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Chemical characterization of the cultivar ‘Vinhão’ (*Vitis vinifera* L.) grape pomace towards its circular valorisation and its health benefits

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ABSTRACT

Among the most significant and productive agroindustries worldwide is the wine business. This agroindustry generates millions of tons of biological residues, carrying huge negative impacts related to the disposal of these materials into the environment. Nevertheless, these byproducts present a high potential for developing new products, contributing to the circularity of this economic sector. In this study, the chemical composition of the grape pomace (GP) obtained from the vinification process (2018 campaign).) of red grapes of the Portuguese cultivar ‘Vinhão’ was determined. The analyses of the pH, proximate composition (moisture content, proteins, lipids, ashes, fibers, and carbohydrates), the total phenolic compounds (Folin Ciocalteau method), and the mineral and fatty acid composition were carried out in triplicate, and the results expressed on a dry matter basis. After dehydration of ‘Vinhão’ GP, this very acidic by-product showed the following proximate composition (%): moisture, 3.43 ± 0.86; ash, 8.20 ± 0.017; lipids, 3.38 ± 0.11; proteins, 9.85 ± 0.51 and 3.28 ± 0.04; dietary fiber, 49.37 ± 1.75; carbohydrates, 35.47 ± 2.35. The content of total phenolic compounds was 35.35 ± 3.61 mg GAE/g. GP also revealed to be an excellent source of K, Ca, Fe, Mn, as well as of arachidic acid. The results obtained in this study suggest that GP from the Portuguese cultivar ‘Vinhão’ is feasible to enrich the nutritional value of food and feed. Moreover, the integration of pomace flour in food and feed is a viable option for reducing the environmental impact and adding value to the by-product, responding to the circular economy challenges.

1. Introduction

The wine sector is one of the world’s most productive and important agroindustry. Every year, 259.9 million hectoliters of wine are produced in the world, being responsible for the generation of 20 million of tons of biological residues. These bioresidues carry on huge sustainability concerns due to the negative impacts related to the disposal of these materials into the environment due to their low degree of biodegradability, acidification potential and excess of polyphenols in water and soil [1–3]. Green Chemistry processes and Circular Economy principles can play a major role on the reduction of these negative impacts and have a very high economic return [4,5], through the conversion of these residues into high-added value products for food, feed, cosmetics or pharmaceutical industry. Among the numerous solid residues of the grape wine industry, grape pomaces – including the peels (grape skin) and seeds - are considered byproducts with extreme potential nutritional value. Indeed, grape pomaces are known as a potential source of high-added-value functional ingredients, such as dietary fibers and bioactive compounds [6–8]. The presence of these high-value compounds in grape pomace [8], led to an increasing interest of companies and research groups towards its valorization for new applications such as novel green, sustainable, and renewable products [9–11]. With these objectives, several recent studies have been carried out to worldwide characterize grape pomace. Some examples such as Ferreira & Santos [12] conducted a study using samples of grape pomace and seeds purchased from Alfândega da Fé, Bragança, Portugal, from three different grape varieties: Touriga Nacional, Touriga Francesa, and Tinta-roriz. The study showed that phenolic extracts possess strong antioxidant

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properties and are effective in protecting creams from degradation and oxidation. Furthermore, there is a possibility that these extracts could replace synthetic ingredients like BHT and act a natural antioxidant agents. Red grape pomace from the red grape varieties (*Vitis vinifera* L.) was collected from the Emilia Romagna region of Italy. The pomace extracts were obtained through extraction of NADES (natural deep eutectic solvents) and have potential for future applications in the cosmetic industry. This research aims to uncover new possibilities for utilizing these extracts in cosmetic products [13,14]. Caponio et al. [14] conducted a study on grape cultivars Aglianico and Nero di Troia from two vineyards in the Corato region of Puglia, Italy. Their findings confirmed that extracts from grape pomace in red wine are a significant source of antioxidant compounds that remain bioaccessible even after undergoing gastrointestinal digestion. Another study was conducted on pomace samples from wineries in Galicia. The purpose of the study was to valorize grape pomace as a nutrient-rich biofertilizer, and to produce a marketable brandy, as well as a mixed fraction composed mainly of seeds. From this mixed fraction, an extract rich in polyphenols and oil in fatty acids can be obtained [15]. Another investigation was conducted utilizing grape pomace (GP), wherein the inclusion of GP (15 and 25%) derived from the Syrah variety in Argentina was undertaken. This resulted in enhancements in the nutritional profile of the muffins with increasing GP content, notably elevating the levels of protein and crude fiber.The authors also verified the good level of acceptability by consumers [16]. In the study of Cilli et al. [17] after incorporating 1% and 2% grape pomace flour into salmon burgers, the authors of the study concluded that the flour could inhibit the secondary compounds of lipid oxidation. As well as in the study of de Alencar et al. [18], determined the best level of winemaking by-product flour (FBM) (1 g.100 g^{-1}) as a natural antioxidant to replace butyl hydroxytoluene (BHT) in beef burgers. In another related study on the utilization of grape pomace for animal feed, alternative sources have been identified to enhance the quality of food products, such as sheep meat. Researchers have observed an exceptional profile of fatty acids and an increase in the oxidative stability of sheep meat, attributing such effects to the presence of phenolic compounds originating from the consumption of grape pomace by these animals [19].

Commercially, grape pomace is used in a variety of ways, including the production of brandies, such as aguardente bagaceira, a high-alcohol Portuguese drink. In addition, it is used in the manufacture of substrates and fertilizers, exemplified by the company Beifiur. Portuguese companies, such as Soalheiro and Tintex, located in Alto Minho, innovate by using grape pomace in new applications. They developed an alternative fabric to leather, made with organic cotton and grape pomace, for use in clothing and bottle labels. This initiative promotes sustainability and innovation by reducing agricultural waste in the food and textile industry [20–22].

Among these varieties and cultivars, 'Vinhão', also known as 'Souzão' in Porto Wine region, is an important cultivar to produce red wine in the Controlled designation of origin region of "Vinhos Verdes". 'Vinhão' is known by the biting acidity and dark and opaque color of wine, conferred by the red pulp and skin of their grapes. Despite the importance of this cultivar, the potential valorisation of 'Vinhão' grape pomace is still hindered by the lack of knowledge on the composition of this by-product of winemaking process. Only few studies can be found in the literature related to the characterization of grape pomace of this cultivar, such as the study on anthocyanins content of grape pomace or that of the seed composition [23,24].

The objective of this investigation was to evaluate the Portuguese cultivar's proximate composition, color parameters, total phenolic content, mineral composition, fatty acid profiles, and Fourier transform infrared spectroscopy (FTIR). The present study points to the great potential of valorization of "Vinhão" grape pomace into the development of high added-value and nutritional products for human or animal consumption, responding to the principles of the circular economy.

2. Material and methods

2.1. Sample collection

In this study, it was used the grape pomace of "Vinhão", a by-product from the bagasse that resulted from the vinification process of red grapes of the Portuguese cultivar "Vinhão" (2018 campaign). The frozen material was supplied by Quinta de Azevedo (Lama, Barcelos, Portugal). The bagasse was thawed, and the grape pomace, which included a mixture of pulp, skins and seeds were distributed in aluminum trays and dried in an oven with forced air circulation at 40 °C for 24 h. After drying, the grape pomace was ground in a mill (Retsch, Germany; impact rotor mill SR 300, sieves with 0.50 mesh opening and round hole 3.00 mm). The powders were sieved in a 35-mesh sieve and stored in a plastic bag, to be later used for proximate composition determination and phenolic, mineral and fatty acid profiling.

2.2. Determination of proximate composition

Analyses of the proximate composition of moisture(AOAC 934.06), proteins (AOAC 960.52), lipids (AOAC 920.39), dietary fibers (AOAC 2011.25) and ash-dried (AOAC 936.03) grape pomace were performed based on the Association of Official Analytical Chemistry [25] methods. The total carbohydrate content was obtained by difference, subtracting from 100 the sum of the values obtained for moisture, proteins, lipids, fibers, and ash percentages [26,27]. All the results of the proximate composition were expressed in g 100 g^{-1} dry basis (100 g^{-1} DB). Physical parameters such as pH were measured using the pH-meter micro pH 2002 (CRISON, Spain) in triplicate.

2.2.1. Identification and quantification of sugars by HPLC

The soluble sugars of the pomace samples were extracted using 85% (v/v) ethanol with a 1:50 of solid to liquid ratio and incubated for 30 min with constant shaking in a water bath at 50 °C [25]. The extraction procedure was repeated twice, and after centrifugation (16,639 × g, 10 min), the supernatants were combined and placed in a rotary evaporator at 50 °C to remove ethanol [28]. The chromatographic separation was carried out using a Beckman Coulter HPLC equipment coupled to IR (K-2301) and UV detector (K2501) (Knauer, Berlin, Germany). Then 20 µL of sample were analyzed using an Aminex HPX-87H column (BioRad, Hercules, CA, USA) operated at 40 °C with 5 mM H_2SO_4 as mobile phase at a constant flow of 0.6 mL/min. Data acquisition and analysis were accomplished using Clarity software. The detection of simple sugars was obtained by the IR and UV detectors, as reported previously by Gomez-García et al. [29]. Qualitative analyses of uronic acids were also performed with the same parameters. All determinations were made in triplicate.

2.2.2. Total energy value

The total energy was calculated based on the energy nutrient results obtained using the conversion factors of Atwater, as described by Osborne and Voogt [30], considering 4 kcal/g for carbohydrates, 4 kcal/g for protein, and 9 kcal/g for lipids [31].

2.3. Conventional extraction of polyphenols

The extraction of polyphenols from grape pomace was performed, according to the adapted methodologies [32–34]. A total of 3 g of grape pomace powder was resuspended in a total volume of 60 mL of a cold mixture of ethanol and water (50/50, v/v), and then stirred at 1.26 x g for 120 min at room temperature, using a stirring incubator (MaxQ™ 6000 - Stackable Incubated Shaker MaxQ SHKE6000JPN; Thermo Scientific™, USA). Afterwards, the sample suspension was centrifuged (Hettich Universal 320R; Germany) at 5000 rpm for 5 min. The supernatant was collected and filtered through a Buechner funnel, using filter paper, then the samples were hermetically sealed to prevent oxygen ingress and then the hydroalcoholic extracts were recovered and stored at −18 °C for further analysis.

2.3.1. *Total phenolic content (TPC)*

Total phenolic content was determined using the Folin-Ciocalteu reagent method [35] in a 96-well microplate as described by Coscueta et al. [36]. An aliquot of 30 μL of extract (and for each corresponding extract of pomace) was added to 100 μL of Folin-Ciocalteu reagent and then mixed. Then, 100 μL of sodium carbonate (7.4% w/v) was added to the mixture, and let the plate with the extracts to be incubated for 30 min at 25 °C. Finally, the absorbance was measured at 750 nm with a UV 1240-UV-Vis spectrophotometer (Shimadzu, Japan). Gallic acid was used as the calibration curve standard (0.025–0.200 mg mL^{-1}) and the results were expressed as milligrams of gallic acid equivalent per gram of dry matter (mg GAE g^{-1} DB). All measurements were performed in triplicate.

2.4. *Color analysis*

The color of the samples was determined in a digital colorimeter model Chroma Meter CR-700 (Konica Minolta. Osaka. Japan). using the CIELab scale to determine L*, a* and b* color parameters [37]. The sample was poured into a Petri dish with a 5 cm diameter covering the entire bottom of the dish and the reading was performed at 10 different points. The total color difference (ΔE*) was calculated about fresh vine stalks. according to Eq. (1).

$$\Delta \mathbf{E} = \sqrt{\Delta \mathbf{L}^2 + \Delta \mathbf{a}^2 + \Delta \mathbf{b}^2} \tag{1}$$

In this study. L*0. a*0 and b*0 stand for the values of the color parameters of fresh stalk vine and L*. a* and b* for the values of the color parameters of the sample in each step.

2.5. *Mineral determination*

For mineral analyses, 0.5 g of each sample was weighed and then digested in nitric acid 5% (HNO_3), in a closed microwave system MARS 1 (CEM Corporation, NC, USA) on stream for 30 min/200 °C. After digestion. the extract obtained was used for the analysis of the following minerals: calcium (Ca), iron (Fe), manganese (Mn), phosphorus (P), lead (Pb), cobalt (Co), nickel (Ni), aluminum (Al), sodium (Na), cadmium (Cd), zinc (Zn), molybdenum (Mo), magnesium (Mg), copper (Cu) and potassium (K). The quantification of the minerals was performed by inductively coupled plasma atomic emission spectrometry (ICP-OES/optima 7000DV; Perkin Elmer, USA) with external standardization by preparing standard curves for each of the elements analysed [25]. All measurements were performed in triplicate and the results were expressed in mg 100 g^{-1} dry basis (DB).

2.6. *Fatty acid profile*

The analysis of fatty acid profile (only two replicates) was determined according to Machado et al. [38] with minor modifications. For the fatty acid profile, 300 mg of the sample was weighed in a glass tube, and then added 200 μL of tridecanoic fatty acid (1.5 mg mL^{-1}), 800 μL of hexane, and 2.26 mL of methanol. and 240 μL of sodium methoxide 5.4 M. The tubes were brought to a thermal block at 80 °C for 10 min after being vortexed for 30 s. Once cooled in ice, 1.25 mL of dimethylformamide and 1.25 mL of H_2SO_4 /MeOH 3M were added, and the tubes were taken again to a thermal block at 60 °C for 30 min, after which they were cooled in ice. Then, 1 mL of hexane was added to the mixture and vortexed for 30 s. After centrifuging for 5 min at 1.250 g, the upper phase, containing the fatty acids methyl esters, was collected into vials. The analysis was carried out in a gas chromatograph Agilent 8860 (Agilent, USA) equipped with a flame ionization detector and a BPX70 capillary column (60 m × 0.25 mm × 0.25 μm; SGE Europe Ltd, Courtaboeuf, France). Analysis conditions were as follows: injector temperature 250 °C, split 25:1, injection volume 1 μL; detector (FID) temperature 275 °C; hydrogen was carrier gas at 20.5 psi; oven temperature program: started at 60 °C (held 5 min), then raised at 15 °C/ min to 165 °C (held 1 min) and finally at 2 °C/ min to 225 °C (held 2 min). Fatty acids were identified by comparing the retention times of fatty acids present in the Supelco 37 Component FAME Mix (Sigma-Aldrich,Branch office in Portugal; REF: CRM47885). Results were expressed as milligrams of fatty acid per 100 gs of dried milled grape pomace (mg 100 g^{-1} DB).

2.7. *Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR)*

The samples were analyzed on a PerkinElmer Paragon 1000 FTIR (Waltham. MA. USA) with an ATR accessory (Diamond/ZnSe). Spectra were obtained in the wavenumber range of 4000–550 cm^{-1}. with a resolution of 4 cm^{-1} accumulating 16 scans. The FTIR-ATR vibrational bands were identified based on the literature [39].

2.8. *Statistical analysis*

Each assay was run in triplicate are reported as mean ± standard deviation (sd). Kolmogorov–Smirnov *test tested the normality of data distribution ($p < 0.05$).* STATISTICA™ v10.0 software (Statsoft. Inc., USA) for Windows was used for data treatment.

3. Results and discussion

For the chemical characterization of the grape pomace of cultivar ‘Vinhão’ from Quinta de Azevedo (Lama, Barcelos, Portugal), it was performed an analysis of the proximate composition, phenolic content, and mineral and lipidic profiles.

3.1. *Proximate composition of ‘Vinhão’ grape pomace powder*

The proximate composition, the dietary fiber content, and the pH of the ‘Vinhão’ grape pomace powder are presented in Table 1.

The pH value of grape pomace of the cultivar ‘Vinhão’ analysed in this study can be classified as an acidic product (pH 3.69). This result was in agreement to that found by Nicolai et al. [40] who obtained pH between 3.77 and 4.52, for grape pomace of other cultivars of *Vitis vinifera* L., collected from two Portuguese locations, Herdade da

Table 1
Physicochemical characterization of grape pomace of ‘Vinhão’cultivar (g 100 g^{-1}DB).

Parameter (mean ± standard deviation)	
pH	3.69 ± 0.01
Moisture content (%)	3.43 ± 0.86
Ashes (%)	8.20 ± 0.02
Lipids (%)	3.38 ± 0.11
Proteins (%)	9.85 ± 0.51
Dietary Fiber (%)	49.37 ± 1.75
Carbohydrates (%)	35.47 ± 2.75
Fructose (g.100 ± g^{-1})	13.97 ± 0.78
Glucose (g.100 ± g^{-1})	21.21 ± 0.47
Uronic acid (%)	11.18 ± 0.48
Total Calories (Kcal.100 g^{-1})	211.70
TPC**	35.35 ± 3.61
Color Parameters	
L*	21.19 ± 0.38
a	21.36 ± 0.05
b	0.25 ± 0.06
C*	21.36 ± 0.05
ΔH*	20.86 ± 0.05

** TPC=Total phenolic compounds (mg GAE g^{-1}).
L*= luminosity; C * (Chroma); ΔH* (Tonal Angle).

Malhadinha Nova (Alentejo) and Herdade de Vila Chã (Ribatejo).

Moisture content represents the total water content of the food, being one of the most influential parameters in the stability evaluation of a product [41]. The moisture content found in 'Vinhão' grape pomace powder was similar to the content of other grape pomaces reported in the literature. For example, Bicas, [42] reported 2.03 g 100 g^{-1} for the grape pomace of the species *Vitis labrusca*, cultivar 'Bordô'. On the other hand, in the study of Kruger et al. [43], grape pomace, where it was collected from wineries located in Vale dos Vinhedos, Serra Gaúcha, Rio Grande do Sul, Brazil, from this residue grape pomace flour was obtained, in which moisture content of 4.5%–3.1% was found, and this study (Table 1)was within the margin of the literature, despite being different locations.

The low moisture content after dehydration and the low pH of 'Vinhão' grape pomace from Quinta de Azevedo suggest an increase in the stability of this product, thus an enhancement of the viability of their conservation and stockage for further upcycling processes.

The ash content corresponds to the mineral content of the sample [44]. Ash content of 'Vinhão' grape pomace found in this study was 8.20 ± 0.02%, almost similar to that of 7.13% and 8.36%, reported by Pereira et al. [45] for the cultivars 'Syrah' and 'Cabernet Sauvignon' (*V. vinifera L*) in Alentejo (Portugal). However, it was four-times higher to that reported by Coelho [46], for the grape pomace 'Vinhão' (supplied by another producer farm from Northern Portugal) (2.44% of ash) or that reported by Madadian et al. [47] for 'Concord' grape pomace powder from grapes growing in Canada (*V. labrusca*) (3.3% ash content).

The protein content found for 'Vinhão' grape pomace from Quinta de Azevedo was 9.85%, being higher than that found by Bender et al. [48] and Spinei & Oroian, [49], 6.78 g 100 g^{-1} g and 8.49 g 100 g^{-1}, respectively, but lower than the 14.65% obtained by Ferreira [50] for the grape pomace of cultivar 'Isabel' (*V. labrusca*). Nevertheless, the protein content observed for the 'Vinhão' grape pomace analysed in study was relative low, but relatively high compared with other vegetable flours [49].

The lipid content obtained in this study for grape pomace was 3.38%. This result was similar to that of Bender et al. [48] that reported 5.13% of lipids for grape peel flour. In another study [51] with cv . 'Malbec' (Vitis vinifera L.) found 8.50% of grape pomace lipids. However, this value was below to that found by Huerta [52] (9,83%) and Oliveira et al. [53] (7.66% and 6.76% lipids for grape pomace and the grape flour of 'Cabernet Sauvignon', respectively). The decreased amount of seeds in the flour may cause of the decrease in lipid content seen in the grape pomace of "Vinhão" [54].

In this work, the total dietary fiber content was 49.37% for grape pomace flour. This dietary fiber was similar to that reported by Spinei and Oroian [48] for the dietary fiber in the grape pomace of the species *V. vinifera* L (46.17%). However, these values were lower compared to the high total dietary fiber content (71.53 g 100 g^{-1}DB) found for grape seed meal of 'Syrah' varieties (59–62 g 100 g^{-1} DB) [55] and higher for grape pomace of 'Cabernet Sauvignon` (37 g 100 g^{-1} DB) in the research of Oliveira et al. [56]. The 'Vinhão' grape pomace contained 35.47 g 100 g^{-1} DB of carbohydrates, higher than those obtained by Machado et al. [57] who used 'Isabel' grape residue flour (26.78 g 100 g^{-1} DB). With remarkable results concerning the composition of grape pomace (Table 1), compared to the literature, it can be stated that the valorization of grape pomace is proving its importance [11].

Grape pomace is a residue rich in carbohydrates, most of these carbohydrates are soluble monosaccharides, such as glucose and fructose, and cell wall polysaccharides [58].There are several methods to determine sugar components by high-performance liquid chromatography globally [59]. In this study, the sugars identified were Glucose (21.21 ± 0.47 g 100 g^{-1}) and fructose (13.97 ± 0.78 g 100 g^{-1}), they are the simple sugars present in the grape pomace. There were no significant values for sucrose. When compared to the literature, Sousa et al.'s study [31],on grape pomace (Vitis vinifera L.), Benitaka variety, cultivated in the semiarid region of Northeast Brazil, reported glucose and fructose values of 7.95 and 8.91 g·100 g^{-1}, respectively. These figures are lower than those observed in the current study with 'Vinhão' (Table 1).

Consequently, fructose and glucose emerge as the most predominant sugars in most fruits, playing a significant role in contributing to the sensory profile of grapes. Thus, the ratio of glucose to fructose in grape pomace can exhibit considerable variations, influenced by various factors such as grape variety, cultivation region, adopted agricultural practices, and the process of wine or grape juice production, among others [60,61].

Uronic acids (acidic sugars), which have the carboxyl group, one of their main components being galacturonic acid, the main pectin-forming monomer (polyuronides) present in the cell wall of seeds. We estimated this amount by measuring the concentration of uronic acids (UA) as shown in Table 1 Uronic acid contents of the ''Vinhão'' grape pomace soluble fractions, may fluctuate based on various factors, including grape variety, growing conditions, winemaking procedures, and juice extraction methods. Moreover, uronic acids could impact the technological characteristics of grape pomace flour in food applications, including water retention and gel formation [62–64].

Concerning the total energy value, the main macronutrients in grape pomace are proteins, lipids, and carbohydrates. Thereby, carbohydrates and proteins are essential for human health, as the lipids of grapes are mainly concentrated in their seeds and are made up of about 90% monounsaturated fatty acids, known for their beneficial properties, namely for the cardiovascular system. Carbohydrates are the body's preferred energy source and fuel the central nervous system and other organs in the human body. Proteins are necessary for the body's growth, development, regeneration, and reconstruction and are responsible for producing antibodies, blood cells, hormones, and enzymes [31,65–67].

3.2. Phenolic content of 'Vinhão` dried grape pomace

The content of total phenolic compounds in the hydroalcoholic extracts of 'Vinhão` grape pomace (Table 1) was quantified as a proxy of the content of all compounds belonging to the classes of phenolic compounds in a sample. The total phenolic content of 'Vinhão` grape pomace (35.35 ± 3.61 mg GAE g^{-1}) was found higher than that reported by Sokač et al. [68](29.17 mg GAE g^{-1}) sample for 'Graševina` grape pomace, as well as superior to the study of Abdelhakam et al. [69]., with 4.92 mg GAE g^{-1} for grape pomace polyphenols Red grapes, variety Red Globel from Egypt, but lower than those reported for by Rodrigues et al. [70]. obtained 38.70 mg GAE g^{-1} of phenolic extracts from the grape pomace (GP) was collected from a red wine producer from the central region of Portugal. As well as the values of total phenolic content of 'Vinhão` grape pomace was lower compared to the levels found by Barriga-Sánchez et al. [71] 'Black Borgoña' bagasse (*Vitis labrusca*) (54.36 mg GAE g^{-1}) from Ica (Peru).

Characterizing the wine industry by-residues such as grape pomace is an essential initial step to improve the understanding on the potential of these by-residues as a source of functional ingredients for food/feed and other applications [48]. The analyses were made to determine the potential valorization of this significant and under-valorized subproduct resulting from the vinification of 'Vinhão`, a very important red grape cultivar of *V. vinifera* L of "Vinho Verde" DOC region (Portugal). It should be noted that the chemical composition of grape pomace flour can be highly variable due to factors such as the maturation phase grapes, the time of year, agroclimatic conditions, and the vineyard management system, such as irrigation, use of fertilization and health status of grapes at the time of harvest, and the oenological practices and vinification processes [72–74].

The grape pomace 'Vinhão' proved to be an excellent source of fibers. Dietary fibers from fruits have better nutritional value than those derived from cereals because they are associated with significant amounts of bioactive compounds, such as polyphenols and carotenoids [75]. The total phenolics content present in 'Vinhão' grape pomace indicated that this wine industry by-product has a high potential as a source of polyphenols. When consumption through nutraceuticals or foods, these compounds can bring health benefits as a prevention to combat cardiovascular diseases, cancer, diabetes and osteoporosis among others [76].

3.3. Colorimetric determination

The color of the grape pomace samples was evaluated based on the following parameters: luminosity (L*), chromaticity (chroma) and shade (ΔH), the results of which are shown in Table 1.

The L* parameter is used to quantify the luminosity of the sample, with values ranging from black to white. When analyzing the luminosity variable, values close to the darkest color were observed. In turn, the parameters a* and b* represent coordinates that can be transformed for better understanding. The results indicated that for parameter a* there was a predominance of a color close to red, while for parameter b* it was closer to blue color, as expected due to the raw material having purple color.

Regarding the shade of the color, which is measured by the Hue angle (H°), the samples showed high intensity, the values are closer to zero (0°), which is equivalent to the color red. The hue angle (h°) is considered the qualitative attribute of color, i.e. how colors are traditionally defined. Regarding chromaticity, it measures the intensity of the color of the sample, for this parameter, 21.36 was verified, so the lower the chroma value, the more violaceous the sample [77,78]. Therefore, in chromaticity, the lower the value, the redder the sample, this parameter is a cylindrical coordinate that is related to the coordinates a* and b* and varies from 0° to 360° according to the tone of the color presented. According to the Commission Internationale de L'éclairage (CIE), color is an aspect of the visual perception of a distinct object that an observer distinguishes through size, shape, texture, position and brightness, depending on the spectral position of the incident light and also on the geometry of the viewing optics [79–82]. Portuguese Standard 937/1987 establishes the process for the determination of color and chromatic characteristics of bagasse extracts by the tristimulate method of the International Commission on Illumination (Commission Internationale de L'éclairage, C.I.E.) [82]. Therefore, the importance of color determination in grape pomace may be an indicator of antioxidant quality. Because the color of grapes and their by-products, such as pomace, is mainly attributed to phenolic compounds, such as anthocyanins and flavonoids, which have significant antioxidant properties [83].

3.4. Mineral profile of 'Vinhão`grape pomace

Ash content represents total mineral content in 'Vinhão' grape pomace from Quinta de Azevedo, therefrom composition of minerals presents in the sample were also analysed (Table 2).

Table 2
Composition of minerals present in 'Vinhão' grape pomace (mg 100 g^{-1}) and WHO's recommended daily intake (RDI) (mg d^{-1}) [84].

Minerals	Content (mg. 100 g^{-1})	RDI (mg. d^{-1})
Mo	0.17 ± 0.00	
Zn	3.8 ± 30.00	7–15
Cd	0.08 ± 0.00	
P	656.36 ± 0.28	700–1000
Pb	9.92 ± 0.00	
Ni	1.08 ± 0.00	
Co	0.02 ± 0.00	
Mn	4.30 ± 0.00	2.3–5.0
Fe	36.38 ± 0.01	14
Mg	234.38 ± 0.09	260–350
Ca	946.41 ± 0.24	1000
Cu	3.34 ± 0.00	
Al	23.17 ± 0.01	
Na	1.85 ± 0.00	
K	6061.94 ± 3.42	2000–3500

The main minerals found in this by-product were potassium and calcium, in very high concentrations, followed by phosphorus and magnesium. Compared to previous studies, with some varieties, this study showed higher levels of potassium and phosphorus compared to the literature. For example, in varieties obtained from different provinces in Turkey, the values ranged for potassium (K) between 11.88 and 27.18 mg/g, calcium (Ca) between 5.78 and 7.92 mg/g, and magnesium (Mg) between 1.68 and 6.44 mg/g [85]. Other studies on dehydrated grape pomace varieties from Brazil have also found varying contents, such as potassium between 15.34 and 20.17 mg/g, calcium between 2.64 and 4.21 mg/g, and magnesium between 0.87 and 1.42 mg/g [86]. It is common to find high levels of potassium in grape pomace, representing approximately 80% of all cations, especially in the skins [87].

The content of Mg quantified in 'Vinhão' grape pomace was much lower than that reported by Mahmoud et al. [88] but the content on Zn was 4.4-fold higher in 'Vinhão' grape pomace than that reported by Barriga-Sanchez et al. [71], for "Black Borgoña" from Peru. Additionally, zinc concentrations help the plant's physiological system and can improve its fruit quality by 90% [89]. Regarding the content of Mn, this mineral was 2.2-fold, 2.1-fold and 1.9-fold higher in 'Vinhão' grape pomace than that of 'Cabernet`(1.94 mg·100 g$^{-1}$), 'Grenache` (2.04 mg·100 g^{-1}), and 'Syrah`(2.24 mg·100 g^{-1}), cultivated in that North-Wester Mexico Hernández et al. [90], respectively.

Minerals are fundamental in diverse chemical and biochemical reactions in the organism [91]. The 'Vinhão' grape pomace showed an expressive ash content and high levels in the various minerals, among them K, Ca, P and Mg. Potassium contributes to the metabolism and synthesis of proteins and glycogen, and regulates the water content in the body [76]. Potassium is considered an essential mineral for human homeostasis since they help maintain osmotic balance [92]. Potassium plays an essential role in reducing blood pressure, and reducing the risk of osteoporosis due to the reduced urinary calcium excretion [1]. Calcium, other mineral present in 'Vinhão` grape pomace in high concentrations, is a macromineral, very important for maintaining healthy bones and teeth, besides providing relaxation and contraction of muscles and also collaborating with nerve functioning, blood clotting, blood pressure regulation and immune system health [93]. Phosphorus is a macroelement of fundamental importance, whose functions are related to bone and tooth mineralization, and which also participates in energy metabolism, absorption and nutrient transport, protein activity regulation and acid-base balance [91]. Magnesium has a very important role as a nutrient that has relevant functions, as it is an antioxidant involved in more than 300 metabolic and bioenergetic reactions in the body [94, 95]. Fe is essential for almost all living organisms, participates in a wide variety of metabolic processes [96]. Moreover, and based on the results obtained, 'Vinhão` grape pomace can be considered an excellent source of K, Ca, Mg and P for human nutrition. In fact, the content of these minerals present in 100 mg of 'Vinhão' grape pomace is within the recommended daily intake (RDI) for minerals, being content of potassium 1.7-fold to 3-fold higher to that of RDI for these minerals (Table 2).

3.5. Fatty acids profile of 'Vinhão`grape pomace

The fatty acid (FA) profile of 'Vinhão' grape pomace is presented in Table 3. The most abundant fatty acids found were arachidic acid (C20:0), followed by palmitic acid (C16:0), oleic acid (C18:1Δ9). Amongst all the fatty acids identified, arachidic was the most abundant FA (61.4%) followed by linoleic acid (C18: 2Δ9,12) (0.75%). Thus, 'Vinhão' grape pomace analysed in this study showed a very different FA profile from that found by [97], where linoleic acid (C18:2Δ9,12) was reported to be the most abundant FA (69.79–70.32%) present in the grape pomace of different white-wine grapes ('Green Veltliner' and 'Pinot Blanc') and red-wine grape ('Zweigelt') cultivars of *V. vinifera*.

FAs are determinants in living organisms since they perform countless functions, mainly as energy sources, and structural and modulating

Table 3
Fatty acid composition of ‘Vinhão’ grape pomace (%, mean ± standard deviation).

Fatty Acid Name	%
Myristic acid (C14:0)	0.03 ± 0.01
Pentadecanoic acid (C15:0)	0.05 ± 0.00
Cis-10-Pentadecanoic acid (C15:1)	0.04 ± 0.00
Palmitic acid (C16:0)	11.02 ± 0.03
Palmitoleic acid (C16:1)	0.24 ± 0.00
Cis-10-Heptedecenoic acid (C17:1)	0.12 ± 0.00
Stearic acid (C18:0)	0.03 ± 0.00
Oleic acid (C18:1Δ9)	5.09 ± 0.04
Linoleic acid (C18: 2Δ9,12)	0.75 ± 0.00
alpha-linolenic (C18:3 c9c12c15)	0.52 ± 0.00
Arachidic acid (C20:0)	61.39 ± 0.02
Dihomo-gamma-linolenic acid(20:3)	0.02 ± 0.01
y-Linoleic acid (C18:3n6)	0.18 ± 0.01
Heneicosanoic acid (C21:0)	1.22 ± 0.01
Behenic acid (C22:0)	0.19 ± 0.01
cis-13-16-Docosadienóic(C22:2)	0.47 ± 0.01
Lignoceric acid (C24:0)	0.03 ± 0.00

physiological functions [98,99]. The most abundant fatty acids found in ‘Vinhão` grape pomace were arachidic acid (ARA, C:20), oleic acid (C18:1Δ9) and palmitic acid (C16:0). Its worth’s to note the high content of arachidic acid in ‘Vinhão’ grape pomace (almost 2/3 of FAs content) and very low content in linoleic acid. These results can be justified by one hand by the presence of seeds (rich in arachidic acid) in Vinhão` grape pomace and, in other hand by the conversion of linoleic acid that is found in most vegetable oils and plant seeds into arachidic acid [100]. ARA is believed to be a precursor of prostaglandins and many other eicosanoids, gives flexibility and fluidity to cell membranes, serves as a secondary lipid messenger in cell signaling, acts as an inflammatory mediator and causes vasodilation, as well as the terpenoid that functions as an activity. antimicrobial, which was generated by the oregano plant [101,102]. In addition, ARA influences the activity of cardiac promise pumps that contribute mainly to the excitability of cardiomyocytes [103]. Research on oleic acid revealed its influence on the cardiovascular system [104]. Oleic acid can improve the profile of blood lipids [105], maintain a healthy body weight. Palmitic acid tends to be neutral in the regulation of blood cholesterol in individuals [103].

3.6. Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR)

FTIR (Fourier Transform Infrared Spectroscopy) provides a characteristic spectral representation of the chemical constituents inherent within the analyzed specimen, delineating their molecular vibrational modes such as elongation and bending. FTIR spectra afford both qualitative and quantitative assessments, typically depicted as transmittance (%) plotted against wavenumber (cm^{-1}). Within the spectral range of 4000–500 cm^{-1}, multiple discernible peaks are evident, as illustrated in Fig. 1. The depicted spectra of the grape pomace exhibit numerous discernible peaks, each attributable to specific functional groups and vibrational modes of the constituent entities [5,106,107].

The peak broadband at about 3294 cm^{-1} for grape pomace corresponds to the OH elongation modes assigned to hydroxyl groups of alcohols, phenols (phenolics and sugars) [108]. The asymmetric stretch vibrations of the CH$_2$ groups correspond to the peak 2978, for the hydroalcoholic extracts from bagasse extract are mainly associated with the hydrocarbon chains of lipids or lignins [109,110].

The 1636 cm^{-1} peak is a very information-rich region, but on the other hand, difficult to analyze due to its complexity. This area provides important information about organic compounds, such as sugars, alcohols and organic acids, present in the grape pomace. The spectral band at 1636 cm^{-1} is attributed to the absorption of the C = O bonds of the ester groups and is related to the presence of fatty acids and their glycerides, as well as pectins and lignins or tannins [111,112], or related elongation of C = C bonds and may be attributed to aromatic compounds, possibly lignin or tannins [113–116].

The 1090 band corresponds to phenolic and C—O-C stretching vibrations, characteristic of saccharides and lignin. The 1046 band refers to the C—O (ester) functional group with an asymmetric stretching vibration. The band at the frequency (cm^{-1}) at 880 represents the R = CH (*cis* or trans) functional group with an off-plate bending vibration mode. FTIR analysis not only provides a detailed characterization of the functional groups present in grape pomace, but also confirms the expected chemical composition, thus strengthening the validity of the data presented [117,118].

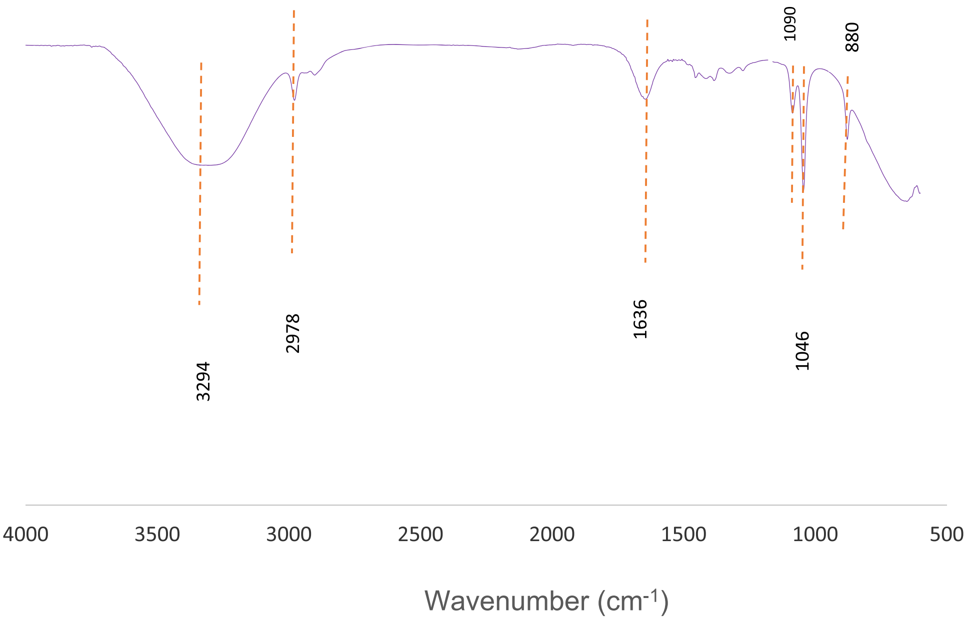

Fig. 1. *Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR)* of grape pomace.

4. Conclusions

This study was the first comprehensive study of ‘Vinhão’ grape pomace. This low-cost by-product from winemaking industry, despite the low nutritional values in terms of proteins, carbohydrates and lipids observed in the ‘Vinhão` grape pomace residue, showed a rich mineral and lipidic profile, a relatively high dietary fibers and polyphenols content. The ‘Vinhão’ grape pomace also show high content of arachidic acid. As the characterization of ‘Vinhão’ grape Pomace was performed only for one campaign (2018 campaign) and one origin (Quinta do Azevedo), further characterization of ‘Vinhão’ grape pomace materials from different origins and campaigns must be performed to better understand the variability of composition and nutritional value of ‘Vinhão’ grape pomace.

Ethics approval

The study did not include trials with humans or animals.

Compliance with ethics requirements

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

CRediT authorship contribution statement

Adriana Rodrigues Machado: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Glenise Bierhalz Voss:** Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **Manuela Machado:** Methodology, Investigation, Conceptualization. **Jorge A.P. Paiva:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Conceptualization. **João Nunes:** Writing – review & editing, Visualization, Validation, Supervision. **Manuela Pintado:** Writing – original draft, Visualization, Validation, Supervision, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data presented in the survey has not been previously shared.

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