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ORIGINAL PAPER

Comparison of Canola Meals Obtained with Conventional Methods and Supercritical CO_2 with and without Ethanol

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Abstract Canola meal is a potentially valuable protein source. Canola meals extracted with supercritical CO_2 (SC-CO_2) were compared to pressed meal and meals extracted with hexane. With regard to the chemical composition, the glucosinolate, phenolic acid, tannin and phosphorus contents were determined in addition to proximate analysis. As for functionality, color, nitrogen solubility index (NSI), water and fat absorption, emulsifying capacity and stability, and overrun were determined. Both hexane- and SC-CO_2-extracted meals had a higher protein content than the pressed meal. The SC-CO_2-extracted meal had lower glucosinolate and higher phosphorus contents than hexane-extracted meal. The phenolic acid contents of hexane- and SC-CO_2-extracted meals were similar, but were higher than those of meals extracted with SC-CO_2 + ethanol. The color values of SC-CO_2- and hexane-extracted meals were similar and both were brighter than commercial meals (pressed and toasted). The NSI levels of SC-CO_2- and hexane-extracted meals were similar, but three times that of the commercial meal. Both hexane- and SC-CO_2-extracted meals had high water holding capacity, oil absorption, emulsifying capacity, emulsion stability and overrun. Canola meal extracted with SC-CO_2 was similar to hexane-extracted meal in terms of both chemical composition and functionality, but was superior to commercial meals.

Keywords Canola meal · Extraction · Functionality · Meal composition · Supercritical CO_2

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Abbreviations

CM	Commercial meal
CEEM	Supercritical CO_2 + ethanol extracted meal
EC	Emulsifying capacity
ES	Emulsion stability
FA	Fat absorption
HDM	Hexane-defatted meal prepared in the laboratory
NSI	Nitrogen solubility index
ODM	Oil-depleted meal
PM	Pressed meal
PDM	Partially-defatted meal
SFE	Supercritical fluid extraction
SC-CO_2	Supercritical CO_2
WA	Water absorption
WHC	Water hydration capacity

Introduction

Canola is the major oilseed crop grown in Canada with an annual production in excess of 9 million tonnes [1]. Although it is mainly utilized for its oil, the residual meal is a valuable source of protein with a well-balanced amino acid composition and it is used as animal feed [2]. But, the presence of hulls and undesired seed components such as glucosinolates (toxins) and phenolics, including tannins (dark color, astringent and bitter taste), precludes the use of canola meal in food products as a protein supplement [3–7]. Furthermore, the residual meal has to be toasted after oil extraction at an elevated temperature (up to 130 °C) to remove the solvent since the oilseed industry traditionally uses hexane. This toasting process severely denatures the proteins, resulting in the poor functionalities of the commercial meal [1].

In order to improve the quality of canola meal in terms of both composition and functionality, numerous processes have been studied, such as dehulling [8], heating [9], solvent treatment [10, 11], and protein isolation [12–16]. The products of these processes could be meals, protein concentrates or isolates, which are typically rich in protein, low in toxins, and have desirable functionalities for various food applications. However, so far none of these processes has been commercialized due to either poor quality or low yield.

Supercritical fluid extraction (SFE) has been explored extensively in the past two decades for oilseed processing. Since hexane poses safety, health and environmental hazards, its replacement has long been sought by the oil industry. As a viable alternative to organic solvents, supercritical carbon dioxide (SC-CO_2) has been investigated specifically for canola oil extraction [17–19]. However, investigations focusing on the evaluation of residual meal following SC-CO_2 extraction have been limited to sunflower meal [20] and soybean flour [21]. Among many advantages of SFE of canola is the elimination of the toasting step so that the proteins remain intact. Thus, the meal functionality could potentially be preserved. However, the quality of canola meal following SC-CO_2 extraction, in terms of chemical composition and functionality, has not been evaluated. In addition, as SC-CO_2 is selective for neutral lipids, it leaves polar compounds like phospholipids in the meal. To remove these compounds, a polar co-solvent, such as ethanol, can be added to SC-CO_2 [22]. While the addition of a polar co-solvent may improve the meal composition, its effect on the functionality was not known. Therefore, the objective of this study was to compare the chemical composition and functionality of canola meals defatted by SC-CO_2 extraction without and with the addition of ethanol as a co-solvent to pressed meal and conventional meals extracted with hexane in the laboratory and industrial setting.

Materials and Methods

Materials

Pressed meal (PM) and commercial meal (CM, prepared by hexane extraction followed by toasting) were kindly provided by a major canola processor and used as is. Canola flakes used to prepare all other meals were also supplied by the same processor.

Carbon dioxide and nitrogen were 99.95 (w/w%) purity, bone dry and obtained from Praxair Canada Inc. (Mississauga, ON). Acetone, methanol, ethyl ether, ethyl acetate, methylene chloride, sulfuric acid, ammonium hydroxide, sodium hydroxide dibasic and sodium phosphate were all from Fisher Scientific (Fair Lawn, NJ). Anthrone, sodium tungstate dehydrate, phosphomolybdic acid, orthophosphoric acid, hydrochloric acid, vanillin, sinapic acid, catechin hydrate, glucose, zinc acetate, ammonium molybdate, ammonium meta-vanadate, trichloroacetic acid, sodium carbonate decahydrate were all from Sigma Chemical Co. (St. Louis, MO). Potassium phosphate monobasic was from ICN Biomedicals, Inc. (Aurora, OH). Citric acid was from Allen & Hanburys (Toronto, ON).

Hexane Extraction

The hexane-defatted meal (HDM) was prepared in the laboratory by refluxing hexane over canola flakes in a Soxhlet apparatus for 24 h. The solvent was removed with air in a fume hood for several days until completely dry.

Supercritical CO_2 Extraction

Two SC-CO_2 extracted canola meals, partially-defatted meal (PDM) and oil-depleted meal (ODM), were prepared at a pilot plant (Norac Technologies Inc., Newlyweds Foods Co., Edmonton, AB) at 51 °C/30 MPa and 56 °C/30 MPa, respectively. The oil depleted meal was further processed with SC-CO_2 plus ethanol as a co-solvent using a laboratory scale SFE unit (Newport Scientific Inc., Jessup, MD) as described previously [22]. The extraction cell was loaded with approximately 10 g meal for each run. Extractions were performed at 70 °C and 40 MPa for 6 h. The flow rate of SC-CO_2 was maintained at 1 L/min (measured at ambient conditions), into which 20% (w/w) of ethanol was introduced continuously. The extract fractions were collected each hour in glass tubes attached to the depressurization valve and held in a circulating bath at −15 °C. A total of six extract fractions were obtained in each run and eight runs were conducted under identical conditions to generate sufficient blended meal for composition and functionality analysis. Ethanol was allowed to evaporate under a fume hood from the supercritical CO_2 + ethanol extracted meal (CEEM).

Chemical Composition

For proximate analyses, moisture was determined gravimetrically by drying samples in an oven (Model 655G, Fisher Scientific, Fair lawn, NJ) at 105 °C overnight. Protein ($N \times 6.25$) was determined using a TruSpec CN Carbon/Nitrogen Determinator (Leco Corporation, St. Joseph, MI). Fat content was analyzed using a Goldfisch Extraction Unit (Labconco, Kansas City, MI) with petroleum ether for 6 h. Ash content was determined gravimetrically by incinerating the samples in a muffle furnace (Model F-A1730, Thermolyne Corp., Dubuque, IA) at 550 °C overnight.

The glucosinolate content of canola meals was determined according to Wetter and Young [23] with a detection limit of 2.2 μmol/g sample. The total phenolic acid content of meals and SC-CO_2 + ethanol extracts was determined using the method of Xu and Diosady [6] and was expressed as mg of sinapic acid equivalents per 100 g sample. Condensed tannins were determined according to Shahidi and Naczk [5] for the meals and SC-CO_2 + ethanol extracts. The total sugar content of the extracts was determined by the method of Trevelyan and Harrison [24]. The phosphorus content of the meals was determined according to the AOAC Official Method 965.17 [25].

Functional Properties

The color of the canola meals was measured using a Labscan XE spectrocolorimeter (Model LSXE/UNI, Hunterlab Associates Laboratory, Inc., Reston, VA). The 'L', '*a*' and '*b*' values, which represent brightness/darkness, redness/greenness, and yellowness/blueness, respectively, were recorded.

For pH measurement, 10% dispersion (w/v) of each sample in de-ionized water was prepared and measured using a pH meter (220 pH meter, Corning Science Products, Corning, NY).

Nitrogen solubility index (NSI) was determined according to AACC Method 46–23 [26]. The effect of pH on NSI was examined for most meals using NaOH or HCl solution to adjust the pH to the targeted values from 2 to 12.

Water absorption (WA) was determined according to Naczk et al. [3]. A 2 g sample was dispersed in 16 mL distilled water in a 50 mL centrifuge tube. The contents were mixed for 30 s every 10 min using a glass rod and centrifuged after seven mixings at 2,000 × *g* for 15 min. The supernatant was decanted. The tube was inverted and drained for 15 min and weighed. Water absorption was expressed as the percentage of sample weight. Based on WA, water hydration capacity (WHC) was measured using AACC Method 88-04 [26].

Fat absorption (FA) was determined according to Sosulski et al. [27]. A 2 g sample was dispersed in 12 mL of canola oil purchased from a local grocery store in a 50 mL centrifuge tube. The contents were stirred for 30 s every 5 min, and after 30 min, the tubes were centrifuged at 1,600 × *g* for 25 min. Free oil was decanted similar to the water in the water absorption tests, and percentage of absorbed oil was determined by weight difference.

In the determination of emulsifying capacity (EC) according to Naczk et al. [3], a 3.5 g sample was homogenized in 50 mL water for 30 s in a plastic bottle using a Polytron PT-2000 homogenizer (Kinematica, Inc., Newark, NJ) at 10,000 rpm. Canola oil (25 mL) was then added and homogenized for 30 s. Another 25 mL of canola oil was added and the mixture was homogenized again for 90 s. The emulsion was then divided evenly into two 50 mL centrifuge tubes and centrifuged at 1,100 × *g* for 5 min. Emulsifying capacity was calculated by dividing the volume of the emulsified layer by the volume of emulsion before centrifugation and multiplying by 100. The emulsion prepared as above was heated at 85 °C for 15 min, cooled and centrifuged. Emulsion stability (ES) was expressed as the percentage of emulsion remaining after heating.

For the determination of overrun (whippability), 50 mL of 3% dispersion of meal in water was homogenized using a Polytron homogenizer at 10,000 rpm for 6 min, which was then immediately transferred into a 250-mL graduated cylinder with the foam volume noted. Overrun was expressed as the percentage increase in volume. The foam volume was measured again after standing for 20, 40, 60 and 120 min to demonstrate foam stability [28].

Statistical Analysis

All chemical and functionality tests were conducted in quadruplets unless otherwise indicated. Analysis of variance was performed at $\alpha = 0.05$ level using the SAS Statistical Software, Version 8 [29]. Tukey's test for multiple comparison of the means as well as regression analysis were also performed with the same software.

Results and Discussion

Chemical Composition

Table 1 shows proximate analysis of pressed canola meal and meals extracted with different solvents (hexane vs SC-CO_2). The proximate composition of the canola meals extracted with hexane (CM and HDM) investigated in this study were in good agreement with the values reported in the Canola Meal Feed Industry Guide [7], with 10% moisture, 35% crude protein, 3.5% fat and 6.1% ash. The CM, HDM and ODM had a significantly ($p \leq 0.05$) lower residual fat content as compared to that of PM and PDM. As a result of this greater fat removal, the protein and ash contents of these meals were higher ($p \leq 0.05$) than those of PM and PDM. As expected, the protein content of the CEEM was the highest ($p \leq 0.05$) but similar to HDM while the protein contents of hexane- and SC-CO_2-extracted meals were higher ($p \leq 0.05$) than that of the pressed meal. The meals obtained by SC-CO_2 extraction (ODM and PDM) contained less ($p \leq 0.05$) moisture than PM and hexane-extracted meals as the water is generally co-extracted with the oil under supercritical conditions [19].

Compared with the amount of ethanol introduced into the system as a co-solvent (at a CO_2 flow rate of 1 L/min,

Table 1 Proximate analyses of canola meals[1]

Sample	Moisture (%)	Ash (%)	Protein (%)	Fat (%)
Pressed meal	6.74 ± 0.05[c]	5.04 ± 0.02[d]	30.5 ± 0.4[e]	26.36 ± 0.05[a]
Hexane extracted				
CM	11.85 ± 0.04[a]	6.58 ± 0.03[b]	37.30 ± 0.03[c]	2.32 ± 0.01[d]
HDM	8.0 ± 0.1[b]	6.51 ± 0.02[b]	40 ± 1[ab]	1.09 ± 0.08[e]
SC-CO_2 extracted				
PDM	3.60 ± 0.08[e]	5.75 ± 0.03[c]	34.8 ± 0.2[d]	17.8 ± 0.1[b]
ODM	3.99 ± 0.07[d]	6.8 ± 0.1[a]	40.0 ± 0.8[b]	2.65 ± 0.04[c]
CEEM	ND	ND	41.8 ± 0.7[a]	ND

ND not determined, *CM* commercial meal, *HDM* hexane-defatted meal prepared in the lab, *PDM* partially-defatted meal, *ODM* oil-depleted meal, *CEEM* supercritical CO_2 + ethanol extracted meal

[1] All results are reported on "as is" basis and as mean ± standard deviation of four replicates

[a–e] Means followed by different letters in the same column are significantly different ($p \leq 0.05$)

ca. 160 mL ethanol was pumped in throughout the 6 h extraction), the amount of crude oil in 10 g of ODM is extremely small (approximately 0.26 g). Therefore, the amount of sample collected after the SC-CO_2 + ethanol extraction was mainly the ethanol added to the system as co-solvent plus a very small amount of oil extracted from the starting material (ODM).

The HDM was higher in glucosinolates ($p \leq 0.05$) than the ODM, indicating that SC-CO_2 extraction removed more glucosinolates (Table 2). However, ethanol addition to SC-CO_2 did not result in further glucosinolate removal, and the CEEM had a higher glucosinolate content than the ODM, which was probably due to the removal of other meal components, such as phenolics and phospholipids. Ethanol removed tannins that might have been complexed with glucosinolates. The glucosinolate contents of the canola meals extracted with hexane (7.3 μmol/g) and SC-CO_2 (5.6–6.8 μmol/g) obtained in this study were similar to those of the *Bronowski* rapeseed meal extracted with methylene chloride (6.9–7.96 μmol/g) at room temperature by Wetter and Young [23].

Table 2 Chemical analyses of canola meals[1]

Sample	Glucosinolates (μmol/g)	Phenolic acids (mg/100 g)	Tannins (mg/100 g)	Phosphorus (mg/g)
Hexane extracted				
HDM	7.3 ± 0.2[a]	1,609 ± 33[a]	121 ± 47[ab]	11.49 ± 0.01[c]
SC-CO_2 extracted				
ODM	5.6 ± 0.5[b]	1,693 ± 45[a]	142 ± 36[a]	12.2 ± 0.2[a]
CEEM	6.8 ± 0.3[a]	1,050 ± 89[b]	92 ± 7[b]	11.84 ± 0.04[b]

HDM hexane-defatted meal prepared in the lab, *ODM* oil-depleted meal, *CEEM* supercritical CO_2 + ethanol extracted meal

[1] All results are expressed as mean ± standard deviation of four replicates

[a–c] Means followed by different letters in the same column are significantly different ($p \leq 0.05$)

The total phenolic acid contents of the three meals (Table 2) were within the range (751 to 2,070 mg sinapic acid equivalents per 100 g sample) reported for canola and rapeseed meals by Naczk et al. [30]. The phenolic acid contents of HDM and ODM were similar ($p > 0.05$), while that for the CEEM was significantly ($p \leq 0.05$) lower, indicating that SC-CO_2 + ethanol extraction removed 37.9% of the phenolic acids from ODM. The phenolic acids removed by SC-CO_2 + ethanol from the starting material were quantified in each fraction throughout the 6-h extraction. As shown in Fig. 1, more phenolic acids were obtained at the later stages of the extraction. The total amount of phenolic acids extracted in 6 h was 11.6 mg sinapic acid equivalents per g extract. All the meals examined in this study (Table 2) had substantially lower tannin contents compared to the values (682–772 mg/100 g of oil free canola meal) reported by Shahidi and Naczk [5]. This may be due to the different varieties of canola studied. The amount of soluble tannins in the SC-CO_2 + ethanol extract was 246.7 mg/g extract, which was substantially higher than the amount remaining in the meals. Although tannins in canola meal tended to form complexes with protein and carbohydrates [31], ethanol was able to break these complexes, thus releasing some tannins into the extract.

The phosphorus contents of the canola meals (Table 2) extracted with hexane and SC-CO_2 (ODM and CEEM) in this study (11.5–12.2 mg/g canola meal) were in the range (11.3–14.3 mg phosphorus per g canola/rapeseed meal extracted with hexane) reported by Naczk et al. [32]. The small, but significant differences ($p \leq 0.05$) in the phosphorus contents determined analytically were likely the result of small differences in the phospholipid contents of meals. As well, since SC-CO_2 extraction did not remove as

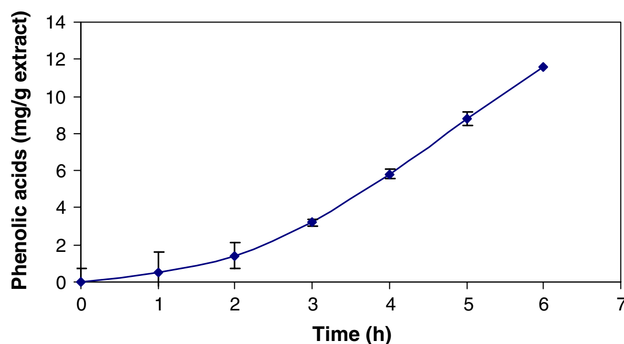

Fig. 1 Extraction curve of phenolic acids from canola meal using SC-CO_2 + ethanol at 70 °C and 40 MPa

much phospholipids as the hexane extraction or SC-CO_2 + ethanol extraction, the ODM had the highest phosphorus content. This finding was in agreement with Fattori et al. [33], who showed that no phospholipids were detected in the oil extracted at 55 °C and 36 MPa from cooked canola seeds. However, it has been demonstrated that the addition of polar ethanol as a co-solvent enhances phospholipid extraction with SC-CO_2 [18, 22]. Indeed, the phosphorus content of the CEEM was lower ($p \leq 0.05$) than that of the starting material, ODM.

The total sugars in the ODM and CEEM were 14.6 and 14.3%, respectively. Apparently, only a small amount of sugars was removed from the starting material, ODM. The total sugar content in the extract was 7.04 mg/g, which was equivalent to 0.92 mg soluble sugar per g meal (0.092%). The concentration of sugars (8%) plus soluble non-starch polysaccharides (1.4%) in canola meal was reported to be 9.4% of canola meal [7]. The protein concentrates of rapeseed were processed with different solvents such as ethanol, methanol and isopropanol [34]. The soluble sugars in the rapeseed protein concentrate prepared by ethanol and methanol were 0.25 and 0.65%, respectively, indicating that ethanol was a good solvent to remove soluble sugars [34]. However, as only 20% of ethanol was added as a co-solvent into SC-CO_2 in this study, it may not be sufficient to remove all the sugars in the meal. Besides, the ethanol introduced into SC-CO_2 was pure ethanol, whereas aqueous ethanol (60–80%) has been used in other studies [34, 35] for sugar removal and the use of such a mixture as co-solvent would require further investigation.

Functional Properties

The appearances of the ODM and PDM were similar to that of HDM, featuring a light yellow color with black speckles from hulls. The PM had a dark yellowish green color, apparently caused by the intensive heat generated during pressing. The CM had a dark brown color due to the extensive toasting process after solvent extraction. The color parameters presented in Table 3 showed differences between these different meals. The HDM, PDM, ODM and CEEM were substantially brighter and more yellow than the CM and PM as the '*L*' and '*b*' values of these four meals were significantly ($p \leq 0.05$) higher than those of CM and PM. Furthermore, the HDM, ODM, PDM and CEEM had a greenish color as the '*a*' values were negative while the CM and PM were reddish with positive '*a*' values. The CM was brighter, more red and yellow compared to the PM as it had higher '*L*', '*a*' and '*b*' values. Among the HDM, ODM, PDM and CEEM, the ODM and PDM seemed to be greener. This might be due to the fact that SC-CO_2 extraction is more favorable for removing the red color pigment than the yellow color pigment as reported earlier [36].

Table 4 presents the different functional properties determined for the canola meals evaluated. The pH values of 10% aqueous dispersions of all meals were between 5.7 and 6.2, which were similar to that of the rapeseed meal (pH 5.9) extracted with diethyl ether [27] and those of canola meals (pH 5.87–6.21) extracted with hexane [3]. However, the pH of the canola/rapeseed meals extracted with methanol/ammonia/water-hexane were all higher than 7 [3, 15].

Nitrogen solubility index is usually the first functional test performed for a protein product as it provides direct information on the effects of processing. When a protein is denatured by certain processing conditions, like heating, its solubility tends to be reduced. Of all the meals examined in this study, the NSI of HDM (40.8%) and ODM (33.0%) was almost twice that of PM (22.2%) and three times that of CM (11.5%). The low NSI of PM and CM was expected

Table 3 Color of different canola meals [1]

Sample	'*L*'	'*a*'	'*b*'
Pressed meal	22.4 ± 0.6^{d}	2.18 ± 0.04^{b}	9.0 ± 0.3^{d}
Hexane extracted			
CM	33.36 ± 0.01^{c}	3.86 ± 0.02^{a}	12.93 ± 0.06^{c}
HDM	70 ± 1^{b}	−2.04 ± 0.04^{c}	17.2 ± 0.7^{b}
SC-CO_2 extracted			
PDM	71.2 ± 0.6^{b}	−4.69 ± 0.08^{d}	20 ± 2^{a}
ODM	73.7 ± 0.1^{a}	−5.35 ± 0.02^{e}	18.83 ± 0.05^{ab}
CEEM	70.6 ± 0.8^{b}	−2.13 ± 0.08^{c}	18.0 ± 0.3^{ab}

'*L*' = brightness/darkness, '*a*' = redness/greenness, '*b*' = yellowness/blueness

CM commercial meal, *HDM* hexane-defatted meal prepared in the lab, *PDM* partially-defatted meal, *ODM* oil-depleted meal, *CEEM* supercritical CO_2 + ethanol extracted meal

[1] All results are expressed as mean ± standard deviation for four replicates

[a–e] Means followed by different letters in the same column are significantly different ($p \leq 0.05$)

Table 4 Functional properties of canola meals[1]

Sample	pH	Nitrogen solubility index (%)	Water absorption (%)	Water hydration capacity (mL water/g)	Fat absorption (%)	Emulsifying capacity (%)	Emulsion stability (%)	Overrun (%)
Pressed meal	6.09 ± 0.05^{b}	22 ± 1^{d}	214 ± 1^{c}	2.6	172.6 ± 0.7^{e}	59 ± 2^{b}	19 ± 1^{d}	166 ± 5^{d}
Hexane extracted								
CM	5.71 ± 0.02^{d}	12 ± 2^{e}	247 ± 4^{b}	2.0	212 ± 6^{d}	8 ± 1^{d}	71.40 ± 0.01^{c}	117 ± 2^{e}
HDM	6.10 ± 0.06^{b}	40.8 ± 0.8^{a}	247 ± 2^{b}	4.0	330.2 ± 0.7^{a}	64 ± 1^{a}	103 ± 2^{a}	253 ± 21^{b}
SC-CO_2 extracted								
PDM	6.21 ± 0.03^{a}	38 ± 3^{a}	206 ± 3^{d}	2.9	262 ± 5^{c}	65 ± 3^{a}	90 ± 8^{b}	275 ± 4^{c}
ODM	5.90 ± 0.03^{c}	33 ± 1^{b}	267 ± 3^{a}	4.9	321 ± 3^{b}	62.1 ± 0.9^{ab}	105 ± 1^{a}	298 ± 19^{a}
CEEM	ND	31 ± 2^{c}	ND	ND	ND	49 ± 1^{c}	94 ± 2^{b}	ND

CM commercial meal, *HDM* hexane-defatted meal prepared in the lab, *PDM* partially-defatted meal, *ODM* oil-depleted meal and *CEEM* supercritical CO_2 + ethanol extracted meal

[1] All results are expressed as mean ± standard deviation of four replicates except for WHC

[a–e] Means followed by different letters in the same column are significantly different ($p \leq 0.05$)

since the heat generated during pressing and the heat applied during toasting likely partially denatured the proteins and thus resulted in lower protein solubility. The PDM and HDM had a similar NSI while the NSI of the ODM was lower, probably due to the use of 56 °C for SC-CO_2 extraction. The NSI of CEEM was even lower than that of ODM, likely a result of 6 h SC-CO_2 + ethanol extraction at 70 °C, which could further denature the meal proteins. In addition, the NSI of the SC-CO_2-extracted meals was similar to the NSI of the ethyl ether extracted rapeseed meal (33.6%) reported by Sosulski et al. [27]. The NSI of the CM (11.5%) was very close to that of the commercial canola meal (13.7%) reported by Naczk et al. [3]. However, the NSI of the hexane-extracted meals of different canola varieties (17.5–27.6%) reported by Naczk et al. [3] was lower than those of the hexane-extracted meals in this study (11.5–40.8%) at pH 6. The NSI values of the different canola/rapeseed meals extracted with methanol/ammonia/water-hexane at pH 7.3 were even lower at 5.3–8.4 and 11.4–14.1% as reported by Naczk et al. [3] and Xu and Diosady [15], respectively.

The effect of pH on NSI of canola meals was also investigated. All solubility curves in Fig. 2 showed a single minimum at pH 4. This finding was in agreement with those of Sosulski et al. [27] for rapeseed meal, indicating a single minimum at pH 4. However, this result contradicted those of Gillberg and Tornell [12] and Naczk et al. [3], who reported that there were two minima in the solubility curves at pH 4.0 and 8.0 for hexane-extracted rapeseed meal, and at pH 4.8 and 7.0 for canola meal, respectively. Such results were likely due to the differences in the specific protein components with varied isoelectric points present in the different canola varieties used. With a pH increase above 4, the NSI of all canola meals in this study increased from pH 6 all the way up to pH 12, with the sharpest increase occurring between pH 10 and 12. Similar sharp increases were observed by Naczk et al. [3] and Gillberg and Tornell [12], except for another sharp increase due to their two minima points, followed by a leveling off at pH 10. Compared to all the other meals, the HDM in this

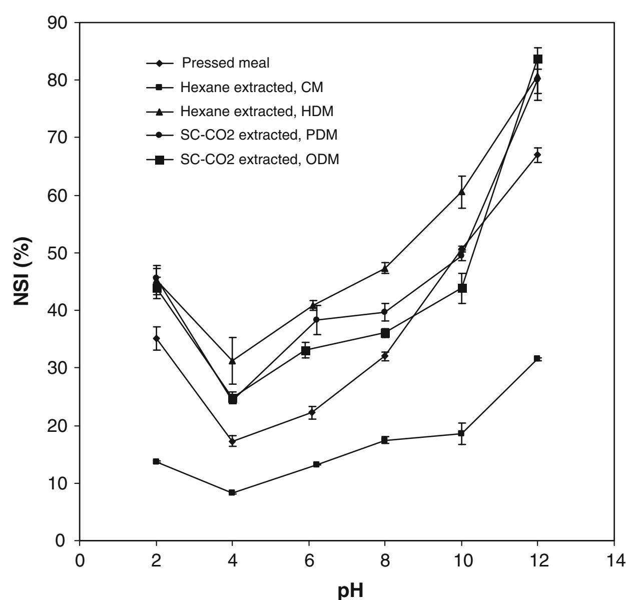

Fig. 2 Effect of pH on NSI of different canola meals (*CM* commercial meal, *HDM* hexane-defatted meal prepared in the lab, *PDM* partially-defatted meal and *ODM* oil-depleted meal)

study had a higher or similar NSI while the CM had the lowest NSI throughout the entire pH range. The NSI of the PM was the second lowest at all pH levels except at pH 10. Again, the low NSI of PM and CM was caused by the partial protein denaturation during commercial processing. The NSI of the CM was much lower than that of the PM, indicating that toasting caused more severe denaturation than pressing. The solubility curves of ODM and PDM obtained by SC-CO_2 extraction were in the middle and exhibited a similar shape, with the ODM having a lower NSI at pH 6, 8 and 10. Compared to PDM, the lower NSI of the ODM might be caused by the longer extraction time.

The water binding properties of a protein product are measured by its interactions with water. Based on the solubility of the protein, these properties can be examined as water absorption for materials with solubilities below 25% or as water hydration capacity for more soluble samples [15]. Unlike the water absorption data in Table 4, which were all means of four replicates, each of the WHC data was only the midpoint of a range as dictated by the method used; hence, no standard deviation was reported. Since the measurement of WHC was based on the results of water absorption, a linear correlation could be found between these properties of canola meals:

$$\mathrm{WHC} = 0.0358 \times [\mathrm{WA}] - 4.765 \quad (r^2 = 0.94) \tag{1}$$

This correlation could not be applied to the CM, which had a very low WHC and relatively high water absorption due to protein denaturation occurring during heat treatment. It can be observed from the data that the presence of fat (Table 1) lowered both water absorption and WHC of the meal (Table 4), possibly due to the immiscibility of water and oil. It was also observed that the fat content affected WHC more considerably than WA. Water absorption was reduced by about 20% while WHC was reduced by more than 40% between the ODM and PDM. This was probably due to the method of analysis. During WHC determination, only enough water was added to saturate the sample without creating a liquid phase; thus, the result was not affected by the solubility of the material. Considering that all meals were soluble to various extents according to the NSI test, WHC apparently better represented the real situation, especially for the CM. Although the water absorption values of the HDM and ODM obtained in this study were somewhat lower than those (327.2–400 and 273–287%, respectively) reported by Naczk et al. [3] for different varieties of hexane-extracted canola meal and Xu and Diosady [15] for protein isolate of Chinese rapeseed, the WHC values of these two meals were substantially higher than theirs (2.89–3.78 and 2.48–2.61, respectively) [3, 15].

Similar to water absorption, fat absorption was also affected by the fat content of the meal. Understandably, a higher fat content in the meal made it less capable of absorbing extra fat. The fat absorption was correlated with the fat content by the following regression equation:

$$\mathrm{FA} = -5.79 \times [\text{fat content}] + 340 \quad (r^2 = 0.95) \tag{2}$$

This correlation cannot be applied to the CM. Although the fat content of the CM was low, it did not have a comparable fat absorption to the ODM and HDM. Both the ODM and HDM were able to absorb oil at a level more than three times their dry weight, and these fat absorption values were higher than those previously reported as 235% for diethyl ether defatted rapeseed meal [7], 188–219% for hexane defatted canola meal [3], and 194–233% for methanol/ammonia/water-hexane defatted rapeseed/canola meal [15].

Emulsification properties of a protein are measured by its simultaneous interactions with both water and fat, and play an important role in determining its food applications. In this study, these properties were examined as emulsifying capacity and emulsion stability. The emulsifying capacity values of HDM, ODM and PDM (Table 4), ranging from 62.1 to 64.9% were comparable to those reported for hexane-extracted canola meals (59.7–63.7%) [3]. The ODM also exhibited an emulsion stability as good as that of HDM, indicating that canola meal defatted by SC-CO_2 extraction has great potential as an excellent emulsifier in food applications. The poor emulsion stability of PM and CM was likely, again, a result of the heat denaturation during processing. The PM had a better emulsifying capacity than CM, while the CM had a better emulsion stability than PM, which may be due to the different extent of denaturation of proteins caused by the different processes.

Foam properties are another important group of functionalities that have a significant impact on the food use of a protein product. They are measured in terms of overrun (whippability) and foam stability. Since overrun was closely related to the protein solubility, the overrun was correlated with the NSI by the following regression equation:

$$\mathrm{Overrun} = 5.49 \times [\mathrm{NSI}] + 61.75 \quad (r^2 = 0.747) \tag{3}$$

The low r^2 value for Eq. 3 is mainly due to the different treatments used on the meals (HDM, PDM and ODM). In addition, the residual fat content of PDM (Table 1) impaired the foaming properties of proteins. The ODM, PDM and HDM (Table 4) showed a higher overrun (253–298%) compared to that of the hexane-extracted canola meals (115–123%) used by Naczk et al. [3], which had a lower NSI of 17.5–27.6% at pH 6. The overrun values of these three meals in this study were lower than that of ethyl ether extracted rapeseed meal (362%) obtained by Sosulski et al. [27]. Figure 3 shows foam stability results for pressed and hexane- and SC-CO_2-extracted canola meals over 120 min. A similar time was used for ethyl ether extracted

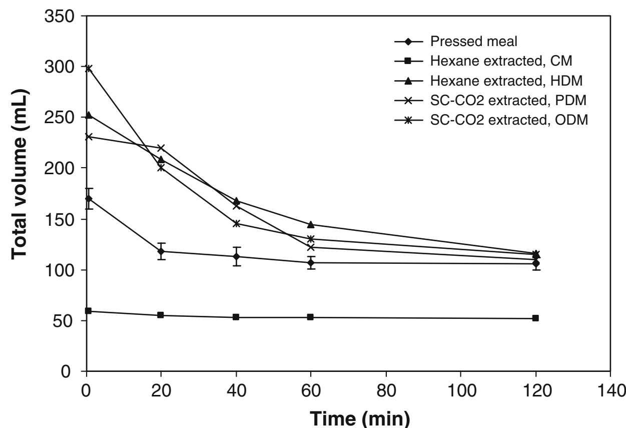

Fig. 3 Foam stability of canola meals (*CM* commercial meal, *HDM* hexane-defatted meal prepared in the lab, *PDM* partially-defatted meal and *ODM* oil-depleted meal)

rapeseed meal by Sosulski et al. [27] and for canola meal treated with methanol/ammonia/water-hexane solution by Xu and Diosady [15]. The curves from all these studies showed similar behavior for foam stability of canola meals. The PM, again due to its proteins being partially denatured, exhibited poorer foam properties than other meals, while the commercial meal had almost no foam forming property at all (Fig. 3).

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