Isolation and Characterization of Defatted Canola Meal Protein

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Protein was extracted and isolated from canola oil processing waste. Canola is an increasingly important oilseed crop worldwide. More than 99% of protein was extracted from crude commercial hexane defatted canola meal when a 5% w/v suspension in 0.4% w/v NaOH was agitated for 60 min at room temperature in baffled flasks on an orbital shaker at 180−200 rpm. Protein recovery was 87.5% upon precipitation with acetic acid. The major proteins in canola seed were glutelins (91.8% soluble in 0.4% w/v NaOH) and globulins (50.9% soluble in 5% w/v NaCl) as measured by separate extractions. Canola proteins had poor solubility between pH 2 and 10 for all dispersion solutions. Solubility of protein isolates was 60% or less. Extraction and precipitation procedure did not produce lysinoalanine. Amino acid profile of isolates indicated high nutritional quality for use in products for 10−12-year-olds and adults.

Keywords: *Canola meal; canola meal protein; protein quality*

INTRODUCTION

Oilseeds are the most valuable agricultural crops in world trade (Shahidi, 1990). Rapeseed is increasingly becoming a major crop worldwide, with 25 million tons produced in 1992. Although oil is highly valued, oil extraction operations depend on revenues from the meal to maintain profitable operations (Howard, 1993). There is a large potential market for high-quality vegetable proteins because of increasing demand by health conscious consumers. Early researchers noted the nutritional quality of rapeseed proteins (Van Etten et al., 1969) as well as the antinutitional components: glucosinolates, hull fiber, tannins, and phenols (Niewiadomski, 1990).

Protein isolation by alkali is commercially used in soybean meal processing. Treatments at pH 8 or 9 cause little or no damage to the protein; however, at high pH the nutritional quality of the isolate is reduced and toxic reaction products may form. Cross-linkage formation between amino acids and protein molecules, amino acid destruction, and racemization may result from extreme alkali treatment. Threonine, lysine, and cystine are most susceptible to destruction at high pH. Methionine, phenylalanine, threonine, serine, aspartic acid, and histidine may be racemized. The cross-linkages of major concern are lysinoalanine formation from lysine and dehydroalanine formation through degradation of cystine or serine. Lysinoalanine reduces protein digestibility. Amino acid destruction and alteration reduce the biological value of proteins by affecting the binding sites of digestive enzymes and/or by reducing absorption (Hurrell and Finot, 1985).

Reports by Ismond and Welsh (1992), Diosady et al. (1984), and Tzeng et al. (1988) indicate 80−95% extraction of canola meal proteins from commercially defatted canola meal but only 30−78.5% protein recovery. Lysinoalanine content has been surveyed in rapeseed protein meals and isolates (Deng et al., 1990), but the effects of extraction at high pH on the amino acid profile of protein isolates have not been reported.

Our objective was to develop an extraction method that maximizes protein recovery and does not adversely affect protein quality.

MATERIALS AND METHODS

Defatted Meal Characterization. Crude commercial hexane defatted canola meal was obtained from CanAmera Foods, Inc. (Hamilton, ON). Proximate analysis was conducted on the canola meal following AOAC methods developed for cereal foods: protein (2.057) N = 6.25, moisture (14.002−14.003), fat (7.056), ash (14.006) and carbohydrate contents were determined by the difference method. P_2O_5 was added to the sulfuric acid to avoid hydrolysis of nitriles and cyanates during the Kjeldahl extraction (Horowitz, 1980).

Protein Solubility Evaluation. The solubility profile of the canola meal protein isolate (CMPI) was determined using the method of Betschart et al. (1977). This method fractionates proteins into four major groups: glutelins, globulins, prolamins, and albumins on the basis of solubility through separate extractions. Solubility of protein in the various solutions was measured using the bicinchoninic acid (BCA) protein analysis method (Sigma Chemical Co., St. Louis, MO) using a modification of the microassay technique (Redinbaugh and Turley, 1986) with 20 μL of sample, 200 μL of dye reagent, 0.1−1.0 $\mu g/\mu L$ bovine serum albumin (BSA) standard, and absorbance determined at 570 nm. Corrections were made for each protein measurement for non-protein nitrogen content by adjusting each for measured BCA protein content of 10% w/v trichloroacetic acid (TCA) extracts (Mannheim and Cheryan, 1990).

The solubility of canola protein was evaluated at ionic strengths of 0.0−1.0 (adjusted with $CaCl_2$) and in 0.1 and 1.0 M NaCl at pH values in the range of 2−12 following the method of Bera and Mukherjee (1989). The soluble protein in the extracts was evaluated using the BCA microassay technique with correction for non-protein nitrogen.

Solubility in NaOH (Mallinckrodt Baker, Inc., Phillipsburg, NJ) solutions ranging from 0.1 to 0.4% w/v was investigated using standard Erlenmeyer and baffled flasks (Bellco Glass, Inc., Vineland, NJ). All flasks were mixed on an orbital shaker (New Brunswick Scientific, New Brunswick, NJ) at 180−200 rpm for 60 min. The weight to volume ratio of meal to extract was kept constant at 5%, and all extractions were conducted at room temperature.

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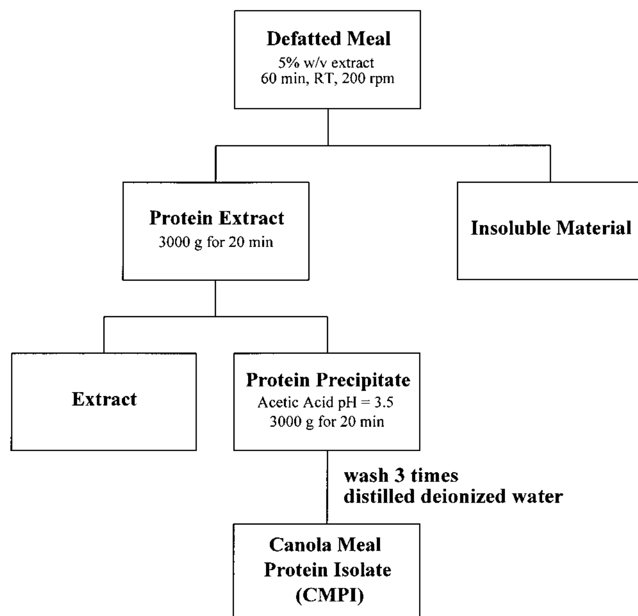

Figure 1. Schematic of canola meal protein isolate extraction method.

Table 1. Canola Protein Solubility Profile

dispersion solution	protein classification	% solubility
0.4% w/v NaOH	glutelins	91.6 ± 1.85
5.0% w/v NaCl	globulins	50.8 ± 1.86
60% v/v ethanol	prolamins	33.9 ± 1.70
distilled deionized water	albumins	31.2 ± 0.40

Protein was extracted from defatted canola meal using a 5% w/v extraction with 0.4% w/v NaOH at room temperature using an orbital shaker at 180−200 rpm for 60 min. Residual solids were discarded following centrifugation at 3000*g* for 20 min at 5−10 °C (Sorvall RC-5B, DuPont, Willmington, DE). Glacial acetic acid (Mallinckrodt Baker) was then added to the protein extract and the pH dropped to 3.5 for protein precipitation. Precipitated protein was separated by centrifugation at 3000*g* for 20 min at 5−10 °C. The protein precipitate was washed three times with distilled deionized water with centrifugation at 3000*g* for 20 min at 5−10 °C between each wash. The final protein isolate (CMPI) obtained was freeze-dried. This extraction method is summarized in Figure 1. Percentage protein extractability was calculated from measured protein solubility data collected using the BCA microassay technique. Values were previously correlated with Kjeldahl results both for CMPI and for BSA. Total protein values were corrected for non-protein nitrogen through BCA analysis of 10% w/v TCA extracts (Mannheim and Cheryan, 1990).

Canola protein extractability was evaluated over time at 5% w/v meal to extract in 0.4% w/v NaOH solution. Protein solubility was evaluated for these time course samples using the BCA microassay technique with corrections for non-protein nitrogen content.

Nutritional Quality Assessment. The amino acid profiles of the protein in the untreated commercial hexane defatted canola meal and the protein isolate were determined at the FDA Southern Region Laboratory (Atlanta, GA) using FDA's standard method employed in the verification of label declarations in food and food supplements. This method included the detection and quantification of lysinoalanine with a 100 ppm detection limit (G. W. Chase, Jr., U.S. FDA SRL, Atlanta, GA, personal communication, 1996). Two 10 μL injections were made for each sample. Following separation with ion exchange HPLC, the amino acids were derevatized postcolumn by the addition of ninhydrin (Robinson, 1978). The absorbance for each sample was measured at 436 and 546 nm. Absorbance values were converted to amino acid content in the sample using the Millennium Software package (Waters Chromatography Division, Millipore Co., Milford, MA).

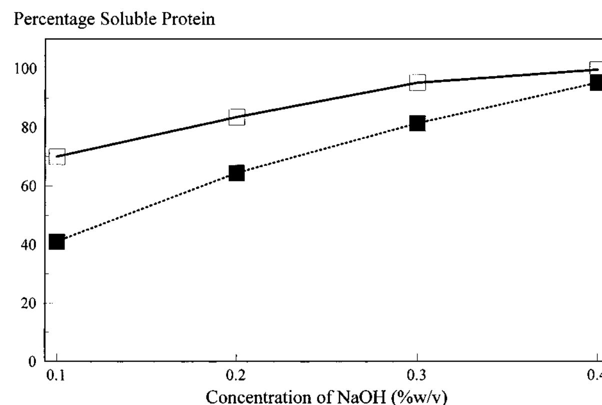

Figure 2. Effect of NaOH concentration and flask type on protein extraction from crude commercial hexane defatted canola meal. Extraction was performed with 5% w/v meal to extract ratio at room temperature for 60 min at 180−200 rpm with baffled (□) and Erlenmeyer (■) flasks.

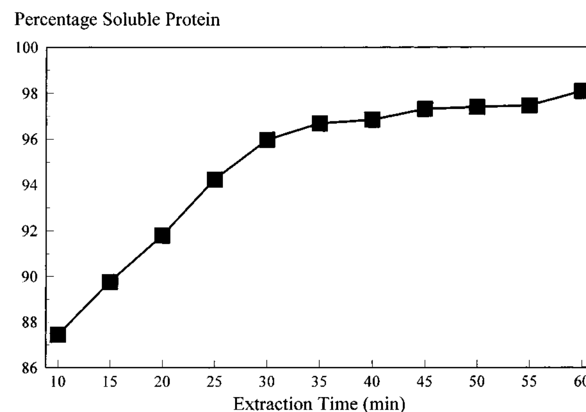

Figure 3. Effect of time on protein extraction from crude commercial hexane defatted canola meal. Extraction was performed with 5% w/v meal to extract in 0.4% NaOH at room temperature and 180−200 rpm.

Protein quality was evaluated through the calculation of PDCAAS values based on four age ranges: (a) average infants, (b) 2−5-year-olds, (c) 10−12-year-olds, and (d) adults (Henley and Kuster, 1994). Digestibility values used in these calculations were assumed to be 93% (Bodwell, 1985).

RESULTS AND DISCUSSION

Proximate analysis revealed that the hexane defatted canola meal contained 12.3% moisture and 32.1% protein, 8.2% ash, 4.4% fat, and 55.4% carbohydrate on a dry weight basis. These measured values are comparable to the specifications provided by the meal manufacturer: 12% moisture, 4% fat on a wet basis, and 34% protein on a dry basis (CanAmera Foods, Inc.).

The majority of the protein was soluble when dispersed in 0.4% NaOH or 5% NaCl (Table 1). This solubility profile indicates isolated canola proteins are primarily glutelins and globulins. Protein extraction in all concentrations of NaOH was significantly increased by using baffled flasks: from 95.2 to 99.6% of total protein in the meal at 0.4% w/v NaOH (Figure 2). Maximum protein extractability was obtained with a 5% w/v meal ratio, 0.4% w/v NaOH extract for 60 min at 180−200 rpm (Figure 3). This represents an increase in protein extractability from defatted canola meal as reported in the literature, 80−95% (Ismond and Welsh, 1992; Diosady et al., 1984; Tzeng et al., 1988). Protein recovery values of 87.5% were obtained as compared to literature values of 33−65% (Gillberg and Tornell, 1976; Rohani and Chen, 1993; Xu and Diosady, 1994).

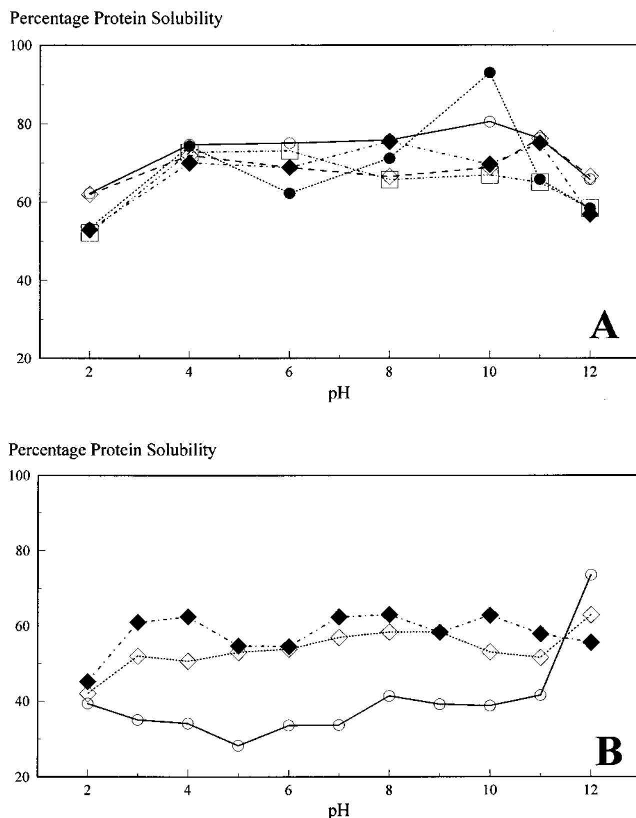

Figure 4. Solubility of CMPI at various pH values with ionic strength (A) and concentration of NaCl (B): ionic strength values of 0.2 (●), 0.4 (▲), 0.6 (□), 0.8 (◇) and 1.0 (○); NaCl concentrations of 0.1 M (◇) and 1.0 M (◆); and distilled deionized water (○).

Protein solubility was <40% in pH-adjusted deionized water and between 50 and 70% when NaCl was added to the solutions at pH values of 3−11 (Figure 4B). At pH 2, solubility was not affected by the presence of NaCl. Generally, solubility leveled off between pH 4 and 11. In this pH range, solubility in the presence of 1 M NaCl at most pH levels was greater than at 0.1 M. Solubility in deionized water was maximum at pH 12 (73.5%).

Solubility in pH-adjusted deionized water in the presence of $CaCl_2$ (Figure 4A) was greater than in the presence of NaCl. At pH 2, adding $CaCl_2$ to an ionic strength of 0.2 increased solubility to 53.3% compared to 39.4% in deionized water. Further increases in ionic strength (0.4−1.0) reduced solubility. Solubility improved slightly in the presence of $CaCl_2$ versus NaCl. The highest solubility of 92.9% was at pH 10 in the presence of $CaCl_2$ at an ionic strength of 0.2 (Figure 4A). If the number of nonpolar regions on the surface of proteins is limited, the solubility will increase at ionic strengths <0.5. This increase in solubility is due to a decrease in ionic activity and has also been shown in β-lactoglobin (Damodaran, 1996). Isolated canola proteins were most soluble in high pH salt solutions but <60% soluble at pH levels below 10 regardless of the salt concentration. This limited solubility in the pH 5−7 range normally encountered in food will need to be improved for application of the protein as a food ingredient.

The amino acid profile of untreated canola meal was similar to that of published values for high erucic acid rapeseed protein (Shahidi, 1990) with lower values for cystine and valine (α = 0.01, Table 2, superscript 5). CMPI contained significantly more leucine, phenylala-

Table 2. Amino Acid Profiles of Defatted Canola Meal and Protein Isolates[a]

amino acid	ref values HEAR[b]	ref values soybean[c]	defatted meal	protein isolate
essential				
histidine	2.7	2.77	2.57	2.45
isoleucine	4.0	4.97	2.82	3.03[3,4,6]
leucine	7.0	7.81	7.22	7.52[4]
lysine	5.8	6.98	5.62	4.74[3,6]
methionine	1.9	1.38	1.74	1.91
cystine	1.7	1.45	0.77[5]	0.63
phenylalanine	3.8	5.41	3.84	4.36[4]
tyrosine	3.1	3.43	2.88	3.02
threonine	4.5	4.40	4.54	4.13
valine	5.0	5.25	3.66[5]	3.77
nonessential				
alanine	4.3	4.66	4.71	4.66
arginine	5.8	7.91	5.38	6.33[4,6]
aspartic acid	7.0	12.80	7.82	8.32[3,4]
glutamic acid	17.5	20.47	17.75	17.28
glycine	4.9	4.57	5.25	5.38
proline	6.0	6.01	5.82	5.00
serine	4.6	5.60	4.81	4.71

[a] Values are given as percentage of total protein. Numbers followed by different superscripts have been shown to be significantly different (α = 0.01) through t-test analysis. Four comparisons were analyzed: [3], canola protein isolate and soybean reference values; [4], canola protein isolate and rapeseed reference values; [5], defatted canola meal and rapeseed reference values; [6], compared defatted meal and protein isolate. [b] High erucic acid rapeseed (Shahidi, 1990). [c] Nehez (1985).

nine, arginine, and asparagine and lower isoleucine than high erucic acid rapeseed reference protein (α = 0.01, Table 2, superscript 4). When compared to defatted canola meal, CMPI contains more isoleucine and arginine and lower lysine (α = 0.01, Table 2, superscript 6). Observed differences between CMPI and published values for rapeseed proteins may indicate changes in seed storage proteins resulting from the genetic manipulations involved in the development of canola varieties of rapeseed, low in both erucic acid and glucosinolate content.

Lysine was the only essential amino acid in CMPI present in significantly lower levels than measured in the crude commercial hexane defatted canola meal (α = 0.01, Table 2, superscript 6). Although there is a decrease in measured lysine, no significant change in serine or cystine content was observed. No lysinoalanine was detected in the isolate, which follows previous reports of lysinoalanine levels <100 ppm in commercial hexane extracted seed (Deng et al., 1990). The levels of isoleucine, lysine, and aspartic acid in CMPI were lower than those in soybean protein isolates (Nehez, 1985) (α = 0.01, Table 2, superscript 3).

Protein quality is indicated by the calculated PDCAAS values (Table 3). PDCAAS scores <1.00 indicate an amino acid deficiency, while scores ≥1.00 are considered equivalent when proteins are compared. All PDCAAS scores for CMPI based on reference values for average infants were <1.00, with lowest scores for methionine and cystine. The lowest essential amino acid PDCAAS score based on requirements for 2−5-year-olds is for lysine. For comparison, the lowest score for soybean protein for this age group is methionine plus cysteine (Henley and Kuster, 1994). All PDCAAS scores calculated for 10−12-year-olds and adults were >1.00.

PDCAAS analysis indicates lower protein quality for CMPI than soy protein for average infants and 2−5-year-olds. If CMPI is to be used in products for infants, blending CMPI with other proteins will be necessary to balance the amino acid profile. The limiting amino

Table 3. PDCAAS Values for CMPI[a]

amino acid	CMPI	CMPI AAS	ref values[b] a	b	c	d	PDCAAS a	b	c	d
His	24.5	22.8	26	19	19	16	0.87	1.20	1.20	1.42
Ile	30.3	28.2	46	28	28	13	0.61	1.01	1.01	2.17
Leu	75.2	69.9	93	66	44	19	0.75	1.06	1.59	3.68
Lys	47.4	44.1	66	58	44	16	0.67	0.76*	1.00*	2.75
Met + cystine	25.4	23.6	42	25	22	17	0.56*	0.94	1.07	1.39*
Phe + Tyr	73.8	68.6	72	63	22	19	0.95	1.09	3.12	3.61
Thr	41.3	38.4	43	34	28	9	0.89	1.13	1.37	4.27
Val	37.7	35.1	55	35	25	13	0.64	1.00	1.40	2.70

[a] Amino acid content values expressed in mg/g of protein. References values are FAO/WHO standards for (a) average infants, (b) 2−5-year-olds, (c) 10−12-year-olds, and (d) adults. Asterisks (*) indicate the lowest amino acid score in each age category. [b] Henley and Kuster (1994).

acids for soy protein and CMPI are complimentary for 2−5-year-olds; the two could be blended for nutritional supplements for this age group. Since all PDCAAS scores for the canola protein isolate are ≥1.00 for both 10−12-year-olds and adults, this protein represents an excellent source of dietary protein for products formulated for both of these age groups. Both CMPI and soy protein have equivalent nutritional quality for these two age groups.

CONCLUSIONS

The reported method for the extraction and isolation of protein from crude commercial hexane defatted canola meal has significantly increased extractability and protein recovery. PDCAAS analysis indicates limited impact of the isolation method on the nutritional quality of CMPI. The nutritional quality of canola protein isolates prepared with other methods has not been reported. CMPI may nutritionally compliment soy protein in products for 2−5-year-old children. The two proteins are nutritionally equivalent for 10−12-year-olds and adults. Thus, CMPI shows great potential for utilization as an alternative protein source for products targeting these age groups currently utilizing soy protein and soy protein hydrolysates. Limited CMPI solubility in the pH 5−7 range will need to be addressed in future investigations before application of the protein as a nutritional and functional food ingredient.

ABBREVIATIONS USED

AAS, adjusted amino acid score; BCA, bicinchoninic acid; BSA, bovine serum albumin; CMPI, canola meal protein isolate; HPLC, high-performance liquid chromatography; PDCAAS, protein digestibility corrected amino acid score; TCA, trichloroacetic acid.

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