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Thermal characteristics of flaxseed (*Linum usitatissimum* L.) proteins

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Abstract

Thermal characteristics of ion-exchange protein fractions from dehulled delipidated meal of four flaxseed cultivars grown at three locations were evaluated by differential scanning calorimetry (DSC) to examine genotypic and environmental effects. DSC was performed in a "dry" state on four, 0.28, 0.35, 0.45 and 0.50 M NaCl lyophilized protein fractions. Flaxseed proteins exhibited two thermal events between 83 and 115 °C with distinct shoulders present in the 0.45 and 0.50 M fractions of all cultivars. Cultivar and location effects were significant for the first thermal transition (T_{d_1}) of the 0.28 and 0.35 M fractions, and the second transition (T_{d_2}) of the 0.35, 0.45 and 0.50 M fractions. The enthalpy of the first transition ΔH_1 of the 0.35 and 0.50 M fractions was cultivar dependent, while that of the second transition ΔH_2 was cultivar and location specific for the 0.35, 0.45 and 0.50 M fractions. The 0.28 and 0.45 M fractions displayed the lowest and highest thermal transition temperatures, respectively. All protein fractions consisted of reversing and nonreversing thermal events as assessed by modulated DSC. Flaxseed cultivars, when grouped by thermal characteristics of protein fractions, can be differentiated by principal component analysis.

Keywords: Flax (*Linum usitatissimum* L); Protein fractions; Differential scanning calorimetry; Thermal transitions; Enthalpy; Cultivars; Locations

1. Introduction

Flaxseed is regaining its status as a functional food after centuries of use as natural medicine. The physiological benefits of flaxseed are generally attributed to its α-linolenic rich oil, lignans and fibre or gum, to a lesser extent. Although flaxseed protein is lately being recognized as an important component, its basic biochemical characteristics have not been fully elucidated despite the advent of proteomics.

Conventional DSC measures the phase transitions arising from conformational changes such as denaturation or unfolding that are specific for individual proteins. Modulated DSC provides the benefits of separating reversible and irreversible thermal events with high precision and sensitivity (Cuq & Icard-Vernière, 2001). One major endothermic transition at 90 °C was observed for purified flaxseed globulins by micro-differential scanning calorimetry at 1.37 °C per min between 30 and 118 °C. Modulated DSC carried out at 5 °C/min from 30 to 130 °C on 10% of the same protein confirmed the single endothermic transition at 90 °C as a cooperative event (Marcone, Kakuda, & Yada, 1998). Whole protein extract from dehulled delipidated flaxseed meal exhibited one major peak with a shoulder, while the major fraction eluted with 0.25 M NaCl showed a single peak at 114.7 °C with a high enthalpy of 16.6 J/g protein (Li-Chan & Ma, 2002). Recently, a single endotherm at 106.4 °C with an enthalpy of 14.16 J/g was observed for protein micellar mass-derived flaxseed protein isolate (90% protein by

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weight) with 78%, 12% and 9% 7S, 12S and 2S, respectively (Green, Milanova, & Logie, 2005). Protein isolate obtained by isoelectric precipitation had an elevated endotherm at 121.5 °C with low enthalpy of 1.2 J/g (Green et al., 2005).

Our previous study (Li-Chan, Sultanbawa, Losso, Oomah, & Mazza, 2002) identified and characterized two fractions eluting at high salt concentrations of 0.45 and 0.50 M NaCl extracted from dehulled and defatted flaxseed cultivar NorMan as metal-binding proteins or phytochelatin-like components. These fractions, although present in trace amounts, had high sulphydryl contents (46 and 41 μmoles SH/g protein for 0.45 and 0.50 M fractions, respectively). Molecular weights of the 0.45 and 0.50 M fractions were estimated by gel permeation chromatography at 90 kDa with minor components at 60 and 30 kDa. The 0.28 M fraction, later changed to 0.25 M fraction (Lei, Li-Chan, Oomah, & Mazza, 2003) was the major protein comprising 64% of the total flaxseed protein representing the 11–12 S storage globulin. This major protein, 0.25 M fraction, has a molecular weight of approximately 350 kDa with 20, 23 and 31 kDa subunits with a relatively low sulphydryl content of 3.4 μmoles SH/g protein (Chung, Lei, & Li-Chan, 2005). It has a high denaturation temperature of 114.7 °C with an enthalpy (ΔH) of 16.8 J/g protein and width at half peak at 10.5 °C (Li-Chan & Ma, 2002).

In food systems, DSC is generally performed in the presence of water, buffer or other solvents, although dry systems often preferred for pharmaceutical products have been used to study wheat proteins (León, Rosell, & Benedito de Barber, 2003; Micard & Guilbert, 2000). The application of DSC to determine the structural stability of dry-heated plant proteins was proposed as a result of a study on dry soluble protein fractions of amaranth and soybean (Gorinstein et al., 2001). In their procedure, dry lyophilized protein samples were scanned at 10 °C/ min from 30 to 120 °C with an empty pan used as reference. DSC measurements of mixed globulins from soybean indicated one transition between 74 and 95 °C with a maximum at 86.2 °C.

The present study is a continuation (an extension) of our earlier investigations (Lei et al., 2003; Li-Chan et al., 2002) aimed at determining the genotypic difference in thermal characteristics of flaxseed proteins using a dry system. The use of modulated DSC allowed the elucidation of the reversing and nonreversing components associated with the heat flow of ion-exchange flaxseed protein fractions. The results provide information on thermal properties of protein fractions essential for evaluating processing especially under low moisture conditions (extrusion, thermomolding), functionality (solubility and stability) and quality considerations of flaxseed proteins for future development of potential functional food and nutraceutical ingredients.

2. Materials and methods

2.1. Source of materials and sample preparations

Tris Ultrapure [Tris-(hydroxymethyl) amino methane] was from ICN Biomedicals, Inc., Costa Mesa, CA. Sodium chloride and hydrochloric acid (ACS certified) were from Fisher Scientific, Nepean, ON, Canada. 2-Mercaptoethanol electrophoresis reagent was from Sigma–Aldrich Canada Ltd., Oakville, ON, Canada. Bicinchoninic acid (BCA) protein assay kit (# 23225) was from Pierce Chemical Company, Rockford, IL. The deionized distilled water (~18 MΩ) used for all experiments was produced by a Barnstead water purification system. DEAE-Sephacel was purchased from Amersham Pharmacia Biotech, Inc., Baie d'Urfe, PQ, Canada.

Five flaxseed cultivars grown at three locations, Morden, Portage La Prairie (referred herein as Portage) and Rosebank in Southern Manitoba in 1997 were obtained from the Agriculture and Agri-Food Canada Cereal Research Center (Morden, MB, Canada). NorMan flaxseed purchased locally as a bulk certified commercial seed grown in 1991 in southern Manitoba, Canada, was the same lot used in our previous investigations (Lei et al., 2003; Li-Chan et al., 2002); it was used as a control for protein extraction and validation of our "wet" DSC method in comparison to those of Li-Chan and Ma (2002). A soy flour sample (Cargill soy protein solutions 100/90, Cedar Rapids, IA) was also used frequently as a daily control for DSC operation for the dry method. Samples were processed essentially as described previously (Lei et al., 2003) by dehulling flaxseed with a Strong Scott Barley Pearler according to Li-Chan et al. (2002) and an air aspirator (Oomah, Mazza, & Kenaschuk, 1996). The dehulled seeds were ground in a small coffee mill and defatted by two changes of hexane and one change of petroleum ether, 1 h each with magnetic stirring, using a seed-to-solvent ratio of 1:10 (w/v). The dehulled defatted meal was air-dried under a fume hood for at least 4 h.

2.2. Protein extraction and fractionation

Protein was extracted from dehulled defatted meal (10 g) according to the method of Li-Chan et al. (2002) with 16 volumes of nitrogen-purged extraction buffer (0.10 M Tris + 0.10 M NaCl + 10 mM mercaptoethanol, pH 8.6) at 4 °C for 16 h with constant magnetic stirring. The extract was centrifuged (12 000*g* for 20 min, Beckman Avanti J25, Beckman Instruments Inc., Palo Alto, CA) at 6 °C, and the supernatant was further centrifuged at 28 000*g* for 25 min.

Ion exchange (IE) chromatography was carried according to the procedure of Li-Chan et al. (2002) using the BioRad automated econo system low pressure

chromatography. The anion-exchange resin DEAE-Sephacel was packed in a column (2.5 × 20 cm) and equilibrated with nitrogen-purged extraction buffer. The protein extract from 10 g of dehulled defatted meal was diluted 10-fold with distilled and deionized water (dd water) and then filtered through a 0.45 μm nylon filter. The extract was placed over the column and the unbound proteins were removed by washing with Tris buffer (25 mM, pH 8.6) until the absorbance at 280 nm was <0.1. Bound fractions were eluted by step gradients of 0.1 M Tris buffer at pH 8.6 containing 0.28, 0.35, 0.45 and 0.50 M NaCl, at a flow rate of 1.5 mL/min. Fractions (10 mL/tube) were collected and monitored for conductivity (Hanna Instruments 3310 conductivity meter, Hanna Instruments, Woonsocket, RI) and absorbance at 280 and 254 nm (SpectraMax Plus^{384} Microplate Spectrophotometer, Molecular Devices Corporation, Sunnyvale, CA). Fractions from each step were pooled, purged with nitrogen and stored in nitrogen-flushed, tightly stoppered containers at −20 °C prior to freeze-drying. Freeze-dried flaxseed fractions were reconstituted with distilled-deionized water, and the salt removed by gel filtration chromatography (Econo-Pac 10DG column, packed with Bio-Gel P-6DG gel, Bio-Rad) according to the manufacturers protocol. The desalted fractions were freeze-dried prior to analysis.

Protein contents of pooled column fractions were measured in triplicate using the bicinchoninic acid (BCA) protein assay (Pierce Chemical Company, Rockford, IL) with bovine serum albumin as the protein standard. Protein ($N \times 6.25$) contents of solid and freeze-dried samples were determined by a nitrogen combustion method (FP-428, LECO Instruments Ltd., Mississauga, ON, Canada). Preliminary experiments with the control NorMan flaxseed indicated good reproducibility (on average <8% relative standard deviation) of the extraction/fractionation protocol replicated over 5 times.

2.3. Thermal analysis

Thermal characteristics of flaxseed protein fractions were investigated using a TA 2910 DSC thermal analyzer (TA Instruments, New Castle, DE) with a refrigerated cooling system (RCS) and modulated capability. The DSC cell and the RCS were purged with dry nitrogen gas (100 ml/min) and helium (150 ml/min), respectively. The DSC cell was calibrated for baseline using empty pans of matched weights and for temperature and heat flow at 10 °C/min with four standards (mercury, $T_m = -38.83$ °C; gallium, $T_m = 29.76$ °C; succinonitrile, $T_m = 56.60$ °C; indium, $T_m = 156.60$ °C and $\Delta H = 28.71$ J/g) of high purity (>99.999%) obtained from Sigma–Aldrich Canada Ltd. (Oakville, ON). Ground freeze-dried protein fractions (approximately 2 mg) were accurately weighed into aluminium pans, hermetically sealed then immediately scanned in the DSC from 25 to 160 °C at 10 °C/min. A sealed empty pan of similar weight was used as reference. Peak transition or denaturation temperature (T_d), enthalpy of denaturation (ΔH) and width at half height ($T1/2$) were computed from the thermogram by the Universal Analysis Program, Version 2.5 H (TA Instruments). When thermograms were comprised of small shoulder close to large peaks, the ΔH of the combined transitions were measured due to difficulties in accurately estimating the partial areas of the overlapping peaks. For modulated DSC, protein samples (ca. 2 mg) were scanned at 1 °C/min with modulation at a period of 60 s and temperature amplitude of 0.16 °C.

Data were subjected to analysis of variance by the general linear models (GLM) procedure, means comparison by Duncan's test, and Principal component analysis according to Statistical Analysis System (SAS Institute, 1990).

3. Results and discussion

Average protein content of flaxseed cultivars grown at three locations in southern Manitoba was 19% ($N \times 6.25$) (Table 1). Flaxseed cultivars grown at Rosebank had significantly lower protein contents in dehulled seeds and dehulled defatted meal than those grown at Morden and Portage resulting in lower protein content of the load. This difference was also reflected in the significantly reduced level of protein in the unbound and 0.45 M fractions. Processing, particularly lipid removal (defatting), increased the protein content more than 2-folds. The protein content of the column load ranged from 58.2 to 61.3 g/100 g and differed significantly among cultivars, NorLin and Normandy, in particular. Cultivar differences were not observed in protein contents of the 0.28, 0.35, and 0.50 M fractions. The 0.35, 0.45 and 0.50 M fractions had less than half the protein content of the 0.28 M fraction.

Reproducibility of the DSC method was evaluated by thermal analysis of a control sample (NorMan) in triplicate. Replicate assay resulted in relative standard deviations (RSD) of 3.9% or less for peak temperature and 7.6% or less for enthalpy of the thermal events (Table 2). The RSD was highest for the 0.28 M fraction while the least variations often encountered in the 0.50 M fraction resulted in the greatest reproducibility for T_{d_2}, ΔH_1 and T_{d_1}. A soy flour (Cargill, 52.4 g/100 g protein) analyzed ($n = 7$) under identical conditions exhibited a single peak with T_d and ΔH values of 65.9 ± 2.09 °C (RSD of 3.2%) and 4.6 ± 0.23 J/g (5.1% RSD) consistent with results reported previously for dry soybean proteins (Gorinstein et al., 2001). A single sharp peak at 114.7 ± 0.3 °C was reported for the major (dialyzed

Table 1
Protein content (g/100 g) of flaxseed and ion-exchange fractions of flaxseed cultivars grown at three locations

Fraction	Cultivar					Location			Mean
	AC Linora	AC McDuff	Flanders	NorLin	Normandy	Morden	Portage	Rosebank	
Seeds	18.7	19.0	19.3	18.2	19.1	19.5x	19.4x	17.7y	18.9tu
Dehulled	20.9	20.8	21.3	20.4	21.7	22.1x	21.5x	19.4y	21.0s
Meal	45.6	45.2	46.1	43.6	47.0	48.1x	46.6x	41.9y	45.5q
Load	60.6ab	60.4abc	58.8bc	58.2c	61.3a	61.6x	60.7x	57.3y	59.9p
Ion-exchange fraction									
Unbound	45.4	45.5	46.8	45.1	46.2	46.7x	46.0xy	44.7y	45.8q
0.28 M	46.8	46.8	44.6	43.5	44.9	46.6	45.0	44.3	45.3q
0.35 M	22.9	24.2	22.9	22.7	21.9	23.0	22.3	23.5	22.9r
0.45 M	19.3ab	19.3ab	19.5a	19.2ab	18.9b	19.5x	19.3xy	18.9y	19.3t
0.50 M	18.1	17.5	17.7	17.8	17.3	18.1	17.5	17.4	17.7u

a–c and x, y Means within a row for cultivars and locations, respectively with different letters are significantly different ($P < 0.05$) by Duncan's multiple range test. p–u Overall means within a column with different letters are significantly different ($P < 0.05$) by Duncan's multiple range test.

Table 2
Reproducibility of thermal characteristics of flaxseed protein fractions

Fraction	$T_{d_{1S}}$	T_{d_1}	ΔH_1	T_{d_2}	ΔH_2
0.28 M					
Mean	73.3	85.7	61.6	106.3	77.1
SD	2.8	1.3	4.7	1.4	5.9
RSD (%)	3.9	1.5	7.6	1.3	7.6
0.35 M					
Mean	89.2	92.5	274.4	112.7	142.0
SD	0.3	1.7	9.5	0.1	3.2
RSD (%)	0.3	1.9	3.5	0.1	2.3
0.45 M					
Mean	88.1	97.1	233.4	114.9	129.7
SD	2.9	2.2	6.4	1.1	1.8
RSD (%)	3.3	2.3	2.7	1.0	1.4
0.50 M					
Mean	87.2	95.5	271.5	111.8	138.6
SD	1.7	0.5	3.8	0.0	4.9
RSD (%)	2.0	0.5	1.4	0.0	3.5

T_d and ΔH (denaturation temperature and enthalpy, respectively) are expressed in °C and J/g protein, respectively ($n = 3$).

0.25 M NaCl) fraction of flaxseed cultivar NorMan (Li-Chan & Ma, 2002) in the presence (1 mg in 10 μL) of Tris buffer (pH 8.6). The desalted 0.28 M fraction of the same NorMan exhibited a single thermal event at 107.5 ± 1.9 °C with enthalpy of 8.5 ± 1.95 J/g protein ($n = 15$) in the presence of distilled water (1 mg in 10 μL) in our laboratory, under conditions similar to those of Li-Chan and Ma (2002) indicating comparable results with the 0.25 M fraction. The T_d of the 0.28 M protein fraction was remarkably similar to those of protein micellar mass-derived flax protein isolate (106.4 °C) reported by Green et al. (2005). In the absence of solvent (distilled water or buffer), the 0.28 M fraction of NorMan displayed the typical shoulder (73.3 ± 2.8 °C) with the two endotherms at 86 and 106 °C, respectively (Table 2). Therefore, DSC of flaxseed proteins in the "dry" state (absence of water or buffer) displayed higher sensitivity by depicting additional thermal events than in the presence of solvents.

The large difference in enthalpy and T_d of the protein fractions between the wet (presence) and dry (absence of solvent) methods is primarily due to the presence of salt in the solvent. This was demonstrated by performing the DSC analysis (wet and dry) of protein fractions desalted using Econo-Pac 10 DG column containing Bio-Gel P-6DG (Bio-Rad), followed by lyophillization prior to DSC analysis. The control NorMan 0.28 M desalted fraction exhibited high T_d and ΔH values for the wet (107.5 ± 1.83 °C and 8.49 ± 1.95 J/g protein, respectively, $n = 15$) compared to the dry method (57.8 ± 0.69 °C and 1.10 ± 0.08 J/g protein, respectively, $n = 3$). The high denaturation temperature observed for the wet method may be attributed to a higher number of hydrophobic bonds stabilizing the protein (Molina, Petruccelli, & Añón, 2004) and for the dry method these interactions may contribute to drastic reduction in ΔH. The difference in T_d and enthalpy between desalted and nondesalted NorMan 0.28 M fraction was negligible (107.5 vs. 109.0 °C and 8.49 vs. 9.0 J/g protein, respectively) when analyzed by the wet method. Salt removal elicited significant reduction in T_d (by over 50%) and ΔH (at least 10-folds) and this trend was consistent for all protein fractions (data not shown) when analyzed by the dry method. The DSC results obtained by the wet and dry methods (Table 3) of desalted 0.28 M fraction (cultivar AC Linora) grown at three locations validate the observed salt effects. Location had a significant effect on ΔH ($P < 0.0001$) and width at half peak height, $\Delta T1/2$ ($P = 0.0009$) of desalted 0.28 M flaxseed protein fraction when analyzed by the wet method (Table 3).

Flaxseed proteins analyzed by the dry method generally exhibited two distinct thermal transitions between 83 and 115 °C (Table 4). A shoulder before the first transition was often observed between 66 and 90 °C, probably a result of partial transition (annealing) occurring

Table 3
Thermal transition properties of 0.28 M desalted protein fraction of flaxseed cultivar AC Linora grown at three locations

Location	T_d (°C)[a]	ΔH (J/g protein)[b]	ΔT 1/2 (°C)[c]
Morden	107.58a	5.39c	9.69c
Portage	106.63a	19.20b	14.03b
Rosebank	107.92a	39.94a	22.22a
Morden (dry)	43.79b	0.42d	16.60b

a–d Means in a column followed by the same letter are not significantly different by Duncan's multiple range test at the 5% level ($n = 3$).
[a] Denaturation temperature.
[b] Enthalpy.
[c] Width at half peak height.

due to the heating rate at 10 °C, amorphous crystallization between different protein isoforms or representing peak overlap (transitions within 1–2 °C). The shoulders for the 0.28 M fraction were present only in cultivars AC McDuff (Rosebank), Flanders and Normandy (Portage) and Normandy (Morden). The first transition (T_{d_1}) of the 0.28 M protein fraction differed significantly among cultivars ($P < 0.0001$) and locations ($P < 0.005$) with a mean endothermic peak temperature at 83.4 °C. The cultivar-location interaction for the 0.28 M fraction was also significant ($P < 0.0001$) according to statistical analysis (data not shown). The second transition (T_{d_2}) of the 0.28 M protein fraction occurred at about 103 °C, with significant variations expressed by two groups of cultivars at about 101 °C (AC Linora and Normandy) and the other group at about 104 °C. Only one transition was observed for the 0.28 M fraction of cultivars AC Linora and AC McDuff at all locations (except Rosebank for AC McDuff). The presence of a single thermal transition at high temperature for the 0.28 M fraction of AC Linora suggests an extremely stable native structure. Only one protein fraction (0.28 M) of cultivar AC McDuff was evaluated for thermal characteristics due to inadequate samples of the other protein fractions (0.35 M, 0.45 M and 0.50 M). It had a shoulder, T_{d_1}, and T_{d_2}, at 62.8, 82.6, and 104.4 °C, respectively.

The 0.35 M protein fraction exhibited two major thermal transitions at 94 and 113 °C. Cultivars Flanders (all locations) and NorLin (only at Morden) also displayed a shoulder at 90 °C. The 0.35 M fraction of all cultivars grown at Portage was characteristically devoid of the shoulder peak indicating an environmental influence. Cultivar and location differences ($P < 0.0005$) and cultivar-location interaction ($P = 0.001$) were significant for T_{d_1} and T_{d_2} of the 0.35 M fraction according to statistical analysis.

The 0.45 and 0.50 M fractions were characterized by the presence of a distinct shoulder peak for all cultivars at all locations. However, cultivar or location had no significant effect on the transition of the shoulder peak for the 0.45 M protein fraction. Location had a significant ($P = 0.0001$) effect on T_{d_1} of 0.45 M protein fraction. Differences among cultivars and cultivar-location interaction ($P < 0.0001$), and locations ($P = 0.005$) were significant for T_{d_2} of the 0.45 M protein fraction. Cultivars Normandy and AC Linora had the lowest T_{d_1} and T_{d_2} values (92.9 and 112.8 °C, respectively) among all cultivars.

The shoulder peak of the 0.5 M protein fraction showed no significant difference among cultivars,

Table 4
Thermal denaturation temperature (°C) of ion-exchange fractions of flaxseed cultivars grown at three locations

Fraction	Cultivar				Location			Mean
	AC Linora	Flanders	NorLin	Normandy	Morden	Portage	Rosebank	
$T_{d_{1S}}$								
0.28 M	nd	67.5a	68.8a	66.0a	66.9x	67.1x	nd	67.1f
0.35 M	nd	89.6a	89.9a	nd	90.2x	nd	88.7x	89.7e
0.45 M	88.9a	88.7a	89.6a	88.8a	89.0x	89.3x	89.1x	89.1e
0.50 M	88.4a	89.9a	89.3a	89.7a	88.2y	88.3y	90.7x	89.2e
T_{d_1}								
0.28 M	nd	81.2c	85.8a	83.2b	82.8y	84.6x	82.8y	83.4g
0.35 M	94.4ab	94.8a	93.5b	91.5c	95.3x	92.7y	92.7y	93.6e
0.45 M	93.5bc	94.2ab	94.7a	92.9c	94.3x	92.5y	94.8x	93.8ef
0.50 M	93.6a	92.9a	93.8a	91.8b	92.7y	92.0y	94.3x	93.0f
T_{d_2}								
0.28 M	99.5b	104.7a	104.8a	101.0b	103.2x	102.6x	101.7x	102.5h
0.35 M	111.2b	115.3a	112.3b	112.0b	111.4y	114.7x	111.9y	112.7f
0.45 M	112.8c	117.0a	114.8b	114.7b	114.5y	115.7x	114.4y	114.8e
0.50 M	110.2d	113.7a	111.8b	111.0c	111.3y	112.2x	111.5y	111.7g

a–d and x–z Means within a row for each denaturation temperature (T_d) of cultivars and locations, respectively with different letters are significantly different ($P < 0.05$) by Duncan's multiple range test. e–h Overall means for each denaturation temperature (T_d) in a column followed by the same letter are not significantly different ($P < 0.05$).

although cultivars grown at Rosebank had significantly higher $T_{d_{1S}}$ ($P = 0.005$) than those grown at Morden or Portage. Cultivars AC Linora and NorLin had the highest T_{d_1} of the 0.5 M protein fraction. Differences in T_{d_1} were highly significant ($P < 0.0001$) for locations and cultivar-location interaction with the highest values observed for cultivars grown at Rosebank. Cultivar differences were significant for the second thermal transition with cultivars Flanders and AC Linora exhibiting the highest and lowest T_{d_2} of all protein fractions. Similarly, cultivars grown at Rosebank exhibited the lowest T_{d_2} of all protein fractions whereas T_{d_2} for Portage was always the highest except for the 0.28 M fraction.

Generally, location had no significant effect on the enthalpy of the first thermal transition (ΔH_1) of all protein fractions. The 0.28 M protein fraction had the lowest enthalpy for both the first and second thermal events (ΔH_1 and ΔH_2; Table 5). ΔH_1 of the 0.28 M protein fraction showed no significant differences among cultivars or locations, although the cultivar-location interaction proved significant ($P = 0.002$). Cultivar and cultivar-location interaction were significant ($P < 0.0001$) for ΔH_1 of the 0.35 and 0.50 M fractions – fractions with the highest mean enthalpy. The highest variation in ΔH_1 was observed for the 0.35 M fraction (25% between cultivars NorLin and Flanders), while variations for the 0.45 and 0.50 M fractions were similar (about 14%). Cultivar, location (except for the 0.28 M fraction), and their interaction were highly significant ($P < 0.0001$) for the enthalpy of the second transition (ΔH_2) for all fractions (data not shown). The 0.28 M fraction displayed the greatest variation in ΔH_2 with AC Linora exhibiting 66% higher enthalpy than Normandy. This difference for ΔH_2 for 0.35, 0.45 and 0.50 M protein fractions, although significant, was only 25% between cultivars with the highest and lowest enthalpy. Generally cultivars grown at Portage were likely to have the highest ΔH_2, while those grown in Morden had the lowest enthalpy, particularly for the 0.35, 0.45 and 0.50 M fractions. Cultivars Flanders and AC Linora exhibiting the highest and lowest T_{d_2} also had the highest and lowest enthalpy ΔH_2, respectively.

Principal component analysis was performed on the thermal parameters (T_{d_1}, T_{d_2} and ΔH_1, ΔH_2) of protein fractions. When these four parameters for each cultivar at each location were entered in the PCA program, one factor was returned that met the criteria of eigenvalue exceeding 1.0 (3.13 for components 1), while component 2 had an eigenvalue of 0.48. It is customary to consider only those components that have an eigenvalue of 1.0 or greater for selecting principal component. The two components accounted for 90% of the total variance. The first component had large positive loadings for T_{d_2} (0.530), ΔH_2 (0.526), T_{d_1} (0.475) and ΔH_1 (0.465). The second component was primarily influenced by positive loadings of ΔH_1 (0.621) and T_{d_1} (0.425) and negative effects of ΔH_2 (−0.473) and T_{d_2} (−0.457).

The biplot of the first two principal components (Fig. 1) differentiated the thermal events when grouped by origin of the sample (identified by cultivar and location) and protein fraction. Since the first component is governed primarily by T_{d_2} and ΔH_2 and the second component by ΔH_1, the PCA plot discriminates the fractions mostly by the ratio of enthalpy to the T_d of the fractions – often referred to as relative stability. For example, the 0.28 M fractions designated by the prefix 2, generally with low T_d and ΔH_2, were located diagonally from those with high T_d and ΔH_1 (the 0.35 M fraction designated with the prefix 3). The protein fractions were unambiguously discriminated with most of the 0.50 M and 0.45 M protein fractions located on the left side of the median, just below the 0.35 M fraction. The 0.35 M protein fractions were segregated based on location between cultivars grown in Morden and Rosebank (upper right quadrant) and Portage (lower right quadrant). This suggests that flaxseed protein fractions when

Table 5
Enthalpy (ΔH, Joules/g protein) of ion-exchange fractions of flaxseed cultivars grown at three locations

Fraction	Cultivar				Location			Mean
	AC Linora	Flanders	NorLin	Normandy	Morden	Portage	Rosebank	
ΔH_1								
0.28 M	65.7a	48.8b	45.7b	51.1b	51.2x	51.4x	47.9x	50.3h
0.35 M	274.4b	245.0c	307.8a	301.8a	280.1x	276.5x	290.1x	282.2f
0.45 M	262.7a	256.4ab	232.3b	233.2b	249.2x	252.2x	237.0x	246.1g
0.50 M	304.7b	283.1c	294.0bc	323.9a	301.1x	301.3x	302.0x	301.4e
ΔH_2								
0.28 M	103.6a	65.9b	66.8b	54.9c	66.7y	68.7xy	73.7x	70.0h
0.35 M	129.0d	164.0a	138.9c	145.7b	131.9z	161.1x	140.2y	144.4g
0.45 M	148.9c	183.6a	174.5b	181.4a	163.6z	181.3x	171.4y	172.1e
0.50 M	130.7c	169.8a	142.1b	147.2b	140.7y	158.8x	142.9y	147.4f

a–d and x–z Means within a row for each enthalpy (ΔH) of cultivars and locations, respectively with different letters are significantly different ($P < 0.05$) by Duncan's multiple range test. e–h Overall means for $\Delta H_{1,2}$ in a column followed by the same letter are not significantly different ($P < 0.05$).

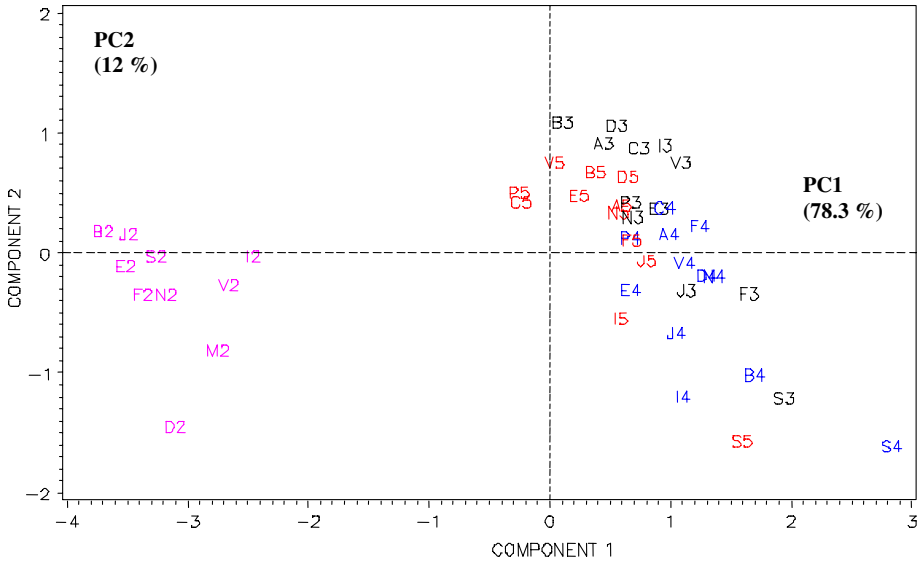

Fig. 1. Classification of flaxseed protein fractions grouped by thermal characteristics according to principal components 1 and 2. The protein 0.28, 0.35, 0.45 and 0.50 M fractions are represented by the numbers 2, 3, 4 and 5, respectively. Cultivars grown at Rosebank, Morden and Portage are denoted respectively, by: (A, C, P) for AC Linora, (F, D, S) for Flanders, (N, I, V) for NorLin, and (B, E, J) for Normandy.

grouped by cultivar and location can be distinguished based on their enthalpy.

Modulated DSC was performed to obtain detailed thermal characterization of the protein fractions. The benefits of higher accuracy and sensitivity of MDSC compared to the conventional DSC is illustrated in Fig. 2. Total heat flow thermograms of the 0.28 M fraction displayed a single endothermic peak at 118 °C consisting of both an irreversible and nonreversing components (about 2/3 and 1/3 of the total enthalpy, respectively) (Fig. 3) suggesting high degree of crystallinity. The 0.35 M fraction exhibited three thermal transitions between 87 and 117 °C comprising both reversing and nonreversing components. The reversing component of the 0.35 M fraction contributed about 17%, 48% and 56% of the total enthalpy of the first, second and third transitions, respectively. Both the 0.45 M and 0.50 M fractions displayed two irreversible (95–98, and 107 °C) and three nonreversible transitions localized between 81 and 115 °C. The first transition of the 0.45 and 0.50 M fractions (86 and 81 °C, respectively) was completely nonreversing, indicative of kinetic events, probably a result of secondary molecular ordering undergone by amorphous proteins (molecular

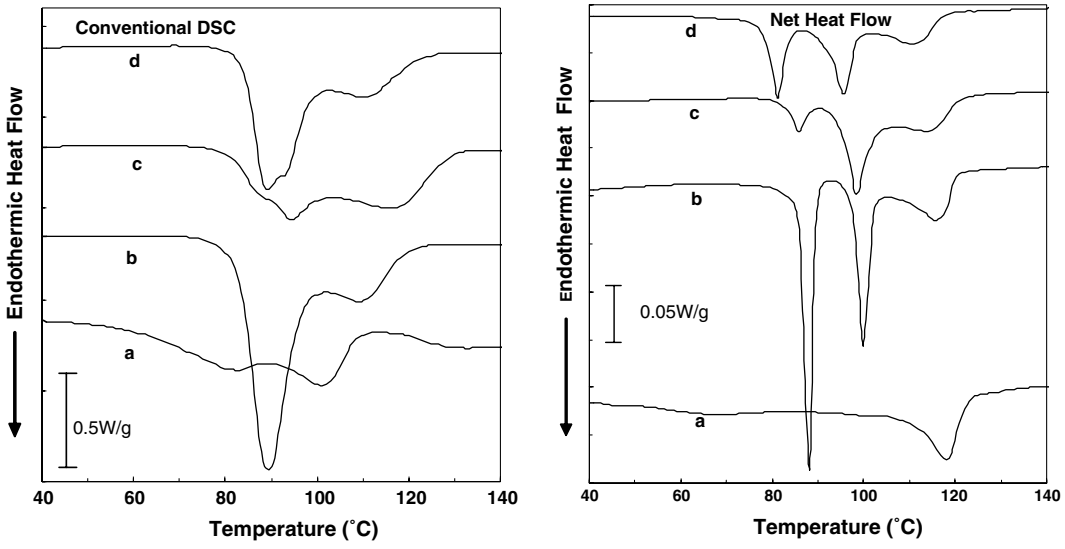

Fig. 2. Conventional and modulated differential scanning calorimetry (MDSC) of the net heat flow of flaxseed protein 0.28, 0.35, 0.45 and 0.50 M fractions (cv. Normandy grown at Rosebank) denoted by curves a, b, c and d, respectively.

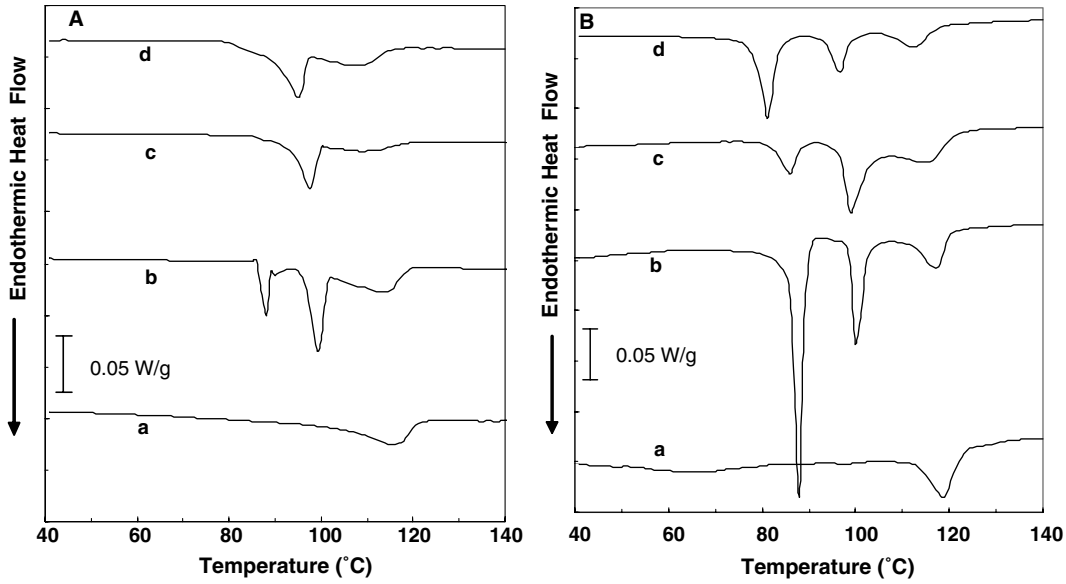

Fig. 3. Modulated differential scanning calorimetry (MDSC) of reversing (A) and nonreversing (B) heat flow of flaxseed protein 0.28, 0.35, 0.45 and 0.50 M fractions (cv. Normandy grown at Rosebank) denoted by curves a, b, c and d, respectively.

relaxation). The two reversing components of the 0.45 M fraction constituted only about 1/3 of the total enthalpy while those of the 0.50 M fraction contributed to over 1/2 (about 68% and 57%) of the total enthalpy indicating partial crystallization and reversible glass transition or aggregation due to protein unfolding. The large nonreversible endotherm observed for the first transition of the 0.35 and 0.50 M fractions (179 and 124 J/g protein) and the second transition of the 0.45 M fraction (169 J/g protein) may be associated with hydrogen bond disruption within proteins similar to those reported for gelatin (Bell & Touma, 1996). The irreversibility of the first transition of the 0.50 M fraction was confirmed by its disappearance on re-running the sample after cooling to room temperature using conventional DSC. Thermal reversibility may be considered as a parameter inversely related to stability. Hence, the irreversibility and low stability of the first transition of the 0.50 M fraction suggest an amorphous trait.

Overall, the 0.28 M fraction had the lowest thermal transition temperatures (for $T_{d_{1S}}$, T_{d_1} and T_{d_2}) whereas the 0.45 M protein fraction exhibited the highest T_{d_1} and T_{d_2} at 93.8 and 115 °C, respectively. The relative physical stabilities of the protein fractions calculated as the ratio of ΔH_2 to T_{d_2} were 0.68, 1.28, 1.50 and 1.34 for 0.28, 0.35, 0.45 and 0.50 M fractions, respectively. Each of the individual protein fractions displayed different thermal behaviors depending on cultivars and locations with the 0.28 and 0.45 M fractions exhibiting the least and most stability. This is consistent with and proportional to their sulfhydryl and disulfide contents that relate to high content of disulfide bonds present in these two protein fractions (Chung et al., 2005). This study provides multi-state thermal signatures of flaxseed protein fractions obtained by a rapid, robust and efficient method. The dry method amplifies differences in thermal properties of flaxseed proteins observed among cultivars and location without additional purification steps such as desalting. Taken together, this technique can be used to interpret thermal transformations of proteins expressing genetic and environmental variability or as a quality control in manufacturing operations and practices.

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