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ORIGINAL PAPER

Effects of pulsed electric field (PEF) pretreatment on the extraction yield and characteristics of rapeseed cake protein

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Abstract
Rapeseed cake (RC), a protein rich by-product of oil extraction, is an underutilized resource with potential for plant-based food applications. This study investigated the effects of pulsed electric field (PEF) pretreatment on the extraction, functional, and structural properties of rapeseed protein concentrate (RPC). RC was treated under four PEF conditions, combining two pH values (7.0 and 11.5) and two specific energy levels (24 and 71 kJ/kg). PEF treatments caused notable changes in the secondary structure of rapeseed protein, particularly a reduction in α-helix content, which was associated with enhanced protein solubility and increased viscosity under higher specific energy, neutral pH conditions (PEF 4). Oil-holding capacity and emulsifying properties remained largely unchanged. These findings demonstrate that PEF can selectively modulate protein structure to improve specific functional properties, revealing a condition-dependent effect that can be exploited in protein extraction and formulation. By linking structural alterations to functional outcomes, this work provides novel insights into the use of non-thermal PEF technology for valorizing RC and tailoring plant protein functionality. Overall, the study demonstrates how PEF influences protein properties, offering a foundation for future research on improving plant protein functionality and exploring potential applications in plant-based and hybrid food products.

Keywords Plant protein · Functional properties · Non-thermal processing · Protein structure · Rapeseed cake · Pulsed electric field

Introduction

Rapeseed, commonly known as canola, is an important vegetable crop that serves as a rich source of oil and protein for both food and industrial applications. Rapeseed Cake (RC) is one of the most important food industrial by-products. According to the USDA Production, Supply and Distribution database, global rapeseed meal production in the 2023/24 marketing year was approximately 49.1 million metric tons [1]. The dry base protein content of RC is particularly high, ranging between 30% and 40% [2]. This equates to approximately 18 million tons of protein annually. While rapeseed protein (RP) have long been used primarily in animal feed, rapeseed protein isolate is also accepted as generally recognized as safe (GRAS) by Food and Drug Administration (FDA) and is increasingly utilized in food applications, such as meat analogues, bakery products, sausages, mayonnaise, and nutritional formulations [3]. In 2013, the European Food Safety Authority (EFSA) also recognized rapeseed protein isolate, derived from RC, as a novel food [4].

The recycling of RC presents numerous economic and sustainable opportunities for the food industry. Cold-pressed RC typically contains an average of 30.6% protein, 28.2% nitrogen-free extracts, 11.2% dietary fiber, and 17.8% crude oil [5]. The ratios of these components, as well as their nutrient content, may vary depending on the technologies employed in oil extraction processes. Recycling RC protein isolates reduces waste and supports circular economy

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principles. A major challenge in the economic production of rapeseed protein isolate is the low extractability of proteins from conventional residues [6]. Low protein extractability from conventional rapeseed residues is mainly due to heat-induced denaturation and aggregation, strong interactions with cell wall fibers, formation of protein-phenolic complexes, covalent cross-linking, the naturally low solubility of RPs near neutral pH, and physical entrapment within the compact, oil-containing cake matrix [7]. The research studies conducted so far have focused on alkaline, saline, acidic, and ultrasonic pre-treated extraction methods, as well as the effects of ultrafiltration methods to improve RP extraction yields [8].

The functional properties of components are essential for the texture, taste, and consistency of the final food product. Consequently, plant proteins, which generally exhibit poor quality functional properties compared to meat proteins, often require modification to achieve competitive market viability [9]. Innovative methods such as PEF, cold plasma (CP), high-pressure processing (HPP), and high-pressure homogenization (HPH) provide advanced approaches to increasing protein yield and modifying protein properties [10]. The application of these techniques induces physical modifications that lead to conformational changes in protein structures without the need for specific chemicals. Among these various non-thermal modification technologies, PEF processing is considered an efficient, reliable, and clean method with relatively low operating costs. PEF applies short pulses of high-voltage electric fields to food materials, resulting in targeted structural effects. When applied to proteins, PEF can induce conformational changes that enhance functional properties such as solubility, emulsification, and gelation [11]. However, despite these advantages, PEF also has certain limitations: its effectiveness depends greatly on the electrical conductivity and moisture content of the sample, and achieving uniform treatment in heterogeneous materials can be challenging. Furthermore, generating a high-intensity electric field requires the flow of an electric current, which may cause side effects such as electrochemical reactions and temperature increases due to the Joule effect, potentially affecting food components such as proteins [12]. These factors can limit the scalability of PEF in industrial applications, as ensuring consistent treatment and maintaining product quality across large volumes requires careful process optimization and specialized equipment design.

Although previous studies have focused on full pH-shifting treatments combined with PEF, the principle remains similar: PEF permeabilizes cells, while pH alters protein solubility and conformation [13]. To our knowledge, no studies have applied pH-adjusted PEF to RC. In this study, RC dispersions were treated at different pH values prior to PEF, followed by alkaline extraction and isoelectric precipitation, to evaluate the effects on protein extraction and functional, rheological, and structural properties.

Materials and methods

Sample preparation

The cold-pressed rapeseed meal was provided by the SAVI ITALO SRL factory and milled in the Experimental Station for the Food Preservation Industry – Research Foundation (Parma, Italy) and kindly provided for this study. The approximate composition of rapeseed meal was determined by proximate analysis: protein content by the Kjeldahl method (ISO 937:1991), lipid by the Soxhlet method (ISO 1444:2010), and moisture and ash by the reference methods UNI ISO 1442:2010 and UNI 10590:1997, respectively. Carbohydrate and fiber contents were determined according to AOAC (2000) procedures [14]. The RC contained 31.8% protein, 18.75% carbohydrates, 34.02% fiber, 7.07% fat, 6.69% ash, and 1.67% moisture.

Pulsed electric field (PEF) treatment

The PEF pretreatment was carried out using a batch-mode laboratory-scale PEF system (EPULSUS® LBM1A-15, EnergyPulse Systems, Lisbon, Portugal). RC was dispersed in distilled water at a 1:20 ratio, and the pH of each dispersion was separately adjusted to 7 and 11.5 with 1 M NaOH. Treatments were performed in a 1 L PEF chamber, with 15 g of RC in 300 mL solution per run. Two independent variables were evaluated: pH and specific energy. Specific energy was controlled by the number of rectangular, bipolar pulses applied, ranging from 24 to 71 kJ/kg. The electric field strength was set at 1.7 kV/cm, with a pulse width of 20 µs, a pulse frequency of 20 Hz, and solution conductivity of 1.8–2.6 µS/cm depending on pH. Each combination of variables was used to produce the PEF-treated samples. A broad PEF-specific energy range (24–71 kJ/kg) was selected because established values for oilseed cakes are not available in the literature and to ensure that the final temperatures of the treated samples remained below 40 °C, thus preventing thermal denaturation. Detailed experimental parameters, including specific energy calculations, are summarized in Table 1.

The electric field and the specific energy are calculated using the following equation:

$$E = \frac{V}{d} \qquad W = \frac{I \times V \times np \times \tau}{m}$$

Table 1 PEF pretreatment parameters

Sample name	Electric field (kV/cm)	Specific energy (kJ/kg)	pH
PEF1	1.7	28	11.5
PEF2	1.7	71	11.5
PEF3	1.7	24	7
PEF4	1.7	65	7

E: field strength (V/m), V: voltage (V), d: electrode distance(m), W: specific energy (J/kg), I: electric current (A), np: pulse number, τ: pulse width (s), m: weight of the solution (kg).

Protein extraction

After PEF pretreatment, all the samples' pH was adjusted to 11.5. The extraction process was then carried out by stirring the samples for 3 h at 35 °C. The pH was checked every 20 min and adjusted as necessary using 1 M NaOH. The solutions were centrifuged with Avanti J-26SXP (Beckman Coulter, US) at 4000 rpm at 25 °C for 20 min. In this way, the supernatant was purified from residual fat, carbohydrates and fibers in pellet form and the supernatant was collected. The pH of the supernatant was adjusted to pH 4.5 with 1 M HCl and kept overnight at 4 °C for isoelectric precipitation. After isoelectric precipitation, the solutions were centrifuged at 4000 rpm at 4 °C for 10 min. The protein was then obtained as a pellet. The pellet was dispersed in distilled water, and the pH was adjusted to 7.0 with 1 M NaOH before freeze-drying with the LIO 2000P (5 Pa, Italy). A diagram illustrates the entire experimental process (Fig. 1).

Protein characterization

Extraction yield

Protein content was determined by the Kjeldahl method using a nitrogen-to-protein conversion factor of 5.3, according to AOAC (1990), and is expressed in g/L [15]. From the protein concentrations and masses of the samples, the yield of protein extraction in the rapeseed cake was calculated by the following equations:

$$\text{Protein extraction yield } (\%) = \frac{g \text{ rapeseed protein in concentrate}}{g \text{ protein in rapeseed cake}} \times 100$$

$$\text{Protein yield } (\%) = \frac{g \text{ rapeseed protein in concentrate}}{g \text{ rapeseed cake}} \times 100$$

Color

The color of the samples was measured using a ColorFlex EZ colorimeter (HunterLab, USA) to obtain L*, a*,

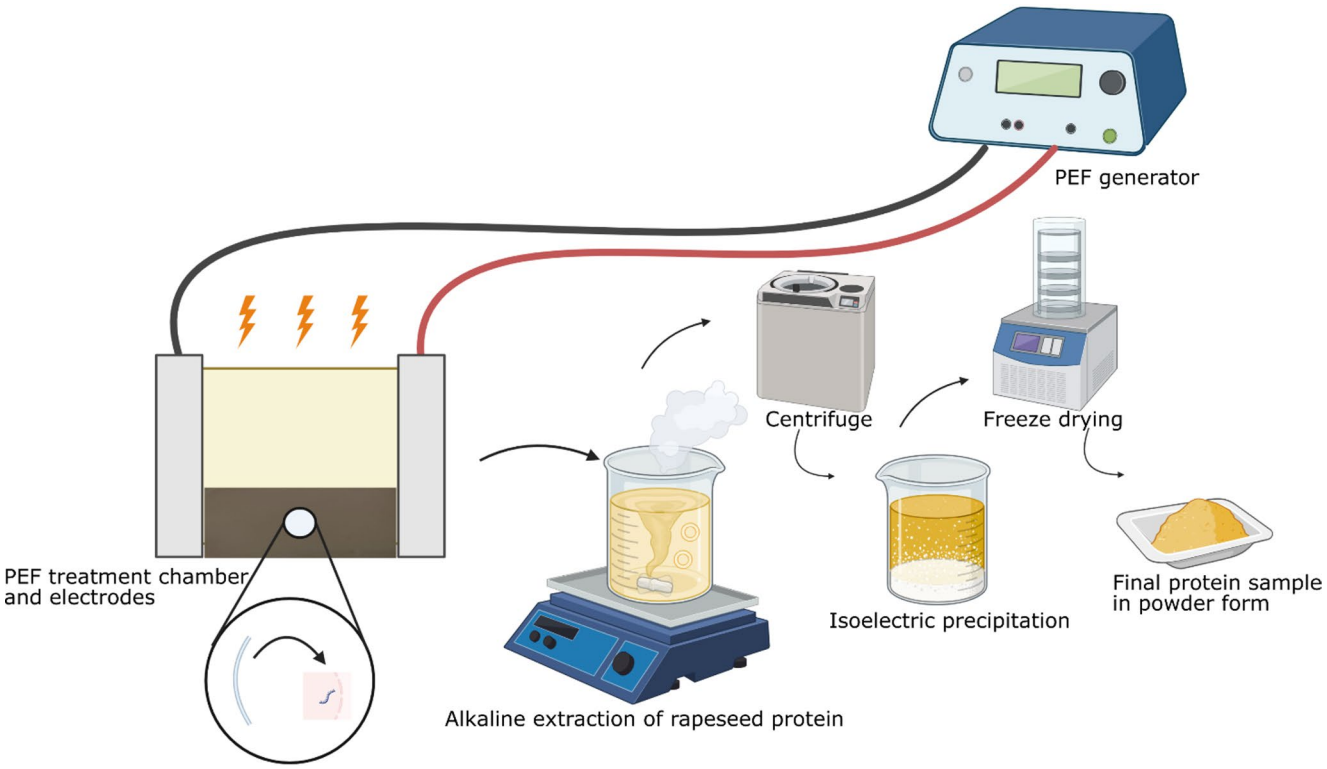

Fig. 1 The experimental workflow for rapeseed protein extraction includes PEF treatment, alkaline extraction, centrifugation, isoelectric precipitation, freeze-drying, and collection of the final protein powder

and b* values. Hue angle (h°), chroma (C), and total color difference (ΔE) were calculated according to the method described by Bashir et al. [16]

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were analyzed using a PerkinElmer Spectrum One spectrophotometer with an ATR accessory in the amide I region (1600–1700 cm^{-1}) at 4 cm^{-1} resolution with 32 scans, and four spectra were recorded per sample. After background and baseline correction, secondary structure composition was determined using Peakfit software by applying Gaussian curve fitting to the amide I band and calculating the relative peak areas corresponding to α-helix (1650–1659 cm^{-1}), β-sheet (1618–1640 and 1689–1698 cm^{-1}), β-turn (1660–1688 cm^{-1}), and unordered structures (1641–1649 cm^{-1}) based on the second-derivative spectra [17].

Differential scanning calorimetry (DSC)

Thermal properties were measured by a DSC (DSC-Q20; TA, New Castle, USA), following the procedure described by Jia et al. [18] with slight modifications. The DSC was calibrated with indium for temperature calibration. RP (2 mg) was directly weighted into the Tzero aluminum pan and 10 µL distilled water was added with a micro syringe. Distilled water was added to dissolve the protein and avoid any interference from impurities that might affect the DSC results. Pans were sealed and allowed to stand overnight at room temperature for even distribution of water. The scanning temperature and the heating rates were 20–200 °C and 5 °C/min, respectively. An empty pan was used as a reference for all measurements; every measurement was performed in triplicate, and the onset temperature (T_0), denaturation temperature (T_d) and enthalpy (ΔH) were obtained using TA universal analysis 2000 software.

Rheological analysis

The rheological analysis of the protein was determined using an MCR 102 rheometer (Anton Paar GmbH, Graz, Austria) with the plate cell, following the procedure described by Zhang N. et al. [19] with slight modifications. Due to the gelling properties of the sample, RP was prepared at a concentration of 20%. The measurement started after the gap was set to 0.0 mm and the sample holder was covered to prevent evaporation. Viscosity, shear rate, and shear stress were automatically calculated by the RheoCompass software (version 1.15, Anton Paar GmbH, Graz, Austria).

Functional properties

Water and oil holding capacity (WHC and OHC)

The WHC and OHC analysis was performed using a modification of the methods of Zhang et al. [20]. To determine the WHC, 0.1 g of the sample was placed in a 15 ml falcon of known weight. Then 5 ml of distilled water was added, and the prepared solutions were mixed with a vortex for 30 s. The samples were centrifuged using a PK110 centrifuge (Thermo, US) at 3000 rpm for 15 min. After centrifugation, the samples rested for 20 min, then the supernatant was drained. For OHC, the same procedure was used, adding 5 ml of soybean oil instead of water and centrifuging the samples at 3000 rpm for 20 min. Finally, the weight of the samples was measured again, and the WHC and OHC were calculated according to the following formula:

$$\mathrm{WHC} = \frac{(g_{W\ \mathrm{pellet}} - g_{W\ \mathrm{sample}})}{g_{W\ \mathrm{sample}}} \qquad \mathrm{OHC} = \frac{(g_{O\ \mathrm{pellet}} - g_{O\ \mathrm{sample}})}{g_{O\ \mathrm{sample}}}$$

$g_{w\ pellet}$: weight of the wet pellet, $g_{w\ sample}$: weight of the dry sample.

Emulsifying activity (EA) and emulsifying stability (ES)

EA an ES were measured using a modification of the method of Georgiev R et al. [21]. In a 50 mL Falcon tube, 1 mg of sample was weighed, followed by the addition of 10 mL distilled water and 10 mL sunflower oil. The emulsion was homogenized using Ultra Turrax (T18 digital, IKA, Germany) at 10,000 rpm for 1 min and centrifuged with Centrifuge PK110 (Thermo, US) at 1100 rpm for 5 min, then the emulsified layer and total sample height were measured in cm by using a ruler. The EA was calculated as the ratio between the height of the emulsified layer and the height of the entire contents of the tube and multiplied by 100 to give the percentage.

The samples were then placed in an 80 °C water bath for 30 min. Afterwords, they were placed in an ice bath for 5 min and centrifuged at 1100 rpm for 5 min, and the emulsifying layer was measured again. ES was calculated as the ratio between the emulsified layer after boiling and the first emulsified layer, and multiplied by 100 to express the percentage.

Protein solubility (PS)

To determine the solubility of RP, the Bradford method was followed [22]. Firstly, a calibration curve was prepared. Then, 50 mg of the sample was added to a Falcon tube containing 25 ml of distilled water. The solution had a pH of

around 6. The solutions were vortexed before centrifugation at 4000 rpm for 10 min. After centrifugation, 50 µL of the sample was mixed with 1.5 mL of reagent and waited for 20 min in the dark. The solubility was then measured with the UV-visual spectrometer UV-1601 at a wavelength of 595 nm (Shimadzu, Japan) [22].

Statistical analysis

To assess the statistical differences between the treatments, a parametric analysis of variance was performed. Post-hoc Tukey ANOVA was used ($p<0.05$), using a JMP software (SAS Institute Inc., Cary, NC).

Results and discussion

Extraction yield

Table 2 reports the protein content of concentrate (P%), protein extraction yield, and total protein yield from rapeseed cake subjected to different PEF pretreatments and control conditions. In terms of protein extraction yield, samples pretreated with PEF 2 conditions showed the highest value (31.81 ± 0.38%), followed by the control (CNT, 29.27 ± 1.10%). Among the three measured parameters, only protein extraction yield showed a significant difference (PEF2 > others, $p<0.05$), while protein content in the concentrate and total protein yield did not differ significantly between treatments ($p>0.05$). This indicates that the specific parameters applied in PEF2 were the most effective for promoting protein extraction. Despite the differences in protein extraction yields, the total protein yield, calculated as the amount of protein recovered per 100 g of cake, was relatively similar for all treatments, ranging from 8.11% to 9.32% (Table 2). Samples pretreated with PEF1 condition had the highest yields (9.32 ± 0.01%), slightly higher than the control (9.02 ± 0.66%), while PEF4 had the lowest yield (8.11 ± 0.06%). The protein content in the concentrates remained below 60%, which is typical for concentrates rather than isolates. This is due to incomplete extraction of all protein fractions and the presence of residual carbohydrates, fibers, and lipids, which limit the maximum protein content achievable under the applied extraction conditions.

Compared with other protein extraction and pretreatment technologies for oilseed cakes, the protein yields obtained in this study (27.6–31.8%) fall within the lower to moderate range reported in the literature. More intensive strategies, such as ultrasound-assisted and enzymatic extraction, can substantially increase recovery, with sunflower meal yielding up to 54.26% protein and rapeseed press cake achieving 40–60%, depending on enzyme type and extraction conditions. Electrotechnologies with higher energy inputs have also improved yields, for example, 240 kJ/kg for optimal protein recovery or 83 kJ/kg with pulsed electric fields or high-voltage discharges. The moderate yields observed are consistent with mild, low-intensity approaches that prioritize protein quality and functionality over maximum recovery [23].

Overall, both extraction and total protein yield values were comparable to those of the control, indicating that the applied PEF conditions did not significantly improve protein extraction from RC. This suggests that the electric field strength and energy input used in this study may have been insufficient to induce the degree of cellular disruption necessary for improved protein release. Supporting this interpretation, previous studies have shown that low-intensity PEF treatments (e.g., 5 kV/cm) did not significantly improve protein extraction from rapeseed green biomass (stems), a fresh, high-moisture material that differs from rapeseed cake (stems), a fresh, high-moisture material that differs from rapeseed cake. A significant improvement (28% higher in protein yield) was only observed when the electric field strength was increased to 8 kV/cm [24]. These findings are consistent with our results and suggest that the electric field strength used in our experiment was insufficient to significantly improve protein release. In addition, higher electric field strengths (≥ 8 kV/cm) were not selected because RC has low moisture content and is inherently highly conductive compared with fresh biomass. Under these conditions, applying strong electric fields can cause rapid Joule heating, electrical arcing, and material degradation. Additionally, adjusting the treatment solution to alkaline pH increases ionic strength and conductivity, which further intensifies heating at higher field strengths.

Table 2 Protein content in concentrate (*P* %), protein extraction yield, and total protein yield from rapeseed cake subjected to different PEF pretreatments and control conditions

Sample	*P* % (Protein in concentrate)	Protein extraction yield (%) (g protein in concentrate /100 g protein in cake)	Total Protein yield (%) (g protein in concentrate /100 g in cake)
PEF1	56.93 ± 1.05[a]	27.83 ± 0.04 [b]	9.32 ± 0.01 [a]
PEF2	54.56 ± 0.65 [a]	31.81 ± 0.38[a]	9.02 ± 0.11 [a]
PEF3	57.08 ± 0.43 [a]	27.57 ± 0.21 [b]	9.23 ± 0.07 [a]
PEF4	53.38 ± 0.42 [a]	28.61 ± 0.22 [b]	8.11 ± 0.06 [a]
CNT	55.21 ± 1.48 [a]	29.27 ± 1.10 [b]	9.02 ± 0.66[a]

[a–b] Values with different letters within the same column indicate significant differences ($p<0.05$)

In addition, according to Patra et al., these low extraction yields can be attributed to such limitations. Residual oil (approximately 6–7%) in RC can also affect protein extractability. In this study, the RC used contained 6.69% residual oil. Partial defatting can improve selectivity for cruciferin- or napin-rich extracts, but excessive oil removal reduces protein flexibility, increases rigidity, and lowers yield. Mild extraction methods help preserve protein quality while maximizing recovery [25].

Color

For plant-based proteins such as RP, color influences consumer acceptance in products like meat alternatives and supplements [26]. Intrinsic components such as phenolics, chlorophylls, and carotenoids affect color and are sensitive to processing and pH [27]. PEF pretreatment slightly altered the color parameters of RPC (Table 3), with higher energy treatments (PEF2 and PEF4) increasing yellowness (b*) and chroma, and reducing redness (a*), while lightness (L*) remained largely unchanged. These changes may result from pigment release or physico-chemical transformations induced by PEF [28, 29]. However, the total color difference (ΔE) remained below the visual perceptual threshold (ΔE < 2), indicating that the changes are not visually discernible [30]. Overall, PEF-induced color changes are minor and unlikely to affect protein functionality or product quality.

Fourier transform infrared spectroscopy (FTIR)

RPCs were analyzed to investigate their secondary structural changes after PEF pretreatment. That was analyzed by ATR-FTIR in the amide I region (1600–1700 cm^{-1}), assigning the bands to the conformations α-helix, β-turn, β-sheet, and random coil according to [17]. Secondary structure content was quantified by Gaussian curve fitting of multiple peaks, which are summarized in Fig. 2(A–E). Under all conditions, β-sheet was the predominant structure in all samples (44.76–46.71%). In the samples pretreated with PEF, the β-sheet content of the resulting RPC showed a slight increase but remained statistically unchanged, with no significant difference observed (Fig. 2F). Similar findings were reported [20] who observed that PEF pretreatment of rapeseed seeds caused structural changes in rapeseed proteins (RPs), including a slight increase in β-sheet structures and a decrease in α-helix content. However, in this study, the α-helix content in RPC tended to increase (13.79% and 14.00%, respectively) compared with the control (12.73%), whereas higher energy treatments (PEF2, PEF4) did not increase α-helix content (12.16% and 11.90%) in samples pretreated with low specific energy, specifically PEF1 (28 kJ/kg) and PEF3 (24 kJ/kg), regardless of the pH used during treatment. This increase in α-helix content may be attributed to slight reorganization and refolding of the polypeptide chain induced by PEF pretreatment [20, 31]. However, when the specific energy increased (PEF2 and PEF4), α-helix content remained almost unchanged, suggesting that high energy inputs during PEF pretreatment may limit further conformational rearrangement in these regions. β-turn content ranged from 25.62% to 28.52%, and unordered structures from 13.23% to 15.55%, with no significant changes due to PEF.

The persistence of the content of β-sheet, β-turn, and unordered structure content across all treatments suggests that the PEF-induced structural changes are primarily restricted to the more flexible α-helical regions. While the β-sheet-rich structure of RPC appears to exhibit strong structural resilience under the specific PEF conditions used in this study.

Differential scanning calorimetry (DSC)

DSC was used to evaluate the thermal behavior of RPC from PEF-pretreated RC.

[a–b] Values with different letters within the same column indicate significant differences ($p < 0.05$).

The data in Table 4 show the onset temperature (T_0), the denaturation temperature (T_d) and the denaturation enthalpy (ΔH) of the native (CNT) and rapeseed protein samples obtained from RC pretreated with PEF. All samples exhibit a denaturation peak occurred between 130 and 134 °C. This result is consistent with a previous study by [32], in which the temperature of the denaturation peak of native RPC was 132.47 °C. No significant differences were observed in denaturation temperatures among samples; however, a slight decrease in onset temperature and ΔH for PEF4 suggests a trend toward reduced thermal stability in proteins extracted from RC pretreated with higher specific energy. A

Table 3 Color parameters of RPC obtained from PEF pre-treated and control samples

Sample	L*	a*	b*	ΔE	C	h°
CNT	52.17 ± 0.12[a]	7.00 ± 0.03[a]	23.11 ± 0.08[c]	0.12 ± 0.02	24.15 ± 0.08[b]	73.16 ± 0.06[c]
PEF1	51.76 ± 0.19[b]	7.10 ± 0.04[a]	22.29 ± 0.07[d]	0.94 ± 0.10	23.39 ± 0.07[c]	72.34 ± 0.09[e]
PEF2	52.22 ± 0.35[a]	6.83 ± 0.09[b]	23.93 ± 0.12[a]	0.88 ± 0.12	24.88 ± 0.14[a]	74.07 ± 0.13[a]
PEF3	51.31 ± 0.20[b]	7.00 ± 0.06[a]	22.56 ± 0.14[d]	1.04 ± 0.08	23.62 ± 0.15[c]	72.75 ± 0.03[d]
PEF4	52.25 ± 0.12[a]	6.84 ± 0.05[b]	23.44 ± 0.10[b]	0.39 ± 0.04	24.42 ± 0.10[b]	73.72 ± 0.04[b]

[a–d] Values with different letters within the same column indicate significant differences ($p < 0.05$)

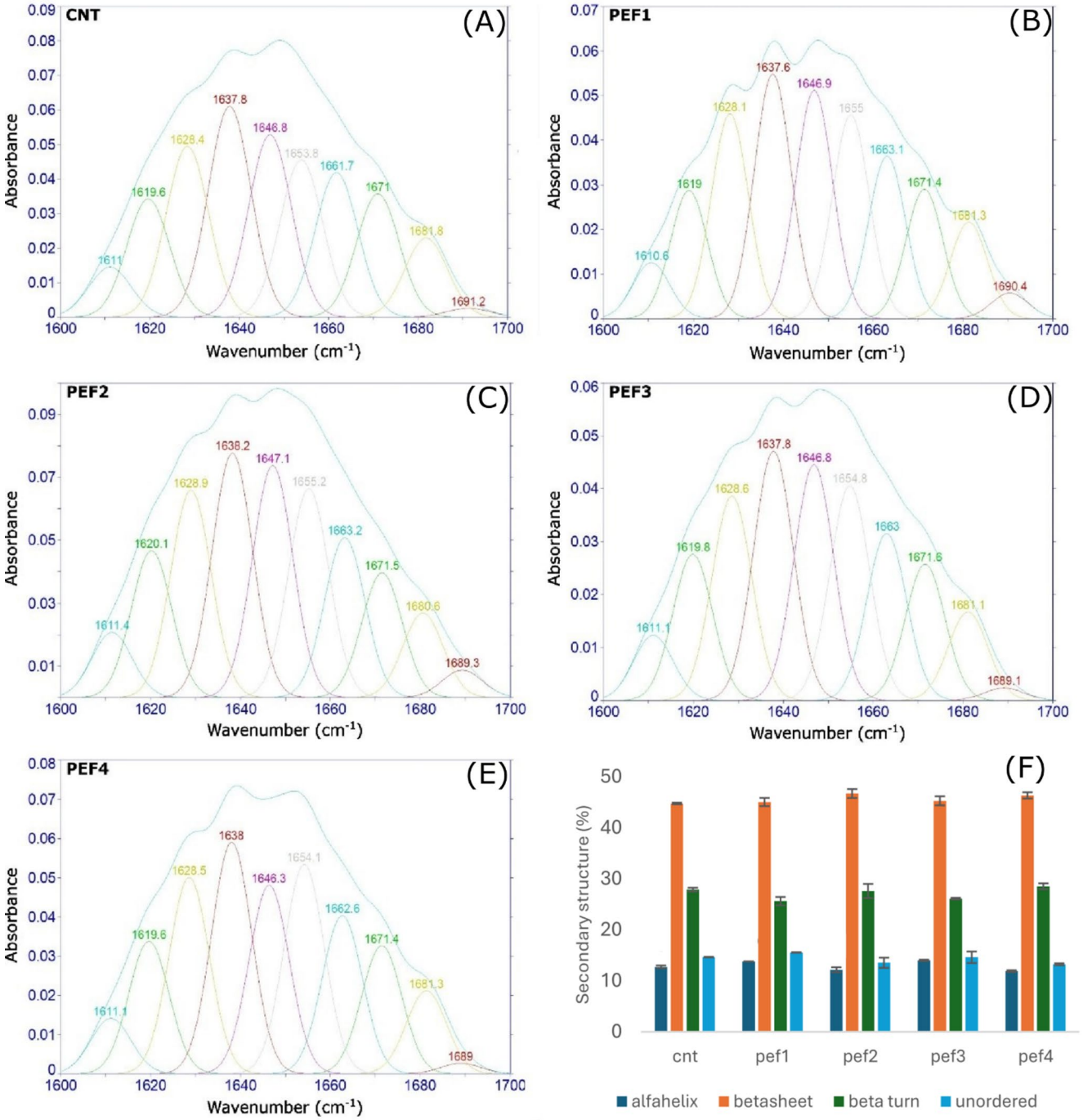

Fig. 2 Fourier-transform infrared (FTIR) spectra and secondary structure analysis of rapeseed protein (RP). Upper panels (**A**–**E**): FTIR spectra in the amide I region for native RP (**A**, CNT) and PEF-treated RP at different pH values and specific energies: **B** (PEF1), **C** (PEF2), **D** (PEF3), **E** (PEF4). Bottom panel: Gaussian fitting curves of the amide I region for the corresponding samples, used to deconvolute secondary structure components. Panel **F**: Quantitative distribution of secondary structures (α-helix, β-sheet, β-turn, random coil) for control (CNT) and PEF-treated samples (PEF1–PEF4)

Table 4 Effect of PEF treatment on onset temperature (T_0), denaturation temperature (T_d) and enthalpy (ΔH) of rapeseed proteins (RPs)

Sample	T_0 (°C)	T_d (°C)	ΔH (J/g)
CNT	133.89 ± 1.23[a]	134.06 ± 1.26[a]	2.56 ± 0.75[a]
PEF1	132.07 ± 2.10[ab]	132.23 ± 2.09[a]	2.20 ± 0.08[a]
PEF2	132.16 ± 1.28[ab]	132.45 ± 1.33[a]	2.45 ± 0.34[a]
PEF3	131.49 ± 0.94[ab]	132.18 ± 1.48[a]	2.68 ± 0.34[a]
PEF4	129.72 ± 0.62[b]	130.24 ± 0.87[a]	1.56 ± 0.41[a]

[a-b] Values with different letters within the same column indicate significant differences ($p < 0.05$)

reduced enthalpy might reflect a more flexible or partially unfolded structure, which could facilitate higher protein solubility due to the exposure of hydrophilic residues [33].

Rheological analysis

Figure 3 shows the flow curves (shear stress vs. shear rate Fig. 3A) and viscosity curve (apparent viscosity vs. shear rate Fig. 3B) of RP, extracted from PEF-pretreated RC. The apparent viscosity values of the RP samples decreased with increasing shear rate. All samples, including the untreated control (CNT), exhibit a typical shear-thinning behavior, characterized by a decrease in apparent viscosity with increasing shear rate. These results were consistent with those of the study [19], who reported similar effects in rapeseed protein isolate treated with dynamic high-pressure micro fluidization. This non-Newtonian behavior is commonly observed in protein dispersions and indicates structural reorganization under shear stress [34, 35].

RP extracted from control and PEF-treated samples generally exhibited a slight decrease in apparent viscosity of 0.7–1.6 log mPa·s from 0 to 100 shear rate (1/s), likely due to PEF-induced changes in the cake matrix that altered the protein environment prior to extraction and affected protein-protein interactions and molecular mobility. However, viscosity increased significantly under PEF4 conditions (68 kJ/kg at pH 7.0). Thus, CNT samples had a viscosity of 29.2 mPa·s while PEF 4 had a viscosity of 44.0 mPa·s at a shear rate of 100 (1/s). Similarly, gel strength increased by PEF treatment directly on pea protein isolate with the treatment conditions of 5, 10, and 20 kV/cm and frequencies of 50 Hz and 20 kHz [36]. This contrasting behavior can be directly attributed to the effect of pH on protein solubility and aggregation. At pH 7.0, near the solubility minimum of RP, proteins have reduced net charge, which decreases electrostatic repulsion and favors hydrophobic interactions. Under these conditions, the high PEF-specific energy likely induces protein unfolding and exposes hydrophobic regions, promoting protein aggregation rather than solubilization, resulting in higher viscosity [29, 37, 38].

Overall, these results highlight the critical role of pH in determining whether PEF treatment promotes protein solubilization or aggregation, which directly influences the rheological properties of RP. The interplay between pH and PEF energy underscores the need for further studies to optimize PEF parameters for functional protein extraction from plant matrices [31, 39].

Water and oil holding capacity (WHC and OHC)

Proteins have the unique ability to bind both oil and water, which is essential for their role as stabilizers and emulsifiers in the food industry. This ability improves the texture, stability and sensory properties of food. Recent research has shown that treatment with PEF can alter these binding properties by changing the protein structure, exposing hydrophobic regions and disrupting intermolecular bonds [11].

The WHC of RP was significantly affected by the different PEF pretreatment conditions (Table 5). The CNT showed the highest WHC (4.82 ± 0.22 g/g). A significant decrease in WHC was observed with PEF2 (3.68 ± 0.06 g/g) and PEF3 (3.96 ± 0.03 g/g), both of which were significantly different from the control. This decrease in WHC could be due to an increase in hydrophobicity caused by protein dissociation

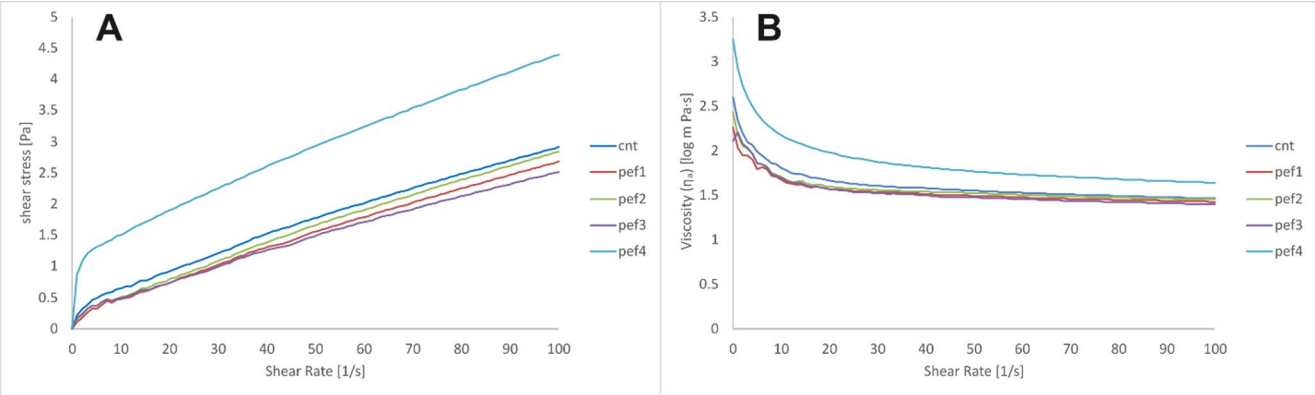

Fig. 3 Rheological characteristics of RP pretreated by PEF and control samples **A** Flow Curves (Shear Stress vs. Shear Rate), **B** Apparent Viscosity (log mPA. s)vs. Shear Rate

Table 5 Techno-functional properties of the RP extracted from PEF-pretreated cake and untreated samples (CNT)

[a-c]Values with different letters within the same column indicate significant differences ($p < 0.05$)

SAMPLE	WHC (g/g)	OHC (g/g)	PS (%)	EA (%)	ES(%)
CNT	4.82 ± 0.22[a]	5.31 ± 0.10[a]	12.67 ± 0.30[b]	42.35 ± 2.8[a]	98.33 ± 2.89[a]
PEF1	4.63 ± 0.34[ab]	5.81 ± 0.38[a]	12.38 ± 0.43[b]	42.84 ± 1.40[a]	92.88 ± 3.45[a]
PEF2	3.68 ± 0.06[c]	5.69 ± 0.63[a]	12.98 ± 0.48[b]	42.39 ± 2.99[a]	91.74 ± 2.61[a]
PEF3	3.96 ± 0.03[c]	5.73 ± 0.41[a]	12.34 ± 0.43[b]	42.64 ± 1.34[a]	98.25 ± 3.04[a]
PEF4	4.12 ± 0.29[bc]	5.86 ± 0.36[a]	20.26 ± 0.43[a]	40.99 ± 0.88[a]	98.33 ± 2.89[a]

induced by PEF application. Additionally, PEF would alter the ability of the protein to interact with the solvent due to pH influenced processes. On the other hand, according to [40], a moderately intensive PEF treatment of peas, rice and gluten at different pH values (at pH 5 and 6) can lead to the gluten proteins interacting with water near the isoelectric point mainly via exposed hydrophilic groups. At lower pH values, water is also trapped in the protein network due to interactions with buried groups. Furthermore, the reduction in WHC may be attributed to the complex composition of the RC matrix. PEF causes electroporation, which facilitates protein release and promotes the co-extraction of insoluble fibers and phenolic compounds, such as sinapic acid [39, 40]. These non-protein constituents can physically block water-binding sites on the protein surface or interfere with the formation of a cohesive hydrated network. The OHC of RPC, instead, was found not to be significantly affected by that PEF pretreatment (Table 5). Zhang et al. [41] showed that OHC of RPC, increased when the voltage was higher than 25 kV, suggesting that the absence of significant changes in the present study is likely due to the comparatively lower electric field strength applied.

Protein solubility

Protein solubility refers to the ability of proteins to disperse in aqueous systems without precipitating or aggregating, and is influenced by factors such as pH, temperature, ionic strength, and the presence of solutes [42]. Improving protein structural properties can enhance water–protein interactions and thus increase solubility.

In this study, RPC extracted from PEF-pretreated RC were evaluated for solubility. Among the pretreatment conditions tested, only PEF4 resulted in a significant improvement in solubility, with an increase of approximately 60% compared to the control, while no significant changes were observed for the other treatments (Table 5). This suggests that solubility enhancement is highly dependent on the specific PEF conditions applied.

Previous studies on soy protein isolate have shown that PEF treatment can induce protein polarization, subunit dissociation, and partial molecular unfolding, which may enhance protein–water interactions and improve solubility [43]. In the present study, the improved solubility observed for PEF4 may be attributed to the combined effects of higher specific energy input and neutral pH, which could promote subtle structural rearrangements and facilitate water penetration by weakening intermolecular interactions.

Studies by Wang et al. [13] and Jiang et al. [44] demonstrated that PEF treatment, particularly in combination with alkaline pH, can significantly enhance protein solubility by promoting partial unfolding and disaggregation into smaller, more soluble particles. However, unlike these studies, which focused on isolated proteins, no significant structural changes or temperature increases were observed in the PEF4-treated samples in the present work. Moreover, only limited precipitation occurred at pH 11, indicating that the alkalinity applied was insufficient to fully dissociate protein subunits, consistent with previous reports [41, 45].

The differences between literature findings and the present results may be attributed to the complex matrix of RC, where proteins are embedded within a heterogeneous structure containing non-protein components that can influence PEF-induced effects. Nevertheless, the observed increase in solubility highlights the potential of PEF4 pretreatment, particularly when combined with optimized pH conditions, to enhance the functional properties of RPCs.

Emulsifying activity and emulsifying stability

EA and ES are important parameters for evaluating the emulsification performance of proteins. Table 5 presents the EA and ES values for the different samples. No statistical differences were observed among the treatments, indicating that the PEF pretreatment conditions did not cause sufficient structural changes to affect the emulsifying properties.

Although no change in EA was observed in soy protein isolate treated with PEF at moderate electric field strengths (5 kV/cm) combined with pH shifts, EA has been reported to improve at higher electric field intensities [13]. This suggests that the electric field applied in this study may not have been sufficient to induce significant changes in surface hydrophobicity or charge distribution, which are critical for modifying emulsification behavior.

While PEF treatment can alter protein conformation, unfolding, or aggregation, as observed in viscosity and rheological measurements, these structural modifications may not always result in changes in EA or ES. One reason is that emulsifying properties are largely governed by the ability of protein molecules to rapidly adsorb and rearrange at the

oil-water interface, forming a cohesive and stable interfacial layer. Even partially unfolded proteins can maintain or enhance interfacial adsorption, compensating for structural alterations elsewhere in the molecule [13].

Additionally, proteins with increased disorder in their secondary structure can maintain fast adsorption kinetics at the interface, facilitating stable emulsions even when some conformational changes occur [46]. Therefore, despite observable structural modifications in other assays, the combination of limited unfolding, maintained interfacial adsorption capacity, and moderate PEF conditions likely explains why EA and ES remained unchanged in these samples.

Higher protein solubility typically enhances emulsifying capacity; however, in this study, although PEF4 showed increased solubility, its emulsifying properties remained essentially unchanged across all samples. This suggests that PEF treatment enhanced the exposure of hydrophilic residues, improving solubility, while the hydrophobic regions responsible for stabilizing oil-water interfaces, and thus emulsifying capacity, were largely unaffected.

Principal component analyses (PCA)

PCA was used to evaluate the relationships among RP samples subjected to different PEF treatments, based on their techno-functional and structural properties, including secondary structure composition, WHC, OHC, EA, ES, PS, and Td. The score plot (Fig. 4A) revealed a clear separation of samples according to treatment conditions. The PEF4 sample was positioned in the first quadrant, while the CNT sample was located in the fourth quadrant, indicating distinct differences between untreated and PEF-treated samples.

Along Dim1, CNT was negatively associated with PEF2 and PEF4, whereas along Dim2, CNT showed a positive association with PEF4. PEF treatment at neutral pH resulted in greater separation from the CNT sample, particularly at low specific energy.

The loading plot (Fig. 4B) showed that the first principal component (Dim1), accounting for 54.8% of the total variance, was positively correlated with OHC, β-sheet content, PS, β-turn content, and ES, and negatively correlated with unordered structures, EA, α-helix content, WHC, and Td. The second principal component (Dim2), explaining 23.3% of the variance, was positively correlated with Td, WHC, ES, β-turn content, and PS, and negatively correlated with unordered structures, EA, α-helix content, OHC, and β-sheet content. Together, Dim1 and Dim2 explained 78.1% of the total variability among samples. The overall mechanism diagram of the PEF effect, together with pH adjustment, is shown in Fig. 5.

Conclusion

This study demonstrates that PEF pretreatment combined with pH adjustment during the treatment of RC can effectively modify the functional and structural properties of RP. In particular, PEF pretreatment at high specific energy applied at neutral pH (pH 7.0), near the isoelectric point of RP, significantly enhanced protein solubility and resulted

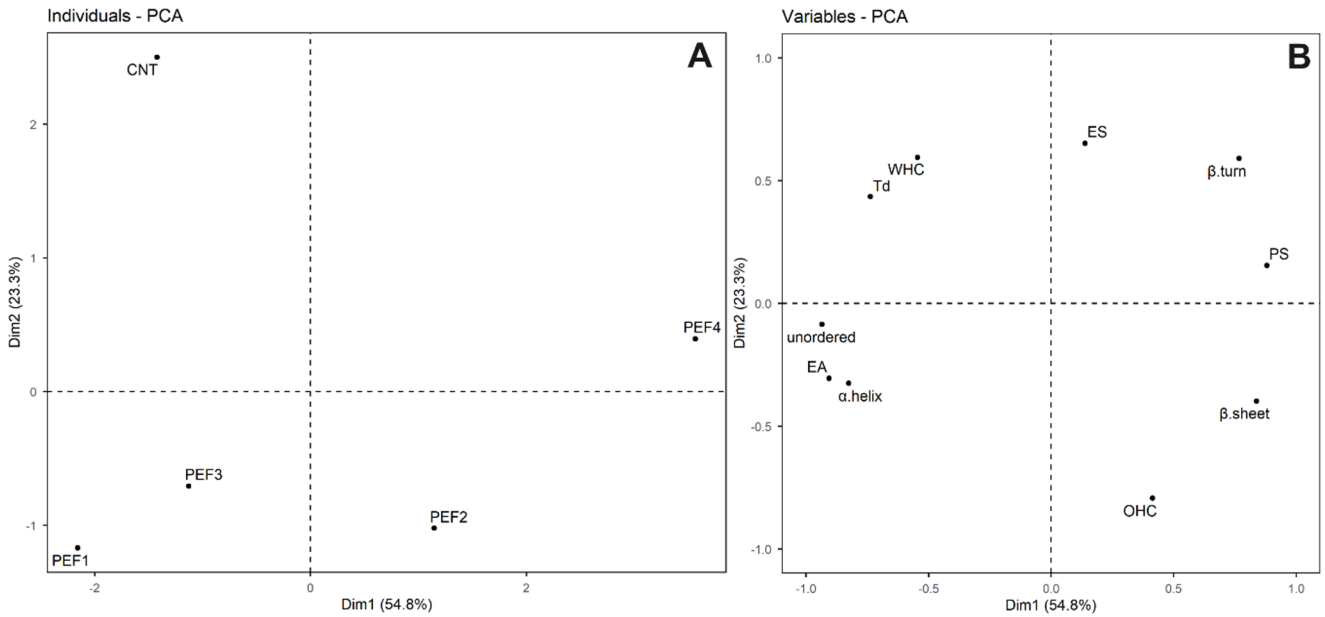

Fig. 4 The principal component analysis of native RP and modified RPs formed by PC1 and PC2: factor loading (**A**) and factor scores (**B**)

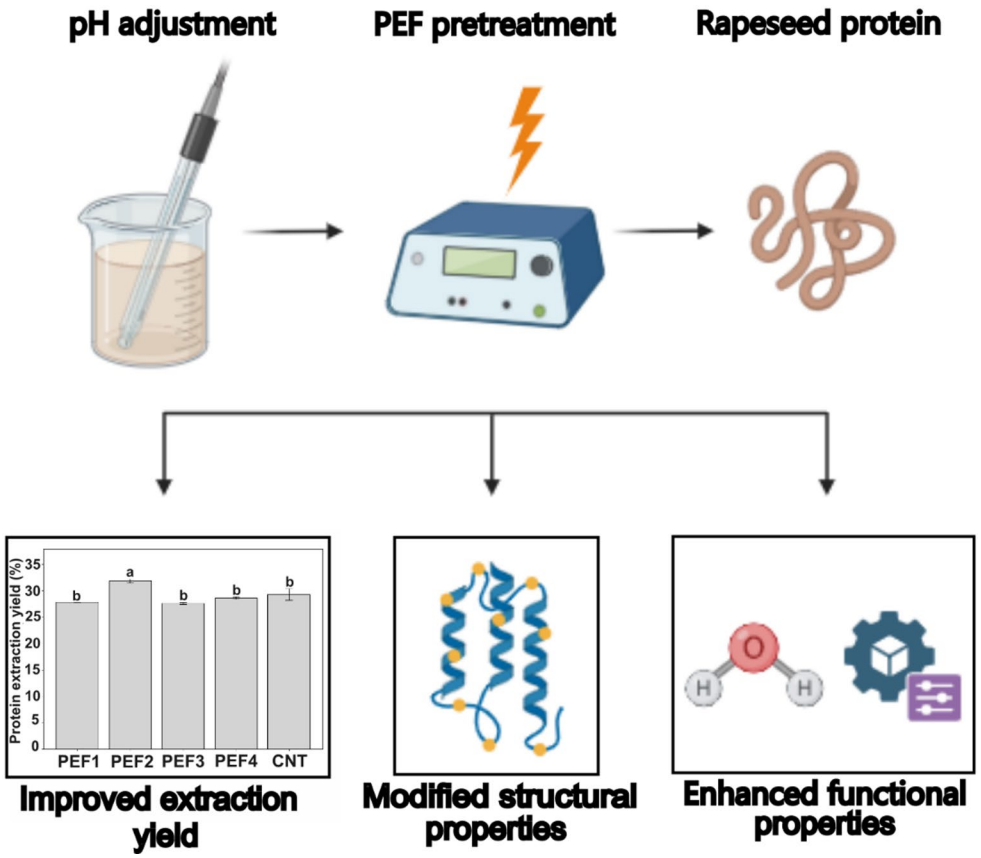

Fig. 5 Mechanism diagram showing the effect of PEF pretreatment combined with pH adjustment on the extraction yield, structural, and functional properties of rapeseed protein

in higher viscosity, likely due to a combination of moderate electrostatic repulsion and partial protein unfolding, as indicated by the observed decrease in α-helix content. This unfolding exposes hydrophilic residues to the solvent, promoting solubility, while other properties, such as WHC, were reduced, and OHC, EA, and ES remained largely unchanged. These results indicate that PEF can selectively influence specific functional properties of RP while leaving others unaffected.

From an application perspective, the improved solubility and viscosity of RP achieved through PEF pretreatment are particularly relevant for industrial food formulations where protein dispersibility and flow behavior are critical, such as in beverages, liquid emulsions, sauces, and plant-based dairy alternatives. Enhanced solubility can facilitate protein incorporation, reduce sedimentation, and improve processing stability without compromising emulsifying performance. Furthermore, the ability to tailor functional properties without extensive chemical modification aligns with current industrial demand for mild, clean-label, and sustainable processing technologies.

However, the precise molecular mechanisms by which PEF-induced electroporation, in combination with pH effects, modulates protein structure and functionality remain to be fully elucidated. Future studies applying PEF to extract protein, along with detailed structural analyses such as circular dichroism, fluorescence spectroscopy, or molecular dynamics simulations, could provide deeper insights and guide the optimization of processing conditions. Overall, these findings support the potential of PEF treatments to develop rapeseed protein products with tailored functionalities for industrial applications.

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Data availability The data that supports the findings of this study are available on reasonable request from the corresponding author.

Declarations

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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