

Article

Nigella sativa L. Press Cake: Effect of Pre-Treatment Methods on Chemical Composition and Functional Properties After Cold Pressing

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Featured Application

Cold press oil extraction of *Nigella sativa* L. seeds results in a protein-, fiber-, and antioxidant-rich press cake (i.e., pulp) that is often disposed of by the manufacturers as process-food waste. Hence, selecting an appropriate seed pretreatment allows this process by-product to be adapted for direct rework in functional foods and animal feed. Thus, results indicate which pretreatment methods may be chosen by the manufacturers to gain a desired functional profile of the press cake. Therefore, turning the press cake into a fully reworked product, aligning with the zero-waste production applicability within oil processing industries.

Abstract

This study evaluates the effects of four pretreatment methods: convection heating, microwaving, ultrasonication, and pulsed electric fields (i.e., PEF), on the chemical composition, color profile, functional properties, phenolic profile, antioxidant activity, fatty acid composition, and protein content of *Nigella sativa* L. (i.e., black cumin) cold-pressed cake. Seeds were subjected to each pretreatment prior to cold press extraction. Microwave, ultrasonication, and pulsed electric field pretreatments produced the lowest press cake yields (63.61–65.54%), statistically equivalent to one another. Also, the microwave-treated and control samples retained the highest protein content on a dry matter basis (30.81 and 30.79 g·100 g^{−1}, respectively). Ultrasonication yielded the highest total phenolic and total flavonoid contents. Analysis of the press cake identified salicylic acid and catechin as the predominant bioactive compounds, suggesting potential antimicrobial and antioxidant applications. These findings provide evidence-based guidance for selecting *Nigella sativa* L. seed pretreatment strategies that at the same time optimize cold press oil extraction and



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the nutritional, functional, and bioactive value of the residual press cake. Thus, supporting its utilization as a sustainable by-product for food and feed applications.

Keywords: *Nigella sativa* L.; black cumin; cold-press extraction; press-cake; pretreatment methods

1. Introduction

Nigella sativa L. is an annual herbaceous species of the family Ranunculaceae cultivated across western Asia, North Africa, Southern Europe and India [1]. Its seeds and oil have an extensive history of use in traditional medicine and cuisine. It possesses antimicrobial, antioxidant, anti-inflammatory, antidiabetic, anticancer and immunomodulatory effects [1–4]. Chemically, the seeds are characterized by a fixed oil content of approximately 30%, together with 0.40–0.45% volatile oil [5]. The oil yield ranges from 25% to 40%, with variants influenced by seed genotype, moisture content, pretreatment conditions, and the extraction method applied [6].

Owing to their rich profile of lipids, proteins, and bioactive phytochemicals, *Nigella sativa* L. seeds and oils [7,8] are increasingly being used as part of functional foods [8,9].

Cold-press extraction is a widely used method for producing high-quality oils from seeds without the use of extensive heat or chemicals. Thus, retaining the nutritional and bioactive components [9–12]. The principal limitation of cold pressing is its relatively low extraction efficiency; hence, the investigation of seed pretreatments capable of improving oil recovery and quality is motivated [6,13,14].

Cold pressing generates a large amount of residual press-cake, which is often unused despite containing large amounts of proteins, dietary fiber, residual lipids, minerals, and phenolic compounds [15,16]. Studies on press-cakes from black cumin and other oilseeds, such as flax and hemp, show that these by-products can contain up to 30% of protein, 15 to 30% of fiber, and high levels of minerals, emphasizing their potential use as ingredients in functional foods and animal feeds [17,18]. Over the past five years, research background has shifted from compositional description towards direct applications. Black cumin press cake proteins have been proposed as a sustainable alternative to commercial protein ingredients, with their antioxidant and functional behaviour targeted against commercial proteins in vegan cheese formulations [15]. Additionally, the press cake has been evaluated as a functional ingredient within sustainable food systems [17], included in durum wheat pasta, where it altered the chemical composition, glycemic index, antioxidant activity and cooking quality of the product [19], and used as the base material for a non-dairy beverage fermented with kefir grains [20].

Interest in the feed sector has developed along similar lines. *Nigella sativa* L. seed press cake has been included in broiler diets at 20 to 60 g·kg^{−1}, with the higher inclusion levels significantly reducing cecal populations of coliforms and *Escherichia coli* [21]. Moreover, recent studies confirm that black cumin, supplied as whole seeds or oil, can improve feed conversion ratio and antioxidant-immune status, positioning it as a candidate replacement for antibiotic growth promoters [22,23]. Together, these findings enable the functional value of press cake in both food and feed.

Typically, mechanical and mild thermal treatments, including convection heating and microwaving, as well as emerging methods such as ultrasonication and pulsed electric fields (i.e., PEF), can modify cell structures and improve oil extraction [10], while influencing the composition and functional properties of the remaining cake [11].

The proteins of oilseed cakes are a primary basis for presenting emulsifying capacity, water and oil binding ability and foaming behavior, all of which influence the texture and stability of formulated foods [24,25].

Thermal and non-thermal approaches act through different physical mechanisms: thermal softening and moisture redistribution during convection heating; rapid internal moisture expansion under microwave irradiation; cavitation during ultrasonication and electroporation of cell membranes under PEF. Each of which disrupts seed cell structures and facilitates the release of oil and associated bioactive compounds.

Convection heating applies thermal energy through hot air conduction, causing progressive protein denaturation and promoting PUFA oxidation, during which polyunsaturated fatty acid content decreases while saturated fatty acids generally increase [26], alongside Maillard reaction-driven antioxidant product formation. Microwave pretreatment acts through rapid heating from within the seed matrix, while largely preserving protein secondary structure due to short exposure duration [27]. Ultrasonication operates through acoustic cavitation, disrupting cell walls mechanically, improving phenolic compound release, and modifying protein conformation through localized pressure gradients without thermal input [28]. PEF acts through electroporation via selectively permeabilizing cell membranes under high-intensity electric pulses without applying heat. Thus, preserving heat sensitive bioactive compounds while improving oil and phenolic extractability. Fatty acid composition remains largely unaffected by PEF, while phenolic fractions including flavonoids and nonflavonoids increase significantly [29].

Despite growing interest in the use of black cumin, currently available scientific data remains focused on the oil, while the press cake is largely uncharacterized. Pretreatment methods are already known to alter other oilseeds, such as sesame [14] and grape seed [29], but these effects are matrix-specific and oil fraction-centered. Nonetheless, *Nigella sativa* L. press cake carries a distinctive profile of salicylic and gallic acids. Thus, their response to pretreatment cannot be conditioned from other seeds. Existing studies also tend to test a single method in isolation. Meanwhile, this research addresses this gap by applying four mechanistically distinct pretreatments: convection heating, microwave irradiation, ultrasonication and pulsed electric fields, to a single seed batch. Therefore, allowing them to be compared directly and linked to the specific quality attributes. This turns pretreatment selection for black cumin cake from a trial-and-error approach into evidence-based direction.

Consequently, the present study evaluates the effect of seed pretreatment methods applied prior to cold pressing on the chemical composition and functional properties of *Nigella sativa* L. press cake.

2. Materials and Methods

2.1. Materials

Seeds of black cumin (*Nigella sativa* L.) were harvested in Poland in autumn 2022 from an integrated productivity plantation on sandy-clay soil with a neutral pH. The black cumin seeds were collected at full maturity (BBCH stage 89) using a combine harvester with air drying and mechanical cleaning. The seeds prepared in this way were stored in paper bags in a dry, cool, and shaded warehouse until the experiment.

2.2. Seeds Pretreatment Methods

The seeds were subjected to various pretreatment methods, including:

2.2.1. Convection Heating

Heating at +180 °C for 5 min in a laboratory oven (Memmert Universal Oven, UN 55 Plus, Memmert GmbH, Schwabach, Germany). The chosen temperature and heating

duration were based on previous studies by Farmanov J. (2021) [30] and pilot trials were carried out. It was observed that temperatures above +180 °C or longer heating times above 5 min led to excessive drying of the seeds and therefore reduced the cold press extraction performance due to a low amount of residual moisture.

2.2.2. Ultrasonication

Seeds were placed in vials with distilled water added to cover them. Treatment proceeded for 60 min at a power of 90 W and 55 kHz using an ultrasonic water bath (Argo Lab AU-65, Argo Lab Ltd., London, UK) [31].

2.2.3. Microwave Irradiation

This method was performed at 540 W for 3 min using a commercial microwave oven. The seeds were used without prior hydration, maintaining their moisture content to evaluate the effect of microwave treatment on dry seeds [32–34].

2.2.4. Pulsed Electric Fields

PEF pretreatment with an electric field intensity of 2.5 kV/cm (charged voltage) and a pulse length of 99 µsec for 45 s was performed with an electroporation system (BTX, Rigol, DS1102E, Biochrom Ltd., Cambridge, UK) [35]. Prior to performing PEF pretreatment, black cumin seeds were moisturized to advance the consistency of the methods and improve cell structure softening for the next extraction phase. The moisturizing step included mixing phase of the *Nigella sativa* L. seeds with distilled water at a seed-to-water volume ratio of 1:1.5. Afterwards, the mixture was left to stand at room temperature (+24 °C) for 60 min. Subsequently the seeds were drained through a cloth mesh sieve to remove excess water. This phase was based on previous pilot trials which showed that usage of moderate moisture conditions of the seeds boosts efficiency of PEF without excessive seed clumping.

2.2.5. Additional Pre-Treatment Notices

After completion of the PEF and Ultrasonication treatments, the moisturized seeds were dried at +50 °C for 120 min until their moisture content returned to the level of the untreated seeds ($5.85 \pm 0.02\%$). Moisture was verified prior to the pressing stage. Thus, all pretreatment groups entered the cold press at an equivalent moisture content. Pretreatment parameters, including temperature, power, duration, voltage, and pulse width, were selected based on previous studies on *Nigella sativa* L. and related seeds [13,30,31,36], validated through pilot trials. As the pretreatment methods act through different physical variables (i.e., temperature, cavitation and electric field intensity), no common parameter can be fixed across all methods, as each was therefore applied at its own established optimum, with the untreated control as the shared reference point.

2.3. Oil Extraction and Press Cake Formation

Prior to cold-press extraction, all seeds exhibited a moisture content of $5.85 \pm 0.02\%$, which falls within the range considered optimal for cold pressing [10].

Cold-press extraction was performed using a PR-H100/1 cold press machine (Oilpressparts, PR-H100/1, Oilpressparts GmbH and Co. KG, Niederkrüchten, Germany) at ambient temperature of +24 °C. For the control sample, seeds directly went to the cold-extraction stage, without any pretreatment.

The cold-press chamber temperature was set to remain below +40 °C during the extraction process. The screw speed was set at a consistent 30 rpm and the feed rate at 100 g/min. Thus, ensuring a stable flow of seeds into the press chamber. The seeds were processed in single-pass mode through the cold-press screw and the resulting oil and residual press cake were collected separately.

The press cake was stored in plastic bags at $-35\text{ }^{\circ}\text{C}$ until further analysis.

2.4. Determination of the Chemical Content of Press Cake

Moisture content was determined by drying the samples at $+105\text{ }^{\circ}\text{C}$ in a laboratory oven (Memmert Universal Oven, UN 55 Plus, Memmert GmbH, Schwabach, Germany) to constant weight [37]. The total protein content was calculated from the nitrogen content using a conversion factor of 6.25 and determined according to the Kjeldahl method using a Büchi Distillation Unit K-355 (Büchi Labortechnik AG, Athens, Greece) [38,39]. Fat content was determined according to the standard method by means of the Soxhlet method in a Büchi B-811 apparatus (Büchi Labortechnik AG, Flawil, Switzerland) with the use of diethyl ether after hydrolysis of the sample with 4 N HCl [40,41]. Total fiber content was determined following international standards according to the enzymatic–gravimetric method [42]. The ash content was determined by burning the samples in a muffle oven at $+550\text{ }^{\circ}\text{C}$ for 4 h [43]. Chemical composition results are expressed on a dry matter (DM) basis.

2.5. Color Profile of Press Cake

The color profile of *Nigella sativa* L. (black cumin) press cake was determined using a colorimeter (ColorFlex EZ Spectrophotometer, HunterLab, Sunset Hills Road, Reston, VA, USA) in the CIE $L^*a^*b^*$ color space [44].

Color measurements were performed on ground press cake. Approximately 10 g of each sample was ground to a fine powder using a laboratory mill (Model Retsch ZM200, Haan, Germany).

The colorimeter was calibrated against a standard white tile before each set of measurements. Five readings were taken to ensure accuracy and the average values were calculated for L^* , a^* , and b^* . The lightness (L^*) value ranges from 0 (black) to 100 (white), with higher L^* values indicating lighter samples. The a^* value ranges from negative (green) to positive (red), and the b^* value ranges from negative (blue) to positive (yellow). Hue angle (h°) and chroma (C^*) were also calculated [45–47].

2.6. Physicochemical Properties of Press Cake

The water holding capacity (WHC) and swelling capacity (SC) of black cumin cold-pressed cake were evaluated using methods adapted from Jūrevičiūtė et al. (2022) [48] with minor adjustments. For WHC analysis, 0.5 g of *Nigella sativa* L. cold-pressed cake was mixed with 10 mL of deionised water and left to hydrate at $+21\text{ }^{\circ}\text{C}$ for 24 h. The hydrated samples were then centrifuged (Clinispin Horizon, 755 VES, Drucker Diagnostics Manufacturing, Shady Lane, Philipsburg, PA, USA) at $686\times g$ for 20 min. After centrifugation, the residue was carefully separated and weighed, and the WHC was calculated.

Briefly, to determine swelling capacity, 0.7 g of black cumin cold-pressed cake was placed in a graduated tube with 0.1 mL increments, and the initial sample volumes (in mL) were recorded. The samples were then mixed with 10 mL of deionised water and kept at $+21\text{ }^{\circ}\text{C}$ for 24 h. Following the hydration period, the samples were centrifuged (Clinispin Horizon, 755 VES, Drucker Diagnostics, Port Matilda, PA, USA) at $686\times g$ for 20 min, and the supernatants were carefully discarded.

The water-binding capacity (WBC) was determined according to the method described by Timilsena et al. (2016), with slight modifications [49]. Briefly, 0.5 g of sample was weighed into a test tube, to which 10 mL of distilled water was added. The resulting mixture was shaken using a laboratory shaker (Heidolph Vibramax 100, 31 W, Retsch GmbH, Haan, Germany). After 15 min, the solution was shaken again for 30 s. The precipitate was then separated from the supernatant using a centrifuge ($420\times g$; 15 min; MPW-351R, MPW Med. Instruments, Warsaw, Poland). The unabsorbed water was carefully poured out and the remaining drops were dried with tissue paper. The separated solid was then oven-dried at

+50 °C for 30 min. Water absorption was expressed as the mass of water (g) absorbed per 1 g of sample, as determined in three analytical replicates.

$$\text{WBC} = \left(\frac{C - B}{A} \right) - 1 \text{ (g water / g sample)}$$

where

A—weight of a sample (g);

B—weight of the empty centrifuge tube (g);

C—mass of test tube with wet sediment after drying (g).

2.7. Determination of Fatty Acids Profile of Press Cake

Fatty acid methyl esters (FAMES) were prepared following the procedure described by the AOAC (Association of Analytical Communities, AOAC Official Method, Gaithersburg, 1996) [39]. A 0.1 mL aliquot of lipid extract from each sample was esterified with 2 mL of methanolic 0.4 M NaOH solution by refluxing at +80 °C for 10 min. After the addition of 4 mL of BF₃-etherate, the samples were boiled for 5 min. The FAMES were extracted with hexane (2.0 mL). For drying, 1 mL of NaCl was added and allowed to settle to clarify the hexane layer, after which the upper phase was transferred to a specific vial. Qualitative and quantitative analysis of fatty acid composition was performed by gas chromatography using an Agilent 7820A gas chromatograph (Agilent, Santa Clara, CA, USA).

Fatty acid methyl esters were prepared with BF₃ in methanol as the methylating agent. A ZB-WAX capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness; Phenomenex, Torrance, CA, USA) was used. The detector (FID) temperature was set at 280 °C, and the injector temperature at +260 °C with a split ratio of 1:100. The column temperature was held initially at +100 °C, increased to +180 °C at 2 °C/min, then raised to +240 °C at 6 °C/min and held for 10 min. Helium was used as the carrier gas. Peaks were identified by comparison of their retention times with those of authentic fatty acid methyl ester standards.

2.8. Determination of Amino Acid Composition in Press Cake

The samples were acid-hydrolyzed [50] and analyzed in an AAA400 automatic amino acid analyzer (INGOS s.r.o., Prague, Czech Republic). A two-wavelength photometer (440 and 570 nm) was used as the detector. The length of the metallic column packed with ion exchanger Ostion LG ANB (INGOS s.r.o., Prague, Czech Republic) was 250 × 4.0 mm, and the column temperature was maintained at +40 to +70 °C, and the detector temperature was kept at +121 °C.

The amino acids were quantified by the ninhydrin method. Glutamine and asparagine were expressed as glutamic acid and aspartic acid, respectively. Tryptophan was not determined. Calculations were performed using Chromulan software v.0.60.3 (Pikron s.r.o., Prague, Czech Republic).

2.9. Determination of Total Phenolics and Flavonoid Contents in Press Cake

The total phenolic content (TPC) of *Nigella sativa* L. cold-press cake was determined using methanol as the extraction solvent. Methanol solution was selected due to its high efficiency in extracting polyphenolic compounds from dark-colored cold-pressed cake. Initially used ethanol (75% and 96%) was found to be less effective for cold-pressed cake.

The TPC was determined using the Folin–Ciocalteu method, following the protocol of Singleton et al. [51]. In brief, 10 mL of the methanol extract was mixed with 5 mL of distilled water and 20 µL of Folin–Ciocalteu reagent. After a reaction time of 6 min, 1 mL of 20% sodium carbonate solution was added to the mixture. The samples were then

incubated in the dark at room temperature for 30 min. The absorbance of the blue-colored solution was measured at 765 nm using a Spectro UVD-3200 spectrophotometer (Spectro UV-VIS Double Beam PC, Labomed, Los Angeles, CA, USA). The TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry matter [52–55].

The total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method. Here, 1 mL of methanol extract was mixed with 10 mL of a 10% aluminum chloride solution, 2 mL of 96% ethanol, and 1 mL of 1 M sodium acetate. The resulting mixture was incubated in the dark at room temperature for 40 min. After the incubation period, the absorbance was measured at 415 nm using the Spectro UVD-3200 spectrophotometer. The TFC was reported as milligrams of quercetin equivalents (QE) per gram of dry matter [53,54].

2.10. Determination of Antioxidant Activity of Press Cake

The antioxidant activity of *Nigella sativa* L. press cake was assessed using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) scavenging assay. A 0.3 mL aliquot of the methanol extract was combined with 5 mL of 0.1 mM DPPH• solution in methanol. The mixture was shaken and incubated in the dark at room temperature for 30 min. The decrease in absorbance reflecting the scavenging of DPPH• radicals was measured at 517 nm using the Spectro UVD-3200 spectrophotometer [55]. Antioxidant capacity expressed as a percentage allows a clear comparison of radical-scavenging abilities among samples [56].

2.11. Assessment of Individual Polyphenols in Press-Cake

Polyphenolic compounds in *Nigella sativa* L. press cake were identified and quantified by HPLC. Approximately 50 mg of the analytical material was weighed into individual test tubes. Each sample was extracted with 5 mL of 80% methanol (Sigma-Aldrich, Darmstadt, Germany) and mixed using a Micro-Shaker 326 M vortex mixer (Premed, Marki, Poland) for 10 s. The tubes were next placed in an ultrasonic bath (PolSonic, Warsaw, Poland) operating at 6000 Hz and +30 °C for 10 min to support extraction efficiency.

After ultrasonication, samples were centrifuged at $3780 \times g$ for 10 min at +5 °C. The supernatant (1 mL) was collected and transferred into HPLC vials for chromatographic analysis. The HPLC system (Shimadzu, Tokyo, Japan) consisted of two LC-20AD pumps, a CMB-20A system controller, an SIL-20AC autosampler, an SPD-20AV UV-Vis detector, a CTD-20AC column oven, and a Phenomenex Fusion-RP 80A column (250 × 4.6 mm).

The mobile phase consisted of two solvents: phase A (deionised water with 10% acetonitrile) and phase B (deionised water with 55% acetonitrile). Both phases were acidified with orthophosphoric acid (85%) to achieve a stable pH of 3.0. The separation was performed under gradient elution at a flow rate of 1 mL min^{-1} according to the following program: 1.00–22.99 min at 95% phase A and 5% phase B; 23.00–27.99 min at 50% phase A and 50% phase B; 28.00–30.99 min at 80% phase A and 20% phase B; 31.00–42.00 min at 95% phase A and 5% phase B.

Detection was carried out at two wavelengths: 250 nm for phenolic acids and 370 nm for flavonoids. Calibration curves were generated using pure phenolic standards (Sigma-Aldrich, Darmstadt, Germany), and compound identification was based on comparison of their retention times with those of the standards.

2.12. Statistical Methods

Analyses were performed in triplicate ($n = 3$), except for the color properties of the press cake, which were measured in quintuplicate ($n = 5$). Data are expressed as mean ± standard deviation. The data were analyzed using one-way analysis of variance (ANOVA); post hoc comparisons were performed using Fisher's least significant difference

(LSD) test at a significance level of $p < 0.05$ using ‘Statistica 12’ software (Statistica; StatSoft, Inc., Tulsa, OK, USA, Version 12). Correlation analysis was performed to assess the relationships between variables.

3. Results and Discussion

3.1. Press Cake Formation

The amount of by-product produced was significantly affected by the pretreatment method. The lowest amount was obtained when the seeds were pretreated by microwave, ultrasonication or pulsed electric fields (Table 1). These three methods did not differ significantly from one another ($p > 0.05$) but were significantly lower than the control and convection-heated samples.

Table 1. Press cake yield after cold-press oil extraction, %.

Seed Pretreatment Method	Press Cake Yield
Control sample	72.15 ± 1.09 ^b
Convection heating	71.85 ± 1.84 ^b
Microwave	65.54 ± 1.57 ^a
Ultrasonication	64.99 ± 0.98 ^a
Pulsed electric fields	63.61 ± 1.42 ^a

Values are means ± SD ($n = 3$). Lowercase letters “a, b” indicate significant differences ($p < 0.05$, Fisher’s LSD test).

A strong negative Pearson’s correlation between oil yield [13] and press-cake formation was confirmed ($r = -0.9929$, $p < 0.01$), indicating that pretreatment-induced improvements in oil extraction yield were directly and consistently reflected in reduced press-cake yield. Average process losses across pretreatments were $2.43\% \pm 0.70\%$, with non-thermal methods (ultrasonication and PEF) showing higher losses (2.97–2.99%) than convection heating (1.14%).

These results demonstrate that the seed pretreatment method affects not only the oil yield and quality, but also the amount of press cake produced, with variations of up to 8.54% observed between pretreatments (Table 1).

The seed pretreatments were chosen primarily to improve cold-press oil yield. The corresponding oil yield and quality from the same seed batch are reported in the accompanying study [13]. This research instead examines the chemical composition of the residual press cake as a potential secondary raw material for food and feed.

3.2. Proximate Composition of the Press Cake

The press cake, a by-product of the cold press oil extraction process described above, retains substantial nutritional and functional components, making it a valuable material for applications in food, animal feed and pharmaceutical industries. The moisture content of the black cumin press cake varied significantly with the pretreatment method, ranging from 8.59% in the ultrasonicated samples to 10.52% in the PEF-treated samples (Table 2). The higher moisture content of the PEF-treated press cake may be advantageous for certain applications, such as fermentation or use as a substrate in food processing, but could also compromise storage stability and shelf-life duration [57].

The fat content of the pressed cake was statistically the highest in the control sample at 26.92%, indicating that a significant amount of oil remained in the cake after extraction. All assessed pretreatments resulted in significantly lower fat content than the control sample, suggesting that convection heating, microwave, ultrasonication, and PEF treatments are comparably effective at improving oil extraction efficiency from black cumin seeds. This finding is consistent with previous studies in which thermal and mechanical pretreatments improved oil extraction efficiency [6].

Table 2. Proximate composition of press cake, %.

Component	Control Sample	Convection Heating	Microwaves	Ultrasonication	Pulsed Electric Fields
Moisture	10.10 ± 0.18 ^d	9.18 ± 0.02 ^c	8.85 ± 0.11 ^b	10.52 ± 0.14 ^e	8.59 ± 0.07 ^a
Fat	26.92 ± 1.17 ^b	23.17 ± 0.53 ^a	22.93 ± 0.42 ^a	24.30 ± 0.53 ^b	24.10 ± 0.68 ^b
Fiber	9.17 ± 1.12 ^a	10.10 ± 1.39 ^a	8.24 ± 0.47 ^a	11.14 ± 1.41 ^a	10.98 ± 0.63 ^a
Ash	6.61 ± 0.04 ^c	6.27 ± 0.10 ^b	6.71 ± 0.12 ^c	5.50 ± 0.09 ^a	6.32 ± 0.03 ^b
Protein	30.79 ± 0.18 ^c	29.87 ± 0.44 ^b	30.81 ± 0.19 ^c	26.75 ± 0.25 ^a	29.57 ± 0.14 ^b

Values are means ± SD ($n = 3$). Lowercase letters “a, b, c, d, e” indicate significant differences ($p < 0.05$, Fisher’s LSD test). The same letters (e.g., “a” and “b”) in a row indicate no statistically significant difference ($p > 0.05$, Fisher’s LSD test).

The protein content of the press cake was the highest in the microwave-pretreated and control samples, which yielded statistically equivalent values ($p > 0.05$), suggesting that microwave pretreatment does not cause significant protein degradation despite the thermal energy involved. This may be explained by the rapid heating mechanism of microwaves, which raises temperature too quickly without prolonged heat exposure, thereby minimizing thermal protein denaturation [27]. The two treatments did not involve the same approach of water contact. The ultrasonicated seeds were treated while immersed in distilled water for 60 min, whereas the PEF seeds were additionally moisturized (i.e., soaked) and then subjected to only 45 s of pulses. Under prolonged cavitation, water-soluble proteins are driven out of the submerged seeds into the surrounding water, which was discarded before pressing, while the conventional soaking before PEF treatment on drained seeds leaches far less material. This explains why the ultrasonicated press cake retained less protein than the PEF cake. It also showed the lowest ash content (Table 2), indicating loss of soluble constituents in ultrasonicated press cake [20,58].

The fiber content on a dry matter basis showed no significant differences across pretreatment methods ($p > 0.05$).

Ash content on a dry matter basis, indicative of the mineral composition of the press cake, varied significantly across pretreatments. Microwave and control samples were statistically equivalent and highest, whereas ultrasonication resulted in the lowest ash amount.

3.3. Color Properties of Press Cake

Color is an important quality attribute in food products, influencing consumer perception and product acceptance.

The L^* value, representing lightness, was significantly lower in the PEF-treated press cake, indicating a darker color than the control and other pretreatment methods (Table 3). If black cumin press cake were incorporated into a formulation, a lighter color (such as within ultrasonication press cake) may be more appealing in certain applications, such as bakery products, or where it serves as a feed-ingredient substitute [35,59]. Although the darker PEF-treated press cake may suit products where a deeper coloration is needed.

Table 3. Color properties of press cake.

Color Parameter	Control Sample	Convection Heating	Microwaves	Ultrasonication	Pulsed Electric Fields
Color coordinate L^*	31.19 ± 0.30 ^c	28.97 ± 0.09 ^b	29.39 ± 0.57 ^b	31.43 ± 1.13 ^c	26.20 ± 0.03 ^a
Color coordinate a^*	0.87 ± 0.13 ^a	1.48 ± 0.03 ^c	1.27 ± 0.23 ^b	1.13 ± 0.05 ^b	1.15 ± 0.02 ^b
Color coordinate b^*	3.83 ± 0.19 ^a	5.50 ± 0.09 ^c	4.25 ± 0.42 ^b	4.48 ± 0.35 ^b	4.44 ± 0.13 ^b
Chroma, C^*	3.93 ± 0.21 ^a	5.69 ± 0.08 ^c	4.43 ± 0.47 ^{ab}	4.62 ± 0.34 ^b	4.58 ± 0.12 ^b
Hue angle, h°	77.20 ± 1.95 ^c	74.94 ± 0.37 ^{ab}	73.36 ± 1.24 ^a	75.84 ± 1.22 ^{bc}	75.48 ± 0.47 ^{bc}

Values are means ± SD ($n = 5$). Lowercase letters “a, b, c” in a row indicate significant differences ($p < 0.05$, Fisher’s LSD test). The same letters (e.g., “a” and “b”) in a row indicate no statistically significant difference ($p > 0.05$, Fisher’s LSD test).

The a^* value, which indicates position on the red-green axis, was lowest in the control sample, suggesting a least reddish hue (i.e., most neutral), whereas the convection heating samples showed a higher a^* value, indicating a shift towards red. The greater redness in the convection heating process could be due to Maillard reaction products, residual oils and pigment changes formed during heating [60].

The b^* value, representing the yellow-blue axis, was highest in the convection-heated press cake, indicating a more yellowish hue, whereas the control sample had the lowest b^* value, indicating a more bluish hue. The yellowish color of the convection heating sample could also be attributed to the Maillard reaction and caramelization of sugars, which are known to impart yellow to golden-brown colors to food products [60]. Such color changes have been shown to affect the visual appeal and marketability of food products, making the choice of pretreatment method important for maintaining desirable color properties [60,61].

The hue angle (h°), a measure of perceived color, ranged from 73.36° in the microwave-treated samples to 77.20° in the control sample. The significantly lowest hue angle, observed in microwave-treated samples, is consistent with the Maillard reaction and caramelization processes generated by rapid heating, processes known to promote pigment degradation and enhance yellow-red coloration in heat-treated food materials [60,62].

Chroma (C^*), which represents color intensity or saturation, was significantly highest in the heat-treated samples (5.69), indicating a more vivid, saturated color that may be advantageous in applications requiring bold coloration. In contrast, the control samples showed lower chroma values, reflecting less saturated tones [63].

Overall, these color differences are relevant because they can influence the final appearance of products made from the press-cake ingredients, such as baked goods, snacks, feed or supplements. For instance, a lighter, more natural colored press cake may be preferred where color consistency is important, whereas a more pigmented press cake might be desirable in products intended to highlight the natural color of the ingredients [20,64].

3.4. Water Holding, Binding and Swelling Capacity

The water holding capacity (WHC), water binding capacity (WBC) and swelling capacity (SC) of the press cake are functional properties that influence its applicability in food formulations, particularly for products requiring moisture retention and structural stability.

Statistical analysis indicated no significant differences ($p > 0.05$) in WHC and WBC values among the pretreatment methods, indicating that it was largely unaffected by the pretreatment method under the conditions tested (Table 4).

Table 4. Water holding, binding and swelling capacity of press cake.

Properties	Control Sample	Convection Heating	Microwaves	Ultrasonication	Pulsed Electric Fields
WHC, %	3.81 ± 0.33^a	4.18 ± 0.03^a	3.96 ± 0.26^a	4.12 ± 0.30^a	3.94 ± 0.38^a
WBC, %	3.17 ± 0.16^a	2.85 ± 0.45^a	2.79 ± 0.21^a	2.60 ± 0.16^a	2.64 ± 0.76^a
SC (mL/g)	1.65 ± 0.16^{abc}	1.40 ± 0.15^{ab}	1.68 ± 0.11^{bc}	1.88 ± 0.15^c	1.35 ± 0.26^a

Values are means $\pm$ SD ($n = 3$). Lowercase letters "a, b, c" in a row indicate significant differences ($p < 0.05$, Fisher's LSD test). The same letters (e.g., "a" and "b") in a row indicate no statistically significant difference ($p > 0.05$, Fisher's LSD test).

The water binding capacity (WBC) represents the ability of the press cake to absorb and retain water under centrifugal force, an important functional property for food applications requiring moisture retention and textural stability [25].

Previous studies have shown that thermal and mechanical treatments can cause protein denaturation and alter the hydrophilic properties of amino acid residues, potentially reducing the overall water binding efficiency [24,65,66]. The relatively stable WBC values

observed suggest that *Nigella sativa* L. press cake maintains consistent hydration properties regardless of the processing approach, which is advantageous for industrial applications where predictable water retention behavior is crucial for product formulation and quality control [25,67].

The SC values ranged from 1.35 mL/g in the PEF-treated samples to 1.88 mL/g in the ultrasonicated samples (Table 4). The ultrasonicated samples showed the highest SC value, significantly greater than the PEF and convection heating samples but not significantly different from the control or microwave-treated ones [28,68].

The stability of WHC and WBC across all treatments suggests that black cumin press cake maintains consistent hydration behavior regardless of how seeds are processed. This is also a practically useful characteristic for food and feed manufacturers who need reliable raw material performance [25]. SC, however, shows a different approach. Given the notably lower protein content of ultrasonicated press cake, this increase in SC can be explained by cavitation-driven modifications to the dietary fiber rather than by greater availability of protein [28]. These contrasting results highlight that pretreatment selection should be guided by the functional demands of the targeted application.

3.5. Fatty Acids Profile of Press Cake

The fatty acid composition of the black cumin press cake varied significantly among the pretreatment methods. The results show that the pretreatment method significantly influenced the distribution of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) in the residual press cake (Table 5). It should also be noted that the fatty acid data (Table 5) are expressed as relative proportions (% of total fatty acids) rather than as absolute contents ($\text{mg}\cdot\text{g}^{-1}$ cake).

The results indicated that saturated fatty acids represented the smallest fraction of the total fatty acid profile, ranging from 16.39% in the ultrasonicated samples (not significantly different from the control sample), to 17.72% in the microwave pretreated samples. Palmitic acid (C16:0) was the dominant saturated fatty acid across all treatments, accounting for 12.52–13.44% of the total fatty acid content. The elevated SFA proportion observed in microwave-treated press cake may be linked to thermal oxidation and degradation of polyunsaturated fatty acids during microwave heating, which relatively increases the proportion of thermally stable saturated fatty acids remaining in the press cake.

During heat processing of oilseeds, complex chemical reactions occur, including lipid oxidation affecting unsaturated fatty acids, as reported by Zhang et al., 2024 [62]. This is consistent with Kaseke et al., 2020 [69], who found that palmitic acid and saturated fatty acids increased after microwave pretreatment of oilseeds. In contrast, the ultrasonicated and control samples showed statistically equivalent and the lowest SFA values, in agreement with the non-thermal nature of ultrasonic cavitation, which disrupts cell membranes to facilitate oil release without inducing the thermal oxidation reactions responsible for PUFA degradation and the consequent relative SFA enrichment [28]. Notably, microwave pretreatment resulted in the highest relative proportion of palmitic acid (13.44%), consistent with the range reported for *Nigella sativa* L. oil (7–13%) [70]. Stearic acid (C18:0) was the second most abundant saturated fatty acid, ranging from 3.35% in the control sample to 3.70% in the microwave pre-treated sample. The minor saturated fatty acids, including myristic acid (C14:0) and arachidic acid (C20:0), were present at below 1% of total fatty acids. Other authors have also reported varying levels of myristic and arachidic acids, ranging from 0.1% to 20.0% [71–73], which may be attributable to differences among black cumin varieties. The oil fatty acid profile here reflects not only possible thermal modification of PUFA but also selective partitioning of lipid fractions between the expressed oil and the press cake. As the composition of the expressed oil was not analyzed in this research,

these two mechanisms cannot be fully separated. Thus, observed shifts likely reflect their combined effect.

Table 5. Fatty acid profile of the press cake, % of total fatty acids.

Fatty Acid	Control Sample	Convection Heating	Microwaves	Ultrasonication	Pulsed Electric Fields
C14:0	0.21 ± 0.01 ^{ab}	0.24 ± 0.01 ^c	0.21 ± 0.01 ^{ab}	0.20 ± 0.01 ^a	0.22 ± 0.01 ^b
C16:0	12.52 ± 0.06 ^a	13.08 ± 0.00 ^c	13.44 ± 0.05 ^d	12.52 ± 0.01 ^a	12.71 ± 0.03 ^b
C18:0	3.35 ± 0.02 ^a	3.51 ± 0.00 ^b	3.7 ± 0.03 ^c	3.36 ± 0.00 ^a	3.37 ± 0.01 ^a
C20:0	0.26 ± 0.01 ^{ab}	0.28 ± 0.00 ^{cd}	0.29 ± 0.00 ^d	0.25 ± 0.00 ^a	0.27 ± 0.01 ^{bc}
C22:0	0.09 ± 0.01 ^b	0.10 ± 0.01 ^b	0.09 ± 0.01 ^b	0.07 ± 0.01 ^a	0.09 ± 0.01 ^b
SFA	16.42 ± 0.11 ^a	17.21 ± 0.02 ^c	17.72 ± 0.09 ^d	16.39 ± 0.00 ^a	16.65 ± 0.06 ^b
C16:1	0.22 ± 0.02 ^{ab}	0.24 ± 0.01 ^b	0.21 ± 0.00 ^{ab}	0.21 ± 0.01 ^{ab}	0.19 ± 0.04 ^a
C18:1	25.51 ± 0.05 ^c	25.82 ± 0.03 ^d	27.17 ± 0.02 ^e	25.24 ± 0.02 ^b	25.13 ± 0.01 ^a
C20:1	0.35 ± 0.01 ^a	0.37 ± 0.00 ^a	0.54 ± 0.06 ^b	0.34 ± 0.00 ^a	0.35 ± 0.01 ^a
C22:1	0.09 ± 0.01 ^a	0.18 ± 0.00 ^c	0.33 ± 0.00 ^d	0.07 ± 0.02 ^a	0.12 ± 0.02 ^b
MUFA	26.16 ± 0.08 ^b	26.61 ± 0.02 ^c	28.24 ± 0.04 ^d	25.85 ± 0.01 ^a	25.78 ± 0.06 ^a
C18:2	54.48 ± 0.18 ^c	53.09 ± 0.00 ^b	51.10 ± 0.05 ^a	54.83 ± 0.00 ^d	54.60 ± 0.01 ^c
C18:3 n3	0.21 ± 0.00 ^a	0.37 ± 0.00 ^a	0.19 ± 0.00 ^a	0.20 ± 0.00 ^a	0.25 ± 0.00 ^a
C20:2	2.75 ± 0.01 ^{bc}	2.73 ± 0.00 ^a	2.76 ± 0.01 ^c	2.74 ± 0.01 ^{ab}	2.73 ± 0.00 ^a
PUFA	57.43 ± 0.18 ^c	56.19 ± 0.00 ^b	54.04 ± 0.04 ^a	57.77 ± 0.01 ^d	57.58 ± 0.01 ^c

Values are means ± SD ($n = 3$). Different lowercase letters “a, b, c, d, e” in a row indicate significant differences ($p < 0.05$, Fisher’s LSD test). Values presented as 0.00 indicate standard deviations below the reporting precision after rounding and do not represent zero variability.

The monounsaturated fatty acid profile varied significantly among treatments, ranging from 25.78% in the PEF-pretreated samples (equivalent to the control) to 28.24% in the microwave-pretreated samples. Microwave pretreatment yielded the highest MUFA share, whereas the PEF and ultrasonicated samples formed a statistically homogeneous group with the lowest MUFA relative content ($p < 0.05$).

Oleic acid (C18:1) was the dominant monounsaturated fatty acid, representing approximately 95% of the total MUFA. Microwave pretreatment also gave the highest oleic acid (27.17%), whereas PEF gave the lowest (25.13%) relative content. These values align with previous studies reporting oleic acid content in *Nigella sativa* L. ranging from 15 to 30% [69].

The higher oleic acid content of the microwave-treated samples may be linked to thermal degradation of polyunsaturated fatty acids, particularly linoleic acid (C18:2), which is more susceptible to oxidation under heat than monounsaturated fatty acids, thereby increasing the relative oleic acid fraction in the residual press cake. This approach is supported by microwave-pretreatment studies in which microwave heating increased oleic acid by 16–42% while decreasing linoleic acid by 17–19% [74], confirming an inverse relationship between the two fatty acids under thermal conditions. Likewise, decreases in total unsaturated and polyunsaturated fatty acids accompanied by an increase in monounsaturated fatty acids have been reported in microwave-heated samples [75]. Hence, supporting the observed pattern in black cumin press-cake [76].

Polyunsaturated fatty acids constituted the largest fraction of the profile, accounting for 54.04–57.77% of the total fatty acids. Linoleic acid (C18:2) was the most abundant fatty acid across all treatments, representing over 90% of the PUFA fraction. The ultrasonicated samples showed the highest linoleic acid relative content (54.83%), followed by the PEF pre-treated sample (54.60%). This predominance of linoleic acid is characteristic of black cumin, with literature values typically ranging from 40 to 70% [11,77]. The high linoleic acid content is nutritionally important, as this omega-6 fatty acid is essential for human health and cannot be synthesized by the body [78,79].

Alpha-linolenic acid (C18:3n-3), the primary omega-3 fatty acid, was also detected; it occurred in relatively small amounts across all pretreatments, with no significant differ-

ences among them. The presence of omega-3 fatty acids further contributes to the overall nutritional value of the press cake [80].

Although the pretreatment method (i.e., microwave) shows measurable shifts in individual fatty acid fractions, the dominant nutritional character of the press cake was sustained across all treatments. Polyunsaturated fatty acids consistently accounted for more than 54% of the total fatty acid profile, with linoleic acid as the principal component, confirming that cold press cake retains significant fatty acid nutritional value regardless of the seed pretreatment applied [60,78,79].

3.6. Total Proteins and Amino Acids of Press Cake

The amino acid composition of black cumin press cake revealed a thorough protein profile with promising nutritional implications for both human food and animal feed applications (Table 6). The total protein content varied among pretreatment methods, ranging from $24.48 \text{ g} \cdot 100 \text{ g}^{-1}$ in the convection-heated sample to $27.53 \text{ g} \cdot 100 \text{ g}^{-1}$ in the PEF-treated sample, indicating that the pretreatment method can influence protein concentration and, potentially, amino acid content. The resulting values derived from amino acid content analysis are systematically lower than the Kjeldahl-based values shown previously in Table 2. This is an expected methodological difference: the Kjeldahl procedure [38] quantifies total nitrogen in the sample and converts it to protein using a fixed factor. Meanwhile, amino acid analysis directly measures the actual amino acid content after protein hydrolysis and is therefore a more specific indicator of protein. Nonetheless, both applications confirm that pretreatment influences the nitrogen-containing fractions of the press cake.

The essential amino acid composition varied significantly across pretreatment methods, with several amino acids showing distinct patterns. Lysine (LYS) ranged from $8.06 \text{ mg} \cdot \text{g}^{-1}$ in the ultrasonicated sample to $9.04 \text{ mg} \cdot \text{g}^{-1}$ in the control sample, representing a significant decrease under ultrasonication. Previous studies have reported lysine contents in *Nigella sativa* L. of 2.66 to $4.18 \text{ mg} \cdot \text{g}^{-1}$ of protein, indicating that the press cake retains high lysine levels despite processing [81–83].

Leucine (Leu) was one of the most abundant essential amino acids across all pretreatments, although ultrasonication caused a significant decrease relative to the other treatments and the control. Leucine represented the most abundant essential amino acid across all pretreatment methods. Its concentration was nonetheless considerably lower than those of the predominant non-essential amino acids, glutamic acid and aspartic acid, within the total amino acid profile. Values for these two acids were notably higher than previously reported for *Nigella sativa* L. (6.65 – $8.21 \text{ mg} \cdot \text{g}^{-1}$) [15,81,84].

Phenylalanine (Phe) ranged from $8.46 \text{ mg} \cdot \text{g}^{-1}$ in the ultrasonicated sample to $9.52 \text{ mg} \cdot \text{g}^{-1}$ in the microwave-pretreated samples. Phenylalanine combined with tyrosine (Tyr) is particularly important for its antioxidant properties and its role in neurotransmitter synthesis [85]. The total aromatic amino acid content (phenylalanine and tyrosine) was consistently high across all treatments, reaching up to $18.19 \text{ mg} \cdot \text{g}^{-1}$ in the microwave-treated sample, which is beneficial for both nutritional and functional applications [85].

The other essential amino acids, including threonine (Thr), valine (Val), isoleucine (Ile), and histidine (His)—showed similar patterns, with ultrasonication generally resulting in lower concentrations than the other methods [86,87].

Glutamic acid (Glu) was the most abundant amino acid across all pretreatments, ranging from $56.88 \text{ mg} \cdot \text{g}^{-1}$ in the ultrasonicated samples to $67.19 \text{ mg} \cdot \text{g}^{-1}$ in the PEF-pretreated samples. This is consistent with previous research identifying glutamic acid as the predominant amino acid in black cumin, typically consisting of 19 to 24% of total amino acids [81,85]. Its high content contributes to the umami flavor and functional properties of

the cold-press cake, which may be advantageous for food applications where enhanced flavor is desirable.

Table 6. Amino acid content in press cake of *Nigella sativa* L., mg·g^{−1} DM.

Criteria	Control Sample	Convection Heating	Microwaves	Ultrasonication	Pulsed Electric Fields
ASP	24.84 ± 0.39 ^c	24.34 ± 0.13 ^{bc}	24.10 ± 0.58 ^b	21.14 ± 0.35 ^a	23.74 ± 0.22 ^b
THR	9.03 ± 0.09 ^b	8.88 ± 0.01 ^b	8.99 ± 0.29 ^b	8.27 ± 0.15 ^a	9.08 ± 0.20 ^b
SER	10.20 ± 0.04 ^b	10.00 ± 0.03 ^b	10.28 ± 0.39 ^b	9.30 ± 0.22 ^a	10.40 ± 0.27 ^b
GLU	64.23 ± 1.19 ^c	61.00 ± 0.53 ^b	66.48 ± 1.89 ^{cd}	56.88 ± 1.22 ^a	67.19 ± 1.38 ^d
PRO	11.22 ± 0.42 ^{ab}	12.26 ± 0.14 ^b	10.88 ± 1.75 ^{ab}	9.43 ± 2.13 ^a	11.18 ± 0.88 ^{ab}
GLY	13.64 ± 0.20 ^{bc}	13.31 ± 0.14 ^b	13.82 ± 0.46 ^c	12.36 ± 0.17 ^a	13.90 ± 0.25 ^c
ALA	11.30 ± 0.29 ^b	11.63 ± 0.08 ^b	11.59 ± 0.16 ^b	10.62 ± 0.09 ^a	11.36 ± 0.37 ^b
CYS	3.00 ± 0.24 ^a	2.77 ± 0.09 ^a	2.46 ± 1.56 ^a	2.74 ± 0.08 ^a	3.07 ± 0.42 ^a
VAL	11.23 ± 0.05 ^c	11.03 ± 0.12 ^{bc}	10.71 ± 0.45 ^b	10.00 ± 0.18 ^a	11.18 ± 0.28 ^c
MET	3.43 ± 0.04 ^c	2.85 ± 0.01 ^a	3.36 ± 0.12 ^c	3.09 ± 0.01 ^b	3.89 ± 0.15 ^d
ILE	8.64 ± 0.17 ^b	8.41 ± 0.08 ^b	8.39 ± 0.26 ^b	7.75 ± 0.09 ^a	8.57 ± 0.25 ^b
LEU	14.62 ± 0.35 ^b	14.21 ± 0.07 ^b	14.45 ± 0.50 ^b	13.21 ± 0.20 ^a	14.68 ± 0.46 ^b
TYR	8.55 ± 0.29 ^{ab}	8.28 ± 0.02 ^{ab}	8.54 ± 0.33 ^{ab}	8.09 ± 0.27 ^a	8.67 ± 0.24 ^b
PHE	9.18 ± 0.47 ^b	9.23 ± 0.03 ^b	9.52 ± 0.05 ^b	8.46 ± 0.11 ^a	9.47 ± 0.22 ^b
HIS	5.97 ± 0.04 ^b	5.90 ± 0.04 ^b	5.96 ± 0.21 ^b	5.33 ± 0.09 ^a	6.12 ± 0.16 ^b
LYS	9.04 ± 0.06 ^b	8.85 ± 0.10 ^b	8.87 ± 0.33 ^b	8.06 ± 0.12 ^a	8.98 ± 0.18 ^b
ARG	20.86 ± 0.28 ^b	20.40 ± 0.13 ^b	20.53 ± 0.27 ^b	18.88 ± 0.26 ^a	20.69 ± 0.65 ^b
Sum (mg g ^{−1})	238.98 ± 4.62 ^b	233.34 ± 1.73 ^b	239.43 ± 8.91 ^b	213.60 ± 1.46 ^a	242.16 ± 6.57 ^b
Total protein (g 100 g ^{−1})	25.45 ± 0.39 ^{bc}	24.48 ± 0.26 ^a	26.29 ± 0.83 ^c	25.22 ± 0.43 ^{ab}	27.53 ± 0.24 ^d

Values are means ± SD ($n = 3$). Different lowercase letters “a, b, c, d” in a row indicate significant differences ($p < 0.05$, Fisher’s LSD test). The same letters (e.g., “a” and “b”) in a row indicate no statistically significant difference ($p > 0.05$, Fisher’s LSD test).

Aspartic acid (Asp), the second most abundant amino acid within this research, varied from 21.14 mg·g^{−1} in the ultrasonicated samples to 24.84 mg·g^{−1} in the control samples, in agreement with literature values reporting aspartic acid as a major component of black cumin protein [73,81,85].

The total amino acid sum ranged from 213.60 mg·g^{−1} in the ultrasonicated samples to 242.16 mg·g^{−1} in the PEF-pretreated samples, corresponding to total protein values of 25.22 g·100 g^{−1} and 27.53 g·100 g^{−1} respectively. These differences indicate that pretreatment selection can significantly impact the nutritional quality and potential applications of the press cake [58].

Ultrasonication consistently showed lower amino acid concentrations for most amino acids, which may reflect protein denaturation or structural modification induced by cavitation. In contrast, PEF generally preserved or increased amino acid content, suggesting that electroporation may enhance protein extractability without causing degradation [67,88].

3.7. Antioxidant Activity and Bioactive Compounds

Phenolic compounds and flavonoids are recognized for their health benefits, including anti-inflammatory, anticancer, and cardioprotective effects [1,4,10,89]. The TPC was highest in the ultrasonicated samples, at 56.77 mg GAE·g^{−1} (Table 7), indicating that ultrasonication as a non-thermal approach is more effective at preserving heat-sensitive nutrients during cold extraction [32]. The relatively high TPC reflects strong antioxidant properties, which are important for maintaining the stability and shelf life of food and feed products [33,90].

The TFC was highest in the ultrasonicated press cake, reaching 14.28 mg QE·g^{−1} DM, whereas convection heating and PEF significantly reduced it. Thus outcome of TFC values varies and does not indicate one particular method as the best solution.

Flavonoids themselves are crucial for their antioxidant properties, which help to protect cells from oxidative stress and reduce the risk of chronic diseases [12]. Remaining stable flavonoid content in the ultrasonically treated press-cake makes it a valuable method for functional foods, feed and nutraceuticals where antioxidant activity may be a key product’s

selling point [35]. Studies have also highlighted the role of flavonoids in improving the sensory and functional properties of food products, further supporting the use of cold-press cake in food formulations [25,81].

Table 7. Total phenols, total flavonoids and antioxidant activity of press cake.

Criteria	Control Sample	Convection Heating	Microwaves	Ultrasonication	Pulsed Electric Fields
Total phenolic content (TPC), mg GAE·g ⁻¹ DM	54.00 ± 0.68 ^b	50.05 ± 0.48 ^a	49.17 ± 0.47 ^a	56.77 ± 0.11 ^c	54.37 ± 0.40 ^b
Total flavonoid content (TFC), mg QE·g ⁻¹ DM	13.43 ± 0.35 ^b	9.78 ± 0.52 ^a	13.51 ± 0.25 ^b	14.28 ± 0.18 ^c	9.79 ± 0.30 ^a
DPPH•, %	61.61 ± 2.47 ^a	73.55 ± 1.49 ^c	75.22 ± 0.76 ^c	69.81 ± 1.31 ^b	62.18 ± 1.85 ^a

Values are means ± SD (*n* = 3). Different lowercase letters “a, b, c” in a row indicate significant differences (*p* < 0.05, Fisher’s LSD test).

A similar case was observed whilst analyzing TPC values, which indicated significant differences. The output showed that convection heating and usage of microwaves as pre-treatments significantly decreased remaining TPC concentrations. Meanwhile PEF retained similar values as the control sample. However, TPC values within ultrasonicated samples significantly increased to 56.77 mg GAE·g⁻¹ dry matter.

The DPPH• radical scavenging activity, a measure of antioxidant capacity, was significantly highest in microwave and convection heating pretreated samples, which formed a statistically homogeneous group (*p* < 0.05). Therefore, indicating that both thermal pretreatment methods comparably improved the antioxidant capacity of the black cumin cold-press cake relative to non-thermal approaches. This indicates that both thermal pretreatments increased the measured radical-scavenging capacity of the black cumin cold-press cake relative to the non-thermal approaches, despite lowering its phenolic content. Especially, the ability of microwave treatment to increase antioxidant activity has been reported in other studies [32,69]. The influence of thermal pretreatment on the antioxidant activity of seeds and their by-products has likewise been reported by other authors [91,92]. The retention of antioxidant activity in the press-cake is particularly important for its potential use in functional foods, feed mixtures and dietary supplements, where antioxidant properties are highly valued [64].

A strong positive correlation between TPC and DPPH• scavenging activity was found in the control sample (*r* = 0.956, *p* < 0.05), validating that phenolic compounds are primary contributors to radical scavenging capacity in untreated press-cake. However, this relationship weakened in thermally pretreated samples.

This discrepancy most likely arises from the differing chemical principles on which the three assays are based. The Folin–Ciocalteu and aluminum chloride methods quantify total reducing capacity and TFC, respectively, while the DPPH• assay measures hydrogen and electron-donating ability. As these pools overlap only partially, each pretreatment shifted them in a different direction: ultrasonication gave the highest TPC and TFC (56.77 mg GAE·g⁻¹ and 14.28 mg QE·g⁻¹) but only intermediate DPPH• activity (69.81%). However, convection heating and microwave treatment gave the lowest TPC values (50.05 and 49.17 mg GAE·g⁻¹) together with the highest radical-scavenging activities (73.55 and 75.22%).

In the thermally pretreated samples, two contrasted processes occurred at once. Native phenolics were moderately degraded (as reflected in the reduced TPC and in the individual phenolic profile (Table 8)). At the same time non-enzymatic browning possibly generated melanoidins, reductones and N-heterocyclic compounds. These are applicable hydrogen donors in the DPPH• assay but were not detected by the Folin–Ciocalteu nor the aluminum chloride method [93,94]. Their formation in heat-treated *Nigella sativa* L. is consistent with the reported generation of Maillard-derived pyrazines, furans and pyrroles during black

cumin roasting [95]. Moreover, an additional potential contribution of thymoquinone (which is redox-active but quantified by neither of the assays [11,82,91]) cannot be excluded. Since these two antioxidant constituents respond differently to the assays and change in opposite directions during heating, the linear TPC-DPPH• relationship is necessarily degraded. This is expressed quantitatively by the DPPH•/TPC ratio, which remained unchanged after PEF treatment (1.14) relative to the control (1.14), increased slightly after ultrasonication (1.23), and rose by 29–34% after convection heating (1.47) and microwave treatment (1.53). Thermal pretreatment therefore did not increase the total reducing capacity of the press cake but altered its composition, replacing degraded phenolics with non-phenolic antioxidants of higher specific activity. Neither the browning products nor thymoquinone were measured in the present work, so this mechanism is presented as a probable contributor that remains to be confirmed by further targeted analysis.

The non-thermally pretreated samples showed the opposite trend. The Folin–Ciocalteu reagent also reacts with proteins, thiols, reducing sugars and aromatic amino acids [51,96,97]. Therefore, possibly Folin–Ciocalteu reactive proteins and amino acids retained in the cake compensated for the loss of phenolics without influence on radical scavenging (DPPH• 62.18%) in PEF-treated press cake. Moreover, radical-scavenging efficiency is governed by phenolic structure. In particular, the *o*-dihydroxy and *o*-trihydroxy configurations, rather than by concentration alone [98]. Catechin was markedly depleted in all pretreated samples (from 9.16 to 0.22–0.43 mg·g^{−1}), presumably reflecting oxidation of its catechol B-ring by polyphenol oxidase and, under ultrasonication, by cavitation-derived hydroxyl radicals [99]. This altered the composition of the phenolic pool while largely preserving its Folin–Ciocalteu reactivity.

Beyond these effects, the cake's bioactive composition depended on the pretreatment applied, rather than on the extent of oil removal, as residual fat did not correlate significantly with TPC, TFC or DPPH• ($r \leq 0.79$, $p > 0.11$). Comparison with the black cumin oil data [13] indicates that phenolics partitioned almost entirely into the solid fraction. This outcome is similar to earlier scientific reports, stating that the cold-press process leaves phenolic compounds in the cake, rather than in the oil [65,100,101].

These findings indicate that the choice of pretreatment method can significantly influence the bioactive compound content and antioxidant potential of the black cumin cold-press cake [65,81].

Table 8. Phenolic compounds in the press cake, mg·g^{−1} DM.

Phenolic Groups	Compound	Control	Convection Heating	Microwaves	Ultrasonication	Pulsed Electric Fields
Hydroxybenzoic acids	Gallic acid	1.59 ± 0.01 ^e	0.84 ± 0.01 ^a	1.30 ± 0.01 ^c	1.38 ± 0.01 ^d	0.94 ± 0.01 ^b
	p-Hydrobenzoic acid	0.04 ± 0.01 ^c	0.04 ± 0.01 ^b	0.04 ± 0.01 ^b	0.03 ± 0.01 ^a	0.04 ± 0.01 ^b
	Salicylic acid	7.29 ± 0.11 ^d	2.93 ± 0.02 ^b	7.99 ± 0.70 ^d	4.15 ± 0.04 ^c	2.45 ± 0.01 ^a
	Benzoic acid	0.04 ± 0.01 ^b	0.01 ± 0.01 ^a	0.07 ± 0.01 ^b	0.02 ± 0.01 ^b	0.06 ± 0.01 ^b
Hydroxycinnamic acids	Chlorogenic acid	0.42 ± 0.01 ^c	0.23 ± 0.01 ^d	0.09 ± 0.01 ^a	0.11 ± 0.01 ^b	0.18 ± 0.01 ^c
	Caffeic acid	0.03 ± 0.01 ^a	0.03 ± 0.01 ^a	0.03 ± 0.01 ^a	0.03 ± 0.01 ^a	0.03 ± 0.01 ^a
	p-Coumaric acid	0.12 ± 0.05 ^b	0.03 ± 0.01 ^a	0.22 ± 0.03 ^b	0.05 ± 0.01 ^b	0.17 ± 0.01 ^b
Flavan-3-ols	Epigallocatechin	0.06 ± 0.01 ^c	0.05 ± 0.01 ^b	7.27 ± 0.06 ^d	8.27 ± 1.47 ^d	0.04 ± 0.01 ^a
	Catechin	9.16 ± 0.10 ^c	0.27 ± 0.01 ^a	0.43 ± 0.01 ^b	0.22 ± 0.01 ^a	0.25 ± 0.01 ^a
	Epigallocatechin gallate	0.11 ± 0.01 ^a	0.11 ± 0.01 ^a	0.18 ± 0.01 ^c	0.11 ± 0.01 ^a	0.14 ± 0.01 ^b
Flavonols	Quercetin-3-O-rutinoside	0.04 ± 0.01 ^d	0.03 ± 0.01 ^c	0.01 ± 0.01 ^a	0.04 ± 0.01 ^d	0.02 ± 0.01 ^b
	Kaempferol-3-O-glucoside	0.03 ± 0.01 ^a	0.04 ± 0.01 ^a	0.03 ± 0.01 ^a	0.06 ± 0.01 ^b	0.03 ± 0.01 ^a
	Myricetin	0.01 ± 0.01 ^c	0.01 ± 0.01 ^a	0.01 ± 0.01 ^c	0.02 ± 0.01 ^d	0.01 ± 0.01 ^b
	Quercetin	0.04 ± 0.01 ^d	0.03 ± 0.01 ^c	0.02 ± 0.01 ^a	0.03 ± 0.01 ^b	0.02 ± 0.01 ^a
	Quercetin-3-O-glucoside	0.54 ± 0.01 ^a	2.00 ± 0.01 ^c	0.51 ± 0.01 ^a	2.15 ± 0.12 ^c	1.93 ± 0.01 ^b
Flavones	Apigenin	0.03 ± 0.01 ^c	0.02 ± 0.01 ^b	0.03 ± 0.01 ^c	0.02 ± 0.01 ^a	0.48 ± 0.01 ^d

Values are means ± SD ($n = 3$). Different lowercase letters “a, b, c, d, e” in a row indicate significant differences ($p < 0.05$, Fisher's LSD test).

3.8. Individual Phenolic Compounds in the Press Cake

To better assess the industrial applicability of black cumin press cake, further analysis was conducted to quantify individual phenolic compounds in *Nigella sativa* L. press cake (Table 8).

A total of 16 phenolic compounds were detected. Salicylic acid and catechin dominated in the press cake. The control samples generally retained the highest levels of gallic acid and catechin, whereas PEF treatment consistently gave the lowest levels of most phenolic acids. Microwave pretreatment preserved relatively high levels of *p*-coumaric acid, and ultrasonication increased epigallocatechin concentrations.

Flavonoid behavior differed from that of the phenolic acids, showing a more even distribution across treatments but significant variation in kaempferol-3-O-glucoside and quercetin-3-O-glucoside. Convection heating reduced chlorogenic and *p*-coumaric acids, whereas microwave and ultrasonication pretreatments showed selective retention of specific flavonoids. This may be because flavonoids are more thermally stable than free phenolic acids: ultrasonication can accelerate flavonoid release through cavitation, while the rapid heating of microwave treatment limits degradation [32].

Salicylic acid was the predominant phenolic compound in the black cumin press cake. Among major plant phenolics, salicylic acid has been shown to inhibit bacterial growth most strongly, with activity directed predominantly against Gram-negative bacteria, such as *Escherichia coli* and *Pseudomonas aeruginosa* [102].

Gallic acid acts as an antibacterial, antioxidant, anticancer, antiviral, and anti-inflammatory agent; its antimicrobial mechanisms involve modification of cytoplasmic membrane function and disruption of intracellular processes [103]. Gallic acid exhibits antioxidant and oxygen scavenging behavior, so its incorporation into packaging materials can enhance their antimicrobial properties [104].

3.9. Suitability for Food and Feed Applications

The combined compositional and functional results allow the cold press-cake of black cumin to be assessed for two potential end uses. The first is partial flour replacement in baked and cereal products. Press cake is rich in protein (up to $27.53 \text{ g} \cdot 100 \text{ g}^{-1}$, within PEF pretreatment) and retains lysine ($8.06\text{--}9.04 \text{ mg} \cdot \text{g}^{-1}$, between pretreatments), an amino acid limiting in wheat. Meanwhile, water holding and binding capacities remained statistically unchanged across all pretreatments, ensuring predictable dough hydration regardless of the method applied.

The second is use as a functional ingredient in meat and other emulsion-based products, where ultrasonicated press cake is the most suitable. For instance, showing the highest swelling capacity ($1.88 \text{ mL} \cdot \text{g}^{-1}$), reflecting cavitation-driven fiber change that supports water retention and texture. Thus, together with the highest phenolic and flavonoid contents, offering protection against lipid oxidation. Nonetheless, its slightly lower protein content is a minor trade-off where fiber and antioxidant functionality dominate. Moreover, pretreatment can be matched to the intended use: ultrasonication for fiber and antioxidant-focused functionality, whilst microwave treatment for antioxidant capacity with protein retention (DPPH• of 75.22%), and PEF for maximal nutritional preservation (e.g., total amino acids of $242.16 \text{ mg} \cdot \text{g}^{-1}$).

4. Conclusions

This study systematically assessed the effects of four black cumin seed pretreatment methods: convection heating, microwave, ultrasonication and pulsed electric fields, on the composition, functional properties, and nutritional quality of cold-pressed *Nigella sativa* L. press cake.

Microwave, ultrasonication, and PEF pretreatments produced statistically equivalent results and the lowest press-cake yields (63.61–65.54%; $p < 0.05$). Among these, microwave pretreatment retained high raw protein content (30.81%, statistically equivalent to the control), gave the highest DPPH• radical scavenging activity (75.22%) and specific polyphenols, such as salicylic acid (7.99 mg·g^{−1}). PEF retained higher glutamic acid (67.19 mg·g^{−1}) contents. Whereas ultrasonication yielded the highest total phenolic and flavonoid contents (56.77 mg GAE·g^{−1} and 14.28 mg QE·g^{−1}, respectively) and the highest swelling capacity (1.88 mL/g), making it well suited for antioxidant, fiber- and protein-rich functional foods or feeds.

The non-thermal methods, particularly PEF, preserved the amino acid profiles (e.g., glutamic acid up to 67.19 mg·g^{−1}), indicating minimal nutritional degradation.

The dominance of polyunsaturated fatty acids (>54%) persisted across all pretreatments, indicating that the nutritional value of the fatty acid fraction was broadly retained.

Overall, these findings identify microwave, ultrasonication, and PEF as the most promising pretreatments for improving press cake quality. Each of them offers evident functional advantages. Thus, the results provide an evidence-based view on why food and feed manufacturers should select applicable pretreatment of *Nigella sativa* L. seeds for cold press oil extraction. Hence, treating the press cake not as a food/process waste, but as a valuable by-product which can be reworked. Thereby, supporting zero-waste production goals, rather than relying on trial-and-error optimization. Future studies should focus on combining these pretreatments to optimize bioactive retention and sensory quality, providing a basis for pilot-scale testing in selected food and feed products.

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Abbreviations

The following abbreviations are used in this manuscript:

SC	Swelling capacity
TPC	Total phenolic content
TFC	Total flavonoid content
DPPH•	Radical scavenging activity
SFAs	Saturated fatty acids
MUFAs	Monounsaturated fatty acids
PUFAs	Polyunsaturated fatty acids

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