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ORIGINAL ARTICLE

Nutraceuticals based on Portuguese grape pomaces as a potential additive in food products

Nutracêuticos à base de bagaços de uva portuguesa como potencial aditivo em produtos alimentares

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Abstract

Portuguese wine industry by-products are often undervalued but constitute a potential source of bioactive phenolic compounds that can be applied as a natural additive in several industries. In this context, the present study aimed to evaluate the nutritional composition, and the phenolic profile of two Portuguese *Vitis vinifera* L. grape pomaces (Touriga Nacional (red variety) and Alvarinho (white variety), and to correlate their chemical characterizations with their antioxidant activities. Strong correlations were observed between the presence of phenolic compounds and antioxidant activities, which enhances the application of pomace extracts in food and pharmaceutical areas. The high content of total phenolic compounds (25 - 41 g/ kg dry extract) and of flavonoids (9.2 - 18 g/ kg dry extract) found in both samples make these pomaces excellent candidates as food additives in food products, as well as antioxidant agents, such as natural dyes. Some polyphenols were identified by High Performance Liquid Chromatography (HPLC), being rutin and catequin the highest compounds found in red grape pomace (Touriga Nacional) while quercetin was only quantified in white grape pomace (Alvarinho). *Cis*-resveratrol was quantified in both grape pomace, which opens horizons for its use since this compound has considerable chemopreventive effects in the three main gains of carcinogenesis. As expected, the anthocyanin content was significantly higher in red grape pomace (37 g/ kg dry extract), emphasizing its interest as a natural food additive. Based on the findings, it is possible to conclude that these by-products have additional value, making them potentially useful in the food, pharmaceutical, and cosmetic industries.

Keywords: *Vitis vinifera* L.; Autochthonous grape pomaces; Nutritional composition; Polyphenols; HPLC; Antioxidant activity; Food additives.

Resumo

Os subprodutos da indústria vitivinícola portuguesa são muitas vezes subvalorizados, mas constituem uma potencial fonte de compostos fenólicos bioativos que podem ser aplicados como aditivos naturais em diversas indústrias. Neste contexto, o objetivo do presente estudo foi avaliar a composição nutricional, o perfil fenólico de dois bagaços de uvas portuguesas *Vitis vinifera* L. − Touriga Nacional (variedade tinta) e Alvarinho (variedade branca) − e correlacionar as suas caracterizações químicas com as suas atividades antioxidantes. Fortes correlações foram observadas entre a presença de compostos fenólicos e a atividade antioxidante, o que potencializa a aplicação dos extratos dos bagaços na área alimentar e farmacêutica. Os altos teores de compostos fenólicos totais (25 - 41 g/kg extrato seco) e de flavonoides (9,2 - 18 g/kg extrato seco) encontrados em ambas as amostras tornam estes bagaços excelentes candidatos a aditivos alimentares em produtos alimentícios, como agentes antioxidantes ou corantes naturais. Alguns fenólicos foram identificados por Cromatografia Líquida de Alta Eficiência (CLAE), sendo a rutina e a catequina os compostos maioritariamente encontrados no bagaço de uva tinta (Touriga Nacional), enquanto a quercetina foi apenas quantificada no bagaço de uva branca (Alvarinho). O *cis*-resveratrol foi quantificado em ambos os bagaços de uvas, o que abre horizontes para seu uso, uma vez que este composto possui consideráveis efeitos quimiopreventivos nos três principais estágios da carcinogênese. Como esperado, o teor de antocianinas foi significativamente maior no bagaço de uva tinta (37 g/kg extrato seco), enfatizando o seu interesse como aditivo alimentar natural. Com base nos resultados obtidos, pode-se concluir que estes subprodutos têm valor agregado, o que lhes confere potencial aplicabilidade nas indústrias alimentícia, farmacêutica e cosmética.

Palavras-chave: *Vitis vinifera* L.; Bagaços de uvas autóctones; Composição nutricional; Polifenóis; CLAE; Atividade antioxidante; Aditivos alimentares.

Highlights

- In the nutritional composition of the two Portuguese grape pomaces, carbohydrates stand out
- Catechin, epicatechin and epigallocatechin were the phenolic compounds with the highest concentration in both grape pomace
- High amount of bioactive compounds correlated with high antioxidant activity in both grapes pomaces
- The composition and antioxidant activity of these by-products make their applicability in the food and pharmaceutical fields very promising

1 Introduction

Grapes are one of the most produced crops worldwide. According to the International Organisation of Vine and Wine (Organização Internacional da Vinha e do Vinho, 2021), in 2020 the world area under vines, corresponding to the total surface area planted with vines for different purposes (wine and juices, table grapes, and raisins), is estimated at 7.3 mha. Moreover, in 2020, world wine production, excluding juices and musts, is estimated at 260 mhl, thus marking a slight increase of almost 3 mhl (+1%), compared to 2019 (Organização Internacional da Vinha e do Vinho, 2021). In addition to the wine industry expanding, also many scientific studies have shown that the consumption of grapes contributes to human health due to the large content of bioactive compounds (Safraid et al., 2022; Balbinoti et al., 2020; Milincic et al., 2020; Bender et al., 2017). With the increase in grape production, there is an increase in the industrial production of wine, which promotes the generation of huge amounts of by-products. In fact, approximately 75% of produced grapes are intended for wine production, out of which 20% to 30% represent waste products (Balbinoti et al., 2020; García-Lomillo & González-Sanjosé, 2017). Among industrial waste grape pomace, which is the predominant one, consists of skins, remaining pulp, seeds, and stalks. The disposal of this waste creates environmental concerns due to its capacity to pollute the soils and reduce the availability of other ingredients (Pereira et al., 2020).

Different types of high-added-value components have been recovered from grape pomace, such as antioxidant components, carbohydrates, sugars, pectins, proteins, and phenolic compounds (Castellanos-Gallo et al., 2022; Canalejo et al., 2021; Rezaei et al., 2021). However, the phenolic composition of *Vitis vinifera* L. fruits is greatly dependent on the grape type. As a result, the by-products of each grape species may contain qualitatively and quantitatively distinct chemical substances. These added-value by-products are now widely used in the food industry as functional ingredients and natural additives, particularly in animal-derived meals that lose color throughout the food preparation process. Therefore, antioxidants are used to reinstate free radicals thereby retarding lipid oxidation, delay the development of unpleasant-flavors, and improve color stability. The application of synthetic antioxidants to mitigate oxidative damage may consider unsafe for consumers. Some studies highlight the incorporation of bioactive compounds in foods and beverages as functional ingredients (Costa et al., 2022; Machado et al., 2021; Cence et al., 2020; Romeo et al., 2020). Furthermore, the recent growth in the understanding of consumers about these hazards resulted in the replacement of synthetic antioxidants with natural bioactive compounds. Several studies provide plant materials, including grape pomace, that are rich sources of bioactive phenolic compounds; hence they can be an effective alternative to synthetic antioxidants. The primary goal of this experiment is to investigate the nutritional and antioxidant properties of two Portuguese grape pomaces. As a secondary goal, and based on our findings, this work proposed to promote the use of grape pomaces as natural additives, improving nutritionally enrich foods, increasing their quality and/or extending their shelf-life validity.

2 Material and methods

2.1 Material

Two by-products of the wine industry were studied. Touriga Nacional pomace (red grape) and Alvarinho pomace (white grape) were collected in October 2021, in the wine presses of the Sociedade Agrícola Trigo de Negreiros Lda, Bragança, Portugal (wine producer). After collecting a significant amount (~10 kg), samples were immediately frozen (-20 °C) and subsequently freeze-dried (Telstar, Cryodos, Spain). Subsequently, the dehydrated samples were ground in a mill (GM Grindomix 200, Retsh, Germany) for 20 seconds at a speed of 5000 rpm to obtain a fine powder. After homogenization, samples were stored in hermetically sealed flasks, protected from light, for future analysis.

2.2 Proximate analysis

Moisture was determined using an infrared balance (Scaltec model SMO01, Scaltec Instruments, Heiligenstadt, Germany). All other nutritional analyses were performed according to AOAC procedures (Association of Official Analytical Chemists, 2019). Briefly, ashes were quantified after incineration at 500 ºC. Total lipids and protein were determined by the Soxhlet and the Kjeldahl methods, respectively. Total carbohydrates were calculated by difference.

2.3 Extracts preparation

Samples (~$1x10^{-3}$ kg) were extracted with 0.05 L of ethanol for 1 hour at 45 °C on a hot plate (Mirak, Thermolyse, USA) under constant stirring (600 rpm) (Costa et al., 2014). The ethanolic extracts were gravity filtered through Whatman No. 1 1 filter paper; concentrated under a vacuum (Vaccum Controller V-800, Büchi, Switzerland) at 40 °C. All extracts were stored at −20 °C for future analysis.

2.4 Phenolic profile by hplc

The qualitative analysis of the phenolic compounds extracted from the pomaces was achieved with a High Performance Liquid Chromatography (HPLC) equipped with an online diode array detector (HPLC-DAD), and the procedure adapted from Vinha et al. (2002). $2x10^{-5}$ L of acetonitrile (ACN) and $1.8x10^{-4}$ L of 0.2% (v/v) acetic acid solution were added to each dry extract, prepared as previously indicated, and later $2x10^{-5}$ L of the resulting solution were injected into the HPLC equipment (Jasco, Großumstad, Germany; detector MD-2010, Plus, Jasco Instruments, Großumstad, Germany; Gemini® column 5 µm C18 110 Å, LC Column 150 x 4.6 mm, Ea, Phenomenex; pre-column SecurityGuard Ea, Phenomenex). The solvent gradient used was: 0 min ACN/0.2% acetic acid v/v pH 3.0 (9:91 v/v); 3 min ACN/0.2% acetic acid (9:91 v/v); 8 min ACN/0.2% acetic acid (14:86 v/v); 10 min ACN/0.2% acetic acid (16:84 v/v); 13 min ACN/0.2% acetic acid (20:80 v/v); 17 min ACN/0.2% acetic acid (37:63 v/v); 24 min ACN/0.2% acetic acid (37:63 v/v); 27 minutes ACN/0.2% acetic acid (100:0 v/v); 29 min ACN/0.2% acetic acid (100:0 v/v); 33 min ACN/0.2% acetic acid (9:91 v/v); and 37 min ACN/0.2% acetic acid (9:91 v/v). Final chromatograms were analyzed at five different wavelengths, by recording the retention times (tr): 270 nm to determine gallic acid (GA) (tr=5 min.), protocatechuic acid (tr=8.2 min), epigallocatechin (EGC) (tr=11 min), catechin (tr=13.5 min), vanillic acid (tr=15 min), syringic acid (tr=16.3 min), epicatechin (EC) (tr=17 min), epigallocatechin gallate (tr=18.8 min), vanillin (tr=20 min), epicatechin gallate (tr=22.8 min) and *trans*-cinnamic acid (tr=30 min); 285 nm to determine *cis*-resveratrol (tr=24.5 min) and naringenin (tr=32 min); 305 nm for *p*-coumaric acid (tr=21.5 min) and *trans*-resveratrol (tr=26.8 min); 323 nm for chlorogenic acid (tr=15 min), caffeic acid (tr=15.8 min), sinapic acid (tr=23 min), ferulic acid (tr=23.5 min); 365 nm to rutin (tr=22.5 min), myricetin (tr=25.6 min), quercetin (tr=28.5 min) and kaempferol (tr=33.8 min). All compounds were identified and quantified by comparison to known internal standards. For the identification of each compound, the known spectra and retention times were taken into account. After identifying and using the peak area in relation to the area and concentration of the standards, it was possible to quantify their presence in the samples studied through a series of calculations involving the molecular weight of the sample, the dilution used, the amount of grape pomaces used and the initial volume of extraction. Data were expressed as g of compound/ kg of dry pomace.

2.5 Antioxidant profile

2.5.1 Total phenolic content

Total phenolic content was determined spectrophotometrically according to the Folin-Ciocalteu procedure described by Costa et al. (2018). $3x10^{-5}$ L of grape pomace ethanolic extracts were mixed with $5x10^{-5}$ L of Folin-Ciocalteu reagent, previously diluted (1:10, v/v) and $1.2x10^{-4}$ L of Na_2CO_3 (7.5%) solution were added. In addition, the solution was incubated for 1800 seconds at room temperature. The absorbance measurements were performed at 765 nm using a Synergy HT GENS5 Microplate Reader (BioTek Instruments, Inc., Winooski, VT, EUA). Gallic acid was used as a standard to obtain a calibration curve (linearity range: $5x10^{-3}$-0.1 g/ kg; R^2 = 0.9981). The total phenolic content of the extracts was expressed as grams of gallic acid equivalents (GAE) *per* kilogram of grape pomace on a dry weight basis (dw).

2.5.2 Flavonoid content

Total flavonoid content was determined using a colorimetric assay previously validated (Costa et al., 2014). Briefly, $3x10^{-5}$ L of each extract was mixed with $7.5x10^{-5}$ L of distilled water and $4.5x10^{-5}$ L of 1% (w/v) $NaNO_2$ solution. After 300 seconds, $4.5x10^{-5}$ L of 5% (w/v) $AlCl_3$ solution were added, and after 60 seconds, $6x10^{-5}$ L of NaOH (1 M) and $4.5x10^{-5}$ L of distilled water were also added. The absorbance was read at 510 nm using the Synergy HT Microplate Reader. Catechin was used as a reference to plot the standard curve (linearity range = $5x10^{-3}$ – 0.3 g/ kg, R^2 = 0.9975). Total flavonoid content was expressed as g of catechin equivalents (CAE) *per* kg of grape pomace on dw.

2.5.3 Anthocyanin content

The quantification of total anthocyanin content was based on a spectrometry test, based on the preparation of two solutions with different acidity values, according to Vinha et al. (2022). Briefly, two solutions of the same grape pomace extract were prepared: i) 1x10^{-3} L of grape pomace extract + 0.01 L of ethanol with 0.1% concentrated HCl + 0.01 L of buffer solution at pH = 3.5; ii) 0.01 L of sample + 0.01 L of ethanol with 0.1% concentrated HCl + 0.01 L of 2% HCl (pH = 0.6). Absorbance measurements were performed at 520 nm and the total anthocyanin content was calculated by the formula: Total anthocyanin content (g/ kg) = 400 x (Absii -Absi).

2.5.4 *In vitro* antioxidant activity

2.5.4.1 DPPH free radical scavenging assay

The DPPH assay was used to evaluate the free radical scavenging activity of grape pomace extracts. The reaction mixture was prepared directly on a 96 well plate between different sample concentrations (3x10^{-5} L) and an ethanolic solution (2.7x10^{-4} L) containing DPPH radicals (6×10^{-5} M) in each well. The reduction of the DPPH radical was determined at 517 nm at 120-second intervals, for 1800 seconds (Costa et al., 2014). Inhibition of free radical DPPH$^{\bullet}$ was expressed as Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalents (mol TE)/ kg dw.

2.5.4.2 Ferric-reducing antioxidant power

FRAP assay was carried out, according to Vinha et al. (2016). Briefly, 9x10^{-5} L of a diluted extract (1:10) were mixed with 2.7x10^{-4} L of distilled water and 2.7x10^{-3} L of FRAP solution (freshly prepared by mixing 0.3 M acetate buffer, 0.01 M TPTZ solution, and 0.02 M of ferric chloride). The mixture was kept in the dark for 1800 seconds at 37 ºC and, afterwards, absorbance was measured at 595 nm. The results were compared with a standard curve prepared with different concentrations of ferrous sulfate (0.05 – 0.5 g/ L, R^2 = 0.9986), and reducing antioxidant power was expressed as Trolox equivalents (mol TE)/ kg dw.

2.6 Statistical analysis

All experimental analyses were performed in triplicate and means, and standard deviations were calculated. Statistical analysis of the data was carried out using IBM SPSS Statistics Version 24.0 (SPSS, Inc. Chicago, IL). One-way Analysis of Variance (ANOVA) was used to evaluate the differences between the two grape pomaces for all assays. Post hoc comparisons of the means were performed according to Tukey's HSD test. In all cases, $p < 0.05$ was accepted as denoting significance.

3 Results and discussion

In view of the potential use of Portuguese grape pomace in industrial applications, it should be highlighted that it is often difficult to ensure that the raw materials are obtained from the same edaphic and climatic conditions. According to that, it is mandatory to evaluate each considered grape variety independently of its phylogenetic origin, i.e., understand its potential regardless of the species in the analysis. Likewise, it might also be useful to find which grape species possess the highest suitability for a determined purpose. Table 1 describes the nutritional composition of the two autochthonous Portuguese grape pomaces analysed: Touriga Nacional (red grapes variety) and Alvarinho (white grapes variety).

Table 1. Proximate analysis of two Portuguese grape pomace based on dry weight (g/ kg). Touriga Nacional (red grape pomace) and Alvarinho (white grape pomace).

Physico-chemical parameters	Red grape pomace	White grape pomace
Ash	48.2 ± 0.05	47.6 ± 0.03
Proteins	85.1 ± 0.02*	66.3 ± 0.04**
Lipids	62.0 ± 0.01	63.7 ± 0.02
Carbohydrates	311.8 ± 0.04*	292.0 ± 0.05**

*; **Repeated measures ANOVA followed by Turkey's post-hoc test, comparing red and white grape pomaces as independent experiments, denote a significant difference between mean values.

According to Antonić et al. (2020), the most important constituents of grape pomace are carbohydrates (fibers), what is in line with the results obtained in our work, as well as polyphenolic compounds, colorants (anthocyanins), and minerals. In addition, the oily part of the grape pomace, mostly due to seeds, is rich in unsaturated fatty acids, colorants, and minerals. The study aimed to determine the nutritional and chemical characterization of two grape pomace varieties to use the by-product as a natural additive. Regarding Table 1, some differences were observed in our studied samples. Ash determines the total mineral content present in a food. Thus, this amount may vary depending on soil and climatic conditions, viticulture practices, and vinification processes, among others. Ash contents were similar in both grape varieties ($p > 0.05$), significantly inferior to those reported by Strapasson (2016) with values close to 7% for Syrah and Bordeaux varieties and 8% for Tannat grape variety. However, our results are similar to those described for Arinto, Aragonese, and Talia grape pomace varieties (Nicolai et al., 2018). Touriga Nacional (red grape pomace) presented higher protein and carbohydrate contents (85.1 g/ kg and 311.8 g/ kg, respectively) exhibiting an identical lipid profile with Alvarinho (white grape pomace). The high protein levels found in both samples potentiate the use of these by-products in the enrichment of food products. For example, fortification of fish products was studied by Cilli et al. (2019) that have added 1% and 2% of grape pomace to the salmon burger mixture. As previously mentioned, in our grape pomace samples significant differences in carbohydrate content were observed ($p < 0.05$), thus agreeing with the results reported by other authors (Spinei & Oroian, 2021; Beres et al., 2017). According to Antonić et al. (2020) and, in agreement with our results, dietary fibers (carbohydrates) are the main compounds described in red grape pomaces, verifying lower values for white grape ones. Despite the differences found between red and white pomaces, both are promising as functional ingredients for incorporation in foods. Together with considerable protein and carbohydrate contents, polyphenols are the most valuable compounds of grape pomace, presenting health beneficial properties, such as the maintenance of intestinal health and the prevention of chronic diseases and cancer (Averilla et al., 2019). The main representatives of polyphenolic compounds in grape pomace are anthocyanins (only in red grapes), catechins, flavonol glycosides, phenolic acids, and alcohols (Messina et al., 2021). Table 2 and 3 present the content of bioactive compounds found in the two studied samples, as well as the *in vitro* antioxidant activity.

Table 2. Quantification of phenolic compounds extracted from the two grape pomaces analysed (g/ kg dry extract), using the HPLC chromatographic technique.

Phenolic compound	Red grape pomace	White grape pomace
Gallic acid	0.15 ± 0.05*	0.25 ± 0.02**
Protocatechuic acid	0.01 ± 0.01	0.02 ± 0.01
Epigallocatechin	0.50 ± 0.07*	0.89 ± 0.09**
Catequin	0.75 ± 0.04*	0.64 ± 0.06**
Vanillic acid	0.18 ± 0.01	nq
Syringic acid	0.09 ± 0.02	nq
Epicatechin	0.30 ± 0.01*	0.25 ± 0.10**
cis- Resveratrol	0.08 ± 0.11*	0.02 ± 0.15**
Rutin	0.63 ± 0.08*	0.10 ± 0.03**
Quercetin	nq	0.40 ± 0.01

*; **Repeated measures ANOVA followed by Turkey's post-hoc test, comparing red and white grape pomaces as independent experiments, denote a significant difference between mean values. Mean ± Standard Deviation. nq: not quantified.

Table 2 shows a broader spectrum of bioactive compounds, as well as higher levels in red grape pomace (Touriga Nacional variety). Deepika & Maurya (2022) stated that some phenolics, such as vanillic acid and syringic acid, were found only in red grape pomace, while quercetin was found only in white grapes (Alvarinho), in agreement with our results. Quercetin content, found only in white grape pomace (0.40 g/ kg), shows the importance of applying this by-product as a functional ingredient and/or natural preservative (Batiha et al., 2020). Xu et al. (2019) noticed that quercetin has a series of activities, namely antioxidant, anti-inflammatory, and anti-cancer in various cell and animal models, as well as in humans, through the modulation of signaling pathways and gene expression involved in these processes. As observed in Table 2, catechin, epicatechin, and epigallocatechin were the major phenolic compounds found in both grape pomaces, reinforcing their use as bioactive compound, duo to these compounds are associated with various properties, such as antibacterial activity (catechin and epicatechin) and free radical scavenging and anti-inflammatory activities (catechin) (Luo et al., 2022). Rutin was the predominant flavonoid presented in red grape pomace (Touriga Nacional), which is known as vitamin P, and being recognized for its antineoplastic, antioxidant, antidiabetic, anti-inflammatory, antibacterial, antifungal, neuroprotective, cardioprotective, hepatoprotective, nephroprotective and hematoprotective properties (Prasad & Prasad, 2019).

As far as we know, polyphenolic compounds and anthocyanins are the main responsible for the grape pomace antioxidant potential, and therefore, a large number of food, cosmetic, and pharmaceutical applications of grape pomace extracts have been reported in the literature, since those compounds act as multipurpose ingredients or additives in different fields of industry (Wasilewski et al., 2022; Spinei & Oroian, 2021; Hassan et al., 2019). Table 3 shows the concentrations of these chemicals in the two grape pomaces examined. Also, as a preliminary indicator of the potential bioactivity of the studied grapes, the antioxidant activity was evaluated by measuring the scavenging activity against DPPH radicals and the ferric reducing power (FRAP).

Table 3. Total phenolic, flavonoid, and anthocyanin contents in the two grape pomace extracts (Touriga Nacional – red grape and Alvarinho – white grape) (g/ kg dw), and antioxidant activity (DPPH and FRAP in mol Trolox equivalents (TE)/ kg dw).

	Red grape pomace	White grape pomace
Total phenolics	41 ± 0.9[a]	25 ± 3[b]
Total flavonoids	18 ± 0.3[a]	9.2 ± 0.8[b]
Total anthocyanins	37 ± 0.7[a]	2.6 ± 2[b]
	Antioxidant activity	
DPPH	2.54 ± 3[a]	1.25 ± 2[b]
FRAP	1.21 ± 2[a]	0.69 ± 4[b]

DPPH (free radical scavenging assay); FRAP (Ferric-reducing antioxidant power).

Total phenolic content values of the two grape pomaces closely matched the data reported by several authors (Negro et al., 2021; Marinelli et al., 2015; Zhang & Zhu, 2015) which have described values between 20 and 70 mg/ g dw. The polyphenols mainly present in the grape varieties are phenolic acids and flavonoids. Significantly (p < 0.05) lower total flavonoid contents were detected in both grape pomaces, being approximately lesser than total phenolic concentration in both samples. These results are according to other studies in different grape by-products. For instance, Sochorova et al. (2020) reported 14 antioxidants (gallic acid, caffeic acid, coumaric acid, coutaric acid, ferulic acid, fertaric acid, trans-piceid, trans-piceatannol, rutin, quercetin-3-β-D-glucoside, quercitrin, myricetin, catechin, and epicatechin) in ten vine seeds varieties, being the phenolic content significantly higher than the flavonoids. Regarding the DPPH test, the red grape marc showed the highest value (25.4 mol TE/ kg dw). The other cultivar (white grape pomace) showed a lower value (12.5 mol TE/ kg dw). The FRAP test provided similar values: the best antioxidant activity values were observed in red grape marc (12.1 mol TE/ kg dw), while white grape pomace extract showed lower activity (6.9 mol TE/ kg dw). From a nutraceutical and industrial perspective, the simultaneous presence of compounds deriving from grapefruit metabolism and molecules modified by yeasts during fermentation makes grape pomace a very interesting by-product; indeed, it has been suggested that the anthocyanins which have many substitute groups are more resistant to degradation during heating and at the same time are less affected by pH variations (Slavu et al., 2020). Anthocyanins are natural pigments and, currently natural colorants have emerged as an alternative to their synthetic counterparts due to an existing health concern of these later. Nowadays, natural-food colorants have found their niche for valuable food applications. Single-phase coloring systems such as baking products, meat, and fish products (solid phase) or drinks (liquid phase) have been successfully assayed with natural colorants such as carotenoids or anthocyanins (Vinha et al., 2020; Lin et al., 2018; Vinha et al., 2018).

The results of the DPPH and FRAP tests showed that the red grape pomace possessed the highest antioxidant activity among the examined samples ($p < 0.05$), which may, presumably, be due to the large number of bioactive compounds detected in red grape pomace. Furthermore, such compounds as syringic acid, vanillic acid, and rutin are characterized to possess high antioxidant activity (Enogieru et al., 2018). Yet, white grape pomace (Alvarinho) also showed relevant antioxidant activity, even with lower levels of anthocyanins, this by-product stood out for the presence of quercetin, which is recognized as a flavonoid with, as mentioned, high antioxidant action, with pivotal role in treatment of diabetes, cancers, and some cardiovascular diseases (Magar & Sohng, 2020; Lesjak et al., 2018; Ozgen et al., 2016).

4 Conclusions

Europe, including Portugal, is considered the largest producer of wine worldwide, showing a high market potential. In this context, the interest in redirecting the use of these residues has increased due to overproduction, great availability, and low costs. The utilization of grape pomace derived from the wine industry would economically benefit the producers while mitigating the environment impacts. These by-products can be expected to different pre-treatments (physical, chemical, and biological) for the partition of different compounds with high industrial interest, reducing the waste of agro-industrial activities and increasing industrial profitability.

Results on the nutritional value and chemical composition of the two grape pomaces provided guidance for developing innovative industrial applications. The high content of total phenolic and flavonoid compounds makes them excellent candidates as food additives which provide food commodities with high antioxidant activity and natural colorants. With the identification and quantification of some polyphenols in both samples, white Alvarinho grape pomace appeared to contain a high content of quercetin, which opens horizons, not only for the food industry but also for the pharmaceutical and cosmetic industries. Finally, red grape pomace (Touriga Nacional) provides high natural colorants contents (anthocyanins) enhancing its application in the food area in addition to pharmaceuticals and cosmetics. Thus, recycling of winery by-products constitutes an opportunity for providing valuable materials to food, pharmaceutical, cosmetic, and nutraceutical industries.

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