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Use of Oak Fragments during the Aging of Red Wines. Effect on the Phenolic, Aromatic, and Sensory Composition of Wines as a Function of the Contact Time with the Wood

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Received: 31 October 2018; Accepted: 29 November 2018; Published: 5 December 2018

Abstract: The use of oak fragments allows wine cellars to reduce costs and the length of wine aging compared to traditional aging in oak barrels in the winery. The main objective of this work was to study the effect of the use of oak fragments on the volatile, phenolic, and organoleptic characteristics of Tempranillo red wines, as a function of the contact time between the wood and the wine. The results showed important changes in the wines' colorimetric parameters after two months of contact time. Extraction kinetics of volatile compounds from the wood was highest during the first month of contact for chips, variable for staves, and slower and continuous over time for barrels. Wines macerated with fragments showed the best quality in short periods of aging, while barrel-aged wines improved over the time they spent in the barrel. In addition, the results allowed an analytical discrimination between the wines aged with oak fragments and those aged in oak barrels, and between chips and staves, just as at the sensory level with triangular tasting tests. In conclusion, the use of oak fragments is a suitable practice for the production of red wines, which may be an appropriate option for wines destined to be aged for short periods.

Keywords: oak fragments; oak barrels; volatile compounds; phenolic compounds; sensory analysis; triangular tasting

1. Introduction

The aging of red wines in oak barrels is a technique commonly used in wineries to increase wine stability and complexity. During this process, an organoleptic improvement of the wines is achieved as a consequence of the contribution of oak wood compounds, and the phenolic and aromatic modifications that take place [1,2]. This practice involves long aging periods and represents a high economic cost for the wineries.

The use of pieces of oak wood during winemaking, as an alternative to traditional aging in oak barrels, is an enological practice authorized by the International Vine and Wine Organisation (OIV) and included in the International Enological Codex [3,4]. This practice was approved by the European Community [5], and is subject to regulation [6,7].

Currently, there is a varied range of commercial products available, and therefore their effects on wine quality can be very variable, since they are influenced by numerous factors (size of fragments, oak wood origin, toasting degree, manufacturing process, dose, contact time with wine, etc.) [8,9]. Due to the large contact surface of these materials with the wine, the extraction of compounds is much faster than in barrels. Additionally, the cost of the process is lower than that of the classic aging in barrels.

The shape and size of the oak fragments available vary: powder, chips, pieces of medium size (cubes or beans, dominoes, blocks or segments), or larger pieces (staves) to put in the tanks. These products are made with oak from different oak origins (American, French, Spanish, Hungarian, etc.), using different toasting processes (direct fire, convection by hot air, or infrared radiation), and different levels of toasting (medium, strong, light, or untoasted). These effects have been largely investigated, but the influence of the length of the aging process needs further studies.

Depending on the characteristics of the final product desired, the contact time of the wood with the wine can vary from a few days to several weeks, and even months. This contact time will depend on the type of wood, the fragment size, the dose, and the sensory profile expected to be achieved in the wines.

Most previous studies about the influence of the length of the aging process have dealt with a particular subgroup of compounds (volatile or phenolic), which is clearly a limiting condition to obtain a more comprehensive view of the subject. The main objective of this work was to study for the first time the evolution of the aromatic and phenolic composition of wine during the contact time with oak wood, employing four different accelerated aging strategies: chips and staves, with and without micro-oxygenation. In addition, the impact of these treatments on the wine organoleptic characteristics was evaluated. These aging procedures were compared with the traditional oak barrels aging method.

2. Materials and Methods

2.1. Wine and Wood

To carry out this study, a red wine of the Tempranillo variety (*Vitis vinifera* L.) was used. Once the malolactic fermentation (MLF) was finished, the wine was divided into eight stainless steel tanks of 250 L capacity and two 225 L oak barrels. In four tanks, oak chips were added at a dose of 4 g/L, while staves were placed in the other four. In this case, the dose was adjusted so as to have a contact surface with the wine of 0.4 m^2/hL. All the fragments were of American oak with medium toasting. Simultaneously, two of the tanks with chips and two with staves were micro-oxygenated with a dose of 2 mL/L/month during the first two months, and 1 mL/L/month during the following two months. Thus, the total dose of oxygen during 4 months was 6 mL/L. Micro-oxygenation treatments were carried out using a Micro-Ox-3V system (Intec, Verona, Italia) connected to the stainless steel tanks. These tanks (Herpanor S.A., Laguardia, Spain) were 2 m in height and a diameter of 0.45 m. The dimensions of the tanks were chosen because of the necessity to achieve the height recommended for micro-oxygenation, and to reproduce the height to diameter ratio of common red wine tanks used in industrial-scale production. These dimensions were necessary so that the small oxygen bubbles produced during micro-oxygenation would have a sufficient displacement height to guarantee their complete dissolution into the wine. Oxygen was provided through a diffuser composed of a porous ceramic membrane placed 10 cm above the bottom of the tank. The contact period of the oak fragments with the wine was 6 months, and then, the wines were bottled to be stored for 18 months. In addition, the same wine was simultaneously aged for 12 months in American oak barrels with medium toasting, manufactured in the same cooperage as the chips and staves. After this aging period, the wine was bottled to be bottle-aged for another year (Figure 1). In this way, all the tests were carried out in duplicate.

The chemical parameters of the wine before aging were: alcoholic strength 13.7% *v*/*v*, pH 3.81, total acidity 4.88 g/L, and volatile acidity 0.51 g/L. These parameters were evaluated using the analytical methods established by the European Community [10].

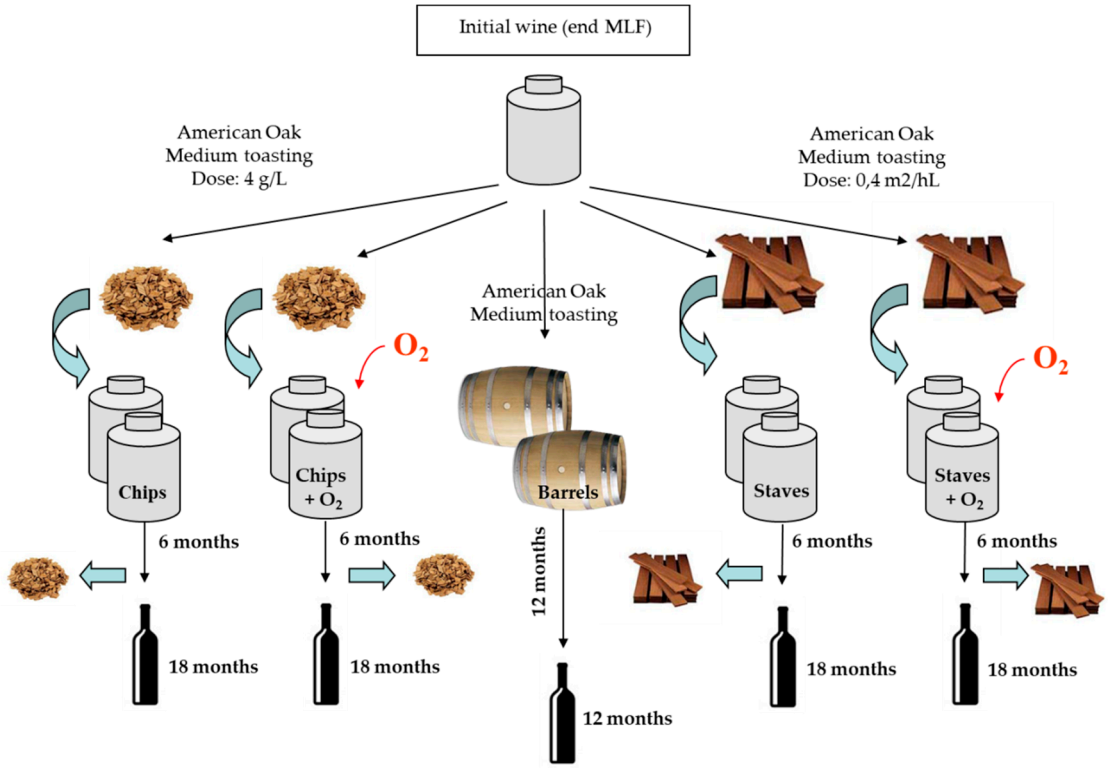

Figure 1. Experimental design.

2.2. Chemical Analysis

2.2.1. Color Parameters Measurements

Color intensity (CI) was determined according to the EEC Regulation 2676/90 [10]. Percentage of yellow, red, and blue components (% yellow, % red, and % blue, respectively) were evaluated according to the methodology described by Glories [11]; while the total polyphenol index (TPI) and total anthocyanins were determined by the methods described by Ribéreau-Gayon et al. and Ribéreau-Gayon and Stonestreet, respectively [1,12]. Color parameter measurements were made before starting the treatments, and after 2, 4, 6, 12, and 24 months.

2.2.2. Wine Volatile Compounds from Oak Wood

The analysis of the volatile compounds in wine coming from oak wood was carried out by Gas Chromatography (GC), with a method based on that described by Ortega et al. [13], under optimized conditions. To carry out the extraction of the volatile compounds, in a glass tube were added: 5 mL of wine, previously centrifuged at 4000 rpm at 0 °C for 10 min; 9.5 mL of supersaturated ammonium sulfate solution; 15 µL of a solution of 2-octanol and 3,4-dimethylphenol (internal standards) in ethanol at a concentration of 50 mg/L; and 0.2 mL of dichloromethane. These tubes were shaken vigorously, first manually, and then horizontally in an orbital agitator at 400 rpm for 60 min. Next, the tubes were centrifuged at 2500 rpm and 0 °C for 10 min. The supernatant aqueous phase was removed with a Pasteur pipette and the organic phase was collected. This extract was transferred to a microtube and centrifuged at 13,000 rpm and 0 °C for 5 min, to break up any possible emulsions formed. Finally, the organic phase was collected with a syringe and transferred to a vial with an insert, in order to analyze in the gas chromatograph.

The separation and detection of the compounds was carried out in a gas chromatograph HP-6890 series II equipped with an automatic injector and a flame ionization detector (FID). The column was a DB-WAX (50 m × 0.20 mm × 0.2 µm thick film, J and W Scientific, Folsom, CA, USA), using nitrogen

as a carrier gas with a flow rate of 0.6 mL/min. The injection was carried out in splitless mode (0.5 min) with an injection volume of 2 µL. The chromatographic conditions used were the following: injector temperature, 250 °C; detector temperature, 275 °C (H_2 flow, 40 mL/min; air flow, 450 mL/min; auxiliary gas, N_2 at 40 mL/min); and initial oven temperature, 75 °C (5 min), 3.7°C/min to 240 °C (maintained for 15 min), and a post time of 10 min at 240 °C.

The identification of the volatile compounds was performed by comparison with the retention times of the standard substances, and the concentration of each substance was measured by comparing it with calibrations made with the pure compounds analyzed under the same conditions.

Wine volatile compounds from oak wood analysis were made before starting the treatments, and after 1, 2, 4, 6, 12, and 24 months.

2.2.3. Low Molecular Weight Phenols

Low molecular weight phenols were analyzed by HPLC-DAD with direct injection of the 30 µL of sample, according to Martínez and Rubio-Bretón [14]. A column Zorbax Eclipse Plus C18 (300 mm × 150 mm × 3.9 µm) was used. The eluents used in the mobile phase were: A (water/acetic acid, 98/2, *v*/*v*), B (water/acetonitrile/acetic acid, 78/20/2, *v*/*v*/*v*), and C (methanol), with a constant flow of 0.9 mL/min according to the following program: 0 to 80% of B from 0 to 65 min; 80% B from 65 to 85 min; 100% C from 86 to 90 min. The wine samples were centrifuged (4000 rpm/0 °C/10 min) and filtered by 0.45 µm before injection into the equipment. The identification of the compounds was carried out by comparing the retention times and the spectral parameters of the chromatographic peaks with those of the standards.

The wine was analyzed before starting the treatments, and after 1, 2, 4, 6, 12, and 24 months.

2.3. Sensorial Analysis

The organoleptic study was carried out during the process at 2, 4, 6, 12, and 24 months. Ten wine tasters carried out the sensory analysis, all of them with extensive experience as wine tasters. The samples were evaluated comparatively by a blind tasting system and served in a random order. A sufficient amount of wine samples were presented without any identification according to regulation UNE 87-022-92 [15]. All the wines were served at room temperature and were evaluated in individual booths. The score sheet used was based on that used in some wine competitions and is considered official in some designations of origin. According to this model sheet the wines are evaluated based on the absence of defects, so the lower the score, the higher the quality of the wine. The sensory attributes valued were visual, olfactory and taste phases, and harmony. Quantitative evaluation of aromatic descriptors was also carried out on an intensity scale of 1 to 10 (fruity, varietal, spices, wood-toasted, almond-caramel, vanilla, and smoked) as well as the taste characteristics (structure, persistence, retronasal aroma, and astringency).

Triangular tasting tests were also carried out, at 6 and 12 months, to determine the existence of detectable differences between two different samples, according to regulation UNE 87-006-92 [15]. To conduct these tests, three coded samples were presented simultaneously to the tasters, two of which were the same, so that the taster could identify which was the different sample. Although the aim of this technique is not the determination of preferences, the preferred sample in each series was also specified. The percentage of preferences was calculated with the correct answers, discarding the preferences of the incorrect tests.

2.4. Statistical Analysis

Canonical discriminant analysis (CDA) was performed with the concentrations of volatile and phenolic compounds in the different samples for all the moments studied. The "IBM SPSS Statistics 22" statistical program was used.

3. Results and Discussion

3.1. Color Parameters of Wine

Figure 2 shows the evolution of the main colorimetric parameters over aging time. In general, all the samples followed a similar chromatic evolution, independent of the treatment carried out.

CI of the wines increased during the first two months, mainly in the micro-oxygenated wines and in the wines aged in barrels. From that moment, this parameter decreased in all the treatments, maintaining the initial differences until the end of the aging process (Figure 2a). These results coincide with those obtained by other authors [16,17]. Furthermore, a higher CI in micro-oxygenated wines was also observed by other authors [18,19], which could be due to an increase in the blue color by the contribution of pigments with ethyl bridges. As noted by these authors, a pronounced increase in the blue color percentage in micro-oxygenated wines was observed (Figure 2f).

In the same way as CI, the percentage of red color increased in all wines until two months, with a notable decrease observed from that moment (Figure 2d). Del Álamo et al. [20] also observed a loss of CI, especially in the red component, from the third month of aging with fragments and barrels.

The percentage of yellow color in wines followed a trend similar to the tonality (data not shown), appreciating a decrease during the first two months, and then a significant increase until the end of the period studied (Figure 2b). Pérez-Prieto et al. [21] attributed the increase in yellow tones to the extraction of color compounds from the oak wood during aging. Our results also coincided with those of Cadahía et al. [22], who observed a decrease in the percentage of red color and an increase in the percentage of yellow color and tonality, while these authors did not observe significant variations in the CI in wines aged for 12 months in oak barrels.

A decrease in TPI was observed over the aging period (Figure 2c). This parameter, at 4 and 12 months of aging, was higher in barrels compared to the other aging systems, but at 24 months, the highest TPI value corresponded to wine with staves and micro-oxygenation. In the case of total anthocyanins, the decrease was more pronounced in micro-oxygenated wines during the first 12 months, although these differences disappeared at 24 months (Figure 2e). Tavares et al. [23] also observed a decrease in anthocyanins during wine contact with chips, probably due to anthocyanin condensation and polymerization reactions, and the precipitation of these compounds during wine aging.

3.2. Volatile Compounds from Oak Wood in Wine

Figure 3 shows the evolution of furanic compounds, benzoic aldehydes, and oak lactones during the period of aging. Furanic compounds, with the exception of furfuryl alcohol, are formed during wood toasting through degradation of carbohydrates [24]. Wines in contact with staves and aged in barrels had a much higher concentration of these compounds than those treated with chips (Figure 3a–c). Moreover, their evolution was similar, with their content increasing during the first 6 months and then decreasing sharply, until practically disappearing in the case of furanic aldehydes.

Towey and Waterhouse [25] also observed that the concentration of these compounds decreased significantly after 7 months of barrel aging. Garde-Cerdán and Ancín-Azpilicueta [26] also found a decrease of furfural and 5-methylfurfural from the sixth month of aging in new barrels, as well as 5-hydroxymethylfurfural from the ninth month. Other authors [27] also observed a maximum extraction in furfural at 6 months of barrel aging and a decrease from that moment. The degradation of these compounds can be due to their reduction to the corresponding alcohols by biological mechanisms [28], although they can also participate in other reactions that contribute to the decrease of their concentrations in free form in wines (such as the formation of 2-furanmethanethiol [29] or of color adducts with the wine catechin [30]). For a short time of aging, extraction of these compounds from the wood is greater than their degradation so that they are accumulated in wine. However, when the aging time increases, degradation reactions may exceed the extraction, so that their concentration tends to decrease [24,31,32].

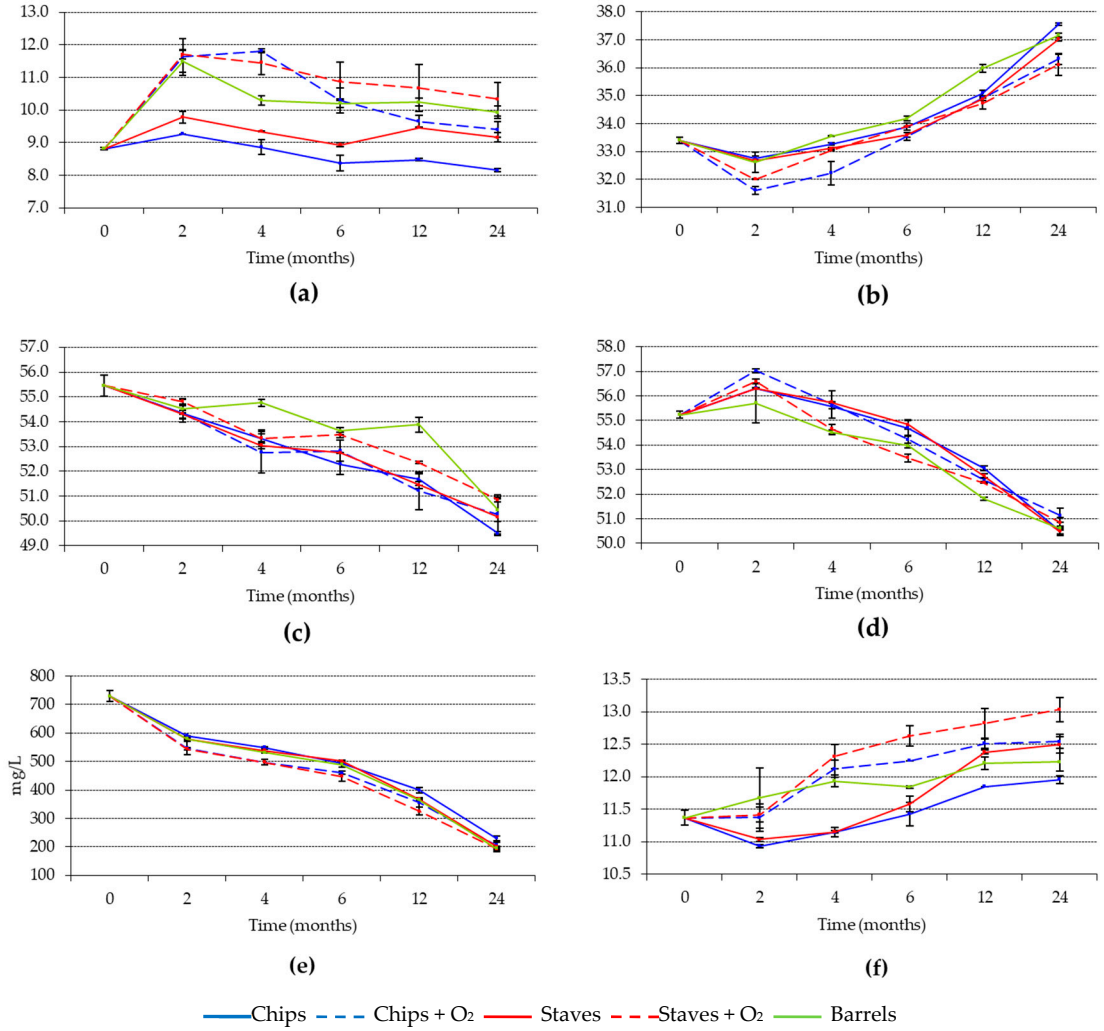

Figure 2. Evolution of the main chromatic parameters over time: (**a**) color intensity (CI); (**b**) percentage of yellow color; (**c**) total polyphenol index (TPI); (**d**) percentage of red color; (**e**) total anthocyanins (mg/L); and (**f**) percentage of blue color. Mean ± standard deviation (n = 2).

On the other hand, in wines treated with oak chips, hardly any extraction of furfural or 5-methylfurfural was observed, while the content of 5-hydroxymethylfurfural increased during the first month of contact with the wood and remained practically constant until 6 months, at which point it disappeared. Similar results were obtained by Fernández de Simón et al. [33], who observed the highest amount of furanic aldehydes in wines treated with chips at 30 days, with the concentration of these also being lower than those treated with staves.

The concentration of furfuryl alcohol also reached higher values in wines treated with staves and aged in barrels (Figure 3d). This compound originates from the microbiological reduction of furfural, even after the alcoholic and malolactic fermentations have been completed [34], and its concentration depends on factors that affect enzymatic reactions, such as pH, temperature, or residual microbiological activity [35].

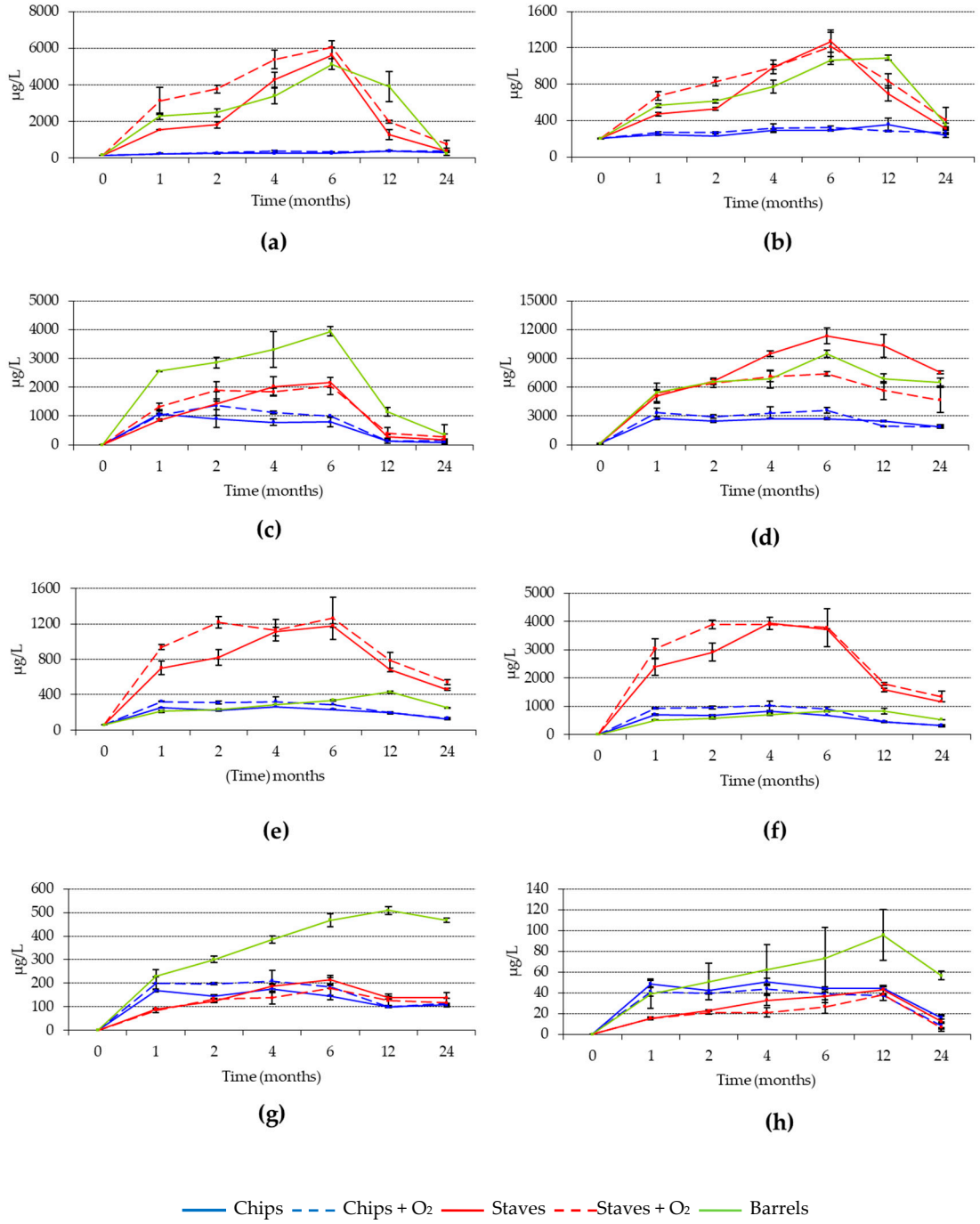

Figure 3. Evolution over time of furanic compound concentration (µg/L): (**a**) furfural, (**b**) 5-methylfurfural, (**c**) 5-hydroxymethylfurfural, and (**d**) furfuryl alcohol; of benzoic aldehyde concentration (µg/L): (**e**) vanillin, and (**f**) syringaldehyde; and of oak lactone concentration (µg/L): (**g**) *cis*-β-methyl-γ-octalactone, and (**h**) *trans*-β-methyl-γ-octalactone. Mean ± standard deviation (n = 2).

Benzoic aldehydes (vanillin and syringaldehyde) are formed during the thermal degradation of lignin. These compounds achieved their maximum concentration during the first month in wines with oak chips, and between 2 and 4 months in the case of wines treated with staves, which obtained the highest concentrations throughout the process studied (Figure 3e–f). In both cases, the concentration decreased from 6 months, probably due to the microbiological reduction to the corresponding alcohols [31,36,37]. On the other hand, the wines aged in barrels presented a lower content of these compounds than the wines in contact with staves, increasing until 12 months of aging, and decreasing

later during the bottle-aging period. Coinciding with our results, different authors [24,32,38] found that the concentration of benzoic aldehydes was at maximum after 10–12 months of barrel aging. Like furanic aldehydes, vanillin accumulates in wine during short aging times, since initially the extraction is high due to the different concentration between wine and wood [26]. However, when the aging time is prolonged, it can be transformed into vanillic alcohol, so that the vanillin concentration can decrease.

In wines treated with oak chips, the maximum extraction of the two isomers of β-methyl-γ-octalactone occurred during the first month of contact with the wood. In the case of staves, *cis*-β-methyl-γ-octalactone was extracted during the 6 months of contact time, while the *trans* isomer was extracted up to 12 months (Figure 3g–h). Other authors [39–41] also observed that the accumulation of *cis* and *trans* oak lactones increased with the aging time of wines in barrels. Meanwhile, wines aged in barrels showed a higher content of these compounds than those macerated with fragments, with these two isomers increasing during the 12 months of contact with the wood, and then decreasing during bottle-aging. The decrease in the concentration of these compounds could be due to the wine undergoing different chemical transformations [42].

The evolution of volatile phenols during the aging time is shown in Figure 4. These compounds are formed from the thermal degradation of the lignin at a high temperature. In general, a greater extraction of these compounds was observed in the wines in contact with the staves, mainly in the case of 4-methylguaiacol, *trans*-isoeugenol, and syringol.

Guaiacol concentration increased throughout the process, obtaining higher values in wines treated with staves and in those aged in barrels, reaching its maximum concentration at 12 months of aging, at which point its content remained practically constant (Figure 4a). These results coincide with those obtained by Garde-Cerdán et al. [38]. The extraction kinetics of 4-methylguaiacol showed major differences between treatments, finding the highest concentration in wines in contact with staves at 6 months, while in wines aged with chips and in oak barrels, it was achieved during the first month of contact (Figure 4b). Pérez-Prieto et al. [43] observed that 4-methylguaiacol reached its maximum concentration at 3 months, while the guaiacol extraction continued for up to a maximum of 9 months of aging in barrels. Although the staves had medium toasting, their appearance indicated that they had undergone a stronger toasting than the chips, probably because the toasting system applied was different. This fact could explain the higher content of guaiacol and 4-methylguaiacol in wines in contact with staves, since they are compounds that are formed at high temperatures of toasting [26]

As can be seen in Figure 4c,d, the wines aged in barrels sharply increased the levels of ethylphenols (4-ethylguaiacol and 4-ethylphenol) during their aging in bottles. These compounds can be extracted from wood in very low concentrations, but mainly they are formed during the aging of wines by microbiological transformations of cinnamic acids carried out by *Brettanomyces/Dekkera* yeast contaminants [44]. The concentrations of ethylphenols found in wines aged in barrels at the end of the process exceeded those considered harmful to the wine aroma, 140 and 620 μg/L for 4-etilguaiacol and 4-ethylphenol, respectively [45]. This fact could be due to contamination of the wines aged in barrels during the bottling process.

The concentration of 4-vinylguaiacol tended to decrease progressively up to 12 months, when the concentration in wines with chips and staves remained practically constant (Figure 4e). However, in wines aged in barrels, its concentration decreased sharply, probably as a consequence of its reduction to 4-ethylguaiacol by the effect of contamination by *Brettanomyces/Dekkera*. The 4-Vinylphenol content decreased slightly or remained constant in all the wines up to 12 months. In the case of wines aged in barrels, its concentration remained practically constant from this moment, and yet, in wines in contact with chips and staves, its content increased until 24 months (Figure 4f).

The concentration of phenol increased until 12 months in all treatments (Figure 4g). From that moment, its concentration continued to increase in wines in contact with staves, while its level decreased slightly in wines aged with chips and more sharply in wines aged in oak barrels. Garde-Cerdán et al. [38]

found the highest concentration of this compound at 10 months of aging in barrels and they also observed a decrease in its content from that moment.

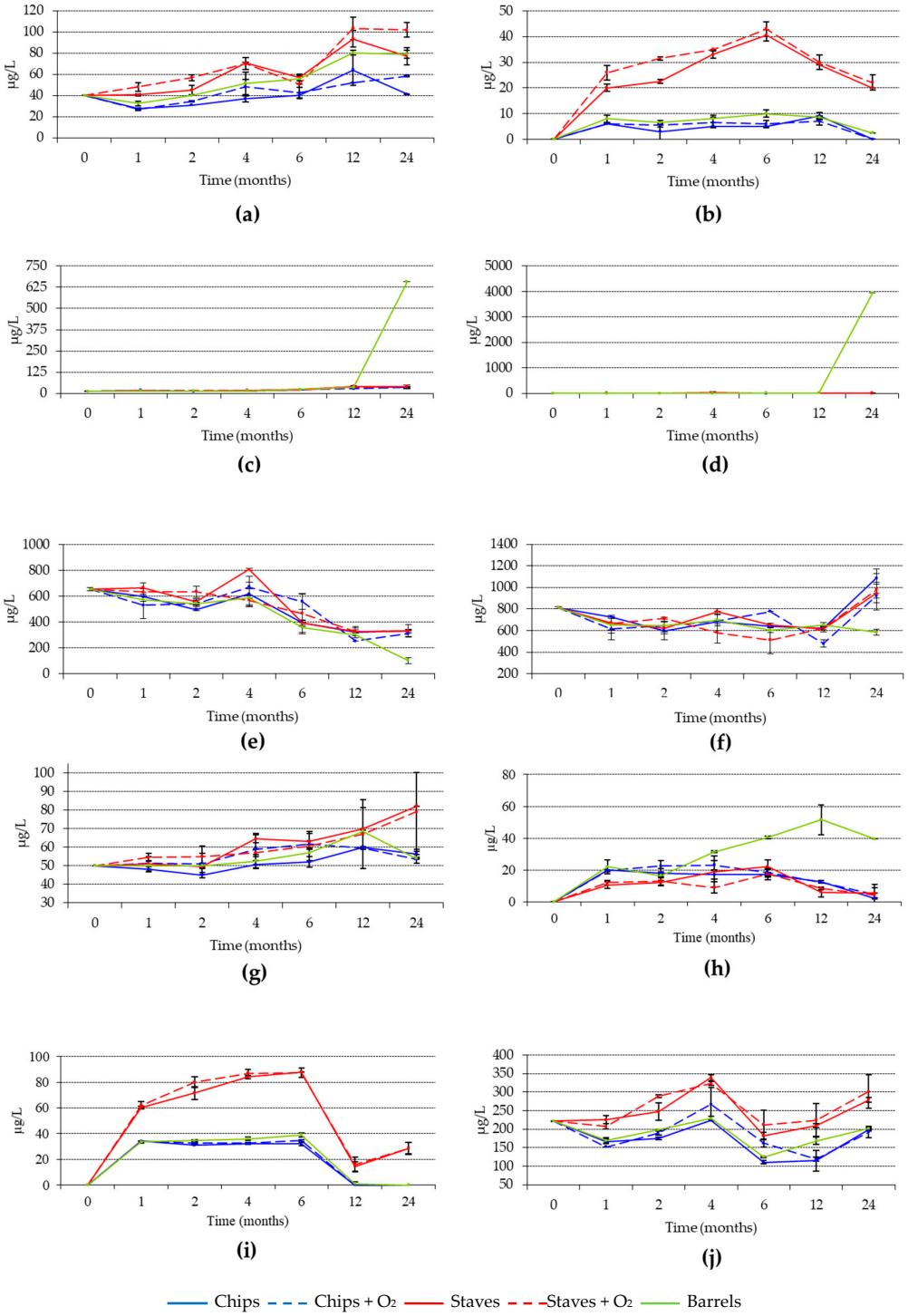

Figure 4. Evolution of volatile phenols concentration (µg/L) over time: (**a**) guaiacol; (**b**) 4-methylguaiacol; (**c**) 4-ethylguaiacol; (**d**) 4-ethylphenol; (**e**) 4-vinylguaiacol; (**f**) 4-vinylphenol; (**g**) phenol; (**h**) eugenol; (**i**) *trans*-isoeugenol; and (**j**) syringol. Mean ± standard deviation (n = 2).

On the other hand, the maximum extraction of eugenol was reached in wines during the first month of contact with chips, and after 6 months in the wines macerated with staves. In addition, eugenol was extracted constantly during the contact time of wines with the oak barrels, thus obtaining

a maximum concentration at 12 months, and its concentration was much higher than that obtained in wines in contact with fragments (Figure 4h). Similar results regarding the increase in barrels of this compound were observed by other authors [25,37,40,41].

The *trans*-Isoeugenol reached its maximum concentration in the first month of contact in wines aged in the barrel and with chips, and at 4–6 months in those treated with staves, which was much higher than in the other wines. In all the wines, a decrease in the concentration of this compound was observed after 6 months, disappearing in wines aged in oak barrels and with chips (Figure 4i). Finally, syringol presented a similar evolution in all the treatments, reaching the maximum concentration at 4 months, which was higher in wines treated with staves throughout the process (Figure 4j). Different results were obtained by Fernández de Simón et al. [33], who observed an increase in the concentrations of *trans*-isoeugenol and syringol until the end of aging in wines treated with alternative oak products.

3.3. Low Molecular Weight Phenols

Benzoic acids are constituents of wood, and its content in wine increased as a result of contact with it (Figure 5). These results are in agreement with those observed by other authors [46,47].

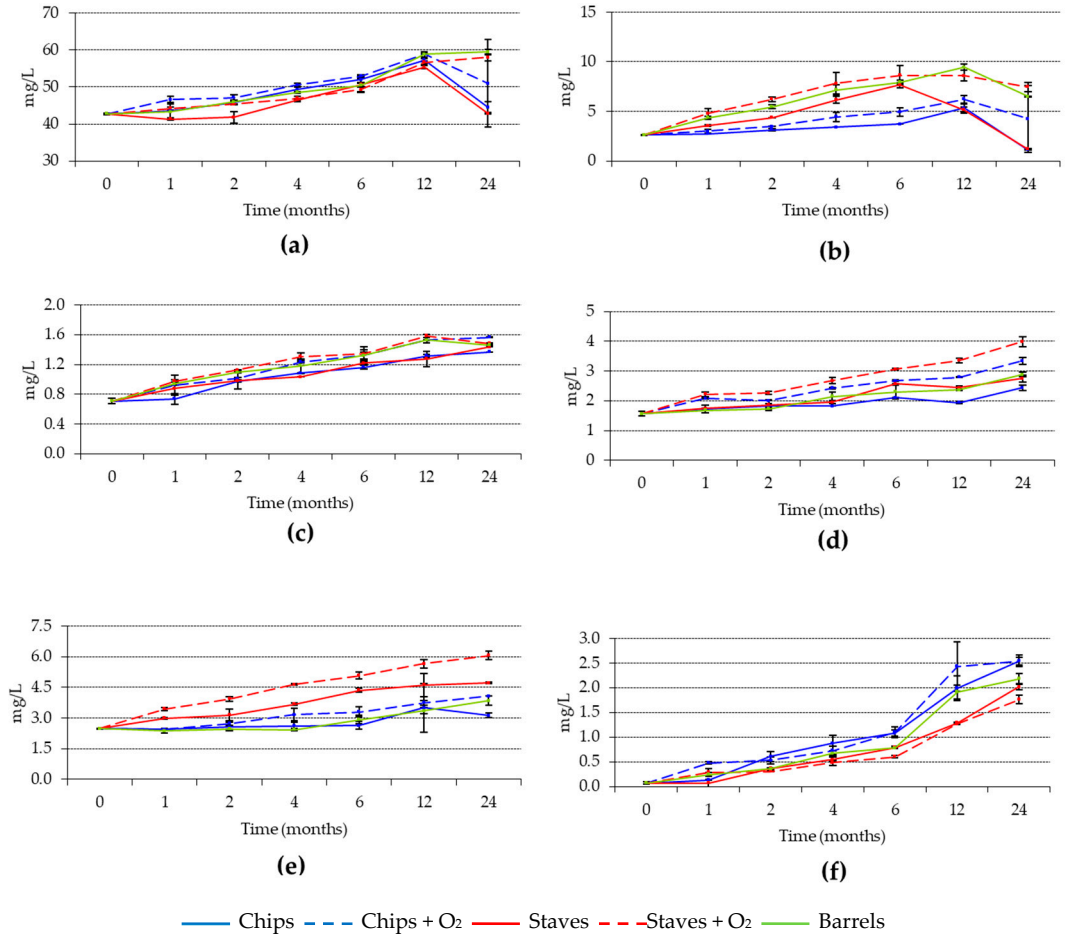

Figure 5. Evolution of benzoic acids concentration (mg/L) over time: (**a**) gallic acid; (**b**) protocatechuic acid; (**c**) p-hydroxybenzoic acid; (**d**) vanillic acid; (**e**) syringic acid; and (**f**) ellagic acid. Mean ± standard deviation (n = 2).

Gallic and ellagic acids are very important compounds due to their strong antioxidant activity, even at very low concentrations [48]. The concentration found for both compounds was slightly higher in wines treated with chips in most of the process (Figure 5a,f), probably due to its greater contact surface and a less intense toasting than in the staves, since gallic acid is a compound which is sensitive

to thermal degradation. Similar results were obtained by Alañón et al. [46] when comparing chestnut wood chips and barrels.

Protocatechuic acid concentration increased more in wines in contact with staves and in wines aged in barrels than in those treated with chips at almost every moment (Figure 5b). This may be because this compound is generated during the toasting process, as a consequence of the thermal degradation of lignin [49], and as has been noted previously the toasting seemed more intense in the staves than in the chips.

Higher concentrations of p-hydroxybenzoic and vanillic acids were detected in micro-oxygenated wines (Figure 5c,d), as well as syringic acid in wines treated with staves, compared to those aged in the barrel and in contact with chips (Figure 5e). The content of these three compounds increased in the wines during the entire study period.

Cinnamic acids were detected in the wines in their free forms (caffeic, coumaric, and ferulic acids) and in their respective tartaric esters (caftaric, coutaric, and fertaric acids). The concentration of these acids in wine depends on grape variety and winemaking technique, but they are not found in oak wood. The evolution of their content over time is shown in Figure 6.

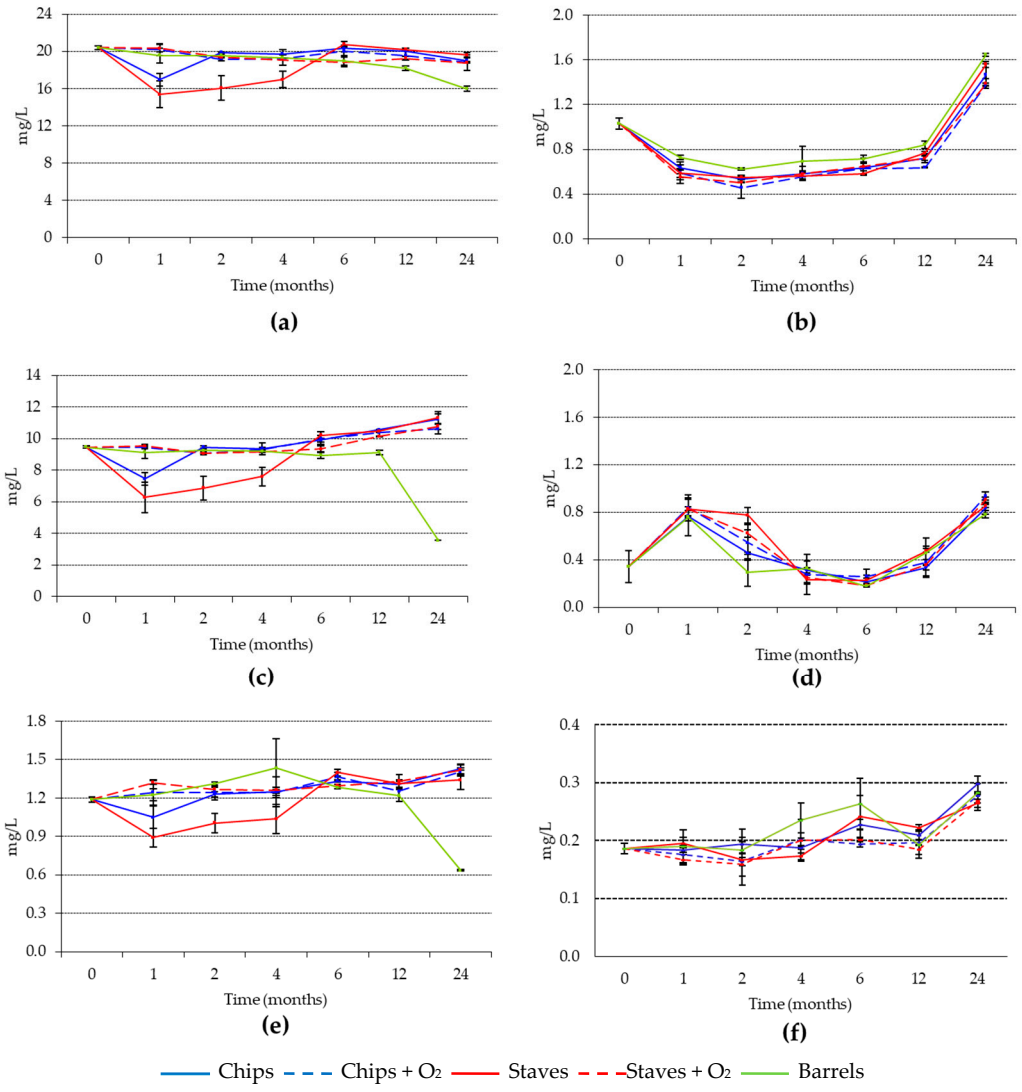

Figure 6. Evolution of cinnamic acids concentration (mg/L) over time: (**a**) caffeic acid; (**b**) caftaric acid; (**c**) coumaric acid; (**d**) coutaric acid; (**e**) ferulic acid; and (**f**) fertaric acid. Mean ± standard deviation (n = 2).

Coumaric acid concentration in wines may decrease in the presence of the enzyme cinnamate decarboxylase, by transformation into 4-vinylphenol, which can be transformed into 4-ethylphenol in the presence of a vinylphenol reductase. In the same way, ferulic acid can decrease by decarboxylation, transforming into 4-vinylguaiacol, and in the presence of the enzyme vinylphenol reductase can form 4-ethylguaiacol [45].

Caffeic, coumaric, and ferulic acids presented a more or less stable concentration up to 12 months, with a similar evolution for all treatments (Figure 6a,c,e). It is important to note the decrease of these acids at 12 months in wines aged in barrels, which could be explained by the decarboxylation reactions described in the previous paragraph. The decrease in coumaric and ferulic acids at 12 months coincided with a significant increase in 4-ethylphenol and 4-ethylguaiacol (Figure 4c,d), which as explained above, could be due to contamination of the wine by *Brettanomyces* at the time of bottling. On the other hand, Cadahía et al. [50] justified the variations of the concentration of caffeic acid due to their involvement in esterification reactions to give caftaric acid, as well as in the co-pigmentation reactions that allow the color of anthocyanins to stabilize.

There was no clear relationship between the content of the free cinnamic acids and their esterified forms. While the free form concentrations remained relatively constant or increased slightly, their corresponding esters had a heterogeneous evolution. Caftaric and coutaric acids diminished or maintained their concentration constantly during the first months, whereas the fertaric acid content remained practically stable. In these compounds there was a notable increase from 12 to 24 months (Figure 6b,d,f). Tartaric esters (caftaric and coutaric acids) decrease during aging because they are very reactive compounds and participate in oxidation processes. The only differences between treatments were observed in the caftaric acid, which had a slightly higher concentration in wines aged in oak barrels (Figure 6b), probably because this type of container encourages the esterification processes [41].

The evolution of flavanols and flavonols in wines can be seen in Figure 7. As can be observed, the concentrations of catechin and epicatechin decreased slightly until 12 months, producing a greater decrease in the case of the epicatechin from 12 to 24 months (Figure 7a,b). These results coincide with those obtained by other authors [18,22,41,50,51] who also obtained a decrease in these phenols over the aging period. This is related to their participation in the oxidative processes, polymerization, and condensation reactions with other compounds, favored in barrels by the continuous diffusion of oxygen [52]. On the other hand, Del Barrio-Galán et al. [53] found lower concentrations of flavanols (catechin and epicatechin) in wines and in model solutions treated with chips than in control wines, corroborating the theory that these compounds can be adsorbed on the surface of the wood. Modifications of the flavanols content between treatments during aging could be due to the reactivity of these compounds. Thus, their concentration may decrease due to oxidation and polymerization reactions and may increase due to the hydrolysis of higher oligomers [54].

Quercetin concentration was higher in wines without micro-oxygenation than in the micro-oxygenates and those aged in barrels, over the whole time studied (Figure 7c). The values of this compound decreased in wines aged in oak barrels and micro-oxygenated wines during the first two months, remaining practically constant during the rest of the process. Likewise, in wines without micro-oxygenation, its concentration remained more or less constant throughout the time studied. Cejudo-Bastante et al. [55] also found lower concentrations in wines aged with chips and micro-oxygenated, compared to those treated with chips but without micro-oxygenation. Castellari et al. [56] also observed a decrease of this compound in micro-oxygenated wines with respect to the control one.

Rutin concentration decreased significantly during the first month in all the wines, decreasing later in the micro-oxygenated wines and wines in barrels, and remaining practically constant in the non-micro-oxygenated wines during the rest of the time studied (Figure 7d). Fernández de Simón et al. [51] also observed a decrease in some flavonols in wines aged for 21 months in oak barrels of different origins. This decrease could be due to the fact that flavonols can react with anthocyanins in co-pigmentation reactions [57,58].

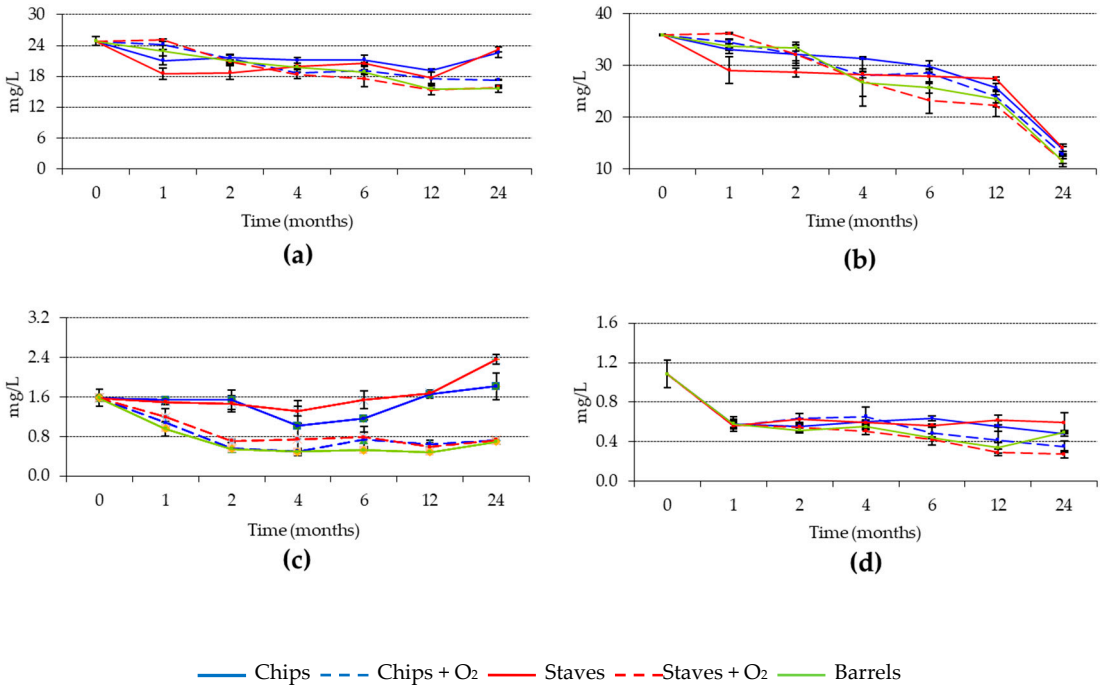

Figure 7. Evolution over time of flavanols concentration (mg/L): (**a**) catechin and (**b**) epicatechin; and of flavonols concentration (mg/L): (**c**) quercetin and (**d**) rutin. Mean ± standard deviation (n = 2).

Figure 8 shows the evolution of the concentration of *trans*-resveratrol and its glycoside, *trans*-piceid. The concentration of *trans*-piceid was hardly modified throughout the process, with no important differences being observed between the treatments (Figure 8a). Alañón et al. [46] did not find differences in the stilbene concentration between wines aged in chestnut wood barrels and wines in contact with chips.

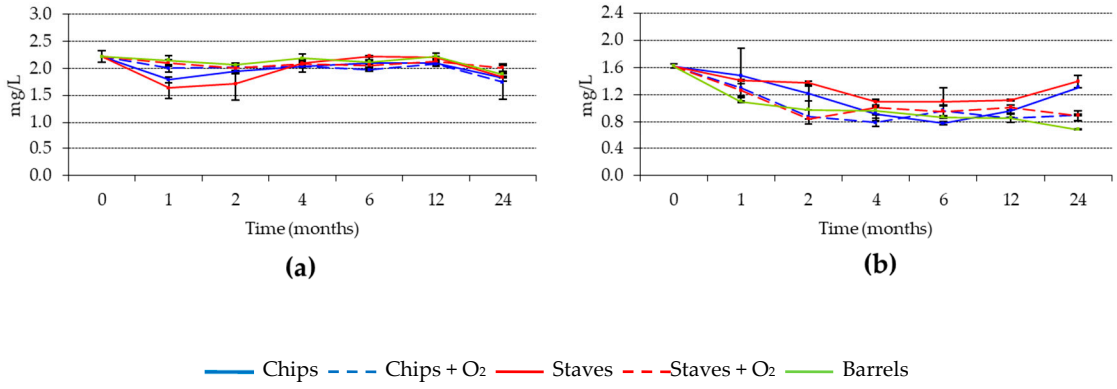

Figure 8. Evolution of stilbenes concentration (mg/L) over time: (**a**) *trans*-piceid; (**b**) *trans*-resveratrol. Mean ± standard deviation (n = 2).

Finally, the concentration of *trans*-resveratrol decreased in all treatments, more in micro-oxygenated wines and in those aged in oak barrels compared to non-micro-oxygenated wines (Figure 8b). Barrera-García et al. [59] estimated an average rate of decrease of *trans*-resveratrol of 50% in a model wine in the presence of oak wood, partly due to adsorption mechanisms on the surface of oak. This coincides with our results when considering micro-oxygenated wines and wines aged in barrels. The decline of stilbenes during aging was also observed by other authors [41,51,60].

3.4. Sensorial Analysis

Figure 9 shows the sensory evaluation of wines during the aging period considered. After 2 months, the most highly valued wines (lowest score) were those treated with staves, followed by those aged in barrel, while wines with chips scored worse. However, at 4, 6, and 12 months, the wines aged in barrels obtained better scores than those treated with fragments, and of these, the wines with staves were better scored than those with chips in most cases.

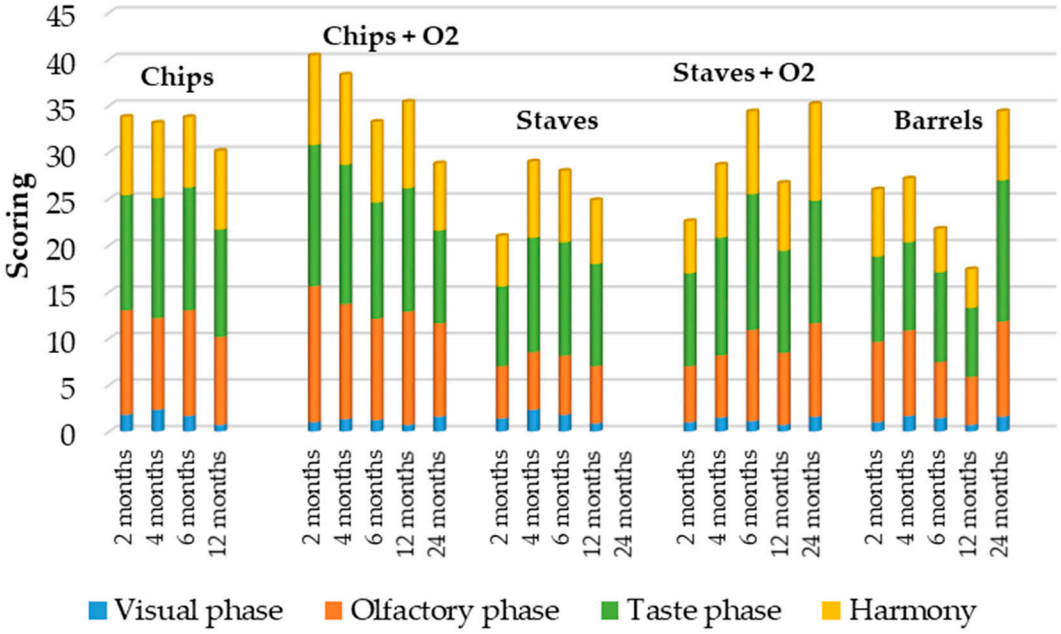

Figure 9. Sensory average valuation of wines over time.

Cano-López et al. [61] obtained the best sensory results in wines with fragments in the form of cubes compared to those aged in barrels, after 3 and 6 months of contact. For this reason, they affirmed that the use of oak chips could be a good choice for short aging wines.

At 24 months, only the sensory analysis of the micro-oxygenated wines and those aged in barrels was carried out. At this time the valuation changed, wines with chips obtaining the best score, followed by wines aged in barrels, and finally those treated with staves. The organoleptic deterioration of wine aged in barrels could be due to its contamination by *Brettanomyces* during bottling. In addition, in wines with staves a high volatile acidity was detected in the organoleptic analysis (data not shown).

Figure 10 shows the sensory profiles of the wines at the different moments studied. Fruity and varietal notes were hardly appreciated in the wines at any of the moments. Up to 6 months, notes related to wood were perceived more intensely in wines treated with staves (toasted, almond, caramel, vanilla, smoked), while at 12 months they were perceived equally in wines aged in barrels and treated with staves. This greater intensity of tertiary aromas in the wines with staves could possibly have been due to a more intense toasting of these, since these aromas come from compounds generated during the wood toasting. Casassa et al. [62] also found an increase in notes related to wood (toast, clove, vanilla, etc.) as the degree of toasting of the chips increased.

With respect to the gustatory phase, wines with chips were perceived as less structured, persistent, astringent, and with a lesser retronasal aroma after 2 months. However, at 4 months they were the ones considered best in this phase. Wines which were micro-oxygenated and in contact with staves obtained the best evaluations at 6 and 12 months, with scores at this time similar to those for wines aged in barrels.

Sensory evaluation of wines at 24 months could be considered invalid due to the strong impact that ethylphenols had on wines aged in barrels and the high volatile acidity in wines treated with staves, which did not allow an adequate evaluation of the rest of the attributes (data not shown).

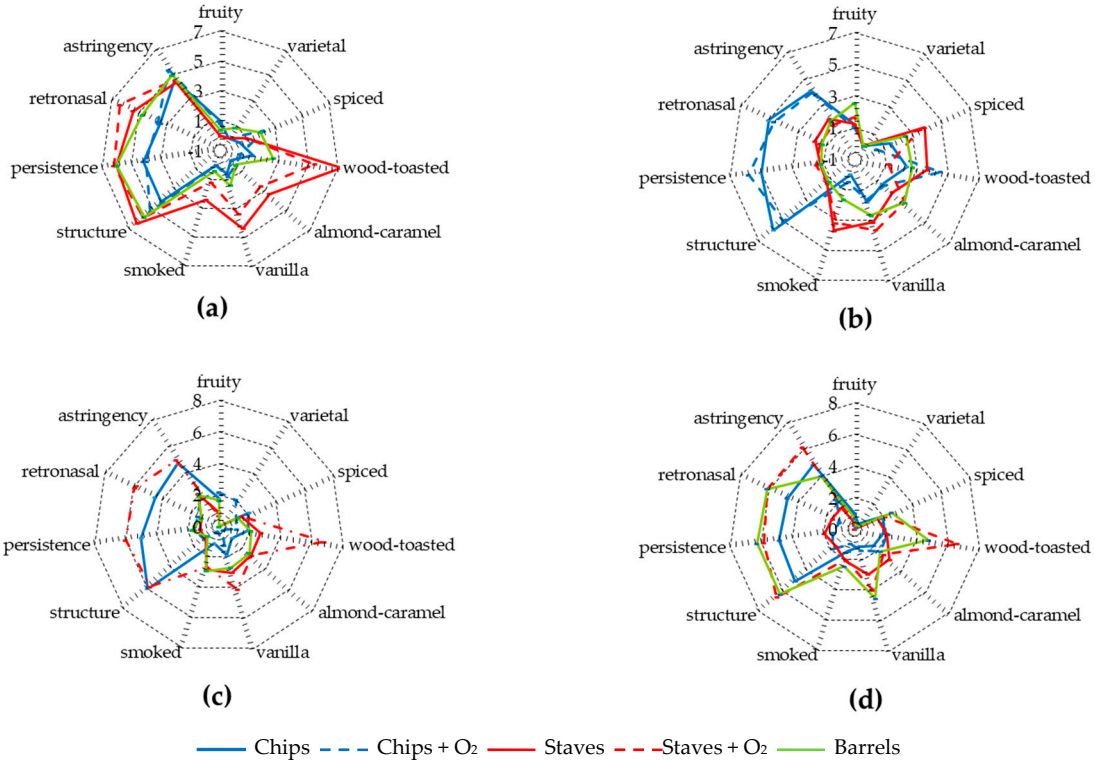

Figure 10. Sensory profiles of wines at: (**a**) 2 months; (**b**) 4 months; (**c**) 6 months; and (**d**) 12 months.

Regarding triangular tests, the results obtained in the sensory assessment at 6 and 12 months are shown in Table 1. At 6 months, wines added with fragments were clearly discriminated (with a level of significance of 99.9%) from those aged in barrels, independently of the size of the fragments. However, an adequate level of significance was not obtained for their discrimination at 12 months. When comparing the size of the fragments (chips vs. staves), it was possible to differentiate between the treatments at both moments (6 and 12 months) with a level of significance of 95% and 99% respectively.

As regards preferences, at 6 months, the preferred wines were those aged in barrels (72.2%), while at 12 months they did not opt for any of the two types of aging. In relation to the size of the fragments, the staves were preferred at 6 months (57.1%), and both treatments were evaluated equally at 12 months.

Table 1. Results of the sensory evaluation by triangular tests.

Moment	Variable	Mean Right Answer (Based on 10)	Preference (%)
6 months	Fragments-Barrel	10.0 (***)	72.2 barrel
	Chips-Staves	7.78 (*)	57.1 staves
12 months	Fragments-Barrel	6.43	50.0
	Chips-Staves	8.57 (**)	50.0

Significance level: $p < 0.05\%$ (*); $p < 0.01\%$ (**); $p < 0.001$ (***).

3.5. Treatments Classification

Two canonical discriminant analyses (DCA) were carried out to classify the different treatments based on the volatile compounds from oak wood and the low molecular weight phenols (Figure 11a,b, respectively). To carry out the discriminant analysis, the data from all the moments and all the trials

studied were used in order to obtain as many variables as possible and try to achieve better sample classification. The graphs obtained present the distribution of the samples in the plane formed by the first two discriminant functions. When trying to discriminate the wines, a good separation was achieved between the three treatments (chips, staves, and barrels) for both groups of compounds.

In the case of the volatile composition (Figure 11a), discriminant function 1 explained 88.8% of the variance and discriminant function 2 explained 11.2%, representing 100% of all variance. In this graph, with 100% of cases correctly classified, the separation between the two types of aging was carried out by means of function 1. Wines aged in barrels were correlated with a high concentration of 5-hydroxymethylfurfural and 5-methylfurfural; and wines in contact with staves were correlated with a high content of vanillin and *trans*-isoeugenol, with these results coinciding with those shown in Section 3.2.

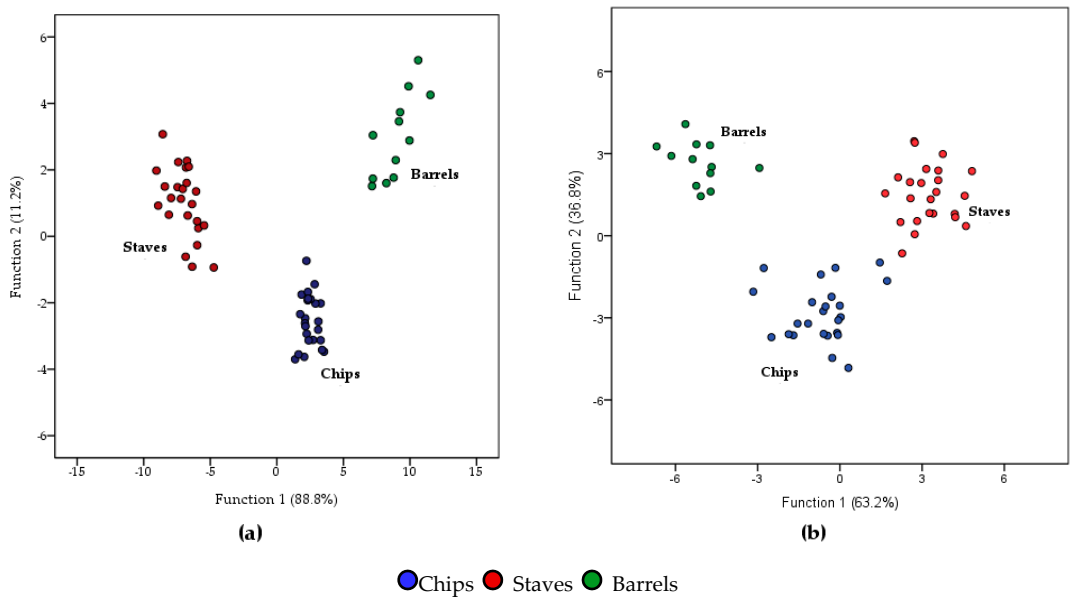

Figure 11. Canonical discriminant analysis: (**a**) volatile composition contributed by oak wood; and (**b**) low molecular weight phenols.

In the graph of low molecular weight polyphenols (Figure 11b), the percentage of accumulated variance explained by the first two functions was 100%, with discriminant function 1 explaining 63.2% of the variance, and discriminant function 2 the 36.8%. Additionally, 100% of the cases were correctly classified. Function 1 allowed us to differentiate between fragments and barrels, while function 2 separated the wines treated with chips from those aged in barrels and with staves. The variables with the highest discriminant power in function 1 were syringic acid and quercetin with a positive character (related to staves), and caftaric acid with a negative sign (associated with barrels). On the other hand, the discriminant function 2 correlates the caftaric acid with the wines aged in barrels and with fragments in the positive part of the axis, and the gallic acid with the wines treated with chips in the negative part. These results coincide with those obtained in Section 3.3.

4. Conclusions

The use of oak fragments and barrels during the aging of red wines influenced the evolution of the wines' chromatic parameters, which was similar between treatments over the aging time. Moreover, an improvement in volatile and phenolic compounds coming from wood was observed in all the wines. Due to the chromatic evolution of the wines and the contribution of substances which came from the wood, the optimal contact time between fragments and wine could be estimated as being 2 months.

The best sensory evaluation of the wines macerated with staves was obtained in short periods of aging, while for those aged in barrels, their sensory quality improved with time. Wines aged with

chips were evaluated more poorly than the wines with other treatments at all times. Good sensory discrimination was also achieved between wines in contact with fragments and those aged in barrels.

The canonical discriminant analyses carried out with the volatile compounds and the low molecular weight phenols showed a good separation between the two types of aging (fragments and barrels), as well as between the two types of fragments (chips and staves).

The results of this study about the contact time with wood during the aging of red wines with oak wood fragments (chips and staves) indicated that the use of these fragments is a suitable practice for aging red wines, which can be an appropriate option for wines destined for short aging periods.

Author Contributions: Conceptualization and funding acquisition, J.M.; methodology, P.R.-B. and J.M.; data curation, P.R.-B. and J.M.; writing—original draft preparation, P.R.-B.; writing—review and editing, P.R.-B., T.G.-C. and J.M.; visualization and supervision, J.M. and T.G.-C.

Funding: This research was funded by Government of La Rioja for regional Projects: P.R.-12-06, P.R.-13-07, P.R.-11-08, P.R.-11-09, P.R.-11-10 and P.R.-15-11.

Acknowledgments: P.R.-B. would like to thank her predoctoral grant of Government of La Rioja. We would like to thank the wine professionals for their participation in sensory evaluation.

Conflicts of Interest: The authors declare no conflict of interest.

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