SOXHLET DEFATTING AND ITS EFFECT ON THE ANTIOXIDANT CAPACITY OF COLD-PRESSED OILSEED CAKES

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ABSTRACT

Cold pressing of oilseeds such as poppy, pumpkin, and sesame yields high-quality edible oils and generates nutritionally rich oilseed cakes that retain a certain proportion of residual oil. This study aimed to quantify the residual oil content in cold-pressed poppy, sesame, and pumpkin seed cakes and to evaluate the effect of its complete removal by Soxhlet extraction on their antioxidant capacity. The results showed that the highest proportion of residual oil remained in pumpkin seed cake, while the lowest was found in sesame cake. In terms of antioxidant properties, poppy seed cake exhibited the highest antioxidant capacity, whereas sesame cake showed the lowest. Following Soxhlet defatting, antioxidant capacity increased in all samples. The most pronounced enhancement was recorded in poppy seed cake, while the smallest increase was observed in pumpkin seed cake. These findings indicate that removing residual oil improves antioxidant capacity; however, this effect is not directly proportional to the amount of oil removed. Although pumpkin seed cake had the highest residual oil content and underwent the greatest oil extraction, it showed the smallest change in antioxidant activity. This suggests that factors other than residual oil content, such as the composition of phenolic compounds, play a significant role in determining the antioxidant potential of defatted oilseed cakes. Overall, Soxhlet defatting can be considered a useful step in enhancing the functional properties of oilseed cakes, supporting their potential application as value-added ingredients in food and nutraceutical formulations.

Keywords: pumpkin seed, sesame, poppy seed, DPPH, ABTS

INTRODUSTION

Cold pressing is a widely used technology for producing edible vegetable oils because it preserves thermolabile bioactive compounds and avoids the use of organic solvents during extraction. In addition to high-quality oils, this process generates substantial amounts of oilseed

cakes, which remain rich in proteins, dietary fibers, minerals, phenolic compounds, and residual oils. Nowadays, there is a growing demand for cold-pressed plant oils, which are produced with relatively simple mechanical devices that can be used locally on the farm or by rural cooperatives. Cold-pressed oils are anticipated to reach the highest growth rate in the next few years [1], [2]. Cold-press processing lies below 50 °C, as opposed to 50–100 °C typically reached during expelling, retaining most of the bioactive and nutritional value of oils [3].

Oilseed by-products generated after cold pressing have attracted increasing attention as sustainable raw materials for the development of value-added functional ingredients. In contrast to solvent extraction, cold pressing preserves a substantial proportion of naturally occurring bioactive compounds while minimizing chemical processing, resulting in press cakes with high nutritional and functional potential [4], [5]. Among various oilseeds, sesame (*Sesamum indicum L.*), pumpkin (*Cucurbita pepo L.*), and poppy (*Papaver somniferum L.*) seeds represent particularly valuable sources of proteins, dietary fiber, and phytochemicals with well-documented antioxidant properties.

Cold-pressed cakes obtained from these seeds contain considerable amounts of high-quality proteins, typically ranging from approximately 20 % to more than 60 %, depending on the botanical origin and processing conditions. Pumpkin seed cake generally exhibits the highest protein content, frequently exceeding 50 %, making it an attractive ingredient for protein enrichment of functional foods [4]. Besides proteins, these by-products provide significant quantities of insoluble and soluble dietary fiber, residual lipids, minerals, and numerous antioxidant constituents, including phenolic acids, flavonoids, lignans, tocopherols, and other secondary metabolites that contribute to their biological activity [4], [6]. Consequently, cold-pressed oilseed cakes are increasingly recognized not merely as industrial residues but as valuable ingredients within the framework of sustainable food systems and circular bioeconomy strategies [7].

Unlike solvent-extracted meals, cold-pressed cakes retain a relatively high proportion of residual oil because mechanical pressing does not completely disrupt the oil-bearing cellular structures. Depending on the seed type and processing parameters, a substantial fraction of lipids remains entrapped within the plant matrix, thereby increasing the caloric value of the material while simultaneously affecting its physicochemical properties [4]. The presence of residual oil may also influence the extraction of hydrophilic phytochemicals by limiting solvent penetration, reducing matrix wettability, and restricting the diffusion of polar compounds into aqueous extraction media. Furthermore, hydrophobic interactions between lipids and phenolic constituents may decrease the overall recovery of antioxidant molecules during water-based extraction procedures [8].

To overcome these limitations, a defatting step is frequently introduced prior to the extraction of bioactive compounds. Soxhlet extraction using non-polar solvents such as hexane remains one of the most widely employed laboratory methods for efficient lipid removal from plant materials. Elimination of residual oil not only improves solvent accessibility to the intracellular matrix but may also increase the release of protein-bound and cell wall-associated phenolic compounds by disrupting hydrophobic interactions and enhancing mass transfer [9], [10]. As a result, defatted oilseed cakes often exhibit improved extraction yields of phenolics and enhanced antioxidant activity in aqueous extracts compared with their non-defatted counterparts. Nevertheless, the magnitude of this effect depends on the specific botanical source, extraction conditions, and the chemical composition of the plant matrix, highlighting the need for systematic evaluation of defatting as a pretreatment strategy for different cold-pressed oilseed cakes. Therefore, the aim of this study was to determine the residual oil content in cold-pressed sesame, pumpkin, and poppy seed cakes and to evaluate the effect of Soxhlet defatting on antioxidant activity measured by DPPH and ABTS radical scavenging assays.

METHODOLOGY SECTION

Materials

Cold-pressed sesame, pumpkin, and poppy seed cakes obtained after industrial oil pressing from Linum Ltd., Serbia, were used in this study. 2,2-difenil-1-pikrilhidrazil (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and all other chemicals used in this study were purchased from Sigma-Aldrich Chemie GmbH and were of analytical grade.

Sample Preparation

Oilseed cakes were ground in a ball mill MM400-Retsch (Verder Scientific, German) and sieved through a sieve with a pore size of 0.6 mm in order to obtain homogeneous samples suitable for analysis.

Soxhlet Extraction of Residual Oil

Residual oil was extracted using hexane in a Behr E4 (Labor-Technik, German) Soxhlet apparatus at 80°C until complete defatting was achieved. The residual oil content was calculated gravimetrically.

Determination of Antioxidant Activity

Antioxidant activity was evaluated using DPPH and ABTS radical scavenging assays in aqueous extracts prepared before and after Soxhlet defatting.

DPPH free radicals scavenging activity assay

The oilseed cake powder was taken in a dilution of 1 % (w/v) and mixed with 0.1 mM–ethanolic DPPH free radical, in a ratio 1 : 18. Mixtures were homogenized and left in the dark for 30 min. The absorbance was measured at 517 nm using a UV-visible spectrophotometer (Ultrospec 3300 pro, Amerischam Bioscienc). For making control, ethanol was used instead of the sample. The antioxidant activity was expressed as a percentage of DPPH activity calculated as:

$$DPPH\ capacity\ (\%) = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100 \quad (1)$$

ABTS free radical scavenging activity assay

The ability of the probiotic powder to scavenge ABTS radical was tested according to Re et al. [11].ABTS radical cation was produced in the reaction between ABTS (7 mM) and potassium persulfate (2.45 mM final concentration). The reaction mixture was kept in the dark at room temperature for 16 h before use. Further, ABTS solution was diluted with 5 mM phosphate-buffered saline (pH 7.4) until the absorbance of 0.700 (± 0.010) was reached. Diluted ABTS solution was mixed with samples in a ratio 99 : 1; absorbance was measured at 734 nm after 5 min. The antioxidant activity was calculated as follows:

$$ABTS\ capacity\ (\%) = \frac{Absorbance\ of\ blank - Absorbance\ of\ sample}{Absorbance\ of\ blank} \times 100 \quad (2)$$

Statistical analysis

All experiments were performed in triplicate, and the results are presented as means with standard deviations. The Tukey test was used to determine significant differences between samples ($p < 0.05$). Data analysis was conducted using Microsoft Excel (Microsoft Office 2013 Edition, USA) and OriginPro 8 (Origin Lab Co., Northampton, USA).

RESULTS AND DISCUSSION

Residual Oil Content in Cold-Pressed Oilseed Cakes

The residual oil content varied considerably among the investigated oilseed cakes, as shown in Table 1. Pumpkin seed cake contained the highest amount of residual oil (28.8 %), followed by poppy seed cake (20.9 %), while sesame cake showed the lowest residual oil content (12.12 %).

Table 1. The residual oil content

	Sesame seed cake	Pumpkin seed cake	Poppy seed cake
Residual oil, %	12.12±0.22	28.8±0.41	20.9±0.35

The relatively high residual oil content confirms that cold pressing, although environmentally friendly and suitable for preserving oil quality, is less efficient at recovering lipids than solvent extraction methods. Significant quantities of oil therefore remain trapped within the cake matrix, potentially affecting the extractability of hydrophilic bioactive compounds.

Changes in Radical Scavenging Activity

The antioxidant capacity measured by both DPPH and ABTS assays increased markedly in sesame and poppy seed cakes after extraction (Figure 1). In sesame cake, DPPH radical scavenging activity increased from 5.4 % to 14.2 %, while ABTS activity rose from 6.1 % to 14.6 %. In poppy seed cake, even more pronounced increases were observed, with DPPH activity rising from 16.4 % to 25.4 % and ABTS activity increasing from 18.9 % to 46.7 %. These results indicate that the removal of residual oil enhanced the extractability and accessibility of hydrophilic antioxidant compounds, particularly phenolic constituents associated with the protein–polysaccharide matrix.

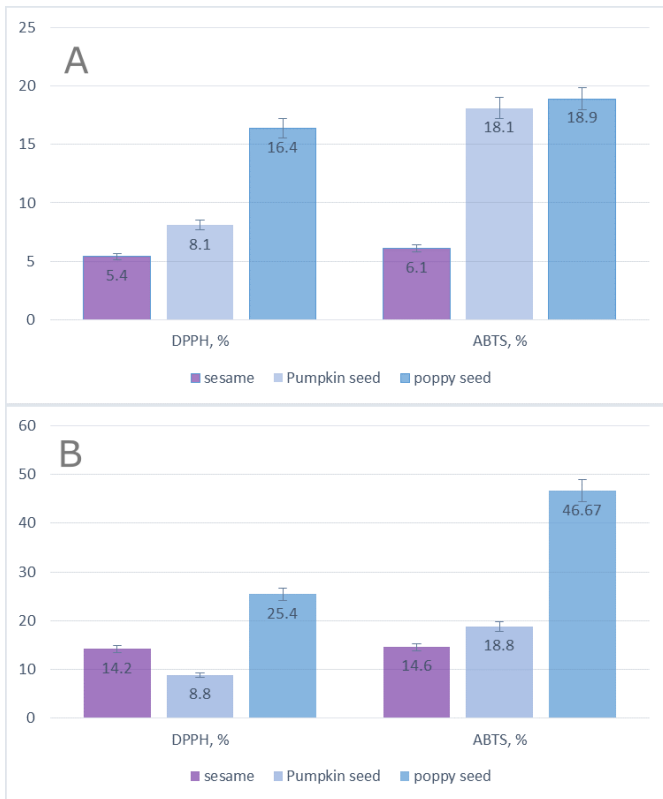


Figure 1. Antioxidant capacity of oilseed cake before (A), and after (B) defatting

Previous studies have shown that phenolic compounds in oilseeds are often bound to cell wall polysaccharides or proteins and may be partially masked by residual lipids, which hinder solvent penetration and reduce extraction efficiency [8], [12]. Removal of lipids improves matrix porosity and facilitates the release of phenolic acids, lignans, and flavonoids into polar extraction solvents used for antioxidant assays [8], [12]. In addition, non-lipid components such as proteins and peptides may contribute to radical scavenging activity through hydrogen atom donation, metal chelation, and electron transfer mechanisms [13].

Sesame seeds are especially rich in lignans such as sesamin, sesamolin, and sesamol, compounds recognized for their potent antioxidant activity and ability to protect against oxidative deterioration [14]. Although some lignans are lipophilic, their oxidative transformation products and associated phenolics can become more readily extractable after oil removal, explaining the significant increase in antioxidant activity observed in the defatted sesame cake.

Poppy seeds contain a variety of phenolic acids, including p-coumaric, ferulic, and caffeic acids, as well as tocopherols and other antioxidants [15]. The dramatic enhancement in ABTS scavenging activity after extraction suggests that many of these phenolics are concentrated within the solid matrix and become substantially more available once the oil fraction is removed. The larger increase in ABTS compared with DPPH may reflect the greater sensitivity of the ABTS assay to both hydrophilic and moderately lipophilic antioxidants. In contrast to sesame and poppy seed cakes, pumpkin seed cake exhibited an increase in DPPH activity (from 8.1 % to 8.8 %) and ABTS activity (from 18.1 % to 18.8 %). Pumpkin seed oil is known to contain high concentrations of tocopherols, carotenoids, phytosterols, and squalene, which exhibit strong antioxidant properties [16]. Since these compounds are predominantly lipophilic, they are largely removed during Soxhlet extraction with hexane.

CONCLUSION

The present study demonstrated that residual oil content differs substantially among cold-pressed sesame, pumpkin, and poppy seed cakes, with pumpkin seed cake retaining the highest amount of oil and sesame cake the lowest. Defatting significantly enhanced the measured antioxidant capacity of sesame and poppy seed cakes, as evidenced by increased DPPH and ABTS radical scavenging activities, indicating improved accessibility and extractability of hydrophilic antioxidant compounds following lipid removal. These findings suggest that residual oil may limit the extraction efficiency of phenolic constituents by reducing solvent penetration and restricting the release of matrix-bound bioactive compounds. In contrast, only marginal changes in antioxidant activity were observed in pumpkin seed cake after defatting, most likely due to the removal of lipophilic antioxidants naturally present in pumpkin seed oil. Overall, the results demonstrate that the effect of defatting on antioxidant potential is strongly dependent on the chemical composition of the oilseed matrix and the distribution of antioxidant compounds between the lipid and non-lipid fractions. Defatting therefore represents an important pretreatment for maximizing the recovery of hydrophilic antioxidants from sesame and poppy seed cakes, while its benefits appear limited for pumpkin seed cake. These findings provide valuable guidance for the valorization of cold-pressed oilseed by-products as sustainable sources of natural antioxidants and functional food ingredients.

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DECLARATIONS OF INTEREST STATEMENT

The authors affirm that there are no conflicts of interest to declare in relation to the research presented in this paper.

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