Effect of size, seasoning and toasting level of *Quercus pyrenaica* Willd. wood on wine phenolic composition during maturation process with micro-oxygenation

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Abstract

Seasoning and toasting treatments carried out in cooperage influence the chemical composition of the oak, and these treatments affect different oak species in different ways. For this reason, numerous studies have focused on the evaluation of both the oak wood materials and the resulting aged wines in order to optimize the different cooperage processes. Seasoning involves immobilizing the wood for long periods of time, leading to an increase in the price of the final product. When using alternative products instead of barrels, in addition to the seasoning and toasting of the wood, the size of the wood pieces is a determining factor in the characteristics of the final wine. Therefore, the objective of this work was to evaluate the polyphenolic composition of the same red wine aged with different alternative products and a small amount of oxygen for 120 days. Specifically, the effect of 2 types of seasoning and 3 types of toasting of chips and staves made from the wood of *Q. pyrenaica* Willd. was studied. The results showed that the size of the alternative product was the factor that determines the phenolic composition of the wines over the entire ageing process, independent of the seasoning or toasting method carried out on the wood.

Keywords: chips, staves, oxygen, wine, phenolic composition, *Quercus pyrenaica* Willd.

1. Introduction

Since wine market is saturated, winemakers are continually seeking ways to differentiate the wines, always prioritizing the final quality. In oenology, oak barrels have an important effect on the aroma, colour and stability of wine, and they also help to clarify the wine, demonstrating that the type of wood used has an important effect on the final quality of the wine. For this reason, oenologists are becoming increasingly innovative and are looking for new types of wood. In addition, there is an imbalance in the amount of oak wood available and the number of barrels produced due to a change in ageing habits towards the use of newer oak barrels, which means that coopers are forced to look for a greater variety of products (Nevares & del Alamo-Sanza, 2017, Gómez-Plaza & Bautista-Ortín, 2019). The current situation has led to the use of the same species of oak used in the traditional way (*Q. petraea*, *Q. robur* and *Q. alba*) (Díaz-Maroto, Guchu, Castro-Vázquez, de Torres, & Pérez-Coello, 2008, in addition to other species, such as *Q. pyrenaica, Q. faginea, Q. humboldtti, Q. oocarpa* and *Q. frainetto* (Fernández de Simón, Cadahía, del Álamo, & Nevares, 2010; Martínez-Gil, del Alamo-Sanza, Sánchez-Gómez, & Nevares, 2018; Martínez-Gil, del Alamo-Sanza, Gutiérrez-Gamboa, Moreno-Simunovic, & Nevares, 2018; Martínez-Gil et al., 2019), as well as other wood types, including *Robinia pseudoacacia, Castanea sativa, Prunus, Fraxinus* and *Morus* (Chinnici, Natali, Bellachioma, Versari, & Riponi, 2015; Delia, Jordao, & Ricardo-Da-Silva, 2017; Martínez-Gil et al., 2018; Jordao, Lozano, & González-SanJosé, 2019).

Q. pyrenaica is, after the traditional woods, the second most widely studied, since has been shown that it has an adequate composition and imparts good characteristics to the wine (Fernández de Simón et al., 2008; Del Álamo, Nevares, Gallego, Fernández de Simón, & Cadahía, 2010; Fernández de Simón, Cadahía, del Álamo, et al., 2010; Fernández de Simón, Cadahía, Muiño, del Álamo, & Nevares, 2010; Gallego et al., 2012; Rodríguez-Bencomo, Ortega-Heras, Pérez-Magariño, & González-Huerta, 2009; Sánchez-Gómez, Nevares, Martínez-Gil, & del Alamo-Sanza, 2018; Tavares, Jordão, & Ricardo-Da-Silva, 2017). Since 1996, there have been studies on the volatile composition and low molecular weight and ellagitannin compounds of *Q. pyrenaica* from different origins and treatments: green, seasoned and toasted (Canas et al., 2000; Fernández de Simón, Cadahía, del Álamo, et al., 2010; Fernández de Simón, Sanz, Cadahía, Poveda, & Broto, 2006) in order to adapt these treatments to obtain the greatest potential of this wood. The current forest mass of *Q. pyrenaica* that can possibly be used for the manufacture of barrels is low due to the high number of poor-quality trees for cooperages. However, given the appropriate characteristics of this wood for use with wine, its use has especially been in the form of alternative oak products.

Alternative oak products have been widely used for a long time in wine-producing countries. However, their use has spread since the main wine producer, Europe, changed its

legislation allowing the use of these products (CE 1507/2006; OENO 9/2001). One of the main differences between barrel-aged and alternative wines is that a barrel acts as an active vessel, providing mild oxygenation that improves the integration between the compounds of the wine and those released by the wood (del Alamo-Sanza & Nevares, 2017). Therefore, when using alternatives, oxygen is essential for reactions similar to those occurring inside the barrel, and the use of these alternatives must be complemented with the micro-oxygenation (MOX) technique.

In the use of alternatives, along to the oak geographical origin used and silvicultural treatments, there are others factors such as the size of the piece and the processes carried out in the cooperage to obtain them. Toasting degree of oak during manufacture and the method of seasoning (natural or alternative), are factors influencing oak's chemical composition. The European regulation CE 1507/2007 specify that the pieces of wood used for the wine aging must have a dimension greater than 2 mm. In response to this, there are different shapes and forms for the alternative oak products in the market: powder, chips, cubes, beans or staves. The size of wood pieces used is a key factor defining the final characteristics of wines. It is known that the smaller the piece of wood, the further the evolution undergone by wine and therefore more differences will be from that aged in barrels (del Alamo et al., 2004, 2008). Like wood used to build barrels, wood for obtaining alternative oak products is seasoned and toasted in a different way, adapted to its shape and size. Wood seasoning allows mainly reducing the high percentage of humidity in wood and simultaneously the fibber contraction. The most used seasoning in cooperage usually happens under natural conditions in the wood yard in open air during a variable time period between 18 and 36 months. During this time, the wood matures, reducing bitterness and astringency and increasing aromatic properties by means of changes in its chemical composition (Cadahía et al., 2007; Fernández de Simón et al., 1999; Li & Duan, 2019). In the meantime, alternative drying results in a different evolution of the chemical composition of the wood, and is usually carried out in a dryer or in a mixed method combining drying in the open air and in a dryer. Toasting process is the stage with the greatest influence on the final wood chemical composition, as heat causes a series of chemical transformations and the degradation of wood (lignin, polyosides, polyphenols and lipids). In general, wood toasting produces a significant decrease in astringency, accompanied by an increase in aromatic substances, some of which are newly formed as a result of the wood thermolysis and others, which already exist in oak and are increased by toasting (Cadahía et al., 2003; Dumitriu et al., 2017; Watrelot, Badet-Murat & Waterhouse 2018). The toasting process for the production of alternative oak products consists of reproducing the toasting in the barrels. However, depending on the size of the alternative product, it is essential to adapt this process (Nevares & del Alamo-Sanza, 2016). Thus, small pieces such as chips or cubes are usually toasted by convection and conduction, while larger pieces such as planks or staves are toasted by convection (Chatonnet 2007). Previous studies have investigated the effects of wine in contact with alterative types of *Q. pyrenaica* (Del

Álamo et al., 2010; Fernández de Simón, Cadahía, del Álamo, et al., 2010; Fernández de Simón, Cadahía, Muiño, et al., 2010; Gallego et al., 2012; Rodríguez-Bencomo et al., 2009; Sánchez-Gómez et al., 2018; Tavares et al., 2017). However, despite the importance of oxygen, there are only few studies in which both the alternatives and MOX were used (Del Álamo et al., 2010; Fernández de Simón, Cadahía, del Álamo, et al., 2010; Fernández de Simón, Cadahía, Muiño, et al., 2010; Gallego et al., 2012; Sánchez-Gómez et al., 2018).

Phenolic compounds determine the colour of wines and other organoleptic characteristics, such as smell and taste, which are decisive for the quality of wine. However, it has not yet been studied how the different toasting and seasoning processes carried out on *Q. pyrenaica* wood can affect these compounds in wines. The main objective of this work was to evaluate the effect of different alternative products of *Q. pyrenaica* and micro-oxygenation on the phenolic compounds found in a red wine. The alternative products were studied with different sizes (chips and staves), different seasoning methods (traditional and alternative), and different types of toasting (light, medium and heavy).

2. Materials and methods

2.1. Oak woods

Q. pyrenaica oak alternative products from trees cultivated in Salamanca (Spain) were used with different sizes, seasoning and toasting characteristics. The two sizes were chips (1 cm x 0.5 cm) and staves (100 cm x 8 cm x 1 cm). The seasoning was subjected to two different conditions. (1) Natural seasoning: this is carried out over a period of two years as for the size usually used for a barrel. After seasoning, the wood was cut into the two sizes (staves and chips). This process was carried out in Tonelería Intona, SA (Navarra, Spain), where the average annual temperature and the total precipitation were 14.4 ºC and 504 mm^3/year, respectively. The wood was watered daily during the first 2 months and during the summer; the rest of the time, every 15 days. (2) Alternative seasoning: the process during the first four months was the same as natural seasoning. The wood was then cut to obtain staves and chips. The staves were washed with unchlorinated water through immersions (7 immersions, 20 min each). Between each immersion, the staves were dry stacked to enable air circulation and allowed to dry for 10 days. The chips were washed by immersion in unchlorinated water for 10 min at ambient temperature, centrifuged for 4 min, and dried in an oven at 50 ºC for 24 h. This process was repeated seven times.

After seasoning, all the wood was toasted in an industrial-scale convection oven, with supports specially adapted to staves or special oven trays for the chips, at three intensity levels: light (10 min at 190 ºC), medium (15 min at 200 ºC), or heavy (20 min at 210 ºC).

The surface/volume ratio of a 225 L barrel was calculated to determine the quantity of chips and staves necessary for the same wine/wood surface proportion in 210 L stainless steel tanks. Therefore, the staves were numbered and measured individually to use the exact wood

surface area as in barrel-aging, adding 11 staves by tank, and the in the case of chips 6,5 g/L were added.

2.2. Wine

The wine used was a red single-variety wine (cv. Tinta del País) produced in a cellar of a Spanish denomination of origin (DO): Ribera del Duero. The chemical parameters of the wine were as follows: total acidity 4.9 g/L, pH 3.76, volatile acidity 0.56 g/L, sugars 1.6 g/L, and alcoholic degree 13.5% (v/v). These parameters were evaluated before the wine was transferred into the tanks according to OIV (International Organisation of Vine and Wine) methods (OIV, 2003). The wines were aged at the same wine cellar, where humidity and temperature conditions were controlled at 65–75% and 15–16 ºC during the ageing period.

Twenty-four 210 L stainless steel tanks were used to study the influencing factors in different combinations (alternative products size-seasoning-toasting level): 1) chips-traditional-light; 2) chips-traditional-medium; 3) chips-traditional-heavy; 4) chips-alternative-light; 5) chips-alternative-medium; 6) chips-alternative-heavy; 7) staves-traditional-light; 8) staves-traditional-medium; 9) staves-traditional-heavy; 10) staves-alternative-light; 11) staves-alternative-medium; 12) staves-alternative-heavy. All test conditions were carried out in duplicate; therefore, two tanks were used for each numbered condition. The experimental design is shown in Figure 1. All tanks were micro-oxygenated using an Eco2 device (Oenodev, France) and ceramic diffusers with dosage rates between 1.5 and 2 mL/L·month. Samples were taken from each tank after 30, 60, 90 and 120 days of ageing.

2.3. Wine analysis

2.3.1. Phenolic global parameter determination and index

Phenolic compounds, such as total phenols (TP), were determined by the method of Folin and Ciocalteu (1927), and low polymerized phenols (LPP) were determined by the method of Masquelier and Michaud (1965). High-polymerized phenols (HPP) were calculated using the difference between TP and LPP. Catechins (CAT, as mg/L of D-catechin) were analysed following the method described by Swain and Hillis (1959). Total anthocyanins (ACY, as mg/L of malvidin-3-O-glucoside) were analysed by means of colour changes according to the pH of the medium (Paronetto, 1977), and tannins (TAN, as g/L of cyanidin chloride) were analysed using the Ribéreau-Gayón and Stonstreet (1965) method. The ionization index (Ioln) was analysed by the Somers and Evans (1977) method, and the gelatine index (Gln) by the Ribéreau-Gayón, Glories, Maujean, and Dobourdieu (2003) method. All analyses were carried out in a Shimadzu Spectrometer (Japan) in duplicate for every sample.

2.3.2. Colour, chemical age and copigmentation determination

178 Colour parameters were determined by a direct measurement of wine absorbance to 420,
179 520 and 620 nm in a 1 mm cell with a Shimadzu UV/Vis spectrophotometer (Japan). The colour
180 intensity (CI), hue (T) and %dA (percentage of free or combined ion flavilium anthocyanin) were
181 also calculated. Other calculated variables were the percentages of red, yellow and blue. All of
182 these measurements were carried out according to Glories (1984).
183 The chemical age was defined according to the parameters (i), (ii), (i/ii), (α) and (αα)
184 using the Somers and Evans (1977) method: (i) indicates the relation between polymeric
185 pigments and wine ACY content; (ii) reflects the relation between polymeric pigments and wine
186 anthocyanins measured in their ion flavilium form; (i/ii) is the relation of wine anthocyanins in
187 flavilium ion form with respect to wine anthocyanins; (α) represents the percentage of
188 anthocyanins found as flavilium ions; and (αα) is the percentage of anthocyanins as flavilium ions
189 after removal of the decolorizing effect of SO_2.
190 The copigmentation parameter was determined according to the method proposed by
191 Boulton (2001) via the following parameters, where the colour was due to: (FC) the estimation of
192 the content of flavanol cofactors; (COP) copigmentation; (AL) free anthocyanins; and (PP)
193 polymeric pigment.
194 All the parameters were measured in duplicate in every sample.
195
196 *2.3.3. Individual anthocyanin analysis*
197 Anthocyanins, including delphinidin-3-O-glucoside (Df-3-Gl), cyaniding-3-O-glucoside
198 (Cn-3-Gl), petunidin-3-O-glucoside (Pt-3-Gl), peonidin-3-O-glucoside (Pn-3-Gl), malvidin-3-O-
199 glucoside (Mv-3-Gl), vitisin A (Mv-3-Gl-Py), malvidin-3-O-glucoside ethyl-epicatechin (Mv-3-
200 Gl-Ethyl), peonidin-3-O-acetylglucoside (Pn-3-Gl-Ac), malvidin-3-O-acetylglucoside (Mv-3-Gl-
201 Ac), delphinidin-3-O-p-coumaroylglucoside (Df-3-Gl-Cm), cyanidin-3-O-p-coumaroylglucoside
202 (Cn-3-Gl-Cm), *cis*-malvidin-3-O-p-coumaroylglucoside (Mv-3-Gl-Cm C), petunidin-3-O-p-
203 coumaroylglucoside (Pt-3-Gl-Cm) and *trans*-malvidin-3-O-p-coumaroylglucoside (Mv-3-Gl-Cm
204 T), were analysed by an Agilent 1100 HPLC-DAD. Anthocyanin identification was carried out by
205 comparison of their spectra and retention times according to the method described by del Álamo
206 Sanza, Nevares Domínguez, and García Merino (2004). These compounds were quantified with
207 Mv-3-Gl as standard, because this is the most representative anthocyanin in wine. All samples
208 were analysed in duplicate.
209
210 *2.3.4. Individual low molecular weight phenol (LMWP) analysis*
211 The different phenolic compounds (hydroxybenzoic acids, including gallic acid,
212 protocatechuic acid, vanillic acid and syringic acid; hydroxycinamic acids including *trans*-caftaric
213 acid, *trans*-coutaric acid, caffeic acid and *p*-coumaric acid; aldehydes, including protocatechuic
214 aldehyde and vanillic aldehyde; catechins, including catechin and epicatechin; and coumarins,

such as scopoletin) were quantified in wine by an Agilent 1100 HPLC-DAD according to the method described by del Álamo, Casado, Hernández, and Jiménez (2004). These compounds were quantified with their respective standards, except *trans*-caftaric and *trans*-coutaric acids, which were identified according to wavelengths and retention times following the method of Monagas, Bartolomé, and Gómez-Cordovés, (2005), and were quantified with caffeic and *p*-coumaric acid, respectively. All samples were analysed in duplicate.

2.3. Sensory analysis of the wines

A group of 10 panelists with previous experience in wine analysis were trained in recognizing wine aromatic characteristics. In this training, all the compounds were presented to the participants at their threshold concentration in water and in test wines. Wines were presented in random order. No information was given to the judges about the origin of the samples. The wine tasting took place in an air-conditioned room (21ºC) with isolated booths, located in University of Valladolid (Palencia, Spain). At the end of the aging process, each wine was assessed by judges using a tasting evaluation sheet that included descriptors grouped by visual phase (colour intensity, clarity, blue, red, yellow, green), taste phase (volume, acidity, tannic intensity, sweet tannin, green tannin and astringency, bitternes) and global parameters (harmony, persistency and final value).

The panelists tasted the different wines, noted the specific descriptors perceived and rated the intensity of each sensory descriptor on a ten-point scale, where 1 indicated that the descriptor was not perceived (absence) and 10 value correspond to a very strong perception. All the sensory evaluations were carried out under Spanish Standardization Rules (6658:2017, 1997)

2.5. Statistical analysis

A statistical analysis on different parameters was performed using SPSS Version 21.0 statistical package for Windows (SPSS, Chicago, USA) one-way or multifactorial analysis of variance (ANOVA) at an alpha level of 5% with Tukey post-hoc least significant difference. Principal component analysis (PCA), evaluated by Statgraphics Centurion XVI (Statistical Graphics Corporation, Virginia, United States) version 16.2.04 for Windows, was used to examine the relationship between different compounds and alternative product types.

3. Results and discussion

3.3. Global phenolic parameters

The global phenolic parameters studied showed significantly differences when the two format used (chips + MOX and staves + MOX) were compared (Table 1). Figures 1S and 2S show the evolution of different compounds in relation to their initial content in the wine,

252 expressed as the ratio between the content at each moment less initial content and the initial
253 content. After 120 days of ageing, wines from staves + MOX had higher levels of total
254 polyphenol content, high polymerized phenols and catechins (TP, HPP and CAT), but lower
255 contents of low polymerized phenols, total anthocyanins and tannins (LPP, ACY and TAN) than
256 those aged with chips + MOX (Table 1), as it can be also seen in the evolution reported in Figure
257 1S, for TP, HPP, ACY and TAN. The results related to TP, HPP and LPP were the same than
258 those reported by Gallego et al. 2012 when *Q. pyrenaica, Q. alba* and *Q. petraea* were studied
259 and those observed for TP by Laqui-Estaña et al., 2018 when *Q. petraea* oak products were
260 considered. The extraction of phenolic compounds and their polymerization was greater in wines
261 aged with staves + MOX than with chips + MOX (Table 1). This fact could be explained by the
262 higher oxygen consumption by wines aged with staves + MOX rather than those with chips +
263 MOX, since, it is known that oxygen determines the reactions between phenolic compounds (Del
264 Álamo et al. 2010), so it is logical to expect further polymerization processes in wines treated
265 with staves + MOX (Sánchez-Gómez et al., 2018; Gómez-Plaza & Bautista-Ortín, 2019).
266 The total anthocyanins (ACY) decrease markedly in the 90 and 120 day samples with
267 respect to the content at 60 days (Figure 1S). This is due to the oxidation, condensation and
268 polymerization processes that transform them into other compounds and has an important effect
269 on the modification of the wine's colour (Laqui-Estaña et al., 2018). In fact, the evolution of
270 anthocyanins is closely related to oxygen consumption, presenting a negative correlation the
271 higher the oxygen consumption is, the lower the anthocyanin wine final content (Sánchez-Gómez
272 et al., 2018). Total anthocyanins (ACY) was lower in wines aged with staves + MOX than in
273 those aged with chips+MOX, as Del Alamo Sanza & Nevares Domínguez, 2006 and Laqui-
274 Estaña et al., 2018 reported. The content of catechins (CAT) in wines was lower when using chips
275 + MOX than when using staves + MOX. This means a faster loss of catechins when wines were
276 aged with chips + MOX rather than staves + MOX, corroborating the results of Del Álamo et al.
277 (2010). Wines aged with staves + MOX had the lowest tannins (TAN) content, which also
278 presented the lower ACY levels (Table 1). Both, TAN and ACY, are closely related to oxygen
279 consumption in wine, as many of the reactions in which these compounds are involved are
280 determined by the level of dissolved oxygen (Del Álamo et al., 2010), to form stable pigments,
281 such as the so-called pyranoanthocyanins (Quaglieri et al., 2017). Oxidative polymerization is
282 important for the combination of tannins with other compounds, where acetaldehydes can play an
283 important role in the formation of acetaldehyde-bridged molecules, assisting in the formation of
284 tannin-tannin complexes and other tannin-anthocyanin reactions (Nevares & del Alamo-Sanza,
285 2016; Laqui-Estaña et al., 2018). Therefore, the greater oxygen consumption in the wines aged
286 with staves + MOX (Del Álamo et al., 2010) may have increased these tannin reactions.
287 From the data in Tables 2 and 3, it is observed that toasting affected the content of TAN
288 in wines aged with staves + MOX, yielding a higher TAN content with lower toasting. This is due

to the wood tannins degradation with heat, which increases with temperature, a fact that has been observed both in the wood of traditional species (Watrelot et al., 2018) as well as in *Q. pyrenaica.* ~~(Jordão et al., 2007).~~ When seasoning methods were compared for chips + MOX (Table 2), wines from alternative seasoning presented a significantly higher content.

3.4. Index, colour characteristics, chemical age and copigmentation parameters

Table 1 shows that the size of the alternative products affected the ionization index (IoIn) of the wines. This index, which indirectly indicates the percentage of anthocyanins that contribute to the colour of wines, was higher in those aged with staves + MOX, which are likely to maintain their base red colour better than wines aged with chips + MOX. Although the ACY of wines aged with staves was lower than those aged with chips, it was observed that the %520 value was higher. This result indicates that the analysis of ACY does not include substances that respond to the IoIn measurement and are responsible for the red colour of wines measured at 520 nm, possibly pigments involving other molecules linked to anthocyanins. For wines aged with staves + MOX, it was observed that seasoning also influenced this index, giving higher IoIn and %520 values when staves were used that were alternately seasoned (Table 3). These wines also had more astringency, which was estimated by the capacity of the tannins to precipitate the saliva protein, parameter known as gelatine index (Gln). The polymerization and depolymerization of tannins and anthocyanins, as well as their involvement with proteins and polysaccharides, have a great impact on the sensory characteristics of the wine, including astringency. In Table 3 it can observed that the type of seasoning had an effect on the Gln: wines aged with staves + MOX with alternative seasoning showed higher levels than when traditionally seasoned woods were used (Table 3).

The chromatic data analysis showed that the alternative size had a definite influence on the final colour of the wines (Table 1). In all of them, an increase in colour intensity over time (30, 60, 90 and 120 days) was observed (data not shown). The wine aged with staves + MOX presented a higher intensity of colour (IC), with a greater predominance of red (%520) and blue (%620) and lower of yellow (%420) than wines aged with chips + MOX, which presented a higher tonality (T) because the value of the yellow component (%420) was higher compared to the red component (%520). Fact that can be explained to the increased polymerization (HPP), since the condensation between anthocyanins and tannins, and copigmentation, lead to the formation of new stable pigment compounds that provide a greater intensity of colour, being more resistant to degradation, and increasing the blue tones at the expense of the yellow ones (Vivas & Glories, 1996). Separating the wines according to the size of the alternative used (chips or staves), it was observed that the type of seasoning affected the final colour in the case of wines aged with staves + MOX (Table 3): those from alternatively seasoned presented higher %520 but lower of %620. Neither toasting nor seasoning had no effect on wines aged with chips + MOX (Table 2).

Regarding copigmentation and chemical age (Table 1), wines aged with staves + MOX presented a higher level of colour fraction to the presence of polymeric pigment (PP) and a lower level of colour fraction to copigmentation (COP), which related to a higher (α), (i) and (ii) and lower (i/ii) values. Such differences had been reported that increased in significance during bottle period (Gallego et al., 2012). Furthermore, the toasting factor affected wines aged with staves + MOX in two of the copigmentation parameters studied: lower toasting led to a higher colour due to the estimated flavanol cofactor (FC) content and a lower colour due to free anthocyanins (AL) (Table 3).

3.3. Anthocyanins

Respect to the anthocyanins content of wines after 120 days of ageing, it was observed that the size of the alternative product (chips or staves) was a determining factor (Table 1), fact previously published by Nevares & del Álamo, 2008 and Del Álamo et al., 2010. Although it has also been reported that the MOX process significantly affected the evolution of anthocyanins in wine (Gómez-Plaza & Bautista-Ortín, 2019; Quaglieri et al., 2017; Sánchez-Gómez et al., 2018). Mv-3-Gl, Pt-3-Gl and Df-3-Gl were the major anthocyanins in the wines (concentrations greater than 10 mg/L), however, their concentration was not affected by the type of alternative used (chips or staves) (Table 1). The rest of the anthocyanins were found in wines at concentrations lower than 10 mg/L, and the content was affected by the size of the alternative product used. Thus, wines aged with chips + MOX presented a significantly higher content of Cn-3-Gl, Pn-3-Gl, Mv-3-Gl-Ethyl, Mv-3-Gl-Ac and Pt-3-Gl-Cm than wines aged with staves + MOX. These results have been observed throughout the evolution as described in Figure 2S for the example of Cn-3-Gl and Pn-3-Gl. Meanwhile, the wines aged with staves + MOX had significantly higher levels of Mv-3-Gl-Py, Pn-3-Gl-Ac and Df-3-Gl-Cm compared to those aged with chips + MOX. Regarding the importance of the seasoning and toasting process (Tables 2 and 3), wines aged with alternative seasoning chips had higher levels of Mv-3-Gl-Py, Mv-3-Gl-Ac and Mv-3-Gl-Cm C compared to wines aged with traditional seasoning chips, but this situation was reversed for Mv-3-Gl-Ethyl and Cn-3-Gl-Cm. Contrary to Dumitriu et al., 2016, toast degree not affected the anthocyanins content. In the case of wines aged with staves + MOX, only Cn-3-Gl-Cm was affected by seasoning process (Table 3).

3.4. Low molecular weight phenols (LMWP)

The size of the alternative product was the primary influencing factor when differentiating the wines based on their LMWP content (Table 1) after 120 days of ageing. In wines aged with chips + MOX, the only LMWP that presented different concentrations depending on the type of seasoning and toasting of the wood was gallic acid (Table 2). However, when

362 ageing with staves + MOX, the content of some of LMWP in wines was significantly influenced
363 by wood seasoning and toasting (Table 3).
364 Gallic acid, which is one of the main compounds typical of wine aged with oak, was the
365 principal low molecular weight phenol found in the different wines. In general, during contact
366 with the wood (30, 60, 90 and 120 days), the gallic acid content in the wine increased (Figure 2S),
367 due to hydrolysis of the elagitannins released from wood. This increment has been observed by
368 other authors when wines were aged with other oak species (Gallego et al., 2011; Oberholster et
369 al., 2015). The wines aged in contact with staves + MOX showed significantly higher levels of
370 gallic acid than those aged with chips + MOX (Table 1), as Gallego et al., 2011; Oberholster et
371 al., 2015 observed using wood from other species. Such differences were presented not only at
372 120 days but also during all evolution (Figure 2S), which could be explained by a greater
373 degradation of this compound in wines in contact with chips. The average concentration of gallic
374 acid was also affected by the degree of wood toasting: its content in both chips-aged and staves-
375 aged wines was higher when lower toasting levels were applied (Tables 2 and 3). This result was
376 to be expected, since gallic acid is a compound thermally sensitive, whose concentration
377 decreased with the degree of toasting in wood *Q. pyrenaica* (Canas et al., 2000). Meanwhile,
378 wood seasoning did not influence the gallic content (Tables 2 and 3).
379 Other compounds that have also been found in higher concentrations in wines aged with
380 staves + MOX compared to chips + MOX were *trans*-caftaric and *trans*-coutaric acids as Gallego
381 et al. 2011 reported using alternative oak products made of American wood. These variations
382 have been described in the bibliography because of reactions between tartaric acid and *trans*-
383 caffeic and *trans*-coumaric acids (Gallego et al., 2011) or to their hydrolysis intro their
384 corresponding acids (Gómez Gallego, Gómez García-Carpintero, Sánchez-Palomo, González
385 Viñas, & Hermosín-Gutiérrez, 2013).
386 The levels of other hydroxybenzoic acids (protocatechuic acid, vanillic acid and syringic
387 acid) and hydroxycinnamic acids (caffeic acid and *p*-coumaric acid), as well as *p*-vanillin and
388 protocatechuic aldehyde, epicatechin and scopoletin, were higher in wines aged with chips +
389 MOX than in those aged with staves + MOX. The highest content of these compounds was not
390 observed only at 120 days as can be seen in the case of vanillic acid, also for 60 and 90 days
391 (Figure 2S). These results corroborate those obtained by Gallego et al. 2011 for all compounds,
392 except for vanillic acid and protocatechuic aldehyde, and, in case of Laqui-Estaña et al. 2018, the
393 results related to vanillic acid. Catechin was the only LMWP whose content in the wine was
394 similar whether aged with chips + MOX or staves + MOX using *Q. pyrenaica* (Table 1).
395 However, when other oak species are used, it was observed that the concentration of this
396 compound in wines was different depending on the size of the product (Gallego et al., 2011;
397 Laqui-Estaña et al., 2018).

The syringic acid and protocatechuic aldehyde content of wines aged with staves + MOX increased with increasing levels of wood toasting, which was in accordance with what was described by Canas et al. (2000) for *Q. pyrenaica* wood. The protocatechuic aldehyde content has not been previously studied in *Q. pyrenaica* wood, but, in other woods such as chestnut, cherry and oaks from different origins (American, Hungarian, French, Romanian and Russian), it has been found that this compound increased as the level of toasting increased, a result attributed to lignin degradation with increasing temperature (Alañón, Castro-Vázquez, Díaz-Maroto, Gordon, & Pérez-Coello, 2011; Soares, Garcia, Freitas, & Cabrita, 2012).

The wood seasoning method affected the content of vainillic acid in wines aged with staves + MOX, since those aged with the wood from alternative seasoning method showed a higher content of vainillic acid. Contrary to this, Fernández de Simón et al. (2010) described that the seasoning method did not affect the vanillin content. For scopoletin and epicatechin these same wines showed significant differences also for wood seasoning: wines aged with the wood from seasoning alternative method showed a higher content of scopoletin and lower concentration of epicatechin respect to the wines aged with wood from the traditional seasoning. Therefore, the content of LWMP in aged wood wines seem to be markedly influenced by the wood size, independently of toasting and seasoning, being the contact time also an important factor.

In general, no studies regarding how the size of the alternatives could affect wines aged with *Q. pyrenaica* wood were found. However, there are some studies in the literature that used other oak species, such as *Q. alba, Q. robur,* and *Q. petraea,* where a greater evolution of the wines has been described related to the smaller the size of the product used (Gallego, Nevares, Fernández, & Del Álamo, 2011; Laqui-Estaña, López-Solís, Peña-Neira, Medel-Marabolí, & Obreque-Slier, 2018; Oberholster et al., 2015).

3.5. Principal component analysis (PCA)

Figure 2 was performed with anthocyanins, index, colour analysis and copigmentation parameters, and Figure 3 was carried out with low molecular weight phenols (LMWP), global phenolic parameters and chemical age. In these analyses, all of the wines resulting from ageing with different sizes, toasting and seasoning levels have been included in the different sampling time points (30, 60, 90 and 120 days) in order to see the importance of the compounds and parameters analysed in the different treatments over time. In all the graphs obtained with this analysis, it can be observed that the size of the alternative used was the factor that defined the greater percentage of variance of the studied wine variables, independent of the seasoning or toasting carried out on the wood. In all cases, component 1 separates all of the wines aged with chips + MOX from those aged with staves + MOX, explaining more than 47% of the variance in all sampling time points (Figures 1 and 2; Supplementary Tables 1 and 2). However, components 1 and 2 could not separate the wines from woods of different degrees of toasting or seasoning.

435 Therefore, the phenolic composition of the different wines was essentially influenced by the size
436 of the alternative used. These results would indicate that *Q. pyrenaica* could be used to shorten
437 the process of obtaining alternative products through alternative seasoning methods, since
438 traditional seasoning is a long process and does not have an important effect on the phenolic
439 composition of wines. In this way, Fernández de Simón et al. (2010) studied how the size,
440 seasoning and toasting factors of *Q. pyrenaica* could affect the volatile composition of wines aged
441 with their alternative products, observing the same than the results obtained in this work: the
442 influence of seasoning was much lower than the influence of the size of the alternative or toasting
443 intensity.
444 Figure 2 shows that wines from ageing with chips + MOX (1, 2, 3, 4, 5, 6) were located
445 in the positive side of first principal component axis at all-time points. In Supplementary Table 1,
446 it was observed that this component was positively defined by Cn-3-Gl, Pn-3-Gl, Mv-3-Gl-Ac,
447 Mv-3-Gl-ethyl and Pt-3-Gl-Cm. Other compounds also define component 1 positively, but with
448 lower weights, such as Mv-3-Gl-Cm T, Mv-3-Gl, Pt-3-Gl, Df-3-Gl, Cn-3-Gl-Cm and Mv-3-Gl-
449 Cm C, especially in the last sample (120 days). Therefore, the anthocyanins Cn-3-Gl, Pn-3-Gl,
450 Mv-3-Gl-Ac, Mv-3-Gl-Ethyl and Pt-3-Gl-Cm were positively related to chips + MOX at all of the
451 sampled times (30, 60, 90 and 120 days). With the exception of the sample at 30 days, wines from
452 chips + MOX were also related to the absorbance at 420, i.e., with yellow tones. However, staves
453 + MOX were more related to the absorbance at 620 (blue tones). In addition, the wines from
454 staves + MOX presented higher colour intensity and a higher colour fraction due to the polymeric
455 pigments in all the samples.
456 Figure 3 showed that the wines aged with staves + MOX (7, 8, 9, 10, 11, 12) were located
457 in the positive side of first principal component axis for all of the samples (30, 60, 90 and 120). In
458 Supplementary Table 2 it was observed that, regardless of the sampling, this component was
459 positively defined by gallic acid and *trans*-coutaric acid content and negatively defined by
460 protocatechuic acid, syringic acid and scopoletin.
461
462 *3.6. Sensorial analysis*
463 The sensory characteristics related to the analytical parameters studied, attributes that
464 define the wines obtained in the visual and gustatory phases, are presented in Figure 4. The
465 sensory analysis of the wines obtained after maturation with wood and micro-oxygenation
466 indicates that the most significant differences are due to the size of the alternative product used.
467 However, tasters have found no differences between wines matured with traditional wood and
468 those obtained after contact with alternative wood seasoning process. Statistically significant
469 differences have only been found in wines aged with light toasted and alternative seasoning *Q.*
470 *pyrenaica* staves, which present greater clarity and harmony than those treated with light toasted
471 and traditionally seasoning *Q. pyrenaica* staves. Figure 5 shows the principal component bi-plot,

illustrating the simultaneous projection of the wines tested and the sensorial descriptors. The two principal components accounted for 68.41% of the total variance. The first principal component represented by the axis "x" separated wines aged with chips + MOX from the wines aged with staves + MOX. Wines treated with chips + MOX are defined with greater tannic intensity, volume, persistence and harmony, while in the case of wines treated with staves + MOX, tasters found sweet tannins, acidity and wines with greater colour intensity defined by the yellow tones and the base red colour. These results are in agreement with some of them obtained in the wine chemical composition since, wines from staves + MOX had less Gln value and higher colour intensity and %520. Meanwhile, wines aged with chips + MOX presented higher values of TAN and Gln.

4. Conclusions

The size of the oak wood alternative (chips or staves) was the studied factor that most affected the content of all analysed parameters (anthocyanins, low molecular weight compounds, global phenolic parameters, index, colour, copigmentation and chemical age), determining the entire ageing process of the wine, not only at 120 days of ageing, but also at 30, 60 and 90.

The alternative seasoning of *Q. pyrenaica* Willd allowed a shortened wood maturation process without significantly affecting the phenolic evolution and sensorial properties of aged wines. This can lead to an important reduction in the process of obtaining alternative products from this wood.

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Figure captions

Figure 1. Experimental design. Twenty-four stainless steel tanks were used, distributed in the following ways: 1) Chip-traditional-light; 2) Chip-traditional-medium; 3) Chip-traditional-heavy; 4) Chip-alternative-light; 5) Chip-alternative-medium; 6) Chip-alternative-heavy; 7) Staves-traditional-light; 8) Staves-traditional-medium; 9) Staves-traditional-heavy; 10) Staves-alternative-light; 11) Staves-alternative-medium; and 12) Staves-alternative-heavy.

Figure 2. Principal component analysis (PCA) performed with anthocyanins, index, colour analysis and copigmentation parameters in the wines aged with alternative products of oak *Q.*

509 *pyrenaica* of different sizes (chips and staves), seasoning methods (traditional and alternative),
510 and toasting levels (light, medium and heavy) at different ageing times: a) 30 days; b) 60 days; c)
511 90 days; and d) 120 days. The number indicates the different wines studied (format, seasoning
512 method and toasting level): 1) Chips-traditional-light; 2) Chips-traditional-medium; 3) Chips-
513 traditional-heavy; 4) Chips-alternative-light; 5) Chips-alternative-medium; 6) Chips-alternative-
514 heavy; 7) Staves-traditional-light; 8) Staves-traditional-medium; 9) Staves-traditional-heavy; 10)
515 Staves-alternative-light; 11) Staves-alternative-medium; and 12) Staves-alternative-heavy.
516
517 **Figure 3**. Principal component analysis (PCA) performed with low molecular weight phenols,
518 global phenolic parameters and chemical age in wines aged with alternative products of oak *Q.*
519 *pyrenaica* of different sizes (chips and staves), seasoning methods (traditional and alternative),
520 and toasting levels (light, medium and heavy) at different ageing times: a) 30 days; b) 60 days; c)
521 90 days; d) 120 days. The number indicates the different wines studied (format, seasoning method
522 and toasting level): 1) Chips-traditional-light; 2) Chips-traditional-medium; 3) Chips-traditional-
523 heavy; 4) Chips-alternative-light; 5) Chips-alternative-medium; 6) Chips-alternative-heavy; 7)
524 Staves-traditional-light; 8) Staves-traditional-medium; 9) Staves-traditional-heavy; 10) Staves-
525 alternative-light; 11) Staves-alternative-medium; and 12) Staves-alternative-heavy.
526
527 **Figure 4**. Sensorial descriptors mean scores of wines aged with alternative products of oak *Q.*
528 *pyrenaica* of different sizes (chips and staves) and seasoning methods (traditional ——— and
529 alternative ------) at 120 days. Values with ** or *** denoted significant differences at $p<0.01$
530 and $p<0.001$ respectively.
531
532 **Figure 5**. Principal component analysis (PCA) performed with sensorial parameters of wines
533 aged with oak *Q. pyrenaica* of different sizes (chips and staves) and seasoning methods
534 (traditional and alternative) 120 days. ChTL: Chips-traditional-light; ChTM: Chips-traditional-
535 medium; ChTH: Chips-traditional-heavy; ChAL: Chips-alternative-light; ChAM: Chips-
536 alternative-medium; ChAH: Chips-alternative-heavy; StTL: Staves-traditional-light; StTM:
537 Staves-traditional-medium; StTH: Staves-traditional-heavy; StAL: Staves-alternative-light;
538 StAM: Staves-alternative-medium; and StAH: Staves-alternative-heavy.
539
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Table 1. Composition of wines after 120 days in contact with chips and staves of *Q. pyrenaica.*

		Chips	*Staves*	*F and p values*
Anthocyanins (mg/L)	Df-3-Gl	27.24±1.34	27.54±1.36	0.29
	Cn-3-Gl	6.12±0.08	5.85±0.13	**38.95*****
	Pt-3-Gl	24.26±1.11	24.44±1.14	0.15
	Pn-3-Gl	9.30±0.31	8.66±0.45	**16.63*****
	Mv-3-Gl	68.19±4.02	67.73±3.87	0.08
	Mv-3-Gl-Py	5.88±0.50	6.71±0.11	**31.38*****
	Mv-3-Gl-Ethyl	5.39±0.09	5.06±0.18	**32.96*****
	Pn-3-Gl-Ac	4.60±0.03	7.53±1.00	**102.89*****
	Mv-3-Gl-Ac	6.30±0.12	6.12±0.15	**10.69****
	Df-3-Gl-Cm	5.94±0.17	6.23±0.12	**24.08*****
	Cn-3-Gl-Cm	4.58±0.09	4.54±0.06	1.11
	Mv-3-Gl-Cm C	4.66±0.04	4.66±0.05	0.08
	Pt-3-Gl-Cm	5.53±0.06	5.44±0.09	**7.61****
	Mv-3-Gl-Cm T	8.29±0.30	8.19±0.37	0.56
LMWP (mg/L)	Gallic acid	102.13±8.23	127.83±5.93	**76.06*****
	Protocatechuic acid	5.51±0.38	5.05±0.40	**8.35****
	Vanillic acid	7.40±0.61	4.86±0.57	**112.10*****
	Syringic acid	5.96±0.55	5.16±0.53	**13.10****
	trans-Caftaric acid	25.97±1.67	31.90±1.04	**108.96*****
	trans-Coutaric acid	11.01±0.59	12.29±0.52	**32.46*****
	Caffeic acid	5.85±0.35	5.41±0.37	**8.96****
	p-Coumaric acid	7.84±0.59	6.24±0.89	**27.13*****
	p-Vanillin	3.02±0.12	2.40±0.26	**58.16*****
	Protocatechuic aldehyde	6.03±0.36	0.63±0.13	**2155.12*****
	Catechin	36.10±11.31	34.78±4.48	0.14
	Epicatechin	12.01±2.44	4.28±2.46	**59.59*****
	Scopoletin	3.58±0.53	2.92±0.34	**13.28*****
Global phenolic parameters	TP (mg/L)	2208.25±45.61	2414.15±22.25	**199.17*****
	LPP (mg/L)	1275.70±62.99	1063.13±28.11	**103.99*****
	HPP (mg/L)	932.55±79.23	1351.88±30.60	**292.52*****
	CAT (mg/L)	740.37±60.33	847.63±45.67	**24.11*****
	ACY (mg/L)	562.63±19.48	490.37±15.02	**103.54*****
	TAN (g/L)	2.40±0.35	2.02±0.21	**10.65****
Index	Gln	65.62±5.95	51.59±4.20	**44.57*****
	IoIn	24.12±30.3	28.80±1.28	**24.30*****
Colour analysis	IC	16.86±0.89	17.62±0.57	**6.11***
	T	0.69±0.01	0.65±0.01	**83.68*****
	%A420	34.63±0.20	33.21±0.28	**205.61*****
	%A520	50.00±0.36	50.83±0.53	**20.21*****
	%A620	15.36±0.20	15.99±0.31	**30.40*****
	%dA	50.00±0.71	51.63±1.04	**26.26*****
Copigmentation	FC	9.48±0.20	9.67±0.46	1.80
	COP	0.35±0.03	0.23±0.02	**184.68*****
	AL	0.34±0.02	0.35±0.02	3.77
	PP	0.32±0.01	0.43±0.02	**242.39*****
Chemical age	α	25.71±2.85	30.08±4.18	**8.94****
	αα	29.45±1.37	29.57±1.64	0.03
	i	0.34±0.02	0.44±0.01	**297.37*****
	ii	0.12±0.01	0.17±0.01	**203.26*****
	i/ii	2.82±0.10	2.62±0.11	**19.32*****

Table 2. Composition of wines after 120 days in contact with chips of *Q. pyrenaica* from different toasting level (light, medium and heavy) and seasoning way (traditional and alternative)

		Light	*Medium*	*Heavy*	*F and p values*	*Traditional*	*Alternative*	*F and p values*
Anthocyanins (mg/L)	Df-3-Gl	26.98±0.64	27.18±0.93	27.57±2.25	0.13	26.99±1.20	27.50±1.54	0.26
	Cn-3-Gl	6.12±0.04	6.14±0.06	6.11±0.13	0.12	6.14±0.09	6.11±0.08	0.22
	Pt-3-Gl	24.10±0.52	24.27±0.82	24.39±1.89	0.04	24.17±1.02	24.34±1.29	0.04
	Pn-3-Gl	9.25±0.16	9.35±0.23	9.31±0.51	0.06	9.29±0.27	9.32±0.37	0.01
	Mv-3-Gl	67.32±2.66	68.20±2.58	69.04±6.61	0.12	67.46±3.26	68.92±4.86	0.26
	Mv-3-Gl-Py	5.84±0.59	5.71±0.27	6.09±0.62	1.02	5.55±0.10	6.21±0.53	**8.53***
	Mv-3-Gl-Ethyl	5.43±0-03	5.39±0.09	5.34±0.11	1.82	5.44±0.05	5.33±0.08	**7.73***
	Pn-3-Gl-Ac	4.59±0.02	4.61±0.04	4.60±0.03	0.38	4.60±0.02	4.60±0.04	0.06
	Mv-3-Gl-Ac	5.88±0.10	5.96±0.14	5.98±0.27	0.65	5.83±0.08	6.05±0.17	**7.86***
	Df-3-Gl-Cm	6.28±0.08	6.35±0.11	6.27±0.16	0.47	6.34±0.10	6.26±0.13	0.96
	Cn-3-Gl-Cm	4.58±0.10	4.60±0.12	4.54±0.03	**5.30***	4.64±0.07	4.51±0.02	**86.65*****
	Mv-3-Gl-Cm C	4.65±0.02	4.66±0.04	4.67±0.05	0.77	4.63±0.02	4.68±0.03	**9.71***
	Pt-3-Gl-Cm	5.53±0.03	5.53±0.04	5.52±0.11	0.02	5.52±0.06	5.53±0.07	0.10
	Mv-3-Gl-Cm T	8.26±0.11	8.38±0.25	8.23±0.48	0.17	8.27±0.27	8.31±0.34	0.05
LMWP (mg/L)	Gallic acid	110.19±8.55	100.24±4.05	95.95±4.07	**7.16***	103.68±10.04	100.57±6.51	0.98
	Protocatechuic acid	5.49±0.65	5.50±0.31	5.53±0.14	0.02	5.65±0.40	5.36±0.34	3.33
	Vanillic acid	7.19±0.73	7.43±0.37	7.57±0.78	0.25	7.42±0.72	7.37±0.54	0.01
	Syringic acid	5.63±0.46	6.00±0.61	6.24±0.53	1.38	5.83±0.42	6.08±0.68	0.68
	trans-Caftaric acid	27.13±2.26	25.01±1.24	25.78±0.70	1.86	26.25±2.05	25.70±1.32	0.36
	trans-Coutaric acid	11.32±0.93	10.68±0.23	11.02±0.25	1.38	11.09±0.71	10.92±0.48	0.28
	Caffeic acid	5.87±0.43	5.68±0.08	5.98±0.46	0.59	5.87±0.28	5.82±0.44	0.05
	p-Coumaric acid	7.87±0.44	7.68±0.31	7.98±0.95	0.32	7.70±0.41	7.99±0.73	0.85
	p-Vanillin	3.01±0.09	3.04±0.19	3.03±0.10	0.06	2.96±0.11	3.09±0.11	3.65
	Protocatechuic aldehyde	6.28±0.46	5.82±0.23	5.98±0.27	2.97	6.01±0.46	6.05±0.28	0.06
	Catechin	38.17±2.49	28.52±16.64	41.61±7.94	1.46	35.66±2.96	36.53±16.50	0.02
	Epicatechin	12.09±3.07	12.72±2.31	11.22±2.35	1.80	11.65±2.52	12.37±2.54	1.23
	Scopoletin	3.53±0.47	3.41±0.43	3.81±0.71	1.01	3.49±0.47	3.67±0.61	0.59
Global phenolic parameters	TP (mg/L)	2210.84±54.35	2225.70±32.16	2188.20±51.86	0.70	2202.84±46.09	2213.65±48.81	0.17
	LPP (mg/L)	1288.20±77.27	1289.89±83.97	1249.01±9.96	0.48	1249.24±40.07	1302.17±73.77	1.87
	HPP (mg/L)	922.64±97.13	935.81±107.05	939.19±43.77	0.04	953.60±67.67	911.49±90.36	0.62
	CAT (mg/L)	788.29±74.35	739.18±31.71	693.64±28.96	**13.06****	770.43±70.74	710.31±28.91	**15.80****
	ACY (mg/L)	558.25±12.52	577.28±8.25	552.34±26.72	1.57	562.04±16.52	563.21±23.68	0.01
	TAN (g/L)	2.40±0.09	2.35±0.46	2.47±0.48	0.14	2.21±0.19	2.60±0.39	4.30
Index	Gln	62.38±5.45	67.91±2.96	66.56±8.30	1.70	63.15±6.31	68.09±4.83	3.74
	IoIn	24.79±2.72	22.34±2.98	25.24±3.29	1.33	25.06±2.78	23.19±3.21	1.43
Colour analysis	IC	16.65±1.27	16.63±0.76	17.30±0.56	0.64	16.64±1.08	17.08±0.67	0.67
	T	0.69±0.01	0.70±0.01	0.69±0.01	0.27	0.69±0.01	0.69±0.01	0.12
	%A420	34.64±0.23	34.69±0.20	34.58±0.19	0.26	34.64±0.15	34.63±0.25	0.01
	%A520	50.03±0.48	49.87±0.33	50.10±0.28	0.31	50.09±0.28	49.92±0.42	0.46
	%A620	15.33±0.25	15.44±0.15	15.32±0.22	0.38	15.27±0.16	15.46±0.21	1.99
	%dA	50.06±0.97	49.73±0.67	50.20±0.56	0.30	50.17±0.57	49.83±0.85	0.47
Copigmentation	FC	9.61±0.19	9.33±0.20	9.50±0.13	2.26	9.53±0.16	9.42±0.23	0.94
	COP	0.34±0.02	0.36±0.01	0.34±0.04	0.70	0.35±0.02	0.34±0.03	0.47
	AL	0.34±0.01	0.33±0.01	0.34±0.02	1.41	0.33±0.01	0.34±0.02	0.64
	PP	0.32±0.01	0.31±0.01	0.32±0.02	0.16	0.31±0.01	0.32±0.01	0.28
Chemical age	α	25.13±3.03	24.73±3.18	27.27±2.34	0.87	25.56±3.46	25.86±2.41	0.03
	αα	29.46±1.08	29.56±2.21	29.34±0.89	0.04	29.89±1.49	29.01±1.19	2.09
	i	0.34±0.01	0.32±0.02	0.35±0.01	2.35	0.34±0.02	0.34±0.02	0.00
	ii	0.12±0.00	0.11±0.01	0.12±0.01	2.37	0.12±0.01	0.12±0.01	0.52
	i/ii	2.81±0.06	2.84±0.15	2.80±0.08	0.22	2.78±0.09	2.85±0.10	2.44

Significant level of aging system factor: *p< 0.01, **p< 0.05 and ***p< 0.001.

Table 3. Composition of wines after 120 days in contact with staves of *Q. pyrenaica* from different toasting level (light, medium and heavy) and seasoning way (traditional and alternative)

		Light	*Medium*	*Heavy*	*F and p values*	*Traditional*	*Alternative*	*F and p values*
Anthocyanins (mg/L)	Df-3-Gl	28.01±1.69	27.03±0.89	27.58±1.56	0.38	28.02±1.42	27.06±1.21	1.10
	Cn-3-Gl	5.92±0.16	5.82±0.04	5.83±0.16	1.08	5.85±0.16	5.86±0.10	0.04
	Pt-3-Gl	25.35±1.25	23.86±0.42	24.10±1.15	1.74	24.40±1.28	24.47±1.10	0.01
	Pn-3-Gl	9.04±0.34	8.51±0.20	8.44±0.55	4.36	8.50±0.57	8.82±0.25	3.04
	Mv-3-Gl	71.39±3.90	65.79±1.23	66.00±3.38	3.16	67.14±4.02	68.31±4.01	0.32
	Mv-3-Gl-Py	6.74±0.14	6.67±0.02	6.71±0.15	0.29	6.75±0.12	6.67±0.11	1.01
	Mv-3-Gl-Ethyl	5.13±0.06	4.98±0.23	5.05±0.22	0.91	4.97±0.23	5.14±0.06	3.40
	Pn-3-Gl-Ac	7.60±1.23	7.22±0.58	7.79±1.27	0.35	7.35±1.03	7.72±1.03	0.44
	Mv-3-Gl-Ac	6.33±0.14	6.19±0.03	6.19±0.12	1.57	6.23±0.13	6.24±0.11	0.05
	Df-3-Gl-Cm	6.25±0.15	6.05±0.04	6.06±0.15	2.93	6.09±0.18	6.16±0.12	0.93
	Cn-3-Gl-Cm	4.52±0.07	4.57±0.05	4.55±0.07	0.68	4.58±0.06	4.51±0.03	5.36
	Mv-3-Gl-Cm C	4.68±0.06	4.65±0.05	4.66±0.05	0.71	4.68±0.04	4.65±0.06	3.07
	Pt-3-Gl-Cm	5.52±0.11	5.39±0.04	5.40±0.05	3.35	5.42±0.09	5.45±0.10	0.30
	Mv-3-Gl-Cm T	8.55±0.35	8.00±0.10	8.01±0.33	4.09	8.09±0.42	8.29±0.32	1.21
LMWP (mg/L)	Gallic acid	133.65±2.67	126.79±5.10	123.04±4.29	**7.87***	126.30±5.99	129.35±5.98	1.90
	Protocatechuic acid	4.81±0.58	5.11±0.27	5.22±0.23	4.89	5.16±0.21	4.94±0.53	3.97
	Vanillic acid	4.47±0.38	5.01±0.72	5.09±0.45	2.29	4.54±0.40	5.18±0.55	**7.97***
	Syringic acid	4.63±0.46	5.32±0.40	5.52±0.28	**6.06***	5.14±0.46	5.17±0.63	0.02
	trans-Caftaric acid	32.36±0.46	31.35±1.57	31.99±0.77	2.80	31.34±1.20	32.46±0.46	**9.84***
	trans-Coutaric acid	12.54±0.16	12.01±0.84	12.33±0.24	**7.11***	12.05±0.62	12.54±0.25	**18.55****
	Caffeic acid	5.57±0.19	5.32±0.53	5.32±0.35	1.12	5.24±0.43	5.58±0.21	4.56
	p-Coumaric acid	5.68±1.36	6.44±0.33	6.60±0.55	1.75	6.62±0.49	5.87±1.08	3.07
	p-Vanillin	2.30±0.15	2.54±0.35	2.35±0.23	1.35	2.36±0.21	2.44±0.31	0.39
	Protocatechuic	0.50±0.02	0.63±0.10	0.75±0.09	**7.61***	0.61±0.14	0.64±0.13	0.17
	Catechin	35.37±3.37	35.18±6.81	33.79±3.72	0.27	32.68±4.69	36.88±3.40	4.75
	Epicatechin	3.15±0.62	5.71±3.10	3.98±2.77	2.31	5.66±2.93	2.91±0.44	**7.68***
	Scopoletin	3.04±0.35	2.93±0.41	2.80±0.29	1.63	2.65±0.17	3.19±0.21	**24.63****
Global phenolic parameters	TP (mg/L)	2430.00±23.94	2412.50±18.87	2402.50±19.20	2.42	2416.25±30.58	2413.75±12.25	0.06
	LPP (mg/L)	1070.00±35.62	1061.25±33.23	1058.13±20.55	0.45	1085.00±17.80	1041.25±16.51	**16.88****
	HPP (mg/L)	1360.00±35.77	1351.25±23.67	1344.38±37.82	6.35	1331.25±25.43	1372.50±19.81	**7.27***
	CAT (mg/L)	888.80±21.04	835.80±57.54	818.30±17.31	**6.79***	828.13±45.39	867.13±40.19	5.75
	ACY (mg/L)	500.72±14.01	485.84±9.38	484.53±18.11	1.20	493.79±12.82	486.94±17.44	0.52
	TAN (g/L)	2.25±0.17	1.93±0.12	1.88±0.04	**21.00****	1.97±0.14	2.07±0.26	3.59
Index	Gln	52.05±2.71	53.02±5.73	49.70±4.08	1.27	49.03±2.60	54.15±4.03	**8.56***
	IoIn	29.75±0.73	28.53±1.60	28.12±1.00	4.22	28.09±1.36	29.51±0.74	**8.82***
Colour analysis	IC	17.92±0.65	17.57±0.52	17.35±0.53	1.01	17.48±0.67	17.75±0.48	0.68
	T	0.64±0.01	0.66±0.01	0.66±0.01	**6.64***	0.66±0.01	0.65±0.01	**10.17***
	%A420	33.00±0.24	33.26±0.32	33.37±0.20	3.03	33.32±0.29	33.10±0.24	3.20
	%A520	51.28±0.37	50.74±0.56	50.48±0.40	**11.21****	50.50±0.44	51.17±0.41	**23.14****
	%A620	15.72±0.29	16.00±0.25	16.14±0.30	**10.80***	16.18±0.22	15.73±0.22	**35.02*****
	%dA	52.49±0.70	51.45±1.09	50.95±0.78	**11.18****	50.98±0.85	52.29±0.79	**23.12****
Copigmentation	FC	10.19±0.24	9.46±0.39	9.37±0.18	**11.43***	9.56±0.55	9.79±0.38	2.23
	COP	0.23±0.02	0.23±0.03	0.22±0.01	0.23	0.23±0.02	0.22±0.01	1.68
	AL	0.33±0.02	0.35±0.01	0.36±0.01	**6.25***	0.35±0.01	0.35±0.02	0.08
	PP	0.44±0.03	0.42±0.02	0.42±0.01	1.19	0.42±0.03	0.43±0.01	0.77
Chemical age	α	32.85±6.44	28.91±2.17	28.47±1.54	1.39	28.63±2.65	31.53±5.14	1.51
	αα	28.81±1.64	29.87±1.22	30.02±2.12	0.69	30.09±2.09	29.04±0.92	1.31
	i	0.44±0.01	0.44±0.00	0.45±0.01	0.90	0.44±0.01	0.44±0.01	0.45
	ii	0.16±0.01	0.17±0.01	0.17±0.01	0.82	0.17±0.01	0.17±0.00	1.07
	i/ii	2.68±0.14	2.60±0.08	2.60±0.13	0.63	2.60±0.15	2.65±0.07	0.77

Significant level of aging system factor: *p< 0.01, **p< 0.05 and ***p< 0.001.

Table 1S. Principal component analysis (PCA) results carried out with anthocyanins, index, colour analysis and copigmentation parameters in wines aged with alternative products of oak *Q. pyrenaica* after different aging time (30, 60, 90 and 120 days).

	Variance PC1 58.2% **Variance PC2 18.7%**		**Variance PC1 69.7%** **Variance PC2 9.0%**		**Variance PC1 61.8%** **Variance PC2 14.8%**		**Variance PC1 47.0%** **Variance PC2 22.4%**	
	30 days		**60 days**		**90 days**		**120 days**	
	Compounds	**Weight**	**Compounds**	**Weight**	**Compounds**	**Weight**	**Compounds**	**Weight**
Chips + MOX	%dA	0.2438	Mv-3-Gl	0.2309	Pn-3-Gl	0.2339	COP	0.2777
	%A520	0.2438	Pt-3-Gl	0.2285	Cn-3-Gl-Cm	0.2322	%A420	0.2706
	Cn-3-Gl-Cm	0.2329	Df-3-Gl	0.2284	Mv-3-Gl-Cm C	0.2279	T	0.2605
	Mv-3-Gl-Ac	0.2314	Pn-3-Gl	0.2272	Mv-3-Gl-Ac	0.2259	Cn-3-Gl	0.2574
	COP	0.2288	%A420	0.2240	%A420	0.2254	Pn-3-Gl	0.2262
	Pn-3-Gl	0.2213	COP	0.2227	%A520	0.2246	Mv-3-Gl-Ac	0.2252
	Pt-3-Gl-Cm	0.2077	Pt-3-Gl-Cm	0.2227	%dA	0.2242	Mv-3-Gl-Ethyl	0.2109
	Cn-3-Gl	0.1957	T	0.2184	Pt-3-Gl-Cm	0.2240	Gln	0.2079
	IoIn	0.1922	Mv-3-Gl-Ac	0.2153	FC	0.2224	Pt-3-Gl-Cm	0.2038
	Df-3-Gl	0.1763	Df 3-Gl Cm	0.2053	Mv-3-Gl-Cm T	0.2172	Mv-3-Gl-Cm T	0.1262
	Pt-3-Gl	0.1752	Mv-3-Gl-Ethyl	0.2032	Mv-3-Gl	0.2152	Mv-3-Gl	0.0937
	Mv-3-Gl-Ethyl	0.1679	Mv-3-Gl Py	0.2004	AL	0.2110	Pt-3-Gl	0.0670
	Pe 3 Gl A	0.1624	Cn-3-Gl	0.1960	Cn-3-Gl	0.1993	Df-3-Gl	0.0549
	Mv-3-Gl-Cm T	0.1464	IoIn	0.1830	Mv-3-Gl Py	0.1984	Cn-3-Gl-Cm	0.0492
	Mv-3-Gl Py	0.1356	Mv-3-Gl-Cm C	0.1658	Pt-3-Gl	0.1690	Mv-3-Gl-Cm C	0.0236
	Mv-3-Gl	0.1255	Mv-3-Gl-Cm T	0.1581	Mv-3-Gl-Ethyl	0.1675		
	Mv-3-Gl-Cm C	0.0620	Pe 3 Gl A	0.1486	Pe 3 Gl A	0.1159		
	Df 3-Gl Cm	0.0276	Cn-3-Gl-Cm	0.1197	Df-3-Gl	0.1087		
					T	0.0730		
Staves + MOX	%A620	-0.2442	AL	-0.2271	%A620	-0.2348	PP	-0.2743
	T	-0.2420	FC	-0.2154	PP	-0.2226	IoIn	-0.2356
	PP	-0.2290	%A620	-0.2108	IC	-0.2024	Mv-3-Gl-Py	-0.2288
	AL	-0.2222	%dA	-0.1942	COP	-0.1645	Pn-3-Gl-Ac	-0.2242
	FC	-0.2112	%A520	-0.1930	Gln	-0.0864	%dA	-0.2164
	%A420	-0.2026	PP	-0.1814	Df 3-Gl Cm	-0.0543	%A520	-0.2159
	IC	-0.2021	IC	-0.1056	IoIn	-0.0197	IC	-0.1924
	Gln	-0.1748	Gln	-0.0379			%A620	-0.1875
							Df 3-Gl Cm	-0.1529
							AL	-0.1367
							FC	-0.0905

The weight represented is that corresponding to component 1

Table 2S. Principal component analysis (PCA) results carried out with low molecular polyphenols. global phenolic parameters and chemical age in wines aged with alternative products of oak *Q. pyrenaica* from different size (chips and staves), seasoning way (traditional and alternative) and toasting level (light, medium and heavy) after 30, 60, 90 and 120 days.

	Variance PC1	**60.9%**	**Variance PC1**	**59.0%**	**Variance PC1**	**55.3%**	**Variance PC1**	**61.9%**
	Variance PC2	**11.7%**	**Variance PC2**	**13.8%**	**Variance PC2**	**15.3%**	**Variance PC2**	**9.2%**
	30 days		**60 days**		**90 days**		**120 days**	
	Compounds	**Weight**	**Compounds**	**Weight**	**Compounds**	**Weight**	**Compounds**	**Weight**
Staves + MOX	*p*-Coumaric acid	0.2497	*p*-Vanillin	0.2440	ii	0.2679	HPP	0.2500
	Epicatechin	0.2473	Gallic acid	0.2316	i	0.2528	ii	0.2451
	ii	0.2431	α	0.2277	*trans*-Caftaric acid	0.2336	TP	0.2450
	i	0.2321	i/ii	0.2239	Gallic acid	0.2301	i	0.2447
	Gallic acid	0.2308	*trans*-Caftaric acid	0.1780	α	0.2257	*trans*-Caftaric acid	0.2350
	Vanillin acid	0.2231	TAN	0.1749	*p*-Vanillin	0.1727	Gallic acid	0.2326
	CAT	0.2173	*trans*-Coutaric acid	0.1748	CAT	0.1724	*trans*-Coutaric acid	0.1974
	HPP	0.2098	Caffeic acid	0.0981	*trans*-Coutaric acid	0.1608	CAT	0.1970
	trans-Coutaric acid	0.2016			αα	0.1380	α	0.1594
	αα	0.1714					αα	0.0287
	Catechin	0.0773						
	TP	0.0577						
	α	0.0537						
	Protocatechuic acid	0.0390						
Chips + MOX	Scopoletin	-0.2547	Catechin	-0.2614	Protocatechuic aldehyde	-0.2701	Protocatechuic aldehyde	-0.2540
	Syringic acid	-0.2522	i	-0.2595	TP	-0.2600	LPP	-0.2383
	p-Vanillin	-0.2511	ii	-0.2560	ACY	-0.2555	ACY	-0.2344
	Caffeic acid	-0.2309	ACY	-0.2551	LPP	-0.2496	Vanillin acid	-0.2335
	TAN	-0.2286	LPP	-0.2422	Vanillin acid	-0.2484	*p*-Vanillin	-0.2311
	LPP	-0.2263	TP	-0.2410	Catechin	-0.2478	Epicatechin	-0.2270
	Protocatechuic aldehyde	-0.2237	Scopoletin	-0.2289	Scopoletin	-0.2342	Coumaric acid	-0.2120
	i/ii	-0.2018	Vanillin acid	-0.2121	i/ii	-0.2225	i/ii	-0.1906
	ACY	-0.1787	Syringic acid	-0.2036	TAN	-0.1634	Syringic acid	-0.1787
	trans-Caftaric acid	-0.1203	CAT	-0.1919	Protocatechuic acid	-0.1463	Scopoletin	-0.1678
			Protocatechuic aldehyde	-0.1807	Syringic acid	-0.1378	TAN	-0.1518
			αα	-0.1793	HPP	-0.1188	Protocatechuic acid	-0.1504
			HPP	-0.1542	*p*-Coumaric acid	-0.0996	Caffeic acid	-0.1455
			Epicatechin	-0.1180	Epicatechin	-0.0956	Catechin	-0.0173
			Protocatechuic acid	-0.1033	Caffeic acid	-0.0746		
			p-Coumaric acid	-0.1025				

The weight represented is that corresponding to component 1

Figure 1.

Chips (1 cm x 0.5 cm)

Seasoning way

Natural seasoning

Toasting

Light 1 1

Medium 2 2

Heavy 3 3

Alternative seasoning

Toasting

Light 4 4

Medium 5 5

Heavy 6 6

Staves (100 cm x 8 cm x 1 cm)

Seasoning way

Natural seasoning

Toasting

Light 7 7

Medium 8 8

Heavy 9 9

Alternative seasoning

Toasting

Light 10 10

Medium 11 11

Heavy 12 12

Figure 2

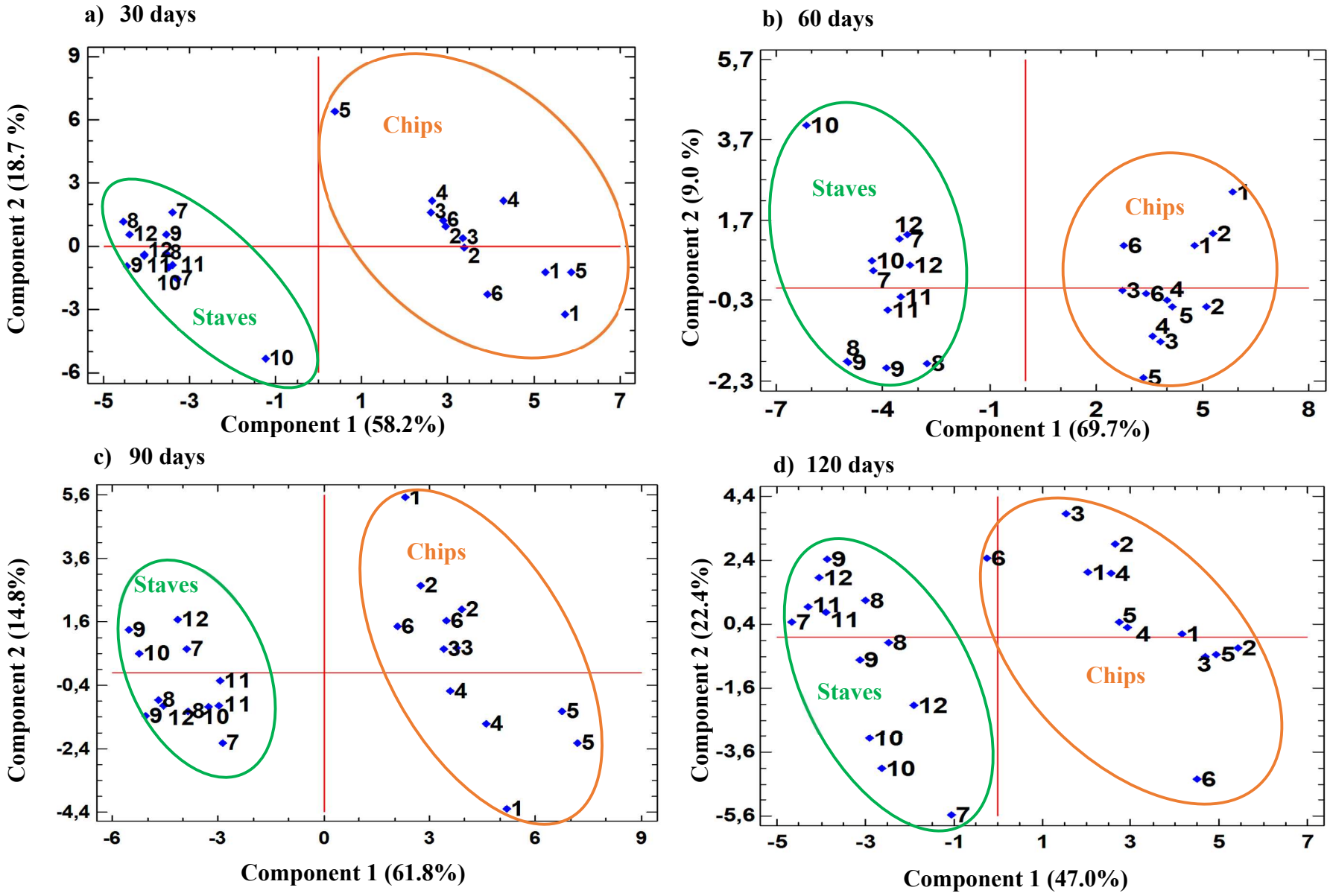
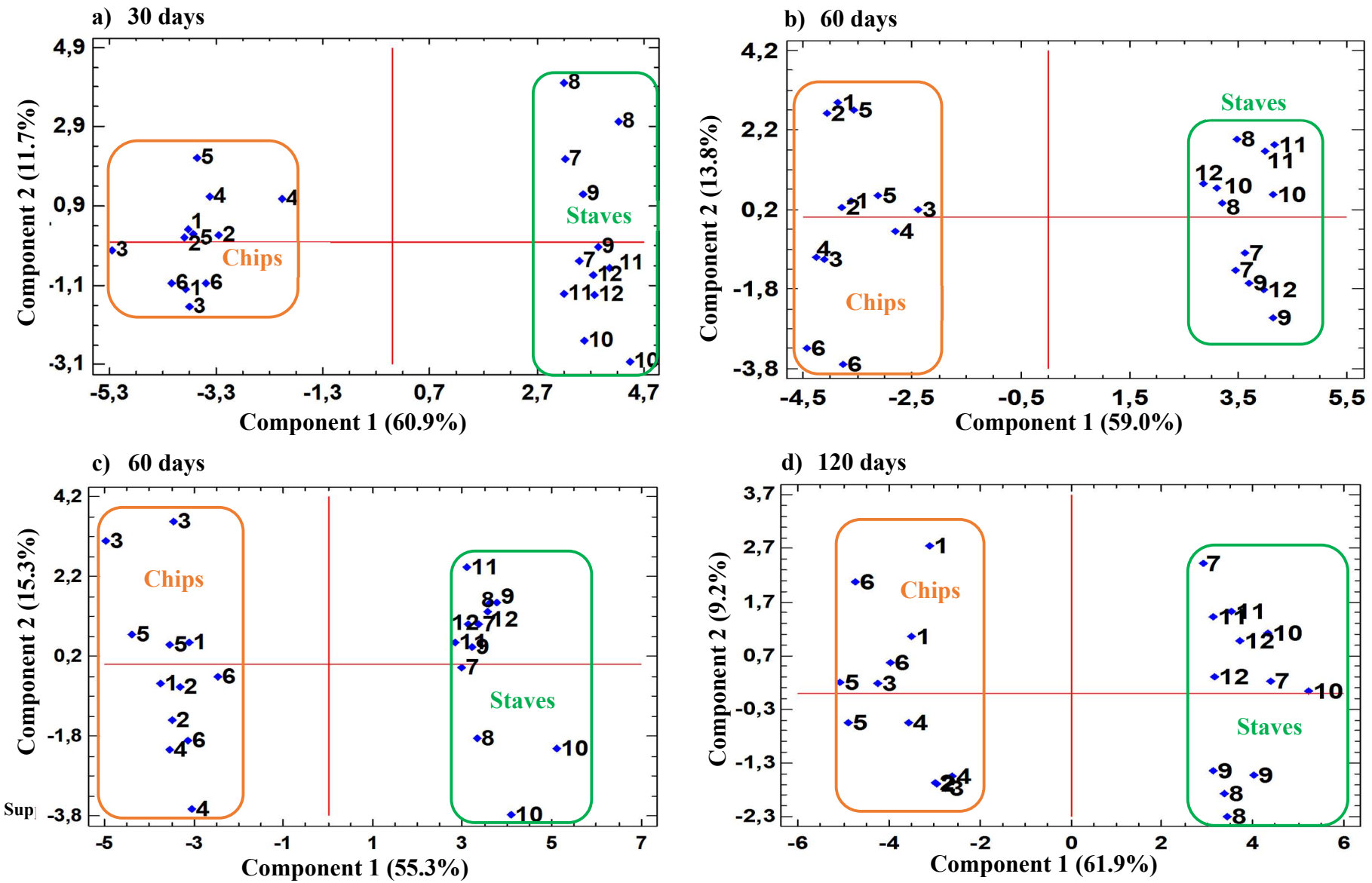
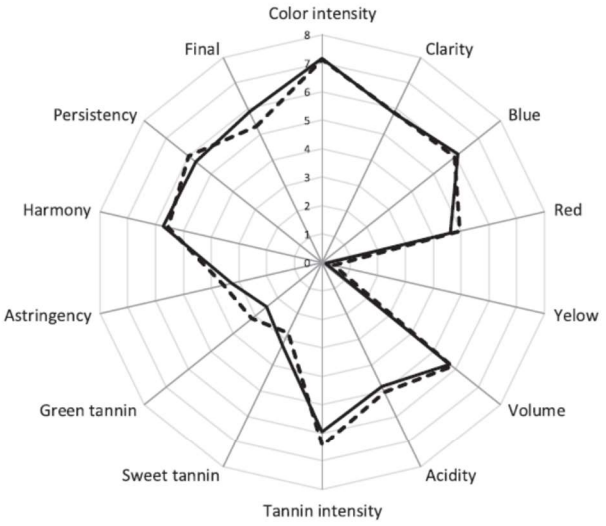

a) 30 days
Component 2 (18.7 %)
Component 1 (58.2%)
Chips
Staves
b) 60 days
Component 2 (9.0 %)
Component 1 (69.7%)
Chips
Staves
c) 90 days
Component 2 (14.8%)
Component 1 (61.8%)
Chips
Staves
d) 120 days
Component 2 (22.4%)
Component 1 (47.0%)
Chips
Staves

Figure 3

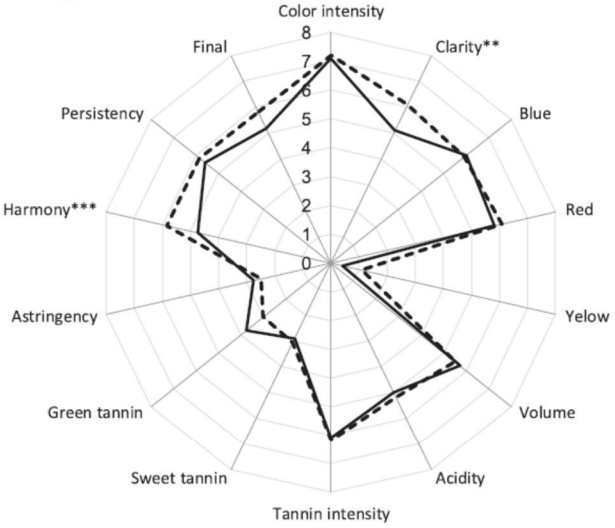

a) 30 days
Component 2 (11.7%)
Component 1 (60.9%)
Chips
Staves
b) 60 days
Component 2 (13.8%)
Component 1 (59.0%)
Chips
Staves
c) 60 days
Component 2 (15.3%)
Component 1 (55.3%)
Chips
Staves
d) 120 days
Component 2 (9.2%)
Component 1 (61.9%)
Chips
Staves

CHIPS

Light toasting level



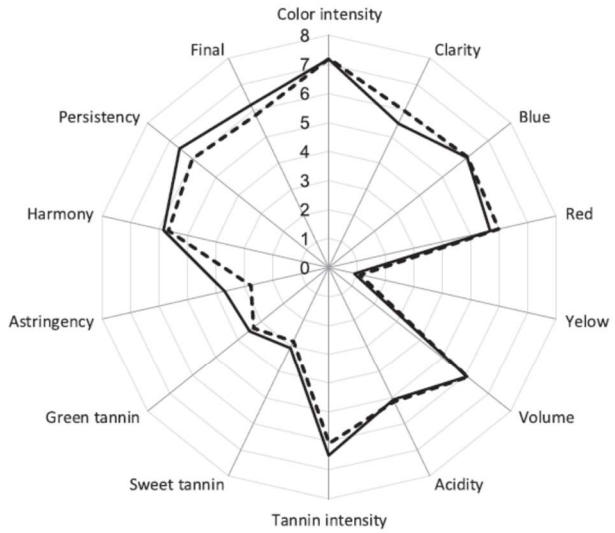
STAVES



CHIPS

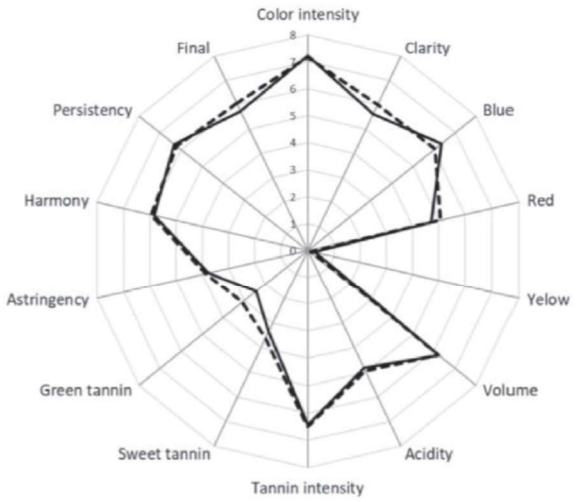
Medium toasting level

STAVES

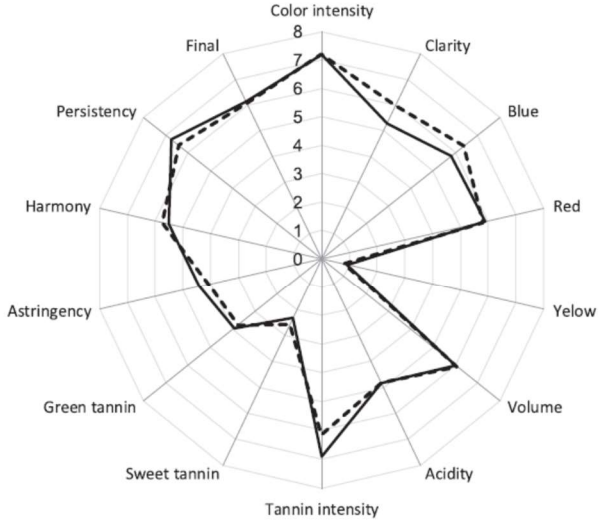


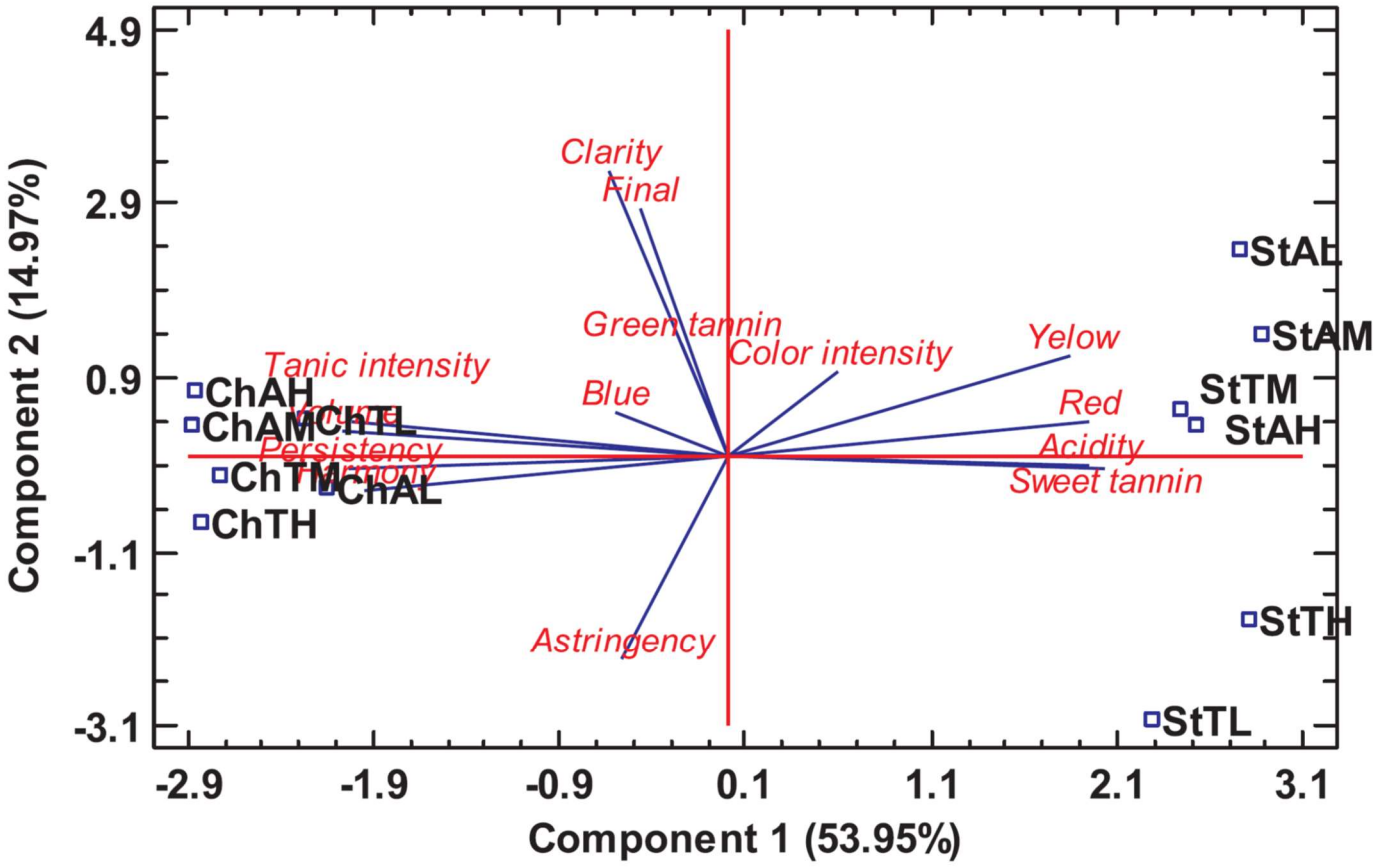
CHIPS

Heavy toasting level



STAVES




4.9
2.9
0.9
-1.1
-3.1
-2.9
-1.9
-0.9
0.1
1.1
2.1
3.1
Component 2 (14.97%)
Component 1 (53.95%)
Clarity
Final
Green tannin
Color intensity
Yelow
Tanic intensity
Blue
Red
Acidity
Sweet tannin
Astringency
ChAH
ChAM
ChTL
ChTM
ChAL
ChTH
StAL
StAM
StTM
StAH
StTH
StTL