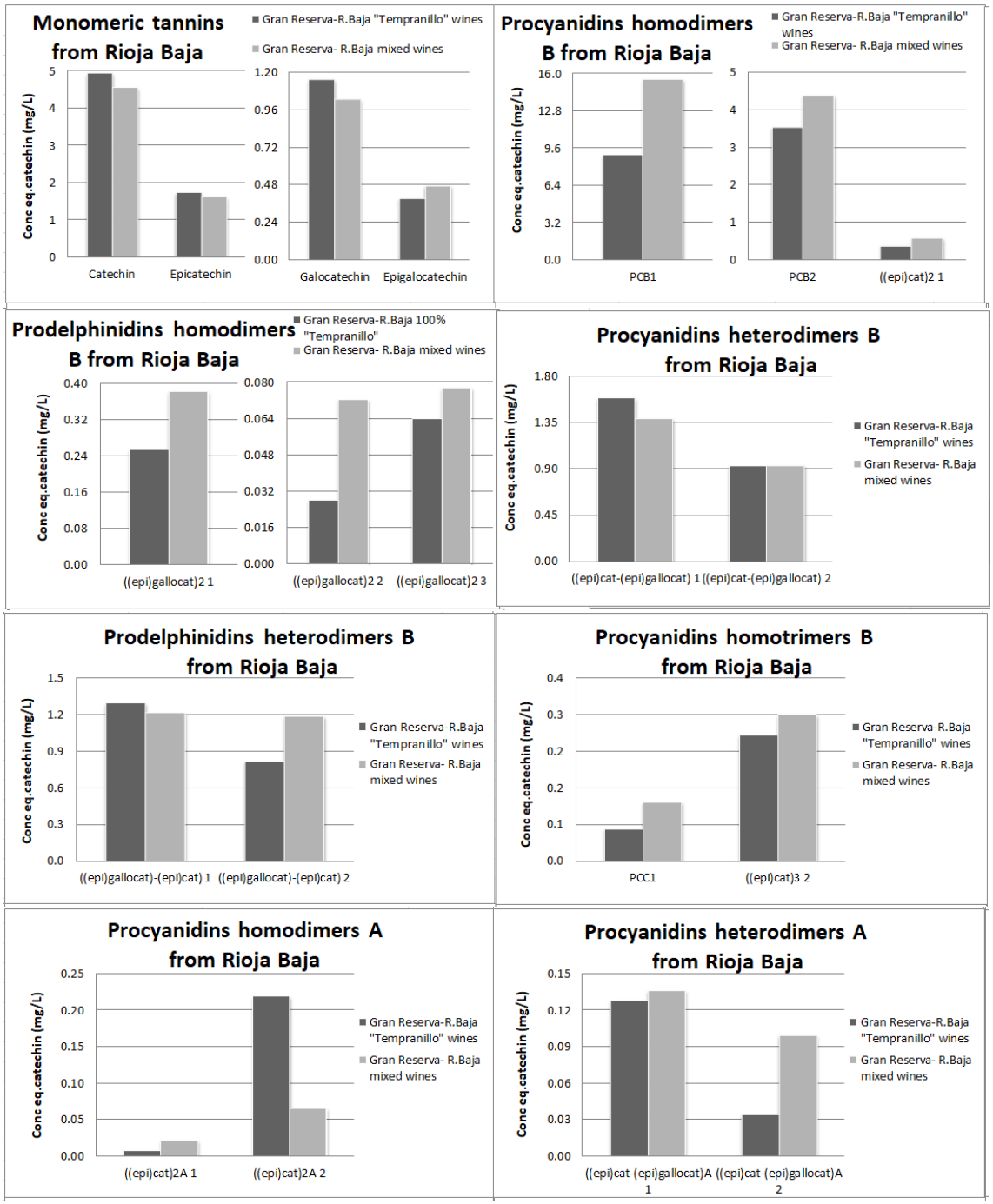



Figure Annex II.27 Mean concentrations of different tannins for *Tempranillo* and mixed *Gran Reserva* wines from Rioja Baja.

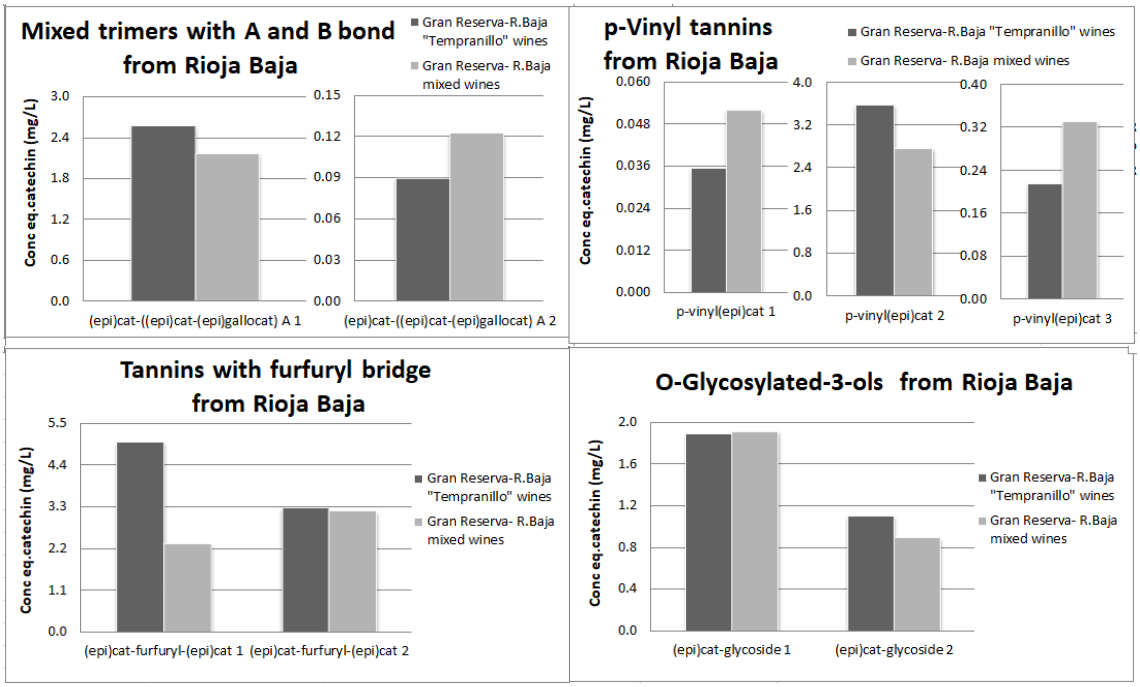



Figure Annex II.27 Cont.

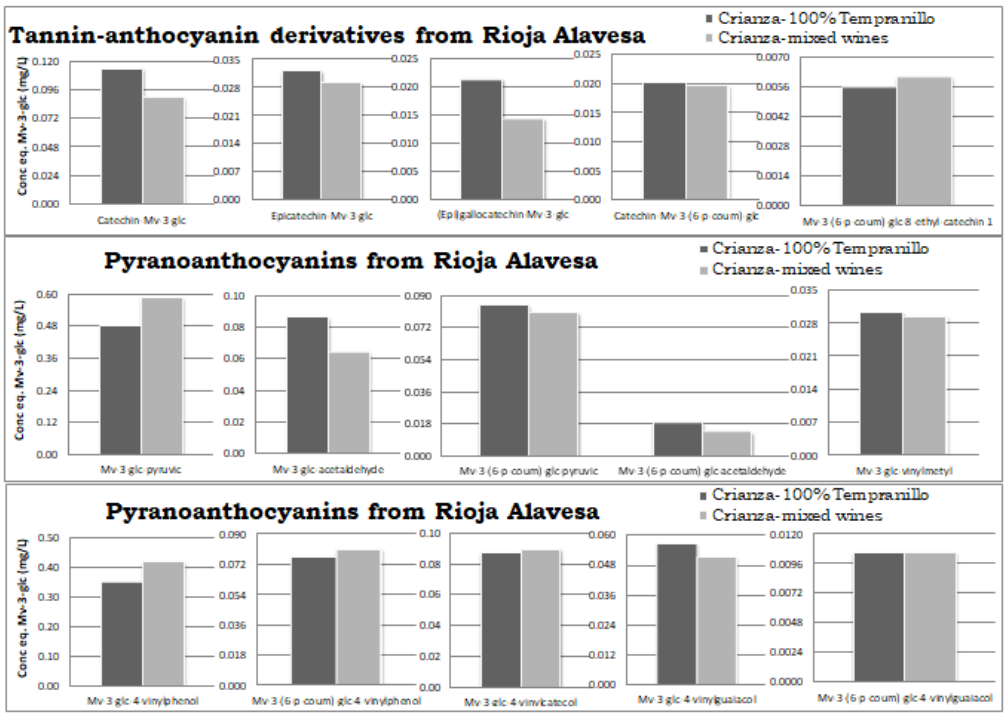



Figure Annex II.28 Mean concentrations of anthocyanin derivatives for *Tempranillo* and mixed *Crianza* wines from Rioja Alavesa.

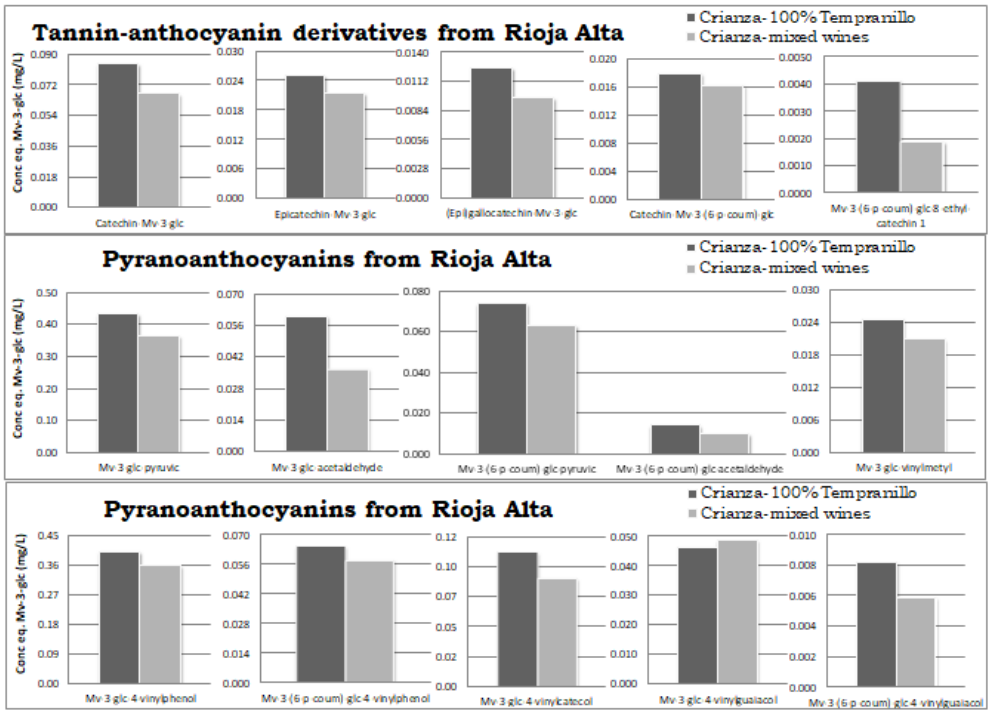



Figure Annex II.29 Mean concentrations of anthocyanin derivatives for *Tempranillo* and mixed *Crianza* wines from Rioja Alta.

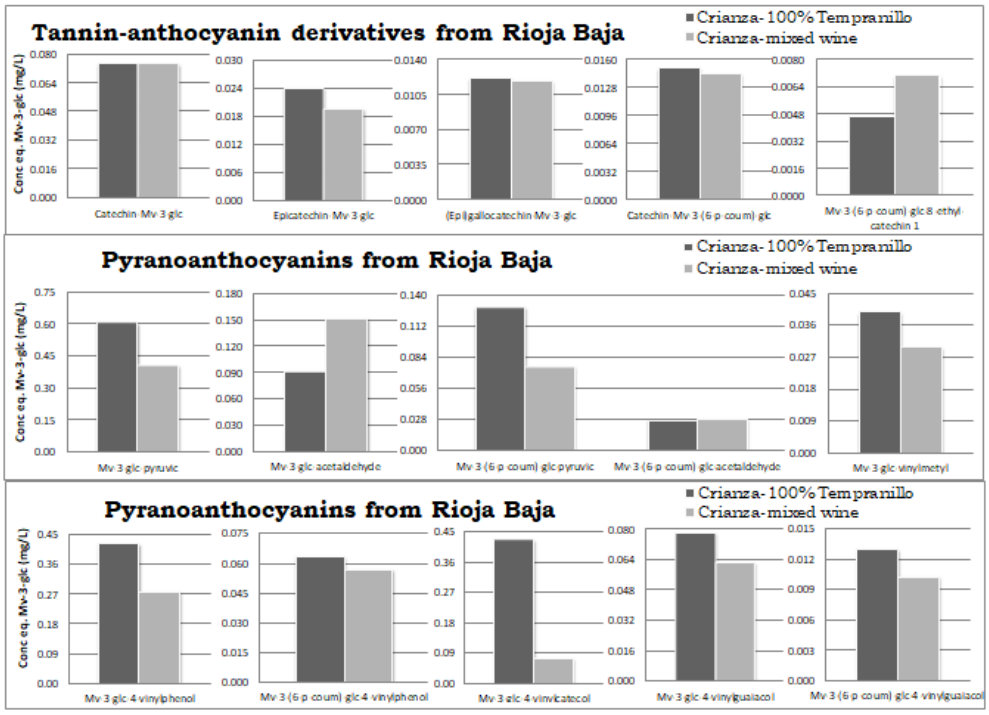



Figure Annex II.30 Mean concentrations of anthocyanin derivatives for *Tempranillo* and mixed *Crianza* wines from Rioja Baja.

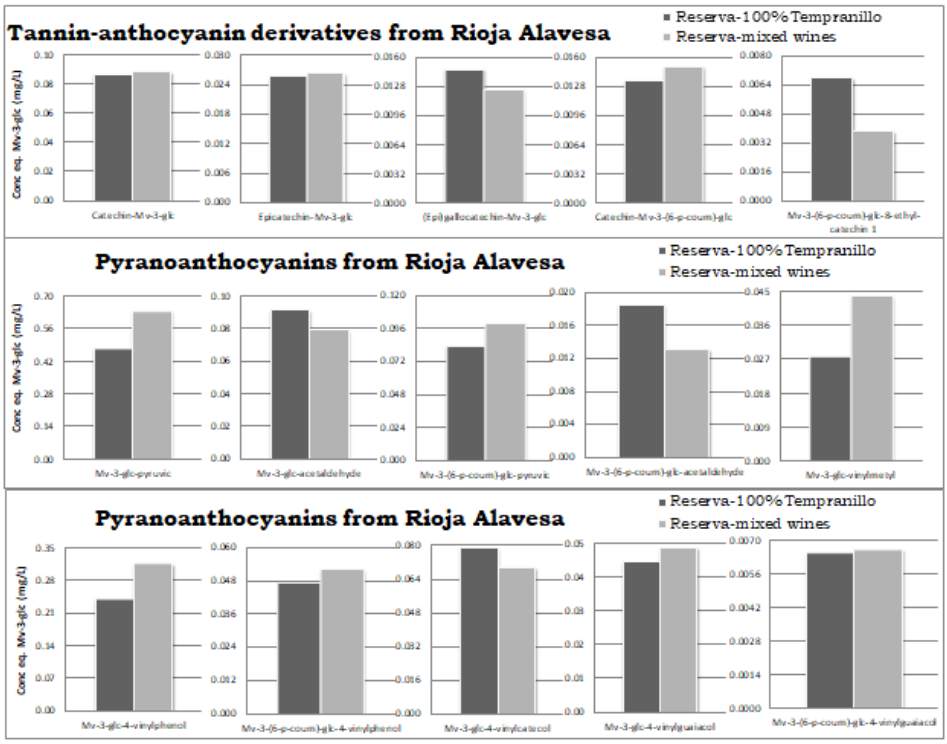



Figure Annex II.31 Mean concentrations of anthocyanin derivatives for *Tempranillo* and mixed *Reserva* wines from Rioja Alavesa.

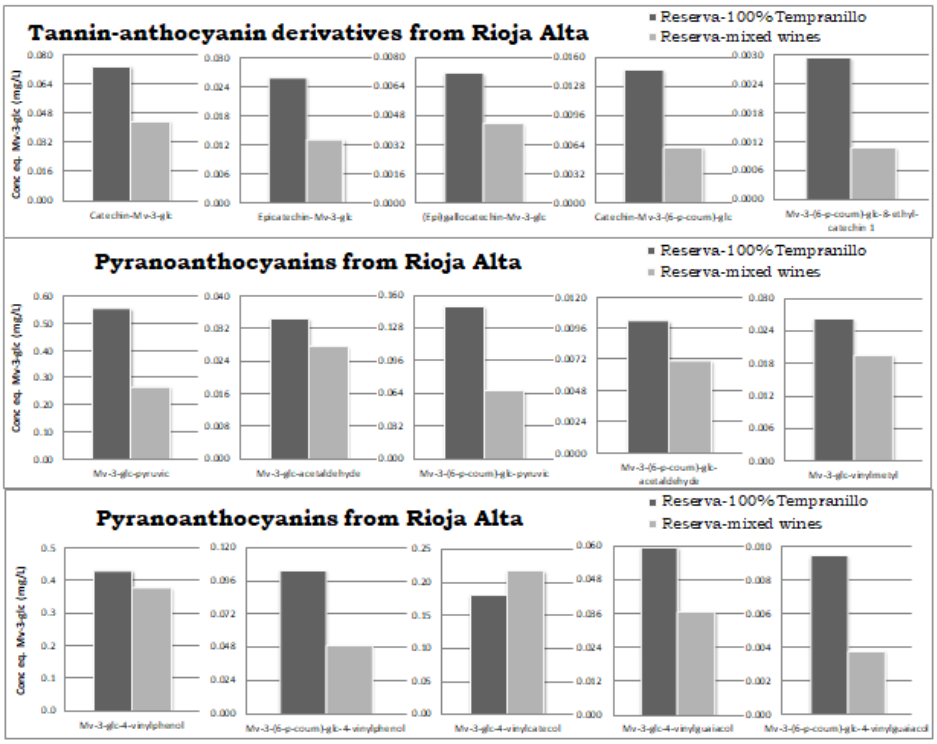



Figure Annex II.32 Mean concentrations of anthocyanin derivatives for *Tempranillo* and mixed *Reserva* wines from Rioja Alta.

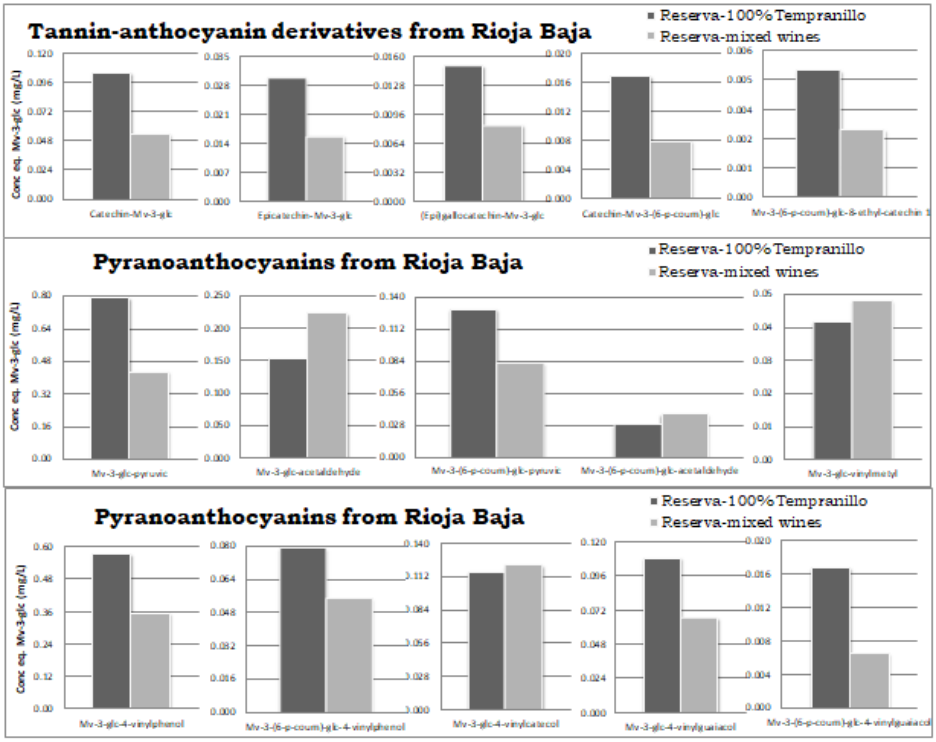



Figure Annex II.33 Mean concentrations of anthocyanin derivatives for *Tempranillo* and mixed *Reserva* wines from Rioja Baja.

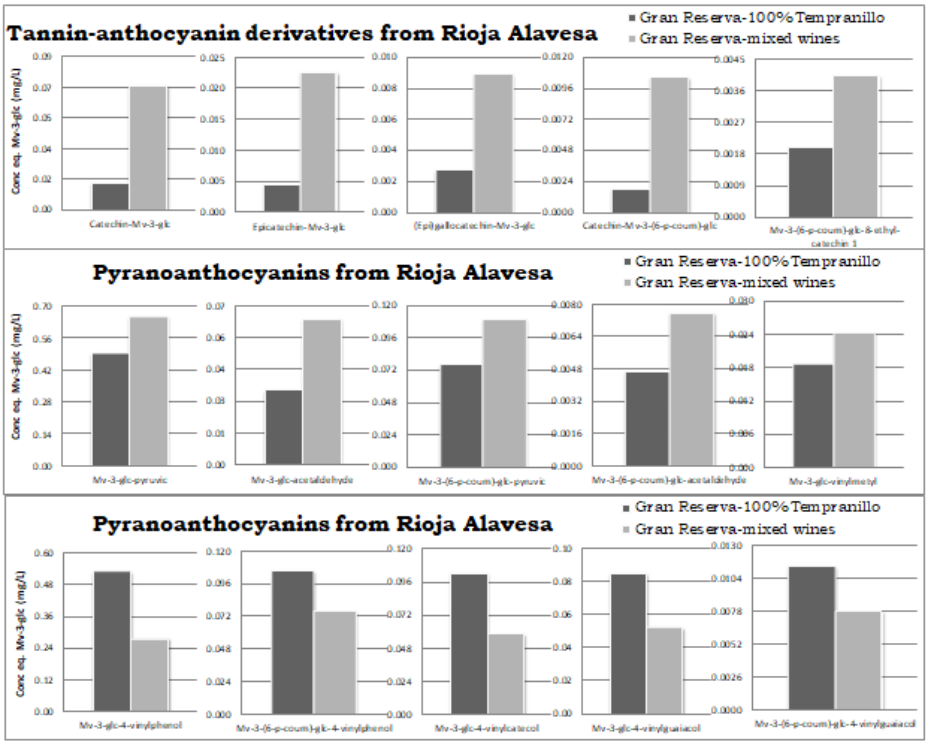



Figure Annex II.34 Mean concentrations of anthocyanin derivatives for *Tempranillo* and mixed *Gran Reserva* wines from Rioja Alavesa.

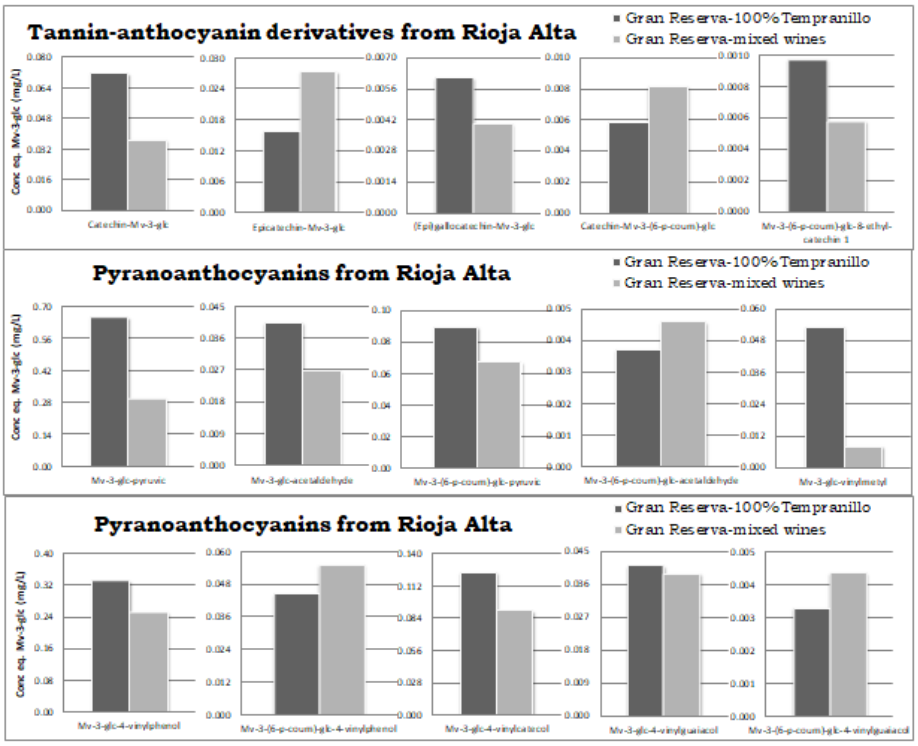



Figure Annex II.35 Mean concentrations of anthocyanin derivatives for *Tempranillo* and mixed *Gran Reserva* wines from Rioja Alta.

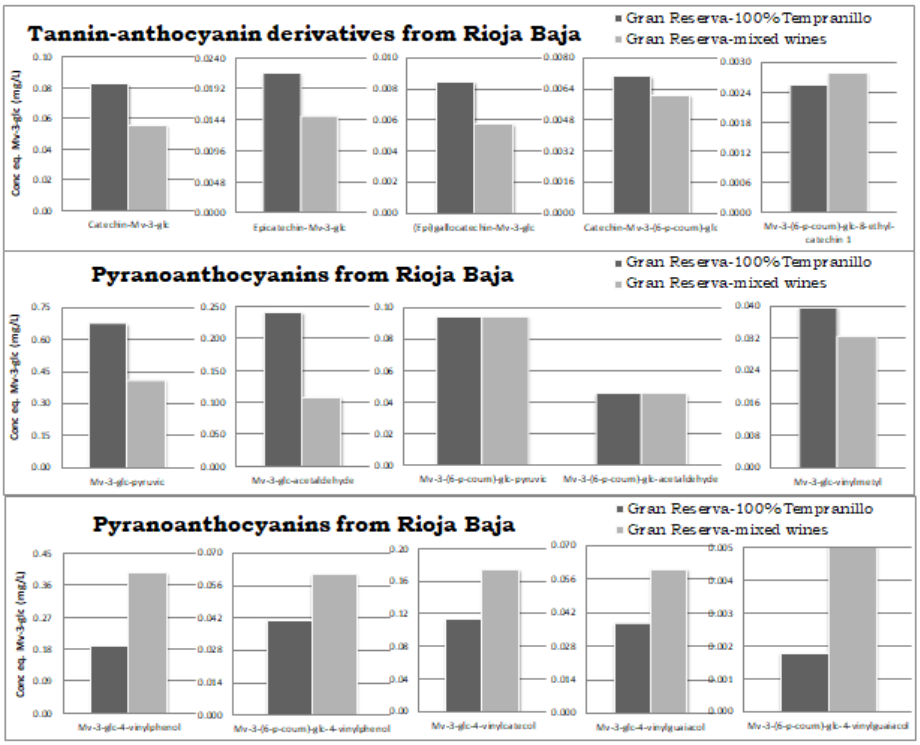



Figure Annex II.36 Mean concentrations of anthocyanin derivatives for *Tempranillo* and mixed *Gran Reserva* wines from Rioja Baja.

Table Annex II.41 Anthocyanin concentration of Rioja wines aged in American and French oak barrel.

| Sample | Dp-3-glc | Cy-3-glc | Pt-3-glc | Pn-3-glc | Mv-3-glc | Dp-3-(6-Ac)-glc | Pt-3-(6-Ac)-glc | Pn-3-(6-Ac)-glc | Mv-3-(6-Ac)-glc | Pt-3-(6-p-coum)-glc | Pn-3-(6-p-coum)-glc | Mv-3-(6-p-coum)-glc |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RV_A-T-Am-C-1 | 8.2007 | 0.3689 | 11.5763 | 2.3628 | 49.1515 | 1.1772 | 0.4569 | 0.2510 | 5.0119 | 0.3463 | 1.3482 | 0.2328 |
| RV_A-T-Fr-C-2 | 4.5961 | 0.5646 | 4.5325 | 1.6247 | 17.4556 | 2.5294 | 0.0773 | 0.0694 | 1.8631 | 0.3055 | 0.7633 | 1.6552 |
| RB-TG-Am-C-3 | 2.9222 | 0.3740 | 4.1378 | 1.5116 | 16.5171 | 2.0742 | 0.0944 | 0.0865 | 1.8549 | 0.0609 | 0.2837 | 1.4791 |
| RB-TG-Fr-C-4 | 0.1833 | 0.0333 | 0.2983 | 0.1192 | 1.7964 | 1.0875 | 0.0819 | 0.0123 | 0.3332 | 0.0248 | 0.2524 | 0.1906 |
| RV_A-TG-Am-C-5 | 8.5758 | 0.3412 | 12.8584 | 2.7582 | 51.3616 | 0.8339 | 0.5581 | 0.3005 | 5.9547 | 0.2299 | 0.8448 | 7.4092 |
| RV_A-TG-Fr-C-6 | 4.3183 | 0.1745 | 5.1053 | 1.3431 | 18.7243 | 0.9344 | 0.1716 | 0.1505 | 2.3431 | 0.0845 | 0.3987 | 2.3857 |
| RB-T-Am-R-7 | 0.1644 | 0.0300 | 0.2189 | 0.0871 | 1.4283 | 2.0488 | 0.2240 | 0.0530 | 0.6862 | 0.0202 | 0.1767 | 0.1534 |
| RB-T-Fr-R-8 | 0.1476 | 0.0254 | 0.2066 | 0.0694 | 1.3355 | 0.8747 | 0.0490 | 0.0103 | 0.2932 | 0.0162 | 0.2262 | 0.1090 |
| RV_A-T-Am-R-9 | 6.7099 | 0.4242 | 7.3348 | 1.8148 | 25.6767 | 2.6107 | 0.1891 | 0.1331 | 2.8650 | 0.0760 | 0.4329 | 2.7411 |
| RV_A-T-Fr-R-10 | 1.5421 | 0.1236 | 1.4423 | 0.6010 | 5.1060 | 2.1562 | 0.1090 | 0.0490 | 0.7553 | 0.0307 | 0.2546 | 0.4082 |
| RB-TG-Am-R-11a | 4.2255 | 0.3077 | 6.0507 | 2.0894 | 26.6210 | 2.4938 | 0.1331 | 0.1563 | 3.1384 | 0.1076 | 0.6076 | 3.5987 |
| RB-TG-Am-R-11b | 0.6505 | 0.1251 | 0.8791 | 0.7983 | 5.9324 | 1.6235 | 0.0681 | 0.0379 | 0.6767 | 0.0418 | 0.2357 | 0.6120 |
| RB-TG-Fr-R-12 | 0.6367 | 0.0891 | 0.8339 | 0.5588 | 4.9572 | 1.9388 | 0.1680 | 0.0313 | 0.6731 | 0.0648 | 0.1687 | 0.6869 |
| RV_A-TG-Am-R-13 | 2.4073 | 0.2735 | 2.3558 | 0.7808 | 8.4677 | 2.5255 | 0.1345 | 0.0655 | 1.2128 | 0.0307 | 0.2808 | 0.7670 |
| RV_A-TG-Fr-R-14 | 0.6367 | 0.0825 | 0.8681 | 0.6563 | 4.6953 | 1.1272 | 0.0510 | 0.0234 | 0.5261 | 0.0346 | 0.2444 | 0.3812 |

Table Annex II.42 Anthocyanin derivatives concentration of Rioja wines aged in American and French oak barrel.

| Sample | Mv-3-glc-acetaldehyde | Mv-3-glc-vinylmetyl | Mv-3-glc-pyruvic | Mv-3-glc-4-vinylphenol | Mv-3-glc-4-vinylcatechol | Mv-3-glc-4-vinylguaiacol | Mv-3-(6-p-coum)-glc-acetaldehyde | Mv-3-(6-p-coum)-glc- pyruvic | Mv-3-(6-p-coum)-glc-4-vinylphenol | Mv-3-(6-p-coum)-glc-4-vinylguaiacol | Mv-3-glc-4-vinylcatechin | Mv-3-glc-4-vinylepicatechin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RV_A-T-Am-C-1 | 0.3182 | 0.0707 | 1.0039 | 0.8993 | 0.1963 | 0.0865 | 0.0484 | 0.1387 | 0.0700 | 0.0160 | 0.0045 | 0.0023 |
| RV_A-T-Fr-C-2 | 0.1997 | 0.1894 | 2.2762 | 1.0298 | 0.8371 | 0.1815 | 0.0352 | 0.3970 | 0.1891 | 0.0311 | 0.0267 | 0.0218 |
| RB-TG-Am-C-3 | 0.4117 | 0.0969 | 0.9964 | 0.3932 | 0.1244 | 0.0332 | 0.0619 | 0.2220 | 0.0666 | 0.0046 | 0.0239 | 0.0206 |
| RB-TG-Fr-C-4 | 0.0988 | 0.0682 | 0.5168 | 0.3740 | 0.2100 | 0.0676 | 0.0163 | 0.1074 | 0.0521 | 0.0103 | 0.0272 | 0.0269 |
| RV_A-TG-Am-C-5 | 0.0793 | 0.0475 | 0.5779 | 0.7317 | 0.1346 | 0.1079 | 0.0270 | 0.1459 | 0.1545 | 0.0233 | 0.0033 | 0.0014 |
| RV_A-TG-Fr-C-6 | 0.0762 | 0.0585 | 0.6035 | 0.7996 | 0.1690 | 0.1494 | 0.0283 | 0.2117 | 0.1767 | 0.0296 | 0.0063 | 0.0073 |
| RB-T-Am-R-7 | 0.0638 | 0.0497 | 1.4221 | 0.2530 | 0.1532 | 0.0376 | 0.0172 | 0.3826 | 0.0361 | 0.0046 | 0.0236 | 0.0274 |
| RB-T-Fr-R-8 | 0.1254 | 0.0622 | 0.5786 | 0.2732 | 0.2296 | 0.0333 | 0.0160 | 0.1346 | 0.0300 | <0.001 | 0.0242 | 0.0253 |
| RV_A-T-Am-R-9 | 0.2501 | 0.1414 | 1.4442 | 0.6879 | 0.1752 | 0.0841 | 0.0478 | 0.3391 | 0.1184 | 0.0170 | 0.0343 | 0.0273 |
| RV_A-T-Fr-R-10 | 0.0325 | 0.0832 | 1.0826 | 0.2747 | 0.1558 | 0.0345 | 0.0061 | 0.1660 | 0.0278 | 0.0015 | 0.0204 | 0.0181 |
| RB-TG-Am-R-11a | 0.0924 | 0.0796 | 1.2601 | 0.3783 | 0.1862 | 0.0550 | 0.0279 | 0.2698 | 0.0695 | 0.0127 | 0.0145 | 0.0137 |
| RB-TG-Am-R-11b | 0.6856 | 0.1475 | 0.7322 | 0.6441 | 0.1317 | 0.0655 | 0.0822 | 0.2043 | 0.1144 | 0.0153 | 0.0536 | 0.0817 |
| RB-TG-Fr-R-12 | 0.2226 | 0.1291 | 0.7887 | 0.3601 | 0.0719 | 0.0540 | 0.0311 | 0.1506 | 0.0443 | 0.0065 | 0.0625 | 0.0839 |
| RV_A-TG-Am-R-13 | 0.1298 | 0.0830 | 1.5777 | 0.4115 | 0.1591 | 0.0483 | 0.0226 | 0.2156 | 0.0456 | 0.0044 | 0.0174 | 0.0139 |
| RV_A-TG-Fr-R-14 | 0.0760 | 0.0813 | 0.5266 | 0.5119 | 0.1664 | 0.0478 | 0.0197 | 0.1349 | 0.0565 | 0.0052 | 0.0154 | 0.0154 |

Continuation of Table Annex II. 42

| Sample | Mv-3-(6-*p* -coum)-glc-4-vinylcatechin | Mv-3-(6-*p* -coum)-glc-4-vinylepicatechin | Mv-3-glc-4-vinylgallocatechin | Mv-3-glc-4-vinylepigallocatechin | Mv-3-(6-*p* -coum)-glc-4-vinyl(epi)gallocatechin 1 | Mv-3-(6-*p* -coum)-glc-4-vinyl(epi)gallocatechin 2 | Catechin-Mv-3-glc | Epicatechin-Mv-3-glc | (Epi)Gallocatechin-Mv-3-glc | Catechin-Mv-3-(6-p-coum)-glc | Mv-3-(6-p-coum)-glc-8-ethyl-(epi)catechin 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| RV_A-T-Am-C-1 | 0.0015 | | <0.001 | <0.001 | 0.0128 | | 0.6364 | 0.0855 | 0.0944 | 0.0516 | 0.0029 |
| RV_A-T-Fr-C-2 | 0.0163 | | 0.0050 | 0.0026 | 0.0130 | | 0.3910 | 0.0820 | 0.0639 | 0.0421 | 0.0097 |
| RB-TG-Am-C-3 | 0.0200 | | 0.0058 | 0.0054 | 0.0096 | | 0.2353 | 0.0432 | 0.0416 | 0.0282 | 0.0083 |
| RB-TG-Fr-C-4 | 0.0226 | | 0.0022 | <0.001 | 0.0014 | | 0.1319 | 0.0253 | 0.0187 | 0.0059 | 0.0016 |
| RV_A-TG-Am-C-5 | 0.0024 | | <0.001 | <0.001 | 0.0144 | | 0.5487 | 0.0850 | 0.0787 | 0.0813 | 0.0035 |
| RV_A-TG-Fr-C-6 | 0.0085 | | <0.001 | <0.001 | 0.0137 | | 0.4249 | 0.0772 | 0.0616 | 0.0635 | 0.0048 |
| RB-T-Am-R-7 | 0.0212 | | <0.001 | <0.001 | <0.001 | | 0.0690 | 0.0223 | 0.0046 | 0.0046 | <0.001 |
| RB-T-Fr-R-8 | 0.0174 | | 0.0019 | <0.001 | 0.0012 | | 0.0680 | 0.0161 | 0.0051 | 0.0023 | <0.001 |
| RV_A-T-Am-R-9 | 0.0269 | | 0.0030 | 0.0010 | 0.0140 | | 0.4236 | 0.0907 | 0.0739 | 0.0569 | 0.0140 |
| RV_A-T-Fr-R-10 | 0.0063 | | 0.0009 | <0.001 | 0.0010 | | 0.2301 | 0.0490 | 0.0325 | 0.0167 | <0.001 |
| RB-TG-Am-R-11a | 0.0102 | | <0.001 | <0.001 | 0.0085 | | 0.3582 | 0.0575 | 0.0427 | 0.0422 | 0.0028 |
| RB-TG-Am-R-11b | 0.0433 | | 0.0053 | 0.0023 | 0.0033 | | 0.1409 | 0.0404 | 0.0210 | 0.0140 | 0.0141 |
| RB-TG-Fr-R-12 | 0.0337 | | 0.0069 | 0.0026 | 0.0028 | | 0.1290 | 0.0355 | 0.0157 | 0.0091 | 0.0044 |
| RV_A-TG-Am-R-13 | 0.0064 | | 0.0018 | <0.001 | 0.0015 | | 0.2757 | 0.0582 | 0.0376 | 0.0228 | 0.0016 |
| RV_A-TG-Fr-R-14 | 0.0075 | | <0.001 | <0.001 | 0.0028 | | 0.1354 | 0.0302 | 0.0197 | 0.0134 | 0.0013 |

Continuation of Table Annex II. 42

| Sample | (Epi)Gallocat-Mv-3-glc-A-(Epi)Cat 1 | (Epi)Gallocat-Mv-3-glc-A-(Epi)Cat 2 | (Epi)Cat-Mv-3-glc-A-(Epi)Cat 1 | (Epi)Cat-Mv-3-glc-A-(Epi)Cat 2 | (Epi)Cat-A-(Epi)Cat-Mv-3-glc | Mv-3-glc-(Epi)Cat A-(Epi)Cat | (Epi)Cat-(Epi)Gallocat Mv-3-glc | Di-(Epi)Cat-Mv-3-glc | Mv-3-glc-Cat | Mv-3-glc-Epicat | Cat-Mv-3-glc | Epicat-Mv-3-glc | Mv-3-glc-A-Cat | Mv-3-glc A-Epicat |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RV_A-T-Am-C-1 | <0.001 | | 0.0020 | <0.001 | <0.001 | <0.001 | 0.0151 | 0.0507 | <0.001 | | 0.0042 | | <0.001 | |
| RV_A-T-Fr-C-2 | 0.0070 | | 0.0088 | 0.0052 | 0.0008 | <0.001 | 0.0059 | 0.0301 | <0.001 | | 0.0026 | | <0.001 | |
| RB-TG-Am-C-3 | 0.0023 | | 0.0052 | 0.0035 | <0.001 | <0.001 | <0.001 | 0.0148 | <0.001 | | <0.001 | | <0.001 | |
| RB-TG-Fr-C-4 | 0.0015 | | 0.0073 | 0.0041 | <0.001 | <0.001 | <0.001 | 0.0030 | <0.001 | | <0.001 | | <0.001 | |
| RV_A-TG-Am-C-5 | 0.0049 | | 0.0078 | 0.0049 | <0.001 | <0.001 | 0.0141 | 0.0547 | <0.001 | | 0.0028 | | <0.001 | |
| RV_A-TG-Fr-C-6 | 0.0043 | | 0.0048 | 0.0052 | 0.0017 | <0.001 | 0.0058 | 0.0226 | <0.001 | | 0.0072 | | 0.0017 | |
| RB-T-Am-R-7 | <0.001 | | 0.0019 | 0.0012 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | | 0.0009 | | <0.001 | |
| RB-T-Fr-R-8 | <0.001 | | 0.0013 | <0.001 | <0.001 | <0.001 | <0.001 | 0.0010 | <0.001 | | <0.001 | | <0.001 | |
| RV_A-T-Am-R-9 | 0.0044 | | 0.0076 | 0.0042 | <0.001 | <0.001 | 0.0051 | 0.0329 | <0.001 | | 0.0062 | | <0.001 | |
| RV_A-T-Fr-R-10 | 0.0024 | | 0.0068 | 0.0037 | <0.001 | <0.001 | 0.0024 | 0.0227 | <0.001 | | 0.0021 | | <0.001 | |
| RB-TG-Am-R-11a | 0.0015 | | 0.0054 | 0.0048 | <0.001 | <0.001 | 0.0047 | 0.0243 | <0.001 | | 0.0033 | | <0.001 | |
| RB-TG-Am-R-11b | 0.0028 | | 0.0086 | 0.0129 | <0.001 | <0.001 | <0.001 | 0.0052 | <0.001 | | 0.0072 | | 0.0061 | |
| RB-TG-Fr-R-12 | 0.0011 | | 0.0071 | 0.0076 | <0.001 | <0.001 | <0.001 | 0.0060 | <0.001 | | 0.0034 | | 0.0019 | |
| RV_A-TG-Am-R-13 | 0.0019 | | 0.0057 | 0.0019 | <0.001 | <0.001 | 0.0033 | 0.0291 | <0.001 | | 0.0030 | | <0.001 | |
| RV_A-TG-Fr-R-14 | <0.001 | | 0.0027 | <0.001 | <0.001 | <0.001 | <0.001 | 0.0139 | <0.001 | | 0.0025 | | <0.001 | |

Table Annex II.43 Tannins concentration of Rioja wines aged in American and French oak barrel.

| Sample | Catechin | Epicatechin | PCB1 | PCB2 | (epi)cat$_2$ 1 | PCC1 | (epi)cat$_3$ 2 | Gallocatechin | Epigallocatechin | (epi)gallocat$_2$ 1 | (epi)gallocat$_2$ 2 | (epi)gallocat$_2$ 3 | (epi)cat-(epi)gallocat) 1 | (epi)cat-(epi)gallocat) 2 | (epi)gallocat)-(epi)cat) 1 | (epi)gallocat)-(epi)cat) 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RV_A-T-Am-C-1 | 4.1728 | 1.9118 | 157.8086 | 1.9972 | 14.9759 | 1.6108 | 2.6316 | 2.2818 | 0.5727 | 12.3016 | 1.1919 | 0.5148 | 61.8137 | 9.2399 | 39.3992 | 7.1903 |
| RV_A-T-Fr-C-2 | 4.2582 | 1.2733 | 70.2041 | 0.5888 | 6.1899 | 0.9813 | 1.7613 | 2.7454 | 0.6145 | 7.3408 | 1.1712 | 0.3957 | 45.9708 | 7.0317 | 28.4297 | 4.8438 |
| RB-TG-Am-C-3 | 6.0069 | 2.6072 | 99.0444 | 0.8205 | 6.3078 | 1.2001 | 1.1635 | 2.5665 | 0.7207 | 3.7133 | 0.5116 | 0.2477 | 26.1736 | 8.3656 | 17.2129 | 4.1972 |
| RB-TG-Fr-C-4 | 7.0155 | 3.3798 | 72.4284 | 0.2992 | 3.9288 | 0.6306 | 0.6950 | 2.7983 | 0.5148 | 2.9813 | 0.4955 | 0.1223 | 16.4525 | 3.1562 | 15.3181 | 4.8113 |
| RV_A-TG-Am-C-5 | 8.1013 | 3.7580 | 131.4778 | 1.0779 | 13.5501 | 2.0826 | 2.4689 | 3.8068 | 0.9942 | 13.8859 | 1.5580 | 0.5051 | 61.7693 | 9.4189 | 46.0025 | 7.8288 |
| RV_A-TG-Fr-C-6 | 7.4994 | 3.0220 | 116.3638 | 0.1834 | 8.7967 | 1.3993 | 1.7979 | 3.9004 | 0.8977 | 10.2891 | 1.7532 | 0.6017 | 51.5856 | 7.9386 | 37.5551 | 7.3408 |
| RB-T-Am-R-7 | 2.1270 | 1.2704 | 14.8951 | 0.0388 | 1.2904 | 0.2365 | 0.1380 | 1.3303 | 0.1183 | 0.7906 | 0.0872 | 0.0359 | 2.8708 | 0.9483 | 2.4484 | 1.5978 |
| RB-T-Fr-R-8 | 2.3205 | 1.2517 | 9.7933 | 0.0520 | 0.9023 | 0.1336 | 0.0919 | 1.3350 | 0.1489 | 0.5300 | 0.0568 | 0.0340 | 2.1082 | 0.5585 | 1.7750 | 0.8366 |
| RV_A-T-Am-R-9 | 3.6710 | 1.9533 | 145.8723 | 0.5081 | 3.5419 | 0.9001 | 1.3491 | 2.2091 | 0.6264 | 3.4516 | 0.8257 | 0.2081 | 10.7084 | 3.2193 | 8.7807 | 3.1289 |
| RV_A-T-Fr-R-10 | 3.7520 | 2.1927 | 144.7474 | 0.4227 | 3.3742 | 0.9198 | 1.3444 | 2.4168 | 0.4227 | 3.5783 | 0.6702 | 0.1818 | 15.0238 | 2.3417 | 9.5762 | 3.1782 |
| RB-TG-Am-R-11a | 4.2999 | 2.8145 | 141.0772 | 0.7249 | 3.3836 | 1.2974 | 1.2798 | 1.9287 | 0.5541 | 2.6127 | 0.7622 | 0.1336 | 8.2692 | 2.8403 | 7.5182 | 3.5889 |
| RB-TG-Am-R-11b | 4.0570 | 2.6549 | 47.3117 | 0.4030 | 2.0495 | 0.5410 | 0.2081 | 1.7386 | 0.4993 | 1.3021 | 0.2738 | 0.0492 | 3.0632 | 1.6553 | 3.2533 | 2.6725 |
| RB-TG-Fr-R-12 | 5.3165 | 3.1311 | 45.4335 | 0.4277 | 1.7275 | 0.4368 | 0.5149 | 1.4801 | 0.3550 | 0.7385 | 0.0961 | 0.0519 | 3.4379 | 1.2624 | 3.3851 | 2.7616 |
| RV_A-TG-Am-R-13 | 4.8299 | 1.8726 | 125.1112 | 0.2950 | 3.2383 | 0.3550 | 0.8330 | 2.4252 | 0.4568 | 2.6792 | 0.4240 | 0.0670 | 11.5161 | 2.1910 | 10.1128 | 2.9695 |
| RV_A-TG-Fr-R-14 | 3.0602 | 1.2558 | 35.2655 | 0.0627 | 0.9784 | 0.1896 | 0.4350 | 0.8584 | 0.2368 | 0.7203 | 0.0950 | 0.0605 | 3.0948 | 1.1651 | 3.0602 | 1.3053 |

Continuation of Table Annex II. 43

| Sample | (epi)cat-glycoside 1 | (epi)cat-glycoside 2 | (epi)cat)$_2$A 1 | (epi)cat)$_2$A 2 | (epi)cat-(epi)gallocat)A 1 | (epi)cat-(epi)gallocat)A 2 | (epi)cat-((epi)cat-(epi)gallocat) A 1 | (epi)cat-((epi)cat-(epi)gallocat) A 2 | p-vinyl(epi)cat 1 | p-vinyl(epi)cat 2 | p-vinyl(epi)cat 3 | (epi)cat-furfuryl-(epi)cat 1 | (epi)cat-furfuryl-(epi)cat 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RV_A-T-Am-C-1 | 17.2256 | 1.1716 | 0.1480 | 1.1004 | 0.1802 | 0.1158 | 15.2557 | 0.7013 | 0.5598 | 4.8211 | 0.0692 | 107.0054 | 24.6164 |
| RV_A-T-Fr-C-2 | 9.2196 | 1.0425 | 0.0982 | 0.4022 | 0.0815 | 0.0399 | 6.3940 | 0.3266 | 0.5140 | 2.0848 | 0.1015 | 10.0833 | 4.2088 |
| RB-TG-Am-C-3 | 7.2432 | 1.5783 | 0.1480 | 1.2123 | 0.0732 | 0.1093 | 4.5685 | 0.3190 | 0.5140 | 5.5521 | 0.1584 | 12.5639 | 5.6554 |
| RB-TG-Fr-C-4 | 4.5226 | 2.4242 | 0.0954 | 0.6178 | 0.1094 | 0.0343 | 15.3836 | 0.5751 | 0.5713 | 2.7239 | 0.6936 | 22.5370 | 10.5617 |
| RV_A-TG-Am-C-5 | 12.5044 | 3.8434 | 0.1158 | 0.9073 | 0.1802 | 0.0787 | 12.6851 | 0.6057 | 0.6745 | 5.3914 | 0.0508 | 98.8358 | 22.7052 |
| RV_A-TG-Fr-C-6 | 8.6625 | 1.3261 | 0.1093 | 1.0586 | 0.2317 | 0.0815 | 22.2813 | 0.8886 | 0.7586 | 3.7343 | 0.0785 | 58.4455 | 17.6380 |
| RB-T-Am-R-7 | 1.4957 | 0.9396 | 0.0207 | 0.1071 | 0.0606 | 0.0283 | 11.1522 | 0.3837 | 0.6528 | 2.5885 | 0.4925 | 13.3631 | 8.7610 |
| RB-T-Fr-R-8 | 1.3502 | 0.4512 | 0.0302 | 0.1033 | 0.0188 | 0.0150 | 3.7570 | 0.1719 | 0.2091 | 0.8990 | 0.3379 | 3.3269 | 2.9184 |
| RV_A-T-Am-R-9 | 0.4402 | 4.1931 | 0.0682 | 0.3986 | 0.0492 | 0.0653 | 6.3050 | 0.3035 | 0.4324 | 6.2238 | 0.0776 | 17.1151 | 7.3869 |
| RV_A-T-Fr-R-10 | 0.3373 | 4.0289 | 0.0539 | 0.1033 | 0.0663 | 0.0464 | 8.1118 | 0.4123 | 0.0448 | 1.4659 | 0.1232 | 68.1503 | 22.0652 |
| RB-TG-Am-R-11a | 0.3438 | 3.9901 | 0.0663 | 0.2519 | 0.0625 | 0.0435 | 9.1668 | 0.6299 | 0.5870 | 3.6948 | 0.2377 | 63.7099 | 18.3710 |
| RB-TG-Am-R-11b | 0.0634 | 2.0953 | 0.0283 | 0.1005 | 0.0511 | 0.0302 | 9.3155 | 0.4553 | 1.3036 | 2.7616 | 1.3604 | 6.4186 | 5.3934 |
| RB-TG-Fr-R-12 | 2.5555 | 0.8312 | 0.0293 | 0.0853 | 0.0304 | 0.0368 | 4.8634 | 0.2847 | 1.1566 | 1.6930 | 1.6051 | 6.0996 | 4.5367 |
| RV_A-TG-Am-R-13 | 5.4270 | 0.5495 | 0.0541 | 0.2677 | 0.0293 | 0.0368 | 7.3095 | 0.6000 | 0.3726 | 3.2882 | 0.0766 | 27.9259 | 10.0962 |
| RV_A-TG-Fr-R-14 | 2.0640 | 0.2332 | 0.0368 | 0.0907 | 0.0336 | 0.0239 | 2.5022 | 0.1021 | 0.2568 | 1.4309 | 0.1024 | 3.6628 | 2.1353 |

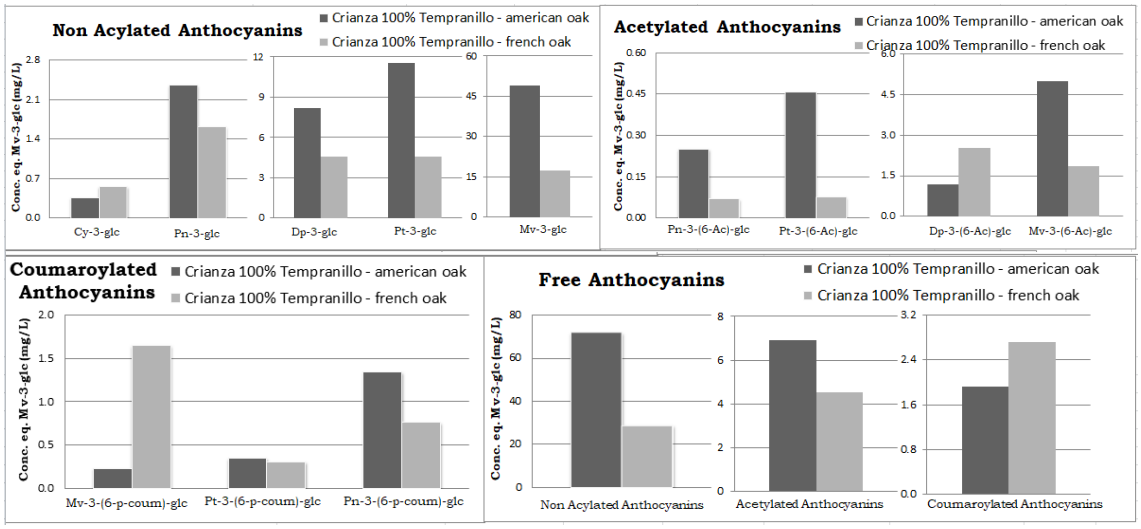



Figure Annex II.37 Grafics of the different families of free anthocyanins for 100% *Tempranillo Crianza* wines aged in American and French oak barrel from Rioja Alavesa and Alta.

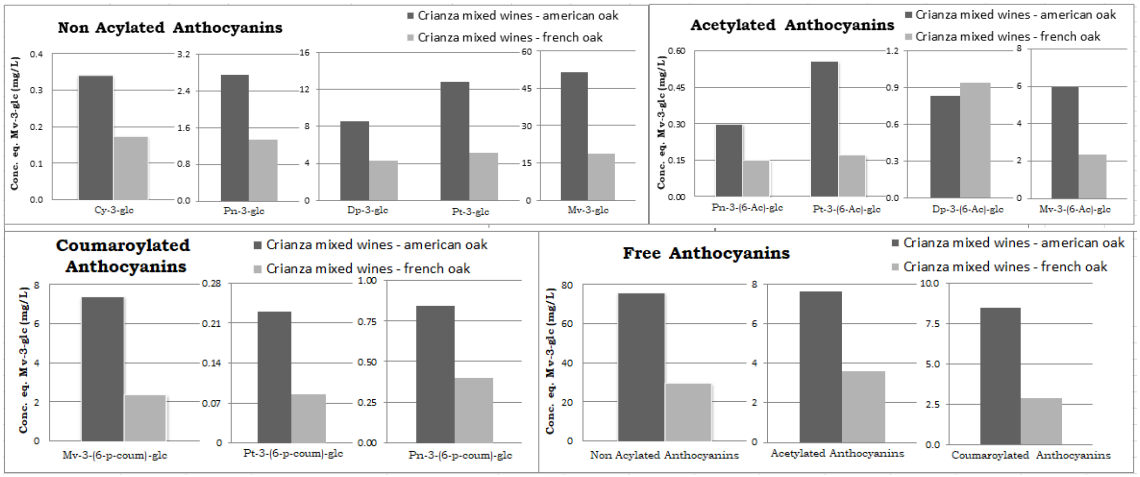



Figure Annex II.38 Grafics of the different families of free anthocyanins for *Crianza* mixed wines aged in American and French oak barrel from Rioja Alavesa and Alta.

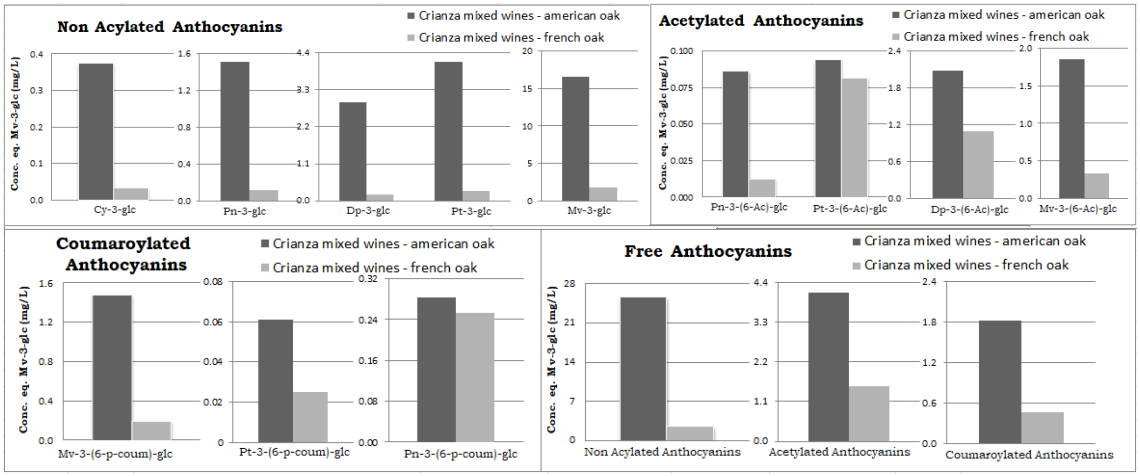



Figure Annex II.39 Grafics of the different families of free anthocyanins for *Crianza* mixed wines aged in American and French oak barrel from Rioja Baja.

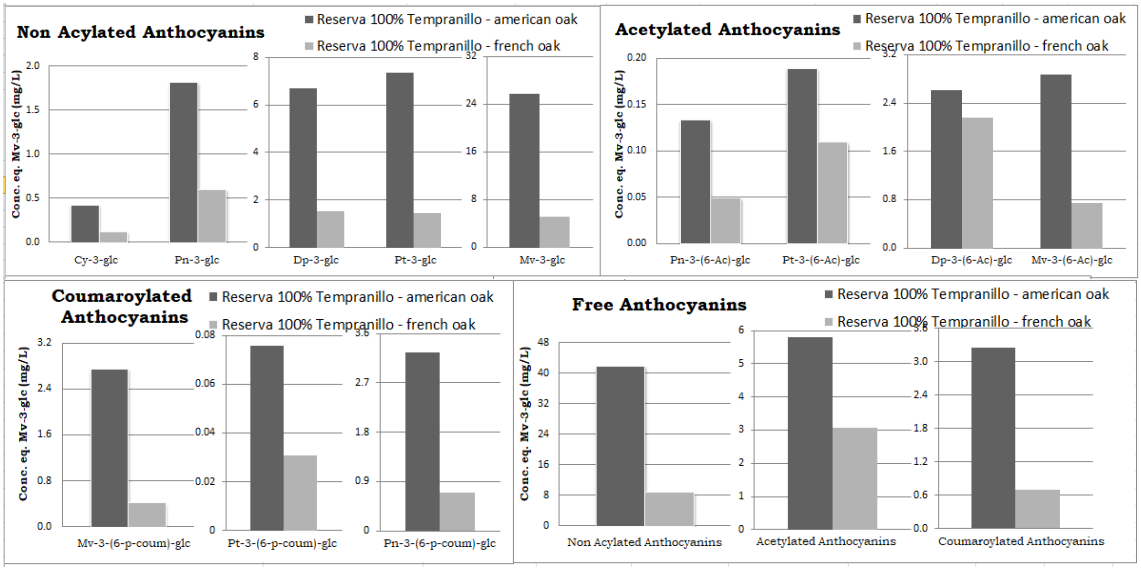



Figure Annex II.40 Grafics of the different families of free anthocyanins for 100% *Tempranillo Reserva* wines aged in American and French oak barrel from Rioja Alavesa and Alta.

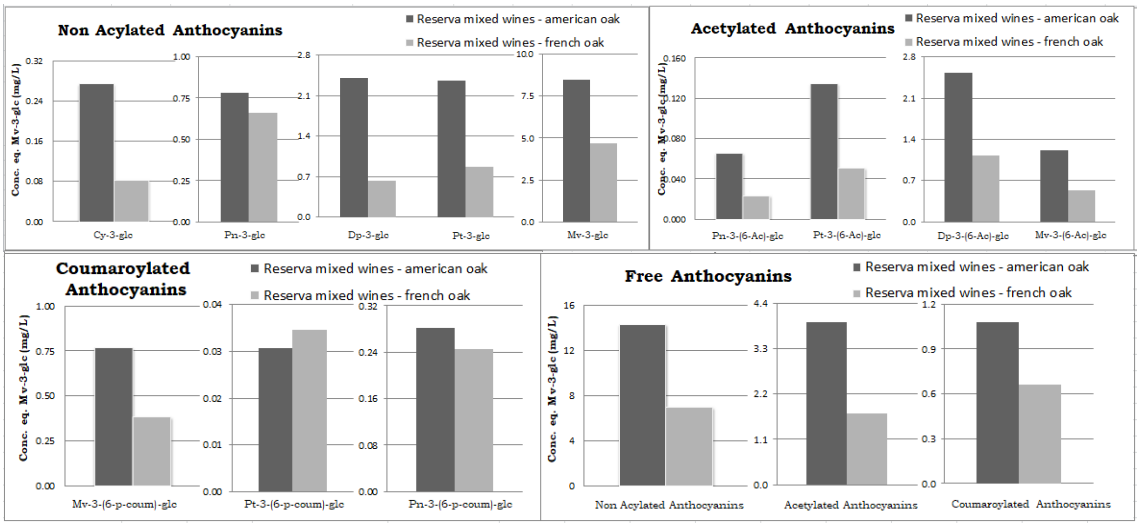



Figure Annex II.41 Grafics of the different families of free anthocyanins for *Reserva* mixed wines aged in American and French oak barrel from Rioja Alavesa and Alta.

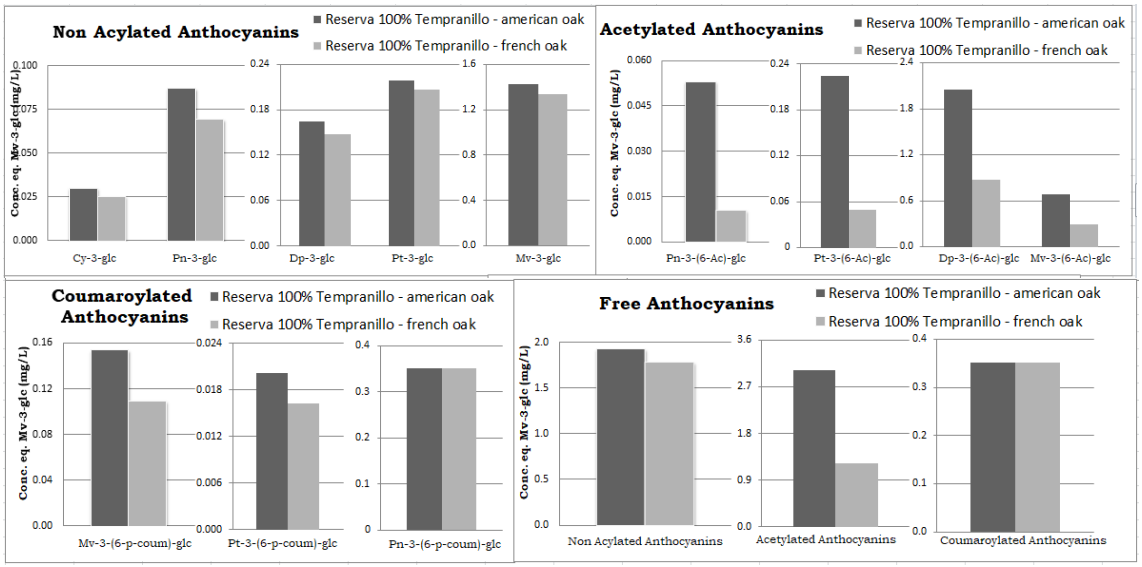



Figure Annex II.42 Grafics of the different families of free anthocyanins for 100% *Tempranillo Reserva* wines aged in American and French oak barrel from Rioja Baja.

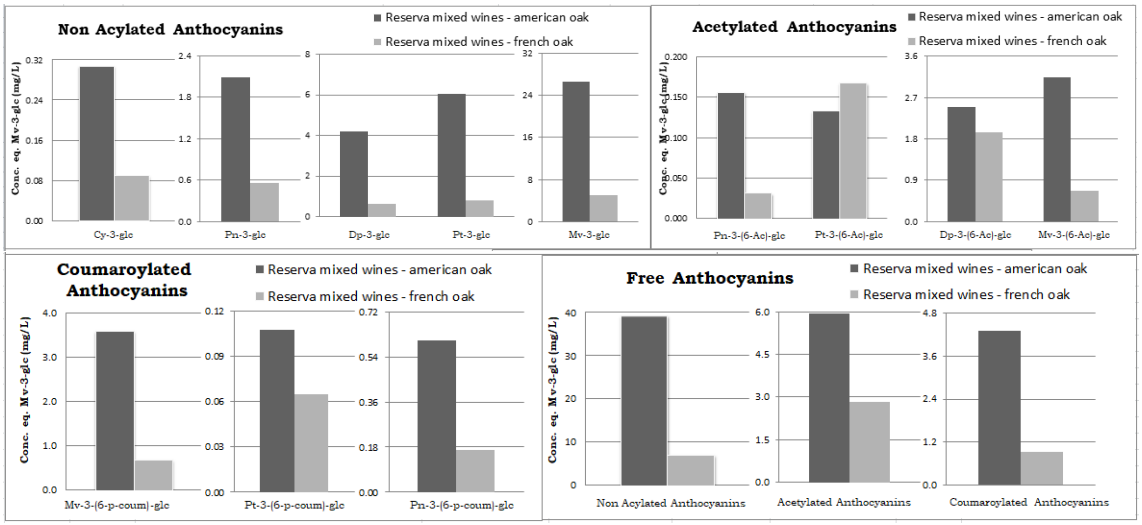



Figure Annex II.43 Grafics of the different families of free anthocyanins for *Reserva* mixed wines aged in American and French oak barrel from Rioja Baja.

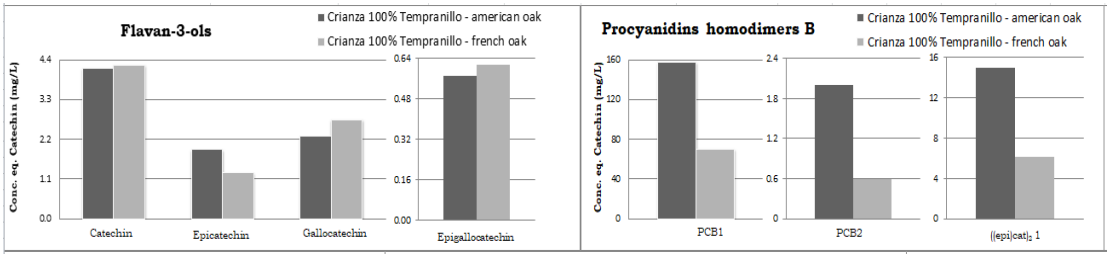



Figure Annex II.44 Grafics of the different families of tannins for 100% *Tempranillo Crianza* wines aged in American and French oak barrel from Rioja Alavesa and Alta.

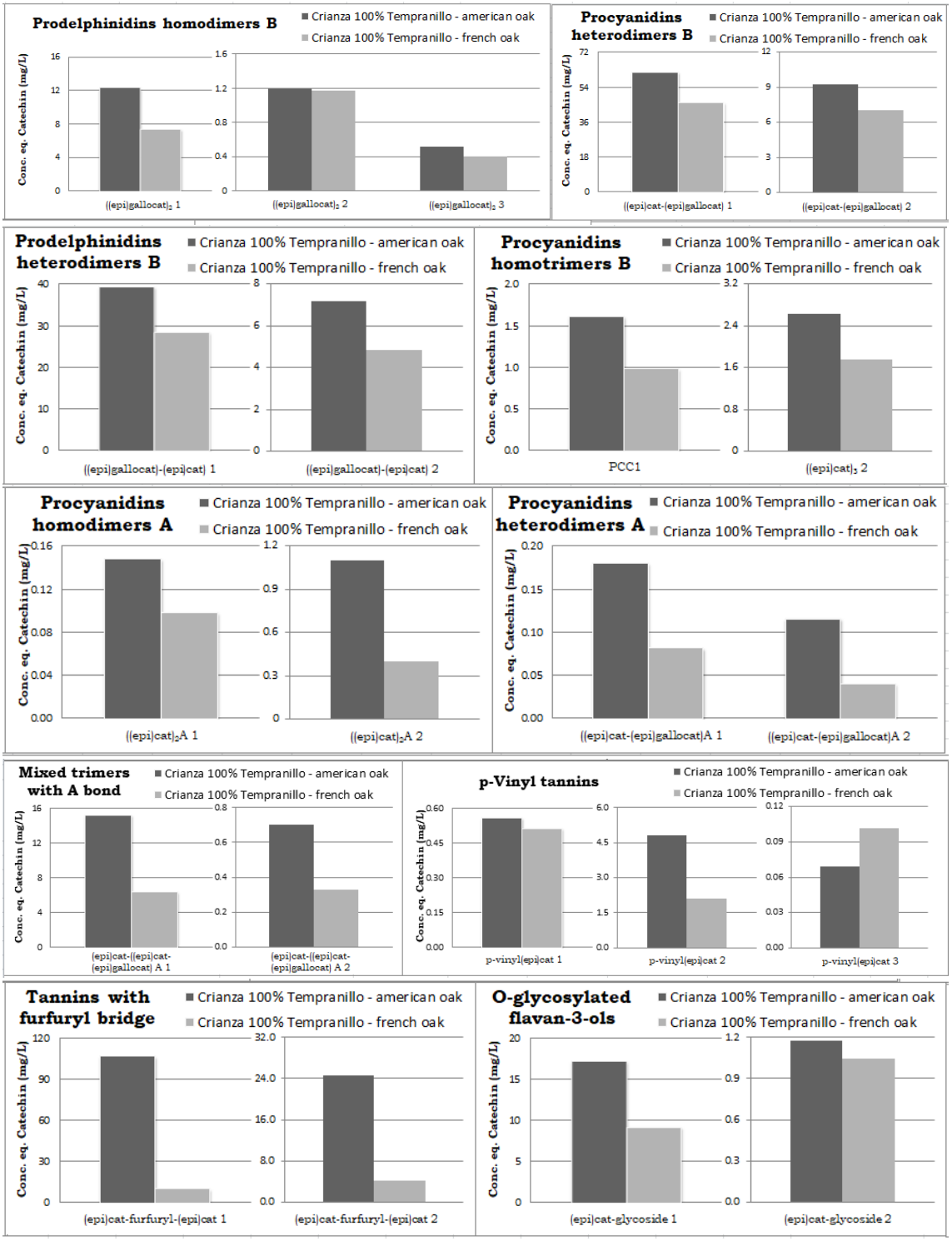



Figure Annex II.44 Cont.

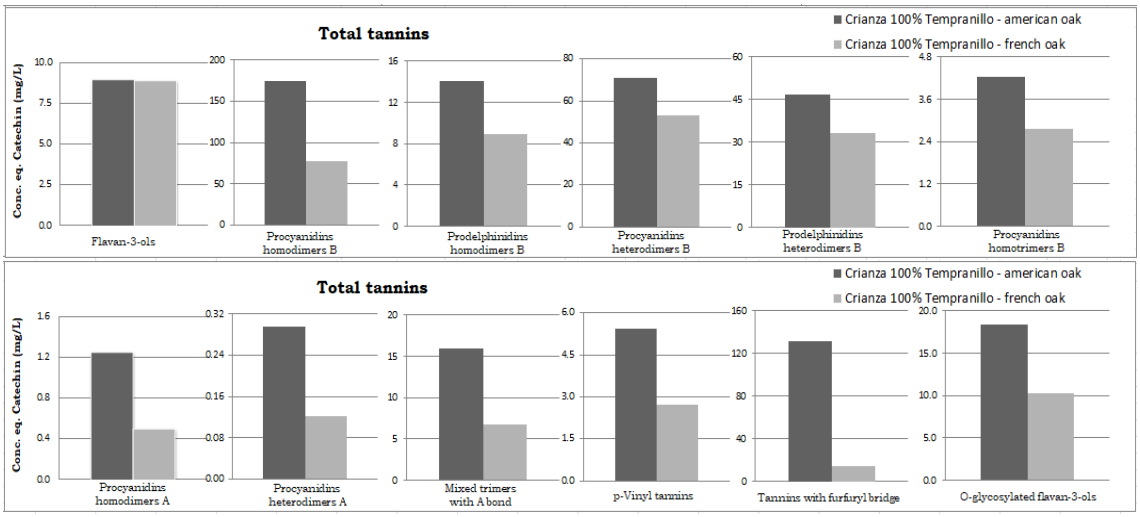



Figure Annex II.44 Cont.

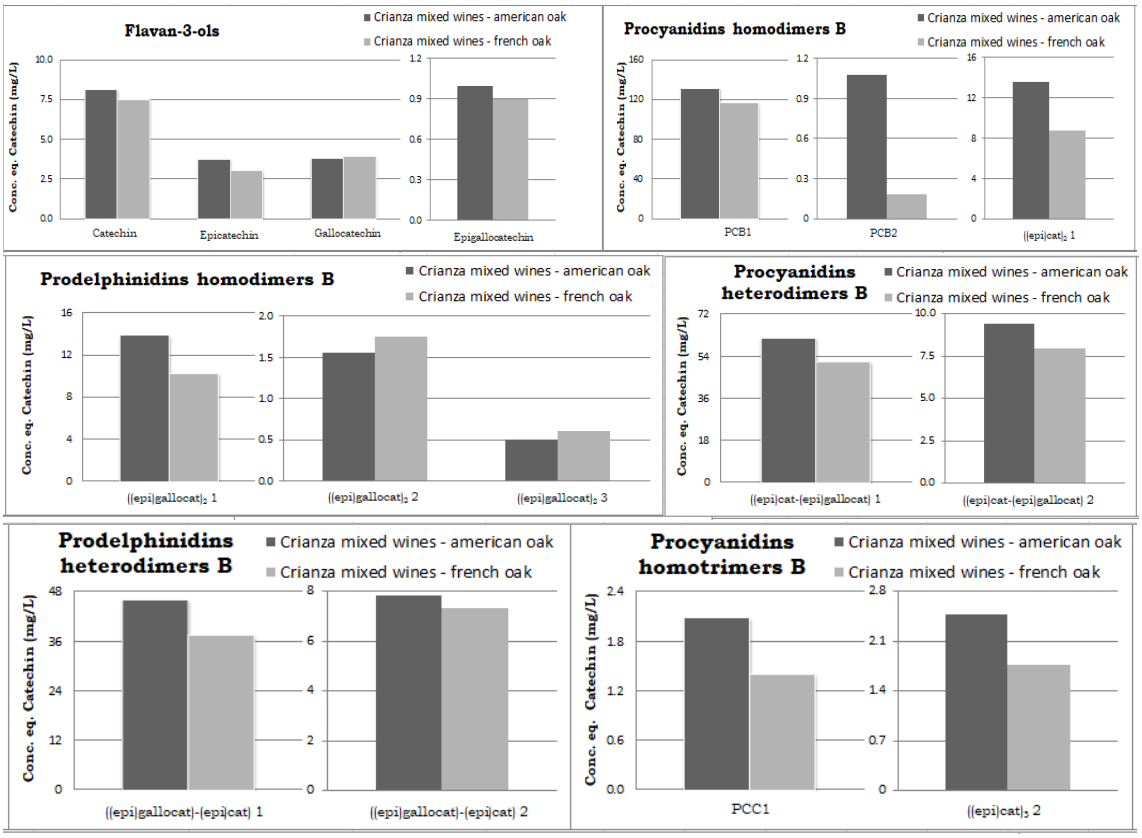



Figure Annex II.45 Grafics of the different families of tannins for *Crianza* mixed wines aged in American and French oak barrel from Rioja Alavesa and Alta.

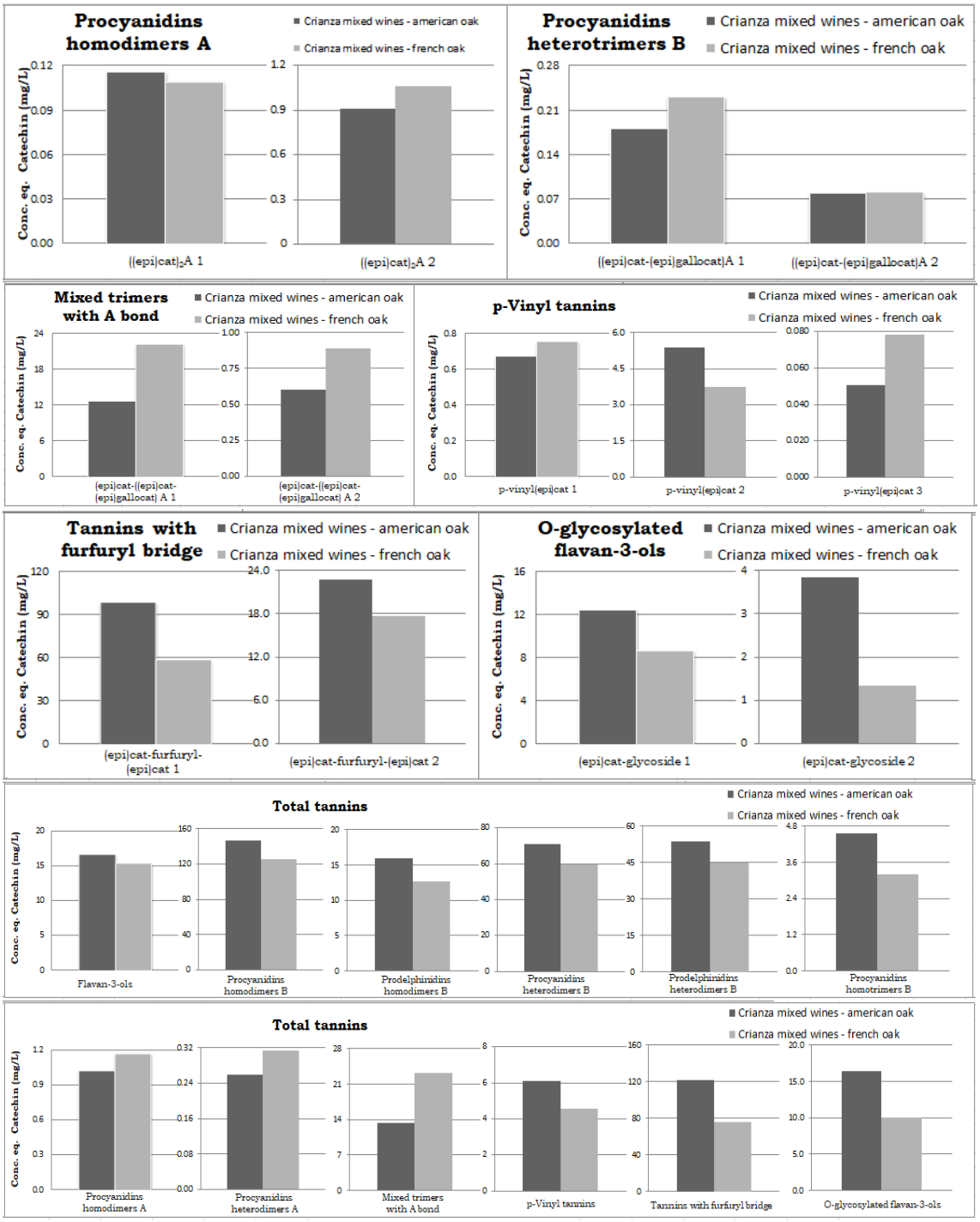



Figure Annex II.45 Cont.

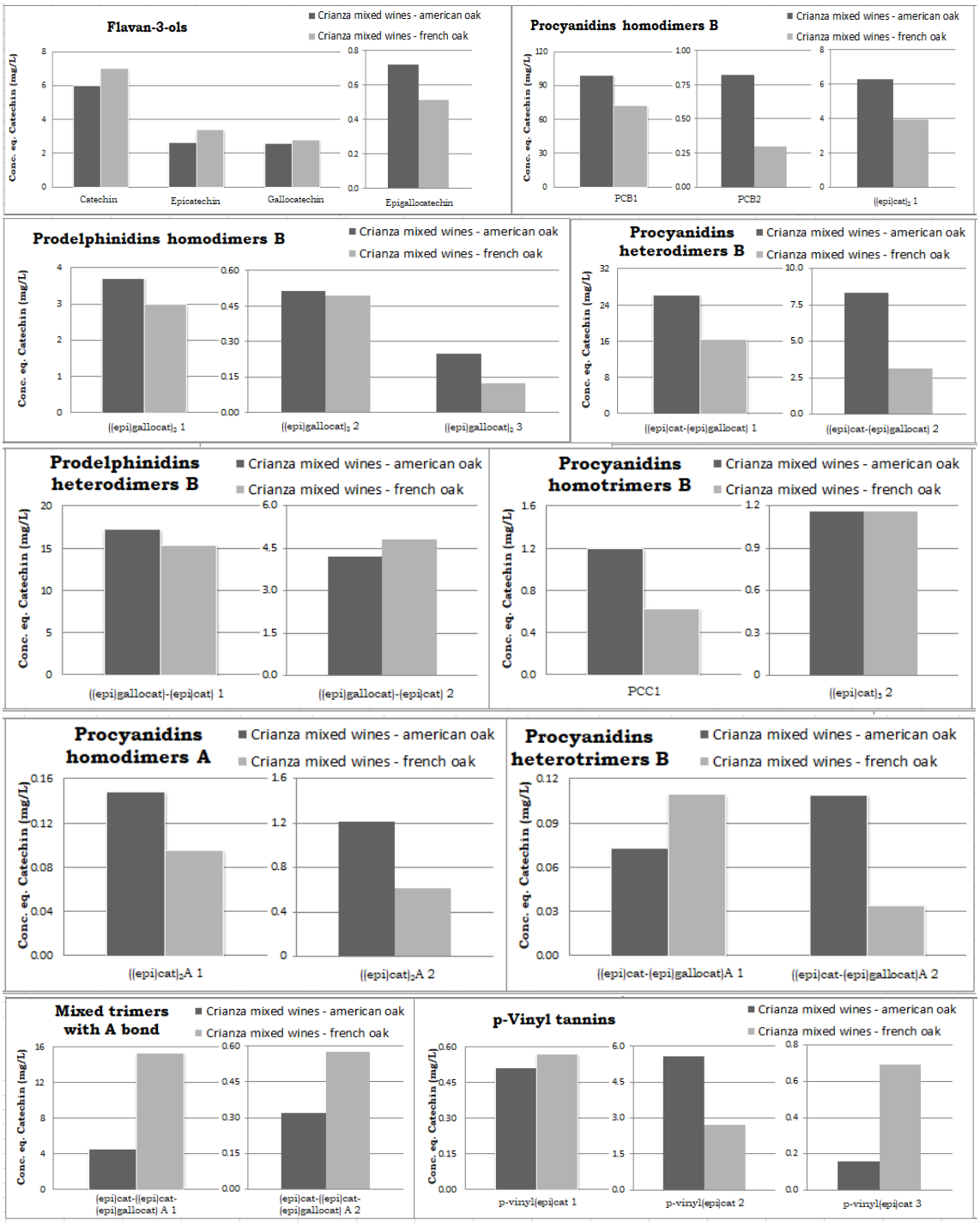



Figure Annex II.46 Grafics of the different families of tannins for *Crianza* mixed wines aged in American and French oak barrel from Rioja Baja.

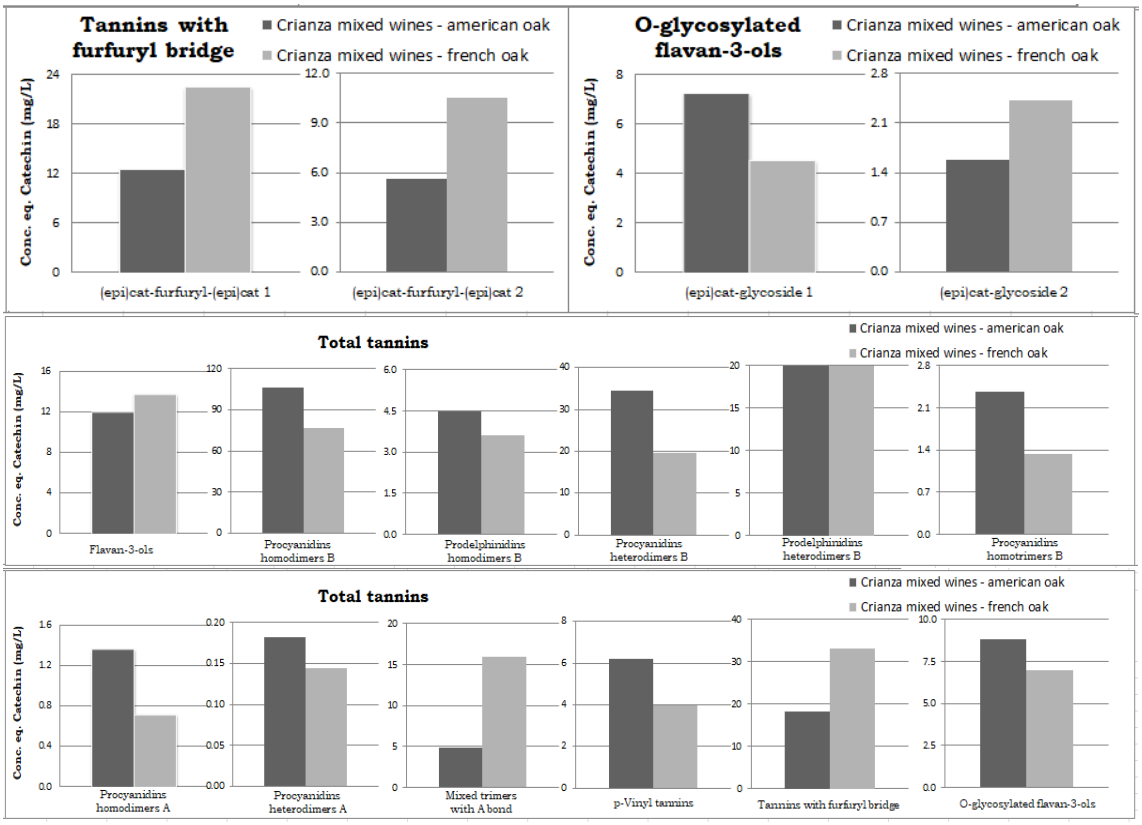



Figure Annex II.46 Cont.

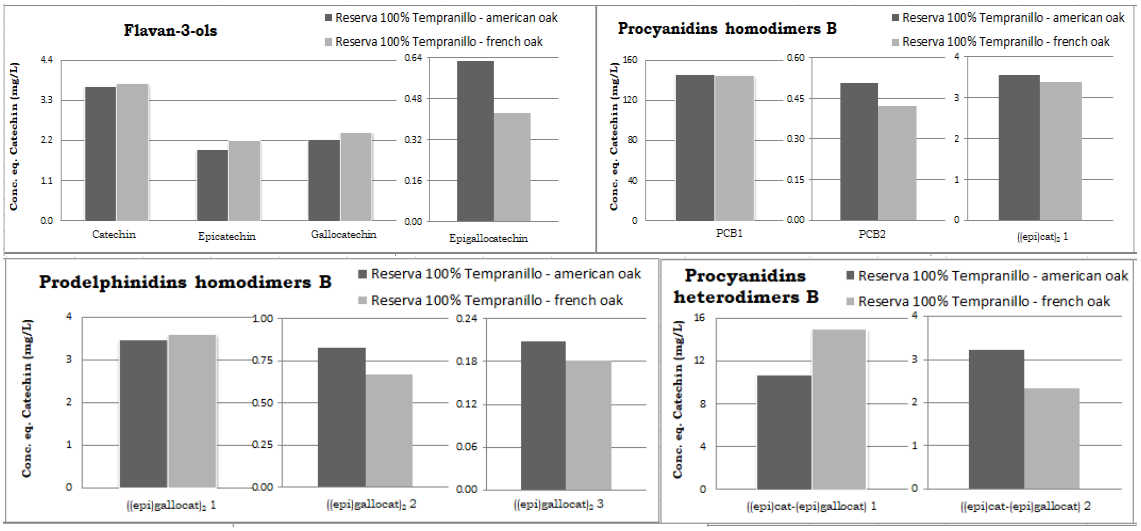



Figure Annex II.47 Grafics of the different families of tannins for 100% *Tempranillo Reserva* wines aged in American and French oak barrel from Rioja Alavesa and Alta.

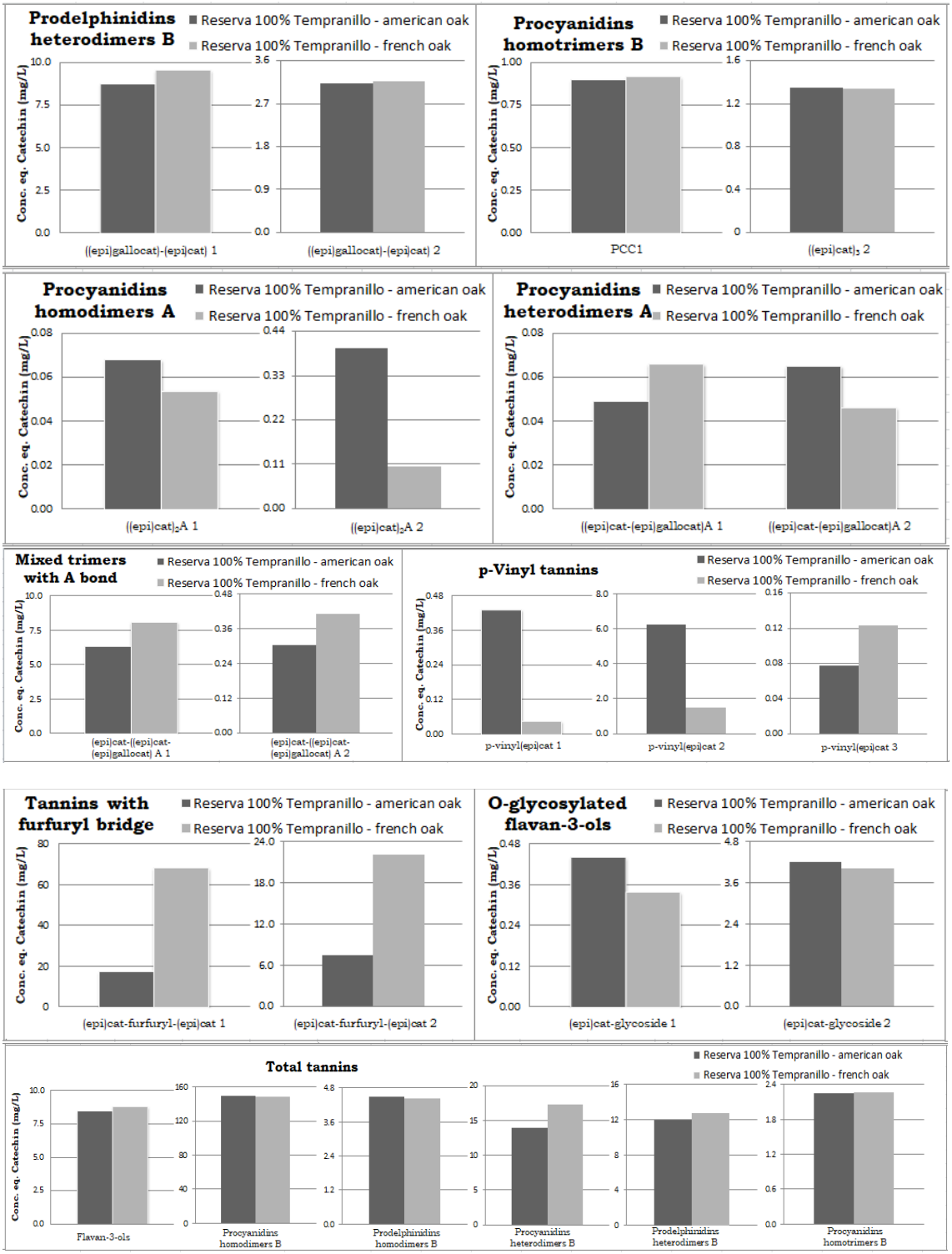



Figure Annex II.47 Cont.

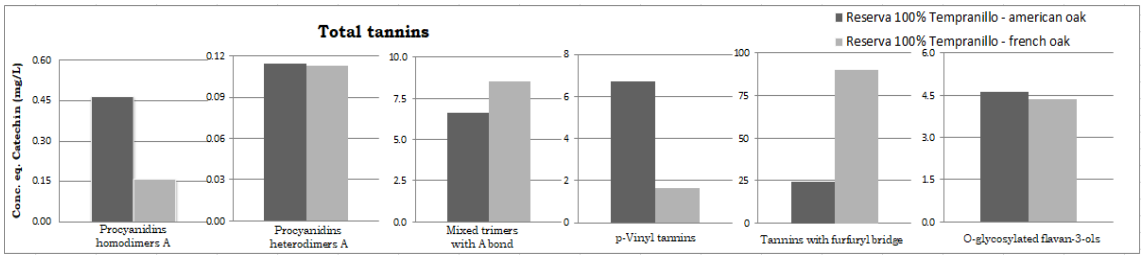



Figure Annex II.47 Cont.

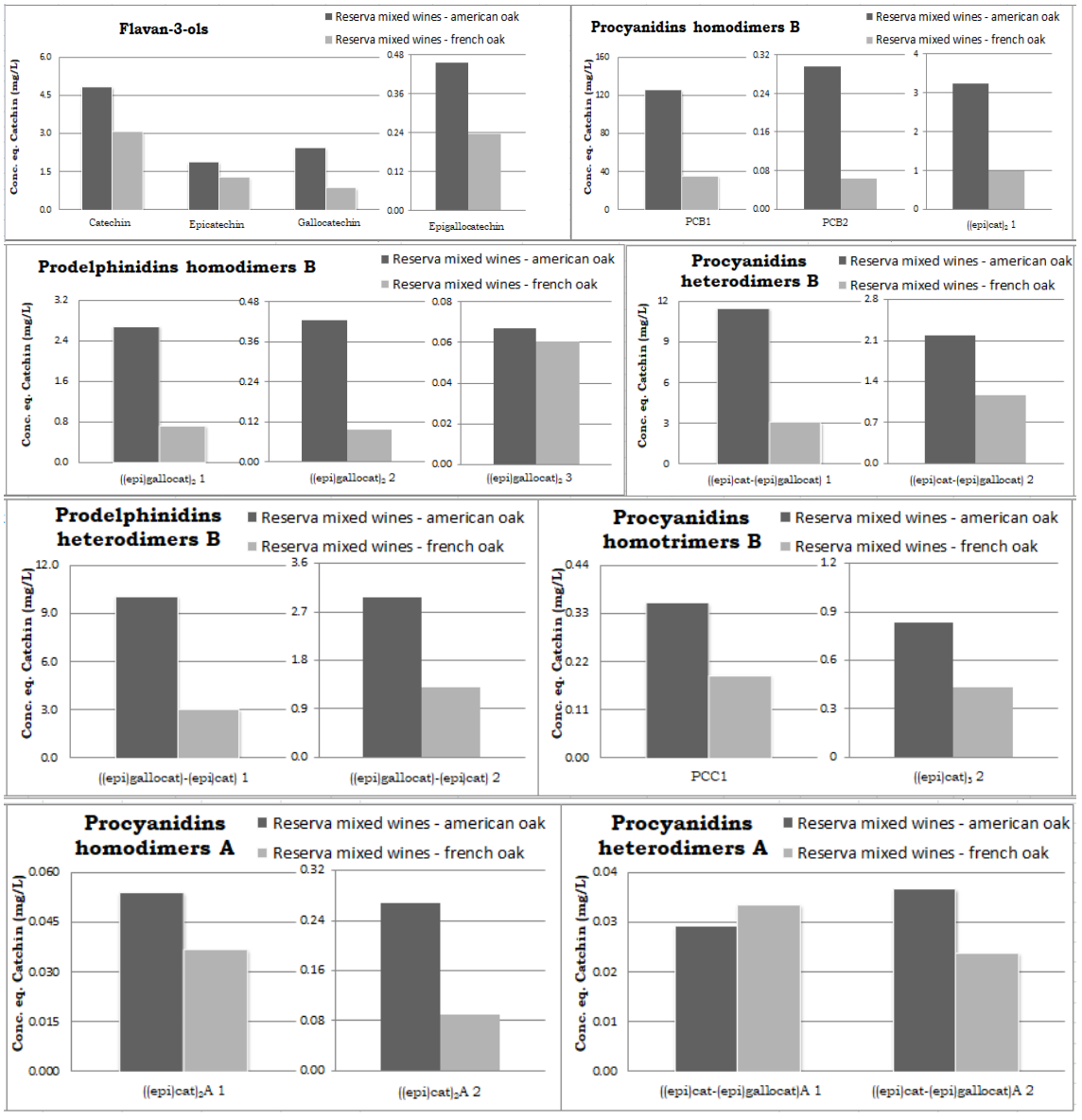



Figure Annex II.48 Grafics of the different families of tannins for *Reserva* mixed wines aged in American and French oak barrel from Rioja Alavesa and Alta.

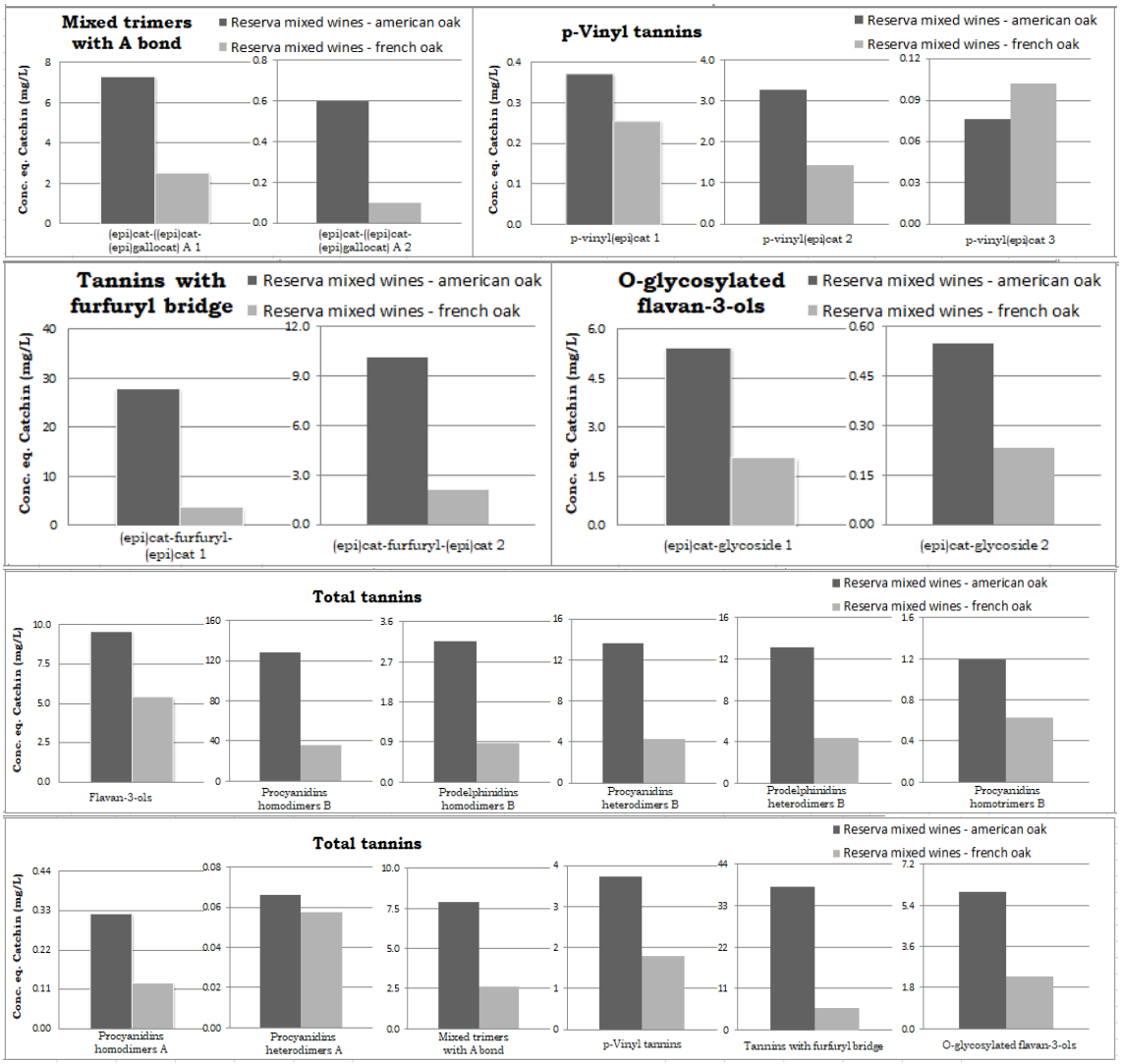



Figure Annex II.48 Cont.

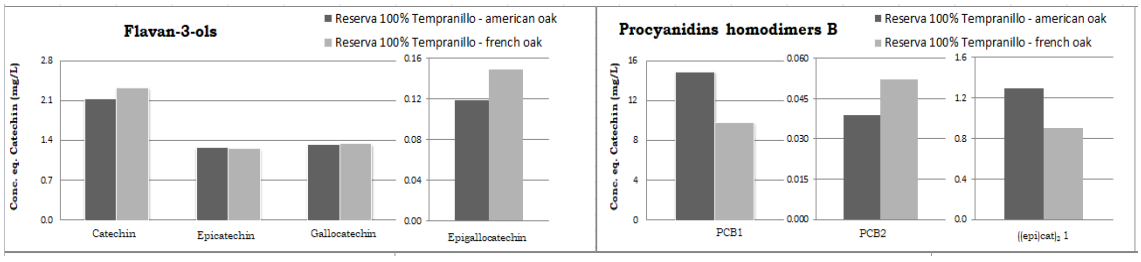



Figure Annex II.49 Grafics of the different families of tanninss for 100% *Tempranillo Reserva* wines aged in American and French oak barrel from Rioja Baja.

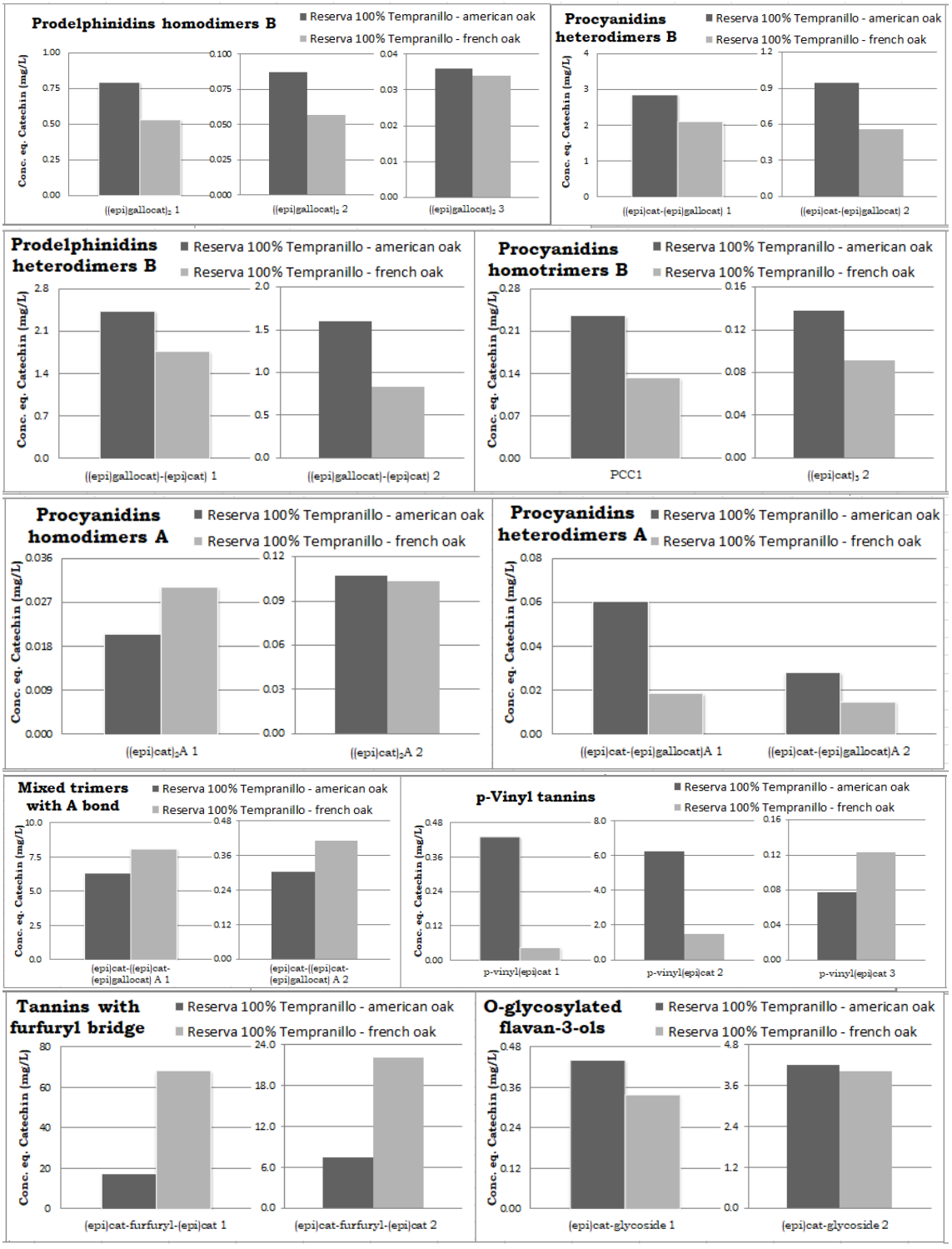



Figure Annex II.49 Cont.

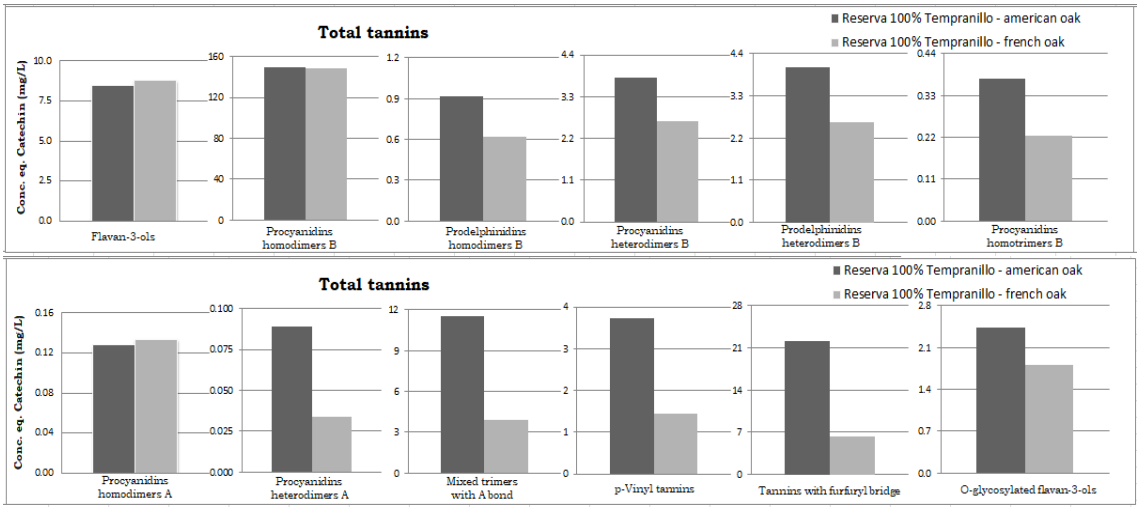



Figure Annex II.49 Cont.

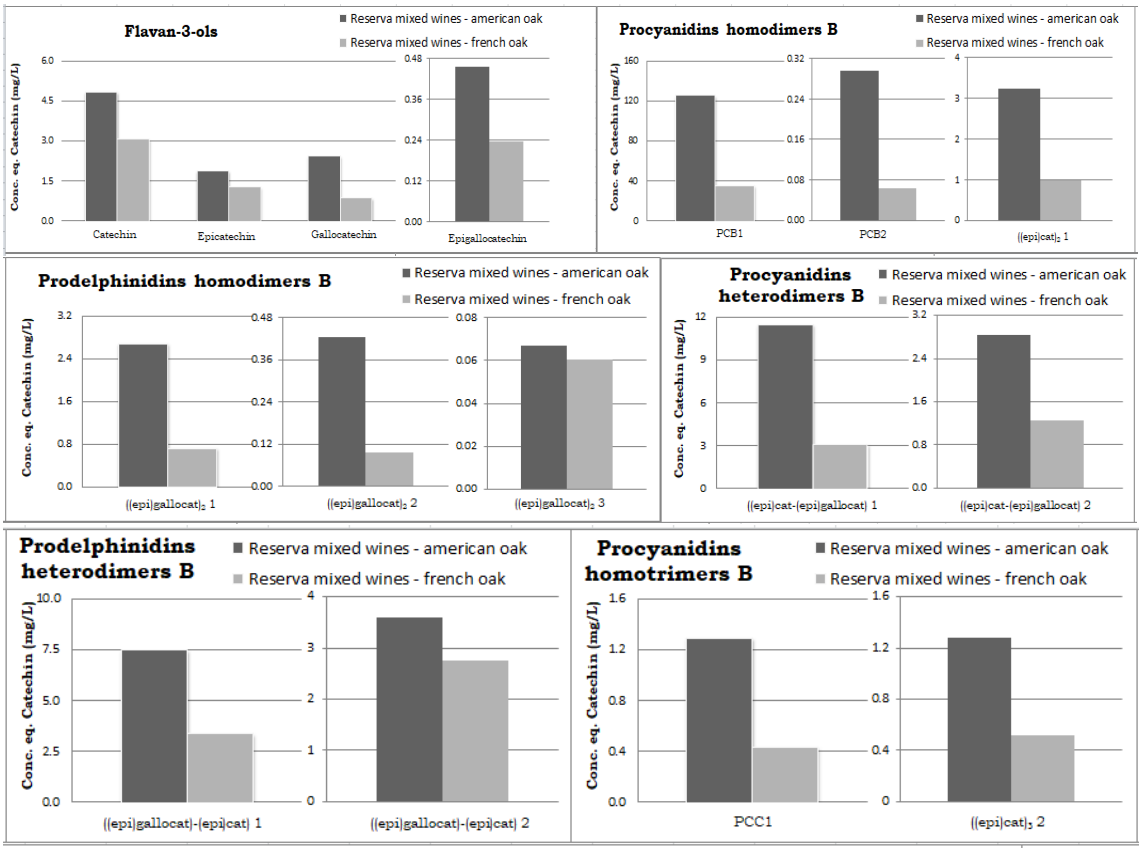



Figure Annex II.50 Grafics of the different families of tannins for *Reserva* mixed wines aged in American and French oak barrel from Rioja Baja.

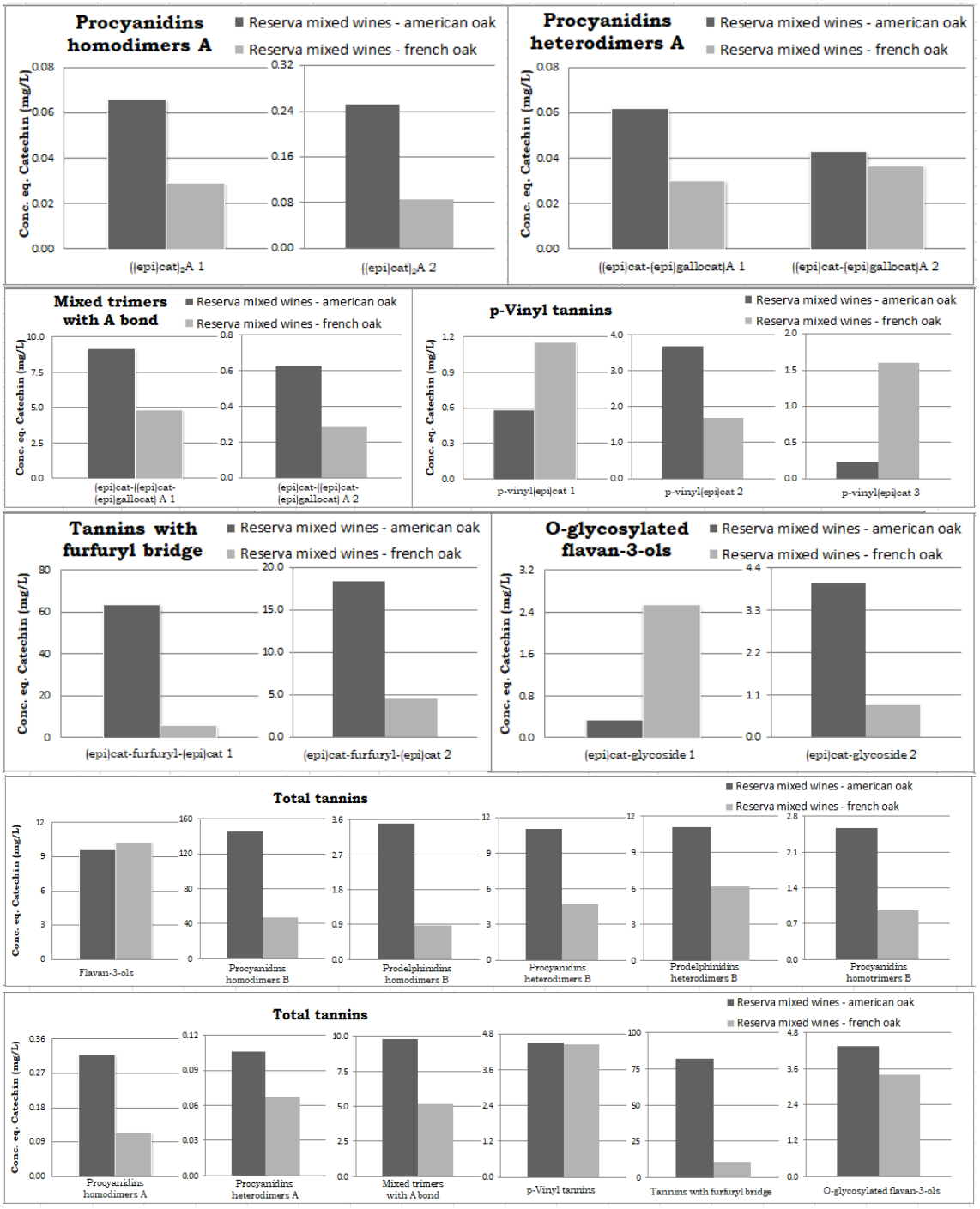



Figure Annex II.50 Cont.

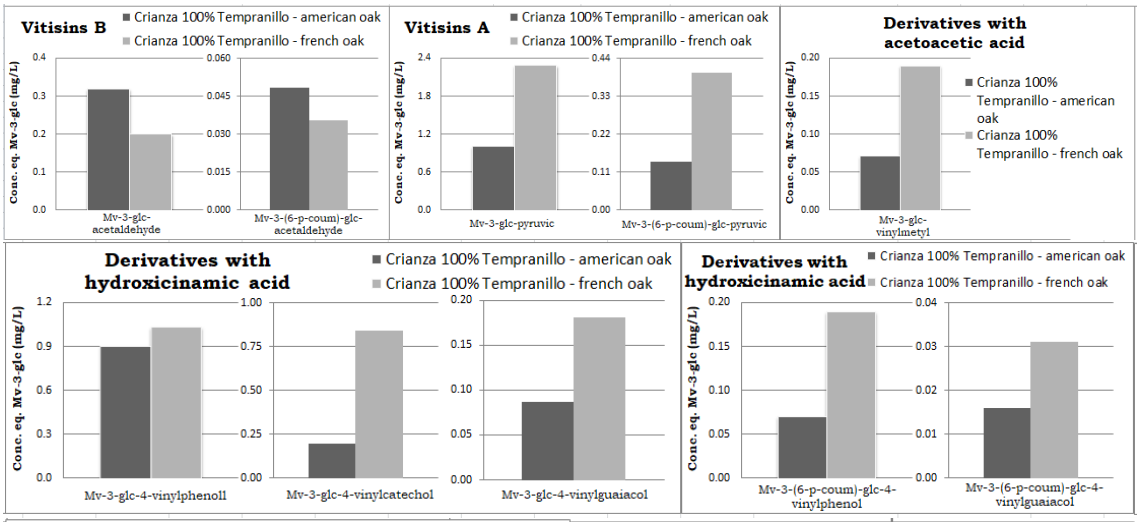
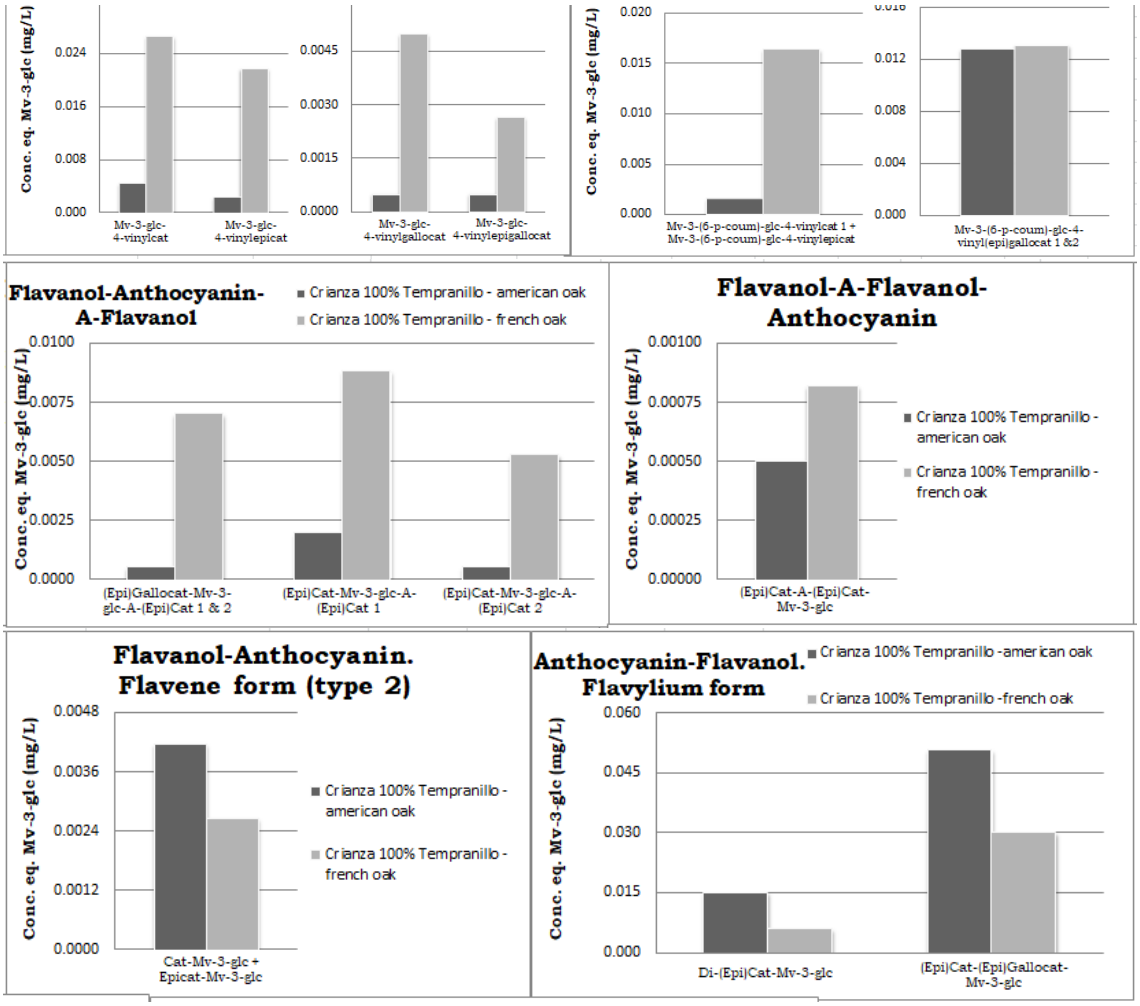



Figure Annex II.51 Grafics of the different families of anthocyanin derivatives for 100% *Tempranillo Crianza* wines aged in American and French oak barrel from Rioja Alavesa and Alta.



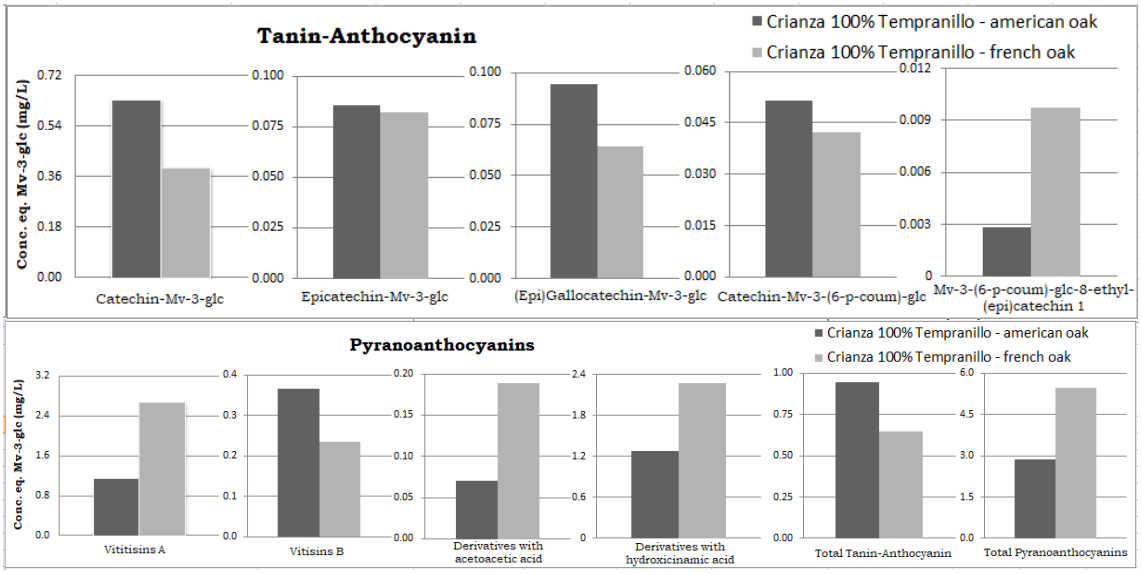
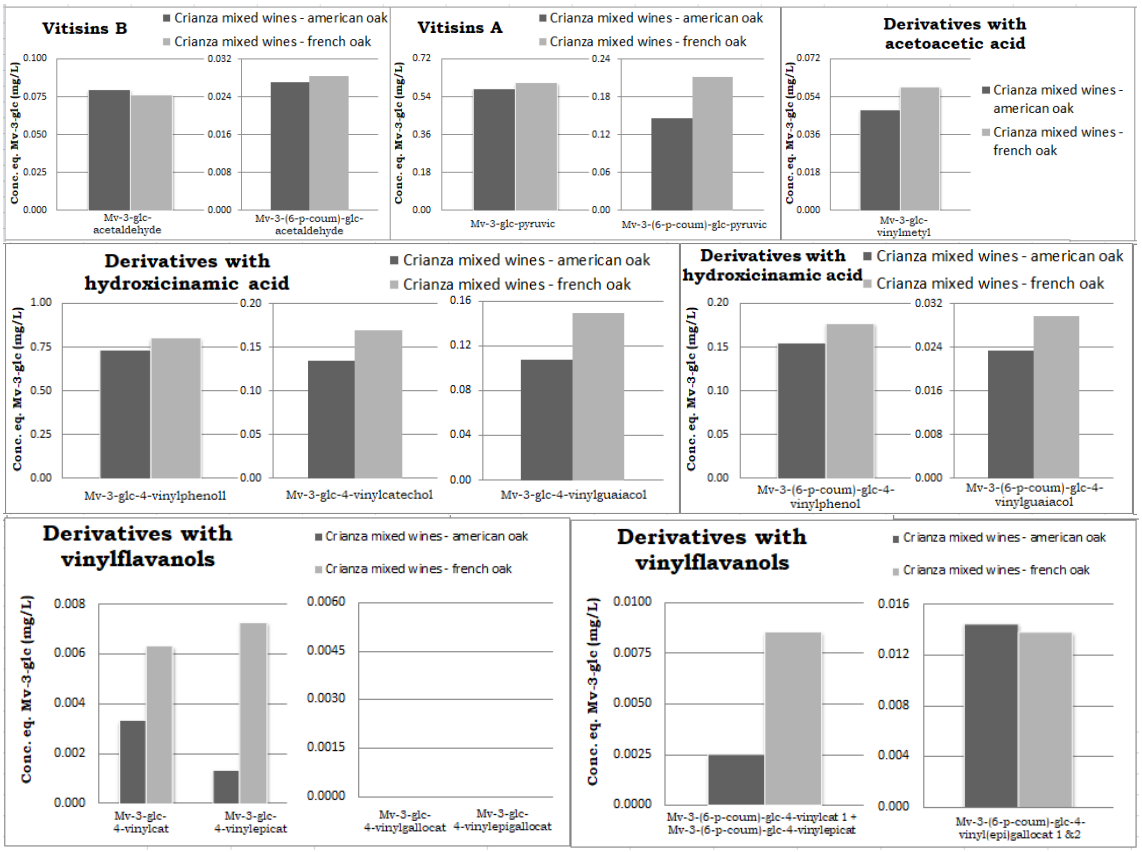



Figure Annex II.51 Cont.



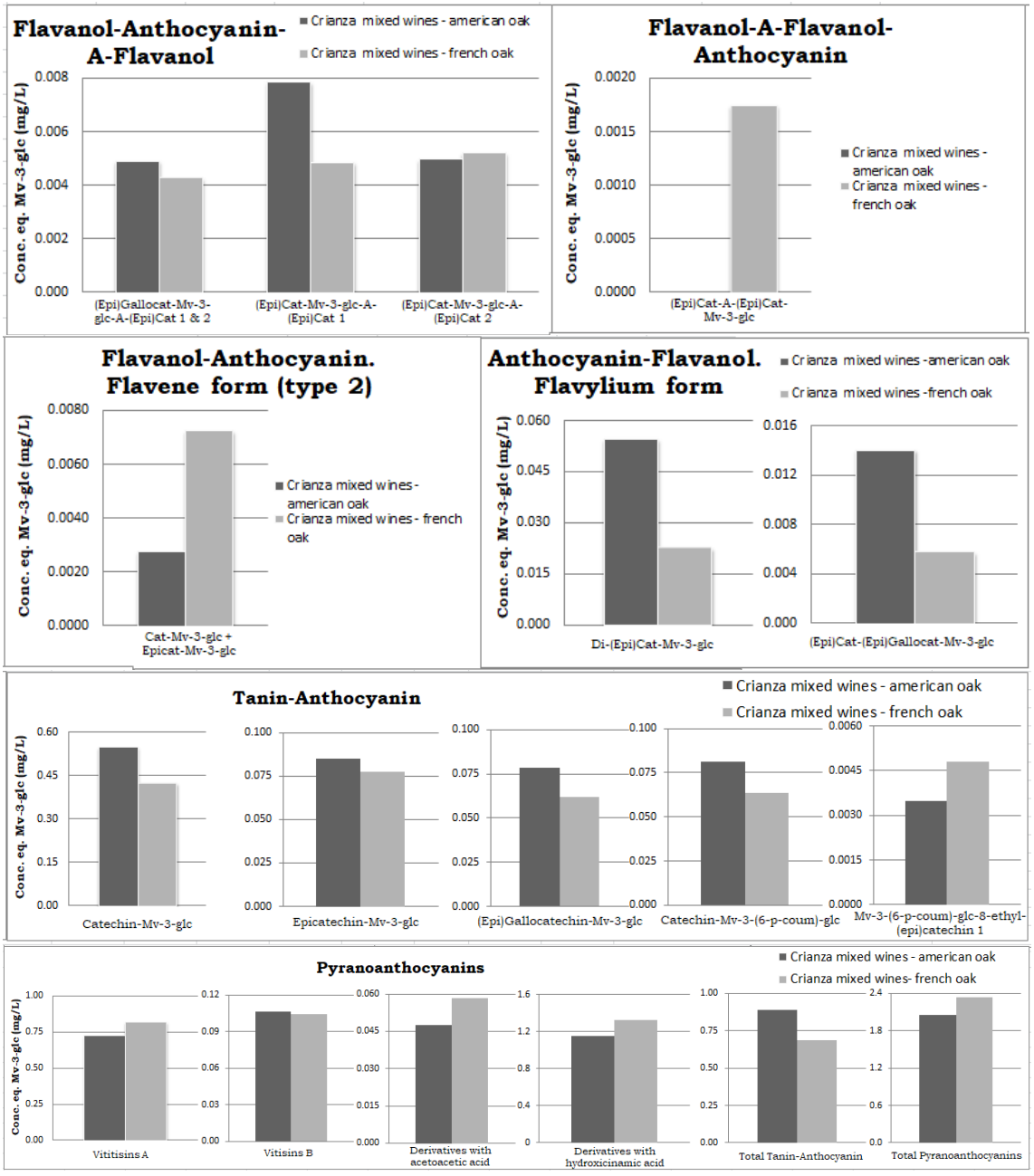



Figure Annex II.52 Cont.

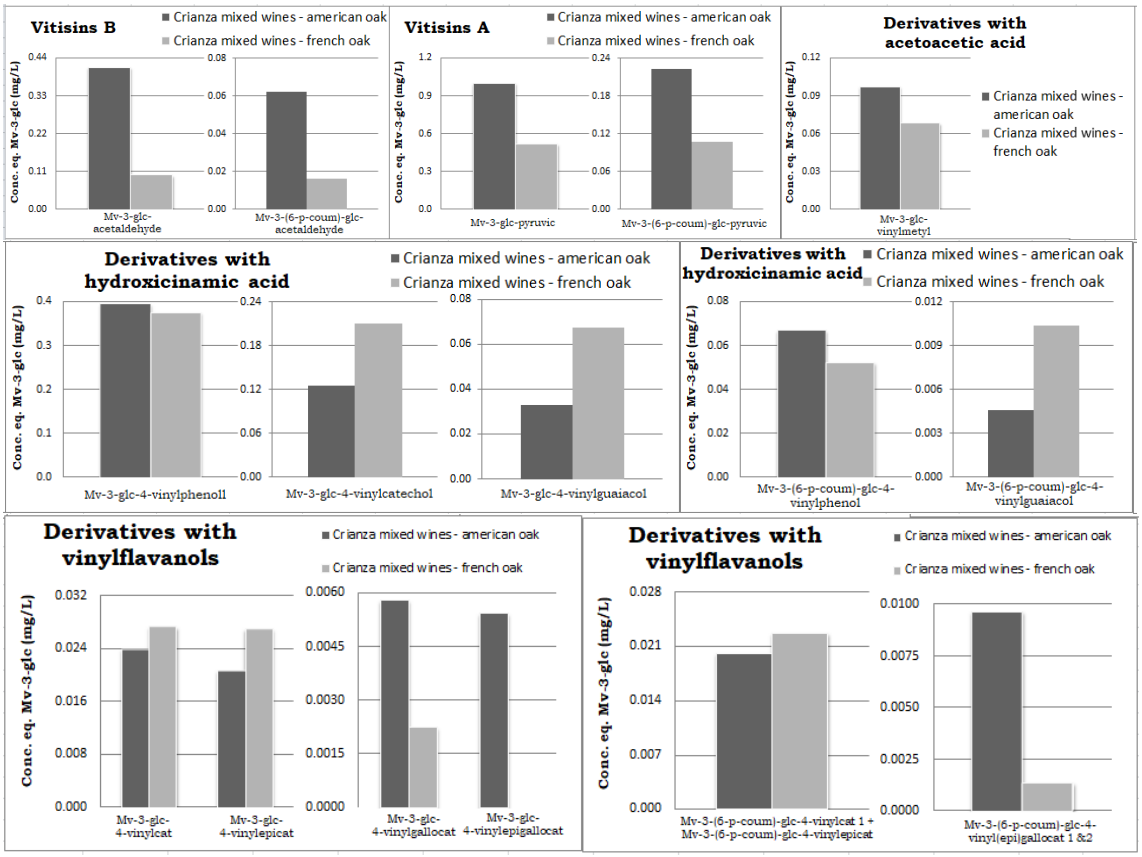
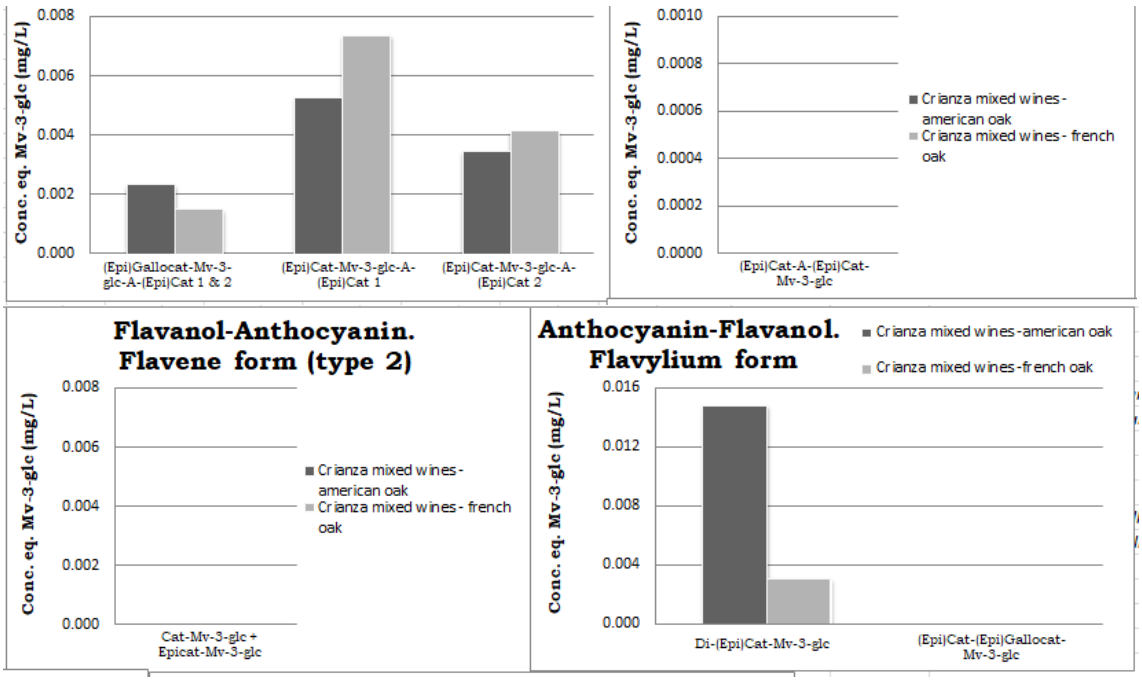



Figure Annex II.53 Grafics of the different families of anthocyanin derivatives for *Crianza* mixed wines aged in American and French oak barrel from Rioja Baja.



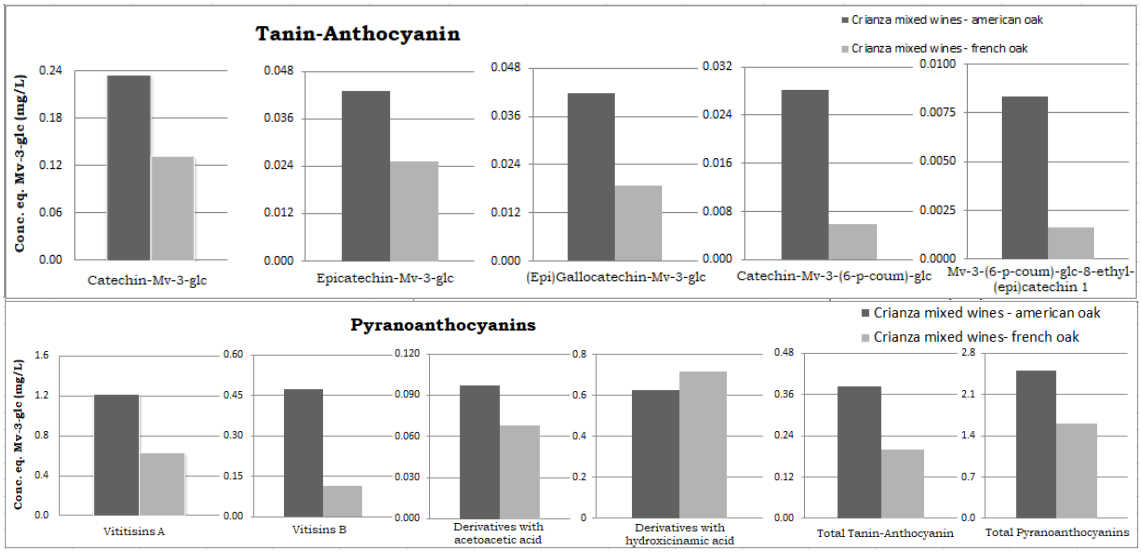
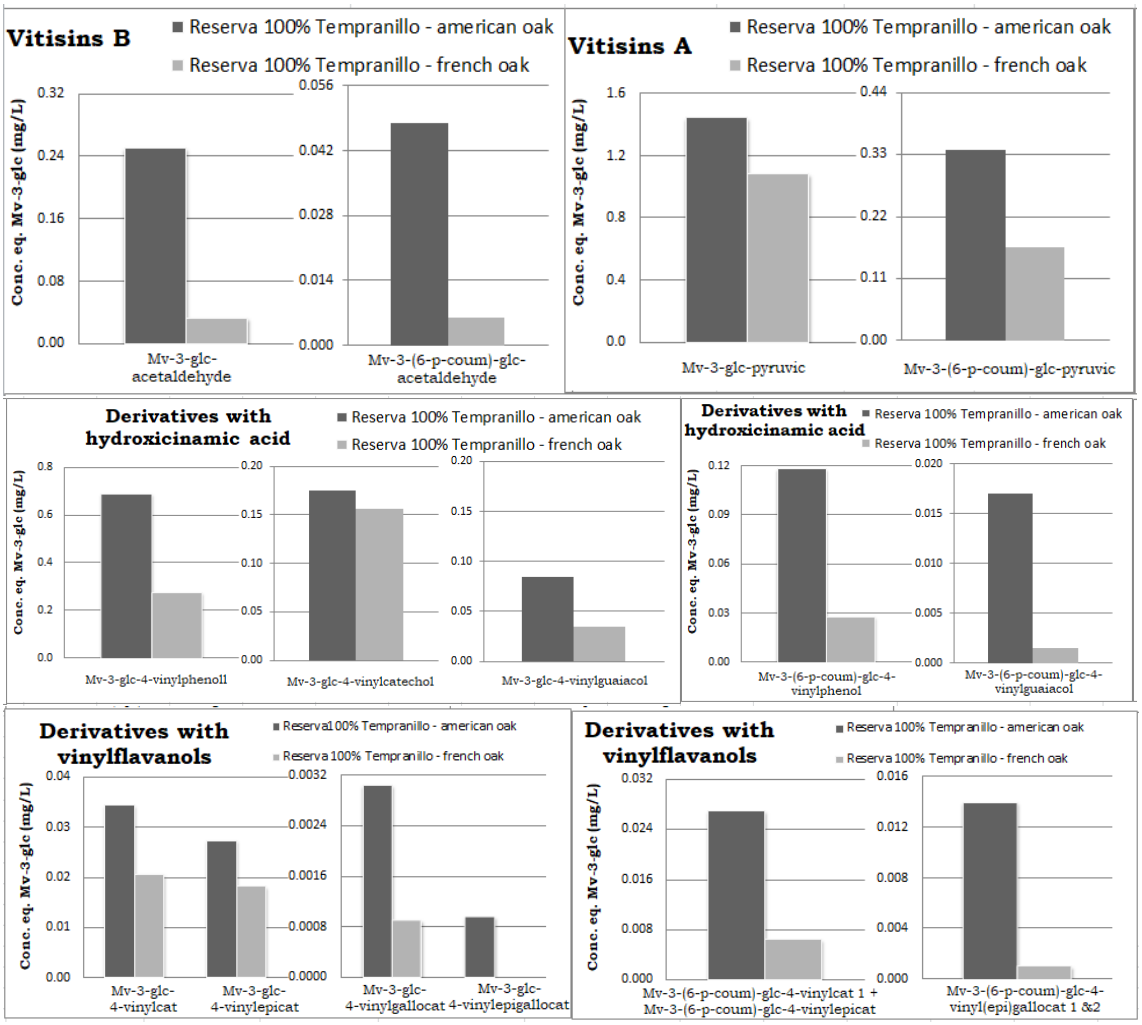



Figure Annex II.53 Cont.



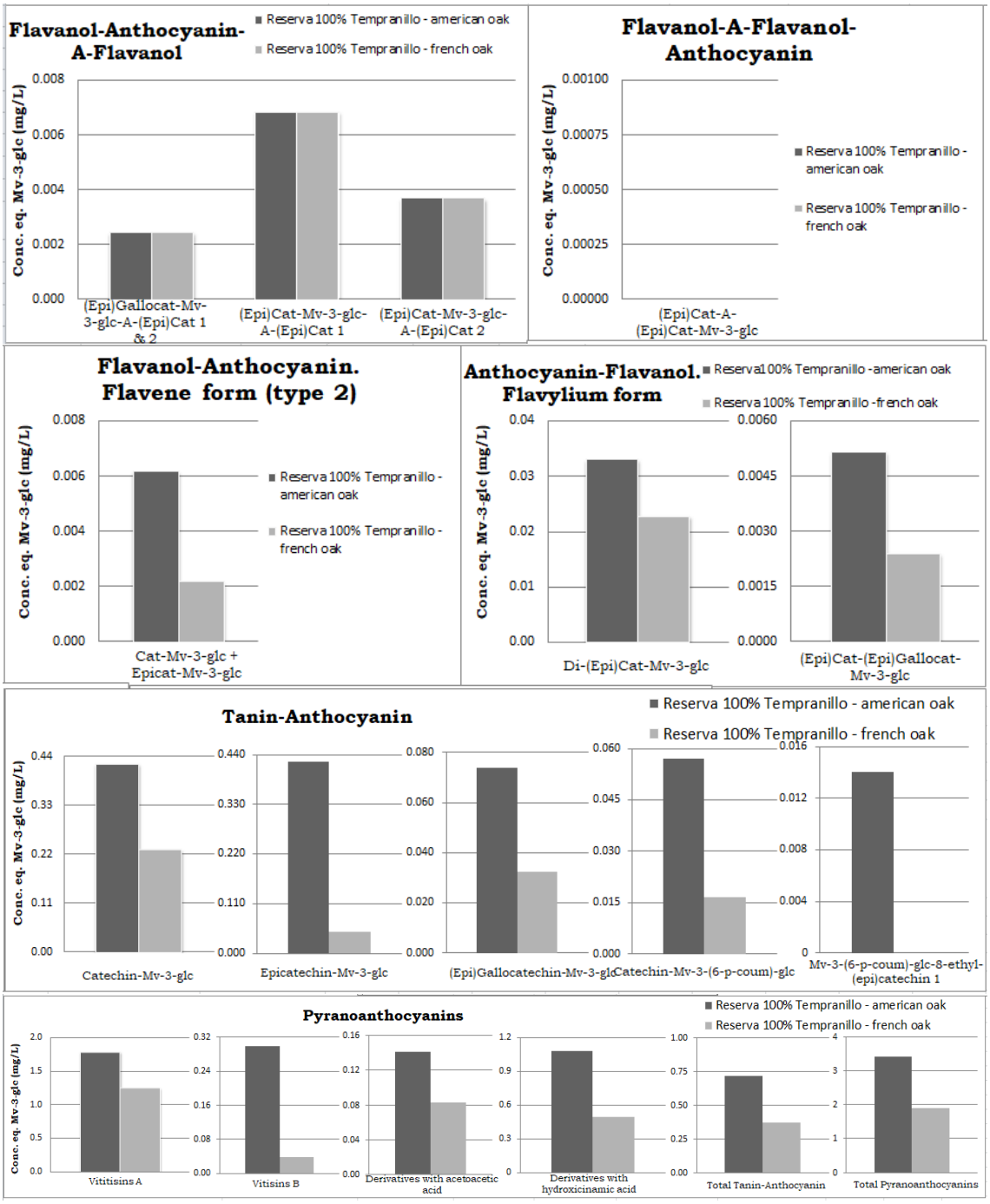



Figure Annex II.54 Cont.

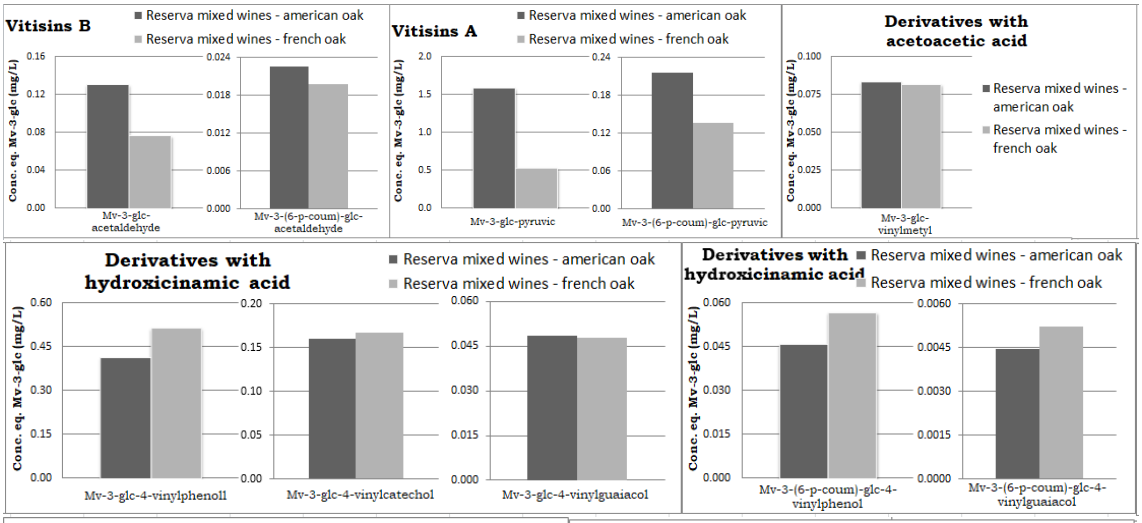
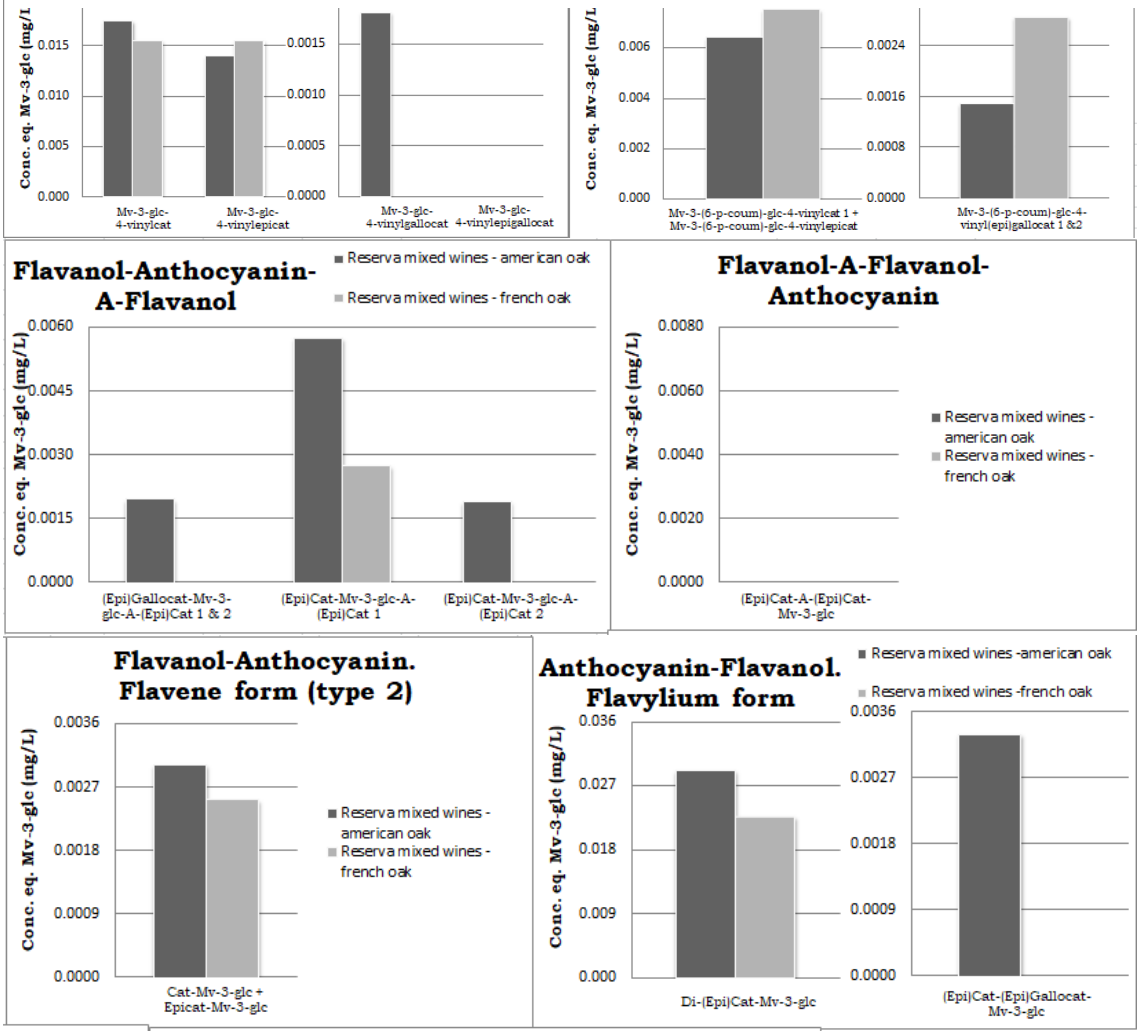



Figure Annex II.55 Grafics of the different families of anthocyanin derivatives for *Reserva* mixed wines aged in American and French oak barrel from Rioja Alavesa and Alta.



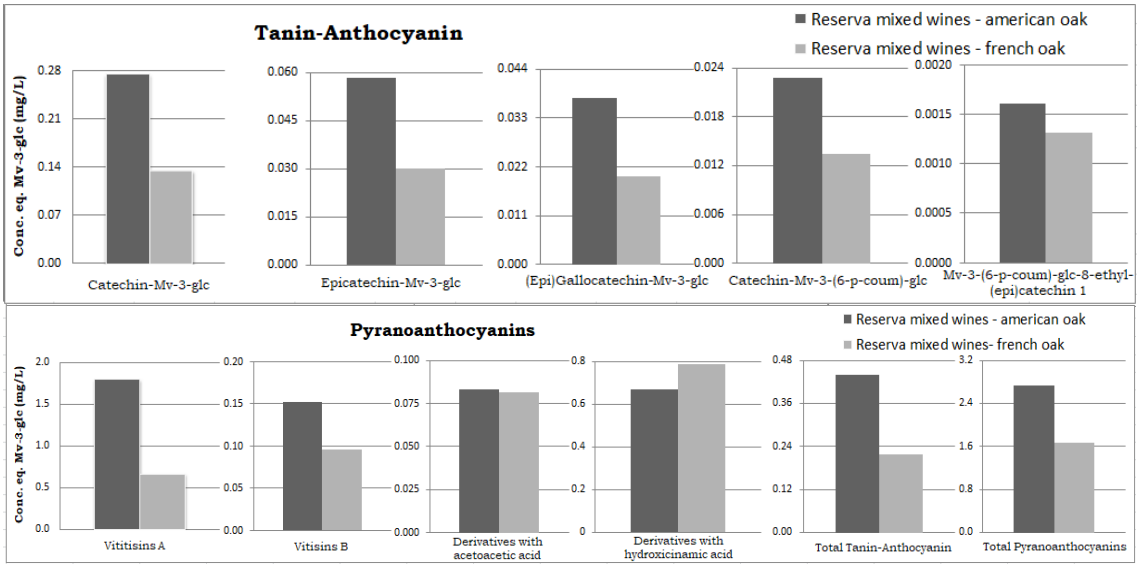
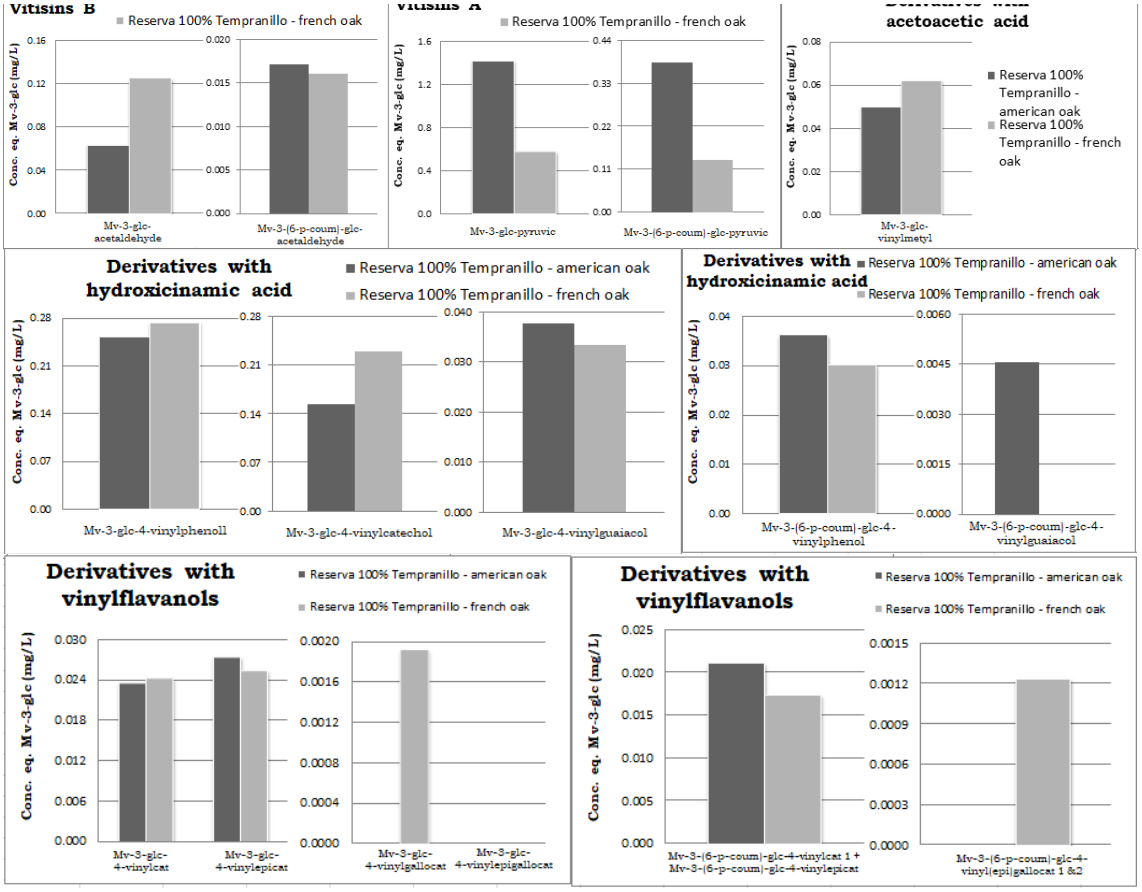



Figure Annex II.55 Cont.



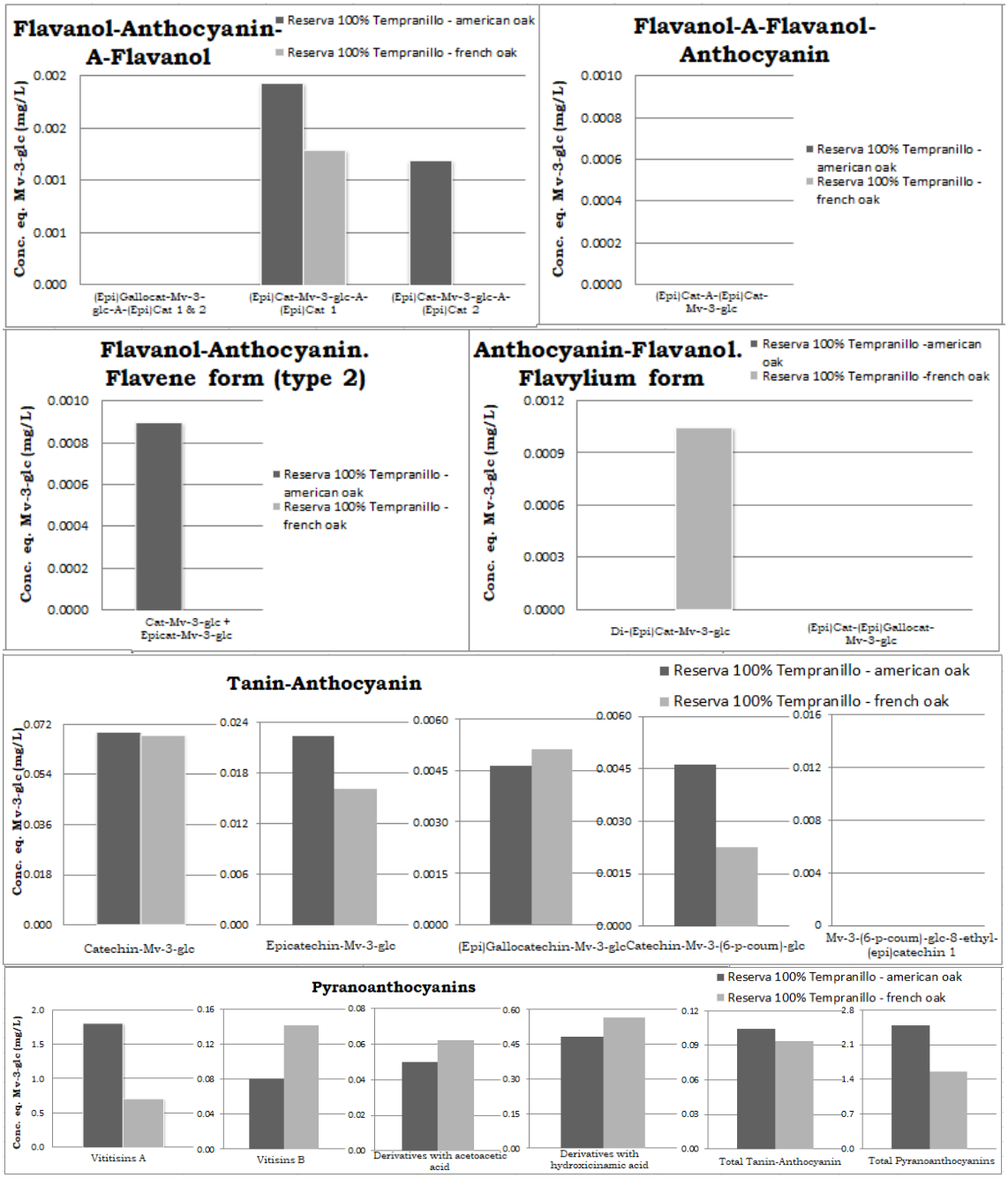



Figure Annex II.56 Cont.

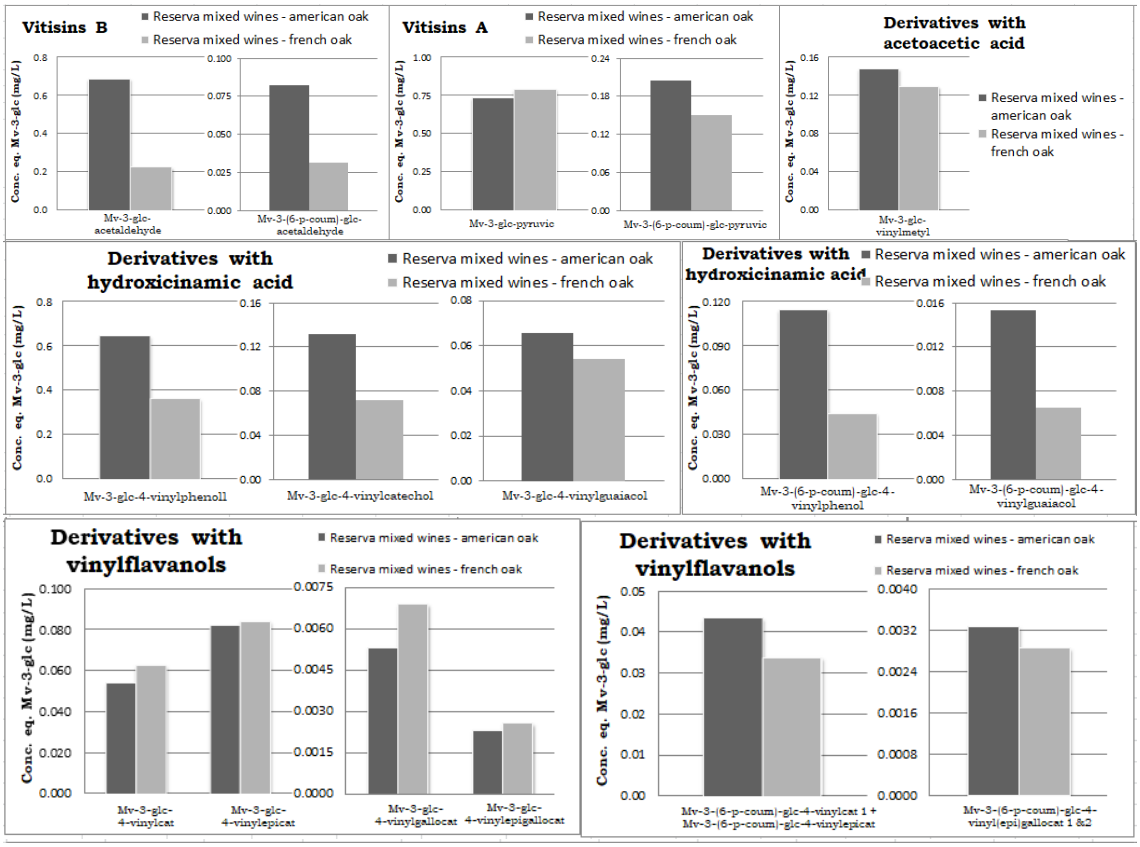
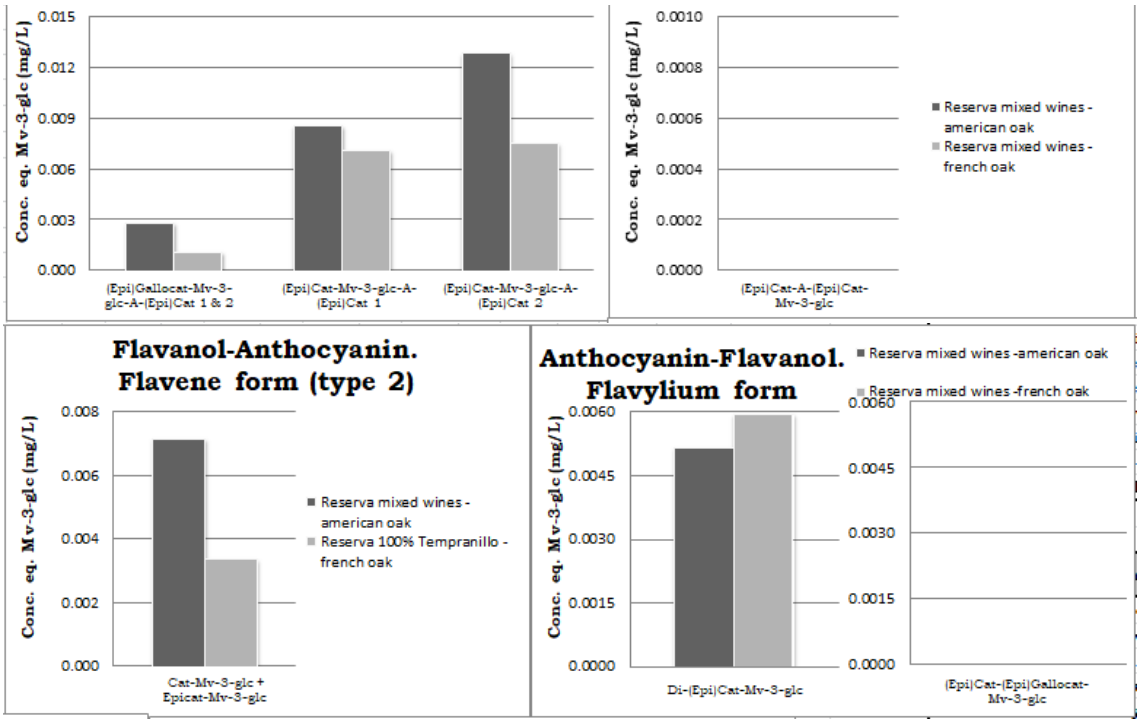



Figure Annex II.57 Grafics of the different families of anthocyanin derivatives for *Reserva* mixed wines aged in American and French oak barrel from Rioja Baja.



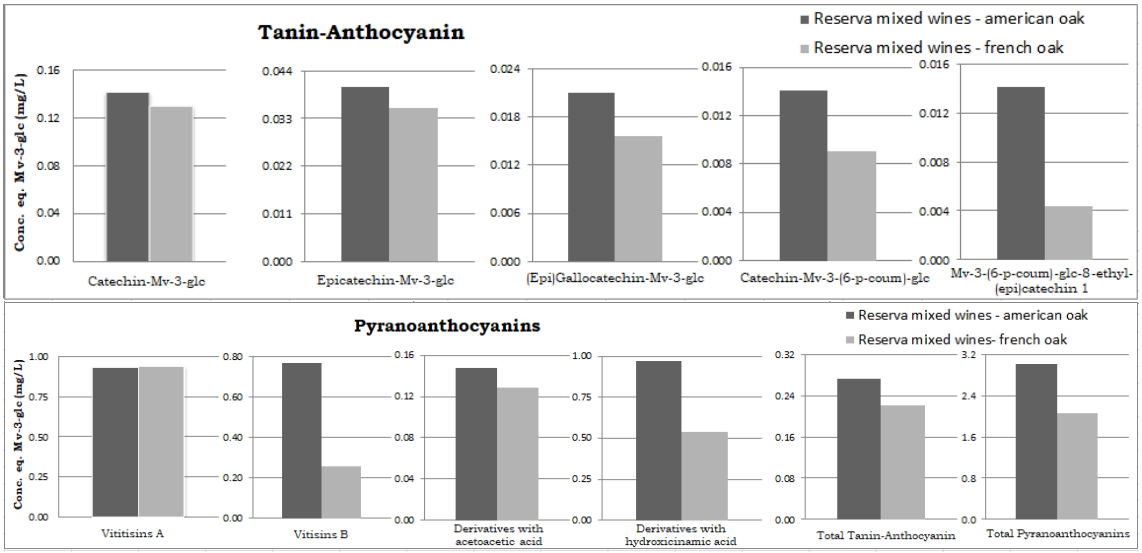



Figure Annex II.57 Cont.