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Characterisation of fibre-rich ingredients obtained from defatted cold-pressed rapeseed cake after protein extraction

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ABSTRACT

Fibre-rich ingredients obtained after protein isolate production from cold-pressed rapeseed meal using alkali extraction at pH 12 with ethanol pretreatment (ingredient 1), and natural deep eutectic solvent (NDES) extraction with choline chloride:urea (ChCl:urea) (ingredient 2) and betaine:citric acid (ingredient 3) were compared in terms of chemical profile, minerals, amino acids, antinutrients, and colour. All ingredients were rich in dietary fibres (46.9 ± 2.36–63.0 ± 1.44 %) and proteins (22.5 ± 0.24–35.5 ± 0.66 %). Minerals, particularly potassium, magnesium, zinc, manganese, and iron, were significantly present. The ChCl:urea system provided the highest lysine and methionine content, with methionine detected only in NDES-extracted ingredients. Glucosinolates were absent in all ingredients. Alkali extraction minimized total phenolic content and tannins, while betaine:citric acid extraction reduced phytates most effectively. NDES systems resulted in better colour parameters. Fibre-rich ingredients exhibit significant potential as functional components for enriching food products, combining high nutritional value with minimized antinutritional factors.

1. Introduction

Rapeseed is primary grown for oil production. During 2023/2024, 87.44 million tons of rapeseed were produced globally (Statista, 2025a), while 34.47 million tons of rapeseed oil were produced in the same period (Statista, 2025b). These data suggest that large quantities of waste are generated during rapeseed oil production.

During cold-pressed rapeseed oil production, rapeseed cake is generated as a by-product, which can be used as a feedstuff or as a raw material for reutilisation to achieve nutrient recycling (Borrello et al., 2017).

After defatting the cold-pressed rapeseed cake, a protein-rich rapeseed meal is produced, containing more than 40 % protein with a well-balanced amino acid composition (Aider & Barbana, 2011). This meal represents a promising source for plant-based protein isolate production (Chmielewska et al., 2021).

The applications of rapeseed proteins have been known for many years in the production of functional foods, including bakery products, beverages, dairy and egg substitutes, processed meat products, salad dressings, sauces, and flavorings (Kotecka-Majchrzak et al., 2020). Gluten-free bread has been supplemented with rapeseed protein isolate to enrich, structure, and improve its sensory characteristics (Korus et al., 2021). Additionally, the use of rapeseed proteins as a supplement in texture-modified food for the elderly was investigated by Di Lena et al. (2023).

Alkali extraction (pH 9–12) followed by acid precipitation (pH 2.5–5.5) is a traditional and efficient method for rapeseed protein isolate production (Zhang et al., 2020), resulting in proteins with better functional properties than those of other plant proteins, such as soybean, pea, and wheat proteins (Ghodsvali et al., 2005).

However, the presence of antinutritional factors in rapeseed meal, such as glucosinolates, phytates, and polyphenols, limits its utilization in protein isolate production, as they reduce both the nutritional value and sensory acceptance of rapeseed proteins (Zhang et al., 2020, 2024). Specifically, the formation of phenolic compound-protein complexes (Wanasundara, 2011) and insoluble phytate-protein complexes (Albe-Slabi et al., 2022) reduces the bioavailability of the proteins. Additionally, the bitter/astringent taste poses a major problem for food-grade rapeseed proteins (Aider & Barbana, 2011; Hald et al., 2019), as does the dark color associated with sinapate esters and phenolic-protein complexes in rapeseed meal (Zhang et al., 2024).

To overcome these limitations, the effects of different treatments on

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removing antinutritional compounds have been investigated (Akbari & Wu, 2015). Pretreating rapeseed meal with an aqueous ethanol solution (Georgiev et al., 2021) or with phytase/ethanol (Chen et al., 2024; Xiong et al., 2022) has been shown to reduce the content of phenolics, glucosinolates, and phytates in protein isolates. Although alkali extraction remains the most commonly applied procedure, new methods for rapeseed protein extraction have recently been explored, including weakly acidic salt extraction (Zhang et al., 2024) and extraction using natural deep eutectic solvents (NDESs) (Karimi et al., 2024).

NDESs are considered green extraction alternatives for many biomolecules, including proteins, due to their non-toxic nature, composition from naturally occurring compounds, and biodegradability (Boateng, 2023). Several studies have explored the use of NDESs for plant protein extraction, with some specifically focusing on protein extraction from rapeseed meal (Grudniewska et al., 2018; Karimi et al., 2024).

After protein extraction, the residual meal remains. To align with the principles of a circular economy, valorization of this fibre-rich by-product is essential. One option is its bioconversion into thermoplastic and biodegradable polymers, such as polyhydroxyalkanoates (PHA) (Wongsirichot et al., 2019). Georgiev et al. (2021) noted that the waste remaining after protein isolation from ethanol-treated rapeseed meal resulted in a product with a relatively high water absorption capacity, making it suitable for functional food applications. Another approach is using the waste for pyrolysis, converting it into energy (Gallorini et al., 2023).

To the best of the authors' knowledge, a comparison of fibre-rich products obtained through alkali extraction and NDESs has not been previously studied. Nutrient profiling of these products is crucial for defining their potential applications in the feed or food industry. Therefore, the aim of this work was to examine the residues obtained after protein isolate production from cold-pressed rapeseed meal using alkali extraction at pH 12 and NDES extraction (choline chloride:urea and betaine:citric acid) (Fig. 1) in terms of: 1) basic chemical profile, 2) minerals, 3) amino acid profile, 4) antinutrient content, and 5) colour.

2. Material and methods

2.1. Rapeseed cake

The rapeseed cake was obtained from Serbian vegetable oil producer specialized for the production of cold-pressed oil (Suncokret d.o.o., Hajdukovo) in 2024. It was stored at −20 °C until defatted. After the defatting process using hexane, the rapeseed meal was stored at 4 °C until analysis.

2.2. NDESs preparation

A mixture of choline chloride (ChCl) and urea in a 1:2 mass ratio, with 10 % added water, was used to prepare NDES 1, while a mixture of betaine and citric acid in a 1:1 mass ratio, with 20 % added water, was used for NDES 2. The components of each NDES were placed in an Erlenmeyer flask and stirred at 60 °C for 60 min to achieve a colorless solution. The solution was allowed to cool to room temperature and left for further analysis.

2.3. Rapeseed fibre-rich ingredient production

The rapeseed cake was defatted twice at 30-min intervals using hexane extraction (1000 rpm, 30 min, 25 °C) with a solid-to-liquid ratio (w/v) of 1:5. Afterward, the cake was left overnight (24 h) to allow the residual hexane to evaporate.

2.3.1. Alkali extraction

The defatted cake (rapeseed meal) was treated with 84 % ethanol at a ratio of 1:60 (w/v) and mixed on a magnetic stirrer (IKA C-Mag HS7, Staufen, Germany) at 1000 rpm for 30 min. The mixture was then placed in an ultrasonic bath (TI-H-10 2.2gal Multi Frequency Ultrasonic Bath, Elma Schmidbauer GmbH, Germany) and occasionally stirred with a glass rod at a frequency of 45 Hz at room temperature for 15 min. The suspension was decanted to separate the sediment, which was further used for protein extraction.

Fig. 1. Study workflow.

For alkaline extraction, the prepared material was suspended in water adjusted to pH 12 at a ratio of 1:20. During mixing, the pH was continuously adjusted with 1 M NaOH. After 60 min of extraction, the mixture was again placed in the ultrasonic bath and stirred with a glass rod at a frequency of 45 Hz at room temperature for 30 min. After extraction, the suspension was filtered to remove the insoluble material, which was analyzed in this study. The obtained material was dried in a vacuum dryer (Binder VD 115, Tuttlingen, Germany) at 40 °C for 24 h (ingredient 1). Afterward, the dried material was ground to a powder using laboratory mill (Knife mill KN 295 Knifetec, FOSS Analytical, Denmark) to pass through a sieve with square openings of 200 μm.

2.3.2. NDES extraction

Rapeseed meal (6 g) was mixed with 60 g of DES solution in a beaker, maintaining a meal-to-solvent mass ratio of 1:10. Protein extraction was carried out at 60 °C for 1 h using a magnetic stirrer. The mixture was then subjected to ultrasonic treatment at 60 °C for 15 min to enhance the extraction process. After sonication, the mixture was stirred at 60 °C for an additional hour using the magnetic stirrer. Finally, the mixture was left to stand overnight at room temperature.

After the extraction process, the mixture was transferred to a centrifuge tube and centrifuged at 9000 rpm for 10 min. The precipitate was then washed five times with distilled water to remove any residual DES and dried in a vacuum dryer at 36 °C for 24 h (ingredients 2 and 3). The dried materials were ground to the powder using laboratory mill to pass through a sieve with square openings of 200 μm.

2.4. Determination of basic chemical composition

Basic chemical composition of defatted rapeseed meal and fibre-rich ingredients was analyzed using the methods of AOAC (2000), which included protein (Official Method No. 950.36), fat (Official Method No. 935.38), total, soluble and insoluble dietary fibre (Official Method No. 991.43), and moisture (Official Method No. 926.5) determinations.

2.5. Determination of mineral content

A 5 g sample was prepared via dry ashing at 550 °C, with repeated HNO_3 treatment until clean ash was obtained. The ash was dissolved in 1:1 HCl, diluted to 50 mL with water, with previous Cs and La addition. Metal concentrations (K, Na, Ca, Mg, Mn, Zn, Cu, Fe, Pb, Cd) were analyzed using atomic absorption spectrometry (Varian SPECTRAA-10) with standard calibration curves ($R^2 = 0.999$).

2.6. Amino acid profiling

Amino acid analysis were performed using ion exchange chromatography with a Biochrom 30+ amino acid analyzer (Biochrom, UK) according to Spackman et al. (1958). Samples were hydrolyzed in 6 M HCl at 110 °C for 24 h, cooled, filtered, and made up to 25 mL in sodium loading buffer (pH 2.2). The prepared samples were filtered through a 0.22 μm PTFE filter and transferred to HPLC vials. Amino acid peaks were identified by comparison with a standard (Sigma-Aldrich). Results were expressed as grams of amino acid per 100 g sample.

2.7. Determination of antinutrients

Total phenolic content (TPC) of the rapeseed meal and fibre-rich ingredients was determined spectrophotometrically using Folin-Ciocalteu method described by Singleton et al. (1999). Condensed tannins were detected using the vanillin method described by Herald et al. (2014).

The glucosinolate content was determined according to the MSZ-08-1908 standard.

The phytic acid content was measured following the protocol provided in the phytic acid assay kit from Megazimes.

2.8. Colour analysis

The colour of the fibre-rich products was assessed using a Minolta Chromameter (Model CR-400, Minolta Co., Osaka, Japan). The samples were placed in 20-mm-thick holders, and the chromameter was calibrated before each measurement using a white calibration plate. All measurements were conducted at room temperature (23 ± 1 °C). Colour measurements were taken at five points on each sample (one at the center and four at the corners). Each measurement was repeated 25 times.

2.9. Statistical analysis

The data were processed statistically using the software package XLSTAT 2024 (Lumivero Denver, CO, USA). Results were presented as the mean ± standard deviation of the triplicate analyses for all measurements, except for the colour determination of the samples, which was performed in 5 repetitions. Analysis of variance (ANOVA) and Tukey's HSD test ($\alpha = 0.05$) were used for comparison of sample means.

3. Results and discussion

3.1. Physicochemical characterization of fibre-rich ingredients

The fibre-rich ingredients obtained from cold-pressed rapeseed meal after alkali extraction of proteins at pH 12 with ethanol pretreatment (ingredient 1), and NDES extraction using choline chloride:urea (ingredient 2) and betaine:citric acid (ingredient 3), were characterized based on their basic chemical profile and mineral content, as shown in Table 1. Both alkali and NDES extractions resulted in residues with considerable protein content, due to the insufficient protein extractability from rapeseed meal (Karimi et al., 2024). The protein content of ingredient 1 (22.5 ± 0.25 %) is comparable to that of deproteinized ethanol-treated rapeseed meal (16.79 ± 0.15 %) reported by Georgiev et al. (2022).

The significantly higher ($p \leq 0.05$) protein content in the materials remaining after NDES extraction is due to the lower extraction efficiency of NDESs compared to alkali extraction, as previously reported by Karimi et al. (2024). The better extraction efficiency of alkali extraction results from the lower water viscosity and density, which enhance diffusion properties, molecular mobility, and protein interactions with water in the alkaline medium (Zhang et al., 2020). Additionally, NDESs differ significantly in properties important for protein extraction, such as polarity, viscosity, and density (Boateng, 2023).

Rapeseed contains fibres (9–20 %) made up of insoluble fibres (cellulose and lignin) and soluble fibres (hemicellulose, pectin, gum, β-glucan, mucilage) (Tofanica et al., 2011). After protein extraction, high amounts of dietary fibre remain in the residues (Georgiev et al., 2022; Wickramasuriya et al., 2015). The residue remaining after NDES extraction contained 46.9 ± 2.36 % and 48.4 ± 1.17 % total fibre (ingredients 2 and 3, respectively), while the residue after alkali extraction (ingredient 1) was significantly higher ($p \leq 0.05$) – 63.0 ± 1.44 % total fibre (Table 1) and consistent with the 67.59 % reported by Georgiev et al. (2022) for deproteinized ethanol-treated rapeseed meal. Insoluble dietary fibres dominate in total dietary fibre content in all ingredients, as previously reported (Georgiev et al., 2022).

Georgiev et al. (2022) investigated deproteinized ethanol-treated rapeseed meal, revealing its relatively high water absorption capacity, which is linked to proteins bound in the residue. Additionally, the abundance of fibres in fibre-rich ingredients offers an opportunity for modification, such as through peroxide ion treatments (Sakač et al., 2009), to produce materials with enhanced properties (colour and water-holding capacity) for food applications.

Table 1
Basic chemical profile and mineral contents of defatted cold-pressed rapeseed cake and fibre-rich ingredients obtained after protein isolate production using alkali extraction at pH 12 with ethanol pretreatment (ingredient 1), and natural deep eutectic solvent (NDES) extraction with choline chloride:urea (ingredient 2) and betaine:citric acid (ingredient 3).

Basic chemical composition[a]	Defatted cold-pressed rapeseed cake	Fibre-rich ingredient 1	Fibre-rich ingredient 2	Fibre-rich ingredient 3
Moisture (%)	7.55 ± 0.18	5.30 ± 0.12^{a}	4.69 ± 0.46^{a}	5.19 ± 0.79^{a}
Proteins (%)[a]	37.4 ± 0.16	22.5 ± 0.25^{a}	32.7 ± 0.40^{b}	35.5 ± 0.66^{c}
Fat (%)[a]	1.16 ± 0.04	–	–	–
Total fibre (%)[a]	38.0 ± 1.22	63.0 ± 1.44^{b}	46.9 ± 2.36^{a}	48.4 ± 1.17^{a}
Soluble fibre (%)[a]	2.89 ± 0.20	2.73 ± 0.21^{a}	5.37 ± 0.44^{b}	3.22 ± 0.14^{a}
Insoluble fibre (%)[a]	35.1 ± 1.02	60.3 ± 1.23^{b}	41.5 ± 1.92^{a}	45.2 ± 1.54^{a}
Minerals[a]				
Potasium (g/kg)	4.73 ± 0.08	13.5 ± 0.22^{b}	2.43 ± 0.04^{a}	<0.75
Sodium (g/kg)	10.1 ± 0.17	0.14 ± 0.00^{a}	0.14 ± 0.00^{a}	0.32 ± 0.01^{b}
Calcium (g/kg)	2.44 ± 0.04	3.22 ± 0.05^{a}	6.11 ± 0.09^{b}	1.17 ± 0.02^{a}
Magnesium (g/kg)	3.78 ± 0.06	6.55 ± 0.10^{c}	3.79 ± 0.06^{b}	0.21 ± 0.00^{a}
Manganese (mg/kg)	39.1 ± 6.03	78.2 ± 12.0^{b}	2.99 ± 0.46^{a}	5.12 ± 0.79^{a}
Zinc (mg/kg)	22.9 ± 3.53	104 ± 16.1^{c}	63.0 ± 9.71^{b}	9.75 ± 1.50^{a}
Copper (mg/kg)	4.76 ± 0.73	8.77 ± 1.34^{b}	5.59 ± 0.86^{a}	5.08 ± 0.78^{a}
Iron (mg/kg)	40.7 ± 6.28	56.7 ± 8.74^{a}	97.8 ± 15.1^{b}	101 ± 15.6^{b}
Lead (mg/kg)	<0.06	0.22 ± 0.03	<0.06	<0.06
Cadmium (mg/kg)	<0.01	<0.01	<0.01	<0.01

Values are means of three determinations ± standard deviation.
Means in the same row with different superscript are statistically different ($p \leq 0.05$).
Fibre-rich ingredient 1 – obtained after alkali extraction at pH 12 with ethanol pretretment.
Fibre-rich ingredient 2 – obtained after NDES extraction (choline chloride:urea 1:2, 10 % H_2O, pH 7.60, T = 38.4 °C).
Fibre-rich ingredient 3 – obtained after NDES extraction (betaine:citric acid, 20 % H_2O, pH 2.45, T = 37.6 °C).
[a] on dry basis.

3.2. Mineral content

The fibre-rich ingredients were rich in minerals (Table 1). Potassium content was highest in ingredient 1 compared to ingredients 2 and 3, with magnesium also dominating. Among micro minerals, zinc was present in the highest amount (104 ± 16.1 mg/kg) in ingredient 1, followed by manganese (78.2 ± 12.0 mg/kg) and iron (56.7 ± 8.74 mg/kg). Georgiev et al. (2022) reported that iron content was highest in deproteinized ethanol-treated rapeseed meal, while zinc and manganese were also present. Lead was detected only in ingredient 1. Cadmium levels were below 0.01 mg/kg in all ingredients, which aligns with the cadmium content in deproteinized ethanol-treated rapeseed meal, as reported by Georgiev et al. (2022).

3.3. Amino acid profile

The amino acid composition of the fibre-rich ingredients is presented in Table 2. All amino acids were detected in all samples, except for cysteine in ingredient 1. The highest amino acid content was observed in ingredient 2, which correlates with its protein content. Ingredients 1–3 were found to be good sources of essential amino acids (EAAs). The ratio of EAA/NEAA (essential to non-essential amino acids) was greater than 0.6 in all samples, indicating that they meet the daily requirements for human consumption based on FAO/WHO recommendations for different age groups and physiological needs (WHO, 2007).

Table 2
Amino acid profile of defatted cold-pressed rapeseed cake and fibre-rich ingredients obtained after protein isolate production using alkali extraction at pH 12 with ethanol pretreatment (ingredient 1), and natural deep eutectic solvent (NDES) extraction with choline chloride:urea (ingredient 2) and betaine:citric acid (ingredient 3).

Amino acid (g/100 g sample)[a]	Defatted cold-pressed rapeseed cake	Fibre-rich ingredient 1	Fibre-rich ingredient 2	Fibre-rich ingredient 3
EAA				
Valine	1.68 ± 0.07	1.30 ± 0.03^{a}	1.65 ± 0.04^{c}	1.50 ± 0.05^{b}
Methionine	0.66 ± 0.03	0.46 ± 0.02^{b}	1.05 ± 0.06^{c}	0.12 ± 0.01^{a}
Isoleucine	1.86 ± 0.09	0.90 ± 0.07^{a}	1.60 ± 0.08^{b}	0.87 ± 0.04^{a}
Leucine	3.18 ± 0.18	1.42 ± 0.11^{a}	1.75 ± 0.12^{b}	1.63 ± 0.06^{b}
Phenylalanine	1.34 ± 0.13	0.98 ± 0.05^{a}	1.35 ± 0.10^{b}	1.11 ± 0.09^{a}
Histidine	1.61 ± 0.11	0.47 ± 0.03^{a}	0.70 ± 0.07^{b}	0.46 ± 0.04^{a}
Lysine	2.35 ± 0.16	1.22 ± 0.09^{a}	1.60 ± 0.13^{b}	1.72 ± 0.13^{b}
Threonine	1.50 ± 0.07	1.18 ± 0.09^{a}	2.10 ± 0.05^{b}	1.33 ± 0.02^{a}
NEAA				
Aspartic acid	2.73 ± 0.05	1.95 ± 0.07^{b}	1.20 ± 0.07^{a}	2.70 ± 0.08^{c}
Arginine	3.52 ± 0.23	2.22 ± 0.14^{b}	2.05 ± 0.15^{b}	1.25 ± 0.12^{a}
Serine	1.63 ± 0.06	1.19 ± 0.07^{a}	1.50 ± 0.04^{b}	1.41 ± 0.10^{b}
Glutamine	4.87 ± 0.11	3.03 ± 0.12^{a}	4.10 ± 0.18^{b}	4.24 ± 0.13^{b}
Proline	3.56 ± 0.10	1.09 ± 0.08^{b}	1.40 ± 0.07^{c}	0.45 ± 0.01^{a}
Glycine	1.48 ± 0.09	1.07 ± 0.08^{a}	2.05 ± 0.13^{c}	1.45 ± 0.07^{b}
Alanine	1.44 ± 0.08	1.00 ± 0.06^{a}	2.20 ± 0.12^{c}	1.29 ± 0.07^{b}
Cystine	0.63 ± 0.04	n.d.	1.00 ± 0.06^{b}	0.36 ± 0.02^{a}
Tyrosine	0.93 ± 0.03	0.76 ± 0.03^{b}	0.95 ± 0.05^{c}	0.63 ± 0.04^{a}
EAA	**14.1**	**7.93**	**11.8**	**8.74**
NEAA	**20.8**	**12.3**	**16.4**	**13.8**
n EAA/NEAA	**0.68**	**0.64**	**0.72**	**0.63**
TAA	**34.9**	**20.2**	**28.2**	**22.5**

Values are means of three determinations ± standard deviation.
Means in the same row with different superscript are statistically different ($p \leq 0.05$).
Fibre-rich ingredient 1 – obtained after alkali extraction at pH 12 with ethanol pretretment.
Fibre-rich ingredient 2 – obtained after NDES extraction (choline chloride:urea 1:2, 10 % H_2O, pH 7.60, T = 38.4 °C).
Fibre-rich ingredient 3 – obtained after NDES extraction (betaine:citric acid, 20 % H_2O, pH 2.45, T = 37.6 °C).
NEAA – nonessential amino acids.
EAA – essential amino acids.
[a] on dry basis.

Furthermore, ingredient 2 exhibited the highest EAA/NEAA ratio, which was 0.72.

Previous studies suggest that the extraction procedure influences the amino acid composition, a finding consistent with our results (Ivanova et al., 2017; Kumar et al., 2021). Among the essential amino acids, valine, leucine, and threonine were the most abundant across all ingredients. Notably, ingredient 2 had higher levels of valine and methionine compared to ingredients 1 and 3.

Among the non-essential amino acids, glutamine, glycine, and alanine were the most abundant. The amino acid profiles suggest that all three fibre-rich ingredients can serve as valuable sources of essential amino acids, further reinforcing their potential as not only fibre-rich but also protein-containing ingredients.

3.4. Antinutrient profile

Rapeseed meal contains high amounts of antinutritional components, such as glucosinolates, polyphenols, and phytic acid. These compounds can have undesirable effects on human and animal health or reduce the nutritional and sensory quality of the final product (Arrutia et al., 2020). Therefore, various elimination strategies have been employed to reduce their content in rapeseed protein isolate production

(Karabulut et al., 2025). Despite these efforts, some antinutrients remain in the obtained fibre-rich ingredients (Table 3).

Glucosinolates were detected only in defatted cold-pressed rapeseed cake (28.8 ± 4.70 μmol/g), but were not found in the fibre-rich ingredients 1–3. Georgiev et al. (2022) reported low amounts of glucosinolates (3.70 ± 0.13 μmol/g) in deproteinized ethanol-treated rapeseed meal.

Defatted rapeseed meal contains approximately 2 % (w/w) phenolic acids, with sinapine being the predominant compound in free, bound, and esterified forms (Karabulut et al., 2025). The polyphenol content in defatted cold-pressed cake (2.33 ± 0.07 g GAE/100 g, Table 3) aligns with the value reported in the literature.

Phenolic compounds in rapeseed interact with proteins through both covalent and non-covalent bonding, especially during protein isolation. These interactions can lead to dark coloration, off-flavors, and reduced protein functionality and digestibility. To reduce polyphenol content, pretreatments of rapeseed meal with ethanol prior to protein extraction have been applied (Chen et al., 2024; Xiong et al., 2022). Fibre-rich ingredient 1 was obtained after pretreatment with ethanol, and its TPC was 0.01 ± 0.00 g GAE/100 g (Table 3). Fibre-rich ingredients obtained after NDES extraction (ingredients 2 and 3) had significantly higher ($p \leq 0.05$) polyphenol contents (0.13 and 0.14 g GAE/100 g, respectively), but their obtaining did not involve the use of pretreatment.

Beyond sinapine and other phenolic acids, rapeseed contains tannins, with levels varying from 1.5 to 3.0 % in whole seeds and reaching up to 6 % in defatted cold-pressed cake (Karabulut et al., 2025). Tannins interact with mineral ions through chelation, reducing their bioavailability, and form insoluble complexes with proteins, inhibiting nutrient absorption. The oxidation and polymerization of tannins under alkaline or neutral conditions, which occur during alkali extraction, result in dark coloration and off-flavors (Yang et al., 2022). The occurrence of these processes may explain the significantly lower ($p \leq 0.05$) tannin content in ingredient 1 compared to ingredients 2 and 3 (Table 3).

Rapeseed contains 1–2 % phytate, which can increase to 2–5 % in defatted rapeseed meal (Yang et al., 2022), and our result for phytic acid in defatted cold-pressed rapeseed cake aligns with this content. During the alkaline and NDES extraction of rapeseed proteins, most of the phytate remained in the fibre-rich ingredients, particularly in ingredient 2 (Table 3). It can be assumed that treatment with phytases prior to protein extraction would significantly reduce phytic acid content in both the protein isolates and the residues. This approach is supported by Chen et al. (2024), who improved the flavour of rapeseed protein isolate using phytase/ethanol treatment. Given the crucial antinutrient effects of phytic acid, which tends to form phytate-protein and phytate-mineral complexes (Albe-Slabi et al., 2022), this strategy should be considered.

Table 3
Antinutritional factors of defatted cold-pressed rapeseed cake and fibre-rich ingredients obtained after protein isolate production using alkali extraction at pH 12 with ethanol pretreatment (ingredient 1), and natural deep eutectic solvent (NDES) extraction with choline chloride:urea (ingredient 2) and betaine: citric acid (ingredient 3).

Antinutrient[a]	Defatted cold-pressed rapeseed cake	Fibre-rich ingredient 1	Fibre-rich ingredient 2	Fibre-rich ingredient 3
Glucosinolates (μmol/g)	28.8 ± 4.70	n.d.	n.d.	n.d.
Tannins (mg catechin/g)	2.53 ± 0.31	0.72 ± 0.05^{a}	1.49 ± 0.12^{b}	1.75 ± 0.21^{b}
Polyphenols (g GAE/100 g)	2.33 ± 0.07	0.01 ± 0.00^{a}	0.13 ± 0.04^{b}	0.14 ± 0.03^{b}
Phytic acid (g/100 g)	1.72 ± 0.04	1.37 ± 0.08^{b}	2.24 ± 0.07^{c}	1.05 ± 0.06^{a}

GAE – gallic acid equivalent.
Values are means of three determinations ± standard deviation.
Means in the same row with different superscript are statistically different ($p \leq 0.05$).
Fibre-rich ingredient 1 – obtained after alkali extraction at pH 12 with ethanol pretretment.
Fibre-rich ingredient 2 – obtained after NDES extraction (choline chloride:urea 1:2, 10 % H_2O, pH 7.60, T = 38.4 °C).
Fibre-rich ingredient 3 – obtained after NDES extraction (betaine:citric acid, 20 % H_2O, pH 2.45, T = 37.6 °C).
[a] on dry basis.

3.5. Colour of fibre-rich ingredients

Colour is an important parameter for consumer acceptance, especially when a new ingredient is considered for food applications. In rapeseed protein extraction, NDESs have proven to be more effective, as they yield isolates with higher extraction efficiency, brighter color, lower antinutrient content, better functionality, and preservation of the protein's native structure compared to alkaline treatments (Karimi et al., 2024). Additionally, NDESs are a better choice in terms of colour parameters of the residues (Table 4). Specifically, fibre-rich ingredients 2 and 3 were significantly lighter ($p \leq 0.05$) compared to ingredient 1 (Fig. 2), likely due to the oxidation and polymerization of tannins under alkaline extraction conditions (Yang et al., 2022). The a^* color coordinate (red tone) varied across all ingredients, being significantly higher ($p \leq 0.05$) in those obtained via NDES extraction, while the yellow tone (b^* color coordinate) was similar in both ingredients 2 and 3 (Table 4).

4. Conclusions

This study evaluated fibre-rich ingredients obtained from cold-pressed rapeseed meal using alkali extraction at pH 12 and NDES extraction (ChCl:urea and betaine:citric acid). The results showed that all ingredients were rich in dietary fibres and proteins, with significant levels of minerals. The ChCl:urea-based ingredient (ingredient 2) had the highest levels of lysine and methionine. Alkali extraction resulted in the lowest TPC and tannins, while NDES systems outperformed alkali extraction in terms of colour.

The study highlights the potential of NDES extraction for producing high-quality, functional ingredients from rapeseed cake, which could enrich food products. The utilization of residues obtained after rapeseed protein extraction represents a valid approach that supports the principles of a circular economy.

However, the study is limited by the scope of the NDES extraction methods and their scalability. Future research should focus on optimizing NDES extraction for large-scale production to produce fibre-rich products with the best properties, including minimal antinutrient content and optimal sensory characteristics.

Table 4
Colour parameters of fibre-rich ingredients obtained after protein isolate production using alkali extraction at pH 12 with ethanol pretreatment (ingredient 1), and natural deep eutectic solvent (NDES) extraction with choline chloride: urea (ingredient 2) and betaine:citric acid (ingredient 3).

Colour parameters	Fibre-rich ingredient 1	Fibre-rich ingredient 2	Fibre-rich ingredient 3
L^*	44.1 ± 0.02^{a}	51.1 ± 0.05^{b}	56. 9 ± 0.08^{c}
a^*	2.58 ± 0.05^{a}	4.21 ± 0.02^{b}	5.48 ± 0.02^{c}
b^*	9.72 ± 0.08^{a}	12.0 ± 0.03^{b}	12.9 ± 0.02^{c}

Values are means of five determinations ± standard deviation.
Means in the same row with different superscript are statistically different ($p \leq 0.05$).
Fibre-rich ingredient 1 – obtained after alkali extraction at pH 12 with ethanol pretretment.
Fibre-rich ingredient 2 – obtained after NDES extraction (choline chloride:urea 1:2, 10 % H_2O, pH 7.60, T = 38.4 °C).
Fibre-rich ingredient 3 – obtained after NDES extraction (betaine:citric acid, 20 % H_2O, pH 2.45, T = 37.6 °C).

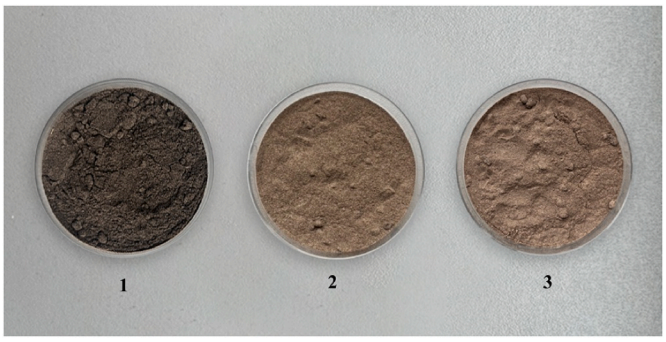

Fig. 2. Appearance of fibre-rich ingredients obtained after protein isolate production using alkali extraction at pH 12 with ethanol pretreatment (ingredient 1), and natural deep eutectic solvent (NDES) extraction with choline chloride:urea (ingredient 2) and betaine:citric acid (ingredient 3).

Declaration of interest: None.

CRediT authorship contribution statement

Marijana Sakač: Writing – original draft, Supervision, Conceptualization. **Aleksandar Marić:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Branislava Đermanović:** Validation, Methodology, Formal analysis, Data curation. **Damjana Tomić:** Visualization, Validation, Software, Formal analysis. **Danka Dragojlović:** Methodology, Investigation, Data curation. **Bojana Šarić:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Pavle Jovanov:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

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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Data availability

Data will be made available on request.

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