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Red Wine Aging by Different Micro-Oxygenation Systems and Oak Wood—Effects on Anthocyanins, Copigmentation and Color Evolution

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Abstract: The micro-oxygenation (MOX) of aged wine in contact with pieces of wood is a technique widely used for aging wines as an alternative to barrels. The available range of passive MOX systems is very wide and offers a behavior closer to that of barrels because it uses materials with a similar permeability to oxygen. The aim of this work has been to age the same red wine for 6 months using the main passive MOX systems and compare them with the classic MOX in stainless steel tanks and with barrels as a reference, in order to evaluate phenolic composition and establish its influence. The quantity and the way in which oxygen is incorporated into wine have been found to determine its evolution and final properties. Wine from barrels could be distinguished throughout the aging period since a better level of individualized anthocyanins was maintained, whereas stainless steel + MOX and PMDS (polydimethylsiloxane) wines presented more bluish hues.

Keywords: active micro-oxygenation; anthocyanins; barrel; oxygen; passive micro-oxygenation; staves

1. Introduction

Red wines have been aged traditionally in oak barrels, although micro-oxygenation (MOX) together with adding wood has been well-known for over 20 years. This technique is used in many wineries all over the world as they represent a good alternative, producing wines comparable to those aged in barrels but in less time and at a lower cost [1,2]. In the last few years, MOX systems adding oxygen to wine in a similar way to barrels have appeared. These systems can be classified in two large groups, active MOX or passive MOX [2,3]. The former inject the wine with oxygen at a controlled pressure thanks to a single porous ceramic [4]. This is usually done in stainless steel tanks where the wine is matured in contact with wood and small doses of oxygen are added by volume (mL/L) or by mass (mg/L). In both cases, it is crucial to ensure that all the oxygen dosed is dissolved in the wine before it reaches the surface, for which a minimum column height of 2 m is recommended [5]. In contrast, passive MOX systems are based on a container constructed using materials which, as in the case of oak wood barrels, are permeable to oxygen and add oxygen from the air to the wine by diffusion over its entire surface [2,3]. The microbubbles characteristic of the MOX systems using injection thus disappear. However, unlike oak barrels, the materials used in passive MOX systems do not modify their permeability in contact with wine and add constant doses of oxygen over time [6]. These systems use two types of materials: synthetics such as high-density polyethylene (HDPE) or natural ones such as stoneware tanks. In addition, passive MOX systems based on using polydimethylsiloxane (PDMS)

are available—not having the right characteristics to be used for building tanks, a tube of this material is inserted inside, so that, by varying its length and thickness, the required doses can be added [2,3,6].

The main difference between the passive systems and barrels is that the oxygen transmission rate (OTR) is constant throughout the aging process, while barrels have a dynamic OTR [7,8]. When wood is added to the passive MOX systems, their OTR is dynamic due to the fact that it contains air trapped in its porosity and this oxygen is transferred to the wine when it becomes soaked during aging [9–11]. This oxygen release kinetic depends on various factors such as size or botanic origin of the material used [12].

Wine color is determined by many variables throughout aging, oxygen being one of those with most influence on its evolution. In addition, the extraction of different compounds from oak, such as ellagitannins, phenolic acids, and furanic and phenolic aldehydes, leads to an increase in intensity and color stability due to the formation of pigments derived from anthocyanins and polymerized pigments [13,14]. Copigmentation is also pointed out as the first interaction between anthocyanins and other wine components, leading to the formation of new colored compounds during red wine aging [14]. However, the copigmentation process is considered the first step towards forming polymeric pigments, since, when increases, copigmented anthocyanins decrease [15,16]. During wine aging, anthocyanins participate in several reactions (reduction, oxidation, and polymerization) and are the precursors of new compounds and, therefore, involve other wine molecules, leading to the formation of more stable anthocyanin-derived compounds [14]. Thus, the color of wines changes from red/violet to brick red hues (orange) due to several chemical reactions among anthocyanins and other compounds [17], which result in the formation of new pigments. Thus, due to the occurrence of various chemical reactions the concentration of individual anthocyanins and copigments decreases, resulting in the decreased influence on wine color expression. However, the new polymerized pigments continue to increase and is becoming increasingly important in modifying red wine color during aging [18].

The relevance of the wine aging process means that new techniques alternative to barrels need to be studied. The development of different passive micro-oxygenation systems and their use with wood product alternatives to barrels opens up new opportunities for aging wine. The aim of this study was to evaluate the phenolic compounds and red wine color during aging with these new passive MOX systems using wood, comparing them with the classic systems of active MOX (stainless steel tanks with micro-oxygenation and oak wood) and with traditional aging in barrels, in all cases using *Quercus petraea* French oak.

2. Materials and Methods

2.1. Aging Systems

Five different passive MOX aging systems were studied, comparing them with the traditional active MOX system and with barrels. As regards the passive MOX systems, a 400-L vessel made with stoneware from natural materials (Clayver S.r.l., Vado Ligure SV, Italy; hereinafter, StW), with a tested oxygen transmission rate (OTR) of 11.4 mg/L·year [3] was used. Three different HDPE passive MOX tanks made from synthetic materials were also considered: two 1000-L cube tanks (Flexcube, Victoria, Australia) with different already tested oxygen transmission rates of 9.3 mg/L·year (hereinafter, H-HDPE) and of 6.2 mg/L·year (hereinafter, L-HDPE) [3]; and one 1125 L HDPE Egg model OVOÏD™ 11.25 (hereinafter, Ö-HDPE), with a nominal OTR of 13 mg/L·year according to the company (Flextank, Vancouver, Washington, USA). In addition, a 600-L HDPE tank with a PDMS infusion tube (RedOaker, West Lakes Shore, South Australia, Australia; hereinafter, PDMS) was used with a tested OTR of 9.2 mg/L·year [3]. The active MOX system consisted of a 210-L and 2-m-high stainless steel tank together with a MOX device and porous ceramic micro diffuser (Parsec S.r.l., Florence, Italy), which was regulated to work with a variable oxygen dose (hereinafter, MOX), as previously done to simulate the dynamic OTR of a new barrel [12]. The oxygen contribution released by the wood when submerged in wine (auto-oxygenation) was considered as well as the respective OTRs for all the alternative systems to barrels. These contributions were quantified depending on the type of oak wood (porosity and

density) and the quantity added: values were 23–26 mg/L of O_2 and kg of wood stave [11]. In this trial, the wood used was determined to have contributed 25.7 mg/L·kg during the first 23 days in contact with the wine. The OTR of the barrel is known (OTR = 2.52 ± 0.28 hPa/day), since it belongs to the same batch of previously tested commercial barrels [19].

2.2. Barrel and Alternative oak Wood Products

New barrels and staves (100 × 8 × 1 cm), both from the same batch of *Q. petraea* French oak, were supplied by the Tonelería INTONA, SL (Navarra, Spain) cooperage. The wood was naturally seasoned for 26 months in the cooperage wood yard in the usual way and toasted at medium intensity. The toasting process for the barrel was carried out by fire radiation: it was heated from room temperature to 180 °C and then maintained at this temperature for 40 min—20 min on each side. Prior to that, the barrel was submitted to a 30-min preheating fire process to give it its typical curved shape. For the staves, the toasting process was carried out by convection in an air circulation oven at a temperature of 220 °C for 30 min. The surface/volume ratio of a barrel (2.01 m^2/225 L) was calculated to determine the quantity of staves necessary for the same wine/wood contact surface according to [12] for each aging system. For this purpose, the staves were numbered and measured individually to use the exact wood surface area in barrel-aging, taking into account the volume of each aging system tank.

2.3. Wine

A red wine (cv Tinta de Toro) from the 2018 vintage produced according a traditional fermentation on Spanish appellation of origin region Toro, was aged using different systems for a period of 6 months.

The chemical parameters of the wine before transfer into the different aging systems were evaluated according to OIV (International Organization of Vine and Wine) methods [20]: total acidity 4.62 g/L (expressed as tartaric acid), pH 3.73, volatile acidity 0.58 g/L (expressed as acetic acid), sugars 1.40 g/L, alcohol degree 14.63%, color intensity 13.8, total polyphenol index 54, dry extract 29.8 g/L, relative density 0.993, glucose + fructose 0.15 g/L, free sulfur dioxide 12 mg/L, total sulfur dioxide 23 mg/L, malic acid <0.09 g/L and potassium 1280 mg/L.

The wine was aged in different aging systems in the same wine cellar, where humidity and temperature conditions were controlled at 65–75% and 13–17 °C.

2.4. Wine Analysis

Samples were taken from each aging system after 45, 90, 135 and 180 days of aging and all analyses were measured in duplicate for every sample, so a total of 57 wines samples were analyzed.

2.4.1. Anthocyanin Global Determination and Index

Total anthocyanins (ACY) as mg/L of Malvidin-3-O-glucoside (Extrasynthese, Genay Cedex, France) were analyzed by means of color changes according to the pH of the medium [21]. The ionization index (Io-ln) was analyzed by the Somers and Evans method [22]. All analyses were carried out in a LAMBDA 25 UV/Vis Spectrophotometer (PerkinElmer's, Waltham, MA, USA).

2.4.2. Color Analysis

Visible spectra were obtained using a quartz cell with a path-length of 1 mm and a LAMBDA 25 UV/vis Spectrophotometer (PerkinElmer's, Waltham, MA, USA) interfaced to a computer. The spectra of all samples were obtained by measuring the absorbance in the range of 380–770 nm at 5 nm intervals. Pure water was used for the reference scan.

Color intensity was determined by the sum of the absorbances at 420, 520 and 620 nm and red, yellow and blue percentages were calculated according to Glories method [23]. The CIELab space was used to describe wine color using all visible spectra and the parameters were calculated using the "Method OIV-MA-AS2-11: Determination of chromatic characteristics according to CIELab" [24].

These parameters were: L*, describing the lightness from black to white; b*, from blue to yellow; a*, from red to green; C*, chroma or saturation; and H*, hue angle.

2.4.3. Copigmentation Determination

Copigmentation was determined according to the method proposed by Boulton [25] using the following parameters: color due to copigmented anthocyanins (C); color fraction due to copigmentation (COP); color due to total anthocyanins (TA); color fraction due to free anthocyanins (AL); color due to polymeric pigment (Ep); color fraction due to polymeric pigment (PP); estimation of flavanol cofactor content (FC); and estimation of the total phenol content (TP).

2.4.4. Anthocyanin Individual Determination

The anthocyanins were analyzes in wines by an Agilent 1100 HPLC-DAD (Agilent, Santa Clara, CA, United States) following the method described by [26]. Chromatographic separation was performed in a Fortis C18 column (Sugelabor, Spain) particle size 5 µm (250 mm, 4.6 mm). The phases used were: A: Formic acid/H_2O (15:85, v/v); B: Formic acid/Methanol/H_2O (10:45:45, v/v); C: Methanol/H_2O (90:10 v/v). The anthocyanins were eluted using a flow rate of 0.8 mL/min in a gradient of solvents A, B and C, with a column temperature of 30 °C. Fifteen milliliters of each sample were concentrated by using a rotary evaporator (Büchi, Plawil, Switzerland), then they were reconstituted with 3 mL of synthetic wine and filtered with 0.2 µm PTFE filters (Labbox, Barcelona, Spain). The injected sample volume was 20 µL. A scan was performed between 220–740 nm and 5 wavelengths (373, 528, 570, 676 and 730 nm) were monitored for detection, although quantification was carried out at 528 nm as it was the predominant one. The identification of anthocyanins was carried out by comparing their spectra and retention times according to the method described above [26]. The anthocyanins analyzed were: delphinidin-3-O-glucoside (Df-3-Gl), cyaniding-3-O-glucoside (Cn-3-Gl), petunidin-3-O-glucoside (Pt-3-Gl), peonidin-3-O-glucoside (Pn-3-Gl), malvidin-3-O-glucoside (Mv-3-Gl), malvidin-3-O-glucoside pyruvic acid (vitisin A, Mv-3-Gl-Py); malvidin-3-O-acetyl glucoside pyruvic acid (Mv-3-Gl-Ac-Py); cyanidin-3-O-acetylglucoside (Cn-3-Gl-Ac); ethyl-linked malvidin-3-O-glucoside ethyl-epicatechin (Mv-3-Gl-Ethyl); petunidin-3-O-acetylglucoside (Pt-3-Gl-Ac); malvidin-3-O-acetylglucoside (Mv-3-Gl-Ac) and malvidin-3-O-*p*-coumaroyl glucoside (Mv-3-Gl-Cm); and (Figure 1).The quantitative analysis was done through the external standard method based on malvidin-3-O-glucoside (Mv-3-Gl) (Extrasynthese, Genay Cedex, France), because this is the most representative anthocyanin in wines.

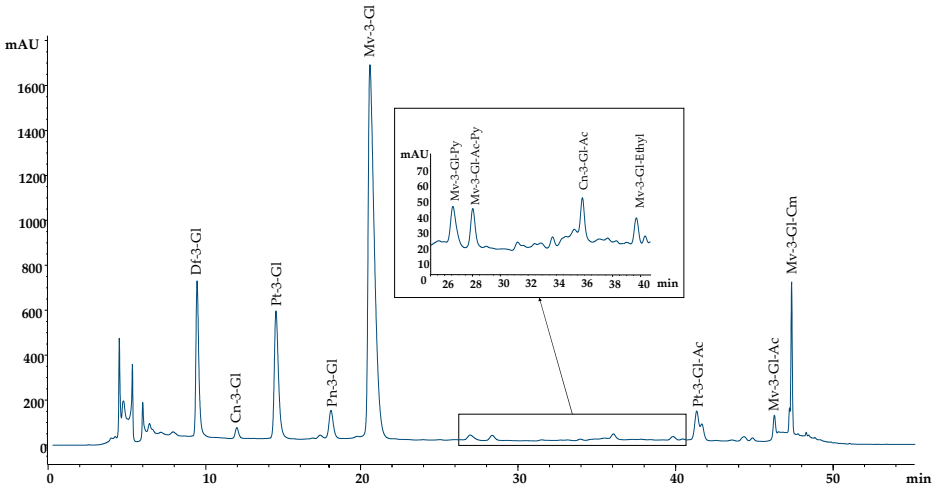

Figure 1. Chromatogram with anthocyanin peaks identification.

2.5. Statistical Analysis

The descriptive analysis results were analyzed by analysis of variance (ANOVA) to determine the differences among the samples. The Fisher's least significant difference was used when a significant difference was found among the samples (p value < 0.05). Principal Component Analysis (PCA) was performed to summarize the results for the chemical parameters and Pearson's correlation analysis was summarized to investigate the relationship between oxygen consumption and analyzed compounds. Statistical analyses were conducted using the Statgraphics Centurion statistical program (version 18.1.12; StatPoint Technologies, Inc., Warrenton, VA, USA) and Statistica (v12; Statsoft Europe GmbH, Hamburg, Germany).

3. Results and Discussion

3.1. Oxygen Management

Figure 2 shows the evolution of total oxygen dosed to red wine in each of the MOX systems (active and passive) for 180 days (about 6 months), according to the OTR values obtained in previous studies and detailed above (see Materials and Methods), and the oxygen released by the wood of the staves. In the case of MOX, the accumulated oxygen dosage responds to the doses injected into the wine (0.049 mg/L·day the first week, 0.037 mg/L·day from day 8 to 30, 0.025 mg/L·day from day 31 to 120 and 0.017 mg/L·day until the end) and to the oxygen released by the air trapped in the porosity of the wood added to the tank. In the case of the PDMS, the atmospheric oxygen was added to the wine by diffusion, as occurs with barrels, through a tube of controllable length and characteristics. However, the diffusion surface/wine volume relationship was much less than in a Bordeaux cask (2.01 m^2/225 L) [3]. The HDPE (H, L and Ö) and the StW systems have walls with an oxygen permeability similar to that of an oak barrel, so they are the molecular oxygen diffusion dosing system [3]. All passive MOX systems (PDMS, HDPE and StW) have constant OTR despite being in contact with wine, which allows the oxygen dosed at each aging time to be known, an aspect that is an apparent advantage of these systems and one that does not occur with oak barrels [7,27]. When the goal is to achieve a behavior similar to that occurring in barrels, understanding it as a natural MOX system, this advantage becomes a disadvantage, although it can be modified thanks to the oxygen that the wood yields, and thus simulate the dynamic behavior of the barrel's OTR.

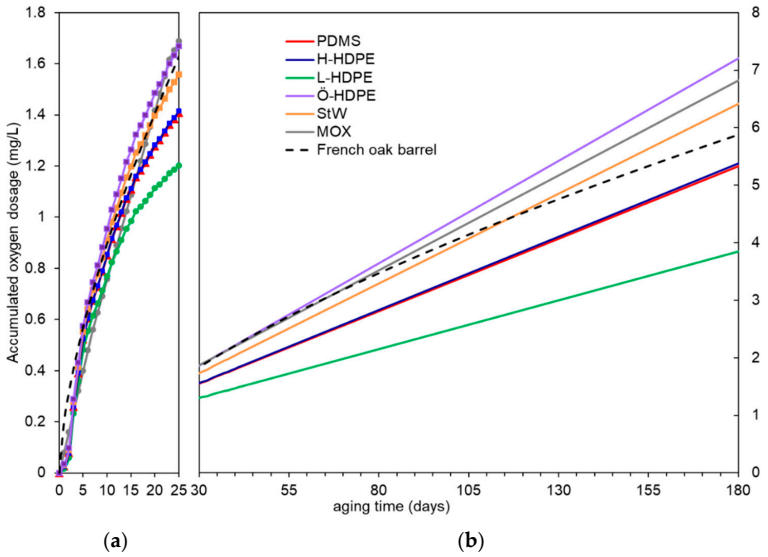

Figure 2. Graphic representation of the measurements of dissolved oxygen content accumulated (mg/L) in wines aged in different aging systems: (**a**) First days; (**b**) Whole experimental period.

Table 1 summarizes dosages during the aging period (mg/L) in the different aging systems. The wines that receive the most oxygen were those aged in Ö-HDPE at a constant dose during the process of 13 mg/L·year followed by MOX and StW at doses of 12 and 11.4 mg/L·year, respectively. The PDMS, H-HDPE and barrel wines received very similar amounts, close to 5.5 mg/L during the 180 days of the study (5.33, 5.38 and 5.88 mg/L), while the L-HDPE aged wines received the lowest dose of oxygen, a total of 3.85 mg/L, which indicates that they received 53% less oxygen than the Ö-HDPE ones. Both in the barrel and with the other alternative aging systems, oxygen was supplied from the wood due to degassing [7,28]. However, this phenomenon differed between the usual method of aging (barrels) and the alternative (other systems), Figure 2a. The oxygen gradient from the outside of the barrel to the inside, which is full of wine, acts as a driving force for the rapid degassing of the staves. However, degassing the wood when it is immersed in wine is produced by the displacement of the air contained when it is flooded with wine. This is thus a slower process which affects the evolution of the wine more gradually. Therefore, when active oxygenation (MOX) was used, the oxygen dosage was adapted over time to suit the barrel situation, as indicated in Figure 2b. In this experiment, French oak staves with the same barrel processing were added to all the alternative systems. The wood dose maintained the same wood surface/wine volume ratio as in a barrel (2.01 m^2/225L, which meant a 0.00898 m^2 of wood surface for each liter of wine). Therefore, Ö-HDPE wines received 7.2 mg/L, the highest amount of oxygen (Figure 2b). If this total contribution of oxygen received by the wine from the barrel (5.9 mg/L) is considered as 100%, it can be seen that L-HDPE wine received 65.5% (3.8 mg/L), PDMS 90.7% (5.3 mg/L), H-HDPE 91.5% (5.4 mg/L), Ö-HDPE 122.5%, StW 109.1% (6.4 mg/L) and finally MOX wines, 116% (6.8 mg/L).

Table 1. Dosages of oxygen (mg/L) carried out during the aging period in the different aging systems.

Aging Time (Days)	Aging Systems						
	PDMS [1]	H-HDPE [1]	L-HDPE [1]	Ö-HDPE [2]	StW [1]	MOX	Barrel [1]
45	1.92	1.94	1.55	2.39	2.20	2.36	2.39
90	3.06	3.08	2.32	4.00	3.60	3.85	3.75
135	4.19	4.23	3.08	5.60	5.01	5.33	4.87
180	5.33	5.38	3.85	7.20	6.41	6.82	5.88

Aging systems: PDMS (polydimethylsiloxane tube); H-HDPE, L-HDPE and Ö-HDPE (high-density polyethylene); StW (stoneware); MOX (stainless steel with micro-oxygenation); and barrel (French oak barrel). [1] see [3]. [2] according supplier.

3.2. Wine Evolution

Table 2 shows the final composition (after 180 days of aging) of each wine from the different systems studied. In order to visualize the evolution of some parameters and/or compounds throughout the aging process, Figure 3 has been included. Moreover, Table 3 summarizes the correlation values (r) found between oxygen consumed by wine aging in the different systems and each analyzed variable in wines. Theses correlations were calculated with the values of the oxygen supplied to the wine (mg/L) and the variation of each analyzed parameter regarding the initial value of each period studied, known as Delta values. Thus, correlation values typed in bold were significant at different *p* values, as indicated in Table 3. Positive correlations indicated an increase in the analyzed variable, whereas the negatives ones indicated a decrease.

Table 2. Differences among the wines aging in the different systems for 180 days.

	PDMS	H-HDPE	L-HDPE	Ö-HDPE	StW	MOX	Barrel	F [1]
				Copigmentation				
ACY	319 ± 7	306 ± 14	318 ± 6	320 ± 3	315 ± 12	319 ± 2	318 ± 6	0.67
Io-In	29.53 ± 1.13	31.39 ± 1.58	30.20 ± 0.16	28.80 ± 1.29	30.03 ± 1.05	29.08 ± 0.81	27.85 ± 0.32	2.45
C	0.64 ± 0.03 c	0.34 ± 0.01 a	0.35 ± 0.03 a	0.55 ± 0.06 b	0.54 ± 0.00 b	0.80 ± 0.04 d	1.00 ± 0.05 e	**89.00** ****
COP	0.10 ± 0.01 c	0.05 ± 0.00 a	0.05 ± 0.00 a	0.08 ± 0.01 b	0.07 ± 0.00 b	0.11 ± 0.00 d	0.13 ± 0.01 e	**78.44** ****
TA	3.68 ± 0.04 a	4.26 ± 0.01 cd	4.31 ± 0.01 d	4.31 ± 0.02 d	4.24 ± 0.01 c	3.71 ± 0.01 a	4.10 ± 0.06 b	**178.35** ****
AL	0.55 ± 0.01 b	0.59 ± 0.00 de	0.60 ± 0.01 e	0.58 ± 0.01 cd	0.57 ± 0.00 c	0.52 ± 0.01 a	0.53 ± 0.01 ab	**32.67** ****
Ep	2.48 ± 0.01 a	2.58 ± 0.01 b	2.57 ± 0.01 b	2.62 ± 0.01 bc	2.61 ± 0.01 bc	2.67 ± 0.01 c	2.66 ± 0.08 c	**7.25** ***
PP	0.37 ± 0.01 cd	0.36 ± 0.00 bcd	0.36 ± 0.01 abc	0.35 ± 0.00 ab	0.35 ± 0.00 ab	0.37 ± 0.00 d	0.35 ± 0.01 a	**7.56** ***
FC	8.25 ± 0.04 a	8.88 ± 0.00 de	8.85 ± 0.01 d	8.96 ± 0.03 e	8.85 ± 0.07 d	8.55 ± 0.01 b	8.65 ± 0.01 c	**105.67** ****
TP	58.47 ± 0.21 a	61.84 ± 0.00 e	61.96 ± 0.00 e	60.68 ± 0.03 b	61.53 ± 0.01 d	61.03 ± 0.07 c	63.76 ± 0.03 f	**688.91** ****
				Color				
Abs420	4.25 ± 0.01 d	4.23 ± 0.01 d	4.16 ± 0.01 c	4.08 ± 0.01 b	4.16 ± 0.02 c	4.38 ± 0.01 e	3.97 ± 0.03 a	**140.27** ****
Abs520	6.77 ± 0.01 d	6.79 ± 0.00 d	6.74 ± 0.01 c	6.69 ± 0.01 b	6.74 ± 0.01 c	6.87 ± 0.01 e	6.36 ± 0.03 a	**350.33** ****
Abs620	1.74 ± 0.01 c	1.74 ± 0.01 d	1.71 ± 0.01 c	1.67 ± 0.01 b	1.73 ± 0.01 c	1.99 ± 0.01 e	1.57 ± 0.02 a	**173.27** ****
Color intensity	12.76 ± 0.02 d	12.75 ± 0.01 d	12.60 ± 0.03 c	12.44 ± 0.01 b	12.62 ± 0.04 c	13.22 ± 0.01 e	11.89 ± 0.07 a	**264.06** ****
Abs420%	33.32 ± 0.00 e	33.15 ± 0.01 d	33.00 ± 0.00 b	32.80 ± 0.06 a	32.96 ± 0.05 b	33.08 ± 0.00 c	33.38 ± 0.01 e	**96.69** ****
Abs520%	53.09 ± 0.05 b	53.25 ± 0.06 bc	53.46 ± 0.01 d	53.76 ± 0.11 e	53.36 ± 0.15 cd	51.94 ± 0.04 a	53.48 ± 0.10 d	**93.22** ****
Abs620%	13.60 ± 0.05 bc	13.61 ± 0.05 c	13.55 ± 0.01 bc	13.44 ± 0.06 b	13.69 ± 0.10 c	14.98 ± 0.04 d	13.14 ± 0.11 a	**144.28** ****
L*	7.41 ± 0.13 b	7.48 ± 0.13 b	7.81 ± 0.02 bc	8.32 ± 0.19 c	7.40 ± 0.45 b	4.34 ± 0.07 a	9.17 ± 0.43 d	**68.58** ****
a*	37.03 ± 0.23 b	37.18 ± 0.23 b	37.71 ± 0.01 bc	38.49 ± 0.30 cd	36.94 ± 0.87 b	27.11 ± 0.31 a	39.51 ± 0.67 d	**141.68** ****
b*	12.74 ± 0.22 b	12.85 ± 0.21 b	13.40 ± 0.03 bc	14.27 ± 0.32 c	12.70 ± 0.77 b	7.44 ± 0.12 a	15.71 ± 0.74 d	**68.20** ****
C*	39.16 ± 0.29 b	39.34 ± 0.29 b	40.02 ± 0.01 bc	41.05 ± 0.39 c	39.06 ± 1.07 b	28.75 ± 0.33 a	42.52 ± 0.90 d	**118.66** ****
H*	18.98 ± 0.19 b	19.07 ± 0.18 b	19.57 ± 0.02 bc	20.34 ± 0.27 c	18.96 ± 0.65 b	15.00 ± 0.06 a	21.67 ± 0.59 d	**63.61** ****
				Anthocyanins				
Df-3-Gl	14.96 ± 0.29 b	15.57 ± 0.42 b	16.40 ± 0.63 b	15.17 ± 2.28 b	16.19 ± 0.55 b	12.65 ± 0.78 a	20.53 ± 0.50 c	**22.49** ****
Cy-3-Gl	1.09 ± 0.01 b	1.13 ± 0.03 b	1.12 ± 0.04 b	1.07 ± 0.11 b	1.10 ± 0.02 b	0.98 ± 0.05 a	1.31 ± 0.02 c	**15.78** ****
Pt-3-Gl	19.84 ± 0.17 b	20.82 ± 0.70 b	21.58 ± 0.82 b	19.97 ± 2.68 b	21.17 ± 0.54 b	17.35 ± 1.04 a	26.12 ± 0.45 c	**19.68** ****
Pn-3-Gl	4.06 ± 0.05 b	4.20 ± 0.09 bc	4.29 ± 0.08 c	4.22 ± 0.29 bc	4.23 ± 0.07 bc	3.78 ± 0.14 a	4.97 ± 0.09 d	**26.20** ****
Mv-3-Gl	75.01 ± 1.28 b	79.08 ± 3.22 b	81.83 ± 3.33 b	74.70 ± 12.06 b	80.65 ± 2.53 b	66.66 ± 5.02 a	100.10 ± 2.41 c	**14.47** ****
Mv-3-Gl-Py	0.66 ± 0.03 a	0.70 ± 0.05 a	0.70 ± 0.05 a	0.70 ± 0.12 a	0.70 ± 0.02 a	0.62 ±0.03 a	0.88 ± 0.05 b	**7.29** ****
Mv-3-Gl-Ac-Py	0.65 ± 0.01 cd	0.66 ± 0.03 d	0.60 ± 0.03 bc	0.56 ± 0.05 b	0.58 ± 0.05 b	0.49 ± 0.03 a	0.72 ± 0.02 e	**18.78** ****
Cy-3-Gl-Ac	0.12 ± 0.01 a	0.12 ± 0.01 a	0.11 ± 0.01 a	0.13 ± 0.02 a	0.13 ± 0.02 a	0.12 ± 0.02 a	0.16 ± 0.02 b	**4.92** ***
Mv-3-Gl-Ethyl	0.18 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.17 ± 0.02	0.17 ± 0.02	0.17 ± 0.02	0.19 ± 0.01	1.64
Pt-3-Gl-Ac	0.50 ± 0.02 ab	0.54 ± 0.03 bc	0.56 ± 0.02 c	0.52 ± 0.04 bc	0.56 ± 0.02 c	0.47 ± 0.03 a	0.71 ± 0.02 d	**28.68** ****
Mv-3-Gl-Ac	3.77 ± 0.02 b	3.90 ± 0.06 bc	3.97 ± 0.07 bc	3.82 ± 0.28 bc	3.99 ± 0.11 c	3.50 ± 0.19 a	4.71 ± 0.09 d	**27.30** ****
Mv-3-Gl-Cm	7.33 ± 0.06 b	7.62 ± 0.21 b	7.69 ± 0.16 b	7.32 ± 0.58 b	7.58 ± 0.14 b	6.76 ± 0.28 a	9.03 ± 0.05 c	**26.43** ****

All parameters are given as mean ± standard deviation; Aging systems: PDMS (polydimethylsiloxane tube); H-DPE, L-HDPE and Ö-HDPE (high-density polyethylene); StW (stoneware); MOX (stainless steel with micro-oxygenation); and barrel (French oak barrel). Different letters indicate significant differences among the aging systems according to the Fisher's least significant difference (LSD) test. Significant values[1] are typed in bold according to: * p value < 0.1; ** p value < 0.05; *** p value < 0.01; **** p value < 0.001.

Table 3. Correlation coefficients between oxygen supplied and analyzed parameters of wines from different aging systems.

	PDMS	H-HDPE	L-HDPE	Ö-HDPE	StW	MOX	Barrel
			Copigmentation				
ACY	**−0.9923** ****	**−0.9749** ****	**−0.9734** ****	**−0.9775** ****	**−0.9887** ****	**−0.9634** ****	**−0.9468** ***
Io-In	−0.2515	−0.1560	−0.4266	−0.5613	−0.2989	−0.4182	−0.0128
C	−0.5125	−0.6085	−0.6595 *	−0.5566	−0.6029	−0.4828	−0.4727
COP	**−0.6564** *	**−0.7686** **	**−0.8116** **	**−0.7391** **	**−0.7693** **	**−0.6388** *	−0.6807
TA	−0.0041	0.3415	0.4143	0.3415	0.3345	−0.0228	0.4036
AL	0.5779	**0.7978** **	**0.8418** ***	**0.7522** **	**0.7818** **	0.4380	0.6197
Ep	**0.6780** *	**0.7792** **	**0.7699** **	**0.7946** **	**0.8072** **	**0.7685** **	**0.7889** ***
PP	**0.6848** *	**0.6837** *	**0.7380** **	**0.6625** *	**0.6876** *	**0.7159** **	0.6572
FC	−0.2351	0.5387	0.4366	0.5004	0.4536	−0.0297	0.4848
TP	−0.1717	0.4065	0.3727	0.3208	0.3666	0.2737	0.4668
				Color			
Abs420	−0.3307	−0.4393	−0.4701	**−0.6311** *	**−0.6370** *	−0.1894	**−0.6644** *
Abs520	**−0.6593** *	−0.3523	−0.5829	−0.4712	−0.3917	−0.4920	**−0.9019** ***
Abs620	−0.4458	0.1847	0.1144	−0.4393	−0.1156	**0.6218** *	**−0.7879** **
Color intensity	−0.5508	−0.3668	−0.5613	−0.6124	−0.6038	−0.1783	**−0.8324** **
Abs420%	0.5253	−0.3303	−0.2679	−0.4971	−0.4601	−0.1672	**0.9288** ****
Abs520%	−0.2262	−0.0169	−0.0250	0.3439	0.2213	−0.4692	0.1141
Abs620%	−0.1147	**0.8473** ***	**0.6788** *	0.2663	0.4170	**0.8822** ***	**−0.7144** **
L*	−0.3471	−0.5279	**−0.7222** **	0.2176	−0.5438	**−0.9486** ****	**0.7509** **
a*	**−0.6418** *	−0.5670	**−0.8158** **	0.1305	−0.6204	**−0.9343** ****	**0.7444** **
b*	−0.3704	−0.5424	**−0.7441** **	0.1904	−0.5591	**−0.9521** ****	**0.7512** **
C*	−0.5673	−0.5594	**−0.7968** **	0.1534	−0.6053	**−0.9378** ****	**0.7448** **
H*	−0.2597	−0.5196	**−0.7013** *	0.2219	−0.5233	**−0.9537** ****	**0.7619** **

Table 3. *Cont.*

	PDMS	H-HDPE	L-HDPE	Ö-HDPE	StW	MOX	Barrel
				Anthocyanins			
Df-3-Gl	**−0.5660** **	**−0.5251** **	**−0.4952** *	**−0.5696** **	−0.3318	**−0.6885** ***	**−0.6039** **
Cy-3-Gl	**−0.5416** **	**−0.4958** *	−0.4186	**−0.5001** **	−0.3778	**−0.6914** ***	**−0.4714** *
Pt-3-Gl	**−0.5812** **	**−0.5290** **	**−0.5128** **	**−0.5956** **	−0.3766	**−0.6995** ***	**−0.6445** ***
Pn-3-Gl	**−0.6220** **	**−0.5634** **	**−0.5813** **	**−0.6056** **	**−0.4445** *	**−0.7268** ***	**−0.6738** ***
Mv-3-Gl	**−0.6199** **	**−0.5779** **	**−0.5790** **	**−0.6379** ***	**−0.4348** *	**−0.6902** ***	**−0.6991** ***
Mv-3-Gl-Py	**−0.7692** ****	**−0.5677** **	**−0.7206** ***	**−0.7399** ***	**−0.6566** ***	**−0.8321** ****	**−0.6976** ***
Mv-3-Gl-Ac-Py	0.0062	0.3695	−0.2610	**−0.4811** *	−0.0883	**−0.8527** ****	0.2966
Cy-3-Gl-Ac	−0.3649	−0.1744	−0.3223	0.0730	−0.1063	0.0214	0.1501
Mv-3-Gl-Ethyl	**−0.7183** ***	**−0.6789** ***	**−0.6863** ***	**−0.7144** ***	**−0.6794** ***	**−0.6759** ***	**−0.7446** ****
Pt-3-Gl-Ac	**−0.6684** ***	**−0.5957** **	**−0.5647** **	**−0.5858** **	**−0.4562** *	**−0.6664** ***	**−0.6242** ***
Mv-3-Gl-Ac	**0.7295** ***	**0.7333** ***	**0.7435** ***	**0.7531** ****	**0.7806** ****	**0.7582** ****	**0.7217** ***
Mv-3-Gl-Cm	**−0.7317** ***	**−0.6816** ***	**−0.7055** ***	**−0.7364** ***	**−0.5900** **	**−0.7922** ****	**−0.7636** ****

Aging systems: PDMS (polydimethylsiloxane tube); H-DPE, L-HDPE and Ö-HDPE (high-density polyethylene); StW (stoneware); MOX (stainless steel with micro-oxygenation); and barrel (French oak barrel). Significant correlation values are typed in bold according to: * p value < 0.1; ** p value < 0.05; *** p value < 0.01; **** p value < 0.001.

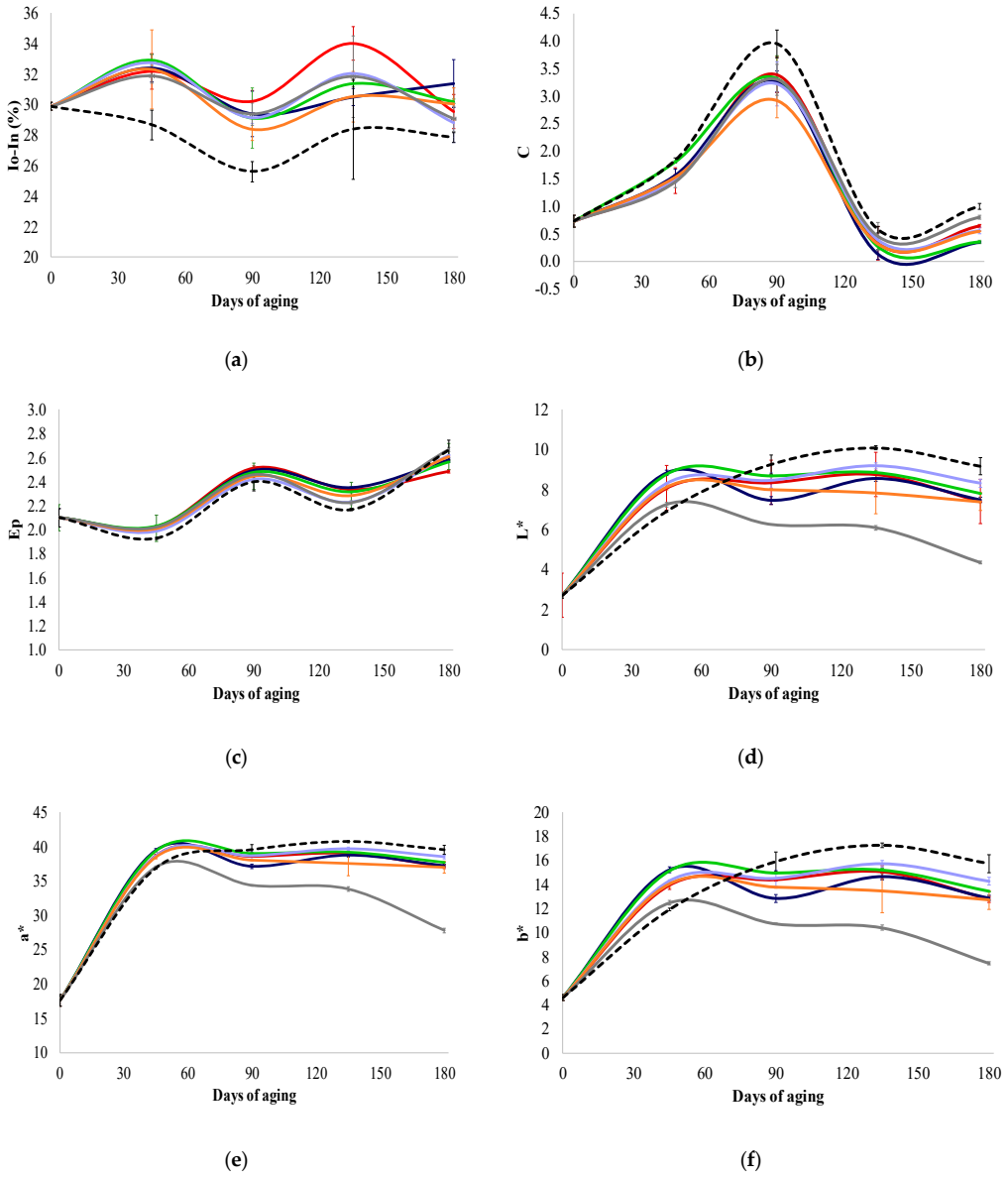

Figure 3. *Cont.*

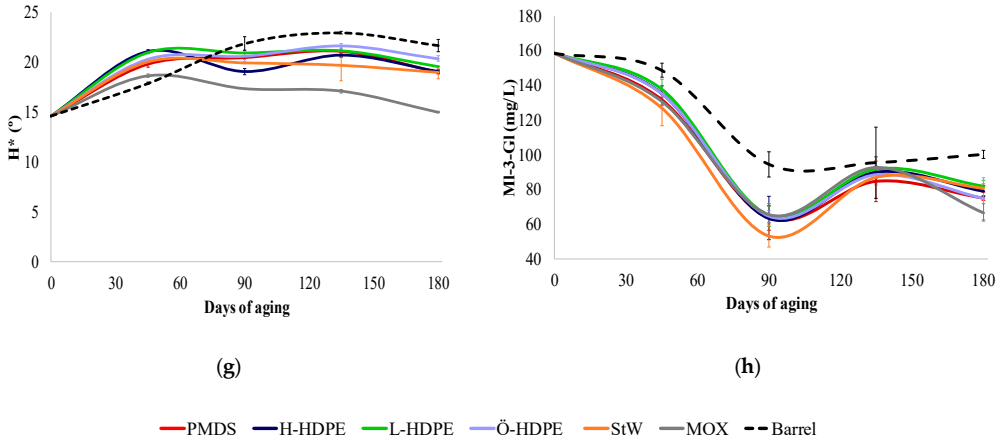

Figure 3. Evolution of some compounds during the wine aging period (180 days) using different systems. (**a**) Ionization index (Io-In); (**b**) Color due to copigmented anthocyanins (C); (**c**) Color due to polymeric pigment (Ep); (**d**) Lightness (L*); (**e**) Red/green (a*); (**f**) Yellow/blue (b*); (**g**) Hue (H*); (**h**) Malvidin-3-O-glucoside (Mv-3-Gl). Aging systems: PDMS (polydimethylsiloxane tube); H-HDPE, L-HDPE and Ö-HDPE (high-density polyethylene); StW (stoneware); MOX (stainless steel with micro-oxygenation); and barrel (French oak barrel).

The ionization index (Io-In) in the all wines was around 30% with the wines aged in barrels presenting the lowest values of this index throughout the entire aging process (Figure 3a), but there were no significant differences among the wines studied at the end of the process (Table 2). Somers and Evans [22] observed that Io-In increased with age, indicating that this index ranged from 10–30% in young wines to 80–90% in those with long aging processes. Copigmentation phenomena are defined by many factors, such as the type of anthocyanin and cofactor, the presence of metals, pH and ethanol, among others [29,30]. The color due to copigmented anthocyanins (C) and color fraction due to copigmentation (COP) were significantly different in wines from different systems after 180 days of aging (p value < 0.001). Barrel wines followed by those aged in MOX showed the highest values for both parameters (C and COP), whereas the rest of the aging systems (PDMS, HDPE and StW) had significantly lower values (Table 2). In addition, wines from barrel and MOX systems also presented a higher color due to polymer pigments (Ep), without presenting any statistically significant differences when compared with wines from Ö-HDPE and StW. The evolution of C (Figure 3b) indicated that it increased in all cases in the first 90 days (3 months), and then decreased until reaching values similar to the initial ones. This evolution could be due to the fact that, in a first phase, anthocyanins undergo copigmentation processes that evolve towards the formation of polymeric pigments [15]. As can be seen in Figure 3c, the color of the wines due to the polymer pigment (Ep) tended to increase after the first 90 days of aging in all the systems studied. These results corroborate what was described by Darias-Martín et al. [31], who observed an increase in the polymer pigment during aging time, followed by a subsequent decrease in the copigmented and free anthocyanins. Over the aging period the wine aged in oak barrels generally had slightly more C and COP values than those aged in the rest of the aging systems. The same results were observed by Zhang et al. [32] when comparing wines aged in barrels and in stainless steel tanks. Additionally, the difference in copigmentation results among all of the aging systems could be due to the copigmentation effect of phenolic substances extracted from oak wood during wine aging [33]. Whereas the Delta C parameter only presented a significantly negative correlation with oxygen dosage in wine aged in L-HDPE (Table 3), Delta COP correlated negatively with oxygen dosage in all the aging systems studied. The wines with the highest Delta COP values had the lowest negative correlation value between this parameter and the oxygen dose (MOX, barrel and PDMS) (Table 3). On the other hand, the Delta Ep and PP (color fraction due to polymeric pigment) showed significantly positive correlations, indicating that the formation of the polymer pigment was favored by the higher amount of oxygen available in the wine. The wine that received the least amount

of oxygen (L-HDPE) was that presenting the highest negative correlation value for Delta COP (−0.8116) and the highest positive correlation value for Delta PP (0.7380). Comparing the three types of HDPE systems (L, H and Ö), it was observed that a lower oxygen dose was correlated with lower COP values and higher PP values. Therefore, it is interesting to note that the greater or lesser development of the copigmentation and polymerization processes not only depend on the availability of oxygen to the wine, but also, and very significantly, on how the oxygen is made available to the wine, as well as how the phenolic compounds are extracted from oak wood during aging. The wines treated in HDPE (H, L and Ö) and StW systems presented the lowest values of C and COP and the highest values of TA (color due to total free anthocyanins) and AL (color fraction due to free anthocyanins) at the end of the aging process when compared to the wines from the other systems studied (Table 2). It was found that in wines with lower AL levels, aged in the PDMS, MOX and barrel systems, Delta AL did not present a statistically significant correlation with the level of oxygen dosed (Table 3). However, in the case of wines treated in H-HDPE, L-HDPE, Ö-HDPE and StW systems, with lower COP and high levels of AL, a positive correlation was found between the increase in Delta COP and the oxygen dosage increment (Table 3). In addition, these (H-HDPE, L-HDPE, Ö-HDPE and StW) presented the highest content of flavanol cofactors (FC) (Table 2) indicating lower copigmentation reactions. Total phenol (TP) content increased over aging for all systems with barrel-aged wine presenting the highest values, possibly due to the different types of toasting in barrels and in staves [34] and to the different wine–wood interaction–impregnation [35]. The wood in the barrels is toasted by radiation, while the staves are toasted in convection ovens. It is also important to note that wine aged in barrels is in contact only with the inner face of the wood, the toasted side. However, the staves are immersed and therefore the wine accesses the wood from all sides (longitudinal, transversal and radial) and all are toasted. This can lead to a greater interaction between wine and wood when staves are used and therefore a faster evolution causing a greater loss of phenolic structure in wines treated with alternatives, as already described in previous studies [36].

In relation to color evolution, it was observed that after 180 days of aging, color intensity (CI) decreased for all the systems, with wine from the MOX system having the highest value and wine aged in barrels the lowest (Table 2). Specifically, MOX wine showed the highest levels of Abs620% and the lowest levels of Abs520% (Table 2). Therefore, MOX (active) seems to have a greater effect on the formation of blue compounds. However, the wine from the barrel had the highest Abs420%. Figure 3 shows the evolution of the CIELab parameters: lightness, L* (Figure 3d), red/green, a* (Figure 3e), yellow/blue, b* (Figure 3f) and hue, H* (Figure 3g). In wines aged in PDMS, HDPE (H, L and Ö) and StW, an increase in color was observed for up to 45 days followed by a certain stabilization of all the parameters studied. However, in the MOX wine, after the increase in the first few weeks, a slight decrease was observed during the 180 days of aging. On the other hand, the evolution of the barrel-aged wine was slightly different, showing a continuous increase up to 135 days, which was maintained until the end of the aging process. Thus, the wines aged in barrels for 6 months showed: (i) higher L*, due to the clarification processes that occur more clearly in the barrel than in other systems; (ii) a higher level of b*, related to the greater significance of yellow shades in the color, as opposed to blue ones; (iii) higher levels of H*, an aspect corroborated by the b* results found in these barrel wines (Table 2). The wines from the MOX system presented significantly lower CIELab values than those from the other systems (Table 2). This result is related to achieving wines with more bluish hues, less browning and more color intensity, as has already been reported by several authors [12,37,38].

In the case of MOX wines, negative correlations were found between the highest oxygen supply and Deltas of L* (−0.9486), a* (−0.9343) and b* (−0.9521). This meant an increase in blue shades, also reflected in the positive correlations found in Abs620 (0.6218) and Abs620% (0.8822), showing the higher formation of new blue hue pigments mentioned above (Table 2). It is interesting to note that wine aged in Ö-HDPE with passive micro-oxygenation received more oxygen throughout the aging process (7.20 mg/L, Figure 2 and Table 1) than MOX wine. However, the higher overall amount of oxygen was not similarly reflected in the color of the wine, meaning that how the oxygen is dosed

influences the modification of the wine's properties. In the case of wine aged in L-HDPE, it was found that the oxygen dosage correlated to the parameters described in the case of MOX wine, although less significantly. Therefore, lower oxygen inputs supplied by the constant passive micro-oxygenation of L-HDPE also caused the formation of new blue hue pigments. However, the oxygen supply to barrel-aged wine was positively correlated to the variation in all CIELab parameters, and Abs420%, while it was negatively correlated to CI and absorptions 420, 520 and 620 as well as Abs620% (Table 3). Therefore, the oxygen received by barrel-aged wine is related to the increase in lightness, red and yellow tones, chroma and hue as well as the loss of blue tones. This result indicates the spontaneous precipitation typical of the barrel-aging process reflected in the increase in the lightness of wines as well as the formation of new compounds with red and yellow tones.

The anthocyanin content decreased throughout the aging process in all wines, as can be seen in Figure 3h, where the evolution of the main anthocyanin (Mv-3-Gl) is showed. This decrease is probably due to the copigmentation and polymerization reactions inherent in the aging process [2], which involve the loss of anthocyanin monomer due to interaction with various wine compounds, thus causing the generation of other colored ones [14]. In all aging systems, a statistically significant correlation between the increase in oxygen dosage and the Delta ACY was found, with negative correlation values greater than 0.9468 (Table 3). The high correlations found in the case of new systems, PMDS, HDPE (H, L and Ö) and StW, were noteworthy (Table 3).

After 180 days of aging, differences were observed between the anthocyanin monoglucoside content of the wines from the different aging systems. The barrel wine had the highest content (p value < 0.001) of almost all anthocyanins studied, except for Cy-3-Gl-Ac, with a p value < 0.01 and Mv-3-Gl-Ethyl, which showed no significant differences (Table 2 and Figure 3h). This indicates that the passive but dynamic MOX of barrels attenuates the loss of these compounds compared to the rest of the passive systems. However, wines from active MOX showed the lowest concentrations of most anthocyanin monoglucoside at the end of aging, as oxygen dosage increased (Tables 1 and 2). This is reflected in the highest negative correlation values between almost all of the Delta anthocyanins studied, and the oxygen supplied (Table 3). This result highlights the lack of stability of the coloring matter of MOX-aged wines compared to those treated in other alternative systems. A significant and negative correlation was found between the loss of anthocyanin monoglucosides and the oxygen supplied, except for Mv-3- Gl-Ac with positive correlation, Cy-3-Gl-Ac without correlation and Mv-3-Gl-Ac-Py which depended on the system used (Table 3). Therefore, as aging progressed and oxygen doses increased, there was a generalized loss of these compounds which participated in copigmentation phenomena in the first stage, followed by the formation of polymeric pigments.

3.3. Principal Component Analysis

Figure 4 shows the projection of the wines in the factor-plane as a result of the Principal Component Analysis (PCA) performed with the 28 parameters analyzed in the different wines. Table 4 also summarizes the weight of the variables for each of the PCAs, which were carried out to reduce the number of linear combinations of variables that explain the greater variability of the data. The first two main components were chosen to visualize the layout of the samples at the variable level. The PCAs carried out were: (i) the whole aging process, identified by time (Figure 4a) or according to the aging system (Figure 4b); and, (ii) for different times, 45 days (Figure 4c), 90 days (Figure 4d), 135 days (Figure 4e) and 180 days (Figure 4f) from the beginning of the aging process.

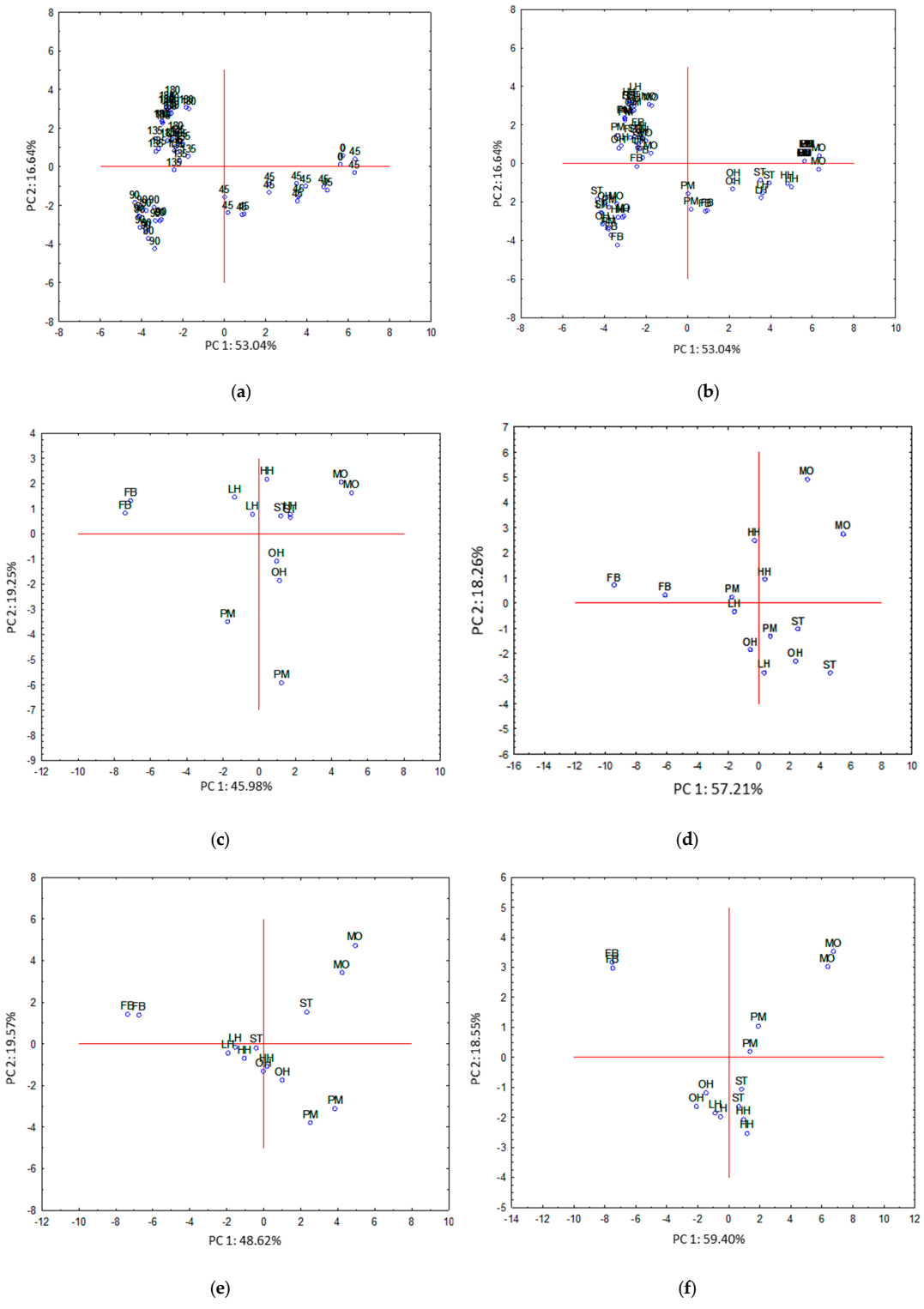

Figure 4. Principal Component Analysis (PCA) performed with copigmentation, color parameters and individual anthocyanins in wines from different aging systems. Evolution of wine during the time (**a**) and different systems (**b**); Differentiation of wines in the sampling performed: (**c**) 45 days; (**d**) 90 days; (**e**) 135 days; and (**f**) 180 days from the beginning of aging. Aging systems: PM (polydimethylsiloxane tube); HH, LH and ÖH (high-density polyethylene); ST (stoneware); MOX (stainless steel with micro-oxygenation); and FB (French oak barrel).

Table 4. Principal component analysis (PCA) results carried out with copigmentation, color parameters and individual anthocyanins in wines aged with different aging systems after different aging times (45, 90, 135 and 180 days).

	Evolution Over the Time		**After 45 Days of Aging**		**After 90 Days Of Aging**		**After 135 Days of Aging**		**After 180 Days of Aging**	
	PC 1	**PC 2**	**PC 1**	**PC 2**	**PC1**	**PC 2**	**PC 1**	**PC 2**	**PC 1**	**PC 2**
% variance	53.04	14.64	45.98	19.25	57.21	18.26	48.62	19.57	59.40	18.55
ACY	**0.7401**	−0.6009	0.6574	0.1055	0.5975	−0.2809	−0.6680	0.3440	−0.0470	0.2805
Io-In	0.2389	0.1565	−0.5706	−0.0815	−0.4826	−0.1941	0.6021	−0.3828	0.3546	−0.6425
COP	−0.1387	**−0.8916**	**0.8093**	0.4356	**0.7977**	−0.3725	−0.1299	0.4369	−0.1666	**0.9542**
AL	0.0248	**0.7927**	−0.6542	−0.4185	−0.6690	0.4173	−0.3335	−0.2848	−0.1375	**−0.9611**
PP	0.3116	**0.8926**	**−0.7848**	−0.3731	**−0.7364**	0.1435	0.6610	−0.3656	**0.8234**	0.2119
FC	0.6919	0.5674	**−0.8122**	−0.3134	−0.6036	0.3185	−0.3109	−0.4095	−0.2534	−0.6231
Tp	−0.6585	0.1166	−0.1430	−0.1744	−0.4389	0.3004	−0.4496	0.4332	−0.5781	0.1288
Color intensity	0.5009	−0.0556	**−0.7655**	0.4912	**−0.7718**	−0.2704	**0.7718**	0.1410	**0.9935**	−0.0319
Abs420%	−0.6207	0.3458	−0.1047	0.5314	0.6243	−0.4551	−0.5253	−0.0863	−0.2519	0.5639
Abs520%	−0.5779	−0.2740	**0.6829**	−0.6311	0.3286	**0.8823**	−0.6148	**−0.6931**	**−0.7705**	−0.5924
Abs620%	**0.7708**	0.1476	**−0.7126**	0.6015	−0.6862	−0.6481	**0.7449**	0.6480	**0.8643**	0.4004
L*	**−0.8706**	−0.0614	**0.7190**	−0.6572	**0.7450**	0.6583	**−0.7928**	−0.5761	**−0.9174**	−0.3247
a*	**−0.9071**	−0.0055	**0.7139**	−0.6673	0.6702	**0.7254**	**−0.7473**	−0.6483	**−0.8284**	−0.4766
b*	**−0.8729**	−0.0613	**0.7171**	−0.6596	**0.7419**	0.6616	**−0.7900**	−0.5810	**−0.9155**	−0.3281
C*	**−0.9045**	−0.0135	**0.7168**	−0.6687	0.6939	**0.7074**	**−0.7624**	−0.6276	**−0.8496**	−0.4467
H*	**−0.7996**	−0.1130	0.6701	−0.5922	**0.7530**	0.6489	**−0.7886**	−0.5814	**−0.9364**	−0.2785
Df-3-Gl	**0.9236**	−0.0212	**0.8884**	0.3980	**0.9576**	−0.2194	**−0.9220**	0.3262	**−0.9830**	0.1403
Cy-3-Gl	**0.8980**	−0.0382	0.6306	0.2083	**0.8873**	−0.2236	**−0.9236**	0.1985	**−0.9529**	0.2054
Pt-3-Gl	**0.9293**	−0.0316	**0.8833**	0.4123	**0.9302**	−0.2942	**−0.9149**	0.3666	**−0.9771**	0.1385
Pn-3-Gl	**0.9268**	−0.0706	**0.9053**	0.2681	**0.9391**	−0.2453	**−0.8731**	0.4632	**−0.9648**	0.2256
Mv-3-Gl	**0.9344**	−0.0604	**0.8842**	0.4348	**0.9506**	−0.2537	**−0.8813**	0.4467	**−0.9613**	0.2091
Mv-3-Gl-Py	**0.9065**	−0.1921	0.4112	0.2585	**0.8850**	−0.2968	**−0.7840**	0.2885	**−0.8211**	0.1936
Mv-3-Gl-Ac-Py	−0.1071	−0.4129	0.0282	−0.5557	0.5247	−0.0572	−0.6313	0.1687	**−0.7505**	0.0563
Cy-3-Gl-Ac	−0.4424	−0.4475	0.3801	0.3868	**0.8876**	−0.2126	−0.1555	**0.7415**	−0.5757	0.6470
Mv-3-Gl-Ethyl	**0.8557**	−0.1744	0.3674	0.0639	0.5289	0.3201	0.0167	0.3018	−0.4417	0.2134
Pt-3-Gl-Ac	**0.9470**	−0.0338	**0.7578**	0.2177	**0.9117**	−0.1966	−0.6836	0.3648	**−0.8838**	0.2454
Mv-3-O-Gl-Ac	−0.2176	0.6731	0.5146	0.1025	**0.9450**	−0.1613	**−0.8731**	0.0582	**−0.9341**	0.2696
Mv-3-O-Gl-Cm	**0.9442**	−0.1155	**0.8972**	0.3561	**0.9345**	−0.2443	**−0.8814**	0.3496	**−0.9614**	0.2372

Variables with the highest weight have been typed in bold.

For the whole aging process, the first two components explained 67.68% of the total variability of the original data, accounting for 53.04% for the first component and 14.64% for the second one. Three groups related to the aging time (45 days, 90 days, and the last 3 months) could be distinguished (Figure 4a), but no differentiation was observed as regards the aging system (Figure 4b). This result agrees with those described in other studies, in which time was indicated as the most influential factor in the changes during aging, more so than the use of different woods or even different systems [38–40]. Thus, in this PCA, the greater distance, and therefore a higher evolution, was observed for wines from the first (45 days) and second (90 days) samplings, while there was almost no differentiation between 90, 135 and 180 days of aging according to the first component (PC1), though 90 days could be differentiated from 135 and 180 according to the second component (PC2) (Figure 4a). When all the aging period was considered, the wine evolved in a very different way depending on the aging system in the first 45 days; however, these differences disappeared with aging time (Figure 4b). The parameters that defined the PC1 were Abs620%, ACY and individual anthocyanins (Df-3-Gl, Cy-3-Gl, Pt-3-Gl, Pn-3-Gl, Mv-3-Gl, Mv-3-Gl-Py, Mv-3-Gl-Ethyl, Pt-3-Gl-Ac and Mv-3-O-Gl-Cm), in positive, and the CIELab parameters in negative (Table 4). The variables that most contributed to the differentiation in PC2 were the copigmentation parameters AL and PP, in positive, and COP in negative (Table 4). According to the distribution of the samples, the youngest wines (the initial ones and after 45 days of aging) were defined by the individual anthocyanins, but also by a higher Abs620%, since they were located in the positive PC1. During aging, there was a loss of monomeric anthocyanin [17], with the consequent interaction with other wine compounds, causing the generation of other colored compounds [14].

As aging progressed (after 90 days), wines maintained their L*, C* and H*. Additionally, such wines seemed to be related to the COP parameter, which could also be explained by parameter a*. Wines at the end of the aging period (after 180 days) were located in the negative PC1 and positive PC2, defined by AL and PP. This could be due to the decrease in anthocyanins and copigments [18,41].

In differentiating wines after 45 days of aging (Figure 4**c**), the first two main components explained 65.23% of the variance (45.98% and 19.25% for PC1 and PC2, respectively). PC1 allowed for the differentiation of barrel-aged wines from the rest, especially MOX wine. The most contributed variables were Abs620%, COP, CIELab parameters (L*, a*, b* and C*) and some individual anthocyanins (Df-3-Gl, Pt-3-Gl, Pn-3-Gl, Mv-3-Gl, Pt-3-Gl-Ac and Mv-3-O-Gl-Cm) (Table 4). This indicated that the impact of the aging system was very different in wines with passive (barrel) or active (MOX) micro-oxygenation during the first 45 days.

In the next two samplings (90 and 135 days of aging), the previously described situation was maintained: PC1 was responsible for the differentiation of barrel and MOX wines (Figure 4d,e). After 90 days of aging (Figure 4d), the first two components explained 75.47% of the variance. The first, which accounted for 57.21%, was defined in positive by anthocyanin content, COP, and CIELab parameters (L*, b* and H*). The PC2, responsible for 18.26% of the variance, were defined positively by the CIELab parameters (a* and C*) and Abs520%. After 135 days of aging (Figure 4e), PC1 explained 48.62% of the variance, where the individual anthocyanins Df-3-Gl, Cy-3-Gl, Pt-3-Gl, Pn-3-Gl, Mv-3-Gl-Py, Mv-3-Gl-Ac and Mv-3-Gl-Cm and the CIELab parameters were those contributing the most to the differentiation of barrel wines from MOX and PDMS ones, which were defined by high color intensity and Abs620%. StW wines and all those from HDPE tanks were in an intermediate position. PC2 explained 19.57% of the variance and allowed the differentiation of PDMS and MOX wines, with the rest of those studied being between the two. Abs520% in negative and Cy-3-Gl-Ac in positive were the most important contributors to the differentiation in PC2 (Table 4). According to Figure 3h, barrel wine suffered the loss of individual anthocyanins more slowly and in its color Abs620% made a lower contribution. In addition, the way in which the oxygen was incorporated into barrels caused a smaller decrease in individual anthocyanins over time, resulting in a color with a greater contribution of Abs520%. This was favored by the processes of copigmentation that can be related to wine–wood interaction and how the release of compounds from the wood is produced [35]. In the case of MOX wines, the higher contribution of Abs620% reflected the higher amount of new blue hue pigments whose formation was mediated by oxygen, as previously reported by other authors [42]. This result shows the importance of oxygen dosing in the formation of new pigments, such as ethyl linked pigments, whose absorbance at 620 nm was higher than that of the original anthocyanins [43].

At the end of the aging period (180 days), the PCA explained 77.95% of the variance, where PC1 accounted for 59.40% and PC2 18.55% (Figure 4f). As with the overall sampling moment, PC1 allowed for the differentiation of barrel wines from the other systems studied, being defined by the CIELab parameters, Abs520% and almost all of the individual anthocyanins. Among alternative systems, the MOX wines were significantly distanced from the barrel ones and defined by color intensity with Abs620% and PP standing out, confirming what was described in the previous aging period (Figure 4e). However, in relation to the PC2, both barrel and MOX wines were located on the positive axis and defined by COP, while StW and HDPE (H, L and Ö) wines were defined mainly by color fraction due to free anthocyanins (AL) (Table 4). Thus, at the end of the aging process it was possible to establish four groups of wines: (i) barrel, (ii) MOX, (iii) HDPE (H, L and Ö) and StW, and (iv) PDMS.

Therefore, throughout aging, but more so after 180 days, wines aged in barrels had very different characteristics from those treated in the other systems, especially MOX. Besides the variables indicated before, the wines from barrel were defined by greater copigmentation, and oxidized tones (Abs420%) but fewer blue ones (Abs620%) (Table 2). The wines from the other systems were positioned between the MOX and barrel wines, with PDMS and H-HDPE wines, then L-HDPE and finally Ö-HDPE which, according to PC1, are closest to those in barrel. Wines from the latter two systems (L-HDPE and Ö-HDPE), located in the negative part of PC1, were considered somewhat less evolved, due to the

participation in color of the anthocyanin monoglucosides, and were also defined by higher values of Abs520%, lower values of Abs620% and also lower PP. The variable that allowed their differentiation in the PC2 of the barrel wines was the color fraction due to free anthocyanins (AL), whose values were higher. As the previous wines, H-HDPE and StW, were also located in the negative part of PC2, they were mainly defined by the AL parameter, as well as a higher color intensity and Abs620%, due to their position in the positive axis of PC1. For PDMS and MOX wines, although the amount of oxygen received was significantly lower in the first case (Table 1), both were in the positive part of PC1 and PC2. In addition to this difference in terms of the amount of oxygen dosed, these are different MOX systems (passive and active, respectively). The method of incorporating the oxygen into PDMS differs from the rest of the passive systems, since the diffusion surface/wine volume relationship is much less [2]. Nevertheless, it could be similar to that of MOX in some ways since oxygen is incorporated into the wine from a point inside the tank.

4. Conclusions

The quantity and the way that the oxygen is dosed into the wine during aging define its evolution and final properties. Thus, most micro-oxygenation (MOX) systems (among them HDPE, PMDS and StW) have been considered suitable for wine aging since wines were similar to those obtained with the classic MOX in stainless steel tanks and with barrels, although with some differentiation allowing them to be distinguished from an analytical point of view. Additionally, the oxygen dosages of passive MOX systems were different but similar to that of barrels, regardless of the type of MOX (active or passive). Thus, it can be said that, in this trial, passive MOX allowed the production of wines closer to that in barrels than with classic active MOX, despite the use of variable doses.

Throughout the aging period, it was possible to differentiate the barrel wine from the MOX one and both from the rest. As the sampling progressed, it was also possible to differentiate wines that aged in PDMS, HDPE (H, L and Ö) and StW. Thus, at the end of the aging period, the wine that aged in barrels maintained a better level of individualized anthocyanins, whereas those from MOX and PDMS tanks presented more color intensity and bluish hues.

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