EXTRACTION OF BIOACTIVE COMPOUNDS FROM FLAXSEED USING SEQUENTIAL GREEN TECHNOLOGY: MICROWAVE AND ULTRASOUND

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Research into sustainable methods for extracting bioactive compounds has led to advancements in green technologies. This research explores the integrated employment of microwave and ultrasound technologies to boost the concentration of beneficial plant compounds. Flaxseeds were pretreated with microwaves before undergoing ultrasound assisted extraction. The impact of various parameters on extraction efficiency was examined. Results showed that 80% ethanol yielded the highest ($p<0.05$) polyphenols concentration and antioxidant capacity, with optimal values ($p<0.05$) at 15 min and 45 °C. The sequential use of microwave and ultrasound significantly outperformed separate techniques and conventional extraction, proving high efficiency and eco-friendliness.

Keywords: polyphenols, flaxseed, microwave pretreatment, ultrasound.

1. Introduction

Selecting a suitable extraction technique for isolating phytocompounds from plant materials requires careful consideration of both traditional and advanced techniques. While conventional solid-liquid extraction is capable of yielding a satisfactory content of polyphenols, it also presents several drawbacks. These include the need for large volumes of solvents, extended extraction times, and restrictions on solvent choices due to potential toxicity [1]. As a result, there is growing interest in exploring more efficient and environmentally friendly extraction methods that can overcome these limitations while maintaining high

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extraction yields. Examples include ultrasound assisted- (UAE) [2], microwave assisted- [3], supercritical fluid- [4], deep eutectic solvent extractions [5] etc.

In the pursuit of green extraction processes for bioactive compounds, the combined use of microwave and ultrasound extraction emerges as a promising approach. This technology combines the advantages of both processes. UAE improves mass transfer through the process of cavitation, which is described by generating bubbles that implode once they reach a certain dimension. When this collapse occurs near solid matter, it may become asymmetrical, causing the breakdown of wall layers of the cell. This, in turn, allows the solvent to infiltrate the plant matrix more effectively, enhancing the overall extraction efficiency [6]. Conversely, due to the non-uniform structure of plant material, microwaves heating may occur selectively, limiting mass transfer rate [7]. This drawback can be mitigated by integrating microwave and ultrasound technologies.

Integrating the microwave technique as a pretreatment for raw material in a combined method can enhance the extraction efficiency of active principles, such as polyphenolic compounds. The presence of polyphenols in plants varies unevenly throughout different tissues: some are localized in the wall layers of the cell, whereas others are contained inside the vacuolar compartments [8]. The effectiveness of the extraction process is determined by the interaction between the plant matrix and bioactive compounds, along with the solvent's ability to diffuse through the sample matrix [9]. Given that the material's structure is a critical factor in extraction efficiency, any method that alters the structure to improve extraction is of great interest. It is acknowledged that pretreatment of the sample has a major impact on the microstructure of plant material and the release of bioactive compounds [10, 11].

Flax (*Linum usitatissimum* L.) is a variety of plants that is a member of the Linaceae family. Flaxseeds and their products are known for the many beneficial properties that lead to the reduction of risks associated with various forms of cancer, such as: breast [12], skin [13] or colon [14] cancers. In addition, flaxseeds have more than 10% fermentable dietary fiber. Consuming the latter is associated with a reduction in high triglycerides [15]. Also, linseed soluble mucilage could be incorporated into nutrition plan to promote weight regulation [16]. More than that, in addition to reducing cholesterol levels [17], the polyphenols rich extract from flaxseeds has a strong antibacterial effect on *Escherichia coli*, *Staphylococcus aureus*, *Salmonella, Listeria monocytogenes*, and *Pseudomonas fluorescens* [18]. The results presented by Draganescu et al. show that linseed extract could be used to control diabetes, as it offers benefits like lowering glycemia, regulating weight, and improving dietary intake [19]. In addition, histopathological investigations have shown that flaxseed extract partially recovers the functions of the pancreas, liver and kidneys, thus reducing the damage associated with the diabetic state [19]. Wang et al. identified 14 phenolic acids (the main ones being ferulic, vanillic, 4-hydroxybenzoic, sinapic, and gallic acids) and 15 flavonoids (the main ones being apigenin, quercitrin, vitexin, kaempferol, and (+)-dihydroquercetin) in flaxseeds, with quercetin and sinapic acid together representing more than 42.67% of their content [18].

In this work, a combination of two advanced extraction techniques was proposed to enhance the yield of phytocompounds extracted from flaxseeds. To the extent of our knowledge, while studies have examined microwave pretreatment of flaxseeds or UAE of polyphenols from flaxseeds, none have investigated the combined use of microwave pretreatment and UAE for polyphenols extraction from flaxseeds.

2. Materials and Methods

2.1. Materials

Flaxseeds (*Linum usitatissimum* L.), supplied by Hofigal S.A. (Bucharest, Romania), were subjected to microwave pretreatment, or used as such. Both pretreated and untreated flaxseeds were processed by grinding and sieving to obtain particles under 500 μm in size. The ground flaxseeds were kept at 4–5° C before being used to extract polyphenols.

The chemicals employed for extracts analysis were gallic acid for total phenolic content (TPC) assessment and Trolox for antioxidant activity (AA). Both were of high purity and purchased from Sigma-Aldrich Co, Bucharest, Romania. From the same supplier, ammonium acetate, neocuproine, copper chloride, sodium carbonate, Folin–Ciocalteu reagent, and ethanol, all of analytical grade, were procured.

2.1. Microwave pretreatment of flaxseeds

A specialized laboratory setup was specifically designed and built for microwave pretreatment of plants prior to extracting bioactive compounds. Detailed information about the apparatus is provided in our previous work [20]. Prior to grinding, part of the flaxseeds was submitted to microwave pretreatment for 100 s at a temperature of 85 °C and a microwave power of 200 W.

2.2. Ultrasound assisted extraction procedure

The polyphenols were extracted from pretreated and untreated flaxseeds using UAE with the help of a Hielscher UP200H ultrasonic probe (Hielscher, Teltow, France). The extractions were carried out using ethylic alcohol-water mixtures as solvent at concentrations of 50%, 80%, and 96%, and were conducted at temperatures ranging from 30 to 60 ºC, with increments of 15 ºC. The flaxseeds relative to solvent was maintained at 1:20 (w/v) throughout all the experiments, and the process was conducted for 5, 15, and 30 min with a stirring rate of 900 rpm. Sonication was applied continuously, with the amplitude set at 40%.

Comparative experiments, under the same extraction conditions, were also performed using a conventional method with both pretreated and untreated flaxseeds. Finally, all extraction mixtures were subjected to centrifugation at 4000 rpm for 10 min. The extracts were freshly analyzed in order to determine TPC and AA. All experiments were conducted threefold.

2.3. Total phenolic content determination

The polyphenol content in the extracts was quantified using the Folin-Ciocalteu assay, as outlined in our earlier work Draghici-Popa et al. [21].

2.4. Antioxidant activity determination

The antioxidant capacity of the flaxseed extracts was quantified through the Cupric Reducing Antioxidant Capacity method, as outlined in our earlier work Draghici-Popa et al. [21].

2.5. Statistical analysis

Statistical analysis was carried out through the one-way analysis of variance, as outlined in our earlier work Staicu et al. [22].

3. Results and Discussion

3.1. Influence of ethanol concentration on the extraction efficiency

UAE improves the mass transfer rate through the process of cavitation, which involves the production of bubbles that expand and then implode once they reach a certain size. When these bubbles collapse near a solid surface, they can do so asymmetrically. This collapse disrupts the wall layers of the cell, enabling the solvent to infiltrate more effectively into the plant structure and thereby increasing the mass transfer rate of the extraction process [6].

A key factor in extracting polyphenols is the choice of solvent and its concentration in water. Besides the solvability of active principles in the solvent, various solvent properties, such as viscosity, vapor pressure, and surface tension, play a significant role in the success of UAE. These characteristics influence acoustic cavitation and the cavitation threshold [23]. Cavitation occurs when the pressure during the rarefaction cycle exceeds the cohesive forces between liquid molecules. Low vapor pressure solvents are more desirable, as they result in more intense bubble collapse [24]. Despite its flammability and potential explosiveness, ethanol is commonly used for extractions due to its classification as a green solvent [25]. Additionally, it is suitable for UAE.

In comparison with both 50% and 96%, the use of 80% ethanol concentration led to a significant increase (p<0.05) in both TPC (Fig. 1A) and AA (Fig. 1B), regardless of the type of flaxseeds used (microwave pretreated flaxseeds and UAE – noted MW+US, untreated flaxseeds and UAE – noted US).

As shown in Fig. 1, all unconventional methods (MW+US, US, and microwave pretreated flaxseeds and conventional extraction – noted MW) resulted in better outcomes compared with conventional extraction (untreated flaxseeds and conventional extraction – noted Conv). Furthermore, UAE of seeds pretreated with microwave led to higher yields compared to phytocompounds extraction from untreated seeds, irrespective of ethanol concentration (Fig. 1). This behavior can be due to the microwave pretreatment of flaxseeds prior to UAE. During microwave pretreatment, rapid heating of intracellular water and polar compounds within the seeds cells occurs. As a result, the water evaporates, and the osmotic pressure created inside the vegetable particle generates the rupture of the cell wall. This process also helps the solvent infiltration into the cells, allowing the targeted compounds to diffuse more easily out of the plant matrix [7].

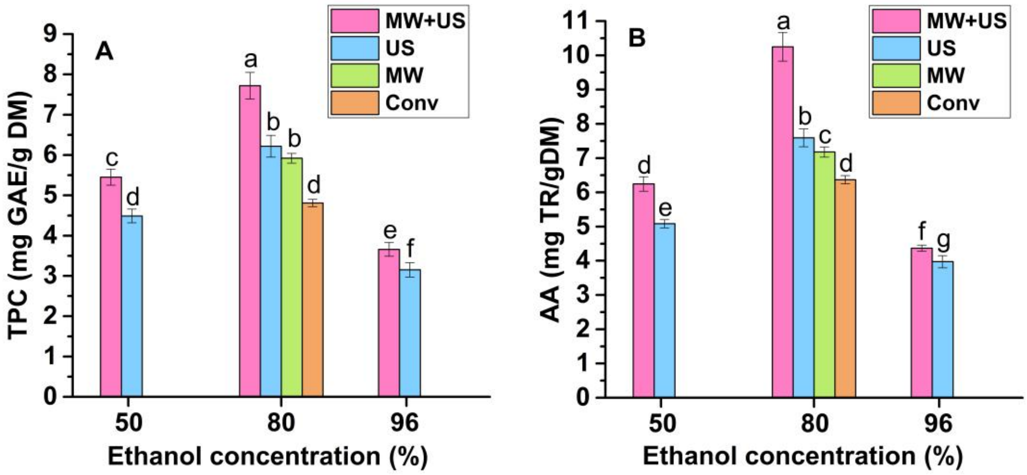

Fig. 1. Evaluation of TPC (A) and AA (B) at different ethanol concentrations – extraction time of 15 min, temperature of 45 °C.

3.2. Influence of temperature on the extraction efficiency

Temperature is a crucial parameter in the extraction of polyphenols. To avoid the degradation of heat-sensitive compounds, the temperature should be carefully selected based on the specific components being targeted [26]. Higher temperatures decrease the interfacial tension and viscosity of the extraction medium, boost the solubility of components, promote molecular interactions at the interface, increase vapor pressure, and improve mass transfer rate [27]. During cavitation, the rise in vapor pressure creates a cushioning effect from vapors inside the bubbles, resulting in an inverse relationship between cavitation intensity and temperature [28].

ANOVA analysis indicated a non-significant increase in TPC within the range of 30 to 60 °C, irrespective of the type of flaxseeds used (MW+US and US)

–Fig. 2A. There is a significant increase for AA from 30 to 45 °C ($p<0.05$) only when microwave pretreated flaxseeds were used (Fig. 2B). This can be explained by the less violent collapse of cavitation bubbles at high temperature, meaning that a 30 °C could be enough to extract polyphenols from flaxseeds by UAE.

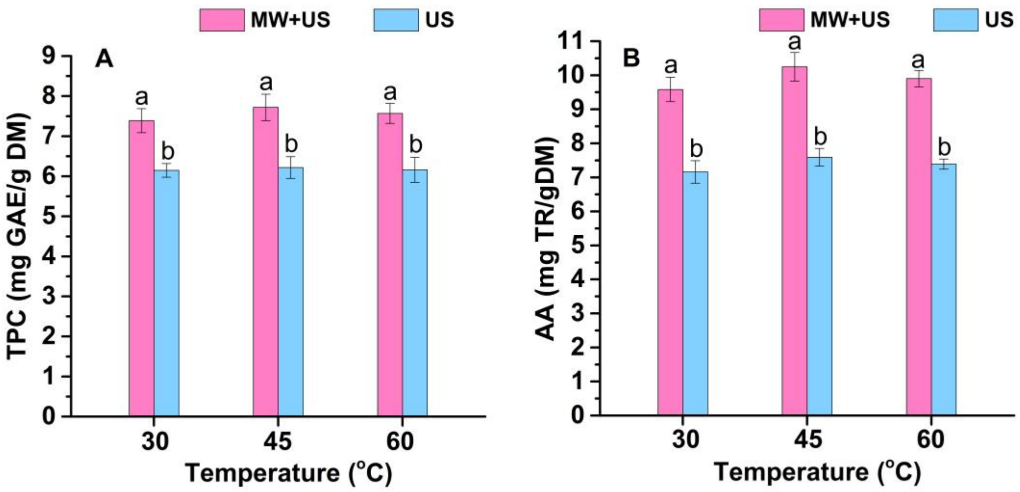

Fig. 2. Evaluation of TPC (A) and AA (B) at various temperature – extraction time of 15 min, ethanol concentration of 80%.

3.3. Influence of time on the extraction efficiency

Extraction time is another critical factor which affects the yield of extracted compounds. The quantity of bioactive compounds extracted gradually increases with time until an equilibrium is achieved between the compounds dissolved in the solvent and those still retained within the plant cells [29].

Fig. 3 shows the results obtained at different times. Both TPC and AA increased significantly in the first 15 min (in accordance with ANOVA analysis, $p< 0.05$), irrespective of the type of flaxseeds used (MW+US and US). After 15 min of extraction a slight decrease of polyphenols occurs. These results can be explained by the degradation of bioactive compounds at long extraction times. Extending the extraction duration allows cavitation to fully break down all plant cells, leading to an increase in bioactive compounds content [30]. However, the solubilized constituents can be readsorbed on the crushed particles of plant material, reducing the recovery of bioactive compounds. Therefore, prolonging the extraction time beyond the point of maximum yield becomes unnecessary [31].

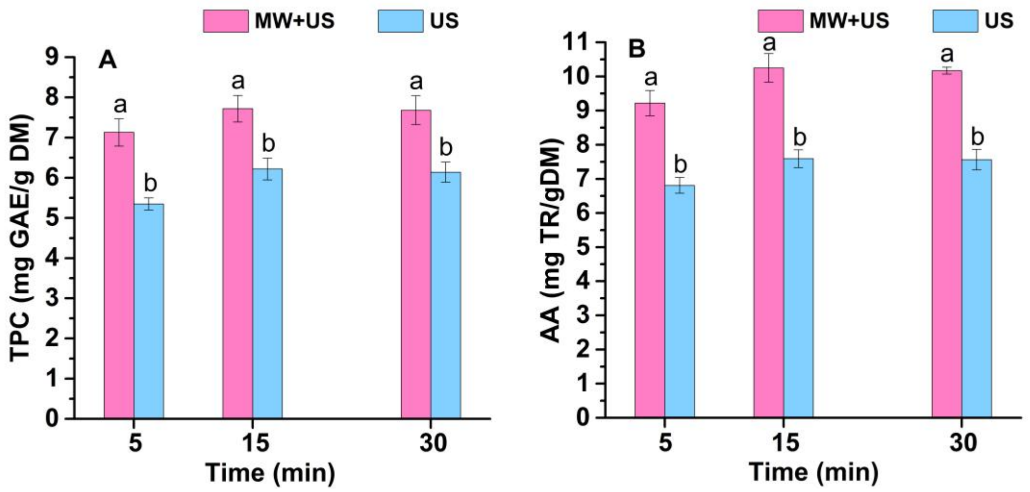

Fig. 3. Evaluation of TPC (A) and AA (B) at different extraction times – ethanol concentration of 80%, temperature of 45 °C.

4. Conclusions

The drive for environmentally sustainable processing of plant materials has spurred research into green extraction processes of phytocompounds. In this context, this study investigated the integrated use of ultrasound and microwave technologies to increase the content of bioactive compounds and improve antioxidant capacity. Flaxseeds were subjected to microwave pretreatment followed by UAE of polyphenols. Comparative extractions were conducted utilizing a classical procedure and the individual techniques. The effect of key factors (temperature, ethanol concentration in water, and extraction time) on TPC and AA was investigated. Compared with 50% and 96% ethanol concentration, a significant enhancement ($p<0.05$) in AA and polyphenols content with 80% ethanol was achieved. The extraction was highly efficient after 15 min, with extended time leading to slight polyphenols degradation. A significant increase in TPC and AA was observed between 30 and 45 °C ($p<0.05$). However, no notable difference was observed between 45 and 60 °C ($p>0.05$, as confirmed by ANOVA analysis). The combined microwave and ultrasound approach (MW+US) outperformed the individual methods, yielding a 24% increase over ultrasound (US), a 30% improvement over microwave (MW), and a 60% enhancement compared with conventional extraction (Conv). This strategy is not only more efficient but also more environmentally friendly than traditional methods. The resulting polyphenol-rich extracts have potential applications in pharmaceuticals, food, agriculture, and other industries as alternative antioxidant sources.

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