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Evolution of Phenolic Composition of Red Wine during Vinification and Storage and Its Contribution to Wine Sensory Properties and Antioxidant Activity

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ABSTRACT: The objective of this work was to study the evolution of the phenolic composition of red wine during vinification and storage and its relationship with some sensory properties (astringency and bitterness) and antioxidant activities. Thus, red wine was made by a classic vinification method with Castelão and Tinta Miúda grapes (*Vitis vinifera* L.) harvested at maturity (3:2; w/w). Samples were taken at 2 and 7 days of maceration, at second racking, at the time of bottling and at 6 and 14 months after bottling. The total polyphenols extract (TPx) in each sample was isolated by column chromatography. The phenolic composition (anthocyanins and proanthocyanidins), in vitro antioxidant activity, and sensory property (astringency, bitterness) of the isolated TPx from different winemaking stages were evaluated through high-performance liquid chromatography−diode array detection, 1,1-diphenyl-2-picrylhidrazyl radical test, ferric reducing antioxidant power assay, total phenolic index, MWI (polyphenol molecular weight index), TSA (tannin specific activity), and sensory panel tasting. The results showed that the phenolic composition of red wine varied significantly during winemaking. The intensity of astringency (IA) and the intensity bitterness (IB) of the isolated TPx from different winemaking stages increased from 2 days of maceration until second racking and then decreased. Furthermore, MWI and TSA are positively correlated with IA and IB. The in vitro antioxidant activity of the isolated TPx from different winemaking stages maintained unchanged after alcoholic fermentation, which was independent of the variation of phenolic composition and sensory properties.

KEYWORDS: Red wine, phenolic composition, vinification, sensory property, antioxidant activity

INTRODUCTION

Phenolic compounds play a very important role in enology because of their contribution to wine sensory properties[1−6] and antioxidant activities.[7−13] Moderate consumption of red wine can reduce mortality from coronary diseases, and the key compounds responsible for this beneficial effect are widely accepted to be phenolic compounds present in red wine.[8,14,12] Polyphenols, particularly proanthocyanidins (or condensed tannins), are also responsible for the astringency of plant-derived foods (including wines) because of their complexation with salivary proteins. Astringency is one of the most important characteristics of red wine. Chemically, this sensation is mostly related to the ability of wine proanthocyanidins to precipitate human salivary proteins.[1,15] Bitterness, which can be produced by a wide range of chemical compounds, such as heavy metal salts, ethanol, amino acids, peptides, and alkaloids, appears to be primarily elicited by phenolic compounds, especially by procyanidins.[16] For this reason, numerous works have been conducted over the years toward the determination of phenolic levels and evaluation of sensory properties and antioxidant activities of red wines and establishment of their relationship. Phenolic levels in wine can be affected by several factors including the grapevine varieties used and their richness in polyphenols,[17−19] the winemaking technology,[5,17,18,20,21] and the conditions of wine aging and storage.[22,23] Variation in winemaking technologies can modify significantly the polyphenols concentration in red wine. By using the same grapevine varieties, red wine made by skin fermentation with stem (stem-contact wines) contained a much higher concentration of proanthocyanidins than nonstem-contact wines,[5,17,21] because of the important transference of these compounds from grape stems to wines during fermentation.[20] Carbonic maceration wines are less colored than classic wines,[5,21] but they may contain higher levels of proanthocyanidins than stem-contact wines[18,21] and nonstem-contact wines[17,18,21] or lower levels of these compounds than classic wines,[5] depending on the state of grape maturation and the conduct of carbonic maceration. During the vinification, the phenolic composition of red wine changes significantly, and a considerable amount of phenolics are degraded or oxidized.[24] After malolatic fermentation, racking, and bottling, the wine becomes more stable, but the phenolic composition is continuously modified during wine storage and aging because of several interactions involving these phenolic compounds.

Many authors have attempted to establish the relationship between phenolic compounds and sensory properties of food products;[6,25−30] the majority of these studies have been conducted in model solutions by using individual phenolic compounds. In the case of red wine, anthocyanins and their derivatives

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are responsible for color perception, while proanthocyanidins play an important role in astringency and, to a lesser extent, bitterness.[6,29] Earlier works[25,31] have shown that cider bitterness and astringency were associated with procyanidins: There appeared to be a maximum response for bitterness at the level of the tetrameric procyanidin, whereas the response for astringency appeared to continue to increase with a degree of polymerization up to 6−7 and then decrease due to the insolubility of higher polymers.[31] However, the latter assumption was not supported by more recently published works because highly polymerized proanthocyanidins were soluble in winelike medium and precipitated by salivary proteins.[29] Reported that in a winelike medium, the astringency perception of proanthocyanidins increased as the increase of their mDP (up to 70) and their percentage of galloylation (up to 22%); the presence of epigallocatechin units in the proanthocyanidin could decrease the coarse perception.

Concerning antioxidant activities of red wine phenolic compounds, it has been well documented that red wine phenolic compounds possess potent antioxidant activities.[7−13] Some authors reported that there was no correlation between the total concentrations of phenolic compounds and the antioxidant activity of wines,[32,33] but others observed a positive correlation between the phenolic content of wine and its antioxidant activity.[34−37] In fact, both antioxidant activity and sensory properties depend on not only the amount but also the type and structural features of polyphenols.[6,28,38]

Furthermore, although various works have been carried out on the quantification of phenolic compounds in grapes and in red wines,[17−19,39−41] most of these works have involved either quantification of total phenolic compounds[17,19,42] or simple phenolics (anthocyanins, catechins, oligomer procyanidins, flavonols, and phenolic acids) by high-performance liquid chromatography (HPLC).[18,21,39−41] On the other hand, although it is well-known that the phenolic composition of red wines varies significantly during vinification and storage, the effect of such variation on wine sensory properties has not been well elucidated. Furthermore, there are no available data concerning the variation of antioxidant activity during winemaking and storage. As a consequence, the objective of this work was to study the evolution of phenolic composition of red wine during vinification and storage and its contribution to wine sensory properties (astringency and bitterness) and antioxidant activity.

MATERIALS AND METHODS

Preparation of Red Wine. Red wine was made by classic vinification method[21] with *Vitis vinifera* varieties (Castelão:Tinta Miúda; 3:2; w/w) harvested at maturity. The grape clusters were crushed using a destemmer-crusher (Gandra, Vila Nova de Famalicão, Portugal) and collected in stainless steel tanks. The must was treated with sulfur dioxide (80 mg/L) prior to undergoing skin fermentation at 25 °C. The cap was punched down twice daily until it remained submerged. After 7 days of maceration when alcoholic fermentation was finished, the mash was pressed. Free-run and press wines were combined and stored in vessels at room temperature. After 3 months of storage, the wines were racked, treated with sulfur dioxide (30 mg/L), and stored at room temperature. The second racking was carried out at 6 months of conservation with the addition of sulfur dioxide (30 mg/L). After another 4 months of maturation, the wine was bottled and stored at room temperature. Winemaking was performed in triplicate with each sampling, and analysis was at least duplicated. Samples were taken at 2 and 7 days of maceration, immediately at the second racking, at the time of bottling, and at 6 and 14 months after bottling. Each wine sample was subjected to column chromatography to isolate total polyphenols (TPs), as described below.

Isolation of TPs from Wine Samples. About 80 mL of the wine samples was evaporated under vacuum at less than 30 °C to remove ethanol and then loaded onto an open column (200 mm × 25 mm i.d.) packed with LiChroprep RP-18 (25−40 μm particle size) already preconditioned with distilled water. The column was washed with 200 mL of distilled water, followed by 150 mL of methanol to recover TPs. The methanol fraction was added with an equal volume of distilled water, evaporated under vacuum at less than 30 °C to remove methanol, and then lyophilized to obtained TPs extract (TPx). The obtained polyphenol extracts from the wine at 2 days of maceration (TPx-MT2), at 7 days of maceration (TPx-MT7), immediately at the second racking (TPx-2nd racking), at the time of bottling (TPx-bottling), at 6 months after bottling (TPx-6M), and at 14 months after bottling (TPx-14M) were stored at 0−4 °C, away from light, and under nitrogen until further analysis. The purity of each of these extracts in polyphenols was verified to be ≥91%, using the methods previously described.[12]

HPLC Analysis of Individual Catechins and Procyanidins. Quantification of individual catechins and procyanidins (dimers and trimers) was performed by prefractionation of TPx samples on Sep-Pak C18 cartridges, followed by HPLC analysis. Briefly, 1 mL of TPx (8 mg/mL in water) was loaded onto the two water-preconditioned Sep-Pak cartridges connected in series: The superior one is tC18 Sep-Pak, and the inferior is C18 Sep-Pak. Elution was carried out with 25 mL of ethyl acetate to elute oligomeric polyphenols including essentially catechins, oligomeric proanthocyanidins, phenolic acids, and stilbenes. The polymeric polyphenols, anthocyanins, and their derivatives were fixed on the top of column, which could be washed with methanol acidified by 0.1% HCl. The ethyl acetate fraction (i.e., oligomeric polyphenols fraction) was evaporated to dryness and recovered by a mixture solution of 0.1 mL of methanol and 0.9 mL of 2% formic acid in water, prior to HPLC−diode array detection (DAD) analysis. The HPLC-DAD apparatus used in this work was a Waters system, equipped with a quaternary pump (Waters 600), a controller (Waters 600), a thermostat controlling the column temperature, an autosampler (Waters 717 plus), and a photodiode array detector (Waters 996) coupled to a data processing computer—Millennium 32. The detection ranged from 200 to 600 nm and was 280 nm for detection of individual catechins and procyanidins. The column was a cartridge of LiChrospher 100 RP18 (5 μm; 250 mm × 4 mm; Merck). The column temperature was 30 °C. The flow rate was fixed at 1 mL/min. Two elution solvents A (water/formic acid; 98:2; v/v) and B (acetonitrile/water/formic acid; 70:28:2; v/v/v) were used with the following elution program: gradient elution from 0 to 6% B in 40 min, from 6 to 13% B in 6 min, from 13 to 20% B in 24 min, from 20 to 30% B in 10 min, from 30 to 50% B in 5 min, isocratic elution with 50% B in 10 min, from 50 to 100% B in 5 min, and isocratic elution with 100% B in 15 min, followed by conditioning column to initial conditions. Calibration curves were established with corresponding catechins (catechin and epicatechin) and procyanidins (procyanidin dimers B1, B2, B3, B4, B1-3-O-gallate, B2-3-O-gallate, and B2-3′-O-gallate, trimers C1 and T2) standards. The latter was isolated from grape seed extract as described previously.[43]

Quantification of Total Individual Phenolics (TIPs) and Polymerized Polyphenols. Quantification of TIPs (i.e., all HPLC-detectable individual phenolic compounds) and TPs was carried out by direct injection of TPx samples (1.5 mg/mL in water) to HPLC without sample preparation. The HPLC equipment and elution conditions were identical to those described above. The TIP was determined by measuring all individual peak areas and using (+)-catechin as a reference standard. Quantification of TP was performed by integration of total baseline peak areas throughout the elution period, and the amount was expressed using

(+)-catechin as a reference standard. Furthermore, the % of polymerized polyphenols could be calculated by [(TP − TIP)/TP] × 100%.

HPLC Analysis of Anthocyanins and Their Derivatives. Anthocyanins and their derivatives were determined by direct injection of TPx samples (1.5 mg/mL in water) to HPLC without sample preparation. The HPLC equipment was identical to that described above. The column (250 mm × 4 mm) was a cartridge of 4 μm Superspher 100 RP18 (Merck). The mobile phase flow rate was fixed at 0.7 mL/min. Detection ranged from 200 to 650 nm, with 525 nm for detection of anthocyanins and their derivatives. The column temperature was set at 30 °C. The flow was fixed at 0.7 mL/min. Elution conditions were as follows: solvents A (formic acid/water; 5:95; v/v) and B (acetonitrile/water/formic acid; 30:65:5; v/v/v) were used; gradient elution from 25 to 85% B in 70 min, isocratic elution with 85% B in 15 min, gradient elution from 85 to 98% B in 11 min, followed by washing and re-equilibration of the column to the initial conditions. Each anthocyanin and its derivatives were quantified by using malvidin-3-glucoside as a reference standard.

Scavenging Activity on 1,1-Diphenyl-2-picrylhidrazyl Radical (DPPH•). The scavenging effects of the isolated TPx samples from different winemaking stages on DPPH• were carried out as previously described,[12] with slight modification. Briefly, a 0.08 mL aliquot of the isolated wine polyphenol fractions in methanol (different concentrations) and 3.12 mL of DPPH• solution in methanol (60 μM) were added directly to 10 mm cell with stopper. The mixture was immediately shaken vigorously for 10 s by a vortex mixer. Absorbance at 515 nm (A_{515}) was recorded continuously against methanol as a blank reference, using a Cary 100 Bio UV−vis spectrophotometer (Varian, Australia) for 60 min (until the reaction reached steady state). The initial concentration of DPPH• was calculated for every experiment from a calibration curve made by measuring the absorbance at 515 nm of standard samples of DPPH• at different concentrations. The percentage of the DPPH• remaining at the steady state, which was calculated as % $DPPH^{\bullet}_{rem} = 100[DPPH^{\bullet}]_T/[DPPH^{\bullet}]_{T=0}$, was plotted against the amount of sample divided by the initial concentration of DPPH•. Each point was acquired in triplicate. A dose−response curve was obtained for every polyphenol extract sample. For the different samples, the antiradical activity unit was defined as the amount, able to consume 50% of the initial amount of free radical (EC_{50}). The results were expressed as antiradical power ($ARP = 1/EC_{50}$).

Ferric Reducing Antioxidant Power (FRAP). The FRAP assay developed by Benzie and Strain[44] was performed with some modifications. The reaction was performed in a microplate reader of 96 well plates (Biotek Powerwave XS with software KC4). The reaction was carried out on the plate wells with a temperature of 37 °C. In short, FRAP reagent [10 parts of 300 mM acetate buffer, pH 3.6 + 1 part of 10 mM TPTZ (2,4,6-tris(2-pyridyl)-*s*-triazine) in 40 mM HCl + 1 part of 20 mM $FeCl_3$] was diluted to one-third with acetate buffer. A 270 μL amount of this solution was added in each well together with 30 μL of the polyphenol extract solution (0.8 mg/mL). The blank assay was performed using 270 μL of FRAP reagent and 30 μL of methanol. The absorbance at 593 nm was measured in time 0 and 4 min. The results were expressed as Trolox equivalents.

Total Phenolic Index (TPI). The TPI was determined by measuring the absorbance of the polyphenol extract solutions at 280 nm according to the method described in the literature.[45]

Polyphenol Molecular Weight Index (MWI). The assay for MWI was performed according to the procedure described in the literature.[46] A 5.0 mL amount of polyphenol extract solution (0.8 mg/mL) was introduced into dialysis tubing (cellulose; 6 mm i.d.; nominal molecular weight cutoffs of 12000−16000; average porous radius of 25 Å) and placed into a vial with 50.0 mL of 12% aqueous ethanol solution (5 g/L tartaric acid, pH 3.2). In the second vial, 5.0 mL of extract solution was diluted directly with the same hydroalcoholic solution up to 50.0 mL (reference solution − do). Both vials were closed and stored at room temperature for 24 h. The difference between the two absorbance values at 280 nm corresponded to the higher phenolic compounds, which were retained inside the dialysis tubing [index = (do − *d*)/do, where *d* is the absorbance of the assay and do is the blank assay]. The membrane permits the diffusion of lower molecular weight compounds in the hydroalcoholic solution through the walls but retain higher molecular weight compounds.

Tannin Specific Activity (TSA). The TSA of the polyphenol extract solutions (0.8 mg/mL) toward bovine serum albumin (BSA) was assessed using nephelometry, according to the procedure previously developed.[46] Briefly, each polyphenol extract solution was diluted to 1:50 with a 12% aqueous ethanol solution at two different pH values (pH 4.0 and 5.0). Furthermore, an excess of protein solution was added to 4 mL of the solution in a test tube for each assay; the mixture was shaken and stored at room temperature. The haze formation of the solution increased with time, and after 40 min, the formation of the complexes stopped, and the haze stabilized. The TSAs were expressed in nephelos turbidity units (NTU) per L of solution. All experiments were performed in quadruplicate.

Sensory Evaluation. To study effect of phenolic compositional changes on some sensory attributes (i.e., astringency and bitterness) of red wine during maturation and storage, the isolated polyphenols extracts from different winemaking stages were dissolved in distilled water and evaluated by a sensory panel composed of 10 judges who participated, at least once a week, in the wine sensory sessions. Because alcohol, tartaric acid, and metal ions—components composing model wine solution—can strengthen or alter significantly astringency and bitterness intensity, we used distilled water instead of a model wine solution for the preparation of polyphenol solutions to avoid any possible interferences. The judges were requested not to smoke or eat for 1 h before each sensory session.

Five separate sensory sessions were carried out, respectively: The first session, corresponding to training, was performed using commercial grape seed procyanidins with different concentrations (150, 300, 600, 900, and 1200 mg/L in water); the second to the fourth sessions were performed using polyphenol extracts TPx-MT7, TPx-bottling, and TPx-14M, respectively, each with different concentrations (150, 300, 600, 900, and 1200 mg/L in water); the fifth session was performed using the isolated polyphenols from different winemaking stages in equivalent concentrations (900 mg/L in water). The judges were asked to score the aqueous polyphenol solutions according to their astringency and bitterness and flavor persistence intensity. Flavor persistence is defined as the duration of good sensation after tasting.

Statistical Analysis. Winemaking was performed in triplicate with sampling, and analyses at least were replicated. Each sensory session was performed in triplicate. One-way analysis of variance and comparison of means [least significant difference (LSD), 99% level] were carried out using Statistica v98 edition (StatSoft Inc., Tulsa, OK).

RESULTS AND DISCUSSION

Polyphenolic Characterization. It is well-known that during maturation and the storage period, the total amount of polyphenols (on weight basis) in red wine is not obviously changed, but its phenolic composition varies significantly due to various enzymatic and chemical reactions involved.[22,47−49] Furthermore, although polyphenols are well accepted to be important compounds responsible for some sensory properties (astringency and bitterness) and antioxidant activities of red wine, many other wine constituents may also contribute to such sensory properties[15] and antioxidant activities.[50] To verify the effect of phenolic compositional changes during wine maturation on its sensory properties and antioxidant activities, in this work, TPs in

Table 1. Composition of Anthocyanins and Their Derivatives in the Lyophilized Polyphenol Extracts (mg/g Dry Matter) from Red Wine at Different Winemaking Stages[a]

anthocyanins	TPx-MT2	TPx-MT7	TPx-2nd racking	TPx-bottling	TPx-6M	TPx-14M
Dp 3-glc	9.87 e ± 0.25	5.26 d ± 0.05	3.00 c ± 0.05	1.94 b ± 0.12	1.58 b ± 0.01	0.61 a ± 0.01
Cy 3-glc	4.05 d ± 0.13	0.92 c ± 0.13	0.49 b ± 0.00	0.26 ab ± 0.03	0.23 ab ± 0.00	0.07 a ± 0.01
Pt 3-glc	9.61 f ± 0.27	6.38 e ± 0.07	3.98 d ± 0.01	2.08 c ± 0.08	1.61 b ± 0.00	0.65 a ± 0.03
Pn 3-glc	34.86 e ± 0.48	11.01 d ± 0.26	6.51 c ± 0.09	2.85 b ± 0.10	2.08 b ± 0.01	0.87 a ± 0.04
Mv 3-glc	76.94 f ± 1.54	53.43 e ± 1.31	32.24 d ± 0.14	14.69 c ± 0.51	11.35 b ± 0.14	4.79 a ± 0.10
Mv 3-glc py	ND	0.64 e ± 0.00	0.50 d ± 0.00	0.29 b ± 0.00	0.37 c ± 0.03	0.36 c ± 0.01
Pn 3-acglc	0.92 d ± 0.01	0.56 c ± 0.02	0.21 b ± 0.06	0.04 a ± 0.02	0.03 a ± 0.04	ND
Mv 3-acglc	2.92 e ± 0.11	1.90 d ± 0.05	1.03 c ± 0.02	0.39 b ± 0.02	0.34 b ± 0.02	ND
Pn 3-cmglc	2.19 d ± 0.03	1.45 cd ± 0.06	1.02 bc ± 0.57	0.10 a ± 0.02	0.21 ab ± 0.00	0.06 a ± 0.01
Mv 3-cmglc	5.32 c ± 0.04	3.54 b ± 0.15	2.56 b ± 1.12	0.40 a ± 0.00	0.68 a ± 0.02	0.26 a ± 0.03

[a] Abbreviations: Dp 3-glc, delphinidin-3-glucoside; Cy 3-glc, cyanidin-3-glucoside; Pt 3-glc, petunidin-3-glucoside; Pn 3-glc, peonidin-3-glucoside; Mv 3-glc, malvidin-3-glucoside; Mv 3-glc py, malvidin-3-glucoside pyruvic derivative; Pn 3-acglc, peonidin 3-acetylglucoside; Mv 3-acglc, malvidin 3-acetylglucoside; Pn 3-cmglc, peonidin 3-coumaroylglucoside; Mv 3-cmglc, malvidin 3-coumaroylglucoside, and ND, not detected. TPx-MT2, TPx-MT7, Px-2nd racking, TPx-bottling, TPx-6M, and TPx-14M are abbreviations of TPxs from red wine at 2 and 7 days of maceration, at the time immediately after 2nd racking, at the time of bottling, and 6 and 14 months of storage in bottle, respectively. Mean values followed by the same letter in a row are not significantly different (LSD, 99.9% level of significance).

Table 2. Composition of Catechins and Procyanidin Dimers and Trimers in the Lyophilized Polyphenol Extracts (mg/g Dry Matter) from Red Wine at Different Winemaking Stages[a]

flavan-3-ol	TPx-MT2	TPx-MT7	TPx-2nd racking	TPx-bottling	TPx-6M	TPx-14M
B3	1.38 b ± 0.02	1.22 b ± 0.06	0.65 a ± 0.02	1.03 ab ± 0.00	1.18 b ± 0.30	1.06 ab ± 0.06
B1	9.22 b ± 0.00	10.85 d ± 0.28	5.67 a ± 0.10	10.80 d ± 0.12	10.19 c ± 0.03	10.38 cd ± 0.05
Cat	9.72 b ± 0.02	13.74 d ± 0.11	9.86 b ± 0.01	11.73 c ± 0.24	7.00 a ± 0.66	5.90 a ± 0.15
T2	3.03 c ± 0.06	3.59 c ± 0.06	1.48 a ± 0.32	3.14 c ± 0.10	2.12 b ± 0.07	1.88 bc ± 0.13
B4	4.69 e ± 0.08	4.06 d ± 0.13	3.01 c ± 0.00	3.66 d ± 0.25	2.09 b ± 0.04	1.62 a ± 0.02
B2	4.92 a ± 0.00	8.32 c ± 0.97	6.86 bc ± 0.05	7.13 bc ± 0.10	6.24 ab ± 0.36	7.37 bc ± 0.19
B2-3-O-G	1.72 bc ± 0.04	2.23 d ± 0.02	1.47 b ± 0.13	1.97 cd ± 0.03	0.59 a ± 0.02	0.74 a ± 0.09
Epi	6.98 d ± 0.09	8.84 e ± 0.00	5.78 b ± 0.26	6.36 c ± 0.04	3.23 a ± 0.01	2.96 a ± 0.22
B2-3′-O-G	2.11 d ± 0.03	1.32 c ± 0.15	0.79 b ± 0.25	1.03 bc ± 0.01	ND	ND
B1-3-O-G	2.41 b ± 0.02	3.18 c ± 0.12	2.37 b ± 0.34	2.71 bc ± 0.03	ND	ND
C1	2.08 c ± 0.01	4.97 e ± 0.00	2.77 cd ± 0.46	3.08 d ± 0.02	ND	ND

[a] Abbreviations: B3, procyanidin dimers B3; B1, procyanidin dimers B1; Cat, (+)-catechin; T2, procyanidin trimers T2; B4, procyanidin dimers B4; B2, procyanidin dimers B2; B2-3-O-G, procyanidin dimers B2-3-*O*-gallate; Epi, (−)-epicatechin; B2-3′-O-G, procyanidin dimers B2-3′-*O*-gallate; B1-3-O-G, procyanidin dimers B1-3-*O*-gallate; C1, procyanidin trimers C1; and ND, not detected. TPx-MT2, TPx-MT7, TPx-2nd racking, TPx-bottling, TPx-6M, and TPx-14M are abbreviations of TPxs from red wine at 2 and 7 days of maceration, at the time immediately after 2nd racking, at the time of bottling, and 6 and 14 months of storage in bottle, respectively. For each row, the means value followed by the same letter are not significantly different (LSD, 99.9% level of significance).

wine at each of winemaking stages were immediately isolated when samples were taken. The lyophilized phenolic powders thus obtained present high purity in polyphenols (≥91%; w/w) verified according to the methods described previously[12] and have very good stability during a long time, verified by HPLC analysis, and thus allowed to perform chemical and sensory analyses directly on these samples. Table 1 presents the anthocyanins composition of the isolated polyphenols extracts from red wine at different winemaking stages.

It can be seen that nearly all anthocyanins and their derivatives present the highest amount in TPx-MT2 (i.e., at the beginning of alcoholic fermentation), with the exception of malvidin 3-glucoside-pyruvic derivative, which is only formed during alcoholic fermentation and present in the largest amount at the end of alcoholic fermentation (0.64 mg/g polyphenols). During alcoholic fermentation and maturation period, the relative amount of all analyzed anthocyanins in TPx decreased significantly. In TPx-14M, all major anthocyanins were presented in very low concentrations (<1 mg/g dry polyphenols extract); acetylated derivatives disappeared, and coumaroylated derivatives were presented in only trace amounts. The compositions of catechins and dimer and trimer procyanidins in the polyphenolic extracts from the red wines of different winemaking stages are given in Table 2.

It can be seen, from Table 2, that the concentrations of catechins and nearly all analyzed procyanidins in TPx-MT7 are higher than those in TPx-MT2. The reason for this is because of the important transfer of these phenolic compounds from solid parts of grape into wine during alcoholic fermentation.[24] However, the concentrations of all of these compounds decreased significantly in TPx-2nd racking as compared with those in TPx-MT7. The reason for this was probably due to the combination or adsorption of these phenolic compounds with solids, proteins, or even yeasts and lactic acid bacteria, polymerization, or oxidation with other phenolic or nonphenolic compounds during

Table 3. Relative Percentage of Different Class of Phenolic Compounds of the Lyophilized Polyphenol Extracts from Red Wine at Different Winemaking Stages[a]

	phenolic composition (% w/w)			
polyphenol extract	anthocyanins	catechins and procyanidin di- and trimers	TIPs	polymerized polyphenols
	**	**	*	**
TPx-MT2	14.00 d ± 1.03	4.61 a ± 0.28	54.96 c ± 1.10	47.58 a ± 1.82
TPx-MT7	8.43 c ± 0.46	6.17 b ± 0.26	33.22 b ± 1.53	67.11 bc ± 0.41
TPx-2nd racking	5.62 b ± 0.17	4.45 a ± 0.32	26.76 a ± 0.06	70.78 c ± 1.72
TPx-bottling	2.54 a ± 0.15	5.81 b ± 0.10	25.73 a ± 0.23	71.62 c ± 0.47
TPx-6M	2.42 a ± 0.01	4.27 a ± 0.08	25.78 a ± 0.04	66.30 bc ± 0.64
TPx-14M	1.02 a ± 0.06	4.39 a ± 0.18	30.46 ab ± 2.67	59.32 b ± 4.86

[a] TPx-MT2, TPx-MT7, TPx-2nd racking, TPx-bottling, TPx-6M, and TPx-14M are abbreviations of TPxs from red wine at 2 and 7 days of maceration, at the time immediately after 2nd racking, at the time of bottling, and 6 and 14 months of storage in bottle, respectively. Symbols * and ** signify that the data given in a column are not significantly different at 95 and 99.9% levels (LSD).

this period, which alter or precipitate an important part of these compounds.[20] On the other hand, it can be noted that the concentrations of catechins and the major analyzed di- and trimer procyanidins in TPx-bottling were significantly higher than those in TPx-2nd racking, which may be explained by the interflavanic bond cleavage of oligomer and polymer proanthocyanidins in mild acidic conditions (as encountered in wine),[51] yielding lower molecular mass species (or higher molecular mass species). However, it is important to note that after bottling, the concentrations of these compounds continued to decline, with the exception of procyanidins B1, B2, and B3. Several procyanidins, including, procyanidins B2-3′-O-G, B1-O-3-G, and C1 were not detected in TPx-6M and TPx-14M.

The relative percentages of TIPs and polymerized polyphenols in TPx samples are presented in Table 3. For comparison purposes, the percentages of total anthocyanins and total procyanidin dimers and trimers are also given in this table. Among the polyphenol extracts, that from 2 days of maceration (TPx-MT2) contains the highest percentage of total anthocyanins (14%) and the highest percentage of TIPs (55%), and that from 7 days of maceration (TPx-MT7) contains the highest percentage of total di- and trimer procyanidins (6%). These results are in agreement with our previous work,[24] which reported that the highest diffusion rate of anthocyanins from grape skins to wine was observed before the beginning of alcoholic fermentation, whereas the highest diffusion rate of procyanidins oligomers from the solid parts of grapes to wine had been reached at the end of alcoholic fermentation. Statistic analysis indicates that total anthocyanins in TPx samples are highly correlated to TIPs (correlation coefficient $r = 0.8817$).

The polyphenol extracts from second racking (TPx-2nd racking) and at the time of bottling (TPx-bottling) present a higher percentage of polymerized polyphenols (70−72%) than TPx-MT2 and TPx-MT7 (48−67%) and TPx-6M and TPx-14M (59−66%). These results may suggest that the highest rate of polyphenol polymerization or condensation reactions occurred during the period after the end of fermentation up to at the time of bottling and thus contributed more polymerized polyphenols to wine during this period.

Antioxidant Activity. The in vitro antioxidant activities of the TPx samples evaluated by DPPH• test and FRAP assay are presented in Table 4. By using both DPPH• test and FRAP assay, the in vitro antioxidant activity of TPx-MT2 is significantly lower than those of the other TPx samples. The reason for this may be explained by its highest percentage of anthocyanins as shown in Table 3, not like catechins and procyanidins, which has a catechol type structure acting as a reducing agent, the major anthocyanins in red wine, that is, malvidin 3-*O*-glucoside and peonidin 3-*O*-glucoside lack such reducing structural features.[13]

Table 4. In Vitro Antioxidant Activity of the Lyophilized Polyphenol Extracts from Red Wine at Different Winemaking Stages[a]

	antioxidant activity	
polyphenol extract	DPPH· ($1/EC_{50}$)	FRAP (μM Trolox equivalent)
	**	*
TPx-MT2	4.83 a ± 0.02	10.76 a ± 0.77
TPx-MT7	6.42 bc ± 0.05	14.83 bc ± 0.67
TPx-2nd racking	6.29 bc ± 0.05	13.98 b ± 0.84
TPx-bottling	6.75 c ± 0.20	16.37 c ± 0.57
TPx-6M	6.78 c ± 0.31	12.83 b ± 1.27
TPx-14M	5.97 b ± 0.15	16.11 c ± 0.82

[a] TPx-MT2, TPx-MT7, TPx-2nd racking, TPx-bottling, TPx-6M, and TPx-14M are abbreviations of TPxs from red wine at 2 and 7 days of maceration, at the time immediately after 2nd racking, at the time of bottling, and 6 and 14 months of storage in bottle, respectively. Symbols * and ** signify that the data given in a column are not significantly different at 95 and 99.9% levels (LSD).

Furthermore, it should be especially noted that by using DPPH• test and FRAP assay, the in vitro antioxidant activities of TPx-MT7, TPx-2nd racking, TPx-bottling, TPx-6M, and TPx-14M are not significantly different, although their phenolic compositions are obviously different as described above. This would indicate that once fermentation/maceration was finished, changes in composition of phenolic compounds did not alter significantly the antioxidant activity of the wine. In other words, during wine maturation and storage, the major reactions among polyphenols or reactions between polyphenols and other nonphenolics would not modify the in vitro antioxidant activity of the constitutive phenol moieties. These results are in good agreement with those obtained from the interaction between malvidin 3-glucoside and epicatechin in model wine solution.[13]

Astringency and Bitterness. It is generally accepted that polyphenols are major compounds responsible for wine astringency and bitterness. These phenolic compounds are considered

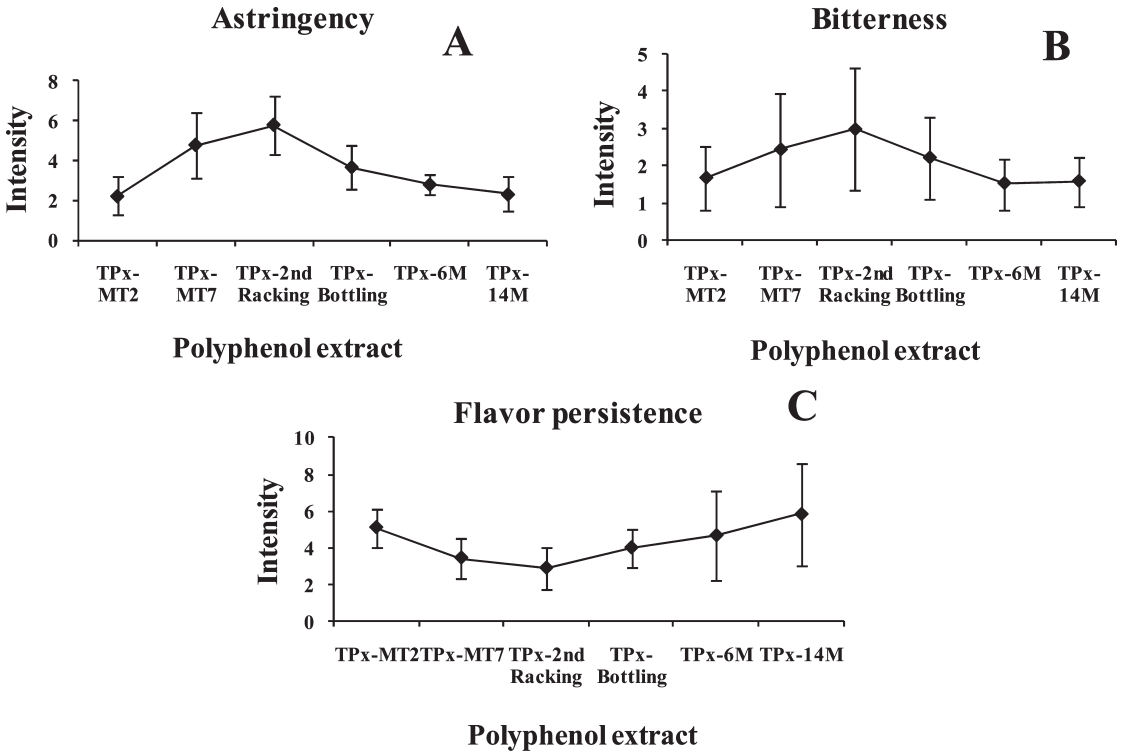

Figure 1. Intensities of astringency and bitterness of the polyphenol extracts from red wine at different winemaking stages with equal concentrations (900 mg/L in water). TPx-MT2, TPx-MT7, TPx-2nd racking, TPx-bottling, TPx-6M, and TPx-14M are abbreviations of TPxs from red wine at 2 and 7 days of maceration, at the time immediately after 2nd racking, at the time of bottling, and 6 and 14 months of storage in bottle, respectively. Vertical bars represent the standard deviation (n = 3).

to react with salivary proteins, resulting in dryness sensation in the palate—astringency.[52−54] To evaluate these sensory properties, both panel sensory analysis and physical-chemical methods were used.

For sensory analysis, a preliminary training session was first carried out with grape seed procyanidins and isolated polyphenol extracts from red wine. The results have shown that both for grape seed procyanidins and for each polyphenol extract isolated from different winemaking stages, their intensity of astringency and bitterness (in water solution) is positively correlated to their concentration, all of which have a correlation coefficient >0.95 with a concentration ranging from 150 to 1200 mg/L in water. The official tasting session was realized by using the same concentration of different polyphenol extracts isolated from different winemaking stages (900 mg/L in water). The results are presented in Figure 1.

It can be seen from Figure 1A that the intensity of astringency of the isolated polyphenolic extracts increases from 2 days of maceration, reaches a maximum at the time of second racking, and then decreases. A similar trend was found for bitterness (Figure 1B), although TPx-6M presented similar intensity of bitterness to that of TPx-14M. These results indicate that the intensity of astringency and bitterness depends on the structural modification of the polyphenols. As mentioned above, polyphenol molecules are modified continuously throughout the winemaking process. The decrease in the intensities of astringency of the polyphenolic extracts from second racking until the end of experiment period (14 months after bottling) should be mainly due to the reaction of proanthocyanidins with anthocyanins. Because the latter reaction is the major phenolic reaction during wine storage, it was expected that the intensity of astringency of the wine polyphenols will be continuously decreased during wine aging in bottle.

It is interesting to note that in spite of large error ranges of each of the sensory attributes, calculation of the correlation by mean values indicated that the intensity of flavor persistence was negatively correlated to the intensity of astringency (correlation coefficient r = −0.9947) and to the intensity of bitterness (correlation coefficient r = −0.9825). From these results, it may be thus expected that the flavor persistence of the wine polyphenols will be improved as aging time increases.

The results obtained by using physical-chemical methods, that is, TPI, MWI, and TSA analyses, are presented in Table 5. Although TPI of the isolated polyphenolic extracts was not changed obviously, their MWI and TSA varied significantly. Interestingly, the profiles of variation in MWI and TSA were very similar to those of variation in astringency and bitterness evaluated by sensory analysis. For the polyphenolic extracts from TPx-MT2 to second racking, MWI values are highly correlated with astringency intensity (correlation coefficient r = 0.9898), highly correlated with bitterness intensity (correlation coefficient r = 1.0000), and highly correlated with flavor persistence (correlation coefficient r = −0.9844); TSA values are correlated with astringency intensity (correlation coefficient r = 0.8544), highly correlated with bitterness intensity (correlation coefficient r = 0.9230), and correlated with flavor persistence (correlation coefficient r = −0.8364). For TPx-14M, the values of MWI and TSA are highly correlated to bitterness intensity, with correlation coefficients r = 0.8524 and 0.8123, respectively. In other words, the results obtained by the physical-chemical methods are

Table 5. TPI, MWI, and TSA of the Lyophilized Polyphenol Extracts (mg/g Dry Matter) from Red Wine at Different Winemaking Stages[a]

polyphenol extract	TPI	MWI	TSA (NTU/mL)
TPx-MT2	25.3 a ± 0.1	0.093 a ± 0.003	5.9 a ± 0.7
TPx-MT7	22.6 b ± 0.4	0.285 d ± 0.007	8.3 b ± 0.3
TPx-2nd racking	24.9 a ± 0.6	0.418 c ± 0.009	16.2 c ± 0.3
TPx-bottling	24.1 a ± 0.1	0.422 c ± 0.006	13.7 d ± 0.4
TPx-6M	20.4 c ± 0.5	0.320 d ± 0.020	9.1 d ± 0.1
TPx-14M	21.8 b ± 0.1	0.380 d ± 0.010	12.1 d ± 0.3

[a] TPx-MT2, TPx-MT7, TPx-2nd racking, TPx-bottling, TPx-6M, and TPx-14M are abbreviations of TPxs from red wine at 2 and 7 days of maceration, at the time immediately after 2nd racking, at the time of bottling, and 6 and 14 months of storage in bottle, respectively. For each column, the mean values followed by the same letter are not significantly different (LSD, 99.9% level of significance).

obviously in good agreement with those by the panel sensory analysis.

It should be mentioned that although the evolution profile of red wine astringency and bitterness during wine storage has been well-known and this phenomena has been considered, for a long time, to be polyphenolic structural transformations with time due to oxidation reactions and interactions, it is for the first time, according to our knowledge, that such sensory properties, together with their antioxidant activities, were verified directly by using TPxs isolated from different winemaking stages.

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