https://doi.org/10.31925/farmacia.2023.6.7 **ORIGINAL ARTICLE**

OPTIMIZING MICROWAVE-ASSISTED EXTRACTION OF POLYPHENOLS FROM MUSTARD SEED MEAL

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Abstract

Nowadays, the management of agro-industrial by-products has become a pressing concern. The global production of 1.3 billion tons of agricultural residues presents a significant challenge, as a considerable portion of these by-products is wasted, leading to substantial economic losses estimated at around 990 billion dollars. These by-products can serve as valuable raw materials in various industries, including food, pharmaceuticals and textiles. Among these compounds, polyphenols stand out as prominent bioactive constituents found within agro-industrial residues, with numerous beneficial applications. However, the industrial utilization of polyphenols derived from agro-industrial by-products faces significant obstacles, particularly in terms of reducing extraction and purification costs and minimizing the use of hazardous solvents and reagents. In this context, this study aims to investigate the optimization of microwave-assisted extraction for polyphenols from mustard seed meal as an eco-friendly method to obtain valuable products from agro-industrial by-products. Various parameters, such as solvent type, concentration, vegetal material: solvent ratio, irradiation time and extraction temperature, are analysed to determine the optimal conditions for maximizing polyphenol extraction from mustard seed meal. Furthermore, the findings of this research have the potential to contribute to the development of solutions that promote a circular economy and minimize losses associated with agro-food industry activities, while also reducing the environmental impact and generating substantial revenue benefits.

Rezumat

În prezent, gestionarea deșeurilor agroindustriale constituie o preocupare prioritară. Producția globală anuală de 1,3 miliarde de tone de reziduuri agricole reprezintă o provocare, o parte considerabilă din aceste reziduuri fiind irosită si generând astfel pierderi economice substanțiale, estimate la aproximativ 990 de miliarde de dolari. Aceste reziduuri pot servi însă drept materii prime valoroase în diverse industrii, precum industria alimentară, farmaceutică și textilă. Printre compușii frecvent întâlniți în deșeurile agroindustriale, polifenolii se evidențiază drept constituenți bioactivi remarcabili, cu numeroase potențiale aplicații benefice omului. Cu toate acestea, utilizarea la nivel industrial a polifenolilor proveniți din reziduurile agroindustriale se confruntă cu numeroase provocări, cum ar fi reducerea costurilor de extracție și purificare, precum și minimizarea utilizării solvenților și a reactivilor toxici. Așadar, acest studiu își propune să investigheze optimizarea metodei de extracție a polifenolilor cu ajutorul microundelor (MAE), pentru polifenolii din componența șrotului de semințe de muștar, ca metodă ecologică de obținere a produselor bioactive din deșeurile agroindustriale. Diverși parametri, precum tipul de solvent, concentrația solventului, raportul material vegetal:solvent, timpul de iradiere și temperatura de extracție, sunt analizați pentru a determina condițiile optime de maximizare a extracției de polifenoli din șrotul de semințe de muștar. Totodată, rezultatele acestei cercetări pot contribui la dezvoltarea unor soluții ce favorizează o economie circulară și minimizează pierderile asociate activităților industriilor agroalimentare, reducând in același timp impactul asupra mediului și generând beneficii economice substanțiale.

Keywords: mustard seed meal, microwave assisted extraction, eco-friendly, agro-industrial residues, polyphenols, bioactive compounds

Introduction

The importance of agro-industrial by-products

Agro-industrial by-products refer to the residual materials or waste generated during the processing or production of agricultural commodities or food products. However, agro-industrial by-products often have potential value or utility and they can be used for various applications such as animal feed, bioenergy production, composting, or the extraction of valuable compounds [12].

The management of agricultural residues has emerged as a critical concern in contemporary times. Agro-industrial residues have significant implications for greenhouse gas (GHG) emissions. According to the Food and Agricultural Organization (FAO), food waste is responsible for approximately 26% of global GHG emissions annually, equivalent to 3.3 gigatons of CO_2 [8]. Given the escalation of demand for food, it is foreseeable that the generation of agro-industrial by-products will continue to increase. Thus, urgent action is required to effectively manage these by-products by adopting sustainable practices that facilitate efficient resource recovery, minimize food wastage and reduce environmental impact [2, 8].

It has been widely demonstrated that food by-products serve as abundant sources of bioactive molecules, holding tremendous potential for future applications and integration into industrial value chains, thereby facilitating the implementation of a circular economy [8]. Therefore, the market for biotechnology-derived products, including those derived from agro-industrial residues, is designed to experience substantial growth, with an anticipated increase of 9.3% until 2027 [2].

Role of polyphenols in industries

Polyphenols stand out as prominent bioactive constituents within agro-industrial residues [2, 8]. Phenolic compounds, which are exclusively synthesized by plants [3], offer a wide range of advantages to various industries, including the health and food sectors [15]. Frequent consumption of polyphenols has shown an inverse correlation with the incidence of cancer, diabetes and cardiovascular diseases [3]. Moreover, polyphenols serve as valuable sources of dyes in the textile industry, finding application in fabric dyeing and the chemical treatment of leather during the tanning process [3, 7]. However, the industrial utilization of polyphenols derived from agro-industrial by-products faces significant challenges, primarily concerning reducing extraction and purification costs and minimising hazardous solvents and reagents. Hence, these challenges require the development of cost-effective and environmentally friendly methods for the extraction and purification of polyphenols to facilitate their widespread industrial applications [8].

Microwave-assisted extraction (MAE)

The microwave-assisted extraction (MAE) technique is demonstrated to be an efficient extraction method for polyphenols from plant material by using the heating effect of microwaves on the solvent. The application of microwave energy offers distinct advantages over conventional extraction methods. One advantage is the ability to control the transfer of polyphenols by adjusting the microwaves' frequency and irradiation time. Additionally, the extraction time is significantly reduced compared to conventional methods. The precise control of microwave sources and pressure during the extraction process further contributes to the selectivity of the extraction. Furthermore, the microwave extraction method demonstrates a reduced solvent requirement in comparison to conventional methods. This reduction in solvent quantity is beneficial from an environmental perspective and can lead to cost savings [4, 5, 21].

Mustard seed meal

Mustard seed meal is the residue obtained from the pressing of mustard seeds to extract mustard oil. In many instances, mustard seed meal serves as a soil fertilizer, with the added benefits of inhibiting weed growth and providing protection to plants against pathogens [16]. Mustard plants contain numerous bioactive compounds that have been utilized as therapeutic tools in combating various diseases such as cancer, obesity, depression and diabetes. Furthermore, the adaptability of mustard plants to diverse climatic conditions has facilitated their cultivation in various environments, resulting in low maintenance and sale costs. Consequently, the production of bioactive compounds with therapeutic potential from mustard plants and mustard by-products holds significant advantages in terms of sustainability [17].

Aim of the study

The objective of this study was to optimize the microwave-assisted extraction (MAE) method for extracting polyphenols from mustard seed meals in a sustainable manner. This method offers a sustainable approach to obtaining polyphenolic compounds from mustard seed meals, which can be further used by various industries, thereby contributing to the principles of a circular economy.

Materials and Methods

Raw materials consisting in mustard meal, origin Poland, Republique of Moldavia and Ukraine, that was subjected to extraction and used in this study, were provided as dried material by local distributors (CT International S.R.L., Cluj Napoca S.C. OLEOMET S.R.L., Bucharest and 2-EPROD S.R.L., Bucharest). Microwave-assisted extractions were conducted using the Ethos Easy – Milestone equipment. For the extraction process, 2 g of each meal was mixed with different solvents, such as methanol and ethanol, at various irradiation times, temperatures, concentrations and mustard seed meal: solvent ratios. The irradiation power of 500 W remained constant throughout the experiments, being the only consistent parameter. The obtained extracts were filtered under vacuum and preserved at a temperature of -80°C for subsequent analysis to determine the concentration of polyphenols.

Determination of total polyphenol concentration

The quantification of the total polyphenol concentration in the seed meal extract was carried out using the Folin-Ciocâlteu method. 10 μL aliquot of the seed meal extract was diluted with 790 μL of double-distilled water, and then 50 μL of Folin-Ciocâlteu reagent was added. After one minute, 150 μL of 20% calcium carbonate solution was added, and the mixture was kept in the dark for two hours. The polyphenolic concentration was determined by measuring the absorbance at 750 nm. The final concentrations were expressed as mg/mL gallic acid equivalents (GAE).

Statistical analysis

The experiments were conducted in triplicate, and the data obtained were subjected to statistical analysis using GraphPad Prism 9.1.2 software. The One-way ANOVA test was employed to analyse the data, and multiple comparison tests including Tukey and Dunnett were used to assess differences between groups.

Results and Discussion

Influence of region

The first parameter investigated was the influence of mustard seed meal provenance on polyphenol content. Mustard seed meals obtained from three different countries, namely Poland, Moldavia and Ukraine, were analysed (Figure 1). The results revealed significant variations in total polyphenol (TP) content among the different countries. Notably, the mustard seed meal from Ukraine exhibited the highest polyphenol content, with a remarkable increase of 352% compared to the mustard seed meal from Moldavia ($p < 0.001$) and Poland ($p < 0.001$). No statistically significant differences were observed in the polyphenol content between the mustard seed meals from Moldavia and Poland.

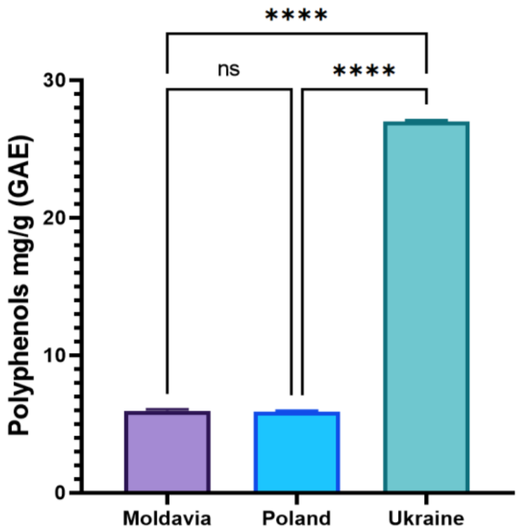

Figure 1.
The comparison of total polyphenol concentrations obtained through the microwave-assisted extraction (MAE) method using mustard seed meal from various regions

The variations observed in the polyphenol concentrations of mustard plants cultivated in different regions can be attributed to the diverse environmental conditions in which the plants were grown. Factors such as climate, soil composition and even the methods employed for harvesting, storage and processing can have an impact on the polyphenol content within the plant [17]. For instance, under conditions of water stress and elevated temperatures, grapes have been observed to exhibit increased concentrations of polyphenols [14].

Influence of type and concentration of solvent

Considering the results obtained in the previous experiments, for the following studies only the Ukraine mustard seed meals was selected. The subsequent parameter investigated was the influence of the solvent type and concentration on TPC. The sleeted solvents were methanol and ethanol, while the concentrations investigated in the study included methanol-water 50% (1:1), methanol-water 80% (1:4), ethanol-water 50% (1:1) and ethanol-water 80% (1:4) (Figure 2). The results revealed significant differences in the polyphenol concentrations among the tested solvents and their concentrations. The most powerful solvent for extraction efficiency was methanol in both percentage either 80% (24.49 mg/g GAE) or 50% (26.35 mg/g GAE). No significant difference was observed between methanol 50% and methanol 80%, or between ethanol 50% and ethanol 80%. However, a significant difference was found between methanol 50% and ethanol 50% ($p = 0.0002$), with methanol 50% being 20.13% more efficient than ethanol 50%. Additionally, a significant difference was observed between methanol 80% and ethanol 80% ($p = 0.0013$), with methanol 80% being 20.96% more efficient than ethanol 80%.

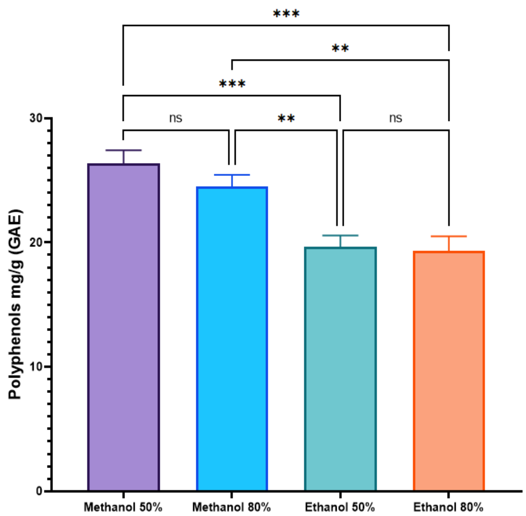

Figure 2.
The differences in polyphenol extraction efficiency from mustard seed meal using the MAE method with different concentrations of methanol (50% and 80%) and ethanol (50% and 80%)

These findings underscore that the selection of an appropriate solvent is crucial for achieving an efficient extraction process. The choice of solvent in microwave-assisted extraction (MAE) is influenced by factors such as the solubility of the target analyte, the interaction between the solvent and the plant matrix, and the dielectric constant of the solvent [17].

Furthermore, it is important to note that each class of polyphenols exhibits a specific affinity for certain solvents. Flavonoids, for example, display different levels of polarity, thereby necessitating the use of highly polar or less polar solvents. Less polar solvents, such as benzene, chloroform, ether and ethyl acetate, are commonly used for extracting less polar aglycone flavonoids, including isoflavones, flavanones, dihydro-flavonols, flavones and flavonols, which are characterized by a high degree of methylation. On the other hand, more polar flavonoid glycosides, including hydroxylated flavones, flavonols, biflavonyls, aurones and chalcones, are typically extracted using acetone, alcohol, or water [13].

In the context of microwave extraction, both polar aglycone and flavonoid glycosides can be effectively extracted using solvents such as methanol or ethanol, often in combination with water at various concentrations [11]. Moreover, other studies have underscored the notable influence of solvent concentration on flavonoid extraction. For instance, Prasetyaningrum *et al.* investigated the extraction of flavonoids from Moringa leaves using various solvents. They found that 95% ethanol yielded the highest concentration of total flavonoids, while 50% ethanol resulted in a lower flavonoid concentration [11]. Conversely, water extraction exhibited the lowest flavonoid yield, likely due to its limited capacity to solubilize these compounds

Influence of irradiation time

The subsequent parameter investigated was the effect of time irradiation of the mustard seed meal (Figure 3). Four different time periods were tested: 20, 30, 40 and 60 minutes on sample extracted in methanol 50%. Methanol 50% was selected as the solvent due to its cost-effectiveness, and there were no substantial differences observed in the extraction efficiency between methanol 50% and methanol 80%. The results revealed that the most effective irradiation periods were 20 and 30 minutes, exhibiting a statistically significant increase of 26.55% (p = 0.0003) in extraction efficiency compared to the 40- and 60-minutes periods. Namely, 20 minutes compared with 40 minutes 23.69% (p = 0.0007), 20 minutes compared with 60 minutes 27.09% (p = 0.0003), 30 minutes compared with 40 minutes 25.68% (p = 0.0003), 30 minutes compared with 60 minutes 29.21% (p = 0.0001). However, no significant differences were observed between the 40- and 60-minutes time periods or between 20- and 30-minute time periods.

It has been noticed that the influence of solvent on irradiation time is an important consideration in the extraction process. Solvents such as ethanol and methanol have the potential to experience significant heating during prolonged exposure, posing a risk to the stability of thermolabile constituents [18]. A correlation between irradiation time and the quantity of solvent employed in extractions was set up. Typically, a smaller solvent volume necessitates a shorter irradiation duration to attain the optimal extraction temperature. Consequently, extended irradiation periods tend to diminish extraction efficiency due to the degradation of polyphenols [1, 21]. In the conducted experiment, a solvent volume of 30 ml of 50% methanol was employed, and the optimal extraction temperature was reached within irradiation durations of 20 to 30 minutes. Polyphenol degradation occurred during irradiation periods of 40 and 60 minutes.

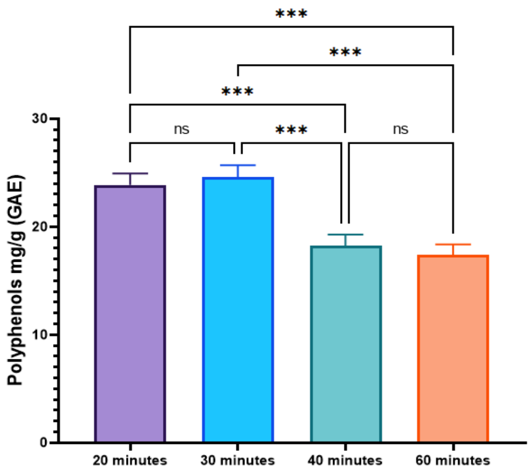

Figure 3.
Comparative analysis of the mustard seed meal polyphenols extraction efficiencies achieved with different irradiation times

Influence of temperature

The influence of temperature, with the experimental conditions set at 70°C, 100°C and 125°C (Figure 4) on mustard meal sample extracted in methanol 50%, with an irradiation time of 20 minutes was further investigated. The findings revealed that the most efficient extraction temperatures were 100°C and 125°C, exhibiting improvements of 21.17% (p = 0.0025) and 27.28% (p = 0.0006), respectively, compared to the temperature of 70°C. Moreover, no statistically significant differences were observed between the temperatures of 100°C and 125°C.

The literature data shows that higher temperatures can lead to a decrease in solvent viscosity, enhancing its mobility and solubility and consequently improving the extraction efficiency [13]. In a study conducted by Liazid *et al.* to investigate the stability of phenolic compounds during microwave extraction, it was found that compounds with a greater number of hydroxyl-type substituents were more susceptible to degradation under high-temperature conditions [10]. Among the 22 compounds analysed, all remained stable at 100°C, but significant degradation occurred for epicatechin, resveratrol and myricetin at 125°C [13]. Similar findings were reported by Xiao *et al.*, who observed a remarkable increase in the yield of flavonoids with temperatures ranging from 90°C to 110°C [19]. However, above 110°C, the increase in yield became gradual, and the extract started to show signs of scorching [13]. Consequently, the results of this study align with those in the literature, establishing that the optimal temperatures for polyphenol extraction from mustard seed meal were 100°C and 125°C, respectively. Analogous results were also reported by Grygier. who investigated the impact of different processing methods on white mustard and its polyphenol content. They observed that exposure to temperatures ranging from 120°C to 140°C led to the highest tocopherol extractions, with an increase of 7 - 9% [9].

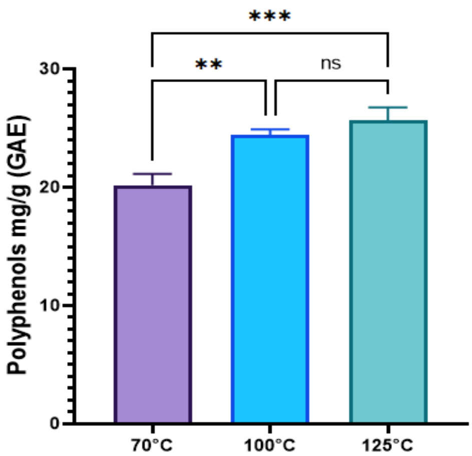

Figure 4.
Comparative analysis of the mustard seed meal polyphenols extraction efficiencies achieved with different temperatures

Influence of meal:solvent ratio

The subsequent parameter investigated in this study was the ratio between the quantity of sample and solvent, with ratios of 1:7, 1:10, 1:20, 1:30, 1:35 and 1:40 (Figure 5). Mustard seed meal was subjected to extraction in 50% methanol, at a temperature of 100°C, with an irradiation time of 20 minutes. The results revealed that the concentration of polyphenols increased with the quantity of meal in a dose-dependent manner. The most effective extraction ratios were 1:30, 1:35 and 1:40, with no significant differences observed among them. However, the 1:20 ratio was found to be 14.07% ($p = 0.0183$) more effective than the 1:10 ratio, while being 12.77% ($p = 0.0251$), 17.96% ($p = 0.0015$) and 17.89% ($p = 0.0018$) less effective than the 1:30, 1:35 and 1:40 ratios, respectively. Furthermore, the 1:10 ratio exhibited a 17.59% ($p = 0.0133$) higher efficiency compared to the 1:7 ratio.

In other studies, Zhao *et al*. observed that plant material:solvent ratios ranging from 1:30 to 1:40 yielded the most effective results [22]. Moreover, Dahmoune *et al*. specifically highlighted the efficacy of a 1:30 ratio for extracting polyphenols from *Myrtus communis* leaves [6]. Furtheremore, these findings are in concurrence with those of Yu et al., who found that a ratio of 1:40 was particularly effective for extracting flavonoids from *Osmanthus fragrans* flowers [20].

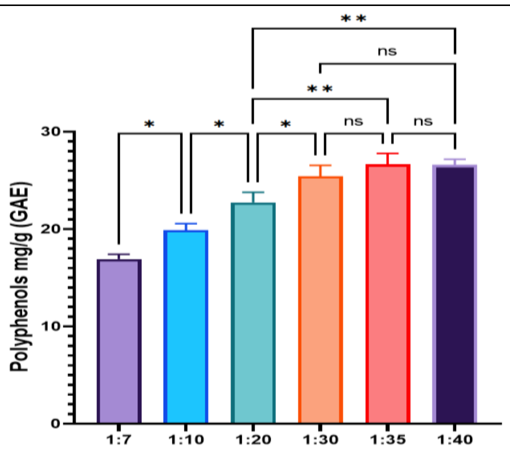

Figure 5.
Comparative analysis of the mustard seed meal polyphenols extraction efficiencies achieved with different ratios. Methanol served as the solvent

Optimizing eco-friendly MAE extraction

Consequently, in the context of flavonoid extraction, it is important to consider the appropriate amount of extracting solvent to avoid excessive energy and time requirements during subsequent steps such as condensation and purification [18].

Although methanol was the most effective solvent for the extraction of polyphenols from mustard meal, it is known to be toxic. That is why, additionally, this study examined the optimal parameters required for the eco-friendly extraction of polyphenols from mustard seed meal using ethanol as the solvent, and the MAE method.

Influence of irradiation time

In the ecological extraction method of polyphenols from mustard seed meal using the MAE method, irradiation time was once again investigated as the primary variable. The experimental periods of 20, 30, 40 and 60 minutes were examined (Figure 6).

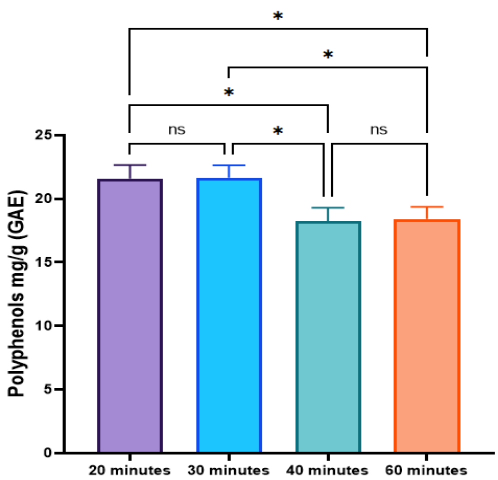

Figure 6.
Comparative analysis of the mustard seed meal polyphenols eco-friendly extraction efficiencies achieved with different irradiation times

The findings were similar to the ones obtained in the case of methanolic extraction, demonstrating that the optimal irradiation durations were 20 and 30 minutes, and exhibiting a statistically significant enhancement of extraction efficiency by 15.35% (p = 0.016) compared to the 40- and 60- minutes periods. However, no statistically significant differences were observed between the 40- and 60- minutes, as well as between the 20- and 30- minute time periods.

Influence of temperature

Keeping ethanol as solvent, the next parameter examined was temperature, with experimental conditions set at 70°C, 100°C and 125°C (Figure 7). The results demonstrated that the optimal extraction temperatures were 100°C and 125°C, showing significant enhancements of 7.64% (p = 0.0014) and 7.73% (p = 0.0013), respectively, compared to the temperature of 70°C. Furthermore, no statistically significant differences were observed between the temperatures of 100°C and 125°C. The results obtained are consistent with those obtained when 50% methanol was used as the solvent. Therefore, temperatures of 100°C and 125°C appear to be optimal for extraction in both types of solvent.

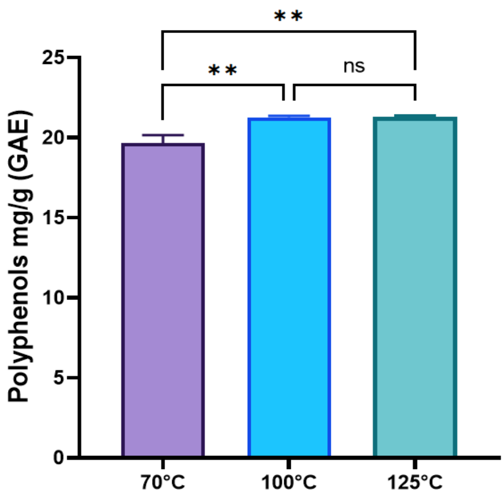

Figure 7.
Comparative analysis of the mustard seed meal polyphenols eco-friendly extraction efficiencies achieved with different temperatures

Influence of meal:solvent ratio

The last parameter investigated in this study was the ratio between mustard seed meal and solvent, with ratios of 1:7, 1:10, 1:20, 1:30, 1:35 and 1:40 (Figure 8). The results revealed that the most effective extraction ratios were 1:20, 1:30, 1:35 and 1:40, with no significant differences observed among them. However, the 1:10 ratio was found to be non-significant compared to the 1:7 ratio, while exhibiting a decrease in effectiveness by 31.84% (p = 0.0035), 33.74% (p = 0.0023), 27.68% (p = 0.0103) and 26.43 % (p = 0.0149) compared to the 1:20, 1:30, 1:35 and 1:40 ratios, respectively.

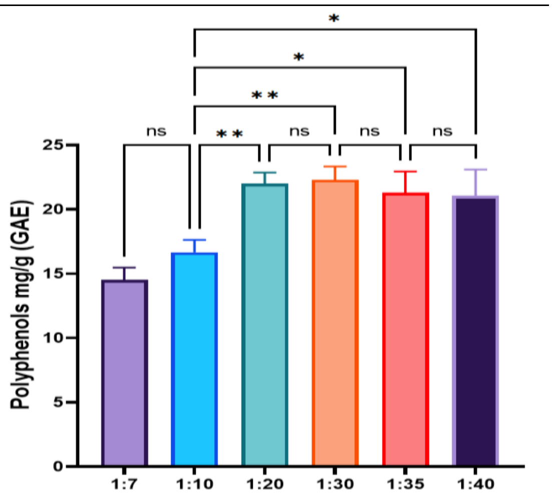

Figure 8.
Comparative analysis of the mustard seed meal polyphenols eco-friendly extraction efficiencies achieved with different ratios. Ethanol served as the employed solvent

Conclusions

This paper emphasizes the efficiency of microwave extraction as a method for extracting polyphenols from mustard seed meal. The findings of this study have implications for various industries, as they contribute to the advancement of sustainable processes and the efficient utilization of resources. The most efficient polyphenol extraction was achieved from Ukrainian mustard seed meal using 50% and 80% methanol as the solvent, with irradiation times of 20 and 30 minutes, temperatures of 100°C and 125°C, and meal-to-solvent ratios of 1:30, 1:35 and 1:40. Moreover, under eco-friendly conditions, optimal polyphenol extraction was observed from Ukrainian mustard seed meal using ethanol as the solvent. The most efficient extraction conditions were achieved with ethanol concentrations of 50% and 80%, irradiation times of 20 and 30 minutes, temperatures of 100°C and 125°C, and meal-to-solvent ratios of 1:20, 1:30, 1:35 and 1:40. However, while methanol extraction yielded a higher amount of polyphenols (25.1 mg/g GAE) compared to ethanol extraction (22.3 mg/g GAE), ethanol extraction is still considered the more favourable method. This preference arises from the cost-effectiveness of ethanol and its environmentally friendly characteristics, which outweigh the slight reduction in extraction yield. The novelty of this study lies in its innovative approach, addressing aspects that have not been previously explored in the optimization of polyphenol extraction from mustard seed meal using the microwave method. Besides, to the best of our knowledge, there have been no studies conducted to optimize the microwave-assisted extraction method for polyphenols from mustard seeds. Furthermore, this research presents eco-friendly extraction conditions, further aligning with environmentally conscious practices.

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Conflict of interest

The authors declare no conflict of interest.

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