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## Pumpkin peel phenolic extracts: optimized extraction and potential use as food preservatives

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In the food industry, byproducts generated from food processing can be a significant source of valuable compounds that can be used for different applications, including food preservation. The aim of this study was to extract phenolic compounds from pumpkin peels of the Greek variety 'Leuka Melitis' in order to valorize this byproduct generated during pumpkin processing and promote sustainability and circular economy. Two different extraction techniques were compared: heat-assisted (HAE) and ultrasound-assisted (UAE) extraction. The extraction processes were optimized using response surface methodology (RSM) based on the Box-Behnken experimental design, using extraction time, temperature (HAE) or power (UAE), and ethanol concentration in the solvent as independent variables; whereas extraction yield (dry residue) and total phenolic content (Folin-Ciocalteu method) were used as dependent variables. In addition, to validate the potential application of the obtained compounds as food preservatives, the phenolic profile (HPLCDAD-ESI/MS), the antioxidant, antimicrobial, and cytotoxic properties of the optimal extract were also evaluated.

The optimization study demonstrated that UAE was more effective than HAE in both responses. This technique resulted in a two-fold increase in the concentration of phenolic compounds compared to the HAE, yielding 307 mg/g dw and 135 mg/g dw of total phenols, respectively, in the individual optimal variable conditions. In the global optimal conditions, although the UAE method required the highest power tested (400 W), it allowed the lowest extraction time (5 min) and only the use of water as solvent (0% ethanol), resulting in 1.1 g/100 g of dry residue and 120 mg/g dw of total phenols. On the other hand, the conventional extraction (HAE) facilitated energy and solvents saving, demanding only 30 °C and water as solvent, despite the increased extraction time (67 min), resulting in a yield of 0.9 g/100 g of dry residue and 106 mg/g dw of total phenols. Through the RSM, it was suggested that the ethanol concentration in the extraction solvent had the most significant impact on both dependent variables, with higher ethanol concentrations resulting in lower extraction yields, but higher phenolic content in the extracts. Regarding the experimental validation of the global optimal conditions by UAE, the results were satisfactory, since the extract presented a heterogenous profile of phenolic compounds, with six tentatively identified molecules, including three flavonoids, two phenolic acids, and one flavan-3-ol, in a total concentration of  $1.525 \pm 0.004$  mg/g of extract. The extract revealed antihemolytic activity ( $IC_{50}$ :  $540 \pm 15$  µg/mL) and inhibited lipid peroxidation ( $IC_{50}$ :  $2510 \pm 147$  µg/mL), while it was effective against four bacteria and one fungus (at the maximum tested concentration of 10 mg/mL). Furthermore, it did not reveal cytotoxicity in a primary culture of non-tumor porcine liver cells, up to 400 µg/mL.

The results of this study demonstrate the potential use of pumpkin byproducts from the food industry to obtain extracts with a high content of bioactive compounds. In addition, the study highlights the efficiency of alternative extraction techniques in reducing byproduct waste, and solvents and energy consumption, while at the same time improve the added value of pumpkin crop within the circular economy context.

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