Sustainable Food Technology

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/d6fb00093b

Impact of canola seed dehulling and pressing temperature on the microstructure of canola meals, protein structure within the meals, and protein isolate extraction and properties

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Canola hulls are rigid structures rich in lignocellulosic polymers and tannins. With the growing demand for sustainable proteins and advances in dehulling technology and hull utilization, dehulling canola seeds before pressing could be a viable way to improve the meal and protein quality. This study examined the combined effects of dehulling and pressing temperature (40, 80, and 120 °C) on canola meals and their derived protein isolates. The removal of ~80–85% of hulls reduced heat-induced aggregation and preserved more of the meal's native microstructure during pressing, especially at 120 °C. Moreover, the secondary and tertiary structures of meal proteins were better preserved, particularly at higher treatment temperatures. These improvements were attributed to the reduction of friction-induced effects and covalent modifications of proteins during the press resulting from dehulling. The above-mentioned observations directly corresponded with protein extraction efficiency values under alkaline (pH 11) and salt (0.8 M NaCl) conditions. Dehulling significantly increased the extraction efficiency of alkaline and salt isolates by an average of 10.49% and 8.12%, respectively, with the differences between non-dehulled and dehulled meals becoming more pronounced as the pressing temperature increased. The observed improvements were associated with higher extraction of unfolded cruciferins and aggregates from dehulled meals, particularly under alkaline conditions. Dehulling also improved the isolate protein content; however, its effects on isolate color, protein structure and aqueous solubility were more nuanced. Overall, dehulling can be an effective technique for preserving the structure of canola meal, increasing protein thermal stability during pressing, and improving the isolate extraction efficiency and protein content.

Received 25th March 2026
Accepted 24th May 2026

DOI: 10.1039/d6fb00093b

rsc.li/susfoodtech

Sustainability spotlight

The recent surge in research on the utilization of canola as a sustainable protein source for human consumption has highlighted the need for a focused investigation into the combined effects of dehulling and pressing temperature to improve the qualitative and structural properties of canola meals and their derived protein isolates. We believe that our results provide novel insights relevant to the optimization of canola oil extraction processes, the utilization of canola meal for food and food-related applications, and the production of protein isolates from the meal. These insights align with the United Nations' Sustainable Development Goals, specifically Goal 2 (Zero Hunger), Goal 12 (Responsible Consumption and Production), and Goal 13 (Climate Action).

1. Introduction

The increasing demand for sustainable protein ingredients has led to growing interest toward agricultural by-products, among which canola/rapeseed meal holds a great potential as an underutilized yet abundant source of high-quality protein.[1] This potential has been increasingly recognized over the past decade, sparking a surge in studies on canola meal and its protein for diverse food science applications, ranging from biomaterials and packaging films to protein isolates and food products.[2,3] While this trend is encouraging, the majority of these studies have used non-pressed or cold-pressed canola meals as their starting materials. This has created a discrepancy with current industrial reality, where canola meal is predominantly produced from seeds exposed to multiple high-temperature (up to 140 °C) steps during oil extraction including cooking, pressing, solvent extraction and desolventizing-toasting.[4] Protein from desolventized-toasted canola meal is known to exhibit poor nutritional and functional properties due to extensive denaturation and chemical

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modification during the above-mentioned steps, creating a need for the implementation of milder oil extraction conditions.[1] On the other hand, complete elimination of heat from the oil extraction process is unlikely to be feasible, since elevated temperatures offer several benefits, such as reduced oil viscosity (which improves its flow and recovery during pressing), and enable enzymatic and microbial deactivation.[5] Therefore, to enable the successful scale-up of canola-based foods and materials from laboratory to industrial production, which will require large quantities of high-quality canola meal, it is essential to explore less severe and balanced heat treatment conditions, along with complementary strategies to better preserve the quality of meal and its protein.

Canola seeds consist of two main anatomical components: the embryo and the hull. The embryo accounts for ~82% of the seed weight and serves as the primary storage tissue for nutrients, containing approximately 97% of total seed oil and 89% of total seed protein.[6] It is also rich in phenolic compounds, containing most of the sinapine and sinapic acid in the seed.[7] In contrast, the hull comprises ~18% of the seed weight and serves as a protective layer surrounding the embryo. Although low in oil and protein, it is rich in polysaccharides and lignin, accounting for around 73% of neutral detergent fiber (NDF), 80% of acid detergent fiber (ADF) and 95% of acid detergent lignin (ADL) of the seed.[6] The hull also contains nearly all of the condensed tannins in canola while exhibiting much lower contents of sinapine and sinapic acid than the embryo.[7,8] The mechanical stress imposed by the rigid hull during seed pressing, together with the potential interactions of hull components with proteins during both oil and protein extraction, can deteriorate the quality of canola proteins.[9] Therefore, dehulling canola seeds may be a beneficial complementary strategy to low-temperature oil pressing and improve the quality of canola meal and its proteins.

Although commonly performed on major oilseeds such as soybean and sunflower, dehulling has not been widely implemented in the canola oil industry due to inefficient hull–kernel separation techniques, concerns about oil retention in the hull fraction, potential reductions in the efficiency of mechanical pressing, and the historically low value of canola meal.[9] However, recent research has proposed specific seed conditioning followed by rolling-based dehulling that can efficiently separate hulls and kernels, minimize kernel fragmentation, and achieve near-complete hull removal.[10] Other separation techniques tailored for canola have reported the removal of up to 95% of hulls, with an associated oil loss of only around 10%.[11] Moreover, the hull fraction can be subjected to separate oil extraction,[12] or be valorized in novel applications such as the production of fuel pellets[13] and insulation boards.[14] Furthermore, although dehulling may reduce oil recovery during pressing, not only can the remaining oil be extracted efficiently through solvent extraction[15] but dehulling may also improve the efficiency of solvent extraction.[16] Beyond the technical aspects, dehulling can improve the nutritional quality of canola meal for animal feed by removing hull-associated anti-nutritional factors.[17,18] Furthermore, dehulling has been shown to reduce the content of phenolic compounds and phytic acid and improve the *in vitro* protein digestibility of canola protein isolates targeted for food applications.[19] In parallel to the above-mentioned advancements, the growing interest in sustainable proteins may transform canola protein from a by-product of oil extraction into a co-product and valuable source of revenue for the canola industry, making efforts to preserve it through low-temperature processing and dehulling highly worthwhile. This highlights a need for a focused investigation into the combined effects of dehulling and pressing temperature on the qualitative and structural properties of canola meals and their derived protein isolates, which, to the best of the authors' knowledge, has never been systematically studied before.

Based on the aforementioned gaps and opportunities, the two main hypotheses of this study were as follows: (1) dehulling of canola seeds would enhance the preservation of the meal structure and its proteins, especially at higher temperatures, and (2) dehulling would also improve the extraction efficiency of canola protein isolates, with these improvements becoming more pronounced as the heat treatment temperature increases. To test these hypotheses, non-dehulled and dehulled canola seeds were subjected to twin-screw pressing at three temperatures of 40 °C, 80 °C and 120 °C. The resulting meals (six samples) were evaluated for qualitative and structural characteristics, followed by protein extraction using both alkaline and salt (NaCl) methods to produce twelve protein isolates. The isolates were then evaluated for extraction efficiency, protein content, color, aqueous solubility, and compositional and structural properties.

2. Materials and methods

2.1. Materials

Canola seeds were purchased from Highwood Crossing Foods Ltd (AB, Canada). All other chemicals were purchased from Sigma Aldrich (ON, Canada) and used without further purification.

2.2. Canola meal preparation

Canola seeds (6.03 ± 0.12% moisture) were fed into a Dimo's Barley Pearler (Dimo's Laboratories, MB, Canada) to split the seeds and detach hulls from embryos. To produce dehulled seeds, an air current was then used to remove the majority of hull components (~80–85%). Next, to simulate the shear environment of screw-pressing at controlled temperatures and evaluate the combined effects of shear and temperature on canola protein, non-dehulled and dehulled seeds were processed using a parallel twin-screw extruder with barrel temperature control (Process 11, Thermo Fischer Scientific, MA, USA) at 40, 80, and 120 °C. The screw speed was set to 130 rpm, resulting in ~30 s of seed exposure to heat and shear. The resulting press cakes were defatted with *n*-hexane at room temperature to produce canola meals and stored at −18 °C until further analyses. The following six canola meal samples were produced: non-dehulled meals treated at 40 °C (ND40), 80 °C (ND80) and 120 °C (ND120) and dehulled meals treated at 40 °C (D40), 80 °C (D80) and 120 °C (D120).

2.3. Canola protein extraction

Protein isolates from non-dehulled and dehulled canola meals were obtained using alkaline and salt extraction. For alkaline extraction, meals were dispersed in an aqueous alkaline solution (1 : 10, meal : solvent) and stirred at 40 °C and 800 rpm, with the pH maintained at 11 using 0.1 and 1 M NaOH or HCl. After 2 h, mixtures were centrifuged at 3350 × *g* for 10 min at 25 °C, and the supernatants were dialyzed (3.5 kDa MWCO) at 4 °C. After 4 days, the contents of the dialysis bags were freeze-dried and stored at −18 °C until analysis. The following six protein isolates were produced *via* alkaline extraction (NDA and DA groups, where A stands for alkaline extraction): ND40A, ND80A, ND120A, D40A, D80A and D120A.

Salt extraction followed the same procedure, except that a 0.8 M NaCl solution was used as the extraction medium without chemical addition. The following six protein isolates were produced *via* salt extraction (NDS and DS groups, where S stands for salt extraction): ND40S, ND80S, ND120S, D40S, D80S and D120S.

2.4. Protein content and extraction efficiency

Protein content of the meals and isolates was measured using a Nitrogen Analyzer model FP-428 (Leco Corporation, MI, USA) with a protein conversion factor of 5.7.[20] The protein extraction efficiency of the isolates was calculated according to the following equation:

$$\text{protein extraction efficiency (\%)} = \frac{\text{isolate protein weight}}{\text{meal protein weight}} \times 100$$

2.5. Color assessment

Color analysis of the meals and isolates was conducted based on the CIELAB color space using a CR-410 chroma meter (Konica Minolta Inc., Tokyo, Japan). Instrument calibration was performed with a standard white plate, and the L^* (lightness), a^* (green [−] to red [+]) and b^* (blue [−] to yellow [+]) values were recorded at room temperature.

2.6. Particle size distribution

Particle size distribution of canola meal suspensions (10 mg mL^{-1}, shaken overnight at room temperature) was measured using a Mastersizer 3000 (Malvern Instruments Ltd, Malvern, UK). Measurements were performed with the refractive indices of the meal and dispersant (water) set to 1.45 and 1.33, respectively.

2.7. Scanning electron microscopy (SEM)

Canola meals were mounted on conductive carbon tapes and coated with a thin layer of gold (~5 nm) using a sputter-coater (SEMprep 2, Nanotech, UK). Imaging was performed using a Zeiss Sigma 300 VP field-emission scanning electron microscope (Carl Zeiss AG, Germany) at an accelerating voltage of 5 kV and magnifications of 200× and 1000×.

2.8. Fourier-transform infrared (FT-IR) spectroscopy

Ground mixtures of KBr and canola meal or protein isolates were pressed into transparent pellets and analyzed using a Nicolet 6700 FT-IR spectrophotometer (Thermo Fisher Scientific Inc., MA, USA). Spectra were collected over 128 scans at a resolution of 4 cm^{-1}. The spectrum of a pure KBr pellet was also recorded and subtracted as background. Fourier self-deconvolution of the amide I region (1700–1600 cm^{-1}) was performed using Omnic 8.1 software with a bandwidth of approximately 25 cm^{-1} and an enhancement factor of 2.5. The percentages of protein secondary structure were calculated according to the analysis of second derivative and peak fitting using PeakFit v4.12 (SeaSolve Software Inc., Framingham, USA).

2.9. Intrinsic fluorescence spectroscopy

Protein suspensions (1 mg mL^{-1}) were prepared in phosphate-buffered saline (PBS, pH 7.4) and subjected to fluorescence spectroscopy using a SpectraMax M3 spectrophotometer (Molecular Devices, CA, USA) at an excitation wavelength of 280 nm. Emission spectra were recorded from 290 to 400 nm with a slit width of 2 nm at 25 °C.

2.10. Size-exclusion high-performance liquid chromatography (SE-HPLC)

Apparent molecular weight (M_w) distributions of the protein isolates were carried out using a method described by Defaix *et al.*[21] with minor modifications. Protein solutions (1 mg mL^{-1}) were prepared in a mobile phase containing 54.9% water, 45% acetonitrile and 0.1% trifluoroacetic acid (TFA). After 2 h of shaking at room temperature and mild sonication, protein solutions were filtered through 0.45 μm syringe filters. SE-HPLC analysis was performed using an Agilent series 1100 system (Agilent Technologies, CA, USA) equipped with a BioSep-SEC-S2000 column (300 × 7.8 mm, 5 μm, Phenomenex, CA, USA) over 30 min at a flow rate of 0.6 mL min^{-1} and a detection wavelength of 214 nm. A protein standard solution was also analyzed under identical conditions, and a logarithmic calibration curve was plotted using the retention times of the standards to calculate apparent molecular weights. The percentages of protein fractions were determined by calculating the area under the identified protein peaks using Origin 2024b software (OriginLab, MA, USA).

2.11. Aqueous solubility

The aqueous solubility of protein isolates was determined according to Yang *et al.*[22] with minor modifications. Protein suspensions (10 mg mL^{-1}, 200 mL) were prepared, and their pH was adjusted to 7 using 0.1 M and 1 M NaOH and HCL solutions. The suspensions were shaken vigorously at room temperature for 2 h and then centrifuged at 3250 × *g* for 30 min at 25 °C. The supernatants were collected and freeze-dried, and their nitrogen contents were measured using a nitrogen analyzer (FP-428, Leco Corporation, St. Joseph, MI, USA). The aqueous solubility was calculated using the following equation:

$$\text{aqueous solubility (\%)} = \frac{\text{nitrogen content of the dried supernatant}}{\text{nitrogen content of the protein isolate}} \times 100$$

2.12. Statistical analyses

Experiments were performed at least in triplicate, and data are presented as mean ± standard deviation. Statistical analyses were conducted using Origin 2024b (OriginLab, MA, USA). Differences among means were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's test at $p < 0.05$, and the terms "significant" and "significantly" used in the paper refer to this level of significance.

3. Results and discussion

3.1. Properties of canola meals

In this section, the first three subsections focus on the effects of dehulling and pressing temperature on the properties of canola meals, whereas the underlying mechanisms responsible for the observed changes are discussed in Section 3.1.4.

3.1.1. Color and protein content. Fig. 1 shows the protein content and visual appearance of canola meals, as well as the visual appearance of canola protein isolates, while Table 1 provides the color parameters of canola meals and protein isolates.

Removal of hulls noticeably increased the lightness and yellowness of canola at all treatment temperatures and also affected the meal color progression as the treatment temperature increased. In non-dehulled meals, lightness decreased from 73.88 (ND40) to 59.99 (ND120) and greenness from −2.78 to −0.44, whereas the dehulled meals retained their original color much better, with lightness decreasing from 80.69 (D40) to 77.88 (D120) and greenness from 2.81 to −0.82. Visually, ND120 developed a light brown color, while D120 retained a bright yellow color close to D80 and D40. These results indicate that dehulling both improved canola meal color and enhanced color stability at elevated temperatures.

In addition, dehulling increased meal protein content by an average of 6.28%, reflecting the higher protein content of the canola embryo compared to the hulls.[6]

3.1.2. Particle size distribution and microstructure. Fig. 2A and B illustrate the volume-weighed particle size distribution of non-dehulled and dehulled canola meals, respectively, with distribution parameters summarized in Table 2. Non-dehulled meals exhibited multimodal distributions at ND40 and ND80, with a dominant peak at ~500 μm and additional peaks at ~100, 40, and 7 μm. Increasing the temperature from 40 to 80 °C intensified the ~100 μm peak at the expense of 40 and 7 μm peaks, while the ~500 μm peak remained largely unchanged. At 120 °C, however, the ~500 μm peak nearly disappeared, and the distribution shifted to a single dominant peak at ~100 μm. These trends were also reflected in the distribution parameters. Increasing the temperature from 40 °C to 80 °C increased all parameters; however, at 120 °C, D[3,2] and D10 continued to increase, while D50 and, to a much larger extent, D[4,3] and D90, were noticeably decreased. D[3,2] and D10 represent the finer and smaller particles in the distribution, which, in the context of canola meal, can be mostly attributed to the cotyledon tissue. In contrast, D[4,3] and D90 (and the peak at ~500 μm) represent the coarser and larger particles, which primarily represent the hulls.[23] Therefore, while the hulls remained stable

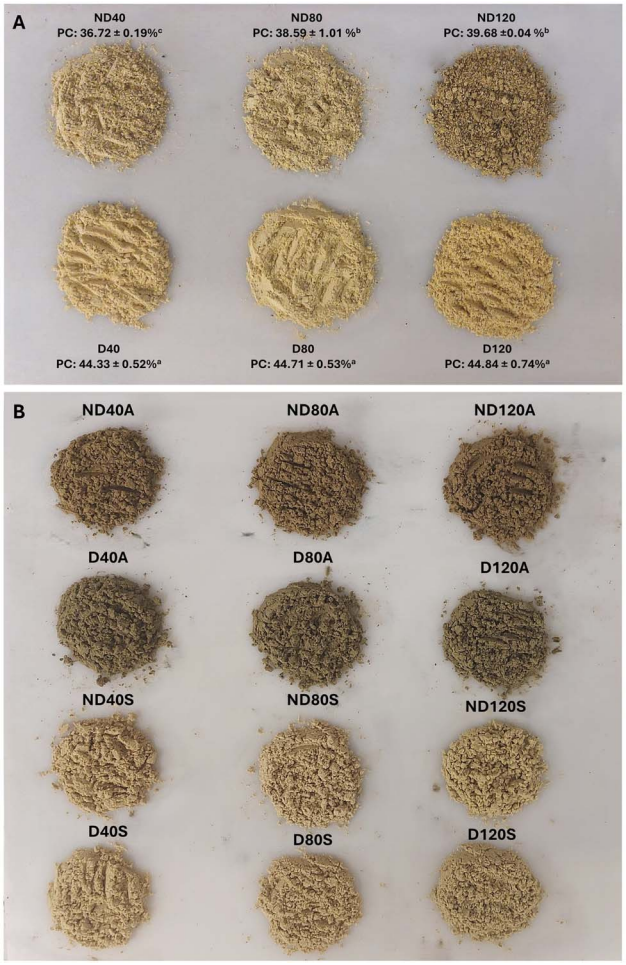

Fig. 1 (A) Protein content (PC) and visual appearance of non-dehulled (ND) and dehulled (D) canola meals treated at 40 °C (ND40 and D40), 80 °C (ND80 and D80) and 120 °C (ND120 and D120). Different letters (a–c) indicate significant differences ($p < 0.05$) between the protein contents of the meals; (B) visual appearance of isolates extracted from ND and D meals through alkaline (ND40A, ND80A, ND120A, D40A, D80A and D120A) and salt (ND40S, ND80S, ND120S, D40S, D80S, and D120S) extraction.

up to 80 °C, exposure to 120 °C combined with strong shear forces of the screw press possibly caused their collapse and fragmentation into smaller particles, resulting in the disappearance of the ~500 μm peak, reduction of D[4,3] and D90 values, and a potentially severe aggregation behavior.

On the other hand, while D40 and D80 also exhibited multimodal distributions, the dominant ~500 μm peak observed in their non-dehulled counterparts was replaced by a smaller peak at ~320 μm, likely due to the removal of most large hull fractions. Furthermore, increasing the treatment temperature from 40 to 120 °C led to a consistent increase in all distribution parameters, with no signs of collapse in the larger fractions of D120, indicating a milder aggregation behavior compared to ND120. Moreover, parameters representing finer cotyledon particles (D[3,2], D10, and partly D50) at lower treatment temperatures showed notably lower values in D40 compared to ND40, which could be attributed to reduced non-

Table 1 Color parameters of canola meals and protein isolates. Different letters (a–e for canola meals, f–j for alkaline extracted isolates and v–z for salt extracted isolates) indicate significant differences ($p < 0.05$) between the values of each color parameter[a]

Canola meals and protein isolates	L^* (lightness)	a^* (green [−] to red [+])	b^* (blue [−] to yellow [+])
ND40	73.88 ± 0.28^{d}	-2.78 ± 0.04^{c}	23.24 ± 0.03^{d}
ND80	74.91 ± 0.51^{c}	-2.96 ± 0.05^{d}	24.17 ± 0.18^{c}
ND120	59.99 ± 0.08^{e}	-0.44 ± 0.01^{a}	22.20 ± 0.06^{e}
D40	80.69 ± 0.22^{a}	$-2.81 \pm 0.04^{c,d}$	29.57 ± 0.30^{b}
D80	81.23 ± 0.35^{a}	$-2.84 \pm 0.08^{c,d}$	29.62 ± 0.15^{b}
D120	77.88 ± 0.38^{b}	-0.82 ± 0.11^{b}	32.24 ± 0.22^{a}
ND40A	41.55 ± 0.12^{h}	2.21 ± 0.04^{h}	18.00 ± 0.15^{g}
ND80A	42.98 ± 0.09^{f}	2.22 ± 0.06^{g}	18.78 ± 0.18^{f}
ND120A	43.32 ± 0.25^{g}	2.82 ± 0.02^{f}	17.51 ± 0.03^{h}
D40A	$41.84 \pm 0.12^{g,h}$	-0.45 ± 0.02^{j}	15.29 ± 0.02^{i}
D80A	43.44 ± 0.13^{f}	-0.34 ± 0.01^{i}	14.97 ± 0.16^{i}
D120A	43.38 ± 0.33^{f}	-0.51 ± 0.01^{j}	15.23 ± 0.16^{i}
ND40S	62.83 ± 0.11^{d}	2.12 ± 0.01^{a}	26.62 ± 0.15^{c}
ND80S	66.08 ± 0.10^{b}	1.71 ± 0.01^{b}	25.23 ± 0.09^{c}
ND120S	63.83 ± 0.09^{c}	-1.04 ± 0.01^{e}	25.08 ± 0.20^{b}
D40S	65.83 ± 0.32^{b}	1.26 ± 0.01^{c}	24.34 ± 0.28^{a}
D80S	66.70 ± 0.40^{a}	1.02 ± 0.04^{d}	23.87 ± 0.44^{b}
D120S	65.56 ± 0.05^{b}	-1.71 ± 0.04^{f}	22.39 ± 0.15^{c}

[a] Abbreviations: ND40, ND80, and ND120: non-dehulled meals treated at 40, 80, and 120 °C, respectively; D40, D80, and D120: dehulled meals treated at 40, 80, and 120 °C, respectively; ND40A, ND80A, ND120A, D40A, D80A, and D120A: protein isolates extracted from non-dehulled and dehulled meals *via* alkaline extraction; ND40S, ND80S, ND120S, D40S, D80S, and D120S: protein isolates extracted from non-dehulled and dehulled meals *via* salt extraction.

covalent interactions between hull tannins and proteins from the cotyledon tissue.[24] In contrast, changes in D[4,3] and D90 at higher treatment temperatures were likely associated with heat-induced covalent interactions.

Evaluation of the microstructure of canola meals captured with a SEM at 1000× magnification (Fig. 3A) further supported the trends observed in particle size distribution. In ND40, smooth, spherical cotyledon particles were clearly separated

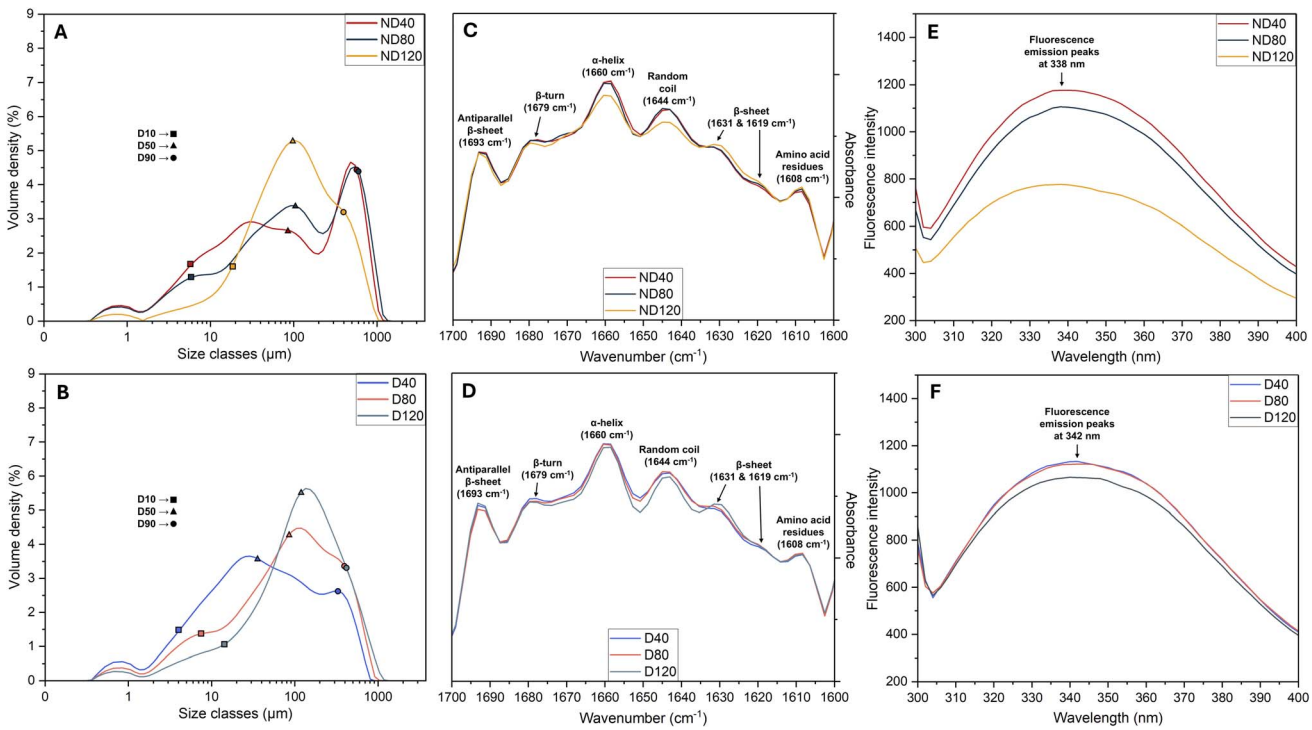

Fig. 2 Volume-weighted particle size distribution (A) and (B), as well as Fourier-transform infrared spectra (C) and (D) and intrinsic fluorescence spectra (E) and (F) of non-dehulled (ND) and dehulled (D) canola meals treated at 40 °C (ND40 and D40), 80 °C (ND80 and D80) and 120 °C (ND120 and D120).

Table 2 Particle size distribution parameters of non-dehulled and dehulled canola meals. Different letters in each column indicate significant differences ($p < 0.05$) between the meals[a]

Canola meals	Particle size distribution parameters (μm)				
	D[3,2]	D[4,3]	D10	D50	D90
ND40	14.78 ± 0.47^{d}	207.60 ± 16.34^{b}	6.14 ± 0.06^{d}	82.86 ± 7.00^{d}	595.2 ± 40.78^{b}
ND80	16.36 ± 0.16^{c}	228.40 ± 9.83^{a}	6.80 ± 0.06^{c}	103.4 ± 2.50^{b}	647.2 ± 29.67^{a}
ND120	29.50 ± 0.57^{a}	158.60 ± 5.68^{d}	19.13 ± 0.05^{a}	96.73 ± 2.28^{c}	404.8 ± 17.70^{d}
D40	11.62 ± 0.83^{e}	107.60 ± 5.22^{e}	4.98 ± 0.06^{e}	39.08 ± 0.71^{e}	332.8 ± 18.52^{e}
D80	16.70 ± 0.26^{c}	151.20 ± 5.97^{d}	7.06 ± 0.59^{c}	88.52 ± 2.68^{d}	$402.2 \pm 17.42^{c,d}$
D120	25.06 ± 0.37^{b}	182.40 ± 9.39^{c}	14.80 ± 0.02^{b}	120.2 ± 2.28^{a}	447.8 ± 28.76^{c}

[a] Abbreviations: ND40, ND80, and ND120: non-dehulled meals treated at 40, 80, and 120 °C, respectively; D40, D80, and D120: dehulled meals treated at 40, 80, and 120 °C, respectively.

from rough, irregular hull fragments, with no obvious signs of heat-induced aggregation. In ND80, while the onset of aggregation was evident by the presence of melted and fused fragments (yellow arrows),[25] a clear separation between the cotyledon and hull tissues was still maintained. However, in ND120, the distinct cotyledon and hull fractions were almost completely converted into much larger and irregularly fused structures, indicating extensive heat-induced aggregation. For dehulled meals, both D40 and D80 exhibited clear separation between the cotyledon and hull components, with the onset of

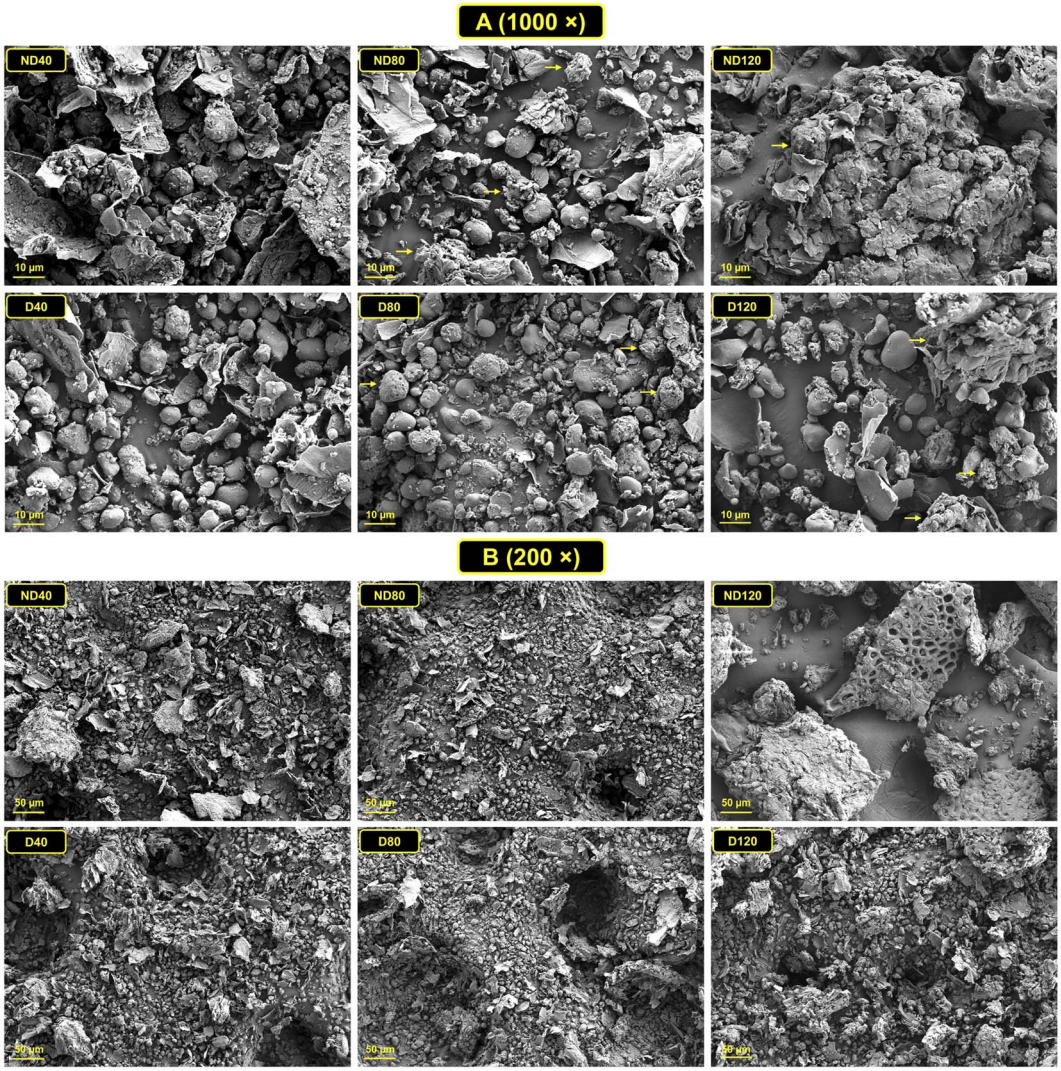

Fig. 3 SEM images of non-dehulled (ND) and dehulled (D) canola meals treated at 40 °C (ND40 and D40), 80 °C (ND80 and D80) and 120 °C (ND120 and D120) at 1000× (A) and 200× (B) magnification. Arrows show the areas with clear signs of heat-induced aggregation.

aggregation observed in D80. In D120, although aggregation was clearly more pronounced, separation between cotyledon and hull components was still largely retained, unlike in ND120. Fig. 3B illustrates the SEM images at a lower magnification (200×) and further highlights the substantially altered morphology and microstructure of ND120 compared with the other meals. Overall, these observations suggest that dehulling allowed for a milder heat-induced aggregation and better preservation of the original meal microstructure at higher temperatures.

3.1.3. Protein structure. Fig. 2C and D illustrate the FT-IR spectra of non-dehulled and dehulled canola meals, respectively, while Table 3 provides the percentages of protein secondary structure components. Spectral deconvolution was performed in the amide I region (1700–1600 cm^{-1}), where peaks arise from C═O stretching vibrations of the peptide backbone and are highly sensitive to changes in hydrogen bonding, enabling the assessment of protein secondary structure.[26] The meal spectra exhibited seven distinct peaks corresponding to anti-parallel β-sheets at 1693 cm^{-1}, β-turns at 1679 cm^{-1}, α-helix at 1660 cm^{-1}, random coil at 1644 cm^{-1}, β-sheets at 1631 and 1619 cm^{-1} and amino acid residues at 1608 cm^{-1}.[27,28] While heat treatment did not alter peak positions or generate new peaks, it noticeably affected some peak intensities. For non-dehulled meals, ND40 and ND80 showed roughly similar spectra, whereas ND120 exhibited reduced α-helix and random coil intensities with a corresponding increase in the intensity of β-sheet. The loss of α-helix and random coil structures, along with the increase in β-sheet structures is frequently associated with protein denaturation.[29] Dehulled meals showed a similar trend, with D40 and D80 exhibiting almost similar spectra and D120 showing the most noticeable changes. However, the extent of intensity changes in α-helix, random coil and β-sheet peaks was clearly less pronounced in D120 compared to ND120. These observations were also reflected in the percentages of protein secondary structure components (Table 3). In non-dehulled meals, increasing the temperature from 40 to 120 °C resulted in a β-sheet increase from 30.40% to 36.81% and an α-helix reduction from 20.43 to 18.57%, whereas the dehulled meals exhibited milder alterations with a β-sheet increase from 30.22% to 33.02% and an α-helix reduction from 20.66% to 19.27%. These results indicate that dehulling mitigated the extent of heat-induced alterations in canola protein secondary structure at elevated pressing temperatures.

Fig. 2E and F illustrate the intrinsic fluorescence spectra of non-dehulled and dehulled canola meals, respectively. Fluorescence emission intensity reflects changes in the protein tertiary structure, as it correlates with the exposure of aromatic amino acids, mainly tryptophan, to the hydrophilic environment.[28] For non-dehulled meals, the fluorescence intensity decreased with increasing treatment temperature, with ND120 showing a substantial reduction. This could be due to tryptophan degradation, or increased protein aggregation leading to the burial of tryptophan residues, both associated with protein thermal denaturation.[29] In contrast, D40 and D80 exhibited nearly identical fluorescence intensities, while D120 showed an intensity reduction that was much less pronounced compared to ND120. These observations suggest that, similar to its effects on the secondary structure, dehulling also mitigated heat-induced alterations in canola protein tertiary structure at elevated pressing temperatures.

Table 3 Percentages of protein secondary structure components in canola meal and protein isolates. Different letters (a–c) in each group (meals, alkaline-extracted isolates, and salt-extracted isolates) indicate significant differences ($p < 0.05$) among the group samples[a]

Meal and protein isolates	Protein secondary structure (%)				
	β-Sheet	Random coil	α-Helix	β-Turn	Anti-parallel β-sheet
ND40	30.40 ± 0.53^{a}	16.31 ± 0.39^{a}	$20.43 \pm 0.49^{a,b}$	25.40 ± 0.26^{a}	7.46 ± 0.72^{a}
ND80	30.90 ± 0.26^{c}	$15.40 \pm 0.39^{a,b}$	20.93 ± 0.71^{a}	25.76 ± 0.29^{a}	$6.96 \pm 0.22^{a,b}$
ND120	36.81 ± 0.06^{c}	14.13 ± 0.07^{b}	18.57 ± 0.72^{b}	24.54 ± 0.70^{a}	5.92 ± 0.04^{b}
D40	30.22 ± 0.53^{c}	16.38 ± 0.31^{a}	$20.66 \pm 0.27^{a,b}$	25.62 ± 0.19^{a}	$7.20 \pm 0.23^{a,b}$
D80	30.77 ± 0.10^{c}	$15.66 \pm 0.19^{a,b}$	$20.74 \pm 0.18^{a,b}$	25.52 ± 0.14^{a}	$7.32 \pm 0.31^{a,b}$
D120	33.02 ± 0.52^{b}	14.58 ± 0.66^{b}	$19.27 \pm 0.76^{a,b}$	25.54 ± 0.13^{a}	7.60 ± 0.32^{a}
ND40A	33.04 ± 0.30^{a}	12.54 ± 0.59^{b}	17.68 ± 0.43^{a}	22.01 ± 0.16^{c}	14.69 ± 0.05^{a}
ND80A	$32.63 \pm 0.27^{a,b}$	$13.52 \pm 0.05^{a,b}$	17.76 ± 0.33^{a}	$23.07 \pm 0.27^{a,b}$	13.02 ± 0.26^{b}
ND120A	30.23 ± 0.16^{c}	14.36 ± 0.26^{a}	18.57 ± 0.32^{a}	23.76 ± 0.22^{a}	13.07 ± 0.11^{b}
D40A	$32.56 \pm 0.39^{a,b}$	$13.42 \pm 0.12^{a,b}$	17.72 ± 0.26^{a}	$23.53 \pm 0.33^{a,b}$	12.68 ± 0.31^{b}
D80A	$32.81 \pm 0.20^{a,b}$	$13.72 \pm 0.28^{a,b}$	17.52 ± 0.11^{a}	$22.78 \pm 0.31^{a,b,c}$	13.11 ± 0.29^{b}
D120A	31.89 ± 0.17^{b}	14.05 ± 0.22^{a}	18.24 ± 0.37^{a}	$22.66 \pm 0.18^{b,c}$	13.16 ± 0.61^{b}
ND40S	$30.38 \pm 0.03^{a,b}$	15.16 ± 0.40^{a}	21.79 ± 0.27^{a}	22.66 ± 0.28^{b}	9.99 ± 0.15^{b}
ND80S	$30.86 \pm 0.46^{a,b}$	$14.27 \pm 0.21^{a,b}$	21.49 ± 0.59^{a}	22.76 ± 0.06^{b}	$10.68 \pm 0.79^{a,b}$
ND120S	29.99 ± 0.30^{b}	13.44 ± 0.32^{b}	21.12 ± 1.16^{a}	$23.47 \pm 0.63^{a,b}$	11.99 ± 0.45^{a}
D40S	$30.22 \pm 0.15^{a,b}$	13.32 ± 0.15^{b}	20.74 ± 0.35^{a}	24.75 ± 0.34^{a}	$10.97 \pm 0.37^{a,b}$
D80S	31.43 ± 0.52^{a}	13.60 ± 0.54^{b}	20.46 ± 0.06^{a}	24.23 ± 0.11^{a}	10.25 ± 0.14^{b}
D120S	$30.26 \pm 0.36^{a,b}$	$13.53 \pm 0.49^{a,b}$	20.95 ± 0.23^{a}	$23.89 \pm 0.13^{a,b}$	$10.42 \pm 0.24^{a,b}$

[a] Abbreviations: ND40, ND80, and ND120: non-dehulled meals treated at 40, 80, and 120 °C, respectively; D40, D80, and D120: dehulled meals treated at 40, 80, and 120 °C, respectively; ND40A, ND80A, ND120A, D40A, D80A, and D120A: protein isolates extracted from non-dehulled and dehulled meals *via* alkaline extraction; ND40S, ND80S, ND120S, D40S, D80S, and D120S: protein isolates extracted from non-dehulled and dehulled meals *via* salt extraction.

3.1.4. Mechanistic interpretation of meal properties. The composition of canola hulls (and embryo) has been well established over the past few decades;[4,6–8] therefore, the focus of this study was on the impact of hulls on canola meal and protein isolates, rather than their direct measurement of the hull components. The hulls contain substantial levels of red-brown condensed tannins and other hull pigments, the removal of which could have caused the notable enhancement in the color of dehulled meals.[7,8] While this likely represents the primary reason for color improvement, the changes observed in other meal properties require consideration of additional underlying mechanisms, namely friction-induced effects and covalent interactions. As noted in the Introduction, canola hulls are mainly composed of polysaccharides and lignin, giving them a rigid, coarse structure compared to the much softer tissue of cotyledon. Therefore, in addition to the shear and heat generated by the twin-screw press, the hull fragments can create extra mechanical friction, further elevating local shear stress and temperatures within the meal.[30,31] These localized thermal and mechanical stresses may further exacerbate heat-induced aggregation and disrupt native intramolecular interactions within proteins. Therefore, the reduction of these stresses through dehulling could have contributed to the better preservation of the meal microstructure, as well as protein secondary and tertiary structures.[9]

In addition, condensed tannins, lignin and polysaccharides can interact with proteins through several well-established mechanisms. Tannins can strongly bind proteins through non-covalent interactions and cause protein aggregation and precipitation.[24] This can explain the lower values of distribution parameters for finer cotyledon particles in D40 compared to ND40. In addition, tannins and lignin can undergo heat-induced oxidation and form highly reactive quinone moieties that covalently bind nucleophilic amino acids of proteins.[32] Furthermore, thermal degradation of hull polysaccharides may also generate reducing sugars that participate in Maillard reactions.[33] These reactions, both independently and in synergy with friction-induced effects, may have contributed to the observed changes in the meal properties at 80 and 120 °C, including changes in color, aggregation behavior and microstructure, and protein secondary and tertiary structures. The synergic impacts were especially pronounced in ND120, where the collapse and fragmentation of hull particles and the subsequent expansion of their surface area not only amplified the friction effects within the meal but also increased the exposure of protein to covalent modification by the hull components.

Conclusively, dehulling notably enhanced the qualitative attributes of the meals and enabled better protein preservation, especially under high-temperature treatments. Previous studies have mainly considered dehulling as a strategy to improve meal nutritional quality for animal feed by removing hull-associated anti-nutritional factors.[17,18] However, the implications of the present study extend beyond feed applications due to their direct relevance to meal structure and its proteins. Recently, there has been a surge in research regarding the direct utilization of canola meal in food and food-related applications, including packaging films,[34] renewable materials,[35,36] stabilizer particles,[37] and pressed gels.[38] In parallel, increasing attention has been given to canola protein isolation from meals[19,39,40] and to the use of canola protein concentrates and isolates in food applications.[41] While improved meal quality due to dehulling can clearly benefit all these applications, the following section focuses specifically on protein isolate production from the meals.

3.2. Properties of canola protein isolates

3.2.1. Extraction efficiency and protein content. Fig. 4 presents the protein extraction efficiency and protein content of isolates obtained from non-dehulled and dehulled meals using alkaline and salt extraction. For alkaline extraction, ND40A showed an extraction efficiency of 66.32% ± 2.35, followed by a decrease to 63.75% ± 0.04 for ND80A and a further, substantial decrease to 48.42% ± 2.31 for ND120A. On the other

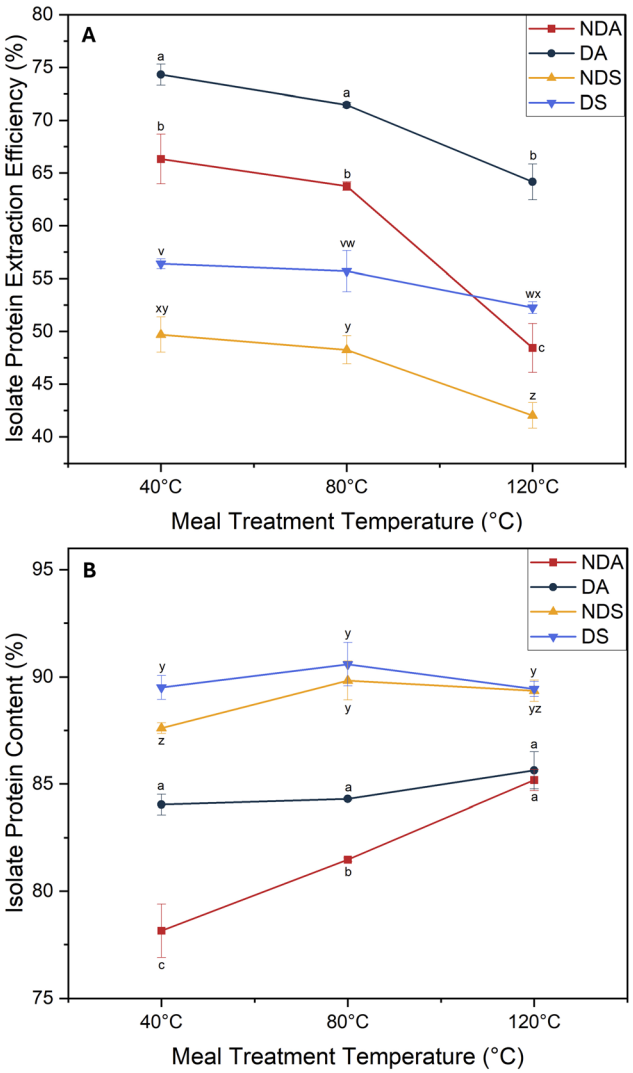

Fig. 4 (A) Protein extraction efficiency of isolates obtained from non-dehulled (ND) and dehulled (D) meals using alkaline (with A at the end) and salt (with S at the end) extraction methods, plotted against the meal treatment temperatures; (B) protein content of the isolates. Different letters (a–d for alkaline extracted isolates and v–z for salt extracted isolates) indicate significant differences ($p < 0.05$) between the extraction efficiency and the protein content of the isolates.

hand, dehulling significantly improved the alkaline extraction efficiency, reaching 74.30% ± 0.99 for D40A and 71.46% ± 0.23 for D80A. While the extraction efficiency was decreased to 64.16% ± 1.69 for D120A, this reduction was far less severe than that observed for ND120A. Alkaline extraction is a harsh process that can substantially affect the protein structure and other components in the medium. In that sense, similar to the effects of heat and shear during meal pressing, alkaline conditions can promote oxidation of tannins and lignin to quinones and subsequent covalent protein modification.[42] Therefore, removal of these components by dehulling likely reduced such reactions, leading to higher extraction efficiencies. The improvements in extraction efficiency were clearly observable between isolates extracted from non-dehulled and dehulled meals treated at 40 and 80 °C but became most evident at 120 °C. At this temperature, the combined impact of prior friction-induced structural changes and covalent modifications during pressing, together with subsequent alkaline extraction, likely caused extensive protein aggregation and reduced extraction efficiency in ND120, whereas dehulling mitigated these effects in D120 and allowed higher protein recovery. Additionally, removal of condensed tannins and the subsequent reduction in non-covalent protein aggregation and precipitation may have further contributed to the higher extraction efficiency of dehulled meals.[43]

Furthermore, while all salt extraction treatments showed lower extraction efficiencies than their alkaline counterparts, their overall trends were highly similar. The extraction efficiency of ND40S was measured at 49.69% ± 1.66, followed by reductions to 48.25% ± 1.33 and 42.02% ± 1.21 for ND80S and ND120S, respectively. On the other hand, isolates obtained from dehulled meals showed significantly higher extraction efficiencies, reaching 56.39% ± 0.46 for D40S and 55.70% ± 1.95 for D80S. The lowest efficiency in this group was observed at 52.25% ± 0.56 for D120S; however, the extent of this reduction was considerably less severe than that observed for ND120S. Compared to alkaline extraction, NaCl extraction is a milder method, in which the existing structure of the proteins is largely preserved. Therefore, the higher extraction efficiencies of dehulled meals likely reflect their improved tolerance to heat treatment and the removal of condensed tannins through dehulling.[44] In another study by Rommi *et al.*[24] on canola protein extraction from cold-pressed non-dehulled and dehulled meals (meals obtained from two different companies rather than produced in-house under controlled conditions; protein extraction yield calculated based on the remaining protein in the solid fraction), dehulling was reported to increase the extraction yield from ∼34% to ∼48% during water extraction. Furthermore, 48 h of enzymatic treatment of the meal with Pectinex increased these efficiencies to ∼56% for non-dehulled meals and ∼78% for dehulled meals, respectively. Overall, dehulling improved the extraction efficiency of canola protein isolates, and the improvements became more noticeable as the meal treatment temperature increased.

Regarding protein content (Fig. 4), NDA isolates showed a sharp increase from 78.14% in ND40A to 85.17% in ND120A, whereas DA isolates exhibited only a mild increase, remaining within 84.04–85.63%. Alkaline extraction may have effectively solubilized the hull components in meals treated at low temperatures, contributing to the relatively lower protein content of ND40A. However, increasing the treatment temperature likely caused these components to aggregate and precipitate or degrade into smaller molecules that were removed during the dialysis, resulting in higher protein contents in ND80A and ND120A.[45] In addition, salt-extracted isolates showed higher protein contents than their alkaline counterparts, with DS isolates maintaining slightly higher values (89.50–90.59%) than NDS isolates (87.60–89.83%). Conclusively, similar to the extraction efficiency, dehulling also increased the protein content of the isolates.

3.2.2. Color. NDA isolates (Fig. 1) exhibited a consistent dark-brown color typical of canola protein isolates produced by alkaline extraction.[5] DA isolates, however, showed a considerable decrease in a^* values (redness) from an average of 2.41 to −0.43 and a relatively milder decrease in b^* values (yellowness), resulting in a dark-green color (Table 1). In contrast, all salt-extracted isolates displayed more desirable color attributes (higher L^* and b^* and lower a^* values) compared to alkaline isolates, with both NDS and DS exhibiting a bright-yellow color. However, compared to NDS, DS isolates showed a decrease in a^* values from an average of 0.93 to 0.19, a trend similar to that observed in NDA *vs.* DA isolates.

The lower a^* values observed in DA and DS isolates can be attributed to meal dehulling and the subsequent removal of red-brown tannins and other hull pigments. The difference was more pronounced in the alkaline isolates, as alkaline conditions enhance the solubility and extraction of hull pigments.[42] Moreover, alkaline conditions promote the oxidation of phenolic compounds into quinones, which can either self-polymerize or covalently bind to the nucleophilic protein groups. These reactions can generate more strongly colored pigments, thereby contributing to the darker color of alkaline isolates compared to salt isolates.[3] Overall, while dehulling clearly improved the color of canola meals, its effects on the color of protein isolates were more nuanced.

3.2.3. Molecular weight and percentage of extracted fractions. Canola protein consists mainly of two storage fractions, cruciferin and napin. Cruciferin is a 12S globulin with a hexameric structure and a molecular weight of 300–350 kDa. Each cruciferin monomer (∼50 kDa) is composed of two polypeptides, α (∼30 kDa) and β (∼20 kDa), linked by disulfide bonds. Napin is a 2S albumin with a monomeric structure and a molecular weight of 12–16 kDa. It is composed of two polypeptides, long (∼9 kDa) and short (∼4 kDa), also connected by disulfide bonds.[46] Aside from the storage fractions, canola also contains minor oil-body proteins, including steroleosins (∼40 kDa), caleosins (∼30 kDa) and oleosins (∼18 kDa). Since the oil-body proteins constitute only a minor fraction of canola protein isolates, they were not accounted for the calculations of the percentage of protein fractions.[20]

Fig. 5A and B demonstrate the SE-HPLC chromatograms of alkaline and salt-extracted isolates, respectively. The highly acidic mobile phase (pH ∼ 2) promoted the dissociation of protein subunits, resulting in the elution of seven, well-

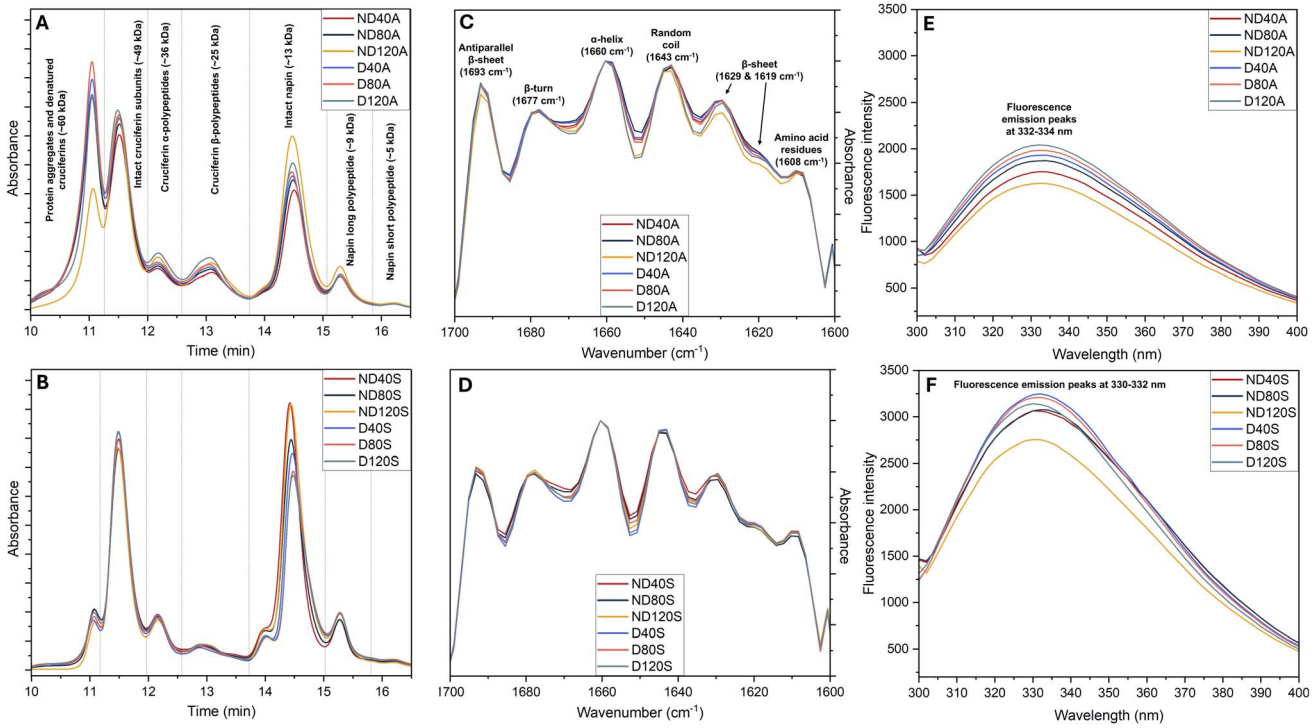

Fig. 5 Size-exclusion high-performance liquid chromatograms (A) and (B), Fourier-transform infrared spectra (C) and (D), and intrinsic fluorescence spectra (E) and (F) of canola protein isolates obtained *via* alkaline (NDA and DA) and salt (NDS and DS) extraction methods.

separated peaks that were assigned to protein fractions based on their estimated molecular weights.[21] The first peak, corresponding to a molecular weight of ~60 kDa was attributed to protein aggregates and unfolded cruciferin subunits eluting earlier than intact subunits.[45] The next three peaks corresponded to the molecular weights of ~49, ~36 and ~25 kDa and were assigned to intact cruciferin subunits, cruciferin α-polypeptides and cruciferin β-polypeptides, respectively. The final three peaks corresponded to the molecular weights of ~13, ~9 and ~5 kDa and were assigned to intact napin, napin long polypeptides and napin short polypeptides, respectively.

Table 4 provides the percentages of extracted fractions in each isolate. For alkaline-extracted isolates, ND40A and ND80A showed a similar composition, with the most notable fractions being approximately 30% aggregates and unfolded cruciferins, 25% intact cruciferin and 21% napin. However, in ND120A, the percentage of aggregates and unfolded cruciferins was substantially reduced to around 15%, accompanied by a sharp

Table 4 Percentage of extracted protein fractions in each canola protein isolate. Different letters (a–d for alkaline extracted isolates and v–z for salt extracted isolates) indicate significant differences ($p < 0.05$) between the values for each fraction[a]

Protein isolates	Protein aggregates and unfolded cruciferins (%)	Intact cruciferin subunits (%)	Cruciferin α-polypeptides (%)	Cruciferin β-polypeptides (%)	Intact napin (%)	Napin long polypeptides (%)	Napin short polypeptides (%)
ND40A	30.94 ± 0.62^{a}	25.48 ± 0.62^{a}	6.16 ± 0.27^{b}	10.03 ± 0.13^{b}	21.03 ± 1.24^{b}	5.19 ± 0.18^{b}	1.14 ± 0.01^{a}
ND80A	30.31 ± 1.04^{a}	25.90 ± 0.84^{a}	6.20 ± 0.26^{b}	10.09 ± 0.07^{b}	21.50 ± 0.61^{b}	$4.88 \pm 0.32^{b,c}$	1.08 ± 0.04^{a}
ND120A	15.12 ± 1.02^{c}	27.01 ± 0.85^{a}	8.38 ± 0.43^{a}	11.66 ± 0.25^{a}	30.37 ± 0.43^{a}	6.25 ± 0.15^{a}	1.18 ± 0.01^{a}
D40A	31.08 ± 0.70^{a}	26.07 ± 0.66^{a}	6.34 ± 0.32^{b}	9.87 ± 0.57^{b}	21.05 ± 0.91^{b}	4.60 ± 0.08^{d}	0.95 ± 0.01^{b}
D80A	31.57 ± 0.51^{a}	25.61 ± 1.09^{a}	6.70 ± 0.29^{b}	9.96 ± 0.53^{b}	20.95 ± 0.39^{b}	4.49 ± 0.08^{c}	0.90 ± 0.03^{b}
D120A	25.98 ± 0.67^{b}	26.72 ± 0.79^{a}	8.01 ± 0.46^{a}	11.30 ± 0.66^{a}	22.32 ± 0.08^{b}	$4.72 \pm 0.09^{b,c}$	0.91 ± 0.01^{b}
ND40S	7.03 ± 0.17^{y}	$30.20 \pm 0.61^{y,z}$	$7.92 \pm 0.18^{y,z}$	$6.63 \pm 0.17^{y,z}$	39.66 ± 0.73^{w}	7.20 ± 0.15^{z}	1.57 ± 0.02^{y}
ND80S	6.68 ± 0.19^{y}	$30.73 \pm 0.81^{x,y,z}$	$7.99 \pm 0.07^{y,z}$	$6.67 \pm 0.38^{y,z}$	$38.55 \pm 0.92^{w,x}$	$7.71 \pm 0.27^{y,z}$	$1.77 \pm 0.03^{x,y}$
ND120S	4.30 ± 0.33^{z}	29.01 ± 0.60^{z}	7.34 ± 0.06^{z}	$6.63 \pm 0.14^{y,z}$	43.14 ± 1.21^{v}	$8.09 \pm 0.91^{x,y,z}$	1.31 ± 0.16^{z}
D40S	6.67 ± 0.09^{y}	$31.74 \pm 0.56^{x,y}$	7.28 ± 0.19^{z}	6.38 ± 0.25^{y}	$37.22 \pm 0.57^{x,y}$	$8.78 \pm 0.33^{x,y}$	$1.90 \pm 0.03^{w,x}$
D80S	7.05 ± 0.70^{y}	$31.71 \pm 0.99^{x,y}$	$7.72 \pm 0.49^{y,z}$	7.02 ± 0.08^{y}	$35.29 \pm 0.57^{y,z}$	9.15 ± 0.12^{x}	2.01 ± 0.05^{w}
D120S	7.45 ± 0.17^{y}	32.34 ± 0.14^{x}	8.20 ± 0.29^{y}	7.12 ± 0.13^{y}	34.21 ± 0.51^{z}	$8.84 \pm 0.35^{x,y}$	$1.82 \pm 0.02^{w,x}$

[a] Abbreviations: ND40A, ND80A, ND120A, D40A, D80A, and D120A: protein isolates extracted from non-dehulled and dehulled meals *via* alkaline extraction; ND40S, ND80S, ND120S, D40S, D80S, and D120S: protein isolates extracted from non-dehulled and dehulled meals *via* salt extraction.

increase in intact napin to nearly 30%. The alkaline isolates from dehulled meals demonstrated a comparable trend, with D40A and D80A containing approximately 31% of aggregates and unfolded cruciferins, 26% of intact cruciferins and 21% intact napins. However, in D120A, the percentage of aggregates and unfolded cruciferins was only moderately reduced to around 26%, accompanied by a more uniform increase in all other fractions, particularly cruciferin α and β subunits. Due to the larger size and more hydrophobic structure of cruciferins compared to napins, these fractions are more likely to be affected by the friction and covalent modification effects of the hulls (explained in Section 3.1.4), as well as the harsh conditions of alkaline media (explained in Section 3.2.1).[42,47] While cruciferins in the meals treated at 40 °C and 80 °C tolerated these conditions, the combined effects of 120 °C treatment and the above mechanisms possibly overwhelmed their structure and triggered their aggregation and precipitation during extraction, resulting in their reduced contents in ND120A and D120A. Napins, being smaller and more soluble, were possibly less affected, leading to their higher proportions in ND120A and D120A. However, since D120A retained a much higher concentration of unfolded cruciferins and aggregates compared to ND120A, it can be concluded that the beneficial effects of dehulling mostly impacted cruciferins rather than napins. This may also explain the reduced protein extraction efficiency observed with increasing meal treatment temperature, which could be due to the precipitation of unfolded cruciferins and aggregates.

Compared to alkaline-extracted isolates, all salt-extracted isolates contained notably lower percentages of aggregates and unfolded cruciferins (4–7%), which may explain their lower extraction efficiency compared to alkaline treatments. Instead, salt-extracted isolates exhibited higher percentages of intact cruciferins (29–32%) and napins (34–43%). In NDS isolates, the percentage of aggregates and unfolded cruciferins decreased from approximately 7% (ND40S) to 4% (ND120S), while the percentage of napin increased from 39% to 43%. This trend was not observed in DS isolates, and their overall composition was relatively similar. Since salt-extraction hardly affects the existing structure of proteins compared to alkaline extraction, these observations further highlighted the effects of meal treatment temperature and the presence of hulls on cruciferins and aggregates. Overall, dehulling could increase the extraction of unfolded cruciferins and aggregates, especially under alkaline conditions, thereby contributing to higher protein extraction efficiency.

3.2.4. Protein structure. Fig. 5C and D demonstrate the FT-IR spectra of alkaline and salt-extracted isolates, respectively. Peak positions in both figures closely resembled those of canola meals, with only minor shifts in β-turns (1679 → 1677 cm^{-1}), random coils (1644 → 1643 cm^{-1}) and β-sheets (1631 → 1629 cm^{-1}), likely due to compositional differences between meals and isolates. Alkaline isolates demonstrated substantially higher percentages of anti-parallel β-sheets (12–15% in alkaline extracted isolates *versus* 5–8% in meal proteins), slightly higher percentages of β-sheets, and lower percentages of α-helix and random coil compared to the protein in the meals (Table 3). This could be related to both the high contents of cruciferins (rich in β-structures) and aggregates in alkaline-extracted isolates (Table 4), as well as alkaline-induced unfolding and re-aggregation, which can promote β-structure formation.[48] Among alkaline isolates, ND40A, ND80A, D40A, and D80A exhibited similar spectra, while ND120A and D120A showed an increase in the intensity of α-helix and random coil alongside a decrease in the intensity of β-sheets, with the effects being more pronounced in ND120A. These trends were also reflected in the calculated secondary structure percentages, but to a lesser magnitude. These observations were most likely related to the higher napin content, ND120, and to a lesser extent, D120,[28] and correspond to the percentages of protein fractions provided in Table 4.

Compared to alkaline-extracted isolates, salt-extracted isolates exhibited lower percentages of anti-parallel β-sheets (10–12% in salt-extracted isolates *versus* 12–15% in alkaline extracted isolates and 5–8% in the meal proteins), along with higher percentages of α-helix and slightly higher percentages of random coil. This can be attributed to the milder conditions of salt extraction, which possibly did not promote β-structure formation to the same extent as alkaline extraction. Another reason could be that salt extraction was less efficient in solubilizing β-rich proteins, as reflected by the higher α-helix content and greater relative presence of napins (rich in α-helical structure) in salt-extracted isolates.[49] Among salt-extracted isolates from non-dehulled meals, a slight increase in the percentages of β-turns and anti-parallel β-sheets, accompanied by a slight decrease in α-helix and random coil, was observed as the meal treatment temperature increased, whereas isolates from dehulled meals showed very similar secondary structure compositions.

Fig. 5E and F demonstrate the intrinsic fluorescence spectra of alkaline and salt-extracted isolates, respectively. Compared to meal spectra, both types of isolates exhibited a blue shift in λ_{max} to 332–334 nm for alkaline and 330–332 nm for salt isolates. This shift likely reflected the compositional differences between meals and isolates, particularly the removal of non-protein fractions, rather than structural changes alone.[28]

Beyond the peak position, the emission intensities revealed clearer trends regarding protein tertiary structure. For both extraction methods, the dehulled isolates consistently showed higher fluorescence intensities than non-dehulled isolates, possibly indicating improved preservation of the tertiary structure due to hull removal, regardless of the extraction method. Furthermore, all alkaline-extracted isolates showed lower fluorescence intensities than salt-extracted isolates, likely due to tryptophan degradation or its burial within aggregates under harsh alkaline conditions.[45] In addition, the relative order of emission intensities differed between extraction methods: alkaline isolates showed altered intensity rankings compared with meals, whereas salt isolates largely retained the intensity order observed in the corresponding meals. This further highlighted the milder condition of salt extraction, which allowed the protein to maintain a closer structural resemblance to meal proteins. Additionally, while ND120 showed a substantially lower emission intensity compared to other meals, the isolates

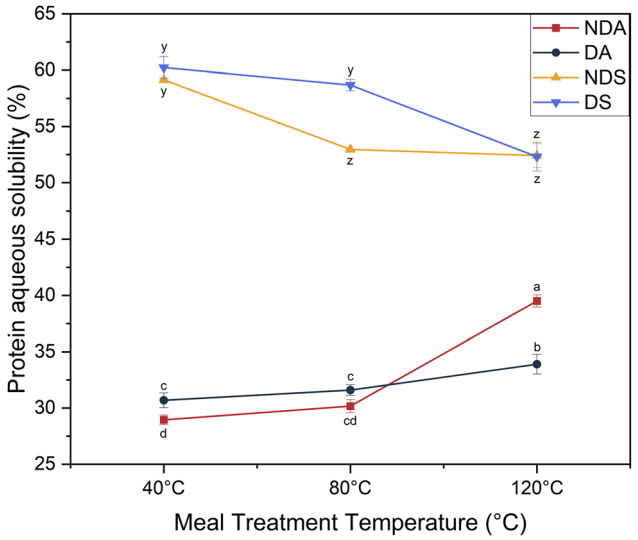

Fig. 6 The aqueous solubility of protein isolates obtained from non-dehulled (ND) and dehulled (D) meals *via* alkaline (NDA and DA) and salt (NDS and DS) extraction methods plotted against the meal treatment temperatures. Different letters (a–d for alkaline extracted isolates, and y and z for salt extracted isolates) indicate significant differences ($p < 0.05$) between the values for each group.

extracted from this meal (ND120A and ND120S) exhibited much closer emission intensities to the rest of the isolates. This pattern could suggest that proteins undergoing severe tertiary structural denaturation in the meal precipitated during extraction and were therefore absent from the isolates obtained by either extraction method. Alternatively, the extraction and purification process may have allowed partial structural reorganization of the remaining proteins, resulting in emission intensities more similar to those of other isolates.[50]

3.2.5. Aqueous solubility. Alkaline-extracted isolates showed aqueous solubilities in the range of 28.95–39.49% (Fig. 6). In both NDA and DA isolates, solubility improved with meal treatment temperature, with a pronounced increase in ND120A. This pattern could be explained by the compositional shift observed in the SE-HPLC data, which demonstrated that higher treatment temperatures reduced extraction of larger unfolded cruciferins and aggregates in favor of smaller, more water-soluble napins, particularly in ND120. In contrast, the aqueous solubility of salt-extracted isolates was substantially higher compared to alkaline-extracted isolates and ranged from 60.22% to 52.31%, likely due to their lower contents of unfolded cruciferins and aggregates, higher napin proportions, and the mild, non-denaturing nature of salt extraction.[44,51] Furthermore, increasing the meal treatment temperature reduced the solubility of both NDS and DS isolates, which can be linked to heat-induced structural changes during meal preparation. Moreover, with the exception of ND120A, dehulling only slightly improved the aqueous solubility of isolates regardless of the extraction method. This suggests that while dehulling can strongly influence solubility during the extraction of proteins from the meal (especially due to the removal of condensed tannins), it has a minor effect on the solubility of protein isolates upon re-solubilization in water.

4. Conclusions

This study evaluated the effects of canola seed dehulling on the quality of meals treated at 40, 80 and 120 °C, as well as the quality of protein isolates obtained from the meals *via* alkaline and salt extraction. The dehulled meals demonstrated better color, milder aggregation behavior and better-preserved meal microstructure and protein secondary and tertiary structures compared to non-dehulled meals, with differences becoming more pronounced at higher treatment temperatures. These patterns directly corresponded with protein extraction efficiency, as dehulling significantly improved the protein extraction efficiency regardless of the extraction method, with the difference becoming more pronounced as the meal treatment temperature increased. Dehulling also influenced the percentages of extracted protein fractions mostly in favor of cruciferins; however, its effects on isolate color, protein structure and aqueous solubility were more nuanced. Overall, dehulling can be an effective tool for improving canola meal quality and increasing the protein extraction efficiency. It can also improve the preservation of embryo proteins at elevated processing temperatures, potentially allowing oil extraction to benefit from higher temperatures while maintaining protein quality.

Although this study focused on the protein in canola seeds to address the associated knowledge gap, canola oil remains the main revenue source for the industry. Therefore, future studies should focus on developing integrated oil and protein processing solutions aimed at maximizing the recovery and quality of both products. These efforts could be further complemented by exploring valorization strategies for canola hulls, as well as economic studies weighing the additional costs of incorporating dehulling and low-temperature oil extraction against the potential revenue gains from the enhanced quality of the residual protein and its derived products.

Author contributions

Abouzar Karimi: conceptualization, data curation, formal analysis, investigation, methodology, validation, writing – original draft. Tos Phuangmarayat: formal analysis, investigation. Pankaj Bhowmik: funding acquisition, supervision. Anusha Samaranayaka: funding acquisition, supervision. Lingyun Chen: conceptualization, funding acquisition, methodology, project administration, resources, supervision, validation, writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting the findings of this study are available within the article and its supplementary information (SI).

Additional data will be provided by the corresponding author upon reasonable request. Supplementary information is available. See DOI: https://doi.org/10.1039/d6fb00093b.

Acknowledgements

The authors are grateful to the Natural Sciences and Engineering Research Council of Canada (NSERC-Discovery) and National Research Council Canada (Collaborative R&D Initiative HQP Grant). Professor Lingyun Chen would like to thank the Natural Sciences and Engineering Research Council of Canada (NSERC) Canada Research Chairs Program for its financial support.

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