Extraction of phenolic acids from distillery stillage

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One of the directions of sustainable development is the recovery and recycling of materials, which increases the value of products and resources in the economy. The reuse of agro-industrial waste can give rise to new products with added value. In this way, modern industries focus on reducing the environmental impact of by-products (Farcas *et al* 2015).

As consumer awareness of the use of food additives increases and the attention is gained from functional foods, there is a need to identify alternative and natural sources of food antioxidants. The distillery stillage can be used to reuse bioactive products such as polyphenols, which shows great potential as a valuable, and at the same time cheap and waste material (Bustamante *et al* 2009). These compounds are plant secondary metabolites with beneficial effects on health due to their antioxidant, antimicrobial, antiviral, and anti-inflammatory properties (Sun *et al* 2006). Phenolic compounds extracted from distillery stillage can be used in various branches of the pharmaceutical, cosmetic, and food industries. The distillery industry is a key contributor to the development of the global economy, but the industry is also considered to be one of the world's major sources of environmental pollution. For 1 L of the spirit produced, 9 to 14 L of distillery stillage are produced. The distillery stillage is characterized by a high content of organic matter susceptible to biodegradation (COD varies between 15 and 176 g O_2/L) (Fito *et al* 2019). Therefore, it is very important to purify distillery wastes because they can cause serious environmental problems. However, more attention should be paid to the recovery of bioactive compounds from by-products from the distillery industry.

Many factors influence the content of bioactive phenolic acids in distillery stillage. It mainly depends on the type and variety of raw materials used during the alcoholic fermentation, as well as the storage conditions and the method of processing. Additionally, an important element is a method of extracting phenolic acids. These bioactive compounds differ in terms of structure. Their chemical structure and interactions with other components are not fully known, and this is a very important aspect when choosing solvents and determining the conditions of the extraction process. Phenolic acids are also susceptible to oxidation. High temperatures and an alkaline environment cause their degradation (Chandrasekar *et al* 2015). For these reasons, the preparation of samples for extraction and the parameters of the process are very important factors that require special attention.

The present study aimed to compare the effect of different concentrations of alcohols (ethanol, methanol) in the extraction of phenolic acids from distillery stillage.

Materials and methods

The recovery of phenolic acids from the stillage was carried out using ultrasonically assisted extraction. Distillery stillage from the production of concentrated unpurified ethyl alcohol from cereals (a company in north-east Poland) was used. It was characterized by the following concentrations of pollutants: 43600 -50400 mg COD/L, 4345 mg Ntot/L, 8.4 mg N-NH_4/L, 280 mg Ptot/L, and 788.6 mg CH_3COOH/L. Two experimental series of extraction were carried out. In the first series, methanol (60%, 70%, 80%, 90%, 100%) was used, and in the second series ethanol (60%, 70%, 80%, 90%, 100%) was used. The extraction of phenolic acids was performed by using 1 g of freeze-dried distillery stillage and 30 mL of alcohol. Then, whole mixture sonication for 20 min using an ultrasonic bath was performed. After extraction, samples were centrifuged in an Eppendorf centrifuge for 10 min. at 15000 rpm. Supernatants were collected and evaporated to dryness at a temperature below 50°C in a vacuum evaporator. Then, the dry extracts were collected and re-dissolved in 1 mL of methanol and subjected to chromatographic separation. Chromatographic separation was performed by the High-Pressure Liquid Chromatography (Varian ProStar) fitted with a UV-Vis detector equipped with Supelcosil C18 column (150 mm × 4.6 mm, 5 μm) working at 30°C. A gradient elution program was employed, using 1 mL water/formic acid (99.85/0.15, v/v) (solvent A) and acetonitrile/formic acid (99.85/0.15, v/v) (solvent B) as elution solvents. The detection was performed at the wavelength of 260 nm (p-OH-benzoic, vanillic, syringic acid) and 320 nm (p-coumaric, ferulic, sinapic acid).

Results

The phenolic acids present in the extracts were quantitatively characterized by HPLC. The effect of the solvent type and its concentration on the extraction efficiency was investigated. Figure 1 shows the total concentration of phenolic acids that were recovered from the distillery stillage using 60%, 70%, 80%, 90%, and 100% ethanol and methanol solutions. The most effective solvent turned out to be an 80% ethanol solution. After

extraction, the concentration of total phenolic acids was 0.0546 μg/mL. The presence of ethanol in the extraction medium improved the extraction efficiency of phenolic compounds compared with methanol. The highest extraction efficiency equal to 75% was when 80% ethanol was used. Also, a beneficial effect of using water and alcohol mixture as compared to pure solvents was observed. 100% ethanol and methanol turned out to be the least effective solvents. Phenolic acid recovery from distillery stillage using 100% ethanol and methanol was 0.0394 μg/mL and 0.0327 μg/mL, respectively.

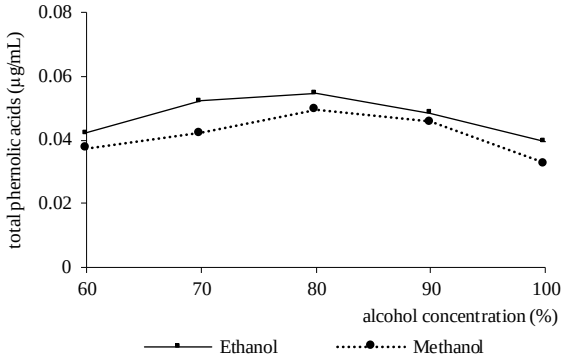

Figure 1. The total concentration of phenolic acids after extraction from the distillery stillage depending on the alcohol concentration.

Both in ethanol and methanol extracts, p-coumaric acid was the phenolic compound of the highest concentration. Using 60%, 70%, 80%, 90% and 100% ethanol, the following concentrations of p-coumaric acid were recovered: 0.0155 μg/mL, 0.0175 μg/mL, 0.0182 μg/mL, 0.0169 μg/mL, 0.0161 μg/mL. When methanol was used in concentrations of 60%, 70%, 80%, 90%, and 100%, p-coumaric acid was recovered at the level of 0.0155 μg/mL, 0.0164 μg/mL, 0.0172 μg/mL, 0.0168 μg/mL, and 0.0124 μg/mL, respectively. The phenolic acid that was recovered from the distillery stillage in the smallest amount was sinapic acid. When using 100% ethanol, the concentration of sinapic acid was 0.00189 μg/mL, while when using 100% methanol it was 0.00138 μg/mL.

Conclusions

Distillery stillage is a source of polyphenolic compounds. Optimizing the extraction method is important before reusing or managing the distillery stillage in the environment. Optimization of the extraction process from distillery stillage performed in this work allows the production of high-value extracts without pretreatment, only using available in any distillery solvents. The extract produced may be labeled as a “natural product”. From the ecological point of view, the direction of further research on the valorization of stillage should be the search for new techniques for recovering valuable polyphenolic compounds that meet the requirements of "green extraction".

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