Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 90 (2005) 271–279
Food Chemistry
www.elsevier.com/locate/foodchem

Isolation and structural characterization of the major protein fraction from NorMan flaxseed (*Linum usitatissimum* L.)

M.W.Y. Chung, B. Lei, E.C.Y. Li-Chan *

The University of British Columbia, Faculty of Agricultural Sciences, Food Science Building, 6650 NW Marine Drive, Vancouver, BC, Canada V6T 1Z4

Received 21 January 2003; accepted 14 July 2003

Abstract

Proteins extracted from dehulled and defatted flaxseed (NorMan cultivar) were fractionated by anion exchange chromatography to yield a major fraction with molecular weight of 365,000 Da, as determined by Sephacryl S-300 gel permeation chromatography. Reducing and non-reducing SDS–PAGE revealed three predominant bands (20, 23 and 31 kDa) and two predominant bands (40 and 48 kDa), respectively, as well as several minor bands. Isoelectric focusing separated three components having isoelectric points (p*I*) of 4.7, 5.1, and 5.6, with acidic (p*I* 4.5, 5.9, 6.1) and basic (p*I* 9.6) components being observed under reducing and denaturing conditions. The flaxseed major fraction had high disulfide but low sulfhydryl content, high contents of glutamate (or glutamine) and aspartate (or asparagine), and lower lysine/arginine ratio than soy or canola globulins. FT-Raman spectroscopy indicated high β-sheet content and a strong band near 1065 cm^{-1}, which is typical of intermolecular sheet interactions, supporting the oligomeric nature of the protein.

Keywords: Flaxseed; Protein; Subunits; Structural properties

1. Introduction

Flaxseed, also known as linseed, is enjoying an upsurge in popularity as a result of reports on its benefits to human health and its potential to reduce the risk of certain diseases (Oomah & Mazza, 2000). However, the current market for edible flaxseed, as well as recent research on its role in human health, has focussed on the whole intact flaxseed and its oil component (Oomah, 2001). The defatted meal, which is the residue after extraction of flaxseed oil, is presently being used as livestock feed, and limited attention has been given to the physicochemical and functional properties of the constituent proteins. This information is essential for increasing the utilization of flaxseed proteins, which have the potential to become important value-added products from the edible oil industry, as evidenced by the successful entry of soy proteins into the functional food ingredient market.

Flaxseed proteins were first isolated by Osborne in 1892, who reported the presence of a globulin with 18.6% nitrogen and an albumin-like protein with 17.7% nitrogen (Vassel & Nesbitt, 1945). Thereafter, research on flaxseed proteins has been concerned primarily with methods for the extraction of protein from the oil-crushed meal (Dev, Quensel, & Hansen, 1986a; Madhusudhan & Singh, 1983; Oomah, Mazza, & Cui, 1994; Painter & Nesbitt, 1946; Smith, Johnson, & Beckel, 1946; Sosulski & Bakal, 1969; Vassel & Nesbitt, 1945; Wanasundara & Shahidi, 1996, 1997).

Only a few studies have been conducted on the characteristics and functionality of the protein components fractionated from flaxseed. These studies reported that flaxseed consists of two major storage proteins, a predominant salt-soluble fraction with high molecular weight (11–12S), and a water-soluble basic component with low molecular weight (1.6–2S) (Dev, Sienkiewicz, Quensel, & Hansen, 1986b; Dev & Sienkiewicz, 1987; Madhusudhan & Singh, 1983, 1985a, 1985b; Marcone,

* Corresponding author. Tel.: +1-604-822-6182/3404; fax: +1-604-822-3959.
E-mail address: ecyl@interchange.ubc.ca (E.C.Y. Li-Chan).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2003.07.038

Kakuda, & Yada, 1998a; Youle & Huang, 1981), and suggest similarity between the properties of the major storage protein of flaxseed and those of other important oilseeds (Madhusudhan & Singh, 1985c, 1985d; Marcone, Kakuda, & Yada, 1998b, 1998c; Oomah & Mazza, 1993). However, most of the properties of the flaxseed major protein fraction are still awaiting investigation (Marcone, 1999; Oomah, 2001).

The main objective of this study was to isolate and characterize the major protein fraction from flaxseed of the NorMan cultivar, with the long-term objective to obtain basic information for understanding the structure–function relationship and potential applications of flaxseed proteins in foods. The molecular characteristics and structural properties that were investigated include the molecular weight of the major protein fraction and its constituent polypeptides, isoelectric point, amino acid composition, content of sulfhydryl groups and disulfide bonds, and molecular structure as analysed by FT-Raman spectroscopy.

2. Materials and methods

2.1. Materials

Flaxseed of the NorMan cultivar was obtained from the Agriculture and Agri-Food (AAFC) Diversification Research Centre, Morden, Manitoba. The seeds were dehulled at the AAFC Pacific Agri-Food Research Centre in Summerland, BC, and ground delipidated powder was prepared as previously described (Li-Chan & Ma, 2002; Li-Chan, Sultanbawa, Losso, Oomah, & Mazza, 2002b).

All chemicals used were of analytical reagent grade, and Tris buffers were prepared from ultra-pure grade Tris buffer salt (ICN Biochemical Inc., Costa Mesa, CA).

2.2. Extraction of flaxseed proteins

Flaxseed proteins were extracted from the dehulled, delipidated powder using 0.10 M NaCl in 0.10 M Tris buffer at pH 8.6, with a 1:16 (w/v) seeds to buffer ratio and magnetic stirring at 4 °C for 16 h (Li-Chan & Ma, 2002). The extract was then passed through a double layer of cheesecloth and centrifuged at 20,400*g* for 30 min at 8–10 °C. The protein extract thus obtained is hereinafter referred to in this study as the "whole extract".

2.3. Isolation of the major fraction of flaxseed proteins

The whole extract was loaded onto a 5×20 cm chromatographic column (Bio-Rad Laboratories, Hercules, CA) packed with 225 ml of DEAE-Sephacel (Amersham Pharmacia Biotech Inc., Quebec) that was pre-equilibrated with 0.10 M NaCl in 0.10 M Tris at pH 8.6 (conductivity of 10–11 mS/cm). The equilibrating buffer was used to wash out the unbound fraction (termed "flow through" fraction in this study). Preliminary experiments indicated that protein compositions were similar for fractions eluted by 0.15, 0.20 and 0.25 M NaCl and therefore, a single step elution of 0.25 M NaCl in 0.10 M Tris at pH 8.6 (conductivity of 19–22 mS/cm) was employed to elute the fraction which comprised 63.7% of the total proteins in flaxseed (termed the "major protein fraction" or simply "major fraction" in this study). Absorbance at 280 nm (Unicam UV2 Spectrophotometer, Analytical Technology Inc, Cambridge, UK) and conductivity (Yellow Springs Instrument Conductivity Bridge YSI Model 31, Yellow Springs, Ohio) were measured to monitor the progress of elution.

The major fraction was lyophilized (LABCONCO® model 75018, Labconco Corporation, Kansas City, MO), reconstituted with distilled–deionized water, and desalted by dialyzing against 5 mM Tris buffer at pH 8.6 using Spectra/Por® Molecularporous dialysis membrane (Spectrum®, Laguna Hills, CA) with molecular weight cut off of 6–8 kDa. The major fraction prepared according to the above-mentioned protocol in six replicate processes were pooled for subsequent analyses, after SDS–PAGE analysis confirmed the similarity of protein composition in the six preparations.

2.4. Analyses

Protein contents of solid samples were determined by the Leco nitrogen combustion method using a LECO FP-428 (LECO Cooperation, Joseph, MI) calibrated with ethylenediaminetetraacetic acid. Protein contents of liquid samples were analysed by the bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL) with bovine serum albumin as the protein standard.

The molecular weight (MW) of the major fraction of flaxseed protein was determined by size exclusion chromatography on Sephacryl S-300 (Amersham Pharmacia Biotech Inc., Quebec) according to Marcone et al. (1998a). The pooled 0.25 M fraction was dialysed against phosphate buffer (32.5 mM K_2HPO_4, 2.6 mM KH_2PO_4 and 0.4 M NaCl, pH 7.5); 40 mg protein was loaded to the S-300 gel filtration column which was pre-equilibrated with the same buffer, and eluted at a flowrate of 0.5 ml/min. Blue dextran and sodium azide were used to evaluate the void volume (V_o) and total elution (V_t) volume, respectively, and the elution volumes (V_e) of thyroglobulin, ferritin, catalase, alkaline phosphatase and α-lactalbumin with MW of 669, 440, 232, 140 and 14 kDa, respectively, were used to establish the calibration curve ($K_{av} = -0.3715 \log \mathrm{MW} + 1.3182$, $R^2 = 0.9966$). The available

partition coefficient value K_{av} was calculated as $(V_e - V_o)/(V_t - V_o)$.

Non-denaturing polyacrylamide gel electrophoresis (Native PAGE) was carried out according to the PhastSystem™ Separation Technique with PhastGel® gradient 8–25; the relative intensity of stained bands was measured using the PhastImage Analyzer (Amersham Pharmacia Biotech Inc., Quebec). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) was carried out with PhastGel® 10–15 and 8–25 gradient gels. BSA A-4503, ovalbumin A-5503, β-lactoglobulin L-6879 (Sigma, St. Louis, MO) and bovine IgG 55917 (ICN Biomedicals, Inc., Costa Mesa, CA) were electrophoresed as MW standards, and the MW of flaxseed protein samples was estimated from a Ferguson plot of log MW versus relative mobility. Isoelectric focusing (IEF) was carried out using PhastGel® pH 3–9 with Sigma IEF-Mix 3.6–9.3, I-3018 (Sigma, St. Louis, MO) as the standard markers. Approximately 2 μg of sample was loaded on each lane, and samples were loaded near the centre of the gels for isoelectric focussing. All PhastGels® were Coomassie stained according to the PhastSystem™ Development Technique.

Amino acid composition was analysed at the University of Victoria Protein Chemistry Centre (Victoria, BC). Samples were hydrolysed and applied to an Applied Biosystems Model 420 derivatizer/analyser system for analysis of PTC-derivatives by RP-HPLC. Amino acid compositions were expressed in picomoles and mole %.

The reactive and total sulfhydryl (SH) contents of the major fraction and the whole extract were determined using Ellman's reagent (5,5′-dithiobis-2-nitrobenzoic acid or DTNB), by modification of the methods of Beveridge, Toma, and Nakai (1974) and Mine (1997). Due to the high protein blank readings arising from the turbidity of samples in this assay, the values should be viewed with some caution. The total sulfhydryl plus disulfide (SH + SS) content was determined using the method of Thannhauser, Konishi, and Scheraga (1983) as modified by Damodaran (1984). The SH content and total SH + SS content were determined from the measured A_{412} values using an extinction coefficient of 13,600 M^{-1} cm^{-1}, and the SS content was calculated as the difference between the SH content and the total SH + SS content. Results of the SH and SS determination were also used to estimate the content of cysteine in the whole flaxseed protein extract and the major fraction, by assuming that each unit of SH was equivalent to one unit of cysteine and each unit of SS was equivalent to two units of cysteine.

The FT-Raman spectrum of the lyophilized major fraction was analysed on a ThermoNicolet Nexus 670 FTIR with FT-Raman module, with 1.064 μm laser excitation and InGaAs detector. The spectrum of Tris buffer salt was also measured and subtracted from the sample spectrum. The assignment of bands was based on the reported literature (Li-Chan, Ismail, Sedman, & van de Voort, 2002a; Lin-Vien, Colthup, Fateley, & Grasselli, 1991).

3. Results and discussion

3.1. Isolation by DEAE-Sephacel chromatography

A typical elution profile of the whole extract upon DEAE-Sephacel chromatography in 0.1 M Tris buffer at pH 8.6 is shown in Fig. 1. Approximately 1/3 of the protein extract was recovered in the flow-through fraction in the buffer containing 0.1 M NaCl, and a minor peak was obtained in the presence of 0.5 M NaCl. The majority (63.7%) of the proteins, or the "major protein fraction", was eluted by the buffer containing 0.25 M NaCl.

3.2. Molecular weight by gel permeation chromatography

Sephacryl S-300 chromatography of the major fraction yielded one predominant peak with V_e/V_o of 1.52 and K_{av} value of 0.366 (Fig. 2). In contrast, Marcone

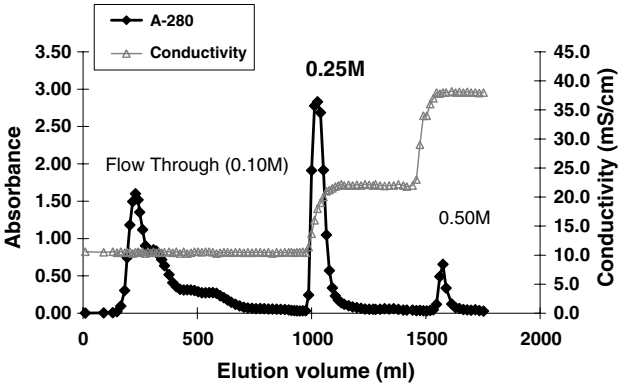

Fig. 1. Typical elution profile of flaxseed proteins on DEAE-Sephacel chromatography.

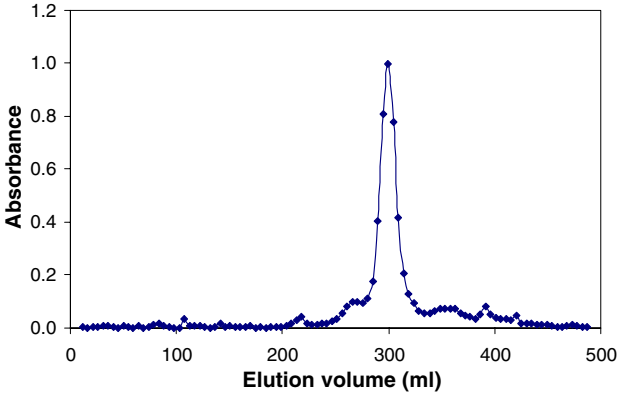

Fig. 2. Sephacryl S-300 gel permeation chromatographic profile of the major fraction of flaxseed proteins.

et al. (1998a) noted four peaks upon Sephacryl S-300 chromatography of the crude globulin preparation from flax – in addition to the major peak corresponding to 11S globulin, three other peaks were observed, including a turbid peak eluting near the void volume which was suggested to be nucleic acids and other non-identifiable high molecular weight components, a second peak that was thought to be polymerized aggregates of the 11S globulin and a final peak that was the 7S globulin. The observation of a single peak in the present study indicates the suitability of the anion exchange chromatography procedure used, to isolate 11S globulin from flaxseed protein extracts.

Based on the calibration curve constructed from proteins of known MW, the MW of the major fraction was estimated to be approximately 365 kDa. This value is slightly higher than the value of 320 kDa reported for salt-soluble globulins from flax by Marcone et al. (1998a), but well within the range of MW values typically reported for 11S globulins of other oilseeds. For example, MW in the 309–380 and 410 kDa ranges have been reported for 11S soybean and pea globulins, respectively (Marcone, 1999).

3.3. Polyacrylamide gel electrophoretic analyses

The native PAGE profile of the major fraction of flaxseed proteins (Fig. 3) showed a major component (relative intensity of $93.4 \pm 3.8\%$; $n = 6$) and two minor components ($1.0 \pm 0.3\%$ and $3.9 \pm 1.1\%$). Some material did not enter the gel and remained either at the point of sample application ($2.0 \pm 1.3\%$), or in the boundary between the stacking and the resolving gel ($0.8 \pm 0.4\%$). Marcone et al. (1998a) also reported the presence of a minor protein band of slightly higher relative electrophoretic mobility during native PAGE of peanut, buckwheat, alfalfa, caraway, cumin, amaranth, and soybean globulins, and attributed this to electrostatic repulsion between subunits of non-covalently linked protein subunits.

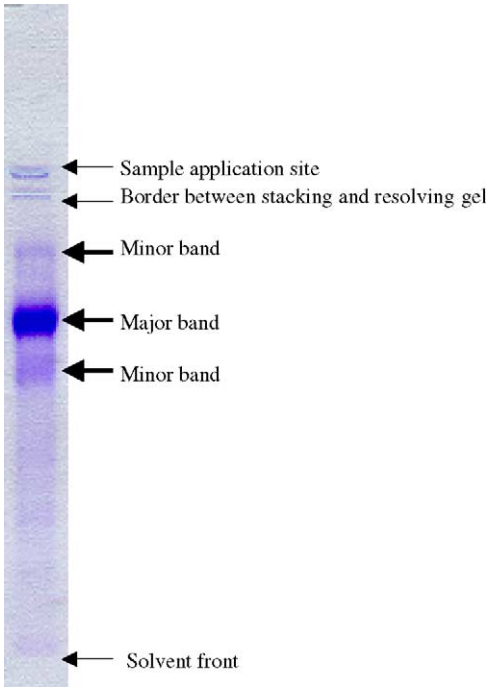

Fig. 3. Native PAGE of the major fraction of flaxseed proteins.

Fig. 4(a) shows the reducing SDS–PAGE profiles of the major fraction. Three predominant bands corresponding to MW of 20 ± 1, 23 ± 2 and 31 ± 1 kDa were observed, but at least 11 bands could be distinguished, including a number of minor bands with MW between 9 and 17 kDa and another minor band at 47 ± 6 kDa. Oomah and Mazza (1998) observed the presence of 4 predominant polypeptides in flaxseed meal products with MW of 14, 24, 25, and 34 kDa, as well as a number of other minor bands. The band intensities varied depending on whether the soluble proteins were extracted from undefatted versus defatted seed, flake, cake or meal. Marcone et al. (1998a) reported five components from flax globulin, with MW of 14.4, 24.6, 30.0, 35.2 and 50.9 kDa. The 24.6 kDa component was reported to correspond to basic subunits while the 30.0 and 35.2 kDa components were identified as the acidic subunits. The basic subunits (MW of 20–27 kDa) of plant globulins could be distinguished from the acidic subunits (30–39 kDa) based on amino acid sequence homologies and immunological cross reactivity (Marcone et al., 1998c).

Fig. 4(b) shows the non-reducing SDS–PAGE profiles of the major fraction. Among the 15 bands that were observed, those corresponding to approximate MW of 40 and 48 kDa were the most intense. The shifts in the intensities of bands from relatively higher MW components under non-reducing conditions (Fig. 4(b)) to lower MW components under reducing conditions (Fig. 4(a)) suggests the dissociation of disulfide-linked components into smaller subunits by reducing agents.

Madhusudhan and Singh (1985a) reported that SDS–PAGE of the 12S protein from flaxseed showed at least five non-identical subunits with MW of 11, 18, 29, 42 and 61 kDa, with the 18 and 42 kDa bands being more intense. Dev and Sienkiewicz (1987) carried out SDS–PAGE on the total protein as well as crude and purified 11S globulin from flaxseed, and found nine bands in the total protein samples, of which four were distinctly predominant over the others. The total number and MW of bands obtained in the crude and purified globulin were not reported; however, two and three predominant bands were found respectively, and "the crude and purified globulin was an oligomeric protein".

It should be noted that the subunit composition could differ depending on the variety or cultivar of flaxseed

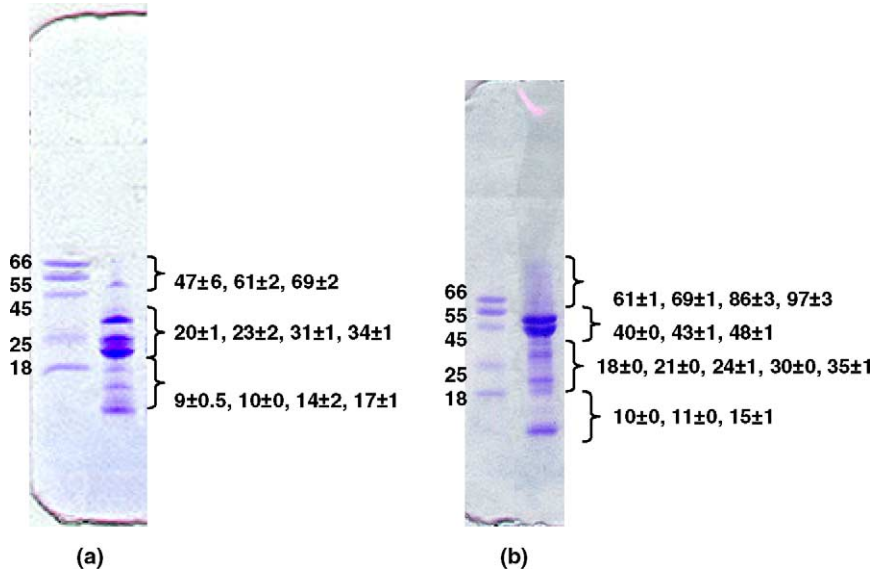

Fig. 4. SDS–PAGE of the major fraction of flaxseed proteins under: (a) reducing and (b) non-reducing conditions. The molecular weight (MW, in kDa) of the standard protein markers in lane 1 are shown on the left, while the values on the right are the MW values calculated for the bands in lane 2, representing the major fraction of flaxseed proteins, expressed as mean ± standard deviation ($n = 6$).

under investigation. In the present study, the major fraction was isolated from flaxseed of the NorMan cultivar. The studies of Madhusudhan and Singh (1983, 1985a, 1985b, 1985c, 1985d) used "linseed, Khategean variety", while no variety was specified in the reports of Oomah and Mazza (1998), Marcone et al. (1998a, 1998b, 1998c) or Dev and Sienkiewicz (1987). As reviewed by Utsumi, Matsumura, and Mori (1997), soy glycinin exhibits polymorphism of subunit composition among cultivars, and the amino acid sequence of each subunit may also differ among cultivars. The molecular heterogeneity leads to various molecular species with different subunit composition and molecular mass, which may be responsible for differences in functional properties among cultivars. Polymorphism and heterogeneity are also likely to exist for the major storage proteins found in different flaxseed cultivars, but to our knowledge, there have been no published reports on this aspect of flaxseed proteins.

3.4. Isoelectric focussing

IEF of the major fraction in 5 mM Tris at pH 8.6 resulted in two main bands with isoelectric points (p*I*) of 5.1 ± 0.0 and 5.6 ± 0.1, and a third less intense band with p*I* of 4.7 ± 0.0 ($n = 4$; data not shown). Vassel and Nesbitt (1945) also reported a p*I* at 4.75 for the linseed 12S protein. IEF conducted under denaturing and reducing conditions (Fig. 5, lane 1) indicated basic subunits with an extrapolated p*I* value of 9.6 ± 0.0, and acidic subunits with p*I* of 6.1 ± 0.0 and 5.9 ± 0.0 ($n = 3$). Minor components were observed, including two components with p*I* of 6.6 ± 0.0 and 4.5 ± 0.0.

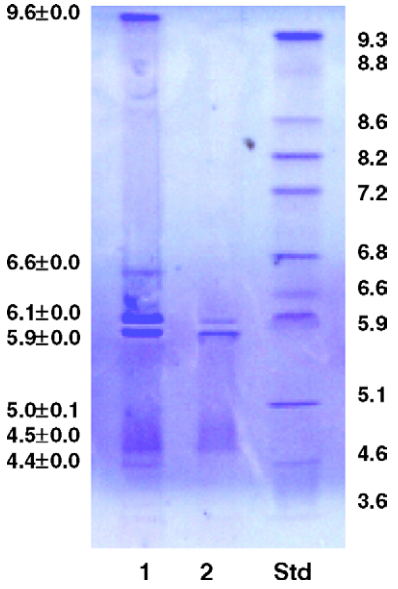

Fig. 5. Isoelectric focussing of the major fraction of flaxseed proteins. The isoelectric point (p*I*) of the standards (Std) are shown on the right, while the values on the left are the p*I* values calculated for the bands in lane 1, representing the major fraction of flaxseed proteins in the presence of 6 M urea and 10 mM dithiothreitol, expressed as mean ± standard deviation ($n = 3$). Lane 2 shows the major fraction profile in the presence of 6 M urea without dithiothreitol.

To our knowledge, the p*I* values of the acidic and basic subunits of flaxseed globulin have not been reported previously. However, the values observed from Fig. 5 are similar to the ranges reported for oat globulin by Peterson and Brinegar (1982), which were approximately 5.9–7.2 for the acidic subunits and 8.7–9.2 for the basic subunits.

Table 1
Amino acid compositions (g/100 g protein)[a] of the major protein fraction as compared with the whole extract from flaxseed, as well as reported values for 11 or 12S globulins from flaxseed, soybean and canola protein

Amino Acid		Flaxseed 12S protein	Flaxseed salt-soluble protein	Flaxseed 11S globulin	Soybean 11S glycinin	Soybean 11S glycinin	Canola 12S globulin	Major Fraction[b]	Whole extract[b]
		Madhusudhan and Singh (1985a, 1985b, 1985c, 1985d)	Dev and Sienkiewicz (1987)	Marcone et al. (1998a, 1998b, 1998c)	Garcia, Torre, Marina, and Laborad (1997)	Wolf and Nelsen (1996)	Gruener and Ismond (1997)	This study	This study
Aspartic acid	D	11.3	8.28	12.4	13.9	12.7	8.48	12.3 ± 0.09[c]	10.3 ± 0.12[c]
Glutamic acid	E	19.8	24.5	24.3	25.1	15.5	19.0	21.8 ± 0.94[c]	21.5 ± 0.59[c]
Serine	S	5.10	3.98	3.1	6.5	5.30	5.04	4.58 ± 1.64	6.36 ± 0.54
Glycine	G	4.80	5.55	5.4	5.0	7.73	6.01	5.61 ± 0.69	10.9 ± 0.98
Histidine	H	2.50	3.53	2.4	2.6	1.80	3.27	2.52 ± 0.12	1.78 ± 0.04
Arginine	R	12.5	10.2	12.6	8.9	5.51	8.31	11.9 ± 0.16	8.38 ± 0.37
Threonine	T	3.90	3.08	3.6	4.1	3.71	3.43	3.07 ± 0.86	3.93 ± 0.14
Alanine	A	4.80	3.83	5.5	4.0	5.55	4.24	5.67 ± 0.66	6.86 ± 0.22
Proline	P	4.50	4.09	0.0	6.9	6.17	8.29	4.17 ± 0.15	3.94 ± 0.34
Tyrosine	Y	2.30	3.16	2.4	4.5	2.77	2.91	2.44 ± 0.24	1.70 ± 0.25
Valine	V	5.60	4.32	5.1	4.9	5.72	3.87	4.65 ± 0.99	4.71 ± 0.80
Methionine	M	1.70	1.89	1.3	1.3	1.56	1.76	1.25 ± 0.33	1.31 ± 0.51
Cysteine	C	1.40	n.a[d]	0.9	1.7	0.70	1.78	(0.58 ± 0.14)[e]	(1.37 ± 0.05)[e]
Isoleucine	I	4.60	4.15	5.6	4.9	4.61	3.42	4.55 ± 0.38	4.16 ± 0.40
Leucine	L	5.80	4.54	5.9	8.1	7.04	8.18	5.83 ± 0.44	5.86 ± 0.43
Phenylalanine	F	5.90	4.30	6.3	5.5	4.30	5.22	5.81 ± 0.41	4.07 ± 0.48
Lysine	K	3.10	4.37	3.1	5.7	4.23	4.66	3.24 ± 0.12	3.44 ± 0.39

[a] Tryptophan was not analysed.
[b] Values are reported as means ± standard deviations of three analyses.
[c] Values for aspartic and glutamic acid may include asparagine and glutamine, respectively.
[d] n.a = data not available.
[e] Cysteine was not derivatized before analysis. Refer to text for estimation of Cys content by colorimetric assay.

3.5. Amino acid analysis

Table 1 shows the amino acid composition of the major protein fraction and whole extract from flaxseed in this study, in comparison to the composition of flaxseed, soybean and canola proteins reported in the literature. Flaxseed proteins, particularly the major fraction, contained high content of arginine, glutamate (and/or glutamine) and aspartate (and/or asparagine). An amino acid composition with high nitrogen content was reported to be important for supplying nitrogen for germination (Youle & Huang, 1981). Protein sources rich in arginine and glutamine have recently gained popularity because of the potential preventative functions of arginine against heart disease (Pszczola, 2002) and of glutamine in supporting the immune system (Oomah, 2001) and improving athletic performance (Blenford, 1996). The lysine/arginine ratio was 0.27 for the major fraction and 0.34 for the whole extract in this study, compared to 0.88 for both soybean and canola proteins as reported by Oomah and Mazza (2000), suggesting that flaxseed protein may be less lipidemic and atherogenic than either soybean or canola proteins (Oomah, 2001).

3.6. Sulfhydryl and disulfide contents

Table 2 shows the reactive SH, total SH, and SS contents for the major fraction and the whole extract. The flaxseed proteins were characterized by low SH content and high SS content, consistent with the observation from reducing and non-reducing SDS–PAGE that some of the components were disulfide-linked. Intermediary disulfide linked subunits have been reported in globulins from dicotyledonous plant seeds (Marcone et al., 1998a). The values for the flaxseed major fraction are similar to, but slightly lower than, the SH and SS contents of 15.84 and 104.64 μmol/g protein, respectively, reported for soy 11S protein (Peng, Quass, Dayton, & Allen, 1984).

Based on these SH and SS contents, the calculated cysteine contents of the major fraction and whole extract were 1.53 ± 0.05 and 2.93 ± 0.02 g/100 g proteins, respectively. The cysteine content of the major fraction was similar to the value (1.4 g/16 g nitrogen) reported for 12S linseed globulin by Madhusudhan and Singh (1985a).

3.7. FT-Raman spectroscopic analysis

Fig. 6 shows the FT-Raman spectrum of the lyophilized major fraction of flaxseed protein. The tentative assignments of the major bands are listed in Table 3.

A strong S–S stretching band near 525 cm^{-1}, with a shoulder at 505 cm^{-1}, is consistent with the high content of disulfide bonds measured by colorimetric assay (Table 2), while the presence of sharp bands at 3292 and 3350 cm^{-1} assigned to N—H stretching may be attributed to either arginine side chains or amide groups of asparaginyl and glutaminyl residues. The high intensity of bands assigned to aromatic side chains of tryptophan

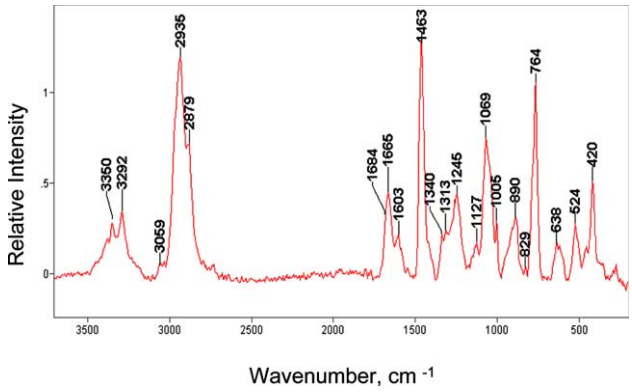

Fig. 6. FT-Raman spectrum of the major fraction of flaxseed proteins.

Table 3
Tentative assignment of bands in the FT-Raman spectrum of lyophilized major protein fraction from flaxseed

Wavenumber (cm^{-1})	Tentative assignment
420	*n*-alkane chain expansion
505 (s), 524	S—S stretch
638	C—S stretch
829	Tyrosine
764, 890, 1340	Tryptophan
1005	Phenylalanine
1069	Backbone
1245	Amide III
1453	C—H bend
1603	Tyrosine or phenylalanine
1665, 1684	Amide I
2879, 2935	C—H stretch
3059	=C—H stretch
3292, 3350	N—H stretch (amines or amides)

Table 2
Total and reactive sulfhydryl (SH) and disulfide (SS) contents[a] of the major fraction and whole protein extract from flaxseed

Sample	Reactive SH (μmol/g protein)	Total SH (μmol/g protein)	SS (μmol/g protein)
Major fraction	0.62 ± 0.09	3.40 ± 0.06	61 ± 2
Whole extract	0.71 ± 0.08	3.25 ± 0.10	121 ± 1

[a] Values reported are means ± standard deviations ($n = 2$ for SH, $n = 3$ for SS).

(764, 890 and 1340 cm^1), phenylalanine (1005 cm^{-1}) and tyrosine (829 and 1603 cm^{-1}), and to the C—H stretching (2879 and 2935 cm^{-1}) and C—H bending (1463 cm^{-1}) vibrational motions of aliphatic side chains, suggest the involvement of these residues in hydrophobic interactions of the flaxseed major protein fraction.

The locations of the Amide III band (near 1245 cm^{-1}) and of the Amide I band (centred at 1665 cm^{-1} with a shoulder at 1684 cm^{-1}) are indicative of low content of α-helix, and high contents of β-sheet and disordered structures. These observations are similar to those based on circular dichroic measurements of dilute solutions of flaxseed, showing 4.0% helix, 62.8% β-sheet, 16.2% β-turn and 17.0% random structure (Marcone et al., 1998b). A strong broad band near 1069 cm^{-1} is suggestive of aggregates or oligomeric nature of the protein; a similar feature that was observed in a heated lysozyme aggregate was assigned to C–C and C–N skeletal stretching vibrations typical of intermolecular sheet interactions (Howell & Li-Chan, 1996).

4. Conclusions

This study demonstrates the complex oligomeric nature of the major protein fraction of flaxseed. This fraction was shown to be composed of acidic and basic polypeptide chains that are linked by disulfide bonds and interact through hydrophobic groups as well as intermolecular sheet structures, producing an oligomeric protein with MW of 365 kDa. Careful investigation of the SDS–PAGE and IEF profiles revealed a number of minor components in addition to several predominant bands, suggesting molecular heterogeneity of the subunit composition of the protein referred to in earlier literature as the flaxseed 11S or 12S globulin. While the present study focussed only on the major protein fraction from flaxseed of a single cultivar (NorMan), it may be speculated that molecular heterogeneity and polymorphism may be exhibited by other flaxseed cultivars. Future research should be carried out to characterize the molecular structure and to determine the possible correlation between structure and functionality of the proteins from different flaxseed cultivars.

Acknowledgements

The authors wish to thank Drs B.D. Oomah, G. Mazza and J. McPherson of Agriculture and Agri-Food Canada for the gift of dehulled NorMan flaxseed and for helpful discussions related to this work. The technical assistance of Dr. G.T. Meng in collecting the FT-Raman spectral data is gratefully acknowledged. This research was partially funded by the Flax Council of Canada, the Natural Sciences and Engineering Research Council of Canada (NSERC), and Agriculture Agri-Food Canada, and by NSERC postgraduate scholarships to M.W.Y. Chung and B. Lei.

References

Beveridge, T., Toma, S. J., & Nakai, S. (1974). Determination of SH- and SS-groups in some food proteins using Ellman's reagent. *Journal of Food Science, 39*, 49–51.

Blenford, D. E. (1996). Use of amino acids and peptides in sports nutrition. *International Food Ingredients, 3*, 20–23.

Damodaran, S. (1984). Estimation of disulfide bonds using 2-nitro-5-thiosulfobenzoic acid: Limitations. *Analytical Biochemistry, 145*, 200–204.

Dev, D. K., Quensel, E., & Hansen, R. (1986a). Nitrogen extractability and buffer capacity of defatted linseed (*Linum usitatissimum* L.) flour. *Journal of the Science of Food and Agriculture, 37*, 199–205.

Dev, D. K., Sienkiewicz, T., Quensel, E., & Hansen, R. (1986b). Isolation and partial characterization of flaxseed proteins (*Linum usitatissimum* L.). *Die Nahrung, 30*, 391–393.

Dev, D. K., & Sienkiewicz, T. (1987). Isolation and subunit composition of 11S globulin of linseed (*Linum usitatissimum* L.). *Die Nahrung, 31*, 767–769.

Garcia, M. C., Torre, M., Marina, M. L., & Laborad, F. (1997). Composition and characterization of soybean and related products. *Critical Reviews in Food Science and Nutrition, 37*, 361–391.

Gruener, L., & Ismond, M. A. H. (1997). Effect of acetylation and succinylation on the physicochemical properties of the canola 12S globulin. Part I. *Food Chemistry, 60*, 357–363.

Howell, N., & Li-Chan, E. (1996). Elucidation of interactions of lysozyme with whey proteins by Raman spectroscopy. *International Journal of Food Science and Technology, 31*, 439–451.

Li-Chan, E. C. Y., & Ma, C.-Y. (2002). Thermal analysis of flaxseed (*Linum usitatissimum*) proteins by differential scanning calorimetry. *Food Chemistry, 77*, 495–502.

Li-Chan, E. C. Y., Ismail, A. A., Sedman, J., & van de Voort, F. R. (2002a). Vibrational spectroscopy of food and food products. In J. M. Chalmers & P. R. Griffiths (Eds.), *Handbook of vibrational spectroscopy* (Vol. 5, pp. 3629–3662). Chichester: Wiley.

Li-Chan, E. C. Y., Sultanbawa, F., Losso, J. N., Oomah, B. D., & Mazza, G. (2002b). Charaacterization of phytochelatin-like complexes from flax (*Linum usitatissimum*) seed. *Journal of Food Biochemistry, 26*, 271–293.

Lin-Vien, D., Colthup, N. B., Fateley, W. G., & Grasselli, J. G. (1991). *The handbook of infrared and Raman characteristic frequencies of organic molecules*. San Diego: Academic Press.

Madhusudhan, K. T., & Singh, N. (1983). Studies on linseed proteins. *Journal of Agricultural and Food Chemistry, 31*, 959–963.

Madhusudhan, K. T., & Singh, N. (1985a). Isolation and characterization of the major fraction (12S) of linseed proteins. *Journal of Agricultural and Food Chemistry, 33*, 673–677.

Madhusudhan, K. T., & Singh, N. (1985b). Isolation and characterization of a small molecular weight protein of linseed meal. *Phytochemistry, 24*, 2507–2509.

Madhusudhan, K. T., & Singh, N. (1985c). Effect of detoxification treatment on the physiochemical properties of linseed proteins. *Journal of Agricultural and Food Chemistry, 33*, 1219–1222.

Madhusudhan, K. T., & Singh, N. (1985d). Effect of heat treatment on the functional properties of linseed meal. *Journal of Agricultural and Food Chemistry, 33*, 1222–1226.

Marcone, M. F. (1999). Biochemical and biophysical properties of plant storage proteins: A current understanding with emphasis on 11S seed globulins. *Food Research International, 32*, 79–92.

Marcone, M. F., Kakuda, Y., & Yada, R. Y. (1998a). Salt-soluble seed globulins of various dicotyledonous and monocotyledonous plants.

I. Isolation/purification and characterization. *Food Chemistry, 62*, 27–47.

Marcone, M. F., Kakuda, Y., & Yada, R. Y. (1998b). Salt-soluble seed globulins of dicotyledonous and monocotyledonous plants. II. Structural characterization. *Food Chemistry, 63*, 265–274.

Marcone, M. F., Kakuda, Y., & Yada, R. Y. (1998c). Immunochemical examination of the physio-chemical properties of various dicotyledonous and monocotyledonous plants. II. Structural characterization. *Food Chemistry, 63*, 85–95.

Mine, Y. (1997). Effect of dry heat and mild alkaline treatment on functional properties of egg white proteins. *Journal of Agricultural and Food Chemistry, 45*, 2924–2928.

Oomah, B. D. (2001). Flaxseed as a functional food source. *Journal of the Science of Food and Agriculture, 81*, 889–894.

Oomah, B. D., & Mazza, G. (1993). Flaxseed proteins – a review. *Food Chemistry, 48*, 109–114.

Oomah, B. D., Mazza, G., & Cui, W. (1994). Optimization of protein extraction from flaxseed meal. *Food Research International, 27*, 355–361.

Oomah, B. D., & Mazza, G. (1998). Compositional changes during commercial processing of flaxseed. *Industrial Crops and Products, 9*, 29–37.

Oomah, B. D., & Mazza, G. (2000). Bioactive components of flaxseed: Occurrence and health benefits. In F. Shahidi & C. T. Ho (Eds.), *Phytochemicals and phytopharmaceuticals* (pp. 105–120). Champaign: AOCS Press.

Painter, E. P., & Nesbitt, L. L. (1946). Nitrogenous constituents of flaxseed. *Industrial and Engineering Chemistry, 38*, 95–98.

Peterson, D. M., & Brinegar, A. C. (1982). Oat storage proteins. In F. H. Webster (Ed.), *Oats: Chemistry and Technology* (pp. 153–203). St Paul: American Association of Cereal Chemists, Inc.

Peng, I. C., Quass, D. W., Dayton, W. R., & Allen, C. E. (1984). The physicochemical and functional properties of soybean 11S globulin – A review. *Cereal Chemistry, 61*, 480–490.

Pszczola, D.E. (2002). Genes and diet: The specialized role ingredients may play. *Food Technology, 54*(10), 82–84, 96.

Smith, A. K., Johnson, V. L., & Beckel, A. C. (1946). Linseed proteins: Alkali dispersion and acid precipitation. *Industrial and Engineering Chemistrry, 38*, 353–356.

Sosulski, F. W., & Bakal, A. (1969). Isolated proteins from rapeseed, flax, and sunflower meals. *Canadian Institute of Food Science and Technology Journal, 2*, 28–32.

Thannhauser, T. W., Konishi, Y., & Scheraga, H. A. (1983). Sensitive quantitative analysis of disulfide bonds in polypeptides and proteins. *Analytical Biochemistry, 138*, 181–188.

Utsumi, S., Matsumura, Y., & Mori, T. (1997). Structure–function relationships of soy proteins. In S. Damodaran & A. Paraf (Eds.), *Food proteins and their applications* (pp. 257–290). New York: Marcel Dekker.

Vassel, B., & Nesbitt, L. L. (1945). The nitrogenous constituents of flaxseed. II. The isolation of a purified protein fraction. *Journal of Biological Chemistry, 159*, 571–584.

Wanasundara, P. K. J. P. D., & Shahidi, F. (1996). Optimization of hexametaphosphate-assisted extraction of flaxseed proteins using response surface methodology. *Journal of Food Science, 61*, 604–607.

Wanasundara, P. K. J. P. D., & Shahidi, F. (1997). Removal of flaxseed mucilage by chemical and enzymatic treatments. *Food Chemistry, 59*, 47–55.

Wolf, W. J., & Nelsen, T. C. (1996). Partial purification and characterization of the 15S globulin of soybeans, a dimer of glycinin. *Journal of Agricultural and Food Chemistry, 44*, 785–791.

Youle, R. J., & Huang, H. C. (1981). Occurrence of low molecular weight and high cysteine containing albumin storage proteins in oilseeds of diverse species. *American Journal of Botany, 68*, 44–48.