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Effect of sieve size on chemical composition and functional properties of canola meal (*Brassica napus*) protein fractions as fishmeal replacement

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Canola meal (CM) use in animal nutrition is limited due to the inclusion of various antinutritional factors (ANF). This study aimed to evaluate the effect of sieving on nutrient and ANF contents of CM. Five sieves with mesh size (diameter) of 16, 18, 20, 30 and 40 were used in this regard. With an increase in the mesh size, the recovery percentage significantly decreased (73.7 vs. 18.3), while crude protein content (370.7 vs. 378.1 g/kg), gross cost (0.47 vs. 1.91 US\$/kg) and protein unit cost (1.27 vs. 1.66 US\$/kg) were significantly increased ($P < 0.05$). In the next stage, only the sieved canola meal obtained through mesh size 16, 18 and 20 was selected for further investigation. The amounts of crude protein, crude fat and total NSP of the processed products were not significantly differed. However, the amounts of acid detergent fiber (218.2 g/kg vs. 206.7 g/kg) and neutral detergent fiber (330.8 g/kg vs. 319.1 g/kg) significantly decreased by increasing mesh size. Glucosinolates (20.67 vs. 21.82 $\mu\text{mol/g DM}$) and phytate (40.6 g/kg vs. 62.0 g/kg) had significant increment ($P < 0.05$) on the other hand. Considering the weighted averages of different measured variables from PCA, the mesh size 16 was selected for use in the production of a canola protein concentrate.

Key words: Canola meal, sieving, chemical composition, anti-nutritional factors, functional properties.

INTRODUCTION

Globally, canola is one of the most important oilseeds as it ranked second after soybean in 2010/2011 (USDA, 2010). Production of rapeseed/canola in 2010 to 2011 was 58 million metric tones and accounted for 13% of global oilseed production. It provides a relatively high crude protein (350 to 360 g/kg), a balanced amino acid profile and a source of several minerals and vitamins

(NRC, 1993; Pastuszewska et al., 2000) for fish. Useful functional properties include high water and oil absorption capacities, high nitrogen solubility (Ghodsvali et al., 2005; Moure et al., 2006; Khattab and Arntfield, 2009), emulsifying, foaming and whipping. Additionally, it has been proposed that hydrolyzed CM has pre-biotic effects (Kiarie et al., 2008).

The use of canola meal in human and animal nutrition has been restricted due to the inclusion of some antinutritional factors (ANFs) including phytate, tannins, glucosinolate and sinapine (Bell, 1993). High crude fiber content is due to the high proportion of hull; typically 16% of seed and 30% of the meal. The main carbohydrates of canola meal are neutral detergent fiber (NDF), acid detergent fiber (ADF), non-starch polysaccharides (NSP) and crude fiber, respectively (Newkirk, 2009). Selective breeding programs have reduced some undesirable compounds such as phytate, glucosinolate, sinapine and

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Abbreviations: CM, Canola meal; ANF, antinutritional factors; NDF, neutral detergent fiber; ADF, acid detergent fiber; NSP, non starch polysaccharide; ADL, acid detergent lignin; AIA, acid insoluble ash; TNSP, total non starch polysaccharide; INSP, insoluble non starch polysaccharide; PCA, principal component analysis.

tannin. However, the effort for producing three-zero variety (low in erucic acid, glucosinolate and fiber) has not yet been successful (Jensen et al., 1995; Mailer et al., 2008). Fishmeal remains a key ingredient in aquafeeds and despite considerable research efforts, opportunity remains to replace it with plant meals. The cost of fishmeal is much higher than that of soybean meal and canola meal. The ratios of soybean meal and canola meal to fishmeal price were 0.23 and 0.13, respectively. This is partly due to the low production efficiency in fishmeal (22.5%) (USDA, 2010). The unit cost of canola meal protein is about 50% of that of fishmeal and, any reduction in fiber and increase in protein will be advantageous (Drew et al., 2007). Dehulling (Thakor et al., 1995; Farhangi and Carter, 2001), fermentation (with *Aspergillus*, *Bacillus* etc.) (Singhania et al., 2009), germination (Zieliński et al., 2006), extrusion (Kraugerud et al., 2007), various carbohydrase enzymes (oligosaccharidase, xylanase, β -glucanase etc) and fractionation, using sieving (Mwachireya et al., 1999; Farhangi and Carter, 2007; Mushtaq et al., 2007), have been used to reduce polysaccharide content. Sieving is the most convenient technique for canola meal and requires the correct mesh sizes in order to optimize the economic recovery of high protein fractions. Surprisingly, this critical issue was not examined in previous studies (McCurdy and March, 1992; Mwachireya et al., 1999; Glencross et al., 2004; Khattab and Arntfield, 2009). The objective of this study was to improve the quality of canola meal by developing an appropriate sieving method in order to facilitate the production of a canola protein concentrate.

MATERIALS AND METHODS

Materials

Double-zero brown canola meal (*Brassica napus*, Hyola 401, a warm climate variety) from Behpak Oilseeds Company (Golestan Province, Iran) was used throughout the experiment. Canola meal was sieved using five laboratory standard sieves with mesh sizes of (diameter, mm) 16 (1.19 mm), 18 (1.00 mm), 20 (0.841 mm), 30 (0.595 mm) and 40 (0.420 mm) (ASTM, 2004), respectively. To determine the chemical composition of the samples, simple-point random sampling was applied (Lichon, 1996). The sieved fractions were then compared in terms of economic indices, chemical composition and functional properties via two screening steps. Firstly, the products from the five sieves were compared with an intact sample as a control in terms of economic indices and protein content. Secondly, only the fractions from mesh sizes 16, 18 and 20 were screened and then compared in relation to fiber compounds (crude fiber, ADF, NDF, NSP etc), ANFs (phytate, tannin, glucosinolate and myrosinase activity) and functional properties (nitrogen solubility, water and oil absorption capacities and viscosity).

Chemical composition

Dry matter, crude protein, crude fat, crude fiber and ash were determined using standard methods and nitrogen free extract (NFE)

was calculated by difference (dry matter minus protein, fat, crude fiber and ash content) (AOAC, 2005). Fiber compounds including NDF, ADF, acid detergent lignin (ADL), ash insoluble acid (AIA), hemicellulose and cellulose were measured sequentially with a fiber automatic analyzer (Fibertec System, M, Tecator, Hoganas, Sweden) (van Soest et al., 1991). NDF was determined without α -amylase, without sodium sulfite and expressed with residual ash. ADF was also expressed inclusive of residual ash. Lignin was measured by solubilization of cellulose with sulphuric acid. To determine the quantity of total and insoluble NSP (TNSP and INSP, respectively), samples were divided (Englyst et al., 1994) into two parts in Englyst's Reference Laboratory. Then, the quantities of constituent neutral and acidic sugars were measured using gas-liquid chromatography and spectrophotometry at 400 and 450 nm, respectively. TNSP and INSP were obtained from summation of neutral and acidic sugars of each part. Soluble NSP (SNSP) was calculated via subtraction of TNSP and INSP. Then, the profiles of neutral and acidic TNSP and SNSP were defined using the internal standards and weighing the difference between free monosaccharides and residue polysaccharides. Since 87% of the glucose in the NSP profile originates (Slominski and Campbell, 1990) from cellulose, the profiles of non-cellulosic polysaccharides were calculated after discounting cellulose. Phytate was determined through the extraction of the samples with hydrochloric acid and sodium sulphate and absorbance measured at 660 nm (De Boland et al., 1975). Tannin was determined using the method of vanillin-hydrochloric acid and absorbance was measured at 500 nm (Price et al., 1978). Glucosinolate was measured as glucose following hydrolysis with myrosinase using HPLC (Quinsac et al., 1991). Total phosphorus was measured through preparing ash, digesting with hydrochloric acid (McQuaker et al., 1979) and then measuring absorbance with ICP-AES. Myrosinase activity was determined via the differences between glucosinolate (Slominski and Campbell, 1987) of initial and wet samples at 40°C. One unit of myrosinase activity was defined as the amount of enzyme (Slominski and Campbell, 1987) that hydrolyzed one micromole aliphatic glucosinolate.

Economic assessments

The primary cost of canola meal was 0.35 US\$/kg. The selected indices were calculated according to McCurdy and March (1992):

Recovery (%) = $100 \times (\text{sieved product weight, g}) / (\text{primary weight, g})$

Gross Cost (US\$/kg) = $100 \times (\text{primary cost, US$}) / (\text{recovery, \%})$

Protein Unit Cost (US\$/kg protein) = $100 \times \text{gross cost (US$/Kg)} / \text{protein content, \%}$

Functional properties

Nitrogen solubility was determined following the extraction and digestion of the samples with potassium hydroxide, sulphuric acid and hydrogen peroxide, respectively and centrifuging at 821 \times g for 15 min (Araba and Dale, 1990). Oil absorption capacity was measured after mixing 1 g of samples with 10 ml refined soybean oil and centrifuging at 15000 \times g for 15 min (Beuchat, 1977). Water absorption capacity was calculated after mixing 1 g of the samples with 10 ml distilled water and centrifuging at 5000 \times g for 30 min (Adebowale et al., 2005). Least gel concentration was measured with suspensions of 2 to 20 g samples in 100 ml distilled water. 10 ml of dispersion was transferred into test tube, heated in a boiling water bath for 60 min and rapidly cooled in a cold-water bath. Then least gel concentration was determined when the samples inverted the test tube without slipping or falling (Coffman and Garcia, 1977). The viscosity of samples was measured at 22°C through mixing

Table 1. Comparison (mean \pm standard deviation) of the recovery percentage, gross cost and protein unit cost of sieved canola meal with different mesh size (n=3).

Mesh size		16	18	20	30	40	P-value
Recovery (%)	-	73.7 \pm 1.5 ^a	62.3 \pm 2.1 ^b	56.0 \pm 2.6 ^c	30.0 \pm 2.0 ^d	18.3 \pm 1.5 ^e	0.0001
Gross cost (US\$/Kg)	0.35 \pm 0.17 ^a	0.47 \pm 0.0 ^b	0.56 \pm 0.02 ^b	0.62 \pm 0.03 ^b	1.16 \pm 0.10 ^c	1.91 \pm 0.16 ^d	0.0001
Crude protein (g/kg)	349.2 \pm 2.8 ^a	370.7 \pm 1.0 ^b	371.2 \pm 2.1 ^{bc}	374.8 \pm 2.1 ^{bcd}	376.8 \pm 3.3 ^{cd}	378.1 \pm 2.2 ^e	0.001
Protein unit cost (US\$/Kg)	1.00 \pm 0.21 ^a	1.27 \pm 0.04 ^b	1.50 \pm 0.09 ^c	1.66 \pm 0.09 ^d	3.07 \pm 0.10 ^e	5.07 \pm 0.12 ^f	0.0001

* Values in the same row with different superscript letters are significantly different ($P \leq 0.05$).

canola meal/distilled water (with the ratio of 3: 5) and with digital plate-cone viscometer (LVDV-I+) connected to RV3 spindle at 10 rpm for 20 s (Refstie et al., 2005).

Statistical analysis

All percentage data were transformed using arcsine method. After confirming the homogeneity of variance and normality of the data using Leaven and Kolmogorov-Smirnov tests (Zar, 1999), respectively, one-way ANOVA was used to compare the treatments. Duncan test was applied to compare significant differences among the treatments ($P < 0.05$). The regression and correlation between variables were studied by Sigmaplot™ version 11. Prioritizing of the treatments was determined through principal component analysis (PCA) and weighted averages and treatments were grouped at the statistical levels of 0.05. Using the PCA, new variables were built from the initial variables. Covariance matrix and varimax rotation method were selected. The first variable was considered as the case that showed the most cumulative variance (Varmuza and Filmoser, 2009). To screen the most appropriate treatment, data were compared using PCA. Then, the main components were defined through a covariance matrix and significantly independent variables such as gross cost, ADF, NDF, crude fiber, phytate, glucosinolate, total polysaccharides and insoluble polysaccharides. Among the eight measured principal components, the first component showed the highest cumulative variance (96.7%).

RESULTS

Economic assessments and chemical composition

The recovery percentage decreased significantly ($P < 0.05$) with increasing mesh size (Table 1). Gross cost was significantly higher using mesh sizes of 30 and 40 compared with mesh sizes of 16, 18 and 20. Gross cost was significantly higher for mesh sizes 30 and 40 than for sizes 16, 18 and 20 (Table 1). Crude protein content for all mesh sizes were significantly higher than the control and it was significantly ($P < 0.05$) higher using mesh size 40 compared with the other mesh sizes (Table 1). Crude protein content from mesh sizes 16, 18 and 20 were similar whilst the protein unit costs showed significant ($P < 0.05$) differences (Table 1). The protein unit costs and gross costs from mesh size 30 and 40 were high and the recovery was low compared with mesh size 16, 18 and 20. Consequently, only the chemical composition from the 16, 18 and 20 sieves was determined in the next

stage.

With an increase in the mesh size, crude fiber, ADF, NDF and cellulose were significantly decreased ($P < 0.05$) (Table 2). Crude fat, NFE and hemicellulose of the products of 16, 18 and 20 sieves, did not show any significant differences. Phytate and total phosphorus content were significantly increased with an increase in the mesh size from 16 to 18 ($P < 0.05$), whereas tannin content remained unchanged (Table 2). Glucosinolate and myrosinase activity significantly increased ($P < 0.05$) with an increase in the mesh size from 16 to 20 (Table 2). There were significant positive correlations between glucosinolate and myrosinase (97.6%) and phosphorus or phytate and crude protein content (both of them 96.6%).

Sieving did not affect NSP content (Table 3). The range of total, soluble and insoluble NSP of the sieved products obtained through all mesh size (16, 18 and 20) were 173 to 177, 24 to 29 and 148 to 150 g/kg, respectively. Soluble fraction constituted about 15% of total NSP. Arabinose, glucose and galacturonic acid constituted the main sugars in the insoluble fraction of NSP. Arabinose and galactose were the most abundant sugars in the soluble fraction (about 14.9%). Arabinose, xylose and galactose from neutral sugars and galacturonic acid constituted the most abundant sugars in the non-cellulosic NSP (Table 4).

Functional properties

Viscosity and water absorption capacity increased with an increase in mesh size (Table 5) whereas oil absorption capacity significantly decreased ($P < 0.05$). There was a negative correlation between oil and water absorption capacities and positive correlations between water absorption capacity and viscosity ($r = 90.7\%$) and between crude fat and oil absorption capacity ($r = 95.4\%$).

Prioritizing the treatments and selecting the most appropriate treatment

The highest value of cumulative variance (96.7%)

Table 2. Comparison (mean \pm standard deviation) of chemical composition (g/kg dry matter) of sieved canola meal with different mesh no (n=3).

Mesh number	16	18	20	P-value
Dry matter	899.7 \pm 0.1 ^a	903.0 \pm 0.1 ^a	899.6 \pm 0.7 ^a	0.20
Crude protein	371 \pm 1.3 ^a	371 \pm 2.1 ^a	375 \pm 0.2 ^a	0.19
Crude fat	56.0 \pm 1.4 ^a	53.0 \pm 1.4 ^a	52.5 \pm 0.7 ^a	0.12
Crude fiber	55.1 \pm 1.1 ^{ab}	61.6 \pm 5.7 ^b	48.1 \pm 2.5 ^a	0.05
Ash	76.0 \pm 1.1 ^a	75.5 \pm 0.4 ^a	80.4 \pm 2.6 ^a	0.10
NFE	341.8 \pm 2.5 ^a	341.7 \pm 1.6 ^a	343.8 \pm 4.4 ^a	0.76
ADF	218.2 \pm 4.4 ^b	213.0 \pm 0.9 ^{ab}	206.7 \pm 3.1 ^a	0.04
NDF	330.8 \pm 2.7 ^b	327.5 \pm 6.7 ^b	319.1 \pm 9.1 ^a	0.05
Acid detergent lignin	91.8 \pm 5.7 ^a	106.4 \pm 4.7 ^a	94.1 \pm 7.8 ^a	0.19
Acid insoluble ash	5.2 \pm 0.4 ^a	4.7 \pm 1.1 ^a	4.3 \pm 0.6 ^a	0.55
Hemicellulose	112.6 \pm 1.7 ^a	114.5 \pm 7.6 ^a	112.4 \pm 6.0 ^a	0.10
Cellulose	126.4 \pm 0.8 ^b	106.6 \pm 4.9 ^a	112.6 \pm 5.4 ^a	0.04
Lignin	86.6 \pm 5.2 ^a	101.7 \pm 5.8 ^a	89.8 \pm 8.5 ^a	0.21
Total phosphorus	58.0 \pm 7.1 ^a	60.3 \pm 7.1 ^a	88.6 \pm 9.5 ^b	0.03
Phytate	40.6 \pm 3.5 ^a	42.2 \pm 3.5 ^a	62.0 \pm 4.7 ^b	0.02
Tannin	24.9 \pm 0.5 ^a	23.7 \pm 0.4 ^a	22.3 \pm 1.8 ^a	0.21
Glucosinolate (μ mol/g)	9.60 \pm 0.45 ^a	11.30 \pm 0.58 ^b	12.50 \pm 0.51 ^c	0.04
Myrosinase activity (μ mol/min)	2.05 \pm 0.35 ^a	3.65 \pm 0.49 ^b	4.55 \pm 0.21 ^b	0.04

*Values in the same row with different superscript letters are significantly different ($P \leq 0.05$).

belonged to the first component. The first main component was the weighted average of eight selected variables that described 96.7% of variance of eight measured variables. Consequently, this main component was used to compare the treatments. The values of functional properties were not included in the model as the main objective in this study was to facilitate the production of canola protein concentrate through reducing antinutritional factors (phytate, glucosinolate, tannin) and fiber compounds (ADF, NDF, total and insoluble polysaccharides). As shown in Table 6, the lowest value of weighted averages belonged to mesh 16. Weighted average was a resultant of eight variables included into the model. As a result, its lower value means that the sieving improved all the variables in a way to finally produce canola protein concentrate.

DISCUSSION

Economic assessment

Dehulling is considered as one of the most appropriate methods for reducing the fiber content of grain legumes such as soybean, lupin, field pea, faba bean and chick pea (Egounlety and Aworth, 2003; Farhangi, 2003). However, this method has not been used extensively in the canola oil extraction industry due to the small size of the canola seeds, oil loss via fiber separation and the strong connection between hull and cotyledon (Thakor et

al., 1995; Ikebudu et al., 2000). Due to the small size as well as the size variation of canola seed, its harvesting coincides with entering large amounts of straw to downstream processes after oil extraction. As a result, sieving is considered as a primary processing technique that has economic justification despite the simplicity of its use in small as well as in large scales conditions. Using sieves with mesh size of 10 (diameter, 2 mm) and 12 (diameter, 1.7 mm), the recovery percentages of 100 and 90% respectively, were achieved (Gattinger, 1990). However, economic indices and the nutritional value of the processed canola meal were not assessed (Gattinger, 1990). The cost of protein in fishmeal, soybean meal and canola meal were estimated as 2.60, 0.95 and 0.63 US\$/kg, respectively (USDA, 2010). Therefore, considering the current limitations in terms of the availability of animal proteins and the low cost of canola meal, any effort to produce canola protein concentrate as a renewable protein source is of paramount importance for the production of sustainable aquafeeds (Forster et al., 1999) and organic aquaculture industry (Mente et al., 2011).

Chemical composition

Crude protein, crude fiber, NDF and ADF

The crude protein content of canola meal fractions in this study significantly increased with an increase in sieve

Table 3. Comparison (mean \pm standard deviation) of quantity and profile of NSP of sieved canola meal with different mesh no ($n = 3$) (Because of non significant differences, the quantity of total, insoluble and soluble NSP are presented in one column).

Mesh	NSP (g/100g)	NSP profile (g/Kg)									
		Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose	Glucuronic acid	Galacturonic acid	
No 16	Soluble	29 \pm 1	ND ¹	ND	11	4	3	6	2	ND	3
	Insoluble	148 \pm 1	1	1	31	17	6	11	57	ND	24
	Total	177 \pm 2	1	1	42	21	9	17	59	ND	27
No 18	Soluble	22 \pm 3	1	0	6	1	4	4	4	ND	2
	Insoluble	150 \pm 3	1	2	35	17	3	11	56	ND	25
	Total	172 \pm 3	2	2	41	18	7	15	60	ND	27
No 20	Soluble	25 \pm 1	1	1	9	3	3	5	0.0	ND	3
	Insoluble	150 \pm 1	1	1	34	17	3	12	59	ND	23
	Total	175 \pm 5	2	2	43	2	6	17	59	ND	26

¹ND: Not Detected.**Table 4.** Comparison the mean quantity and profile of non- cellulosic NSP of sieved canola meal with different mesh no ($n = 3$) (Because of non significant differences, the quantity of non-cellulosic non-starch polysaccharide are presented without standard deviation).

Mesh	Cellulose (g/kg)	Non-cellulosic NSP profile (g/Kg)							
		Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose	Galacturonic acid
No 16	131.6	8	8	341	170	73	138	43	219
No 18	111.3	17	17	350	153	60	128	45	230
No 20	116.9	16	16	354	165	49	140	44	214

size (from 16 to 40) and were in agreement with previous studies (McCurdy and March, 1992; Mwachireya et al., 1999). Protein contents of 371 g/kg with sieve size 40 (Mwachireya et al., 1999) and 467 g/kg with sieve size 70 (McCurdy and March, 1992) were significantly higher than the respective controls. Sieving significantly decreased crude fiber, ADF and NDF contents and similar trends were observed in other studies (McCurdy and March, 1992; Mwachireya et al., 1999). However, the crude fiber content of 55 g/kg obtained in this study was much lower than 100 to 130 g/kg reported in previous studies (Spragg and Mailer, 2007; Newkirk, 2009). The quantity of ADF of canola meal in this study (average of 3 meshes; 212.6 g/kg) was similar to the canola meal that is cultivated in China (219 g/kg), but it was significantly higher than the canola meal from other regions (164 to 182 g/kg). The NDF content of canola meal in this study (averages of 3 mesh; 325.8 g/kg) was lower than Chinese canola meal (351 g/kg), similar to European canola meal (281 g/kg) and higher than canola meal from Canada and Australia (207 to 241 g/kg) (Spragg and Mailer, 2007; Newkirk, 2009). Seed size, ratio of hull to

embryo, composition of the hull (Bell, 1993), color (Jensen et al., 1995), variety, maturity conditions and cultivation site (Mailer et al., 2008) are different characteristics that may affect the amount and nature of fiber. Due to intrinsic differences between fiber contents, varieties cultivated in various regions should be used for different target animals.

Phytate, tannin, glucosinolate and NSP

Phytate, tannin and glucosinolate content of canola meal in this study were 40.6 to 62.0 g/kg, 22.3 to 24.9 g/kg and 9.6 to 12.5 μ mol/g, respectively. Sieving caused significant differences in terms of phytate and glucosinolate (using mesh size 18 and 20). The amounts of phytate and tannin in canola meal were 30 to 60 g/kg and 15 to 30 g/kg, respectively (Bell, 1993). The canola meal used in this study was prepared from Golestan Province, the biggest producer of canola in Iran. The phytate content of the Iranian canola meal appears high compared with the value reported by other researcher

Table 5. Comparison of (mean \pm standard deviation) functional properties of sieved canola meal with different mesh (n = 3).

Variable	16	18	20	P-value
Viscosity (centipoises)	479.5 \pm 0.70 ^a	484.00 \pm 1.40 ^a	543.50 \pm 2.12 ^b	0.03
Oil absorption capacity (g/kg)	2940.0 \pm 57.0 ^b	2840.0 \pm 14.1 ^{ab}	2790.0 \pm 28.3 ^a	0.01
Water absorption capacity (g/kg)	3120.0 \pm 4.25 ^a	3265.0 \pm 35.4 ^b	3400.0 \pm 28.3 ^c	0.00
Nitrogen solubility (g/kg)	130.5 \pm 7.8 ^a	134.0 \pm 4.2 ^a	138.5 \pm 3.5 ^a	0.12
Least concentration of gelation (g/kg)	136.0 \pm 7.1 ^a	133.0 \pm 5.6 ^a	131.5 \pm 9.2 ^a	0.15

Values in the same row with different superscript letters are significantly different ($P \leq 0.05$).

Table 6. Comparison of weighted (mean \pm standard deviation) of experimental treatments using principal component analysis.

Treatment	Weighted average (g/Kg)
Mesh no 16	7958.6 \pm 22.0 ^a
Mesh no 18	9403.7 \pm 53.8 ^b
Mesh no 20	10361.7 \pm 57.8 ^c

*Values in the same column with different superscript letters were significantly different ($P \leq 0.05$).

(Bell, 1993). Phytate in canola exists as a spherical crystal located in the protein bodies in radicles and mainly inside the cotyledon (Thompson, 1990). In contrast, tannin content was in the same range reported in other studies (Bell, 1993; Mwachireya et al., 1999). Tannin is mainly distributed in the hull (Bell, 1993), that is why with an increase in mesh size; the amount of tannin remained unchanged. Lower glucosinolate content of canola meal used in this study (9.6 μ mol/g dry matter) compared to the varieties from other regions (from 11 to 34 μ mol/g dry matter) (Mailer et al., 2008) confirms that the Iranian canola was produced appropriately. Glucosinolate is mainly distributed in the cotyledon and partly in canola seed hull (Bell, 1993). The results of this study agree with other reports (McCurdy and March, 1992; Mwachireya et al., 1999) that showed sieving increased the concentrations of phytate and glucosinolate and decreased the tannin content in the processed products of canola meal. It has been reported that the quantity of phytate depends on variety, maturation stage and the location of their distribution in the seed (Reddy, 2002). The quantities of glucosinolate and tannin in canola meal are affected by climate, cultivation time, variety (Tripathi and Mishra, 2007) and the type of tannin (Kozłowska et al., 1990).

There is limited information regarding NSP, its soluble and insoluble fractions, and the sugars in canola meal. The average amounts of total (175 g/kg), soluble (25 g/kg) and insoluble NSP (149 g/kg) in the canola meal used in this study were slightly higher than the values that have been reported in previous study (157, 14 and 144 g/kg, respectively) (Newkirk, 2009). However, they were similar to those of canola meal cultivated in Canada (Slominski et al., 1994). Polysaccharides are present in

larger amounts in the hulls of canola. The polysaccharides in canola meal and hulls are composed of cellulose, hemicelluloses (Naczka and Shahidi, 1990), pentosans and pectins. Information regarding the constituent sugars of NSP is an important prerequisite for designing the appropriate processing method for producing canola protein concentrate. Comparing the constituents' sugars in this study (average of three mesh size) and that of brown canola meal showed lower rhamnose (10 vs. 11 g/kg), fucose (8 vs. 12 g/kg), arabinose (238 vs. 252 g/kg) and galacturonic acid (155 vs. 242 g/kg) and higher quantities of xylose (113 vs. 90 g/kg), mannose (42 vs. 22 g/kg) and galactose (95 vs. 93 g/kg), respectively. It seems that a major part of arabinose and galactose, that were not associated with pectic compounds originated from arabinan and arabinogalactan. The high amount of xylose indicated the existence of xylose and xyloglucans in canola meal. Xyloglucans of canola meal contained xylose, glucose and galactose (amyloid) and the presence of these sugars together with fucose (fucoamyloids) were reported in previous study (Siddiqui and Wood, 1977). The profile of monomer sugars in this study is in agreement with the results reported in the previous studies (Slominski and Campbell, 1990). Although, sieved CM with mesh 16 was selected for the next experiments via PCA, other products (mesh 18 and 20) may have other characteristics that deserve to be further assessed in the future studies.

Functional properties

A number of studies have been conducted regarding the

chemical composition of canola meal and the effect of different processing methods on canola (McCurdy, 1990; Moure et al., 2006; Gatlin et al., 2007; Khattab and Arntfield, 2009). However, to date, a holistic approach that takes account of economic indices, chemical composition and functional properties has not been taken to produce canola protein concentrate. In spite of the suitable protein quality of canola meal and its superior properties (oil absorption capacity, emulsification and foaming) compared to soybean meal, very few studies have been carried out on the functional properties of canola meal (Ghodsvali et al., 2005; Khattab and Arntfield, 2009). The water absorption capacity of canola meal used in this study (3120 to 3400 g/kg) was near to the values reported in another study (3350 g/kg) (Giger-Reverdin, 2000). The oil absorption capacity of canola meal in this study (2790 to 2940 g/kg) was higher but in the range of those reported by other researchers 1880 to 2030 g/kg and 2650 to 2815 g/kg (Naczka et al., 1985; Ghodsvali et al., 2005). Increased oil and water absorption capacities help to improve the binding capacities, enhance flavor retention, improve mouth feel and reduce moisture and fat losses of food products (Sreerama et al., 2008). The inverse relationship between water and oil absorption capacities in this study is in agreement with the results of other researchers (Dench et al., 1981). Increased oil absorption properties were associated with heat dissociation of proteins and denaturation, which is expected to unmask the non-polar residues from the protein molecules (Kinsella, 1976). The nitrogen solubility in this study (130 to 140 g/kg) was lower than that of the values reported (370 to 660 g/kg) in previous study (Khattab and Arntfield, 2009). Nitrogen solubility is considered as an effective functional property for emulsification, foaming and gelation. It should be noted that the nitrogen solubility is affected by temperature and pH (McCurdy, 1990). Furthermore, the values reported in these studies were collected from laboratory (pilot) studies. High soluble protein is required for the production methods where emulsification, whipping and film formation are important. Low protein solubility is desirable when there is a need for high protein content diets (Kolar et al., 1985). Considering the increasing demand of the aquaculture feed industry for nutrient dense diets and the limited solubility of nitrogen, this issue is of great importance in aquafeed production (Cho and Bureau, 2001).

Conclusions

Sieving significantly increased the crude protein content of CM. With an increase in sieve size (from 16 to 20), the amounts of ADF and NDF significantly decreased. In contrast, the amounts of phytate and glucosinolate were significantly increased. Considering the weighted averages of different measured variables from PCA, the

mesh size 16 (1.19 mm) was selected for use in the production of a canola protein concentrate.

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