Food Research International 75 (2015) 374–384

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Pre-drying and submerged cap winemaking: Effects on polyphenolic compounds and sensory descriptors. Part I: BRS Rúbea and BRS Cora

Maurício Bonatto Machado de Castilhos [a,*], Odinéli Louzada dos Santos Corrêa [b], Mauro Celso Zanus [b], João Dimas Garcia Maia [c], Sérgio Gómez-Alonso [d,e], Esteban García-Romero [f], Vanildo Luiz Del Bianchi [a], Isidro Hermosín-Gutiérrez [d]

[a] Vinification and Bioprocess Laboratory, São Paulo State University, São José do Rio Preto, São Paulo, Brazil
[b] Brazilian Agro-farming Research Agency EMBRAPA Grape and Wine, Bento Gonçalves, Rio Grande do Sul, Brazil
[c] Brazilian Agro-farming Research Agency EMBRAPA Grape and Wine, Jales, São Paulo, Brazil
[d] Instituto Regional de Investigación Científica Aplicada (IRICA), Universidad de Castilla-La Mancha, Avda. Camilo José Cela s/n, 13071 Ciudad Real, Spain
[e] Parque Científico y Tecnológico de Albacete, Paseo de la Innovación, 1, 02006 Albacete, Spain
[f] Instituto de la Vid y el Vino de Castilla-La Mancha, Carretera de Albacete s/n, 13700 Tomelloso, Spain

ARTICLE INFO

Article history:
Received 1 April 2015
Received in revised form 20 May 2015
Accepted 27 May 2015
Available online 30 May 2015

Chemical compounds studied in this article:
The list of up to 10 names of chemical compounds studied in this article is described below:
Delphinidin 3,5-diglucoside (PubChem CID: 10100906)
Cyanidin 3,5-diglucoside (PubChem CID: 12305316)
Petunidin 3,5-diglucoside (PubChem CID: 71587075)
Peonidin 3,5-diglucoside (PubChem CID: 44256843)
Malvidin 3,5-diglucoside (PubChem CID: 12312725)
Malvidin 3-(6″-*p*-coumaroyl)-glucoside-5-glucoside (PubChem CID: 44256995)
Myricetin 3-glucoside (PubChem CID: 22841567)
Laricitrin 3-glucoside (PubChem CID: 44259475)
Catechin (PubChem CID: 9064)
Proanthocyanidin B1 (PubChem CID: 11250133)

Keywords:
Red wine
Submerged cap
Drying
Polyphenols
Sensory analysis
Antioxidant capacity

ABSTRACT

In contrast to the most worldwide used grape varieties, wine production in Brazil is mainly devoted to the elaboration of table wines from American grapes and hybrids. These grapes show initial disadvantages such as low soluble solids content in their optimal stage of ripening and poor color quality. Based on this, the Brazilian Agency EMBRAPA Grape and Wine has developed BRS type cultivars in order to enhance the quality of the table wines. This study analyzed the phenolic composition and sensory profile of BRS Rúbea and BRS Cora red wines elaborated by traditional and two alternative winemaking technologies: grape pre-drying and submerged cap of chaptalized musts. Pre-dried wines presented low concentrations of anthocyanins/pyranoanthocyanins and flavonols, suggesting that they were partially degraded by the thermal treatment (60 °C). These wines were described as bitter and full-bodied because of their higher flavan-3-ols content, suggesting that these compounds were not greatly influenced by thermal degradation. Submerged cap was described as persistent to the palate and with an intense violet hue due to its high anthocyanin and flavonol concentrations. The antioxidant capacity presented a weak relationship with the anthocyanins and stilbenes, but was intensely related to the % of galloylated flavan-3-ols.

* Corresponding author at: Engineering and Food Technology Department, São Paulo State University, Cristóvão Colombo Street, 2265 São José do Rio Preto, São Paulo, Brazil.
E-mail address: mbonattosp@yahoo.com.br (M.B.M. de Castilhos).

http://dx.doi.org/10.1016/j.foodres.2015.05.056
0963-9969/

1. Introduction

Brazilian viticulture is increasing in the worldwide panorama and in 2013 more than 1.4 million tons of grapes were produced, with approximately 50% dedicated to the production of juices and wines. In addition to the South of Brazil, already a well-known wine producing region, other locations are excelling in their wine production such as the state of São Paulo, which is responsible for approximately 12.5% of the national grape production (Rebello et al., 2013). In contrast to the worldwide production of wines, Brazil stands out in the production of wines elaborated from *Vitis labrusca* grapes and their hybrids, known as table wines (Biasoto, Netto, Marques & Da Silva, 2014).

In this context, the Brazilian Agro-farming Research Agency EMBRAPA Grape and Wine has been developing new cultivars aimed at producing red wines with unique features, highlighting the 'BRS' type cultivars. Of these one can highlight 'BRS Rúbea', which was a result of the cross between 'Niagara Rosada' and 'Bordô' grapes, producing red wines with an intense color and foxy flavor (Camargo & Dias, 1999), and 'BRS Cora', which originated in 1992 from the cross between 'Muscat Belly A' and 'H.65.9.14', reaching 18 to 20 °Brix under its normal growth conditions and producing wines with an intense color (Camargo & Maia, 2004).

Phenolic compounds are important to red wines because they have a relevant impact on the color and sensory mouth feel and also present antioxidant activity. They have thus been the focus of studies that evaluate their concentration through the variation in winemaking processes such as the influence of grape drying (De Torres, Díaz-Maroto, Hermosín-Gutiérrez & Pérez-Coello, 2010; Figueiredo-González, Cancho-Grande & Simal-Gándara, 2013; Marquez, Serratosa, Lopez-Toledano & Merida, 2012; Marquez, Serratosa & Merida, 2013; Rivero-Pérez, Pérez-Magariño & González-San José, 2002), and of submerged cap maceration on the concentration of the phenolic compounds (Bosso et al., 2011). Browning in wines can be a result of enzymatic reactions due to the action of the polyphenol oxidase (PPO) on the polyphenols, as a result of damage and structural changes caused to the grape skin, facilitating contact between the substrates and the PPOs (Marquez et al., 2012). Non-enzymatic reactions can also be responsible for browning in wines due to the formation of melanoidins, which are the final product of the Maillard reactions. The presence of amino acids and monosaccharides in grapes, especially due to the high concentration resulting from drying of the grapes, facilitates the Maillard reaction during the drying process. In addition, the formation of melanoidins is especially favored at temperatures above 50 °C (Rivero-Pérez et al., 2002).

The above-mentioned research studies mainly reported on the influence of these variations on wines elaborated by *Vitis vinifera* grapes, and are focused on the chemical and phenolic identification/quantitation and presented no sensory data. Few studies present data related to the aging of Brazilian red table wines at different temperatures (Lago-Vanzela et al., 2014), and some studies presented no results concerning the polyphenolic compounds (De Castilhos, Cattelan, Conti-Silva & Del Bianchi, 2013; De Castilhos, Conti-Silva & Del Bianchi, 2012). Thus, research involving correlations between polyphenolic compounds, sensory data and antioxidant activity is practically non-existent.

Based on this, the aim of the present research was to evaluate the detailed composition of the most relevant phenolic compounds in 'BRS Rúbea' and 'BRS Cora' red table wines prepared by traditional winemaking methods (T) and two alternative winemaking processes: pre-drying (PD) and submerged cap (SC). In addition to the expected differences in chemical composition among the treatments, the antioxidant activity and descriptive sensory analysis data were collected, generating a chemometric approach that allowed for a relationship among the phenolic/chemical and sensory profiles of the red wines.

2. Material and methods

2.1. Chemicals

All solvents were of HPLC quality, all chemicals were of analytical grade (>99%) and the water was of Milli-Q quality. The following commercial standards from Phytolab (Vestenbergsgreuth, Germany) were used for the identification of the phenolic compounds: malvidin 3-glucoside, malvidin 3,5-diglucoside, peonidin 3,5-diglucoside, *trans*-piceid, *trans*-caftaric acid, (−)-epigallocatechin and (−)-gallocatechin, as also the following commercial standards from Extrasynthese (Genay, France): cyanidin 3-glucoside, cyanidin 3,5-diglucoside, procyanidins B1 and B2, kaempferol, quercetin, isorhamnetin, myricetin, syringetin and the 3-glucosides of kaempferol, quercetin, isorhamnetin and syringetin. In addition, the following commercial standards from Sigma Aldrich (Tres Cantos, Madrid, Spain) were used: *trans*-resveratrol, caffeic acid, (+)-catechin, (−)-epicatechin, (−)-epicatechin 3-gallate and (−)-gallocatechin 3-gallate. Other non-commercial flavonol standards such as myricetin 3-glucoside, quercetin 3-glucuronide and laricitrin 3-glucoside were previously isolated from Petit Verdot grape skins (Castillo-Muñoz et al., 2009). Procyanidin B4 was kindly supplied by Prof. Fernando Zamora (Department of Biochemistry and Biotechnology, Universitat Rovira i Virgili, Spain). The *trans* isomers of resveratrol and its 3-glucosides (piceid) were converted into their respective *cis* isomers by UV irradiation (366 nm light for 5 min in quartz vials) of 25% MeOH solutions of the *trans* isomers.

All the standards were used for identification and quantitation by calibration curves covering the expected concentration ranges. When a standard was not available, the quantitation was done using the calibration curve of the most similar compound: malvidin 3,5-diglucoside for 3,5-diglucoside anthocyanin type and malvidin 3-glucoside for the 3-glucoside type, quercetin 3-glucoside for flavonol 3-glycosides and their free aglycones, caffeic acid for hydroxycinnamic acid derivatives, (+)-catechin for polymeric flavan-3-ols (total proanthocyanidins), and individual flavan-3-ol monomers and dimers by their corresponding standards considering their total sum as (+)-catechin equivalents.

2.2. Winemaking

Six red wines were produced: Traditional Rúbea wine (RUBT), Pre-dried Rúbea wine (RUBPD), Submerged Cap Rúbea wine (RUBSC), Traditional Cora wine (CORAT), Pre-dried Cora wine (CORAPD) and Submerged Cap Cora wine (CORASC). The grapes were harvested in the city of Jales (20° 16′ 7″ South and 50° 32′ 58″ West), São Paulo state, Brazil, at their usual complete maturity levels and in good sanitary conditions. The Rúbea and Cora grapes presented, at the start of the winemaking procedure, soluble solids contents of 18.1 ± 1.6 °Brix and 17.2 ± 1.2 °Brix, and pH values of 3.18 ± 0.13 and 3.34 ± 0.08, respectively.

All the treatments followed the standard winemaking procedure described by De Castilhos et al. (2013), which started with de-stemming and manual crushing of the grapes allowing the release of the juice. The must and pomace were then inserted into 10 L fermentation vessels, sulfur dioxide was added to the must by adding 150 ppm of potassium metabisulfite and alcoholic fermentation was induced by the inoculation of active dry *Saccharomyces cerevisiae* Y904 (Amazon Group®) in the proportion of 200 ppm.

The submerged cap treatment provided the effect of the constant maceration of the grape's solid parts by using stainless steel screens to maintain the cap at the bottom of the fermentative vessel, avoiding its rise due to the production of carbon dioxide. The screen was arranged in a way that its friction with the walls of the fermentation vessel was sufficient to inhibit the movement of the solid part to the upper side of the vessel, which is caused by the formation of carbon dioxide resulting from the alcoholic fermentation. Traditional and submerged

cap were chaptalized by the addition of 37.8 $g \cdot L^{-1}$ and 45.0 $g \cdot L^{-1}$ of sugar for Rúbea and Cora wines, respectively.

The pre-drying treatment consisted of drying the grapes to 22 °Brix to avoid chaptalization and obtain wines with an alcoholic strength between 8.6 and 14 °GL, as required by Brazilian legislation (Brasil, 2005). This winemaking process was carried out using a convective drying method with a tray dryer at 60 °C and airflow of 1.1 $m \cdot s^{-1}$ (De Castilhos et al., 2013). At the end of the drying procedure, the Rúbea and Cora wines presented 22.3 and 22.0 °Brix, respectively, with 14.7% and 17.4% of the water evaporated in relation to the initial weight. All the winemaking trials, including both the standard and alternative processes, were carried out in duplicate.

The results obtained for the oenological parameters measured according to the official analysis for wine of the Association of Official Analytical Chemists (2005) and Brasil (2005), for the Rúbea and Cora wines were expressed in Table 1. Rúbea wines presented significant differences for the alcohol content and reducing sugars, traditional and submerged cap samples showed higher values for these parameters, and also presented differences for dry extract and total phenolic content, highlighting the pre-dried samples. The different winemaking procedures influenced the pH, dry extract and reducing sugars and the pre-dried Cora wine presented the higher values for these oenological parameters; traditional Cora sample presented the higher value for alcohol content, as this property also presented significant differences ($P < 0.05$).

2.3. Analysis of the phenolic compounds

2.3.1. Preparation of the wine for the determination of the non-anthocyanin phenolic compounds

The flavonol fractions were isolated from diluted wine samples following the procedure described by Castillo-Muñoz, Gómez-Alonso, García-Romero, and Hermosín-Gutiérrez (2007), using Bond Elute Plexa PCX solid phase extraction cartridges (Agilent; 6 cm^3, 500 mg of adsorbent). The flavan-3-ols (monomers, B-type dimers and polymeric proanthocyanidins) and stilbenes were isolated following the procedure described by Rebello et al. (2013), using SPE C18 cartridges (Waters® Sep-Pak Plus, filled with 820 mg of adsorbent).

2.3.2. HPLC-DAD-ESI-MS^n analysis of the phenolic compounds

The HPLC separation, identification and quantitation of the phenolic compounds were carried out on an Agilent 1100 Series HPLC system (Agilent, Germany) equipped with DAD (G1315B) and a LC/MSD Trap VL (G2445C VL) electrospray ionization mass spectrometry (ESI-MS^n) system, coupled to an Agilent ChemStation (version B.01.03) data-processing unit. The mass spectra data were processed using the Agilent LC/MS Trap software (version 5.3).

The anthocyanin and non-anthocyanin compounds were analyzed according to a previously described method (Lago-Vanzela, Da-Silva, Gomes, García-Romero & Hermosín-Gutiérrez, 2011). The wine samples were injected (10 μL for anthocyanin analysis and 20 μL for non-anthocyanin flavonol analysis) onto a Zorbax Eclipse XDB-C18 reversed-phase column (2.1 × 150 mm; 3.5 μm particle; Agilent, Germany) with the temperature controlled at 40 °C.

For identification, the ESI/MS-MS was used in both the positive (anthocyanins) and negative (flavonols and hydroxycinnamic acid derivatives) ionization modes set for the following parameters: dry N_2 gas with a flow of 8 $L.min^{-1}$ at a drying temperature of 325 °C; and N_2 nebulizer at 50 psi. The ionization and fragmentation parameters were optimized by direct injection of the appropriate standard solutions (malvidin 3,5-diglucoside solution in the positive ionization mode; quercetin 3-glucoside and caftaric acid in the negative ionization mode) using a scan range of 50–1200 *m/z*. Identification was based on the spectroscopic data (UV–vis and MS/MS) obtained from the aforementioned authentic standards or using previously reported data (Barcia, Pertuzatti, Gómez-Alonso, Godoy & Hermosín-Gutiérrez, 2014; Lago-Vanzela et al., 2013, 2014; Nixdorf & Hermosín-Gutiérrez, 2010; Rebello et al., 2013). For quantitation, the DAD chromatograms were extracted at 520 nm for anthocyanins, 360 nm for flavonols and 320 nm for the hydroxycinnamic acid derivatives (HCAD). The analyses were carried out in duplicate.

2.3.3. Identification and quantitation of the flavan-3-ols and stilbenes using Multiple Reaction Monitoring (MRM) HPLC-ESI-MS/MS

The analysis was carried out using a HPLC Agilent 1200 series system equipped with DAD (Agilent, Germany) and coupled to an AB Sciex 3200 TRAP (Applied Biosystems) with triple quadrupole, turbo spray ionization (electrospray assisted by a thermonebulization) mass spectroscopy system (ESI-MS/MS). The chromatographic system was managed an Agilent ChemStation (version B.01.03) data-processing unit, and the mass spectra data was processed using the Analyst MSD software (Applied Biosystems, version 1.5).

Structural information concerning the proanthocyanidins was obtained using the pyrogallol-induced acid-catalyzed depolymerization method (Bordiga, Coïsson, Locatelli, Arlorio & Travaglia, 2013). The reaction consisted of adding 0.50 mL of the pyrogallol solution (100 $g \cdot L^{-1}$ pyrogallol plus 20 $g \cdot L^{-1}$ of ascorbic acid in 0.3 N HCl) to 0.25 mL of the sample in MeOH and incubating 40 min at 30 °C. The hydrolysis reaction was stopped by adding 2.25 mL of sodium acetate (67 mM). An aliquot of 2 mL of the reacted sample was placed in a vial and injected directly into the equipment for analysis.

The samples, before and after the acid-catalyzed depolymerization reaction, were injected (20 μL) onto an Ascentis C18 reversed-phase column (150 mm × 4.6 mm with 2.7 μm of particle size), with the temperature controlled at 16 °C. The solvents and gradients used for this analysis and the two MS scan types used (Enhanced MS — EMS and Multiple Reaction Monitoring — MRM) as well as all the mass transitions (*m/z*) for identification and quantitation were according to the methodology reported by Lago-Vanzela et al. (2011).

2.4. Determination of the antioxidant capacity by the DPPH assay

The procedure consisted of adding 100 μL of wine diluted in methanol to 2.9 mL of a methanolic DPPH (2,2-diphenyl-1-picrylhydracyl,

Table 1
Results (mean ± standard deviation) of the conventional enological parameters. Abbreviations: RUBT, Traditional Rúbea wine; RUBPD, Pre-dried Rúbea wine; RUBSC, Submerged cap Rúbea wine; CORAT, Traditional Cora wine; CORAPD, Pre-dried Cora wine; CORASC, Submerged cap Cora wine. Different letters in the same column indicate significant differences (ANOVA, Tukey's post-hoc test, $\alpha = 0.05$).

Wines	Enological parameter						
	Total acidity ($g \cdot L^{-1}$)	Volatile acidity ($g \cdot L^{-1}$)	pH	Alcohol content (%v/v)	Dry extract ($g \cdot L^{-1}$)	Reducing sugar ($g \cdot L^{-1}$)	Total phenolic content ($mg \cdot L^{-1}$)
RUBT	9.42 ± 1.48 a	0.66 ± 0.28 a	3.18 ± 0.13 a	12.15 ± 0.20 a	26.98 ± 1.83 b	2.95 ± 0.38 a	737.9 ± 87.4 b
RUBPD	9.79 ± 1.63 a	0.52 ± 0.08 a	3.22 ± 0.07 a	10.65 ± 0.08 b	32.95 ± 3.74 a	2.38 ± 0.34 b	975.1 ± 33.6 a
RUBSC	11.18 ± 0.45 a	0.45 ± 0.07 a	3.15 ± 0.03 a	12.03 ± 0.19 a	31.48 ± 0.84 a	2.54 ± 0.33 ab	706.1 ± 18.4 b
CORAT	10.34 ± 1.97 a	0.70 ± 0.20 a	3.34 ± 0.08 ab	11.15 ± 0.24 a	28.14 ± 2.03 b	1.62 ± 0.12 b	380.1 ± 46.5 a
CORAPD	10.60 ± 0.14 a	0.72 ± 0.06 a	3.42 ± 0.06 a	9.70 ± 0.08 c	31.09 ± 0.67 a	1.86 ± 0.22 a	369.1 ± 15.8 a
CORASC	11.60 ± 1.90 a	0.61 ± 0.09 a	3.27 ± 0.13 b	10.43 ± 0.63 b	28.82 ± 2.29 ab	1.64 ± 0.08 ab	366.1 ± 66.7 a

Table 2
Anthocyanin and pyranoanthocyanin profiles determined by HPLC/MS/MS (mean value ± standard deviation) for BRS Rúbea and BRS Cora young red wines. Abbreviations: Dp, delphinidin; Cy, cyanidin; Pt, petunidin; Pn, peonidin; Mv, malvidin; 3,5-diglc, 3,5-diglucosides; 3-acglc-5-glc, 3-(6″-acetyl)-glucoside-5-glucoside; 3-cmglc-5-glc, 3-(6″-*p*-coumaroyl)-glucoside-5-glucoside; 3-glc, 3-glucoside; 3-acglc, 3-(6″-acetyl)-glucoside; 3-cmglc, 3-(6″-*p*-coumaroyl)-glucoside; 10-HP, 10-*p*-hydroxyphenyl; 10-DHP, 10-*p*-dihydroxyphenyl; RUBT, Traditional Rúbea wine; RUBPD, Pre-drying Rúbea wine; RUBSC, Submerged cap Rúbea wine; CORAT, Traditional Cora wine; CORAPD, Pre-drying Cora wine; CORASC, Submerged cap Cora wine; ND, not detectable; NQ, not quantifiable. Different letters in the same row indicate significant differences (ANOVA, Tukey's post-hoc test, $\alpha = 0.05$).

Anthocyanidins and pyranoanthocyanins	Peak	R_t (min)	Molecular ion; product ions (m/z)	RUBT	RUBPD	RUBSC	CORAT	CORAPD	CORASC
Anthocyanins ($mg \cdot L^{-1}$)				550.89 ± 5.54 a	105.56 ± 1.80 c	269.00 ± 5.78 b	274.54 ± 1.66 a	56.74 ± 3.25 b	287.74 ± 4.35 a
Dp-3,5diglc	1	4.5	627;465,303	235.22 ± 4.93 a	36.46 ± 0.15 c	115.15 ± 2.18 b	144.86 ± 0.84 b	27.49 ± 2.90 c	153.35 ± 1.37 a
Cy-3,5diglc	2	6.5	611;449,287	51.46 ± 0.92 a	23.37 ± 1.32 c	35.86 ± 0.93 b	14.43 ± 0.27 a	7.41 ± 0.39 b	17.00 ± 1.34 a
Pt-3,5diglc	3	9.5	641;479,317	24.65 ± 0.42 a	5.57 ± 0.04 c	13.00 ± 1.00 b	3.85 ± 0.06 a	1.45 ± 0.05 b	4,58 ± 0.78 a
Pn-3,5diglc	4	12.1	625;463,301	7.86 ± 0.90 a	2.44 ± 0.02 b	3.17 ± 0.24 b	1.45 ± 0.49 a	0.87 ± 0.00 a	1.53 ± 0.11 a
Mv-3,5diglc	5	14.0	655;493,331	37.78 ± 1.39 a	6.97 ± 0.32 c	17.68 ± 0.30 b	11.06 ± 0.11 b	2.75 ± 0.01 c	11.52 ± 0.04 a
Dp-3acglc-5glc	6	14.1	669;507,303	NQ	NQ	NQ	NQ	NQ	NQ
Cy-3acglc-5glc	7	16.3	653;491,287	7.04 ± 0.30 a	2.79 ± 0.00 b	3.40 ± 0.04 b	ND	ND	ND
cis-Dp-3cmglc-5glc	8	19.2	773;611,465,303	8.23 ± 0.16 a	3.59 ± 0.01 c	6.23 ± 0.01 b	8.43 ± 0.03 a	1.82 ± 0.00 c	4.66 ± 0.02 b
trans-Dp-3cmglc-5glc	11	23.6	773;611,465,303	138.23 ± 0.41 a	11.09 ± 0.04 c	53.98 ± 0.76 b	76.93 ± 0.49 b	10.29 ± 0.00 c	79.81 ± 0.58 a
Cy-3cmglc-5glc	12	25.8	757;595,449,287	30.08 ± 0.48 a	8.09 ± 0.04 c	13.07 ± 0.16 b	7.81 ± 0.23 b	3.12 ± 0.00 c	8.49 ± 0.04 a
Pt-3cmglc-5glc	14	27.2	787;625,479,317	7.36 ± 1.51 a	2.89 ± 0.01 b	5.29 ± 0.13 ab	4.48 ± 0.15 a	1.51 ± 0.00 b	4.75 ± 0.01 a
Pn-3cmglc-5glc	17	29.6	771;609,463,301	1.38 ± 0.00 a	1.20 ± 0.00 b	1.04 ± 0.03 c	1.14 ± 0.01	ND	0.88 ± 0.01
Mv-3cmglc-5glc	18	30.5	801;639,493,331	1.36 ± 0.01 a	1.05 ± 0.02 b	1.08 ± 0.00 b	NQ	ND	0.95 ± 0.00
Dp-3acglc	9	20.3	507;303	0.21 ± 0.01	NQ	NQ	0.08 ± 0.00	NQ	0.18 ± 0.00
Dp-3cmglc	15	27.7	611;303	ND	ND	ND	NQ	ND	NQ
Pyranoanthocyanins ($mg \cdot L^{-1}$)				50.32 ± 0.12 a	43.11 ± 0.76 b	41.40 ± 1.44 b	26.82 ± 0.01 a	12.10 ± 0.02 c	25.53 ± 0.14 b
10carboxy-pyrcy-3cmglc	10	21.9	663;355	4.84 ± 0.62 a	2.54 ± 0.08 b	4.47 ± 0.01 a	4.01 ± 0.02 a	2.00 ± 0.00 c	3.12 ± 0.00 b
10DHP-pyrdp-3glc	13	26.7	597;435	NQ	NQ	ND	2.56 ± 0.06 a	2.53 ± 0.00 a	2.50 ± 0.01 a
10DHP-pyrcy-3glc	16	28.9	581;419	3.25 ± 0.00 a	2.12 ± 0.00 c	2.69 ± 0.02 b	2.07 ± 0.06	NQ	2.26 ± 0.00
10DHP-pyrdp-3cmglc	19	31.2	743;435	2.43 ± 0.00	NQ	ND	1.93 ± 0.00	ND	2.03 ± 0.00
10HP-pyrdp-3acglc	20	31.3	623;419	4.21 ± 0.01	ND	NQ	4.28 ± 0.01	ND	4.12 ± 0.00
10HP-pyrcy-3glc	21	32.8	565;403	NQ	NQ	NQ	ND	ND	ND
10HP-pyrdp-3cmglc	22	35.1	727;419	5.14 ± 0.01 a	4.17 ± 0.01 c	4.46 ± 0.07 b	4.22 ± 0.00	ND	4.15 ± 0.00
10HP-pyrcy-3acglc	23	35.5	607;403	NQ	NQ	NQ	ND	ND	ND
10HP-pyrcy-3cmglc	24	38.6	711;403	NQ	NQ	NQ	ND	ND	ND
10HP-pyrpn-3cmglc and 10HP-pyrmv-3cmglc (coelution)	25	41.9	725;417/755;447	30.43 ± 0.46 b	34.27 ± 0.69 a	29.77 ± 1.32 b	7.73 ± 0.15 a	7.55 ± 0.02 a	7.32 ± 0.14 a

Fluka Chemie) radical solution (6×10^{-5} mol L^{-1}) (Brand-Williams, Cuvelier & Berset, 1995). After 25 min, the decrease in the percent absorbance at 515 nm was measured. For this measurement, the range should be between 20 and 80% of the initial DPPH absorbance and thus the dilution of the wine with methanol was adjusted in order to enter this range; for red wines the usual dilution factors are between 1/10 and 1/20. Quantitation of the antioxidant capacity was achieved using calibration curves obtained with methanolic solutions of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Fluka, Chemie).

2.5. Sensory analysis

Descriptive analysis was used to profile the six red table wines (two grapes × three treatments). Eight panelists (EMBRAPA Grape and Wine, Brazil) with more than 15 years of wine tasting experience took part in a session using representative wine samples and reference standards. After a discussion, a list of eleven attributes was established, two attributes for appearance (color intensity, violet hue) and nine attributes for taste (sweetness, acidity, bitterness, flavor intensity/body, structure/tannins, herbaceous taste, astringency, pungency and persistence). The evaluation sessions took place in a sensory analysis room with individual booths under daylight at ambient temperature. Aliquots of 30 mL of the red wines at 18 °C were poured into transparent glass cups and for each wine, the panelists evaluated each descriptor on a horizontal unstructured 9 cm scale anchored by the minimum and maximum extremes. All the samples were coded with three random digits and were presented in a monadic and randomized form. The panelists evaluated the samples in triplicate (Girard, Yuksel, Cliff, Delaquis & Reynolds, 2001). The Ethical Issues regarding the sensory analysis were approved by the Ethics in Research Committee of the Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University (process n. 15159913.3.0000.5466).

2.6. Data analysis

All the data were treated using a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test (when P value < 0.05) and the relationship between the chemical properties and the sensory attributes was determined using the Principal Component Analysis (PCA). All the statistical tests were applied at a significance level of 0.05 using the Statistica 10 software (StatSoft Inc., Tulsa, OK).

3. Results and discussion

3.1. Anthocyanin and pyranoanthocyanin profiles

The data concerning the anthocyanins (Lago-Vanzela et al., 2011; Nixdorf & Hermosín-Gutiérrez, 2010) and pyranoanthocyanins were easily identified by MS/MS and UV–vis spectroscopic data (Blanco-Vega, López-Bellido, Alía-Robledo & Hermosín-Gutiérrez, 2011). The 3,5-diglucosides of the five expected wine anthocyanidins (delphinidin, cyanidin, petunidin, peonidin and malvidin) were identified and quantitated by UV–vis spectra according to the aforementioned studies, with the different forms of delphinidin as the principal anthocyanidin (Table 2, Fig. 1). This result was expected since American grapes (non-*Vitis vinifera*) and their hybrids contain large amounts of anthocyanidin 3,5-diglucosides as opposed to the 3-monoglucosides, and this profile is transferred to their red wines (Lago-Vanzela et al., 2011; Nixdorf & Hermosín-Gutiérrez, 2010). The absence of ion product signals at *m/z* 271 indicated that no pelargonidin-based anthocyanin could be found in those red wines, although pelargonidin 3-glucoside has been found

in some non-*Vitis vinifera* or hybrid grape cultivars (Wang, Race & Shrikhande, 2003).

The 3-(6″-coumaroyl)-glucoside-5-glucoside (3-cmglc-5-glc) derivatives of the five aforementioned anthocyanidins were also detected. They gave rise to three signals in their MS^2 spectra, corresponding to different combinations of the independent losses of the glucose and 6″-coumaroyl-glucose moieties — $[M\text{-}glc]^+$, $[M\text{-}cmglc]^+$ and $[M\text{-}glc\text{-}cmglc]^+$. It was assumed that the glucose moiety was linked to the C-5 position and the 6″-coumaroyl-glucose moiety to the C-3 position as previously reported (Mazzuca, Ferranti, Picariello, Chianese & Addeo, 2005). It was possible to detect the *cis* (19.2 min) and *trans*-delphinidin-3-(6″-*p*-coumaroyl)-glucoside-5-glucoside (23.6 min) forms in both red wines, the latter at high concentrations, and this result corroborates the findings of Nixdorf & Hermosín-Gutiérrez (2010), who reported the presence of a *cis* isomer of a 3-(6″-*p*-coumaroyl)-glucoside-5-glucoside for the first time, showing that both presented the same MS^2 spectra and a slight difference in the UV–vis zone at approximately 310–314 nm. The same study also showed that the *cis*-isomer eluted before the corresponding *trans*-isomer.

Regarding the occurrence of anthocyanidin 3-glucosides, none of the non-acylated derivatives were detected in the Rúbea and Cora wines, and the only acylated anthocyanidin 3-glucoside found, as minor compounds, were the 3-(6″-acetyl)-glucoside and 3-(6″-*p*-coumaroyl)-glucoside of delphinidin. This result can be explained due to the formation of the pyranoanthocyanins, which can only be formed from anthocyanidin 3-glucosides. The grape anthocyanins, when transferred to the wine during the maceration period, can react with yeast metabolites such as pyruvic acid and acetaldehyde, giving rise to other types of pigment known as A- and B-type vitisins, respectively. In addition, anthocyanins can react with hydroxycinnamic acids or their decarboxylation products mediated by yeast activity during the alcoholic fermentation, allowing for the formation of hydroxyphenyl-pyranoanthocyanins (Blanco-Vega et al., 2011).

These wine pigments are formed by the coupled reaction between the above-mentioned compounds and the anthocyanin via the C-4 position and its –OH substituent at the C-5 position, giving rise to a newly formed pyrano ring over the previous anthocyanin skeleton (pyranoanthocyanins). Since the major anthocyanins found in red wines made from non-*Vitis vinifera* grapes or their hybrids are 3,5-diglucosides, both reaction positions are sterically hindered or blocked and this fact could explain the discreet amounts of these compounds in non-*Vitis vinifera* red wines as previously reported (Lago-Vanzela et al., 2013; Nixdorf & Hermosín-Gutiérrez, 2010). However, up to 11 different pyranoanthocyanins derived from delphinidin, cyanidin, peonidin and malvidin anthocyanidins were detected by means of their MS, MS/MS and UV–vis characteristics, most of them being delphinidin and cyanidin-derivative pigments in their glucoside, 6″-acetyl glucoside or 6″-*p*-coumaroyl glucoside forms (Blanco-Vega et al., 2011).

Despite the quite similar anthocyanin profiles of the two red wines, the total number of anthocyanins (anthocyanidin glucosides) and pyranoanthocyanins varied according to the winemaking process, some not being detected in the pre-drying treatment. In both the red wines, the pre-drying winemaking process decreased the pigment contents, very likely due to the thermal degradation of these compounds. It has been suggested that non-enzymatic browning (Maillard reaction) would have occurred to a greater extent when using 60 °C as the drying temperature, since PPO is denatured by mild heating, for example, a blanching step at 50 °C (Patras, Brunton, O'Donnell & Tiwari, 2010). Anthocyanin degradation could occur by oxidation, cleavage of the covalent bonds or by deglycosylation of the anthocyanin 3-glucosides, resulting in the formation of different compounds such as phloroglucinaldehyde and 4-hydroxybenzoic acid from the cyanidin 3-glucoside (Patras et al., 2010). In addition, the product obtained after drying depends on factors such as berries' size, content of reducing sugars, degree of ripeness and skin thickness. The drying process could increase the color intensity by the extraction or production of brown-colored compounds mainly due to enzymatic or non-enzymatic reactions (Marquez et al., 2013). Since the drying process causes an irreversible damage in grape skin allowing the diffusion of colored compounds to the berry, the heat could cause a decrease on anthocyanin content and, on the other hand, could increase the brown-colored compounds, determining a gain/loss balance of the colored compounds (Marquez et al., 2012; Patras et al., 2010).

Submerged cap winemaking presented significant differences when compared to traditional winemaking with regard to some of the anthocyanin derivatives, mainly in the Rúbea wines, and in most cases they assumed intermediate concentrations, i.e., between those found for traditional winemaking (higher) and those found for the pre-drying treatment (lower). This result is in agreement with the findings reported for Barbera red wines, since the extraction of the phenolic compounds during fermentative maceration using submerged cap was lower than the traditional floating-cap maceration, suggesting that the submerged cap did not increase the phenolic concentrations in the wine due to the limited effect of the pumped must on the solid parts of the berries during the alcoholic fermentation (Bosso et al., 2011).

3.2. Profile of the flavonols and hydroxycinnamic acid derivatives (HCAD)

The 3-glucosides (3-glc) of the six expected aglycones (Q, quercetin; M, myricetin; L, laricitrin; S, syringetin; I, isorhamnetin and K, kaempferol) were detected and quantitated in both red wines (Table 3, Fig. 2). In addition, the 3-glucuronides (3-glcU) of M and Q; 3-galactosides (3-gal) of M, Q and K; and the free forms of M, Q and L could be detected. The 3-glucoside forms of M, L and Q and the free form of Q presented the highest concentrations in both red wines. The latter results differed from those reported for other red wines made from the hybrid cultivar Isabel (Nixdorf & Hermosín-Gutiérrez, 2010), BRS Violeta red wines (Lago-Vanzela et al., 2013) and Bordô grapes (Lago-Vanzela et al., 2011), that were defined by high concentrations

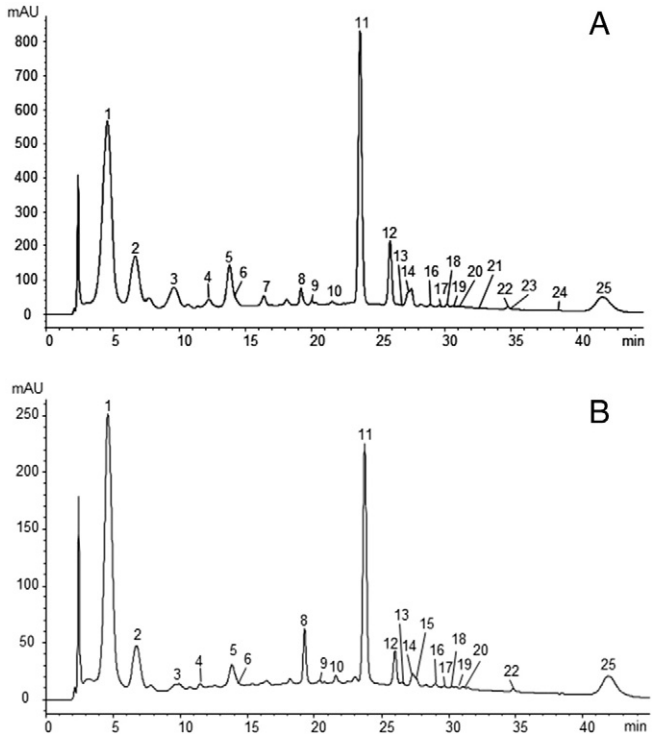

Fig. 1. HPLC DAD-chromatogram (detection at 520 nm) of BRS Rúbea (A) and BRS Cora (B) young red wines anthocyanins. For peak assignation see Table 2. Peak n. 15 was not detectable for Rúbea samples. Peaks n. 7, 21, 23 and 24 were not detectable for Cora samples.

of the myricetin and quercetin-forms. In the Rúbea and Cora red wines, the M-3glc was the most important type of flavonol, followed by L-3glc, which did not present relevant amounts in the latter aforementioned studies. It was possible to observe significant differences for Q-3-gal and L-3-glc (Rúbea wines) and for M-3-glc, Q-3-gal, L-3-glc and K-3-glc (Cora wines) when the winemaking treatments were compared. The pre-drying treatment presented low concentrations for all the aforementioned compounds, except for the Q-3-gal, which presented significant differences when compared to the submerged cap treated Rúbea red wine and for both treatments (traditional and submerged cap) with the Cora red wines.

With regard to the hydroxycinnamic acid derivatives (HCAD), larger amounts of caftaric, caffeic and *p*-coumaric acids were observed for both red wines (Table 3, Fig. 3). An important piece of information was extracted from these data: traditional and submerged cap wines, regardless of the cultivar, presented high concentrations of caftaric acid, but almost all the caftaric acid was degraded in the pre-dried wines, giving rise to large amounts of caffeic acid, which was probably not degraded by the use of heat. This result corroborates with the findings of Barcia et al. (2014), who mentioned the reduction of caftaric acid when the winemaking by-products of BRS Violeta and BRS Lorena grapes were analyzed by drying at 50 °C. Furthermore, they also described that the drying process apparently did not affect the caffeic acid and its derivatives. No significant differences were observed in the comparison of the winemaking procedures for Rúbea red wines, but for Cora red wines, pre-drying resulted in low concentrations of *p*-coumaroyl-glucose-1 and -2, *p*-coumaric acid and ethyl *p*-coumarate, indicating that this treatment possibly gave rise to chemical oxidations and thermal degradation (Patras et al., 2010). The submerged cap treatment presented the same behavior as seen for the anthocyanin pigments, i.e., differing from the traditional treatment in some cases, but usually resulting in intermediate values.

3.3. Profile of the flavan-3-ols and stilbenes

Catechin (C), epicatechin (EC), epicatechin gallate (ECG), proanthocyanidin B1 (PB1), proanthocyanidin B2 (PB2) and proanthocyanidin B4 (PB4) were detected in both the Rúbea and Cora red wines, the Rúbea red wines accounting for higher concentrations of these compounds (Table 4). An interesting behavior was detected in the flavan-3-ols profile when the winemaking procedures were compared, since the pre-dried red wines presented the highest concentrations of all the flavan-3-ols and proanthocyanidins, and when the difference was significant, the pre-dried wines accounted for higher concentrations than the traditional and submerged cap wines. It has been suggested that the grapes lost their physiological integrity during dehydration, thus favoring the diffusion of anthocyanins and flavan-3-ols from the grape skin to the pulp (Figueiredo-González et al., 2013); however this could also promote the well-known reaction between anthocyanidin 3-glucosides and flavan-3-ols, giving rise to polymeric pigments with a parallel decrease in the total flavan-3-ol content, but this reaction was probably handicapped in the case of hybrid grapes, since the main anthocyanins are 3,5-diglucosides.

Flavan-3-ols can take part in several reactions including non-enzymatic browning, and oxidation by enzymes such as polyphenol oxidases and peroxidases, which could possibly reduce their concentration in wines (Macheix, Sapis & Fleuriet, 1991). However, flavan-3-ols derivatives of high molecular weight could suffer depolymerization reactions and result in lower molecular weight phenols, accounting for the increase in the flavan-3-ols concentration in red wines (Dallas, Ricardo-da-Silva & Laureano, 1995). Furthermore, the condensation of catechins/proanthocyanidins and monomeric anthocyanins could lead to the formation of polymeric compounds (Budic-Leto, Lovrić, Kljusuric, Pezo & Vrhovsek, 2006) and, based on these, the evolution of the flavan-3-ols during the drying process of the grapes could result in a

Table 3
Flavonol and HCAD profile determined by HPLC/MS/MS (mean value ± standard deviation) for BRS Rúbea and BRS Cora young red wines. Abbreviations: M, myricetin; Q, quercetin; L, laricitrin; K, kaempferol; S, syringetin; I, isorhamnetin; glcU, glucuronide; gal, galactoside; glc, glucoside; RUBT, Traditional Rúbea wine; RUBPD, Pre-drying Rúbea wine; RUBSC, Submerged cap Rúbea wine; CORAT, Traditional Cora wine; CORAPD, Pre-drying Cora wine; CORASC, Submerged cap Cora wine. Different letters in the same row indicate significant differences (ANOVA, Tukey's post-hoc test, $\alpha = 0.05$).

Flavonols and HCAD	Peak	R_t (min)	Molecular ion; product ions (m/z)	RUBT	RUBPD	RUBSC	CORAT	CORAPD	CORASC
Flavonols ($mg \cdot L^{-1}$)				103.69 ± 33.40 a	62.91 ± 10.2 a	88.90 ± 6.99 a	55.62 ± 3.05 a	38.73 ± 6.79 a	50.73 ± 5.67 a
M-3-glcU	26	20.0	493;317	2.16 ± 0.50 a	1.62 ± 0.26 a	2.22 ± 0.56 a	1.01 ± 0.18 a	0.55 ± 0.20 a	0.85 ± 0.21 a
M-3-gal	27	20.4	479;317	NQ	NQ	NQ	NQ	NQ	NQ
M-3-glc	28	21.5	479;317	54.30 ± 19.50 a	31.44 ± 6.48 a	51.58 ± 3.04 a	17.36 ± 0.48 a	3.95 ± 0.99 b	17.08 ± 1.96 a
Q-3-gal	29	28.2	463;301	8.87 ± 0.86 ab	14.06 ± 1.97 a	8.04 ± 0.18 b	9.51 ± 0.60 b	22.28 ± 2.82 a	10.61 ± 1.83 b
Q-3-glcU	30	28.6	477;301	NQ	NQ	NQ	NQ	NQ	NQ
Q-3-glc	31	29.9	463;301	6.20 ± 3.48 a	6.55 ± 0.56 a	6.54 ± 1.00 a	3.53 ± 0.05 a	2.70 ± 0.47 a	3.25 ± 0.13 a
L-3-glc	32	33.0	493;331	19.69 ± 4.40 a	2.22 ± 1.54 b	10.96 ± 3.70 ab	11.38 ± 1.61 a	2.71 ± 1.18 b	7.53 ± 2.57 ab
Free M	33	33.2	317	NQ	NQ	NQ	NQ	NQ	NQ
K-3-gal	34	34.0	447;285	NQ	NQ	NQ	NQ	NQ	NQ
K-3-glc	35	37.0	447;285	0.76 ± 0.03 a	0.70 ± 0.21 a	1.01 ± 0.16 a	1.40 ± 0.04 a	0.66 ± 0.14 b	1.54 ± 0.06 a
I-3-glc	36	40.1	477;315	0.62 ± 0.11 a	0.76 ± 0.51 a	1.29 ± 0.18 a	0.74 ± 0.42 a	0.44 ± 0.12 a	0.58 ± 0.04 a
S-3-glc	37	41.6	507;345	1.26 ± 0.47 a	1.66 ± 0.44 a	0.44 ± 0.00 a	0.70 ± 0.22 a	0.44 ± 0.01 a	0.52 ± 0.12 a
Free Q	38	45.0	301	9.48 ± 4.00 a	3.66 ± 3.25 a	6.60 ± 0.92 a	9.97 ± 2.64 a	4.98 ± 1.28 a	8.72 ± 1.49 a
Free L	39	48.7	331	0.39 ± 0.18 a	0.20 ± 0.03 a	0.19 ± 0.01 a	NQ	NQ	NQ
Hydroxycinnamic acid derivatives (*HCAD*) ($mg \cdot L^{-1}$)				265.27 ± 60.9 ab	135.63 ± 4.33 b	306.26 ± 8.53 a	259.56 ± 113.8 a	90.91 ± 26.20 a	245.96 ± 112.0 a
Caftaric acid	40	4.1	311;179,149,135	126.80 ± 134.80 a	1.46 ± 0.51 a	220.92 ± 7.09 a	105.00 ± 145.00 a	16.70 ± 19.20 a	92.30 ± 129.20 a
trans-Coutaric acid	41	6.1	295;163,149,119	8.65 ± 9.07	NQ	16.52 ± 0.72	8.16 ± 10.32 a	1.60 ± 0.00 a	14.75 ± 0.00 a
cis-Coutaric acid	42	6.5	295;163,149,119	1.69 ± 1.97 a	3.00 ± 0.31 a	3.44 ± 0.11 a	3.42 ± 0.00 a	0.60 ± 0.14 a	1.12 ± 1.37 a
Caffeic acid	43	7.8	179;135	58.80 ± 69.90 a	62.70 ± 3.70 a	8.34 ± 0.55 a	87.50 ± 39.00 a	36.67 ± 5.49 a	85.10 ± 26.10 a
p-Coumaroyl-glucose-1	44	9.0	325;163,145	21.43 ± 2.38 a	30.89 ± 9.09 a	19.96 ± 1.25 a	9.24 ± 1.40 ab	4.62 ± 0.76 b	12.79 ± 2.30 a
p-Coumaroyl-glucose-2	45	11.6	325;163,145	7.85 ± 1.68 a	10.43 ± 1.87 a	8.20 ± 0.11 a	3.80 ± 0.14 b	1.76 ± 0.48 c	5.63 ± 0.08 a
p-Coumaric acid	46	14.4	163;119	38.20 ± 18.30 a	26.02 ± 2.71 a	27.39 ± 1.65 a	40.72 ± 3.19 a	29.24 ± 0.68 b	38.27 ± 0.14 a
Ethyl caffeate	47	46.1	207;179,135	0.36 ± 0.00 a	0.28 ± 0.21 a	0.07 ± 0.00 a	0.32 ± 0.01	NQ	0.29 ± 0.13
Ethyl *p*-coumarate	48	55.8	191;163,119	1.68 ± 0.50 a	0.85 ± 0.23 a	1.42 ± 0.18 a	2.62 ± 0.22 a	0.46 ± 0.04 b	3.11 ± 0.43 a

balance between reactions that increase their concentrations and others that result in their loss (Marquez et al., 2012). Proanthocyanidins B1, B2 and B4 accounted for the highest values in the pre-dried wines and presented significant differences when the winemaking procedures were compared in the Cora wines. This result corroborates the findings of Dallas et al. (1995) who reported goo stability and low reactivity for these compounds, suggesting they were not affected by thermal degradation during drying.

With respect to stilbenes, *cis*-resveratrol, *trans*-piceid and *cis*-piceid were detected, and the latter presented high concentrations in both red wines. The results showed that the use of heat promoted the total or almost complete degradation of these compounds and this result corroborates with the findings of Barcia et al. (2014), who detected and quantitated these compounds in grape skins and, after drying, no longer detected them.

It is well known that the stilbenes and other phenolic compounds have been the focus of several studies since they present antioxidant properties. However, it is also known the wine that has the highest concentration of phenolic compounds does not always show the greatest antioxidant activity, i.e., it has been suggested that the antioxidant properties are more related to the types of phenolic compound existent in the wines than to their global amounts (Rivero-Pérez, Muñiz & González-San José, 2007). Thus the antioxidant activity (AA) was measured, and the Rúbea red wines showed higher AA than the Cora wines, although there were no significant differences in antioxidant activity for the Rúbea wines when the winemaking treatments were compared. Conversely, for the Cora wines, the AA for traditional wines was higher than for the pre-dried wines. These results showed that the drying process influenced the decrease in AA for the Cora red wines, although this result was the opposite of that shown for the AA of the Rúbea wines, which presented high AA for the pre-dried wines. This difference is probably due to the balance in the reactions that produce more antioxidant compounds and, at the same time, produce losses in antioxidant grape polyphenols, i.e., while drying could cause the degradation of phenolic compounds, correlated with antioxidant efficiency in wines (Makris, Kallithraka & Kefalas, 2006), it could also be responsible for the formation of new compounds that present AA, such as the melanoidins resulting from the Maillard reaction (Delgado-Andrade & Morales, 2005).

It was expected that the phenolic contents of the pre-dried wines would be higher than those of the other treatments due to the effect of water evaporation. However, since these compounds are transferred from the skin to the pulp during drying, there is a need to assess the balance between the optimization of the extraction of these compounds promoted by the use of the heat and the occurrence of chemical oxidation. Probably, the results obtained showed that the above-mentioned balance was more effective on the oxidation side, since both the anthocyanins and the flavonols suffered decreases in their concentrations by the use of the heat.

3.4. Sensory assessment

As can be seen in Table 5, the comparison of the winemaking treatments provided relevant differences with respect to color intensity and violet hue for both Rúbea and Cora red wines, and the differences for sweetness and persistence were also significant for the Cora red wines. RUBPD wines showed greater color intensity than the RUBT and RUBSC wines, and for the other aforementioned descriptors, the traditional and submerged cap wines differed from the pre-dried wine for both Rúbea and Cora wines. Comparing the two wines, the Rúbea samples showed higher scores for color intensity, violet hue, body, tannins and persistence, whereas the Cora wines showed higher scores for acidity. The other sensory descriptors presented similar scores for the two wines.

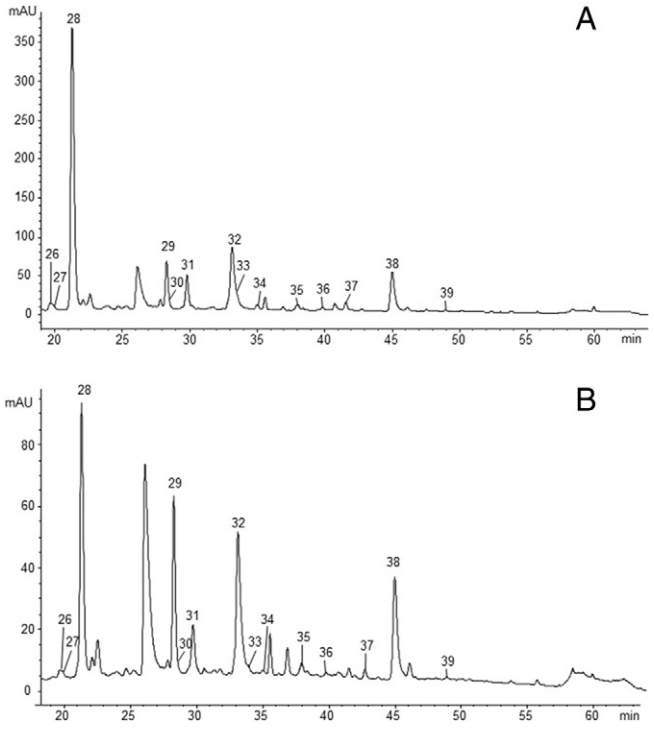

Fig. 2. HPLC DAD-chromatogram (detection at 360 nm) of BRS Rúbea (A) and BRS Cora (B) young red wines flavonols. For peak assignation see Table 3.

3.5. Chemometric approach

The objective of the chemometric approach was to evaluate the relationship between the chemical profiles and the data from the descriptive sensory assessment, using multivariate statistical tools.

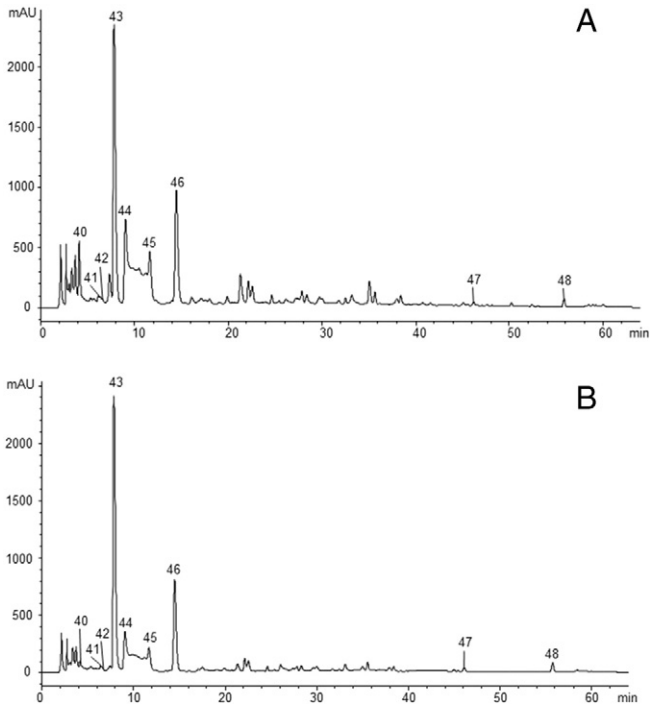

Fig. 3. HPLC DAD-chromatogram (detection at 320 nm) of BRS Rúbea (A) and BRS Cora (B) young red wines hydroxycinnamic acid derivatives (HCAD). For peak assignation see Table 3.

Table 4
Flavan-3-ol/stilbene profiles determined by HPLC-ESI-MS/MS (MRM) and antioxidant capacity determined by DPPH radical scavenging (mean value ± standard deviation) for BRS Rúbea and BRS Cora young red wines. Abbreviations: C, catechin; EC, epicatechin; ECG, epicatechin gallate; PB1, proanthocyanidin B1; PB2, proanthocyanidin B2; PB4, proanthocyanidin B4; mDP, mean degree of polymerization; RUBT, Traditional Rúbea wine; RUBPD, Pre-drying Rúbea wine; RUBSC, Submerged cap Rúbea wine; CORAT, Traditional Cora wine; CORAPD, Pre-drying Cora wine; CORASC, Submerged cap Cora wine. Different letters in the same row indicate significant differences (ANOVA, Tukey's post-hoc test, $\alpha = 0.05$).

Flavan-3-ols and stilbenes	RUBT	RUBPD	RUBSC	CORAT	CORAPD	CORASC
Flavan-3-ol monomers and dimers ($mg \cdot L^{-1}$)	104.74 ± 101.0 a	127.45 ± 74.20 a	45.59 ± 6.60 a	60.78 ± 4.82 b	233.42 ± 20.50 a	65.51 ± 0.62 b
C	36.30 ± 28.90 a	43.40 ± 21.80 a	21.56 ± 2.90 a	24.45 ± 0.27 b	85.74 ± 2.90 a	26.18 ± 2.18 b
EC	13.55 ± 12.19 a	14.14 ± 6.84 a	7.72 ± 1.02 a	17.39 ± 3.72 b	60.71 ± 1.82 a	19.12 ± 0.00 b
ECG	0.76 ± 0.58 a	1.41 ± 0.08 a	0.22 ± 0.19 a	0.04 ± 0.06 a	0.00 ± 0.00 a	0.07 ± 0.10 a
PB1	27.70 ± 30.20 a	36.20 ± 23.70 a	7.83 ± 1.28 a	7.33 ± 0.10 b	27.56 ± 8.03 a	7.74 ± 1.12 b
PB2	23.00 ± 25.40 a	28.20 ± 18.70 a	7.38 ± 1.23 a	9.30 ± 0.68 b	43.07 ± 11.39 a	9.30 ± 1.45 b
PB4	3.39 ± 3.68 a	4.10 ± 3.22 a	0.86 ± 0.04 a	2.25 ± 0.64 b	16.35 ± 2.15 a	3.08 ± 0.12 b
Proanthocyanidin total content ($mg \cdot L^{-1}$)	111.30 ± 48.20 a	203.69 ± 43.00 a	82.33 ± 23.40 a	52.89 ± 4.91 b	88.46 ± 1.21 a	51.42 ± 6.20 b
Proanthocyanidin structural characterization						
mDP	1.18 ± 0.08 a	1.16 ± 0.03 a	1.12 ± 0.00 a	1.48 ± 0.00 a	1.65 ± 0.18 a	1.44 ± 0.07 a
% galloylation	8.57 ± 1.51 ab	13.11 ± 1.49 a	3.87 ± 2.67 b	4.49 ± 0.28 a	3.05 ± 2.63 a	3.76 ± 0.88 a
% prodelphinidin	3.85 ± 1.79 a	1.10 ± 0.27 a	2.44 ± 0.41 a	1.61 ± 0.35 a	0.65 ± 0.03 b	1.46 ± 0.14 ab
Stilbenes ($mg \cdot L^{-1}$)	2.35 ± 0.21 a	1.66 ± 0.07 b	2.67 ± 0.08 a	3.11 ± 0.34 a	1.35 ± 0.63 a	2.70 ± 0.81 a
cis-Resveratrol	0.14 ± 0.04 a	0.05 ± 0.05 a	0.13 ± 0.03 a	0.42 ± 0.18 a	0.14 ± 0.01 a	0.37 ± 0.02 a
cis-Piceid	1.27 ± 0.24 a	1.13 ± 0.11 a	1.62 ± 0.07 a	2.32 ± 0.72 a	0.95 ± 0.44 a	1.74 ± 0.49 a
trans-Piceid	0.93 ± 0.02 a	0.48 ± 0.02 b	0.91 ± 0.02 a	0.36 ± 0.20 a	0.26 ± 0.18 a	0.58 ± 0.34 a
Antioxidant capacity ($mmol \cdot L^{-1}$ of Trolox equivalents)	7.68 ± 1.09 a	8.35 ± 1.09 a	6.85 ± 1.04 a	5.40 ± 0.54 a	4.34 ± 0.17 b	4.86 ± 0.08 ab

This approach allowed the observation of results that were not observed in the univariate approach. According to the PCA results (Fig. 4A/B), 72.46% of the total variance was explained by the first two components, PC1 explained 48.80% and PC2 explained 23.66%. PCA was successfully applied as it provided information about the differences between the winemaking treatments (PC1) and grape cultivars (PC2). PC1 allowed observing the differences between two groups of winemaking treatments, regardless the grape cultivar, i.e., the traditional (T) and submerged cap (SC) winemaking samples located at the left side of the bidimensional graph and the pre-dried (PD) samples located at the right side of the graph. Additionally, the PC2 allowed to differ the variables related to the grape cultivar, regardless the winemaking procedure, i.e., Cora samples located above and Rúbea samples located below the origin horizontal line. This result suggested that the variables related to the PC1 were influenced by the winemaking treatments and the intrinsic grape cultivar features influenced the variables related to the PC2.

Two groups of variables mainly explained the first PC. The first group was composed of the anthocyanins 3,5-diglc, 3-cmglc-5-glc and pyranoanthocyanins, the flavonols myricetin, laricitrin, kaempferol, caftaric, coutaric and coumaric HCAD, ethyl esters, stilbenes and three sensory descriptors, violet hue, sweetness and persistence. The wines covered by these features were the submerged cap and traditional (RUBSC, CORASC, RUBT and CORAT) (Fig. 4B). This result indicated that the intense violet hue was linked to the presence of the anthocyanin 3-cmglc-5glc, which had a strong tendency to form inter and intra co-pigmentation complexes, enhancing the violet hue of the red wines. Furthermore, persistence was probably correlated with the high concentration of HCAD. Gonzalo-Diago, Dizy, and Fernández-Zurbano (2014) reported that the perceived persistence of a wine in the mouth may be influenced by the presence of acids, when astringency and bitterness present low scores. This is a possible explanation for the association of persistence with the HCAD compounds and, at the same time, by the low scores for bitterness and astringency.

The second group was composed of the flavonol quercetin, the flavan-3-ols catechin and epicatechin, the three proanthocyanidins, the degree of polymerization (mDP) and one sensory descriptor, bitterness. The wines that presented a distinct connection with these parameters were the pre-dried ones (RUBPD and CORAPD). There is a tendency to presuppose that bitterness is closely linked to the monomer flavan-3-ols content and the low molecular weight proanthocyanidins, while the high molecular weight proanthocyanidins are related to astringency (Chira, Pacella, Jourdes & Teissedre, 2011), and the PCA projection corroborated these findings.

Two groups explained the total variance of the PC2: the first was composed of the flavan-3-ols % galloylation, antioxidant capacity and four sensory descriptors, color intensity, body, structure/tannins and pungency. Rúbea wines were described by these properties. This result

Table 5
Descriptive sensory profile (mean ± standard deviation) for Rúbea and Cora red wines. Abbreviations: RUBT, Traditional Rúbea wine; RUBPD, Pre-drying Rúbea wine; RUBSC, Submerged cap Rúbea wine; CORAT, Traditional Cora wine; CORAPD, Pre-drying Cora wine; CORASC, Submerged cap Cora wine. Different letters in the same row indicate significant differences (ANOVA, Tukey's post-hoc test, $\alpha = 0.05$).

Sensory attributes	Wines					
	RUBT	RUBPD	RUBSC	CORAT	CORAPD	CORASC
Appearance						
Color intensity	7.25 ± 0.76 ab	7.77 ± 0,96 a	6.95 ± 0.97 b	4.95 ± 1.15 a	4.12 ± 0.87 b	4.91 ± 1.29 a
Violet hue	6.70 ± 1.03 a	5.41 ± 2.06 b	6.48 ± 1.06 a	3.87 ± 1.63 a	0.60 ± 1.18 b	4.56 ± 1.61 a
Taste						
Sweetness	1.87 ± 1.15 a	1.98 ± 1.39 a	2.16 ± 1.42 a	1.75 ± 1.42 a	0.85 ± 0.71 b	1.75 ± 1.09 a
Acidity	5.79 ± 1.17 a	5.83 ± 1.26 a	5.85 ± 1.39 a	6.70 ± 1.17 a	6.89 ± 1.83 a	6.60 ± 1.01 a
Bitterness	2.41 ± 2.17 a	2.58 ± 1.65 a	2.52 ± 1.65 a	2.62 ± 1.76 a	2.89 ± 2.14 a	2.48 ± 1.70 a
Flavor intensity/body	5.64 ± 0.86 a	5.50 ± 1.25 a	5.29 ± 1.56 a	3.97 ± 1.55 a	3.50 ± 1.33 a	4.23 ± 1.56 a
Structure/tannins	4.02 ± 1.57 a	4.12 ± 0.97 a	3.75 ± 1.68 a	2.70 ± 1.21 a	2.18 ± 1.10 a	2.95 ± 1.73 a
Herbaceous taste	2.58 ± 1.21 a	2.31 ± 0.97 a	2.27 ± 1.12 a	3.43 ± 2.07 a	2.70 ± 1.49 a	2.77 ± 1.28 a
Astringency	2.58 ± 1.44 a	2.45 ± 1.21 a	2.04 ± 0.99 a	2.35 ± 1.20 a	2.23 ± 1.58 a	2.25 ± 1.48 a
Pungency	5.06 ± 1.07 a	4.91 ± 1.12 a	4.75 ± 1.18 a	4.23 ± 1.33 a	3.66 ± 1.30 a	4.43 ± 1.27 a
Persistence	5.79 ± 0.86 a	5.56 ± 0.94 a	5.56 ± 1.10 a	4.70 ± 1.04 a	3.25 ± 1.50 b	4.60 ± 1.26 a

showed that the sensory descriptors of body, structure and pungency, which are usually connected with the flavan-3-ols concentration, were closely related to the % galloylation. There is evidence that the increase in galloylation (flavan-3-ols esterified with gallic acid) accentuates the rough or coarse attributes as well as the dryness of wines, which may contribute to structure, body and pungency (Vidal et al., 2003).

With regard to the antioxidant capacity (AA), some authors have described a positive correlation between the polyphenolic content and AA (Fernández-Pachón, Villaño, García-Parrilla & Troncoso, 2004; Villaño, Fernández-Pachón, Moya, Troncoso & García-Parrilla, 2007). However, this relationship was questioned in a more recent study, and the newer findings suggested that the AA was more related to the types of phenolic compounds present in the wines rather than with their global content. Furthermore, the flavonoids, flavan-3-ols (tannins) and anthocyanin fractions are responsible for the high AA of red wines (Makris et al., 2006; Rivero-Pérez et al., 2007). In the present study, the AA was related to the % of galloylated flavan-3-ols, giving rise to an alternative interpretation of the AA as a nutritional property of the red wines.

The second group of the PC2 was composed only of the sensory descriptor acidity, and the Cora wines were the representative samples. None of the chemical properties studied were related to this

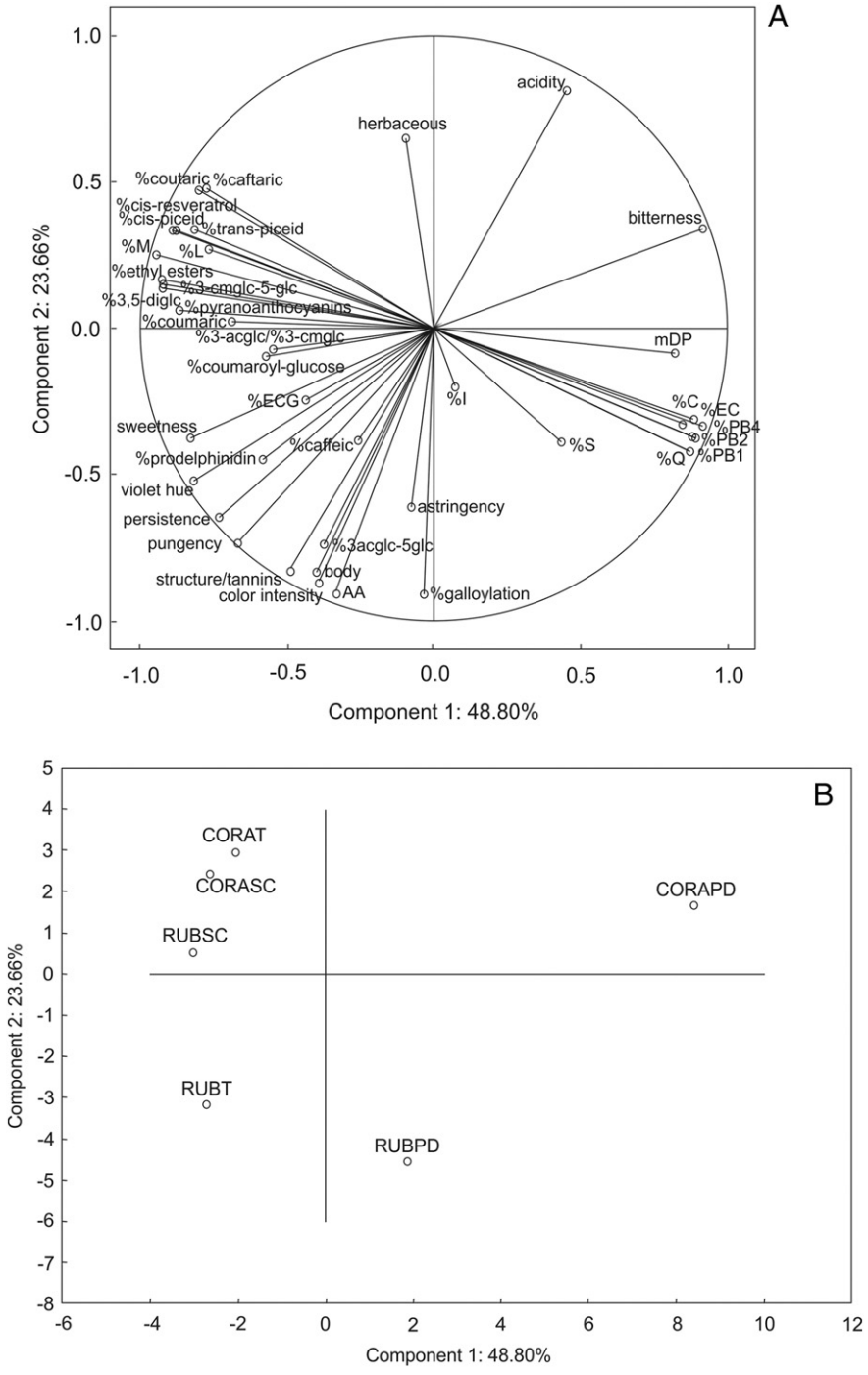

Fig. 4. Projection of the phenolic profile and sensory descriptors (A) and wine samples (B) using PCA. Abbreviations: 3,5-diglc, 3,5-diglucosides; 3-acglc, 3-(6″-acetyl)-glucoside; 3-cmglc, 3-(6″-*p*-coumaroyl)-glucoside; 3-cmglc-5-glc, 3-(6″-*p*-coumaroyl)-glucoside-5-glucoside; M, myricetin; Q, quercetin; L, laricitrin; K, kaempferol; S, syringetin; I, isorhamnetin; C, catechin; EC, epicatechin; ECG, epicatechin gallate; PB1, proanthocyanidin B1; PB2, proanthocyanidin B2; PB4, proanthocyanidin B4; mDP, mean degree of polymerization; RUBT, Traditional Rúbea wine; RUBPD, Pre-drying Rúbea wine; RUBSC, Submerged cap Rúbea wine; CORAT, Traditional Cora wine; CORAPD, Pre-drying Cora wine; CORASC, Submerged cap Cora wine.

attribute. The % of the anthocyanins 3-acglc and 3-cmglc, the contents of the flavonols syringetin and isorhamnetin, caffeic acid and coumaroyl-glucose, the flavan-3-ol epicatechin gallate (ECG) and the % prodelphinidin presented weak representation for the PCs, and two sensory descriptors were not linked to any chemical property, herbaceous taste and astringency.

In general, according to the chemometric approach, pre-drying winemaking provided bitter wines due to the higher flavan-3-ols content. The submerged cap wines were characterized by enhancement of the anthocyanin features, which were responsible for the intense violet hue and also for the high scores for persistence that were connected to the high HCAD content.

4. Conclusion

The chemical analyses provided essential information about the BRS Rúbea and BRS Cora red wines and settled the question of the relationship between them and the sensory attributes. Evidence was found that the anthocyanin contents and also the flavan-3-ol contents in the case of the Cora red wines, were the chemical profiles that presented relevant differentiation between the winemaking treatments. The HCAD, flavonols and stilbenes appeared to be less influenced by these treatments. The sensory profiles showed that the winemaking procedures were responsible for significant differences between the appearance features (color intensity and violet hue), and the chemometric approach established that the pre-drying winemaking treatment provided greater bitterness, due to the flavan-3-ols content, while the submerged cap wines were described as more persistent and with an intense violet hue due to the anthocyanin 3-cmglc-5-glc derivatives. The antioxidant capacity showed a connection with the galloylated flavan-3-ols content and no correlation with the stilbenes. These findings indicate the potential of the drying process in order to obtain more structured red wines, and the submerged cap technique as an alternative to obtain colorful red wines.

Acknowledgments

The author De Castilhos, M.B.M. thanks the Coordination for the Improvement of Higher Level Personnel (CAPES — Brazil) for the scholarship in the Overseas Doctoral Sandwich Program (PDSE). The authors are also grateful to the Brazilian Agro-farming Research Agency EMBRAPA Grape and Wine (Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA Uva e Vinho) and all the wine experts who helped us in the sensory analysis. Author Gómez-Alonso, S. thanks the Fondo Social Europeo and the Junta de Comunidades de Castilla-La Mancha for co-funding his contract via the INCRECYT program. Also, the authors Gómez-Alonso, S. and Hermosín-Gutiérrez, I. are grateful to the Spanish Ministerio de Economía y Competitividad for financial support (project AGL2011-29708-C02-02).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.foodres.2015.05.056.

References

Association of Official Analytical Chemists (2005). *Official methods of analysis of the AOAC International* (18th ed.) (Washington: Gaithersburg (Chapter 28)).

Barcia, M.T., Pertuzatti, P.B., Gómez-Alonso, S., Godoy, H.T., & Hermosín-Gutiérrez, I. (2014). Phenolic composition of grape winemaking by-products of Brazilian hybrid cultivars BRS Violeta and BRS Lorena. *Food Chemistry*, *159*, 95–105.

Biasoto, A.C.T., Netto, F.M., Marques, E.J.N., & Da Silva, M.A.A.P. (2014). Acceptability and preference drivers of red wines produced from *Vitis labrusca* and hybrid grapes. *Food Research International*, *62*, 456–466.

Blanco-Vega, D., López-Bellido, F.J., Alía-Robledo, J.M., & Hermosín-Gutiérrez, I. (2011). HPLC-DAD-ESI-MS/MS characterization of pyranoanthocyanins pigments formed in model wine. *Journal of Agricultural and Food Chemistry*, *59*, 9523–9531.

Bordiga, M., Coïsson, J.D., Locatelli, M., Arlorio, M., & Travaglia, F. (2013). Pyrogallol: An alternative trapping agent in proanthocyanidins analysis. *Food Analytical Methods*, *6*, 148–156.

Bosso, A., Panero, L., Petrozziello, M., Follis, R., Motta, S., & Guaita, M. (2011). Influence of submerged-cap vinification on polyphenolic composition and volatile compounds of Barbera wines. *American Journal of Enology and Viticulture*, *62*, 503–511.

Brand-Williams, W., Cuvelier, M.E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft & Technologie*, *28*, 25–30.

Brasil (2005). *Altera dispositivos da Lei n. 7678 de 8 de novembro de 1988.* Brasília: Diário Oficial da União.

Budic-Leto, I., Lovrić, T., Kljusuric, J.G., Pezo, I., & Vrhovsek, U. (2006). Anthocyanin composition of the red wine Babić affected by maceration treatment. *European Food Research and Technology*, *222*, 397–402.

Camargo, U.A., & Dias, M.F. (1999). *BRS Rúbea.* Bento Gonçalves: Comunicado Técnico, 33.

Camargo, U.A., & Maia, J.D.G. (2004). *BRS Cora: Nova cultivar de uva para suco adaptada a climas tropicais.* Bento Gonçalves: Comunicado Técnico, 53.

Castillo-Muñoz, N., Gómez-Alonso, S., García-Romero, E., Gómez, M.V., Velders, A.H., & Hermosín-Gutiérrez, I. (2009). Flavonol 3-O-glycosides series of *Vitis vinifera* cv. Petit Verdot red wine grapes. *Journal of Agricultural and Food Chemistry*, *57*, 209–219.

Castillo-Muñoz, N., Gómez-Alonso, S., García-Romero, E., & Hermosín-Gutiérrez, I. (2007). Flavonol profiles of *Vitis vinifera* red grapes and their single-cultivar wines. *Journal of Agricultural and Food Chemistry*, *55*, 992–1002.

Chira, K., Pacella, N., Jourdes, M., & Teissedre, P. -L. (2011). Chemical and sensory evaluation of Bordeaux wines (Cabernet-Sauvignon and Merlot) and correlation with wine age. *Food Chemistry*, *126*, 1971–1977.

Dallas, C., Ricardo-da-Silva, J.M., & Laureano, O. (1995). Degradation of oligomeric procyanidins and anthocyanins in a Tinta Roriz red wine during maturation. *Vitis*, *34*, 51–56.

De Castilhos, M.B.M., Cattelan, M.G., Conti-Silva, A.C., & Del Bianchi, V.L. (2013). Influence of two different vinification procedures on the physicochemical and sensory properties of Brazilian non-*Vitis vinifera* red wines. *Lebensmittel-Wissenschaft & Technologie*, *54*, 360–366.

De Castilhos, M.B.M., Conti-Silva, A.C., & Del Bianchi, V.L. (2012). Effect of grape pre-drying and static pomace contact on physicochemical properties and sensory acceptance of Brazilian (Bordô and Isabel) red wines. *European Food Research and Technology*, *235*, 345–354.

De Torres, C., Díaz-Maroto, M.C., Hermosín-Gutiérrez, I., & Pérez-Coello, M.S. (2010). Effect of freeze-drying and oven-drying on volatiles and phenolics composition of grape skins. *Analytica Chimica Acta*, *660*, 177–182.

Delgado-Andrade, C., & Morales, F.J. (2005). Unraveling the contribution of melanoidins to the antioxidant activity of coffee brews. *Journal of Agricultural and Food Chemistry*, *53*, 1403–1407.

Fernández-Pachón, M.S., Villaño, D., García-Parrilla, M.C., & Troncoso, A.M. (2004). Antioxidant activity of wines and relation with their polyphenolic composition. *Analytica Chimica Acta*, *513*, 113–118.

Figueiredo-González, M., Cancho-Grande, B., & Simal-Gándara, J. (2013). Effects on colour and phenolic composition of sugar concentration processes in dried-on- and dried-off-vine grapes and their aged or not natural sweet wines. *Trends in Food Science & Technology*, *31*, 36–54.

Girard, B., Yuksel, D., Cliff, M.A., Delaquis, P., & Reynolds, A.G. (2001). Vinification effects on the sensory, colour, and GC profiles of Pinot noir wines from British Colombia. *Food Research International*, *34*, 483–499.

Gonzalo-Diago, A., Dizy, M., & Fernández-Zurbano, P. (2014). Contribution of low molecular weight phenols to bitter taste and mouthfeel properties in red wines. *Food Chemistry*, *154*, 187–198.

Lago-Vanzela, E.S., Da-Silva, R., Gomes, E., García-Romero, E., & Hermosín-Gutiérrez, I. (2011). Phenolic composition of the edible parts (flesh and skin) of Bordô grape (*Vitis labrusca*) using HPLC-DAD-ESI-MS/MS. *Journal of Agricultural and Food Chemistry*, *59*, 13136–13146.

Lago-Vanzela, E.S., Procópio, D.P., Fontes, E.A.F., Ramos, A.M., Stringheta, P.C., Da-Silva, R., Castillo-Muñoz, N., & Hermosín-Gutiérrez, I. (2014). Aging of red wines made from hybrid grape cv. BRS Violeta: Effects of accelerated aging conditions on phenolic composition, color and antioxidant capacity. *Food Research International*, *56*, 182–189.

Lago-Vanzela, E.S., Rebello, L.P.G., Ramos, A.M., Stringheta, P.C., Da-Silva, R., García-Romero, E., Gómez-Alonso, S., & Hermosín-Gutiérrez, I. (2013). Chromatic characteristics and color-related phenolic composition of Brazilian young red wines made from the hybrid grape cultivar BRS Violeta ("BRS Rúbea" × "IAC 1398-21"). *Food Research International*, *54*, 33–43.

Macheix, J.J., Sapis, J.C., & Fleuriet, A. (1991). Phenolic compounds and polyphenoloxidase in relation to browning in grapes and wines. *Critical Reviews in Food Science and Nutrition*, *30*, 441–448.

Makris, D.P., Kallithraka, S., & Kefalas, P. (2006). Flavonols in grapes, grape products and wines: Burden, profile and influential parameters. *Journal of Food Composition and Analysis*, *19*, 396–404.

Marquez, A., Serratosa, M.P., Lopez-Toledano, A., & Merida, J. (2012). Colour and phenolic compounds in sweet red wines from Merlot and Tempranillo grapes chamber-dried under controlled conditions. *Food Chemistry*, *130*, 111–120.

Marquez, A., Serratosa, M.P., & Merida, J. (2013). Anthocyanin evolution and color changes in red grapes during their chamber drying. *Journal of Agricultural and Food Chemistry*, *61*, 9908–9914.

Mazzuca, P., Ferranti, P., Picariello, G., Chianese, L., & Addeo, F. (2005). Mass spectrometry in the study of anthocyanins and their derivatives: differentiation of *Vitis vinifera* and hybrid grapes by liquid chromatography/electrospray ionization mass spectrometry and tandem mass spectrometry. *Journal of Mass Spectrometry*, *40*, 83–90.

Nixdorf, S.L., & Hermosín-Gutiérrez, I. (2010). Brazilian red wines made from the hybrid grape cultivar Isabel: Phenolic composition and antioxidant capacity. *Analytica Chimica Acta*, *659*, 208–215.

Patras, A., Brunton, N.P., O'Donnell, C., & Tiwari, B.K. (2010). Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends in Food Science & Technology*, *21*, 3–11.

Rebello, L.P.G., Lago-Vanzela, E.S., Barcia, M.T., Ramos, A.M., Stringheta, P.C., Da-Silva, R., Castillo-Muñoz, N., Gómez-Alonso, S., & Hermosín-Gutiérrez, I. (2013). Phenolic composition of the berry parts of hybrid grape cultivar BRS Violeta (BRS Rubea × IAC 1398-21) using HPLC-DAD-ESI-MS/MS. *Food Research International*, *54*, 354–366.

Rivero-Pérez, M.D., Muñiz, P., & González-San José, M.L. (2007). Antioxidant profile of red wines evaluated by total antioxidant capacity, scavenger capacity, and biomarkers of oxidative stress methodologies. *Journal of Agricultural and Food Chemistry*, *55*, 5476–5483.

Rivero-Pérez, M.D., Pérez-Magariño, S., & González-San José, M.L. (2002). Role of melanoidins in sweet wines. *Analytica Chimica Acta*, *458*, 169–175.

Vidal, S., Francis, L., Guyot, S., Marnet, N., Kwiatkowski, M., Gawel, R., Cheynier, V., & Waters, E.J. (2003). The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. *Journal of the Science of Food and Agriculture*, *83*, 564–573.

Villaño, D., Fernández-Pachón, M.S., Moya, M.L., Troncoso, A.M., & García-Parrilla, M.C. (2007). Radical scavenging ability of polyphenolic compounds towards DPPH free radical. *Talanta*, *71*, 230–235.

Wang, H., Race, E.J., & Shrikhande, A.J. (2003). Characterization of anthocyanins in grape juices by ion trap liquid chromatography–mass spectrometry. *Journal of Agricultural and Food Chemistry*, *51*, 1839–1844.