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Innovation and Winemaking By-Product Valorization: An Ohmic Heating Approach

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Abstract: The by-products of the winemaking process can represent chances for the development of new products. This study focused on the "zero waste" strategy development for by-products generated within winemaking from white and red grape varieties cultivated in the north of Portugal. The phytochemical properties of by-products were identified and characterized. Ohmic heating (OH) as a green extraction method was also applied to grape pomace due to their unknown effects on centesimal and phytochemical compositions. Both protein and carbohydrates were shown to be higher in grape bagasse than in stems. Additionally, red bagasse is richer in bioactive compounds (BC) than white bagasse. The sugar content was 21.91 and 11.01 g/100 g of DW in red and white grape bagasse, respectively. The amount of protein was 12.46 g/100 g of DW for red grape bagasse and 13.18 g/100 g of DW for white. Regarding the extraction methods, two fractions were obtained, a liquid fraction and solid (the remainder after the methodology application). OH presented a higher antioxidant capacity than a conventional (CONV) method. In addition, both extracts presented similar contents of anthocyanins, e.g., delphinidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, and peonidin-3-*O*-glucoside. The solid fraction presented higher amounts of protein and phenols bound to fiber than CONV, which allows its use as a functional ingredient. In conclusion, OH can be an alternative extraction method compared with CONV methods, avoiding non-food grade solvents, thus contributing to circular economy implementation.

Keywords: grape pomace; valorization; fractionation; food ingredients

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1. Introduction

Vitis vinifera l. (grape) are one of the most harvested fruits in the world. Approximately 78 million tons (mt) of grapes are produced annually, with 37% produced in Europe, 34% in Asia, and 19% in America, representing a global vineyard surface area of 7.4 million hectares [1]. Global grape production is approximately 78 mt. It is additionally one of the most variously used fruit crops worldwide: while practically half of the grapes are utilized to make wine, 33% is expended as new food, while the remaining part is dried, consumed as grapes or stored as grape musts (regardless of whether concentrated or not) [2,3]. For each 1.32 kg of grape, one hectoliter of wine is produced. In 2018, world wine production reached a record volume of 293 million hectoliters [1]. Nine million tons of grape by-products are produced annually globally, representing 20% (*w*/*w*) on average of the overall grapes used for wine production. The main by-product is bagasse, which is the remaining solid after pressing that usually contains the skins, pulp, seeds, and stems of the fruit [4,5]. Grape skins represent, on average, 65% of the overall material of grape bagasse, and they are rich

in BC, depending on the vinification process and extraction method employed (solvent temperature, time, and other factors) [4]. Although the phytochemical profile of this by-product has been extensively studied, which showed that these by-products have a high potential, there is a lack of proper valorization operations [6–15]. Currently, its primary destination is to be used as compost or discarded in public fields, possibly causing environmental issues. Additionally, most thermal treatments, such as pasteurization, extraction or blanching, are related to a decrease in nutritional properties and BC losses because of oxidation, filtering and different actions that lessen these antioxidant properties [16,17].

Several studies explore the use of grape bagasse, a source of healthy and technological compounds that could be applied in animal feed, the pharmaceutical, cosmetic, or food industries to improve stability and nutritional characteristics, and in the cosmetic industry, where grape seed oil is widely used [18,19].

However, many of the by-products generated by the winemaking industry remain without a sustainable recovery solution implemented. Such waste streams are partly valorized in various added-value levels (spread on land, animal feed, composting), whereas the first volumes are overseen as natural misuse, with applicable negative impacts on the sustainability of the agro-industry [20,21]. Furthermore, by-products usually comprise high amounts of protein, sugar, and fiber, making them a source of cheap nutrients and BC [22].

Grape bagasse is a fibrous and tannin-rich material used in the oil and gas industry as a lost circulation material in oil-based drilling muds. Grape skin by-products have also been used as compost to regenerate vineyards [23].

The European Union's (EU) goal is to achieve zero food by-products by 2030, and obviously, this includes food loss reduction along food supply chains. Thus, it is essential to find alternatives to reuse or reintroduce by-products into the supply chain.

The actual modus operandi to extract or make synthetic BC uses a large number of organic solvents, which are very toxic, prejudicial for both the environment and health, and with higher costs when associated with cleaning solvents to use the materials [4,24,25]. Thus, to make this a more sustainable process, it is necessary to break the linear economy. Thus, it is vital to use greener alternatives with lower impacts and allow direct by-products to develop new products further. Therefore, several technologies have been studied to reach these objectives. Ohmic heating (OH) has been highlighted as a good alternative due to its environmentally friendly character, faster and more homogenous processing properties [26–28]. OH, also known as joule heating or resistive heating, consists of the passage of an alternating electric current through a food sample that acts as a semi-conductor, thus producing internal heat within a given material. OH can be used in various unit operations, such as pre-treatment or thermal processing, blanching, evaporation, dehydration, fermentation, recovery, sterilization, and pasteurization. A disadvantage of this technique is that it depends on the product's electrical conductivity—for example, it does not occur in non-conducting materials, as these materials do not allow the passage of electrical current.

Regarding winemaking by-products, OH is used as a pre-treatment, and only in a few cases are its phytochemical impacts evaluated. Ref. [26] showed that POH (pulsed Ohmic heating) causes cell wall disruption in red grape bagasse. The study has shown that permeabilization increases with an increasing temperature and electrical field power intensity. Polyphenol extraction yields were 36% higher than in untreated samples, for a current of 400 V/cm, during 60 min, at 50 °C in 30% of aqueous ethanol solution. Nevertheless, few studies about the impact of OH on the nutritional composition of the residual winemaking by-product flour obtained after polyphenol extraction.

This study aimed to valorize winemaking by-products. Firstly, we evaluate the phytochemical potential of by-products from two Portuguese cultivars, Vinhão and Loureiro, red and white grape cultivars, respectively. Secondly, an alternative technology, OH, was used to improve the extraction yields of BC compared to conventional (CONV) methods that use chemical solvents to recover the same compounds.

Overall, this work aims to create a new winemaking strategy that considers the benefits of a waste management policy.

2. Materials and Methods

2.1. Chemicals

The 2,20-azo-bis-(2-methylpropionamidine)-dihydrochloride (AAPH), fluorescein, 2,2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS diammonium salt), potassium sorbate, sodium carbonate, and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma-Aldrich (Sintra, Portugal). Hexane, ethanol, Folin–Ciocalteu's reagent, and potassium persulfate were purchased from Merck (Algés, Portugal). Standards of ascorbic acid, trolox, gallic acid, rutin, *p*-coumaric, and 4-hydroxybenzoic acid were purchased from Sigma-Aldrich (Sintra, Portugal), while kaempferol, β-carotene, lycopene, zeaxanthin, and lutein (Extrasynthese, France) were purchased from Extrasynthese (Lyon, France).

2.2. Sample Preparation

Grape bagasse (seeds, skins, pulp) and stems from two grape cultivars, "Vinhão" and "Loureiro" grape cultivars were kindly provided by two farms in the north of Portugal that produce white and red wine, respectively. These by-products were collected three times after production and were transported under refrigeration until they reached the laboratory. After collecting these three batches, the samples were homogenized, packed in polyethene flasks, and stored at −80 °C until analysis.

2.3. Fractionation of Grape By-Products

White (WB) and red bagasse (RB), and white (WS) and red stems (RS) were dried in an oven at 55 °C until levels of moisture of ca. 5% were attained, and then milled in a kitchen robot Thermomix TM5, obtaining a flour with a particle size of <1 mm for bagasse and <1.5 mm for stems. All batches were mixed, with each 100 g of the mixture being stored in sampling bags in a dark and dry place at room temperature until the analysis.

2.4. Characterization of the Raw Material

The production of solid by-products worldwide is dramatically increasing every year, and most of them are composed of food by-products rich in BC with high potential. Thus, a complete and preliminary characterization of each grape by-product was performed to envisage the value of new functional ingredients. Additionally, after by-product evaluation, a green recovery process, Ohmic heating technology (OH), was used and compared with conventional methods, which use organic solvents.

2.4.1. Proximate Composition

All by-products from cultivars were submitted to nutritional and phytochemical characterization with referenced methods. The moisture content of the grape by-product bagasse (composed of seeds, peels, pulp) and stems (fibrous parts) was determined according to the oven method (Association of Official Agricultural Chemists (AOAC) No. 934.06) [29] in fresh samples. The calculation was presented as follows:

$$Moisture\ (dry\ matter)(\%) = \frac{W1 - W2}{W1} \times 100\% \quad (1)$$

where $W1$ is the weight (g) of the sample before drying and $W2$ is the weight (g) of the sample after drying.

The ashes were determined from the resulting inorganic residue weight after ignition in a Muffle furnace at 550 ± 25 °C overnight.

Total nitrogen was obtained according to the Kjeldahl method, and protein content was then calculated using a conversion factor of 6.25 in all fresh samples. The fat content was determined gravimetrically using the Soxhlet method with petroleum ether (boiling point 60–80 °C), according to the method described in AOAC 920.152. The crude fiber

content was determined with an acid/alkaline hydrolysis of insoluble by-products, as described in AOAC 2000. The total carbohydrates were measured by the phenol–sulfuric acid method [30].

All methodologies followed the recommendations of the Official Methods of Analysis [29]. All measurements were completed in triplicate. The content of each parameter was expressed as g/100 g of dry weight (DW).

2.4.2. Total Pectin

The total pectin content of stems and bagasse from red and white grapes was calculated based on the method described by [29] consisting of the sum of three fractions: water-soluble pectin (WSP), chelator-soluble pectin (CSP), and hydroxide-soluble pectin (HSP).

2.4.3. Cellulose, Hemicellulose, and Lignin

For the determination of the crude grape by-products, the WS, RS, WB, RB fraction tests were used. The technique of Sluiter et al. was followed for cellulose (as glucose), hemicellulose (as arabinose, mannose, galactose and xylose) and lignin (soluble and insoluble). The extractors were already extracted with ultra-pure water and absolute ethanol (SER 148, Velp, Usmate Velate MB, Italy) as a solvent in two stages. Then, two steps of acid sequence hydrolysis were submitted for free-extractive samples, further cellulose determination/quantification, and high-performance liquid chromatography (HPLC) method utilizing a diode array detector (DAD), which achieved hemicellulose quality. Structural carbohydrates were calculated using HPLC-DAD (micro guard column: Amenex Carbo-P, Bio-Rad (Berkeley, CA, USA); carbohydrate analysis column: Aminex HPX-87P heavy metal, 300 × 7.8 mm, Bio-Rad; flowrate: 0.6 mL/min; detector: refractive index) and were used to measure the contents of the cellulose (as glucose) and hemicellulose (as arabinose, mannose, galactose, and xylose) and the column for carbohydrates: Aminex HPX-87P, heavy metal, 300 × 7.8 mm, bio-Wheel. Following hydrolysis residue filtration, the insoluble amount of lignin was gravimetrically measured, and the soluble lignin was determined using spectrophotometry for UV (ultraviolet) at 340 nm [31]. The findings have been shown in g/100 g of DW.

2.4.4. Soluble Sugars

In ultrapure water extracts, soluble sugars were measured using the total carbohydrate phenol–sulphuric acid technique [32]. The sugar content was determined by combining 80 µL of the soluble sugar solution with 2 mL, 98% H_2SO_4 and 320 µL of 5% phenol. The reaction occurred at 100 °C for 15 min and was measured at the absorption of 490 nm, using a D-(+)-glucose-grading curve and the results were expressed as g glucose equivalents/100 g DW. Free sugar profiles were defined at 55 °C, with 35 mM H_2SO_4 as a mobile phase (flow rates: 0.5 mL/min), using HPLC combined with a refractive index detector using an Aminex 87-H column (Bio-Rad) [33]. Sugars have been identified by comparing the retention time of glucose and mannitol peaks and the results were expressed in g/100 g of DW.

2.5. Extraction Methodologies to Phenolic Compound Recovery

2.5.1. CONV Method

In the case of white bagasse and stems, an aqueous methanolic solution at 80% (25 mL) was added to 2.5 g of grape by-products and homogenized with an ultra-turrax (IKA T18, Wilmington, DC, USA) operated at 12,879× *g* for 2 min [34]. In the case of red bagasse and stems, the same method was used to obtain the anthocyanin extraction, with a slight modification. The solvent used was 80% of acidified aqueous methanol solution (methanol:water:hydrochloric acid, 12 N: 800 mL: 150 mL: 50 mL) [35]. After the extracts were centrifuged at 4000× *g*, 4 °C for 10 min, the supernatants were filtered through a 0.45 mm cellulose acetate filter (Orange Scientific, Braine-l'Alleud, Belgium) and used

for total activity measurements. The remaining solid fractions (red and white) were also characterized for fibers, bound protein, and bound and free polyphenols.

2.5.2. OH Technology

Based on the results of previous proximal characterization, both bagasses were chosen for further extraction procedures since they presented the highest polyphenol content, i.e., the level of free polyphenols in the stems was significantly reduced. In addition, electrical conductivity was 0.03 ms/s, whereas bagasse presented a conductivity between 1.0 ms/s and 8 ms/s.

OH was carried out according to [27]. Briefly, 2.5 g of grape by-products and 12 mL of water were added to the reactor (composed of a double-walled water-jacketed cylindrical glass tube vessel, with a 30 cm total length, and a 2.3 cm inner diameter; the electrodes had a gap of 5 cm). A function generator to supply voltage (1–25 MHz and 1 to 10 V, Agilent 33220A, Penang, Malaysia) connected to an amplifier system (Peavey CS3000, Meridian, MS, USA) controlled the sample heating with an electrical frequency at 25 kHz.

After 10 min of reaction, the extracts were centrifuged (4000 rotations per minute (RPM), 10 min), and the liquid fraction (LF) and pellets corresponding to the solid fraction (SF) were also kept.

The total antioxidant activity and the quantitative phenolic compounds profile were analyzed on the liquid fractions obtained from the grape bagasse extractions. The solid fractions were analyzed as previously described, and results were compared against a CONV method of extracting phenolic compounds [36].

2.6. Bioactive Characterization

All samples were characterized concerning the content and profile of main polyphenols and the related antioxidant.

2.6.1. Total Antioxidant Capacity and Total Phenolic Content

After CONV extraction and the OH extraction of bagasse from red and white grapes, LF and SF were obtained. In the LF, the total antioxidant activity (AA) was measured through the ABTS method, according to [37] with slight modifications. A sample (10 µL) was added to a colored solution of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid radical cation) ($ABTS^{\bullet+}$), with an optical density (OD) measured at 734 nm and adjusted to 700 ± 0.020 in a spectrophotometric microplate reader (Sunrise Tecan, Grödig, Austria). After 6 min of reaction, the final OD was read, and the results were given in mg of the ascorbic acid equivalent per 100 g of DW.

As described elsewhere [37,38], the total polyphenolic content of LF was evaluated through the Folin–Ciocalteu (TPC) spectrophotometric method. A reaction with a sample (5 µL), *Folin–Ciocalteu* reagent (15 µL), sodium carbonate at 7.5 g/L (60 µL of), Sigma-Aldrich and distilled water (200 µL) were performed, and the solutions were mixed. After the samples were heated at 60 °C for 5 min, the OD was read at 700 nm using a spectrophotometric microplate reader (Sunrise Tecan, Grödig, Austria). TPC was expressed as a milligram of the gallic acid equivalent per dry weight of material (mg GAE/g). The analyses were performed in triplicate, and standard deviation was calculated.

2.6.2. Bound Phenols

The red grape bagasse (1 g) and the extraction-derived solid fractions were washed with ethanol 3 times to eliminate the free polyphenols. An extraction residue was obtained after 4 h of reaction in 20 mL of NaOH (4 M). The solution obtained before was acidified with HCl (6 M) at pH 1.5 to 2.0 and centrifuged for 30 min at $12{,}879\times g$. An extraction was then performed with ethyl-acetate, for 15 min, 5 times. The supernatant was concentrated in a vacuum evaporator, resuspended with 10 mL of ethanol, and the phenols obtained were analyzed using HPLC-DAD.

2.6.3. Phenolic Compound Quantification Using HPLC-DAD

Polyphenol characterization (quantitative and qualitative) was carried out, according to [37]. Analysis was conducted on a HPLC-DAD (Waters Series 600. Mildford, MA, USA). A Symmetry® C18 column (Waters, Wexford, Ireland), 250 × 4.6 mm i.d. 5 µm particle size and 125 Å pore size with a guard column (Waters, Wexford, Ireland), was used and solvent elution consisted of solvent A: Acetonitrile (98%) with 0.2% TFA; and Solvent B: acetonitrile/water (5:93 v/v) (Merck pure grade and pure water) with 0.2% TFA (Carlo erba, Val-De-Reuil Cedex, France). Samples were analyzed in triplicate. Calibration curves were obtained at a detection wavelength of 280 nm to flavan-3-ols and 320 nm to flavonols. Standards solutions over the concentration ranging from 0.10 to 100.00 mg/L were prepared to identify and quantify the following compounds. In essence, rutin, naringenin, kaempferol gallic acid, protocatechuic acid, catechin, vanillic acid, syringic acid, *p*-coumaric acid, and phloretin (Sigma, Sintra, Portugal) were expressed as µg per mL of dry weight (DW) biomass of grape. All calibration curves were linear over the concentration ranges tested, with correlation coefficients of 0.999.

2.7. Statistical Analysis

An SPSS v. 19 (Chicago, IL, USA) evaluated the statistical differences using the non-parametric Mann–Whitney test. All experiments were performed in triplicate, and the results were expressed as the mean of the triplicated analysis and the respective standard deviations. Differences were considered significant at a 5% confidence level ($p < 0.05$).

3. Results

3.1. Fractionation Approach

A higher water loss occurred during the drying of stems and the bagasse fraction from white cultivar, due to their higher moisture (WGS: 76.42 ± 0.09, WGB: 72.71 ± 0.35) than the stems and bagasse obtained from red grape by-products (RGS: 69.37 ± 0.22, RGB: 68.99 ± 0.47).

Two separate flour fractions were produced after the drying phase and the dry fractionation (milling and sieving) of the stem and bagasse. For raw grape by-products (0.21–0.23 kg of 1 kg of wet grape), the bagasse percentage of both samples was around 69% and 73% of DW (WGB and RGB samples) for both samples.

Authors developed a strategy for the integrated use of white grape skin bagasse [39]. This strategy comprises a consistent or simultaneous removal of grapes with neutral organic solvent and reflux water. Bio-extract is a good raw source of oleanolic acid recovery. The aqueous extract (about 50% w/w) is primarily hexose constituted and is adapted to produce high yields (up to 51%) of bioethanol at a peak cell growth rate (max) of 0.29 h^{-1}. The grape skin remaining is the structural polysaccharide complex. Ref. [14] tested an extract from grape stems to identify its potential for replacing and/or reducing SO_2 in vinification with antioxidant and antibacterial activity in model wines. The results showed significant antioxidant activity, suggesting a strong antioxidant extracts from grape stem.

The authors claimed to valorize grape bagasse using ultrasound phenol extraction [40–44]. They examined the drying behavior and the kinetics of total phenolic degradation in the drying process of that winery by-product. The effectiveness of the diffusive properties was assessed by the slope technique of the curve of dryness (working at 60–85 °C). It examined the influence on phenol recovery yields from solvent type, extract temperature, solvent/solid ratio, amplitude level, and pulse duration/pulse interval ratio. They demonstrated that the drying rate was enhanced when the temperature was raised, but drying was primarily completed during the decreasing rate phase.

Neither study provided the output of each of the fractions produced by the fractionation of the grape bagasse. In addition, the previous techniques have not explored the full potential of the grape bagasse fractions, affecting the quality of the value-added products and the "zero waste" objective. In contrast, the fractionation approach proposed in this paper seems to provide a promising, sustainable alternative for the production, without any consumption of water or chemicals, of various added-value products from grape bagasse

biomass and, first, for higher value usages and, secondly, for the utilization of energy according to cyclical principles for bioeconomic efficiency.

3.2. Proximate Composition

Regarding the grape's minerals content, the stem's is higher than the one found in the grape bagasse, representing a difference between samples of approximately 4 g/100 g of DW. These results follow the reported literature [22,45]. Additionally, the white grape stems presented more minerals than the red grape stems. Relative to grape bagasse, significant differences ($p < 0.05$) were found between cultivars. The red grape bagasse had a mineral content higher than white grape bagasse, 2.50 g/100 g to 1.23 g/100 g, respectively. The differences could be explained by the winemaking process. Red bagasse is obtained after the fermentation of grape skins and must, while white bagasse is obtained before fermentation [46,47].

Regarding the protein content in stems, no differences were found between cultivars. Nevertheless, protein content in the bagasse samples ranged from 8.31 to 8.52 g/100 g, which compared well with the literature [48–53]. The literature also refers to these higher amounts of protein in peels than in the seed content, which depends on the cultivar [22,24].

Carbohydrates and dietary fibers, as predicted in material rich in plant cells, have greater levels in bagasse samples than stems. Additionally, the red grape bagasse presented more carbohydrates than the white bagasse ($p < 0.05$).

Since 1995, grape by-products have been shown as dietary fiber sources [4,11,54,55]. Dietary fiber is composed of two fractions: soluble fibers, e.g., fructooligosaccharides, and pectin (soluble in water), which are readily fermented in the colon; and insoluble fibers (non-soluble in water), e.g., cellulose, hemicellulose, and lignin, which are inert to digestive enzymes providing bulking, and may be poorly or non-fermented in the colon [4,52].

The results showed that the white cultivar had higher values of dietary fiber than the red cultivar. Concerning white stems, they had a significantly higher total fiber value than red stems (Table 1)—i.e., 55.91 to 50.28 g/100 g—containing more cellulose than lignin ($p < 0.05$). Relative to both bagasses, it was possible to observe that they were mainly constituted by lignin. In general, bagasse contained more insoluble fiber than stems. The fiber content is in agreement with reported in the literature [11,15,22,49,50,56]. Valiente and colleagues studied the dietary fiber composition present in red grape bagasse, and they reported that total dietary fiber represents 77.89% of the dry matter. Additionally, they found that 90% of insoluble dietary fiber consists mainly of cellulose. The authors also reported the importance of seedless grape bagasse as an ingredient with a good source of fiber for industry and their potential beneficial effects on the regulation of bowel functions and water retention [11].

Authors found an amount of grape fiber ranging from 56.8 g/100 g to 83.6 g/100 g. Both grape cultivar, climacteric, and processing conditions influence the dietary fiber amount [56]. The results obtained for grape bagasse showed that it could be a good substitute due to its soluble dietary fiber content and low caloric content, with a better insoluble/soluble ratio and better functional properties than cereal, which represents the leading fiber supplier.

In addition, bagasse use contributes to a high economic value and supports the circular economy [57].

Relative to the pectin present in grape by-products, higher values of water-soluble pectin (WSP) were found in stems from both cultivars compared with bagasse. Additionally, significant differences ($p < 0.05$) were found in the red grape stems with higher CSP values than white grape stems. Relatively to grape bagasse, no differences ($p > 0.05$) were found between cultivars; nevertheless, the tendency is the same as in the stems. The difference of pectin fractions is related to their structure, which influences functionality and application. The lowest CSP and HSP in white grape bagasse and stems indicates the small capacity to recover high-esterified pectin. Following previous studies, winemaking procedures did not significantly impact pectin distributions, except for the WSP that might be reduced due to the compression step [30,58].

Table 1. Chemical composition of raw grape bagasse (C-GB) and grape fractions (LF-GB and SF-GB) obtained after OH and CONV extractions (g/100 g DW).

Chemical Components		R-GB				LF-GB				SF-GB			
		WGS	**RGS**	**WGB**	**RGB**	**W_OH**	**W_CONV**	**R_OH**	**R_CONV**	**W_OH**	**W_CONV**	**R_OH**	**R_CONV**
Proximate composition (g/100 g)	Moisture	0.98 ± 0.12	0.97 ± 0.19	0.97 ± 0.10	0.92 ± 0.09	2.97 ± 0.32	3.03 ± 0.21	2.70 ± 0.17	2.96 ± 0.15	0.58 ± 0.04	0.72 ± 0.08	0.63 ± 0.05	0.76 ± 0.08
	Ash	6.40 ± 0.02	6.30 ± 0.01	1.23 ± 0.01	2.50 ± 0.01 *	13.50 ± 0.09	12.71 ± 0.12	6.21 ± 0.08	10 ± 0.07	1.29 ± 0.09	1.20 ± 0.01	1.93 ± 0.01	2.44 ± 0.01
	Protein	7.31 ± 0.01	7.30 ± 0.02	8.34 ± 0.02	8.51 ± 0.02	5.02 ± 0.32	2.18 ± 0.12	5.05 ± 0.15	4.26 ± 0.05	8.51 ± 0.02	11.3 ± 0.5	8.34 ± 0.02	9.95 ± 0.25
	Fat	2.15 ± 0.12	2.96 ± 0.15	14.14 ± 0.17	12.58 ± 0.23	1.25 ± 0.14	2.49 ± 0.08	3.08 ± 0.12	4.87 ± 0.03	15.02 ± 0.45	12.18 ± 0.2	11.86 ± 0.31	9.46 ± 0.21
	Crude Fiber	76.91 ± 0.80 **	72.28 ± 0.73	57.82 ± 0.76 *	55.98 ± 0.96	21.02 ± 0.54	16.98 ± 0.25	20.98 ± 0.23	15 ± 0.76	68.98 ± 0.69	70.56 ± 0.45	70 ± 0.44	71.02 ± 0.53
	Carbohydrates	6.33 ± 0.04	7.80 ± 0.13 **	11.03 ± 0.29	14.28 ± 0.05 *	56.26 ± 0.35	62.58 ± 0.34	62.03 ± 0.12	62.55 ± 0.23	5.76 ± 0.09	4.06 ± 0.12	6.98 ± 0.13	8 ± 0.15
Structural Carbohydrates	Cellulose (as glucose)	16.33 ± 0.04	17.33 ± 0.69 **	5.42 ± 0.68	6.77 ± 0.04 *	0.50244	ND	ND	ND	6.03 ± 0.96	5.41 ± 0.68	8.12 ± 0.68	7.69 ± 0.40
	Hemicellulose	6.70 ± 0.44	6.85 ± 0.28	6.74 ± 0.16	8.38 ± 0.09 *	ND	ND	ND	ND	7.83 ± 0.65 *	6.73 ± 0.16	11.23 ± 0.51 *	9.52 ± 0.11
	Lignin	30.54 ± 0.10	30.24 ± 0.09	40.46 ± 0.09	40.84 ± 0.40	ND	ND	ND	ND	21.53 ± 1.81 *	18.44 ± 0.72	21.97 ± 1.8	21.37 ± 4.00
	Insoluble	21.24 ± 0.39	21.07 ± 0.04	22.31 ± 0.08	22.41 ± 0.22	ND	ND	ND	ND	20.45 ± 0.10	16.91 ± 0.09	17.53 ± 0.07	17.59 ± 0.07
	Soluble	9.33 ± 0.15	9.17 ± 0.10	18.15 ± 0.12	18.42 ± 0.16	ND	ND	ND	ND	1.57 ± 0.08	1.52 ± 0.04	4.44 ± 0.17	3.78 ± 0.15
Pectins	TSP	13.72 ± 0.50	14.21 ± 0.60	7.75 ± 0.15	8.77 ± 0.19	4.09 ± 0.21	2.12 ± 0.23	5.49 ± 0.12	6.08 ± 0.41	3.75 ± 0.22	5.75 ± 0.49	3.77 ± 0.36	2.77 ± 0.24
	WSP	7.84 ± 0.75	6.53 ± 0.73	3.04 ± 0.18	3.12 ± 0.06	1.98 ± 0.41	1.24 ± 0.17	2.12 ± 0.31	1.41 ± 0.12	4.80 ± 0.32 *	3.00 ± 0.18	5.03 ± 0.14 *	3.51 ± 0.18
	CSP	4.42 ± 0.19	6.47 ± 0.61	1.51 ± 0.06	2.88 ± 0.08	ND	ND	ND	ND	3.20 ± 0.69 *	1.50 ± 0.06	4.76 ± 0.12 *	3.25 ± 0.24
	HSP	1.46 ± 0.06	1.02 ± 0.06	3.20 ± 0.15	2.80 ± 0.28	ND	ND	ND	ND	4.20 ± 0.23 *	3.18 ± 0.15	4.53 ± 0.56	3.14 ± 0.81
Soluble sugars	total soluble sugars	6.74 ± 0.3	1.04 ± 0.98	10.57 ± 1.76	17.24 ± 1.25	12.36 ± 0.85	11.01 ± 1.3	21.91 ± 0.56	23.5 ± 0.31	1.79 ± 0.03	0.44 ± 0.01	4.67 ± 0.08	6.26 ± 0.06

R-GB—raw grape by-products; LF-GB—liquid fraction from grape bagasse; SF-GB—solid fraction from grape bagasse; WGS—grape stems from white grape by-products; RGS—grape stems from red cultivars; WGB—grape bagasse from white cultivars; RGB—grape bagasse from red cultivars; W_OH—white bagasse with OH application; W_CONV—white bagasse with CONV technique application; R_OH—red bagasse with OH application: R_CONV—red bagasse with CONV application. NDF—neutral detergent fiber; ADF—acid detergent fiber. TSP—total soluble pectins; WSP—water-soluble pectins; CSP—chelator-soluble pectins; HSP—hydroxide-soluble pectins. ND—Not detected. Data were expressed as mean ± SD (n = 3). The different superscripts in the same row represent significant differences between samples (* $p < 0.05$; ** $p < 0.01$).

In contrast, a high-esterified pectin structure for red grape stems indicates a high potential for food applications. This pectin could be used on confection jellies, make a friendly gel system with a clean taste, and confer a great flavor. It may also be used to strengthen acidic protein beverages, e.g., drinkable food, improving the mouthfeel and the flesh stability in juice beverages, and as a fat substitute in baked goods [30,39,59,60].

3.3. Bioactivity Characterization

Antioxidant Capacity and Total Polyphenolic Content after CONV Extraction

Significant differences ($p < 0.05$) in antioxidant capacity were found between the different by-products. Red grape stems showed higher antioxidant capacity values than white grape stems; the same was observed relative to red and white grape bagasse (Table 2).

Table 2. Total phenolic compounds and antioxidant activity of bagasse and stems from red and white grape by-products in LF (mg/100 g DW).

Samples	Bagasse		Stems	
	White Grape	**Red Grape**	**White Grape**	**Red Grape**
Total phenolic compound	4.0 ± 0.0	6.6 ± 0.1 *	3.0 ± 0.1	9.8 ± 0.0 *
Total antioxidant activity	5.2 ± 0.66	22.6 ± 0.8 *	8.3 ± 0.7	34.6 ± 0.9 *

* $p < 0.05$ between white and red cultivars.

Phenolic compounds have been related to beneficial health effects, namely antioxidant, anti-inflammatory, anti-tumoral, anti-obesogenic effects, and the prevention of therapeutic neurodegenerative and cardiovascular diseases [61,62]. These properties depend upon the number of phenols available in the lower parts of the digestive tract (bioaccessibility) or on the quantity effectively absorbed.

Results showed a significant difference between the grape bagasse's total phenolic content from red and white grapes (Table 2). This is in line with previous studies about the total phenolic content of grape by-products [49,63–66].

The red grape by-products displayed higher values than the white ones, with significant differences ($p < 0.05$). The results are justified by the grape composition and the vinification process described in the literature [30,67,68]. In red wine production, bagasse is produced after free juice is poured, leaving behind dark blackish-red debris consisting of grape skins and stems. The color of red wine is derived from skin contact during the maceration period, which sometimes includes partial fermentation. The resulting bagasse is more alcoholic and tannic than bagasse produced from white wine production. In white wine production, the grapes are crushed and quickly pressed to avoid skin contact with the juice, resulting in a pale greenish-brown pressed by-product [49,63–66].

Furthermore, the cultivar and the weather conditions determine the total phenolic compounds obtained for stems since they were isolated from grapes before the winemaking and did not endure any procedure [4,54].

The results observed show the directed correlation obtained between polyphenol content and antioxidant capacity (r 0.93). Other authors also reported these correlations [69,70].

Therefore, grape bagasse may be used in the food industry as a food preservative, changing or preventing the decay of nutrients by aerobic mechanisms and as an antimicrobial agent restricting the development of spoilage and pathogenic microorganisms.

3.4. Impact of OH vs. CONV Method on Phytochemical Composition of Grape Bagasse

In this study, the use of OH was evaluated and compared against chemical extraction to evaluate if it can be an efficient alternative for the reuse/use of red grape bagasse with quality and safety without damaging the inherent BC (Table 3).

Table 3. Phenol quantitative profile identified from HPLC-DAD in the red and white grape bagasse (μg/g of DW). Free polyphenols were measured in LF and bound phenols in SF.

Compound (μg/g)c	White Grape Bagasse		Red Grape Bagasse	
Free Phenolic Compound (LF)	**CONV**	**OH**	**CONV**	**OH**
(-)-Epicatechin	145.30 ± 18.10 *	38.95 ± 2.01	n.d.	n.d.
Syringic acid	1.14 ± 0.33	0.98 ± 0.03	n.d.	n.d.
Ferulic acid	0.39 ± 0.07 *	n.d.		
p-coumaric acid	0.80 ± 0.02 *	n.d.	n.d.	n.d.
Caffeic acid	0.79 ± 0.01 *	n.d	n.d	n.d
Gallic acid	7.46 ± 2.37	8.63 ± 1.81	10.83 ± 1.85	28.64 ± 0.96 *
Esculin	n.d.	n.d	n.d.	1.77 ± 0.06 *
Catechin hydrate			0.98 ± 0.07	1.63 ± 0.35 *
Vanillic acid			0.23 ± 0.32	1.36 ± 0.05
Delphinidin-3-o-glucoside	n.d.	n.d.	0.02 ± 0.01	0.02 ± 0.01
Petunidin-o-glucoside	n.d.	n.d.	132.64 ± 1.45	133.80 ± 0.16
Peonidin-3-o-glucoside	n.d.	n.d.	0.02 ± 0.001	0.02 ± 0.01
	bound phenolic compound (SF)			
Gallic Acid	15.30 ± 0.98	21.57 ± 1.84	26.71 ± 1.15	54.20 ± 3.24 *
Protocatechuic acid	n.d.	n.d.	5.35 ± 0.21 *	n.d.
Catechin	n.d.	n.d.	13.40 ± 0.52	32.70 ± 2.50 *
Vanillic acid	n.d.	n.d.	4.67 ± 0.30	6.94 ± 0.61 *
Caffeic acid	n.d.	n.d.	n.d.	0.03 ± 0.01
Syringic acid	n.d.	n.d.	0.88 ± 0.01	1.76 ± 0.01
p-coumaric acid	n.d.	n.d.	2.62 ± 0.03	3.06 ± 0.01
Rutin	n.d.	n.d.	0.04 ± 0.01	0.01 ± 0.01
Phloretin	n.d.	n.d.	1.24 ± 0.01	n.d.

n.d.—not detected. The different superscripts in the same row were significantly different (* $p < 0.05$).

3.4.1. Protein Content

Regarding protein content in the SF after extractions (Table 1), the values showed a protein content range from 8.6 to 14.65 g/100 g with the application of CONV methods, which are in agreement with the literature [11,49,50,52–54,71,72]. In the SF of white grape bagasse, a significant increase ($p < 0.05$) of protein content was observed after OH treatment application compared with the CONV method, at 13.18 and 15.02 g/100 g, respectively. A similar result was obtained for red grape bagasse when OH was compared to the CONV method, at 13.86 and 12.46 g/100 g, respectively. Solvents and extraction techniques used during the extraction process could explain the protein content differences obtained from extraction methods [4,53,72,73].

Furthermore, both methodologies used in this study have different approaches. The CONV extraction method used 80% of a MeOH solution, while water was used in OH extraction. It is known that MeOH is a non-food grade solvent, and alternative methods, based on solvent-free and new technologies, such as pulsed electric field and high pressure of extraction, have been studied and could be used in a similar way to OH. The OH extraction method causes cell-wall electroporation but does not cause protein denaturation. Additionally, the temperature increased but was not sufficient to cause protein denaturation. Furthermore, at concentrations higher than 40% of this solvent, denaturation of the proteins can occur [74]. Besides that, MeOH molecules adjacent to the protein side-chain can generate van der Waals interactions, which reduce intra-protein nonpolar interactions and lead to the full extension of protein tertiary structures [75]. Additionally, water molecules can establish hydrogen links with proteins and impact the strength of intra-protein hydrogen bonds, which increase protein stability and decrease the extracted capacity in SF comparing with the CONV method [76–79].

3.4.2. Dietary Fiber

SF found no significant dietary fiber content differences between methods applied to the grape by-products and cultivars studied (Table 1). To the best of our knowledge, there are no studies about the OH extraction method's effects on dietary fiber in grape by-products. This study shows a more significant increase in insoluble dietary fiber in SF after OH treatment than in the SF obtained from the CONV method ($p < 0.05$). The increase in temperature during OH (100 °C) intensifies the Maillard reaction and the quantity of the products, quantified as insoluble dietary fiber [80]. Only a few researchers refer to the thermal effects on dietary fiber, and these also depend on the type of fiber source and the processing method [81–83].

The soluble fiber in SF is higher in the OH application than CONV; oppositely, there was less soluble fiber in the LF treated with OH than in the CONV method. The CONV method may increase the extraction of arabinoxylans (soluble in water) from the cell wall included in the soluble fiber quantification [84,85]. The CONV method has water with an organic solvent, allowing the breakage of cell wall and arabinoxylans release from the pericarp under alkaline conditions.

3.5. Bioactivity Characterization

3.5.1. Total Phenolic Content and Antioxidant Activity of Free and Bound Phenolics

As previously mentioned, the application extraction methods resulted in an LF rich in phenolic compounds. The SF of red grape bagasse presented a higher content in total phenolic compounds (Table 2) with antioxidant capacity than the SF from white grape bagasse (Figure 1). Nevertheless, the remaining SF also contained polyphenols linked to fibers.

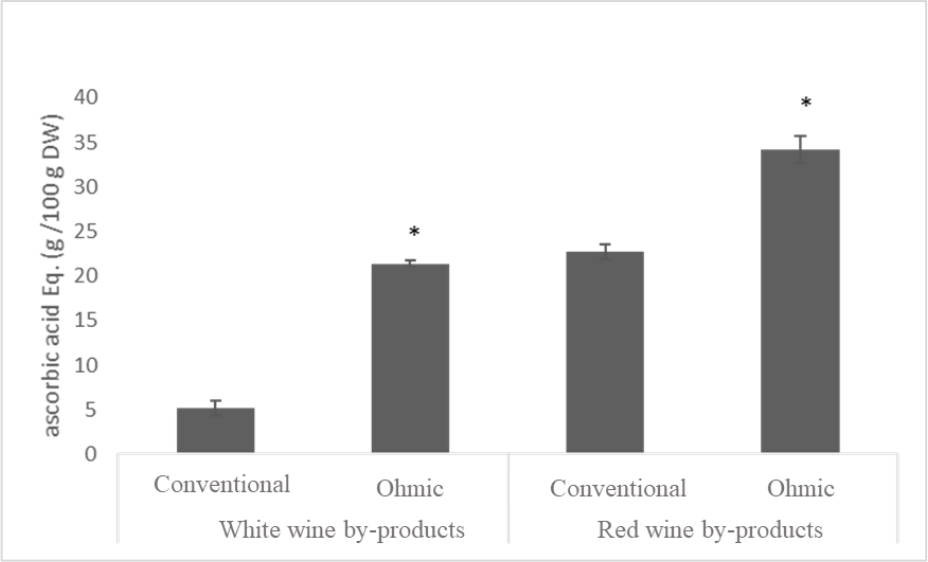

Figure 1. Total antioxidant activity of white and red wine by-products of LF submitted to CONV and OH treatments. * $p < 0.05$ OH compared with the CONV method.

The total phenolic compounds in the LF from white and red grape bagasse observed in Figure 2 were significantly higher ($p < 0.05$) with OH than the CONV method. OH allows increased polyphenol extraction due to its improved diffusion kinetics, resulting from electro-heating effects and membrane alteration. Furthermore, this method is fast, and depending on the voltage applied, it may be used to extract heat-sensitive and unstable compounds, e.g., anthocyanins [26,28,38,86]. Researchers applied OH to grape bagasse and obtained a higher extraction yield of 36% than the CONV methods, with a hydroalcoholic solution used to extract polyphenols [26].

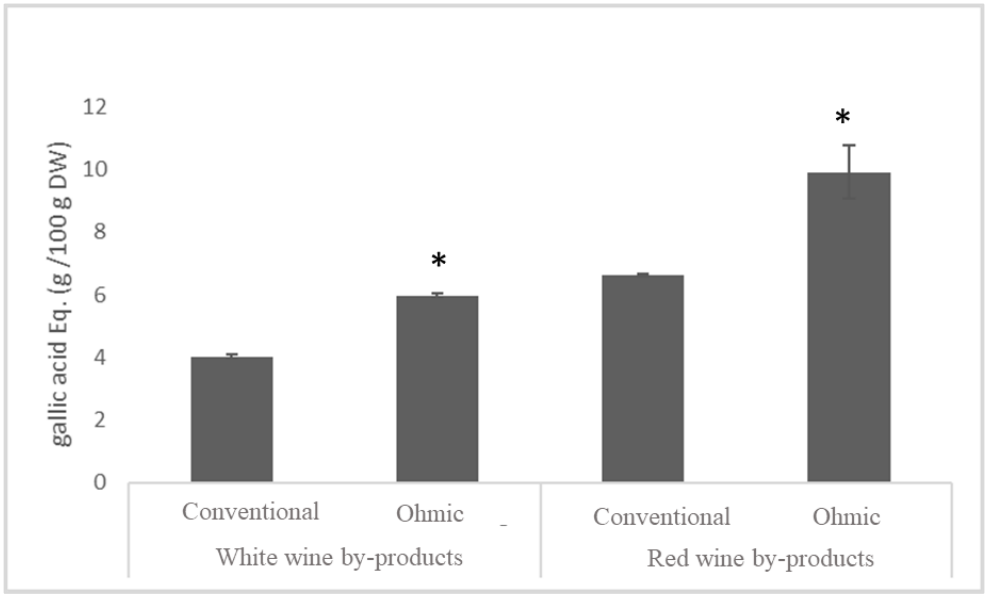

Figure 2. Total phenolic compound content of white and red wine byproducts after CONV and OH treatments. * $p < 0.05$ OH compared with the CONV method.

3.5.2. Identification of Phenolic Compounds

It is relevant to evaluate OH's impact on the extraction of heat-sensitive polyphenols compared to the CONV extraction method. The individual compounds found for red grape are shown in Table 3. The main phenolic compounds identified and quantified by HPLC-DAD in the LF of both cultivars were: (-) epicatechin, gallic acid, *p*-coumaric acid, syringic acid, ferulic acid, and caffeic acid. Significant differences were found between the methods applied ($p < 0.05$). In both methods, the majority of free compounds present were phenolic acids. Regarding bound phenolic in SF, gallic acid, catechin, and vanillic acid were the most predominant ones (32.7–54.2 µg/g DW).

In white grape bagasse, more individual compounds were identified in the CONV method than OH, mainly epicatechin, syringic, ferulic, *p*-coumaric, caffeic, and gallic acid, while OH identified epicatechin, syringic, and gallic acid. Regarding red LF, the content of the individual compounds from OH are similar to the CONV samples. Additionally, higher values were found for bound phenols in SF obtained from OH when compared with the CONV method, indicating that the OH preserves these compounds in the final product [85]. The red grape bagasse is richer in individual phenols than white samples.

The results showed grape bagasse extracted with OH rich in dietary fiber bound to phenols compounds, allowing their utilization as an effective enhancer in the food industry, improving drinks, or even as an element of a dried-out organic product increased in phenolic content. In addition, the OH allowed the obtaining of an LF rich in phenols, including anthocyanins. Regarding anthocyanins, they are considered sensitivity compounds. No differences were obtained between the methods of extraction. The recovery yields of anthocyanins (e.g., delphinidin-3-*o*-glucoside, petunidin-3-*o*-glucoside, and peonidin-3-*o*-glucoside) were similar in the LF from the two extraction methods. The results obtained can also be explained by the difference in the solvents used. Additionally, in OH extraction, acidified water was used. It is a more polar solvent than methanol which promotes higher solubilization of the anthocyanins following the "like dissolves like" principle [86,87]. Furthermore, free and bound phenolic compound differences in OH extracts could be explained by the deliverance of bound phenolic acids just as the cell constituents of plant cells broke down or mellowed, caused by thermal application, prompting increased BC accessibility [88].

Other authors also corroborate using OH as an alternative to organic solvents [4,89,90].

The results also showed that OH could be a selective method of extraction of phenolic compounds. The remaining bagasse is also rich in phenolic compounds bound to fiber, conferring a functional ingredient. Thus, OH can be an excellent solvent-free alternative to extract these compounds.

4. Conclusions

The results show that the cultivar and type of grape by-product have different phytochemical properties. Bagasse presents with higher protein, phenolic compounds (such as anthocyanins) and carbohydrates contents than stems, which could be helpful for food applications aiming at a health impact The OH technology could allow integral valorization obtaining two valuable ingredients: one liquid fraction resulting from the extraction from the by-product and the correspondent solid fraction resulting from the leftovers after extraction. With OH application, it is also possible to obtain higher amounts of phenolic compounds, including anthocyanins, compared with the CONV method. In this way, this method may be applied during the separation process and applied directly in bagasse as a continuous process improving compound extraction and reusing the remaining solid fraction of a new product. In addition, this study unveils that the resulting solid by-products are a rich source of fiber linked to polyphenols, making them an ingredient with health benefits, and may be used as a potential ingredient.

Grape by-products and their extracted BC have higher commercial potential as an ingredient or an integral product. Furthermore, the OH could be applied in the winemaking process, allowing valuable compounds to be extracted, and contributing to the circular economy.

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References

1. Intergovernmental Organisation OIV. *2019 Statistical Report on World Vitiviniculture*; Organisation Internationale de la Vigne et du Vin Publication: France, Paris, 2019; pp. 3–20.
2. Fereres, E.; Goldhamer, D.A.; Sadras, V. Yield Response to Water of Fruit Trees and Vines: Guidelines. FAO Irrigation and Drainage Paper 66. 2012. Available online: https://www.oiv.int/public/medias/5116/booklet-fao-oiv-grapes-focus.pdf (accessed on 1 October 2022).
3. FAO FAOSTAT. Available online: http://www.fao.org/faostat/en/#home (accessed on 9 December 2022).

4. Coelho, M.C.; Pereira, R.N.; Rodrigues, A.S.; Teixeira, J.A.; Pintado, M.E. The Use of Emergent Technologies to Extract Added Value Compounds from Grape By-Products. *Trends Food Sci. Technol.* **2020**, *106*, 182–197. [CrossRef]
5. Teixeira, A.; Baenas, N.; Dominguez-Perles, R.; Barros, A.; Rosa, E.; Moreno, D.A.; Garcia-Viguera, C. Natural Bioactive Compounds from Winery By-Products as Health Promoters: A Review. *Int. J. Mol. Sci.* **2014**, *15*, 15638–15678. [CrossRef] [PubMed]
6. Guerrero, R.F.; Biais, B.; Richard, T.; Puertas, B.; Waffo-Teguo, P.; Merillon, J.-M.; Cantos-Villar, E. Grapevine Cane's Waste Is a Source of Bioactive Stilbenes. *Ind. Crops Prod.* **2016**, *94*, 884–892. [CrossRef]
7. Delgado Adámez, J.; Gamero Samino, E.; Valdés Sánchez, E.; González-Gómez, D. In Vitro Estimation of the Antibacterial Activity and Antioxidant Capacity of Aqueous Extracts from Grape-Seeds (*Vitis vinifera* L.). *Food Control* **2012**, *24*, 136–141. [CrossRef]
8. Lee, H.S.; Coates, G.A. Characterization of Color Fade during Frozen Storage of Red Grapefruit Juice Concentrates. *J. Agric. Food Chem.* **2002**, *50*, 3988–3991. [CrossRef] [PubMed]
9. Dharmadhikari, M. Composition of Grapes. *Vineyard VInt. Age View Mo. State Univ.* **1994**, *9*, 3–8.
10. Prozil, S.O.; Evtuguin, D.V.; Lopes, L.P.C. Chemical Composition of Grape Stalks of *Vitis vinifera* L. from Red Grape Pomaces. *Ind. Crops Prod* **2012**, *35*, 178–184. [CrossRef]
11. Valiente, C.; Arrigoni, E.; Esteban, R.M.; Amado, R. Grape Pomace as a Potential Food Fiber. *J. Food Sci.* **1995**, *60*, 818–820. [CrossRef]
12. Zacharof, M.-P. Grape Winery Waste as Feedstock for Bioconversions: Applying the Biorefinery Concept. *Waste Biomass Valorization* **2017**, *8*, 1011–1025. [CrossRef]
13. Abdrabba, S.; Hussein, S. Chemical Composition of Pulp, Seed and Peel of Red Grape From Libya. *Glob. J. Sci. Res.* **2015**, *3*, 6–11. [CrossRef]
14. Ruiz-Moreno, M.J.; Raposo, R.; Cayuela, J.M.; Zafrilla, P.; Piñeiro, Z.; Moreno-Rojas, J.M.; Mulero, J.; Puertas, B.; Giron, F.; Guerrero, R.F.; et al. Valorization of Grape Stems. *Ind. Crops Prod.* **2015**, *63*, 152–157. [CrossRef]
15. González-Centeno, M.R.; Rosselló, C.; Simal, S.; Garau, M.C.; López, F.; Femenia, A. Physico-Chemical Properties of Cell Wall Materials Obtained from Ten Grape Varieties and Their Byproducts: Grape Pomaces and Stems. *LWT—Food Sci. Technol.* **2010**, *43*, 1580–1586. [CrossRef]
16. Minatel, I.O.; Borges, C.V.; Ferreira, M.I.; Gomez, H.A.G.; Chen, C.-Y.O.; Lima, G.P.P. Phenolic Compounds: Functional Properties, Impact of Processing and Bioavailability. In *Phenolic Compounds—Biological Activity*; Soto-Hernández, M., Ed.; IntechOpen: London, UK, 2017.
17. Guida, V.; Ferrari, G.; Pataro, G.; Chambery, A.; di Maro, A.; Parente, A. The Effects of Ohmic and Conventional Blanching on the Nutritional, Bioactive Compounds and Quality Parameters of Artichoke Heads. *LWT—Food Sci. Technol.* **2013**, *53*, 569–579. [CrossRef]
18. Ianni, A.; Martino, G. Dietary Grape Pomace Supplementation in Dairy Cows: Effect on Nutritional Quality of Milk and Its Derived Dairy Products. *Foods* **2020**, *9*, 168. [CrossRef] [PubMed]
19. Kalli, E.; Lappa, I.; Bouchagier, P.; Tarantilis, P.A.; Skotti, E. Novel Application and Industrial Exploitation of Winery By-Products. *Bioresour Bioprocess* **2018**, *5*, 46. [CrossRef]
20. Truong, L.; Morash, D.; Liu, Y.; King, A. Food Waste in Animal Feed with a Focus on Use for Broilers. *Int. J. Recycl. Org. Waste Agric.* **2019**, *8*, 417–429. [CrossRef]
21. Jimenez-Lopez, C.; Fraga-Corral, M.; Carpena, M.; García-Oliveira, P.; Echave, J.; Pereira, A.G.; Lourenço-Lopes, C.; Prieto, M.A.; Simal-Gandara, J. Agriculture Waste Valorisation as a Source of Antioxidant Phenolic Compounds within a Circular and Sustainable Bioeconomy. *Food Funct.* **2020**, *11*, 4853–4877. [CrossRef]
22. Hanušovský, O.; Gálik, B.; Bíro, D.; Šimko, M.; Juráček, M.; Rolinec, M.; Zábranský, L.; Philipp, C.; Puntigam, R.; Slama, J.A.; et al. The Nutritional Potential of Grape By-Products from the Area of Slovakia and Austria. *Emir. J. Food Agric.* **2020**, *32*, 1–10. [CrossRef]
23. Kurosawa, A. Multigas Mitigation: An Economic Analysis Using GRAPE Model. *Energy J.* **2006**, *27*, 275–288. [CrossRef]
24. Vidović, S.; Vladić, J.; Nastić, N.; Jokić, S. Subcritical and Supercritical Extraction in Food By-Product and Food Waste Valorization. In *Innovative Food Processing Technologies*; Elsevier: Amsterdam, The Netherlands, 2021; pp. 705–721. ISBN 9780081005965.
25. Galanakis, C.M. *Food Waste Recovery*; Elsevier: Chania, Greece, 2014. [CrossRef]
26. el Darra, N.; Grimi, N.; Vorobiev, E.; Louka, N.; Maroun, R. Extraction of Polyphenols from Red Grape Pomace Assisted by Pulsed Ohmic Heating. *Food Bioproc. Tech.* **2013**, *6*, 1281–1289. [CrossRef]
27. Pereira, R.N.; Rodrigues, R.M.; Genisheva, Z.; Oliveira, H.; de Freitas, V.; Teixeira, J.A.; Vicente, A.A. Effects of Ohmic Heating on Extraction of Food-Grade Phytochemicals from Colored Potato. *LWT—Food Sci. Technol.* **2016**, *74*, 493–503. [CrossRef]
28. Maroun, R.G.; Rajha, H.N.; Vorobiev, E.; Louka, N. Emerging Technologies for the Recovery of Valuable Compounds from Grape Processing By-Products. In *Handbook of Grape Processing By-Products: Sustainable Solutions*; Galanakis, C.M.B.T., Ed.; Academic Press: Cambridge, MA, USA, 2017; ISBN 9780128098714.
29. AOAC. *Official Methods of Analysis of AOAC International*, 21st ed.; AOAC International: Gaithersburg, MD, USA, 2019; ISBN 0-935584-89-7.
30. Deng, Q.; Penner, M.H.; Zhao, Y. Chemical Composition of Dietary Fiber and Polyphenols of Five Different Varieties of Wine Grape Pomace Skins. *Food Res. Int.* **2011**, *44*, 2712–2720. [CrossRef]

31. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Nrel, D.C. Determination of Structural Carbohydrates and Lignin in Biomass. In *NREL Laboratory Analytical Procedures*; Golden, CO, USA, 2012; pp. 1–3. Available online: https://www.nrel.gov/docs/gen/fy13/42618.pdf (accessed on 1 October 2022).
32. Ribeiro, T.B.; Oliveira, A.L.; Costa, C.; Nunes, J.; Vicente, A.A.; Pintado, M. Total and Sustainable Valorisation of Olive Pomace Using a Fractionation Approach. *Appl. Sci.* **2020**, *10*, 6785. [CrossRef]
33. Nielsen, S.S. Phenol-Sulfuric Acid Method for Total Carbohydrates. In *Food Analysis Laboratory Manual*; Springer: Boston, MA, USA, 2010.
34. Oliveira, A.; Alexandre, E.M.C.; Coelho, M.; Gomes, M.H.; Almeida, D.P.F.; Pintado, M. Effect of Modified Atmosphere on Polyphenols during Storage of Pasteurised Strawberry Purées. *LWT—Food Sci. Technol.* **2015**, *60*, 377–384. [CrossRef]
35. Oliveira, A.; Alexandre, E.M.C.; Coelho, M.; Lopes, C.; Almeida, D.P.F.; Pintado, M. Incorporation of Strawberries Preparation in Yoghurt: Impact on Phytochemicals and Milk Proteins. *Food Chem.* **2015**, *171*, 370–378. [CrossRef]
36. Silva, S.; Costa, E.M.; Calhau, C.; Morais, R.M.; Pintado, M.M.E. Production of a Food Grade Blueberry Extract Rich in Anthocyanins: Selection of Solvents, Extraction Conditions and Purification Method. *J. Food Meas. Charact.* **2017**, *11*, 1248–1253. [CrossRef]
37. Gião, M.S.; González-Sanjosé, M.L.; Rivero-Pérez, M.D.; Pereira, C.I.; Pintado, M.E.; Malcata, F.X. Infusions of Portuguese Medicinal Plants: Dependence of Final Antioxidant Capacity and Phenol Content on Extraction Features. *J. Sci. Food Agric.* **2007**, *87*, 2638–2647. [CrossRef]
38. Coelho, M.; Pereira, R.; Rodrigues, A.S.; Teixeira, J.A.; Pintado, M.E. Extraction of Tomato By-Products' Bioactive Compounds Using Ohmic Technology. *Food Bioprod. Process.* **2019**, *117*, 329–339. [CrossRef]
39. Mendes, J.A.S.; Xavier, A.M.R.B.; Evtuguin, D.V.; Lopes, L.P.C. Integrated Utilization of Grape Skins from White Grape Pomaces. *Ind. Crops Prod.* **2013**, *49*, 286–291. [CrossRef]
40. Goula, A.M.; Thymiatis, K.; Kaderides, K. Valorization of Grape Pomace: Drying Behavior and Ultrasound Extraction of Phenolics. *Food Bioprod. Process.* **2016**, *100*, 132–144. [CrossRef]
41. Rocha, C.B.; Noreña, C.P.Z. Microwave-Assisted Extraction and Ultrasound-Assisted Extraction of Bioactive Compounds from Grape Pomace. *Int. J. Food Eng.* **2020**, *16*, 20190191. [CrossRef]
42. Wang, W.; Jung, J.; Tomasino, E.; Zhao, Y. Optimization of Solvent and Ultrasound-Assisted Extraction for Different Anthocyanin Rich Fruit and Their Effects on Anthocyanin Compositions. *LWT—Food Sci. Technol.* **2016**, *72*, 229–238. [CrossRef]
43. da Porto, C.; Porretto, E.; Decorti, D. Comparison of Ultrasound-Assisted Extraction with Conventional Extraction Methods of Oil and Polyphenols from Grape (*Vitis vinifera* L.) Seeds. *Ultrason. Sonochem.* **2013**, *20*, 1076–1080. [CrossRef] [PubMed]
44. Bonfigli, M.; Godoy, E.; Reinheimer, M.A.; Scenna, N.J. Comparison between Conventional and Ultrasound-Assisted Techniques for Extraction of Anthocyanins from Grape Pomace. Experimental Results and Mathematical Modeling. *J. Food Eng.* **2017**, *207*, 56–72. [CrossRef]
45. Ribeiro, T.P.; De Lima, M.A.C.; Alves, R.E.; Gonçalves, A.L.D.S.; Souza, A.P.C. Chemical Characterization of Winemaking Byproducts from Grape Varieties Cultivated in Vale Do São Francisco, Brazil. *Food Sci. Technol.* **2018**, *38*, 577–583. [CrossRef]
46. Lachman, J.; Hejtmánková, A.; Hejtmánková, K.; Horníčková, Š.; Pivec, V.; Skala, O.; Dědina, M.; Přibyl, J. Towards Complex Utilisation of Winemaking Residues: Characterisation of Grape Seeds by Total Phenols, Tocols and Essential Elements Content as a by-Product of Winemaking. *Ind. Crops Prod.* **2013**, *49*, 445–453. [CrossRef]
47. Çetin, E.S.; Altinöz, D.; Tarçan, E.; Göktürk Baydar, N. Chemical Composition of Grape Canes. *Ind. Crops Prod.* **2011**, *34*, 994–998. [CrossRef]
48. Bravo, L.; Saura-Calixto, F. Characterization of Dietary Fiber and the in Vitro Indigestible Fraction of Grape Pomace. *Am. J. Enol. Vitic.* **1998**, *49*, 135–141. [CrossRef]
49. Llobera, A.; Cañellas, J. Dietary Fibre Content and Antioxidant Activity of Manto Negro Red Grape (*Vitis vinifera*): Pomace and Stem. *Food Chem.* **2007**, *101*, 659–666. [CrossRef]
50. Sousa, E.C.; DuarteAlexandrino, C.; Carioca, J.O.B.; Rodrigues, S.P.; Uchôa-Thomaz, A.M.A.; Rodrigues, L.L.; Lima, A.; Ferreira, P.A.T.; Morais, S.M.; Rodrigues, A.L.M.; et al. Chemical Composition and Bioactive Compounds of Grape Pomace (*Vitis vinifera* L.), Benitaka Variety, Grown in the Semiarid Region of Northeast Brazil. *Food Sci. Technol.* **2014**, *34*, 135–142. [CrossRef]
51. Apolinar-Valiente, R.; Romero-Cascales, I.; Williams, P.; Gómez-Plaza, E.; López-Roca, J.M.; Ros-García, J.M.; Doco, T. Oligosaccharides of Cabernet Sauvignon, Syrah and Monastrell Red Wines. *Food Chem.* **2015**, *179*, 311–317. [CrossRef] [PubMed]
52. Karnopp, A.R.; Margraf, T.; Maciel, L.G.; Santos, J.S.; Granato, D. Chemical Composition, Nutritional and in Vitro Functional Properties of by-Products from the Brazilian Organic Grape Juice Industry. *Int. Food Res. J.* **2017**, *24*, 207–214.
53. Tahergorabi, R.; Hosseini, S.V. Chapter 2—Proteins, Peptides, and Amino Acids A2—Galanakis, Charis M. BT—Nutraceutical and Functional Food Components. In *Nutraceutical and Functional Food Components*; Academic Press: Cambridge, MA, USA, 2017; pp. 15–38. ISBN 978-0-12-805257-0.
54. Llobera, A.; Cañellas, J. Antioxidant Activity and Dietary Fibre of Prensal Blanc White Grape (*Vitis vinifera*) by-Products. *Int. J. Food Sci. Technol.* **2008**, *43*, 1953–1959. [CrossRef]
55. Costa, J.R.; Amorim, M.; Vilas-Boas, A.; Tonon, R.V.; Cabral, L.M.C.; Pastrana, L.; Pintado, M. Impact of: In Vitro Gastrointestinal Digestion on the Chemical Composition, Bioactive Properties, and Cytotoxicity of *Vitis vinifera* L. Cv. Syrah Grape Pomace Extract. *Food Funct.* **2019**, *10*, 1856–1869. [CrossRef]

56. Karovičová, J.; Kohajdová, Z.; Minarovičová, L.; Kuchtová, V. The Chemical Composition of Grape Fibre. *Potravinarstvo* **2015**, *9*, 53–57. [CrossRef]
57. Mendes, J.A.S.; Prozil, S.O.; Evtuguin, D.V.; Lopes, L.P.C. Towards Comprehensive Utilization of Winemaking Residues: Characterization of Grape Skins from Red Grape Pomaces of Variety Touriga Nacional. *Ind. Crops Prod.* **2013**, *43*, 25–32. [CrossRef]
58. Sauvignon, C.; Words, K.E.Y.; Morgues, A.; Sauvi, C.; Valley, N.; Napa, T.; Division, C. Changes in Pectin Content of Cabernet Sauvignon Grape Berries During Maturation. *Water* **1990**, *41*, 111–115.
59. Güzel, M.; Akpınar, Ö. Valorisation of Fruit By-Products: Production Characterization of Pectins from Fruit Peels. *Food Bioprod. Process.* **2019**, *115*, 126–133. [CrossRef]
60. van Buggenhout, S.; Sila, D.N.; Duvetter, T.; van Loey, A.; Hendrickx, M. Pectins in Processed Fruits and Vegetables: Part III—Texture Engineering. *Compr. Rev. Food Sci. Food Saf.* **2009**, *8*, 105–117. [CrossRef]
61. Cory, H.; Passarelli, S.; Szeto, J.; Tamez, M.; Mattei, J. The Role of Polyphenols in Human Health and Food Systems: A Mini-Review. *Front. Nutr.* **2018**, *5*, 87. [CrossRef]
62. Koch, W. Dietary Polyphenols-Important Non-Nutrients in the Prevention of Chronic Noncommunicable Diseases. A Systematic Review. *Nutrients* **2019**, *11*, 1039. [CrossRef] [PubMed]
63. Alonso, Á.M.; Guillén, D.A.; Barroso, C.G.; Puertas, B.; García, A. Determination of Antioxidant Activity of Wine Byproducts and Its Correlation with Polyphenolic Content. *J. Agric. Food Chem.* **2002**, *50*, 5832–5836. [CrossRef] [PubMed]
64. Spigno, G.; De Faveri, D.M. Antioxidants from Grape Stalks and Marc: Influence of Extraction Procedure on Yield, Purity and Antioxidant Power of the Extracts. *J. Food Eng.* **2007**, *78*, 793–801. [CrossRef]
65. Doshi, P.; Adsule, P.; Banerjee, K.; Oulkar, D. Phenolic Compounds, Antioxidant Activity and Insulinotropic Effect of Extracts Prepared from Grape (*Vitis Vinifera* L.) Byproducts. *J. Food Sci. Technol.* **2015**, *52*, 181–190. [CrossRef] [PubMed]
66. Anastasiadi, M.; Pratsinis, H.; Kletsas, D.; Skaltsounis, A.L.; Haroutounian, S.A. Grape Stem Extracts: Polyphenolic Content and Assessment of Their in Vitro Antioxidant Properties. *LWT—Food Sci. Technol.* **2012**, *48*, 316–322. [CrossRef]
67. Thimothe, J.; Bonsi, I.A.; Padilla-Zakour, O.I.; Koo, H. Chemical Characterization of Red Wine Grape (*Vitis vinifera* and *Vitis Interspecific Hybrids*) and Pomace Phenolic Extracts and Their Biological Activity against Streptococcus Mutans. *J. Agric. Food Chem.* **2007**, *55*, 10200–10207. [CrossRef]
68. Comuzzo, P.; Voce, S.; Grazioli, C.; Tubaro, F.; Marconi, M.; Zanella, G.; Querzè, M. Pulsed Electric Field Processing of Red Grapes (Cv. Rondinella): Modifications of Phenolic Fraction and Effects on Wine Evolution. *Foods* **2020**, *9*, 414. [CrossRef]
69. Iylia Arina, M.Z.; Harisun, Y. Effect of Extraction Temperatures on Tannin Content and Antioxidant Activity of Quercus Infectoria (Manjakani). *Biocatal. Agric. Biotechnol.* **2019**, *19*, 101104. [CrossRef]
70. Abreu, J.; Quintino, I.; Pascoal, G.; Postingher, B.; Cadena, R.; Teodoro, A. Antioxidant Capacity, Phenolic Compound Content and Sensory Properties of Cookies Produced from Organic Grape Peel (*Vitis labrusca*) Flour. *Int. J. Food Sci. Technol.* **2019**, *54*, 1215–1224. [CrossRef]
71. Ueno, T.; Masuda, H.; Ho, C.-T. Formation Mechanism of P-Methylacetophenone from Citral via a Tert-Alkoxy Radical Intermediate. *J. Agric. Food Chem.* **2004**, *52*, 5677–5684. [CrossRef]
72. Ferreira, R.B.; Piçarra-Pereira, M.A.; Monteiro, S.; Loureiro, V.B.; Teixeira, A.R. The Wine Proteins. *Trends Food Sci. Technol.* **2001**, *12*, 230–239. [CrossRef]
73. Burin, V.M.; Ferreira-Lima, N.E.; Panceri, C.P.; Bordignon-Luiz, M.T. Bioactive Compounds and Antioxidant Activity of *Vitis Vinifera* and Vitis Labrusca Grapes: Evaluation of Different Extraction Methods. *Microchem. J.* **2014**, *114*, 155–163. [CrossRef]
74. Kayser, J.J.; Arnold, P.; Steffen-Heins, A.; Schwarz, K.; Keppler, J.K. Functional Ethanol-Induced Fibrils: Influence of Solvents and Temperature on Amyloid-like Aggregation of Beta-Lactoglobulin. *J. Food Eng.* **2020**, *270*, 109764. [CrossRef]
75. Shao, Q. Methanol Concentration Dependent Protein Denaturing Ability of Guanidinium/Methanol Mixed Solution. *J. Phys. Chem. B* **2014**, *118*, 6175–6185. [CrossRef]
76. Pereira, R.N.; Teixeira, J.A.; Vicente, A.A. Exploring the Denaturation of Whey Proteins upon Application of Moderate Electric Fields: A Kinetic and Thermodynamic Study. *J. Agric. Food Chem.* **2011**, *59*, 11589–11597. [CrossRef]
77. Pereira, R.N.; Vicente, A.A. Environmental Impact of Novel Thermal and Non-Thermal Technologies in Food Processing. *Food Res. Int.* **2010**, *43*, 1936–1943. [CrossRef]
78. Rodrigues, R.M.; Martins, A.J.; Ramos, O.L.; Malcata, F.X.; Teixeira, J.A.; Vicente, A.A.; Pereira, R.N. Influence of Moderate Electric Fields on Gelation of Whey Protein Isolate. *Food Hydrocoll.* **2015**, *43*, 329–339. [CrossRef]
79. Pereira, R.N.; Souza, B.W.S.; Cerqueira, M.A.; Teixeira, J.A.; Vicente, A.A. Effects of Electric Fields on Protein Unfolding and Aggregation: Influence on Edible Films Formation. *Biomacromolecules* **2010**, *11*, 2912–2918. [CrossRef]
80. Huda, M.S.; Drzal, L.T.; Mohanty, A.K.; Misra, M. The Effect of Chemical Treatments of Fibers on the Mechanical and Thermo-Mechanical Properties of the Pineapple Leaf Fiber (PALF) Reinforced Poly(Lactic Acid) (PLA) Laminated Composites. In Proceedings of the American Society for Composites—21st Technical Conference of the American Society for Composites 2006, Dearborn, MI, USA, 17–20 September 2006; Volume 2, pp. 888–900.
81. Chang, M.-C.; Morris, W.C. The Effect of Heat Treatments on Dietary Fiber as Assessed by Scanning Electron Microscopy. *J. Food Process. Preserv.* **1990**, *14*, 335–343. [CrossRef]
82. Elleuch, M.; Bedigian, D.; Roiseux, O.; Besbes, S.; Blecker, C.; Attia, H. Dietary Fibre and Fibre-Rich by-Products of Food Processing: Characterisation, Technological Functionality and Commercial Applications: A Review. *Food Chem.* **2011**, *124*, 411–421. [CrossRef]

83. Ain, H.B.U.; Saeed, F.; Ahmed, A.; Khan, M.A.; Niaz, B.; Tufail, T. Improving the Physicochemical Properties of Partially Enhanced Soluble Dietary Fiber through Innovative Techniques: A Coherent Review. *J. Food Process. Preserv.* **2019**, *43*, e13917. [CrossRef]
84. Jesus, M.S.; Ballesteros, L.F.; Pereira, R.N.; Genisheva, Z.; Carvalho, A.C.; Pereira-Wilson, C.; Teixeira, J.A.; Domingues, L. Ohmic Heating Polyphenolic Extracts from Vine Pruning Residue with Enhanced Biological Activity. *Food Chem.* **2020**, *316*, 126298. [CrossRef]
85. Ramírez-Jiménez, A.K.; Rangel-Hernández, J.; Morales-Sánchez, E.; Loarca-Piña, G.; Gaytán-Martínez, M. Changes on the Phytochemicals Profile of Instant Corn Flours Obtained by Traditional Nixtamalization and Ohmic Heating Process. *Food Chem.* **2019**, *276*, 57–62. [CrossRef] [PubMed]
86. Roda-Serrat, M.C.; Andrade, T.A.; Rindom, J.; Lund, P.B.; Norddahl, B.; Errico, M. Optimization of the Recovery of Anthocyanins from Chokeberry Juice Pomace by Homogenization in Acidified Water. *Waste Biomass Valorization* **2021**, *12*, 1815–1827. [CrossRef] [PubMed]
87. de Sousa Sabino, L.B.; Alves Filho, E.G.; Fernandes, F.A.N.; de Brito, E.S.; da Silva Júnior, I.J. Optimization of Pressurized Liquid Extraction and Ultrasound Methods for Recovery of Anthocyanins Present in Jambolan Fruit (*Syzygium cumini* L.). *Food Bioprod. Process.* **2021**, *127*, 77–89. [CrossRef]
88. Khan, M.K.; Ahmad, K.; Hassan, S.; Imran, M.; Ahmad, N.; Xu, C. Effect of Novel Technologies on Polyphenols during Food Processing. *Innov. Food Sci. Emerg. Technol.* **2018**, *45*, 361–381. [CrossRef]
89. Loypimai, P.; Moongngarm, A.; Chottanom, P.; Moontree, T. Ohmic Heating-Assisted Extraction of Anthocyanins from Black Rice Bran to Prepare a Natural Food Colourant. *Innov. Food Sci. Emerg. Technol.* **2015**, *27*, 102–110. [CrossRef]
90. Salari, S.; Jafari, S.M. The Influence of Ohmic Heating on Degradation of Food Bioactive Ingredients. *Food Eng. Rev.* **2020**, *12*, 191–208. [CrossRef]