Subcritical water extraction of phenolic acids from distillery stillage

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The distillery industry is an example of an industry that makes a significant contribution to the world economy while generating huge amounts of by-products (termed distillery stillage). From 1 L of produced alcohol, about 8–15 L of distillery stillage is produced, which is characterized by a low pH, high content of organic matter, and a dark brown color (Fito et al. 2019). Due to the serious environmental problems that distillery by-products can cause, it is important to develop a management strategy for it. The emphasis is now on the concept of a circular economy in which by-products stay in circulation for as long as possible. Thus, the priority is to process and valorize the distillery stillage by giving its value to produce economically useful products. One of the methods of valorization of distillery stillage is the recovery of bioactive compounds (phenolic compounds), which will not only reduce the pollution generated during alcohol production but also contribute to the sustainable development of the distillery industry.

Polyphenols present in distillery stillage are secondary metabolites of cereals and include flavonoids and non-flavonoids (mainly phenolic acids) (Alara et al. 2021). Phenolic acids are classified as hydroxybenzoic acids (including p-OH benzoic, vanillic, and syringic acid) or hydroxycinnamic acids (including p-coumaric, ferulic, and sinapic acids) based on C1-C6 and C3-C6 backbones, respectively (Kim et al. 2006). Phenolic acids are the main antioxidants in the human diet. Phenolic compounds have a beneficial effect on health due to their anti-inflammatory, analgesic, anticancer, and antimicrobial (antifungal and antiviral) properties (Araujo et al. 2015). Due to the potential health benefits, the importance of polyphenols is strongly related to their industrial use in many areas, such as cosmetics, pharmaceuticals, agriculture, etc. This explains the global emphasis on the search for environmentally friendly polyphenol extraction methods based on the use of green technologies that avoid the use of toxic solvents.

In recent years, various methods of recovering bioactive compounds from by-products have been proposed, e.g., ultrasound- and microwave-assisted extraction, high-pressure processing, or subcritical water extraction (SWE) (Barba et al. 2016). Among these techniques, SWE has proven to be an environmentally friendly alternative because the use of high pressure in combination with high temperatures allows the use of water as a liquid solvent above its boiling point under atmospheric pressure (Carabias-Martinez et al. 2005). However, attention should be paid to the chemical structure of phenolic acids and their interaction with other components, which is a very important aspect when optimizing the conditions for their effective recovery. Phenolic acids are also prone to oxidation and high temperatures that cause their degradation (Chandrasekar et al. 2015). For these reasons, the preparation of samples for extraction and the process parameters are very important factors for optimizing the extraction.

There is a lack of information on the recovery of high-quality bioactive compounds from distillery stillage employing green extraction procedures. Therefore, this study investigated the effect of SWE conditions on the efficiency of recovery of phenolic acids from distillery stillage.

Materials and methods

This study used distillery stillage from the production of concentrated crude ethyl alcohol from cereals (a company from north-eastern Poland). The distillery stillage samples had the following characteristics: 47,000±5,300 mg COD/L, 4,345±5 mg N_{tot}/L, 280±2 mg P_{tot}/L, 789±3 mg CH_3COOH/L.

Extraction of phenolic acids was performed with SWE using a microwave system. 1 g of distillery stillage and 30 mL of water were used for the extraction. The process was carried out for 15, 30, and 60 min at temperatures of 80, 140, and 200°C and the pressure of 600 psi. After extraction, the samples were centrifuged for 10 min at 10,000 rpm. The supernatants were collected, evaporated to dryness, and then dissolved in 1 mL of methanol and analyzed via High-Pressure Liquid Chromatography (HPLC).

The chromatographic separation was performed by Chromatograph (Varian ProStar) equipped with a UV-Vis detector equipped with a Supelcosil C18 column (150 mm×4.6 mm, 5 μm) operating at 35°C. The mobile phase consisted of acetonitrile containing 0.15% formic acid (solvent A) and water containing 0.15% formic acid (solvent B). The separation was performed for 42 min with the following elution gradient ranges: 0–18 min, 1–96% B; 18–35 min, 96–82% B; 35–40 min, 82–75% B. The detection of phenolic acids was performed at 260 nm (p-OH benzoic, vanillic, and syringic acids) and 320 nm (p-coumaric, ferulic and sinapic acids).

Results

Phenolic acids present in the extracts were quantified by HPLC. The influence of the temperature and SWE time on the recovery efficiency was investigated. Figure 1 shows the heatmap which represents the concentrations of individual phenolic acids that were recovered from the stillage with 15-, 30-, and 60-min SWE at 80, 140, and 200°C. The best conditions were obtained with 30-min SWE at 140°C, which allowed to obtain the total concentration of phenolic acids of 2.88 μg/g DM. Extending the SWE time from 15 to 30 minutes resulted in obtaining extracts with 1.38 times higher concentrations of phenolic acids. Further extending the SWE time to 60 min gradually decreased the extraction efficiency. Increasing the extraction temperature of SWE from 80 to 140°C increased the yield and recovery by about 20%. However, a further increase in temperature from 140 to 200°C slightly reduced the recovery of phenolic acids by about 13%. The least effective conditions turned out to be 15-min SWE at 80ºC.

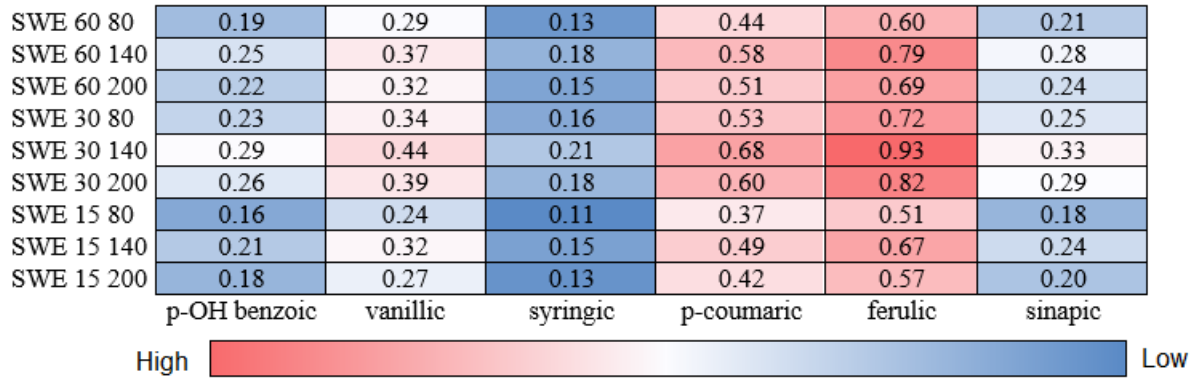

Figure 1. Heatmap of the concentrations of phenolic acids in distillery stillage extracts obtained with SWE. In the abbreviations, the values after SWE represent the extraction time and then extraction temperature. The concentrations of phenolic acids in the table are expressed as microgram per gram dry mass (μg/g DM). The content of individual phenolic acids is represented by a color (red indicates a high content; blue indicates a low content).

In the extracts, ferulic and p-coumaric acids predominated, constituting from 29 to 32% and from 20 to 24% of all detected phenolic acids, respectively, depending on the extraction condition (Figure 1). In turn, p-OH benzoic, vanillic, and sinapic acids accounted for about 8–10%, 13–16%, and 12–14% of the total, respectively. Syringic acid had the smallest share of all phenolic acids in the distillery stillage extracts (about 5–7%).

Conclusions

The results obtained in this study showed that SWE successfully recovers phenolic compounds from distillery stillage. This makes it possible to develop green extraction techniques to recover bioactive compounds from various wastes. In this extraction process, the elimination of the organic solvent allows the production of natural products that can be used in the production of organic functional foods and nutraceuticals. Therefore, SWE can be considered a cost-effective and environmentally friendly process to extract antioxidants from waste biomass.

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