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Winery By-Products as Source of Bioactive Compounds for Pharmaceutical and Cosmetic Industries

By Irene Gouvinhas and Ana Barros

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Abstract

It is well established in the scientific community that agro-food wastes represent economic advantages and contribute to circular economy. For instance, wine industries of Região Demarcada do Douro involve the production of large quantities of by-products, such as stem, pomace, trimmed vine shoots, or wine lees, presenting a remarkable valuable composition in phytochemicals with putative health-promoting qualities. Nevertheless, the bioactive compounds obtained from these natural sources depends on the extraction process employed. In order to reduce production costs and optimize processes, new technologies—such as ultrasound-assisted extraction (UAE)—have been employed to decrease energy consumption and increase the product or process safety/control and quality. This work aims to characterize the phenolic compounds extracted from winery by-products (WBPs), namely grape stems, grape pomace, and wine lees of two grape (*Vitis vinifera* L.) varieties (Sousão and Tinta Barroca) from the same geographical site, as well as the antioxidant capacity. Wine lees and grape stems presented the highest concentration of phenolic compounds and the highest antioxidant capacity for Tinta Barroca variety, while grape pomace presented the highest values of these parameters for Sousão variety, demonstrating the high influence of the variety studied. Furthermore, wine lees revealed to be the winery by-product with the lowest antioxidant capacity and content of phenolics. These by-products revealed to be a rich source of phenolic compounds with high antioxidant capacities revealing to be of interest for pharmaceutical and cosmetic industries.

Keywords

Winery by-products

Ultrasound-assisted extraction

Bioactive compounds

Antioxidant capacity

Valorization

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1. Introduction

The main strategies for the valorization of food wastes are related to their biotechnological transformation into chemicals or even the recovery of important substances, such as polyphenols that typically appear in the winery by-products (WBPs). Currently, the implemented alternatives for reducing the environmental impact of agronomic residues involves the development of new feeds and their use as soils amendments. Actually, these are the primary alternatives considered. Given their low added-value there is a need to search for new valorization alternatives. Based on these premises, the content of bioactive phytochemicals of agro-food materials in general has allowed envisaging their use as donors of these kind of molecules to obtain materials that could contribute to enhance medical/nursing treatments.

Given the relevance of the winemaking companies, particularly at Douro region, and the high amount of underexploited wastes produced, the development of innovative applications for these materials urges [1]. On these materials ongoing research (also relevant studies developed by the research group) has revealed the valuable quantitative profile of bioactive compounds in WBPs, namely a variety of (poly)phenols and stilbenes that could be responsible for remarkable biological activities, such as anti-inflammatory, antioxidant, and antibacterial, among others [2, 3, 4, 5].

However, for envisaging new applications for these materials, it is important to be aware about the close dependency of the phytochemical composition and therefore the biological power on an array of factors, namely the geographical growing conditions [3], the cultivar studied [4] and, most important, the extraction methodology employed [6]. In fact, the extraction methodology not just condition the phytochemical compounds obtained from a given plant material, but also is associated to environmental constraints, as well as to economic and toxicological issues depending on the solvent used [7]. To overcome these limitations, special attention has been paid to the extraction methods for bioactive compounds [8]. So, the use of eco-friendly techniques blended with reusable and non-toxic solvents is gaining a wide acceptance, due to its contribution to minimizing costs, health related risks, and environmental impacts. As a valuable alternative to the traditional extraction methods, UAE arises as exceptional option to extract (poly)phenols, revealing to be an environment-friendly technology that offers several advantages over the conventional and non-conventional ones, such as a lower cost, versatility, and easily scale-up [7]. This technique has been already employed in diverse plant matrices and the outcomes reported have revealed it as one of the best alternatives to extract phenolic compounds from winery wastes [7, 9].

Based on these compositional features, potential applications for these materials, and specifically for grape stems, have been described by the research team, such as the spirits production, leading to an industrial alternative to traditional distilled spirits [10]. Beyond this application, recently, a preliminary study developed by the team demonstrated the stem extracts capacity to inhibit the growth of foot wound ulcers multidrug resistance bacteria (*S. aureus* and *Enterobacter aerogenes*) through disc diffusion and minimum inhibitory concentration assays [5, 11]. Whereby, WBPs are valuable candidates as wound healing agents for instance. Additional studies of our group also revealed that the quantitative (poly)phenolic profile of grape stems remains almost constant during storage for months, leading to the possibility to access this by-product all year-round, due to the preservation of the phytochemical composition [2], and thus, the biological activity expected.

In this work, we intend to generate new knowledge on the potential ability of WBPs (wine lees, grape pomace, and grape stems) bioactive compounds to be further used in pharmaceutical and cosmetic industries, using a sustainable and green extraction way, namely ultrasound-assisted extraction (UAE), enhancing the regional and circular economy.

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2. Material and methods

2.1 Chemicals

Folin–Ciocalteu's reagent, 3,4,5-trihydroxybenzoic acid (gallic acid), acetic acid, both extra pure (>99%), and sodium hydroxide were purchased from Panreac (Panreac Química S.L.U., Barcelona, Spain). Sodium nitrate, aluminum chloride, and sodium carbonate, all extra pure (>99%), and methanol were acquired from Merck (Merck, Darmstadt, Germany). Sodium molybdate (99.5%) was purchased from Chem-Lab (Chem-Lab N.V., Zedelgem, Belgium). The compounds 2,2-diphenyl-1-picrylhydrazyl radical (DPPH[•]), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS^{•+}), and potassium phosphate were obtained from Sigma-Aldrich (Steinheim, Germany), as well as the standards compounds for the chromatographic separation. Additionally, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Fluka Chemika (Neu-Ulm, Switzerland).

Ultrapure water was obtained using a Millipore water purification system. Chromatography solvents were of HPLC grade according to the analysis performed.

2.2 Sampling

The present work was carried on WBPs, namely, grape stems, grape pomace and wine lees of two varieties of *Vitis vinifera* L. (Sousão and Tinta Barroca), which are traditionally cultivated in the *Região Demarcada do Douro*, in northern Portugal. Plant material came from a farm located in Cima Corgo sub-region (Upper Corgo) (Sanfins do Douro - GPS: 41.1656, -7.2912, Average Altitude.: 730 m, vineyard altitude from 690 to 730 m), as demonstrated in [Figure 1](#), where geology is essentially characterized by schist formations with occasional outcrops of granite in Mediterranean-like climatic conditions [12]. No irrigation was applied in the field trial of this investigation. WBPs were collected in 2020 growing season, at the wine company which possesses this vineyard. Once collected, samples were lyophilized, grounded to a fine powder, and stored, protected from light, at room temperature until analysis.

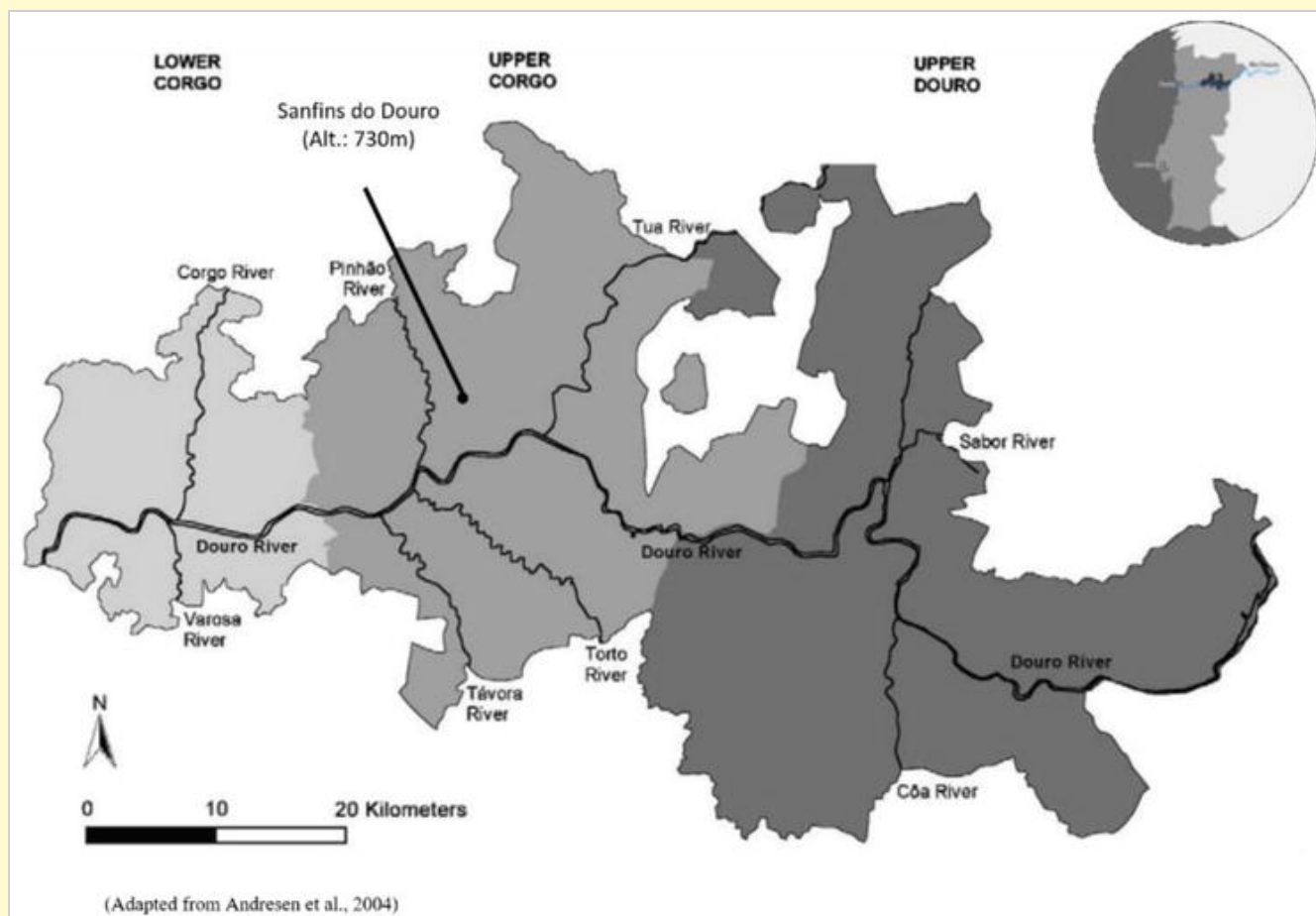


Figure 1.

Geographical origin of WBPs from the Região Demarcada do Douro (Portugal).

2.3 Ultrasound-assisted extraction (UAE) of bioactive compounds from olive seeds

For the phenolic compounds extraction, the protocol used was previously described by Lameirão et al. with some modifications [8]. The UAE was performed with an ultrasonic apparatus (VCX 500 Vibra-Cell™, Newtown, Connecticut, USA), using a 13 mm diameter tip with amplitude, temperature and time controller. The amplitude was employed at 50%. The powdered samples (2.5 g) were extracted with 50 mL of methanol:water (70:30, v/v) into the ultrasonic apparatus during 40 min and at 70°C. After

ultrasonic extraction, the methanolic extracts were centrifuged (13,000 rpm, 4°C) for 15 min (Sigma Centrifuges 2–16 K, Germany) and filtered. Samples were stored at 4°C until analysis.

2.4 Phenolic content

The content in total phenols, flavonoids, and *ortho*-diphenols was determined according to spectrophotometric methodologies previously reported [13].

Briefly, the content of total phenolics in olive seed extracts was evaluated by the Folin–Ciocalteu spectrophotometric method, using gallic acid as standard, being the results expressed as mg of gallic acid per gram of dry weight (mg GA g⁻¹ DW).

The content of *ortho*-diphenols in olive seeds was determined by adding Na₂MoO₄ (50 g L⁻¹) to the samples appropriately diluted, reading the absorbance at 375 nm. For the quantification, the gallic acid was used as standard. Results were expressed as mg GA g⁻¹ DW.

For the assessment of flavonoid content, the aluminum complex method was performed, using catechin as standard. Results were expressed as mg of catechin per gram of dry weight (mg CAT g⁻¹ DW).

All the assays were performed using 96-well micro plates (Nunc, Roskilde, Denmark) and an Infinite M200 microplate reader (Tecan, Grödig, Austria). For all analyses, three replicates (n = 3) of each sample were assessed.

2.5 Antioxidant capacity assays

The free radical scavenging capacity was determined by ABTS and DPPH spectrophotometric methods, according to the method described by [14]. FRAP methodology was also applied to measure ferric antioxidant power of WBPs extracts.

These assays were also performed using 96-well micro plates (Nunc, Roskilde, Denmark) and an Infinite M200 microplate reader (Tecan, Grödig, Austria), being the results expressed in mmol Trolox per gram of dried sample (mmol Trolox g⁻¹ DW). All the analyses were made in triplicate (n = 3) for each sample [15].

2.6 Identification and quantification of phenolic compounds by RP–HPLC–DAD

The polyphenolic profile of WBPs extracts was assessed by Reverse Phase - High Performance Liquid Chromatography - Diode Array Detector (RP-HPLC-DAD), in an Agilent HPLC 1100 series equipped

with a photodiode array detector and a mass detector in series (Agilent Technologies, Waldbronn, Germany), in accordance with the method previously described [13]. The equipment consisted of a photodiode array detector (model G1315B), an autosampler (model G1313A), a binary pump (model G1312A), and a degasser (model G1322A). The HPLC system was controlled by Xcalibur software (Agilent, version 08.03). A C18 column (250 x 4.6 mm, 5 μ m particle size; ACE, Aberdeen, Scotland) was used, being the reverse phase HPLC method based on a polar mobile phase with the mixture of solvent A: H₂O/HCOOH (99.9:0.1, v/v), and solvent B: CH₃CN/HCOOH (99.9:0.1, v/v). The following linear gradient scheme was used (t in min; %B): (0; 5%), (15; 15%), (30; 30%), (40; 50%), (45; 95%), (50; 95%) and (55; 5%). At this last time (55 min), return to 5% of B to stabilize and prepare the column for the next sample. The analysis was performed at 25°C, with a flow rate of 1.0 mL/min and a sample injection volume of 20 μ L. All samples were injected in triplicate. For the quantification of the identified compounds, the respective standards were used at 280 nm. Concentrations were expressed in mg g⁻¹ of dry weight (mg g⁻¹ DW).

2.7 Statistical analysis

The results are presented as mean (n = 3) \pm standard deviation (SD). The data obtained were subjected to variance analysis (ANOVA) and a multiple range test (Tukey's test) for a p value <0.05, using IBM SPSS statistics 21.0 software (SPSS Inc., Chicago, IL, USA).

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3. Results and discussion

3.1 Phenolic content of wine lees, grape pomace and grape stems

In the present work, the determination of the phenolic composition and the antioxidant capacity of wine lees, grape stems, and grape pomace extracts of two grape (*Vitis vinifera* L.) varieties (Sousão and Tinta Barroca) were performed. The phenolic content of these samples collected from Douro region (Northern Portugal) was presented in [Table 1](#). As it can be observed, in general, grape stems were the WPBs with the highest content of total phenols (168.75 mg GA g⁻¹ DW, on average), *ortho*-diphenols (166.39 mg GA g⁻¹ DW, on average), and flavonoids (152.31 mg CAT g⁻¹ DW, on average), followed by grape pomace and

wine lees. Concerning this last winery by-product, it can be stated that the samples from Sousão variety showed the lowest content of these three studied parameters, being significantly different from the other variety and WBPs ($p < 0.05$). In fact, wine lees and grape stems presented the highest values of phenolic content in Tinta Barroca samples which has not been observed for the grape pomace extracts, which can be explained by the different phenolic compounds present in these WBPs.

	Phenolic content				
	Total phenols		<i>Ortho</i> -diphenols		Flavonoids
	Sousão	Tinta Barroca	Sousão	Tinta Barroca	Sousão
Wine lees	$\underline{Z}15.44 \pm 1.25^a$	125.39 ± 1.12^b	118.91 ± 0.95^a	136.03 ± 1.10^b	18.50 ± 0.89^a
Grape pomace	153.70 ± 0.53^d	135.32 ± 2.76^c	151.78 ± 1.89^c	138.70 ± 1.42^b	144.81 ± 1.75^c
Grape stems	156.81 ± 1.29^e	180.68 ± 2.77^f	162.53 ± 1.01^d	170.24 ± 1.88^e	143.90 ± 2.13^c
<i>P</i> -value	\underline{Y}^{***}		***		***

Table 1.

Total phenols (mg GA g⁻¹ DW), *ortho*-diphenols (mg GA g⁻¹ DW), and flavonoids (mg CAT g⁻¹ DW) content and antioxidant capacity (mmol Trolox g⁻¹ DW) of WBPs from Sousão and Tinta Barroca varieties.

^Z Data presented as Mean (n = 3) ± SD values for the same parameter evaluated followed by different superscript lowercase letters are significantly different at $p < 0.001$, according to Tukey's test.

^Y Level of significance: N.s.: not significant ($p > 0.05$); *significant at $p < 0.05$; **significant at $p < 0.01$; ***significant at $p < 0.001$.

Romero et al. obtained similar values in wine lees of total phenols (38–254 mg CAT g⁻¹) and flavonoids (16–146 mg CAT g⁻¹) content from the Tempranillo variety, with these ranges caused by the extraction solvent employed by these authors [16]. However, Pérez-Serradilha et al. [17] obtained higher values in this WBP of total phenols content (364 mg g⁻¹) than those obtained in this work after a microwave-assisted extraction optimized. Our research group have analyzed the phenolic content of grape stem extracts prepared with conventional extraction methods (hydro-methanolic solvents) [3, 4, 11, 18].

The values ranged between 32 and 123 mg GA g⁻¹ DW for total phenols, between 35 and 116 mg GA g⁻¹ DW for *ortho*-diphenols, and from 34 to 106 mg CAT g⁻¹ DW for flavonoids, depending on cultivar, geographical localization, crop season, among other factors [4, 18, 19]. The values of the present work were slightly higher than the values obtained in those studies, maybe due to the new efficient extraction method performed in this work (UAE). Grape pomace has been also analyzed by other authors concerning its phenolic content, obtaining values around 40 mg GA g⁻¹ DW for total phenols and around 14 mg CAT g⁻¹ DW for flavonoids which are significantly lower than those obtained in this work.

In fact, these contents are particularly dependent of several factors, such as the agronomic conditions [3], cultivar [4] and extraction methods employed [6]. In this sense, in order to reduce production costs and optimize processes, new technologies—such as ultrasound-assisted extraction (UAE) or microwave-assisted extraction (MAE)—have been employed to decrease energy consumption and increase the product or process safety/control and quality. These techniques, already used at large scale, emerged as efficient, energy/time-saving and clean extraction methodologies, providing higher recoveries of bioactive compounds using low amounts of solvent, with particular advantageous for natural sources [8, 20].

3.2 Antioxidant capacity of wine lees, grape pomace and grape stems

The antioxidant capacity of the WBPs investigated in this work was performed by three methods (ABTS, DPPH, and FRAP), being the results presented in Table 1. As expected, due to the correlation of most phenolic compounds with antioxidant capacity, Tinta Barroca samples presented the highest values for all the methods concerning wine lees and grape pomace samples. In contrast, grape pomace samples from Sousão variety showed the highest antioxidant capacity for ABTS (5.54 ± 0.09 mmol Trolox g⁻¹ DW), DPPH (1.64 ± 0.03 mmol Trolox g⁻¹ DW), and FRAP (1.75 ± 0.01 mmol Trolox g⁻¹ DW) methodologies, being significantly different from the Tinta Barroca samples (p < 0.05).

Romero et al. [16] also determined the antioxidant capacity of wine lees extracts from Tempranillo variety, obtaining values ranged between 0.46 and 2.197 mmol Trolox g⁻¹. The values obtained in the present work are in the range of those presented by these authors, concerning FRAP method. In literature, grape stem extracts have been also analyzed concerning this biological property, which present antioxidant capacities of 0.35–0.84 mmol Trolox g⁻¹ DW, 0.15–0.76 mmol Trolox g⁻¹ DW, and 0.33–1.03 mmol Trolox g⁻¹ DW for ABTS, DPPH, and FRAP methodologies [4, 18, 19], which were lower than those presented in the present study essentially due to several factors, such as different extraction methods, extraction solvents or protocols, varieties, among others.

3.3 Phenolic profile of wine lees, grape pomace and grape stems

The phenolic profile of wine lees, grape pomace and grape stems was performed by RP-HPLC-DAD, being the results presented in [Table 2](#). Fifteen compounds were identified, being grape pomace samples the ones with more phenolic compounds identified, including phenolic acids, flavanols, and anthocyanins. In this study, it was possible to observe the same behavior referred above, namely the significant highest content of the phenolic compounds identified in grape pomace extracts from Sousão variety ($p < 0.05$). Similar compounds were identified in wine lees, namely gallic acid, catechin, epicatechin, and malvidin-3-*O*-glucoside, beside others which were found only in this by-product, namely protocatechuic acid ($0.337 \text{ mg g}^{-1} \text{ DW}$, on average), delphinidin-3-*O*-glucoside ($0.190 \text{ mg g}^{-1} \text{ DW}$, on average), and petunidin-3-*O*-glucoside ($0.268 \text{ mg g}^{-1} \text{ DW}$, on average). All these compounds were also determined in higher concentrations in Tinta Barroca samples which is in agreement with the previous results reported above. Grape stem extracts presented three phenolic compounds which were not identified in wine lees and grape pomace, namely isorhammetin-3-*O*-(6-*O*-feruloyl)-glucoside, caftaric acid, and ϵ -viniferin, which were also present in higher concentration in Tinta Barroca samples, being significantly different from Sousão samples ($p < 0.05$).

	Wine lees		Grape pomace	
	Sousão	Tinta Barroca	Sousão	Tinta Barroca
1. Gallic acid	0.422 ± 0.001^a	0.503 ± 0.003^b	0.750 ± 0.002^d	0.641 ± 0.002^c
2. Isorhammetin-3- <i>O</i> -(6- <i>O</i> -feruloyl)-glucoside	nd	nd	nd	nd
3. Caftaric acid	nd	nd	nd	nd
4. Protocatechuic acid	0.216 ± 0.001^a	0.458 ± 0.001^b	nd	nd
5. <i>p</i> -coumaric acid	nd	nd	0.434 ± 0.005^b	0.125 ± 0.002^a
6. Delphinidin-3- <i>O</i> -glucoside	0.123 ± 0.002^a	0.258 ± 0.001^b	nd	nd
7. Catechin	0.559 ± 0.010^a	0.798 ± 0.018^b	1.501 ± 0.025^d	0.993 ± 0.031^c

	Wine lees		Grape pomace	
	Sousão	Tinta Barroca	Sousão	Tinta Barroca
8. Epicatechin	0.498 ± 0.001 ^b	0.660 ± 0.005 ^d	0.601 ± 0.007 ^c	0.412 ± 0.003 ^a
9. Petunidin-3-O-glucoside	0.215 ± 0.005 ^a	0.321 ± 0.007 ^b	nd	nd
10. Malvidin-3-O-glucoside	0.875 ± 0.023 ^a	0.968 ± 0.012 ^c	1.002 ± 0.024 ^d	0.934 ± 0.017 ^b
11. Quercetin-3-O-rutinoside	nd	nd	0,450 ± 0,002 ^c	0.214 ± 0.012 ^a
12. Quercetin-3-O-glucoside	nd	nd	0.458 ± 0,011 ^c	0.385 ± 0.062 ^a
13. Kaempferol-3-O-rutinoside	nd	nd	0.207 ± 0.001 ^d	0.110 ± 0.001 ^c
14. Kaempferol-3-O-glucoside	nd	nd	0.305 ± 0.003 ^c	0.401 ± 0.001 ^d
15. ε-viniferin	nd	nd	nd	nd

Table 2.

Content of individual phenolics (mg g⁻¹ dw) of WBPs from Sousão and Tinta Barroca varieties. Statistical treatment notes: Data were subjected to analysis of variance (ANOVA) and multiple range test (Tukey's test) with a significance of $p < 0.05$.

^Z Data presented as Mean (n = 3) ± SD values in the same row followed by different superscript lowercase letters are significantly different at $p < 0.001$, according to Tukey's test.

^Y Level of significance: N.s.: not significant ($p > 0.05$); *significant at $p < 0.05$; **significant at $p < 0.01$; ***significant at $p < 0.001$. nd: not detected.

Several compounds identified in this work have been also identified by other authors in these WBP, namely gallic acid, catechin, delphinidin-3-*O*-glucoside, epicatechin, *p*-coumaric acid, petunidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, among others [16, 17, 21, 22]. However, it is well known that the extraction method, the cultivars, the geographical conditions, the growing season, the plant diseases, among others, affect the chemical composition of WBPs, namely the secondary metabolites which are highly present in these matrices.

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4. Conclusions

Nowadays, it is well established in the scientific community that wine has an important role in the prevention of some cardiovascular diseases, resulting from their content in bioactive phytochemicals with antioxidant capacity. Many of these compounds are derived from the solid parts of the grape cluster (stem, pomace, trimmed vine shoots, and wine lees). However, during the winemaking process, a complete extraction of these compounds to the juice/wine does not occur, and they may remain at high concentrations in certain wastes, such as in the stems. Indeed, the wine industry involves the production of large quantities of by-products, characterized by a valuable composition in phytochemicals with putative health-promoting qualities. Additionally, in light of the biological properties revealed recently,

the search for natural bioactive compounds has paid attention on these materials as promising alternatives.

In this work, it was possible to observe the high content of phenolic compounds and the high antioxidant capacities demonstrated by several winery by-products, namely wine lees, grape pomace, and grape stems which were subjected to an ultrasound assisted extraction, obtaining higher values than those obtained by conventional extraction methods employed by the research group.

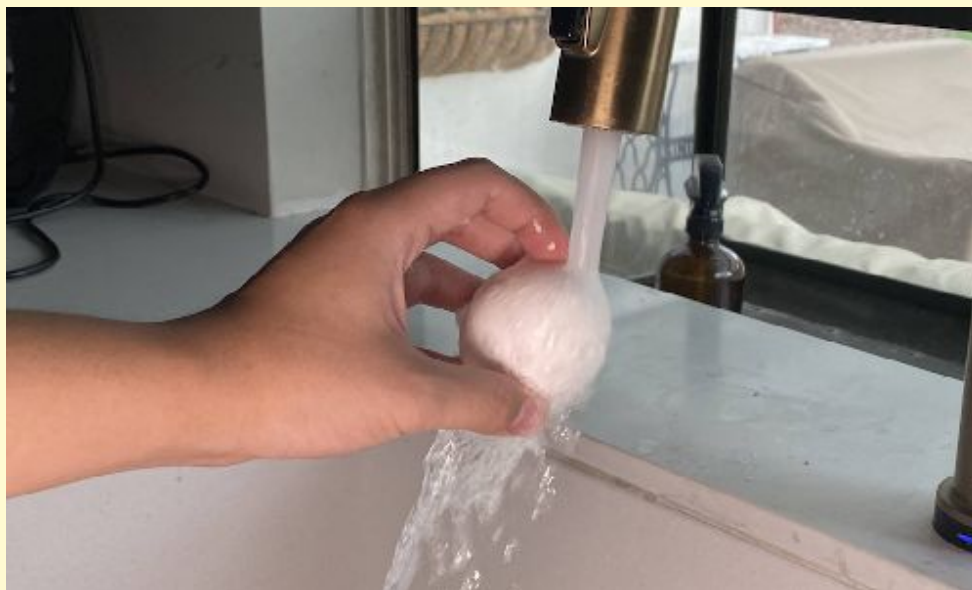
In this sense, the phenolics present in winery by-products may have an added-value to be used as an alternative to synthetic substances employed in distinct industries, giving rise to sustainable agro-industrial activities.

Acknowledgments

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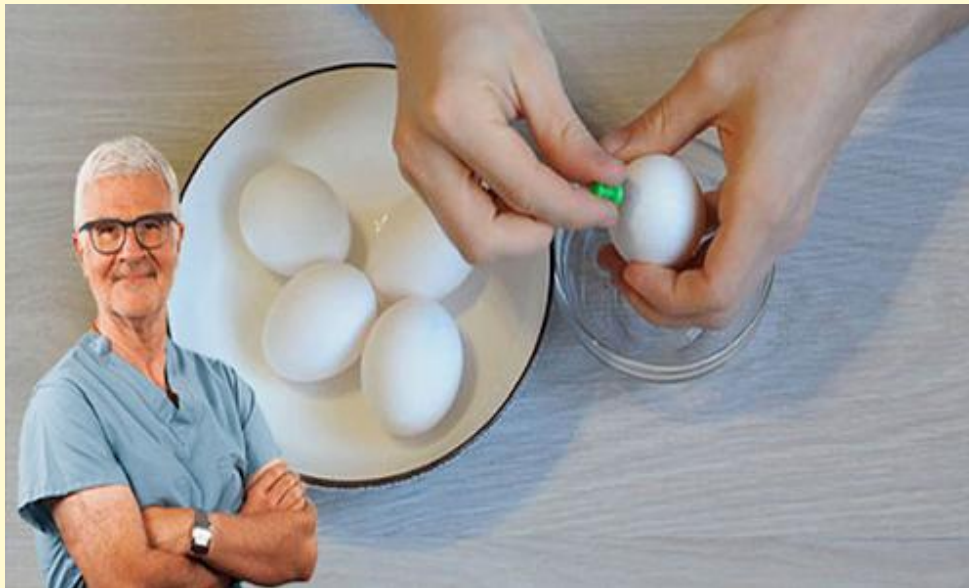
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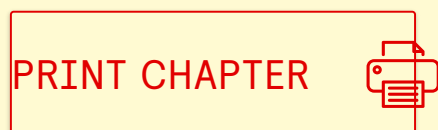
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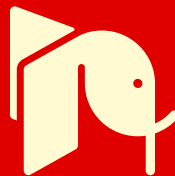
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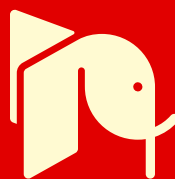
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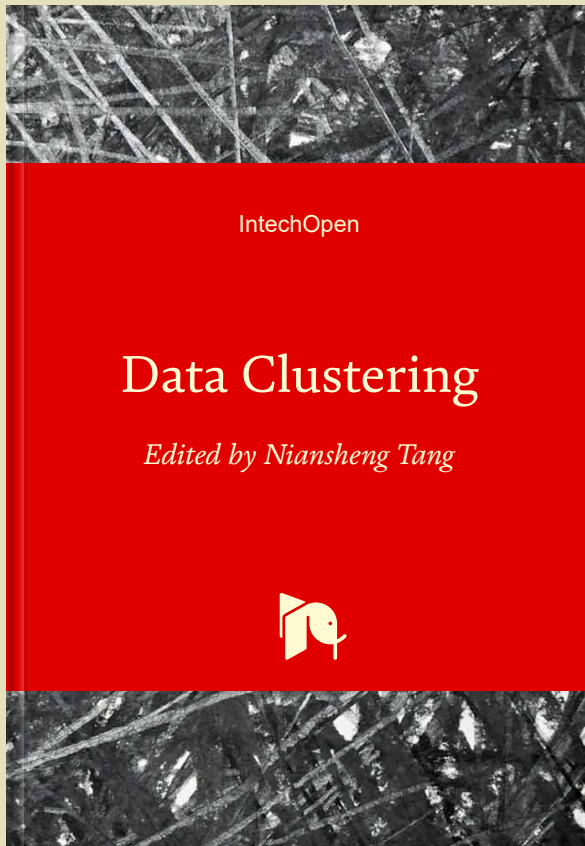
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