LWT - Food Science and Technology 81 (2017) 58–66

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Isabel red wines produced from grape pre-drying and submerged cap winemaking: A phenolic and sensory approach

Maurício Bonatto Machado de Castilhos [a, *], Sergio Gómez-Alonso [b, c], Esteban García-Romero [d], Vanildo Luiz Del Bianchi [a], Isidro Hermosín-Gutiérrez [b]

[a] Universidade Estadual Paulista "Júlio de Mesquita Filho", Cristóvão Colombo street, nº 2265, Bairro Jardim Nazareth, CEP 15054-000 São José do Rio Preto, SP, Brazil
[b] Universidad de Castilla-La Mancha, Instituto Regional de Investigación Científica Aplicada, Camilo José Cela s/nº, 13071 Ciudad Real, Spain
[c] Parque Científico y Tecnológico de Albacete, Passeo de la Innovación, nº 1, 02006 Albacete, Spain
[d] Instituto de la Vid y el Vino de Castilla-La Mancha, Carretera de Albacete s/nº, 13700 Tomelloso, Spain

ARTICLE INFO

Article history:
Received 12 November 2016
Received in revised form
16 March 2017
Accepted 17 March 2017
Available online 20 March 2017

Keywords:
Red wine
Vitis labrusca
Polyphenols
Sensory profile
Antioxidant capacity

ABSTRACT

The aim of this work was to determine the detailed phenolic composition and sensory profile of red wines produced from Isabel grape using two alternative winemaking: grape pre-drying (IPD) and submerged cap (ISC). IPD wines were produced by the grape drying using a tray dryer at 60 °C and 1.1 m/s of airflow and the ISC wines were produced using stainless steel screens inside the fermentation vessel aiming at avoiding the rise of the cap due to the carbon dioxide. As expected by the thermal degradation, IPD wines presented not quantifiable concentration of anthocyanins and were described as bitter, acid, herbaceous and astringent due to their higher content of galloylated (6.94 mg/L), monomeric flavan-3-ols (49.66 mg/L) and proanthocyanidins (6.28 mg/L), which were less affected by thermal degradation. Submerged cap wines were described as colorful, pungent and persistent in mouth probably due to the anthocyanin content (2.43 mg/L) and hydroxycinnamic acid derivatives (280.5 mg/L), respectively. Submerged cap is a promising procedure because these wines presented higher yield and anthocyanin content and color intensity similar to traditional wines; pre-drying winemaking can be considered less promising since it presented lower yield and sensory features that are not very appreciated by the consumers.

1. Introduction

Table wines are produced from American grapes and their hybrids (*Vitis labrusca L.*) and, in Brazil, exceeded the production of wines produced from European grapes. Isabel grape (*Vitis labrusca L.* x *Vitis vinifera*) plays an important role in the production of red wines and derivative products, since it accounts for approximately 50% of Brazilian grape production (Nixdorf & Hermosín-Gutiérrez, 2010). The wine elaborated from this grape cultivar presents foxy and raspberry aroma and flavor, features that are very appreciated by Brazilian consumers (Rizzon, Miele, & Meneguzzo, 2000).

To date, there has been little published research on the Isabel wine phenolic composition and they have revealed a discreet anthocyanin and pyranoanthocyanin content ranging from 149.8 to 212.8 mg/L and 12.9–20.8 mg/L, respectively (Nixdorf & Hermosín-Gutiérrez, 2010) and another study reported the anthocyanin content of Isabel juices ranging from 102.9 to 216.0 mg/L (Yamamoto et al., 2015). The few mentioned studies were focused only on the phenolic composition of Isabel red wines or juices and presented no data related to sensory descriptors. In addition, no studies were found dealing with Isabel red table wines produced from variations in winemaking procedures.

Despite the fruity flavor and rusticity of the American grapes, the low soluble solids content in their optimal stage of ripening and the low color potential of these grape cultivars are problems that need to be solved by the winemakers. In this context, wineries have used alternative winemaking procedures aiming at enhancing the phenolic extraction, mainly anthocyanin, in order to produce colorful red wines, responding positively in the sensory acceptance. In general, winemakers have employed several variations on winemaking by the application of drying process of the grapes (Marquez, Serratosa, Lopez-Toledano, & Merida, 2012) and

* Corresponding author.
E-mail address: mbonattosp@yahoo.com.br (M.B.M. de Castilhos).

http://dx.doi.org/10.1016/j.lwt.2017.03.033
0023-6438/

submerged cap during alcoholic fermentation (Bosso et al., 2011; Suriano, Ceci, & Tamborra, 2012).

Studies dealing with grape drying prior fermentation showed that the heating caused an irreversible damage in the cellular structure of the grape skin increasing the extraction of the phenolic compounds to the wine during maceration (Marquez et al., 2012). In contrast, the thermal degradation of anthocyanins is a well-known phenomenon that could occur in parallel to the phenolic extraction enhancement (Patras, Brunton, O'Donnell, & Tiwari, 2010). Additionally, submerged cap winemaking procedure provides a balance between gains and losses concerning phenolic concentration, i.e., the constant contact between pomace and must causes gains in the phenolic concentration, mainly anthocyanins (Bosso et al., 2011); however, the absence of mechanical pumping or punching-down steps during maceration negatively affects the flavan-3-ol concentrations (Suriano et al., 2012). All the above mentioned studies presented relevant results; however, they presented data regarding *Vitis vinifera* red wines and provided no relationships with sensory data.

In this context, the aim of this work was to evaluate the detailed phenolic composition and the antioxidant activity of Isabel red wines produced from two alternative winemaking procedures: pre-drying and submerged cap in comparison with the traditional winemaking procedure employed in Brazilian wineries. In addition, wine sensory descriptors were associated with the chemical data as a result of a multivariate chemometric approach aiming at assessing the potential of the alternative winemaking procedures.

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. Commercial standards from Phytolab (Vestenbergsgreuth, Germany), Extrasynthese (Genay, France) and Sigma Aldrich (Tres Cantos, Madrid, Spain) were used for analysis. Other non-commercial flavonol standards 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) in methanol:water 250/750 mL/mL solution.

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. Microvinification

Three red wines were produced in duplicate: Traditional Isabel wine (IT), Pre-dried Isabel wine (IPD) and Submerged Cap Isabel wine (ISC). The grapes were harvested in the city of Jales (20° 16′ 7″ South and 50° 32' 58" West), São Paulo state, Brazil, and they presented, at the start of the winemaking procedure, soluble solids content of 16.4 ± 1.0°Brix, pH value of 3.38 ± 0.03 and total acidity of 5.90 ± 0.07 g.L^{-1} as tartaric acid equivalents.

All the treatments followed the standard winemaking procedure described by De Castilhos, Conti-Silva, and Del Bianchi (2012). Two batches of 7 kg grape each were used for the microvinification process which contemplates the addition of sulfur dioxide (0.086 g/L) and dry active *Saccharomyces cerevisiae* yeasts Y904 (Amazon Group®) in the proportion of 0.2 g/L in order to induce the alcoholic fermentation.

The submerged cap treatment maintained the cap at the bottom of the fermentative vessel by using stainless steel screens, avoiding its rise due to the production of carbon dioxide. The pre-drying treatment consisted of drying the grapes to 22°Brix to avoid chaptalization and obtain wines with an alcoholic content between 8.6 and 14%v/v, as required by Brazilian legislation (Brasil, 2004). This winemaking process was carried out using a convective drying method with a tray dryer at 60 °C and airflow of 1.1 m/s (De Castilhos et al., 2012). At the end of drying procedure, Isabel wines presented 22.9°Brix, with 22.2% of the water evaporated in relation to the initial weight. Both traditional and submerged cap de-stemmed grapes were chaptalized by the addition of 52.2 g/L of sugar.

The following conventional enological parameters were measured: total and volatile acidities as g/L tartaric and acetic acid equivalents, respectively and pH (Brasil, 2004); total dry extract (g/L) (AOAC, 2005); reducing sugars (g/L) by the Lane-Eynon method (AOAC, 2005), alcoholic content (% vol/vol) (AOAC, 2005) and total phenolic content using gallic acid as standard (Slinkard & Singleton, 1977).

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, Waldbronn, Germany). 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, Saint-Quentin En Yvelines, France).

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

The HPLC separation, identification and quantitation of the phenolic compounds was carried out on an Agilent 1100 Series HPLC system (Agilent®, Waldbronn, 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, flavonols and hydroxycinnamic acid derivatives (HCAD) 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 anthocyanins and 20 μL for flavonols; flow rate at 0.19 mL/min) 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 anthocyanin and flavonol analysis, the eluents used were as follows: solvent A (water/formic acid/acetonitrile 885/85/30 mL/mL/mL); solvent B (acetonitrile/water/formic acid 500/415/85 mL/mL/mL) and solvent C (methanol/formic acid/water 900/85/15 mL/mL/mL). For both analysis, it was performed a gradient elution; for anthocyanin analysis the gradient was as follows: 0 min (97% A and

3% B), 20 min (72% A and 28% B), 34 min (57% A and 43% B), 36 min (0% A and 100% B), 45 min (97% A and 3% B); and for flavonol analysis, the gradient used was as follows: 0 min (98% A and 2% B), 8 min (96% A and 4% B), 37 min (70% A, 17% B and 13% C), 51 min (50% A, 30% B and 20% C), 51.5 min (30% A, 40% B and 30% C), 56 min (0% A, 50% B and 50% C), 64 min (98% A and 2% B).

The anthocyanin and pyranoanthocyanin identification was based on the spectroscopic data (UV–Vis and MS/MS) obtained from the authentic standards or using previously reported data (Blanco-Vega, López-Bellido, Alía-Robledo, & Hermosín-Gutiérrez, 2011; Lago-Vanzela et al., 2011; Nixdorf & Hermosín-Gutiérrez, 2010). 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

Identification and quantitation of the flavan-3-ols and stilbenes was carried out using a HPLC Agilent 1200 series system equipped with DAD (Agilent®, Waldbronn, Germany) and coupled to an AB Sciex 3200 TRAP (Applied Biosystems, Foster City, USA) with triple quadrupole, turbo spray ionization (electrospray assisted by a thermonebulization) mass spectroscopy system (ESI-MS/MS). Structural information concerning the proanthocyanidins was obtained using the pyrogallol-induced acid-catalyzed depolymerization method (Bordiga, Coïsson, Locatelli, Arlorio, & Travaglia, 2013). The samples were identified and quantified according to the methodology reported by Lago-Vanzela et al. (2011).

2.4. Determination of the antioxidant capacity by the DPPH assay

The antioxidant capacity was determined 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, Fluka Chemie, Madrid, Spain) radical solution (6×10^{-5} mol/L). 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, Madrid, Spain) (Brand-Williams, Cuvelier, & Berset, 1995).

2.5. Sensory analysis

Ten panelists (Embrapa Grape and Wine, Brazil) used descriptive analysis to describe the sensory profile of the Isabel red table wines. They took part in a session using different wines among the produced samples (traditional, pre-dried and submerged cap) and reference standards. After a brief discussion among the panelists, a list of eleven attributes was established, two attributes for appearance (color intensity, violet hue) and nine for taste (sweetness, acidity, bitterness, body, structure, 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. The Ethical Issues regarding the sensory analysis were approved by the Ethics in Research Committee of the Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista "Júlio de Mesquita Filho" (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 or Games-Howell 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). The statistical tests were performed using the Statistica 10 software (StatSoft Inc., Tulsa, OK) and Minitab 17 (Minitab Inc.).

3. Results and discussion

3.1. Conventional enological parameters

According to the obtained results, all the classic enological parameters presented significant differences when compared among the different winemaking treatments ($P < 0.05$) (Supplementary Table 1). The wines produced from pre-drying process (IPD) presented higher total and volatile acidity, dry extract, reducing sugars and total phenolic content when compared to the wines produced from traditional process (IT). IPD volatile acidity and reducing sugar concentration did not differ from the wines produced from the submerged cap process (ISC). This aforementioned result was expected since the grape drying and the consequent water evaporation could be a possible explanation for the concentration of solid compounds in the Isabel grapes, resulting in a significant enhancement of the IPD dry extract. In addition, the submerged cap yield (L of wine produced per kg of grape) (53.4%) was significantly higher from the traditional yield (47.6%) and pre-drying yield (31.3%) presuming that the submerged cap provide an increase of wine production due to the constant contact between the must and pomace.

3.2. Anthocyanin and pyranoanthocyanin profiles

Isabel red wines presented lower quantity of detection and quantitation of anthocyanins in comparison to other wines produced from hybrid grapes (Nixdorf & Hermosín-Gutiérrez, 2010), since it was detected only five anthocyanin forms: peonidin and malvidin 3,5-diglucosides, cyanidin 3-glucoside, malvidin 3-coumaroylated and malvidin 3-coumaroyl-5-glucoside (Table 1, Fig. 1A). Sixteen different types of pyranoanthocyanins were detected derived from the five expected anthocyanidins (cyanidin, peonidin, petunidin, delphinidin and malvidin) as a result of the fragmentation mass spectra (MS and MS/MS) and the UV–visible absorption spectra DAD-chromatograms at 520 nm of these compounds, being mostly determined by the 10-(3″-hydroxyphenyl) and 10-(3", 4″-dihydroxyphenyl) forms (Blanco-Vega et al., 2011).

Additionally to these aforementioned forms, vitisin-type derived forms were detected (carboxy-pyranoanthocyanins) with only one hydrogen atom linked to the carbon 10 (C-10) of the structure (10-H-pyrmv-3glc and 10-H-pyrpt-3glc – Table 1). The formation of vitisin-type derived forms of pyranoanthocyanins results from the reaction between anthocyanidin-3-glucosides and acetaldehyde, pyruvic acid, acetoacetic acid and diacetyl, all secondary metabolites of yeasts as a result of the enolic form of the latter and, after that, their decarboxylation (Rentzsch, Schwarz, & Winterhalter, 2007).

The wines produced from hybrid grapes naturally present higher concentration of diglucoside anthocyanins instead of monoglucoside forms, as reported by Lago-Vanzela et al. (2011), Nixdorf and Hermosín-Gutiérrez (2010), Rebello et al. (2013) and Yamamoto et al. (2015); and this was corroborated in this present study. Additionally, the absence of the detection of monoglucoside anthocyanins could be explained by the formation of different

Table 1
Anthocyanin and pyranoanthocyanin profiles determined by HPLC/MS/MS (mean ± standard deviation) for Isabel young red wines.

Anthocyanidins and pyranoanthocyanins	Peak	R_t (min)	Molecular ion; product ions (m/z)	IT	IPD	ISC
Anthocyanins (mg/L)				3.12 ± 0.27	NQ	2.43 ± 0.01
Pn-3,5diglc	1	12.6	625; 463,301	1.15 ± 0.08	NQ	1.02 ± 0.08
Mv-3,5diglc	2	14.4	655; 493,331	1.97 ± 0.18	NQ	1.41 ± 0.00
Cy-3glc	5	26.2	449; 287	NQ	NQ	NQ
Mv-3cmglc-5glc	10	30.9	801; 639,493,331	NQ	ND	NQ
Mv-3cmglc	14	36.2	639; 331	NQ	ND	ND
Pyranoanthocyanins (mg/L)				31.34 ± 1.25 a	14.21 ± 0.15 c	22.39 ± 0.02 b
10-H-pyrmv-3glc	3	22.1	517; 355	2.18 ± 0.22	ND	NQ
10-carboxi-pyrpn-3glc	4	25.6	531; 369	1.92 ± 0.07	NQ	NQ
10-H-pyrpt-3glc	6	26.9	503; 341	NQ	NQ	NQ
10-carboxi-pymv-3cmglc (cm-vitisin A)	7	27.9	707; 399	NQ	ND	NQ
10-carboxi-pyrdp-3glc	8	28.3	533; 371	1.87 ± 0.00	ND	NQ
10DHP-pyrcy-3glc	9	29.1	581; 419	NQ	NQ	NQ
10-HP-pyrcy-3glc	11	32.9	565; 403	NQ	ND	NQ
10-carboxi-pyrpn-3cmglc	12	34.1	677; 369	2.55 ± 0.03 a	2.57 ± 0.02 a	1.93 ± 0.01 a
10DPH-pyrpn-3glc	13	34.7	595; 433	2.41 ± 0.00	NQ	1.91 ± 0.03
10HP-pyrpn-3cfglc	15	36.6	741; 417	NQ	ND	NQ
10DHP-pyrmv-3glc	16	37.0	625; 463	2.03 ± 0.07 a	2.19 ± 0.00 a	1.78 ± 0.03 b
10HP-pyrpn-3glc	17	38.3	579; 417	NQ	NQ	NQ
10HP-pyrmv-3glc	18	39.8	609; 447	7.69 ± 0.37 a	5.43 ± 0.12 b	6.20 ± 0.00 b
10DHP-pyrmv-3cmglc	19	41.5	771; 463	1.89 ± 0.08	NQ	1.80 ± 0.00
10HP-pyrpn-3cmglc	20	42.2	725; 417	5.29 ± 0.03 a	4.01 ± 0.04 c	4.66 ± 0.08 b
10HP-pyrmv-3cmglc	21	42.4	755; 447	4.40 ± 0.02	NQ	4.07 ± 0.02

Abbreviations: Cy, cyanidin; Pt, petunidin; Pn, peonidin; Mv, malvidin; 3,5-diglc, 3,5-diglucosides; 3-cmglc-5-glc, 3-(6″-*p*-coumaroyl)-glucoside-5-glucoside; 3-glc, 3-glucoside; 3-cmglc, 3-(6″-*p*-coumaroyl)-glucoside; 10-HP, 10-*p*-hydroxyphenyl; 10-DHP, 10-*p*-dihydroxyphenyl; IT, Traditional Isabel wine; IPD, Pre-drying Isabel wine; ISC, Submerged cap Isabel wine; ND, not detectable; NQ, not quantifiable. Different letters in the same row indicate significant differences (ANOVA, Tukey's post-hoc test, $\alpha = 0{,}05$). Number of replicates = 2.

types of hydroxyphenyl-pyranoanthocyanins, since they are formed by the reaction between the monoglucoside anthocyanins and hydroxycinnamic acids, known as 10-(3″-hydroxyphenyl) (10-HP; reaction products with *p*-coumaric acid) or 10-(3", 4″-dihydroxyphenyl) (10-DHP; reaction products with caffeic acid) (Blanco-Vega et al., 2011).

The winemaking process influenced the total content of anthocyanins and pyranoanthocyanins, since some of the anthocyanins and pyranoanthocyanins found in traditional and submerged cap Isabel red wines were not detected in pre-dried Isabel wines and this result was possible explained by the thermal effect that caused the degradation of these compounds, since the anthocyanins present relevant instability and are commonly degraded by heat (Marquez et al., 2012).

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

The 3-glucosides (3-glc) of the six aglycones (Q, quercetin; M, myricetin; L, laricitrin; S, syringetin; I, isorhamnetin and K, kaempferol) were detected and quantified in Isabel wines (Table 2, Fig. 1B). In addition, myricetin and quercetin 3-glucuronides (3-glcU) and the galactoside forms of myricetin, quercetin and kaempferol were also detected in Isabel red wines. The 3-glucoside of Q presented the highest concentrations in Isabel red wines, followed by the 3-glucosides of M and free Q form. This result was in accordance with Yamamoto et al. (2015) and Nixdorf and Hermosín-Gutiérrez (2010) who reported high concentration for the same flavonols for Isabel red wines.

The winemaking procedures influenced the concentration of M-3glc, Q-3gal, Q-3glc and L-3glc, and IPD wines showed lower concentration for all the aforementioned flavonols, except Q-3gal. The glucoside forms of laricitrin, syringetin and free quercetin seemed to be less influenced by the heat effect, since they presented similar concentrations when compared to traditional and submerged cap red wines. In all flavonols detected, except for myricetin-3-glucoside (M-3-glc), the submerged cap wines (ISC) did not differ from the traditional wines (IT), showing a slight similarity in the flavonol profile for both winemaking treatments.

With regard to the hydroxycinnamic acid derivatives (HCAD), larger amounts of caftaric and free *p*-coumaric acids were observed (Table 2, Fig. 1C), thus indicating a high degree of hydrolysis of coutaric acid, the grape native precursor of *p*-coumaric acid, which accounted for minor concentrations. In addition, the same explanation could be applied for IPD wines regarding the caftaric concentration, i.e., despite the absence of significant differences, pre-dried wines showed higher concentration of caffeic acid and, subsequently, lower concentration of caftaric acid, its precursor, the latter being affected by the heat degradation. The data concerning the HCAD showed no significant differences ($P > 0.05$) when the winemaking treatments were compared, suggesting that the submerged cap and the pre-drying did not significantly affect the HCAD concentration.

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

Catechin (C), epicatechin (EC), epicatechin 3-gallate (ECG), proanthocyanidin B1 (PB1), proanthocyanidin B2 (PB2) and proanthocyanidin B4 (PB4) were detected and quantified in Isabel red wines (Table 3). There were no significant differences on the flavan-3-ol contents when the pre-drying and submerged cap winemaking procedures were compared to traditional process and a possible explanation for this aforementioned result is due to a balance between losses and gains. The grape cellular walls lost their physiological integrity during dehydration, thus favoring the diffusion of phenolic compounds and flavan-3-ols from the grape skin to the pulp, increasing their concentration (Figueiredo-González, Cancho-Grande, & Simal-Gándara, 2013); on other hand, the higher content of flavan-3-ols in IPD wine due to the latter reason seems to be counteracted by the also expected thermal degradation of these flavonoids, decreasing their concentration. The apparent balance between these two opposite effects could explain the lack of significant differences in the flavan-3-ol contents when the different winemaking procedure were

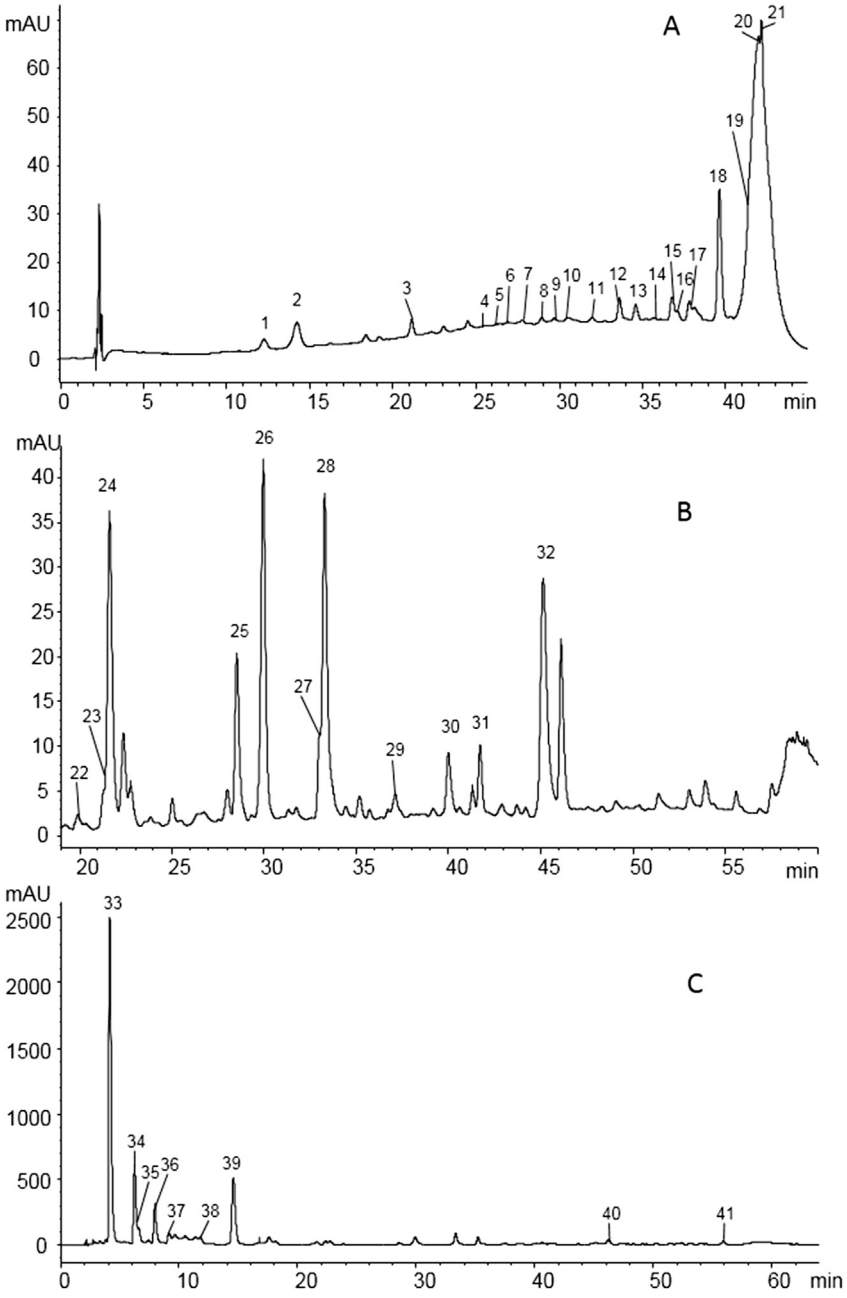

Fig. 1. HPLC DAD-chromatogram (detection at 520 nm) of Isabel young red wines anthocyanins (A): for peak assignation see Table 1; HPLC DAD-chromatogram (detection at 360 nm) of flavonols (B) and HPLC DAD-chromatogram (detection at 320 nm) of hydroxycinnamic acid derivatives (HCAD) (C): for (B) and (C) peak assignation see Table 2.

compared. In addition, flavan-3-ols configuration affects their reactivity and they seem to be more stable to heat than anthocyanins, which is an additional explanation for this result (Jackson, 2008).

In addition, the lower content of tannins and the extraction of almost only monomeric flavan-3-ols in the wines produced from Isabel hybrid cultivar is probably explained by two factors: the grape cultivar itself, since the hybrid grapes present more than 4-fold lower tannin concentration when compared to *Vitis vinifera* grapes; and the binding phenomenon between the tannins and the compounds existent in the skin cell walls of the hybrid grapes such as proteins and, to a lesser extent, pectin (Springer & Sacks, 2014).

Isabel wines presented low values for mDP, indicating that there is low concentration of polymeric tannins (mDP around 1.00). According to Poncet-Legrand et al. (2010) and Bindon, McCarthy, and Smith (2014), the interpretation of this parameter needs some caution because tannins form inter- and intra-molecular bonds that are responsible to increase their resistance to hydrolysis, which can result in a reduction in tannin mDP, without tannin concentration losses. In addition, previous work developed by De Castilhos, Maia, Gómez-Alonso, Del Bianchi, and Hermosín-Gutiérrez (2016) showed that Isabel wines presented low concentration of non-bleachable pigments (from 37.0 up to 67.1 mg/L) and low prodelphinidin content, which are responsible, together, for the formation of polymeric pigments (Bindon, Varela, Kennedy, Holt, & Herderich, 2013). All these facts could possibly explain the low values for Isabel red wines mDP.

With respect to stilbenes, *cis*-resveratrol, *trans*-piceid and *cis*-

Table 2
Flavonol and HCAD profile determined by HPLC/MS/MS (mean value ± standard deviation) for Isabel young red wines.

Flavonols and HCAD	Peak	R_t (min)	Molecular ion; product ions (m/z)	IT	IPD	ISC
Flavonols (mg.L^{-1})				27.94 ± 4.31 a	5.37 ± 1.51 b	17.21 ± 0.58 a
M-3-glcU	22	20.0	493; 317	0.47 ± 0.04	NQ	NQ
M-3-gal	23	20.4	479; 317	NQ	NQ	NQ
M-3-glc	24	21.5	479; 317	5.88 ± 0.13 a	0.51 ± 0.00 b	1.29 ± 0.41 b
Q-3-gal	25	28.2	463; 301	0.50 ± 0.00 b	2.14 ± 0.11 a	0.30 ± 0.06 b
Q-3-glcU	26	28.6	477; 301	3.03 ± 0.01	NQ	1.73 ± 0.01
Q-3-glc	27	29.9	463; 301	7.51 ± 0.38 a	1.26 ± 0.00 b	5.37 ± 1.01 ab
L-3-glc	28	33.0	493; 331	3.84 ± 0.84 a	0.58 ± 0.56 b	3.81 ± 0.01 a
K-3-gal	29	33.2	447; 285	NQ	NQ	0.27 ± 0.08
K-3-glc	30	37.0	447; 285	0.55 ± 0.24	NQ	0.45 ± 0.00
I-3-glc	31	40.1	477; 315	1.27 ± 0.22 a	0.64 ± 0.17 a	0.87 ± 0.28 a
S-3-glc	32	41.6	507; 345	1.10 ± 0.00 a	0.35 ± 0.00 a	0.84 ± 0.16 a
Free Q	33	45.0	301	4.30 ± 2.71 a	0.67 ± 0.10 a	2.23 ± 0.47 a
Hydroxycinnamic acid derivatives (HCAD) (mg.L^{-1})				286.81 ± 12.03 a	72.40 ± 30.30 b	280.50 ± 14.50 a
caftaric acid[1]	34	4.1	311; 179,149,135	229.35 ± 6.22 a	29.20 ± 39.70 a	235.88 ± 0.82 a
trans-coutaric acid	35	6.1	295; 163,149,119	16.47 ± 0.35	NQ	12.76 ± 0.00
cis-coutaric acid	36	6.5	295; 163,149,119	1.27 ± 0.00	NQ	8.14 ± 10.16
caffeic acid[1]	37	7.8	179; 135	11.12 ± 0.77 a	21.97 ± 8.34 a	9.04 ± 0.05 a
p-coumaroyl-glucose-1	38	9.0	325; 163,145	1.73 ± 0.03 a	1.34 ± 0.47 a	1.67 ± 0.00 a
p-coumaroyl-glucose-2	39	11.6	325; 163,145	0.61 ± 0.27 a	0.52 ± 0.47 a	0.80 ± 0.08 a
p-coumaric acid[1]	40	14.4	163; 119	24.66 ± 6.00 a	19.35 ± 1.04 a	17.42 ± 3.56 a
ethyl caffeate	41	46.1	207; 179,135	0.66 ± 0.70	NQ	0.01 ± 0.00
ethyl *p*-coumarate	42	55.8	191; 163,119	1.55 ± 1.35	NQ	1.14 ± 0.47

Abbreviations: M, myricetin; Q, quercetin; L, laricitrin; K, kaempferol; S, syringetin; I, isorhamnetin; glcU, glucuronide; gal, galactoside; glc, glucoside; IT, Traditional Isabel wine; IPD, Pre-drying Isabel wine; ISC, Submerged cap Isabel wine. Different letters in the same row indicate significant differences (ANOVA, Tukey's post-hoc test and Games Howell post-hoc test1 when the variances were different, $\alpha = 0{,}05$). Number of replicates = 2.

Table 3
Flavan-3-ol/stilbenes profiles determined by HPLC-ESI-MS/MS (MRM) and antioxidant capacity (mean value ± standard deviation) for Isabel young red wines.

Flavan-3-ols and stilbenes	IT	IPD	ISC
Flavan-3-ol monomers and dimers (mg/L)[1]	52.66 ± 2.49 a	56.00 ± 72.00 a	2.09 ± 0.28 a
C[1]	37.70 ± 1.23 a	39.90 ± 51.20 a	1.54 ± 0.20 a
EC[1]	7.79 ± 0.44 a	9.05 ± 11.79 a	0.36 ± 0.10 a
ECG	0.30 ± 0.17 a	0.71 ± 0.72 a	0.02 ± 0.02 a
PB1[1]	4.40 ± 0.65 a	4.04 ± 5.38 a	0.10 ± 0.06 a
PB2[1]	2.07 ± 0.03 a	1.87 ± 2.39 ab	0.05 ± 0.00 b
PB4	0.37 ± 0.02 a	0.37 ± 0.53 a	0.00 ± 0.00 a
mDP	1.18 ± 0.01 a	1.12 ± 0.06 a	1.07 ± 0.01 a
% galloylation	4.01 ± 1.00 a	6.94 ± 1.37 a	4.10 ± 1.50 a
% prodelphinidin	0.69 ± 0.21 a	0.06 ± 0.09 b	0.00 ± 0.00 b
Stilbenes (mg/L)[1]	2.06 ± 0.73 a	3.18 ± 2.42 a	0.87 ± 0.05 a
cis-resveratrol	0.32 ± 0.17 a	0.00 ± 0.00 a	0.00 ± 0.00 a
cis-piceid[1]	1.30 ± 0.57 a	2.31 ± 2.37 a	0.36 ± 0.06 a
trans-piceid	0.43 ± 0.02 b	0.86 ± 0.04 a	0.51 ± 0.01 b
Antioxidant capacity (mmol/L of Trolox equivalents)[1]	1.45 ± 0.02 a	2.01 ± 1.36 a	0.39 ± 0.29 a

Abbreviations: C, catechin; EC, epicatechin; ECG, epicatechin gallate; PB1, proanthocyanidin B1; PB2, proanthocyanidin B2; PB4, proanthocyanidin B4; mDP, mean degree of polymerization; IT, Traditional Isabel wine; IPD, Pre-drying Isabel wine; ISC, Submerged cap Isabel wine. Different letters in the same row indicate significant differences (ANOVA, Tukey's post-hoc test and Games Howell post-hoc test1 when the variances were different, $\alpha = 0{,}05$). NQ: not quantifiable. Number of replicates = 2.

piceid were detected and quantified for all Isabel wines. In all wines, the total and individual contents of each stilbene were low and confirmed previous findings suggesting that Isabel grape is a low resveratrol producer (Yamamoto et al., 2015). The content of resveratrol, its glycoside forms (piceids) and the global content of phenolic compounds have been suggested to be significantly correlated with the antioxidant capacity of grapes and wines (Granato, Katayama, & Castro, 2011). Despite this aforementioned correlation, wines presenting high global amounts of stilbenes or phenolic concentration not always show the greatest antioxidant capacity, because this property depends more of the types of the phenolic compounds than their global amounts (Rivero-Pérez, Muñiz, & González-San José, 2007).

3.5. Sensory assessment

The comparison of the winemaking treatments only provided

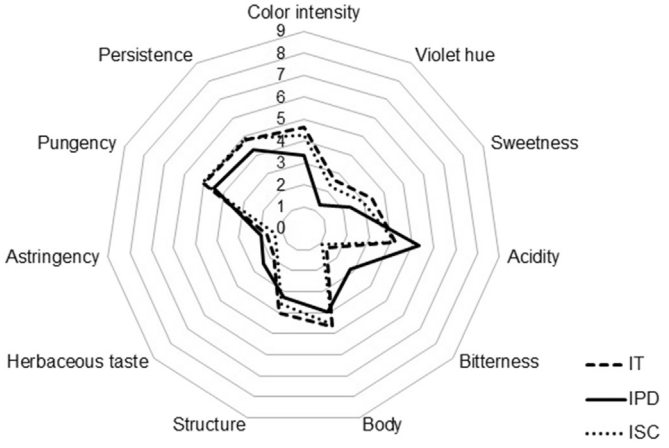

Fig. 2. Spider plot for sensory descriptors of Isabel young red wines using 0–9 scale.

significant differences with respect to the violet hue, color intensity, sweetness, acidity and bitterness (Supplementary Table 2). Pre-dried wine (IPD) showed higher values for both latter descriptive sensory attributes and the traditional wine (IT) higher scores for violet hue and sweetness, presenting no significant differences from submerged cap Isabel wine (ISC). The other sensory descriptors presented similar scores for all the three winemaking procedures (Fig. 2). The lack of significant differences provided the application of Principal Component Analysis (PCA) aiming at analyzing the relationship between the chemical and sensory profiles.

3.6. Sensometric approach

According to the PCA results (Fig. 3), the first two PC explained 85.53% of the total variance (PC1 and PC2 explained, respectively, 57.40% and 28.13% of the total variance). The PC1 provided the differentiation of the traditional (IT) and submerged cap (ISC) wines from those produced from pre-drying winemaking (IPD) and the PC2 allowed for the differentiation of the traditional wines (IT) from the submerged cap ones (ISC).

The relationship between the chemical and sensory profiles was performed by quadrants and, in this context, the variables located

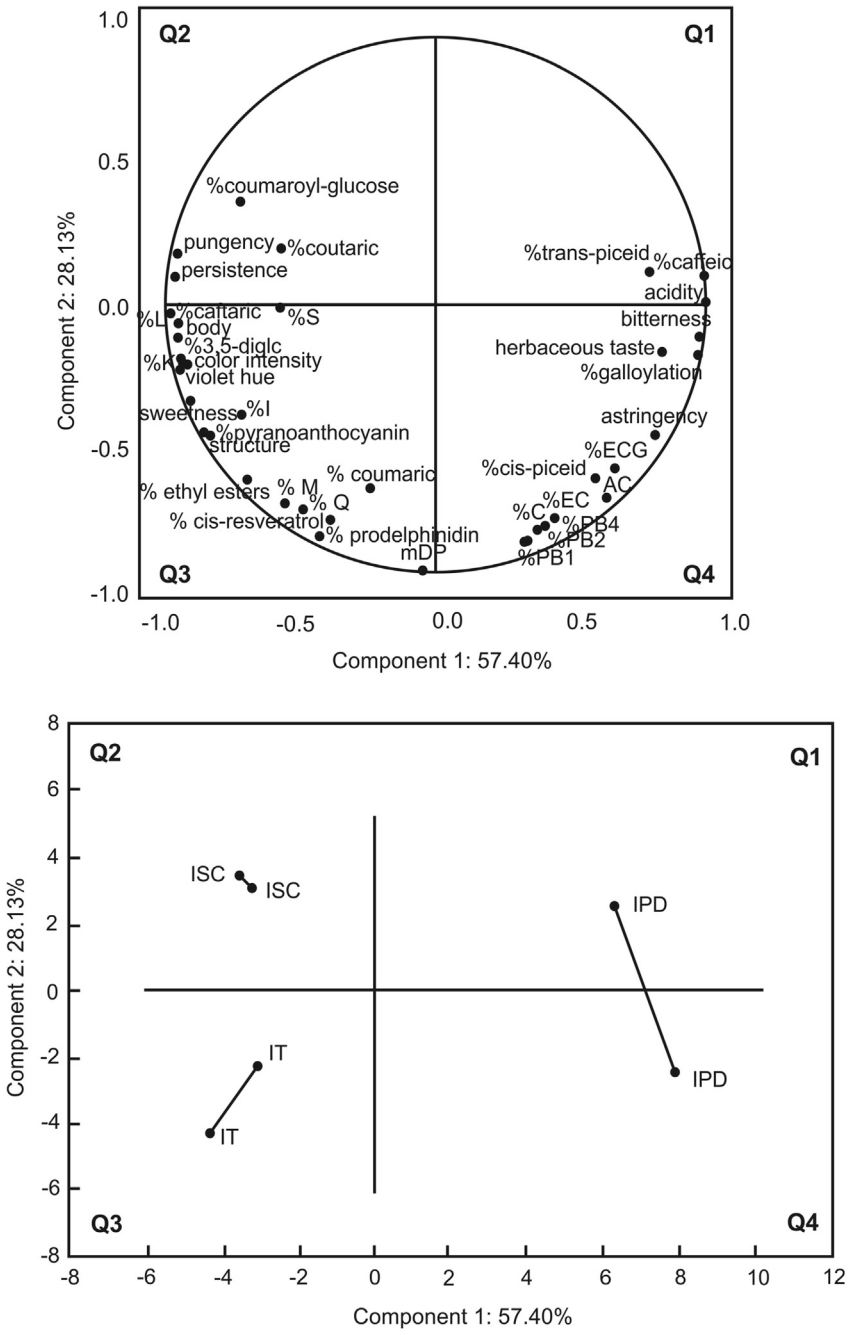

Fig. 3. Projection of the phenolic profile and sensory descriptors (A) and wine samples (B) using PCA.
Abbreviations: 3,5-diglc, 3,5-diglucosides; M, myricetin; Q, quercetin; L, laricitrin; S, syringetin; I, isorhamnetin; K, kaempferol; C, catechin; EC, epicatechin; ECG, epicatechin gallate; PB1, proanthocyanidin B1; PB2, proanthocyanidin B2; PB4, proanthocyanidin B4; mDP, mean degree of polymerization; IT, Traditional Isabel wine; IPD, Pre-dried Isabel wine; ISC, Submerged cap Isabel wine.

in quadrants 1 and 4 were significantly correlated with the pre-dried wines (IPD). Variables located in quadrant 2 provided high relationship with submerged cap red wines (ISC) and the variables located in quadrant 3 were correlated with the traditional wines (IT).

IPD wines were related with the following chemical compounds: caffeic acid, catechin, epicatechin, epicatechin galate, proanthocyanidins B1, B2 and B4, piceids, high galloylated flavan-3-ols and antioxidant capacity. Additionally, the IPD wines were related to the following sensory descriptors: acidity, bitterness, herbaceous taste and astringency. There is a controversy about the contribution of the monomeric and polymeric flavan-3-ols in providing bitterness and astringency; however, some authors reported that wine bitterness is directly related with monomeric flavan-3-ols and astringency with polymeric flavan-3-ols (Chira, Pacella, Jourdes, & Teissedre, 2011; Gonzalo-Diago, Dizy, & Fernández-Zurbano, 2014). These relationships were possible to be observed in the present results concerning IPD wines. Bitterness is considered a basic taste and, as well as astringency, is defined by the interaction between the mouth receptors and phenolic compounds, mainly flavan-3-ols. The galloylated flavan-3-ols significantly contribute for wine bitterness and in a more discreet way for wine astringency corroborating with Narukawa, Noga, Ueno, Sato, Misaka, & Watanabe. (2011).

Herbaceous taste, which constitute a defect, could be correlated with an intrinsic feature of the grape cultivar; however, this sensory descriptor could be explained by phenolic thermal degradation due to the temperature applied. These mechanisms are chemically complex; however, it is well known that the thermal degradation of the phenolic compounds could generate an herbaceous taste and aroma and these features negatively influence the wine final quality (Patras et al., 2010).

IPD wines presented, as well, higher antioxidant capacity (AC) and this result was strongly connected with piceids and flavan-3-ol monomers, dimers and galloylated flavan-3-ols. In addition, these results are in accordance with Rivero-Pérez et al. (2007) who reported that the AC is not always connected with a specific class of chemical compounds, such as stilbenes, but with a several groups of phenolic compounds in the wine sample. In addition, as previously mentioned, the formation of melanoidins, products of Maillard reaction, presented antioxidant properties and possibly contributed with the higher IPD AC (Marquez et al., 2012).

Wines produced from submerged cap winemaking process (ISC) presented high correlation with coutaric acid and *p*-coumaroyl-glucose, which are coutaric esters (coumaric acid + tartaric acid). ISC wines showed relevant connection with pungency and persistence. The perceived persistence of a wine in mouth could be triggered by the presence of acids, when wine is not considered bitterness and astringent (Gonzalo-Diago et al., 2014). In this context, this is a possible explanation for the association between coutaric acid and its esters with wine persistence.

Traditional Isabel wines (IT) were connected with diglucoside anthocyanins, pyranoanthocyanins, flavonols (M, Q, L, I and K), caftaric acid, ethyl esters, *cis*-resveratrol, prodelphinidins and presented the higher mean degree of polymerization (mDP). In addition, these wines showed relationship with the following sensory descriptors: color intensity, violet hue, sweetness, body and structure. The higher scores for color intensity and violet hue were mainly correlated with the diglucoside anthocyanins and the color intensity was also strongly related with wine structure, corroborating the results from Tecchio, Miele, and Rizzon (2007), who reported the high connection between color intensity and structure of Bordô (*Vitis labrusca L.*) red wines.

Body is a mouth-feel closely related to alcohol content, glycerol and reducing sugar content; however, it can also be related with flavan-3-ols such as prodelphinidins and flavan-3-ols with high mean degree of polymerization, which provide a sensation of structured and full-bodied wine (Chira et al., 2011). IT wines also showed high *cis*-resveratrol concentration when compared to the alternative winemaking procedures; however, the high amounts of the aforementioned stilbene provided no relevant relationship with antioxidant capacity, corroborating the results reported by Rivero-Pérez et al. (2007).

4. Conclusion

The chemical and sensory profiles provided essential information about the Isabel red wines submitted to alternative winemaking procedures. It was expected that the thermal degradation of the phenolic compounds due to the pre-drying treatment would decrease the antioxidant capacity of IPD wines; however, the absence of significant differences of the antioxidant capacity between the treatments is possibly explained by the induction of Maillard reaction products, which contain antioxidant properties. The submerged cap was considered an alternative procedure in order to obtain red wines with similar features to traditional winemaking procedure, with a higher yield, being a positive outcome of this treatment. The pre-drying procedure presented lower potential in comparison with traditional and submerged cap procedures, since it showed the limitation of the lower production of wine per amount of grape (lower yield) and, in addition, it presented sensory features that are not very appreciated by the consumers.

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) and Brazilian Agro-farming Research Agency EMBRAPA Grape and Wine (Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA Uva e Vinho). 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 Ministerio de Economía y Competitividad Español for financial support (project AGL2011-29708-C02-02).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2017.03.033

References

Association of Official Analytical Chemists. (2005). *Official methods of analysis of the AOAC international* (18th ed., p. 2590p). Washington: Gaithersburg.

Bindon, K. A., McCarthy, M. G., & Smith, P. A. (2014). Development of wine colour and non-bleachable pigments during the fermentation and ageing of (*Vitis vinifera* L. *cv.*) Cabernet Sauvignon wines differing in anthocyanin and tannin concentration. *LWT – Food Science and Technology, 59*, 923–932.

Bindon, K., Varela, C., Kennedy, J., Holt, H., & Herderich, M. (2013). Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 1. Grape and wine chemistry. *Food Chemistry, 138*, 1696–1705.

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. *LWT-Food Science & Technology, 28*, 25–30.
Brasil. (2004). *Altera dispositivos da Lei n. 7678 de 8 de novembro de 1988. Brasília*. Lei n. 10970 de 11 de novembro de 2004. Diário Oficial da União.
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.
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 Castilhos, M. B. M., Maia, J. D. G., Gómez-Alonso, S., Del Bianchi, V. L., & Hermosín-Gutiérrez, I. (2016). Sensory acceptance drivers of pre-fermentation dehydration and submerged cap red wines produced from *Vitis labrusca* hybrid grapes. *LWT – Food Science and Technology, 69*, 82–90.
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 and Technology, 31*, 36–54.
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.
Granato, D., Katayama, F. C. U., & Castro, I. A. (2011). Phenolic composition of South American red wines classified according to their antioxidant activity, retail price and sensory quality. *Food Chemistry, 129*, 366–373.
Jackson, R. S. (2008). *Wine science: Principles and applications* (3rd ed., p. 776p). San Diego: Academic Press.
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.
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.
Narukawa, M., Noga, C., Ueno, Y., Sato, T., Misaka, T., & Watanabe, T. (2011). Evaluation of the bitterness of green tea catechins by a cell-based assay with the human bitter taste receptor hTAS2R39. *Biochemical and Biophysical Research Communications, 405*, 620–625.
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 and Technology, 21*, 3–11.
Poncet-Legrand, C., Cabane, B., Bautista-Ortín, A. B., Carrillo, S., Fulcrand, H., Pérez, J., et al. (2010). Tannin oxidation: Intra- versus intermolecular reactions. *Biomacromolecules, 11*, 2376–2386.
Rebello, L. P. G., Lago-Vanzela, E. S., Barcia, M. T., Ramos, A. M., Stringheta, P. C., Da-Silva, R., et al. (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.
Rentzsch, M., Schwarz, M., & Winterhalter, P. (2007). Pyranoanthocyanins – an overview on structures, occurrence, and pathways of formation. *Trends in Food Science and Technology, 18*, 526–534.
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.
Rizzon, L. A., Miele, A., & Meneguzzo, J. (2000). Avaliação da uva cv. Isabel para a elaboração de vinho tinto. *Ciência e Tecnologia de Alimentos, 20*, 115–121.
Slinkard, K., & Singleton, V. L. (1977). Total phenol analysis: Automation and comparison with manual methods. *American Journal of Enology and Viticulture, 28*, 49–55.
Springer, L. F., & Sacks, G. L. (2014). Protein-precipitable tannin in wines from *Vitis vinifera* and interspecific hybrid grapes (*Vitis spp.*): Differences in concentration, extractability, and cell wall binding. *Journal of Agricultural and Food Chemistry, 62*, 7515–7523.
Suriano, S., Ceci, G., & Tamborra, T. (2012). Impact of different winemaking techniques on polyphenolic compounds of Nero Di Troia wine. *Italian Food Beverage Technology, 70*, 5–15.
Tecchio, F. M., Miele, A., & Rizzon, L. A. (2007). Características sensoriais do vinho Bordô. *Pesquisa Agropecuária Brasileira, 42*, 897–899.
Yamamoto, L. Y., Assis, A. M., Roberto, S. R., Bovolenta, Y. R., Nixdorf, S. L., García-Romero, E., et al. (2015). Application of abscisic acid (S-ABA) to cv. Isabel grapes (*Vitis vinifera* x *Vitis labrusca*) for color improvement: Effects on color, phenolic composition and antioxidant capacity of their grape juice. *Food Research International, 77*, 572–583.