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Evolution of main polyphenolics during cidermaking

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A R T I C L E I N F O

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A B S T R A C T

This work reports the evolution of polyphenolic compounds content during the cidermaking process in the Basque Country (Northern Spain). Fourteen monovarietal musts were obtained throughout three seasons (13 different, one repeated) using different apple cultivars from the Basque Country. Monovarietal musts were used to cover a wide range of polyphenolic content and to introduce variability. These musts were fermented and matured to obtain 14 monovarietal ciders. The evolution of the musts was monitored during 6–8 months by measuring the polyphenolic profile with an HPLC method throughout 4 or 5 samplings. Chlorogenic acid, 4-*p*-coumaroylquinic acid and (−)-epicatechin showed fluctuations during the alcoholic fermentation (10–40 days), followed by stabilisation. With phloretin 2′-*O*-xyloglucoside, an increase or a stable concentration was observed during the alcoholic fermentation followed by stabilisation. Tyrosol, absent in the initial musts, showed an increase during the alcoholic fermentation and became stable afterwards. These were the only general patterns observed. The rest of the phenolic compounds studied, such as procyanidin B1, procyanidin B2 and phloridzin, did not show any general evolution rule.

1. Introduction

Cider is an alcoholic beverage obtained by fermentation of apple juice and is popular in Europe, North America, Australia and South Africa (Lea, 2010, chap. 1). In Spain (southern Europe) there are two cider-producing areas: Asturias and the Basque Country. There is also a small production of Basque cider in Navarre (Fig. 1). In the Basque Country, natural cider is obtained by spontaneous fermentation of a mixture of musts of different varieties of apple. Alcoholic and malolactic fermentations (AF and MLF) are carried out, respectively, by indigenous yeasts and lactic acid bacteria (LAB). The addition of chemicals is kept to a minimum (sulphite, ascorbic acid) in order to maintain the organoleptic quality of the final product (del Campo, Berregi, Santos, Dueñas, & Irastorza, 2008).

Cider apple varieties contain relatively large amounts of polyphenolic compounds, which can be grouped into several classes (Guyot, Marnet, Laraba, Sanoner, & Drilleau, 1998): hydroxycinnamic acids and their derivatives (esters), flavan-3-ols, either monomeric (catechines) and oligomeric (procyanidines), dihydrochalcones, as well as smaller quantities of flavonols, benzoic acids and volatile polyphenols. These compounds experiment different patterns of evolution during the fermentation processes, but the ciders obtained are beverages rich in polyphenols (Coton, Coton, & Guichard, 2016).

Polyphenolic compounds, particularly procyanidins, are responsible of haze and sediment formation because of their interaction with proteins. (Siebert, 2006). They are also involved in browning processes by the action of polyphenol oxidases (Enzyme Commission number 1.14.18.1). These enzymes catalyse the oxidation of phenolic compounds into unstable ortho-quinones, which subsequently react to give colourless and brown products (Croguennec, 2016, chap. 6). From the organoleptic point of view, polyphenols give bitterness and astringency to the cider and make an important contribution to overall mouthfeel of the beverage, preventing it from becoming too insipid (Lea & Drilleau, 2003, chap. 4). Finally, polyphenols are natural antioxidants, which can prevent cell damages induced by free radicals, giving some protection against heart diseases and cancers (Cerit et al., 2017; Ganesan & Xu, 2017).

In the present work, 14 monovarietal musts were obtained throughout three seasons (13 different, one repeated) by using different apple cultivars from the Basque Country. Monovarietal musts were used to obtain polyphenolic profiles as different as possible. Spontaneous fermentation of these musts resulted in 14 monovarietal ciders. The evolution of the musts was followed for 6–8 months by measuring the concentration of the polyphenolic compounds throughout 4 or 5 samplings.

The evolution of polyphenolic contents during winemaking is an

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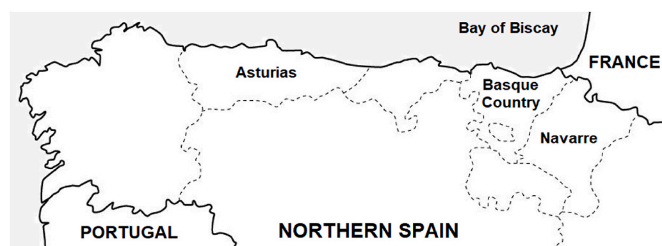


Fig. 1. Location of the Basque Country, Navarre and Asturias in northern Spain (south-west Europe).

extensively covered topic. However, only four articles have been published about the evolution of polyphenols in cidermaking: one about ciders of Asturias (Spain) (Picinelli, Suárez, García, & Mangas, 2000), another about ciders of Bretagne (France) (Nogueira et al., 2008) and two about Chinese ciders (Ye, Yue, & Yuan, 2014; Zuo et al., 2019). No article of this kind has been published about ciders of the Basque Country, and none of the previously mentioned works covers as much different musts as the present one does (fourteen). We therefore believe that this work is an important contribution in this field.

2. Material and methods

2.1. Chemicals and reagents

Avicularin was obtained from LGC Standards (Barcelona, Spain). Caffeic acid, catechol, chlorogenic acid, (−)-epicatechin, gallic acid, hydrocaffeic acid, *p*-coumaric acid, phloridzin, *p*-hydroxybenzoic acid, protocatechuic acid, tannic acid, (trans)-ferulic acid, and tyrosol were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). (+)-Catechin, isoquercitrin, hyperin and quercitrin were supplied by Apin Chemicals (Abingdon, UK). Procyanidin B1, procyanidin B2, procyanidin B5, 4-*p*-coumaroylquinic acid and phloretin 2′-*O*-xyloglucoside were provided by Polyphenols Biotech (Villenave d'Ornon, France). Acetic acid, acetonitrile, ethanol, Folin-Ciocalteu reagent, sodium carbonate and sodium hydroxide were obtained from PanReac Química (Castellar del Vallès, Barcelona, Spain). Methanol for high-performance liquid chromatography (HPLC) was purchased from Romil Ltd (Cambridge, UK). Enzymatic assay kits for the determination of L-malic and acetic acids were supplied by BioSystems S.A. (Barcelona, Spain). Ascorbic acid was provided by Apasa (Astigarraga, Gipuzkoa, Spain). Potassium metabisulfite was obtained from Laffort (Bordeaux, France).

Solutions were prepared with double-distilled water (hereafter, "water").

2.2. Obtaining the musts. Fermentation and maturation process

Throughout the whole article, the word *must* indicates freshly obtained or fermenting apple juice, and the word *cider* indicates the final beverage.

Apple musts were obtained from 300 kg lots of cider apples harvested in September–October 2012, 2013, and 2014 from different orchards located in northeast of the Basque Country (Spain). Each lot was washed and crushed separately and pressed in a pneumatic press (Bucher Vaslin, Chalonnes-sur-Loire, France), and the obtained must was introduced into a 150 L stainless steel tank where the fermentation took place. The temperature was kept between 14 °C and 18 °C. AF and MLF were spontaneously carried out by indigenous microflora. The evolution of AF was monitored by the decrease in density and the MLF by the decrease in L-malic acid content (see subsections 2.4 and 2.7 for methods). The tanks were kept unsealed until the end of AF (density ≈ 1000 g/L). They were then hermetically sealed and connected to a CO_2 cylinder with an overpressure of 2.0×10^4 Pa, in order to protect the ciders from oxidation. After the end of MLF (L-malic acid concentration <0.5 g/L), ciders were racked to eliminate the sediments deposited at the bottom of the tanks. Some months later (2–5), and following the usual practice in Basque Country, 20 mg/L of potassium metabisulphite were added to each tank, as a preservative, and 200 mg/L of ascorbic acid were added to avoid browning of the ciders. Three weeks later, the ciders were bottled. To ensure the absence of organoleptic defects, a sensory analysis was performed on all ciders by a tasting panel of the Fraisoro Agro-Environmental Laboratory (Zizurkil, Basque Country, Spain).

All musts were monovarietal, obtained from the Basque cultivars *Aritza, Frantzes, Gezamina, Goikoetxea, Manttoni, Merabi, Moko, Narbarte-Gorria, Txalaka, Udare-Marroi, Urdin, Urtebi-Haundi* and *Urtebi-Txiki*. These 13 varieties are widely used in the Basque Country to elaborate cider, with the exception of *Narbarte-Gorria*, an extremely acidic and phenolic variety that was found in a private orchard. The considered cultivars covered a wide range of acidity and polyphenolic content, which introduced variability in the study. The varieties used in the 2012 season were *Gezamina, Goikoetxea, Moko, Txalaka, Urtebi-Haundi* and *Urtebi-Txiki*; in the 2013 season, *Merabi, Narbarte-Gorria, Urdin and Urtebi-Txiki*; in the 2014 season, *Aritza, Frantzes, Manttoni,* and *Udare-Marroi*. In the case of *Narbarte-Gorria*, only a few apples were available; thus, 40 L of must could be obtained, which was introduced in a 50 L stainless steel tank. A total of 14 musts were studied, 13 different and one repeated in two seasons: *Urtebi-Txiki*. This repetition was done because of the interesting organoleptic properties of the cider obtained.

When AF became stuck (*Moko*), 30 g/hL of commercial yeasts were added to restart it (*Saccharomyces cerevisiae*, Darnstar Ferment AG, Zug, Switzerland). In the cases where MLF became stuck (*Moko*, *Merabi, Narbarte-Gorria* and *Urtebi-Txiki*-2), it was restarted by adding 5 g/hL of commercial LAB (*Oenococcus oeni*, Chr. Hansen Holding A/S, Hørsholm, Denmark) and increasing the temperature of the tank up to 20 °C, until the end of the fermentation. In the case of *Narbarte-Gorria*, increasing the temperature up to 20–25 °C was sufficient, and no commercial LAB were added. Stuck AF or MLF considerably lengthened the time of fermentation and maturation.

The entire procedure described in this section is based on the usual practices of cider factories in the Basque Country, except that they use a mixture of different apple varieties to obtain the initial musts. Monovarietal musts were used in this work to cover a wider range of polyphenolic content.

Throughout the whole article, the musts or ciders are named after the name of the cultivar. In the case of *Urtebi-Txiki*, *Urtebi-Txiki-1* corresponds to 2012 season, and *Urtebi-Txiki-2* to 2013.

2.3. Sampling

Must or cider samples of about 250 mL were taken from the tanks for 6–8 months each season from September–October to March–June at the following moments: 1) just after pressing the apples; 2) half of AF (density ≈ 1020 g/L); 3) end of AF (density ≈ 1000 g/L); 4) end of MLF (L-malic acid concentration <0.5 g/L), immediately before racking; and 5) immediately before bottling (previous to the addition of potassium metabisulfite and ascorbic acid).

The first sampling corresponded to 0 days of fermentation in all cases, but the following samplings had great variability, as the fermentation rate was different from one variety to another and from one season to another. In the 2014 season, the second sampling was not done because it was found in the previous two seasons that the exact moment of half of AF was difficult to capture. It was also not done with *Narbarte-Gorria* because the available must volume of this variety was limited.

Each sample was homogenized by manual shaking and degasified (except initial musts) by using a stirrer and a vacuum pump. Total acidity (only in initial musts) and density were then measured. An aliquot of 100 mL was centrifuged at 9000×*g* for 20 min at room temperature (20–25 °C) in a centrifuge (Sorvall™ ST 8, Thermo Fisher Scientific, Walthan, MA, USA). A fraction of the centrifuged sample was used to measure Folin-Ciocalteu index (only in initial musts), L-malic

acid and acetic acid contents. Another fraction was filtered through a 0.45 μm nylon filter, distributed in four vials for later determination of polyphenolic compounds by HPLC and ethanol by gas chromatography, and stored in a freezer at −20 °C until analysis. The whole sampling procedure described was performed in one day to prevent the polyphenols from oxidizing and fermentation from continuing.

2.4. Density

Two precision hydrometers with scale 0.990–1.005 g/cm^3 and 1.000–1.050 g/cm^3 (Ludwig Schneider Messtechnik GmbH, Wertheim, Germany) were used to measure the density of the initial musts.

2.5. Total acidity

It was measured only in the initial musts in order to classify the cider apple varieties used in technological groups. It was determined by titration with 0.1 mol/L NaOH, according to the Official Methods of Analysis (OIV, 2013). The total acidity, obtained in milliequivalents per litre (meq/L), was transformed into malic acid g/L, a usual practice in ciders as malic acid is the main acid in apple musts. Following formula was used:

$$\text{Malic acid g/L} = \text{meq/L} \times 10^{-3} \times 134.09/2$$

where 134.09 is the molecular weight of malic acid (diprotic). It has to be remarked that this is just a way to express the total acidity, not the real malic acid concentration, which was determined with an enzymatic method, as explained later.

2.6. Folin-Ciocalteu index

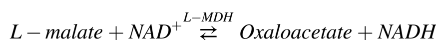
It was determined by means of the Automatic Analyser Y15 from BioSystems S.A. (Barcelona, Spain). The analyser was provided with a halogen lamp, 340, 405, 420, 480, 520, 560, 600, 670 and 750 nm filters, and a reusable methacrylate rotatory tray for absorbance measurements, thermostatised at 37 °C, with 120 wells of 6 mm optical path.

Folin-Ciocalteu index was determined according to the Official Methods of Analysis (OIV, 2013), although some changes were made to adapt the method to the Automatic Analyser Y15. It was measured only in the initial musts in order to classify the cider apple varieties used in technological groups.

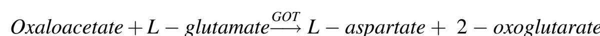
A working Folin-Ciocalteu reagent was previously prepared by diluting 7.5 mL of the commercial reagent to 100 mL with water. The sample (4 μL) was mixed with 400 μL of working Folin-Ciocalteu reagent and 200 μL of 120 g/L Na_2CO_3 solution. After 10 min, absorbance at 750 nm (Folin-Ciocalteu index) was measured. From this absorbance, the result was also calculated in tannic acid g/L units by reference to a calibration curve (absorbance versus tannic acid g/L) generated with tannic acid standards (0–5 g/L).

2.7. L-Malic acid

The concentration of L-malic acid was determined by using the Automatic Analyser Y15 with a commercial enzymatic assay from BioSystems S.A., which uses the two reactions described below. In the first reaction, catalysed by the enzyme L-malate dehydrogenase (L-MDH), L-malic acid in the sample generates NADH that can be measured by its absorbance at 340 nm. The amount of NADH produced is stoichiometric to the amount of L-malic acid in the sample. Nevertheless, the equilibrium of this reaction lies on the side of L-malate. Therefore, the enzyme glutamate-oxoaloacetate transaminase (GOT) must be added together with L-glutamate to convert oxaloacetate to L-aspartate with the second reaction and displace the equilibrium of the first reaction towards NADH.



$$L-malate + NAD^{+} \overset{L-MDH}{\rightleftarrows} Oxaloacetate + NADH$$



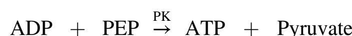
$$Oxaloacetate + L-glutamate \xrightarrow{GOT} L-aspartate + 2-oxoglutarate$$

L-malic acid content was determined as follows: 3 μL of sample were mixed with 240 μL of reagent A (buffer 0.62 mol/L + L-glutamate 60 mmol/L + L-MDH 833 nkat/mL + GOT >167 nkat/mL, pH 9.5), and then, after 1.5 min, 60 μL of reagent B (NAD^{+} > 35 mmol/L) were added. After 10 min, the absorbance was measured at 340 nm. A 1 g/L L-malic acid standard, provided by the commercial enzymatic assay, was used for calibration.

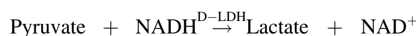
2.8. Acetic acid

This acid, main responsible of the volatile acidity in ciders, was also determined by using the Automatic Analyser Y15 with another commercial enzymatic assay from BioSystems S.A. By means of the three reactions described below, acetic acid in the sample consumes NADH that can be measured by its absorbance at 340 nm. In the first reaction, catalysed by the enzyme acetate kinase (AK), acetic acid generates ADP. In the second reaction, catalysed by the enzyme pyruvate kinase (PK), ADP reacts with phosphoenolpyruvate (PEP) to generate pyruvate, which subsequently reacts with NADH in the third reaction, catalysed by the enzyme D-lactate dehydrogenase (D-LDH). The amount of NADH consumed is stoichiometric to the amount of acetic acid in the sample.

$$\text{Acetate} + \text{ATP} \xrightarrow{AK} \text{Acetylphosphate} + \text{ADP}$$



$$\text{ADP} + \text{PEP} \xrightarrow{\text{PK}} \text{ATP} + \text{Pyruvate}$$



$$\text{Pyruvate} + \text{NADH} \xrightarrow{\text{D-LDH}} \text{Lactate} + \text{NAD}^{+}$$

Acetic acid content was determined as follows: 3 μL of sample were mixed with 240 μL of reagent A (Tris 40 mmol/L + ATP 3 mmol/L + PEP 1.25 mmol/L + NADH 0.56 mmol/L, pH 9.5) and then, after 1.5 min, 60 μL of reagent B (buffer 150 mmol/L + AK > 833 μkat/L + PK > 1667 μkat/L + D-LDH > 667 μkat/L, pH 7.2) were added. The absorbance was measured at 340 nm immediately after the addition of reagent B and 10 min later, to evaluate the consumed NADH. Calibration was done with a 0.50 g/L acetic acid standard, provided by the enzymatic assay.

2.9. Ethanol

This compound was determined using gas chromatography, together with other volatile compounds, like methanol and acetaldehyde, not included in this article. A gas chromatograph was used (Agilent HP 6890N, Agilent Technologies, Santa Clara, CA, USA), provided with a 60 m × 0.53 mm × 1.00 μm capillary column, (Restek-Stabilwax, Restek Corp., Bellefonte, PA, USA), and a flame ionisation detector. Helium at 7 mL/min was the carrier gas. The temperature program was 1 min at 40 °C, an increase to 65 °C at 5 °C/min, 1 min at 65 °C, another increase to 125 °C at 15 °C/min, and 1 min at 125 °C. To clean the column, the temperature was increased to 200 °C and kept there for 4 min. The injector and detector temperatures were 200 and 250 °C, respectively. A 3 μL volume of each previously filtered must or cider sample was directly injected in a 1:15 split mode. Ethanol retention time was 6.0 min, and it was quantified from the peak area using acetonitrile as an internal standard. A calibration graph was previously obtained with ethanol standards (5–75 mL/L).

2.10. Polyphenolic compounds

Twenty-two individual polyphenolic compounds were determined: caffeic acid, chlorogenic acid, *p*-coumaric acid, 4-*p*-coumaroylquinic acid, hydrocaffeic acid and (trans)-ferulic acid (group of

hydroxycinnamic acids); (+)-catechin, (−)-epicatechin, procyanidin B1, procyanidin B2 and procyanidin B5 (*flavan-3-ols*); phloretin 2′-*O*-xyloglucoside and phloridzin (*dihydrochalcones*); avicularin, hyperin, isoquercitrin and quercitrin (*flavonols*); gallic acid, *p*-hydroxybenzoic acid and protocatechuic acid (*benzoic acids*); catechol and tyrosol (*volatile polyphenols*).

The abovementioned 22 polyphenolic compounds were determined by HPLC using the filtered samples after thawing them at room temperature. The HPLC method by Suárez, Palacios, Fraga, and Rodríguez (2005) was employed, as described in a previous work (Zuriarrain et al., 2015). Briefly, a liquid chromatograph (Agilent 1100 Series, Agilent Technologies, Santa Clara, CA, USA), provided with a diode-array detector, was used with a 250 × 4.6 mm × 3 μm C_{18}-column (Nucleosil® 120-3 C_{18}, Macherey-Nagel, Düren, Germany). The flow rate was 0.8 mL/min, the column temperature 25 °C, and the injected volume 50 μL. The elution solvents were aqueous 20 mL/L acetic acid (solvent A) and pure methanol (solvent B). The samples were eluted according to the following gradient: a linear increase from 0 to 45% solvent B in 55 min, followed by a 20 min isocratic step, and finally, a return to the initial conditions (0% solvent B), allowing 5 min for stabilisation. Column effluents were monitored at three wavelengths: 355 nm for flavonols, 313 nm for hydroxycinnamic acids, and 280 nm for flavan-3-ols, dihydrochalcones, benzoic acids and volatile polyphenols. Phenolic compounds were quantified by the external standard method from peak areas. They were identified by means of their retention time and their 190–900 nm spectra, which were previously recorded by injecting pure compounds. Fig. S1, S2 and S3 (supplementary material) show the chromatograms obtained with pure standards, a fresh juice and a finished cider, respectively.

3. Results and discussion

The polyphenolic compounds content obtained throughout the samplings, together with density, ethanol, L-malic acid and acetic acid content, are shown in Table S1 (supplementary material). The polyphenolic compounds content of apples and ciders of the Basque Country was previously reported (Alonso-Salces et al., 2004a), so these data are not discussed here. The point of interest was the evolution of polyphenols throughout the fermentation process.

Fig. 2a shows the evolution of AF by means of the density and Fig. 2b shows the evolution of MLF by means of the L-malic acid content. AF was finished or almost finished in all must in 15–45 days, with the exception of *Moko*, *Merabi* and *Narbarte-Gorria*, while multiple patterns were observed in MLF, but the ciders finally obtained were free from defects. The microbial population present in Basque musts and ciders was previously described (Dueñas, Irastorza, Fernández, Bilbao, & Huerta, 1994; Dueñas, Irastorza, Fernández, Bilbao, & del Campo, 1997). It is relatively high, which can cause alterations such as acrolein, *framboisé*, ropiness, excess of acetic acid (Nogueira & Wosiacki, 2012), or phenolic off-flavour (Buron et al., 2012), but none of them were observed here. The final ethanol content was between 44 ± 2 mL/L (*Aritza*) and 75 ± 3 mL/L (*Gezamina*). The final acetic acid concentration was always under 2.2 g/L, the maximum permitted in Spain.

3.1. Classification of cider apple varieties

According to the English classification of cider apple varieties (Jolicoeur, 2013), the apple cultivars used in this study were included in 4 technological groups, based on the total acidity, expressed as L-malic acid content, and the Folin-Ciocalteu index, expressed as tannic acid content (see Table 1): sweet (L-malic acid <4.5 g/L, tannic acid <2 g/L), bittersweet (L-malic acid <4.5 g/L, tannic acid >2 g/L), sharp (L-malic acid >4.5 g/L, tannic acid <2 g/L) and bittersharp (L-malic acid >4.5 g/L, tannic acid >2 g/L). The apple varieties used for cidermaking in the Basque Country are mostly sharp or bittersharp.

The English classification was used because there is no systematic classification of cider apple varieties in the Basque Country. In the other cider producing area of Spain, Asturias, there exists a systematic classification, but they change it from time to time. In England, by contrast, the classification was established in the early years of the 20th century and has remained unchanged until now.

3.2. Hydroxycinammic acids

This was the most abundant group of polyphenols in all apple musts studied, accounting for 40–100% of the total polyphenols determined. Chlorogenic acid was the main hydroxycinnamic acid of the musts, with great difference over the others, except in *Urdin* and *Manttoni*. The

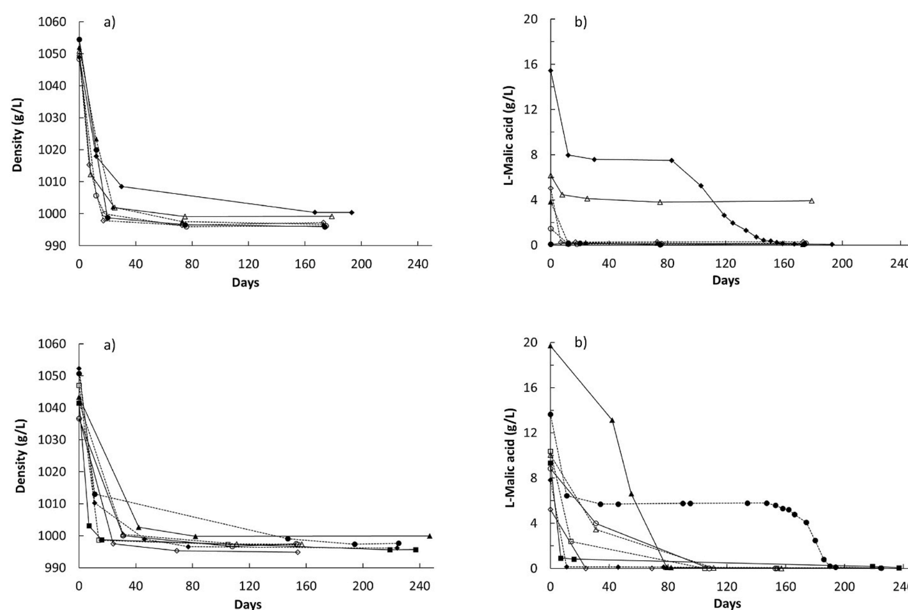


Fig. 2. a) Alcoholic fermentation of the musts followed by the decrease in density; b) Malolactic fermentation of the musts followed by the decrease in malic acid content 2012: *Gezamina* —●—, *Goikoetxea* ···▲···, *Moko* —◆—, *Txalaka* ···○···, *Urtebi-Haundi* —△—, *Urtebi-Txiki-1* ···◇···; 2013: *Merabi* ···●···, *Narbarte-Gorria* —▲—, *Urdin* ···◆···, *Urtebi-Txiki-2* —■—; 2014: *Aritza* —○—, *Frantzes* ···△···, *Manttoni* —◇—, *Udare-Marroi*.···□···.

Table 1
Total acidity, pH and Folin-Ciocaltey index of initial musts[a] (first sampling) and technological group of apple varieties according to English classification.

Season	Apple variety	Total acidity (Malic acid, g/L)	pH	Folin-Ciocalteu index (Tannic acid, g/L)	Technological group
2012	*Gezamina*	2.02 ± 0.03	3.88 ± 0.02	3.01 ± 0.11	Bittersweet
	Goikoetxea	6.69 ± 0.01	3.47 ± 0.01	1.62 ± 0.15	Sharp
	Moko	13.84 ± 0.03	3.09 ± 0.01	3.78 ± 0.09	Bittersharp
	Txalaka	6.68 ± 0.01	3.33 ± 0.06	1.49 ± 0.03	Sharp
	Urtebi-Haundi	10.81 ± 0.01	3.26 ± 0.02	1.06 ± 0.11	Sharp
	Urtebi-Txiki-1	7.82 ± 0.02	3.35 ± 0.01	1.89 ± 0.08	Sharp
2013	*Merabi*	11.08 ± 0.01	3.22 ± 0.02	4.46 ± 0.25	Bittersharp
	Narbarte-Gorria	17.09 ± 0.01	3.14 ± 0.01	3.33 ± 0.34	Bittersharp
	Urdin	6.29 ± 0.01	3.48 ± 0.01	2.08 ± 0.15	Bittersharp
	Urtebi-Txiki-2	7.04 ± 0.01	3.36 ± 0.03	1.36 ± 0.18	Sharp
2014	*Aritza*	7.62 ± 0.04	3.43 ± 0.02	2.27 ± 0.05	Bittersharp
	Frantzes	9.17 ± 0.01	3.40 ± 0.03	1.07 ± 0.08	Sharp
	Manttoni	4.32 ± 0.01	3.66 ± 0.02	0.58 ± 0.07	Sweet
	Udare-Marroi	9.14 ± 0.01	3.42 ± 0.03	0.99 ± 0.08	Sharp

[a] Mean of two measurements ± standard deviation.

general trend (Fig. 3) was an increase or decrease in concentration during the first 10–40 days, coinciding with the most active phase of AF, followed by stabilisation, although slight decreases were observed in some musts. In *Gezamina, Moko, Udare-Marroi, Urtebi-Haundi* and *Urtebi-Txiki-1*, there was an increase during the first 10–15 days, followed by a slight decrease and posterior stabilisation. Similar patterns were registered in *Goikoetxea* and *Urtebi-Txiki-2*, but in the first one, the decrease continued without stabilisation, and in the second one, after stabilisation from day 7 onwards, a new decrease was observed at the end, from day 219 onwards. In *Aritza, Frantzes, Merabi* and *Txalaka*, a decrease took place during the first 10–30 days, followed by stabilisation. In *Narbarte-Gorria* it decreased slightly from the first day to the last day measured. The decrease of chlorogenic acid could be attributed to oxidation processes, as this acid is an important substrate of polyphenol oxidases (Jiang, Duan, Qu, & Zheng, 2016). It was slight because the tanks were hermetically sealed, precisely to protect the musts from oxygen. Regarding the increase, the musts were not filtered after obtaining them. They are never filtered in the Basque Country. Consequently, some pulp remained in the musts, together with the microbial population, which made the observed increase possible, not only with chlorogenic acid but also with other polyphenols. In fact, the turbidity caused by the pulp and the microbial population did not substantially disappear until the musts were racked.

Different trends were observed in *Urdin* and *Manttoni*. In *Urdin*, the chlorogenic acid content decreased rapidly during the first 11 days and slower thereafter, until its total absence on day 46. In *Manttoni*, it was never detected. *Urdin* and *Manttoni* were the only musts where chlorogenic acid disappeared or was not detected at all. The different evolution of phenolic compounds depending on the variety has been reported in previous studies (Alberti et al., 2016; Laaksonen, Kuldjärv, Paalme, Virkki, & Yang, 2017), but the total absence of chlorogenic acid from the first sampling registered in *Manttoni* has not been reported until now, maybe because cider is always done with a mixture of apple varieties. In the case of Urdin, after the disappearance of chlorogenic acid, caffeic acid became the most abundant hydroxycinnamic acid in the third and fourth samplings, with 70–73% of the total, which was subsequently reduced to hydrocaffeic acid, representing 77% of the total in the fifth sampling. It is known that some LAB strains can hydrolyse chlorogenic acid to quinic and caffeic acids, and subsequently, caffeic acid can be reduced to hydrocaffeic acid (Whiting, 1973). This could explain the transformation observed in *Urdin* must, but not the absence of chlorogenic acid in *Manttoni* must in the first sampling, as only 0.6 mg/L caffeic acid was detected in this sample and no hydrocaffeic acid at all.

The second most abundant hydroxycinnamic acid was 4-*p*-coumaroylquinic acid. The evolution of this acid (Fig. 3) showed two main patterns. In the first one, observed in *Gezamina, Goikoetxea, Moko, Txalaka, Urtebi-Haundi and Urtebi-Txiki-1*, 4-*p*-coumaroylquinic acid concentration increased in the first 12 days and then became stable or slightly decreased (only *Gezamina* and *Moko*) and then became stable. In the second one, observed in *Merabi, Urdin, Aritza, Frantzes, Manttoni* and *Udare-Marroi*, it remained almost stable all the time. Only *Narbarte-Gorria* and *Udare-Marroi* followed slightly different patterns. In *Narbarte-Gorria* a decrease was registered in the first 42 days, followed by an increase until day 82, and from this point onwards, it remained stable. In *Urtebi-Txiki-2*, it increased in the first 7 days, then decreased slowly and, from day 219 onwards, it increased again. Some authors reported a possible hydrolysis of 4-*p*-coumaroylquinic acid to give *p*-coumaric acid (Benvenutti et al., 2021), but this was not observed in this study. In fact, the *p*-coumaric concentration was very low in all samples, being the highest values found 6.3 mg/L in *Goikoetxea-4*, and 3.0 mg/L in *Narbarte-Gorria-4*. In addition, since 4-*p*-coumaroylquinic acid is a derivative of *p*-coumaric acid, which is known to be an *o*-diphenol oxidase inhibitor (Walker & Wilson, 1975), it might be a potential inhibitor of PPO, as well (Le Deun, Van der Werf, Le Bail, Le Quéré, & Guyot, 2015). This could explain the stability observed for this acid after finishing of AF.

The sum of chlorogenic acid and 4-*p*-coumaroylquinic acid represented more than 70% of the overall content of hydroxycinnamic acids determined in all the ciders of this work, with the exceptions of *Manttoni* and *Urdin*. In *Manttoni*, chlorogenic acid was not detected and 4-*p*-coumaroylquinic acid was the main hydroxicinnamic acid, representing more than 57% of the total. In the case of *Urdin*, chlorogenic acid was present in the first two samplings, and the sum of this acid and 4-*p*-coumaroylquinic acid represented 100% of the total in the first sampling, and 69% in the second one. However, from the third sampling onwards, chlorogenic acid was not detected, and 4-*p*-coumaroylquinic acid represented only 22–28% of the total.

Urtebi-Txiki-1 and *Urtebi-Txiki-2* musts were obtained from the same variety harvested in two different years, 2012 and 2013, respectively. As usually happens, the two initial musts were similar but not the same, and the polyphenolic content was somewhat lower in *Urtebi-Txiki-1* than in *Urtebi-Txiki-2*. The initial concentrations of chlorogenic and 4-*p*-coumaroylquinic acids were 111 and 42 mg/L, respectively, in *Urtebi-Txiki-1*, and 115 and 61 mg/L in *Urtebi-Txiki-2*. In spite of this difference, the evolution of these compounds was similar in both musts, except for the changes observed from the day 219 onwards in *Urtebi-Txiki-2*. In this must, MLF was almost completed in 7 days (0.91 g L^{-1} L-malic acid), but at this moment, it became stuck. No explanation for this behaviour was found as the initial acidity of this must was not particularly high, and it was even lower when the fermentation stopped. Maybe the amount of indigenous LAB in the initial must was not sufficient to complete the MLF. After waiting for almost 6 months without any change, commercial LAB were added, and the temperature of the tank was increased up to 20 °C. MLF ended on day 219, just 10 days after the addition of LAB. These actions could explain the changes observed in chlorogenic and 4-*p*-coumaroylquinic acids in the last sample.

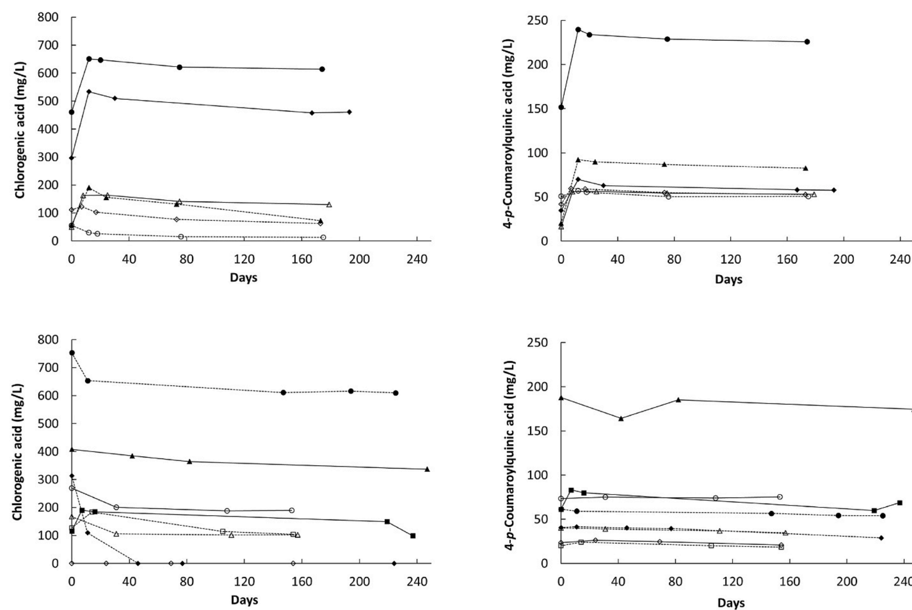


Fig. 3. Evolution of chlorogenic and 4-*p*-coumaroylquinic acids content of the musts throughout the fermentation and maturation process 2012: *Gezamina* —●—, *Goikoetxea* ···▲···, *Moko* —◆—, *Txalaka* ···○···, *Urtebi-Haundi* —△—, *Urtebi-Txiki-1* ···◇···; 2013: *Merabi* ···●···, *Narbarte-Gorria* —▲—, *Urdin* ···◆···, *Urtebi-Txiki-2* —■—; 2014: *Aritza* —○—, *Frantzes* ···△···, *Manttoni* —◇—, *Udare-Marroi*.···□···.

Piccineli et al. (2000) studied two fermenting apple musts in Asturias (Spain) and obtained similar evolution patterns for chlorogenic acid as in the present work. They also reported a final increase in caffeic acid, but they did not determine 4-*p*-coumaroylquinic acid. Nogueira et al. (2008) studied five fermenting monovarietal apple musts in Bretagne (France), and they reported no variation in the concentration of chlorogenic and 4-*p*-coumaroylquinic acids during the whole fermentation process, except in one must, where a decrease in chlorogenic acid content was observed, together with an increase in caffeic acid. These patterns correlate roughly with those described in the present work. It should be noted that the cidermaking process in Asturias is the same as in the Basque Country, with other apple cultivars (Rodríguez-Madrera, Picinelli-Lobo, & Suárez-Valles, 2006), but it is different from that used in Bretagne (France). While in the Basque Country a complete fermentation to dryness is carried out and only a small amount of residual sugar is left in the final ciders, in Bretagne fermentation is not allowed to finish, which produces ciders of different sweetness and lower alcoholic degree (Alonso-Salces et al., 2004b). In spite of this, similar evolution patterns were observed for the hydroxycinammic acids, strongly suggesting that it is a fairly general behaviour.

The studies on ciders of China cannot be taken as a reference here. Ye et al. (2014) used the dessert *Fuji* apple varieties, which are much less phenolic than those used in the present work. Therefore, they reported very low maximum chlorogenic acid levels (22.0 mg/L), and this is probably the reason why they did not report the presence of 4-*p*-coumaroylquinic acid. This kind of apple is rarely used in the Basque Country as more phenolic varieties are preferred. Besides, these authors mixed fresh apple juice with commercial apple juice concentrate, which is never done in Basque Ciders. Zuo et al. (2019) used very phenolic apples, and reported maximum chlorogenic acid levels higher than in the present work (3781 mg/L), but they did not determine 4-*p*-coumaroylquinic acid, and they studied the fermentation-maturation process for only 28 days. On the other hand, the cidermaking process itself was very different in both studies, because they sterilized the initial musts to inhibit bacterial growth. As they only inoculated yeasts to perform the fermentation and no LAB were added, only AF took place. MLF did not occur, and malic acid was not consumed. In the Basque Country, MLF is considered essential because the transformation of malic acid into the less strong lactic acid gives a smoother taste to the cider. Ciders with unfinished MLF are not acceptable to the Basque consumer, who perceives them as immature.

3.3. Flavan-3-ols

This type of polyphenols was the second most abundant in *Aritza, Gezamina, Merabi, Narbarte-Gorria, Txalaka, Urtebi-Txiki-1* and *Urtebi-Txiki-2*, whereas the dihydrochalcones group took this second place in *Frantzes, Goikoetxea, Manttoni, Udare-Marroi, Urdin* and *Urtebi-Haundi*. In *Moko*, both types of polyphenols had similar total concentrations. Monomeric and dimeric flavan-3-ols accounted for 0–45% of the total polyphenols determined.

(−)-Epicatechin was the main flavan-3-ol in most of the musts, with a content between 40% (*Urdin*) and 82% (*Moko*) of the overall flavan-3-ols content in the last sampling. The most frequently observed trends were (Fig. 4), on the one hand, an increase during the first 25 days followed by stabilisation or slight fluctuations (*Gezamina, Goikoetxea, Txalaka, Urtebi-Txiki-1 and Urtebi-Txiki-2*), and on the other hand, stabilisation or slight fluctuations from the first day (*Aritza, Frantzes, Narbarte-Gorria* and *Udare-Marroi*). However, other patterns were also observed. In *Moko*, after a great increase in the first 12 days, a decrease took place before the stabilisation from day 30 onwards. In *Merabi*, the epicatechin content remained almost stable until day 147, then it decreased, and from day 194 onwards, it remained almost stable again. *Urdin* was the only must where all the flavan-3-ols disappeared in the last sampling.

Aritza, Narbarte-Gorria, Manttoni and *Urtebi-Haundi* were different from the rest in the sense that (−)-epicatechin was not always the main flavan-3-ol. In *Aritza*, procyanidin B2 was the main one, with great difference over the others, until its sharp decline in the last sampling. From this point onwards, (−)-epicatechin was the main flavan-3-ol. The same pattern was observed in *Narbarte-Gorria*, but the sharp decline in procyanidin B2 occurred in the penultimate sampling. *Manttoni* was the only must where no flavan-3-ols were detected, except small quantities of (−)-epicatechin (4.0 mg/L) and (+)-catechin (1.7 mg/L) in the third sampling. In *Urtebi-Haundi*, (−)-epicatechin was only detected in the third sampling, and no flavan-3-ols were detected in the first sampling. In the rest of the samplings, (+)-catechin or procyanidin B1 became the

main flavan-3-ols.

The evolution of (−)-epicatechin in *Urtebi-Txiki-1* and *Urtebi-Txiki-2* musts was similar, except at the beginning. In the more phenolic *Urtebi-Txiki-2* the concentration increase was sharper, and it was followed by small decrease, not observed in *Urtebi-Txiki-1*. However, the final content was almost the same in both musts.

As a general pattern, after the most active phase of AF, epicatechin became a fairly stable compound with little changes in concentration. In the cases where it disappeared, as in *Urdin* and *Urtebi-Haundi*, this could be attributed to enzymatic processes, although these are poorly understood (Zhang, Hu, Guo, Wang, & Meng, 2021), since flavan-3-ol monomers do not form precipitates in apple derivatives (Millet, Poupard, Le Quéré, Bauduin, & Guyot, 2017).

In ciders of Asturias (Spain), an increase in (−)-epicatechin at the beginning of the fermentation was reported, followed by a decrease (Piccineli et al., 2000). In the present work, this was observed only in *Urtebi-Haundi* and *Urdin* musts, even though Asturian and Basque cidermaking processes are similar. In ciders of Bretagne (France), a decrease in musts was observed in the cases where (−)-epicatechin was found (Nogueira et al., 2008). On the other side, the absence of (−)-epicatechin was also reported in two of the five monovarietal musts studied, which is in agreement with the *Manttoni* must of the present work.

Fig. 4 also shows the evolution of procyanidin B1 and procyanidin B2. In *Urtebi-Haundi, Urtebi-Txiki-1* and *Frantzes*, only procyanidin B1 was detected; in *Txalaka* only procyanidin B2; in *Gezamina, Goikoetxea, Moko, Merabi, Narbarte-Gorria, Urdin, Urtebi-Txiki-2 and Aritza*, both of them; and in *Manttoni* and *Udare-Marroi*, none of them. When both procyanidins were present, the concentration of procyanidin B1 was higher, except in *Moko*, with similar contents of both procyanidins, and *Aritza* and *Narbarte-Gorria*, where procyanidin B2 was dominant in the first samplings. In *Frantzes*, the small concentrations of procyanidin B1 detected in the first two samplings (2.4 and 5.4 mg/L, respectively) disappeared from day 111 onwards.

The greatest change in procyanidin B2 was observed in *Narbarte-Gorria*, where it decreased from 342 mg/L on day 42 to 32 mg/L on day 82, and remained stable afterwards. In *Aritza*, after being stable for many days, it decreased from 149 mg/L on day 108 to 2.8 mg/L on day 153. In *Merabi*, it decreased from 56 to 45 mg/L in the first 11 days, then remained stable until day 194, and then decreased from 41 mg/L to not detected in the last sampling. In the rest of the musts where procyanidin B2 was present, its concentration was always under 20 mg/L, and small fluctuations were observed without any general rule.

The changes in procyanidin B1 were less important that those observed in procyanidin B2. The maximum contents, around 50 mg/L, were measured in *Merabi* and *Narbarte-Gorria*. In *Frantzes* and *Urdin*, after a small increase at the beginning up to 5.4 and 13.1 mg/L, respectively, it decreased until its disappearance. The greatest increase was registered in *Gezamina*, transitioning from not detected levels to 20.9 mg/L in the first 20 days, followed by a slower increase until 33.5 mg/L on day 174. In the rest of the musts, many different evolution patterns were observed with no general behaviour.

Procyanidin B5 was found in *Urtebi-Haundi, Merabi, Narbarte-Gorria, Urdin, Urtebi-Txiki-2, Aritza* and *Frantzes* but almost always in smaller concentrations than the other two procyanidins.

Many authors have reported the ability of procyanidins to interact with proteins and polysaccharides to give insoluble aggregates. In the case of ciders, procyanidins can represent more than 90% of hazes (Millet et al., 2017; Siebert, 2009, chap. 2). This could account for the dramatic decrease in procyanidin B2 observed in *Aritza* and *Narbarte--Gorria*, and it is likely that most of the procyanidins initially present in the apple juices were removed from the liquid by this kind of precipitation. Besides, the formation of precipitates apparently gives rise to many different evolution patterns, without any general rule.

The small differences observed between *Urtebi-Txiki-1* and *Urtebi-Txiki-2* derived from the more phenolic character of the latter. While in *Urtebi-Txiki-2* a fast increase of procyanidin B1 was observed at the beginning, followed by a little decrease and a stabilisation, in *Urtebi-Txiki-1* the increase began later and was smaller, but it was also followed by a stabilisation. While in *Urtebi-Txiki-2* very little procyanidin B2 was found (less than 7 mg/L), it was totally absent in *Urtebi-Txiki-1*.

Piccineli et al. (2000) observed an increase in procyanidin B1 and B2 at the beginning of fermentation, followed by a decrease, similar as in *Frantzes* and *Urdin* with procyanidin B1, and in *Moko*, *Urdin* and *Urtebi-Txiki-2* with procyanidin B2. Nogueira et al. (2008) reported a decrease in procyanidin B2 and in "other procyanidins", in the same way

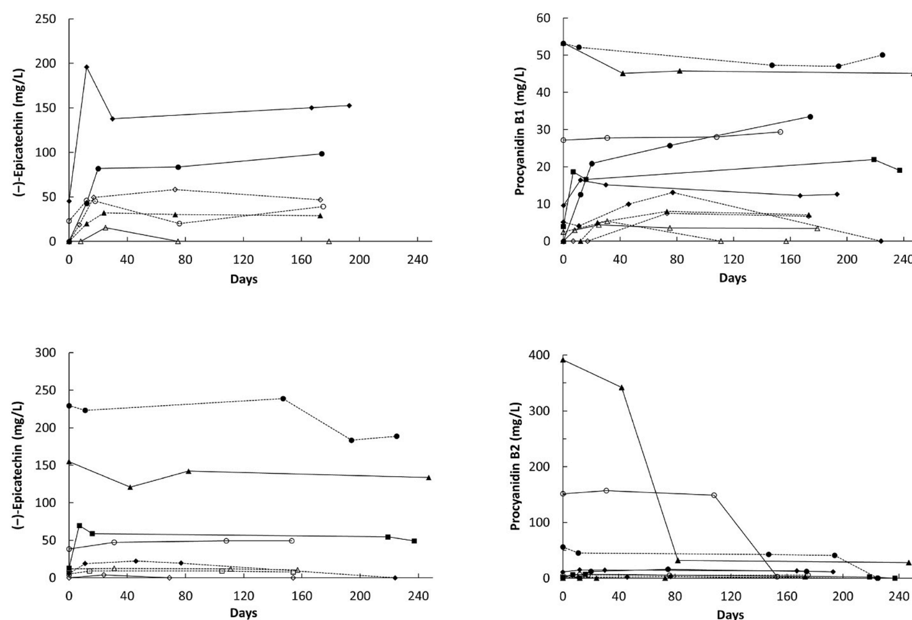


Fig. 4. Evolution of (−)-epicatechin, procyanidin B1 and procyanidin B2 content of the musts throughout the fermentation and maturation process 2012: *Gezamina* —●—, *Goikoetxea* ···▲···, *Moko* —◆—, *Txalaka* ···○···, *Urtebi-Haundi* —△—, *Urtebi-Txiki-1* ···◇···; 2013: *Merabi* ···●···, *Narbarte-Gorria* —▲—, *Urdin* ···◆···, *Urtebi-Txiki-2* —■—; 2014: *Aritza* —○—, *Frantzes* ···△···, *Manttoni* —◇—, *Udare-Marroi*.···□···.

as in *Merabi* with procyanidin B2 but less dramatic than in *Aritza* and *Narbarte-Gorria*. They also reported the absence of procyanidins in two of the five monovarietal musts they studied, the same as in *Manttoni* and *Udare-Marroi* musts of the present work. Although these results were coincident with some of the musts of the present study, with the rest of them they were not.

3.4. Dihydrochalcones

Two polyphenols of this group were determined in this work, both of them characteristic of apples: phloretin 2′-*O*-xyloglucoside and phloridzin (Mangas, Rodríguez, Suárez, Picinelli, & Dapena, 1999). Both are derivatives of phloretin, but this compound was not found in any of the musts or ciders. They accounted for 0–39% of the total polyphenols determined. Phloretin 2′-*O*-xyloglucoside content was always greater than that of phloridzin, except in the last sampling of *Urdin*, where the latter became more concentrated. The general trend observed in phloretin 2′-*O*-xyloglucoside (Fig. 5) was an increase during the first 7–25 days, in the most active phase of the AF, followed by stabilisation, or stable concentration from the beginning. The first pattern was followed in *Gezamina, Goikoetxea, Txalaka, Udare-Marroi, Urtebi-Haundi, Urtebi-Txiki-1* and *Urtebi-Txiki-2*. The second one was observed in *Aritza, Frantzes, Manttoni* and *Narbarte-Gorria*. The evolution in *Urtebi-Txiki-1* and *Urtebi-Txiki-2* was almost the same, but this time the concentration was somewhat higher in *Urtebi-Txiki-1*, except in the first sampling. *Moko* and *Merabi* showed a slightly different behaviour as the increase in the first 11–12 days was followed by a decrease, slower in *Merabi* than in *Moko*, before the stabilisation. A different pattern was followed by *Urdin*. In this must, an increase was observed in the first 11 days followed by a slight decrease until day 77. But from this point onwards, instead of getting stable as in the case of the other musts, it decreased faster from 70 to 4.7 mg/L in the last sampling (day 224), a concentration below the phloridzin content, as mentioned above. It was the only must where this final rapid decrease was registered. This decrease was coincident in time with an increase in phloridzin from 12.5 to 26.1 mg/L, lower than the decrease in phloretin 2′-*O*-xyloglucoside. These data suggest that a part of phloretin 2′-*O*-xyloglucoside (23% according to the mmol/L experimental concentrations) was transformed into phloridzin in this must. However, this kind of transformation has never been reported.

Phloridzin showed fluctuations, quantitatively less important than those observed in the previous compound (Fig. 5). In all the musts, an increase was observed in the first 7–31 days, being the strongest ones those observed in *Goikoetxea* and *Moko*, and the slightest ones those observed in *Aritza* and *Frantzes*. As an exception, phloridzin content remained stable in this period in *Narbarte-Gorria*. However, after this period, the evolution was different in every must, without any clear pattern, as increases, decreases, stabilisations and fluctuations were observed. Even in *Urtebi-Txiki-1* and *Urtebi-Txiki-2* completely different evolutions were observed. These variations were quantitatively less important than the first increase, with the exception of the aforementioned increase observed in *Urdin*, the final increase observed in Gezamina, and the sharp decrease observed in *Urtebi-Txiki-2* from the day 219 onwards. Once again, the last change in *Urtebi-Txiki-2* can be explained by the addition of commercial LAB that was done in this must before the last sampling.

Piccineli et al. (2000) reported again different trends as they observed an increase in both compounds, followed by a decrease. Moreover, in one of the two musts they studied, phloridzin content was much greater than that of phloretin 2′-*O*-xyloglucoside. On the other side, Nogueira et al. (2008) reported little changes in the content of both compounds, which was also observed in some musts of the present study.

3.5. Other polyphenols

Flavonols (avicularin, isoquercitrin, hyperin and quercitrin) were always less than 0.8% of the total polyphenol content determined, and benzoic acids (gallic acid, *p*-hydroxybenzoic acid and protocatechuic acid) less than 3.6%. Consequently, they will not be discussed here. With respect to volatile polyphenols, catechol and tyrosol, the last one showed significant concentrations in some musts, reaching to 25% of total polyphenols in the last sampling of *Manttoni* (17.3 mg/L). It was not present in the initial musts, but its concentration increased in all of them in the first 20–40 days, coinciding with the most active phase of AF (Fig. 6), and remained stable afterwards or showed slight fluctuations, depending on the must (in *Narbarte-Gorria* decreased slowly). The

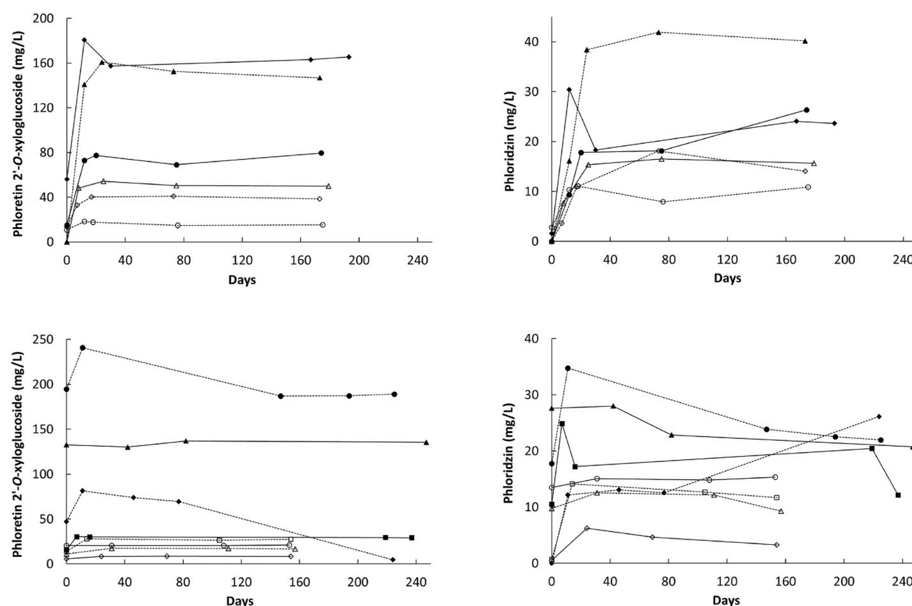
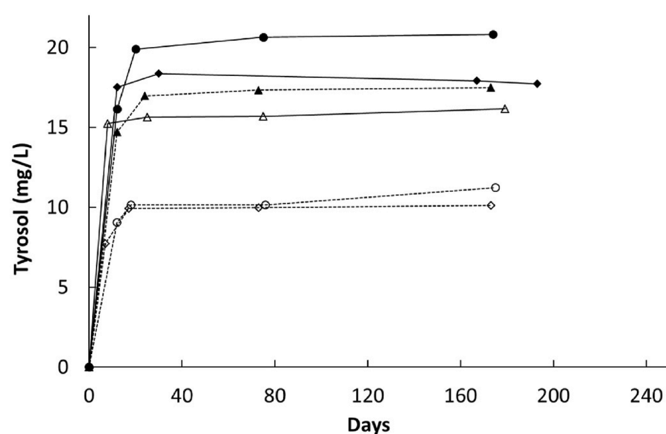


Fig. 5. Evolution of phloretin 2′-*O*-xyloglucoside and phloridzin content of the musts throughout the fermentation and maturation process 2012: *Gezamina* —●—, *Goikoetxea* ···▲···, *Moko* —◆—, *Txalaka* ···○···, *Urtebi-Haundi* —△—, *Urtebi-Txiki-1* ···◇···; 2013: *Merabi* ···●···, *Narbarte-Gorria* —▲—, *Urdin* ···◆···, *Urtebi-Txiki-2* —■—; 2014: *Aritza* —○—, *Frantzes* ···△···, *Manttoni* —◇—, *Udare-Marroi*.···□···.



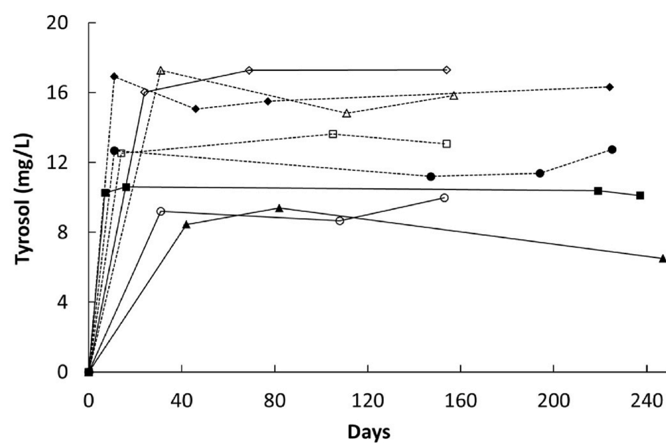


Fig. 6. Evolution of tyrosol content of the musts throughout the fermentation and maturation process 2012: *Gezamina* —●—, *Goikoetxea* ···▲···, *Moko* —◆—, *Txalaka* ···○···, *Urtebi-Haundi* —△—, *Urtebi-Txiki-1* ···◇···; 2013: *Merabi* ···●···, *Narbarte-Gorria* —▲—, *Urdin* ···◆···, *Urtebi-Txiki-2* —■—; 2014: *Aritza* —○—, *Frantzes* ···△···, *Manttoni* —◇—, *Udare-Marroi*.···□···.

maximum concentration, 20.8 mg/L, was measured in the last sampling of *Gezamina*, although it was only 1.8% of the total amount of polyphenols in this must. *Urtebi-Txiki-1* and *Urtebi-Txiki-2* showed similar behaviour with respect to tyrosol, both in content and in evolution.

The evolution observed is explained because tyrosol is a secondary metabolite from the tyrosine formed by yeasts during AF, so it is synthesized always during the fermentation process, as previously reported (Rodríguez-Madrera et al., 2006). The presence of tyrosol in the ciders is interesting due to its potential free radical scavenging, antimicrobial, cardiopreventive, and anticarcinogenic properties (Piñeiro, Cantos-Villar, Palma, & Puertas, 2011).

4. Conclusions

The evolution of the main polyphenols concentration during the cidermaking process in the Basque Country was discussed in detail. The most frequent behaviours observed in the different compounds were the following:

- Chlorogenic acid, 4-*p*-coumaroylquinic acid and (−)-epicatechin: Fluctuations during the most active phase of AF (10–40 days), followed by stabilisation.
- Phloretin 2′-*O*-xyloglucoside. Increase or stable concentration during the most active phase of AF followed by stabilisation.
- Tyrosol: Absence in the initial musts, increase during the most active phase of AF followed by stabilisation.
- Procyanidin B1 and B2: many different evolution patterns with no general rule. Low concentrations probably because of previous precipitation with proteins and polysaccharides.
- Phloridzin. No general evolution patterns.

The changes observed in chlorogenic acid, 4-*p*-coumaroylquinic acid and tyrosol were similar as the previously reported, but this was not the case with the rest of polyphenols. The evolution of most phenolic compounds did not follow any general pattern.

Conflict of interest form

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Andoni Zuriarrain-Ocio: Investigation, Methodology, Data curation, Formal analysis. **Juan Zuriarrain:** Conceptualization, Methodology, Supervision. **Oier Etxebeste:** Investigation, Methodology, Data curation. **María Teresa Dueñas:** Conceptualization, Methodology. **Iñaki Berregi:** Conceptualization, Methodology, Supervision, Writing – original draft.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.113798.

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