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Physicochemical and textural properties of heat-induced pea protein isolate gels

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Abstract

Chemical and thermal properties of pea protein isolates (laboratory prepared or native; PPIn and commercial; PPIc) and textural properties of heat-set gels obtained from pea protein isolates were compared with homologous soy protein isolates (laboratory prepared, or native; SPIn and commercial; SPIc). The protein banding pattern resulting from electrophoresis separation confirmed the presence of predominant storage proteins of pea and soy seeds in the respective protein products. PPIc and SPIc had lower nitrogen solubility than their native counterparts, likely due to their denaturated state which was further confirmed by the absence of distinct endotherms in these commercial materials compared to the laboratory prepared ones. Addition of NaCl at 1.0–2.0% (w/w) to PPIn and SPIn slurries increased thermal transition temperatures for both proteins.

The optimal conditions for formation of strong heat-induced gels from PPIc were 19.6% (w/w) protein content, pH 7.1, 2.0% (w/w) NaCl, and heating at 93 °C. SPIc gels prepared under the same conditions were stronger and more elastic than PPIc gels as denoted by texture indictors.

Keywords: Pea protein isolates; Chemical composition; Protein gelation; Thermal properties; Texture

1. Introduction

Heat-induced gel network formation by globular proteins is of enormous significance to generate texture in food. Heat-induced protein gels simultaneously bind water, fats, flavour, pigments and other ingredients and stabilize them in the dispersed phase thus allowing an interesting product platform to generate new food products. Concentrated forms of seed storage proteins such as soy protein isolates have widely been employed in more sophisticated applications; for example to generate gel structures that give the same texture but with reduced lipid and animal protein content in the final product. Field pea (*Pisum sativum* L.) may be an alternative source of seed proteins for such applications. The province of Saskatchewan contributes ~70% of the total Canadian field pea production (Agriculture & Agri-Food Canada, 2005). Although pea is a widely consumed legume seed, protein-based ingredients of pea are not yet well utilized in food applications.

Like other legume seeds, *Pisum sativum* L. is rich in protein (18–30%, Guéguen, 1991). The protein fraction of pea has been extensively studied (Guéguen, Vu, & Schaeffer, 1984; Hsu, Leung, Morad, Finney, & Leung, 1982; Lampart-Szczapa, 2001; O'Kane, Vereijken, Gruppen, & van Boekel, 2005; Osborne & Campbell, 1898; Sosulski, Garratt, & Slinkard, 1976; Swanson, 1990; Tian, William, & Small, 1999). Pea seed storage proteins are composed mainly of legumin (11S), vicillin (7S) and albumins (2S) and the majority of pea protein isolates contain globular 11S and 7S (Gatehouse, Lycett, Croy, & Boulter, 1982; O'Kane et al., 2005). The ratio of legumin to vicilin in

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pea ranges from 0.2 to 1.5 (Casey, Charman, Wright, Bacon, & Guldager, 1982). Pea protein products have been reported to exhibit comparable and complementary functionality to homologous soybean protein products, however, it has been noted that heat-induced gels of pea proteins were weaker than soy protein gels (Hsu et al., 1982; O'Kane et al., 2005; Soral-Śmietana, Świgoń, Amarowicz, & Sijtsma, 1998; Sosulski et al., 1976).

Previous research suggests that the heat-induced gelation of globular proteins is a multi-stage process requiring thermally-induced unfolding of the native molecules to expose interaction sites, intermolecular interaction of unfolded proteins or aggregation of unfolded molecules, and agglomeration of aggregates to form a network (Clark, Kavanagh, & Ross-Murphy, 2001). Thus gel-forming ability and viscoelastic properties of globular proteins largely depend on modes of interaction and bonding, such as hydrogen and covalent bonds, and electrostatic and hydrophobic interactions (Clark et al., 2001; Matsumura & Mori, 1996). Protein interactions are primarily based on unfolding of the protein chain, which occurs as a thermal transition above the denaturation temperature of the protein. This event is essential prior to the aggregation and gelling events in the multistage process. The pH, presence of ionic species and their strength, heating temperature, and heating time are factors that affect gel network formation by globular proteins (Matsumura & Mori, 1996). Actually these are process conditions that can be manipulated for gel formation.

Although the gelation mechanism of soy proteins and other related plant proteins has received much attention, insight into the gelation of pea protein isolate is limited. Recent studies by O'Kane, Happe, Vereijken, Gruppen, and van Boekel (2004a, 2004b, 2004c) and O'Kane et al. (2005) suggest that pea globulins may not behave in a similar manner to soy globulins in the mechanism of heat-set gel formation. The present study was carried out with a special interest on utilizing pea protein products for vegetable protein-based product development that relies on heat-induced gelation properties of proteins. The objective of this communication was to report the effect of processing conditions and essential ingredients (temperature, pH and NaCl concentration) on the thermal and textural properties of heat-induced pea protein isolate gels.

2. Materials and methods

2.1. Raw materials

Protein isolates were prepared in the laboratory from field pea concentrate (Progress Protein; protein rich flour/concentrate obtained by air classification, Parrheim Foods, Saskatoon, SK) or soy flour (Nutrisoy 7B defatted, Archer Daniels Midland Company, Decatur, IL) by alkaline extraction (pH 8.5) followed by acidic precipitation (pH 4.5) of proteins according to the conditions adopted from Sumner, Nielsen, and Youngs (1981). The protein suspension obtained from the final step of the extraction process was adjusted to pH 6.5 by addition of 1 M NaOH and freeze-dried. These conditions minimized denaturation of proteins and thus the isolates were considered to contain "native" forms of the proteins (pea protein isolate native, PPIn; soy protein isolate native, SPIn). Protein content of the final product was 80.7% and 89.9% ($6.25 \times N\%$) for pea and soy isolates, respectively. Commercial pea protein isolate (PPIc, Propulse®) was kindly provided by Parrheim Foods Limited (Portage la Prairie, MB). Pro-FAM 982 isolated soy protein (soy protein isolate commercial, SPIc) was obtained from Archer Daniels Midland Company (Decatur, IL).

2.2. Characterization of protein isolates

2.2.1. Chemical composition

Total moisture (Method No. 925.1), crude protein (Method No. 920.87) and ash (Method No. 923.03) contents of pea and soy protein isolates, pea concentrate and soy flour were determined by the AOAC (1990) procedures. The total lipid content was determined by AOAC method 960.39 (AOAC, 1990) with a modification of Southgate (1971). Total dietary fiber assay was performed according to Technical Bulletin No. TDFAB-3 (Sigma, Saint Louis, MO).

2.2.2. Nitrogen solubility index (NSI)

The determination of NSI was according to AACC method 46–23 (1982) with a slight modification according to Betschart (1974). The pH was adjusted to values from 3 to 10 with either 0.1 or 1.0 M HCl or NaOH. Samples were placed in a 30 °C shaking water bath (Jeio Tech, BS-10, Korea) at 120 rpm for 2 h. Incubated samples were centrifuged for 10 min at 1500*g* at room temperature and the supernatant was used for determination of soluble nitrogen content.

2.2.3. Thermal properties

The thermal properties of commercial and native pea and soy protein slurries (10% protein concentration, w/w, pH 6.4–6.5) as a result of NaCl (1.0–2.0%, w/w) addition were examined using the method of Arntfield and Murray (1981) with a slight modification. The protein isolate and salt were mixed and dispersed in deionized water using a vortex (Vortex-Genie, Scientific Instruments, Inc. Bohemia, NY). Approximately 10–15 mg of protein slurry was weighed into the aluminum pan. The pan was hermetically sealed and then heated from 20 to 120 °C at a rate of 10 °C/min on a TA Modulated DSC thermal analyzer (TA Instruments, New Castle, DE). A sealed empty pan was used as a reference. Onset temperature (T_o), peak transition temperature or denaturation temperature (T_d), and enthalpy of denaturation (ΔH) were computed from the thermograms using computer software (Universal Analysis Program, Version 2.5H, TA Instruments, New Castle, DE). Temperature calibration was done with indium.

2.2.4. Protein composition using electrophoresis

Protein isolates were suspended in distilled water (5 mg/ml) and used for sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). Samples were prepared according to Laemmli (1970) procedure in a sample buffer containing 0.1 M Tris–HCl at pH 8.0, 10 % (w/v) SDS, 5% (v/v) β-mercaptoethanol. Gradient mini gels (resolving 8–25% T and 2% C, stacking zone 4.5% T and 3% C, $43 \times 50 \times 0.45$ mm, polyacrylamide gels cast on GelBond® plastic backing, buffer 0.112 M acetate, 0.112 M Tris, pH 6.4) were used to separate proteins on a PhastSystem equipped with separation and development capabilities (Pharmacia Biotech AB, Uppsala, Sweden). Approximately 1 μg protein was applied into each lane. The molecular weight standard (Sigma wide range molecular weight markers, 6.5–205 kDa) was applied into a separate lane. Electrophoresis conditions were 250 V, 10 mA throughout at 15 °C, and 45 min running time. The buffer strips were at pH 8.1, composed of 0.2 M Tricine, 0.2 M Tris, 0.55% (w/v) SDS and 3% (w/v) agarose, and served as the discontinuous buffer reservoir. Following separation, the proteins were fixed and stained using the PhastGel blue R (coomasie R-350) and developed to obtain suitable background colour. The gels were scanned and the acquired images were analyzed by the Image Master® (version 3.0, Pharmacia Biotech AB, Uppsala, Sweden) software.

2.3. Preparation of heat-induced pea protein isolate gels

2.3.1. Studies to compare gelation properties of native and commercial proteins

Protein gels (400 g each) were prepared by mixing protein isolates and other ingredients with deionized water for 90 s using a food processor (Braun, UK100, Kronberg, Germany). In all treatments, sodium chloride concentration, protein concentration and pH were fixed at 1.0%, 19.6% and 6.5, respectively. The pH of each sample was adjusted by addition of 1 M NaOH or 1 M HCl if needed. After the pH was measured, the batter was placed in a vacuum bag, and a vacuum was applied twice at maximum vacuum capacity for 2.2 s (Bizerba, Bizerba Canada, Inc., ON) to eliminate air in the batter. Batters were stuffed into cylindrical plastic tubes (30 mm × 115 mm) which were centrifuged at 1300*g* for 3 min (IEC Clinical Centrifuge, International Equipment Company, MA) to prevent air voids. The stuffed tubes were then heated isothermally at either 82 or 92 °C for 45 min in circulating water baths (Haake, D1, Dreieich, Germany). After thermal processing, all samples were stored at 4 °C for 14 h until testing.

2.3.2. Response surface study to determine optimal gelation conditions for commercial PPI

Each experimental run (formulation weight of 1220*g*) was prepared in random order. PPIc (~25% w/w to achieve a protein level of 19.6%) was blended for 90 s with deionized water, NaCl (0–2.0%, w/w) and the required 1 M NaOH or 1 M HCl for pH adjustment at high speed using a food processor (Braun, UK100, Kronberg, Germany). Immediately after preparation, the batter was placed in a vacuum bag. After a single vacuum treatment, the batter was transferred to 400 ml beakers with constant knocking on the beaker to prevent air voids. The beakers were then covered with food packaging film and sealed with an elastic band to prevent moisture losses during thermal processing. Samples were placed in a water bath (Isotemp 205, Fisher Scientific, PA) maintained at 99 °C and thermally processed to center temperatures of 79, 82, 87, 92 and 95 °C. When the central point of each sample reached the desired temperature, the water bath temperature was adjusted and samples were held at that temperature for an additional 25 min. Thermocouples (Omega, HH23 Microprocessor thermometer, Omega Engineering Inc., Stamford, CT) were used to monitor the centre temperature. After heating, samples were immediately transferred to the 4 °C cooler and stored overnight (14 h). The same method was used to prepare gels from commercial soy protein using conditions at the midpoint of the experimental design (1% (w/w) NaCl, pH 7.1 and heating up to 87 °C) at a protein level of 19.6%.

2.4. Characterization of heat-induced pea and soy protein gels

2.4.1. pH and water binding properties

The pH was measured in a slurry prepared by blending 20 g of batter or gel in 80 ml of deionized water for 1 min and using an Accumet® pH meter (Model 915, Fisher Scientific Ltd., Nepean, ON) with a Fisher combination electrode (silver/silver chloride). For water binding properties, the chilled gel after overnight storage was removed from the beakers or tubes, blotted dry with a paper towel and reweighed. Total cook yield was calculated as a percentage based on the raw weight. Expressible Moisture (EM) was determined by using a modification of the method described by Jauregui, Regenstein, and Baker (1981). The sample (1.5 ± 0.3 g) was weighed and then placed in a filter paper thimble for centrifugation (J2-HC Centrifuge, Beckman) for 10 min at 750*g* and 4 °C. All samples were run in duplicate and expressible moisture reported as percent weight reduction from original sample.

2.4.2. Torsional rheometry of gels

Six cores (20 mm in diameter, 28 mm in length) were prepared from each treatment. Disposable polystyrene discs, designed to hold the sample in a shaping apparatus and torsion device, were attached to the ends of the cylindrical samples with instant adhesive (Loctite 404, Loctite Corp., Hartfield, CT). The samples were placed into a sealed container to prevent moisture loss and cooled at 4 °C for 2 h. The samples were then carved into dumbbell-shaped specimens with a 10 mm diameter at the midsection using a grinder apparatus (KCI-24A2, Bodine Electric Company, IL). The shaped specimens were equilibrated to room temperature (approx. 21 °C) and torsion-

ally sheared at 2.5 rev/min to the point of structural failure in a device attached to a Brookfield viscometer (Model DV-I+, Brookfield Engineering Laboratories, Inc., Stoughton, MA). Shear stress (kPa) and shear strain at failure were calculated from torque and angular displacement using the provided software (GelScan®, Gel Consultants Inc., Raleigh, NC).

2.4.3. Texture profile analysis (TPA) of gels

Texture profile analysis (TPA) of gel samples was performed using a TMS-90 Texture Machine (Food Technology Corporation, VA). Five center cores (23 mm in diameter, 15 mm in height) of each gel sample were compressed twice to 25% of their original height at a constant crosshead speed of 0.17 cm/s. The TPA parameters, namely hardness (peak force on first compression, *N*), cohesiveness (ratio of the active work done under the second force–displacement curve to that done under the first compression curve) were computed automatically by the instrument.

2.5. Statistical analysis

Statistical analyses were performed using the program Statistical Analysis System (SAS for windows, Release 8.02, SAS Institute Inc., Cary, NC). For DSC data, a one-way ANOVA was performed with NaCl concentration as an independent variable. For comparison studies (PPIn and PPIc gels) a full factor factorial treatment design was employed and multiple comparisons of means were performed by using least significant differences (LSD).

To study the simultaneous effects of the processing variables (heating temperature, pH and NaCl concentration) and optimum levels of the variables for PPIc gels, response surface methodology (RSM) was used. Five levels of each factor were chosen based on the central composite rotatable design, CCRD (Kuehl, 2000). The levels of the variables were in the range of 79–92 °C for heating temperature, 6.1–8.1 for pH and 0–2.0% (w/w) for NaCl concentration. The following second-order polynomial equation was assumed for the model of CCRD and the same for generating response surface plots:

$$Y = b_o + \sum_{i=1}^{n} b_i x_i + \sum_{i=1}^{n} b_{ii} x_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} x_i x_j$$

where Y is the estimated response, texture parameters; shear stress, shear strain, TPA hardness, and TPA cohesiveness, b_0 is constant and b_i, b_{ii} and b_{ij} are linear, quadratic and interaction regression coefficients, respectively; X_i and X_j are independent variables (heating temperature, pH and salt level). For each experimental factor the variance was partitioned into components; linear, quadratic and interaction in order to assess the adequacy of the second order polynomial function and the relative importance of these components. The significance of the equation parameters for each response variable was assessed by P value ($P < 0.05$). Contour and surface plots were generated to show the effect of two independent variables on a given response at a fixed value of the third independent variable that was set at the center point.

3. Results and discussion

3.1. Chemical composition

The proximate composition of the starting flour/concentrate, commercial isolates and laboratory prepared protein isolates are shown in Table 1. The values obtained for PPIn were in similar range to that reported by Tian et al. (1999, 77% protein on a dry basis) except a higher protein content of 85% (dry basis) was obtained in the present study. An air classified pea concentrate was used for preparing PPIn in this study because it eliminated handling of large volumes of slurries and also minimized interferences from carbohydrates during extraction and isolation of proteins. The ash content of the isolates were higher than the respective starting materials. Sosulski and McCurdy (1987) have pointed out that strong acid and alkali used in pH adjustment during protein extraction may contribute to salt formation and results in high values for ash content in the isolate.

The high total fat content in PPIn compared to SPIn may have contributed to the total protein differences observed although both proteins were isolated by the same process and from starting materials of similar total lipid

Table 1
Chemical composition of protein samples (as is basis)

Material	Protein (%)	TDF[a] (%)	Total lipids (%)	Ash (%)	Moisture (%)
Pea concentrate (air classified)	44.8 ± 0.3[b]	20.1 ± 1.2	5.8 ± 0.3	4.3 ± 0.3	11.8 ± 0.0
PPIn[c] (laboratory prepared)	80.7 ± 0.4	0.7 ± 0.1	9.2 ± 0.1	5.9 ± 0.1	4.3 ± 0.1
PPIc[d] (commercial)	76.9 ± 0.2	2.6 ± 0.4	11.7 ± 0.2	5.0 ± 0.5	5.3 ± 0.2
Soy flour	51.4 ± 0.9	19.3 ± 1.2	5.7 ± 0.1	2.7 ± 0.2	5.7 ± 0.0
SPIn[e] (laboratory prepared)	89.9 ± 0.3	1.1 ± 0.1	3.7 ± 0.1	4.1 ± 0.0	1.4 ± 0.0
SPIc[f] (commercial)	87.1 ± 0.4	0.5 ± 0.0	4.2 ± 0.1	4.9 ± 0.4	3.5 ± 0.1

[a] Total dietary fiber.
[b] Mean ± standard deviation.
[c] Pea protein isolate native.
[d] Pea protein isolate commercial.
[e] Soy protein isolate native.
[f] Soy protein isolate commercial.

content. The differential retention of lipid during extraction could be due to differences in lipid composition and physical location of lipids in the pea concentrate and soy flour matrix. Compared with the pea concentrate, total lipids of PPIn actually increased from 5.8% to 9.2% following extraction. Pea seeds contain 3–4% lipids that can be extracted in hot chloroform–methanol (2:1, v/v) mixture. Pea lipids tend to associate with the protein fraction (Haydar & Hadziyev, 1973). According to Bacon, Noel, and Lambert (1990) alkaline solubilization and isoelectric precipitation tended to concentrate lipid in the pea protein products because of lipid–protein binding during protein extraction.

SPIc contained higher amounts of protein than PPIc. Higher amounts of TDF and total lipids were found in PPIc than SPIc. The ash content of both commercial protein isolates was at similar levels. It is expected that the differences in total lipids and dietary fiber contents between pea and soy protein products may influence gelation properties of native and commercial protein isolates. All these protein isolates had a creamy, light yellow colour.

3.2. Nitrogen solubility index (NSI) of protein isolates

All four protein products showed minimum solubility near pH 4.5 (Fig. 1). The predominant proteins of soy and pea protein isolates likely have isoelectric pH around this value. Both native pea and soybean protein isolates had a higher NSI value than that of commercial pea and soybean protein isolates at pHs higher or lower than the minimum solubility pH (Fig. 1). The SPIn exhibited a higher solubility than the PPIn at all pH levels. The biggest differences in NSI values were found at pH 3 and 7. The SPIn showed only a gradual increase in solubility from pH 7–10. However, at pH 7, SPIn reached 90% NSI while the PPIn exhibited only 59% for NSI. The inherent properties of each protein, such as protein conformation and interactions, and the other components in the isolate might have caused the differences in NSI observed between the soy and pea protein isolates. Sosulski and McCurdy (1987) and Tömösközi, Lásztity, Haraszi, and Baticz (2001) have also observed similar NSI profiles for laboratory prepared pea protein isolates.

Within the pH range of this study, commercially produced pea and soy protein isolates exhibited lower values of NSI than their native counterparts. The low NSI of commercial protein products may have resulted from processing conditions such as high temperatures during spray-drying that led to denaturation and aggregation. The PPIc showed a moderate increase in NSI on both sides of the isoelectric point. Similar values of NSI were observed at pH 7 for both PPIc and SPIc. It is interesting to note that a sharp increase in NSI was observed for SPIc after pH 7 but not for PPIc.

Solubility is a physico-chemical property of a protein that critically affects its functional properties as manifested in foods, mainly emulsifying, foaming, and gel forming abilities (Sikorski, 2001). In heat-induced protein gel formation, soluble aggregation is the second step of the proposed three-step gelation mechanism and the extent of protein that is in soluble form (indicated by NSI) may relate to different degrees of protein–protein interactions during this process. Therefore, the NSI may have an indirect relationship with elasticity or deformation properties of heat-induced protein gels.

3.3. SDS-PAGE analysis of protein isolates

The protein composition of PPIc, PPIn, SPIc and SPIn as separated on SDS-PAGE under reducing conditions is provided in Fig. 2. The protein banding pattern of commercial proteins were identical to the native proteins of

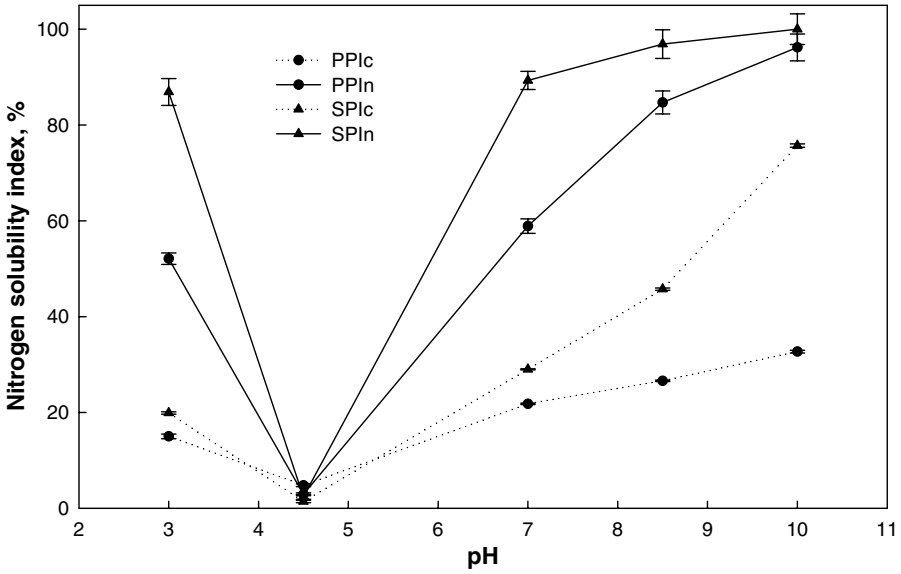

Fig. 1. Nitrogen solubility index (NSI) of native and commercial pea and soy protein isolates as a function of pH.

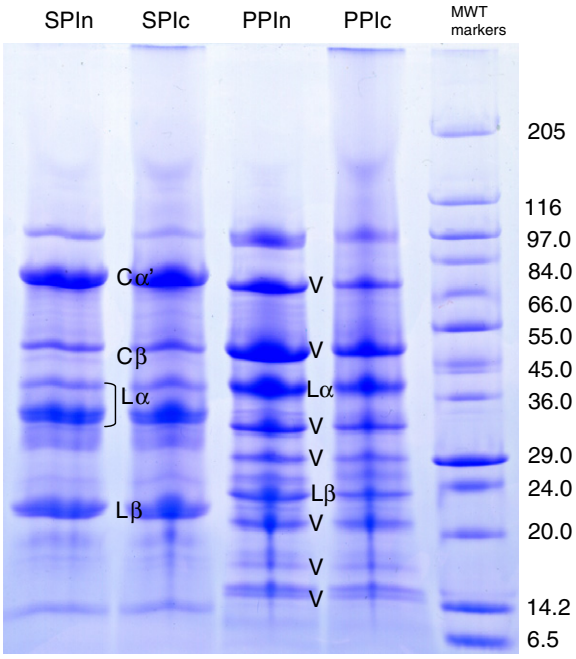

Fig. 2. Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) pattern of pea and soy protein products under reducing conditions. Gels were of 8–25% gradient T and 2% C. In the lanes of PPIc (pea protein isolate commercial) and PPIn (pea protein isolate native), V = bands from vicilin proteins, Lα = legumin acidic subunit and Lβ = legumin basic subunit. For lanes of SPIc (soy protein commercial) SPIn (soy protein isolate native), Cα′ = β-conglycinin α′ subunit, Lα = legumin acidic subunit and Lβ = legumin basic subunit. MWT = molecular weight markers.

the same seed. Electrophoresis of the pea protein showed bands ranging from 99 to 11.8 kDa that originated mainly from legumin and vicilin, which are 11S and 7S globulins, respectively. The molecular weight calculated based on the R_f value was matched with previous work for band identification. A protein, with an apparent MW of ~90 kDa, which was present in pea and soy samples, has been reported to be lypoxygenase (Crévieu et al., 1997). Legumin, a hexameric protein, dissociates into two subunit peptides (α; acidic 38–40 kDa and β; basic 19–22 kDa) when S–S bonds are broken under reducing conditions (Bacon, Noel, & Wright, 1989; Crévieu et al., 1997; Casey, 1979; Gatehouse, Croy, & Boulter, 1980; Gatehouse et al., 1982; Matta, Gatehouse, & Boulter, 1981). Vicilin is a trimeric protein, composed of three heterogeneous subunits of ~50 and ~70 kDa. No S–S bonds are involved in stabilizing the vicilin protein structure (O'Kane et al., 2004a). Gatehouse et al. (1982) have indicated that the major polypeptides of the vicilin fraction (7S) have molecular mass of 71, 50 and 33 kDa with minor components of lower MW (19–12.5 kDa). The 70 kDa protein band has been considered to be convicilin, however, the detailed work of O'Kane and group (2004a) denotes that it is the α-subunit of vicilin. Results of the SDS-PAGE of the soy protein isolates were as expected showing the presence of subunit peptides from β-conglycinin (7S) and legumin (11S) proteins. Protein precipitation at pH 4.5 ensured recovery of most of the proteins solubilized at alkaline pH, therefore the final product was a mixture of 11S and 7S storage proteins of these legume seeds. It should also be noted here the presence of bands above 116 kDa in SPIc and PPIc samples in the gels. These bands may represent polymerized proteins which were formed during commercial processing of the protein isolates.

3.4. Thermal properties of pea protein isolates in comparison with soy protein isolates

Differential scanning calorimetry of protein slurries (10% protein w/w, 10 °C/min heating rate) provided insight into the thermal properties of these proteins. Two major endothermic peaks at 67.1 ± 1.8 °C and 85.1 ± 0.4 °C were observed for PPIn (Fig. 3, Table 2). The second peak of the thermograph had a high enthalpy value and may represent the denaturation of the legumin and vicilin fractions of PPIn. It is possible that the smaller first peak represents a thermal transition for the non-globulin fraction components, such as crude fiber or/and starch. Compositional analysis also support that there are carbohydrate polymers as a contaminant in the protein products which may have contributed to the first endothermic peak. According to Sosulski, Hoover, Tyler, Murray, and Arntfield (1985), the starch of field pea show a peak gelatinization temperature (T_g) at 65 °C with a smaller enthalpy change than that of the protein fraction. The same group speculated that storage proteins, vicilin (7S) and legumin (11S) of pea could account for the multiple endotherms and variation in denaturation temperature (T_d) due to their heterogeneity. However, several studies (Arntfield & Murray, 1981; Bacon et al., 1989; Cserhalmi, Czukor, & Gajzágó-Schuster, 1998) support that the small difference between T_d values of vicilin and legumin results in one major endothermic peak because of overlapping transition temperatures of these two protein molecules. According to Arntfield and Murray (1981), a T_d value of 86 °C was observed for air-classified field pea; Bora, Brekke, and Powders (1994) have reported that the mixed globulins (35.7% legumin and 64.3% vicilin of pea) gave one thermal transition between 74 and 95 °C with a maximum at 86.2 °C under 10 °C / min heating rate. Therefore the second endothermic peak obtained for PPIn may represent the thermal transition of the protein fraction.

In contrast to PPIn, only one endothermic peak (with a T_d at 74.8 °C) was observed in the thermograph of PPIc (Fig. 3). This peak had a slightly higher T_d than peak 1 of PPIn and a low enthalpy value. The absence of any other endothermic peak in PPIc may indicate the possible denaturation of major proteins in this product. Arntfield and Murray (1981) have concluded that the lack of an endotherm might indicate a denatured protein, particularly if the undenatured protein gives an endotherm under usual analytical conditions. Therefore, combined with the relatively low NSI value observed for the commercial PPI, it

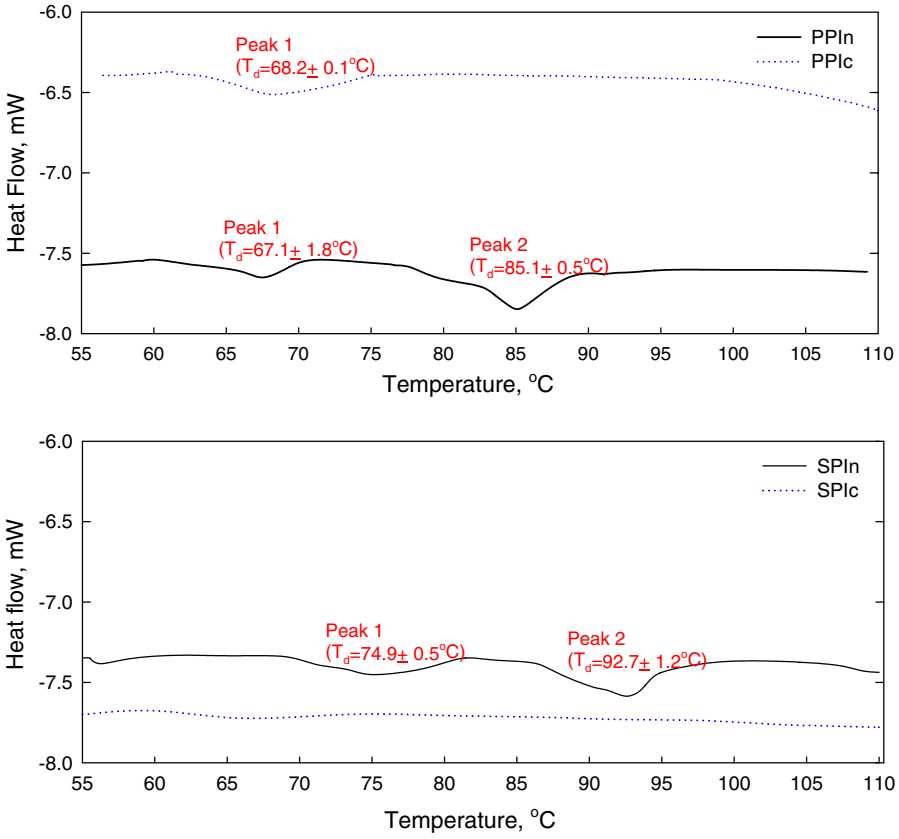

Fig. 3. DSC thermograms of pea and soy protein products (slurry of 10% w/w protein heated at 10 °C/min).

Table 2
Temperature of thermal transitions (T_d) and enthalpy (ΔH) of the combined endotherm of native pea protein isolate (PPIn) and native soy protein isolate (SPIn) with different sodium chloride levels

Treatment	PPIn		SPIn	
	T_d (°C)	ΔH (J/g)	T_d (°C)	ΔH (J/g)
Peak 1				
0% NaCl[a]	67.15 ± 1.83a[b]	0.095 ± 0.006a	74.95 ± 0.56a	0.100 ± 0.019a
1% NaCl	68.15 ± 0.80b	0.070 ± 0.027a	79.56 ± 0.34b	0.133 ± 0.045a
2% NaCl	68.06 ± 2.01a	0.146 ± 0.025a	82.27 ± 1.23c	0.187 ± 0.032a
Peak 2				
0% NaCl	85.07 ± 0.47a	0.725 ± 0.060a	92.71 ± 1.20a	0.494 ± 0.050a
1% NaCl	90.03 ± 0.69b	0.852 ± 0.044a	97.44 ± 0.29b	0.549 ± 0.034a
2% NaCl	92.90 ± 0.30c	0.922 ± 0.105a	99.91 ± 0.29c	0.651 ± 0.031b

[a] NaCl levels are on weight basis.
[b] Means in the same column with the same letter are not significantly different ($P > 0.05$).

can be concluded that the PPIc contains partially or completely denatured proteins.

The thermographs of the SPIn exhibited two peaks corresponding to characteristic endothermic transitions of the glycinin (11S, 92.7 ± 1.2 °C) and β-conglycinin (7S, 74.95 ± 0.5 °C) fractions (Fig. 3). Scilingo and Aňón (1996) have reported a similar finding for native soy protein with denaturation temperatures of 76 and 92 °C for 7S and 11S components, respectively, however higher thermal transition temperatures have been reported by Liu and Xiong (2000) and Yildirim and Hettiarachchy (1997) for individual soy protein fractions. SPIc did not produce distinguishable thermal transition peaks in the thermogram indicating denatured proteins in the product.

3.5. Effect of sodium chloride concentration on thermal properties of protein isolates

Additions of NaCl (w/w) at 1.0% and 2.0% to PPIn slurries had no effect on the first thermal transition peak. However, addition of NaCl resulted in a gradual increase in the T_d values with increasing sodium chloride concentration

(Table 2) demonstrating a stabilizing influence of NaCl on the proteins. The effect of sodium chloride concentration on the shift of thermal transition temperature for pea proteins has not been reported before. As NaCl concentration increased, there was a shift in the transition temperatures of both 7S and 11S fractions of SPIn (Table 2). The effects of NaCl concentration on denaturation temperatures and enthalpy change of PPIc were minor as there were no clearly distinguishable endothermic peaks.

Zheng, Matsumura, and Mori (1993) observed that NaCl addition dramatically increased the denaturation temperature of legumin of broad bean. Meng and Ma (2001) and Arntfield, Murray, and Ismond (1986) have also observed a similar shifting effect on thermal transition temperatures for the globulins of red bean and faba bean, respectively. The enhancement of thermal stability of PPIn in the presence of NaCl might have resulted from increased intramolecular hydrophobic associations (Arntfield, Murray, & Ismond, 1990a, 1990b) and the electrostatic response or alteration of water structure around the protein, which enhanced the hydration of the protein molecules (Meng & Ma, 2001). Protein molecules of PPIc were probably unfolded during processing and may have exposed hydrophobic groups thus decreasing any effect of sodium chloride addition.

3.6. Properties of heat-induced gels

The protein content of gels was kept constant at 19.6% (w/w) to ensure that a self supporting gel was formed. This value was obtained from the preliminary experiments with PPIn and PPIc as described by Ya (2004). O'Kane et al. (2005) have reported that the minimum concentration required for heat-induced gel formation near neutral pH (pH 7.1) is 16% (w/v) for pea protein isolates containing 20–28% legumin and 61–67% vicilins in their composition. The protein gels of PPIn and PPIc were opaque and had an orange-tan tint.

3.6.1. Shear stress and shear strain of heat-induced gels

Effects of final heating temperatures (82 °C was lower and 92 °C higher than the T_d for the second endothermic peak) on textural properties of gels from PPIn and PPIc are presented in Table 3. The gels of PPIc showed relatively higher shear stress (gel strength) than that of PPIn when heated to either 82 or 92 °C. However, PPIn gels showed higher shear strain than PPIc gels at both temperatures, indicating greater elasticity of the PPIn gels. At 82 °C the pea proteins, especially those of PPIn, might not be completely denatured (this temperature was slightly lower than peak 2 T_d, Fig. 2) and not unfolded enough to interact with each other and thus not able to form a strong gel network under these conditions. When heating temperature (92 °C) was higher than T_d of the second peak, the shear stress of PPIn resulted in a sharp increase, most likely due to unfolding of the protein and formation of the strong gel network. At this temperature, a greater extent of denaturation would be found in both legumin and vicilin fractions of PPIn. The structure development usually coincides with the temperature associated with maximum heat flow on T_d for the most prominent protein in the mixture (Arntfield, Murray, Ismond, & Bernasky, 1989).

Table 3
Effect of temperatures on rheological properties of heat-induced gels from PPIn and PPIc (protein content 19.6% w/w, pH 6.5, and 1% w/w NaCl concentration)

Protein	Temperature of heating (°C)	Shear stress (kPa)	Shear strain
PPIn	82	6.93 ± 0.22c[a]	0.75 ± 0.04a
	92	11.05 ± 1.04ab	0.80 ± 0.04a
PPIc	82	10.29 ± 2.24b	0.57 ± 0.10b
	92	12.45 ± 0.87a	0.62 ± 0.05b

[a] Means with the same letter are not significantly different at $P > 0.05$.

For PPIc, the shear stress increased moderately with an increase in the heating temperature (Table 3). At each temperature, higher gel strength (shear stress) was observed for gels from PPIc than from PPIn. There was a 37% change in shear stress of PPIn gels when temperature was increased from 82 to 92 °C and only a 17% change for PPIc gels. This observation may be attributed to the differences in temperature of denaturation and the solubility as indicated earlier. Since PPIc is already denatured as evidenced by DSC, gel strength was less dependent on heating temperature compared with PPIn. This could be an advantage for PPIc in processing applications when lower thermal processing temperatures are used. However, the commercial product produced less elastic gels at either temperature.

The partial or nearly complete denaturation of protein in PPIc must have contributed to the differences in NSI values. The NSI values for PPIn and PPIc were 59% and 22%, respectively, at pH 7 (Fig. 1). According to Lanier (1986), shear strain of protein gels are more sensitive to functional properties of protein including nitrogen solubility. A higher value of shear strain was observed for PPIn at both temperatures which also showed a higher solubility than its commercial counterpart. These results support the concept that higher level of soluble aggregate formation during heat-induced protein gelation may result in an increasing intermolecular interaction needed to create more cross-links. According to Beveridge, Jones, and Tung (1984), gels become more elastic as the increasing numbers of cross-links stiffen the structure of individual aggregates. This possibility may have led to the enhanced elasticity of native pea protein gels that was observed in this study. Whereas for PPIc, the soluble aggregation during gelation was affected by relatively low NSI values and the number and type of cross-linking might be less than in PPIn gels. The shear strain of the gels were not significantly ($P < 0.05$) affected by the difference in heating temperature for both pea protein isolates, although the numbers were higher at 92 °C.

3.6.2. Effects of NaCl concentration, pH, and heating temperature on the textural properties of heat-induced gels from PPIc

Response surface analysis revealed that none of the experimental factors (heating temperature, pH level and salt concentration) had significant ($P > 0.05$) effects on cooking loss and expressible moisture of pea protein gels (data not shown). PPIc gels with 19.6% protein content exhibited a strong water holding capacity under these processing conditions. Only a small moisture loss occurred during cooking and at low speed (750*g*) centrifugation of the heat-induced PPIc gels.

Fig. 4 shows the texture profile analysis (TPA) results for cohesiveness, TPA hardness, shear stress and shear strain of heat-induced PPIc gels mapped as a function of heating temperature and pH (at 1.0% w/w NaCl level). The regression models for shear stress and TPA hardness were significant ($P = 0.031$ and 0.017), while the models for shear strain and TPA cohesiveness explained a lower percentage of variability in the data ($P = 0.059$ and $P = 0.095$). Heating temperature had an appreciable positive effect on the shear stress and TPA hardness, the linear components of the regression being highly significant ($P < 0.05$). Higher shear stress and hardness was observed for gels prepared at higher temperatures than for ones at lower temperatures. This is a well-known property of globular proteins in that when the well-defined secondary and tertiary structures of the compact molecule becomes disrupted on heating, the protein molecules become more reactive towards the neighboring molecules to form a network (Clark et al., 2001).

An optimum pH level of 7.1–7.2 was observed for the highest hardness and shear stress values at all heating temperatures. As a variable, pH exhibited a positive quadratic effect ($P = 0.088$) on the model for TPA hardness ($P < 0.01$) and shear stress. However, both these parameters of the gels were not affected by increased sodium chloride levels. Meng and Ma (2001) reported that at high or low pH, net charges are largely induced and repulsive forces increase, resulting in an unfolding of protein molecules. At high pH levels, the repulsive force of protein molecules are so high that it affects the gel structure and reflects in a decrease in the gel strength.

The polynomial models for shear strain and TPA cohesiveness were not significant, nonetheless the results of model fitting showed that pH was the only variable that

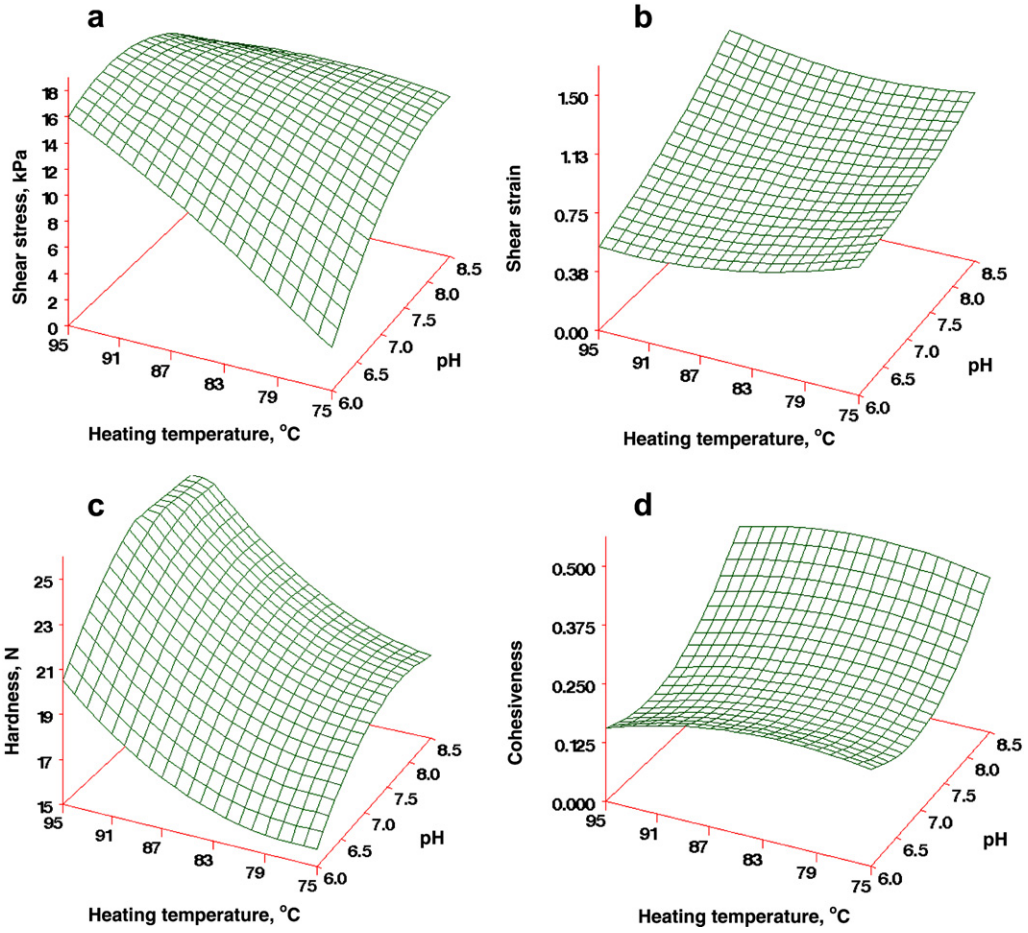

Fig. 4. Effects of temperature (°C) and pH on (a) shear stress, (b) shear strain, (c) TPA hardness and (d) TPA cohesiveness of PPIc heat-induced gels (19.6% w/w protein) at 1% (w/w) NaCl level.

significantly affected these parameters. General linear model (GLM) analysis indicated that there was a positive linear effect of pH level on shear strain ($P < 0.001$, $R^2 = 0.85$) and cohesiveness ($P < 0.001$, $R^2 = 0.91$). The higher the pH, the more cohesive and elastic were the gel samples.

The effect of pH may be related to the nitrogen solubility. As shown earlier, change in the pH considerably modifies the solubility of PPIc and thus may influence the elasticity of heat-induced gels. This observation was supported by results of Hamann and Lanier (1986) who reported that shear strain is influenced mainly by protein quality and protein functionality, such as nitrogen solubility, but not the process conditions of gel formation. Beveridge et al. (1984) reported that increasing elasticity or deformation of protein gels probably results from increasing numbers of cross-links that stiffen the structure of individual aggregates. Cross-link formation may strongly relate to the degree of soluble aggregation of the protein molecules during the heating process.

Textural properties of heat-induced gels from PPIc and SPIc (prepared at 87 °C, pH 7.15 and 1.0%, w/w NaCl) were compared. As a protein ingredient, the gel-forming and water-retention abilities of soy proteins are well recognized. The shear strain and stress of the heat-induced SPIc gel were considerably higher ($P < 0.05$) than those of the PPIc gel, indicating that the SPIc gels had higher elasticity and hardness or stronger rheological properties than PPIc gels under the same gelation conditions provided. Mean shear strain and stress values were 0.78 and 14.8*N* for PPIc gels, and 1.44 and 26.9*N* for SPIc gels. This difference might have resulted from the different functionalities, protein conformation, and compositions of these protein isolates.

Research over many years and involving a range of protein types has suggested that heat-induced gel formation by globular proteins is a multi-stage mechanism. The essential steps are thermally-induced unfolding of the native protein in solution exposing hydrophobic sites (some degree of dissociation of multi-subunits and disruption of well defined 2° and 3° structure of proteins may occur), intermolecular hydrophobic interaction of the unfolded protein or aggregation of unfolded proteins, and then agglomeration of aggregates to form a network structure (Foegeding, Bowland, & Hardin, 1995; Clark et al., 2001). Hermansson (1986) reported that the denaturation of both native conglycinin (7S) and native glycinin (12S) contributed to the gel structure development with the formation of cross-links and ordered structures during heating. The work by O'Kane et al. (2004c) emphasized that the molecular driving forces of the heat-induced gelation of pea legumin (12S fraction) and soy bean glycinin (12S fraction) are the same, however, the contribution from disulfide bonds to the gel network strength is minimum in pea legumin (O'Kane et al., 2005). The same group has observed that the gelation behaviour of isolated pea protein is related to the disulfide bonding ability of legumin, and the extent of repulsive forces on the α-subunits (70 kDa fragment) of vicilin fraction but not to the absolute extent of legumins present. The larger vicillin protein fragment (~70 kDa) has been shown to be responsible for the heterogeneous gelation behaviour of vicilin at near-neutral pH conditions (O'Kane et al., 2004b).

This study shows that pea protein isolate can form heat-induced gels, however the conditions for commercial production of this protein must be carefully manipulated to enhance their gel forming functionality. With the growing availability of large-scale commercial production and its nutritional and physicochemical properties, pea protein isolates may be utilized in a variety of food applications related to heat-induced gels. To further enhance gel properties, careful adjustment of processing conditions should be considered. Addition of other ingredients may also be useful.

4. Conclusions

Pea protein isolates (both commercial and native) had higher total lipid content than soy protein isolates. Commercial protein products showed lower NSI values and also the lack of distinct endothermic peaks during differential scanning calorimetry indicated their proteins have already undergone denaturation to a certain extent. One ($T_d \sim 85$ °C) of the two endothermic peaks observed for PPIn may represent thermal denaturation of the vicilin and legumin fraction. Sodium chloride addition at 1.0% and 2.0% (w/w) to native pea protein showed a significant effect by shifting thermal denaturation temperatures to a higher value, however no influence was observed for commercial protein isolates. Textural properties of the PPIc gels could be modified by adjusting gel forming conditions such as temperature and pH, although changes in NaCl concentration had little effect. Within the limits of this study the optimal process conditions for forming a strong heat-induced gels (19.6% protein content, w/w) from PPIc were at pH 7.1, addition of NaCl (2.0%, w/w), and a heating temperature of 93 °C.

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