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Comparison of extraction methods for exploitation of grape skin residues from ethanol distillation

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ABSTRACT

Four extraction techniques—namely, conventional maceration, ultrasound-assisted extraction, microwave-assisted extraction, and superheated liquid extraction (SHLE)—have been compared to evaluate their suitability to obtain valuable compounds from a raw material traditionally of scant interest: grape skin residues from ethanol-distillation. With this aim, red- and white-grape skins were separated from the rest of the pomace residue and subjected to extraction with 1:1 ethanol–acidic water as extractant in order to obtain the largest possible number of valuable compounds from this material, which has so far been used only as a heat source. The resulting extracts were characterized by the Folin–Ciocalteu and Ferric Reducing Antioxidant Power tests and by liquid chromatography–time-of-flight/mass spectrometry (LC–TOF/MS). The composition of the extracts under each of the optimal conditions was studied by LC–TOF/MS, and the information thus obtained compared by Venn diagrams. These diagrams, together with the extracted base peak chromatograms, were used to assess the optimal working conditions. Tentative identification of compounds was conducted using open-free databases. Grape skins from distillation industries are a source of compounds of interest for the food and nutraceutical fields.

1. Introduction

Grape production worldwide is about 70 million tonnes, 80% of which is dedicated to winemaking and, practically the rest amount to the grape juice industry [1]. There is an unequal use of by-products obtained from vine cultivars or from the wine-making process such as vine-shoots, vinification lees or grape skins that, in any case, allow obtaining high-added value products [2]. Among these by-products, grape skins should be emphasized due to their significant content in monomeric and polymeric molecules such as anthocyanins, flavan-3-ols, flavonols, dihydroflavonols, hydroxycinnamoyl tartaric acids, hydroxybenzoic acids and hydroxystilbenes [3]. Attending to this composition, grape skins are considered a potential source for isolation of natural compounds [4].

The composition of grape skins is characterized by the vine cultivar, with special significance of the grape color. Overall, red grapes possess higher crude protein, fat and ash contents than white grapes [5,6]. They also have higher total extractable pectins, dietary fiber, neutral sugars, condensed tannins and resistant proteins. White grape skins have significantly higher soluble sugars, uronic acid and Klason lignin than red skins [7].

From a nutraceutical point of view, an interesting fraction of compounds in grapes is that formed by phenolic compounds, which are endowed in general terms with a high antioxidant capacity and are at higher concentrations in skin and seeds than in grape pulp [2,8]. Usually, the amount of total phenols in white grape varieties is lower than in red grapes, as the former ones do not synthesize anthocyanins [5]. These are phenolic compounds with flavonoid structure responsible for color of the red grapes [9,10], while the low-molecular weight flavan-3-ols (such as catechins) and procyanidin oligomers are responsible for bitterness [4]. The nutraceutical interest of phenolic compounds is out of doubt. Phenols are considered key compounds contributing to the antioxidant potential of grapes, wine and other fruits, which is responsible of the health benefits attributed to this fraction [11–14].

After the winemaking process, low-quality wines, grape pomace (skins and seeds) and vinification lees are mainly destined to distilleries to produce alcohol. In the case of pomace, this residue is subjected to an extraction proccess (cleaning) with water, yielding clean pomace and pomace extract, called pickax [15]. Currently, this extraction is performed by diffusion in industrial extractors type "broadcaster band" with a processing capacity of around 300,000 kg/day. After extraction, the clean pomace, completely depleted in sugar, is subjected to

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fermentation as a preparation step for alcohol distillation. Following alcohol isolation, the resulting residue is wasted although valorization studies are demanded. One example is the detartration process to isolate tartaric salts present in grape skins. The exploitation strategies have not considered the isolation of phenolic compounds, which could be a challenge taking into account the different exhaustive steps applied to the pomace residue. Phenolic extracts from this residue could increase the value of this residue.

The extraction of phenols remains as a challenge due to the variety of compounds and the chemical complexity of the sample. This step is focused on the removal of phenolic compounds from the vacuolar structures where they are found, either through rupturing plant tissue or through a diffusion process [16].

Maceration is the most commonly used procedure for leaching phenolic compounds from grapes and residues from them, where the solid sample is stirred with a suitable liquid for long times [17]. However, the different treatments to which grape pomace is subjected suggest the possibility of using auxiliary energies to enhance the leaching efficiency of strongly retained compounds. In this sense, the leaching step can be assisted by auxiliary energies such as microwaves [18,19] or ultrasound [20]. One other alternative is to use supercritical fluids, which has been reported for the leaching of relevant phenolic compounds from grape skin [21]; however, the hight cost of the common supercritical extractant—CO_2— and its nonpolar character make its application non-attractive for phenols extraction. In this context, the use of superheated solvents can be an interesting alternative for extracting phenolic compounds from grapes and their residues. The dielectric constant of the extractant decreases by increasing the temperature above its boiling point and polar and relatively non-polar compounds can be extracted by ambient-temperature polar extractants [22].

The main aim of the present research was to compare the present and traditional extraction techniques to propose an optimum method for isolation of priced compounds from a raw material with very scant value: waste from distillery industries using the cake obtained after alcohol distillation from red- and white-grape pomace. Three present extraction techniques (*viz.* microwave-assisted extraction, MAE; ultrasound-assisted extraction, USAE; and superheated-liquid extraction, SHLE) have been selected and used in comparison with conventional maceration extraction, CME, using overall quantitation methods and individual characterization by LC–TOF/MS in order to know both the extraction efficiency of each at a whole, and the compounds extracted by the different working conditions provided by each extraction technique. A final aim was to demonstrate that not only the waste from the winemaking industry can be exploited to obtain valuable compounds, but that these compounds—and even others resulting from the drastic conditions to which grape pomace is subjected for ethanol distillation—can be obtained from the ''waste from the waste'' of this industry.

2. Materials and methods

2.1. Samples

Grape pomace generated in the winemaking process from red and white grapes was collected in an industrial plant (''Alcoholeras Reunidas, S.A.'', Ciudad Real, Spain) after alcohol distillation. Skins from red and white grapes were separated from the corresponding pomace. After separation, both types of skins were dried for 72 h at 35 °C, then milled and sieved with a 40-mm mesh-particle size (less than 0.42 mm d). The unknown and heterogeneous origin of the massive raw materials subjected to distillation prevents from knowing the degradation caused by the drastic conditions of ethanol-distillation through comparison of the materials before and after this step.

2.2. Reagents

Ethanol (96% v/v) PA from Panreac (Barcelona, Spain) and distilled water were used to prepare the different ethanol–water mixtures. LC–MS grade formic acid and acetonitrile (ACN) were purchased from Scharlab (Barcelona, Spain). Deionized water (18 MΩ cm) was obtained from a Millipore (Bedfore, MA, USA) Milli-Q plus system, and *n*-hexane, for liquid–liquid extractions, was from LiChrosolv (Merck, Darmstadt, Germany). TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were from Fluka (Buches, Switzerland). The Folin–Ciocalteu (F–C) reagent, sodium carbonate, gallic acid and AAPH (2,2′-azobis-2-methyl-propanimidamide dihydrochloride) were from Sigma (St. Louis, USA).

2.3. Apparatus and instruments

A grinder (Ball Mill Restch MM301) was used to mill the skins. Superheated liquid extractions were performed by a laboratory-made dynamic extractor [23], consisting of the following units: (a) an extractant supply; (b) a high-pressure pump (Shimadzu LD-AC10), which propels the extractant through the system; (c) a switching valve placed next to the pump to develop static extractions; (d) a stainless-steel cylindrical extraction chamber (550 mm × 10 mm i.d., 4.3 mL internal volume) where the sample is placed. This chamber was closed at both ends with screws whose caps contain cotton made filters to ensure the sample is not carried away by the extractant; (e) a restriction valve to maintain the desired pressure in the system; (f) a cooler made of a stainless steel tube (1 m length, 0.4 mm i.d.) and refrigerated with water; (g) a gas chromatograph oven (Konix, Cromatix KNK-2000) where the extraction chamber was placed and heated. Both chamber and tubing system were of high quality stainless-steel to avoid or minimize corrosion under drastic working conditions.

Microwave-assisted extractions were carried out in a Microdigest 301 digestor of 200 W maximum power by Prolabo (Paris, France), furnished with a microprocessor programmer to control the microwave unit and used to accelerate solid–liquid extraction, and provided.

Ultrasound-assisted extractions were performed with a Branson 450 digital sonifier (20 kHz, 450 W) equipped with a cylindrical titanium-alloy probe (12.70 mm diameter), which was immersed into a lab-made stainless-steel container with eight compartments to place test tubes, which were thus subjected to similar irradiation [20].

The absorbance of the extracts after reaction with the F–C reagent and the FRAP (Ferric Reducing Antioxidant Power) assay was monitored by a Thermo Spectronic Helios Gamma spectrometer (Waltham, MA, USA). Shaking and centrifugation of the extracts were carried out by means of an MS2 Minishaker Vortex (IKA, Germany) and a Mixtasel centrifuge (Selecta, Barcelona, Spain), respectively.

All samples were analyzed by a 1200 Series LC system (Agilent Technologies, Waldbronn, Germany) coupled to an Agilent 6530 TOF mass spectrometer with an electrospray ionization (ESI) source.

2.4. Superheated-liquid extraction

One gram of milled grape skin was placed into the extraction cell installed into the gas chromatograph oven; then, a relative high flow rate (7 mL/min) was used for 1 min to fill the cell rapidly. To ensure the absence of air inside the extraction cell, the restrictor valve was kept open until the first drop of extractant

appeared. At that moment, the restrictor valve was closed and when the desired pressure was reached, the switching valve was closed, the pump was turned off and the oven was switched on. During temperature rising, the switching valve had to be opened at short intervals to prevent the pressure from surpassing the working value. Once the selected temperature and pressure were reached, static extraction was performed for a preset time. Finally, the oven was switched off, the chamber was cooled below the boiling point of ethanol and then, the switching valve and the restrictor valve were switched to enable new extractant to flow through the cell and flush out the extract.

2.5. Extraction protocols with auxiliary energies

Two grams grape skins were placed into the extraction vessel with 20 mL 50% (v/v) aqueous ethanol acidified with 0.8% (v/v) HCl. The suspension was subjected to microwave assistance for 10 min with a power irradiation of 140 W. After extraction, the liquid phase was isolated by centrifugation and stored at −20 °C until analysis.

The same amount of material and extractant were located in a glass vessel that was introduced in a water bath at 25 °C located in a sound-proof chamber. The ultrasonic probe was immersed in the extraction vessel through the upper part of the chamber. Ultrasonic irradiation was applied for 10 min with a duty cycle of 0.5 s/s and 60% of the nominal power of the converter.

2.6. Conventional maceration extraction (CME)

Two grams of grape skins were extracted with 20 mL 50% (v/v) acidified with 0.8% (v/v) HCl aqueous ethanol by stirring at 40 °C for 24 h.

2.7. Determination of total phenols by the F–C method

The total amount of phenolic compounds was quantified by the F–C method using gallic acid as standard. With this purpose, a calibration curve was run using solutions of 1, 10, 25, 50, 75 and 100 mg/L of this acid (Absorbance = 0.0065 Concentration + 0.1286, $R^2 = 0.9909$). A 0.5-mL aliquot of extract, 10 mL of distilled water, 1 mL of F–C reagent and 3 mL of Na_2CO_3 (20%, w/v) were mixed, made to 25 mL with distilled water and heated at 50 °C for 5 min. After heating, the samples were kept at room temperature for 30 min and, finally, the absorbance was measured at 765 nm against a blank solution containing distilled water instead of extract. The concentration of phenolic compounds thus obtained was multiplied by the dilution factor of the extract volume and divided by the amount of grape skins used. The results were expressed as equivalent to milligrams of gallic acid (mg GAE) per mL of grape skin extract (mg GAE/mL of extract) [24].

2.8. FRAP assay

The antioxidant (AOP) potential of grape skin extracts was determined using the FRAP assay, based on reduction of the ferric tripyridyltriazin [Fe(III)–TPTZ] complex to ferrous tri-pyridyl triazin [Fe(II)–TPTZ] at low pH, by the action of antioxidants in the target solution. The ferrous complex [Fe(II)–TPTZ] has an intense blue color that which can be monitored at 593 nm. The assay response was standardized against the antioxidant standard Trolox.

2.9. LC–TOF/MS analysis

An injection volume of 20 μL and a flow rate of 1 mL/min were used. A mobile phase A consisting of 0.2% (v/v) formic acid aqueous solution and a mobile phase B consisting of acetonitrile constituted the chromatographic phases to establish the following gradient method: from 96% to 50% A in 60 min, from 50% to 0% A in 5 min. MassHunter Workstation Data acquisition software (Agilent Technologies) was used to control the instrument. Data were processed using MassHunter Qualitative Analysis software (Agilent Technologies). Extraction of unknown molecular features from raw data was carried out by the Molecular Feature Extraction (MFE) algorithm in MassHunter Qualitative analysis software—taking as molecular feature any molecule, ion, etc. structural and isotopically identifiable as a separately distinct entity. The feature extraction algorithm took all ions into account exceeding 1000 counts with a charge state equal to or above one and a feature had to be composed of two or more ions to be valid (*e.g.* two ions in the isotope cluster). The theoretical formula adjusted to the corresponding isotopic distribution of molecular features was generated with the Molecular Formula Generation software (Agilent Technologies). Using background subtracted data, files in compound exchange format (.cef files) were created for each sample and exported into the Mass Profiler Professional (MPP) software package (version 2.0, Agilent Technologies, Santa Clara, CA, USA) for further processing. In the next step, alignment of RT and m/z values was carried out across the sample set using a tolerance window of 0.2 min and 5 ppm, respectively (Table 1). Analyses were processed using MassHunter Qualitative software and tentative identification of compounds was performed using the METLIN Personal Metabolite Database and PlantCyc [25].

3. Results and discussion

3.1. Comparison of extraction techniques

Four extraction techniques were selected to compare their efficiency for isolation of compounds from grape skins as sample preparation strategy. These were CME as reference, USAE and MAE to check the influence of auxiliary energies for extraction assistance, and SHLE to take benefits from the superheated state of a suited extractant. Proper comparison required to use the same amount of material, volume and composition of the extractant. This was set at 50:50 (v/v) ethanol–water with 0.8% (v/v) HCl, according to preliminary experiments [23]. A hydroalcoholic extractant was selected to favor isolation of polar and mid-polar compounds. Ethanol was used as organic solvent avoiding other toxic solvents such as methanol, acetonitrile, chloroform or hexane, because of the potential use of the extracts for human consumption. Apart from that, an acid pH was required to enhance hydrolysis of polymeric structures and release monomeric metabolites, easily solubilized in this way. Other extraction conditions used were based on previous MAE [18,19,26], USAE [20] and SHLE [23] methods found in the literature for isolation of natural products from vegetal materials. Thus, in the case of MAE and USAE, the irradiation power was set at intermediate values, while the extraction time was planned for 10 min in both cases (0.5 s/s irradiation cycle for USAE). Concerning SHLE, the temperature was set at 180 °C, and 60 min was adopted as extraction time.

Since the same ratio between sample weight and extractant volume was used in the four isolation protocols, the extracts obtained were analyzed by LC–TOF/MS in accurate mode with the same dilution pattern. Supplementary Fig. 1 illustrates the TOF chromatograms (Base Peak Chromatograms, BPC) for extracts from red and white grape skins. As can be seen, the extracts were predominantly rich in polar compounds, mainly eluted within 28 min of the chromatographic gradient described under "LC–TOF/MS analysis". This elution time corresponds to a chromatographic mobile phase with 25% ACN. Visually, in the case of white

Table 1
Identified compounds in the extracts from grape skins.

Flavonoids	**Theoretical *m/z***	**Experimental *m/z***	**Formula**	**Grape skin**	
				Red	White
Anthocyanins					
Malvidin-3-glucoside acetaldehyde (Vitisin B)	528.1035	528.1028	C23H25ClO12	v	χ
Flavanols					
Epicatechin	290.079	290.0807	C15H14O6	χ	v
Catechin	290.079	290.0807	C15H14O6	v	v
Epicatechin-o-gallate	442.09	442.0902	C22 H18O10	v	χ
Epigallocatechin	306.074	306.0725	C15H14O7	v	v
Gallocatechin-o-gallate	458.0849	458.0867	C22H18O11	v	χ
Procyanidin B2	578.1424	578.1436	C30H26O12	v	χ
Flavonols					
Kaempferol	286.0477	286.0489	C15H10O6	v	v
Myricetin	318.0376	318.0395	C15H10O8	v	χ
Quercetin	302.0427	302.0427	C15H10O7	v	v
Isorhamnetin	316.0583	316.0613	C16H12O7	χ	v
Myricetin-3-o-glucoside	480.0904	480.0925	C21H20O3	v	χ
Myricetin-7-o-glucoside	480.0904	480.0902	C21H20O3	v	v
Quercetin-3-o-glucoside	464.0955	464.0964	C21H20O12	v	χ
Non flavonoids					
Phenolic acids					
Cinnamic acid	148.0524	148.0523	C9H8O2	χ	v
Caffeic acid	180.0423	180.0423	C9 H8O4	v	v
o-Coumaric acid	164.0473	164.0476	C9H8O3	v	v
Ferulic acid	194.0579	194.0574	C10H10 O4	χ	v
Gallic acid	170.0215	170.022	C7H6O5	v	v
4-Hydroxybenzoic acid	138.0317	138.0317	C7H6O3	v	v
Protocatechuic acid	154.0266	154.0268	C7H6O4	v	v
Syringic acid	198.0528	198.0527	C9H10O5	v	χ
Stilbenes					
Trans-resveratrol	228.0786	228.0786	C14 H12O3	v	v
α-Viniferin	678.189	678.188	C42H30O9	v	v
Astringinin	244.0736	244.0736	C14H12O4	v	v
Trans-ε-viniferin	454.1416	454.1426	C28H22O6	v	v
Piceid	390.1315	390.1331	C20H22O8	v	v
Trans-pterostilbene	256.1099	256.1097	C16H16O3	χ	χ
Other phenols					
Ethyl ferulate	222.0892	222.0902	C12H14ClO4	v	v
Ethyl protocatechuate	182.0579	182.0583	C9H10O4	v	v
Lignin monomers and derivatives					
Pyrocatechol	110.0368	110.0368	C6H6O2	v	v
Pyrogallol	126.0317	126.0311	C6H6O3	v	v
Syringol	154.063	154.225	C8H10O3	v	χ
4-Methylpyrocatechol	120.0575	120.0578	C8H8O	v	v
4-Vinylguaiacol	122.0368	122.0365	C7H6O2	v	v
Coniferaldehyde	178.063	178.0627	C10H10O3	v	v
Phenolic ketones					
p-Hydroxybenzalacetone	162.0681	162.0683	C10H10O2	v	v
Vanillin	152.0473	152.0472	C8H8O3	v	v
1-3-4-Dihidroxyphenyl-1propanone	166.063	166.065	C9H10O3	χ	v
Methoxy and ethoxy phenols					
2,6-Dimethoxy-4-1-propenyl-phenol	194.0943	194.0941	C11H14O3	χ	v
4-ethoxyphenol	138.0681	138.0878	C8H10O2	χ	χ
Furanic					
5-Acetoxymethylfurfural	168.0423	168.0423	C8H8O4	v	v
2-Furancarboxylic acid ethyl ester	140.0473	140.0466	C7H8O3	v	χ

grape skins extract, higher peaks were obtained with SHLE in the first five min of elution. Since this chromatographic time, the highest signals corresponded to the extracts obtained by CME. The opposite situation was found in the analysis of extracts from red-grape skins. Thus, CME reported higher chromatographic peaks during the first 5 min, but the peaks were, in general, higher for SHLE in the rest of the chromatogram.

The first test for comparison of the efficiency of the extraction alternatives was to compare the molecular features obtained as described under "LC–TOF/MS analysis". For this purpose, data sets obtained by analysis of blanks (extraction solvents) were subtracted from the data set corresponding to the analytical samples in the step for extraction of molecular entities in each raw data file. Venn diagrams are shown in Fig. 1 for each type of grape skin. As can be seen, similar behavior showed the results obtained for white and red grape skins. In both cases, CME and SHLE reported the highest number of detected molecular features (267 and 318 in CME for white and red grape skins, respectively, *versus* 244 and 309 in SHLE for white and red grape skins, respectively). By contrast, MAE reported 195 and 260 molecular features for white

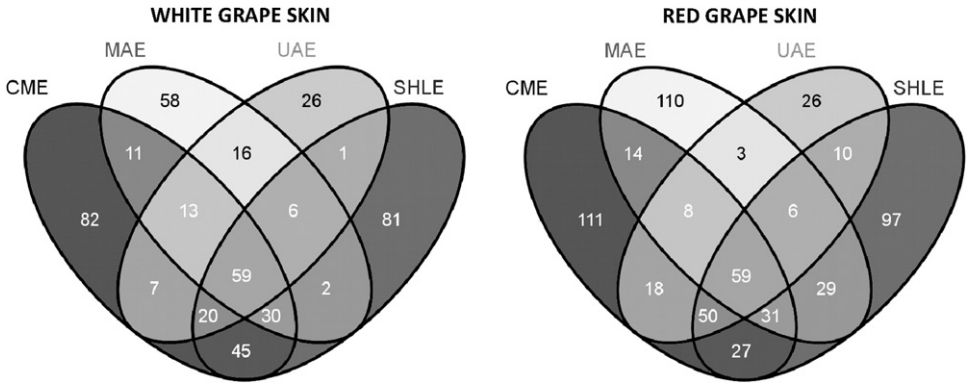

Fig. 1. Venn diagrams obtained after processing the molecular features extracted in the analysis of extracts from white- and red-grape skins showing the number of potential compounds obtained for each extraction method used and those which are common to them.

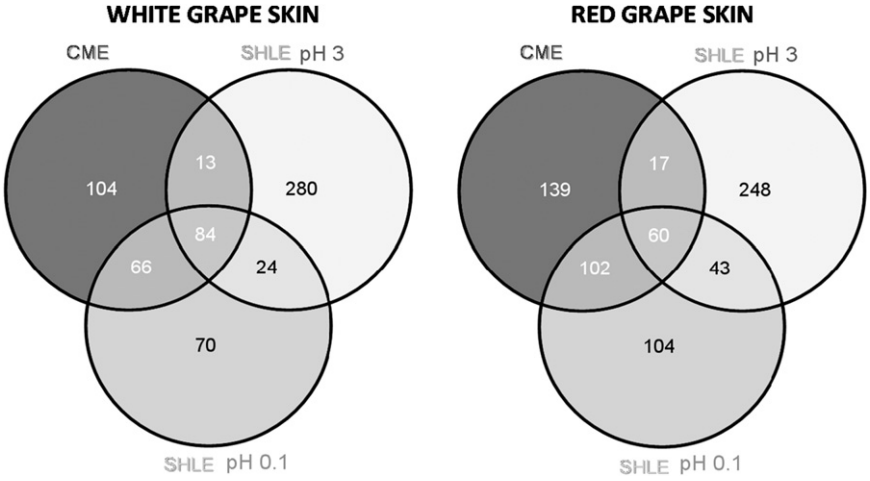

Fig. 2. Venn diagrams obtained after processing the molecular features extracted in the analysis of extracts from white and red grape skins showing the number of potential compounds obtained for SHLE working at two pHs (0.1 and 3.0) and CME, showing common and uncommon compounds.

and red grape skins, respectively, while USAE reported the lowest number of molecular features in both types of samples. It is important to mention that only 59 potential molecular features were constant for the four extraction methods with the maximum similarity for CME and SHLE with 154 and 167 common features for white and red grape skins, respectively. Both SHLE and CME were also characterized by the low number of features common to USAE and MAE. Maceration is the extraction alternative that operates under the softest conditions in terms of temperature or assisted energy. Attending to the profiles of potential molecular features obtained by the different techniques, MAE and USAE seem to alter the composition of the extracts by comparison to CME. On the other hand, SHLE provided an extract with coverage, in terms of molecular entities, quite similar to that obtained by CME. With these premises, MAE and USAE were discarded while SHLE was the preferred technique to optimize exhaustively the extraction conditions.

3.2. Influence of extractant pH on SHLE

The influence of extractant pH on SHLE was studied with the purpose of approaching to the efficiency attained with CME taking into account that less acidic pHs have also been used in SHLE methods for isolation of natural extracts [27]. Fig. 2 plots the Venn diagrams for each type of grape skin used in this study. As can be seen, an improved effect was found by SHLE at pH 3 as compared with lower pH tests. The efficiency of SHLE was considerably enhanced at pH 3, providing more molecular features than maceration. This result could be justified by hydrolysis and degradation of labile compounds at extreme acid pH values.

3.3. Influence of critical SHLE variables

The two most critical variables influencing SHLE (namely: the extraction temperature and processing time) were studied for isolation of high-added value compounds from grape skins. The results obtained after univariate study of both variables are discussed below.

Temperature: this variable was tested from 140 to 200 °C in the case of red-grape skins, while it was forced up to 220 °C for white-grape skins according to the chromatographic profiles obtained. Fig. 3 shows the Venn diagrams representing the influence of the extraction temperature on the metabolites coverage for the two types of grape skins.

The highest number of molecular features (441 and 398 for white- and red-grape skins, respectively) was obtained at the maximum temperatures tested in each case, 220 and 200 °C for white- and red-grape skins, respectively. However, both temperature tests were characterized by a high proportion of molecular features exclusively detected in the extracts from these experiments, 35 and 46% for white- and red-grape skins, which could be indicative of degradation of the target compounds in the extracts

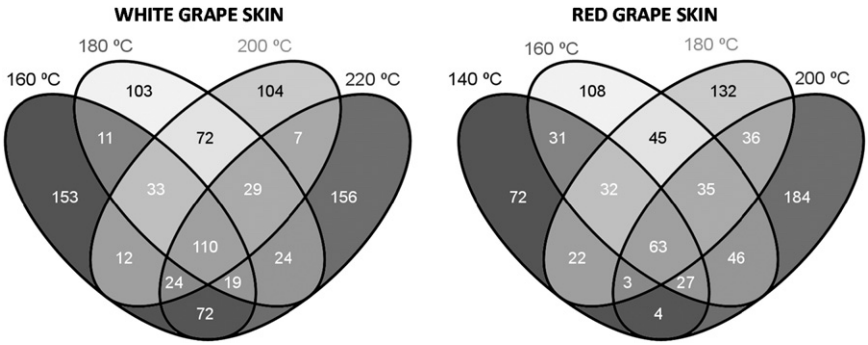

Fig. 3. Venn diagrams obtained after processing the molecular features extracted in the analysis of extracts from white and red grape skins showing both the number of potential compounds obtained at each extraction temperature used, and the common and uncommon compounds.

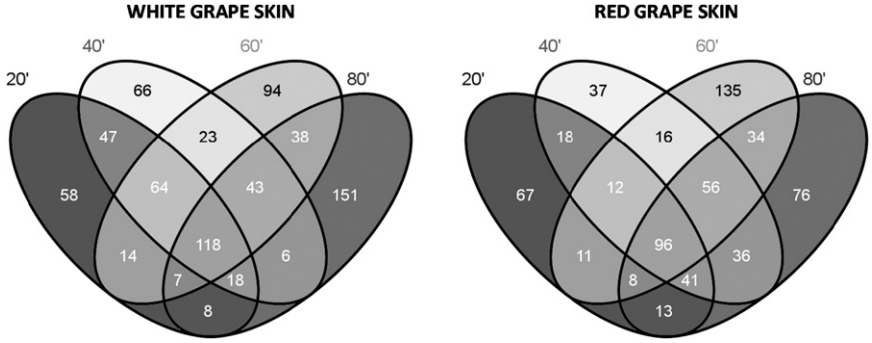

Fig. 4. Venn diagrams obtained after processing the molecular features extracted in the analysis of extracts from white and red grape skins showing both the number of potential compounds obtained for each extraction time and those common and uncommon to them.

as compared to the other tested temperatures. Attending to the balance between number of molecular features and proportion of molecular features detected exclusively in one experiment, the optimum temperatures are 180 and 160 °C for extraction of white- and red-grape skins, respectively. Thus, 401 molecular features were detected in the extract of white-grape skins at 180 °C; 25% of them were detected only in this extract. Similarly, 387 molecular features were detected in the extract from red grape skins at 160 °C, with a 27% detected only in it. Supplementary Fig. 2 illustrates the BPC obtained for each type of grape skins at the tested temperatures, showing the differences between the chromatographic profiles of the extracts.

Time: a kinetics study was developed to set the optimum extraction time; thus, extraction times of 20, 40, 60 and 80 min were tested. The results, as Venn diagrams, are shown in Fig. 4. These diagrams demonstrated that an extraction time of 60 min provided the highest number of molecular features for both matrices (401 and 364 for white- and red-grape skins, respectively). In the case of extracts from white-grape skins, no statistical differences were observed, at 95% confidence level, in the number of molecular features between 40 and 60 min. However, there were significant differences for the extracts from red-grape skins and, for this reason, the selected extraction time was 60 min.

Supplementary Fig. 3 plots BPCs corresponding to the analysis of extracts obtained at different extraction times from the two types of samples.

3.4. Total phenolic content and evaluation of the antioxidant potential by the FRAP assay

Two different tests were applied to evaluate the potential of the two types of grape skins used in this research for isolation of interesting compounds such as phenolic compounds or, more generally, antioxidants. The F–C test revealed that the extracts from white-grape skins reported higher concentrations of total phenols (equivalent to 4.2 mg GAE/mL of extract) than those from red-grape skins, with 1.5 mg GAE/mL. Concerning the antioxidant activity, the same trend as in the total phenols content was observed. Thus, the skin extracts from white grapes reported the highest antioxidant activity with 5.8 mg equivalents of Trolox/mL of extract, *versus* skin extracts from red grapes that gave 2.3 mg equivalents of Trolox/mL.

3.5. Identification of interesting compounds in skins from white and red grapes

Once the extraction conditions were defined for isolation of the polar/mid-polar fraction from the two types of skins from residues obtained after grape pressing and alcohol distillation, characterization of the resulting extracts was demanded for valorization of the residues. Numerous compounds were tentatively identified in skins from white and red grapes as shows Table 1. Identification was supported on mass accurate detection with 10 ppm maximum error in monoisotopic mass taking into account the isotopic distribution. Metabolites were identified by searching on PlantCyc database (www.plantcyc.org) and on a personnel database prepared after review of existing research in the literature about characterization of skin from grapes [27,28]. However, this section will be focused on two main families that should be emphasized because of their nutraceutical and enological interest: phenols and furfural derivatives (the latter produced by sugars degradation). In all cases, more polar than non-polar compounds were extracted from grape skins, which is a consequence of both the polar nature of the extractant used and the high content of polar compounds in the raw material.

The variety of phenolic compounds extracted under superheated-extractant conditions encompassed flavonoid and non-flavonoid

compounds. The former can be classified as follows: (i) anthocyanins, only present in red grapes, were not detected in any extracts, except for the pyroanthocyanin vitisin B (malvidin-3-glucoside acetaldehyde); (ii) flavanols, that included catechin, epicatechin and gallate derivatives, were detected, but also a procyanidin B isomer was tentatively identified; (iii) flavonols, which were detected as free and 3-glucoside derivatives, were present in higher number in extracts from red-grape residues. Concerning non-flavonoid phenols, a great variety of phenolic acids was detected in the extracts from grape-skin residues. Thus, compounds such as caffeic acid, gallic acid or protocatechuic acid were detected in both types of extracts. An interesting fraction because of their biological activity was that formed by stilbenes, indistinctly detected in the residue from both grape varieties. Other phenolic compounds with biological interest such as ethyl esters (ethyl ferulate and ethyl protocatechuate), lignin monomers and derivatives (pyrocatechol, pyrogallol and syringol), aldehydes (coniferaldehyde) and ketones (vanillin), among others, were also tentatively identified in the characterization analysis.

Concerning furanic derivatives, the removal of sugars in the previous industrial steps justified the scant number of furan derivatives identified (among them furfuryl alcohol). Despite the degradation caused by the drastic conditions of the distillation process, many interesting compounds are found in the extract from the waste of this process, which makes it a useful matter for more than as a heating source.

4. Conclusions

The research, the results of which have been exposed and discussed, was aimed at demonstrating that: (i) very different profiles of compounds can be obtained from the extracts from the same raw material depending on the type of energy used to accelerated/improve the extraction step. Therefore, the different options of auxiliary energy should be tested in the light of the target compounds we are looking for before selecting one of them. (ii) Not only the waste from the winemaking industry can be exploited to obtain valuable compounds, but the same compounds—and even others resulting from the drastic conditions to which grape pomace is subjected for ethanol distillation—can be obtained from the "waste from the waste" of this industry.

The results obtained by subjecting the raw material from waste of red and white grapes to four extraction methods allow obtaining the following conclusions:

- In all cases, more polar than non-polar compounds were extracted, which are a consequence of both the polar extractant used and the high content of the former in the raw materials.
- The two extraction techniques based on the use of auxiliary energy (MAE, UAE) yielded lower efficiencies than CME and SHLE.
- The highest number of compounds was detected in the extracts obtained by SHLE and CME.
- The increase of pH also increased the efficiency of SHLE as compared with CME.
- The yield of extraction using ethanol–water mixtures under superheated conditions clearly surpassed that of the conventional method, which used the extractant at ambient temperature.
- The superheated conditions dramatically shortened the extraction time (60 min *versus* 24 h for the conventional method).

The final conclusion from this study is that despite the degradation caused by the drastic conditions of the distillation process, many interesting, valuable compounds are found in the extract from the waste of this process, which make it a useful matter for a better exploitation than as a heating source.

The variety of tentatively identified compounds in the extracts makes them exploitable as additives in the food industry (either as colorants, as flavor modifiers or as antioxidants), and also in the cosmetics and nutraceuticals industries.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.09.028.

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