THE EFFECT OF ULTRASONIC TREATMENT AND THE ETHANOL ON THE EXTRACTION OF POLYPHENOL CONTENT OF APPLE POMACE

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Abstract
Agro-industrial waste poses a significant effect on environmental pollution due to the challenges of waste management. Meanwhile, agro-industrial wastes are rich in bioactive compounds, particularly polyphenols that can be useful in the food, cosmetics and medicine industries. This study investigates the extraction of polyphenols from the industrial apple pomace of the variety Idared for potential use as natural food additives for the development of functional foods. Polyphenols from the dried apple pomace were extracted by the ultrasound-assisted extraction method using food-grade solvents ethanol and water. The amount of polyphenols extracted (Total phenolic content) was quantified by Folin-Ciocalteu's method. Antioxidant activity was quantified by the Ferric reducing ability of plasma (FRAP) method. Sonicating the pomace at 20 kHz for 10 and 35 minutes resulted in the extracts with extraction recovery of 27 – 33 % for all solvents. Total phenolic content (TPC) was found to be 647 – 1430 µg GAE mL^{-1} while antioxidant activity (FRAP) was found to be 436 – 842 µg AAE mL^{-1}. Results suggest that waste products (pomace) from the industrial processing of apples (variety Idared) are valuable resources for extracting useful polyphenols that can be used for functional foods development.

Keywords: Agro-industrial waste, Apple pomace, Polyphenol, TPC, Antioxidant, Functional foods

Introduction
Processing of agricultural produce results in the production of an enormous amount of waste products most of which are discarded into the environment. Apart from contributing to environmental pollution, discarding this waste into the environment results in the loss of valuable bioactive compounds that can be used in other sectors including food, cosmetics and pharmaceuticals. It has been known that there is great potential in utilizing waste from agroindustrial products for various uses such as energy production, chemicals and food additives. Recently concepts such as zero waste production in agroindustrial processing factories are being introduced where a majority of the waste products are being turned into other products [1], [2]. One of the important agroindustrial wastes that can be turned into other products is apple pomace.

Solid waste products from the industrial processing of apples to produce juice commonly referred to as apple pomace are important agroindustrial wastes that are rich in fiber, pectin and polyphenols. A small amount of this waste is being used for animal feed, energy production and as a pectin source [3], [4]. A huge amount of the pomace is still disposed into the environment despite the fact that they are rich in bioactive compounds. Research shows that these pomaces can be used to develop natural food additives such as antioxidants, antimicrobials and food packages [5], [6]. Moreover, they are good candidates for developing functional foods such as bakery products enhanced with dietary fibers as well as health foods enriched with polyphenols for antioxidant enhancement [7], [8]. This study, therefore, focuses on investigating the potential of obtaining polyphenols from the industrial apple pomace of the Idared variety, using food-grade solvents (ethanol and water) for the development of functional foods rich in antioxidants.

Experimental

The apple pomaces were obtained from an apple juice processing company in Hungary. The pomaces were from the apples cultivated in 2020/2021 grown in Hungary and immediately frozen at -20°C after pressing to obtain the juice.

Prior to drying, the pomace were thawed overnight and drying was done using the atmospheric oven at 60°C for 3 hours followed by a vacuum drying oven at 60°, 65 mbar to reach a final moisture content of about 3 - 4%. The dried pomace was ground to make a final powder using a multi-chopper coffee grinder followed by packaging in sealed polythene bags and stored in a dry condition awaiting the extraction.

Extraction was done by ultrasound-assisted extraction method according to [9] with minor modifications. 10 g of the dried apple pomace powder was mixed with 200 mL of the solvent (Water or ethanol) in flasks. The flasks were then sonicated at 20 kHz, power 240 watts at room temperature for 10 and 35 minutes in an ultrasonic water bath. The temperature of water in a sonicating bath was maintained by periodically replacing it with cold water. The pomaces were allowed to stay in contact with the solvent overnight followed by centrifugation at 4500 RPM, followed by filtration using filter paper No. 1 under vacuum. The filtrate was then rotary evaporated to reduce the solvent to about 30% followed by further drying in the atmospheric oven at 60°C for complete drying. The obtained residues were quantified to obtain extraction recovery followed by redissolving in water to make a final concentration of 400 mg/mL.

Total phenolic content (TPC) was analyzed by means of the Folin-Ciocalteu's method according to [10] where Gallic acid was used for the calibration curve and the results were expressed as microgram Gallic acid equivalent per milliliter (μg GAE mL^{-1}). The antioxidant activity was quantified by the means of Ferric reducing ability of plasma (FRAP) according to [11] where Ascorbic acid was used for the preparation of the calibration curve and the results were expressed as microgram Ascorbic acid equivalent per milliliter (μg AAE mL^{-1}).

Results and discussion

Solvent type (Ethanol or water) didn't affect the extraction recovery of the extracts from the pomace where there was no significant difference in the amount of the crude extracts obtained between the two solvents. The recovery of the extracts from the dried pomace ranged from 27% to 33% of the dried pomace for both ethanolic and aqueous extracts (Figure 1). Smaller ranges were seen from other studies where extraction recovery using methanol as extraction solvent was between 8% - 15% in different varieties of the apple pomace [12].

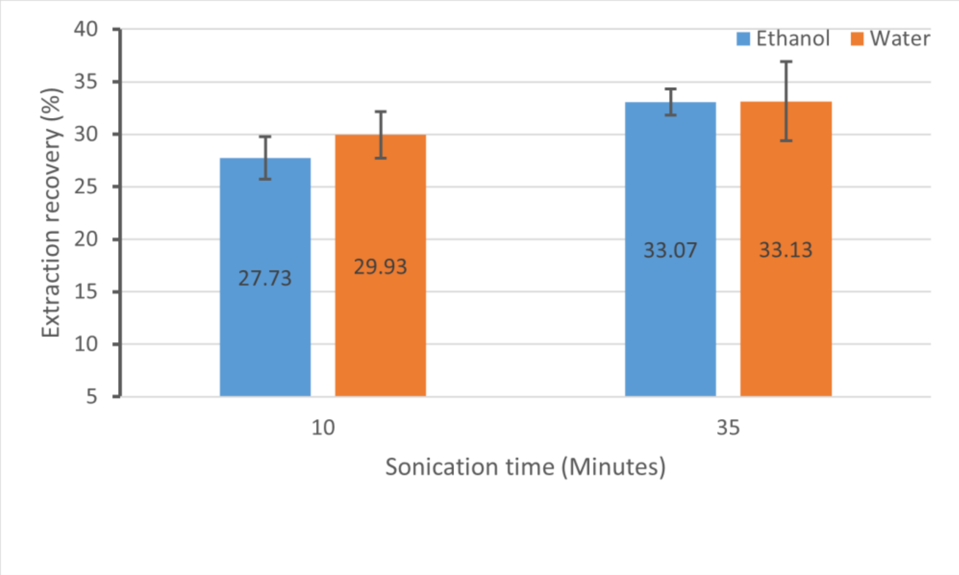

Figure 1. Influence of ethanol and water on extraction recovery of the crude extracts from apple pomace

Solvent type significantly affected the amount of polyphenol phenols extracted from the apple pomace where ethanol resulted in extracts with higher amounts of TPC compared to water. The amount of TPC was approximately 650 µg GAE mL^{-1} for aqueous extracts and 1400 µg GAE mL^{-1} for ethanolic extracts and there was no effect in the TPC when the sonication was done for both 10 and 35 minutes (Figure 2). Similar results were reported by L. Wang et al., 2018 where ethanol displayed higher TPC content compared to water. Differing observations were observed in the study of Silva et al., 2020 where there were no significant differences in TPC when ethanol and water were used as extraction solvents by ultrasound-assisted extraction.

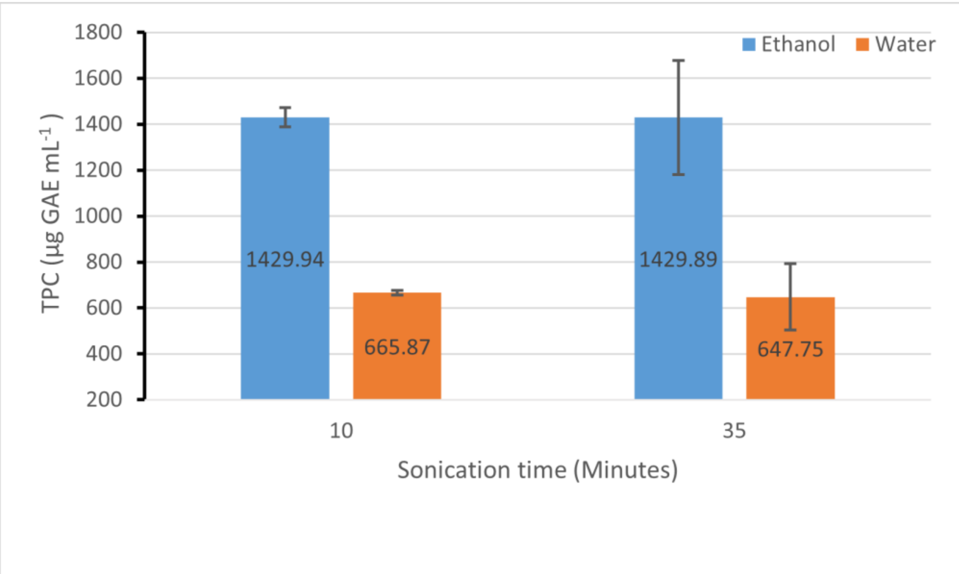

Figure 2. Effect of ethanol and water on TPC of the apple pomace extracts

There was a hugely significant difference in the antioxidant activity of the apple pomace extracts after sonicating for 10 minutes where ethanolic extracts displayed FRAP value of 842 µg AAE/ mL while aqueous extracts displayed 435µg AAE mL^{-1} . Contrarily, there was no significant difference between ethanolic and aqueous extracts when the sonication was done for

35 minutes. Opposite to our study, the study of Gharedaghi et al., 2020 found that water results in extracts with higher antioxidant capacity than ethanol when a conventional extraction method is used.

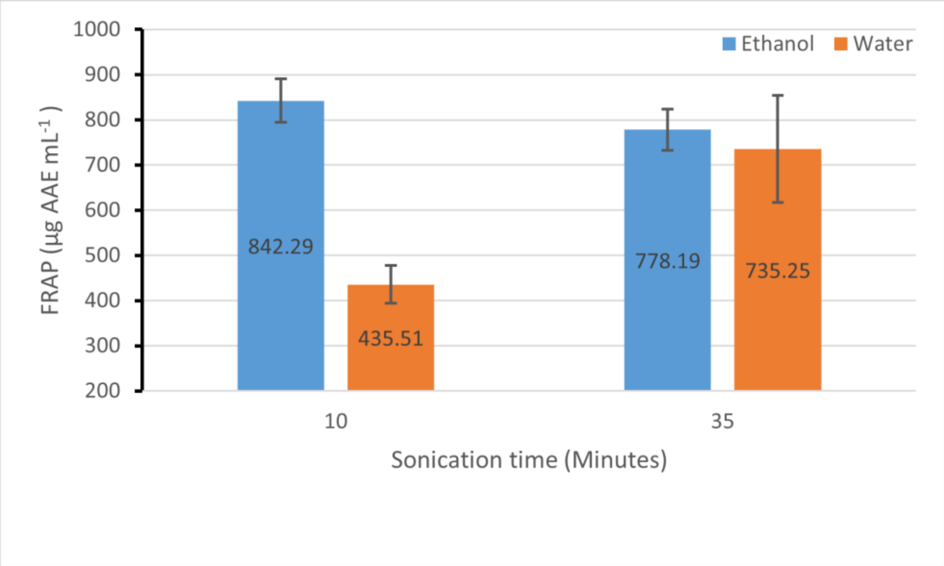

Figure 3. Effect of ethanol and water on antioxidant of the apple pomace extracts.

Conclusion

Agro-industrial wastes are known to have an enormous amount of bioactive compounds mostly, polyphenols that are known to be potentially useful in various sectors such as food and pharmaceuticals. Apple pomace from the industrial processing of the apples into juice contains a huge amount of polyphenols that can be exploited for other uses instead of disposing them into the environment. From this study, it has been seen that ethanol results in higher polyphenols and to some extent antioxidant activity than water. It is recommended that apple pomace, a cheap and reliable resource can be useful in obtaining polyphenols that can be used for the development of functional foods rich in antioxidants using food-grade solvents ethanol and water.

Acknowledgments

The authors acknowledge the support of the Doctoral School of Food Science of the Hungarian University of Agriculture and Life Sciences.

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