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Proceeding Paper

Comparison of Green Extraction Techniques for Bioactive Compounds Recover from Grapevine by-Products †

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Abstract: Different extraction techniques, namely ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), subcritical water extraction (SWE) and conventional extraction (CE), were tested to evaluate their efficiency to recover bioactive compounds from grapevine by-products. SWE was the extraction technique that allowed the highest recovery of polyphenolic compounds, while grape stalk from *Cerceal Branco* variety obtained using SWE at 150 °C had the highest TPC (17.0 ± 0.2 mgGAE/g fw) as well as the highest antioxidant activity by ABTS and FRAP assays (19.9 ± 0.3 and 13.0 ± 0.3 mgAAE/g fw). The phenolic composition revealed high amounts of catechin, epicatechin, chlorogenic and neochlorogenic acids. SWE demonstrated to be a powerful extraction technique for polyphenols recovery from grapevine by-products.

Keywords: grapevine by-products; polyphenols; green extraction techniques; functional products

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1. Introduction

The winemaking sector is one of the most important worldwide, which translates into the production of huge amounts of by-products, such as grape pomace and stalks, with high environmental impacts [1]. The crescent environmental conscience and the government regulations increasingly promote more sustainable production practices creating new challenges, such as the reuse or the destination of the generated waste. These grapevine by-products represent potential sources of natural polyphenols, and due to their recognized health-promoting properties, several studies have focused their efforts on their efficient extraction [2].

In the last years, environmentally friendly extraction techniques, such as such as subcritical water extraction (SWE), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE), has been applied to determine which one is best for recovering bioactive compounds [1,3]. Compared to conventional extraction (CE), these techniques are solvent and time-saving, and the use of organic solvents limits extracts incorporation in food and pharmaceutical products. This work aims to determine the potential of the grapevine by-products, namely grape pomace, stalk and must from two different varieties (*Cerceal Branco* and *Tinta Miúda*), as a source of antioxidants for their possible incorporation in food products. This samples were submitted to different extraction techniques and their total phenolic content (TPC) and antioxidant activities (through ferric reduction antioxidant power (FRAP) and 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS) assays) were screened.

2. Materials and Methods

2.1. Samples

Samples from *Tinta Miúda* and *Cerceal Branco* variety were kindly provided by AVIPE (Vinegrowers Association of the Municipality of Palmela). These were separated according to their nature, must, stalk or pomace, and stored in the freezer until further use.

2.2. Extraction Techniques

All the samples were submitted to different extraction techniques:

- UAE: 2.0 g of sample was mixed with 100 mL of ethanol:water 60:40 (*v*/*v*) for 20 min at 25 °C [4];
- MAE: 1.5 g of sample was mixed with 20 mL of ethanol:water 60:40 (*v*/*v*) for 20 min at 70 °C [5,6];
- SWE: 2.0 g of sample were mixed with 140 mL of water for 20 min at three temperatures (100, 150 and 200 °C) [7];
- CE: 1.5 g of sample were mixed with 10 mL of ethanol:water 60:40 (*v*/*v*) for 60 min at 70 °C [6].

2.3. Total Phenolic Content and Antioxidant Activity

The TPC and antioxidant activity, evaluated by the FRAP and ABTS assays, were performed as previously described [8,9]. Results were expressed as milligrams of gallic acid equivalents (GAE) and ascorbic acid equivalents (AAE) per gram of fresh weight (fw).

2.4. Qualitative and Quantitative Polyphenol Characterization by HPLC-PDA

The phenolic profile of the optimal extract was characterized by HPLC with a photodiode array detector and a C18 column as described in detail by Moreira et al. [3]. The extract was analyzed in triplicate, and the results were expressed as mg of compound/100 g of fw.

3. Results and Discussion

3.1. Total Phenolic Content

Figure 1 presents the TPC obtained for the analyzed samples subjected to the different extraction techniques.

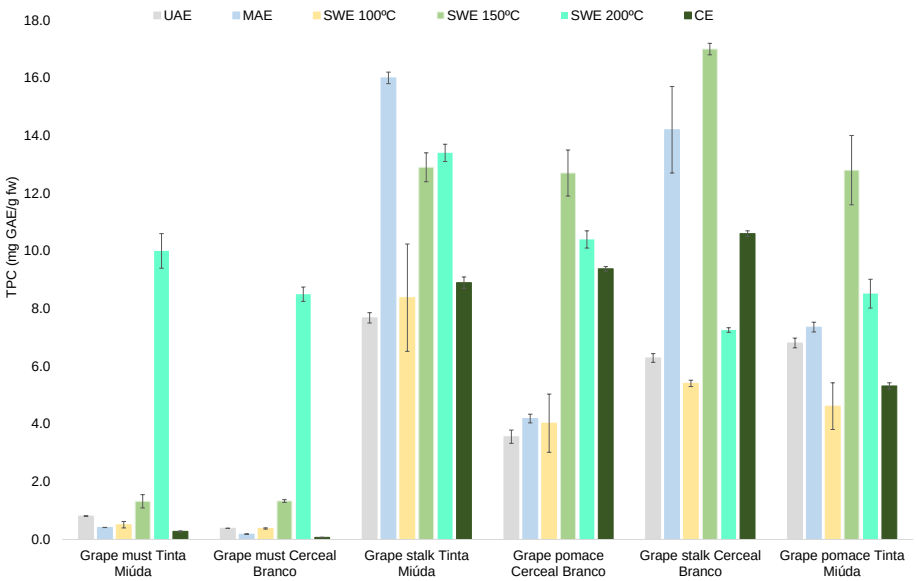

Figure 1. Total phenolic content obtained by different extraction techniques; results are expressed as mg gallic acid equivalents/g fresh weight (mg GAE/g fw), mean ± standard deviation, n = 3.

For all the extraction techniques tested, SWE allowed to recover the highest amount of polyphenols. Regarding the samples analyzed, the extract of grape stalk from *Cerceal Branco* variety obtained using SWE at 150 °C had the highest TPC (17.0 ± 0.2 mg GAE/g fw). On the opposite, the extracts from grape must from both varieties presented the lowest TPC, except for subcritical water extracts at 200 °C (10.0 ± 0.6 and 8.5 ± 0.3 mg GAE/g fw for *Tinta Miúda* and *Cerceal Branco*, respectively). Despite from the obtained results being lower than the ones reported in the literature [10], it must be highlighted that the differences in grape varieties as well as the extraction conditions applied, such as solvents, extraction time and temperature, may exert a huge influence in the amount of phenolic compounds recovered.

3.2. Antioxidant Activity

Figures 2 and 3 shows the obtained results for the antioxidant activity assessed by the ABTS and FRAP assays of samples extracted using different extraction techniques.

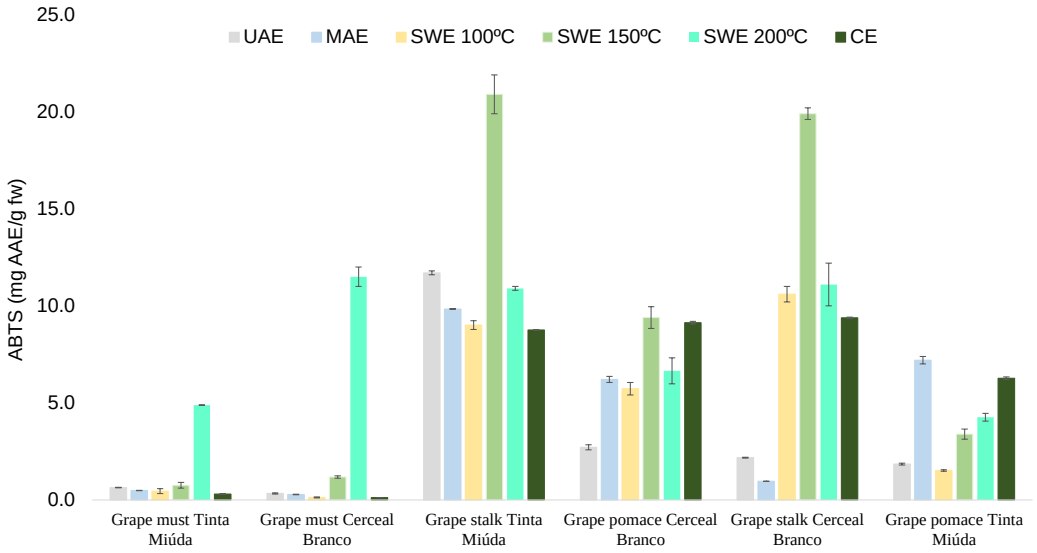

Figure 2. Antioxidant activity evaluated by ABTS assay obtained by different extraction techniques; results are expressed as mg ascorbic acid equivalents/g fresh weight (mg AAE/g fw), mean ± standard deviation, n = 3.

The highest antioxidant activity, evaluated by ABTS and FRAP assays (Figures 2 and 3), was registered for grape stalks extracts from both varieties obtained by SWE at 150 °C. On the contrary, grape must extracts presented the lowest antioxidant activity. The same correlation was observed for the TPC results, demonstrating the close relationship between the different spectrophotometric assays.

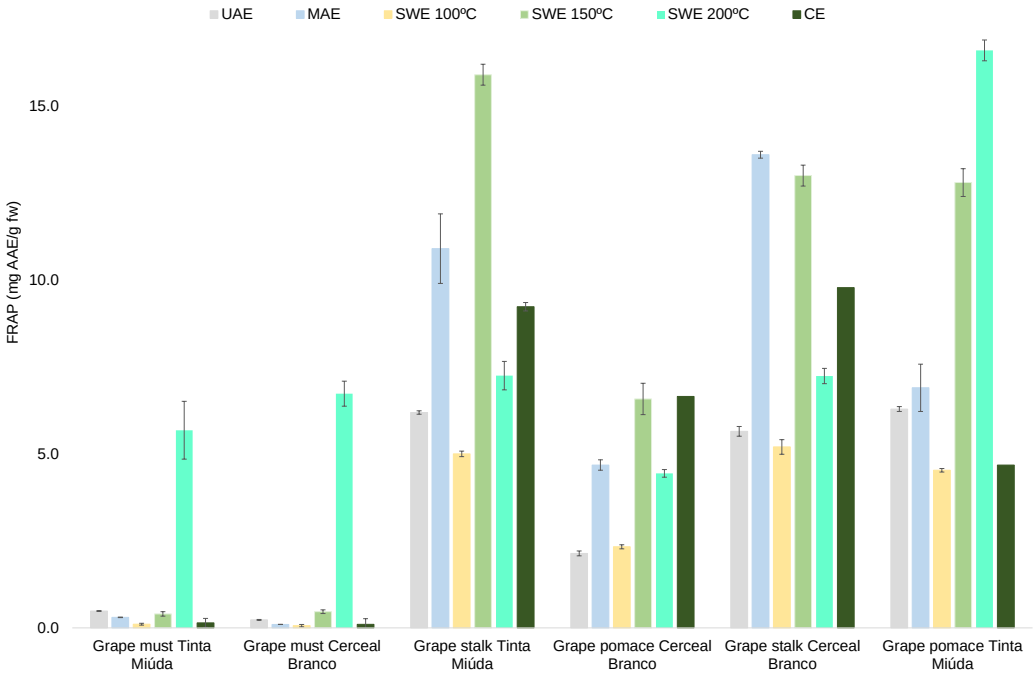

Figure 3. Antioxidant activity evaluated by FRAP assay obtained by different extraction techniques; results are expressed as mg ascorbic acid equivalents/g fresh weight (mg AAE/g fw), mean ± standard deviation, n = 3.

In general, the extracts obtained by the application of SWE technique, namely at 200 °C for grape must and at 150 °C for grape stalk and pomace, presented the highest amount of bioactive compounds, as well as the highest antioxidant activity. Afterwards, in order to identify the individual phenolic compounds of the obtained extracts which can be contributing to the described antioxidant properties, an HPLC-DAD analysis was performed.

3.3. Phenolic Profile by HPLC-DAD Analysis

Grape stalk extract from *Cerceal Branco* variety (obtained by SWE at 150 °C) was analyzed by HPLC-DAD and Table 1 reports the obtained content for the individual phenolic compounds identified.

Table 1. Content of the individual polyphenols in grape stalk extract from *Cerceal Branco* variety obtained with SWE at 150 °C. Results are expressed as mean ± standard deviation (milligrams of compound/100 g fw, n = 3).

Phenolic Compounds	**Mean ± SD (mg of Compound/100 g fw)**
Gallic acid	38.8 ± 1.9
Protocatechuic acid	ND [a]
Neochlorogenic acid	123 ± 6
Caftaric acid	18.5 ± 0.9
(+)-Catechin	467 ± 23
Caffeine	105 ± 5
Chlorogenic acid	154 ± 8
4-O-caffeyolquinic acid	48.0 ± 2.4
Vanillic acid	47.3 ± 2.4
Caffeic acid	21.2 ± 1.1
Syringic acid	30.2 ± 1.5

(−)-Epicatechin	129 ± 6
p-Coumaric acid	8.66 ± 0.43
trans-Ferulic acid	2.67 ± 0.13
Sinapic acid	ND
trans-polydatin	<LOQ [b]
Naringin	4.25 ± 0.21
3,5-di-caffeoylquinic acid	1.30 ± 0.06
Quercetin-3-*O*-galactoside	25.6 ± 1.3
Resveratrol	<LOD[c]
Rutin	ND
Phloridzin	8.42 ± 0.42
Ellagic acid	3.62 ± 0.18
3,4-di-*O*-caffeoylquinic acid	21.4 ± 1.1
Myricetin	7.50 ± 0.38
Cinnamic acid	ND
Kaempferol-3-*O*-glucoside	ND
Kaempferol-3-*O*-rutinoside	ND
Naringenin	ND
trans-epsilon viniferin	22.1 ± 1.1
Quercetin	44.3 ± 2.2
Phloretin	ND
Tiliroside	1.55 ± 0.08
Kaempferol	1.31 ± 0.07
Apigenin	<LOD
Chrysin	ND

[a] ND: not detected; [b] Limit of quantification; [c] Limit of detection.

The phenolic composition determined by HPLC-DAD revealed the presence of compounds belonging to different families, with catechin (467 ± 23 mg/100 g fw), chlorogenic acid (154 ± 8 mg/100 g fw), epicatechin (129 ± 6 mg/100 g fw) and neochlorogenic acid (123 ± 6 mg/100 g fw) being the major contributors to the demonstrated antioxidant properties of grape stalk from *Cerceal Branco* variety. On the contrary, 3,5-di-O-caffeoylquinic acid, tiliroside and kaempferol were present in the lowest amount.

To conclude, the extracts obtained by the application of SWE technique, namely at 200 °C for grape must and at 150 °C for grape stalk and pomace, presented the highest amount of bioactive compounds, as well as the highest antioxidant activity. On the contrary, the extraction technique which revealed to be less efficient was CE, with the extracts from grape must from *Cerceal Branco* variety presenting the lowest TPC (0.071 ± 0.003 mg GAE/g fw), and antioxidant activity (0.12 ± 0.01 and 0.103 ± 0.006 mg AAE/g fw for ABTS and FRAP assays, respectively).

The presented results demonstrated that SWE can be an efficient and green extraction technique for obtaining phenolic compounds from different grapevine by-products, which can be further safely applied to food or cosmetic industries, creating an added value to this residue.

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Conflicts of Interest: The authors declare no conflict of interest.

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