Valorization of rapeseed meal: Influence of ethanol antinutrients removal on protein extractability, amino acid composition and fractional profile

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Abstract

The production of rapeseed oil leads to generation of large quantities of rapeseed meal as a by-product. To increase the applicability of the rapeseed meal in feed and food industries, the content of antinutrient compounds is often reduced by treatment with ethanol. The aim of the study was to evaluate the influence of ethanol pre-treatment of the rapeseed meal on protein extractability, amino acid composition and fractional profile. The ethanol treatment of the rapeseed meal significantly increased the protein content from 37.4% to 42.3% and reduced the lipid concentration from 1.9% to 1.1%. Approximately 4- and 14-fold reductions of the phenols and glucosinolate contents were achieved respectively. Protein yield, however, was diminished from 26.4% to 23.6%. A stronger decrease of the protein yield, from 47.8% to 26.4%, was caused by processing of the rape seeds to rapeseed meal. The process resulted in the reduction of lysine content, while further ethanol treatment of the rapeseed meal affected more amino acids, both essential (threonine, phenylalanine) and non-essential (alanine, tyrosine, arginine, histidine). Comparative fractional protein profiles of rape seeds, rapeseed meal and ethanol treated rapeseed meal exhibited differences in both composition of the fractions and the relative quantity of the proteins. Data suggested that the treatment of the rapeseed meal with ethanol impacted protein solubility, amino acid composition and protein fractional profile. This knowledge is valuable when ethanol treated rapeseed meal is used either as a protein feed additive or as a source for generation of protein-rich ingredients with specific nutritive value and functionality.

Key words: ethanol treatment, amino acid composition, protein extractability, protein fractional profile, rapeseed meal

Statement of Novelty

Rapeseed meal is generated in large quantities as a by-product. However, high levels of antinutrient compounds limit its utilization in feed and food industries thus turning it into a waste. To increase the applicability of the rapeseed meal, ethanol is often used to reduce the content of antinutrients. While most studies are focused on the efficiency of the ethanol treatment on antinutrient reduction, little is known on its influence on the quality of the ethanol treated rapeseed meal protein. Knowledge on amino acid composition, protein solubility and fractional profile is valuable when ethanol treated rapeseed meal is intended for use either as a feed additive or as a source for production of protein-rich ingredients with specific functionality.

Introduction

Rapeseed is an economically valuable technical crop which is primarily used for oil production. In recent years, there is an increasing interest in rape seeds processing due to the use of the rapeseed oil for both food and technical purposes [1]. Currently, the European Union is emerging as a global leader in biodiesel production where rapeseed oil is used as the raw material [2]. The production of rapeseed oil, whether for food or technical applications, involves the generation of large quantities of rapeseed meal as a by-product. According to Ivanova [3], rapeseed meal may reach up to 48% of the mass of the processed seeds. Due to its high protein content (38-48%) and relatively balanced amino acid composition, rapeseed meal is used as a high-protein component in the production of feed. However, its application as a feed additive is limited due to the presence of antinutritional compounds and high fiber content [4]. As a result, large quantities of the rapeseed meal remain unused and thus turn into a waste. Long term storage or disposal of the waste might be associated with additional financial expenses leading to a price increase of the primary product (rapeseed oil).

Alternatively, rapeseed meal could be used as raw material for generation of protein-rich ingredients for food industry [5]. However, high levels of antinutritional factors such as phenolics, tannins, glucosinolates, allyl isothiocyanates, and phytates may worsen the quality of the products and thwart their application. Due to the negative physiological effect of these compounds on animals and humans, a pre-treatment step of the rapeseed meal, aiming at their removal or reduction has been strongly suggested if further use of the biomass for the generation of more protein-rich products with added values is intended [4].

Numerous approaches have been studied for their efficacy to reduce/remove antinutritive compounds from rapeseed meal. They include, but are not limited to, alkaline [6] or thermal [6, 7] treatments, fermentation [8] and extraction with NH_4^+ or Ca^{2+} containing solvents [9, 10].

Purkayastha et al. [11] established high reductive potential of solvents containing acetone or methanol combined with water or an acid which, however, resulted in extracts with high hemolytic activity. Efficient removal of glucosinolates from a commercial rapeseed meal by water extraction on a pilot scale was reported by Liu et al. [4].

According to Adem et al. [12], sufficient reduction of antinutrient compounds in rapeseed meal can be achieved by using aqueous ethanol solution. Ethanol is a polar solvent, allowed for use in the food industry [13], which might explain its broad application as an antinutrient reductant. In addition to glucosinolates, water-ethanol treatment of rapeseed meal may also reduce the phenolic compounds up to 75% [14]. While most studies are focused on the efficiency of the ethanol treatment on antinutrient reduction, little is known on its influence on the quality of the ethanol treated rapeseed meal protein. Ethanol is a denaturing agent and its application may affect protein solubility, amino acid composition and fractional profile and, as a consequence, the functional properties of the rapeseed meal protein [15]. The aim of present study was to evaluate the influence of the ethanol treatment of commercial rapeseed meal on protein extractability, amino acid and protein fractional composition which is valuable information needed for the further application of this by-product either as a protein feed additive or as a source for generation of protein-rich ingredients for food industry.

Material and Methods

Material

Rape seeds were used as a primary material for the study. The rapeseed meal was commercially produced by thermal treatment at 110 – 115 ºC followed by extraction with hexane at 60 – 65ºC for approximately 1 h. Both the rape seeds and the rapeseed meal were provided by a local company. The ethanol treated rapeseed meal was prepared under laboratory conditions as described by Chabanon et al. [14] with some modifications. Briefly, the rapeseed meal was grinded and sifted to collect 0.315 mm particles, which were treated four times with 75% aqueous ethanol solution at a meal to solvent ratio of 25% (w/v) for 30 min at a room temperature. The residues were collected by decanting, dried in air and stored in a closed container. All reagents used were of analytical grade.

Chemical analysis

Total nitrogen was determined by the Kjeldahl method and multiplied by 6.25 to convert to crude protein [16]. Ash content was determined by ICC Standard №104/1[17]. Total lipids and crude fiber were evaluated by standardized methods [18, 19]. Phenols were extracted with 70% aqueous ethanol solution as describe by Petkova et al. [20] and quantified using Folin-Ciocalteu reagent [21]. Total glucosinolates were evaluated as described by Jezek et al. [22]. The method is based on spectrophotometric evaluation of glucosinolates after alkaline hydrolysis and reduction with potassium ferricyanide. Sinigrin was used for standard curve generation.

Amino acid analysis

Samples were hydrolyzed with 6 N HCl at 105°C for 24 h followed by neutralization and filtration [23]. The hydrolysates were derivatized by using AccQ-Fluor TM Reagent kit (Waters Corporation, Milford, MA, USA) following manufacturer’s instructions. The amino acid analyzes were performed on a high performance liquid chromatograph (ELITE LaChrome, Hitachi High Technologies America, Inc., San Jose, CA, USA) equipped with a C18 AccQ-Tag (3.9 mm x 150 mm) reversed-phase chromatographic column and a Diod array detector.

Protein fractionation

Each sample of rape seeds, rapeseed meal and ethanol treated rapeseed meal was sequentially extracted with water, 5% NaCl, 70% ethanol and 0.1 N NaOH to obtain albumin, globulin, prolamin and glutelin fractions respectively [24]. Each fractional extraction was repeated three times with decreasing meal/residue to solvent ratio from the first to the third extraction as it follows: 1:10, 1:5 and 1:2.5. All extractions were performed at room temperature (23°C) for 30 min and under constant agitation. Extraction aliquots were collected and protein contents were evaluated by the Bradford method [25], using bovine serum albumin as a standard. Protein yield was presented as percentage of the amount of the crude protein in the samples determined by the Kjeldahl method. The three extraction aliquots of each fraction were combined and stored at -20°C for further evaluation of protein fractional profiles by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

SDS-PAGE

SDS-PAGE was performed with an omniPAGE mini Cleaver electrophoresis (Model CVS10DSYS, Cleaver Scientific Ltd, United Kingdom) as described by Laemmli [26]. The gel system consisted of a 15% polyacrylamide resolving gel (pH 8.8). Visualization of gels was realized with 0.2% Coomassie Brilliant Blue R-250 dye (Serva Electrophoresis GmbH, Germany) for 20 min and discolored by immerging in a solution containing 10% ethanol and 7% CH_3COOH for overnight. Data were analyzed by using TotalLab1D Analysis software (BioStep GmbH, Germany). To ease readability of data, proteins were provisionally grouped into three categories: low molecular weight proteins with a molecular weight up to 50 kDa (LMW), medium molecular weight proteins with molecular weight ranging from 50 to 150 kDa (MMW) and high molecular weight proteins with a molecular weight above 150 kDa (HMW).

Statistical analysis

Amino acid analyzes were replicated. All remaining experiments were performed in triplicate. Results are presented as means ± standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA) using Statgraphics Centurion statistical program (version XVI, 2009) (Stat Point Technologies, Ins., Warrenton, VA, USA). Mean differences were established by Fisher’s least significant difference test for paired comparison with a significance level α = 0.05.

Results and Discussion

Biochemical characteristics

Ethanol treatment of the rapeseed meal significantly influenced the quantity of all evaluated components (Table 1). While increased protein content to 42% is a desired characteristic, the enhanced fiber level is disadvantageous if the material is intended for direct use as a protein-rich additive in feed industry. The ethanol treatment resulted in a significant decrease of lipid content which favors a further potential protein extraction [27]. Approximately 4- and 14-fold reductions of the phenols and glucosinolates contents were achieved, respectively. The decrease in phenols content (in %) was similar to that previously published by Chabanon et al. [14] and Ivanova et al. [28]. The 93% reduction of glucosinolates level in ethanol treated rapeseed meal was higher than the 85% reduction reported by Adem et al. [12] but lower than the ones achieved by Slawski et al. [29] and Ivanova et al. [28]. Compared to the original sample (non-processed rape seeds), a 94% decrease in the glucosinolate content in the ethanol treated rapeseed meal was observed.

The rapeseed meal, used in our study, contained relatively high amount of glucosinolates when compared to non-processed rape seeds (Table 1). This result was unexpected since thermal instability of these compounds is known. The heat sensitivity of rapeseed meal glucosinolates was previously demonstrated by Jensen et al. [30] and Mansour et al. [31] who reported up to 94% reduction of these compounds after thermal treatment. Still, the influence of temperature on glucosinolates stability is controversial. A temperature as high as 150 °C during the extrusion of mixtures of rapeseed and soya bean was reported to effectively inactivate myrosinase but had little effect on the total and individual glucosinolates contents [32]. Glucosinolates reductions in coarsely ground rape seeds were not achieved after 5-min water treatments at 40, 50 and 80 °C [33]. It should be noted though that processing of rape seeds to rapeseed meal includes multiple factors that might influence glucosinolates content. According to Mosenthin et al. [34], the decrease in glucosinolates contents during processing is affected not only by temperature but also by the combination of factors such as steam pressure, duration of heat treatment, organic solvents used and material moisture which explain the observed variability in the published data. The same authors established that differently processed meals, under standardized and defined conditions in a pilot plant, differed in the contents of total and individual glucosinolates. Therefore, due to a lack of uniformed processing and experimental conditions, direct comparison between this study result and literature data is precluded.

Proximate analyzes of rapeseed and ethanol treated rapeseed meals were previously performed in our laboratory [28, 35]. However, the analyzed samples were derived from a different rape seeds harvest which does not allow statistical evaluation and comparison. Due to the high variability of rape seeds chemical composition and the correspondingly produced rapeseed meals [36 - 38], any conclusion on the influence of the ethanol treatment on rapeseed meal quality without direct comparison to the primary source might be inaccurate and compromised. The rapeseed meal and the ethanol treated rapeseed meal analyzed in this study differed from earlier samples evaluated by Ivanova et al. [28, 35]. The most profound differences were observed in the levels of glucosinolates.

Amino acid composition

The rapeseed meal contained relatively high amounts of both phenylalanine (5.93 g/100 g protein) and threonine (5.06 g/100 g protein) (Table 2). For comparison, the contents of those amino acids in “ideal” protein as determined by FAO [39] were 6.0 and 4.0 g/100 g protein, respectively. Lysine (4.21 g/100 g protein), which is considered first limiting amino acid in rapeseed meal, was in higher concentration than that established by Ivanova et al. [35] but lower than the results reported by Slominski et al. [40] and Tzeng et al. [41]. Glutamate level (14.03 g/100 g protein) was the highest among that of non-essential amino acids (Table 3) and in agreement with reported data [40, 41]. Overall, the quantity of the essential (Table 2) and non-essential (Table 3) amino acids of the rapeseed meal, in the present study, were close to that published by Slominski et al. [40]. However, a direct comparison to data, previously published, is difficult because this commercial rapeseed meal is a result of a mixture of different rapeseed cultivars. Most studies are focused on either specific commercial type meal or a meal produced from a selected rapeseed cultivar under laboratory conditions. The quality of rapeseed meal is highly variable and depends on various factors including rapeseed cultivar and growth conditions. Rape seeds processing as well as storage conditions may also alter protein quality. Variability in biochemical characteristics including amino acid composition of different rapeseed meals was previously reported [37, 40].

The processing of the rape seeds to rapeseed meal resulted in a decrease of lysine (Table 2), glycine and glutamate contents (Table 3) of approximately 1 g/100 g protein. Reduction of lysine content in canola meal, as affected by steam heating, was reported by Anderson-Hafermann et al. [42]. The same authors observed little or no processing effect on remaining amino acids. The treatment of the rapeseed meal with ethanol further reduced the contents of some essential (threonine, phenylalanine) and nonessential amino acids (alanine, tyrosine, arginine, histidine) by 0.5 to 1 g/100 g protein. This result may be explained by the solubility of some proteins and their partial extraction by ethanol during the process. Similarly, few differences were found in the essential amino acid contents due to treatment of rapeseed meals with ammonia in absolute or 95% methanol [9].

Rapeseed meal protein extractability

The Osborne procedure is a well-established method for fractionation of plant proteins into four groups, albumin, globulin, glutelin and prolamin, which is based on their solubility in water, salt solution, alkaline and alcoholic solutions respectively [24]. It allows preparation of proteins with specific characteristics, nutritive values and functional properties which explains its wide application.

Processing of the rape seeds to rapeseed meal significantly decreased the extractability of all protein fractions (Table 4). Approximately a two-fold reduction in yield of albumin, globulin and prolamin fractions was observed. Although statistically significant, the decrease in glutelin yield by absolute value was negligible. Compared to the protein yield, obtained from the rapeseeds, the cumulative protein yield from the rapeseed meal was reduced by 45% (Table 4). The finding is most probably due to elevated temperature and use of organic solvents involved in oil production. These are known to denature proteins and alter their physico-chemical properties [43]. A negative effect of elevated processing temperature on the protein yield from rapeseed meal was observed by Tan et al. [24]. The authors reported the lowest cumulative protein yield for industrial toasted rapeseed meal compared to the other samples tested ((Australian canola (*Brassica napus*) meal, mustard (*Sinapis alba*) meal and pre-toasted industrial meal)), suggesting high influence of thermal load on protein solubility. Studying the effect of drying on nutritional and functional quality of soy flour from sprouted soybean, Agrahar-Murugkar and Jha [44] found the highest protein solubility in the samples subjected to minimal heat treatment and a progressive decrease of protein solubility in response to increasing severity of the heat treatment.

Further treatment of the rapeseed meal with ethanol, aiming the reduction of antinutrients, additionally decreased the total protein yield from 26.4% to 23.6% due to diminished yields of albumin and globulin fractions (Table 4). Ethanol is known to induce conformational changes [45] or formation of disulfide cross - linkages [46] that might lead to protein aggregation, negatively affecting their solubility and extraction yield [12, 47]. Since albumin and globulin fractions contribute most to the nutritive value of the rapeseed proteins [48], reduction of their contents may negatively influence the overall quality of the protein in the rapeseed meal after antinutrients removal with ethanol. Changes in physico-chemical properties and fractional profile of proteins may also be expected which would affect their functional properties and application [49].

Unexpectedly, the yield of the other two fractions, namely prolamin and glutelin, increased (Table 4). Although the increases were less than 1%, the differences were significant ($p < 0.05$). Prolamins are alcohol-soluble proteins which, most probably, were not completely extracted during the 4-step ethanol treatment of the rapeseed meal. The results from Osborne fractionation implied that the ethanol pre-treatment of the rapeseed meal enhanced the extractability of the remaining prolamins which might be due to removal/reduction of non-protein substances such as polyphenols. A similar trend was observed for the glutelin fraction. Polyphenols can interact with proteins via formation of non-covalent bonds leading to generation of transient, unstable complexes [50]. This could partially explain the slight enhancement of prolamin and glutelin yields after ethanol treatment of the rapeseed meal. Although studies clarifying the nature of interactions between polyphenols and specific plant protein fractions are limited, recent investigation by Dai et al. [51] demonstrated hydrogen bonds and van der Waals forces as major interaction means between rice glutelin and gallic acid. Resveratrol (a polyphenol) and zein (maize prolamin) binding was predominantly mediated through hydrogen bonds [52].

Rapeseed protein fractional profile

SDS-PAGE of the albumin, globulin, prolamin and glutelin fractions, obtained by the Osborne procedure, revealed qualitative and quantitative differences in the protein composition of the rape seeds, rapeseed meal and ethanol treated rapeseed meals (Fig. 1). Comparative profile of albumins (Fig. 2), globulins (Fig. 3) and glutelins (Fig. 4) demonstrated the presence of proteins in the rape seeds which were not found either in rapeseed meal or in ethanol treated rapeseed meal. All of them had molecular weights higher than 30 kDa. In contrast, the majority of proteins with lower molecular weights were observed in all three samples. This may partially be due to disruption of non-covalent bonds and formation of smaller protein subunits during oil production. In addition, denaturing conditions and the use of reducing agent in SDS-PAGE most probably contributed to the decomposition of oligomeric proteins maintained by inter-chain disulfide bonds which explains the prevalence of LMW proteins in all fractions of the samples (Table 5). By analyzing protein profile of laboratory-defatted canola meals with SDS-PAGE, under reducing and non-reducing conditions, Aluko and McIntosh [53] observed significant reductions in intensity of the major bands in the presence of mercaptoethanol and suggested formation of smaller monomers after reduction of disulfide bonds. Our results are similar to data obtained by Adem et al. [12] who found 92.6% proteins with molecular weights lower than 50 kDa in rapeseed meal protein concentrate analyzed by SDS-PAGE.

For proteins with molecular weights less than 30 kDa, the comparative analyzes of albumins (Fig. 2), globulins (Fig. 3) and glutelins (Fig. 4) showed presence of proteins with the same molecular weights. For example, proteins with 11, 18 and 30 kDa were observed in albumin, globulin and glutelin fractions obtained from the rape seeds, rapeseed meal and the ethanol treated rapeseed meal. Although the Osborne fractionation is based on solubility difference of the proteins in specific solvents, some of the albumins and globulins, which remained in the solids due to incomplete extraction, might have been extracted with NaOH that is a strong alkali. Aluko and McIntosh [53] also demonstrated that salt-soluble globulins from canola meal were extracted with NaOH. Difficulties in complete separation of albumins and globulins, based on their solubility only, were reported by DuPont et al. [54]. By using 0.5 M NaCl solution, Fu and Sapirstein [55] extracted mixed protein types of albumins and globulins from wheat flour. In addition to LMW proteins, commonly observed in albumin and globulin fractions, a 65 kDa protein, reported by Adem et al. [12] as undefined, was noticed (Fig. 2 and 3). A protein with a similar molecular weight (66 kDa) was reported by Ivanova et al. [28] as a minor band when studying the protein profile of a protein isolate obtained from industrial rapeseed meal. The present study suggests that this specific protein is a part of the proteome of the three samples. However, additional research is needed for its characterization.

Prolamins of the rape seeds, rapeseed meal and ethanol treated rapeseed meal were mainly composed of LMW varying from 11 to 16 kDa (Fig. 5). Although they accounted for the majority of the proteins in this fraction, a minor presence of a 213 kDa protein was also found (Table 5). The same protein was present in both globulin (Fig. 3) and glutelin fractions (Fig. 4) but only made up a small portion of them (Table 5). It is probably a monomeric protein which remained intact regardless of rape seeds processing conditions. This result agrees with Huang [56] who reported that prolamins in rape seeds were predominantly composed of structural proteins with low molecular weights. While variable by relative quantities, proteins in the prolamin fractions of the rape seeds, rapeseed meal and ethanol treated meal differed only slightly in composition.

Conclusion

Ethanol treatment of the rapeseed meal efficiently reduced phenols and glucosinolates contents, while increasing protein level. The solubility of albumins was lowered the most, followed by globulins which resulted in an overall decrease of the protein yield achieved. The comparative fractional protein profile of rape seeds, rapeseed meal and ethanol treated rapeseed meal exhibited differences in both composition of the fractions and the relative quantity of the proteins. Overall, our study demonstrated that after processing of the rape seeds, the treatment of the rapeseed meal with ethanol affected protein solubility, amino acid composition and protein fractional profile. This knowledge is valuable when ethanol treated rapeseed meal is intended for use either as a feed additive or as a source for production of protein-rich ingredients with specific nutritive values and functionality.

Compliance with Ethical Standards

Conflict of interest: The authors declare that they have no conflict of interest.

Human or Animal Context: This article does not contain any studies with human or animal subjects.

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Table 1 Chemical composition of samples

Component	Content*, %		
	Rape seeds	Rapeseed meal	Ethanol treated rapeseed meal
Crude protein	19.44±0.25^{c}	37.41±0.47^{b}	42.25±0.57^{a}
Ash	3.71±0.03^{c}	6.90±0.08^{b}	7.45±0.15^{a}
Total lipids	49.49±0.15^{a}	1.90±0.14^{b}	1.13±0.00^{c}
Total fiber	28.09±0.71^{b}	40.23±0.07^{a}	42.83±0.22^{a}
Phenols	0.50±0.03^{b}	0.70±0.06^{a}	0.18±0.01^{c}
Total glucosinolates**	85.77±4.12^{a}	73.86±1.04^{b}	5.15±0.34^{c}

*Contents are calculated on a dry matter basis (94.20±0.07% for rape seeds, 92.41±0.00% for rapeseed meal, and 87.74±0.70% for ethanol treated rapeseed meal).
**Content of total glucosinolates is presented in μmol/g.
$^{a\text{-}c}$Means in a row with different superscripts differ significantly ($p < 0.05$)

Table 2 Essential amino acid composition of rape seeds, rapeseed meal and ethanol treated rapeseed meal

Amino acids	Amino acid content*, g/100 g protein		
	Rape seeds	Rapeseed meal	Ethanol treated rapeseed meal
Valine	3.78±0.08	3.35±0.11	3.57±0.16
Leucine	0.80±0.13	0.76±0.05	0.81± 0.22
Isoleucine	3.81±0.12	3.55±0.17	3.40±0.09
Threonine	5.34±0.10	5.06±0.16	4.49±0.11
Lysine	5.95±0.06	4.21±0.12	5.15±0.03
Phenylalanine	5.05±0.15	5.93±0.12	4.48±0.16
Methionine	0.62±0.03	0.81±0.14	1.17±0.05

*Contents are calculated on a dry matter basis (94.20±0.07% for rape seeds, 92.41±0.00% for rapeseed meal, and 87.74±0.70% for ethanol treated rapeseed meal).

Table 3 Non-essential amino acids composition of rape seeds, rapeseed meal and ethanol treated rapeseed meal

Amino acids	Amino acid content*, g/100 g protein		
	Rape seeds	Rapeseed meal	Ethanol treated rapeseed meal
Alanine	5.77±0.04	5.73±0.07	5.34±0.05
Tyrosine	4.27±0.08	3.93±0.15	3.30±0.12
Glycine	4.50±0.11	3.51±0.12	3.66±0.10
Arginine	5.72±0.03	5.94±0.07	4.37±0.10
Serine	6.56±0.18	6.93±0.10	6.63±0.13
Aspartate	4.09±0.14	6.78±0.11	5.94± 0.11
Glutamate	15.08±0.02	14.03±0.08	14.32±0.06
Histidine	2.65±0.07	2.91±0.09	2.16±0.09
Prolin	5.07±0.15	4.87±0.05	5.17±0.08
Cysteine	1.48±0.17	1.59±0.09	1.42±0.16

*Contents are calculated on a dry matter basis (94.20±0.07% for rape seeds, 92.41±0.00% for rapeseed meal, and 87.74±0.70% for ethanol treated rapeseed meal).

Table 4 Protein extractability of rape seeds, rapeseed meal and ethanol treated rapeseed meal

Fractions	Extraction, №	Protein yield, %		
		Rape seeds	Rapeseed meal	Ethanol treated rapeseed meal
Albumin	1	12.69±0.51^{a}	5.83±0.04^{b}	2.89±0.02^{c}
	2	4.51±0.25^{a}	1.45±0.05^{b}	0.89±0.01^{c}
	3	1.86±0.06^{a}	0.52±0.03^{b}	0.45±0.01^{c}
	Total	19.06±0.27^{a}	7.80±0.05^{b}	4.23±0.00^{c}
Globulin	1	11.23±0.82^{a}	5.60±0.23^{b}	5.12±0.01^{b}
	2	2.77±0.18^{a}	1.70±0.07^{b}	1.39±0.03^{c}
	3	1.32±0.07^{a}	0.65±0.05^{b}	0.56±0.02^{b}
	Total	15.32±0.57^{a}	7.95±0.12^{b}	7.07±0.03^{c}
Prolamin	1	1.50±0.44^{a}	0.68±0.03^{b}	1.18±0.04^{ab}
	2	0.74±0.13^{a}	0.22±0.01^{c}	0.41±0.03^{b}
	3	0.38±0.10^{a}	0.11±0.01^{b}	0.16±0.00^{b}
	Total	2.62±0.23^{a}	1.01±0.02^{c}	1.75±0.01^{b}
Glutelin	1	7.79±0.26^{a}	4.82±0.31^{b}	5.64±0.06^{b}
	2	2.19±0.10^{b}	3.17±0.02^{a}	3.08±0.04^{a}
	3	0.85±0.06^{c}	1.68±0.11^{b}	1.81±0.03^{a}
	Total	10.83±0.12^{a}	9.67±0.17^{c}	10.53±0.03^{b}
Total		47.83	26.43	23.58

$^{a\text{-}c}$ Means in a row with different superscripts differ significantly ($p < 0.05$).

Table 5 Protein fraction distribution of rape seeds, rapeseed meal and ethanol treated rapeseed meal by molecular weight

Fractions	Proteins	Protein distribution, %		
		Rape seeds	Rapeseed meal	Ethanol treated rapeseed meal
Albumin	LMW	84.67	91.12	94.12
	MMW	15.34	8.87	5.87
	HMW	0	0	0
Globulin	LMW	87.69	95.29	96.14
	MMW	10.22	2.17	2.18
	HMW	2.09	2.54	1.68
Glutelin	LMW	85.76	80.08	78.44
	MMW	10.34	17.01	18.28
	HMW	3.9	2.91	3.28
Prolamin	LMW	92.41	93.88	93.56
	MMW	0	0	0
	HMW	7.59	6.12	6.44

LMW: proteins with molecular weights < 50 kDa;
MMW: proteins with molecular weights from 50 to 150 kDa;
HMW: proteins with molecular weights > 150 kDa.

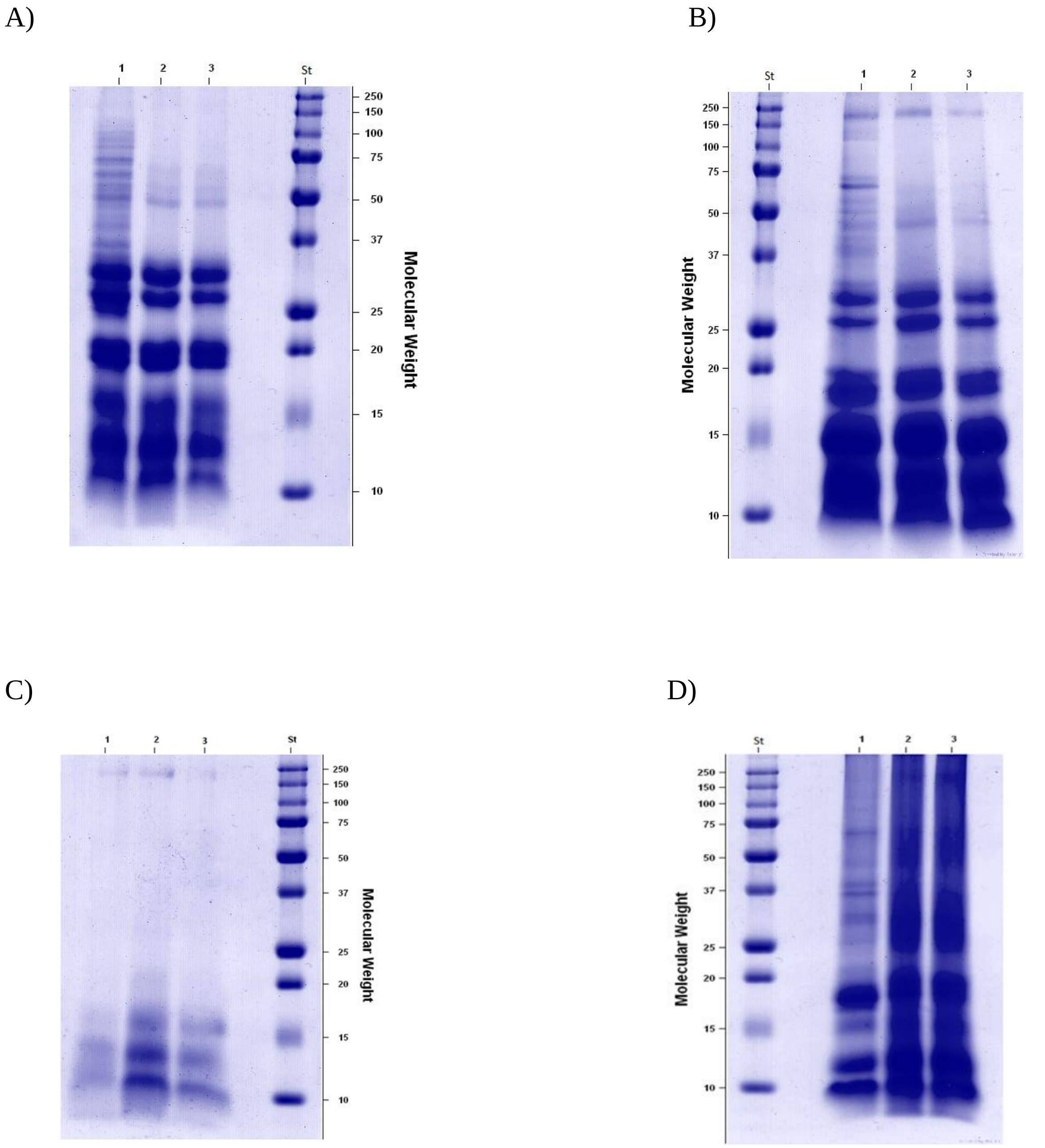

Fig. 1 SDS-PAGE of A) albumin, B) globulin, C) prolamin and D) glutelin fractions of rape seeds (1), rapeseed meal (2) and ethanol treated rapeseed meal (3). St denotes Molecular weight marker

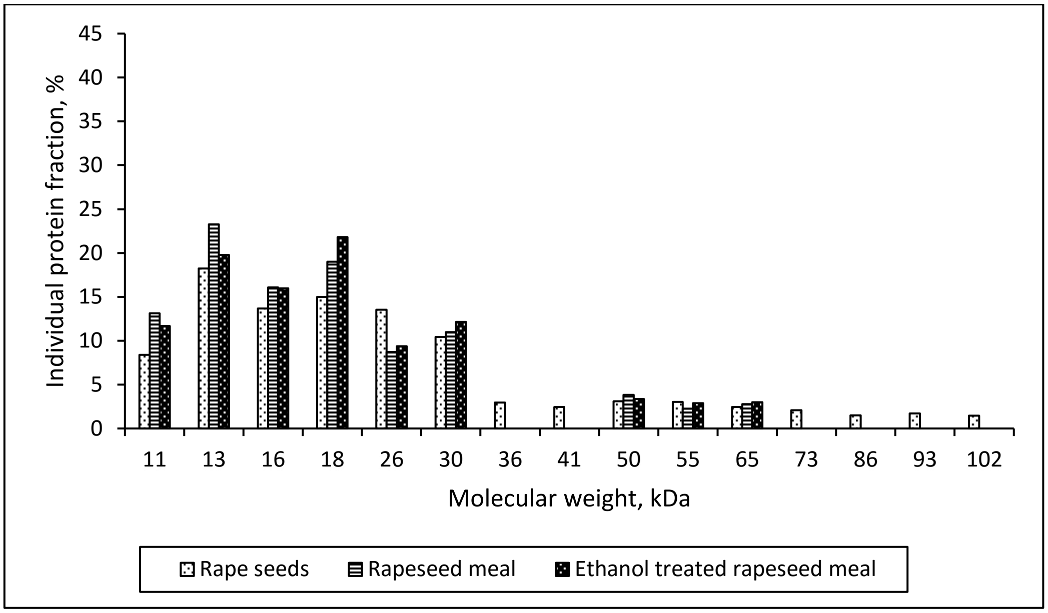

Fig. 2 Comparative albumin profile of rape seeds, rapeseed meal and ethanol treated rapeseed meal

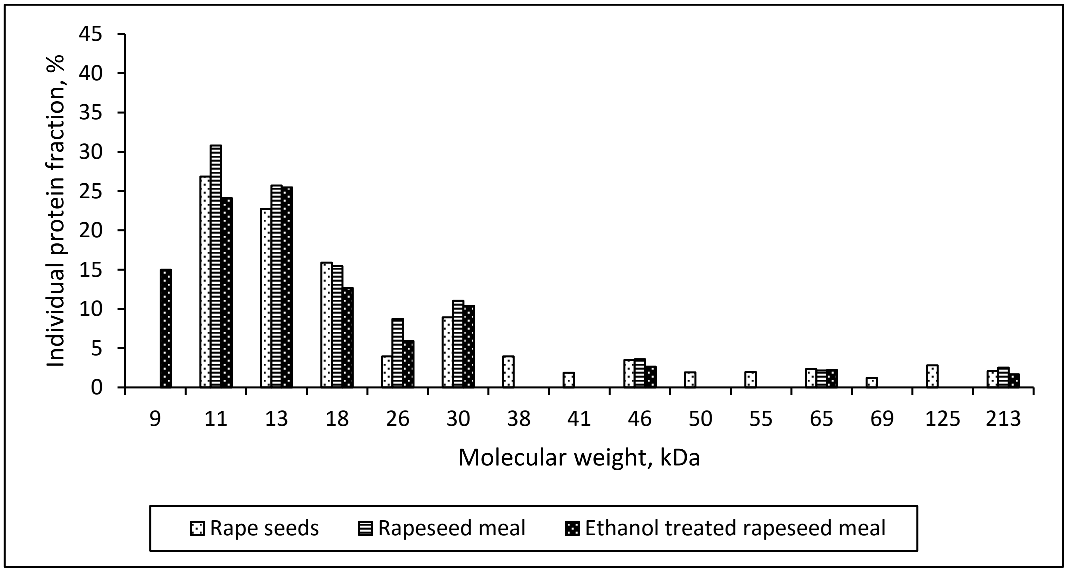

Fig. 3 Comparative globulin profile of rape seeds, rapeseed meal and ethanol treated rapeseed meal

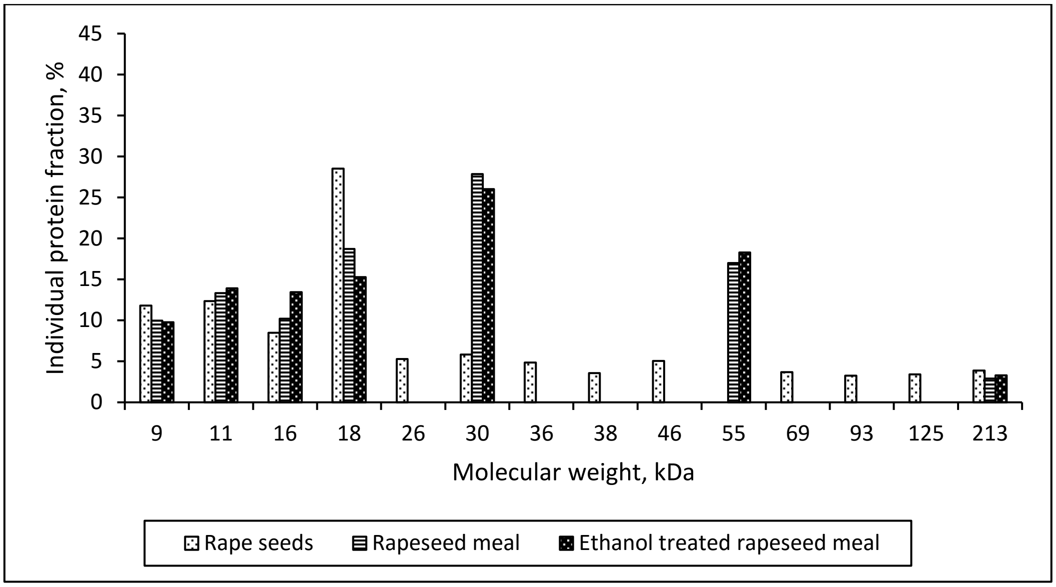

Fig. 4 Comparative glutelin profile of rape seeds, rapeseed meal and ethanol treated rapeseed meal

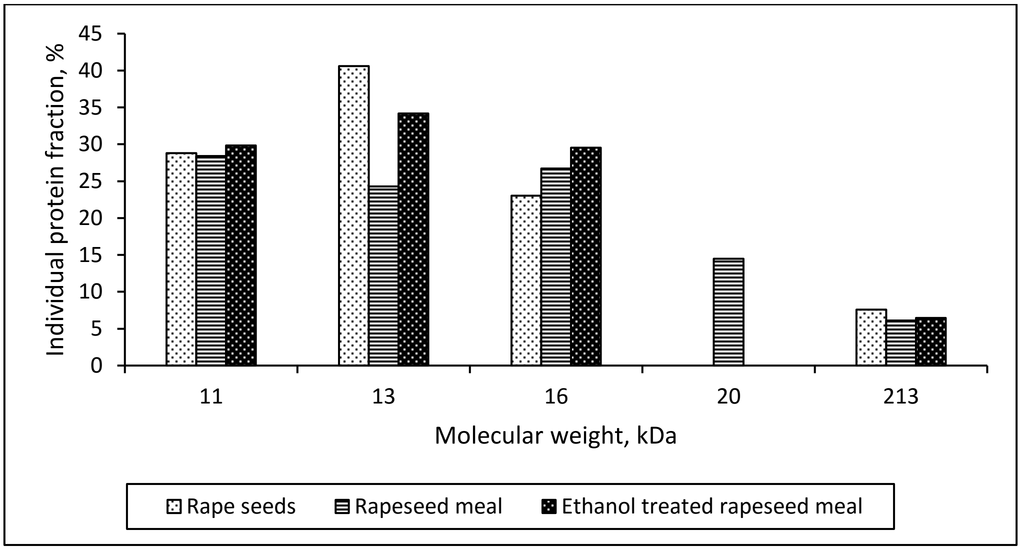

Fig. 5 Comparative prolamin profile of rape seeds, rapeseed meal and ethanol treated rapeseed meal