Pol. J. Food Nutr. Sci., 2019, Vol. 69, No. 2, pp. 129–136
DOI: 10.31883/pjfns-2019-0007
http://journal.pan.olsztyn.pl

Original research article
Section: Food Quality and Functionality

Functional Properties of Protein Isolate and Acid Soluble Protein-Rich Ingredient Co-Produced from Ethanol-Treated Industrial Rapeseed Meal

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Key words: ethanol pre-treatment, rapeseed meal, protein isolate, acid soluble protein, functional properties

Rapeseed meal is produced in large quantities as a by-product of vegetable oil production. To enhance the utility and profitability of the rapeseed meal, it was treated with ethanol and used for concomitant preparation of two protein-rich ingredients, namely protein isolate (PI) and acid soluble protein (ASP). Their functional properties were evaluated in response to two boundary concentrations of NaCl (0.03 and 0.25 mol/L) in a wide pH range (2 to 10). The PI exhibited the lowest protein solubility at isolectric point (pH 4.5) which increased both at lower and higher pH. In contrast, ASP exhibited high protein solubility (>70%) which was negligibly influenced by pH. The addition of 0.03 mol/L NaCl increased its protein solubility to almost 100% at acidic pH. The water holding capacity of PI was positively influenced by the addition of 0.25 mol/L NaCl. The ASP did not exhibit any capacity to hold water but demonstrated higher ability to absorb oil compared to the PI. Both ingredients exhibited different thermal stability in response to salt addition at pH 7 and 8. PI and ASP exhibited completely different pattern of emulsion stability as influenced by pH. While the stability of PI emulsions was close to 100% and only negligibly affected by pH, the ASP emulsion stability significantly varied in response to pH variation. The concomitant production of PI and ASP resulted in products with distinctive techno-functional properties, which makes them suitable for different applications as additives in the formulation of new food products.

INTRODUCTION

Rapeseed meal is a by-product of vegetable oil production [Kreps *et al*., 2014]. In recent years, a steady increase in the generation of rapeseed meal worldwide is observed due to enhanced interest in rapeseed oil as a stock for biofuel production [Zentková & Cvengrošová, 2013]. Its quantity may reach up to 48% of the rape seeds used [Ivanova, 2012]. The most typical application of the rapeseed meal is as a protein-rich additive for the feed industry [Kreps *et al*., 2014]. However, high fiber and anti-nutrient availability limit its inclusion levels due to adverse physiological effect on animals. This is especially valid for rapeseed meal, which, compared to canola meal, contains higher amounts of phytates, allyl isothiocyanates, glucosinolates, and polyphenols [Das Purkayastha *et al*., 2014]. Alternatively, the rapeseed meal can serve as a raw material for preparation of protein-rich ingredients for the food industry [Ivanova *et al*., 2016; Wanasundara *et al*., 2016]. According to Das Purkayastha *et al*. [2014], antinutrients may remain as a part of the final product and negatively affect its nutritive quality and functional properties.

To reduce the antinutrient level in the rapeseed meal and increase the applicability of this by-product, various pre-treatment approaches have been studied [Ghodsvali *et al*., 2005; Gu *et al*., 2011; Das Purkayastha *et al*., 2013]. Among them, treatment with an aqueous ethanol solution is one of the most widely used due to its high reduction efficiency on phenols and glucosinolate and possible use in the food industry as a safe reagent [Chabanon *et al*., 2007]. However, this specific treatment may alter the nutritive quality or functional properties of the protein-rich derived products and, as a consequence, impact their application in the food industry.

The most common approach for preparation of protein isolates includes alkaline extraction of proteins with NaOH followed by isoelectric precipitation. Rapeseed meal protein isolates are characterized with relatively balanced amino acid composition and attractive techno-functional properties because of which they are considered alternatives to plant protein ingredients currently utilized in the food industry [Wanasundara *et al*., 2016]. However, regardless of the advantages of the rapeseed meal protein isolates as protein-rich ingredients, their commercial production is highly limited due to low protein yield recovery (approximately 20%) [Lqari *et al*., 2002; Chabanon *et al*., 2007] and as a consequence, low profitability [Li & Guo, 2017]. Therefore, a concordant production of multiple value-added products would result in enhanced efficiency of the rapeseed meal use, diversified application of rapeseed meal-derived products and overall better economical outcome [Li & Gui, 2017]. The aim of the current

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research was to evaluate functionality of two protein-rich products, a protein isolate (PI) and an acid soluble protein--containing ingredient (ASP), concomitantly produced from ethanol-treated industrial rapeseed meal. Due to the large influence of salts and pH on the functional properties of proteins, the impact of NaCl at two boundary concentrations (0.03 and 0.25 mol/L) in a wide pH range (2.0–10.0) was evaluated as well.

MATERIAL AND METHODS

Co-production of protein isolate (PI) and an acid soluble protein-rich ingredient (ASP)

Industrially manufactured rapeseed meal was provided by a local company. It was produced after thermal treatment of rape seeds at 110–115°C followed by extraction with hexane at 60–65°C for approximately 1 h. Proximate composition of the meal was previously analyzed in our laboratory by Ivanova *et al.* [2016]. The meal was ground and sieved to obtain samples with uniform size particles (≤0.315 mm). To enhance the practical application of the study and achieve more efficient utilization of the rapeseed meal as a by-product, it was not subjected to further modifications, except for treating with ethanol, which aimed at reducing phenol and glucosinolate levels. The procedure included a 4-step treatment with a 75% aqueous ethanol solution (v/v) at a meal to solvent ratio of 25% (w/v) for 30 min at room temperature as described by Chabanon *et al*. [2007]. The residue was collected by decanting, dried in air and stored in a closed container for further use as a source for preparation of the protein-rich ingredients. The PI was produced by extraction of 5% (w/v) meal dispersion with 1 mol/L NaOH (pH 12.0) at 40°C for 60 min under continuous agitation. Extracted proteins were precipitated with 1 mol/L HCl at pH 4.5 which corresponded to the lowest protein solubility. The resulting sediment (PI) was collected by centrifugation for 15 min at 1800×*g* (MPW-251, Med. Instruments, Poland) and dried by lyophilization (Lyovac GT2, Leybold-Heraeus, Germany). The supernatant, containing acid soluble protein, was collected and lyophilized (Lyovac GT2, Leybold-Heraeus, Germany) as well to obtain ASP. The crude protein content of both products was established by Kjeldahl method [AOAC, 1990] with a conversion coefficient of 6.25.

Protein solubility

Protein solubility of PI and ASP in water was determined as described by González-Pérez *et al*. [2007] with some modifications. Samples were dispersed in water to a final protein concentration of 4 mg/mL. The pH was varied from 2 to 8.5 with an increment of 0.5 by using either concentrated or diluted NaOH and HCl solutions as appropriate. NaCl was added to a final concentration of 0.03 or 0.25 mol/L when needed. After 2 h at room temperature, samples were centrifuged for 15 min at 1800×*g* (MPW-251, Med. Instruments, Poland) and supernatants were collected. Protein solubility was calculated as a ratio of the amount of the protein in a supernatant as determined by the biuret method [AACC, 1983] and the initial concentration of the protein used in the test system. The result was multiplied by 100 to express in percentage. Bovine serum albumin was used as a standard protein.

Determination of water- and oil absorption capacity

Water absorption capacity (WAC) was determined as described by Rodriguez-Ambriz *et al*. [2005]. Each protein sample (100 mg) was mixed with 1 mL distilled water. The resulting suspension was centrifuged for 20 min at 1800×*g* (23°C) (MPW-251, Med. Instruments, Poland) and the supernatant was decanted for 10 min at a 45° angle. WAC was calculated by dividing the weight of the absorbed water (g) by the weight of the protein sample (g).

Oil absorption capacity (OAC) was determined by the method of Lin & Zayas [1987]. Each protein sample (100 mg) was mixed with 1 mL sunflower oil and vortexed for 30 s. The emulsion was incubated at room temperature (23°C) for 30 min and subsequently centrifuged for 10 min at 13,600×*g* (MPW-251, Med. Instruments, Poland). The supernatant was decanted and drained for 20 min at a 45° angle. OAC was calculated by dividing the weight of the absorbed oil (g) by the weight of the protein sample (g).

Thermal stability

Thermal stability was determined as described by Tang *et al*. [2012]. Aliquots of 5 mL of protein solutions (2 mg protein/mL) were adjusted to either pH 7 or 8 and were heated for 20 min at temperatures varying from 50 to 90°C with increments of 10°C. After cooling to room temperature (23°C), the turbidity of the solutions was measured at λ=500 nm (Spekol 11, Carl Zeiss Jena, Jena, Germany). Distilled water was used as a control. Thermal stability was evaluated by changes in samples turbidity relative to turbidity of the sample at room temperature (23°C) and multiplied by 100 to express in percentage. Negative values indicate decrease in turbidity.

Emulsifying properties

Emulsifying activity and emulsion stability were determined as described by Neto *et al*. [2001]. Five milliliter protein solution (5 mg protein/mL) was homogenized with 5 mL sunflower oil. The emulsion was centrifuged at 1800×*g* for 5 min and the height of the emulsified layer was recorded. The emulsifying activity was calculated as a ratio of the height of the emulsified layer and the height of the total content of the tube and multiplied by 100 to express in percentage. Emulsion stability was established by heating protein emulsion at 80°C for 30 min followed by centrifugation at 1800×*g* for 5 min. Emulsion stability was calculated by the height of emulsified layer after heating divided by the height of the emulsified layer before heating and multiplied by 100 to express in percentage. NaCl was added to a test system to reach a final concentration of either 0.03 or 0.25 mol/L whenever appropriate. The influence of pH on emulsifying properties was tested by varying pH from 2 to 10 with an increment of 2.0 by using either concentrated or diluted NaOH and HCl solutions as appropriate.

Statistical analysis

Results are presented as means of at least three independent determinations ± standard deviation (SD). Statistical evaluation was performed by using one-way analysis of variance (ANOVA) of the IBM SPSS Statistics program (Somers, NY, USA). Mean differences were established by Fisher's

least significant difference test for paired comparison with a significance level of $\alpha=0.05$.

RESULTS AND DISCUSSION

Protein solubility

Solubility of protein is a key determinant of the other functional properties such as gelling, foaming and emulsification and, as a consequence, their application as food additives [Vioque *et al*., 2000]. Protein solubility is influenced by numerous factors including amino acid composition, protein conformation, interaction with other food compounds, and pH and NaCl, which are the parameters of the highest practical impact [Kinsella *et al*., 1985]. The pH was studied in a wide range of values (from 2 to 8.5), while the two supplementation levels of NaCl were chosen as the most typical boundary concentrations used in food processing [Antova *et al*., 2008; Dragoev *et al*., 2009].

PI and ASP exhibited different protein solubility behavior in response to NaCl supplementation and pH variation (Figures 1A and B). The PI expressed negligible protein solubility (2.80%) at isolectric point (pH 4.5). Improvement of the protein solubility was observed below or above the pI value, but more prominently in alkaline media (Figure 1A). This is a typical trend for solubility of protein isolates obtained from oilseed crops [Xu & Diosady, 1994; Vioque *et al*., 2000; Ogunwolu *et al*., 2009; Ivanova *et al*., 2013]. At pH in the range of 2.5–6, the protein solubility of PI did not exceed 30%. This feature limits the application of the PI as an additive in food processing, since numerous food products have a typical pH from 4.5 to 6.0 [FDA, 2018]. Our results agree with findings reported by Alashi *et al*. [2013] who implied low utilization of canola/rapeseed protein isolates in the food industry due to their poor solubility in neutral and low acidic media. The addition of the lower concentration NaCl (0.03 mol/L) did not change the protein solubility pattern. However, supplementation of the reaction mixture with 0.25 mol/L NaCl resulted in more than a 10-fold increase of protein solubility at the isoelectric point (pH 4.5). The protein solubility in the low acidic area was augmented and reached 51% at pH 6.

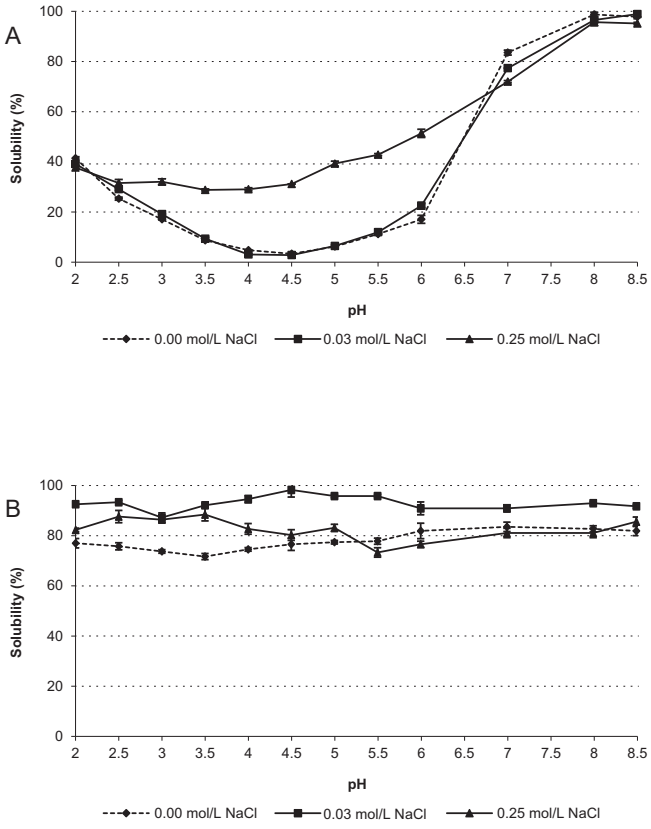

FIGURE 1. Solubility of protein isolate – PI (A) and acid soluble protein ingredient – ASP (B) at different pH values and NaCl concentrations.

In contrast, ASP exhibited protein solubility higher than 70% over the entire pH range studied (Figure 1B). It was slightly influenced by pH in the acidic range and insensitive to alkaline media. A similar influence of pH on solubility of Chinese rapeseed protein isolate, obtained by membrane processing, was observed by Xu & Diosady [1994]. A greater solubility of ultrafiltered protein isolate compared to a precipitated protein isolate was reported by Yoshie-Stark *et al*. [2008] as well. The better protein solubility of the ASP compared to PI is partially due to its protein profile. A previously performed SDS-PAGE study by Ivanova *et al*. [2017] revealed that ASP was composed of proteins with low molecular weights which did not exceed 33 kDa. The PI contained proteins with higher molecular weights which accounted for the higher susceptibility to agglomeration and coagulation near of pI. Our observations agreed with findings reported by Aider & Barbana [2011] who underlined the importance of low molecular proteins for acquiring good solubility. Improved solubility of rapeseed meal protein isolate was achieved by Vioque *et al*. [2000] after partial hydrolysis. Except at pH 3, the protein solubility of ASP increased to more than 90% for all pH values after supplementation with 0.03 mol/L NaCl. The higher ash content of ASP (20.5%) compared to PI (2.3%) [Ivanova *et al*., 2017] is most probably responsible for the higher impact of the low concentration NaCl (0.03 mol/L) (Figure 1B). The addition of 0.25 mol/L NaCl slightly enhanced the protein solubility at acidic pH but not in alkaline media. The higher impact of NaCl addition on protein solubility of both PI and ASP in acidic than in alkaline media may be due to the greater affinity of Cl^- to positively charged proteins in the acidic range than that of Na^+ to negatively charged proteins in alkaline media [Schnepf, 1992]. The influence of salts on protein solubility is complex because it is not only pH dependent but also affected by salt concentration and protein conformation.

Water and oil absorption capacity

The ability of protein-containing ingredients to absorb and retain water and oil is an important feature which influences texture and mouthfeel of food products [Okezie & Bello, 1988]. The PI and ASP, prepared in our study, exhibited significant differences in both WAC and OAC (Table 1). The WAC of the PI (2.00 g H_2O/g sample) was lower than that of commercial soybean protein isolates, such as Purina Protein 500E and 760 [Zayas, 1997], but higher than the WAC of an industrial sunflower meal protein isolate [Ivanova *et al*., 2014], cashew nut protein concentrate [Ogunwolu *et al*., 2009], and a rapeseed protein isolate [Vioque

TABLE 1. Water and oil absorption capacity of protein isolate and acid soluble protein ingredient at different concentrations of NaCl.

Sample	Water absorption capacity (g H_2O/g sample)			Oil absorption capacity (g oil/g sample)		
	NaCl concentration (mol/L)			NaCl concentration (mol/L)		
	0.00	0.03	0.25	0.00	0.03	0.25
Protein isolate	2.00±0.13^{b}	2.16±0.16^{b}	2.70±0.02^{a}	1.29±0.10^{a,B}	1.45±0.22^{a,B}	1.47±0.04^{a,B}
Acid soluble protein	–	–	–	2.77±0.12^{a,A}	2.79±0.00^{a,A}	3.03±0.09^{a,A}

$^{a-b}$Means in a row for a particular functional property with common lowercase superscripts do not differ significantly (p≥0.05). $^{A-B}$Means in a column with common uppercase superscripts do not differ significantly (p≥0.05).

et al., 2000]. The addition of the higher NaCl concentration (0.25 mol/L) significantly increased the WAC to 2.70 g H_2O/g sample, while the lower NaCl level (0.03 mol/L) did not alter the capacity of the PI to retain water. The ASP did not exhibit any capacity to hold water (Table 1). This may be due to the relatively lower protein content of ASP (28.8%) compared to PI (86.8%). Lower WAC of a sunflower protein isolate with a lower protein content compared to a similar protein isolate but with higher protein level was observed by Ivanova *et al*. [2014]. In contrast, the ASP showed higher OAC than PI (Table 1). This feature may be due to differences in the chemical composition and protein fractional profiles of the two protein-containing products as previously established in our laboratory by Ivanova *et al*. [2017]. The authors reported that the ASP was composed of proteins with low molecular weights up to 33 kDa with a prevailing 8 kDa fraction (approximately 35%). According to Vioque *et al*. [2000], small polypeptide chains provide better exposure of amino acid nonpolar sides to hydrocarbon chains, thus contributing to an increase of OAC. Both levels of NaCl supplementations did not alter OAC of either PI or ASP (Table 1).

Thermal stability

Thermal stability of functional protein ingredients is essential when a heat treatment is a part of the food processing scheme. Aiming at comparison between PI and ASP, the thermal stability was evaluated at pH 7 and 8 where the two ingredients exhibited similar protein solubility. PI and ASP showed completely different behavior in response to temperature increase, which was also highly influenced by pH (Table 2). The PI exhibited better thermal stability at pH 7 than at pH 8, regardless of the higher protein solubility of this ingredient at the higher pH (Figure 1A). In contrast, the ASP expressed higher thermal stability at pH 8 than at pH 7, as evidenced by the decrease of turbidity. Since the protein solubility of the ASP at both pH values is not significantly different,

TABLE 2. Thermal stability of protein isolate and acid soluble protein ingredient at pH 7 and 8.

Temperature (°C)	Turbidity alteration (%)					
	Protein isolate			Acid soluble protein		
	NaCl concentration (mol/L)			NaCl concentration (mol/L)		
	0.00	0.03	0.25	0.00	0.03	0.25
pH 7						
50	-0.15±0.1^{bB}	-3.58±0.28^{aC}	15.19±0.11^{eA}	0.57±0.1^{eA}	-3.46±0.52^{eB}	-7.19±0.43^{eC}
60	1.19±0.3^{aB}	-5.69±0.18^{cC}	9.91±0.16^{bA}	6.86±0.1^{dA}	-0.34±0.14^{dB}	-5.42±0.43^{dC}
70	-1.09±0.4^{cB}	-4.50±0.16^{bC}	41.67±0.48^{bA}	15.5±1.2^{cA}	5.99±0.15^{cC}	11.86±0.58^{cB}
80	-1.23±0.5^{cB}	-6.79±0.33^{dC}	12.51±0.21^{dA}	36.38±0.65^{bA}	19.83±0.53^{bC}	22.22±0.58^{bB}
90	1.43±0.4^{aB}	-4.92±0.26^{bC}	42.71±0.27^{aA}	55.56±0.4^{aA}	35.44±0.25^{aC}	26.98±0.16^{aB}
pH 8						
50	0.62±0.3^{cB}	-4.99±0.36^{cC}	20.62±0.36^{eA}	-13.27±0.5^{dA}	-18.82±0.24^{dB}	-19.34±0.15^{eC}
60	0.41 ±0.2^{cB}	-6.83±0.31^{dC}	33.96±0.69^{dA}	-13.61±0.4^{dA}	-19.06±0.71^{dC}	-17.76±0.55^{dB}
70	0.72±0.2^{cB}	-3.97±0.36^{bC}	66.44±0.55^{cA}	-8.98±0.4^{cA}	-12.16±0.49^{cC}	-11.46±0.80^{cB}
80	1.34±0.5^{bB}	-3.85±0.18^{bC}	114.07±0.43^{bA}	-3.81±0.1^{bA}	-6.82±0.23^{bC}	-5.16±0.30^{bB}
90	5.73±0.2^{aB}	-1.93±0.18^{aC}	167.35±0.75^{aA}	4.56±0.6^{aBA}	-2.04±0.36^{aC}	-0.70±0.15^{aB}

$^{a-e}$ Means in a column with same lowercase letter do not differ significantly (p≥0.05). $^{A-B}$ Means in a row for a specific ingredient with same capital letter do not differ significantly (p≥0.05). Negative values indicate decrease in turbidity.

TABLE 3. Emulsifying activity of protein isolate and acid soluble protein ingredient at different pH and NaCl concentrations.

Sample	NaCl concentration (mol/L)	Emulsifying activity (%)				
		pH				
		2	4	6	8	10
Protein isolate	0.00	$59.00 \pm 0.25^{c,A}$	$57.46 \pm 0.19^{d,B}$	$62.20 \pm 0.00^{a,A}$	$58.99 \pm 0.23^{c,A}$	$61.46 \pm 0.39^{b,B}$
	0.03	$57.83 \pm 0.71^{d,A}$	$59.28 \pm 1.34^{cd,B}$	$62.50 \pm 0.00^{a,A}$	$61.41 \pm 0.32^{ab,A}$	$60.48 \pm 0.33^{bc,C}$
	0.25	$60.97 \pm 1.74^{a,A}$	$63.29 \pm 0.00^{a,A}$	$63.81 \pm 1.85^{a,A}$	$62.11 \pm 1.68^{a,A}$	$63.04 \pm 0.13^{a,A}$
Acid soluble protein	0.00	$59.88 \pm 0.87^{b,A}$	$58.13 \pm 0.28^{c,B}$	$61.22 \pm 0.05^{a,A}$	$61.46 \pm 0.39^{a,A}$	$62.05 \pm 0.21^{a,A}$
	0.03	$59.75 \pm 0.36^{b,A}$	$60.12 \pm 0.17^{b,A}$	$60.06 \pm 0.76^{b,AB}$	$59.52 \pm 0.68^{b,B}$	$61.46 \pm 0.39^{a,A}$
	0.25	$59.52 \pm 0.68^{a,A}$	$59.35 \pm 0.75^{a,AB}$	$59.08 \pm 0.62^{a,B}$	$59.39 \pm 0.18^{a,AB}$	$60.59 \pm 0.83^{a,A}$

$^{a-d}$ Means in a row with same lowercase superscripts do not differ significantly ($p \geq 0.05$). $^{A-B}$ Means in a column for a specific sample with same letter do not differ significantly ($p \geq 0.05$).

it cannot account for the different pattern of thermal stability observed. Overall, PI had better thermal stability than ASP at pH 7 but worse at pH 8, regardless of the similar protein solubility (80%) at the higher pH (Figures 1A and B). According to Jiang *et al*. [2010], proteins with higher solubility are supposed to have a better thermal stability. However, our data imply that more factors might be involved in this process. The previously established differences in the chemical composition and protein fractional profile of PI and ASP [Ivanova *et al*., 2017] might affect the number and position of hydrogen bonds, protein density packing and the number and fractional volume of protein cavities formed, which are significant determinants of protein thermal stability [Vogt & Argos, 1997]. Internal van der Waals' packing and interplay of the hydrophobic and electrostatic interactions may also be important for stabilizing proteins against heat [Kaushik & Bhat, 1999]. In addition, variations in long-range protein contacts, influencing the formation of protein-stabilizing centers and the content of stabilization center elements under the studied conditions, may occur [Magyar *et al*., 2016]. According to the same authors, an increase in the latter and, therefore, an increase of thermal stability of thermolabile proteins may occur up to 80°C. This might explain the sharp decrease in thermal stability of both protein-containing ingredients at 90°C under all studied conditions (Table 2).

The addition of NaCl significantly influenced the thermal stability of both ingredients at the two pH values studied, but to a different extent. A decrease in turbidity and, therefore, an increase in thermal stability of PI and ASP, was observed at the lower concentration of NaCl (0.03 mol/L). Fontanari *et al*. [2012] implied that the stabilizing effect of low salt concentrations was due to improved hydration of protein molecules as a result of electrostatic induction and/or structural alteration of water molecules surrounding the protein. In contrast, supplementation with 0.25 mol/L NaCl reduced the thermal stability which was more pronounced for the PI. At a higher ionic strength, the reduced protein stability is rather attributed to nonspecific osmotic effects [Pegram *et al*., 2010] and protein conformational changes [Beauchamp & Khajehpour, 2012]. Different response of PI and ASP to salt addition is most probably due to differences in their protein composition and profile, which in turn leads to variations in electrical charge on the protein surface and interaction with water molecules. Arntfield *et al*. [1986] also observed alteration of the heat resistance of faba bean legumin and vicilin in response to different NaCl load.

Emulsifying properties

Emulsifying activities of PI and ASP are presented in Table 3. For both protein-containing ingredients, the emulsifying activity varied in a narrow range, from 57 to 63%, under all studied conditions. The lowest activity of PI was observed at pH 4, which is in the pH range with the lowest protein solubility for this ingredient (Figure 1A). According to Kinsella [1985], poor emulsifying properties of plant proteins at a pH close to their isoelectric point are due to weak electrostatic repulsive forces. Similar observations were reported by Ivanova *et al*. [2014] and Mao & Hua [2012] who studied the emulsifying properties of protein isolates obtained from sunflower meal and walnut, respectively. The ASP also exhibited the lowest emulsifying activity at pH 4. In contrast to PI, this ingredient was highly soluble over the entire pH range studied (Figure 1B) and, therefore weak electrostatic repulsive forces cannot be a probable explanation. Due to relatively high impurity of the ASP [Ivanova *et al*., 2017], other interactions in addition to the protein-protein ones may also influence the overall emulsifying capacity. The addition of 0.25 mol/L NaCl neutralized the influence of pH on the emulsifying activity of both PI and ASP (Table 3).

PI and ASP exhibited a completely different pattern of emulsion stability as influenced by pH. While the stability of PI emulsions was close to 100% and only negligibly affected by all pH conditions studied (Figure 2), the ASP emulsion stability highly varied depending on pH (Figure 3). The highest stability was observed at pH 4, 8, and 10 (Figure 3). Except at pH 10, the emulsion stability of ASP was lower than that of PI (Figures 2 and 3). Our results differ from these obtained by Xu & Diosady [1994] who reported higher emulsion stability of a soluble protein isolate (111.1%) than that of a protein isolate (98.4%) obtained from Chinese rapeseed meal. This may be due to differences in the methodologies used for their preparation, and, as a consequence, in their chemical composition. The influence of NaCl on

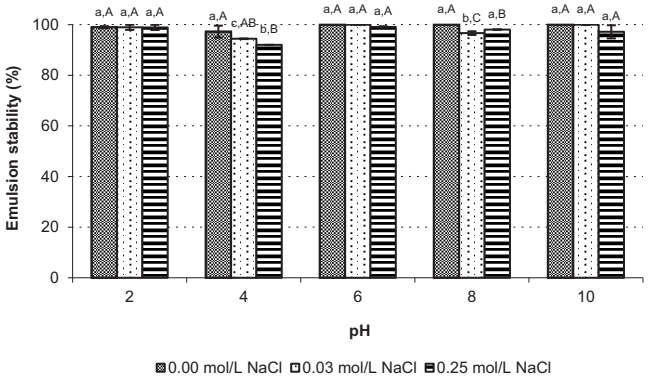

FIGURE 2. Emulsion stability of protein isolate (PI).

[a-c] Means with same lowercase letter for a particular NaCl concentration do not differ significantly (p≥0.05). [A-C] Means with same capital letter for a particular pH do not differ significantly (p≥0.05).

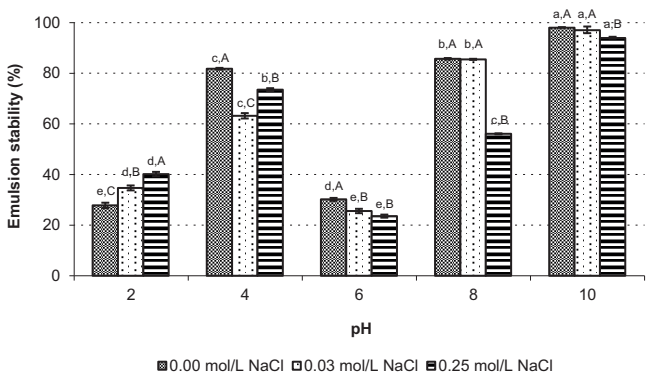

FIGURE 3. Emulsion stability of acid soluble protein ingredient (ASP).

[a-e] Means with same lowercase letter for a particular NaCl concentration do not differ significantly (p≥0.05). [A-C]Means with same capital letter for a particular pH do not differ significantly (p≥0.05).

the emulsion stability of ASP also differed from that of PI (Figures 2 and 3). The addition of 0.25 mol/L NaCl reduced the emulsion stability of ASP at all pH values except at pH 2 (Figure 3). A similar trend was observed by Cheung *et al*. [2015] who observed destabilization of napin protein isolate emulsions after NaCl addition.

The chosen methodology for the evaluation of emulsifying activity and stability of the PI and ASP is a quick approach which allows discrimination between emulsifying capacities of the two protein-containing products. Once the superiority of emulsifying properties of the PI to the ASP is established, additional experiments and analyses, including but not limited to hydrophobicity evaluation, particle size measurement, and determination of emulsion stability from turbidity measurements, are needed to provide a better insight and a more profound explanation of the phenomenon.

CONCLUSION

The study demonstrated the opportunity to concomitantly prepare two protein-containing ingredients from ethanol-treated industrial rapeseed meal with valuable functionality. While PI protein solubility was pH dependent, the one of the ASP was slightly sensitive to pH and remained higher than 70% for the entire pH range studied. Compared to the PI, the ASP demonstrated higher ability to absorb oil and higher thermal stability at pH 8 than at pH 7. The emulsifying activity of PI and ASP varied from 57.46% to 63.81% for all combinations of pH and NaCl. The emulsion stability of the ASP was lower than that of the PI. Distinctive techno-functional properties of the two ingredients define their wide and versatile application in the food industry as additives. The simplicity of the procedure and the lack of purification steps lead to a better and more complete use of the industrially produced rapeseed meal, with the potential for a higher profitability.

ACKNOWLEDGEMENTS

Authors would like to express their appreciation to professor Turid Rustad, Department of Biotechnology and Food Science, Norwegian University of Science and Technology, Trondheim, Norway, for her comments and improvement of manuscript quality.

CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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Submitted: 14 June 2018. Revised: 19 September 2018. Accepted: 16 November 2018. Published on-line: 19 March 2019.