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Proceeding Paper

Microwave-Assisted Ethanolic and Aqueous Extraction of Antioxidant Compounds from Pomegranate Peel and Broccoli Leaves By-Products †

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Abstract: The aim of the present work was to study the effect of solvent (50%:50% ethanol:water; 100% ethanol, and 100% water) on the extraction of total phenolic content (TPC) and total antioxidant activity (TAC) from pomegranate peel and broccoli leaves by microwave-assisted extraction (MWAE). In pomegranate peel, TPC after 100% aqueous MWAE (119.6 g GAE/kg dw) increased 1.6-folder more than 100% ethanolic extraction (74.2 g GAE/kg dw). As to broccoli leaves, TPC after 100% aqueous MWAE (28.4 g GAE/kg dw) increased 3.4-folder more than 100% ethanolic extraction (8.5 g GAE/kg dw). In this sense, TAC reported a similar behavior in broccoli leaves extraction (DPPH and ABTS values ranged between 1.1-3.5 g TE/kg dw and 2.4-3.8 g TE/kg dw, respectively), while no great differences were found among solvents during the pomegranate peel extraction (DPPH and ABTS values ranged between 3.5-3.7 g TE/kg dw and 3.7-4.3 g TE/kg dw, respectively). Therefore, aqueous MWAE could be considered as green technology to recover TPC from horticultural by-products with great interest for the industry, which should contribute to the problem of food loss.

Keywords: green-technology; revalorization; food loss

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1. Introduction

Considering that the food supply chain can be divided into primary production, processing, retail/distribution, and consumption stages, FAO defines 'food loss' as food discarded along the two mentioned first stages. During pomegranate processing stage, more than 50% of the product is discarded, representing up to 43% peel and the carpel membranes as non-edible parts [1]. Pomegranate by-products are very rich in bioactive compounds such as ellagitannins [2]. On the other hand, harvesting broccoli generates a huge number of by-products, mainly leaves, and stalks. Broccoli leaves represent 50% of total plant biomass. Furthermore, compared to broccoli florets or stalks, broccoli leaves have higher total phenolic content (TPC) and other bioactive compounds related to their antioxidant activity than the rest of broccoli by-products (Artés-Hernández et al., 2023).

Green extraction technologies of key compounds from food by-products have been recently developed, including ultrasound-, microwave-, and enzyme-assisted extractions, among others [1,3,4]. These technologies use less non-green solvents, minimizing environmental and health impacts.

In view of the above, the aim of the present work is to observe the effect of green-solvents (50%-50% Ethanol:Water; 100% Ethanol, and 100% Water) on the extraction of TPC and Total Antioxidant Activity (DPPH and ABTS) from pomegranate peel and broccoli leaves by microwave-assisted extraction (MWAE).

2. Materials and Methods

2.1. Materials

Pomegranate fruits cv. Mollar de Elche 'Protected Designation of Origin' were obtained from the Sociedad Cooperativa Cambayas (Elche, Spain). Broccoli leaves (Naxos F1 Hybrid Broccoli, Sakata) were harvested and supplied by GrupoLucas® (El Raal, Murcia, España) in November 2022. Fruits were immediately transported to the pilot plant of the Universidad Politécnica of Cartagena and stored at 5 °C and 90% relative humidity for less than 48 h until processing.

2.2. Microwave-Assisted Extraction (MWAE)

Prior to the MWAE of bioactive compounds, drying and grinding pre-treatments were carried out to obtain a stable and homogeneous raw material. Samples were freeze-dried using a Telstar® LyoBeta (Terrassa, Spain). The fixed variables were: (i) particle size (<56 µm), (ii) solid-liquid ratio (1:10), (iii) time (13 min), and (iv) temperature (50 °C). The continuous variables was the solvent (50%-50% ethanol:water; 100% ethanol, and 100% water). Subsequently, the extraction was carried out by a microwave device (Milestone, Ethos up, Italy). Once the extraction of all the samples was completed, the samples were centrifugated to separate the solid from the extract. The extracts were stored at -80 °C until further analysis. To carry out the experiment, a clear and concise organization was required according to the studied variables (Table 1).

Table 1. Codification and combination of the variables.

Codes	By-Product	Drying Method	% EtOH	% H_20	Ratio S:L	Time (min)	T^a (°C)
P 50:50 EtOH:H_20	Pomegranate	FD	50	50	1:10	13	50
P 100 EtOH		FD	100	0	1:10	13	50
P 100 H_20		FD	0	100	1:10	13	50
B 50:50 EtOH:H_20	Broccoli	FD	50	50	1:10	13	50
B 100 EtOH		FD	100	0	1:10	13	50
B 100 H_20		FD	0	100	1:10	13	50

FD: Freeze-drying.

2.3. Spectrophotometric Analysis

The determination of TPC was carried out according to the method previously described [5] with some modifications [6]. The TPC was calculated using a gallic acid standard and expressed as g of gallic acid equivalent per kg of dried weight (g GAE/kg dw).

The Total Antioxidant Capacity (TAC) was analyzed by DPPH and ABTS assay. For the DPPH assay [7], 194 µL of DPPH solution were added to 21 µL of extract in a 96-well plate. The mixture was incubated for 30 min at room temperature in darkness. The absorbance was measured by changes at 515 nm and data were expressed as g of Trolox Equivalents (TE)/kg dw. The ABTS assay was carried out following the method previously described [7]. For that, 200 µL of the activated ABTS solution (32 µM) was added to 11 µL of extract in a 96-well plate and incubated for 20 min at room temperature in darkness and it was measured by changes in absorbance at 414 nm.

2.4. Statistics

Statistical analysis and comparison among means were carried out using the statistical package. Each sample was analyzed in triplicate for each analysis. One–way ANOVA test using "solvent" for each food by-products. Tukey test was used for means comparison (95% confidence level). Pearson correlation using XLSTAT Premium 2016 (Addingsoft, Barcelona, Spain) was conducted.

3. Result and Discussion

3.1. Total Polyphenolic Content

The effect of the solvent on the TPC extraction by MWAE in pomegranate by-products and broccoli by-products is shown in Figure 1. It should be noted that, because of polarity differences between solvents, the solubility of the key bioactive compounds into the solvent is expected to be different. Significant differences were observed among the studied solvents in both pomegranate and broccoli by-products. Also, it is important to highlight that the use of water as a solvent was the best option for TPC extraction in both food by-products under study. In pomegranate peel, TPC after 100% aqueous extraction increased 1.6-folder more than 100% ethanolic extraction. In broccoli leaves by-products, TPC after 100% aqueous extraction increased 3.4-folder more than 100% ethanolic extraction. In general, previous studies indicated that the effect of the dielectric constants of the solvents impacts on the antioxidant compounds extraction. Methanol and water present higher constants than other solvents as ethanol and acetone. Therefore, the extraction yield depends on the type of antioxidant compounds is interested on and it is necessary to optimize for each fruit and vegetables commodities and derivatives. It is essential to mention that there are other solvents with higher capacity to extract key bioactive compounds but there are not catalogued as green solvents [8,9].

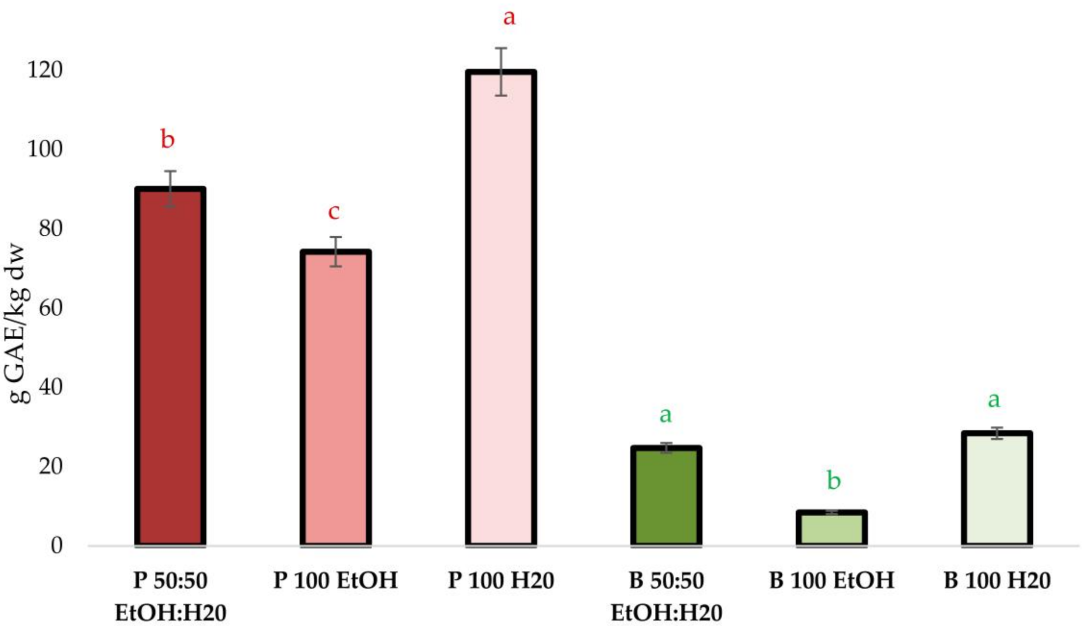

Figure 1. Total phenolic compounds (g gallic acid equivalents/kg dw) by Folin assay (garnet-coloured colums: pomegranate samples; greenish columns: broccoli samples). ‡ Columns with the different letter and same color were significantly different ($p < 0.05$).

3.2. Total Antioxidant Capacity

The behaviour of the effect of the solvent on the TAC by DPPH and ABTS assay was different in pomegranate and broccoli by-products extracts by MWAE. In pomegranate extracts, ethanolic and aquose extracts presented the highest values of DPPH assay, followed by 50:50 Ethanol:Water solvent. No significant differences were observed among solvents in ABTS data in pomegranate peel. On the other hand, the highest DPPH values were observed in the extracts by 50:50 Ethanol:Water solvent, followed by aquose solvent. Brocoli by-products extracts using 50:50 ethanol:water and 100% water solvent presented the highest values of ABTS (Figure 2).

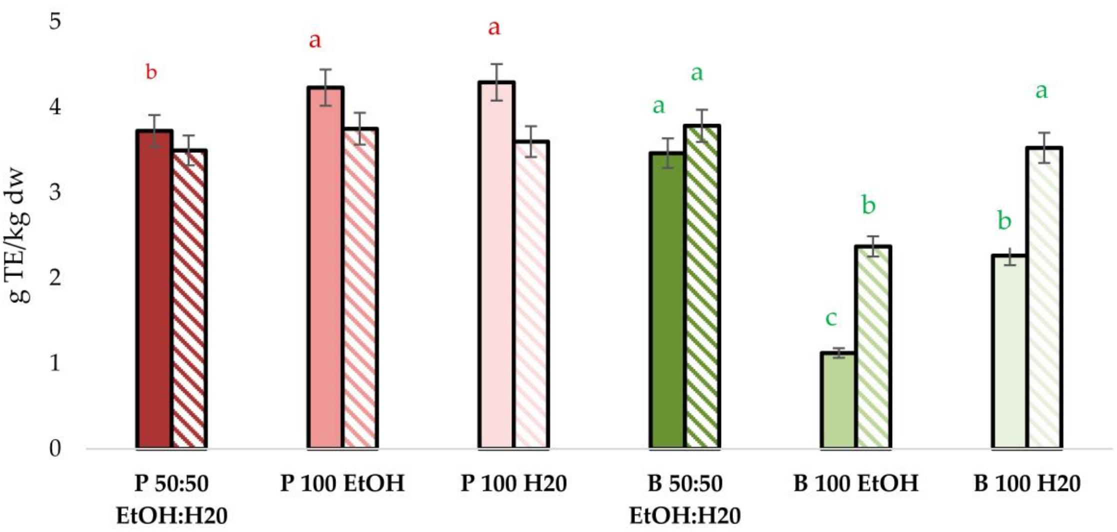

Figure 2. Antioxidant Activity: DPPH (filled columns) and ABTS assay (unfilled columns) ‡ Columns with the different letter and same color were significantly different ($p < 0.05$).

Table 2 shows the correlation among phytochemical parameters (TPC and TAC by DPPH• and ABTS+ assays). It was observed a possitive and significant correlation between TPC and DPPH•, while no significant correlation was observed between TPC and ABTS+.

Table 2. Pearson's correlation coefficients (R) among phytochemical parameters (TPC) and antioxidant activity (DPPH• and ABTS+) assays.

	TPC	ABTS+	DPPH•
TPC	1		
ABTS+	0.483 (p-value = 0.332)	1	
DPPH•	0.817 * (p-value = 0.047)	0.836 *(p-value = 0.038)	1

* Person correlation with significant differences (p-value < 0.05).

4. Conclusions

In general, it can be concluded that the type of solvent and food by-product (pomegranate and broccoli material) influenced the extraction of TPC, being higher in pomegranate by-product than in broccoli by-products. MWAE demonstrated to be a clean, efficient, and a green alternative for the extraction of TPC and bioactive compounds with TAC from pomegranate peels and broccoli leaves. Aqueous MWAE is a green alternative to recover TPC and has a great interest for manufacturers, which should address the problem of food loss, within the goals of the 2030 Agenda for Sustainable Development, the objectives of the "Farm to Fork Strategy" of the European Green Deal, and of the "Four betters" strategy of the FAO Strategic Framework 2022-31.

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