ELSEVIER

Food Chemistry 77 (2002) 495–502

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

Thermal analysis of flaxseed (*Linum usitatissimum*) proteins by differential scanning calorimetry

E.C.Y. Li-Chan[a,*], C.-Y. Ma[b]

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

[b]*The University of Hong Kong, Department of Botany, Hong Kong*

Received 7 July 2001; received in revised form 8 October 2001; accepted 8 October 2001

Abstract

The thermal properties of flaxseed (whole or dehulled), dehulled and delipidated meal, as well as proteins extracted and isolated from the meal, were investigated by differential scanning calorimetry (DSC). A high denaturation temperature (T_d) of 114.7 °C was observed for the major fraction of flaxseed protein isolated by anion-exchange chromatography, representing the 11–12 S storage globulin. Marked decreases in T_d and enthalpy were observed at pH 3 compared with pH of 5 or higher, while the presence of high salt (1.0 M NaCl) resulted in higher thermal stability, enthalpy and greater cooperativity of the transition. Thermal analysis of the major fraction in the presence of chaotropic salts or protein structure perturbants (sodium dodecyl sulfate, urea, dithiothreitol, N-ethylmaleimide, ethylene glycol) suggested the contributions of hydrophobic and ionic interactions, hydrogen bonding as well as disulfide linkages or disulfide–sulfhydryl (SS–SH) interactions, to the thermal stability of flaxseed protein.

Keywords: *Linum usitatissiumum*; Flaxseed; Differential scanning calorimetry; Protein stability

1. Introduction

Flaxseed, also called linseed, is the small flat oval seed from flax (*Linum usitatissimum*), which has been gaining popularity in the health food market because of its reported health benefits and disease preventive properties (Oomah & Mazza, 2000). The current market for edible flaxseed is limited to the whole intact flaxseed and its oil, while the defatted meal, which is the residue after extraction of flaxseed oil, is primarily used as livestock feed. However, with the increasing demand for vegetable sources of proteins, there is a potential for utilizing flaxseed proteins as a food source.

Dietary proteins are proposed to have an effect on various diseases, including coronary heart disease, kidney disease and cancer (Oomah & Mazza, 2000). Similar to other plant storage proteins reported in the literature, the major protein isolated from flaxseed (cultivar NorMan) was shown to have high contents of the amino acids arginine, glutamate/glutamine, and aspartate/asparagine (Chung, 2001). Food sources rich in glutamine have been of interest due to the potential function of this amino acid in supporting the immune system and improving athletic performance (Blenford, 1996; O'Carrol, 1995), while sources rich in arginine have been reported to have potential preventative functions against heart disease (Pszczola, 2000; Sugano & Beynen, 1990). Proteins with low lysine/arginine ratio are suggested to be less lipidemic and artherogenic than proteins with higher ratio (Czarnecki & Kritchevsky, 1992; Park & Liepa, 1982). A lysine:arginine ratio of 0.33 was determined for NorMan flaxseed (Chung, 2001), compared with a ratio of 0.88 for soybean and canola proteins (Oomah & Mazza, 2000).

The protein content ($N\times5.41$) of 109 flaxseed accessions from the world collection was reported as being normally distributed, ranging from 20.9 to 48.1% and with a mean of 34.5% (Oomah & Mazza, 1995). Oomah and Mazza (1993) reviewed the literature on the protein and oil contents of different flaxseed cultivars, and on the amino acid composition, fractionation, and functional properties of proteins from flaxseed. To date,

* Corresponding author. Tel.: +1-604-822-6182; fax: +1-604-822-3959.

E-mail address: ecyl@interchange.ubc.ca (E.C.Y. Li-Chan).

0308-8146/02/$ - see front matter
PII: S0308-8146(01)00365-X

research indicates that flaxseed consists of two major storage proteins, the predominant fraction with high (11–12 S) and a minor component with low (1.6–2 S) molecular weight. Preliminary findings suggest that the 11–12 S protein has properties similar to the major storage proteins of other important oilseeds such as soy and canola. However, most of the properties of this major fraction of flaxseed proteins are still awaiting investigation. More in-depth understanding of the main storage proteins, the 11S globulins, from both mono and dicotyledonous plant seeds is vital for exploitation of plant seeds for nutritional, as well as pharmaceutical or nutraceutical, applications (Marcone, 1999).

Before plant foods including oilseeds and legumes can be consumed, they are usually subjected to various thermal processes. The detoxification of linseed meal by water boiling resulted in a 38% increase in the in vitro digestibility, but was accompanied by a 30% decrease in available lysine content (Madhusudhan & Singh, 1985a). The high molecular weight protein fraction from the detoxified meal was also dissociated (Madhusudhan & Singh, 1985a). Water-boiling reduced nitrogen solubility of linseed meal in water, NaCl and sodium hexametaphosphate; it also diminished the foam capacity and stability, emulsification capacity and fat absorption capacity but increased water absorption capacity of the linseed meal (Madhusudhan & Singh, 1985b).

Differential scanning calorimetry (DSC) can be used to study changes in proteins as a function of temperature. Thermally induced denaturation of proteins may be caused by disruption of various chemical forces. Endothermic changes are associated with the rupture of hydrogen bonds, while exothermic changes result upon weakening of hydrophobic interactions and aggregation of proteins. These changes are reflected in the DSC thermogram. Under controlled conditions of heating rate and protein concentration, thermal stability of a protein can be monitored by the denaturation temperature or peak transition temperature (T_d), while the proportion of undenatured protein or extent of ordered structure is reflected by the area under the endothermic peak, representing the enthalpy change ΔH (Arntfield & Murray, 1981). The sharpness of the transition peak, measured as width at half peak height ($\Delta T_{1/2}$), is an index of the cooperativity of the transition from native to denatured state (Privalov, 1982). Recently, Marcone, Kakuda, and Yada (1998a, 1998b) isolated and characterized a variety of salt-soluble seed storage globulins. Micro differential scanning calorimetric analysis of a 0.05% protein solution, in phosphate buffer at pH 7.5 containing 0.4 M NaCl, run between 30 and 118 °C at a ramp rate of 1.37 °C per min, indicated a T_d of 91.3 °C for the globulins from flax (Marcone et al., 1998b). However, the influence of medium composition or purity of the isolated globulins on the thermal properties was not investigated.

The objective of our investigation was to study the thermal properties of flaxseed proteins using DSC. Whole, dehulled, or dehulled and delipidated flaxseed, and the proteins isolated from the meal, were analyzed. The effects of pH, salt and protein structure perturbants were investigated for the major protein fraction isolated using anion-exchange chromatography. This study will provide information on the role of covalent and non-covalent chemical forces in stabilizing flaxseed protein conformation, with the long-term objective of elucidating factors affecting the functionality and utilization of flaxseed proteins in food and nutraceutical applications.

2. Materials and methods

2.1. Flaxseed

Flaxseed (NorMan cultivar), obtained from the Agriculture and Agri-Food Diversification Research Centre (Morden, Manitoba), was dehulled at the Pacific Agri-Food Research Centre (PARC) in Summerland, BC, by mechanically grinding the seeds through a Strong Scott barley pearler fitted with a 2-mm screen. The ground seeds were passed through an air separator (The Cuthbert Co. Ltd, Winnipeg, MB) to separate the hulls from the seeds. The mechanically dehulled seeds were further manually sorted until no hulls were observed, then ground through a no. 10 sieve with a Wiley Mill (Arthur, H. Thomas Co. Scientific Apparatus, Philadelphia, PA USA).

Delipidation was accomplished by three 1-h solvent extractions using methanol–chloroform, with a 1:10 dehulled seed to solvent ratio (w/v) and 1:1 (v/v) proportion of methanol to chloroform. The dehulled, delipidated flaxseed meal was recovered by filtering through a Whatman® filter paper number two (Whatman International Ltd, Maidstone UK) and air-dried overnight in the fume hood.

2.2. Extraction of flaxseed proteins

Flaxseed proteins were extracted by modification of the method of Oomah, Mazza, and Cui (1994). Extraction of proteins from dehulled, delipidated meal was carried out at 4 °C for 16 h with 0.10 M NaCl in 0.10 M Tris buffer at pH 8.6 as extraction buffer and 1:16 (w/v) seed-to-buffer ratio. The extract was passed through a double layer of cheesecloth to remove large residues, then centrifuged twice (at 10 400 ***g*** for 30 min, then at 20 400 ***g*** for 30 min at 8–10 °C). The protein extracts thus obtained (termed "whole extract" in this study) were dialyzed against 25 mM Tris buffer at pH 8.6 using a Spectra/Por® Molecularporous dialysis membrane (Spectrum®, Laguna Hills, CA) with molecular weight cut off of 6000–8000.

2.3. Isolation of major fraction of flaxseed protein

Dialyzed protein extracts were loaded onto 225 ml of DEAE-Sephacel (Amersham Pharmacia Biotech Inc., Quebec) packed in a Bio-Rad (5×20 cm) column, which was equilibrated with 0.10 M NaCl in 0.10 M Tris at pH 8.6. A step gradient elution of 0.10 M, 0.25 M and 0.50 M NaCl (1000, 500 and 250 ml, respectively) was employed. Absorbance at 280 nm (measured by a Unicam UV/vis Spectrophotometer UV2, Analytical Technology Inc, Cambridge, UK) and conductivity (measured with a Yellow Springs Instrument Conductivity Bridge Model 31, Yellow Springs, Ohio) were used to monitor the progress of elution. Preliminary experiments indicated similar protein compositions of fractions eluted by 0.15, 0.20 and 0.25 M NaCl; thus the major fraction of flaxseed protein, constituting 63.7% of the total proteins, was recovered by a single step elution using 0.25 M NaCl or conductivity of 19–22 mS/cm (Chung, 2001). The recovered fraction (termed "major fraction" in this study) was freeze-dried (LABCONCO® model 75018, Labconco Corporation, Kansas City, MO), reconstituted with distilled–deionized water, and dialyzed against 5 mM Tris buffer at pH 8.6 using a Spectra/Por® Molecularporous dialysis membrane (Spectrum®, Laguna Hills, CA) with molecular weight cut off of 6000–8000.

2.4. Protein determination

Protein content of freeze-dried flaxseed and extracted protein samples was determined as 5.41×nitrogen content, measured by a combustion method (LECO FP-428, LECO Cooperation, Joseph, MI, USA).

2.5. Differential scanning calorimetry

The thermal properties of flaxseed (dehulled and dehulled, delipidated powder) and the extracted proteins (whole extract and major fraction eluted at 0.25 M NaCl from DEAE-Sephacel chromatography) were investigated using a TA 2920 modulated DSC thermal analyzer (TA Instruments, New Castle, DE). The procedure was as described by Meng and Ma (2001), and analyses were conducted in triplicate. Freeze-dried sample containing approximately 1 mg protein was accurately weighed into the aluminium pan, and 10 μl of buffer was added. The pan was hermetically sealed then heated from 25 to 140 °C at a rate of 10 °C/min. A sealed empty pan was used as the reference. The onset temperature (T_m), denaturation temperature (T_d), enthalpy of denaturation (ΔH) and cooperativity, represented by the width at half-peak height ($\Delta T_{1/2}$), were computed from the thermograms by the Universal Analysis Program, Version 1.9 D (TA Instruments).

The pH was adjusted as previously described by Harwalkar and Ma (1987), with the pH values selected to include those (3, 5, and 7) at which other physicochemical and functional properties (surface hydrophobicity, SS and SH contents, foaming, etc.) have been previously measured (Chung, 2001). For experiments involving additives (chaotropic salts and protein structure perturbants), buffers containing the additives were added to the pans, which were then sealed and equilibrated at 25 °C.

All experiments were performed in triplicate and the coefficient of variation ranged from 0.3 to 0.6% for T_m and T_d, and 5–10% for ΔH. Some of the thermograms were comprised of two overlapping peaks, and only the ΔH of the combined transitions were measured, due to difficulties in accurately estimating the partial areas of the overlapping transitions.

3. Results and discussion

3.1. Effects of purification on thermal properties

Fig. 1 shows the thermograms of flaxseed samples at pH 7 and 0.01M NaCl, at different stages of purification. A single peak was observed for the whole seed (Fig. 1a), while two overlapping peaks were observed for the dehulled seed (Fig. 1b) and dehulled, delipidated meal (Fig. 1c). One major peak with a shoulder was observed for the whole extract (Fig. 1d) and a single sharp peak was observed for the major fraction (Fig. 1e).

The purified major fraction was observed to have a much higher enthalpy ΔH value (16.8 J/g protein) than the other samples (10.4–13.3 J/g protein), suggesting that constituents removed from the seed and meal may suppress thermally induced protein unfolding. Hydrophobic interactions between these components and protein would result in an exothermic contribution to the enthalpy value, thus lowering the observed ΔH (Arntfield & Murray, 1981). The increase in enthalpy may also reflect the removal of nonproteinaceous material and some proteinaceous material associated with the cell wall. These materials increase the Kjeldahl nitrogen value used in calculating ΔH but do not actually contribute to the enthalpy change associated with protein denaturation, thus giving an apparently lower ΔH value (Arntfield & Murray, 1990). In a study on the influence of processing on thermal properties of fababean, field pea, soybean and canola preparations, it was observed that ΔH values increased as the protein level increased and the phenolic level decreased (Murray, Arntfield, & Ismond, 1985). It was suggested that ΔH values observed for proteins in complex food systems are affected by the native protein, their environment, association of the proteins through processing, as well as non-proteinaceous components in the system. The solvent used in the current study for delipidation of flaxseed could denature the proteins. However, it has been

observed that hexane extraction of lipids from canola and soybean did not lead to significant change in enthalpy, probably due to the low moisture content in the system (Murray et al., 1985). The conditions used for extraction of flaxseed proteins and isolation of flaxseed protein fractions were relatively mild and should not lead to marked changes in protein conformation.

Little change in T_d of the first transition peak was observed between the dehulled seed and dehulled, delipidated meal. A slight increase in the T_d of the second transition peak in the dehulled seed after delipidation suggests the influence of lipid (which constitutes approximately 40% of flaxseed on a dry weight basis) on the thermal stability of that protein fraction. The exothermic nature of hydrophobic protein–lipid interactions would be expected to result in a lower ΔH value in the presence of lipid, as was indeed observed in Fig. 1b (dehulled seed) compared wtih Fig. 1c (dehulled, delipidated meal).

The two overlapping peaks in the thermograms of the meal probably correspond to the two main protein components of flaxseed protein, the 1.6–2S water-soluble component and the 11–12 S salt-soluble globulin, respectively. As shown in the thermogram of the major fraction isolated by anion-exchange chromatography (Fig. 1e), the 11–12S flaxseed globulin has an exceptionally high T_d of about 114.7–C, which is higher than the values reported for other plant globulins, in the range of 90–105 °C (Marcone et al., 1998b), and oat globulin, with a T_d at 110 °C (Harwalkar & Ma, 1987). According to Privalov (1982), most proteins have insufficient hydrophobic interactions involved in maintenance of the "thermal core", thereby preventing denaturation temperatures above 110 °C. The specific enthalpy and entropy of the conformation transition of proteins from the native to denatured state has an upper limit that is reached above 140 °C and seems to be universal for all compact globular proteins (Privalov & Gill, 1988). The high T_d observed in this study, compared with that (91.3 °C) reported by Marcone et al. (1998b) for globulins from flaxseed, could be due to measurement at a faster heating rate of 10 °C/min in this study. Heating rate has been shown to affect T_d measurements (Wright, 1984). Differences in purification steps could also lead to protein preparations with different DSC characteristics. Nevertheless, these results indicate that the major fraction of flaxseed protein exhibits high thermal stability.

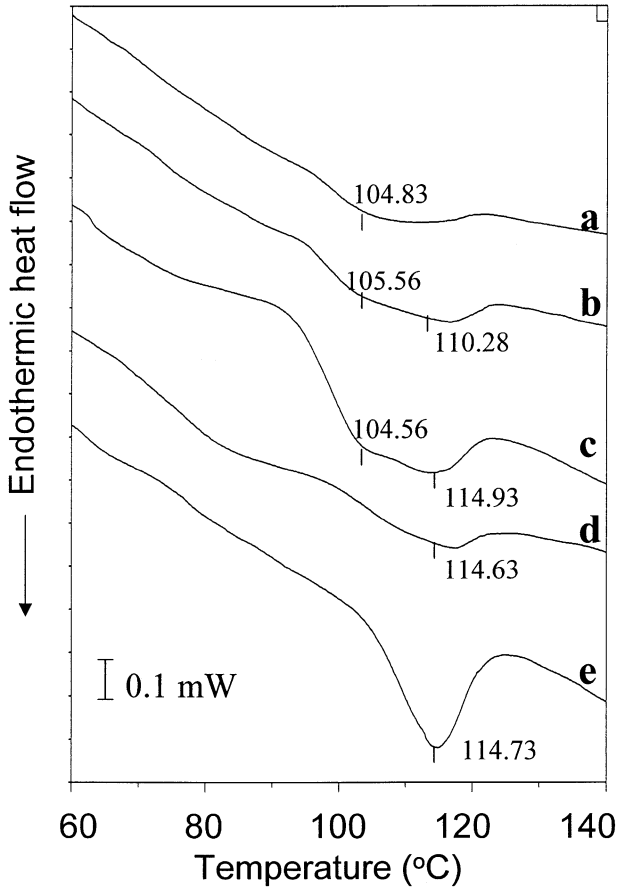

Fig. 1. Differential scanning calorimetric (DSC) thermograms of flaxseed fractions. a, whole seed; b, dehulled seed; c, dehulled, delipidated meal; d, whole extract; e, major fraction.

3.2. Effects of pH and NaCl concentration

Fig. 2 shows the thermal transition properties of the flaxseed major fraction as a function of pH (3–11) and NaCl (0.01 or 1.0 M). At both salt concentrations, the highest T_d and ΔH values were observed at pH 5–7 (Fig. 2a) and at pH 5 (Fig. 2b), respectively, and marked decreases in T_d and ΔH were observed at pH 3. At alkaline pH, there was a slight decrease in T_d (Fig. 2a), while enthalpy was progressively lowered (Fig. 2b). The $\Delta T_{1/2}$ value was increased at both acidic and alkaline pH extremes (pH 3 and 11) at 0.01 M NaCl, indicating decreased cooperativity (Fig. 2c).

The presence of high salt concentration (1.0 M NaCl) resulted in higher thermal stability for the major fraction at each of the pH conditions studied (Fig. 2a). The higher salt condition also resulted in higher enthalpy values (Fig. 2b) and greater cooperativity of the transition, as indicated by much lower $\Delta T_{1/2}$ values (Fig. 2c).

Most proteins are stable over a specific pH range, normally near the isoelectric pH, when the repulsive forces are quite low and therefore the proteins remain in a native state. At high or low pH, large net charges are induced and repulsive forces increase, resulting in unfolding of proteins (Morrissey, Mulvihill, & O'Neill, 1987). The isoelectric point of the major fraction or 12 S flaxseed protein lies close to pH 4.75 (Chung, 2001; Vassel & Nesbitt, 1945), and the DSC data indicate highest stability near this pH range. The unfolding of proteins at extreme pHs may also be attributed to rupture of hydrogen bonds and a breakup of hydrophobic

interactions (Privalov & Khechinashviti, 1974). The pH-induced changes in conformation may lead to a less cooperative system, as indicated by higher $\Delta T_{1/2}$ values. The DSC data are consistent with the trends measured by a fluorescence probe assay, which showed a marked reduction in surface hydrophobicity of the major fraction at pH 3 compared with pH 5 and 7 (Chung, 2001). Similar changes in thermal characteristics with pH were reported for globulins from oat (Harwalker & Ma, 1987) and fababean (Arntfield, Murray, & Ismond, 1986).

The heat stability of proteins is controlled by their balance of polar and nonpolar residues (Bigelow, 1967), with higher heat stability (higher T_d) for proteins having higher proportions of nonpolar residues. Protein conformation can be perturbed by the addition of salts that affect the electrostatic interactions among charged groups and polar groups, and influence the hydrophobic interactions via modification of the structure of water (Arakawa & Timasheff, 1982; von Hippel & Scheich, 1969). At high concentrations of NaCl (>1.0 M), the solubility of protein may decrease due to a "salting-out" phenomenon, which causes the aggregation or precipitation of protein molecules resulting from competition between the protein and ions for water (Morrissey et al., 1987). In this case, a more compact protein conformation is formed with increased thermal stability (higher T_d). The present data indicate that the conformation of flaxseed protein is favoured by higher salt concentration, as indicated by higher enthalpy and lower $\Delta T_{1/2}$ values at 1.0 M NaCl than at 0.01M NaCl at all pHs studied. The pH-induced changes in DSC characteristics were also less marked in the presence of 1.0 than 0.01 M NaCl. Similar changes in thermal stability with salt concentrations were also observed in oat (Harwalkar & Ma, 1987) and some legume (Arntfield et al., 1986; Meng & Ma, 2001) globulins, although enthalpy and half-peak width values were normally not affected. This suggests a greater influence of ionic strength on flaxseed protein than other plant proteins.

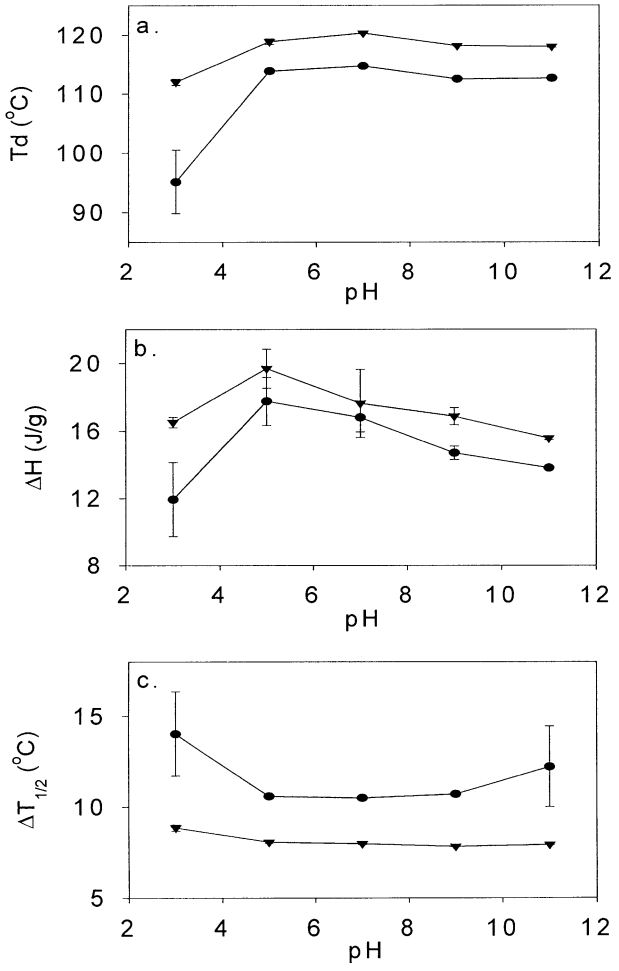

Fig. 2. Effects of pH and salt concentration on differential scanning calorimetric (DSC) characteristics of flaxseed major fraction. a, T_d (°C); b, ΔH (J/g protein); c, $\Delta T_{1/2}$ (°C). ●, 0.01 M NaCl; ▼, 1.0 M NaCl. The error bars represent standard deviations of the means.

3.3. Effects of chaotropic salts

The effects of the sodium salts of chloride, bromide, iodide and thiocyanate (1.0 M) on the thermal transition properties of the major fraction are shown in Table 1. A progressive reduction in thermal stability was observed following the lyotropic series from $Cl^- \rightarrow Br^- \rightarrow I^- \rightarrow SCN^-$ (Hatefi & Hanstein, 1969). The changes in thermal stability were accompanied by a progressive decrease in enthalpy and increase in the width at half-peak height following the lyotropic series.

Chloride and bromide ions promote salting-out and aggregation due to high molar surface tension increments, and high concentrations of these anions resulted

Table 1
Effects of chaotropic salts on the thermal transition properties of the major fraction of flaxseed proteins[a]

	T_m (°C)[b]	T_d (°C)[c]	ΔH (J/g protein)[d]	$\Delta T_{1/2}$ (°C)[e]
Control	104.50±0.38	114.73±0.27	16.8±0.85	10.50±0.06
Cl^-	113.48±0.38	120.34±0.10	17.6±2.01	7.96±0.04
Br^-	108.00±0.02	115.02±0.12	15.6±0.65	8.09±0.02
I^-	99.56±0.79	108.02±0.28	14.1±0.24	9.18±0.49
SCN^-	95.60±0.72	104.03±0.24	13.1±0.21	9.67±0.19

[a] Means±standard deviation of triplicate analyses. The sodium salts of Cl^-, Br^-, I^- and SCN^- were used in 1.0 M concentration. The control contained 0.01 M NaCl in pH 7 buffer.
[b] On-set temperature.
[c] Denaturation temperature.
[d] Enthalpy.
[e] Width at half peak height.

in higher onsets as well as peak denaturation temperatures than the control in 0.01 M NaCl. On the other hand, iodide and thiocyanate ions are destabilizing anions because of their higher hydration energy and steric hindrance, which promote unfolding, dissociation and salting-in of proteins (Boye, Ma, & Harwalkar, 1997), and their de-stabilizing effects were reflected in lower onset and peak denaturation temperatures compared with the control.

Similar effects of these chaotropic salts on thermal stability were observed in globulins from oats (Harwalkar & Ma, 1987) and red bean (*Phaseolus angularis*; Meng & Ma, 2001). For these two proteins, enthalpy values also decreased progressively in the presence of salts following the lyotropic series.

3.4. Effects of protein structure perturbants

The effects of a number of protein structure perturbants, including the denaturing agents sodium dodecyl sulfate (SDS) and urea, and the sulfhydryl reducing or blocking agents dithiothreitol (DTT) and N-ethylmaleimide (NEM), on the thermal transition properties of the major fraction are shown in Table 2.

The incorporation of 20 mM SDS resulted in lower onset and denaturation temperatures, and slight decrease in the enthalpy, as well as broader transition of the major fraction of flaxseed protein (Table 2). SDS is an anionic detergent, which interacts with the hydrophobic regions of protein molecules through its dodecyl hydrocarbon chain, causing unfolding and destabilization (Steinhardt, 1975). Similar effects of SDS on thermal properties were observed in the globulins from oat (Harwalkar & Ma, 1987), fababean (Arntfield & Murray, 1990) and red bean (Meng & Ma, 2001).

Only a slight decrease in thermal stability was observed in the presence of 20 mM DTT, but marked reduction in thermal stability resulted with 100 mM DTT, alone or in conjunction with 20 mM SDS (Table 2). DTT is a reducing agent which reduces the disulfide bond of cystinyl residues to sulfhydryl groups. Our data are consistent with other oligomeric proteins, such as soy glycinin and oat globulin (Brinegar & Peterson, 1982; Wolf & Tamura, 1969), in which the breaking of disulfide linkages by DTT led to dissociation of the oligomers and marked decreases in thermal stability and enthalpy. Like other 11 S globulins, the major fraction of flaxseed protein is composed of polypeptide subunits which are linked by disulfide bonds, with 61.4 μmols S–S/gram protein, as measured by colorimetric reaction with 2-nitro-5-thio-sulfobenzoate (Chung, 2001). The high content of disulfide linkages may contribute to the high thermal stability of flaxseed globulin. It is interesting to note that the T_d (approx. 90 °C) and the enthalpy of denaturation (13.4 J/g protein) remained fairly high, even in the presence of 100 mM DTT and 20 mM SDS, suggesting that the dissociated monomers may also exhibit significant DSC response. N-ethylmaleimide, a sulfhydryl-blocking reagent, also led to marked changes in DSC characteristics, especially at the higher concentration (Table 2). The results indicate that although the flaxseed major fraction has a relatively low sulfhydryl content of 3.4 μmols SH/gram protein (Chung, 2001), SS–SH interchange reactions may play a major role in stabilizing the conformation of the protein molecules.

Table 2
Effects of denaturing and reducing agents on the thermal transition properties of the major fraction of flaxseed proteins[a]

	T_m (°C)[b]	T_d (°C)[c]	ΔH (J/g protein)[d]	$\Delta T_{1/2}$ (°C)[e]
Control	104.50±0.38	114.73±0.27	16.8±0.85	10.50±0.06
20 mM SDS	95.11±1.91	108.65±1.21	15.0±0.78	13.22±1.00
20 mM DTT	101.41±0.27	111.97±0.36	16.8±0.37	10.58±0.17
100 mM DTT	82.19±2.13	92.36±0.80	14.4±0.47	14.90±1.08
20 mM SDS + 100 mM DTT	80.19±1.33	90.98±0.71	13.4±1.12	14.36±1.23
20 mM NEM	96.67±0.53	111.06±0.40	16.7±0.50	14.63±0.87
100 mM NEM	91.31±1.42	106.06±1.14	12.8±0.57	15.67±0.30
6 M Urea	107.03±0.36	117.47±0.48	4.92±0.15	14.10±0.56

[a] Means±standard deviation of triplicate analyses. The control contained 0.01 M NaCl in pH 7 buffer.
[b] On-set temperature.
[c] Denaturation temperature.
[d] Enthalpy.
[e] Width at half peak height.

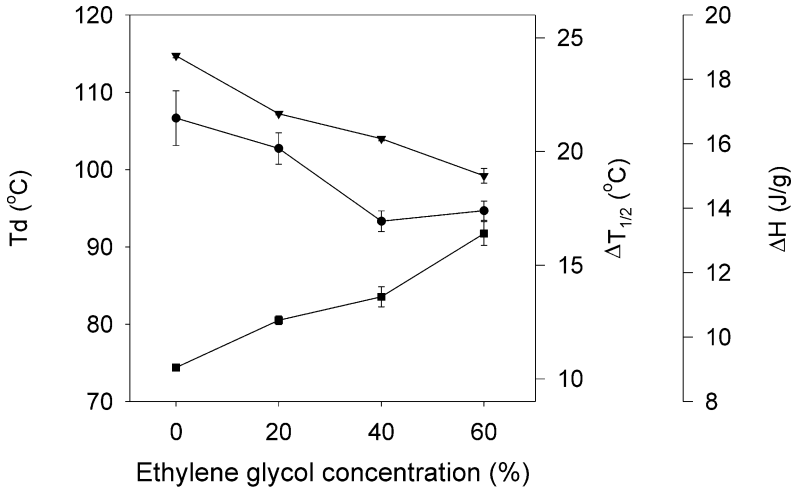

Fig. 3. Effects of ethylene glycol on differential scanning calorimetric (DSC) characteristics of flaxseed major fraction. ▼, T_d; ●, ΔH; ■, $\Delta T_{1/2}$. The error bars represent standard deviations of the means.

The effect of urea on the DSC characteristics of the flaxseed major fraction was also studied. The presence of 6 M urea caused a dramatic decrease in enthalpy and marked increase in half-peak width, suggesting extensive protein unfolding and decrease in cooperativity. However, the transition temperatures were slightly increased (Table 2). Urea can denature proteins due to its ability to destabilize hydrogen bonding and hydrophobic interactions (Kinsella, 1982). Urea also increases the "permittivity" of water (Franks & Eagland, 1975) for the apolar residues causing loss of protein structure. Effects of urea on DSC characteristics were similarly demonstrated in other plant proteins, although thermal stability was significantly decreased in those cases (Harwalkar & Ma, 1987; Meng & Ma, 2001). The urea-denatured flaxseed protein may assume a specific conformation which is more heat-stable than the native protein. SDS, at specific levels, has been shown to increase T_d in some proteins, due to the formation of a bridge between a charged group in one loop of a polypeptide chain with a hydrophobic region in another (Hegg & Löfqvist, 1974; Markus, Love, & Wissler, 1964).

Fig. 3 shows the effect of ethylene glycol (EG) on the DSC characteristics of the flaxseed major fraction. Both the T_d and ΔH values were progressively decreased with increase in EG concentration, indicating lowering of thermal stability and protein denaturation. In contrast, $\Delta T_{1/2}$ was progressively increased with increased EG concentration, showing loss of cooperativity in the denaturation process. EG, a water-miscible solvent, lowers the dielectric constant of the medium, weakening the non-polar interactions between protein molecules and enhancing hydrogen-bonding and electrostatic interactions (Damodaran & Kinsella, 1982; Tanford, 1962). Similar lowering in thermal stability (and partial protein unfolding by EG) was observed for β-lactoglobulin (Harwalkar & Ma, 1992), lysozyme and ribonuclease (Gerlsma & Stuur, 1972), oat globulin (Harwalkar & Ma, 1987) and red bean globulin (Meng & Ma, 2001).

4. Conclusions

The present data show that the DSC characteristics of different flaxseed fractions were mainly attributed to the proteins and their purification during the extraction procedures. Interactions with non-protein components may also affect the thermal properties of the protein fractions. The DSC characteristics of the major protein fraction in flaxseed, the 11–12S globulin, were influenced by environmental factors, including ionic strength, pH, chaotropic salts and protein structure perturbants. The results suggest that hydrophobic and ionic interactions and hydrogen bonding play an important role in stabilizing the conformation of protein molecules. Disulfide linkages and SS–SH interchange also make a significant contribution to the stability of the flaxseed protein.

Acknowledgements

The authors gratefully acknowledge the gift of dehulled NorMan flaxseed from Drs B.D. Oomah and G. Mazza of Agriculture and Agri-Food Canada, preparation of samples by M. W. Y. Chung at the University of BC, and DSC analyses by A. Chan at Hong Kong University. This research was partially funded by the Flax Council of Canada, the Natural Sciences and Engineering Research Council of Canada, and Agriculture Agri-Food Canada, and Hong Kong Research Grant Council grants (HKU 7223/98M and HKU 7089/99M).

References

Arakawa, J., & Timasheff, S. N. (1982). Preferential interactions of proteins with salt in concentrated solutions. *Biochemistry*, *21*, 6545–6552.

Arntfield, S. D., & Murray, E. D. (1981). The influence of processing parameters on food protein functionality. I. Differential scanning calorimetry as an indicator of protein denaturation. *Canadian Institute of Food Science and Technology Journal*, *14*, 289–294.

Arntfield, S. D., & Murray, E. D. (1990). Thermal analysis of food proteins in relation to processing effects. In V. R. Harwalkar, & C.-Y. Ma (Eds.), *Thermal analysis of foods* (pp. 51–91). London: Elsevier Applied Science.

Arntfield, S. D., Murray, E. D., & Ismond, M. A. H. (1986). Effect of salt on the thermal stability of storage proteins from fababean (*Vicia faba*). *Journal of Food Science*, *51*, 371–377.

Bigelow, C. C. (1967). On the average hydrophobicity of proteins and the relationship between it and protein structure. *Journal of Theoretical Biology*, *16*, 187–211.

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

Boye, J. I., Ma, C.-Y., & Harwalkar, V. R. (1997). Thermal denaturation and coagulation of proteins. In S. Damodaran, & A. Paraf (Eds.), *Food proteins and their applications* (pp. 25–56). New York: Marcel Dekker.

Brinegar, A. C., & Peterson, D. M. (1982). Separation and characterization of oat globulin polypeptides. *Archives in Biochemistry and Biophysics*, *219*, 71–79.

Chung, M. W. Y. (2001). *Isolation and characterization of the major fraction of flaxseed proteins*. MSc thesis, The University of British Columbia, Vancouver, BC, Canada.

Czarnecki, S. K., & Kritchevsky, D. (1992). Dietary protein and atherosclerosis. In G. U. Liepa, D. C. Bietz, A. C. Beynen, & M. A. Gorman (Eds.), *Dietary proteins: how they alleviate disease and promote better health* (pp. 42–56). Champaign: American Oil Chemists' Society.

Damodaran, S., & Kinsella, J. E.. In J. P. Cherry (Ed.), *Effects of ions on protein conformation and functionality*, *ACS Symposium Series 206* (pp. 327–357). Washington: American Chemical Society.

Franks, F., & Eagland, D. (1975). The role of solvent interactions in protein conformation. *CRC Critical Reviews in Biochemistry*, *3*, 165–219.

Gerlsma, S. Y., & Stuur, E. J. (1972). The effect of polyhydric and monohydric alcohols on the heat-induced reversible denaturation of lysozyme and ribonuclease. *International Journal of Peptide and Protein Research*, *4*, 377–383.

Harwalkar, V. R., & Ma, C.-Y. (1987). Study of thermal properties of oat globulin by differential scanning calorimetry. *Journal of Food Science*, *52*, 394–398.

Harwalkar, V. R., & Ma, C.-Y. (1992). Evaluation of interactions of β-lactoglobulin by differential scanning calorimetry. In H. Visser (Ed.), *Protein interactions* (pp. 359–378). Weinheim: VCH Publishers.

Hatefi, Y., & Hanstein, W. G. (1969). Solubilization of particulate proteins and nonelectrolytes by chaotropic agents. *Proceedings of the National Academy of Science*, *62*, 1129–1136.

Hegg, P.-O., & Löfqvist, B. (1974). The protective effect of small amounts of anionic detergents on the thermal aggregation of crude ovalbumin. *Journal of Food Science*, *39*, 1231–1236.

Kinsella, J. E. (1982). Relationship between structure and functional properties of food proteins. In P. F. Fox, & J. J. Cowden (Eds.), *Food proteins* (pp. 51–103). London: Applied Science Publisher.

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

Marcone, M. F. (1999). Biochemical and biopohysical 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.

Markus, G., Love, R. L., & Wissler, F. C. (1964). Mechanism of protection by anionic detergents against denaturation of serum albumin. *Journal of Biological Chemistry*, *239*, 3687–3693.

Meng, G.-T., & Ma, C.-Y. (2001). Thermal properties of *Phaseolus angularis* (red bean) globulin. *Food Chemistry*, *73*, 453–460.

Morrissey, P. A., Mulvihill, D. M., & O'Neill, E. M. (1987). Functional properties of muscle proteins. In B. J. F. Hudson (Ed.), *Development in food proteins* (Vol. 5) (pp. 195–256). London: Elsevier Applied Science.

Murray, E. D., Arntfield, S. D., & Ismond, M. A. H. (1985). The influence of processing parameters on food protein functionality. II. Factor affecting thermal properties as analyzed by differential scanning calorimetry. *Canadian Institute of Food Science and Technology Journal*, *18*, 158–162.

O'Carrol, P. (1995). A bio-active future. *World of Ingredients*, *November/December*, 28–30.

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

Oomah, B. D., & Mazza, G. (1995). Functional properties, uses of flaxseed protein. *INFORM*, *6*(11), 1246–1252.

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.

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

Park, M. S., & Liepa, G. U. (1982). Effects of dietary protein and amino acids on the metabolism of cholesterol-carrying lipoproteins in rats. *Journal of Nutrition*, *112*, 1892–1898.

Privalov, P. L. (1982). Stability of proteins: proteins which do not present a single cooperative system. *Advances in Protein Chemistry*, *35*, 1–104.

Privalov, P. L., & Gill, S. J. (1988). Stability of protein structure and hydrophobic interaction. *Advances in Protein Chemistry*, *39*, 191–233.

Privalov, P. L., & Khechinashviti, N. N. (1974). A thermodynamic approach to the problem of stabilization of globular proteins: a calorimetric approach. *Journal of Molecular Biology*, *86*, 665–684.

Pszczola, D. E. (2000). Genes and diet: the specialized role ingredients may play. *Food Technology*, *54*(10), 82 84, 96.

Steinhardt, J. (1975). The nature of specific and non-specific interactions of detergent with protein: complexing and unfolding. In H. Sund, & G. Blauer (Eds.), *Protein–ligand interaction* (pp. 412–426). Berlin: de Gruyter.

Sugano, M., Beynen, A. C. (Eds.). (1990). *Dietary proteins, cholesterol metabolism and atherolsclerosis*. New York: Basel.

Tanford, C. (1962). Contribution of hydrophobic interactions to the stability of the globular conformation. *Journal of American Chemical Society*, *84*, 4240–4247.

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.

von Hippel, P. H., & Scheich, T. (1969). The effect of neutral salts on the structure and conformational stability of macromolecules in solution. In S. N. Timesheff, & G. D. Fasman (Eds.), *Structure and stability of biological macromolecule* (Vol. 2) (pp. 417–574). New York: Marcel-Dekker.

Wolf, W. J., & Tamura, T. (1969). Heat denaturation of soybean 11s protein. *Cereal Chemistry*, *46*, 331–344.

Wright, D. J. (1984). Thermoanalytical methods in food research. *Critical Reports on Applied Chemistry*, *5*, 1–36.