

Research Article

Blueberry Wine Aging: Influence of Bottle Storage Time on Color, Anthocyanin Monomers, and Antioxidant Activity

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In this paper, the influence of bottle storage time on different blueberry wines has been studied. Four blueberry wines with different fruit weight/sugar solution volume ratios and different fermentation times were stored. The storage was conducted for 12 months in darkness and at a constant temperature of 4°C. All the wines showed a similar behavior during the 12-month study period. The concentration of anthocyanins decreased significantly after the storage period. Wines obtained through partial fermentation showed a low concentration of anthocyanin monomers, and in the case of wines from total fermentation, no anthocyanin monomers could be identified. However, the wines at 12 months of storage exhibited a significant contribution to red color. The color intensity experienced a sharp decrease from 0 to 4 months, remaining unchanged until 12 months. Moreover, antioxidant activity increased during the storage process from 0 to 4 months and then remained relatively stable up to 12 months.

1. Introduction

The market launch of a new product requires an exhaustive study of its evolution to verify its stability; otherwise, significant sales and marketing issues may arise. To define the quality of red wines, phenolic compounds are very important because of their influence on organoleptic properties such as color, astringency, and bitterness, as well as aging capacity [1]. The compounds most involved in these properties are anthocyanins, flavanols, and the resulting polymeric forms between anthocyanins and flavanol polymers. The first ones are responsible for the red color, while flavanols and polymeric pigments are responsible for bitterness and astringency. All these compounds present in blueberry and beverages made from them are known for their beneficial properties for health due to their high antioxidant activity [2–4].

It is well known that during the evolution of red grape wines, important changes occur in the phenolic composition, with the appearance of other compounds that are characterized to be more stable and having more complex

structures [5]. Some of the factors that most affect the progress of chemical reactions of phenolic compounds during wine aging are the concentration of anthocyanins, copigments, acetaldehydes, and other yeast metabolites, as well as pH, temperature, and the presence of oxygen and potassium metabisulphite, among others [6]. Many of these phenolic compounds contribute to the color of both grape and blueberry wines. Color is an important attribute of a beverage since it affects the choice of the product by the consumer [7]. The initial color of red wines is mainly due to the anthocyanins that are extracted from the skins of the fruit during the winemaking process. However, during subsequent storage, or aging processes, numerous reactions occur that can modify anthocyanins profile. Among these reactions are oxidation, copigmentation, cycloaddition, condensation, or polymerization processes [8–10]. Likewise, flavanols are involved in oxidative browning reactions and interact with proteins, causing turbidity in the wine [11]. Together, the reactions modify the color, astringency, and bitterness of the wine during the aging period. It is known that red grape wines evolve from red to orange-red tones

[12] and that spontaneous clarification takes place in this process.

Currently, numerous studies have been conducted to understand the evolution of fruits and their derivatives during the production process and storage [13–22]. However, studies examining the evolution of color, anthocyanins, and antioxidant activity during the aging or bottle storage of blueberry wines are limited. In the available literature, only studies on the development of aromas in blueberry wines over a 16-month bottle aging period can be found [23] or a study of only 6 months of aging of bog bilberry syrup wines [7].

The purpose of this study was to evaluate the influence of bottle storage time on color, anthocyanins, and antioxidant activity during bottle storage time of blueberry wines and to check if the winemaking conditions were adequate to keep the wines stable overtime.

2. Materials and Methods

2.1. Material. The blueberries used were grown in southern Spain (Huelva, Andalusia). The variety used was Windsor which belongs to the Highbush blueberry (*Vaccinium corymbosum*). The blueberries were frozen at -20°C until use.

2.2. Reagents. Hydrochloric acid (PubChem CID: 313), metaphosphoric acid (PubChem CID: 3084658), formic acid (PubChem CID: 284), acetic acid (PubChem CID: 176), phosphoric acid (PubChem CID: 1004), methanol (PubChem CID: 887), acetonitrile (PubChem CID: 6342), and potassium dihydrogen phosphate (PubChem CID: 516951) were bought from Merck (Madrid, Spain). Malvidin-3-O-galactoside chloride (PubChem CID: 94409), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (PubChem CID: 40634), DPPH (2,2-diphenylpicrylhydrazyl) (PubChem CID: 2735032), and DTT (DL-dithiothreitol) (PubChem CID: 446094) were purchased from Sigma-Aldrich Chemical Co. (Madrid, Spain). Potassium metabisulphite and potassium bicarbonate for food use were purchased from Agrovín S.A. (Spain).

2.3. Production of Blueberry Wines. The blueberries were added to a sugar solution in a proportion of 1:1 and 2:1 (weight of blueberries/volume of sugar solution) (wt/vol) to obtain an initial juice with a sugar content of 21°Brix. A commercial yeast inoculum *Saccharomyces cerevisiae* (Viniferm CT 007, Agrovín S.A., Spain) was added to the juice with the solid parts of the berries at the dose recommended by the manufacturer (0.3 g/L). These mixtures were divided into 1-liter flasks containing 500 mL of juices with solid parts and immersed in thermostatic water baths at 17°C . Four of the flasks (two 1:1 and two 2:1 wt/vol) were fermented completely and four others' (two 1:1 and two 2:1 wt/vol) fermentation was stopped when the alcohol content reached between 6 and 7% v/v by addition of wine alcohol up to 13% v/v. The fully fermented wines were named TW1 (fully fermented wine 1:1 wt/vol) and TW2 (fully fermented wine 2:1 wt/vol) and the partially fermented wines were named PW1

(partially fermented wine 1:1 wt/vol) and PW2 (partially fermented wine 2:1 wt/vol). Fermentation was monitored by weight loss of CO_2 released during the process using the estimation of alcohol content using formula (1) [24]:

$$\text{percentage of ethanol} \left(\frac{v}{v} \right) = \frac{0.94 \times \text{CO}_2 \text{ (g/L)} + 2.7}{7.89} \quad (1)$$

After fermentation, the final wines were pressed on a vertical press and skin residues separated from the wine.

After 24 hours, potassium metabisulphite (175 mg/L) and potassium bicarbonate (1 g/L) for food use were added to the wines to keep them stable overtime. After this treatment, the wines were kept at rest for 24 hours before bottling in green glass Bordeaux bottles with standard UNE-EN-12726-18.5 mm cork stoppers. The bottles were labeled and stored in the absence of light at 4°C for 12 months. The analyses performed in this study were carried out every four months (0, 4, 8, and 12 months) of stabilization in the bottle. Prior to analysis, the samples were centrifuged at 3000 rpm, filtered through 0.45 μm pore size HA filters from Millipore (Billerica, MA) and all determinations being carried out in triplicate.

2.4. Separation, Extraction, Identification, and Quantification of Anthocyanins by HPLC-DAD. For the separation, extraction, identification, and quantification of anthocyanins, the method proposed by Marquez et al. [22] was used.

2.4.1. Separation and Extraction of Anthocyanins from Wine. A volume of 2 mL of wine was passed through a Sep-Pak C18 cartridge, with 900 mg of filling (Long Body Sep-Pak Plus; Waters Associates, Milford, MA) that was previously activated with 5 mL of methanol and washed with aqueous HCl 0.01% (v/v). The cartridge was washed with 10 mL of 0.01% aqueous HCl and then with 5 mL of ethyl acetate and sequentially anthocyanins were recovered with 5 mL of methanol which was acidified to pH 2 with HCl. The samples were evaporated to dryness using a vacuum centrifuge thermostatic at 30°C and then dissolved in 1 mL of acidified methanol (pH = 2). Samples were passed through a Nylon filter of 0.45 μm pore size for HPLC analysis.

2.4.2. Identification and Quantification by HPLC-DAD of Anthocyanins. A volume of 20 μL of wine was injected into a P4000 HPLC instrument from Spectra-Physics (San Jose, CA). Analyses were carried out on a LiChrospher 100 RP-18 column (250 mm \times 4.6 mm, 5 μm), using 10% aqueous formic acid in HPLC-grade water (solvent A) and 10% formic acid, 45% acetonitrile, and 45% HPLC-grade water (solvent B), as a mobile phase, at a flow rate of 1 mL/min. Anthocyanins were registered at 520 nm, by gradient elution from 15% to 30% B in 17 min, gradient elution up to 73% B in 28 min, gradient elution up to 100% B in 3 min, and isocratic elution in 3 min. Identifications were confirmed by HPLC-ESI-MS on an AQA quadrupole mass spectrometer from Thermo Fisher Scientific. All anthocyanins were quantified as malvidin-3-O-galactoside.

2.5. Spectrophotometric Determinations

2.5.1. Alcohol Content of Blueberry Wines. The alcohol content was determined by vapor of the ethanol contained in the sample following the method proposed by Crowell and Ough [25]. This ethanol was reacted with a solution of potassium dichromate in an acid medium at controlled temperature. A spectrophotometric measurement of Cr^{3+} at 600 nm was performed using a PerkinElmer (Waltham, MA) Lambda 25 spectrophotometer, and the absorbance was compared with that obtained from an ethanol calibration curve. All analyses were performed in triplicate.

2.5.2. Absorbances at 420, 520, and 620 nm. Absorbances at 420 (A420), 520 (A520), and 620 nm (A620) were made on a PerkinElmer (Waltham, MA) Lambda 25 spectrophotometer, using quartz cells of 1 mm light path. The samples were prepassed through 0.45 μm pore size HA filters from Millipore (Billerica, MA). All measurements were adjusted for a path length of 1 cm.

2.5.3. Color Intensity. This parameter was calculated measuring the absorbances at 420, 520, and 620 nm with a quartz cuvette with a path length of 1 mm [26], and the color intensity is given as the sum of the values of the absorbances recorded:

$$\text{color intensity (CI)} = A420 \text{ (a.u.)} + A520 \text{ (a.u.)} + A620 \text{ (a.u.)} \quad (2)$$

2.5.4. Antioxidant Activity. Antioxidant activity was performed by DPPH assay according to Alen-Ruiz et al. [27]. A 45 mg/L solution of DPPH in methanol was prepared and a 80 mg/L solution of Trolox, a vitamin E analogue, was used as a standard. The analytical procedure was as follows: a 200 μL aliquot of diluted wine was placed in a cell and 3 mL of a 45 mg/L solution of DPPH in methanol was then added. A blank (200 μL diluted wine + 3 mL methanol), a control sample (200 μL of 12% ethanol in water + 3 mL of DPPH solution), and a Trolox standard (200 μL of Trolox solution + 3 mL of DPPH solution) were also prepared in parallel. Following vigorous stirring, the absorbance at 517 nm was measured using a PerkinElmer (Waltham, MA) Lambda 25 spectrophotometer of the control sample and blank was also measured. The sample and the Trolox standard were measured under identical conditions after 120 min of incubation at room temperature. The results were expressed in millimoles of Trolox per liter (mmol TE/L).

2.6. pH. The pH was determined by direct measurement with a Crison pH meter model micropH 2001, calibrated with buffer solutions of pH 7.02, 4.00, and 9.21 at 25°C.

2.7. Statistical Procedures. All results obtained from the wines produced were subjected to a one-factor analysis of variance (ANOVA) test in triplicate, at a 95% confidence

level, using the Statgraphics v. 5.0 software package from Statistical Graphics Corp (Statgraphics Technologies, Inc., The Plains, Virginia). This establishes homogeneous groups and allows testing for significant differences.

3. Results and Discussion

3.1. Fermentation Time, Alcohol Content, and pH of Blueberry Wines. The fermentation time and the desired alcohol content of a wine depend on various factors, including nutrient levels, pH of the medium, the type of yeast used, and more. In this context, the blueberry wines produced exhibited different fermentation times primarily due to the initial fruit quantity. PW1 and TW1 wines, prepared with a 1:1 ratio (wt/vol), achieved their desired alcohol content (6.4% v/v and 13.08% v/v, respectively) later than PW2 and TW2 wines (6.2% v/v and 12.95% v/v, respectively). Notably, the difference in fermentation time between the partially fermented wines, PW1 (267.7 hours) and PW2 (229.5 hours), was not as substantial as that observed in the fully fermented wines, TW1 (660.2 hours) and TW2 (497 hours). On the other hand, it is known that the pH is a parameter indicative of the quality of the wine and influences the evolution of the color over time [28]. The pH values of blueberry wines could be related to some of the most important changes associated with the quality of the wines. For example, if the pH increases too much with storage time, there is a greater possibility of oxidation of the wine since the formation of quinones from anthocyanins would be favored and there would be a loss of red color. Moreover, the balance of the different forms of SO_2 is conditioned by pH. Therefore, the increase of pH value could lead to a decrease of free SO_2 , so less antioxidant and preservative action in wines. In addition, if the pH is too low, bacterial contamination would be prevented and oxidation and loss of color would be avoided, but the organoleptic characteristics of the wines would not be adequate. Table 1 shows the pH values of blueberry wines at 0, 4, 8 and 12 months of bottle stabilization. As can be seen, the pH range of blueberry wines studied agrees with the pH of commercially available blueberry wines (2.8–3.7) [29]. Consequently, it could be affirmed that the conditions used for bottle storage of the blueberry wines obtained could be adequate.

As observed, most of the wines showed significant differences with respect to the 0-month-old wine, and not so many differences were found between the wines at 4, 8, and 12 months of bottle storage. Secondly, the most substantial changes in the wines tend to occur during the initial time of aging. This is because a reductive environment is established in the bottle, which means that condensation, oxidation, and polymerization reactions occur to a lesser extent and at slower rates during this period [30].

3.2. Absorbances at 420, 520, and 620 nm. Storage time and temperature are two of the factors that affect wine color [21]. In this study, the storage temperature was constant; therefore, the only variable to be considered is the evolution of the different parameters over time.

TABLE 1: pH values of blueberry wines at 0, 4, 8, and 12 months of bottle storage time ($n = 3$; mean \pm standard deviation and homogenous groups between the same wine).

Blueberry wine	Time (months)			
	0	4	8	12
PW1	3.44 ± 0.010^a	3.34 ± 0.035^b	3.32 ± 0.006^b	3.32 ± 0.006^b
TW1	3.32 ± 0.015^a	3.28 ± 0.006^b	3.24 ± 0.023^c	3.24 ± 0.015^c
PW2	3.03 ± 0.010^a	3.22 ± 0.006^b	3.22 ± 0.010^b	3.29 ± 0.032^c
TW2	2.82 ± 0.010^a	3.32 ± 0.029^b	3.26 ± 0.044^c	3.21 ± 0.006^c

Values in the same row with different superscript letters are significantly different. $p \leq 0.05$.

The absorbance at 420 nm was measured as an indicator of the yellow-brown color and was utilized to evaluate oxidative browning in the beverages. Furthermore, the absorbance at 520 nm offers insights into the red color, attributed to the presence of anthocyanins, as these compounds exhibit an absorption peak at around 520 nm. Lastly, the measurement of absorbance at 620 nm was used to determine the contribution of the blue color in the beverages. Figures 1(a) and 1(b) show the evolution of the absorbances at 420 and 520 nm of blueberry wines studied during the 12 months of storage. These presented similar behavior throughout the process, and it was observed that all the wines displayed a more pronounced presence of brown hues than red prior to bottling. However, it can be noted that from 0 to 4 months, the red and brown tones suffered a marked decrease in the contribution to color. In particular, the wines after 4 months of bottle storage were redder than brown. In this context, if anthocyanins are indeed the compounds responsible for the red color in these wines, this observation may suggest the formation of other compounds, not identified or quantified in this study, that also contribute to the red color in blueberry wines, as it could be vitisins [31] and colored polymers from tannins [32, 33].

Furthermore, beyond the 4-month mark, these two absorbances exhibited noticeable differences as the storage period advanced for each type of wine produced. However, the values of these absorbances underwent minimal changes, remaining almost constant, with no discernible alteration in color. This fact may be since the reactions involving the colored compounds advance rapidly in the first months of storage modifying the color, since once the concentration of reagents (mainly anthocyanins) decreases, the changes in color are smaller. Regarding absorbance at 620 nm (blue tone) (Figure 1(c)), it is noteworthy that the initial wines did not show values of this absorbance, however, there was an increase during the first 8 months, although with low values, decreasing again at 12 months. This could be due to a bathochromic shift produced by condensation reactions of anthocyanins with flavonols (anthocyanin-flavonol copigmentation reaction) [19] and/or with other compounds present in the wine. Because of these reactions, a shift in the absorption maximum occurs, giving rise to bluish-red tones [14].

3.3. Color Intensity. From the point of view of stability and quality of a wine, it is interesting to know if the color intensity of the wines under study is maintained over time

since this parameter can indicate whether the amount of color of a wine is stable. Figure 2 shows the evolution of color intensity from 0 to 12 months of bottle storage of blueberry wines elaborated. As can be seen, the most abrupt change in color intensity occurred between 0 and 4 months, with a marked decrease. Other authors found that in bog bilberry syrup wines, the color intensity decreased from 0 to 6 months of bottle aging [7]. On the other hand, from 4 to 12 months, the color intensity remained practically constant. This fact is of great importance, since it would ensure the stability of the color of the wines over time during their storage and sale period.

The evolution and stabilization of the color of red grape wines have been classically attributed to the formation of polymeric pigments, which explains the decrease in the color intensity of the wines, since being compounds with a high degree of polymerization, they end up precipitating by insolubilization in the wine. In addition, this behavior could be because T-A polymeric pigments (tannin-anthocyanin) are not affected by sulfites, since they have the C-4 position of the pyran ring of the anthocyanin blocked, a position where the sulfite could react, so this type of pigment is stable and would maintain the color of the wine. However, in the case of A-T type pigments (anthocyanin-tannin), they keep this position free and could be discolored. On the other hand, the disappearance of the copigmentation phenomenon (due to the decrease in anthocyanin monomers) would also explain the decrease and stabilization of the color intensity since the bathochromic effect would disappear [34].

3.4. Anthocyanin Concentration. Anthocyanins are natural colorants responsible for the red color of wines and contribute to the development of polymeric pigments during wine aging [35]. During fermentation and the first years of wine maturation, the anthocyanin content decreases due to a variety of reactions and compound associations, resulting in new anthocyanin-derived pigments, which are extremely crucial for the color stability of beverages [36].

Tables 2 and 3 show the anthocyanins identified and quantified in the blueberry wines under study at 0, 4, 8, and 12 months of bottle storage. Sixteen compounds were identified and quantified, of which 5 were galactoside derivatives, 3 were glycoside derivatives, 4 were arabinoside derivatives, and 4 of them were aglycones. Of all of them, the galactoside family was always the most important, with malvidin-3-O-galactoside being the most important compound in all cases. As for the total anthocyanin

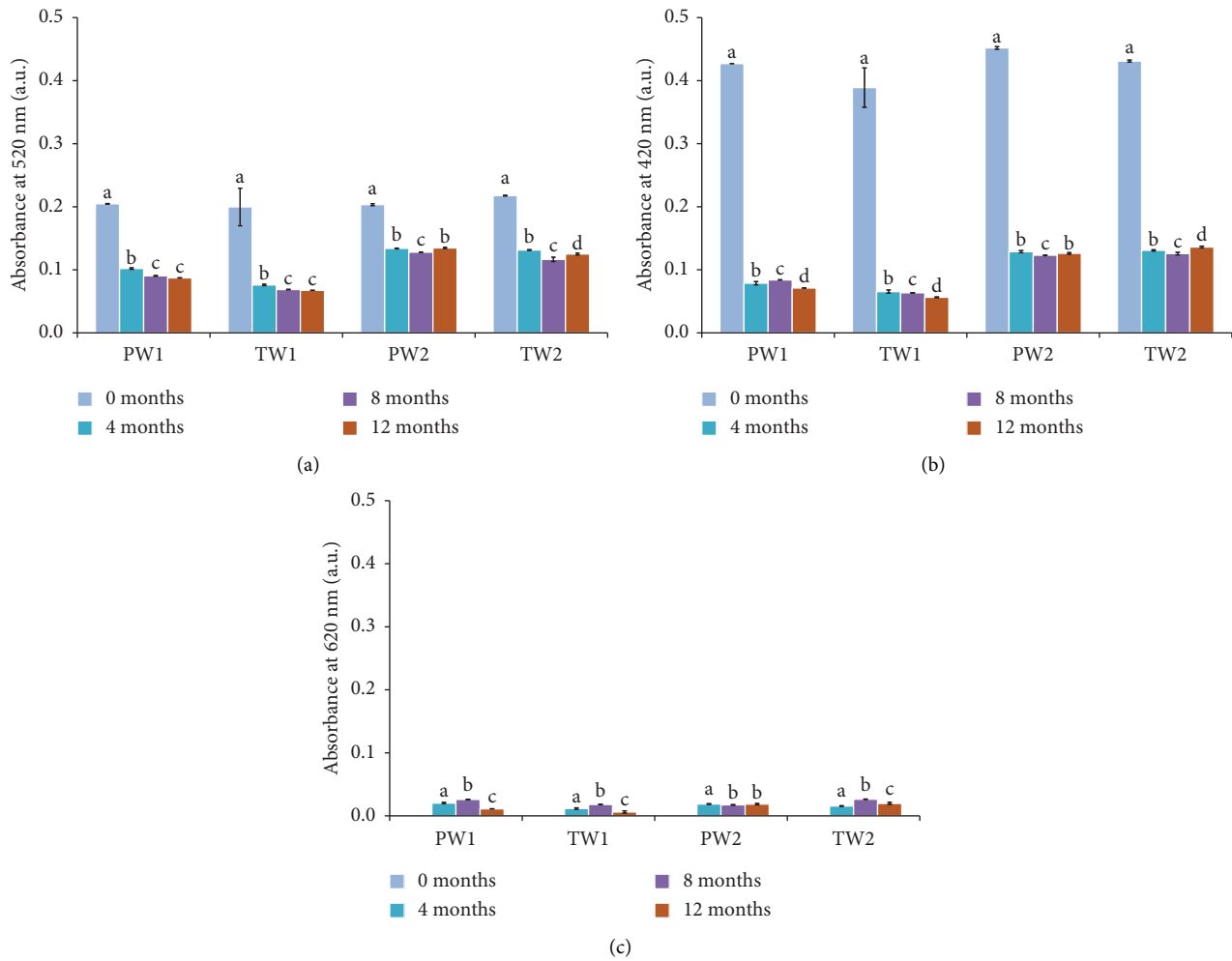


FIGURE 1: Absorbances at 520 nm (a), 420 nm (b), and 620 nm (c) of blueberry wines at 0, 4, 8, and 12 months of bottle storage time. Values in the same type of wine with different letters are significantly different. $p \leq 0.05$. ($n = 3$).

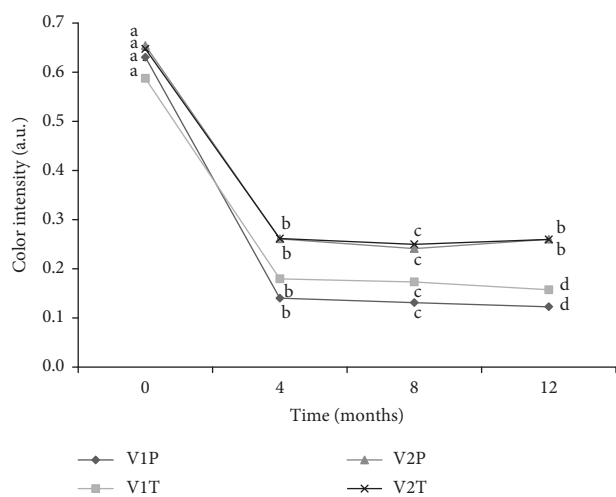


FIGURE 2: Color intensity (a.u.) of blueberry wines at 0, 4, 8, and 12 months of bottle storage time. Values in the same type of wines with different letters are significantly different. $p \leq 0.05$. ($n = 3$).

concentrations obtained from each wine at 0, 4, 8, and 12 months of storage, it can be observed that there was a decrease during aging, finding significant differences with increasing storage time. After 12 months in bottles, only the galactoside, arabinoside, and glycoside derivatives of malvidin could be quantified in the partially fermented wines, which is logical since they were the major compounds from the 0-month-old wines. On the other hand, it was found that the decrease in the concentration of these compounds in the PW1 and TW1 wines was slower than in the PW2 and TW2 wines, although the latter had a higher concentration of anthocyanins.

Figure 3 shows the total concentration of galactoside, arabinoside, and glycoside derivatives during the evolution of blueberry wines in bottles. It can be clearly seen that the concentration of galactoside, arabinoside, and glycoside derivatives decreased with bottle storage time. In general, after 12 months of bottle aging, the partially fermented wines showed a decrease in galactoside derivatives between 70 and 90%, while in the case of the total fermented wines, these

TABLE 2: Anthocyanin concentration (malvidin-3-O-galactoside (mg)/L) of PW1 and TW1 blueberry wines at 0, 4, 8, and 12 months of storage time (mean, standard deviations, and homogenous groups between the same wines).

	PW1				TW1			
	0 months	4 months	8 months	12 months	0 months	4 months	8 months	12 months
Delphinidin-3-O-galactoside	0.479 ± 0.019	0.910 ± 0.005	n.d.	n.d.	n.d.	0.520 ± 0.001	0.469 ± 0.013	n.d.
Cyanidin-3-O-galactoside	0.476 ± 0.001	0.647 ± 0.002	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Petunidin-3-O-galactoside	0.827 ± 0.035	0.831 ± 0.004	1.13 ± 0.008	n.d.	0.667 ± 0.017	0.966 ± 0.057	0.650 ± 0.026	n.d.
Peonidin-3-O-galactoside	0.538 ± 0.018	0.943 ± 0.007	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Malvidin-3-O-galactoside	7.11 ± 0.448	4.04 ± 0.102	4.78 ± 0.067	2.84 ± 0.188	5.11 ± 0.274	4.22 ± 0.030	4.02 ± 0.046	n.d.
Total galactosides	9.43 ± 0.449 ^a	7.37 ± 0.093 ^b	5.91 ± 0.074 ^c	2.84 ± 0.188 ^d	5.78 ± 0.260 ^a	5.71 ± 0.087 ^a	5.14 ± 0.033 ^b	n.d. ^d
Delphinidin-3-O-glucoside	0.383 ± 0.004	0.479 ± 0.003	n.d.	n.d.	0.477 ± 0.003	0.442 ± 0.004	0.533 ± 0.006	n.d.
Cyanidin-3-O-glucoside	0.343 ± 0.006	0.447 ± 0.002	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Malvidin-3-O-glucoside	3.14 ± 0.142	2.69 ± 0.011	2.61 ± 0.007	2.22 ± 0.034	2.32 ± 0.012	2.22 ± 0.005	1.56 ± 0.011	n.d.
Total glucosides	3.87 ± 0.151 ^a	3.61 ± 0.013 ^b	2.61 ± 0.007 ^c	2.22 ± 0.034 ^d	2.80 ± 0.013 ^a	2.66 ± 0.007 ^b	2.09 ± 0.013 ^c	n.d. ^d
Delphinidin-3-O-arabinoside	0.396 ± 0.002	0.831 ± 0.004	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cyanidin-3-O-arabinoside	0.621 ± 0.025	0.734 ± 0.005	0.860 ± 0.004	n.d.	0.831 ± 0.018	0.818 ± 0.079	0.747 ± 0.004	n.d.
Petunidin-3-O-arabinoside	0.611 ± 0.007	0.903 ± 0.004	n.d.	n.d.	0.620 ± 0.012	n.d.	n.d.	n.d.
Malvidin-3-O-arabinoside	5.16 ± 0.233	3.84 ± 0.016	3.99 ± 0.017	1.91 ± 0.012	4.10 ± 0.102	2.99 ± 0.450	2.03 ± 0.081	n.d.
Total arabinosides	6.79 ± 0.217 ^a	6.31 ± 0.019 ^b	4.85 ± 0.020 ^c	1.91 ± 0.012 ^d	5.55 ± 0.108 ^a	3.81 ± 0.372 ^b	2.78 ± 0.078 ^c	n.d. ^d
Cyanidin	0.518 ± 0.002	n.d.	n.d.	n.d.	0.676 ± 0.032	n.d.	n.d.	n.d.
Petunidin	0.668 ± 0.019	n.d.	n.d.	n.d.	0.713 ± 0.006	n.d.	n.d.	n.d.
Peonidine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Malvidin	0.668 ± 0.026	0.809 ± 0.010	0.841 ± 0.014	n.d.	0.490 ± 0.019	0.550 ± 0.016	0.553 ± 0.011	n.d.
Total aglycones	1.85 ± 0.016 ^a	0.849 ± 0.010 ^b	0.841 ± 0.014 ^c	n.d. ^d	1.88 ± 0.007 ^a	0.550 ± 0.016 ^b	0.553 ± 0.011 ^c	n.d. ^d
Total anthocyanins	22.0 ± 0.721 ^a	17.9 ± 0.071 ^b	14.2 ± 0.043 ^c	6.97 ± 0.168 ^d	16.0 ± 0.180 ^a	12.4 ± 0.446 ^b	10.6 ± 0.066 ^c	n.d. ^d

For each type of wine, values in the same row with different superscript letters are significantly different. $p \leq 0.05$.

TABLE 3: Anthocyanin concentration (malvidin-3-O-galactoside (mg)/L) of PW2 and TW2 blueberry wines at 0, 4, 8, and 12 months of storage time (mean, standard deviations, and homogenous groups between the same wines).

	PW2				TW2			
	0 months	4 months	8 months	12 months	0 months	4 months	8 months	12 months
Delphinidin-3-O-galactoside	2.61 ± 0.124	1.04 ± 0.002	0.501 ± 0.025	n.d.	1.19 ± 0.066	0.906 ± 0.002	n.d.	n.d.
Cyanidin-3-O-galactoside	0.874 ± 0.068	1.06 ± 0.004	0.276 ± 0.000	n.d.	1.17 ± 0.105	0.569 ± 0.004	n.d.	n.d.
Petunidin-3-O-galactoside	2.88 ± 0.035	1.96 ± 0.004	1.24 ± 0.009	n.d.	2.50 ± 1.76	1.40 ± 0.006	n.d.	n.d.
Peonidin-3-O-galactoside	1.00 ± 0.021	1.23 ± 0.006	0.780 ± 0.080	n.d.	1.19 ± 0.074	0.890 ± 0.005	n.d.	n.d.
Malvidin-3-O-galactoside	14.1 ± 0.284	9.26 ± 0.038	5.57 ± 0.055	2.86 ± 0.049	11.8 ± 0.380	7.47 ± 0.201	1.67 ± 0.012	n.d.
Total galactosides	21.5 ± 0.122 ^a	14.5 ± 0.049 ^b	8.36 ± 0.046 ^c	2.86 ± 0.049 ^d	17.8 ± 0.790 ^a	11.2 ± 0.197 ^b	1.67 ± 0.012 ^c	n.d. ^d
Delphinidin-3-O-glucoside	1.58 ± 0.001	0.819 ± 0.010	0.543 ± 0.013	n.d.	0.989 ± 0.105	0.834 ± 0.003	n.d.	n.d.
Cyanidin-3-O-glucoside	0.970 ± 0.014	0.942 ± 0.010	0.563 ± 0.018	n.d.	0.899 ± 0.074	0.643 ± 0.007	n.d.	n.d.
Malvidin-3-O-glucoside	8.73 ± 0.228	5.82 ± 0.036	3.18 ± 0.035	1.67 ± 0.015	7.05 ± 0.268	4.48 ± 0.033	0.992 ± 0.016	n.d.
Total glucosides	11.3 ± 0.241 ^a	7.58 ± 0.040 ^b	4.28 ± 0.008 ^c	1.67 ± 0.015 ^d	8.94 ± 0.446 ^a	5.96 ± 0.040 ^b	0.992 ± 0.016 ^c	n.d. ^d
Delphinidin-3-O-arabinoside	1.18 ± 0.021	0.777 ± 0.006	0.563 ± 0.002	n.d.	0.873 ± 0.077	0.652 ± 0.003	n.d.	n.d.
Cyanidin-3-O-arabinoside	2.86 ± 0.041	1.55 ± 0.003	1.06 ± 0.060	n.d.	1.82 ± 0.086	1.47 ± 0.004	n.d.	n.d.
Petunidin-3-O-arabinoside	1.61 ± 0.015	1.17 ± 0.017	0.750 ± 0.024	n.d.	1.25 ± 0.016	0.863 ± 0.007	n.d.	n.d.
Malvidin-3-O-arabinoside	9.10 ± 0.025	4.89 ± 0.157	2.00 ± 0.042	1.30 ± 0.013	7.96 ± 0.225	5.26 ± 0.032	0.927 ± 0.019	n.d.
Total arabinosides	16.7 ± 3.33 ^a	8.39 ± 0.141 ^b	4.37 ± 0.083 ^c	1.30 ± 0.013 ^d	13.3 ± 2.07 ^a	8.24 ± 0.039 ^b	0.927 ± 0.019 ^c	n.d. ^d
Cyanidin	0.967 ± 0.050	n.d.	n.d.	n.d.	0.930 ± 0.041	n.d.	n.d.	n.d.
Petunidin	1.02 ± 0.010	n.d.	n.d.	n.d.	1.38 ± 0.036	n.d.	n.d.	n.d.
Peonidine	0.566 ± 0.006	n.d.	n.d.	n.d.	0.619 ± 0.008	n.d.	n.d.	n.d.
Malvidin	1.15 ± 0.029	1.05 ± 0.005	0.763 ± 0.013	n.d.	1.24 ± 0.051	0.711 ± 0.006	n.d.	n.d.
Total aglycones	3.70 ± 0.081 ^a	1.05 ± 0.005 ^b	0.763 ± 0.013 ^c	n.d. ^d	4.17 ± 0.017 ^a	0.711 ± 0.006 ^b	n.d. ^c	n.d. ^d
Total anthocyanins	51.2 ± 0.266 ^a	30.3 ± 0.184 ^b	18.2 ± 0.111 ^c	5.83 ± 0.072 ^d	43.6 ± 1.62 ^a	25.3 ± 0.163 ^b	3.59 ± 0.043 ^c	n.d. ^d

For each type of wine, values in the same row with different superscript letters are significantly different. ≤0.05.

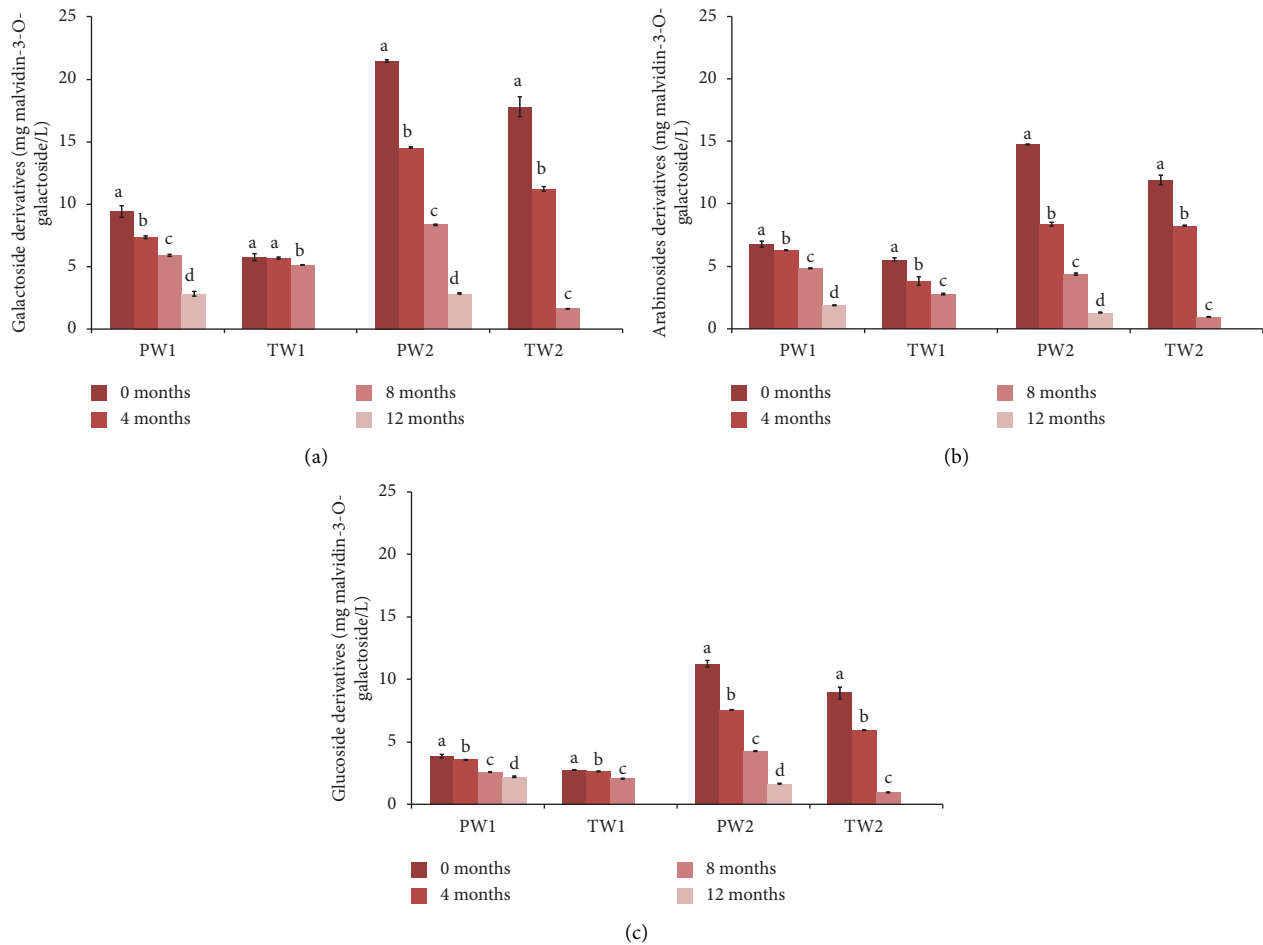


FIGURE 3: Galactoside derivatives (malvidin-3-O-galactoside (mg)/L) (a); arabinoside derivatives (malvidin-3-O-galactoside (mg)/L) (b); and glucoside derivatives (malvidin-3-O-galactoside (mg)/L) (c) of blueberry wines at 0, 4, 8, and 12 months of bottle storage time. Values in the same type of wines with different letters are significantly different. $p < 0.05$. ($n = 3$).

compounds could not be detected. The arabinoside derivatives reduced their concentration from 72 and 92% to 0 and 12 months of storage in the partially fermented wines and disappeared completely in the total fermented wines. Finally, the concentration of glucoside derivatives decreased by 42 to 85% from 0 to 12 months in the partially fermented wines, while they disappeared completely in the wines that completed their fermentation.

The decrease in anthocyanin monomer concentrations during bottle stabilization of grape wines has been studied by numerous authors [27, 37]. Many of these authors have found that the decrease in anthocyanin monomers is due to the transformation into more stable oligomers or polymers [38]. These transformations occur because of oxidation, condensation, and polymerization reactions [30], with reductions of 72% and 85% being measured in wines made from Mencía and Brancellao grapes, respectively, after 12 months of stabilization in bottles [39].

Even with the anthocyanin concentration constantly decreasing over time, red grape wines maintain their red color [36]. This fact is demonstrated in Figure 1(a). In this study, it was possible to verify that the wines made from both total and partial fermentation showed red color at

12 months, although monomeric anthocyanins could not be detected or were quantified in small quantities, as in the case of partially fermented wines. This decrease of anthocyanins and persistence of the color of red wines may be due to reactions of complex mechanisms, such as self-association and copigmentation, or the formation reactions of polymeric pigments resulting from anthocyanins with flavan-3-ol and proanthocyanidins, as well as the formation of new pigments such as pyronoanthocyanins and their more polymerized derivatives, causing the color to change from bluish-red to orange-red [40]. Some authors have found that the red color of red wines is maintained over time due to the formation of vitisins A and B, which are stable pigments [31]. Other authors found pyranthocyanins in bog bilberry syrup wines, although in very low concentrations [7].

3.5. Antioxidant Activity. The DPPH test is widely used as an efficient and simple method of evaluating the antioxidant activity of red wines [41, 42]. Red grape wines contain large amounts of phenols that are involved in many chemical reactions during wine maturation and aging, modifying the characteristics of the wines and the antioxidant activity [30].

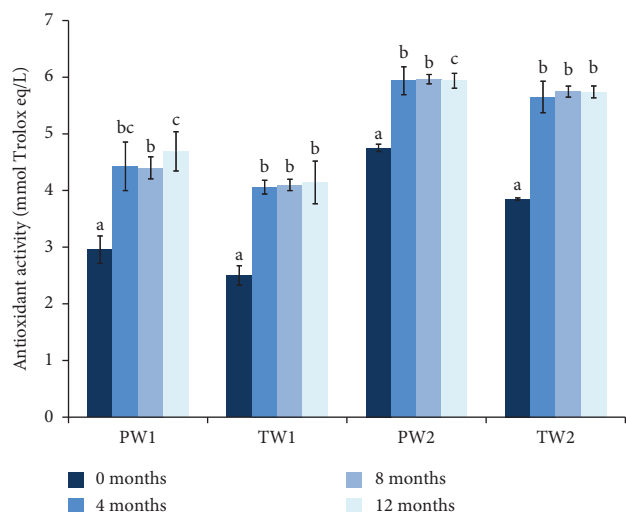


FIGURE 4: Antioxidant activity (mmol TE/L) of blueberry wines at 0, 4, 8, and 12 months of bottle storage time. Values in the same type of wine with different letters are significantly different. $p \leq 0.05$. ($n = 3$).

As it can be seen in Figure 4, the antioxidant activity of the wines obtained was influenced by storage time. In general, antioxidant activity increased with storage time, with PW2 and TW2 showing the highest values. This is logical due to the higher concentration of phenolic compounds in these wines.

The greatest increase occurred in the first 4 months, and it can be noted that this increase was in the case of TW2 wine up to more than double the initial value of antioxidant activity. As could be observed, after four months of bottle storage, the antioxidant activity of the wines remained practically constant in many cases, with no significant differences being found between months 4, 8, and 12 in many of them. It can therefore be affirmed that the storage conditions were adequate for the blueberry wines produced to remain stable, without losing their antioxidant potential. Authors such as Larrauri et al. [43] report that aged wines show higher antioxidant activity than young wines. These authors stated that, although the concentration of anthocyanins decreases in aged wines, there is an increase of the phenol content and the increase of the tannins and that all this could be related to a higher antioxidant efficiency of the aged wines. All the above would agree with the wines studied.

4. Conclusions

In conclusion, the storage conditions of the blueberry wines influence the anthocyanin content, color, and antioxidant activity. However, it could be affirmed that the conditions used in this study are suitable to elaborate a blueberry wine to take advantage of the beneficial health properties outside the harvesting season. Although initially the wines showed a decrease in color intensity, these parameters remained practically stable after 4 months of storage. The antioxidant activity showed an increase from 0 to 4 months of storage, maintaining these values up to 12 months. As for

anthocyanin content, only a few anthocyanin monomers could be identified and quantified in the partially fermented wines, and no compounds were found in the fully fermented wines. However, the wines retained their red color. Therefore, further investigations are necessary to identify and quantify the compounds responsible for this color and study their evolution during the storage period.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest that could have appeared to influence the work reported in this paper.

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