Select functional properties of protein isolates obtained from canola meals modified by solid-state fermentation

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Abstract

The present study investigated the effect of solid-state fermentation (SSF) of cold-pressed (CP) and hexane-extracted (HE) canola meals with Aspergillus niger NRRL 334 and A. oryzae NRRL 5590 on the functionalities of protein products extracted from them. After SSF, proteins were recovered using alkaline extraction-isoelectric precipitation (AE-IP) or salt extraction-dialysis (SE). SSF of the two meal types reduced the protein content of the extracts produced by AE-IP. There were varied effects to solubility, foaming, and emulsifying properties as a result of SSF under the combined influence of functionality pH, strain, meal type, and protein extraction method. The protein isolate produced from CP meal using SE had increased solubility at pH 7 (from 51.8 to 90.7%) when the meal was fermented with A. oryzae. Both strains resulted in an over 2-fold increase in the emulsifying activity index (at pH 7) of AE-IP products from CP meal. For both protein extraction methods, the protein products from A. niger fermented HE meal had better foaming capacity (FC) at pH 7 than the controls (non-fermented), but reduced FC at pH 3. Overall, regardless of meal fermentation, the SE products were richer in protein and had higher oil holding capacity (OHC), whereas the water holding capacity (WHC) was higher for AE-IP isolates. SSF of the meals generally improved the O/WHC of the extracted proteins. The findings suggest that canola protein functionality could be effectively modulated by SSF with different microbial strains under various processing conditions to enhance their applicability in the food industry.

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KEYWORDS

Aspergillus niger, Aspergillus oryzae , canola protein, functionality, protein extraction, solid-state fermentation

1 INTRODUCTION

Canola meal, the by-product of the canola oil extraction process, is gaining tremendous interest in the food industry due to its high protein content (35%-45%) and low cost. The use of canola meals as an alternative protein source can provide more protein options to the plant-based food industry. Canola proteins are dominated by two major proteins, a salt-soluble globulin protein (cruciferin, 12S , MW ˜300 kDa) and a smaller water-soluble albumin (napin, 1.7-2S , MW ˜14–17 kDa). The amino acid composition of canola meal is well balanced with a relatively high protein efficiency ratio (PER) of 2.64 and therefore can be used for human nutrition (Aider & Barbana, 2011). In recent research, canola proteins have presented high solubility, foaming capacity and stability, and comparable or moderate emulsifying properties compared to other plant proteins such as soybean and pea protein (Chang *et al.* , 2015; Cheung *et al.* , 2014; Khattab & Arntfield, 2009; Tan *et al.* , 2011a; Wu &Muir, 2008). These characteristics make canola protein a potential ingredient for the food industry.

Canola oil can be extracted by hexane extraction (HE) or cold-pressing (CP). The conventional way, HE, is a chemical process involving the use of non-polar solvents, e.g., hexane. During HE, the seeds are ground up and washed with hexane under controlled conditions to release oil molecules stored within. A desolventizing step is followed to remove the hexane by distilling at approximately 100-110^{o}C, after which the solvent is recycled and reused (Cheng et al., 2019). The HE process efficiently produces a high yield of oil, however, the use of chemicals and the application of heat lead to considerate damage to the proteins within the meal, resulting in reduced functionality (e.g., emulsifying properties) (Östbring et al., 2019). The CP technique, on the other hand, solely utilizes mechanical forces (e.g., crushing) without the application of organic solvents or high heat for their subsequent removal. The seeds are pressed at low temperatures ($<$40^{o}C), leaving nutrients in the remaining meal in a less disturbed form compared to HE, however, the oil yield was lower in HE with a high level of residual oil (6-20%) (Hickling, 2007; Östbring et al., 2019).

Fermentation is the process accomplished by the metabolism of microorganisms that catalyze nutrients, synthesize secondary metabolites, and complete other physiological activities under anaerobic or aerobic conditions. Solid-state fermentation (SSF) is a popular process to modify the functionality and nutritional composition of protein ingredients. As opposed to submerged fermentation (SmF), there's no free water in SSF, making it suitable for microorganisms that don't require high moisture or high water activity to

grow, for example, fungi. Recently, SSF has been applied to canola meals to improve their quality. Pal Vig and Walia (2001) used *Rhizopus oligosporus* as the fermenting culture to produce a high-protein product from HE canola meal. The results showed a significant decline in contents of glucosinolates (GLS) (˜43%), thiooxazolidones (˜31 %), phytic acid (˜42%) and fibre (˜26%) along with a ˜65% increase in crude protein after 10 days. Croat *et al.* (2016) studied the use of SSF on HE and CP canola meals to modify their nutritional composition. The strains *Trichoderma reesei* , *Aspergillus pullulans* , and *A. pullulans* improved protein content by 22.9, 16.9 and 15.4%, while reducing the total GLS content from 60.6 to 1.0, 3.2 and 10.7 µmol/g, respectively. Significantly higher content of dry matter yield was reported for the HE meal compared to the CP meal, which is mainly due to the high oil residues left by CP. Much work has been done on enhancing the nutritional quality of canola meals, however, the use of SSF to alter the functionality of proteins in HE or CP canola meals has been limited.

Several methods, including alkaline extraction-isoelectric precipitation (AE-IP), salt extraction-dialysis (SE), protein micellation method (PMM), and low pH extraction combined with membrane separation and ultrafiltration (UF), have been widely used for the production of canola protein isolates (Tan *et al.* , 2011a, 2011b; Wanasundara, 2011). The structural composition and functionality of the canola proteins may vary significantly depending on the extraction method used (Can Karaca*et al.* , 2011; Hoglund *et al.* , 1992; Wu & Muir, 2008). In addition, the extraction of canola proteins is especially difficult due to the differences in protein fractions (widely differing isoelectric points of pH 4-11 and molecular weights of 13-320 kDa) and the presence of antinutrients (GLS, phytic acid, and polyphenols), pigments, and fibre in the canola meal (Wu & Muir, 2008). It is expected that pre-treatment, such as fermentation, and specific purification processes may improve protein solubility in the meals and consequently protein extractability.

In our previous study, both CP and HE canola meals had a ˜45% degree of protein hydrolysis upon 72 hours of fermentation with *Aspergillus niger* NRRL 334 and*Aspergillus oryzae* NRRL 5590. In this study, those fermented canola meals were used in the wet fractionation process to produce protein products. Two wet extraction processes, AE-IP and SE, were employed. The resulting protein products were characterized for select functional properties and compared with protein products extracted from meals that were not pre-treated by fermentation. We hypothesize that pre-treatment with SSF has the potential to improve the protein extractability from canola meal because of partial protein hydrolysis and the loosening of protein-carbohydrate interactions while also modifying protein functionality. Moreover, the CP meals are expected to yield protein products with higher functional properties than those processed by HE due to the lack of heat and chemical damage to the proteins. The findings of this work will provide a relatively comprehensive view of the effect of SSF of canola meals on the functionality of extracted proteins with direct comparisons between the test fungal strains, meal type, and protein fractionation techniques.

2 MATERIALS AND METHODS

2.1 Materials

Canola meals (CP from Pleasant Valley Oil Mills (Clive, AB, Canada) or HE from Bunge Canada (Harobe, MB, Canada)) of 150 g were fermented separately with *Aspergillus niger* NRRL 334 and *Aspergillus oryzae* NRRL 5590 (each at 10^7 spores/g meal) for three days (72 h) with moisture maintained at 50% and temperature at 30. The strains were obtained from the Agricultural Research Service (ARS) Culture Collection (Northern Regional Research Laboratory (NRRL), Peoria, IL, USA). The fermented meals were immediately processed for protein extraction using the AE-IP and SE methods (as described in the following sections) to obtain canola protein products. All the reagents and chemicals used were of analytical grade and obtained from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada).

2.2 Preparation of canola protein products

2.2.1 Alkaline extraction-isoelectric precipitation

The fermented (72 h) CP and HE meals (100 g) were extracted at a 1:10 (w/v, meal:water) ratio at pH 11 (adjusted using 2 N NaOH) for a 1-h period at room temperature (21-23) according to Aider and Barbana

(2011). The suspension was then centrifuged (Sorvall RC Plus Superspeed Centrifuge, Thermo Fisher Scientific, Asheville NC, USA) (4,500 × *g* at 4 for 15 min), and the supernatant was vacuum filtered with No. 1 Whatman filter paper (Whatman International Ltd., Maidstone, UK) to remove any floating particles. The supernatant was then adjusted to pH 5 using 2 N HCl to precipitate the proteins. The protein precipitate was collected as a pellet after centrifugation at 4500 x *g* at 4 for 15 min and freeze dried. The crude protein content of meals and protein products was determined using the micro-Kjeldahl method (AOAC 960.52) with a conversion factor of 6.25 (AOAC, 2005).

2.2.2 Salt extraction-dialysis (SE)

SE described by Klassen *et al.* (2011) and Chang *et al.* (2015) was employed with minor modification to obtain protein products from fermented CP and HE canola meals. In brief, a 0.05 M Tris–HCl buffer solution (pH 7.0) containing 0.1 M NaCl was mixed with fermented canola meal at a 1:10 (w/v) ratio and stirred at 500 rpm for 2 h at room temperature (21-23). The supernatant was then collected by centrifuging (Sorvall RC Plus Superspeed Centrifuge, Thermo Fisher Scientific, Asheville NC, USA) at 6,000 x *g* for 30 min, followed by vacuum filtration with No.1 Whatman filter paper (Whatman International Ltd., Maidstone, UK). The filtrate was dialyzed to remove NaCl and other small molecules using a Spectra/Por molecular porous membrane tubing (6–8 kDa cut off, Spectrum Medical Industries, Inc., USA) at 4 for 72 h against distilled water (distilled water was refreshed twice a day). The dialyzed protein extract was then centrifuged at 3,000 x *g* for 30 min at 4. The supernatant was discarded, and the protein pellet was collected and freeze dried. The protein content was determined using the same protocol for AE-IP samples.

2.2.3 Protein product controls

The canola protein products were extracted directly from unfermented CP and HE meals (without defatting) using both the AE-IP and SE methods as described above and used as the control protein products for the study. In addition, a defatted CP meal was also prepared as a control to study the effect of residual oil on protein extractability. In brief, the unfermented CP meal was defatted with a meal:hexane ratio of 1:3 (w:v) over a 1.5 h period of stirring at room temperature, followed by vacuum filtration with No. 1 Whatman filter paper (Whatman International Ltd., Maidstone, UK) to recover the defatted solids. The above defatting process was repeated twice to reach an oil content of <2%, and the resulting meal was used to extract the unfermented defatted CP control (DCP) protein products using both the AE-IP and SE methods. In total, three canola protein product controls were prepared (from HE, CP, and defatted CP meals) for each extraction method (AE-IP and SE) resulting in six total control (unfermented) protein products.

2.3 Functional analysis

2.3.1 Solubility

Protein solubility was studied as a function of pH according to Can Karaca *et al.* (2011) and Chang *et al.* (2015) with minor modifications. In brief, 20 mL of 0.25% (w/w) protein (corrected by protein content) solution at the required pH (3, 5, and 7) was prepared and allowed to stir for 1 h at room temperature (21-23). The protein solution was then transferred to a 15-mL centrifuge tube and centrifuged (VWR clinical centrifuge 200, VWR International, Mississauga, ON, Canada) at 4,180 x *g* for 10 min at room temperature. The supernatant was carefully collected, followed by the Bradford method to determine the protein content. In detail, a 50 µL of supernatant was added to a 1.5-mL centrifuge tube and 1.5 mL of Coomassie dye (Thermo Fisher Scientific, Madison, WI, USA) was added. The mixture was vortexed for 10 s vigorously and then left to stand for 5 min. The absorbance of the sample was measured at 595 nm using a UV-visible spectrophotometer (Genesys 20, Thermo Scientific, Madison, WI, USA), and Milli-Q water was used as a blank while a mixture of 50 µL of 0.5 N NaOH and 1.5 mL of Coomassie dye was used as the sample blank. Different concentrations of bovine serum albumin (BSA) were used to create a standard curve. The total protein content was determined by dissolving 20 mg of dry protein extract in 20 mL of 0.5 N NaOH solution and allowing the solution to stir for 1 h at room temperature, followed by centrifugation at 12,100 ×*g* for 30 min, and the total protein content of the supernatant was determined using the Bradford method. The protein solubility (%) was determined by dividing the protein content in the solutions at different pH

levels by the total protein in the sample (N% × 6.25), multiplied by 100%. All measurements were reported as the mean ± one standard deviation (n=3).

2.3.2 Water and oil holding capacity

The water and oil holding capacity (WHC and OHC) were determined according to Stone *et al.* (2015). In brief, 0.5 g of protein (corrected by protein content) was mixed with 5.0 g of water or oil in a 50 mL screw cap centrifuge tube. The mixture was vortexed for 10 s every 5 min for a total period of 30 min, followed by centrifugation (VWR clinical centrifuge 200, VWR International, Mississauga, ON, Canada) at 1,000 × *g* for 15 min. The remaining pellet was weighed after carefully decanting the supernatant. The WHC and OHC were calculated by dividing the weight gain by the original sample weight (g/g). All measurements were reported as the mean ± one standard deviation (n=3).

2.3.3 Emulsifying properties

The emulsifying activity index (EAI, m^2/g) and emulsion stability index (ESI, min) were determined according to Pearce and Kinsella (1978) and Cheung*et al.* (2014). In brief, a 0.25% (w/v) protein solution (corrected by protein content) was prepared at pH 3, 5, and 7 and stirred at room temperature (21-23) overnight. To prepare the emulsion, 5.0 g of the protein solution was added to 5.0 g of canola oil, followed by homogenization using an Omni Macro Homogenizer (Omni International, Marietta, GA, USA) with a 20 mm saw tooth generating probe positioned at the oil/water interface at speed 4 (˜7,200 rpm) for 5 min. Immediately after homogenization, 50 µL of the emulsion from the bottom of the tube was transferred to 7.5 mL of 0.1 % (w/v) sodium dodecyl sulphate (SDS), followed by vortexing for 10 s. The absorbance of the diluted emulsion was determined using a Genesys 20 UV-visible spectrophotometer (Thermo Scientific, Madison, WI, USA) at 500 nm using plastic cuvettes. A second absorbance reading was taken from the dilution after 10 min. The EAI and ESI were calculated using the following equations:

$$EAI\ (\frac{m^2}{g}) = \frac{2 \times 2.203 \times A_0 \times N\ \times l}{c \times \varphi \times 100} \tag{1}$$

$$\text{ESI}\ (\text{min}) = \frac{A_0}{A} \times t \tag{2}$$

where A_0 is the absorbance of the diluted emulsion immediately after homogenization, N is the dilution factor, l is the 0.01 m path length of the plastic cuvette,c is the weight of protein per volume (g/mL), φ is the oil volume fraction of the emulsion, ΔA is the difference in absorbance between 0 and 10 min (A_0 -A_{10}), and t is the time interval (10 min). All measurements were reported as the mean ± one standard deviation (n=3).

2.3.4 Foaming properties

The foaming properties were determined according to Stone *et al.*(2015). In brief, a 1.0% (w/w) protein solution (corrected by protein content) was prepared at pH 3, 5, and 7 and stirred overnight at 4. For each protein solution, 15 mL (V_{li}) was transferred into a 400 mL tall glass beaker (inner diameter = 69 mm; height = 127 mm; as measured with a digital caliper) and foamed using an Omni Macro homogenizer (Omni International, Marietta, GA, USA) with a 20 mm saw tooth generating probe for a total of 5 min at speed 4 (˜7,200 rpm). The foam was immediately transferred to a 50- or 100-mL graduated cylinder (inner diameter = 26 mm) after homogenization. The foam volume was recorded at time zero (V_{fi}) and after 30 min (V_{ft}) at room temperature. The foaming capacity (FC) and foaming stability (FS) were determined using following the equations:

$$\%FC = \frac{V_{\text{fi}}}{V_{\text{li}}} \times 100\% \tag{3}$$

$$\%FS = \frac{V_{\text{ft}}}{V_{\text{fi}}} \times 100\% \tag{4}$$

where V_{fi} is the volume of foam immediately after homogenization, and V_{ft} is the volume of foam remaining after 30 min.

2.3.5 Statistical analysis

All protein products were prepared in triplicate on separate fermented meals (n=3). The results were reported as mean +- one standard deviation. A two-way analysis of variance (ANOVA) was used to study the statistical differences in protein functionality using a Tukey's test with a significance level of $p < 0.05$. A simple Pearson correlation (r) was used to describe the relationship between the functional properties. The statistical analysis was performed using the IBM SPSS Version 28.0 software (IBM Corp. NY, IL, USA).

3 RESULTS AND DISCUSSION

3.1 Protein content of canola protein products

The protein products obtained using AE-IP from unfermented HE and CP (non-defatted) meals had protein levels of 86.5% and 68.9%, respectively (Table 1). After the defatting process, an 81.6% protein content was achieved for the CP meal, indicating that the high oil content negatively influenced protein extraction by AE-IP. The canola protein products obtained from the CP meal fermented with *A. niger* NRRL 334 and *A. oryzae* NRRL 5590 had protein levels of 62.7% and 57.9%, respectively. The protein products from the HE meals fermented with *A. niger* and *A. oryzae* had protein levels of 56.3% and 58.4%, respectively. Both the CP and HE meals, when fermented with either of the fungal strains, gave products with lower protein content upon enrichment using AE-IP.

In contrast, the SE method resulted in higher protein levels (>90%) for all unfermented and fermented canola protein products than those produced by AE-IP (56.3%-86.5%). The lower protein content found in the AE-IP samples may be due to the heterogeneous nature of canola proteins (cruciferin and napin), which were proven to have different isoelectric points that complicate their precipitation (Wu & Muir, 2008). Fermentation did not increase the protein content of SE products for either type of meal (CP and HE). Of the protein products extracted from fermented meals, the SE process using the CP meal fermented with *A. oryzae* resulted in the highest protein content (99.0%).

As these findings refute the hypothesis that SSF could improve the protein extraction process, a few theories are proposed. Fermentation can lead to partial hydrolysis of the protein, which is hypothesized to increase the protein surface hydrophobicity (decrease in protein solubility) and in turn create stronger protein-lipid interactions. Also, the large quantity of hyphae produced by fungi may act like a 'glue' to connect the solid substrates tightly and affect the protein extraction by limiting the soluble protein dissolving into the surrounding aquatic system. After the SSF, the degradation of fibre might also release insoluble or soluble carbohydrates (polysaccharides or starch) and make it difficult to separate the proteins from the complex (protein-carbohydrate interaction). In addition, the oil could largely affect the protein extraction process, which may be the case for the CP meals (Krause & Schwenke, 2001), although adding a defatting step to the unfermented meal resulted in slightly lower protein content (99.6 *vs.* 96.5%) of the SE products.

3.2 Protein solubility

The protein solubility is one of the most important physicochemical properties, as it is generally related to other functional properties, which may affect the rheological, hydrodynamic, and surface activity characteristics (Zayas, 1997). As shown in Table 2, the AE-IP CP control had a significantly higher ($p < 0.05$) solubility at pH 3 where it was 88.9% soluble protein compared to the defatted CP and HE controls (76.2% and 81.4%, respectively). Similar to the results at pH 3, at pH 5 the AE-IP CP control showed higher solubility (53.5%) than the defatted CP (36.9%) and HE (2.0%) controls, whereas at pH 7 the HE control (70.0%) had a higher solubility than both the CP control (27.7%) and the defatted CP control (37.8%). As for the SE products, all of the controls had a higher solubility ([?]94%) at pH 3 than the controls produced by AE-IP. At pH 5, a higher solubility was found for the SE HE control (29.4%) compared to AE-IP (2.0%), whereas it was lower than the SE CP control (37.7%) and defatted CP control (37.3%), which had similar solubility values. At pH 7, both the SE CP (51.8%) and defatted CP control (49.3%) had a higher solubility

than the respective AE-IP product however, the SE HE control (43.2%) was less soluble than the AE-IP HE control. Overall, the SE products showed higher solubility at pH 3 and 7. Stone *et al.* (2015) also found a lower solubility of canola protein products obtained by AE-IP compared to the SE process. Similar solubility values were found for both the CP and defatted CP controls at every pH and tended to be higher than the HE control.

The canola protein products prepared from fermented meals showed a lower solubility at each pH compared to the control products. This is hypothesized to be due to partial hydrolysis of protein during fermentation which led to the exposure of previously buried hydrophobic groups. At pH 3, all AE-IP and SE controls showed higher solubility than products from fermented meals. However, the solubility of products from *A. oryzae* fermented meals was significantly higher than products from *A. niger* fermented meals. This indicated that fermentation with *A. oryzae* was able to maintain the solubility (pH 3) at a relatively higher level than *A. niger* after SSF. As such, using *A. oryzae* may be favorable to obtain a more soluble protein product at pH 3. After fermentation, most extracted samples showed significant decreases (p <0.05) in solubility compared to the controls at pH 5 and had overall low solubility values ranging from 4.5% to 11.8%. However, there was an increase in solubility at pH 5 from 2.0% for the AE-IP HE control to 11.8% and 5.8% when fermented using *A. niger* and *A. oryzae* , respectively. According to a previous study, the isoelectric point at pH 6.2 and low solubility at both pH 5 (˜37%) and pH 7 (˜23%) were reported for a SE canola protein isolate produced from unfermented HE meal (Chang *et al.* , 2015). Our results indicated that SSF processing decreased the protein solubility at pH 5 which may be due to a change in the isoelectric point (close to pH 5) by partial hydrolysis of the proteins, causing the shift in solubility with pH value.

At pH 7, the solubility of AE-IP canola protein products showed significantly lower (p <0.05) solubility than SE products obtained from CP control meals. The inverse was found for the products extracted from the HE control meal. Unlike at pH 3 and 5, the SSF showed the ability to improve the protein solubility of the extracted products at pH 7. For instance, the AE-IP product from the *A. niger* fermented CP meal showed a significant (p<0.05) increase in solubility (to 47.5%) compared to both the AE-IP CP controls. In addition, the same level of solubility (70.0%) was found for the AE-IP HE control and the HE product from the *A. niger* fermented meal. However, there was a decrease in solubility (p <0.05) for protein products extracted (AE-IP) from *A. oryzae* fermented CP and HE meals compared to the controls, which indicated that *A. niger* was preferred to maintain or increase the solubility of AE-IP protein products at pH 7. Some opposite results were found for SE protein products, as products from *A. oryzae* fermented meals resulted in higher solubility values (90.7% for *A. oryzae* CP and 55.6% for *A. oryzae* HE) compared to the controls (p<0.05). An increase in the solubility of the SE product from *A. niger* fermented HE meal (48.5%) was also observed compared to the HE control (p<0.05). However, for the CP meals a decrease in solubility was reported when using SE on the *A. niger* fermented meal (37.2%) compared to the CP controls. When extracting proteins via SE, both *A. niger* and *A. oryzae* can be acceptable inoculums for meals to maintain or increase the protein solubility at pH 7, whereas *A. niger* might be a better culture choice for AE-IP products at pH 7.

The results above indicate the differences between AE-IP and SE canola protein (protein fraction, the percentage of napin and cruciferin) and the hydrolysis mechanism of *A. niger* and *A. oryzae* including possible different proteinase, length of peptides, structure of hydrolyzed protein, and synthesized metabolism. The protein products extracted using SE showed higher solubility than the AE-IP products mainly due to the low content of non-protein compounds, possible differences in protein fraction, and possible interactions of protein and carbohydrate in the AE-IP products. In addition, the protein products extracted from CP meals showed higher solubility than products from the HE meal. Protein denaturation during the hexane-extraction (heat treatment) could explain the solubility reduction. Heat treatments could result in the exposure of hydrophobic groups, which contribute to the reduction in protein solubility (Khattab & Arntfield, 2009). The partial denaturation of proteins can alternate the balance of protein hydrophobicity/hydrophilicity and further affect solubility (Moure *et al.* , 2006).

3.3 Water holding capacity

The ability of a protein to absorb and retain water is critical in stabilizing the structure, improving flavour

retention and mouth feel, and reducing moisture loss of food products (Khattab & Arntfield, 2009). As shown in Table 3(a), the AE-IP CP control protein product showed higher WHC (2.1 g/g) than the products of the HE control (1.8 g/g), which may be due to the thermal and chemical changes of HE proteins that occurred during the solvent extraction processing, leading to an increase in the protein-protein aggregations and limiting the protein-water or protein-oil interactions. However, the value of the defatted CP control (2.7 g/g) was higher than that of non-defatted ones (2.1 g/g), which was mainly due to the presence of residual oil that impacted the hydrophilic ability. Similar results were found in the SE controls of which the defatted CP control (2.1 g/g) had a higher WHC value than the HE (1.3 g/g) and non-defatted CP controls (1.1 g/g) as shown in Table 3(b).

After a period of 72-h fermentation of the source meal, a significant increase (p <0.05) in the WHC of AE-IP products was found. The WHC increased from 2.1 g/g (CP control) and 2.7 g/g (non-defatted CP control) to 3.1 g/g (*A. niger* fermented) and 3.0 g/g (*A. oryzae* fermented). No significant difference (p >0.05) was found between the strains for AE-IP CP products. In addition, *A. oryzae* showed a higher increase in WHC of AE-IP HE products (3.2 g/g) than *A. niger.* (2.9 g/g). Similar results were found for SE HE products as SSF increased the WHC values from 1.3 g/g to 2.3 g/g (*A. niger*) and 2.7 g/g (*A. oryzae*). The partial protein hydrolysis during the fermentation process may change the compact protein structure and expose more hydrophilic and hydrophobic sites (additional binding sites available for water), which made it easier for the protein to adsorb and hold water (Kinsella, 1982). In contrast, the WHC values of the SE CP products remained stable (p >0.05) after SSF using *A. niger* (1.3 g/g) and *A. oryzae* (1.4 g/g) compared to the non-defatted CP control (1.1 g/g) and decreased when compared to the defatted CP control (2.1 g/g). For the type of strains, *A. oryzae* was preferred to improve the WHC of both AE-IP and SE HE products. In addition, all HE products showed higher increases in WHC than the CP products.

Overall, the defatted CP controls showed higher WHC values than the non-defatted CP controls and the HE controls. As for the difference between the AE-IP and SE products, the AE-IP protein products showed a slightly higher WHC value than the SE ones. This was possibly due to the lower protein level in AE-IP products, which led to a larger sample amount of AE-IP products used for testing than the SE products (corrected by protein content). The non-protein compounds in AE-IP products such as the soluble fibre and polysaccharides may contribute to the ability of water to absorb and bind. Thus, it is important to take the possibly contained fibre and polysaccharides into consideration. The presence of these compounds can enhance the overall water holding capacity of canola products (Aider & Barbana, 2011).

3.4 Oil holding capacity

Fats act as a flavour carrier and mouthfeel enhancer, so the ability of the protein to absorb and retain oil is important in food formulations (Kinsella, 1982). Fat adsorption of proteins mainly depends on the physical entrapment of oil through a capillary-attraction process (Kinsella, 1982). As shown in Table 3(a), the AE-IP CP control showed a higher OHC value (2.9 g/g) than the HE control (2.3 g/g), possibly due to the thermal and chemical changes of proteins that occurred during solvent extraction, leading to an increase in protein-protein aggregations and limited protein-water or protein-oil interactions. Similar results were observed for the products obtained using SE in Table 3(b). The CP control had a higher OHC value (3.2 g/g) than the products of the HE control (2.4 g/g). The OHC value of the AE-IP defatted CP control (2.4 g/g) was lower than that of the non-defatted CP control (2.9 g/g), whereas the SE defatted CP control (3.9 g/g) had a higher OHC value than the CP non-defatted control (3.2 g/g).

After the 72-h SSF, the OHC of AE-IP CP products increased to 2.8 g/g (both *A. niger* and *A. oryzae*) compared to the defatted CP control (2.4 g/g, p< 0.05), whereas it was similar to that of the non-defatting CP control (2.9 g/g, p>0.05). The AE-IP HE products also showed an increase (p<0.05) from 2.3 g/g to 3.1 g/g (*A. niger*) and 3.3 g/g (*A. oryzae*). As for the SE products, an increase in OHC was found for both the AE-IP and the SE protein products compared to the controls (p<0.05). For the SE CP products, the OHC values increased from 3.2 g/g (CP control) and 3.9 g/g (defatted CP control) to 4.6 g/g (*A. niger*) and 4.7 g/g (*A. oryzae*), while the value increased from 2.4 g/g (HE control) to 4.5 /g (*A. niger*) and 3.9 g/g (*A. oryzae*) for HE products. The partial hydrolysis of proteins during fermentation may change the compact

protein structure and expose more hydrophilic and hydrophobic sites, making it easier for proteins to adsorb and hold oil. The above results were agreed by Kinsella (1982) that the increase in fat absorption could be associated with the hydrolysis and denaturation of proteins due to the exposure of non-polar residues buried inside the protein molecules. Comparing the type of strains, there is no significant difference ($p>0.05$) between *A. niger* and*A. oryzae* except for the SE HE products, where *A. niger*was preferred to improve the OHC of products. Similar to the WHC, a higher increase was found in the HE meal compared with the CP meal after SSF.

Whereas the WHC results showed higher capacities with the AE-IP isolates, canola protein products obtained using SE showed higher OHC compared to those prepared by AE-IP. It may indicate that a higher level of protein (˜95% for SE products and ˜60-80% for AE-IP products) is preferred to obtain protein products with enhanced OHC. It could also be concluded that the proteins of SE might be more hydrophilic than those from AE-IP, hence a higher protein solubility for the SE products. The lower solubility and greater OHC suggest that the proteins recovered from AE-IP could obtain a more hydrophobic nature. The partial hydrolysis of proteins during the fermentation process may change the compact protein structure and expose more hydrophilic and hydrophobic sites (additional binding sites available) buried inside the protein molecule, which may decrease the solubility of canola proteins while increasing the ability of the proteins to adsorb and hold water/oil (Kinsella, 1982).

3.5 Emulsifying properties

The emulsifying properties are critical functional attributes of food proteins. Several factors affect the emulsifying properties, including the type and concentration of protein, pH, ionic strength, and viscosity of the solvating medium. Many chemical and physical factors are involved in the formation, stability, and textural properties of oil-water emulsions stabilized by protein (Chang *et al.* , 2015). The EAI is a measurement of the interfacial area coated by protein during the formation of an emulsion and acts as a good predictor for the surface activity of the protein, whereas the ESI is a measurement of the stability of the diluted emulsion over a fixed period of time (Can Karaca *et al.* , 2011).

As shown in Table 4(a), the AE-IP HE control had a higher EAI value (13.2 m^2/g) than the CP control (11.4 m^2/g) and the defatted CP control (8.3 m^2/g) at pH 3, which may indicate a higher hydrophobicity value of the HE products compared to the CP controls at pH 3. Similarly for pH 7, a higher EAI value was found for the HE control (18.3 m^2/g) than the CP control (11.7 m^2/g) and the defatted CP control (11.6 m^2/g) at pH 3. As for pH 5, the defatted CP control (5.0 m^2/g) had a higher EAI value than the CP control (4.1 m^2/g, $p>0.05$) and the HE control (3.0 m^2/g, $p<0.05$). As for the SE products, the defatted CP control (11.0, 9.4, and 14.5 m^2/g at pH 3, 5, and 7, respectively) showed the highest EAI values compared to the CP (5.6, 5.8, and 12.9 m^2/g at pH 3, 5, and 7, respectively) and the HE control (5.9, 6.8, and 13.9 m^2/g at pH 3, 5, and 7, respectively). The difference between the AE-IP and SE products may be due to the different protein fractions and the nature of products resulting from the two methods. As for pH, the EAI values tended to be low at pH 5, ranging from 1.3-5.5 m^2/g due to the low protein solubility. Higher EAI values were found at pH 7 compared to pH 3 for all protein products obtained from unfermented control meals, which was possibly related to the higher solubility at pH 7 than at pH 3.

During SSF, the partial hydrolysis of proteins loosens the compact protein structure and exposes more hydrophobic sites buried inside the protein molecule. A higher hydrophobicity value can be achieved. Townsend and Nakai (1983) suggested a positive correlation between hydrophobicity and the emulsifying properties of proteins. Results of the present study showed that canola products extracted from fermented meals have better or at least unchanged emulsifying properties compared to the controls. Among the AE-IP products, the EAI values increased to 13.8 m^2/g (*A. niger*) and 21.1 m^2/g (*A. oryzae*) at pH 3 for the CP products ($p<0.05$). In addition, only *A. oryzae* improved the EAI of the HE product (17.6 m^2/g), while a decrease (8.5 m^2/g) was found using *A. niger* at pH 3. At pH 7, the AE-IP products from *A. niger* and *A. oryzae*fermented meals had either improved or unchanged EAI values. In detail, the EAI values of AE-IP CP products extracted from *A. niger* and*A. oryzae* fermented CP meals significantly increased ($p<0.05$) to 25.8 and 27.6 m^2/g, respectively. Similar to the AE-IP HE products, the EAI values were significantly

increased to 18.0 and 25.5 m^2/g when fermented using *A. niger* and *A. oryzae* . The EAI values of the AE-IP products at pH 5 remained relatively low, ranging from 1.3-5.5 m^2/g for both the AE-IP CP and the HE products, regardless of whether fermented or not, may be due to the low protein solubility (pH 5). In addition, a low ESI was reported for all protein products and ranged from 1.1-4.5 min. For the SE products, *A. niger* and *A. oryzae* improved the EAI values of the CP products at pH 3 to 8.5 m^2/g (*A. niger*) and 9.7 m^2/g (*A. oryzae*) compared to the CP control (5.6 m^2/g), however, they were lower than the defatted CP control (11.0 m^2/g). The increases in the SE HE product at pH 3 after SSF were also reported as 8.5 m^2/g (*A. niger*) and 11.3 m^2/g (*A. oryzae*) compared to the HE control (5.9 m^2/g). However, SSF decreased the EAI of the CP and HE products to 1.8 m^2/g (CP, *A. niger*), 4.6 m^2/g (CP, *A. oryzae*), 4.1 m^2/g (HE, *A. niger*), and 3.0 m^2/g (HE, *A. oryzae*) at pH 5. At pH 7, a decrease in the EAI of SE CP and HE products was also reported to 12.2 m^2/g (CP, *A. niger*), 14.9 m^2/g (CP, *A. oryzae*), 11.4 m^2/g (HE, *A. niger*), and 7.6 m^2/g (HE, *A. oryzae*). The strain *A. oryzae* was able to more positively modify the EAI values of canola protein products than *A. niger* while the enhanced properties were more frequently observed in AE-IP isolates than SE.

The above EAI and ESI results reported were lower than those from previous studies by Cheung *et al.* (2014) and Chang *et al.*(2015). Cheung *et al.* (2014) suggested that high solubility was positively correlated with the ESI values due to the additional protein precipitation and adherence to the viscoelastic film surrounding the droplets. A lower solubility was reported compared to previous studies on canola protein, which may result in a reduction in ESI. In contrast, Kinsella and Melachouris (1976) explained that high protein solubility and high fat-adsorption capacity were positively correlated with the ability of emulsifying (form and stabilize emulsions). Wu and Muir (2008) and Cheung *et al.* (2014) examined the emulsifying properties of two major canola proteins, cruciferin and napin. It has been reported that the emulsion prepared with cruciferin showed a significantly higher specific surface area and a lower particle size than that with napin. The study by Wu and Muir (2008) indicated that the presence of napin could detrimentally affect the emulsion stability of canola protein isolates. The differences may be a result of the canola cultivar, the preparation of canola meals (oil extraction method) and protein products (extraction methods and conditions), and the analytical methods employed. These differences might result in variations in protein fractions and the levels of protein that further affect the emulsifying properties. In addition, the released short peptides from fermentation may also have an impact on the protein functionality such as a reduction in emulsion activity and stability (Kristinsson & Rasco, 2000).

3.6 Foaming properties

The foaming properties of proteins are important in the production of a variety of foods (Hettiarachchy *et al.* , 2012). Foaming properties can be defined as two parts, the ability to form a two-phase system consisting of air cells surrounded by a thin continuous liquid layer (the lamellar phase) and the ability to stabilize the foams. All canola protein products in this study regardless of the source of meal or fermentation treatment showed FC of 131.1-480.0 % and moderate FS ranging from 68.0-89.0% at pH 3, 5, and 7.

Among the controls, the defatted AE-IP CP control (306.7% at pH 3 and 243.8% at pH 7) had higher FC than the CP (224.4% pH 3 and 165% at pH 7) and the HE control (244.4% at pH 3 and 154.4% at pH 7) possibly due to the residual oil in the CP control and a low protein content (˜60%) of both controls. As for the SE products, the CP control had a higher foaming capacity (322.2%) than the defatted CP (246.7%) and the HE (266.7%) control at pH 3. At pH 7, the SE defatted CP control showed the highest FC in all products (480.0%), higher than the SE CP (464.4%) and the HE (145.6%) control. At pH 5, only the CP (241.1% for AE-IP and 211.1% for SE) and the defatted CP control (131.1% for AE-IP and 222.2% for SE) showed the ability to form stable foams for both AE-IP and SE products. However, the poor solubility at pH 5 still had an adverse impact on the foaming properties compared to the values at pH 3 and pH 7. Besides, the high temperature and the exposure to hexane during oil extraction may be the factors that contribute to the loss of foaming ability of the HE control at pH 5. In addition, the SE products showed significantly higher FC values than AE-IP. This might be due to the higher purity of protein in the SE products (>93%) than AE-IP (<80%) and possible differences in protein composition (napin and cruciferin).

Among the AE-IP products, only the AE-IP CP protein products by *A. oryzae* fermented meals showed the ability to form foams (228.9%) with a low FS of 33.0% at pH 3 after SSF. At pH 7, all fermented AE-IP products showed foaming properties of 230.7% (CP, *A. niger*), 180.0% (CP, *A. oryzae*) 244.2% (HE, *A. niger*), and 136.7% (HE, *A. oryzae*) with comparable FC compared to the controls ranging 72.0-84.6%. The AE-IP HE samples pre-treated with*A. niger* even showed a significantly higher FC value (244.2%) compared to the HE control (154.4%). As for the SE products, all samples were able to form foams at pH 3 and 7. In detail, the FC values of CP products from fermented meals decreased to 196.5% (*A. niger* at pH 3), 175.6% (*A. oryzae* at pH 3), 191.1% (*A. niger* at pH 7), and 153.3% (*A. oryzae* at pH 7) compared to the CP control at pH 3 and 7. For the HE products, decreases were also found for products at pH 3, at 195.6% (*A. niger*) and 135.6% (*A. oryzae*), while significant increases were reported at pH 7 as 306.7% (*A. niger*) and 155.6% (*A. oryzae*) compared to the HE control (266.7% at pH 3 and 155.6% at pH 7). In addition, the FS decreased to ˜52% for all products from fermented meals at pH 3, while they remained relatively unchanged (77.6-88.4%) at pH 7. At pH 5, none of the products extracted from fermented meals showed the ability to form stable foams. All proteins had poor foaming properties at pH 3 and 5 and could only form weak foams with large bubbles that disappeared within 5 min. This was possibly due to the extremely low protein solubility (<10%) of proteins extracted from fermented meals at pH 5. The SSF was found to decrease the solubility of canola protein products to <10% at pH 5, which could have further adverse impacts on the foaming properties. Overall, protein products prepared from the fermented HE meals using both extraction methods showed better FC and FS than the HE controls, while opposite results were found for CP canola meal products.

The above results indicate the potential of SSF for improving the foaming properties at pH 7 of protein products extracted from HE meal, especially when using *A. niger* . In other words, *A. niger*was preferred to provide protein products with better foaming properties compared to *A. oryzae* . The CP products had better foaming properties demonstrated by some foam when non was observed for HE isolates under certain conditions. Although the FC and FS values decreased after SSF, proteins extracted from the fermented CP meal displayed acceptable foaming properties compared to other plant proteins like soybean, pea, and lentil (˜200%) (Chabanon*et al.* , 2007; Stone *et al.* , 2015). However, they still could not produce comparable protein products similar to the ones from unfermented CP meals.

4 CONCLUSIONS

The present study investigated the effect of treating canola meals (CP or HE) with SSF by *A. niger* and *A. oryzae* on the functionality of extracted protein products (AE-IP or SE). In summary of the findings, protein purity in the resulting isolates was negatively influenced by fermentation, and the magnitude of reduction was greater for AE-IP products than SE. The protein content was also lowered in the presence of residual fat in the CP samples compared to HE when the protein products were produced by AE-IP. There were significant differences in canola protein functionalities extracted from fermented meals compared to the controls. For AE-IP proteins, meal fermentation had an overall negative effect on solubility however some improvements were seen with *A. niger* fermentation. Pretreatment with *A. oryzae* increased the protein solubility at pH 7 when extracting proteins via SE. Grouped together, the SE protein products were more soluble than those by AE-IP, while those extracted from CP meals showed higher solubility than those from HE. Fermentation of the meals improved the WHC and OHC of most protein products with strain difference most evident in SE proteins from HE meals. For emulsifying properties, the strain *A. oryzae* was shown to enhance or maintain the EAI of the isolates more effectively than *A. niger* , whereas ESI was largely unchanged upon fermentation, regardless of the strains; the AE-IP prepared isolates responded more positively to SSF than those by SE. Foaming properties at pH 3 and 5 were generally diminished by SSF while some increases at pH 7 were observed. *A. niger* was slightly preferred over *A. oryzae* to improve or minimize the decreases, while the CP products, control or fermented, tended to foam better than those processed by HE. In short, no overarching trend could be reported for the effect of meal fermentation on the resulting functionalities of extracted canola proteins, as most functional attributes were under the combined influence of pH, test strains, and oil and protein extraction techniques. However, the findings indicate that by modifying processing conditions, functionalities of canola meal protein products could be

tailored accordingly to suit potential applications, and SSF by *A. niger* and *A. oryzae* remains a promising practice to expand the utilization of canola proteins.

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AUTHOR CONTRIBUTIONS

Chenghao Li carried out the experiments, analyzed data, and wrote the first draft of the manuscript. Dai Shi and Andrea K. Stone performed additional data analysis and interpretation, and critical revision of the manuscript. Janitha P. D. Wanasundara and Michael T. Nickerson were responsible for the design of the study, supervision of the research, and revision of the manuscript drafts. Takuji Tanaka provided scientific consultation on the results and manuscript-writing.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Table 1 Protein content (%, d.b.) of protein products obtained by alkaline extraction-isoelectric precipitation (AE-IP) and salt extraction-dialysis (SE) prepared from fermented and unfermented (control) cold-pressed (CP) and hexane-extracted (HE) canola meals.

	CP	**CP**	**CP**	**CP**	**HE**	**HE**	**HE**
	Unfermented No defatting	Unfermented With defatting*	Fermented *A. niger*	Fermented *A. oryzae*	Unfermented Control	Fermented *A. niger*	Fermented *A. oryzae*
AE-IP	68.9 ± 0.3^{c}	81.6 ± 1.1^{b}	62.7 ± 0.2^{d}	57.9 ± 0.2^{f}	86.5 ± 0.2^{a}	56.5 ± 0.2^{g}	58.5 ± 0.2^{e}
SE	99.6 ± 1.0^{a}	96.5 ± 1.3^{d}	93.3 ± 2.2^{f}	99.0 ± 1.2^{b}	97.7 ± 3.3^{c}	95.9 ± 2.3^{e}	95.8 ± 4.2^{e}

Notes:

*Defatted CP control - canola protein products extracted from defatted CP meal using AE-IP or SE. Data was reported as mean ± one standard deviation across each extraction process (n=3). Significant difference exists between data with different letters in a row ($p<0.05$).

Table 2 Protein (nitrogen-based) solubility (%) of protein products obtained by alkaline extraction-isoelectric precipitation (AE-IP) and salt extraction-dialysis (SE) from fermented and unfermented (control) canola meals.

	AE-IP	**AE-IP**	**AE-IP**	**SE**	**SE**	**SE**
	pH 3	**pH 5**	**pH 7**	**pH 3**	**pH 5**	**pH 7**
CP control	88.9 ± 3.7^{a}	53.5 ± 2.6^{a}	27.7 ± 2.2^{d}	94.0 ± 0.4^{a}	37.7 ± 0.2^{a}	51.8 ± 1.1^{bc}
DCP control*	76.2 ± 3.6^{cd}	36.9 ± 2.1^{b}	37.8 ± 2.4^{c}	97.1 ± 0.5^{a}	37.3 ± 0.8^{a}	49.3 ± 0.8^{c}
CP *A. niger*	25.9 ± 1.9^{f}	5.9 ± 0.9^{d}	47.5 ± 2.9^{b}	21.3 ± 1.6^{e}	4.5 ± 0.2^{d}	37.2 ± 0.1^{e}
CP *A. oryzae*	72.7 ± 1.2^{d}	7.5 ± 0.5^{cd}	25.6 ± 0.9^{d}	83.4 ± 0.2^{b}	6.6 ± 0.4^{c}	90.7 ± 3.8^{a}
HE control	81.4 ± 0.9^{b}	2.0 ± 0.9^{e}	70.0 ± 1.4^{a}	94.6 ± 1.7^{a}	29.4 ± 2.3^{b}	43.2 ± 1.1^{d}
HE *A. niger*	10.1 ± 1.0^{g}	11.8 ± 1.0^{c}	70.0 ± 1.1^{a}	43.8 ± 0.9^{d}	5.9 ± 0.4^{c}	48.5 ± 1.6^{c}
HE *A. oryzae*	53.0 ± 1.1^{e}	5.8 ± 0.4^{d}	38.5 ± 3.2^{c}	49.8 ± 0.7^{c}	6.5 ± 0.3^{c}	55.6 ± 2.7^{b}

Notes:

*DCP control: canola protein control prepared from defatted unfermented CP meal using AE-IP or SE. Data was reported as mean ± one standard deviation (n=3). Significant difference exists between data with different letters across each pH ($p<0.05$).

Table 3 Water (WHC) and oil holding capacity (OHC) (g/g) of protein products obtained by alkaline extraction-isoelectric precipitation (AE-IP) (a) and salt extraction-dialysis (SE) (b) from fermented and unfermented (control) canola meals.

a) AE-IP

	CP	**CP**	**CP**	**CP**	**HE**	**HE**	**HE**
	Unfermented No defatting	Unfermented With defatting*	Fermented *A. niger*	Fermented *A. oryzae*	Unfermented Control	Fermented *A. niger*	Fermented *A. oryzae*
WHC	2.1 ± 0.0^{d}	2.7 ± 0.1^{c}	3.1 ± 0.1^{ab}	3.0 ± 0.0^{b}	1.8 ± 0.0^{e}	2.9 ± 0.1^{b}	3.2 ± 0.0^{a}
OHC	2.9 ± 0.0^{b}	2.4 ± 0.1^{c}	2.8 ± 0.1^{b}	2.8 ± 0.1^{b}	2.3 ± 0.1^{c}	3.1 ± 0.1^{ab}	3.3 ± 0.1^{a}

b) SE

	CP	**CP**	**CP**	**CP**	**HE**	**HE**	**HE**
	Unfermented No defatting	Unfermented With defatting*	Fermented *A. niger*	Fermented *A. oryzae*	Unfermented Control	Fermented *A. niger*	Fermented *A. oryzae*

	CP	CP	CP	CP	HE	HE	HE
WHC	1.1 ± 0.1^{c}	2.1 ± 0.1^{b}	1.3 ± 0.1^{c}	1.4 ± 0.1^{c}	1.3 ± 0.1^{c}	2.3 ± 0.1^{b}	2.7 ± 0.0^{a}
OHC	3.2 ± 0.1^{d}	3.9 ± 0.1^{c}	4.6 ± 0.1^{a}	4.7 ± 0.0^{a}	2.4 ± 0.1^{e}	4.5 ± 0.0^{b}	3.9 ± 0.1^{c}

Notes:

*Defatted CP control: canola protein product extracted from defatted cold-press meal using AE-IP or SE. Data was reported as mean ± one standard deviation (n=3). Significant difference exists between data with different letters in a row ($p<0.05$).

Table 4 Emulsifying activity index (EAI, m^2/g) and emulsion stability index (ESI, min) of protein products obtained by alkaline extraction-isoelectric precipitation (AE-IP) (a) and salt extraction-dialysis (SE) (b) of fermented and unfermented (control) canola meals.

a) AE-IP

	EAI (m^2/g)	EAI (m^2/g)	EAI (m^2/g)	ESI (min)	ESI (min)	ESI (min)
	pH 3	**pH 5**	**pH 7**	**pH 3**	**pH 5**	**pH 7**
CP control	11.4 ± 0.9^{cd}	4.1 ± 0.3^{a}	11.7 ± 1.5^{c}	4.5 ± 1.4^{ab}	1.4 ± 0.1^{ab}	1.2 ± 0.1^{b}
DCP control*	8.3 ± 0.6^{d}	5.0 ± 0.4^{a}	11.6 ± 1.7^{c}	1.5 ± 0.1^{ab}	1.6 ± 0.1^{ab}	1.4 ± 0.2^{b}
CP *A. niger*	13.8 ± 0.7^{c}	2.1 ± 0.3^{bc}	25.8 ± 1.9^{a}	4.8 ± 2.9^{a}	1.1 ± 0.1^{b}	1.4 ± 0.2^{b}
CP *A. oryzae*	21.1 ± 1.4^{a}	5.5 ± 0.1^{a}	27.6 ± 1.8^{a}	1.8 ± 0.3^{ab}	1.5 ± 0.1^{ab}	1.6 ± 0.1^{ab}
HE control	13.2 ± 1.6^{c}	3.0 ± 0.5^{b}	18.3 ± 1.6^{b}	3.1 ± 0.6^{ab}	2.2 ± 0.7^{a}	2.0 ± 0.3^{a}
HE *A. niger*	8.5 ± 0.8^{d}	1.3 ± 0.1^{c}	18.0 ± 1.9^{b}	1.6 ± 0.4^{ab}	1.3 ± 0.2^{b}	1.3 ± 0.2^{b}
HE *A. oryzae*	17.6 ± 1.9^{b}	4.8 ± 0.6^{a}	25.5 ± 2.5^{a}	1.2 ± 0.1^{b}	1.3 ± 0.1^{b}	1.2 ± 0.2^{b}

b) SE

	EAI (m^2/g)	EAI (m^2/g)	EAI (m^2/g)	ESI (min)	ESI (min)	ESI (min)
	pH 3	**pH 5**	**pH 7**	**pH 3**	**pH 5**	**pH 7**
CP control	5.6 ± 1.1^{c}	5.8 ± 0.5^{bc}	12.9 ± 0.5^{b}	1.7 ± 0.2^{b}	1.2 ± 0.1^{d}	3.4 ± 0.3^{a}
DCP control*	11.0 ± 0.4^{a}	9.4 ± 0.2^{a}	14.5 ± 0.4^{a}	1.2 ± 0.0^{b}	1.2 ± 0.0^{d}	1.6 ± 0.1^{b}
CP *A. niger*	8.5 ± 0.5^{b}	1.8 ± 0.2^{d}	12.2 ± 0.7^{b}	4.2 ± 1.0^{a}	2.8 ± 0.4^{a}	1.8 ± 0.2^{b}
CP *A. oryzae*	9.7 ± 0.4^{ab}	4.6 ± 0.5^{c}	14.9 ± 0.3^{a}	1.2 ± 0.0^{b}	1.2 ± 0.1^{d}	1.7 ± 0.1^{b}
HE control	5.9 ± 0.5^{c}	6.8 ± 0.4^{b}	13.9 ± 0.7^{ab}	1.6 ± 0.2^{b}	2.4 ± 0.3^{ab}	4.3 ± 1.3^{a}
HE *A. niger*	8.5 ± 0.5^{b}	4.1 ± 0.6^{cd}	11.4 ± 0.4^{b}	1.3 ± 0.1^{b}	1.9 ± 0.1^{bc}	1.5 ± 0.1^{b}
HE *A. oryzae*	11.3 ± 0.9^{a}	3.0 ± 0.3^{d}	7.6 ± 0.8^{c}	1.2 ± 0.1^{b}	1.4 ± 0.2^{cd}	1.2 ± 0.0^{b}

Notes:

*DCP control: canola protein product extracted from defatted cold-press meal using AE-IP or SE. Data was reported as mean ± one standard deviation (n=3). Significant difference exists between data with different letters within each pH ($p<0.05$).

Table 5 Foaming capacity (%) and foam stability (%) of alkaline extraction-isoelectric precipitation (AE-IP) (a) and salt-extraction dialysis (SE) (b) canola concentrates and isolates from different fermented and control canola meals.

a) AE-IP

	Foaming capacity (%)	**Foaming capacity (%)**	**Foaming capacity (%)**	**Foam stability (%)**
	pH 3	**pH 5**	**pH 7**	**pH 3**
CP control	224.4 ± 20.4^{c}	241.1 ± 16.4^{a}	165.0 ± 5.2^{bc}	93.0 ± 2.3^{a}
DCP control*	306.7 ± 20.0^{a}	131.1 ± 7.7^{b}	243.8 ± 8.1^{a}	87.0 ± 1.9^{b}
CP *A. niger*	NF	NF	230.7 ± 6.8^{a}	NF
CP *A. oryzae*	228.9 ± 3.8^{c}	NF	180.0 ± 3.3^{b}	33.0 ± 1.1^{c}
HE control	244.4 ± 10.2^{bc}	NF	154.4 ± 6.9^{cd}	17.3 ± 1.6^{d}
HE *A. niger*	NF	NF	244.2 ± 12.4^{a}	NF
HE *A. oryzae*	NF	NF	136.7 ± 8.8^{d}	NF

b) SE

	Foaming capacity (%)	**Foaming capacity (%)**	**Foaming capacity (%)**	**Foam stability (%)**
	pH 3	**pH 5**	**pH 7**	**pH 3**
CP control	322.2 ± 7.7^{a}	211.1 ± 20.4^{b}	480.0 ± 11.5^{a}	87.2 ± 1.5^{a}
DCP control*	246.7 ± 17.6^{b}	222.2 ± 7.7^{a}	464.4 ± 20.4^{b}	84.2 ± 0.4^{a}
CP *A. niger*	196.5 ± 6.6^{c}	NF	191.1 ± 13.9^{d}	54.6 ± 0.1^{c}
CP *A. oryzae*	175.6 ± 10.2^{c}	NF	153.3 ± 8.8^{e}	51.0 ± 1.1^{c}
HE control	266.7 ± 6.7^{b}	NF	145.6 ± 10.2^{e}	53.4 ± 4.9^{c}
HE *A. niger*	195.6 ± 13.9^{c}	NF	306.7 ± 17.6^{c}	51.8 ± 2.0^{c}
HE *A. oryzae*	135.6 ± 10.2^{d}	NF	155.6 ± 3.8^{de}	51.3 ± 1.4^{d}

Notes:

*DCP control: canola protein product extracted from defatted cold-press meal using AE-IP or SE. NF: no foam, or foam disappeared within 5 min. Data was reported as mean ± one standard deviation (n=3). Significant difference exists between data with different letters across each pH ($p<0.05$).