JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Characterization of Polyphenols and Antioxidant Potential of White Grape Pomace Byproducts (*Vitis vinifera* L.)

María Reyes González-Centeno,[†] Michael Jourdes,[‡,§] Antoni Femenia,*[,†] Susana Simal,[†] Carmen Rosselló,[†] and Pierre-Louis Teissedre[‡,§]

[†]Department of Chemistry, University of the Balearic Islands, Crta Valldemossa, Km 7.5, 07122 Palma de Mallorca, Spain

[‡]Univ. Bordeaux, ISVV, EA 4577 Oenologie, 210, Chemin de Leysotte CS 50008, 33882 Villenave d'Ornon Cedex, France

[§]INRA, ISVV, USC 1366 Oenologie, 210, Chemin de Leysotte CS 50008, 33882 Villenave d'Ornon Cedex, France

ABSTRACT: A detailed assessment of the total phenolic and total tannin contents, the monomeric and oligomeric flavan-3-ol composition, the proanthocyanidin profile, and the antioxidant potential of the grape pomace byproducts (considered as a whole, both skins and seeds), derived from four white grape varieties (*Vitis vinifera* L.), was performed. Significant differences ($p < 0.05$) of the total phenolic content, total tannin content, and antioxidant capacity of grape pomace byproducts were observed among the different grape varieties studied. For the first time in the literature, the particular flavan-3-ol composition of the four grape varieties investigated was described for the whole fraction of their grape pomace byproducts. The phenolic composition and antioxidant capacity of grape pomaces were compared to those of their corresponding stems. The global characterization of these white grape varieties provided a basis for an integrated exploitation of both winemaking byproducts as potential, inexpensive, and easily available sources of bioactive compounds for the pharmaceutical, cosmetic, and food industries.

KEYWORDS: *white grape pomace, winemaking byproducts, proanthocyanidins, antioxidant capacity, total phenolic content, mean degree of polymerization*

INTRODUCTION

Over the last few decades, various epidemiological studies have reported a direct relationship between a polyphenol-rich diet and the decreasing risk of developing/suffering certain chronic diseases, such as cardiovascular pathologies, cancer, neurodegenerative disorders, and atherosclerosis, among others.[1−3] Phytochemicals occur naturally in plants as bioactive secondary metabolites due to a response to various forms of environmental stress.[4] Human intake of these compounds with special health benefits mainly takes place through the daily fruit and vegetable consumption. However, phenolic compounds have been found in both the edible and the nonedible parts of the plants. For this reason, agro-industrial byproducts have become valuable raw materials of widespread and increasing interest in terms of exploitation and recovery of their natural bioactive components.[5] The storage, transformation, and/or elimination of the large amounts of agro-industrial residues are a great problem from economic and environmental points of view. Thus, the phenolic and antioxidant characterization of the byproducts is the first step to improving and promoting applications for these materials. In fact, these residues could be an alternative source of natural antioxidants, which are considered completely safe in comparison with synthetic antioxidants, widely used in the food industry but with undesirable effects on human organs.[6]

The winemaking industry produces large quantities of waste residues that reached over the 16 million tons in 2010, when considered that winery byproducts account for over 30% of the grapes used for wine production[7] (10th General Assembly of the International Organization of Vine and Wine, Izmir, 2012). It is well-known that these winemaking byproducts, mainly consisting of grape pomaces and stems, still contain a significant amount of phenolics with beneficial health-related effects, at different concentrations and chemical structures depending on the grape variety considered. Available studies regarding these phenolic compositions are mainly focused on pomace deriving from red grape varieties, whereas little attention has been devoted to the white varieties, which might also contain a wide spectrum of potentially bioactive polyphenols.[8] In fact, to the best of the authors' knowledge, no detailed description of the flavan-3-ol composition of the grape pomaces (skins and seeds considered as a whole) has been previously reported in the literature for any of the white grape varieties investigated in the present research. Thus far, out of the four white varieties considered in the present research, in only one (Chardonnay), the flavan-3-ol profile of separated skin and seed byproducts has already been described.[9−11] There are also very few investigations into the total antioxidant capacity of grape pomace from white grape varieties.[12−14] Furthermore, these reports use a single method, instead of a combination of different assays to give a global vision of the antioxidant properties of the byproduct.

Thus, the aim of the present research was to characterize the phenolic compounds of the grape pomace byproducts of four white different cultivars of *Vitis vinifera* (Chardonnay, Macabeu, Parellada, Premsal Blanc), in order to identify their interesting properties to be used as functional ingredients and to compare

Received: February 16, 2013
Revised: November 4, 2013
Accepted: November 10, 2013
Published: November 10, 2013

dx.doi.org/10.1021/jf403168k | *J. Agric. Food Chem.* 2013, 61, 11579−11587

them with those of their corresponding stems, previously reported by González-Centeno et al.[15] This research consisted of the determination of the total phenolic and total tannin contents of the grape pomace byproducts, the identification and quantification of monomeric and oligomeric (dimers and trimer) flavan-3-ol composition by HPLC-UV-fluo, the determination of the mean degree of polymerization (mDP) of the condensed tannins, and the estimation of their antioxidant capacity by four different procedures, in particular, ABTS, CUPRAC, FRAP, and ORAC assays.

MATERIALS AND METHODS

Chemicals. Copper(II) chloride dihydrate, ammonium acetate, potassium peroxodisulfate, hydrochloric acid, ethyl alcohol, iron(III) chloride hexahydrate, sodium acetate 3-hydrate, glacial acetic acid, Folin Ciocalteau reagent, and gallic acid were purchased from Scharlau (Barcelona, Spain). TPTZ (2,4,6-tri-(2-pyridyl)-s-triazine) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were from Acros Organics (New Jersey, USA). ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) was obtained from Biochemica (Darmstadt, Germany). Sodium dihydrogen phosphate dihydrate, disodium hydrogen phosphate dodecahydrate, fluorescein, AAPH (2,2′-azobis-(2-methylpropionamidine) dihydrochloride, Neocuproine (2,9-dimethyl-1,10-phenanthroline), phloroglucinol, (+)-catechin, (−)-epicatechin, (−)-epigallocatechin (EGC), (−)-epicatechin-3-*O*-gallate (ECG), procyanidin B1 [(−)-epicatechin-(4β-8)-(+)-catechin], and procyanidin B2 [(−)-epicatechin-(4β-8)-(−)-epicatechin] were supplied from Sigma-Aldrich (Saint Quentin Fallavier, France). Acetonitrile (HPLC grade), formic acid (HPLC grade), methanol (HPLC grade), glacial acetic acid (HPLC grade), L-ascorbic acid, and sodium acetate were purchased from Prolabo-VWR (Fontenays/Bois, France). Procyanidin B3 [(+)-catechin-(4α-8)-(+)-catechin], procyanidin B4 [(+)-catechin-(4α-8)-(−)-epicatechin] and trimer C1 [(−)-epicatechin-(4β-8)-(−)-epicatechin-(4β-8)-(−)-epicatechin] were obtained from Polyphenols Biotech (Villenave d'Ornon, France).

Samples. This study was carried out with grape pomace byproducts obtained from the four most representative white grape varieties (*Vitis vinifera* L.) cultivated in the Balearic Islands: Chardonnay, Macabeu, Parellada, and Premsal Blanc. In particular, Parellada and Premsal Blanc are indigenous to Catalonia and the Balearic Islands, respectively, whereas the other two grape varieties considered are well-known and widely cultivated elsewhere. Samples were provided by Pere Seda S.L. winery (Mallorca, Balearic Islands, Spain; latitude 39°33′6″ N and longitude 3°10′30″ E) during the 2009 harvest. To limit the influence of external factors and to allow a better comparison among results, all samples shared the same geographical area, vintage, cultivation system, and viticultural practices. The grapes used were harvested at the optimum technological ripeness, as judged by stabilization of the potential alcohol content and control of the acidity index, the visual lignification degree of seeds, and the adhesion degree of the skins to the seeds, established by the winery.

For the four varieties considered, grape pomace was collected the day of grape harvest after destemming and pressing the grapes under identical conditions. A pneumatic press (Vaslin−Bucher RPS 50, France) was used filled at 75−80% of its capacity. In all cases, the press program applied was as follows: 40 min at a constant pressure of 0.150−0.200 bar (P_{min}) with cycles consisting of 2 min at the inflated position, followed by a rapid deflation and 3 laps; 40 min at an increasing pressure from P_{min} to 1.750−1.800 bar (P_{max}), with an inflation cycle of 3 min, followed by deflation and 2 laps; and then 15 min at P_{max}, with cycles of 3 min at the inflated position, a rapid deflation and 3 laps. After pressing, all the grape pomaces derived from the same variety were combined and homogenized to ensure a representative sampling of the whole grape pomace. All the samples were stored vacuum-packed at −80 °C until analysis.

Experimental data about the phenolic and antioxidant characterization of the corresponding stem byproducts obtained from the same four white grape varieties, geographical origin, and vintage have been previously reported by González-Centeno et al.[15]

Polyphenol Extraction Procedure. Grape pomace byproducts, consisting of both skins and seeds, were first lyophilized and mechanically ground with a ceramic laboratory mortar. An ASE 350 Accelerated Solvent Extraction System (Dionex Corporation, Sunnyvale, CA) was used to extract the phenolic compounds from the obtained powder under the extraction conditions described previously by González-Centeno et al.[15] Briefly, the ground grape pomace (∼10 g) was submitted to eight and three solid/liquid consecutive extractions with acetone/water (80:20, v/v) and with MeOH/water (60:40, v/v) as solvent systems, respectively (each individual extraction used 40 mL of the corresponding solvent system). The ASE experimental variables were pressure (1500 psi), temperature (40 °C), static time (4 min), and preheat time (5 min), by using a N_2 flush to prevent oxidation during extraction. The volume of all collection tubes was combined after extraction and then evaporated under reduced pressure. The obtained solid residue was redissolved in 30 mL of water and lyophilized, prior to final storage as a dry powder under dark conditions until analysis. All samples were extracted in duplicate.

Determination of Total Phenolic Content. The total phenolic content was spectrophotometrically measured according to a modified Folin Ciocalteu method to be applied in 96-well microplates. Stock solutions (10 mg/mL) of the grape pomace extracts were prepared in EtOH/H_2O (25:75, v/v), and a microplate spectrophotometer (MultiSkan Spectrum, Thermo Scientific) was used for the incubation and measurement. Briefly, each well was filled with 184 μL of distilled water and 24 μL of the sample solution, followed by 12 μL of the Folin Ciocalteu reagent and 30 μL of 20% (w/v) Na_2CO_3 solution. Prior to the measurement of the absorbance at 765 nm, the mixture was incubated for 1 h under dark conditions at 25 °C. Gallic acid (0−24 ppm) was used as a standard for calibration. Results, expressed as milligrams of gallic acid per 100 g of grape pomace sample (on a dry matter basis, dm), were a mean of six determinations.

Determination of Total Proanthocyanidins. Total proanthocyanidins were estimated spectrophotometrically through the Bate−Smith reaction, in accordance with Ribereau-Gayon and Stonestreet.[16] Stock solutions of the grape pomace extracts were prepared at a concentration of 0.5 mg/mL by using EtOH/H_2O (10:90, v/v) as solvent. A Varian Cary 300 Bio UV/vis spectrophotometer was used for the absorbance measurements at 550 nm. Total proanthocyanidin determination was performed in triplicate for each grape pomace sample, and results were expressed in milligrams of proanthocyanidins per 100 g of grape pomace sample (dm).

HPLC Analysis of Monomeric and Oligomeric Flavan-3-ols. The equipment used for the HPLC analysis consisted of a Thermo-Finnigan UV−vis detector (Surveyor PDA Plus), a Finnigan fluorescence detector (Surveyor FL Plus Detector), a Finnigan autosampler (Surveyor autosampler Plus), and a Finnigan quaternary pump (Surveyor MS pump Plus) coupled to Xcalibur and ChromQuest software for UV−vis and fluorescence data treatment, respectively. Separation was performed in duplicate on a reversed-phase LiChrospher 100 RP18 (250 mm × 4 mm, 5 μm) column using the method described previously by González-Centeno et al.[15]

Methanolic solutions of the grape pomace extracts (10 mg/mL) were filtered and directly injected. Calibration curves were established using external standards (flavan-3-ol monomers (+)-catechin (C) and (−)-epicatechin (EC); dimers B1, B2, B3, B4; and trimer C1). Results were expressed as milligrams per 100 g of grape pomace sample (dm).

HPLC Analysis of Mean Degree of Polymerization (mDP). Grape pomace extracts were solubilized in MeOH at a concentration of 10 mg/mL. The proanthocyanidin composition, percentage of galloylation (% G), and mDP values of the grape pomace extracts were determined by phloroglucinolysis. Briefly, 200 μL of methanolic solutions of the grape pomace extracts were mixed with 200 μL of the phloroglucinol reagent and left standing to react at 50 °C for 20 min. Finally, 1 mL of 40 mM aqueous sodium acetate was added to stop the reaction. Elution conditions, flow rate, and composition of the mobile phases were fixed as previously described.[15] HPLC separations were

performed on a reversed-phase Waters XTerra RP18 (100 mm × 4.6 mm, 3.5 μm) column by using a Thermo-Accela HPLC instrument including a UV−vis detector (Accela PDA Detector), an autosampler (Accela autosampler), and a quaternary pump (Accela 600 − pump), controlled by Xcalibur data treatment software. All mDP analyses were performed in duplicate. Apparent mDP values were calculated as the ratio between the total number of released subunits and the number of terminal ones, as described by Chira et al.[17] Polymeric proanthocyanidin composition was expressed as weight percentage (wt %).

Evaluation of the Antioxidant Capacity. To achieve a more realistic characterization of the antioxidant properties of the grape pomace byproducts, four different antioxidant capacity assays were applied: ABTS, CUPRAC, and FRAP as spectrophotometric assays, and ORAC, as the fluorometric one. An automated microplate reader was used in all cases, specifically, a MultiSkan Spectrum (Thermo Scientific) for the first three analyses, and a FLUOstar Optima (BMG LabTech), for the ORAC assay.

Modified versions of the original ABTS, CUPRAC, FRAP, and ORAC assays were performed to fit the antioxidant capacity analyses in 96-well microplates according to the procedures described by González-Centeno et al.[15] Trolox standard curves were correlated with the difference in absorbance between a final reading and the reagent blank reading for the spectrophotometric assays, and with the area under the fluorescence curves (AUC) for the ORAC assay. The results were expressed as a mean of six determinations in milligrams of Trolox per gram of grape pomace sample (dm).

For the ABTS, CUPRAC, and FRAP assays, stock solutions of the grape pomace extracts were prepared at a concentration of 0.4 mg/mL by using EtOH/H_2O (25:75, v/v) as solvent. In the case of the ORAC measurement, more diluted stock solutions of the sample extracts (20 mg/L) were considered in 75 mM phosphate buffer (pH 7.4). Trolox standard solutions were then prepared in EtOH/H_2O (25:75, v/v) or 75 mM phosphate buffer (pH 7.4) depending on the analysis performed.

Statistical Analysis. All experimental results were presented as mean values with their corresponding standard deviations. The study of the variability among grape varieties in the polyphenol content and antioxidant properties of their grape pomaces was carried out using the statistical package R version 2.14.2 (R Foundation for Statistical Computing, Wien, Austria). Normality and homoscedasticity of the data were evaluated for all parameters, by using the Shapiro−Wilk Test and Levene's Test, respectively. When populations were distributed normally and presented homogeneity in variance, the parametric ANOVA and Tukey tests were used to evaluate the existence and degree of significant differences. These statistical analyses were substituted, respectively, by the nonparametric Kruskal−Wallis and Pairwise-Wilcox (with BH adjustment) tests, if populations were not distributed normally and/or presented heterogeneity in variance. Correlation between variables and regression analysis were also assessed. Differences at $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Polyphenol Extraction Yields. The use of different reference units (wet or dry matter basis and extract or sample matter basis) hinders accurate comparison of the winemaking byproducts data reported in the literature. For this reason, in order to enhance comparisons with future studies, equivalence factors of polyphenol extraction yields obtained at the present research are given in Table 1, as grams of extract per 100 g of dry matter (dm) and wet matter (wm) of grape pomace samples. Significant differences ($p < 0.05$) were observed among varieties according to their polyphenol extraction yields.

Total Phenolic Content. Table 2 shows the total phenolic content of grape pomaces from the four varieties considered in the present study. The total phenolic results ranged from 3093 ± 266 to 4654 ± 255 mg of GA/100 g dm. The Parellada variety yielded the highest values, followed by Chardonnay, Premsal Blanc, and Macabeu, in that order.

Table 1. Polyphenol Extraction Yields

	polyphenol extraction yield[a]	
	% dm	% wm
Chardonnay	23.3 ± 0.3 a	19.7 ± 0.3 a
Macabeu	32.4 ± 0.5 b	19.5 ± 0.3 a
Parellada	17.2 ± 0.4 c	15.5 ± 0.4 b
Premsal Blanc	33.3 ± 0.1 b	26.2 ± 0.1 c

[a]Results expressed as grams of extract/100 g of dry matter (dm) and wet matter (wm) of grape pomace sample. Letters following the values in each column show the significant differences among grape varieties ($p < 0.05$).

Since all the pomace samples were collected from close vineyards and obtained under the same pressing procedure, the differences observed in total phenolic content among pomace samples are mainly due to the inherent characteristics of each grape variety investigated. As examined in Table 2, except between Chardonnay and Premsal Blanc pomaces, significant differences ($p < 0.05$) were found among all varieties according to their total phenolic content.

Literature data concerning total phenolic content of white grape pomaces as such largely vary, from 1500 mg of GA/100 g dm for Palomino Fino[12] to 4826 mg of GA/100 g dm for Roditis,[8] depending on the grape cultivar, vintage, geographical origin, winemaking practices, and extraction methodology.[18] In any case, all total phenolic values described in Table 2 were in broad agreement with the aforementioned bibliographic range. In particular, a similar total phenolic content was presented (3490 mg of GA/100 g dm) by Llobera et al.[13] for Premsal Blanc pomaces. In the case of seed byproducts, Mandic et al.[19] reported total phenolic values, for both Italian Riesling and Rhine Riesling white varieties, slightly lower than those described for white pomaces considered in the present study. Further, the experimental results of this research are in broad agreement with those found by Anastasiadi et al.[20] for skins and seeds of two native Greek white varieties.

Vine stems are directly discarded during the winemaking process; thus their original phenolic composition is preserved almost intact and, as previously reported, they usually exhibit a noticeably higher phenolic content than the corresponding grape pomaces.[8,13,21] In the present research, when comparing the experimental total phenolic content of both winemaking byproducts, values were, as expected, from 1.2 to 2.5 times lower for the grape pomace extracts.

Total Proanthocyanidin Content. The total proanthocyanidin contents of the grape pomace byproducts, estimated by the Bate−Smith reaction, are described in Table 2. As for the total phenolic content, Parellada exhibited the highest total tannin content (92.1 ± 4.1 mg/g dm), whereas Macabeu pomace presented the lowest value (50.8 ± 0.0 mg/g dm). Significant differences ($p < 0.05$) were observed among the white varieties investigated.

Total proanthocyanidin results obtained in the present research were higher than some values previously reported in the literature for skin and seed byproducts from white and red grape varieties.[6,19,22,23] Nevertheless, Llobera et al.[13] observed total tannin values for Premsal Blanc pomace 2.3-fold higher than those described in the present study for the same white grape variety. These differences could be attributed to the different vintage and viticultural conditions of the samples, as

Table 2. Total Phenolics, Total Proanthocyanidins, and Flavan-3-ol Content of the Grape Pomace Samples

			flavan-3-ol profile[c]						
	total phenolics[a]	total proanthocyanidins[b]	C	EC	B1	B2	B3	B4	C1
Chardonnay	3891 ± 383 a	71.9 ± 2.0 a	47.3 ± 2.2 a	48.8 ± 3.2 a	9.6 ± 0.6 a	13.1 ± 1.0 a	5.0 ± 0.3 a	3.0 ± 0.7 a	9.1 ± 0.3 a
Macabeu	3093 ± 266 b	50.8 ± 0.0 b	15.4 ± 3.0 b	9.5 ± 1.6 b	3.5 ± 0.7 b	4.7 ± 0.8 b	3.9 ± 0.4 a	2.4 ± 0.4 a	2.2 ± 0.6 b
Parellada	4654 ± 255 c	92.1 ± 4.1 c	63.3 ± 7.5 c	22.9 ± 1.3 c	17.0 ± 1.2 c	14.2 ± 1.0 a	14.0 ± 0.5 b	2.1 ± 0.1 a	6.6 ± 0.3 c
Premsal Blanc	3639 ± 200 a	73.2 ± 4.8 a	12.5 ± 1.3 b	12.6 ± 1.2 b	6.7 ± 0.3 d	8.7 ± 0.5 c	5.2 ± 0.1 a	6.0 ± 0.4 b	5.1 ± 1.2 c

[a]Total phenolics expressed as mg of GA/100 g dm. [b]Total proanthocyanidins expressed in mg of tannins/g dm. [c]Flavan-3-ol concentration expressed in mg/100 g dm. C, (+)-catechin; EC, (−)-epicatechin; B1−B4, procyanidin dimers; C1, procyanidin trimer. Letters following the values in each column show the significant differences among grape varieties ($p < 0.05$).

well as to the solvent used during polyphenol extraction and, also, to the standard considered and the analytical technique applied to evaluate the total tannin content.

In contrast with Llobera et al.,[13,21] who reported a higher total proanthocyanidin content for grape pomace byproducts than for the corresponding stems, the present research does not reflect this trend when comparing the experimental results of both winemaking byproducts. In general, total proanthocyanidin contents of grape pomace were lower than those of the corresponding stems, apart from the Chardonnay variety, which presented similar total proanthocyanidin content for both byproducts.

As previously observed,[19] a high significant correlation was found between the total phenolic and total proanthocyanidin contents of the grape pomace extracts ($r = 0.97$, $p < 0.05$).

HPLC Analysis of Monomeric and Oligomeric Flavan-3-ols. The monomeric and oligomeric flavan-3-ol composition of the whole grape pomace byproduct from the four white grape varieties investigated is described in a detailed form in Table 2. All the extracts were analyzed by HPLC to identify and quantify the flavan-3-ols procyanidin B1, procyanidin B3, (+)-catechin, procyanidin B4, procyanidin B2, (−)-epicatechin, and the trimer C1, in this order of elution.

Adding up the individual concentrations of each of the above-mentioned compounds, the total content of flavan-3-ols in grape pomace byproducts ranged from 41.6 to 140.2 mg/100 g dm, for Macabeu and Parellada varieties, respectively. These results consistently agree with the total flavan-3-ol range (29−199 mg/100 g dm) proposed by González-Paramás et al.[24] for the same type of winemaking byproduct. Significant differences were found among the four varieties considered ($p < 0.05$), both Parellada and Chardonnay exhibiting the highest total flavan-3-ol content.

In terms of distribution, both monomers, (+)-catechin and (−)-epicatechin, accounted for 44−71% of the total flavan-3-ol content of grape pomaces depending on the grape variety considered, whereas the dimers represented from 23% to 47% of the total content. Apart from the Premsal Blanc variety, which presented a similar proportion of both fractions, in general, the monomeric fraction was greater than the dimeric one. This observation agrees with that reported by Monrad et al.[25] for Sunbelt red grape pomace (*V. labrusca* L.) and, also, with the results described by different authors for grape seeds (*V. vinifera* L.).[10,18,19,26]

A general ranking order of the individual flavan-3-ol compounds was detected throughout all the varieties investigated. The monomer (+)-catechin was the major flavan-3-ol component of grape pomace byproducts, representing from 49% to 73% of the monomeric fraction and from 22% to 45% of the total flavan-3-ol content. This predominance of the monomer (+)-catechin has been previously observed in the literature for skins and/or seeds of different white grape varieties.[20,26−28] On the other hand, the monomer (−)-epicatechin was the second main component in Macabeu and Parellada varieties, whereas, for Chardonnay and Premsal Blanc, a similar contribution of both (+)-catechin and (−)-epicatechin to their total flavan-3-ol contents was observed. This second behavior was also reported by Escribano-Bailón et al.[29] for seeds of the Tinta del Pais red grape variety.

Regarding the oligomers, the procyanidin B2 was placed as the third major flavan-3-ol component and the most abundant dimer of grape pomace, as previously observed for the same type of winemaking byproduct[24] as well as for seeds[9,27,29,30]

Table 3. Mean Degree of Polymerization (mDP), Percentage of Galloylation (% G), and Structural Composition of Grape Pomace Polymeric Proanthocyanidins[a]

			general composition		terminal subunits			extension subunits	
	mDP	% G	% C	% EC	% C	% EC	% ECG	% C	% EC
Chardonnay	4.5 a	2.0 a	17 a	81 a	44 a	47 a	9 a	9 a	91 a
Macabeu	7.1 b	1.8 a	18 b	80 b	53 b	34 b	13 b	13 b	87 b
Parellada	5.0 c	2.4 b	24 c	74 c	64 c	24 c	12 c	13 b	87 b
Premsal Blanc	10.1 d	1.0 c	16 d	83 d	47 d	43 d	10 d	13 b	87 b

[a]Structural composition expressed as weight percentage (wt %). C, (+)-catechin; EC, (−)-epicatechin; ECG, (−)-epicatechin-3-*O*-gallate. Letters following the values in each column show the significant differences among grape varieties ($p < 0.05$).

from different red and white grape varieties. Some differences occurred in its individual distribution depending on the grape variety: in Chardonnay, Macabeu, and Premsal Blanc, the procyanidin B2 content was clearly major, whereas, in Parellada grape pomace, the procyanidin B1 was predominant. This second behavior was also reported for skins from Verdejo, Malvasia, Sauvignon, Chardonnay, Manzac, and Grenache Blanc white varieties.[9,28] Finally, the oligomers B4 and C1 were minor constituents in all cases, contributing no more than ∼11% of the total flavan-3-ol content.

Significant differences ($p < 0.05$) in terms of quantification of these seven individual compounds revealed a particular monomeric and oligomeric flavan-3-ol composition for pomace byproducts from each white grape variety considered. This observation is in general agreement with the results previously reported for skin[9,11,27,28] and seed[9−11,20,26,27] byproducts separately. Nevertheless, it is important to highlight that no references to the monomeric and oligomeric flavan-3-ol composition of white grape pomace byproducts have been found in the literature, except for the works of Monrad et al.,[25] who investigated the grape pomace from a red *Vitis labrusca* variety rather than a white *Vitis vinifera*, and also the studies of Alonso et al.[12] and Rockenbach et al.,[6] although only (+)-catechin and (−)-epicatechin concentrations were reported.

By comparing the total flavan-3-ol content of white grape pomaces with that of the corresponding stem byproducts, it was generally higher for stems (28.8−354.5 mg/100 g dm).[15] Regarding the monomeric−oligomeric proportion, it was clear that grape pomaces showed a higher percentage of monomers and trimers than stems, but a lower percentage of dimers. Finally, with regard to the ranking order of the individual compounds, the main flavan-3-ols for grape pomaces and stems were the monomer (+)-catechin and the procyanidin B1, respectively. Interestingly, despite being minor constituents, the procyanidin B4 and the trimer C1 were only discerned in grape pomace byproducts. Thus, accordingly to the results previously reported in the literature,[9,27,30] a particular flavan-3-ol profile has been identified for the different winemaking byproducts.

HPLC Analysis of Mean Degree of Polymerization. Results of mDP, % galloylation, and structural composition of grape pomace proanthocyanidins after phloroglucinol reaction are presented in Table 3. Grape pomace mDP values ranged from 4.5 to 10.1, with the Premsal Blanc variety exhibiting the highest value, whereas Chardonnay presented the lowest. Significant differences ($p < 0.05$) were observed among all the four white grape varieties considered.

The literature usually reports data concerning mDP values of skin or seed polymeric proanthocyanidins separately, but no studies for the whole grape pomace as in the present research, apart from that of Torres et al.[31] In general, the mDP of skins is higher than that of seeds,[32] both denoting a large variability depending on the grape variety, vintage, vinegrowing region, and/or analytical technique used. Considering mDP values for grape pomace byproduct as being midway between those of the seed and skin of the literature, the experimental results obtained in this study are in broad agreement with those found by some authors,[22,33−36] but lower than those reported by others.[18,23,37−40] In the case of Parellada grape pomace, the experimental mDP value was higher than that observed by Torres et al.[31] for the whole winemaking byproduct of the same grape variety.

With regard to the polymeric proanthocyanidin profile, (−)-epicatechin was, as expected, the most abundant subunit in grape pomace byproduct, accounting for up to 74 wt % of the total polymeric composition, depending on the grape variety. The (+)-catechin was the second main constituent with concentrations ranging from 16 to 24 wt %. In contrast to grape skin results of a previous report,[39] (−)-epigallocatechin (EGC) was not detected in any of the grape pomace extracts.

In terms of structural composition, the terminal subunits that constituted the grape pomace proanthocyanidins were (+)-catechin and (−)-epicatechin, either in a similar proportion in the cases of Chardonnay and Premsal Blanc grape pomaces or with a higher percentage of (+)-catechin for Macabeu and Parellada. Both behaviors have been previously reported in the literature:[33,36,41] the first one, especially for the seed proanthocyanidin composition, and the second mainly for the skins. Thus, both Chardonnay and Premsal Blanc grape pomaces could be presented as having a higher seed content than the others.

The (−)-epicatechin-3-*O*-gallate (ECG) also contributed as a terminal subunit to the proanthocyanidin profile,[37,39] with concentrations ranging from 9 to 13 wt %, respectively, for Chardonnay and Macabeu grape pomaces. As observed in Table 3, significant differences were found among all grape pomaces according to their terminal subunit composition ($p < 0.05$), a particular terminal polymeric proanthocyanidin profile being shown for each grape variety.

With regard to the extension subunits, as previously observed for red grape skins by Lago-Vanzela et al.[42] and Souquet et al.,[39] (−)-epicatechin participated to a greater extent than (+)-catechin, which only accounted for between 9 and 13 wt % of the total, depending on the grape variety considered. Apart from the Chardonnay variety, which completely differed from the rest ($p < 0.05$), no significant differences ($p > 0.05$) were observed among the other grape pomaces with regard to their proanthocyanidin extension subunit composition.

The grape pomace polymeric proanthocyanidin composition described in this research coincided with previous studies, where (+)-catechin as the main terminal subunit and (−)-epicatechin, as the most abundant extension subunit,

Table 4. Antioxidant Capacity Determined by ABTS, CUPRAC, FRAP, and ORAC Assays for the Grape Pomace Samples[a]

	ABTS	CUPRAC	FRAP	ORAC
Chardonnay	92.4 ± 1.0 a	124.2 ± 9.8 a	76.1 ± 4.8 a	93.7 ± 9.0 a
Macabeu	71.6 ± 1.6 b	106.3 ± 5.1 b	49.0 ± 5.0 b	58.1 ± 7.6 b
Parellada	134.0 ± 4.3 c	209.1 ± 12.8 c	124.8 ± 10.7 c	122.2 ± 7.3 c
Premsal Blanc	93.8 ± 4.0 a	139.7 ± 9.7 d	68.3 ± 6.2 a	62.8 ± 6.2 b

[a]Antioxidant capacities expressed as equivalents of mg of Trolox/g dm. Letters following the values in each column show the significant differences among grape varieties ($p < 0.05$).

were proposed for both skins and seeds from grape pomace byproducts.[18,34,38,39,42]

By comparing both winemaking byproducts, mDP values of grape pomace from Macabeu and Premsal Blanc varieties were significantly higher than those determined for their corresponding stems, as previously observed by Souquet et al.[43]

The extension and terminal subunits found to constitute the polymeric proanthocyanidin profile of grape pomaces and stems were exactly the same. With regard to the extension fraction, a similar composition was observed for both winemaking byproducts, whereas important differences were derived from their terminal fraction. Whereas stem terminal subunits were mainly constituted by (+)-catechin and the presence of (−)-epicatechin and (−)-epicatechin-3-*O*-gallate was very limited, in the case of grape pomaces, the proportion of both terminal (+)-catechin and (−)-epicatechin subunits was more similar, presenting from 2- to 4-fold higher (−)-epicatechin-3-*O*-gallate content than the corresponding stems.

Antioxidant Capacity. Because no single method is able to quantify the total antioxidant capacity of the samples, due to its multifaceted action mechanisms, the use of different assays is becoming a feature of most published studies.[44] Therefore, four different methods were applied in the present research (ABTS, CUPRAC, FRAP, and ORAC) in order to achieve a general view of the antioxidant potential of the white grape pomace byproducts.

The antioxidant capacity results of the grape pomace extracts, measured by the four aforementioned analytical assays, are set out in Table 4. Similar behavior patterns were observed for the results of ABTS, CUPRAC, FRAP, and ORAC assays, regardless of their action mechanism.

Results of the ABTS assay ranged from 71.6 ± 1.6 to 134.0 ± 4.3 mg of Trolox/g dm, for Macabeu and Parellada grape pomaces, respectively. The ABTS values denoted the same ranking order observed in total phenolic content.

Similar results were obtained for CUPRAC, FRAP and ORAC assays, with the Parellada variety yielding the highest antioxidant capacities (209.1 ± 12.8, 124.8 ± 10.7, and 122.2 ± 7.3 mg of Trolox/g dm, respectively). Meanwhile, the Macabeu variety again showed the lowest values, with an antioxidant potential from 2 to 2.5 times smaller than that observed for the Parellada variety, depending on the method used. Further, the CUPRAC assay showed significant differences ($p < 0.05$) among the antioxidant capacity values of all four grape pomaces investigated, whereas, in the ORAC assay, both Macabeu and Premsal Blanc varieties did not differ significantly as having the lowest antioxidant potential ($p > 0.05$).

All the examined white grape pomaces showed considerable antioxidant activity, in particular, the Parellada variety, whose phenolic content was also the greatest among the four white varieties considered. Regardless of the method used, the antioxidant capacity values of Parellada grape pomace were notably high when compared to those of grape pomace from some red varieties.[6,12,45]

A review of the literature concerning antioxidant capacity of winemaking byproducts reveals a quite difficult comparison among the reported data, mainly because of the utilization of different analytical methods (ABTS, CUPRAC, DPPH, FRAP, ORAC, or TRAP, among others), standards, reference units (in a wet or dry matter basis), and/or grape material of reference (grapes, pomaces, or skins and seeds individually). Furthermore, the extraction methodology, the geographical origin of the samples, and the winemaking procedure applied might influence significantly their antioxidant capacity. Nevertheless, regardless of the grape variety and the analytical method considered, the antioxidant capacity values of the grape pomace extracts were of the same order of magnitude as those previously described in the literature. For example, Alonso et al.[12] reported antioxidant capacity ranges measured by ABTS assay in broad agreement with those of the present research (50.1−62.6 mg of Trolox/g dm). Similarly, Sánchez-Alonso et al.[14] reported the antioxidant capacity of Airén white grape pomace, measured by ABTS (71.1 mg of Trolox/g dm) and FRAP assays (116.6 mg of Trolox/g dm), being similar to the experimental results reported in Table 4. Interestingly, in the case of Chardonnay grape pomace, ORAC results from the present research (93.7 ± 9.0 mg of Trolox/g dm) were, as expected, halfway between skin (25.7 mg of Trolox/g dm) and seed (112.8 mg of Trolox/g dm) antioxidant capacities evaluated by Yilmaz et al.[46] for the same white grape variety.

Although the DPPH assay was not performed in this research, since this method shows the same single-electron action mechanism as ABTS, CUPRAC, and FRAP assays, it may be interesting to compare some values reported in the literature for the grape pomace byproducts. For instance, the described DPPH value for Premsal Blanc pomace (173 mg of Trolox/g dm)[13] was 1.8, 1.2, and 2.5 times higher than the ABTS, CUPRAC, and FRAP values, respectively, stated in Table 4 for grape pomace of the same grape variety.

Although both winemaking byproducts exhibited important antioxidant capacities, comparing the experimental results, stems are usually described as having a greater antioxidant potential, regardless of the method used. This phenomenon has been previously observed by other authors.[7,12,13,21] Macabeu, Parellada, and Premsal Blanc varieties showed significant differences between both winemaking byproducts, specifically from 48% to 152% higher antioxidant activity for stems, depending on the grape variety and the method considered. Interestingly, stems of the Premsal Blanc variety were 133, 114, 148, and 128% more active than the corresponding pomaces, when their antioxidant capacity was measured by ABTS, CUPRAC, FRAP, and ORAC assays, respectively. This phenomenon is in agreement with data previously described by Llobera et al.[13] and Makris et al.[7] for stems from Premsal Blanc and Roditis white grape varieties, which presented 68%

and 43% greater potential than their pomaces, respectively. Similar results have also been published for some red grape varieties, with Tempranillo, Cabernet Sauvignon, Syrah, and Manto Negro stems showing, respectively, about 30, 115, 150, and 200% higher antioxidant capacity than their corresponding pomaces.[12,21]

Pearson's correlation coefficients were calculated to evaluate the agreement on the expression of the grape pomace antioxidant capacity among the four assays applied. Regardless of the pair of methods considered, a high, significant and positive correlation was observed ($r \geq 0.84$, $p < 0.05$), suggesting that ABTS, CUPRAC, FRAP, and ORAC assays give comparable and interchangeable antioxidant capacity values for grape pomaces. Correlation coefficients among antioxidant capacities based on ABTS, CUPRAC, and FRAP assays were the highest ($0.97 \leq r \leq 0.99$), whereas ORAC data exhibited lower values ranging from 0.84 to 0.94. The different degree of correlation among these four assays may be due to the different chemical information provided depending on the electron or hydrogen transfer mechanism on which they are based.

These correlation results among antioxidant capacities based on different analytical methods are in broad agreement with those previously reported in the literature for different kinds of winemaking byproducts, such as grape pomaces from Cabernet Sauvignon, Merlot, Bordeaux, and Isabel varieties.[6] In general, ABTS and FRAP antioxidant capacity assays are the most described in the literature, showing a high and positive correlation among them ($r \geq 0.92$, $p < 0.05$), regardless of the food product considered.[44,47,48] With regard to the ORAC assay, the correlation coefficient with ABTS was similar to those observed by Thaipong et al.[48] and González-Centeno et al.,[15] but slightly higher than the value reported by Dudonné et al.[47] for different plant extracts. Meanwhile, when comparing FRAP and ORAC data from Table 4, the correlation degree was significantly higher ($r = 0.94$) than all previously described in the literature for the same pair of antioxidant capacity methods.[47,48]

Further study of the correlation in the present research revealed that total phenolics and antioxidant capacity data were also highly and significantly correlated ($r \geq 0.93$, $p < 0.05$). The most important correlation with total phenolic content was exhibited by the ABTS and FRAP assays ($r = 0.98$, $p < 0.05$), as previously observed in the literature for skins, seeds, and grape pomaces.[6,7,12] When comparing the total tannin content and the antioxidant capacity of the grape pomace extracts (Tables 2 and 4), a high positive correlation was also noted ($r \geq 0.92$, $p < 0.05$), being slightly lower in the case of the ORAC assay ($r = 0.85$, $p < 0.05$).

The present research encloses a detailed and integrated assessment of the phenolic composition (total phenolic and total proanthocyanidin contents, monomeric and oligomeric flavan-3-ol composition, and proanthocyanidin profile) and antioxidant potential of white grape pomace byproducts derived from the vinification process. To the best of the authors' knowledge, no studies addressing this issue in such a detailed form for white varieties have been previously published in the literature.

As observed, the whole fraction of grape pomace byproducts (skins and seeds together) may be considered as an important source of polyphenols and, depending on the end use, separation of skins and seeds in preliminary steps may not be always necessary. A comparison of the phenolic composition and antioxidant properties of grape pomace byproducts with those of their corresponding stems has been carried out in the present research. Although both winemaking byproducts are reported to be potential polyphenol-rich sources, in general, stems are described as having greater phenolic and tannin contents, and a larger antioxidant potential than the grape pomaces from the same grape variety, geographical origin, and vintage.

Along with the dietary fiber profile of both winemaking byproducts described by González-Centeno et al.[49] and the phenolic characterization of the stem byproducts reported by González-Centeno et al.[15] for the same white grape varieties, the phenolic description of the grape pomace byproducts investigated in the present research contributes to a useful database for selecting the most suitable winemaking byproduct and grape variety depending on the phenolic compounds or antioxidant properties required. In this regard, this global characterization may potentially provide the basis for a sustainable process of integrated exploitation of both winemaking byproducts as potential, inexpensive, and easily available sources of bioactive compounds for the pharmaceutical, cosmetic, and food industries.

AUTHOR INFORMATION

Corresponding Author

*E-mail: antoni.femenia@uib.es. Phone: (+34)971173241. Fax: (+34)971173426.

Funding

The authors acknowledge the financial support of the Balearic Government (57/2011 and Research Fellowship FPI09-43165867-L), the Spanish Government (AGL2012-4627 and DPI2012-37466-C03-02), and the European Regional Development Fund (RTA2009-00119-C02-02). The fellowship was cofinanced within the operational program framework by the European Social Fund. The Aquitaine Regional Council participated in the purchase of the ASE 350 Dionex extraction material.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank the Pere Seda S.L. winery for supplying all of the grape pomace samples.

REFERENCES

(1) Gómez-Pinilla, F.; Nguyen, T. T. J. Natural mood foods: The actions of polyphenols against psychiatric and cognitive disorders. *Nutr. Neurosci.* **2012**, *15*, 127−133.

(2) Teissedre, P.-L.; Frankel, E. N.; Waterhouse, A. L.; Peleg, H.; German, J. B. Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wines. *J. Sci. Food Agric.* **1996**, *70*, 55−61.

(3) Weng, C.-J.; Yen, G.-C. Chemopreventive effects of dietary phytochemicals against cancer invasion and metastasis: Phenolic acids, monophenol, polyphenol, and their derivatives. *Cancer Treat. Rev.* **2012**, *38*, 76−87.

(4) Naczk, M.; Shahidi, F. Extraction and analysis of phenolics in food. *J. Chromatogr., A* **2004**, *1054*, 95−111.

(5) Garau, M. C.; Simal, S.; Rosselló, C.; Femenia, A. Effect of air-drying temperature on physico-chemical properties of dietary fibre and antioxidant capacity of orange (*Citrus aurantium* v. Canoneta) byproducts. *Food Chem.* **2007**, *104*, 1014−1024.

(6) Rockenbach, I. I.; Rodrigues, E.; Gonzaga, L. V.; Caliari, V.; Genovese, M. I.; de Souza Schmidt Goncalves, A. E.; Fett, R. Phenolic compounds content and antioxidant activity in pomace from selected

red grapes (*Vitis vinifera* L. and *Vitis labrusca* L.) widely produced in Brazil. *Food Chem.* **2011**, *127*, 174−179.
(7) Makris, D. P.; Boskou, G.; Andrikopoulos, N. K. Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. *J. Food Compos. Anal.* **2007**, *20*, 125−132.
(8) Makris, D. P.; Boskou, G.; Andrikopoulos, N. K. Recovery of antioxidant phenolics from white vinification solid by-products employing water/ethanol mixtures. *Bioresour. Technol.* **2007**, *98*, 2963−2967.
(9) Ricardo-Da-Silva, J. M.; Bourzeix, M.; Cheynier, V.; Moutounet, M. Procyanidin composition of Chardonnay, Mauzac and Grenache Blanc grapes. *Vitis* **1991**, *30*, 245−252.
(10) Fuleki, T.; Ricardo-da-Silva, J. M. Catechin and procyanidin composition of seeds from grape cultivars grown in Ontario. *J. Agric. Food Chem.* **1997**, *45*, 1156−1160.
(11) Rodríguez Montealegre, R.; Romero Peces, R.; Chacón Vozmediano, J. L.; Martínez Gascuena, J.; García Romero, E. Phenolic compounds in skins and seeds of ten grape Vitis vinifera varieties grown in a warm climate. *J. Food Compos. Anal.* **2006**, *19*, 687−693.
(12) Alonso, A. M.; Guillen, D. A.; Barroso, C. G.; Puertas, B.; Garcia, A. Determination of antioxidant activity of wine byproducts and its correlation with polyphenolic content. *J. Agric. Food Chem.* **2002**, *50*, 5832−5836.
(13) Llobera, A.; Canellas, J. Antioxidant activity and dietary fibre of Prensal Blanc white grape (*Vitis vinifera*) by-products. *Int. J. Food Sci. Tech.* **2008**, *43*, 1953−1959.
(14) Sánchez-Alonso, I.; Jiménez-Escrig, A.; Saura-Calixto, F.; Borderías, A. J. Antioxidant protection of white grape pomace on restructured fish products during frozen storage. *LWT* **2008**, *41*, 42−50.
(15) González-Centeno, M. R.; Jourdes, M.; Femenia, A.; Simal, S.; Rosselló, C.; Teissedre, P.-L. Proanthocyanidin composition and antioxidant potential of the stem winemaking byproducts from 10 different grape varieties (*Vitis vinifera* L.). *J. Agric. Food Chem.* **2012**, *60*, 11850−11858.
(16) Ribereau-Gayon, P.; Stonestreet, E. Dosage des tanins du vin rouge et détermination de leur structure. *Chim. Anal.* **1966**, *48*, 188−196.
(17) Chira, K.; Pacella, N.; Jourdes, M.; Teissedre, P.-L. Chemical and sensory evaluation of Bordeaux wines (Cabernet-Sauvignon and Merlot) and correlation with wine age. *Food Chem.* **2011**, *126*, 1971−1977.
(18) Monagas, M.; Gómez-Cordovés, C.; Bartolomé, B.; Laureano, O.; Ricardo-Da-Silva, J. M. Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from *Vitis vinifera* L. cv. Graciano, Tempranillo, and Cabernet Sauvignon. *J. Agric. Food Chem.* **2003**, *51*, 6475−6481.
(19) Mandic, A. I.; Dilas, S. M.; Cetkovic, G. S.; Canadanovic-Brunet, J. M.; Tumbas, V. T. Polyphenolic composition and antioxidant activities of grape seed extract. *Int. J. Food Prop.* **2008**, *11*, 713−726.
(20) Anastasiadi, M.; Pratsinis, H.; Kletsas, D.; Skaltsounis, A.-L.; Haroutounian, S. A. Bioactive non-coloured polyphenols content of grapes, wines and vinification by-products: Evaluation of the antioxidant activities of their extracts. *Food Res. Int.* **2010**, *43*, 805−813.
(21) Llobera, A.; Cañellas, J. Dietary fibre content and antioxidant activity of Manto Negro red grape (*Vitis vinifera*): Pomace and stem. *Food Chem.* **2007**, *101*, 659−666.
(22) Obreque-Slier, E.; Pena-Neira, A.; López-Solis, R.; Zamora-Marin, F.; Ricardo-da-Silva, J. M.; Laureano, O. Comparative study of the phenolic composition of seeds and skins from Carmenere and Cabernet Sauvignon grape varieties (*Vitis vinifera* L.) during ripening. *J. Agric. Food Chem.* **2010**, *58*, 3591−3599.
(23) Travaglia, F.; Bordiga, M.; Locatelli, M.; Coisson, J. D.; Arlorio, M. Polymeric proanthocyanidins in skins and seeds of 37 *Vitis vinifera* L. cultivars: A methodological comparative study. *J. Food Sci.* **2011**, *76*, C742−C749.
(24) González-Paramás, A. M.; Esteban-Ruano, S.; Santos-Buelga, C.; De Pascual-Teresa, S.; Rivas-Gonzalo, J. C. Flavanol content and antioxidant activity in winery byproducts. *J. Agric. Food Chem.* **2004**, *52*, 234−238.
(25) Monrad, J. K.; Howard, L. R.; King, J. W.; Srinivas, K.; Mauromoustakos, A. Subcritical solvent extraction of procyanidins from dried red grape pomace. *J. Agric. Food Chem.* **2010**, *58*, 4014−4021.
(26) Santos-Buelga, C.; Francia-Aricha, E. M.; Escribano-Bailón, M. T. Comparative flavan-3-ol composition of seeds from different grape varieties. *Food Chem.* **1995**, *53*, 197−201.
(27) Bourzeix, M.; Weyland, D.; Heredia, N. Étude des catéchines et des procyanidols de la grappe de raisin, du vin et d'autres dérivés de la vigne. *Bull. OIV* **1986**, *669−670*, 1171−1254.
(28) Escribano-Bailón, M. T.; Guerra, M. T.; Rivas-Gonzalo, J. C.; Santos-Buelga, C. Proanthocyanidins in skins from different grape varieties. *Z. Lebensm.-Unters. Forsch.* **1995**, *200*, 221−224.
(29) Escribano-Bailón, M. T.; Gutiérrez-Fernández, Y.; Rivas-Gonzalo, J. C.; Santos-Buelga, C. Characterization of procyanidins of *Vitis vinifera* variety Tinta del Pais grape seeds. *J. Agric. Food Chem.* **1992**, *40*, 1794−1799.
(30) Ricardo-Da-Silva, J. M.; Rosec, J.-Ph.; Bourzeix, M.; Mourgues, J.; Moutounet, M. Dimer and trimer procyanidins in Carignan and Mourvèdre grapes and red wines. *Vitis* **1992**, *31*, 55−63.
(31) Torres, J. L.; Varela, B.; Garcia, M. T.; Carilla, J.; Matito, C.; Centelles, J. J.; Cascante, M.; Sort, X.; Bobet, R. Valorization of grape (*Vitis vinifera*) byproducts. Antioxidant and biological properties of polyphenolic fractions differing in procyanidin composition and flavonol content. *J. Agric. Food Chem.* **2002**, *50*, 7548−7555.
(32) Pinelo, M.; Arnous, A.; Meyer, A. S. Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. *Trend Food Sci. Technol.* **2006**, *17*, 579−590.
(33) Fernández, K.; Kennedy, J. A.; Agosín, E. Characterization of *Vitis vinifera* L. Cv. Carménère grape and wine proanthocyanidins. *J. Agric. Food Chem.* **2007**, *55*, 3675−3680.
(34) Lago-Vanzela, E. S.; Da-Silva, R.; Gomes, E.; García-Romero, E.; Hermosín-Gutiérrez, I. Phenolic composition of the Brazilian seedless table grape varieties BRS Clara and BRS Morena. *J. Agric. Food Chem.* **2011**, *59*, 8314−8323.
(35) Llaudy, M. C.; Canals, R.; Miquel Canals, J.; Zamora, F. Influence of ripening stage and maceration length on the contribution of grape skins, seeds and stems to phenolic composition and astringency in wine-simulated macerations. *Eur. Food Res. Technol.* **2008**, *226*, 337−344.
(36) Mattivi, F.; Vrhovsek, U.; Masuero, D.; Trainotti, D. Differences in the amount and structure of extractable skin and seed tannins amongst red grape varieties. *Aust. J. Grape Wine Res.* **2009**, *15*, 27−35.
(37) Hanlin, R. L.; Downey, M. O. Condensed tannin accumulation and composition in skin of Shiraz and Cabernet Sauvignon grapes during berry development. *Am. J. Enol. Vitic.* **2009**, *60*, 13−23.
(38) Hernández-Jiménez, A.; Gómez-Plaza, E.; Martínez-Cutillas, A.; Kennedy, J. A. Grape skin and seed proanthocyanidins from Monastrell × Syrah grapes. *J. Agric. Food Chem.* **2009**, *57*, 10798−10803.
(39) Souquet, J. M.; Cheynier, V.; Brossaud, F.; Moutounet, M. Polymeric proanthocyanidins from grape skins. *Phytochemistry* **1996**, *43*, 509−512.
(40) Chira, K.; Schmauch, G.; Saucier, C.; Fabre, S.; Teissedre, P.-L. Grape variety effect on proanthocyanidin composition and sensory perception of skin and seed tannin extracts from Bordeaux wine grapes (Cabernet Sauvignon and Merlot) for two consecutive vintages (2006 and 2007). *J. Agric. Food Chem.* **2009**, *57*, 545−553.
(41) Hanlin, R. L.; Kelm, M. A.; Wilkinson, K. L.; Downey, M. O. Detailed characterization of proanthocyanidins in skin, seeds, and wine of Shiraz and Cabernet Sauvignon wine grapes (*Vitis vinifera*). *J. Agric. Food Chem.* **2011**, *59*, 13265−13276.
(42) Lago-Vanzela, E. S.; Da-Silva, R.; Gomes, E.; García-Romero, E.; Hermosín-Gutiérrez, I. Phenolic composition of the edible parts (flesh

and skin) of Bordo grape (*Vitis labrusca*) using HPLC-DAD-ESI-MS/MS. *J. Agric. Food Chem.* **2011**, *59*, 13136−13146.
(43) Souquet, J. M.; Labarbe, B.; Le Guerneve, C.; Cheynier, V.; Moutounet, M. Phenolic composition of grape stems. *J. Agric. Food Chem.* **2000**, *48*, 1076−1080.
(44) Pellegrini, N.; Serafini, M.; Colombi, B.; Del Rio, D.; Salvatore, S.; Bianchi, M.; Brighenti, F. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *J. Nutr.* **2003**, *133*, 2812−2819.
(45) Rockenbach, I. I.; da Silva, G. L.; Rodrigues, E.; Kuskoski, E. M.; Fett, R. Solvent influence on total polyphenol content, anthocyanins, and antioxidant activity of grape (*Vitis vinifera*) bagasse extracts from Tannat and Ancelota - Different varieties of *Vitis vinifera* varieties. *Cienc. Tecnol. Aliment.* **2008**, *28*, 238−244.
(46) Yilmaz, Y.; Toledo, R. T. Major flavonoids in grape seeds and skins: Antioxidant capacity of catechin, epicatechin, and gallic acid. *J. Agric. Food Chem.* **2004**, *52*, 255−260.
(47) Dudonné, S.; Vitrac, X.; Coutière, P.; Woillez, M.; Mérillon, J.-M. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *J. Agric. Food Chem.* **2009**, *57*, 1768−1774.
(48) Thaipong, K.; Boonprakob, U.; Crosby, K.; Cisneros-Zevallos, L.; Byrne, D. H. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J. Food Compos. Anal.* **2006**, *19*, 669−675.
(49) González-Centeno, M. R.; Rosselló, C.; Simal, S.; Garau, M. C.; López, F.; Femenia, A. Physico-chemical properties of cell wall materials obtained from ten grape varieties and their byproducts: Grape pomaces and stems. *LWT—Food Sci. Technol.* **2010**, *43*, 1580−1586.