LWT - Food Science and Technology 156 (2022) 113057

Contents lists available at ScienceDirect

LWT

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

Revalorization of residues from the industrial exhaustion of grape by-products

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ARTICLE INFO

Keywords:
Grape pomace
Byproduct
Bioactive compounds
Antioxidant activity
Industrial extraction

ABSTRACT

Wine industry produces annually a large amount of grape pomace by-products whose bioactive compounds are extracted and exploited for different technological purposes. In order to verify the efficiency of the extraction, the monitoring of each stage of the industrial processing of grape pomace, not previously considered, was carried out. For that purpose, samples of all stages of the extraction process (grape pomace, grape seed, washing waters, and defatted grape seed flour), from two grape varieties (Airén and Tempranillo) were considered. Attention was focused on the content of protein, lipid, fiber, ash, polyphenols, and antioxidant activity. The data obtained showed that considerable amounts of protein (around 10%), fiber (20%–30%), and non-extracted phenols remain in the by-products of each stage of processing. Therefore, industrial extraction should be optimized to recover greater amounts of components of interest to other industries such as the food or pharmaceutical industry. Finally, the antioxidant activity of these samples was tested by DPPH and ABTS, and a positive correlation was found between phenols and antioxidant activity.

1. Introduction

In recent years, the interest in obtaining natural components from food wastes has increased significantly. This practice does not only allow food industries to reduce their environmental impact by the circular production of by-products, but also entails an economic benefit derived from the reuse of products with added value (Dwyer, Hosseinian, & Rod, 2014). In that concern, the extracted components could be transformed into food ingredients, nutraceutical compounds, biodiesel, and other useful bioproducts, which are the target market of a wide diversity of industries (Maier, Schieber, Kammerer, & Carle, 2009).

Derived from viticulture and enology, approximately seven million tons of grape pomace were originated annually worldwide in the wine industries (Bordiga, Travaglia, & Locatelli, 2019), which is typically used for animal feed, composting, industrial biomass or distillate production (Bordiga, Travaglia, Locatelli, Arlorio, & Daniel Coïsson, 2015). However, grape pomace is a high added-value by-product due to its wide variety of compounds susceptible of extraction and exploitation with the consequent economic benefit. Besides, this fact could contribute to reduce the environmental impact due to large quantities of grape pomace end up in landfills thus generating environmental problems such as water pollution, bad smells, pests, and leaching of tannins and other compounds into groundwater, affecting the vegetation and animals around (Arvanitoyannis, Ladas, & Mavromatis, 2006). Moreover, this fact is aggravated by the slow biological degradation of these wastes due to their acidic pH values and the presence of phenolic compounds, which have phytotoxic and antibacterial properties (Bustamante et al., 2008).

Grape pomace is constituted by skin, pulp, seeds and stems of the bunch of grapes, and its composition is influenced by grape variety, cultivation, climate, or the conditions of grape processing during the winemaking process (Bordiga, 2018). The major component of grape pomace is fiber, which mainly consists of lignin and polysaccharides of grape seeds and skins, and represents between 45% and 75% of the dry weight of the white and red grape pomaces, respectively (Gül, Acun, Sen, Nayir, & Turk, 2013). Grape pomace is also an important source of grape seed oil (7%–15%) (Bordiga et al., 2019), mainly consisting of unsaturated fatty acids such as linoleic (70%) and oleic acids (15%) (Bordiga et al., 2015), and grape seed proteins (6%–15%), characterized by the high percentage of glutamic and aspartic acids and the low

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https://doi.org/10.1016/j.lwt.2021.113057
Received 15 September 2021; Received in revised form 23 November 2021; Accepted 31 December 2021
Available online 3 January 2022
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content of tryptophan and sulfur amino acids (Gazzola et al., 2014). Moreover, phenolic compounds are present in a remarkable quantity in grape pomace (around 70%) (Nunes, Rodrigues, & Oliveira, 2017) due to their incomplete extraction during winemaking (Travaglia, Bordiga, Locatelli, Coïsson, & Arlorio, 2011), and mainly coming from seeds (60%), followed by skin and pulp (30% and 10%, respectively) (Jara Palacios et al., 2012). They belong to different families such as anthocyanins, flavonols, hydroxybenzoic acids, hydroxycinnamic acids, flavan-3-ols, stilbenes, and proanthocyanidins (Bordiga, Coïsson, Locatelli, Arlorio, & Travaglia, 2013). Other compounds present in grape pomace are oligosaccharides and minerals, such as bitartrate, potassium, phosphorus, sulfur, and magnesium (Bordiga et al., 2019).

To accomplish the obtaining of all components of interest, grape pomace processing industries implement different procedures, such as distillation of the water derived from the washing of grape pomace or spent stillages for obtaining alcohol and tartaric salts, respectively, extraction of anthocyanins to be used as food coloring agent or grape seed oil extraction with organic solvents, among others. The so-obtained products are exploited in cosmetics (Wittenauer, Mäckle, Suβmann, Schweiggert-Weisz, & Carle, 2015), pharmaceutical and food industries for use as functional ingredients (Ismail, Salem, & Eassawy, 2016) or with diverse technological purposes (Katalinić et al., 2010).

Despite the economical, technological, and environmental advantages of the industrial processing of grape pomace, there is no published scientific study about the follow-up of the industrial extraction of the different components from grape pomace. Therefore, the present work has twofold aims. The first one is the characterization of the composition of the obtained fractions in the different stages of the industrial extraction process of grape pomace, to revalue it as a source of several substances of interest. On the other hand, the second objective is to verify the extraction effectiveness of each stage of the industrial process, based on whether grape pomace still could contain any component of interest in significant quantity and it would be necessary to improve, consequently, the extraction yield of any stage. To this end, the study was performed using different samples of all stages of the industrial extraction process (grape pomace, grape seed, washing waters, and defatted grape seed flour), not previously considered, from different grape varieties (Airén and Tempranillo). Our interest was focused on several perspectives, the macro and micronutrient amount (protein, lipid, fiber, and ash), and the antioxidant activity related to the content of polyphenol content.

2. Materials and methods

2.1. Chemicals and reagents

Gallic acid, sodium hydroxide, 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and disodium hydrogen phosphate anhydrous were provided by Sigma–Aldrich (Madrid, Spain). 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased by Alfa Aesar (Ward Hill, MA, USA). Tetramethylchromane-2-carboxylic acid (Trolox) was acquired from Fluka (Madrid, Spain). Trichloroacetic acid (TCA), and ethyl ether were purchased from VWR Chemicals (USA). Folin-Ciocalteau's phenol reagent, sodium carbonate, methanol, and ethanol were purchased from Panreac Química S.L.U. (Barcelona, Spain). Potassium persulfate and potassium dihydrogen phosphate were purchased from Merck (Darmstadt, Germany).

2.2. Samples

The samples used in this study were provided by Alvinesa Natural Ingredients, S.A (Ciudad Real, Spain). Solid and liquid samples derived from the different stages of the extraction process of the natural ingredients from grape pomace were considered. The solid samples corresponded to grape pomace (GP), grape seeds (GS), and defatted grape seed flour (DF), from Tempranillo red grapes (R) and Airén white grapes (W). The liquid samples corresponded to picket water (PW) and depletion water (DW), both derived from washing the grape pomace in diffusion bands. Both solid and liquid samples came from only red grapes (RR) or from the mixture of white and red grapes (WR) in a ratio of 55/45, respectively.

In general, the grape pomace that arrives at the industry was washed in diffusion bands to extract the components of interest, generating picket and depletion waters. Subsequently, grape pomace was destemmed and dried and, after that, the grape seeds were separated from the grape pomace, dried, ground and pelletized oil extraction using hexane. Finally, the resulting defatted grape seed meal was desolventized, which, together with the remains of exhausted pomace, are used for the industry's own energy self-supply or are marketed by the industry (Cejudo-Bastante, Oliva-Sobrado, González-Miret, & Heredia, 2022) (Fig. 1). In detail, two different types of GP were used as raw materials: fresh unwashed GP (RRGP0 and WRGP0) and fresh washed GP on diffusion bands (RRGP1 and WRGP1) (Fig. 1). The washing consists of a counter-current diffusion process using sulfur anhydride, water, and steam at 60 °C, to extract the compounds of interest (alcohols, phenols, bitartrate) which will be used for the industry with different purposes. Subsequently, the washed red GP (RRGP) undergone a storage process outdoors for one month, to be further re-washed on diffusion bands under the same conditions as the first washing but at 90 °C to deplete its bitartrate content. Samples were collected before (RRGP2) and after (RRGP3) the re-washing stage (Fig. 1). This second washing was not carried out on WR samples because its GP was already exhausted in the compounds of interest in the first washing.

Consequently, two different liquid samples were obtained: picket waters (RRPW1, RRPW2, and WRPW1) and depletion waters (RRDW1, RRDW2, and WRDW1), both from the washing in diffusion bands of the two types of grape pomace (Fig. 1).

Finally, the washed GPs were dried at 300–350 °C in a rotator trommel and cooling for further separation of the grape seeds (RRGS and WRGS) (Fig. 1). Afterward, the GS oil extraction process was undergone. For that purpose, the grape seeds were ground and submitted to extraction with hexane, thus obtaining the crude oil of grape seeds and, as a residue, the defatted seed flour (DF). Both the defatted flour residue and the extracted seed oil are subjected to a desolventization process at 100–105 °C to eliminate the solvent residue.

The solid samples were lyophilized and finely milled. The liquid samples were centrifuged at 4 °C and 12,000×*g* for 10 min, to separate possible suspended solids. Samples were stored at −20 °C.

2.3. Total protein content

The total nitrogen content of the samples was determined using the standard Kjeldahl method as described by AOAC (1995). Samples were digested using a MicroKjeldahl System (J.P. Selecta, Barcelona, Spain). The percentage of proteins was obtained by multiplying the total nitrogen content by the conversion factor of 5.71 (Chamizo-González, Gordillo, & Heredia, 2021; Gianazza et al., 1989; Zhou, Li, Zhang, Bai, & Zhao, 2010). All tests were performed in triplicate and the results were expressed as percentages with respect to the dry sample (g/100 g).

2.4. Total lipid content

Lipids were extracted from lyophilized samples by Soxhlet method with hexane (AOAC, 1990). The extractions were performed in triplicate, and the results were expressed as percentages with respect to the dry sample (g/100 g).

2.5. Total fiber and ash content

Total fiber and ash contents of the lyophilized samples were determined in triplicate by acid treatment following the procedures described by AOAC (1990). Briefly, a mixture of acetic acid (900 mL),

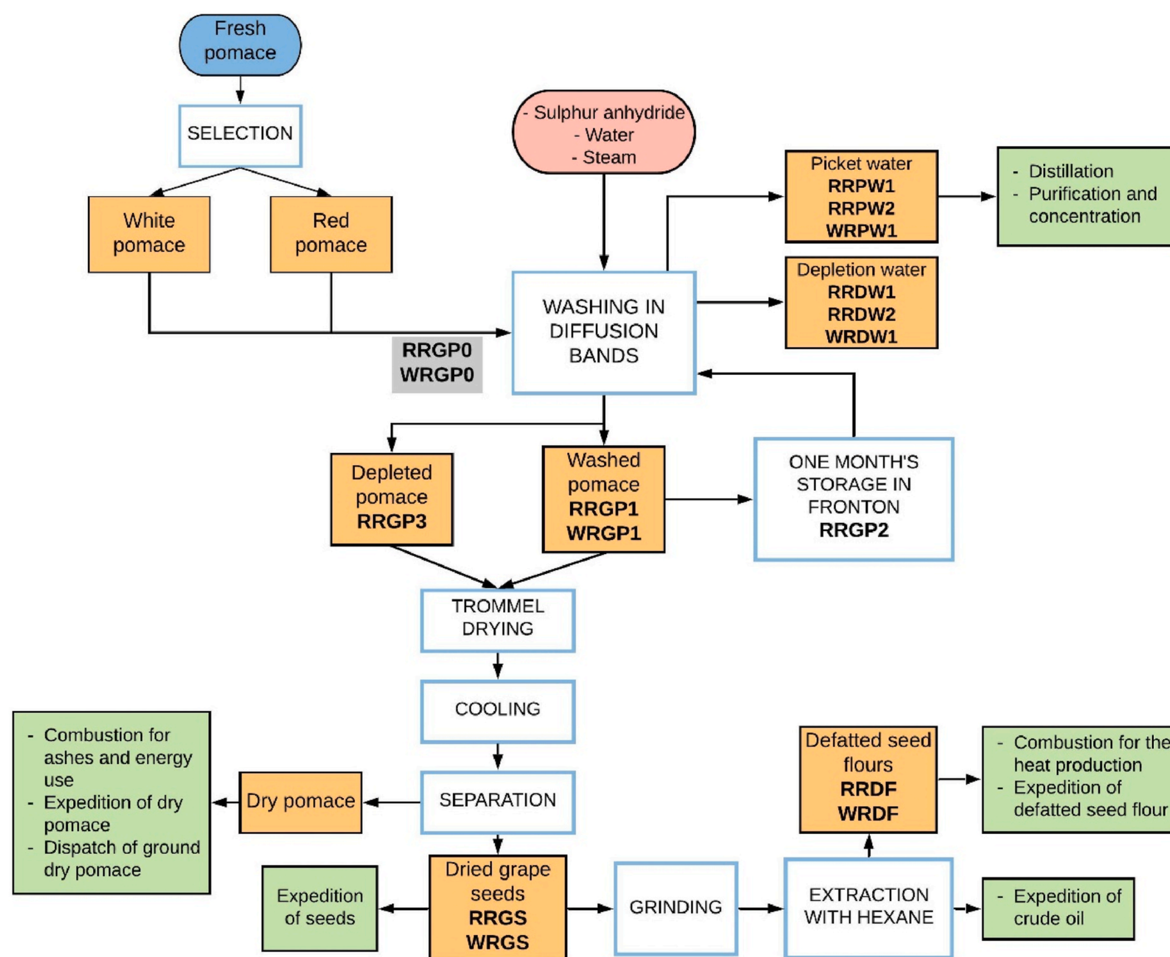

Fig. 1. Flow chart of the extraction process of natural ingredients from grape pomace. RRGP0, fresh unwashed red pomace; WRGP0, fresh unwashed white-red pomace; RRGP1, washed red pomace in the fisrt washing; WRGP1, washed white-red pomace; RRGP2, red pomace washed in the first washing and stored one month in the outdoors: RRGP3, red pomace washed in the second washing; RRPW1, picket waters from the first washing of the red pomace; WRPW1, picket waters from the washing of the white-red pomace; RRPW2, picket waters from the second washing of the red pomace; RRDW1, depletion waters from the first washing of the red pomace; WRDW1, depletion waters from the washing of the white-red pomace; RRDW2, depletion waters from the second washing of the red pomace; RRGS, red grape seeds; WRGS, white-red grape seeds; RRDF, defatted red grape seed flour and WRDF, defatted white-red grape seed flour. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

trichloroacetic acid (24 g), and nitric acid (60 mL) was prepared. The samples were heated (>100 °C) together with 80 mL of the acid mixture for 1 h. Subsequently, samples were filtered and washed with water, ethanol, and ethyl ether solvents for further mineralization at 550 °C. The results were expressed in percentages of dry sample (g/100 g).

2.6. Total phenolic content

The solid samples were extracted with 75% (v/v) methanol according to the methodology described by Jara-Palacios et al. (2014). Liquid samples were directly analyzed.

The Folin-Ciocalteu assay was used to determine total phenolic content (TPC) (Singleton & Rossi, 1965). The solutions were left to stand for 120 min for the reaction to take place. Then, the absorbance was read at 765 nm with a Hewlett-Packard UV–vis HP8452 spectrophotometer (Palo Alto, CA, USA). Different dilutions (1/2, 1/5, 1/10) were used to perform the analysis. Gallic acid was used as a calibration standard and results were expressed as gallic acid equivalents (mg GAE/g of dry matter for solid samples, and mg GAE/L of sample for liquid samples).

2.7. Antioxidant capacity

2.7.1. DPPH assay

The DPPH$^•$ radical scavenging assay was assessed using the method described by Soler-Rivas, Espín de Gea, & Wichers (2000) with some modifications. The phenolic extracts previously obtained from solid samples and the direct washing water samples were analyzed. Different dilutions of each sample were tested (1/10, 1/100, and 1/500) to obtain initial and final absorbance values within the range of accuracy of spectrophotometry.

30 μL of diluted samples were added to 300 μL of 108 μM DPPH$^•$ methanolic solution, the mixture was diluted with 570 μL of 80% (v/v) methanol. The absorbance was measured at 517 nm, using a Hewlett-Packard UV–vis HP8452 spectrophotometer, after 30 min of incubation in the dark at room temperature.

Trolox was used as a standard. Concentrations were calculated from a calibration curve in the range between 0.025 and 0.3 mM Trolox, and the results were expressed as Trolox equivalents (TE)/g of dry matter for solid samples, and as Trolox equivalents (TE)/L of the sample, for liquid samples. Assays were carried out in triplicate.

Table 1
Mean values and standard deviation of protein, lipid, fiber, ash, total phenolic content (TPC), and antioxidant activity in solid samples from 100% red grapes (RR).

	RRGP0	RRGP1	RRGP2	RRGP3	RRGS	RRDF
Protein (g/100 g)	7.93 ± 0.78 [a]	7.64 ± 1.15 [a]	8.87 ± 0.70 [ab]	9.94 ± 1.91 [b]	8.05 ± 0.25 [a]	7.34 ± 0.21 [a]
Lipid (g/100 g)	8.62 ± 2.16 [b]	9.08 ± 2.02 [b]	12.12 ± 3.97 [b]	10.05 ± 0.36 [b]	19.44 ± 3.04 [c]	2.46 ± 0.34 [a]
Fiber (g/100 g)	19.53 ± 4.00 [a]	19.96 ± 2.78 [a]	20.07 ± 4.73 [a]	25.19 ± 4.19 [a]	23.76 ± 0.94 [a]	22.94 ± 3.18 [a]
Ash (g/100 g)	0.55 ± 0.41 [a]	0.97 ± 0.30 [ab]	0.53 ± 0.31 [a]	0.75 ± 0.21 [ab]	0.87 ± 0.05 [ab]	0.99 ± 0.10 [b]
TPC (mg/g)	14.03 ± 2.10 [b]	23.67 ± 3.58 [d]	25.06 ± 2.13 [d]	21.48 ± 3.07 [cd]	18.07 ± 1.27 [c]	10.12 ± 0.31 [a]
DPPH (μmol TE/g)	129.56 ± 11.33 [b]	199.45 ± 36.04 [c]	189.64 ± 7.23 [c]	199.02 ± 14.36 [c]	235.02 ± 12.74 [d]	71.91 ± 28.22 [a]
ABTS (μmol TE/g)	201.69 ± 12.31 [a]	374.35 ± 51.30 [bc]	396.57 ± 17.69 [c]	396.57 ± 17.69 [c]	305.76 ± 41.46 [b]	175.32 ± 19.72 [a]

Mean ± standard deviation is calculated from triplicate samples. Different superscripts in the same row indicate significant differences by ANOVA test ($p < 0.05$). RRGP0, fresh unwashed red pomace; RRGP1, washed red pomace in the first washing; RRGP2, red pomace washed in the first washing and stored one month in the outdoors; RRGP3, red pomace washed in the second washing; RRGS, red grape seeds; RRDF, defatted red grape seed flour.

Table 2
Mean values and standard deviation of protein, lipid, fiber, ash, total phenolic content (TPC), and antioxidant activity in solid samples from 55% to 45% white and red grapes (WR).

	WRGP0	WRGP1	WRGS	WRDF
Protein (g/100 g)	6.86 ± 0.65 [a]	7.27 ± 0.63 [a]	6.69 ± 0.31 [a]	8.75 ± 0.20 [b]
Lipid (g/100 g)	16.07 ± 4.35 [b]	14.93 ± 6.43 [b]	25.92 ± 1.82 [c]	2.29 ± 1.03 [a]
Fiber (g/100 g)	28.93 ± 4.51 [b]	27.23 ± 4.96 [ab]	25.07 ± 2.08 [ab]	21.23 ± 2.44 [a]
Ash (g/100 g)	0.84 ± 0.42 [a]	1.01 ± 0.42 [a]	0.68 ± 0.13 [a]	0.92 ± 0.15 [a]
TPC (mg/g)	9.36 ± 1.66 [c]	5.84 ± 1.00 [b]	2.78 ± 0.44 [a]	3.03 ± 0.12 [a]
DPPH (μmol TE/g)	137.55 ± 18.29 [c]	124.23 ± 22.87 [c]	0.00 ± 0.00 [a]	29.62 ± 1.01 [b]
ABTS (μmol TE/g)	151.52 ± 20.45 [a]	114.64 ± 15.60 [b]	52.84 ± 6.54 [c]	64.27 ± 2.39 [cd]

Mean ± standard deviation is calculated from triplicate samples. Different superscripts in the same row indicate significant differences by ANOVA test ($p < 0.05$). WRGP0, fresh unwashed white-red pomace; WRGP1, washed white-red pomace; WRGS, white-red grape seeds; WRDF, defatted white-red grape seed flour.

Table 3
Mean values and standard deviation of total phenolic content (TPC), and antioxidant activity in liquid samples from 100% red grapes (RR).

	RRPW1	RRDW1	RRPW2	RRDW2
TPC (mg/L)	4885.67 ± 168.25 [c]	1339.00 ± 63.71 [a]	4959.00 ± 368.61 [c]	2815.24 ± 156.43 [b]
DPPH (mmol TE/L)	72.87 ± 17.39 [b]	14.62 ± 2.97 [a]	82.99 ± 2.30 [b]	37.22 ± 6.73 [a]
ABTS (mmol TE/L)	125.42 ± 9.00 [a]	28.46 ± 1.54 [c]	99.78 ± 13.78 [b]	48.15 ± 5.86 [cd]

Mean ± standard deviation is calculated from triplicate samples. Different superscripts in the same row indicate significant differences by ANOVA test ($p < 0.05$). RRPW1, picket waters from the first washing of the red pomace; RRDW1, depletion waters from the first washing of the red pomace; RRPW2, picket waters from the second washing of the red pomace; RRDW2, depletion waters from the second washing of the red pomace.

Table 4
Mean values and standard deviation of total phenolic content (TPC), and antioxidant activity in liquid samples from 55% to 45% white and red grapes (WR).

	WRPW1	WRDW1
TPC (mg/L)	4425.01 ± 373.08 [a]	433.24 ± 21.98 [b]
DPPH (mmol TE/L)	75.61 ± 8.42 [a]	7.50 ± 2.30 [b]
ABTS (mmol TE/L)	105.19 ± 11.40 [a]	11.86 ± 3.10 [b]

Mean ± standard deviation is calculated from triplicate samples. Different superscripts in the same row indicate significant differences by ANOVA test ($p < 0.05$). WRPW1, picket waters from the washing of the white-red pomace; WRDW1, depletion waters from the washing of the white-red pomace.

2.7.2. 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical scavenging assay

The ABTS free radical scavenging activity of each phenolic extract and each washing water sample was determined as described by Re et al. (1999). A 7 mM $ABTS^{\bullet+}$ stock solution was prepared using potassium persulfate 2.45 mM as the oxidizing agent. After 12–16 h stored in the dark at room temperature, this stock solution was diluted with phosphate buffer to form the test reagent, with an absorbance of 0.700 ± 0.02 at 734 nm. 2 mL of this reagent was mixed with 50 μL of sample, or different concentrations of Trolox standard solution (0.03–1.00 mmol/L) and, after 4 min, the absorbance was determined at 734 nm using a Hewlett-Packard UV–vis HP8452 spectrophotometer. The sample that achieved 20–80% inhibition of the blank absorbance was selected for the calculations. For this purpose, different dilutions of samples were necessary (1/25, 1/50, 1/100, 1/200). The results were expressed as Trolox equivalents (TE)/g of dry matter for solid samples, and as Trolox equivalents (TE)/L of sample, for liquid samples. Three replicates from each sample were analyzed.

2.8. Statistical analysis

Statistical analysis of the data was performed using the Statistica v.8.0 software (Statistica, 2007). One-way analysis of variance (ANOVA) was applied to evaluate whether significant differences ($p < 0.05$) exist among the different samples. A T-Student test was used to evaluate the significant differences ($p < 0.05$) between 100% red and 55-45% white-red samples. Furthermore, a T-Student test and Pearson's correlation analysis were applied to evaluate if there is a relation between the assayed parameters.

Table 5
p-Level of comparison between 100% red samples (RR) and 55%–45% white and red samples (WR) for protein, lipid, fiber, ash, total phenolic content (TPC), and antioxidant activity.

	GP0	GP1	GS	DF	PW	DW
Protein	**0.0265**	0.5003	**0.0040**	**0.0010**	–	–
Lipid	**0.0071**	0.0593	**0.0338**	0.8039	–	–
Fiber	**0.0034**	**0.0140**	0.3928	0.4993	–	–
Ash	0.2593	0.8182	0.1435	0.5502	–	–
TPC	**0.0016**	**2.2964 x 10^{-5}**	**3.9652 x 10^{-5}**	**3.4530 x 10^{-6}**	**0.0381**	**0.0017**
DPPH	0.3901	**0.0015**	**0.0010**	0.1221	0.7498	**0.0051**
ABTS	**0.0004**	**0.0000**	**0.0005**	**0.0006**	**0.0327**	**0.0001**

Values of $p < 0.05$ indicate significant differences by T-Student test. GP0, fresh unwashed pomace; GP1, washed pomace in the first washing; GS, grape seed; DF, defatted grape seed flour; PW, picket waters from the first washing of the pomace; DW, depletion waters from the first washing of the pomace.

3. Results and discussion

Tables 1–4 show the content of the parameters analyzed along with the grape pomace industrial processing. The data of the analyzed parameters were compared at the different stages of the process. Furthermore, the comparison of the data between the two types of samples (only red grapes (RR) and mixture of white grapes and red grapes (WR)) in each stage, are shown in Table 5.

3.1. Total protein content

In both types of samples (RR and WR), it is observed that, during the whole process, from the initial product (GP) to the defatted flour (DF), the percentage of protein did not show a great variability (Tables 1 and 2), ranging between 6 and 10%. This means that this protein content is not significantly ($p>0.05$) affected by the different processes carried out in the industry. The percentage of protein in grape pomace was similar to those reported by other authors (Bordiga et al., 2019), establishing between 6% and 15% (dry matter). They have also found that the proportion of protein in grape pomace skins and seeds is similar, but the skins are slightly richer than the seeds extracted from grape pomace (García-Lomillo & González-SanJosé, 2016).

The total protein content of the RR samples ranged between 7% and 10% (Table 1). No significant differences ($p>0.05$) were found between the protein content of the GP before (RRGP0) and after (RRGP1) washing by diffusion bands concluding that the washing did not reduce the protein content. The same is true between RRGS and RRDF, fact that pointed out that the defatting process did not either influence the protein content. After the exit of the GP from the second washing (RRGP3), an increase in the protein percentage of the sample was observed (9.94%), which was significantly different ($p < 0.05$) from the rest of the values except for the one at the entrance of the re-washing (RRGP2). This may be due to many components of interest have already been removed from GP during the first washing (potassium bitartrate, polyphenols, alcohol, sugars, acids, minerals, etc …), thus increasing the proportion of proteins in this sample.

Similar results were obtained for the WR samples (Table 2). No significant ($p>0.05$) differences were observed between the different stages of industrial processing, except for DF samples, whose protein percentage (8.75%) was significantly ($p < 0.05$) higher than in the rest of the samples. As previously explained, the DF is a more purified substance due to the removal of other compounds along with the industrial processing, which makes increase the protein percentage per dry sample.

Comparing the RR and WR samples, in general, the percentage of protein was significantly ($p < 0.05$) higher in RR samples (Table 5). Similar results were reported by Jin et al. (2019), who obtained a higher protein content in red GP than white GP. This could be explained by part of the biomass of the yeasts is retained in the skins and seeds during maceration in red winemaking, increasing the protein content of the respective grape pomace. Therefore, the absence of the maceration in the production of white wine would explain the lower protein content of the WR samples compared to the RR samples. Despite the different extraction procedures carried out in the industry to extract compounds of interest, the stability of the protein content throughout the process is demonstrated. As a result, all solid by-products could be reused as non-allergenic protein sources, offering greater economic profitability and a reduction in the waste generated.

3.2. Total lipid content

Tables 1 and 2 show the values of total lipid content in the RR and WR samples, which ranged between 2% and 26%.

As can be expected, the highest percentage of lipids in the samples was obtained in the GS, regardless of the nature of the grape varieties (WR or RR). In fact, an industrial oil extraction with organic solvents is normally undergone. The percentage obtained in the RRGS was 19.44%, which was significantly ($p < 0.05$) higher than both RRGP and RRDF (Table 1). This is due to GP contains other components apart from the seeds (skin, pulp, and flesh) with low or absence of lipid content. This was also the case for the WRGS where the fat percentage obtained was 25.92% (Table 2). Moreover, as expected, the total lipid content of the defatted flour (RRDF and WRDF) was significantly ($p < 0.05$) lower than GP and GS lipid content (Tables 1 and 2), as DF is the product obtained after lipid extraction. The results obtained in the two types of samples showed that, although lipid extraction significantly reduces the lipid content of GS (about 17%–24%), there is still an appreciable amount of lipids in the DF, so this residue could be reusable, or the lipid extraction process could be improved for higher performance. The lipid percentage of the GS was slightly higher than those described by other authors (Bordiga et al., 2019; García-Lomillo & González-SanJosé, 2016), which ranged between 7% and 15%.

There were no significant differences between the GP samples before and after the washing process (in both RRGP and WRGP samples) (Tables 1 and 2). The same occurred in the re-washing process of the RR samples, as the lipid percentages were 12.12% and 10.05% respectively (Table 1). This means that the lipid content of the pomace is not affected by the washing processes carried out in the industry. With regard to the percentage of lipid in the GP, very similar results have been obtained to those of other authors, who ranged between 4% and 12% (Jin et al., 2019).

Comparing both raw materials, there were significant ($p < 0.05$) differences between RR and WR samples for the lipid percentages in some points of the industrial process. That is the case of GS and GP0 at the entrance to the washing (Table 5). In both cases, the WR samples had higher percentages of lipid content than the RR samples.

3.3. Total fiber content

In general, the values of fiber percentages obtained in the samples (19%–29%) were lower compared to those obtained by other authors (Bordiga et al., 2019).

In WR samples, there were significant differences ($p < 0.05$) only between WRGP0 at the entrance of the bands (28.93%) and WRDF (21.23%) (Table 2). This may occur since, prior to lipid extraction, the

industry performs a sieving process that separates the seeds from the rest of the components (branches, leaves, skins, etc.), which have a higher fiber content. The fiber percentages of the rest of WR samples did not show significant differences between the different stages of their industrial process (Table 2).

The different RR samples (GP, GS, and DF) presented similar fiber contents, which ranged between 19% and 26%. There were no significant differences ($p>0.05$) between them (Table 1).

When comparing RR and WR samples, significant ($p < 0.05$) differences were observed between the GPs, both at the entrance and at the exit of the washing process (Table 5). In both, the percentage of fiber was significantly higher in WR. These results are in contrast to Gül et al. (2013), who stated that red grape pomace is richer in fiber than white grape pomace. After confirming that the washing process does not affect the fiber content of the samples, these results may be due to the heterogeneity of the composition of the samples, as their components (stems, skins, and seeds) may be found in different proportions. In consequence, and as affirmed Dwyer et al. (2014), the white and red grape samples may have a higher proportion of fiber-rich components, such as seeds and skins, compared to the 100% red samples.

3.4. Total ash content

According to the results obtained, the total ash content of the samples did not vary significantly throughout the process (0.5–1.0%). These values are lower than those reported by other authors, with ash content in GP between 4% and 6% (Jin et al., 2019). Similarly, García-Lomillo and González-SanJosé (2016) indicate that potassium salts (mainly potassium bitartrate) are abundant in GP, which are usually found in amounts between 4% and 14% (in dry matter). However, they also stated that these percentages are subject to great variability, as they can be influenced by different factors such as the cultivation practices and the degree of ripening of the grapes (Nurgel & Canbas, 1998).

The ash percentages in the RRGP, both at the exit of the first washing (RRGP1) and the re-washing (RRGP3), were slightly higher (0.97% and 0.75%, respectively) with respect to the GP at the entrance to the washing (RRGP0) and re-washing (RRGP2) (0.55% and 0.53%, respectively) (Table 1). A similar behavior was observed between RRGS and RRDF. The major and significant differences ($p < 0.05$) were found between RRDF and RRGP0 and RRGP2, reaching the highest values the defatted grape seed meal (Table 1). According to Lachman et al. (2013), mineral content depends on numerous factors, such as growing conditions (soil, climate, variety) or the winemaking process itself, which may explain the variability of the results obtained. Similar values were obtained in WR samples (Table 2).

Although some studies affirmed that the grape variety influences the mineral content of the grape and this could be transferred to the pomace (Botelho, Bennemann, Torres, & Sato, 2018), when comparing both RR and WR samples, no significant differences ($p>0.05$) were observed between them throughout the process (GP, GS, and DF) (Table 5).

3.5. Total phenolic content

As Table 1 shown, the percentage of phenols in the RR solid samples decreased from the initial to the final product of the process, in which the extraction of phenols took place to obtain components of interest to other industries. The RRGP0 at the entrance to the washing presented a significantly ($p < 0.05$) lower values of total phenols than at the exit (RRGP1) (Table 1). These data are contradictory, as phenols are extracted in the washing water and therefore the phenolic content of the sample before entering the washing should be higher than after. The result obtained may be due to the heterogeneity of the samples in the industrial process. Due to the large quantities of pomace being processed in a continuous mode in this industry, a follow-up of the same sample through all the extraction processes is difficult to control. This means that the sample at the entrance of the washing process is not exactly the same sample taken at the exit of the washing process, so its chemical composition could vary. This fact could explain the higher phenolic content at the exit of the washing (Peixoto et al., 2018).

The phenolic content of the RRGP1 after washing and the RRGP2 before the second washing was very similar, concluding that the storage time in the fronton did not significantly reduce the phenolic concentration of the GP. In the light of the scarce decrease in the total phenolic content between the latter samples and the RRGP3 at the exit of the second washing (Table 1), it might conclude that the second washing did not exhaust the phenolic content of the GP. Finally, the lowest value of total phenols was ascribed to RRDF, being significantly lower ($p < 0.05$) compared to RRGS (Table 1). This behavior could be related to the seed lipid extraction process, due to the organic solvent and the high temperature used for such extraction could remove and degrade phenols (Bordiga et al., 2019).

In the RR solid samples, the GP samples (with the exception of RRGP0 and RRGP3) had a significantly ($p < 0.05$) higher phenolic content than RRGS (Table 1). This is due to the possible degradation of phenols by the high temperature during the drying process to which the seeds were subjected. The pomace also contains other phenolic-rich components apart from the seeds that contribute to enhance the phenolic content in GP. Furthermore, prior to obtaining the seeds, the pomace has undergone extraction processes for these compounds, which are of interest to other industries, thus reducing the content of these compounds in the seeds. In the RR liquid samples, as expected, the picket waters (PW), both from the first (RRPW1) and second washing (RRPW2) (4885.67 mg/L and 4959.00 mg/L, respectively), presented significantly ($p < 0.05$) higher total phenolic values than the respective depletion waters (RRDW) (1339.00 mg/L and 2815.24 mg/L, respectively) (Table 3). These results confirmed the appropriate industrial performance of the washing process. Although the GP has been heavily depleted by washing, it can be seen that there are still phenolic compounds in the DW, so that the first industrial washing could be improved and the DW, with less organic matter, could be discarded or used for irrigation.

Total phenolic content was significantly higher ($p < 0.05$) in RRDW2 than RRDW1 (Table 3). The heterogeneity of the starting industrial GP and the fact that appreciable amounts of phenolic compounds are still found in the DW, are two more reasons to the need of optimizing the initial washing to improve the extraction of these compounds.

In the WR solid samples, the highest total phenols values were obtained in GP at the entrance of the washing (WRGP0), showing significant ($p < 0.05$) differences with that of the exit (WRGP1) (Table 2), similarly to that observed in RR samples. Moreover, the GPs showed significantly ($p < 0.05$) higher phenolic content (9.36 and 5.84 mg/g dry sample) than WRGS and WRDF (2.78 and 3.03 mg/g dry sample, respectively) (Table 2). Although several studies (Peixoto et al., 2018; Silva et al., 2018) reported the highest phenolic content in the seeds, due to the previous phenolic extraction processes carried out on the WRGPs, these compounds are depleted in the WRGS and in the WRDF, as it has been mentioned in the RR solid samples. Moreover, as previously commented of RR samples, some phenols may have been degraded in the seed and meal due to the effect of the increase in temperature during industrial processing (Bordiga et al., 2019).

As in the case of the (RR) liquid samples, the highest phenols content of (WR) liquid samples was obtained in the WRPW1, with significant differences ($p < 0.05$) between these and the WRDW1, because phenols are extracted in the WRPW1 (Table 4).

Although the decrease in phenolic content has been significant, appreciable amounts are still observed in the DW. When comparing both types of samples, RR and WR samples, RR samples showed a significantly ($p < 0.05$) higher phenolic content than WR samples (Table 5). According to Teixeira et al. (2014), the phenolic content of grape pomace is influenced by numerous factors, including grape variety, agro-climatic conditions or the industrial winemaking process. Despite all these variables, red grapes are richer in polyphenols than white

grapes because the latter lack anthocyanin phenolic compounds (Jin et al., 2019; Puig i Vayreda, 2015).

3.6. Antioxidant activity

Based on data obtained through the DPPH procedure, in the RR solid samples, the GP at the entrance to the washing (RRGP0) showed a significantly ($p < 0.05$) lower antioxidant activity than the rest of the GP samples (Table 1), fact that also explains their consequently lower phenolic content. The highest antioxidant activity was observed in the RRGS, and the lowest in the RRDF, both showing significant differences ($p < 0.05$) between them and all the RRGP samples (Table 1). The higher antioxidant activity of RRGS compared to RRGP, despite their lower amount of phenols (Peixoto et al., 2018), could be attributed to the type of phenols rather than their amount. In fact, grape seed flavanols showed higher antioxidant activity than other grape skin phenols (Yilmaz & Toledo, 2004). Other compounds generated by the Maillard reaction, which may be produced during the drying process of the sample, may also contribute to the increase in the antioxidant activity (Siddhuraju & Becker, 2007). The still appreciable antioxidant activity of the pomace from the re-washing process (RRGP3) indicates that it may contain appreciable quantities of antioxidant compounds that make this pomace suitable for reuse.

According to data obtained in WR solid samples, the significantly ($p < 0.05$) highest antioxidant activity were found in the GPs (WRGP0 and WRGP1) (Table 2). Contrarily, the lowest antioxidant activity was found in the WRDF and WRGS, due to the possible degradation of phenol-related antioxidants by the temperature (Table 2). A significant antioxidant activity was observed in the GP at the exit of the re-washing process (WRGP3), in the case of the RR samples, which means that the industry did not completely deplete phenols during the washing of GP. The same applies to the WRGP1 at the exit of the washing process in the WR samples.

In both RR and WR liquid samples (Tables 3 and 4), the significantly ($p < 0.05$) higher antioxidant activity of the PW, compared to the DW, could be because the PW contained significantly ($p < 0.05$) higher amounts of phenols than the DW, since the latter are in contact with the GP already depleted in phenols. Although antioxidant activity in the DW was low, the results denote that it may still contain antioxidant compounds that may be of interest.

Both the data obtained regarding the phenolic content of the pomace after the washing processes and the washing waters, as well as the data on antioxidant activity in these same samples, indicate that this industrial process could be optimized to deplete these compounds, which are of great interest to other industries nowadays.

According to the data obtained when comparing RR and WR samples, significant ($p < 0.05$) differences were found between GPs from the washing exit (Table 5), the GS and the DW, with higher antioxidant activity recorded in RR samples. This behavior could be due to the occurrence of anthocyanins in the RRGP samples (Puig i Vayreda, 2015), to which is attributable a remarkable antioxidant activity (Kuskoski, Garcia Asuero, García-Parilla, Troncoso, & Fett, 2004).

The data provided by the ABTS methodology suggest that, in both solid and liquid samples and regardless the type of grape, the antioxidant capacity followed the same pattern as the DPPH results (Tables 1–5). In the light of the reported relationship between phenols from grape pomace and antioxidant activity (Peixoto et al., 2018; Silva et al., 2018), Pearson's linear correlation coefficient and the coefficient of determination have been calculated for both antioxidant capacity methodologies used. For that purpose, solid and liquid samples have been separately considered. To confirm that the linear relationship between total phenols and antioxidant activity is not due to chance, the T-Student test was performed.

In the case of DPPH analysis, the correlation coefficients, for both liquid and solid samples, had high values (0.9492 and 0.8176, respectively), indicating a direct correlation between phenols and antioxidant activity. Furthermore, the coefficient of determination of the solid samples (66.85%) indicated a moderate linear relationship between the two variables, contrarily to the better relationship obtained in the liquid samples (90.10%). The fact that, in solid samples, the linear correlation was lower could be due to the presence of other compounds that also contribute to antioxidant activity (Beres et al., 2019; Shinagawa, Santana, Torres, & Mancini-Filho, 2015), such as reducing sugars (such as glucose and fructose), acids (tartaric, malic, lactic or succinic) and glycerins (Scalzo, Morassut, & Rapisarda, 2012). On the other hand, the higher correlation in liquid samples may be due to those other antioxidant components were not soluble in the washing water, thus most of the antioxidant activity is exerted by soluble phenols.

Correlating the phenolic content with their antioxidant activity obtained by the ABTS method, the high direct linear correlation between both parameters was again confirmed. The correlation coefficients obtained for the solid and liquid samples were 0.9627 and 0.9490, respectively, and their respective coefficients of determination were 92,68% and 90,06%. In this case, the solid samples showed a more similar phenol-antioxidant activity relationship to the liquid samples than in the case of DPPH.

In any case, the Student's test ($p < 0.05$) confirmed the existence of a positive linear correlation between phenols and antioxidant activity, rejecting the null hypothesis (no correlation between the two variables). This fact occurred for both liquid and solid samples.

4. Conclusions

Based on the considerable amounts of unextracted phenols and the presence of fiber and proteins in the studied by-products and residues, it is evident that the grape pomace industry could improve the extraction efficiency of some compounds of interest at each stage of the processing, as well as consider the usage of the normally-discarded residues. Concretely, both in the washed and re-washed grape pomaces and in the depletion water, a significant quantity of phenols still remains, together with proteins and fibers in the defatted grape seed meal residue. Therefore, this work could have an important impact on a direct transfer of results to the grape pomace processing industry, to consider and extract new fractions and improve their extraction yields, which lead to an economic benefit and a reduction in the waste management. After this preliminary evaluation, a more exhaustive study of the chemical composition of the samples after industrial processing would be needed to assess more accurately their usefulness and profitability as by-products.

Funding

This work is financially supported by FEDER/Ministerio de Ciencia e Innovación – Agencia Estatal de Investigación [Project AGL2017-84793-C2].

CRediT authorship contribution statement

Ana Belén Mora-Garrido: Investigation, Writing – original draft. **María Jesús Cejudo-Bastante:** Writing – original draft, Writing – review & editing. **Francisco J. Heredia:** Conceptualization, Supervision. **M. Luisa Escudero-Gilete:** Writing – review & editing.

Acknowledgments

The authors thank the technical staff of Biology Service (Servicios Generales de Investigación (SGI), Universidad de Sevilla). The authors also thank Alvinesa Natural Ingredients, S.A (Daimiel, Ciudad Real, Spain) for supplying the by-product samples.

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