J Am Oil Chem Soc (2010) 87:909–915
DOI 10.1007/s11746-010-1616-8

ORIGINAL PAPER

Plastics from an Improved Canola Protein Isolate: Preparation and Properties

Wajira A. R. Manamperi · Sam K. C. Chang · Chad A. Ulven · Scott W. Pryor

Received: 24 September 2009 / Revised: 13 April 2010 / Accepted: 2 June 2010 / Published online: 23 June 2010

Abstract Canola meal proteins were solubilized from canola flour at pH 12 using sodium hydroxide solution. Proteins were then precipitated sequentially at pH values ranging from 11 to 3 in decrements of 1 pH unit. The weight distribution and the properties of these fractions were analyzed. The majority (>76%) of the recovered proteins were precipitated at pH values at or below 7. Another substantial fraction was precipitated at pH 11. The functional and thermal property (differential scanning calorimetry) analyses showed that this protein fraction exhibits the highest water holding capacity and lowest melting point. The plastics prepared with refined protein isolates (with pH 11 fraction removed) showed higher water resistance, tensile, and flexural strength, toughness, and elongation values compared to those prepared with standard canola protein isolates. This shows that mechanical and water resistance properties of protein-based plastics can be enhanced using improved protein isolates.

Keywords Canola proteins · Bioplastics · Acid precipitation · Isoelectric point · Water holding capacity · Thermal properties · Mechanical properties

W. A. R. Manamperi · S. W. Pryor (✉)
Department of Agricultural and Biosystems Engineering (Dept 7620), North Dakota State University, PO Box 6050, Fargo, ND 58108-6050, USA
e-mail: Scott.Pryor@ndsu.edu

S. K. C. Chang
Department of Cereal and Food Sciences, North Dakota State University, Fargo, ND 58108-6050, USA

C. A. Ulven
Department of Mechanical Engineering and Applied Mechanics, North Dakota State University, Fargo, ND 58108-6050, USA

Introduction

Canola (*Brassica napus* L.) is an oilseed crop with a high oil content (45–50%) and a protein content of 30–45% (w/w) in the defatted meal [1, 2]. Canola was developed in the early 1970s by reducing the anti-nutritional components, erucic acid, and glucosinolates, from rapeseed using traditional plant breeding techniques. Rapeseed/canola ranks as second largest oilseed crop produced worldwide after soy [3]. The importance of rapeseed/canola as an oilseed crop is increasing with the growth of the biodiesel industry [4].

High value uses of canola meal protein products such as adhesives, plastics, and biocomposites will enhance the economics of the canola oil industry. Suitable methods for separation and modifications of these proteins will play an important role in developing such higher value products. Exploration of suitable protein components and properties (functional and thermal) for specific applications are also important. Functional properties, such as water holding capacity, and thermal properties, such as glass transition temperature and melting temperature, may be directly related to the product mechanical properties of plastics.

The isolation method for extracting proteins in high-pH solutions and subsequent precipitation at low pH is practiced commercially to produce protein isolates. The solubilization efficiency and precipitation yields of defatted canola meal have been reported to be in the range of 80–95 and 30–79%, respectively [2, 5, 6]. Klockeman et al. [7] stated that isolation of proteins at high pH causes the reduction of protein nutritional value through the cross-linkage of amino acid residues, amino acid destruction, and racemization [7]. It has not been shown, however, that these factors will reduce the quality of protein for use in industrial applications such as adhesives, plastics, and biocomposites. The extraction of canola protein at a high

pH is generally desirable due to the fact that extraction efficiency is high under these conditions [8].

Isoelectric precipitation is a protein purification technique that utilizes pH adjustment of protein-rich solutions to precipitate proteins at their isoelectric points, p*I* [2]. At the p*I*, the negatively charged amino acid residues exactly balance the positively charged residues, so that the proteins possess no net charge and will have maximum hydrophobicity. Solubility of proteins is the lowest at the isoelectric point since the electrostatic repulsion between proteins is smallest at this point. As individual proteins in a single feedstock have a range of isoelectric points, only a fraction of the total proteins will be precipitated at any given pH.

Diosady et al. [9] argued that the isoelectric precipitation technique is not suitable for seeds containing proteins with a wide range of isoelectric points and molecular weights since yields depend largely on the selected precipitation pH value. Canola and rapeseed possess such a wide range of proteins with different isoelectric points (pH 4–11) and molecular weights (13–320 kDa) [9, 10]. Therefore, sequential precipitation of canola proteins at a range of pH values may largely address this issue.

An additional advantage of precipitating proteins at a range of pH values is that these fractions may behave differently with respect to functional, thermal, and mechanical properties when used in industrial applications. Removing some undesirable protein fractions and refining the protein isolates may enhance the properties of plastics prepared from these protein isolates. In this study a stepwise protein precipitation technique was used to maximize the overall protein recovery. The properties of proteins precipitated at each stage were evaluated. The impacts of some of these fractions on the industrial applications were also analyzed.

Materials and Methods

Materials

Raw canola seeds (cv. Invigor 2573, grown in Cavalier County in northeastern North Dakota) were cleaned according to USDA-GIPSA recommendations using a Carter-Day dockage tester (Minneapolis, MN) and hand sieves. A biodegradable co-polyester, Natureplast PBI 001, was purchased from Natureplast Inc. (Caen, France). Glycerol, polyvinylpyrrolidone (PVP), and zinc sulfate ($ZnSO_4$) were used without further treatment as plasticizer, compatibilizer, and crosslinker, respectively, in plastic preparation.

Preparation of Canola Meal Flour

Canola seeds were defatted by screw pressing followed by solvent extraction. The moisture content of canola seeds was determined gravimetrically at 120 °C using an LJ16 moisture analyzer (Mettler Toledo Inc., Columbus, OH). To adjust the moisture content to 7% (wet basis), distilled water was added and mixed uniformly with seeds (in sealed plastic bags) and allowed to equilibrate overnight before screw pressing. Seeds were then fed at 80 g/min to a model S 87G Komet screw press (Monchengladbach, Germany) preheated to 70 °C. Screw rotation speed was kept at 24 rpm. The resulting canola meal was then ground using a Retsch ZM1 mill (Brinkmann Instruments Inc., Westbury, NY) and passed through a 25 mesh screen. The residual oil in the pressed and ground canola flour was removed by solvent extraction with hexane for 24 h using a Soxhlet extraction unit. Fully defatted flour was then desolventized in a fume hood at room temperature for 2 days.

Fractionation of Protein Isolates

Defatted canola meal flour (100 g) was dissolved in 400 ml of distilled water. The pH of the solution was adjusted to 12 using 6 N sodium hydroxide (NaOH) and stirred for 1 h to solubilize the proteins. The solution was centrifuged at 5,000*g* for 30 min to remove fiber and other solids and the protein-rich supernatant was recovered. The pellet was re-suspended in 400 ml of distilled water and adjusted to pH 12 using 6 N NaOH solution to solubilize any residual protein. The solution was again stirred for 1 h and centrifuged at 5,000*g* for 30 min. This supernatant was combined with the original supernatant removed from the first step and the residual solids were discarded.

The combined protein-rich supernatant was precipitated by drop-wise addition of 6 N hydrochloric acid (HCl). The pH of the solution was sequentially lowered by 1 pH unit between pH values of 11 and 3. The protein fraction at each step was recovered by centrifugation at 5,000*g* for 30 min and the precipitated proteins were collected in plastic bottles and frozen for further processing. Further precipitation of proteins at pH values less than three was found to be negligible. All the precipitated protein fractions were suspended in a small volume (5% v/w) of water, neutralized, and lyophilized for functional and thermal property testing. Weights of protein fractions precipitated at each pH were recorded in Table 1. Protein content of each fraction was determined by Kjeldahl method using the nitrogen to protein conversion factor 6.25 [11] and this was used to calculate the weight of pure proteins precipitated. The "Fraction of total initial proteins" was calculated by dividing the amount of pure protein in each fraction by the total initial protein in canola meal flour (45%). The "Fraction of total recovered proteins" was calculated by dividing the weight of pure proteins in each fraction by the sum of pure proteins precipitated at each step.

Table 1 Percentage of proteins precipitated at different pH values

Precipitation pH	Weight of protein isolates (g)	Purity of protein isolates (%)	Weight of pure proteins precipitated (g)	Fraction of total initial proteins (%)	Fraction of total recovered proteins (%)
3	3.0 (0.4)	85.5	2.6	5.8	11.0
4	2.3 (0.1)	87.7	2.0	4.4	8.4
5	3.5 (0.3)	87.2	3.1	6.9	13.0
6	5.8 (0.2)	81.3	4.7	10.4	19.8
7	8.3 (0.5)	80.3	6.7	14.9	28.3
8	0.4 (0.1)	73.3	0.3	0.7	1.3
9	1.1 (0.1)	68.8	0.8	1.8	3.4
10	0.8 (0.1)	65.3	0.5	1.1	2.1
11	4.9 (0.4)	60.8	3.0	6.7	12.7
Total	30.1		23.7	52.5	100.0

Values in parentheses represent sample standard deviations

Preparation of Standard and Refined Protein Isolates

Canola meal flour was solubilized for 1 h at pH 12 using 6 N NaOH and the supernatant was collected as described above. Standard protein isolates were prepared by precipitating the supernatant at pH 5.0 and freeze-drying.

To remove the most hydrophilic protein fraction from the isolates, protein supernatant solubilized at pH 12 was first precipitated at pH 11 using 6 N HCl. After removing this fraction via centrifugation, the supernatant was again precipitated at pH 5.0 as was done for the standard protein isolate. This protein isolate was freeze-dried and labeled as the refined protein isolate. Approximately 500 g of each of these two types of isolates were prepared.

Water Holding Capacity of Protein Isolates

The water holding capacity was measured for all protein fractions sequentially precipitated at different pH values, standard protein isolates, and refined protein isolates according to the method described by Bora (2002) with minor modifications [12]. Protein isolate samples (500 mg) were dissolved in 5 ml of distilled water and placed in 15-ml centrifuge tubes. The tubes were vortexed for 30 s every 10 min for 1 h. The tubes were then centrifuged at 2,000*g* for 15 min and drained for 30 min by inverting the tube. Final weight of each sample was measured and water holding capacity was calculated as the percentage increase of sample weight.

Preparation of Plastic Specimens

Canola protein-based plastic specimens were prepared according to the method described by Mungara et al. [13] with modifications and using the base formula as follows: canola protein isolates (35 parts), plasticizer (15 parts), synthetic co-polyester (40 parts), compatibilizer (2 parts), water (7 parts), and crosslinker (1 part) were mixed mechanically until a homogeneous blend was obtained. Glycerol was used as plasticizer and PVP was used as the compatibilizer to mediate the interaction between protein isolates and synthetic co-polyester. Zinc sulfate was used as a cross-linker. The blend was allowed to equilibrate for at least 24 h before further processing.

The formulation was fed to a Leistritz Micro-18/GL-40D co-rotating twin-screw extruder (American Leistritz Extruder Corp., Somerville, NJ) for extrusion compounding. The extrusion compounding process involved heating, pressure, and shear forces in order to facilitate the reactions between proteins, polyesters, compatibilizers, plasticizers, and cross-linkers. The temperature profile for the extruder was maintained at 95, 116, 126, 136, 136, and 141 °C from feeder to the die. Canola protein blends extruded as polymer strands were then pelletized. The pellet moisture content was analyzed as described above and adjusted to 7% (wet basis) by mixing distilled water and allowing to equilibrate overnight. These pellets were then fed to a Technoplas SIM 5080 injection molder (Technoplas, Inc., Australia) to form dogbone-shaped specimens according to the ASTM standards D-638. Injection molder temperature profile was maintained at 116, 124, 127, 132, and 124 °C from feeder to the die. The injection molded specimens were used for the mechanical tests and water absorption tests.

Mechanical Properties

Mechanical properties (tensile strength, tensile modulus, elongation, toughness, flexural strength, and flexural modulus) of injection molded specimens were analyzed using a model 5567 Instron Universal Testing Machine (Instron Corporation, Canton, MA) according to the ASTM

D638 and ASTM D790 testing methods. Five replicates were analyzed for each of tensile and flexural tests.

Water Absorption of Plastics

Plastics (dogbone-shaped specimens prepared according to ASTM D-638) were used for the water absorption test. Water absorption of specimens was determined according to the ASTM D570 testing method with minor modifications. Plastic specimens were conditioned in a convection oven at 50 °C for 24 h and cooled to room temperature in a desiccator and weighed. Specimens were then immersed in distilled water for 2 h, and weighed after removing surface water using paper towels. Specimens were again submerged in distilled water for a total period of 24 h and weighed again after removing surface water. The samples were then reconditioned following the same initial drying method and the final weights were recorded to account for the loss of water-soluble matter during soaking. Water absorption was calculated as a percentage weight increase of the samples after accounting for the loss of water-soluble matter.

Thermal Properties of Protein Isolates and Plastic Specimens

Thermal properties (glass transition temperature and melting temperature) were measured for all protein fractions using a Q 1000 differential scanning calorimeter (DSC) (TA Instruments; New Castle, DE). About 5 mg of the freeze-dried protein isolate powder was weighed into a hermetic DSC pan and sealed. A heat–cool–heat cycle was used with a ramp rate of 10 °C/min within the range of 0–175 °C.

Also the glass transition temperature and melting temperature of the plastic specimens were measured using the same instrument. A 5 mg piece was cut from the plastic specimens and sealed inside a hermetic pan. A heat–cool–heat cycle was used with a ramp rate of 10 °C/min within the range of −100 to 150 °C. The DSC scans were analyzed using Universal Analysis 2000 software and used to determine the glass transition temperature and melting temperature of the protein isolates and protein-based plastics.

Statistical Analysis

Mechanical and functional properties were tested using 5 and 3 replicates, respectively, and analyzed with one-way analysis of variance. Multiple comparisons of means were done using a Tukey's test controlling the overall confidence level at 95%. Statistical analyses were done using Minitab software (Minitab Inc., State College, PA).

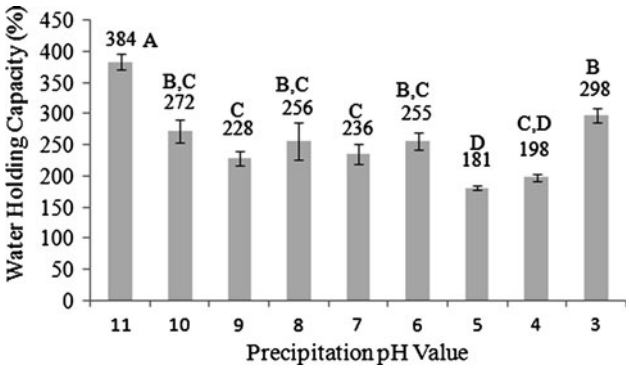

Fig. 1 Water holding capacity of protein fractions precipitated at different pH values. *Letters* indicate significant difference at $p < 0.05$

Results and Discussion

Weight Distribution

The weight distribution of protein fractions isolated through sequential precipitation at different pH values is presented in Table 1. Protein precipitation was comparatively less in alkaline pH values with the exception of pH 11. At this pH, a significant fraction was precipitated compared to the fractions precipitated at pH 10, 9, and 8. This indicates that an isoelectric point of a specific protein fraction existed around this pH value. Berot et al. [14] stated that specific canola proteins, napins, exhibit an isoelectric point around pH 11 and are the second largest fraction of proteins found in canola meal [14]. Therefore, the substantial fraction precipitated at pH 11 is likely to consist largely of napins.

Several reports have stated that the major isoelectric point of proteins from different canola varieties lies between pH 3.5–5.5 [2, 6, 10]. Most of the proteins were precipitated at neutral or acidic pH values. Table 1 shows a substantial cumulative protein yield within the region pH 3–7. However, the quantities precipitated decreased with the decreasing pH with a slight increase in the fraction precipitated at pH 3.

The protein fraction precipitated at pH 7 comprised the largest single fraction representing 28.3% of the total recovered proteins and 14.9% of the total initial protein (Table 1). This agrees well with the fact that the isoelectric point of cruciferins (12S globulins of canola) appears around pH 7.2 [14]. Cruciferins are the largest family of storage proteins found in canola [15].

Water Holding Capacity

Water holding capacities of the stepwise-precipitated different protein fractions are presented in Fig. 1. Proteins precipitated at pH 11 showed significantly higher water

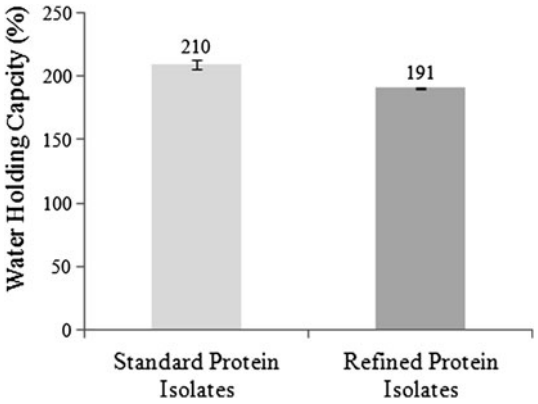

Fig. 2 Water holding capacity of standard and refined protein isolates

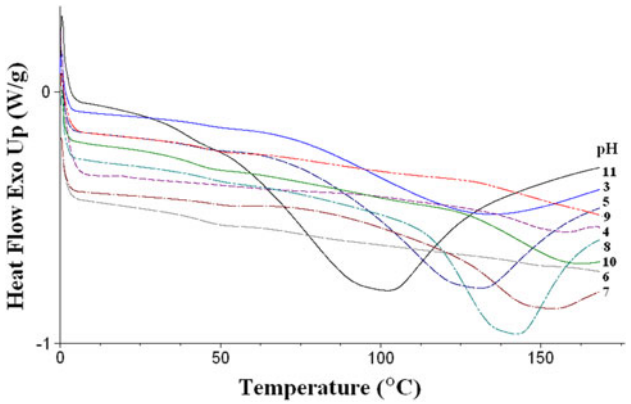

Fig. 3 DSC thermograms of protein fractions precipitated at different pH values (identified with the *curves*)

holding capacity (above 380%) than all other fractions (<300%). The protein fractions precipitated at pH 5 showed the lowest water holding capacity (181%). Most protein fractions exceeded 200% water holding capacity, but there was no clear pattern with respect to different fractions.

One of the largest problems hindering usefulness of protein-based plastics is their high water absorption. Considering this fact, the lower water holding capacity fractions (precipitated at pH 5 and 4) may be particularly suitable for preparation of plastics. Alternatively the higher water holding capacity fractions such as that precipitated at pH 11 could be removed to improve the water barrier properties of the protein products such as protein concentrates and protein isolates. These removed fractions may find different applications such as in the food industry where water holding capacity may be more valuable [1].

The water holding capacity of standard and refined protein isolates were also compared (Fig. 2) and refined isolates showed a 9% improvement in water resistance compared to the standard isolates. This could be attributed to improvement of overall hydrophobicity due to removal of the most hydrophilic protein fraction at pH 11.

Thermal Properties of Protein Isolates

The thermal properties of protein fractions such as glass transition temperature and melting temperature play an important role in industrial applications such as plastics [16]. Glass transition temperature can be used as a measure of plasticization for protein-based plastics. Usually glass transition temperature drops when the proteins are plasticized using a plasticizer such as glycerol. Therefore, determination of glass transition temperature of proteins helps in determining the degree of plasticization in extrusion and molding processes [17]. The melting point of proteins also gives important information in processing of protein products (protein concentrates and isolates) to be used in plastics. Melting point is a direct measure of the denaturation of proteins [18]. DSC thermograms obtained for protein fractions precipitated at different pH values are shown in Fig. 3. Also the glass transition temperature and melting point of protein fractions are presented in Table 2.

Table 2 Thermal properties of protein fractions precipitated at different pH values

Precipitation pH	Glass transition temp (°C)	Melting point/denaturation point (°C)
3	44.8	132.0
4	48.2	155.1
5	40.6	128.7
6	43.0	NA
7	47.1	151.2
8	46.2	142.4
9	43.2	NA
10	41.6	158.6
11	40.3	100.9

At the glass transition temperature, the structure of proteins changes from glass state to a more liquid-like rubbery state [19]. However, interpreting phase transition is a challenge since protein molecules are mostly amorphous. Glass transition temperatures of different protein fractions showed a narrow range between 40 and 50 °C, which is comparable with soy protein isolates [20]. However, the melting point showed a wide range of temperatures from 100 to 160 °C. The endothermic melting peaks at the high temperature range represent a true phase change of the proteins. However, these values are not comparable with soy protein melting peaks that generally appear around 70 and 90 °C for 7S and 11S proteins, respectively. A possible reason for the high temperature range melting peaks could be denaturation of proteins due to processing conditions

Table 3 Properties of plastic specimens prepared using standard and refined protein isolates

Property	Plastics prepared with standard isolates	Plastics prepared with refined isolates
Tensile strength (MPa)	10.3 (0.6)	11.9 (0.4)
Flexural strength (MPa)	11.5 (0.5)	12.9 (0.4)
Tensile modulus (MPa)	192 (18)	200 (8)
Flexural modulus (MPa)	267 (13)	273 (20)
Toughness (J)	3.0 (0.2)	4.9 (0.6)
Elongation (%)	17.5 (0.8)	23.5 (2.2)
Water absorption (%) (2 h)	3.9 (0.3)	2.6 (0.3)
Water absorption (%) (24 h)	6.6 (0.2)	5.9 (0.4)
Glass transition temperature (°C)	−21.9 (0.1)	−22.9 (0.2)
Melting temperature (°C)	111.0 (0.6)	111.5 (1.0)

Values in parentheses represent sample standard deviations

such as contact with NaOH for longer durations, etc. The fact that individual fractions have different denaturation temperatures indicates that each may be more or less suited to specific applications and that sequential protein fractionation may be an effective method for tailoring protein isolates according to end use.

Mechanical Properties of Plastic Specimens

Mechanical properties of plastic specimens prepared using standard and refined protein isolates are shown in Table 3. Significant improvements ($p < 0.05$) of both tensile and flexural strengths were obtained when the refined protein fractions were used with the same base formula containing 35% protein. This could be attributed to the improvement of hydrophobicity in the refined protein isolates after removing the most hydrophilic protein fraction that precipitated at pH 11. When the hydrophobicity is improved, the compatibility and interaction between proteins and synthetic polyesters are enhanced. The increased interactions between hydrophobic moieties in proteins and polyesters can increase both tensile and flexural strength of the plastics. However, tensile and flexural modulus values of these two types of plastic specimens did not show significant differences (Table 3). These modulus results indicate both isolates allow similar elastic deformation to take place based on recoverable molecular movement. However, once non-recoverable plastic flow initiates, the refined protein isolates showed better interaction and flowability. This was evident from results shown in both Fig. 3 and Table 3.

Toughness is related to the elongation or strain of a material since both properties are measures of the ability to move or deform to accommodate external forces. Both toughness and elongation of plastic specimens that were prepared using refined protein isolates showed significant improvements over those prepared using standard isolates (Table 3). This indicates that refined protein fractions also contributed to the improvement of ductility of the plastics prepared using protein-polyester blends. Toughness and ductility are important properties that protein-based plastics typically lack as compared to the petroleum-based plastics.

Thermal Properties of Plastic Specimens

The thermal properties of the plastic specimens are shown in Table 3. The glass transition temperature of the plastics prepared using refined isolates showed a slight decrease compared to that prepared with standard isolates, but there was no significant difference in melting temperature despite the removal of the protein fraction with lowest melting temperatures. The other components of the plastic specimen such as the synthetic polyester appeared to dominate in determining the thermal properties of plastic specimens with no discernable impact from the protein isolates [21]. However, if the protein:polyester ratio in the formulation is increased, the impact of proteins on the plastic thermal properties is expected to be more significant.

Water Absorption of Plastic Specimens

Generally, the plastic specimens showed relatively low water absorption (under 7%) compared to some other reports (up to 70%) [13, 22], which could be attributed to the presence of synthetic polyesters and cross-linkers in the blends. The 2 and 24 h water absorption of the plastic specimens showed that the specimens prepared with refined proteins had significantly lower water absorption (34 and 11%, respectively) compared to that prepared with standard proteins (Table 3). This could be attributed to the improved hydrophobicity of the refined proteins, which helps enhance the water resistance of the plastic specimens. Plastic specimens that were prepared with refined protein isolates also showed less swelling compared to those prepared with standard isolates. This could be attributed to the more compact structures obtained in the plastic specimens that used the refined protein isolates, which is an indication of the higher interaction between the more hydrophobic protein isolates and synthetic polyesters.

In summary, the proteins removed via sequential precipitation at decreasing pH values showed significant differences in functional and thermal properties. Removal of the proteins (precipitated at pH 11) with high water holding capacity and low melting temperature prior to isolation of remaining proteins resulted in a protein isolate with

improved functional properties. The plastic specimens prepared with this refined isolate showed significant improvements in mechanical properties (tensile and flexural strength, toughness, and elongation) and water resistance but did not have a significant impact on thermal properties. Overall it was shown that sequential precipitation could be used for preparing protein isolates with improved properties and that those improvements can have positive impacts on the protein-based products prepared with them.

Acknowledgments We thank Mr. Tom Borgen (Langdon, ND) for providing the canola seeds. Technical support given by Michael A. Fuqua (extrusion and injection molding), Shashika Ratnasinghe and Jaidev Sehrawat (protein isolate preparation), Angela Ostenson (freeze-drying), and Heidi Docktor (thermal analysis) are gratefully acknowledged. This research is supported by the USDA/CSREES NRI program through grant 2008-35504-18667.

References

1. Khattab RY, Arntfield SD (2009) Functional properties of raw and processed canola meal. LWT Food Sci Technol 42:1119–1124
2. Ghodsvali A, Khodaparast MHH, Vosoughi M, Diosady LL (2005) Preparation of canola protein materials using membrane technology and evaluation of meals functional properties. Food Res Int 38:223–231
3. USDA Economic Research Service (2010) Soybeans and oil crops: canola. http://www.ers.usda.gov/Briefing/SoybeansOilcrops/Canola.htm. Accessed June 2010
4. NBB, US (2007) Biodiesel production capacity. National Biodiesel Board, Missouri
5. Ismond MAH, Welsh WD (1992) Application of new methodology to canola protein isolation. Food Chem 45:125–127
6. Tzeng YM, Diosady LL, Rubin LJ (1988) Preparation of rapeseed protein isolate by sodium hexametaphosphate extraction, ultrafiltration, diafiltration, and ion-exchange. J Food Sci 53:1537–1542
7. Klockeman DM, Toledo R, Sims KA (1997) Isolation and characterization of defatted canola meal protein. J Agric Food Chem 45:3867–3870
8. Kumar R, Choudhary V, Mishra S, Varma IK, Mattiason B (2002) Adhesives and plastics based on soy protein products. Ind Crop Prod 16:155–172
9. Tzeng YM, Diosady LL, Rubin LJ (1990) Production of canola protein materials by alkaline extraction, precipitation, and membrane processing. J Food Sci 55:1147–1156
10. Diosady LL, Rubin LJ, Chen BK (1990) Membrane based processes for the production of rapeseed protein isolates. Int Congr Eng Food 3:280–289
11. AOAC (1995) Official methods of analysis, 15th edn. Association of Official Analytical Chemists, Arlington
12. Bora PS (2002) Functional properties of native and succinylated lentil (*Lens culinaris*) globulins. Food Chem 77:171–176
13. Mungara P, Chang T, Zhu J, Jane J (2002) Processing and physical properties of plastics made from soy protein polyester blends. J Environ Polym Degr 10:31–37
14. Berot S, Compoint JP, Larre C, Malabat C, Gueguen J (2005) Large scale purification of rapeseed proteins (*Brassica napus* L.). J Chromatogr B Analyt Technol Biomed Life Sci 818:35–42
15. Salleh M, Maruyama N, Adachi M, Hontani N, Saka S, Kato N, Ohkawa Y, Utsumi S (2002) Comparison of protein chemical and physicochemical properties of rapeseed cruciferin with those of soybean glycinin. J Agric Food Chem 50:7380–7385
16. Rouilly A, Orliac O, Silvestre F, Rigal L (2001) DSC study on the thermal properties of sunflower proteins according to their water content. Polymer 42:10111–10117
17. Wang H, Jiang L, Fu L (2007) Properties of molded soy protein isolate plastics. J Appl Polym Sci 106:3716–3720
18. Uruakpa FO, Arntfield SD (2006) Structural thermostability of commercial canola protein–hydrocolloid mixtures. LWT Food Sci Technol 39:124–134
19. Bengoechea C, Arrachid A, Guerrero A, Hill SE, Mitchell JR (2007) Relationship between the glass transition temperature and the melt flow behavior for gluten, casein, and soya. J Cereal Sci 45:275–284
20. Zhong ZK, Sun XS (2001) Thermal and mechanical properties and water absorption of sodium dodecyl sulfate-modified soy protein (11S). J Appl Polym Sci 81:166–175
21. Lim SW, Jung IK, Lee KH, Jin BS (1999) Structure and properties of biodegradable gluten/aliphatic polyester blends. Eur Polym J 35:1875–1881
22. Zhang J, Mungara P, Jane J (2001) Mechanical and thermal properties of extruded soy protein sheets. Polymer 42:2569–2578