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Transglutaminase treatment of pea proteins: Effect on physicochemical and rheological properties of heat-induced protein gels

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Abstract

Rheological properties of heat-induced pea protein isolate (PPI) gels with added microbial transglutaminase (MTGase) were studied under various reaction conditions. A positive linear relationship was observed between level of MTGase used (0 to 0.7% w/w) and shear stress and shear strain of heat-set commercial pea protein isolate (PPIc) gels at 92 °C following incubation at 50 °C. Use of MTGase allowed for preparation of PPIc gels of similar strength and elasticity as commercial soy protein isolate gels and commercial meat bologna. MTGase treatment did not alter thermal properties of PPI gels. The shear stress and strain of PPIc gels were also improved following low temperature (4 °C) incubation of PPI with MTGase. Enhancement of shear strain or gel elasticity of heat-induced PPI gels with MTGase has not been reported before and provides opportunities for extending the properties of pea proteins when developing new food products.

Keywords: Pea protein isolates; Microbial transglutaminase; Protein gelation; Thermal properties; Texture

1. Introduction

The ability of food proteins to form heat-induced gels imparts important textural properties to food which can be important in food product development. Like other legume (*e.g.*, soy) and cereal proteins (*e.g.*, wheat gluten), pea protein forms a heat-set gel when conditions are satisfied. Pea seed storage proteins are mainly composed of globulins and albumins. The commercial processes that are employed to isolate pea proteins tend to form a product rich in globulins, which is heterogenic and composed of legumins (11S) and vicilins (7S). The chemical and physical mechanistics of formation of heat-induced gels by legumin and vicilin fractions and their mixtures have been recently detailed (O'Kane, Happe, Vereijken, Gruppen, & van Boekel, 2004a, 2004b, 2004c, 2005). Since the content of legumin, vicilin and their subunit composition are cultivar specific, the contribution of each protein to gel forming behaviour and characteristics are also slightly different (O'Kane, Vereijken, Gruppen, & van Boekel, 2005).

Protein crosslinking refers to the formation of covalent bonds between polypeptide chains within a protein i.e., intramolecular crosslinks or between proteins; i.e., intermolecular crosslinks. Crosslinking and aggregation of protein molecules has been cited as one of the most important mechanisms for engineering food structures with desirable mechanical properties (Dickinson, 1997) because crosslinking can change physicochemical properties of a protein in either the native or denatured state (Gerrard, 2003). Therefore, textural and rheological properties of food protein gels such as hardness, cohesiveness and their indicators, shear stress and shear strain may be modified by altering natural crosslinks or by introducing new crosslinks during protein network formation. Exogenous transglutaminase (TGase) has been widely used for catalyzing crosslinking of food proteins and modifying their functional properties (Dickinson, 1997; Kuraishi, Sakamoto, & Soeda, 1998;

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Motoki & Seguro, 1998). Recently, there have been a number of reports about TGase induced polymerization of food proteins, such as β-casein, β-lactoglobulin (Han & Damodaran, 1996), pea legumin (Larré, Kedzior, Chenu, Viroben, & Gueguen, 1992), gelatin (Babin & Dickinson, 2001), soy protein, egg white (Sakamoto, Kumazawa, & Motoki, 1994), and myofibrillar protein (Ramırez-Suárez & Xiong, 2003) to improve physicochemical properties. Nio, Motoki, and Takinami (1985) reported that crosslinks catalyzed by guinea pig liver transglutaminase increased gel strength of casein protein fractions and soy globulins; enhanced gel strength of TGase treatment on thermoreversible gelation of gelatin was reported by Babin and Dickinson (2001); beef muscle protein gels containing microbial transglutaminase (MTGase) exhibited greater hardness and cohesiveness than those produced without TGase addition (Pietrasik, 2003).

Our previous study (Shand, Ya, Pietrasik, & Wanasundara, 2007) showed that commercial pea protein isolate has limited ability to generate strong heat-induced gels. Therefore, it was hypothesized that introduction of new crosslinks by TGase catalysis may be beneficial for strong and stable structure development and modification of other rheological properties of pea protein gels. The objectives of this study were to compare the effect of various reaction conditions of MTGase on rheological properties of pea protein isolate gels and to determine processing conditions that would allow preparation of pea protein isolate gels of similar rheological properties to gels from other protein sources.

2. Materials and methods

2.1. Raw materials

Commercial pea protein isolate (PPIc, Propulse®) was kindly provided by Parrheim Foods Limited (now Nutra-Pea, Portage La Prairie, MB). Commercial soy isolate (SPIc, Pro-FAM 982) was obtained from Archer Daniels Midland Company (Decatur, IL). Native pea protein isolate (PPIn) and soy protein isolate (SPIn) were prepared in the laboratory from field pea protein concentrate (Progress Protein; air classified field pea protein flour/concentrate, from Parrheim Foods, Saskatoon, SK) or soy flour (Nutrisoy 7B defatted, obtained from Archer Daniels Midland Company, Decatur, IL), respectively as described in a previous communication (Shand et al., 2007) and designated as native proteins. Protein content of the isolates was 80.7% and 89.9% (as is basis) for pea and soy, respectively.

Microbial transglutaminase (MTGase), Activa™ TG-TI, was provided by Ajinomoto® USA, Inc. (Ames, IA). Enzyme activity reported by the supplier was 86–135 units/g dry material. This enzyme-containing product consisted of 99% maltodextrin and 1% enzyme on a mass basis. The protein and ash contents of the enzyme product were 0.23% and 0.02%, respectively.

The bologna style vegetarian product used for textural comparison was kindly provided by Yves Veggie Cuisine (Delta, BC). This product contained 24.2% protein, 1.7% NaCl and 1.5% fat on a mass basis. The ingredients of the product included water, soy protein, vital wheat gluten, canola oil, organic evaporated cane juice, salt, carrageenan, wheat and rice starch, wheat germ, citric acid and spices. Commercially produced meat bologna (No name®, Sunfresh Limited, Toronto, ON) was bought at retail. The product contained 11.3% protein, 2.5% NaCl and 22.7% fat (w/w). The ingredients of the product included water, beef, pork, pork back fat, nitrite, sodium erythorbate, sodium chloride and spices.

2.2. Preparation of heat-induced protein gels from commercial PPI

PPIc, NaCl, water, MTGase were added into a food processor (Braun, UK100, Kronberg, Germany) and then blended with deionized water for 90 s. In all treatments, protein (w/w) and salt (w/w) concentrations, and pH level were at 19.6%, 1% and 6.5, respectively. The pH of each sample was adjusted by addition of 1 M NaOH or 1 M HCl if needed. The experiment was designed according to a small composite design (Draper/Lin version of central composite rotatable design CCRD, SAS version 8.02) to study the effects of the variables: level of MTGase (0.00–0.70%, w/w), incubation temperature (22–78 °C) and incubation time (10–80 min). The protein batters obtained at each experiment point were placed in a vacuum bag, and the vacuum was applied at maximum vacuum capacity for 2.2 s (Bizerba, Bizerba Canada, Inc., ON) to eliminate air in the batter. Batters were then 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 remove any remaining air voids. Preparation up to this stage was carried out at room temperature. Batters were incubated at different temperatures for a defined time (chosen according to the CCRD) in a circulating water bath (Haake, D1, Dreieich, Germany) to allow crosslinking reaction. After incubation, the batters were heated for 45 min in a water bath at 92 °C to inactive the enzyme and to induce thermal gelation. Then the gels were stored at 4 °C for 14 h until testing. Selected formulations were prepared with SPIc using the same procedures as described above.

For low temperature incubation MGTase at 0.6% addition was used. The food processor and ingredients were maintained at 4 °C overnight before preparing PPIc and SPIc batters with MTGase. Different incubation time at 4 °C (0, 2, 4, 7 and 18 h) were tested. Other processing steps were as described above.

2.3. Torsional rheometry

Heat-set protein gels from all experimental combinations were evaluated for torsion rheological properties.

Gel sample preparation for torsional rheometry was as described in the previous communication from this group (Shand et al., 2007).

2.4. Differential scanning calorimetry (DSC)

The thermal properties of commercial and native pea protein slurries (10% protein concentration, w/w; pH 6.5) with and without MTGase product addition (0.6%, w/w) and incubation at 50 °C for 0 or 30 min were examined. Instrumental conditions were as described in Shand et al. (2007). Peak transition temperature or denaturation temperature (T_d), and enthalpy of denaturation (ΔH) were computed from the endothermic peaks observed in the thermograms using computer software (Universal Analysis Program, Version 2.5H, TA Instruments, New Castle, DE).

2.5. Dynamic rheological measurements

Slurries (10% protein, w/w) were prepared by dispersing specific amounts of PPIc, PPIn, SPIc or SPIn with deionized water and then mixed by vortexing (Vortex-Genie, Scientific Instruments, Inc. Bohemia, NY). After adjusting the pH to 6.5 by addition of 1 M NaOH or 1 M HCl, protein solutions were incubated with MTGase product (0.6%, w/w) for 0 and 30 min at 50 °C. The MTGase treated samples were subjected to dynamic rheological testing using a model AR1000N rheometer (TA Instruments, New Castle, DE), equipped with a parallel plate measuring system (40 mm diameter). Samples were loaded in the space between parallel plates immediately after incubation, and the exposed rim was covered with a thin layer of mineral oil to prevent dehydration. Samples were heated from 35 to 95 °C at a rate of 2 °C min^{-1}. Oscillatory measurements were made at a fixed frequency of 1 Hz and strain amplitude of 0.02, which was within the linear viscoelastic region as determined in preliminary tests. Changes in the storage modulus (G') and loss modulus (G'') were monitored throughout heating.

2.6. Statistical analysis

Response surface methodology was used to determine the simultaneous effects of experimental variables: incubation temperature, incubation time, and MTGase concentration on PPIc gels. Experimental design and statistical analysis were performed using Statistical Analysis System (SAS for windows, Release 8.02, SAS Institute Inc., Cary, NC) essentially the same as described in Shand et al. (2007). The significance of the equation parameters for each response variable was assessed by P value ($P < 0.05$). Analysis of variance was performed using the General Linear Models (GLM) procedure of SAS on quantitative DSC (T_{max}) data to test statistical significance among treatments. The least significant difference (LSD) test ($P < 0.05$) was used to determine differences between treatment means.

3. Results and discussion

3.1. Effect of reaction conditions for MTGase on heat-induced PPIc gel strength

Fig. 1 provides response surface plots generated for shear stress (A1, B1, C1) and shear strain (A2, B2, C2), the prime indicators of gel characteristics, as a function of MTGase concentration, incubation time and incubation temperature for PPIc gels. Regression models assumed for these two characteristics (responses) of PPIc gels were significant ($P < 0.05$) according to the analyses of variance. Significant linear effects were observed for MTGase concentration on shear stress ($P < 0.01$) and shear strain ($P < 0.01$) of the protein gels. The gels obtained by incubation with increasing MTGase levels exhibited consistently higher values of these parameters in relation to the ones prepared with lower levels of MTGase (Fig. 1).

The dominant reaction catalyzed by MTGase is the formation of isopeptide bonds between glutamine and lysine residues in proteins, which leads to inter- and intramolecular crosslinking and increased gel strength in various food systems (de Jong & Koppleman, 2002). In the present study, we did not measure MTGase-catalyzed crosslinking directly. However, we did observe the formation of large molecular weight compounds on SDS-PAGE gels that were too large to enter the gel (data not presented, refer to Ya, 2004). While pea legumin has been considered a relatively poor substrate for transglutaminase (Larré et al., 1992), recently, Schäfer, Zacherl, Engel, Neidhart, and Carle (2007) reported a 155% increase in gel strength of heat-treated PPI gels (18% w/w) with ~0.2% MTGase product compared to the MTGase treatment without incubation. In their study, content of the newly formed €-(γ-glutamyl)lysine isopeptide was highly correlated with the increase in firmness as measured by a simple penetration test. The present results show that several fundamental rheological properties of PPIc gels were specifically influenced by MTGase treatment, with shear stress and strain of PPIc gels with 0.7% MTGase (45 min incubation at 50 °C) increasing by 260% and 200%, respectively, compared to the control treatment without MTGase.

Incubation temperature influenced textural characteristics of PPIc gels through positive quadratic terms of the model for shear stress ($P = 0.018$) and shear strain ($P = 0.017$). The strongest and most elastic gels resulted when incubation temperatures were between 50 and 60 °C (Fig. 1); above or below this temperature range, gels were weak as reflected by gel strength indicators. These temperatures were within the temperature range for the highest activity of the enzyme as reported by the producer (Ajinomoto, 2005). A higher temperature of incubation than the optimum range may inactivate the enzyme thus causing inadequate crosslinking of the proteins before gelation.

Shear stress and shear strain tended to show a linear increase with incubation time up to 80 min ($P = 0.06$ and

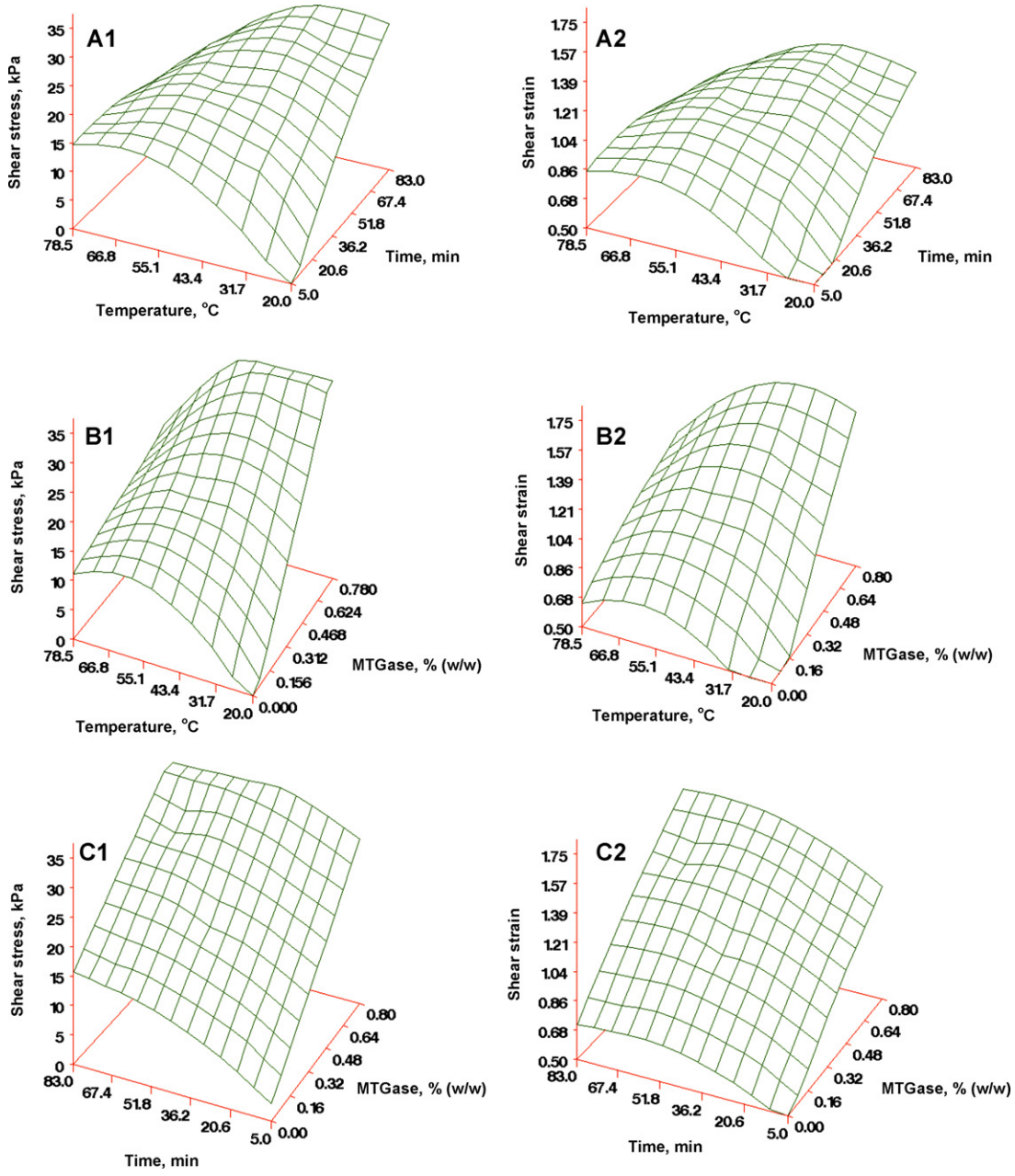

Fig. 1. Response surface plots for shear stress (A1, B1 and C1) and shear strain (A2, B2 and C2) of heat-induced PPIc gels. A1 and A2: Effect of incubation tempertuare and incubation time with 0.35% MTGase treatment, B1 and B2: Effect of incubation temperature (45 min incubation time) and MTGase level, and C1 and C2: Effect of incubation time at 50 °C and MTGase treatment levels.

$P = 0.13$, respectively). It would have been interesting, but perhaps not practical, to consider longer incubation times. Schäfer et al. (2007) incubated commercial PPI with MTGase at 40 °C for up to 240 min and reported that crosslinking of pea protein isolate increased at a linear rate during the first 120 min of incubation.

A texture map was employed to illustrate textural properties of heat-induced PPIc gels prepared with MTGase addition (Fig. 2). The plot of shear stress versus shear strain at failure provides a useful graphical representation of product texture (Truong & Daubert, 2001). When the gel forming conditions were favourable to the crosslinking reaction assisted by MTGase, such as at higher enzyme level (Fig. 2), the gel texture moved towards the tougher region of the map. The strongest and most elastic gel was obtained from the treatment incubated with the highest MTGase concentration used in this study, 0.7% (w/w), for 45 min incubation at 50 °C; on the contrary, the PPIc sample without MTGase addition was the nearest to the mushy quadrant (Fig. 2). Comparison of stress and strain of soy protein isolate gels and commercial products to that of the PPIc gels showed that the 0.7% MTGase treatment of PPIc gave fairly close values for texture indicators to the SPIc treatment without MTGase and to the commercial

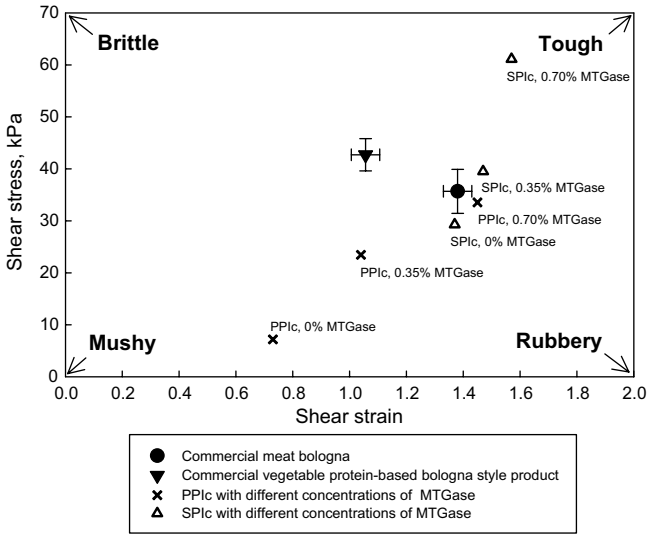

Fig. 2. Texture map (plot of shear stress versus shear strain) for heat-induced PPIc and SPIc gels prepared with different levels of MTGase and incubated for 45 min at 50 °C. Values for commercial meat bologna and vegetarian bologna-type product are also provided.

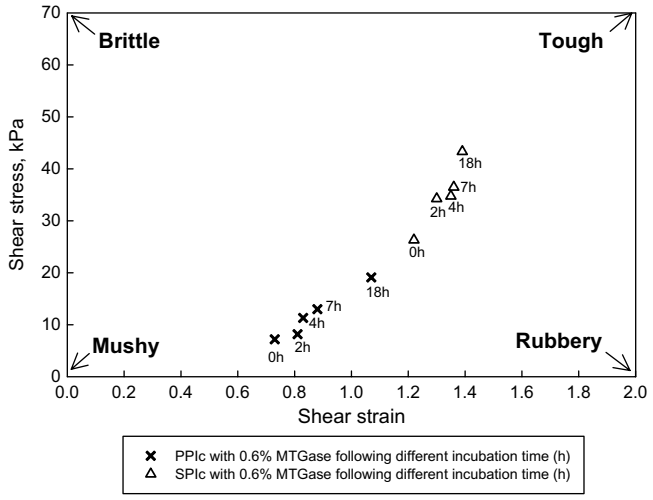

Fig. 3. Texture map of heat-induced PPIc and SPIc gels obtained following incubation with 0.6% MTGase at 4 °C for up to 18 h.

meat bologna, as shown by their positions on the texture map (Fig. 2).

Soy protein isolate is an often-used vegetable protein ingredient in food processing and it provides a good reference for this study. The reaction of SPIc with MTGase was carried out under the same conditions as that for PPIc. SPIc gels showed higher values of shear strain and stress than those of PPIc at each MTGase level (Fig. 2). Gels obtained from SPIc crosslinked with 0.35% MTGase treatment had improved gel strength, however 0.7% MTGase treatment of SPIc produced gels that reached to the "tough" area on the texture map (Fig. 2). It was interesting to note that MTGase increased shear strain at failure of PPIc gels by 0.8 units, while the increase was only 0.2 units for SPIc treatments with 0.7% MTGase product.

The enzyme MTGase is capable of catalyzing a crosslinking reaction at temperatures below ambient (Kuraishi et al., 1998). The texture map for heat-induced gels obtained after low temperature incubation (4 °C) with 0.6% MTGase for varying duration is shown in Fig. 3. It shows that low temperature incubation of PPIc or SPIc with MTGase can also improve textural properties of the final product. Shear stress of SPIc gels increased within a shorter incubation time and reached higher values during low temperature incubation (4 °C) than did PPIc gels, possibly due to their inherent physicochemical differences. Although 18 h incubation at 4 °C did not produce as high of a gel strength and elasticity for PPIc gels as 45 min incubation at 50 °C, this technique provides an alternative to maintaining high temperatures during incubation which is a concern with respect to microbial safety of protein-rich slurries. Thus, low temperature incubation with MTGase would have practical significance for improvement of PPIc gel properties, such as hardness and elasticity, for certain commercial products.

The commercial availability of bacterial TGase (Ca^{2+} independent) has led to successful development of several food products that owe their physical properties to the effect of TGase-catalyzed crosslinking, especially improvements in gelation and emulsification properties. The enhancement in textural properties of the PPIc gels obtained by MTGase treatment may be attributed to the crosslinks formed between the pea protein molecules. Transglutaminase, (R-glutaminyl-peptidyl or amine γ-glutamyl transferase; EC 2.3.2.13) catalyzes the transfer of the γ-carboxyamide group of glutaminyl residues in protein to primary amino groups, especially the lysine residue and hence the number of inter- or intramolecular crosslinks (covalent bonds) in the substrate protein can be increased (Mizuno, Mitsuiki, & Motoki, 2000). Schäfer et al. (2007) determined that a similar concentration of €-(γ-glutamyl)lysine isopeptide formed in either commercial PPI or SPI gels incubated with ~0.2% MTGase (225 and 192 μmol/100 g of protein, respectively), but found differences in subsequent gel strength, indicating that crosslink location is also of importance.

O'Kane, Happe, Vereijken, Gruppen, and van Boekel (2004c) observed that mainly hydrophobic and hydrogen bonds supported network formation of legumin gels of pea and soy, while disulfide bonds had minimum involvement. They also found that soybean glycinin was inherently better able to form a well-structured network than pea legumin (possibly due to the availability of lysysl and glutaminyl residues or accessibility by the enzyme), which was reflected in the textural properties of the heat-induced gel.

It was interesting to note that shear stress and strain values of heat-induced gels of PPIc crosslinked at 0.7% MTGase addition level were between that of gels obtained for SPIc without enzymatic crosslinking and SPIc gels with 0.35% MTGase treatment (Fig. 2) and also close to values for commercial meat bologna. This shows the potential of MTGase-assisted crosslinking to modify the rheological properties of heat-induced gels of PPIc.

The position of the commercial meat and vegetarian bologna on the texture map is illustrated in Fig. 2. They both had slightly different textural properties with the vegetable protein product positioned closer to the center of the map than the meat product due to its lower strain and slightly higher shear stress at failure than the meat bologna. From the perspective of texture, PPIc gels prepared with enzymatic crosslinking at 0.7% MTGase addition level resulted in shear stress (hardness) and shear strain (elasticity) values comparable to those obtained for the commercial meat bologna products, which was what we were trying to achieve. Also, PPIc gels with 0.35% MTGase, although softer (lower shear stress), had similar strain (elasticity) to the commercial vegetarian bologna. Shear stress of gels can easily be manipulated through changes in processing conditions and ingredients (Hamann, 1988). Therefore, adjustment of protein concentration and/or addition of other texture modifiers could be used to increase gel stress of PPIc gels to that of the commercial vegetarian bologna.

Since MTGase treatment of PPI had the capability to increase both shear stress and strain, MTGase could replace some non-meat ingredients, such as carrageenan and starch (Shand, 2000) that enhance hardness or gel strength and vital wheat gluten that is used to increase elasticity (Xu, Bietz, Felker, Carriere, & Wirtz, 2001). The mechanism of gel textural enhancement by MTGase may be different than the contribution from such polysaccharide ingredients, as it enhanced both shear stress and shear strain of pea protein gels. Of particular note was the large improvement to shear strain of PPIc gels with MTGase-assisted crosslinking. Further research is needed to understand the scientific basis for MTGase-induced changes to gel elasticity.

3.2. Differential scanning calorimetry study of protein isolates with MTGase

Differential scanning calorimetry of PPIn and PPIc was performed with and without 30 min of preincubation at 50 °C. No effect of preincubation was observed (data not shown). The control PPIn samples and those treated with MTGase displayed similar thermal curves, all showing two endothermic peaks corresponding to the non-globulin ($T_d = 67$–69 °C) and globulin ($T_d = 85$–86 °C) fractions (endotherms are not shown, refer to Shand et al., 2007). There were no significant differences in T_d or enthalpy of PPIn samples with and without MTGase addition. This indicated that the crosslinking catalyzed by MTGase did not significantly modify the thermal stability and protein conformation of PPIn. However, Yildirim and Hettiarachchy (1997) reported that MTGase could increase the thermal stability of proteins by inter- or intramolecular crosslinking. Similarly, Ramírez-Suárez and Xiong (2003) reported that after MTGase treatment, β-conglycinin and glycinin from soybean proteins showed a small increase in T_d, 1–2 °C and 2–3 °C, respectively. The one major endothermic peak observed in the present study for globulins represented denaturation of both legumin and vicilin fractions. The overlapping transition temperatures for these proteins may have obscured any thermal stability effect due to MTGase.

The thermograms of PPIc lacked an endothermic peak for the globulin protein fraction as reported in our earlier communication. Thermograms of PPIc with MTGase also did not show any endothermic peaks. Therefore, it was not possible to observe thermal transition and enthalpy changes of the commercial PPI samples that were crosslinked by MTGase catalysis.

3.3. Dynamic rheological properties of PPIc and PPIn gels

Heat-induced rheological changes in PPIn with and without MTGase addition are displayed in Fig. 4. The storage modulus (G') of PPIn without enzymatic crosslinking remained unchanged during heating from 20 °C to 71 °C and started to increase after 71 °C. As described by Renkema, Knabben, and van Vliet (2001) for soy proteins, the crossover temperature of G' and G'' can be considered as an indicator of the gel point of soy protein slurries that were at the same pH. For PPIn without crosslinking, crossover of G' (elastic response) with G'' happened at 71 °C and when 0.6% MTGase was present (no incubation) this point was lowered to 65 °C (Fig. 4, inset). Later in the heating cycle the G'' (viscous response) values of the protein slurries also started to increase. This structure development occurred after the globulin fraction of PPIn was denatured ($T_d = 85$ °C) showing a sharp rise in both of these moduli. Above 85 °C, G' values increased because more unfolded protein may have been incorporated into the network leading to further structure development.

When PPIn proteins were allowed to crosslink prior to dynamic rheological testing (30 min preincubation with MTGase at 50 °C), responses for storage and loss modulus were very different and very high values were observed compared to control samples without MTGase. A crossover temperature for these two moduli was not observed for the incubated sample with MTGase within the range of temperature the observations were made. This indicated that there was a network structure formed in the MTGase-incubated sample prior to rheological testing. The earlier onset of gelation for MTGase-crosslinked PPIn when compared to those without prior crosslinking suggests that crosslinked protein has a lower temperature requirement for producing an elastic gel network structure. These results indicate that MTGase facilitated the formation of a rigid gel structure and preincubation was beneficial for producing a more viscoelastic and cohesive gel.

In contrast, commercial PPI (10% protein slurries) with and without MTGase did not produce any significant heat-induced rheological changes during heating up to 95 °C (data not shown). Reduced structure forming ability of PPIc may be due to the fact that proteins of PPIc are partially or completely denaturated which led to their reduced

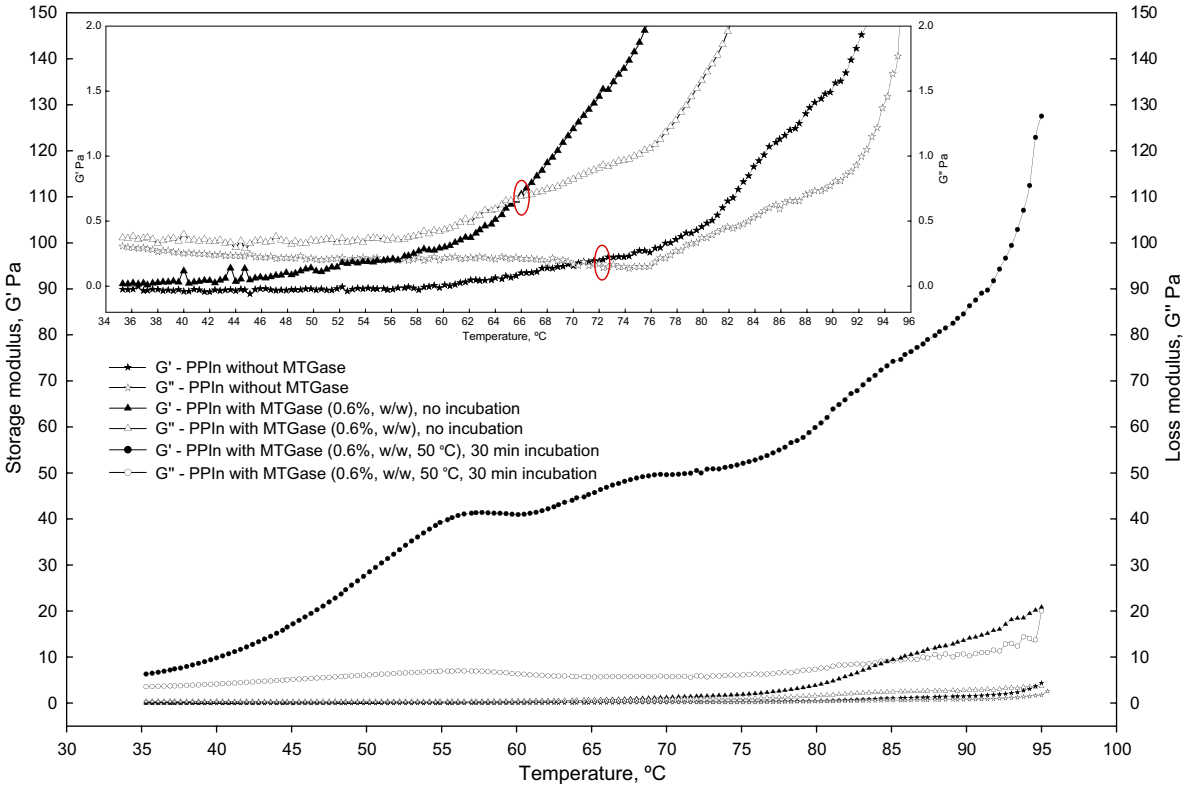

Fig. 4. Changing pattern of storage modulus (G') and loss modulus (G'') during heating of PPIn with and without 0.6% MTGase. All samples contained 10% protein (w/w) and were at pH 6.5. *Inset*: amplified scale of y axis to show changes more clearly, circles enclose crossover points of G' over G''.

solubility as reported in the previous communication (Shand et al., 2007). For heat-induced gel formation, soluble aggregation is the second step of the three-step gelation mechanism (Clark, Kavanagh, & Ross-Murphy, 2001) and thus the reduced solubility could affect the mechanism of gel formation through impaired protein-protein interactions during heat-induced gelation (Sikorski, 2001). Extensive protein denaturation from heat treatment (most likely due to the spray drying process) during commercial production might have resulted in the aggregation and precipitation of pea proteins thereby diminishing their ability to interact or to participate in the MTGase-mediated crosslinking reaction. The soluble protein concentration during dynamic rheological testing of PPIc may also have been insufficient for gelation to occur. These results are in contrast to the large deformation textural data which showed increased stress and strain of PPIc gels (19.6% protein w/w) following incubation with MTGase and heating to 92 °C.

According to Larré et al. (1992), legumin protein of pea in its native form is a poor substrate for transglutaminase-assisted crosslinking because of the close-packed globular structure despite its high content of Glu and Lys residues. However, our study and that of Schäfer et al. (2007) shows that it is possible to modify rheological properties of heat-set pea protein gels following incubation with MTGase. The pea protein isolates we used were mixtures of legumin and vicilin as indicated in our previous study. Wang and Damodaran (1990) indicated that in phenomenological terms, the strength or rigidity of protein gels is related to the number of intermolecular crosslinks formed in the gel network. In general, the higher the number of crosslinks, the greater would be the gel strength. Therefore, the results of this study suggest that non-legumin protein of pea may also provide a significant contribution to the MTGase-induced crosslinking. Further work to identify specific pea proteins that may be involved in the MTGase-assisted crosslinking reactions is underway.

4. Conclusions

This study shows that MTGase treatment of pea proteins was very useful in improving rheological properties of heat-induced PPIc gels, especially that of elasticity (shear strain). A positive linear relationship between enzyme addition and shear stress and strain of PPIc gels was observed within the enzyme product addition range of 0–0.7% (w/w). MTGase treatment enhanced the strength and elasticity of PPIc gels, resulting in shear stress and strain at failure similar to that of homologous SPIc gels and to commercial meat bologna. Incubation of native pea protein isolate with MTGase did not result in significant changes in thermal transition temperatures and enthalpy suggesting the thermal stability of PPIn was not modified by MTGase treatment. Shear stress of SPIc gels increased within a shorter incubation time and reached

higher values following low temperature incubation with MTGase (4 °C) than did PPIc gels. The enhancement of the shear stress and strain of PPIc gels by low temperature incubation with MTGase show the practical significance of the enzyme-assisted crosslinking of legume proteins, despite the relatively long incubation time needed. Adjustment of processing conditions, including use of MTGase, provides new opportunities to extend the range of functional properties of commercial pea proteins in food systems which have not been exploited before.

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