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ORIGINAL PAPER

Characterization of grape marcs from native and foreign white varieties grown in northwestern Spain by their polyphenolic composition and antioxidant activity

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Abstract Northwestern Spain wine production is mainly focused on high-quality white wines, generating substantial quantities of grape marc as a by-product that can be exploited as a source of bioactive polyphenols. An optimized PLE method was applied to the extraction of polyphenols from marc of white grape varieties. The study of the polyphenolic composition of the extracts in addition to the determination of their antioxidant capacity was used to characterize the marcs obtained from autochthonous white grape varieties (Albariño, Caiño blanco, Godello, Loureiro, Torrontes, and Treixadura) cultivated in five protected areas of production (Denomination of Origin, DO). Also, the marcs obtained in the winemaking of non-native varieties grown experimentally in the region (Chardonnay, Gewürztraminer, Pinot blanc, Pinot gris, Riesling, and Sauvignon blanc) were included in the characterization study and compared with the native ones. Three vintage years (2010–2012) were considered to account for climate variability. Significant differences in the phenolic composition and the antioxidant capacity of grape marcs obtained from the white varieties were found, which could be helpful for a selective exploitation of winemaking by-products.

Keywords White grape marc · Winemaking by-products · Polyphenolic composition · Antioxidant activity · Pressurized liquid extraction

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Introduction

In recent years, consumers have increased their demand for natural and safer additives to be used as alternative in food industry, showing also a greater interest in the so-called functional products. In this regard, the winemaking industry wastes are subject of numerous studies for the extraction of bioactive compounds; among them polyphenols were the focus of a great number of them [1, 2]. Epidemiological studies demonstrated a direct relationship between a diet rich in polyphenols and decreased risk of chronic diseases such as cardiovascular diseases, cancer, and certain degenerative diseases [3].

Most studies on polyphenol composition of grapes focused on the red varieties [4], being white varieties less studied [5, 6]. However, white wines produce substantial quantities of grape marc as by-product, with storage and disposal representing both an economic and ecological problem [7]. Currently, grape marc is mainly used for producing ethanol and, in many cases, is directly applied as fertilizer; in this case, a high content of polyphenolic compounds may constitute a problem since polyphenols can inhibit germination of plants [8].

Galicia is a region of northwestern Spain where white wines of very high quality and high price are produced, and thus, no attention has been paid to the winemaking wastes. However, since most of the polyphenol content of the grape is in the skin, it is relevant to obtain information on the phenolic content retained in the white grape marc to try profitable extraction of polyphenols to be used mainly in cosmetics and food industry.

Different techniques for extraction of polyphenols from fruits and vegetables processing industry wastes were explored [9], including solid–liquid extraction by maceration

[10, 11], agitation [12, 13], or ultrasound assisted [14], generally needing long times and high organic solvent volumes to carry out the extraction [15]; also, subcritical water was used [6, 16], and pulsed electric fields were applied [17]. Pressurized liquid extraction (PLE) demonstrated to be suitable for the extraction of polyphenols [18–20], with the advantage over conventional techniques that equal or better recoveries are obtained with an overall reduction in solvent consumption and sample preparation time. Most of the polyphenols extraction methods were applied to red winemaking products (grapes, must, wine, or by-products) supported by their general higher polyphenol content. However, a comparative study of polyphenol screening of red and white grape pomace concluded that besides the lack of anthocyanins in white grape marc, no principal differences between red and white varieties were observed [12]. Even despite this, most applications of PLE to grape marc are up to now, to extract anthocyanins [19] and procyanidins [18], with the exception reported by González-Centeno et al. [21] who applied PLE in the characterization of pomaces obtained from four grape white varieties.

In this paper, a PLE-based method is applied to the extraction of polyphenols from marc of Galician white grape varieties [20]. The study of the polyphenolic composition of the extracts in addition to the determination of their antioxidant capacity is used to characterize the marcs obtained from seven autochthonous white grape varieties (Albariño, Caiño blanco, Godello, Loureiro, Torrontes, and Treixadura) cultivated in five Galician Denomination of Origin (DO). The study also includes the non-autochthonous variety Branco lexitimo (also known as Albarin), a well-adapted variety in the protected region Tierra de Betanzos with increasing commercial interest, as well as six non-native varieties currently grown in Galicia experimentally (Chardonnay, Gewürztraminer, Pinot blanc, Pinot gris, Riesling, and Sauvignon blanc). The ultimate goal is to know whether there are significant differences in the phenolic composition and the antioxidant capacity of grape marcs obtained from different white varieties cultivated in the Galician region, which could be helpful for a selective exploitation of winemaking by-products. To our knowledge, this is the first study on characterization of the winemaking by-products obtained from Galician autochthonous and experimental white grape varieties based on their polyphenolic composition.

Materials and methods

Chemicals

Pure standards of gallic acid 99 % (CAS 149-91-7), (+)catechin 99 % (CAS 154-23-4), (−)epicatechin 97 % (CAS 490-46-0), caftaric acid 98 % (CAS 67879-58-7), (−)epicatechin-gallate 98 % (CAS 1257-08-5), procyanidin B1 98 % (CAS 20315-25-7), procyanidin B2 98 % (CAS 29106-49-8), protocatechuic acid 98 % (CAS 121-33-5), caffeic acid 98 % (CAS 331-39-5), quercetin 98 % (CAS 117-39-5), isoquercetin (quercetin-3-glucoside) 98 % (CAS 482-35-9), rutin (quercetin-3-rutinoside) 98 % (CAS 153-18-4), quercetin-3-glucuronide 98 % (CAS 22688-79-5) were all supplied by Sigma-Aldrich (St. Louis, MO, USA).

Individual standard stock solutions of 2000–8000 μg mL^{-1} were prepared in methanol. Working solutions in water containing the target analytes (1–200 μg mL^{-1}, gallic acid; 5–700 μg mL^{-1} catechin; 25–500 μg mL^{-1} epicatechin) were obtained by appropriate dilution. Solutions were stored at 20 °C protected from light.

Washed sea sand (200–300 μm) was supplied by Scharlau (Barcelona, Spain). Methanol HPLC grade was obtained from Panreac (Castellar del Vallès, Barcelona, Spain); acetone HPLC grade and formic acid (98–100 %) from Merck (Darmstadt, Germany) and acetonitrile LC–MS Chromasolv Fluka from Sigma. Ultrapure water was produced in the laboratory with a Milli-Q gradient system (Millipore, Bedford, MA, USA). The Folin-Ciocalteu phenol reagent was obtained from Sigma-Aldrich (Steinheim, Germany). Other chemicals that are needed to determine the spectrophotometric indexes were DMACA (*p*-dimethylamino-cinnamaldehyde, Sigma), sodium hydroxide (NaOH, Merck), sodium nitrite ($NaNO_2$, PRO-BVS), sodium carbonate (Na_2CO_3, Panreac), and aluminum trichloride ($AlCl_3$, Merck). 2,2-Diphenyil-1-picrylhydrazyl (DPPH, Sigma) was used to determine the scavenging activity of the grape marc extracts.

Samples

Grape marc samples of autochthonous white monovarietal grapes (*Vitis vinifera sp*) were kindly supplied by 22 wineries of Galicia (NW Spain) belonging to five protected DO (Monterrei, Rias Baixas, Riberia Sacra, Ribeiro, and Valdeorras). The Rias Baixas DO samples include the recognized production subareas Condado de Tea, O Rosal, Ribeira do Ulla, and Val do Salnés. Non-autochthonous samples were obtained at the Experimental Station for Viticulture and Winemaking of Ribadumia (Pontevedra), and the Centre of Training and Experimentation in Agroforestry (Guisamo, A Coruña). A total of 115 samples were collected in three vintages (2010–2012). Table 1 shows the samples distributed by variety and vintage. The number of samples obtained from each variety and each geographical area was according to its relevance on the total grape production.

The grape marc samples were collected in the wineries on the day of its production immediately after the pressing process, placed into plastic freezer bags, sealed, and stored at −20 °C.

Table 1 Grape marc samples included in the study

Variety	Vintage year			Total
	2010	2011	2012	
Albariño	17	20	18	55
Branco lexitimo	1	2	1	4
Caiño blanco	3	2	1	6
Godello	2	4	4	10
Loureiro	3	3	2	8
Torrontes	1	1	1	3
Treixadura	4	5	5	14
Experimental varieties	7	3	5	15
Total	38	40	37	115

To calculate the moisture content of samples, 3 g of grape marc was dried in an oven at 105 °C. The sample was weighed before and after the dryness step. This operation was carried out in triplicate. All data were expressed on dry weight (dw). For homogenization, samples were ground with a conventional electric grinder (Moulinex).

Pressurized liquid extraction (PLE)

Extractions were performed on an ASE 150 (Dionex, Co., Sunyvale, CA, USA), equipped with 10-mL stainless steel cells and 60-mL collection vials. The method was previously optimized by the authors [20]. One cellulose filter (Dionex) was placed at each end of the PLE cell. Each sample, previously ground in a mortar with the selected dispersant in a ratio 1:2, was introduced into the extraction cell, where previously 1 g of clean sand (200–300 µm grain size) was placed. Finally, the dead volume of the cell was also filled with sand. The cell was tightly closed and placed into the PLE system. Extractions were performed without preheating the cell. The extraction pressure was set to 1500 psi, the flush volume was 60 %, and the purge time was set to 100 s. The applied experimental conditions in the PLE process were: 2 extraction cycles, 5 min of each cycle, 1 g of sample size, 105 °C of extraction temperature, the dispersant was sand, and the solvent was methanol 65 %. The obtained extract was made level in all cases to a final volume of 25 mL with MeOH (to facilitate the subsequent process of concentration of the extract) and then passed through a 0.45 µm polyvinylidene fluoride (PVDF) filter (Simplepure, Membrane Solutions, TX).

HPLC–DAD

A 5-mL aliquot of each grape marc extract was concentrated to a final volume of 0.5 mL under a N_2 stream (VLM EC1 Sample Concentrator), keeping the extract at a temperature of 40 °C. Finally, the concentrated extract was filtered through 0.22 µm PVDF filter (Simplepure) and analyzed using a Varian Prostar HPLC equipped with a diode array detector (DAD), and a 3.9 mm × 150 mm, 4 µm, 60 Å, Waters Nova-Pak C_{18} column. The injection volume was 20 µL in all cases. The mobile phase solvents were (A) 1 % formic acid/water and (B) 1 % formic acid/methanol. The mobile phase gradient program started with 5 % B, changed to 20 % B at 20 min, and then changed to 100 % B at 25 min. The entire HPLC run time was 25 min with a flow rate of 1.0 mL/min and 50 °C column temperature. Polyphenols were detected at 280 nm and 350 nm and were identified by comparison with the retention times and UV spectra of the correspondent pure standards.

LC–MS–MS

The PLE grape marc extracts were analyzed by LC–MS/MS for an accurate identification of the major polyphenols. The liquid chromatographic system used was a Finnigan Surveyor™ HPLC Thermo Fisher Scientific (Madrid, Spain) equipped with a TSP AS3000 autosampler. The experimental conditions are the same used in the HPLC–DAD methodology depicted before (see Sect. 2.4). Electrospray mass spectrometry was performed with a TSQ Quantum Discovery triple-stage quadrupole mass spectrometer from Thermo Fisher Scientific. Column effluent was monitored using the selected reaction monitoring (SRM).

Most polyphenols were detected in the negative mode using ESI (electrospray ionization) by their $[M - H]^-$ pseudomolecular ions, with the exception of quercetin, quercetin-glucuronide and quercetin-glucoside,which were detected in the positive mode. The ESI–MS/MS was operated with a scanning range of *m/z* 100–600. The capillary voltage was set to 3.0 kV, and the capillary temperature was set to 320 °C. High-purity nitrogen (99.9 %) was used as sheath gas and auxiliary gas at 40 and 10 psi and 350 °C, respectively. Argon was the collision gas at 30 psi. Identification was performed using selected reaction monitoring (SRM) in negative mode (ESI-NI) of precursor > product ion transitions. The *m/z* values for the parent/product ions pairs were 169/125 for gallic acid and 289/205 and 289/245 for both catechin and epicatechin. Only one transition was available for gallic acid; therefore, its identity was confirmed via one transition and the retention time. The corresponding tube lens offset was 90 V, and the collision energies were 20 eV for *m/z* 125 and 245 and 16 eV for *m/z* 205. Table 2 summarizes the detection conditions for each compound.

Table 2 LC–MS/MS analytical parameters

Compound	Retention time (min)	Parent ion (*m*/*z*)	Product ions	Collision energy (eV)
Gallic acid	2.19	169.0 $[M-H]^-$	125	26
Protocatechuic acid	3.56	152.9 $[M-H]^-$	108/109	26/17
Caftaric acid	3.91	310.9 $[M-H]^-$	148.9/174.9/178.9	30/19/26
Procyanidin B1	4.38	577.0 $[M-H]^-$	288.9/407/424.9	26
(+)Catechin	4.88	289.0 $[M-H]^-$	203.1/245	26/15
Procyanidin B2	5.38	577.0 $[M-H]^-$	288.9/407.0/424.9	26
(−)Epicatechin	6.14	289.0 $[M-H]^-$	203.1/245	26/15
(−)Epicatechin-gallate	7.34	441.0 $[M-H]^-$	125/169/289	26
Quercetin-3-glucuronide	11.14	479.0 $[M+H]^+$	302.9/461.5	18/14
Quercetin-3-glucoside	11.30	465.0 $[M+H]^+$	256.9/302.9	41/14
Quercetin-3-rutinoside	10.33	609.1 $[M-H]^-$	178.8/270.9/300	44/56/37
Quercetin	11.47	303.1 $[M+H]^+$	153.0/229.1	33/28

Total polyphenols

Total polyphenol (TP) content in grape marc extracts was determined according to the Folin-Ciocalteu (FC) colorimetric method [22]. TPs were quantified from a calibration curve prepared with gallic acid standard solutions in concentrations ranging from 3 to 20 mg L^{-1} ($R^2 = 0.9982$) and expressed as mg of gallic acid equivalents in the liquid extract (mg L^{-1} GAE). TP sample concentrations were expressed as mg gallic acid per g of dry weight of grape marc (mg gallic/g dw).

DPPH radical scavenging activity

2,2-Diphenyil-1-picrylhydrazyl (DPPH) scavenging activity was determined using a modified method against Trolox® [23]. DPPH 0.1 mM was dissolved in 100 % methanol. The grape marc extracts, 0.1 mL, were added to 3.9 mL of the methanolic DPPH solution. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. The decrease in absorbance of the resulting solution was monitored at 515 nm at 30 min. The antiradical activity (AA) was determined using the following equation ($y = 0.5223x + 0.0276$; $R^2 = 0.999$) obtained from linear regression after plotting the A_{515} of known solutions of Trolox against concentration (0.08–1 mM). The DPPH radical scavenging activity of the PLE extracts was expressed as mM Trolox g^{-1} of grape marc (dw). The radical stock solution was prepared fresh daily.

Statistical analysis

Data analysis was performed using Statgraphics XV Centurion software package (Manugistics Inc, Rockville, MD, USA).

Results and discussion

Polyphenolic composition and antioxidant activity of grape marc from Galician white varieties

The concentration of polyphenolic compounds in grape marc depends on many factors other than variety including the different parts of the grape (seeds, skin, and pedicel) [10, 24], the conduction and the irrigation systems [25], the winemaking process [26], the vintage [27], the maturity of the fruit [28], and climate [29]. All these factors introduce variability in the data, and hence, considering data from different vintages is fundamental to improve the consistency of results [30]. In the present study, three vintage years were considered. An ANOVA was run to check for significant differences among vintages [multiple range test using the Fisher's least significant difference (LSD) at 95 % confidence], and results showed significantly higher values ($p < 0.05$) for the antioxidant activity and the sum of individual polyphenols in the 2012 samples respect to vintages 2010 and 2011, whereas differences among vintages were not found for TP.

The antioxidant activity and polyphenols content of varietal Galician white grape marc samples can be seen in Table 3 as well as the homogeneous groups found by the ANOVA. Values shown are the average of the three vintage years. Catechin was the most abundant compound in the samples, followed in concentration by epicatechin and the procyanidins. These results are in accordance with those previously reported [10, 21, 31]. Phenolic acids, gallic, caftaric, and protocatechuic, were the compounds found in the lowest concentrations, which is in accordance with previous published data on grape seeds and peels [28]. The variety Loureiro together with Albariño and Caiño showed the highest values for the AA and TP, whereas Torrontes and Branco lexitimo showed the lowest ones. Regarding

Table 3 Antioxidant activity and polyphenolic composition of Galician varietal white grape marc samples (mean ± standard deviation, $n = 3$)

	Albariño	Branco lexitimo	Caiño	Godello	Loureiro	Torrontes	Treixadura
AA	$3.65^{b} \pm 0.00$	$2.36^{e} \pm 0.01$	$3.35^{c} \pm 0.01$	$3.65^{bc} \pm 0.01$	$4.27^{a} \pm 0.01$	$1.76^{f} \pm 0.02$	$2.71^{d} \pm 0.01$
TP	$43.4^{a} \pm 0.4$	$31^{c} \pm 1$	$41^{a} \pm 1$	$36^{b} \pm 1$	$44^{a} \pm 1$	$22^{d} \pm 2$	$35^{b} \pm 1$
Gallic acid	$95^{a} \pm 2$	$40^{cd} \pm 6$	$81^{b} \pm 5$	$83^{b} \pm 4$	$87^{ab} \pm 4$	$33^{d} \pm 7$	$87^{b} \pm 3$
Caftaric acid	$24^{c} \pm 1$	$25^{cd} \pm 3$	$79^{a} \pm 2$	$19^{d} \pm 2$	$62^{b} \pm 2$	$19^{cd} \pm 3$	$23^{cd} \pm 1$
Protocatechuic acid	$4.3^{c} \pm 0.1$	$3.4^{d} \pm 0.4$	$5.1^{ab} \pm 0.3$	$5.7^{a} \pm 0.2$	$5.1^{ab} \pm 0.3$	$4.5^{bc} \pm 0.4$	$5.6^{a} \pm 0.2$
(+)Catechin	$2027^{a} \pm 31$	$1488^{bcd} \pm 109$	$1436^{cd} \pm 95$	$1670^{b} \pm 74$	$1592^{bc} \pm 82$	$373^{e} \pm 134$	$1530^{bcd} \pm 62$
(−)Epicatechin	$944^{b} \pm 21$	$736^{cd} \pm 78$	$577^{de} \pm 63$	$737^{c} \pm 48$	$605^{cde} \pm 54$	$196^{f} \pm 88$	$1053^{a} \pm 41$
(−)Epicatechin-gallate	$101^{d} \pm 2$	$198^{a} \pm 8$	$88^{de} \pm 7$	$171^{b} \pm 5$	$79^{ef} \pm 6$	$60^{f} \pm 9$	$122^{c} \pm 4$
Procyanidin B1	$517^{c} \pm 8$	$306^{e} \pm 31$	$793^{a} \pm 25$	$522^{c} \pm 19$	$610^{b} \pm 21$	$223^{e} \pm 35$	$404^{d} \pm 16$
Procyanidin B2	$431^{c} \pm 9$	$294^{d} \pm 33$	$400^{c} \pm 26$	$480^{b} \pm 20$	$510^{b} \pm 23$	$252^{d} \pm 37$	$635^{a} \pm 17$
Quercetin-3-glucoside	$570^{b} \pm 16$	$208^{d} \pm 61$	$507^{bc} \pm 49$	$596^{b} \pm 38$	$569^{b} \pm 42$	$280^{d} \pm 69$	$718^{a} \pm 32$
Quercetin-3-glucuronide	$366^{b} \pm 8$	233 ± 32^{d}	$606^{a} \pm 26$	$315^{c} \pm 20$	$587^{a} \pm 22$	$305^{bcd} \pm 36$	$253^{d} \pm 17$
Quercetin-3-rutinoside	$54^{c} \pm 2$	$28^{d} \pm 8$	$60^{bc} \pm 6$	$66^{b} \pm 5$	$157^{a} \pm 6$	$41^{cd} \pm 9$	$20^{d} \pm 4$
Quercetin	$607^{b} \pm 13$	$326^{d} \pm 49$	$475^{c} \pm 39$	$752^{a} \pm 30$	$478^{c} \pm 34$	$441^{cd} \pm 55$	$684^{a} \pm 26$
Sum of compounds	$5753^{a} \pm 68$	$3909^{c} \pm 260$	$5101^{b} \pm 208$	$5430^{ab} \pm 161$	$5346^{b} \pm 179$	$2225^{d} \pm 293$	$5539^{ab} \pm 135$

Units: TP: mg GAE/g dw; AA: mmol Trolox/g dw; compounds: μg/g dw

Superindices a, b, c, d, e, f indicate the ANOVA and LSD homogeneous groups ($p = 95$ %)

individual polyphenols, Loureiro and Caiño contain more caftaric acid than the rest of varieties. Catechins were quite homogeneously distributed among varieties, with Albariño showing the highest content of catechin, and Branco lexitimo and Godello the highest content of epicatechin-gallate. Gallic acid was found in concentrations lower than the average in Branco lexitimo and Torrontes samples, which also showed the lowest content of procyanidins and quercetin-3-glucoside and also of protocatechuic acid in the case of Branco lexitimo. Godello and Loureiro marc stand out by its content in quercetin and quercetin-3-rutinoside, respectively. Treixadura was the variety showing the highest content of epicatechin, procyanidin B2, and quercetin-3-glucoside. These findings are in agreement with those of Rodríguez Montealegre et al. [28]. TP values ranged from 22 to 44 mg GAE/g dw, which are similar to those found by González-Centeno et al. [21] in marc from white grapes grown in Balear islands also using PLE. Results obtained in the present work can be compared with those available up to now on polyphenolic composition of different grape marcs. Table 4 summarizes these results including the extraction technique applied. As can be seen, most of the procedures described led in general to polyphenol concentrations lower than those obtained by PLE.

Differentiation of Galician native white grape marcs by variety

Discriminant analysis was selected as the tool for classification of grape marc samples based on their varietal origin [32]. Values of TP and AA, as well as the individual polyphenols concentration (acids gallic, protocatechuic, and caftaric; catechin, epicatechin, epicatechin-gallate, procyanidin B1, procyanidin B2, quercetin-glucoside, quercetin-glucuronide, quercetin, and quercetin-rutinoside), were selected as variables. Five autochthonous varieties (Albariño, Caíño, Godello, Loureiro, and Treixadura) were finally considered. Torrontés and Branco lexítimo samples were excluded from this analysis since they were supplied by experimental wineries and their grape marcs were obtained in the laboratory after manually pressing the grapes.

Figure 1 shows the distribution of the samples in the five established groups. The overall percentage of discrimination achieved was 95.29 %. Treixadura was well differentiated from the rest of the varieties; the varieties Caiño and Loureiro grown in O Rosal (sub-zone of Rias Baixas DO) were partially misclassified among Albariño samples, as can be seen in Table 5. Also, a little percentage of Albariño and Godello samples were misclassified in the Godello and Treixadura groups, respectively.

Differentiation of Albariño grape marcs produced in the subareas of DO Rias Baixas

Albariño is the priority variety for wine production in the Rias Baixas DO, and it is the predominant white grape variety produced in Galicia. Hence, Albariño could be considered a priori as the most interesting grape marc for the industrial exploitation of its polyphenolic content. Rias Baixas DO include five production subzones: Salnés, Condado do Tea, O Rosal, Ribeira do Ulla, and Soutomaior, of

Table 4 Polyphenolic composition of white grape marcs, seeds and skins

Sample	Extraction procedure	TP	Gallic acid	Caft. acid	Proto. acid	Catechin	Epicatechin	Epi-gallate	PB1	PB2	Flavanols[a]	Q-3-glucoside	Q-3-glucuronide	References
Grape marc														
	Subcr. water	32												Aliakbarian et al. [16]
	Shaking	8												Aliakbarian et al. [16]
	Microwaves	8												Brahim et al. [6]
	Shaking	4												Brahim et al. [6]
Albillo	Ultrasounds										400			Gonzalez-Paramas et al. [5]
Viura	Ultrasounds										100			Gonzalez-Paramas et al. [5]
Albariño	MSPD	35												Lores et al. [25]
Roditis		48												Makris et al. [10]
Arinto	Shaking	14												Matias et al. [7]
Albariño	Distillation	8												Sanchez et al. [2]
Chardonnay	PLE	39				473	488		96	131				González-Centeno et al. [21]
Macabeu	PLE	31				154	95		35	47				González-Centeno et al. [21]
Parellada	PLE	47				633	229		170	140				González-Centeno et al. [21]
Premsal	PLE	36				125	126		67	87				González-Centeno et al. [21]
Sauvignon blanc	Maceration	19	26	ND	4.2	477	506	39	23	81	1126	ND	ND	De la Cerda-Carrasco et al. [11]
Chardonnay	Maceration	17	13	ND	0.9	195	409	ND	15	66	685	ND	ND	De la Cerda-Carrasco et al. [11]
Sauvignon blanc	PLE	33	42	26	3.5	1276	689	321	176	257	2408	194	277	This work
Albariño	PLE	43	95	24	4.3	2027	944	101	517	431	4020	570	366	This work
Chardonnay	PLE	36	69	21	6.3	1505	872	89	323	471	3260	687	266	This work
Seeds														
Merzling	Shaking		106	9.3	102									Kammerer et al. [12]

Table 4 continued

Sample	Extraction procedure	TP	Gallic acid	Caft. acid	Proto. acid	Catechin	Epicatechin	Epi-gallate	PB1	PB2	Flavanols[a]	Q-3-glucoside	Q-3-glucuronide	References
Riesling	Shaking					79	674	457	1053	506		32	38	Kammerer et al. [12]
	Maceration	111												Makris et al. [13]
Chardonnay			150			3580	4210							Yilmaz and Toledo [33]
Skin														
Merzling	Shaking		15	61	42									Kammerer et al. [12]
Riesling	Shaking					226	134	35	191	91		351	509	Kammerer et al. [12]
	Maceration	10												Makris et al. [13]
Chardonnay			50			600	440							Makris et al. [13]

Units: TP: mg GAE/g dw; compounds: μg/g dw

ND not detected

[a] Comparison purposes, flavanols considered in this work are the sum of catechin, epicatechin, epicatechin-gallate, and procyanidins B1 and B2

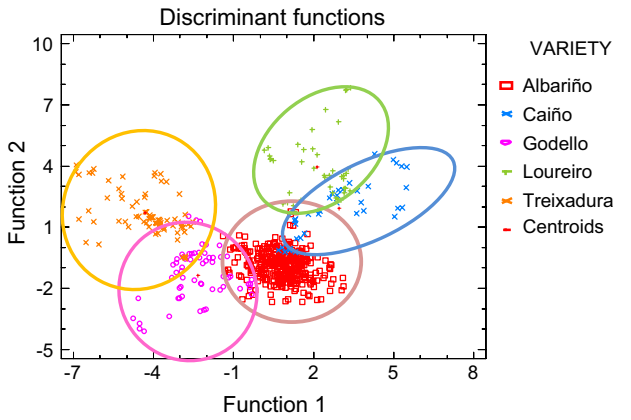

Fig. 1 Graphical representation of the discriminant functions for the autochthonous varietal samples

which Salnés accounts for the 65 % of the Albariño production (Fig. 2). Albariño marc was sampled trying to keep the proportionality with the production by zones. In total, 55 samples have been considered to study whether differences in polyphenolic composition among Albariño grape marc from the different subzones of Rias Baixas DO could be established. Since Soutomaior only accounts for 0.1 % production, this zone has not been sampled.

Table 6 shows the average values of the measured parameters in each of the Rias Baixas subzones, as well as the homogeneous groups found by the ANOVA (95 % confidence level). Samples of all subzones presented similar polyphenolic profiles being Salnés the subarea showing the highest TP value and polyphenols concentration. Great variations in composition among vintages were found in Ribeira do Ulla, which is the Rias Baixas northern subzone, probably due to larger variations in the climate conditions with regard to the southern DO zones.

Several significant differences were found among zones. O Rosal showed higher content of phenolic acids like caftaric and protocatechuic, Salnés higher content of gallic acid, flavanols quercetin-3-glucoside, and quercetin-3-rutinoside. By contrast, the subzone Condado do Tea generally showed the lowest values of all variables, particularly of epicatechin-gallate and quercetin-3-glucuronide.

Albariño marc from grapes grown in each of the different subareas of the DO Rias Baixas was submitted to discriminant analysis, obtaining the results shown in Table 7, with 83 % of the samples correctly classified. Figure 3 shows the overlapping of the four groups. Samples of the subarea Ribeira do Ulla are widely dispersed among Salnés, O Rosal, and Condado do Tea. The exclusion of Ribeira do Ulla samples from the discriminant analysis enhances the differentiation with up to 87 % of samples correctly classified. Consequently, the polyphenols content and antioxidant activity of the samples are not enough

Table 5 Classification of the autochthonous varietal grape marc samples (95.3 % correct classification)

	Albariño (%)	Caiño (%)	Godello (%)	Loureiro (%)	Treixadura (%)
Albariño	97.2	0.00	2.80	0.00	0.00
Caíño	19.4	80.6	0.00	0.00	0.00
Godello	0.00	0.00	93.3	0.00	6.70
Loureiro	11.9	0.00	0.00	88.1	0.00
Treixadura	0.00	0.00	0.00	0.00	100

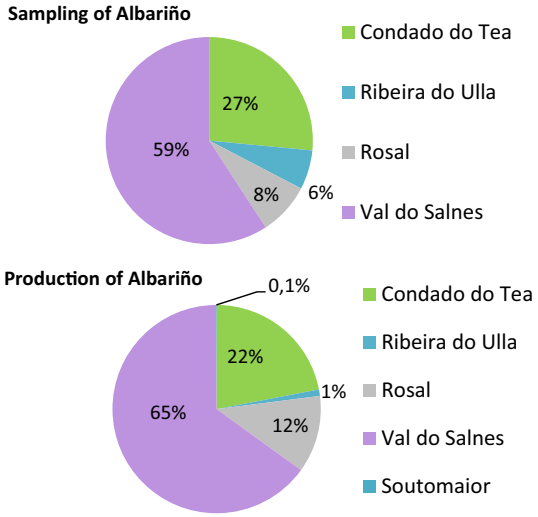

Fig. 2 Distribution of the Albariño grape marc samples according to grape production in the DO Rias Baixas subareas

different to allow complete discrimination by production area of the Rias Baixas DO Albariño grape marcs.

Polyphenolic composition of grape marc from non-native white varieties experimentally cultivated in Galicia

In this study, six varieties were included: Chardonnay, Gewurzträminer, Pinot blanc, Pinot gris, Riesling, and Sauvignon blanc. In total, 15 samples of the 3 vintages were considered (2010–2012). These samples were experimentally cultivated at the Oenological Station in Ribadumia. In most cases, since the harvested grapes of each variety were not enough to be individually pressed to make a monovarietal wine, marc samples were obtained by manually pressing the grapes in the laboratory.

Factors such as climate, geography, culture techniques, preparation of the sample for analysis, and extraction technique were the same for all the samples. Thus, data

Table 6 Antioxidant activity and polyphenolic composition of Albariño grape marc samples (mean ± standard deviation, $n = 3$)

	Condado do Tea	Ribeira do Ulla	Rosal	Val do Salnes
AA	$3.49^{b} \pm 0.06$	$3.63^{ab} \pm 0.12$	$3.66^{ab} \pm 0.10$	$3.67^{a} \pm 0.04$
TP	$41^{b} \pm 1$	$40^{b} \pm 2$	$42^{b} \pm 1$	$45.4^{a} \pm 0.5$
Gallic acid	$87^{b} \pm 3$	$82^{b} \pm 6$	$86^{b} \pm 5$	$99^{a} \pm 2$
Protocatechuic acid	$4.1^{b} \pm 0.2$	$4.2^{b} \pm 0.3$	$5.0^{a} \pm 0.3$	$4.2^{b} \pm 0.1$
Caftaric acid	$21^{c} \pm 1$	$23^{bc} \pm 2$	$33^{a} \pm 1$	$24^{b} \pm 1$
(+)Catechin	$2016^{a} \pm 42$	$2124^{a} \pm 88$	$2095^{a} \pm 72$	$1986^{a} \pm 29$
(−)Epicatechin	$849^{b} \pm 30$	$1030^{a} \pm 63$	$919^{ab} \pm 52$	$958^{a} \pm 21$
(−)Epicatechin-gallate	$91^{c} \pm 3$	$113^{ab} \pm 7$	$119^{a} \pm 6$	$101^{b} \pm 2$
Procyanidin B1	$467^{b} \pm 14$	$479^{b} \pm 29$	$519^{ab} \pm 24$	$556^{a} \pm 9$
Procyanidin B2	$430^{ab} \pm 12$	$384^{b} \pm 24$	$419^{ab} \pm 20$	$444^{a} \pm 8$
Quercetin-3-glucoside	$389^{b} \pm 30$	$499^{b} \pm 62$	$391^{b} \pm 51$	$704^{a} \pm 20$
Quercetin-3-glucuronide	$243^{c} \pm 14$	$379^{ab} \pm 29$	$323^{b} \pm 24$	$424^{a} \pm 9$
Quercetin-3-rutinoside	$27^{c} \pm 3$	$43^{b} \pm 6$	$33^{bc} \pm 5$	$70^{a} \pm 2$
Quercetin	$553^{b} \pm 27$	$495^{b} \pm 55$	$587^{ab} \pm 45$	$634^{a} \pm 18$
Sum of compounds	$5176^{c} \pm 93$	$5655^{ab} \pm 194$	$5529^{bc} \pm 159$	$6006^{a} \pm 63$

Units: TP: mg GAE/g dw; AA: mmol Trolox/g dw; compounds: µg/g dw

Superindices a, b, c, indicate the ANOVA and LSD homogeneous groups ($p = 95$ %)

Table 7 Classification of the Albariño samples by production subarea (82.9 % correct classification)

DO Rias Baixas subarea	Condado do Tea (%)	Ribeira do Ulla (%)	Rosal (%)	Val do Salnes (%)
Condado do Tea	73.6	5.56	15.3	5.56
Ribeira do Ulla	5.56	5.56	11.1	77.8
Rosal	14.8	11.1	70.4	3.70
Val do Salnes	6.13	3.07	1.23	89.6

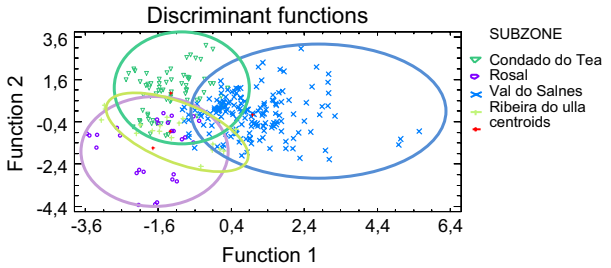

Fig. 3 Discriminant functions for the Albariño produced in the different DO Rias Baixas subareas

obtained are comparable to obtain robust conclusions. Table 8 shows the values of AA, TP, and the individual polyphenols. ANOVA was used to compare each marc variety composition (confidence level of 95 %), allowing classifying them in homogeneous groups. Results showed that Riesling and Sauvignon blanc presented the lowest content of polyphenols considering the sum of the individual compounds. Flavan-3-ols were the predominant compounds in all the samples, with catechin at the highest concentration followed by epicatechin. The results for Chardonnay were in agreement with those previously reported [33], although De la Cerda-Carrasco et al. [11] found that epicatechin concentration was greater than that of catechin in the marcs from Chardonnay and Sauvignon blanc, as well as González-Centeno et al. [21] in the varieties they studied. Regarding procyanidins, similar concentrations were found in all varieties, with PB2 at higher concentration than PB1, according to [20]. A similar relationship was reported [11, 21]. On the other hand, Rodríguez Montealegre et al. [28], who analyzed the polyphenols of peels and seeds in grapes including Riesling, Chardonnay, Sauvignon blanc, and Gewurztträminer, found PB1 at a much higher concentration than PB2. They attribute this fact to variations caused by the mild climate with very hot summers where the analyzed grapes were cultivated. In addition to this, the different extraction systems used could vary the proportions among the polyphenolic compounds. For Pinot gris and Gewurztträminer, no significant differences in procyanidins levels were found. Riesling showed higher caftaric acid, which is in agreement with previous data [28]. For other phenolic acids (gallic and protocatechuic), no

Table 8 Antioxidant activity and polyphenolic composition of experimental non-autochthonous grape marcs (mean ± standard deviation, $n = 3$)

	Chardonnay	Pinot blanc	Pinot gris	Riesling	Sauvignon blanc	Gewürztraminer
AA	$3.16^{a} \pm 0.20$	$3.39^{a} \pm 0.26$	$3.39^{a} \pm 0.35$	$3.07^{a} \pm 0.20$	$2.93^{a} \pm 0.20$	$3.01^{a} \pm 0.25$
TP	$36^{ab} \pm 2$	$38^{b} \pm 2$	$49^{a} \pm 3$	$31^{d} \pm 2$	$33^{cd} \pm 2$	$33^{bcd} \pm 2$
Gallic acid	$69^{a} \pm 8$	$80^{a} \pm 11$	$60^{ab} \pm 14$	$56^{ab} \pm 8$	$42^{b} \pm 8$	$73^{a} \pm 10$
Protocatechuic acid	$21^{c} \pm 6$	$40^{b} \pm 7$	$27^{bc} \pm 10$	$70^{a} \pm 5$	$26^{bc} \pm 6$	$38^{b} \pm 7$
Caftaric acid	$6.3^{a} \pm 0.5$	$3.6^{c} \pm 0.6$	$7.1^{a} \pm 0.9$	$4.7^{bc} \pm 0.5$	$3.5^{c} \pm 0.5$	$5.7^{ab} \pm 0.6$
(+)Catechin	$1505^{bc} \pm 135$	$1518^{bc} \pm 173$	$1843^{ab} \pm 234$	$1009^{d} \pm 131$	$1276^{cd} \pm 135$	$1998^{a} \pm 165$
(−)Epicatechin	$872^{bc} \pm 105$	$1555^{a} \pm 134$	$1132^{ab} \pm 182$	$569^{d} \pm 102$	$689^{cd} \pm 105$	$773^{bcd} \pm 129$
(−)Epicatechin-gallate	$89^{cd} \pm 13$	$140^{ab} \pm 16$	$101^{bcd} \pm 22$	$73^{d} \pm 12$	$176^{a} \pm 13$	$123^{bc} \pm 16$
Procyanidin B1	$323^{c} \pm 35$	$447^{ab} \pm 45$	$507^{a} \pm 61$	$324^{c} \pm 34$	$257^{c} \pm 35$	$348^{bc} \pm 43$
Procyanidin B2	$471^{bc} \pm 44$	$605^{d} \pm 56$	$474^{abc} \pm 76$	$415^{cd} \pm 43$	$321^{a} \pm 44$	$309^{d} \pm 54$
Quercetin-3-glucoside	$687^{a} \pm 44$	$200^{c} \pm 56$	$606^{ab} \pm 76$	$463^{b} \pm 43$	$194^{c} \pm 44$	$697^{a} \pm 54$
Quercetin-3-glucuronide	$266^{b} \pm 14$	$214^{c} \pm 18$	$348^{a} \pm 24$	$330^{a} \pm 14$	$277^{b} \pm 14$	$270^{b} \pm 17$
Quercetin-3-rutinoside	$28^{c} \pm 3$	$31^{c} \pm 4$	$50^{b} \pm 5$	$72^{a} \pm 3$	$16^{d} \pm 3$	$34^{c} \pm 4$
Quercetin	$326^{a} \pm 6$	$229^{c} \pm 8$	$256^{c} \pm 11$	$305^{b} \pm 6$	$323^{ab} \pm 6$	$304^{b} \pm 8$
Sum of compounds	$4662^{a} \pm 300$	$5063^{a} \pm 384$	$5410^{a} \pm 520$	$3691^{b} \pm 292$	$3601^{b} \pm 300$	$4971^{a} \pm 368$

Units: TP: mg GAE/g dw; AA: mmol Trolox/g dw; compounds: µg/g dw

Superindices a, b, c, d, indicate the ANOVA and LSD homogeneous groups ($p = 95$ %)

significant differences were found. Regarding flavonoids, Pinot blanc and Sauvignon blanc showed generally lower concentrations.

Discriminant analysis was applied to the samples, finding that the six varieties could be clearly differentiated by the polyphenolic composition of their grape marcs with a 100 % degree of discrimination, as can be seen in Fig. 4.

Comparison of grape marc obtained from Galician autochthonous and non-autochthonous varieties

Total polyphenols calculated as the sum of the concentration of each compound are displayed in Fig. 5 for all the varieties. It can be seen that native and non-autochthonous varieties cannot be differentiated according to their TP content, which was ≥3500 μg g^{-1} dw for all the samples with the exception of Torrontes grape marc that showed a noticeable lower polyphenolic content. Pinot and Gewurztträminer samples were at the same TP level than Albariño, Godello, Loureiro, Treixadura, and Caiño samples (≥5000 μg g^{-1} dw). Regarding the polyphenols distribution, it was the same in all the varieties, being the flavan-3-ols catechin and epicatechin, the predominant compounds, and the phenolic acids the compounds in lower proportion.

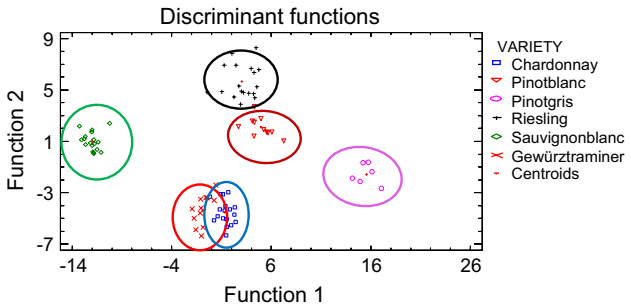

Fig. 4 Discriminant functions for the non-autochthonous varietal grape marc samples experimentally cultivated in Galicia

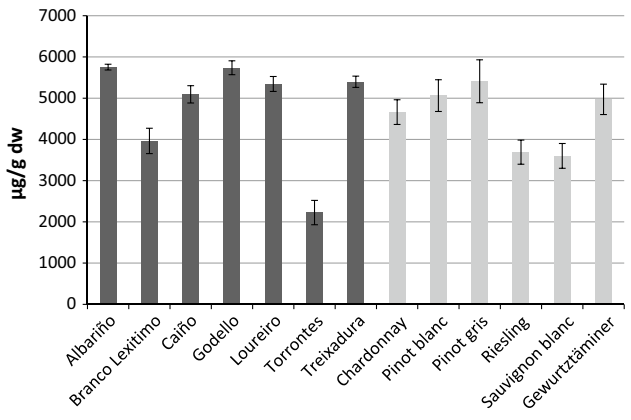

Fig. 5 Comparison of autochthonous and non-autochthonous varietal grape marc according to their polyphenolic content (expressed as the sum of polyphenols)

Conclusions

The phenolic composition and the antioxidant activity of marcs obtained from 13 white grape varieties cultivated in Galicia (115 samples, 22 wineries and 2 experimental centers, 3 vintage years) were determined in order to characterize potential exploitation of these winemaking by-products. Grape marcs from autochthonous and also foreign varieties that are experimentally cultivated in the region were studied. Results indicate that most of the native varietal grape marcs cannot be fully discriminated by its phenolic composition, which would allow a polyphenols extraction of the winemaking wastes independent of the grape variety, an interesting option from an industrial point of view. On the other hand, significant differences found for some of the individual polyphenols among varieties (native and non-native) would allow a varietal selective exploitation of the wastes.

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Compliance with ethical standards

Conflict of interest None.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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