ISOLATION AND CHARACTERIZATION OF PROTEIN FRACTIONS ISOLATED FROM CAMELINA MEAL

N. Li, G. Qi, X. S. Sun, D. Wang, S. Bean, D. Blackwell

ABSTRACT. *Camelina is a new oil crop in North America. Camelina meal, a by-product of the camelina oil extraction process, typically contains 10% to 15% residual oil and 40% crude protein. As camelina oil demand increases, utilization of camelina protein for value-added products is critical to food and biotechnology industries; however, few studies have been conducted on camelina proteins. In this study, camelina protein fractions (albumin, globulins, and glutelins) were isolated from camelina meal based on their solubility using three different sequences: method 0 (S0), method 1 (S1), and method 2 (S2). The proteins' physicochemical properties, including solubility, amino acid profiles, molecular weight, and thermal and morphological properties, were also characterized. Results showed that S1 harvested more protein (88.20%) than S0 (84.05%) and S2 (76.52%). Glutelin was the major fraction (64.64%) in camelina, followed by globulin (17.67%), and albumin (10.54%). Essential amino acids accounted for approximately 40% of the total amino acids in camelina protein. High molecular weight aggregates stabilized by covalent bonds in the glutelin and albumin fractions, as shown in size-exclusion chromatography (SEC), are closely related to larger-size protein aggregates observed in TEM images.*

Keywords. *Albumin, Amino acid profiles, Camelina protein, FTIR, Globulin, Glutelin, Molecular weight, SEC, TEM, TGA.*

Camelina sativa, also known as camelina, gold-of-pleasure, false flax, wild flax, linseed dodder, or German sesame, is an important and ancient oil plant that originated in Germany around 600 B.C. (Budin et al., 1995). In North America, camelina is a new oil crop that was possibly introduced as a weed in flax. Camelina is an annual summer or wintering plant with a short mature period (85 to 100 days) (Budin et al., 1995; Sampath, 2009).

In general, camelina contains 29.9% to 38.3% oil, 23% to 30% protein, 10% carbohydrates, and 6.6% ash, depending on the variety and variations of soil composition and environment (Budin et al., 1995; Sampath, 2009). Camelina oil contains up to 90% unsaturated fatty acid, of which approximately 33.6% is α-linolenic acid (18:3, omega-3), which is lower than flaxseed (45.1%) but far exceeds canola (6.6%), soybean (7.2%), and sunflower (0%) (Budin et al., 1995). The high omega-3 content in camelina offers an opportunity to meet the growing demand for good-quality edible oils. Camelina oil also shows great potential as a source of biodiesel; in particular, it can be used to produce jet fuels that reduce greenhouse gas emissions up to 80% compared to petroleum-based jet fuel (Shonnard et al., 2010). Camelina meal is a by-product of the oil extraction process from camelina seed that typically contains 10% to 15% residual oil, 40% crude protein, 5% minerals, 10% to 12% crude fiber, and a small portion of vitamins (Sampath, 2009). As edible oil demands and biodiesel production increase, utilization of camelina protein for value-added products is critical to food and biotechnology industries.

Compared with other oilseeds such as canola, flaxseed, or soybean, camelina is less investigated for its protein research. However, protein isolation technologies applied to other oil seeds provided a framework for recovering proteins from camelina. According to previous studies, oilseeds usually contain mixed or heterogeneous proteins comprising different protein fractions (Manamperi et al., 2008; Ayad, 2010). Manamperi et al. (2008) isolated four protein fractions from canola meal based on protein solubility at different pH: albumins (water-soluble), globulins (5% NaCl-soluble), prolamins (70% ethanol-soluble), and glutelins (0.1 N NaOH-soluble). Results showed a protein recovery rate of 78.6%, among which albumins were the major fraction (38.7%), followed by globulins (22.0%), glutelins (10.3%), and prolamins (7.6%). These protein fractions were characterized by varied functional properties. Prolamins showed higher fat absorption, whereas globulins were characterized by better emulsifying activity. Ayad (2010) isolated flaxseed protein fractions from defat-

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ted flaxseed meal, and 38.1% albumin, 27.9% globulin, and 22.5% glutelin were extracted.

Camelina proteins are a mixture of protein fractions including albumins, globulins, and glutelins with varied solubility. Research on isolation and characterization of camelina protein fractions has not been reported. Therefore, the objective of this research was to study isolation processes for camelina protein fractions and to characterize the proteins' physicochemical properties, including solubility, morphological characteristics, and thermal properties, as well as amino acid profiles.

MATERIALS AND METHODS

MATERIALS

Camelina meal (CM) with 15% lipids (d.b.), 32.4% crude protein (d.b.), and 11.0% moisture content (w.b.) was provided by Montana Gluten-Free Processors (Belgrade, Mont.). Meal pellets emerged from a screw oil press at approximately 80°C. Hexanes, Bradford assay kit, hydrochloric acid (HCl), and sodium hydroxide (NaOH) were purchased from Fisher Scientific (Fair Lawn, N.J.).

CAMELINA MEAL DEFATTING

Camelina meal with particle size <0.5 mm was obtained by using a cyclone sample mill (Udy Corp., Fort Collins, Colo.). Camelina meal was then defatted with hexane at a solid/liquid ratio of 1:10 (w/v) for 2 h at room temperature in three cycles. The defatted camelina meal (DCM) was placed in a fume hood with a very thin layer (~2 mm) for 24 h to evaporate residual hexane.

MAXIMUM SOLUBILITY pH OF GLUTELIN

A standard curve was created first. Standard protein solutions were prepared using 0, 0.10, 0.25, 0.50, 0.75, and 1.00 mg protein mL^{-1} bovine serum albumin (BSA), and absorption readings of the solutions were measured with a BioMate 3 UV-Vis spectrophotometer (Madison, Wisc.) at 595 nm. The readings and known protein concentrations were interpolated in the calibration curve, and the standard curve was used to determine protein concentration in solutions tested in this study by spectrophotometer readings. The standard curve was reliable only in the range from 0.0 to 1.0 mg mL^{-1} of protein, and protein contents of the samples studied in this part of the experiment remained in this range.

Based on a preliminary test to determine the pH at which camelina glutelin protein has maximum solubility, 10 g of DCM was dispersed in 1000 mL of distilled water for 1 h. The slurry was then adjusted to a pH of 10 using 2 N NaOH and continuously stirred for 2 h. Five milliliters of the slurry was collected and centrifuged at 12,000×*g* for 15 min, and then the supernatant was decanted through a six-layer cheesecloth to remove impurities. All centrifugation conditions mentioned in this article were identical unless otherwise indicated. The remaining slurry was adjusted to pH 10.5, 11.0, 11.5, 12.0, 12.5, and 13.0 with 2 N NaOH, respectively, and then stirred for 2 h. Slurry samples were collected and centrifuged at each pH point. At the specified pH points, 100 µL of the supernatant was mixed with 3 mL of Bradford reagent for 10 min at room temperature, and absorbance of the solution was measured with the BioMate 3 spectrophotometer at 595 nm. Each sample was measured in triplicate.

SOLUBILITIES OF CAMELINA PROTEIN FRACTIONS

Glutelin: 10 g of DCM was dispersed in 1,000 g of distilled water with pH adjusted to 12 using 2 N NaOH. The slurry was stirred for 2 h and then centrifuged. The pH of the supernatant dropped from 12 to 1.0 in increments of 0.5. Slurry samples were collected and centrifuged at each pH point, and the supernatants were used to measure protein content.

Albumin: 10 g of DCM was mixed with 500 g of distilled water for 2 h, with stirring followed by centrifugation. The pH of the supernatant initially dropped to 6.0 and then dropped from 6.0 to 1.0 in increments of 0.5 with pH adjusted using 2 N HCl. Slurry samples were collected and centrifuged at each pH point, and the supernatants were used to measure protein content.

Globulin: 10 g of DCM was mixed with 500 g of 5% NaCl solution for 2 h, and the slurry was stirred and centrifuged. The pH of the supernatant initially dropped to 6.0 and then dropped from 6.0 to 1.0 in increments of 0.5 with pH adjusted using 2 N HCl. Slurry samples were collected and centrifuged at each pH point, and the supernatants were measured protein content.

ISOLATION OF CAMELINA PROTEIN FRACTIONS

Method 0 (S0): With this method, globulin 0 and glutelin 0 were isolated from two batches of DCM with the same solvents and centrifugation procedures as described for S1, respectively, without a degumming step.

Method 1 (S1): Protein can be fractionated into albumins, globulins, glutelins, and prolamins with different solvents (Osborne, 1924). Based on the preliminary testing, three protein fractions (albumin, globulin, and glutelin) were isolated with distilled water, 5% NaCl solution, and NaOH solution, respectively, from DCM. Prolamin was not studied because only trace amounts exist in CM.

As shown in figure 1, DCM samples were mixed with distilled water at a solid/liquid ratio of 1:30 (w/v), stirred for 2 h, and then centrifuged, and the residues were collected for further camelina protein fraction isolation. The supernatant was slowly adjusted to pH 3.0 with 2 N HCl and then centrifuged to precipitate the albumin fractions. The residues mentioned above were resuspended in water at a solid/liquid ratio of 1:30 (w/v), adjusted to pH 12 using 2 N NaOH with continuous stirring for 2 h, and centrifuged. The supernatants were adjusted to pH 4.5 and centrifuged to precipitate the protein fractions referred to as glutelin 1. After centrifugation, the resulting residues were collected for further fraction extraction. The residues were resuspended in 5% NaCl at a solid/liquid ratio of 1:30 (w/v), adjusted to pH 8.0 using 2 N NaOH, stirred for 2 h, and then centrifuged. The supernatants were adjusted to pH 3.0 with 2 N HCl and centrifuged to isolate the protein fractions referred to as globulin 1. All camelina protein fractions except albumins were washed with distilled water for

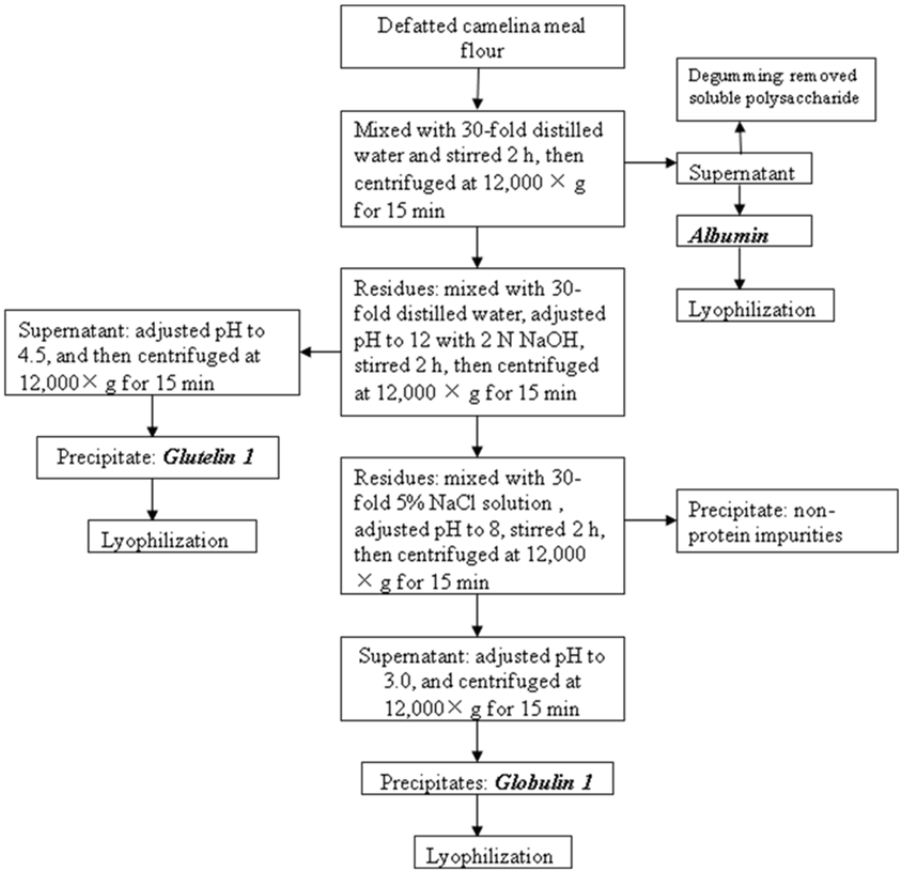

Figure 1. Flowchart of camelina protein fractions extraction procedure (S1).

three cycles, lyophilized, and ground into powder for further analyses.

Method 2 (S2): The main difference between S2 and S1 was that the globulin fractions were isolated after albumin but before glutelin. The protein fractions isolated with this method are referred to as globulin 2 and glutelin 2, respectively.

CHEMICAL ANALYSIS

Moisture content was measured with a V30 Compact Volumetric KF Titrator (Columbus, Ohio). Carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) contents were measured with a PerkinElmer 2400 Series II CHNS/O Elemental Analyzer (Shelton, Conn.). Nitrogen was converted to protein using a factor of 6.25. All tests were performed in duplicate.

AMINO ACID COMPOSITION ANALYSIS

Amino acids profiles in camelina proteins were measured using the method described by Li et al. (2011a). Approximately 100 mg of each sample was weighed and placed in 0.5 mL of 6 N HCl along with the internal standard and hydrolyzed at 110°C for 20 h. An aliquot, typically 10 or 20 µL, was diluted to 250 µL with 0.4 M borate buffer to raise the pH. After precolumn derivatization with o-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC), 1 µL of this diluent was injected into an HPLC system with a C18 column (Hypersil AA-ODS, 2.1 × 200 mm, 5 µm). Mobile phase A was 20 mM sodium acetate buffer containing 0.018% (v/v) triethylamine, 0.05 mM EDTA, and 0.3% tetrahydrofuran with pH adjusted to 7.2 using acetic acid. Mobile phase B was a mixture of 100 mM sodium acetate, acetonitrile, and methanol (20:40:40, v/v). Elution conditions progressed from 100% A to 60% B in 17 min with a flow rate of 0.45 mL min^{-1}. Amino acid derivatives were detected with a fluorescent detector at 340/450 nm (excitation/emission) for primary amino acids and 266/305 nm for secondary amino acids. Human serum albumin was used as a control, and norvaline and sarcosine were used as internal standards.

FOURIER TRANSFORM INFRARED SPECTROSCOPY

Fourier transform infrared (FTIR) data of 0.5 g of dried protein powders were collected in the region of 400 to 4000 cm^{-1} with a PerkinElmer Spectrum 400 FT-IR/FT-NIR spectrophotometer (Shelton, Conn.). Transmission spectra of 32 scans of each sample were collected at a resolution of 1 cm^{-1} in the reflectance mode. All samples were tested with duplications. Information on fat, carbohydrates, and protein in samples was given by absorptions. Because the objective of using FTIR was also to determine the relative amounts of α-helix and β-sheet secondary structure protein, a band shape of each peak resolved by deconvolution needed to be produced that would allow peak area determination as a method of quantitative analysis. Fourier self-peak deconvolution (FSD), the most widely used tool, was used to identify the α-helix and β-sheet in the protein amide I region, and modeling by the Peak Fitting Wizard

tool with Gaussian function in Origin 8.0 data analysis and graphing software was also used (OriginLab Corp., Northampton, Mass.) to obtain areas of individual protein forms. The α-helix and β-sheet content ratio was described as the ratio of peak areas (Wetzel et al., 2003; Yu et al., 2005).

TRANSMISSION ELECTRON MICROSCOPY

Transmission electron microscopy (TEM) was obtained using a model CM 100 TEM (FEI Co., Hillsboro, Ore.) operated at 100 kV. Camelina protein isolates were first dissolved in distilled water with a solids concentration of 0.05% (w/w). Prepared protein samples were absorbed for approximately 30 s at room temperature onto Formvar/carbon-coated 200-mesh copper grids (Electron Microscopy Sciences, Fort Washington, Pa.) and stained with 2% (w/v) uranyl acetate (Ladd Research Industries, Inc., Burlington, Vt.) for 60 s at room temperature before being viewed by TEM.

THERMAL GRAVIMETRIC ANALYSIS

Thermal gravimetric analysis (TGA) of camelina proteins was conducted with a Perkin-Elmer TGA 7 (Norwalk, Conn.) in a nitrogen atmosphere. Approximately 10 mg of ground powder was weighed into a platinum cup and scanned from 25°C to 900°C at a heating rate of 10°C min^{-1}. The maximum degradation rate was calculated as mass (%) at peak temperature divided by peak temperature.

SIZE-EXCLUSION CHROMATOGRAPHY

Size-exclusion chromatography (SEC) analysis was conducted as described by Bean et al. (2006). A high-performance liquid chromatography (HPLC) system (1100, Agilent, Palo Alto, Cal.) with a 300 mm × 7.8 mm BioSep-4000 column and security guard columns (Phenomonex, Torrance, Cal.) was used. The mobile phase was a pH 7 sodium phosphate buffer (50 mM) with 1% SDS added. Column temperature was maintained at 25°C, and flow rate was 1 mL min^{-1}. Samples for SEC analysis were redissolved in a pH 7 sodium borate buffer (12.5 mM) plus 1% SDS with or without 2% β-ME at a constant final protein concentration of 5 mg L^{-1}. Standard proteins, BSA (66 kDa), carbonic anhydrase (29 kDa), and glutathione (307.3 Da), were analyzed to estimate the molecular weight distribution of camelina protein fractions separated by SEC.

STATISTICAL ANALYSIS

Data from experiments carried out at least in duplicate were analyzed through analysis of variance (ANOVA) and least significant difference (LSD) at the 0.05 level according to procedures in the SAS statistical software package (SAS Institute, Inc., Cary, N.C.).

RESULTS AND DISCUSSION

CAMELINA PROTEIN SOLUBILITIES AND PRECIPITATION PROPERTIES

According to the Bradford standard curve, net absorbance at 595 nm showed a linear relationship with bovine serum albumin (BSA) at concentrations from 0.0 to 1.0 mg mL^{-1}. The linear relationship is expressed by equation 1 with R^2 of 0.999:

$$Y = 0.88901X + 0.0226 \quad (1)$$

where Y is absorbance at 595 nm, and X is protein concentration (mg mL^{-1}). As shown in figure 2, solubility of glutelin was highly sensitive to pH values. Solubility of glutelin increased slightly from pH 10.0 to pH 11.0 and then reached its peak value around pH 12.0. Therefore, pH 12 was used for the solubilization of camelina glutelin in this study.

The purpose of characterizing precipitation properties of camelina proteins was to identify the pH value at which the solubility is minimum (MS-pH). As shown in figure 3, the albumin and glutelin fractions exhibited a typical U-shaped solubility profile, whereas the globulin fraction showed a step shape. The lowest protein solubility was observed in the pH range from 2.5 to 3.0 for the albumin fraction and pH 4.0 to 5.0 for the glutelin fraction. For the globulin fraction, protein concentration decreased significantly from pH 6.93 to pH 4.0 and then leveled off from pH 4.0 to 12.0. In this case, the MS-pH values of camelina protein fractions were considered to be pH 3.0, 4.0 to 5.0, and 3.0 for albumin, glutelin, and globulin, respectively.

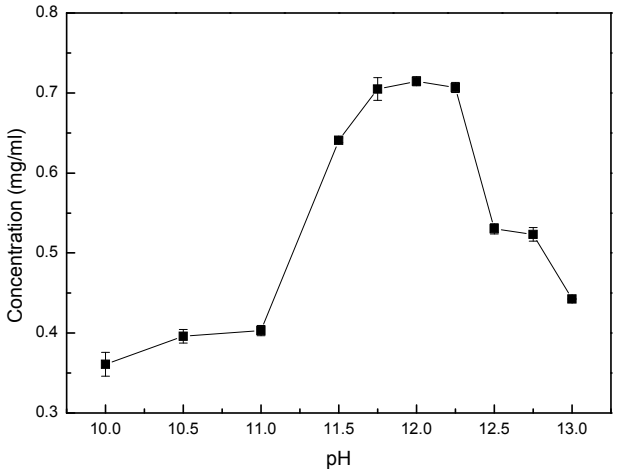

Figure 2. Effect of pH on solubility of glutelin.

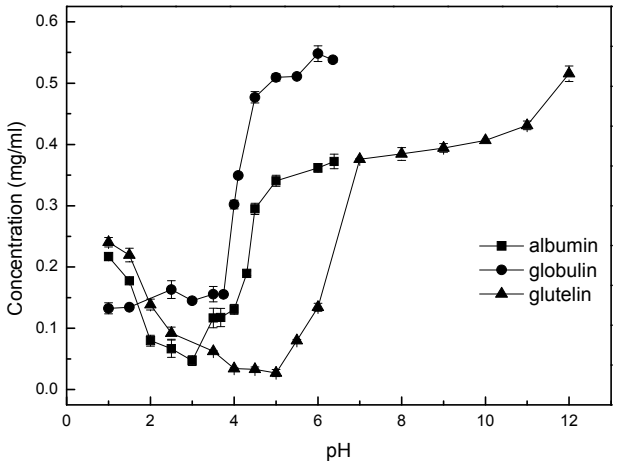

Figure 3. Effect of pH on precipitation properties of protein fractions.

Partial Proximate Analysis and Elemental Composition of Camelina Proteins

Table 1 shows the partial proximate and elemental compositions of camelina fractions. The DCM consisted of approximately 38.12% crude protein. For all three isolation methods, glutelin (48.32% to 64.64%) was the major fraction in protein, followed by globulins (13.03% to 17.67%) and albumins (10.54%).

More protein isolates were recovered with S0 (48.67%) than with S1 (41.54%) and S2 (36.86%); however, the protein purity with S0 (57.56% to 70.81%) was far lower than that of S1 (81.0% to 87.04%) and S2 (83.68% to 86.93%), which could be attributed to the presence of gum in S0. Similarly, the albumin fraction showed lower protein purity (56.57%) than the globulin and glutelin fractions (81.0% to 87.0%) due to gum, which was extracted along with the albumin protein and consequently led to low protein purity. In addition, much purer globulin 2 and glutelin 1 fractions were extracted than globulin 0 and glutelin 0, indicating that the presence of gum negatively affected the protein isolates' purity. The DCM-water slurry was very thick and sticky when those gums were present, possibly causing inefficient solubilization of protein in the slurry and resulting in lower protein extraction yield.

More glutelin was extracted in S1 than in S2, which could be attributed to the effect of NaCl. As described in similar studies, NaCl can negatively affect protein solubility in specific pH ranges. Carbonaro et al. (1997) found that lower solubility of fava bean, lentil, and chickpea proteins (all proteins had a MS-pH around pH 4.0) in NaCl at pH above 7.5 or with pH from 1.0 to 3.0 could be ascribed to increased hydrophobic interaction. Hydrophobic interactions are the driving force for protein-protein aggregation, leading to protein insolubilization. Makkar et al. (2008) also reported that recovery of Jatropha protein decreased with the presence of NaCl at pH 10 or 11 due to an increase in ionic strength and the resulting increase in hydrophobic interaction, or perhaps due to the "salting out" effect of NaCl on protein resulting from competition between charged proteins and salt ions for necessary water for solvation (Badifu and Akubor, 2001). In contrast, Carbonaro et al. (1997) indicated that the shielding of charged groups of dry bean by NaCl resulted in increased electrostatic repulsive force that reduced protein aggregation and therefore improved solubility. In this research, camelina glutelin 2 was solubilized at pH 12, which is far from its MS-pH (pH 4.0 to 5.0) with the presence of NaCl, resulting in a lower recovery rate for glutelin 2 due to strong ionic strength and protein aggregations driven from increased hydrophobic forces, as described by Makkar et al. (2008).

Some protein remained in the residues: 3.45% for S1 and 5.86% for S2. Furthermore, the sum of isolated pure proteins (%) and unextracted proteins in residues (%) was not 100%; instead, the sum was 97.24% for S1 and 91.89% for S2, implying that part of the proteins was lost during the extraction process.

The elemental composition of camelina proteins varied for different protein fractions (table 1). The glutelin and albumin fractions contained higher levels of C (42.33% to 49.22%), H (6.30% to 7.21%), and S (2.03% to 2.34%) than globulins (31.08% to 39.51%, 4.55% to 5.78%, and 1.62% to 1.75%, respectively). The sulfur in protein is known from the side-chains of amino acids methionine and cysteine (Brosnan and Brosnan, 2006). Higher levels of sulfur in albumin and glutelins coincide with higher content of methionine or cysteine in the amino acid profiles of camelina proteins, as shown in table 2.

Amino Acid Composition

Sixteen kinds of amino acids were detected and quantified in camelina proteins (table 2). Tryptophan and cysteine in camelina accounted for only 1.15% and 2.12% of the total amino acids, respectively (Zubr, 2002). However, tryptophan and cysteine were not detected because they were destroyed by the liquid HCl hydrolysis assay during the test, which may be one reason why the total sum of amino acids was lower than the protein content obtained by the nitrogen combustion method (table 1). Another reason may be the presence of non-protein nitrogen (NPN) in camelina. Although no published data are available on NPN content in camelina, NPN is very common in oilseeds. NPN content in soy, rape, and sunflower is up to 12.3%, 29.0%, and 15.4%, respectively, using 1% trichloroacetic acid as the extracting buffer (Bhatty and Finlayson, 1973).

Table 1. Partial proximate and elemental compositions of camelina meal and protein fractions produced by different isolation sequences.[a]

Camelina Protein Samples	Moisture Content (% d.b.)	Weight of Isolates Compared to DCM (% d.b.)	Protein Content (% d.b.)	Weight of Protein Compared to DCM (% d.b.)	Weight of Protein Compared to Total Protein in DCM (% d.b.)	Elemental Composition of Protein Sources (% d.b.)			
						C	H	N	S
DCM	9.35 a	-	38.12	-	-	45.31 b	6.92 a	6.10 af	2.12 c
Albumin	7.30 e	7.10 h	56.57	4.02	10.54	42.33 c	6.30 b	9.05 e	2.27 ab
Globulin 0	7.20 e	10.66 g	57.56	6.14	16.10	31.08 e	4.55 e	9.21 e	1.75 d
Glutelin 0	8.73 c	30.91 d	70.81	21.89	57.42	45.92 b	6.71 ab	11.33 d	2.22 b
Globulin 1	6.57 f	6.13 i	81.00	4.97	13.03	37.96 d	5.54 cd	12.96 c	1.62 e
Glutelin 1	6.87 g	28.31 e	87.04	24.64	64.64	49.22 a	7.12 a	13.93 a	2.34 a
Globulin 2	6.14 h	7.75 h	86.93	6.74	17.67	39.51 d	5.78 c	13.91 a	1.70 de
Glutelin 2	7.54 d	22.01 f	83.68	18.42	48.32	46.03 b	6.71 ab	13.39 b	2.03 c
Residue 1	9.03 b	40.30 b	8.55	3.45	9.04	27.76 f	3.91 f	1.37 h	0.94 g
Residue 2	9.03 b	48.11 a	12.18	5.86	15.37	39.15 d	5.35 d	1.95 g	1.26 f
Sum of S0		48.67 a		32.04	84.05				
Sum of S1		41.54 b		33.62	88.20				
Sum of S2		36.86 c		29.17	76.52				

[a] Means in the same column followed by different letters are significantly different at $p < 0.05$. DCM = defatted camelina meal.

Table 2. Amino acid composition (% of total) of DCM, canola meal, and camelina protein fractions.

Amino Acid[a]	Albumin	Globulin 0	Glutelin 0	Globulin 1	Glutelin 1	Globulin 2	Glutelin 2	DCM[b]	Canola Meal[c]
Essential									
Histidine	2.84	2.97	3.02	3.04	3.04	3.00	3.02	2.90	3.32
Isoleucine*	4.70	4.99	4.85	4.96	5.01	4.93	5.16	4.94	5.80
Leucine*	8.14	9.21	8.66	9.14	8.93	9.37	9.12	8.32	8.86
Lysine	5.88	4.42	5.41	4.36	5.05	4.12	5.93	5.93	5.85
Methionine*	1.85	1.83	2.14	1.68	2.27	1.73	2.46	1.70	1.74
Phenylalanine*	5.77	6.50	5.60	6.64	5.63	6.84	5.41	5.08	5.00
Threonine	5.35	4.74	5.14	4.78	5.05	4.73	5.81	5.26	5.45
Valine	5.75	6.09	5.71	6.09	5.77	6.11	5.84	5.63	6.05
Total essential	40.29	40.75	40.54	40.69	40.75	40.85	42.74	39.75	42.07
Non-essential									
Alanine*	5.87	5.36	5.78	5.29	5.62	5.22	6.26	5.68	5.40
Aspartate	11.45	11.56	9.97	11.19	9.70	11.83	9.68	9.84	8.44
Glutamate	18.46	19.23	19.11	19.03	19.24	18.92	15.65	19.94	22.37
Serine	6.41	5.98	6.12	5.91	6.02	5.92	6.44	6.09	5.47
Arginine	7.71	7.60	8.42	8.16	8.53	7.77	8.09	8.57	6.95
Glycine	6.24	5.88	6.13	5.95	6.12	5.79	6.46	6.40	6.07
Tyrosine	3.58	3.63	3.93	3.79	4.02	3.71	4.69	3.73	3.23
Ornithine	0	0	0	0	0	0	0	0	0
Total non-essential	59.71	59.24	59.46	59.31	59.25	59.15	57.26	60.25	57.93
Total amino acid	100	100	100	100	100	100	100	100	100
Total protein	46.35	51.05	61.84	67.38	68.91	76.10	72.07	34.13	34.84

[a] Asterisks (*) indicate hydrophilic amino acids.
[b] DCM = defatted camelina meal.
[c] Source: Li et al. (2011a).

Camelina proteins are characterized by high content of glutamate (18.46% to 19.23%), aspartate (9.68% to 11.83%), leucine (8.14% to 9.17%), arginine (7.60% to 8.57%), and phenylalanine (5.08% to 6.84%) but low content of ornithine (0%), methionine (1.68% to 2.46%), histidine (2.84% to 3.04%), and tyrosine (3.58% to 4.02%). Globulins showed lower levels of lysine, methionine, threonine, alanine, and glycine and higher levels of leucine, phenylalanine, valine, and aspartate. Notably, methionine, a sulfur-containing amino acid, was higher in glutelins than in globulins, which is attributed to a high sulfur content in glutelins. Compared with globulins, albumin had lower levels of isoleucine, leucine, phenylalanine, valine, and lysine, but albumin exceeded globulins for the content of lysine, threonine, alanine, serine, and glycine. Furthermore, albumin exhibited lower levels of histidine, leucine, methionine, glutamate, and arginine and higher level of lysine, threonine, and aspartate than glutelins.

Amino acids were classified into groups according to their physical, chemical, and structural properties. Nutritionally, camelina proteins contained approximately 40% essential amino acids that cannot be synthesized by human and many farm animals, and approximately 60% non-essential amino acids that can be produced in humans and animals. The percentage of essential amino acids in camelina is slightly lower than in canola protein (42%) (Li et al., 2011a), sorghum protein (48%) (Li et al., 2011b), and soy protein (49%) (Khorasani et al., 1990). The lysine content (4.12% to 5.88%) in all camelina protein fractions and the phenylalanine content (5.08% to 6.50%) in DCM, albumin, and glutelins were lower than World Health Organization (WHO) standards for children at 0.5 years old (6.4% for lysine, 5.9% for phenylalanine), but all essential amino acid contents in camelina protein meet or exceed WHO amino acid requirement standards for children over one year old and adults (WHO, 2007).

Based on hydrophobicity, amino acids can be grouped into hydrophobic (non-polar) and hydrophilic (polar) types. Hydrophobic amino acids have side-chains that do not prefer an aqueous environment. Betts and Russell (2003) reported that these amino acids are generally buried within the hydrophobic core of the protein or within the lipid portion of the membrane. Among the detected amino acids, alanine, methionine, phenylananine, isoleucine, and leucine belong to the hydrophobic group and account for approximately 26.34% to 28.40% of camelina protein fractions (table 2). Albumin showed the lowest hydrophobic property, whereas globulin 2 was the most hydrophobic. Hydrophobic properties of camelina proteins are comparable to canola protein (26%) but lower than soy protein (37%) and sorghum protein (57%) (Li et al., 2011a).

SIZE-EXCLUSION CHROMATOGRAPHY

Molecular weight (MW) distributions of camelina protein fractions were characterized with SEC in the presence and absence of reducing agents (fig. 4a). Under non-reducing conditions for all protein samples, three major peaks were detected at 8 min, 8.75 min, and 9.5 min, respectively. The major peak was around 66 kDa, except in the glutelin 2 fraction, which exhibited a major peak at approximately 9.5 min with MW less than 29 kDa. Globulin 0 and globulin 2 showed stronger peak intensity than globulin 1 at MW around 66 kDa, indicating more protein subunits with larger MW present in globulin 0 and 1. This difference in MW among globulins may be ascribed to effects of the initial protein isolating conditions and sequences. As stated in the protein isolation steps, globulin 0 and globulin 2 were extracted with 5% NaCl solution, whereas globulin 1 was extracted from the pellets already treated with NaOH solution. Some NaOH residue could have remained when performing globulin 1 isolation, so the globulin 1 fraction was extracted as a result of the combined action of both

NaCl and NaOH. In addition, the albumin and glutelin fractions both had a small peak around 5 min, indicating the presence of the high MW protein subunits. However, this peak was barely detected in the globulin fraction except globulin 1, which also could be attributed to the combined action of NaCl and NaOH in the globulin 1 fraction.

In the presence of a reducing agent (fig. 4b), the intensity of the peak around 66 kDa decreased significantly and shifted to MW lower than 29 kDa, indicating that disulfide-bonded cross-linked subunits were present in all protein fractions. Many studies have reported that glutelins from corn and wheat are composed of a high level of subunits linked by disulfide bonds (Nielsen et al., 1970; Masci et al., 1998). The high intensity of the peak at 66 kDa in the globulin 0 and 2 fractions prove that they contained more disulfide linkages; however, according to tables 1 and 2, more sulfur content and methionine were detected in the glutelin fraction, which should have translated into a larger number of disulfide bonds than in the globulin fraction. The reason for this may be due to the fact that NaOH could have destroyed cystine and cleft disulfide bonds during extraction (Nielsen et al., 1970; Tecson et al., 1971), thus leading to reduced disulfide bonds in the glutelin fraction. Furthermore, large MW peaks around 5 min were still detected under the reducing condition, indicating that protein aggregates were stabilized by covalent bonds other than disulfide bonds in those protein fractions.

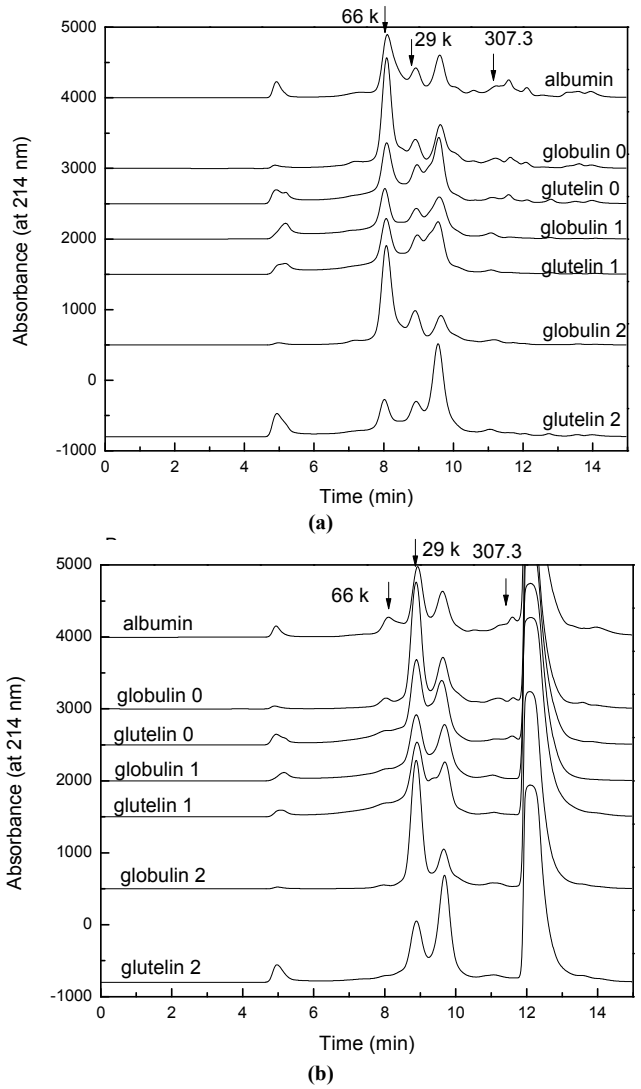

Figure 4. SEC separations of camelina proteins at (a) non-reducing and (b) reducing conditions.

FOURIER TRANSFORM INFRARED SPECTROSCOPY

As shown in figure 5a, typical oil absorption bands localized at 1710 and 1745 cm^{-1} (C=O stretching) and at 2853, 2924, and 3006 cm^{-1} (C-H stretching) were detected in the spectra of DCM (Guillén and Cabo, 1997). The significant diminishment or disappearance of these bands indicated only trace to low oil content in the isolated protein fractions. Absorptions in the range of 900 to 1250 cm^{-1} related to C-O stretching vibrations in polysaccharides were detected for all samples with different intensities. Consistent with previous results, albumin and proteins extracted with S0 with low protein purities (table 1) had higher peak intensity at 1050 cm^{-1}, indicating absorption of polysaccharides, than other fractions. It was reported that camelina seeds contained polysaccharides, which showed good water-binding capacity and were capable of aiding seed germination in dry environments (Grady and Nleya, 2010).

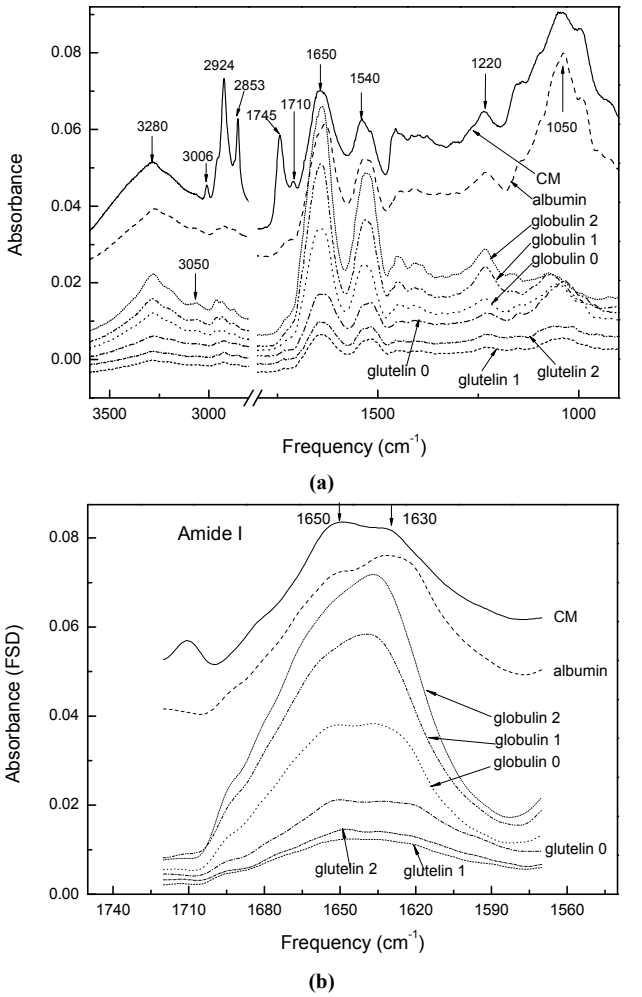

Figure 5. (a) FTIR analysis of camelina meal and proteins and (b) α-helix, β-sheet identification in amide I.

Table 3. Fourier self-peak deconvolution of amide I.

Camelina Protein Samples	α-Helix Frequency (cm^{-1})	α-Helix Area	β-Sheet Frequency (cm^{-1})	β-Sheet Area	Area Ratio of α-Helix to β-Sheet
CM[a]	1650	1.16	1630	1.04	1.12
Albumin	1650	1.09	1630	1.29	0.84
Globulin 0	1650	1.12	1634	1.12	1.00
Glutelin 0	1650	0.66	1628	0.64	1.03
Globulin 1	1650	1.54	1637	1.70	0.91
Glutelin 1	1650	0.39	1626	0.39	1.00
Globulin 2	1650	1.98	1635	2.18	0.91
Glutelin 2	1650	0.42	1630	0.40	1.05

[a] CM = camelina meal.

Protein units give rise to nine characteristic absorption bands, namely, amide A, B, and I to VII, among which the amide I and II bands are the most prominent vibrational bands of the protein backbone (Kong and Yu, 2007). The amide I absorption contains contributions from primarily C=O stretching vibrations (80%) with a minor C-N stretching vibration, whereas the amide II absorption appears to arise from N-H bending vibrations (60%) coupled with C-N stretching vibrations (40%) (Jackson and Mantsch, 1995). Peaks at 1630 and 1520 cm^{-1} are dominated by camelina protein secondary structures amide I and amide II, respectively (Yu et al., 2005). After deconvolution, the α-helix in amide I was shown at 1650 cm^{-1} for all protein samples (fig. 5b). Absorptions of β-sheet were in the frequency range of 1626 to 1637 cm^{-1}. Interestingly, absorption peaks of globulin fractions were at higher frequencies than those of glutelin and albumin fractions and DCM. The ratio of α-helix and β-sheet in amide I was quantified by the peak area (table 3). DCM showed higher a α-helix to β-sheet ratio (1.12) than the other samples, and albumin had the lowest ratio (0.84). Glutelin exhibited a higher α-helix to β-sheet ratio (1.03 to 1.05) than globulin (0.91 to 1.00), indicating a higher α-helix portion in the glutelin fraction.

MORPHOLOGICAL PROPERTIES

TEM images of camelina protein fractions are presented in figure 6 at 130,000× magnification. Albumin showed a spherical shape with diameters from 10 to 85 nm (image A in fig. 6), which is bigger than globulins (images B, D, and F in fig. 6) and glutelins (images C, E, and G in fig. 6) on average. All globulins isolated with various methods exhibited a spherical shape with similar diameters of around 10 nm (images B, D, and F in fig. 6) and distributed uniformly. For the glutelins (images C, E, and G in fig. 6), irregular and highly dense protein clusters were observed that comprised a mixture of spherical and rod-shaped clusters with diameters from less than 10 nm to several hundred nanometers. In short, larger protein aggregates were exhibited in albumin and glutelin fractions compared with globulin fractions.

Generally, aggregates of proteins may arise from several mechanisms and may be classified in numerous ways, including soluble/insoluble, covalent/non-covalent, reversible/irreversible, and native/denatured, thus influencing the amount of aggregate produced during the cell culture and purification process. As discussed earlier in the Size-Exclusion Chromatography section, high MW subunits stabilized by covalent bonds were observed in all glutelin fractions, which may contribute to the larger size of protein aggregates. Cromwell et al. (2006) concluded that disulfide bonds played an important role in protein aggregates and the resulting compact protein structures, but the glutelin fractions contained fewer disulfide bond-linked subunits than the globulin fractions (fig. 4a), indicating that disulfide bonds were insignificant in these larger aggregate formations. Moreover, oxidation of tyrosine may also result in covalent aggregation through the formation of bityrosine (Cromwell et al., 2006). In the amino acid profiles of camelina protein (table 2), glutelins contained more tyrosine than globulins, leading to the possibility that more covalent bonds could be formed through

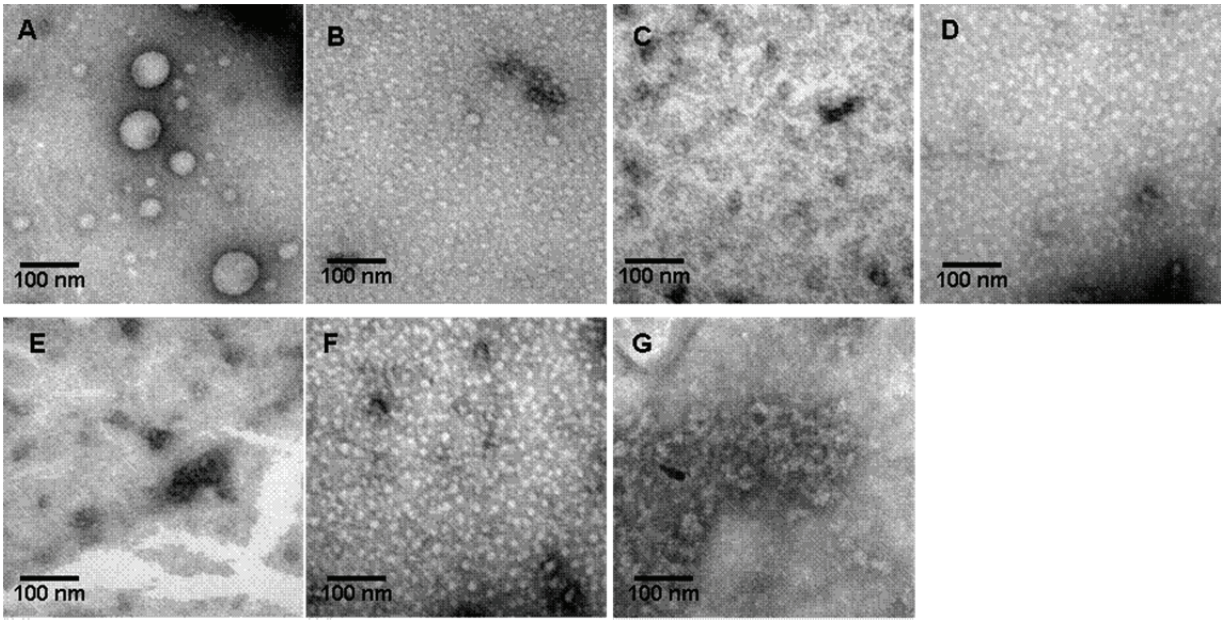

Figure 6. TEM images of DCM and camelina proteins: A = albumin, B = globulin 0, C = glutelin 0, D = globulin 1, E = glutelin 1, F = globulin 2, and G = glutelin 2. All images are at 130,000× magnification.

oxidation. The albumin fraction contained less tyrosine than globulins, suggesting that oxidation of tyrosine may play an insignificant role in protein aggregation.

THERMAL GRAVIMETRIC ANALYSIS

TGA and derivative thermogravimetry (DTG) curves are presented in figure 7 as the weight loss (%) and derivative weight loss rate (% min^{-1}), respectively, and as a function of sample temperature in the range of 25°C to 900°C. The degradation of camelina protein fractions underwent two to four stages with different final mass of retention residues. Noncombustible residues are inorganic materials. Albumin showed four stages (three stages for globulin 0 and glutelin 0, 1, and 2, and two stages for globulin 1 and 2), indicating that albumin had a more complicated composition.

In the first stage, the mass of the protein samples decreased by 3% to 7.5% as the temperature increased from 25°C to around 150°C, which could be ascribed to evaporation of both the free water and physically absorbed water in the samples. In this stage, the maximum mass loss occurred to albumin, indicating that albumin had the highest water absorption ability, possibly because albumin had the most hydrophilic nature (about 73% of hydrophilic amino acid, table 2) of the camelina protein fractions. Hydrophilic amino acid side-chains are known to be charged or polar and capable of attracting water molecules involved in the formation of hydrogen bonds. They are also predominantly found on the exterior surfaces of proteins (King, 2011). The second peak at 206.4°C for albumin was probably due to the degradation of water-soluble gum, which coexisted with albumin, as mentioned previously. Similarly, peaks with similar temperatures were also observed for the globulin 0 and glutelin 0 fractions extracted without the degumming procedure. In addition, the larger mass loss at 206.4°C for globulin 0 indicated a high content of gum, which is consistent with the low protein purity of globulin 0, as shown in table 1. Notably, peaks were detected at around 280°C to 290°C for all glutelin reactions, probably due to thermal breakage of weak non-covalent or covalent bonds.

The major peaks observed around 356°C for all the protein fractions are believed to be protein degradation, a process that involves breakage of intermolecular and intramolecular hydrogen bonds and electrostatic bonds, decomposition of protein side-chains, and rupture of weak bonds such as C-N, C(O)-NH, C(O)-NH_2, and NH_2 (Mo et al., 2011). Glutelins had a lower degradation peak (around 348°C) than globulins (around 352°C), with the exception of glutelin 2 (356°C). As explained previously, NaCl increased the ionic strength and resulted in enhanced hydrophobic interactions, thus improving the thermal stability of globulins and glutelin 2 in terms of the higher degradation peak. Molecular conformation can also affect the protein degradation rate. Again, the globulin fraction had higher mass retention at approximately 350°C (64.66% to 66.42%), which also suggests a higher thermal stability for globulin.

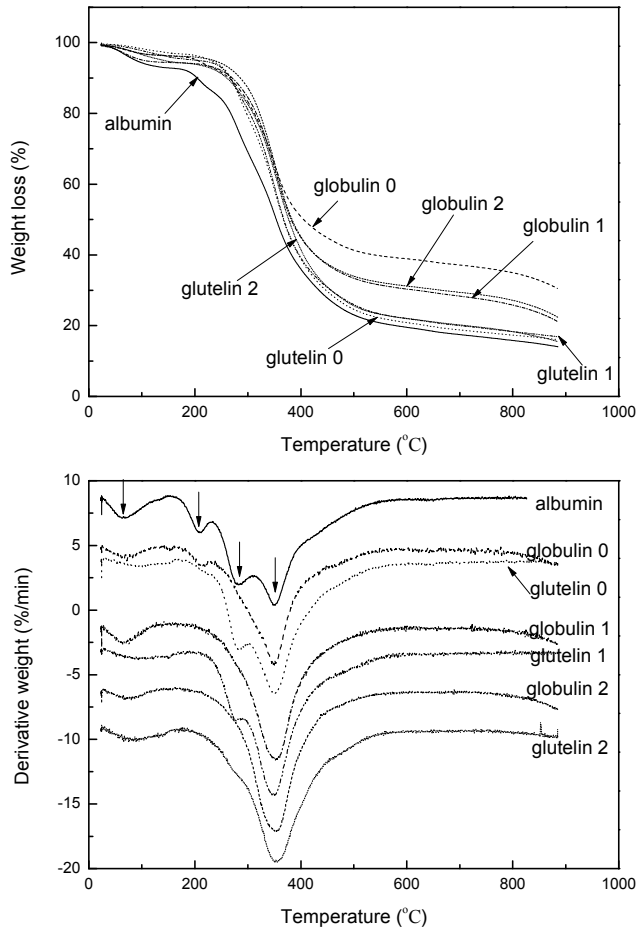

Figure 7. Thermogravimetric and derivative thermogravimetric curves of camelina proteins.

CONCLUSIONS

Physicochemical properties of camelina protein fractions, including solubility and precipitation abilities, amino acid profiles, molecular weight distributions, secondary structures, morphological properties, and thermal properties, varied in different protein fractions. The MS-pH of albumin, globulin, and glutelin were found at pH 3.0, 3.0, and 4.5 to 5.0, respectively. S0 extracted the highest amounts of protein isolates but the lowest protein purities due to the presence of gum. S1 was more effective than S0 and S2 in terms of protein recovery and purities. Essential amino acids accounted for approximately 40% of total amino acids, and essential amino acid profiles met or exceeded WHO standards for children over one year old and adults. Camelina proteins had 26% to 28% hydrophobic amino acids, which is lower than canola, soy, and sorghum proteins. Glutelins exhibited higher α-helix to β-sheet ratios (1.03 to 1.05) than the globulin fractions (0.91 to 1.00) and albumin (0.84). Studying the isolation process of camelina protein fractions and the proteins' physicochemical properties is vital to understanding camelina's unique functionality and thus exploring its applications in food and industrial areas such as biodegradable adhesives, plastics, or films.

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