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Physicochemical properties and microstructures of soy protein isolate gels produced using combined cross-linking treatments of microbial transglutaminase and Maillard cross-linking

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ABSTRACT

Soy protein isolate (SPI) was incubated with microbial transglutaminase (MTG) enzyme for 5 (SPI/MTG(5)) or 24 (SPI/MTG(24)) h at 40 °C and the gel obtained was freeze-dried, and heated with 2% (w/v) ribose (R) for 2 h at 95 °C to produce combined-treated gels. Control experiments were run with sucrose. Stress relaxation experiments indicated that combined-treated gels were higher in the compressive gel strength, viscoelasticity properties (K_1 and K_2), solidity (E_a), and were lower in % deformation as compared to single-treated or control gels (gels produced without cross-linking treatment). At most of pHs tested, the solubility of single-treated or control gels were always higher than that of the combined-treated gels. The pH ranges at which the gels were least soluble were wider in the combined-treated gels compared to those of other gels. Field emission scanning electron microscope (FESEM) analysis revealed a denser and finer network of combined-treated gels as compared to that of other gels. The occurrence of covalent cross-links within the network of the gels as well as changes in net charge of soy protein had all affected the physicochemical properties and the microstructure of the combined-treated gels.

1. Introduction

Modification of soy protein for functionality improvements has been carried out via physical means such as heat treatment (Renkema & van Vliet, 2002) and application of pressure (Torrezan, Tham, Bell, Frazier, & Cristianini, 2007) or via chemical means such as acidification (Tay, Xu, & Peter, 2005), addition of salts (Puppo & Añón, 1999) and by the Maillard reaction induced cross-linkings (Md Yasir, Sutton, Newberry, Andrews, & Gerrard, 2007b). However, one of the most popular methods of protein modification in industry involves the application of transglutaminase enzyme (Md Yasir, Sutton, Newberry, Andrews, & Gerrard, 2007a; Tang, 2007). MTGase (protein-glutamine: amine γ-glutamyltransferase, E.C. 2.3.2.13) works by catalyzing an acyl-transfer reaction between the γ-carboxyamide group of peptide-bound glutamine residues (acyl donors) and variety of primary amines (acyl acceptors), including the ε-amino group of lysine residues to form an ε-(γ-glutamyl)lysine isopeptide bond (Motoki & Seguro, 1998). This treatment has been used in meat products (Trespalacios & Pla, 2007), fish products (Jongjareonrak, Benjakul, Visessanguan, & Tanaka, 2006), dairy products (Lorenzen, 2007), legume products (Tang, Li, Wang, & Yang, 2007) and wheat products (Caballero, Gómez, & Rosell, 2007) in enhancing the texture and functional properties.

The Maillard or non-enzymatic browning reaction comprises the reaction between reducing sugars and amino groups of amino acids and proteins. "Maillard cross-link" has been shown to produce cross-linked protein and improved protein gel strength and texture of protein-based products (Gerrard & Brown, 2002; Hill & Easa, 1998; Md Yasir et al., 2007b). Probably for that reason, it has been used for modification of globular proteins (Oliver, Melton, & Stanley, 2006).

MTGase cross-linked high protein products are available in the market. To further enhance the functionalities of cross-linked protein it is possible for technologists to combine it with an additional cross-linking treatment. On the other hand, there could be situations when the cross-linked products are subjected to heating treatments (cooking, retort cooking, blanching or drying) with reducing sugars or other ingredients capable of undergoing the Maillard reaction. The effect of this on physicochemical and microstructure properties of the protein has not been studied. The objective of this paper has been to evaluate SPI gels, a model industrial grade protein, formed as a result of the combined cross-linking treatments using microbial transglutaminase, followed by a heating treatment with ribose that induces "Maillard cross-linkings". Ribose, an emerging nutraceutical ingredient (Hellsten, Skadhauge, & Bangsbo, 2004), is also known for its high reactivity in terms of

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reacting with protein via the Maillard reaction (Ashoor & Zent, 1984) and capability to cross-link proteins (Graham, 1996). Other than the potential "Maillard cross-link", ribose may also produce other changes that is related to the Maillard reaction, such as charge modification and pH adjustment (Easa, 1996; Yaylayan, 1997).

2. Materials and methods

2.1. Materials

Commercial grade soy protein isolate (SPI) with 90% protein content was purchased from Sim Company Sdn. Bhd., Penang, Malaysia. A Ca^{2+} independent microbial transglutaminase (ACTIVA TG-K) was a gift from Ajinomoto Co., Inc. (Tokyo, Japan). D-(−)-Ribose was purchased from Sigma–Aldrich company. Other chemicals (analytical grade) used in this study were obtained from Sigma–Aldrich company and Fluka company.

2.2. Sample treatments

2.2.1. MTGase cross-linking

SPI (10 g) was dispersed in 100 mL of distilled water and then reacted with MTGase (4.0 U). The mixtures were incubated at 40 °C with constant shaking (90 rpm) in LM-570R Orbital Shaker (Yih Der, Taiwan) for 5 and 24 h. The non-MTGase incubated samples (0 h) considered as a control sample. The enzyme was subsequently deactivated by heating at 95 °C for 30 min in a water bath. The mixture was then cooled to room temperature in running water followed by freezing at −20 °C prior to lyophilization (Labconco, Kansas City, US). The lyophilized samples were ground and sieved (w = 0.5 mm, d = 0.32 mm). All samples were stored in glass sample bottles in a desiccator prior to further treatment.

2.2.2. "Maillard cross-linking"

MTGase incubated and non-incubated SPI dispersions (0.1 g/mL) with 2% (w/v) ribose or 2% (w/v) sucrose (as control) were prepared and subjected to a heating treatment at 95 °C for 2 h in WB22-16X2501 water bath (Memmert, Germany). The mixtures were then cooled to room temperature in running water. The gels formed were stored in SJ-D21T refrigerator (SHARP, Osaka, Japan) at ~2 °C overnight prior to analysis.

The sample treatments/preparations and their designations are summarized in Fig. 1. Sample definitions and descriptions are: SPI/C/S(2): non-MTGase incubated sample heated with 2% (w/v) sucrose solution for 2 h; SPI/MTG(5)/S(2): MTGase incubated sample (incubated for 5 h) heated with 2% (w/v) sucrose solution for 2 h; SPI/MTG(24)/S(2): MTGase incubated sample (incubated for 24 h) heated with 2% (w/v) sucrose solution for 2 h; SPI/C/R(2): non-MTGase incubated sample heated with 2% (w/v) ribose solution for 2 h; SPI/MTG(5)/R(2): MTGase incubated sample (incubated for 5 h) heated with 2% (w/v) ribose solution for 2 h; SPI/MTG(24)/R(2): MTGase incubated sample (incubated for 24 h) heated with 2% (w/v) ribose solution for 2 h.

2.3. Analysis

2.3.1. Stress relaxation under compression

Four different gels were prepared at protein concentration of 15% (w/v): control gel [SPI/C/S(2)], single-treated gel using MTGase [SPI/MTG(24)/S(2)], single-treated gel using ribose [SPI/C/R(2)] and combined-treated gel [SPI/MTG(24)/R(2)].

For the four different gels, stress relaxation experiments were performed at a constant strain level (25% deformation, 5 mm). The gel was initially compressed at a rate of 2 mm/s and decay in force was followed for 150 s (Armstrong, Hill, Schrooyen, & Mitchell, 1994). The relaxation curves obtained were then normalized and linearized using the method of Peleg (1979). The equation employed was

$$\frac{F_0 t}{F_0 - F(t)} = K_1 + K_2 t, \tag{1}$$

where F_0 and $F(t)$ are the initial and momentary force and K_1 (s) and K_2 (dimensionless) are constants.

The asymptotic residual modulus (E_A) was calculated using the following equation (Nussinovitch, Kaletunc, Normand, & Peleg, 1990):

$$E_A = \frac{F_0}{A(\varepsilon)\varepsilon}\left[1 - \frac{1}{K_2}\right], \tag{2}$$

where ε is the imposed strain and $A(\varepsilon)$ is the corresponding cross-sectional area of relaxing specimen, i.e.:

$$A(\varepsilon) = \frac{A_0 - L_0}{L_0 - \Delta L}, \tag{3}$$

where L_0 is the height of the original gel, A_0 is the cross-sectional area and ΔL is the degree of compression.

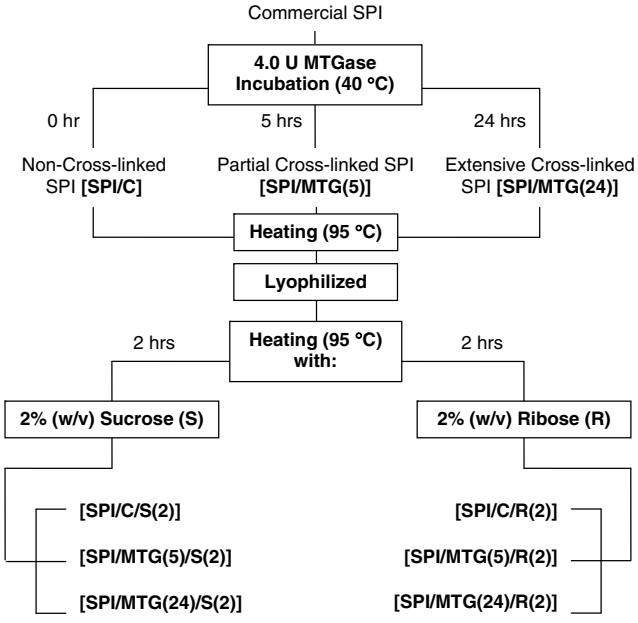

Fig. 1. Summary of sample preparations/treatments and their designations.

2.3.2. Gel solubility in different pH

Gels (6 g) were extracted with 50 ml of 0.2% NaOH pH 12 solution and centrifuged at 8000 × g for 20 min according to Lqari, Vioque, Pedroche, and Millán (2002). Aliquots (1 ml) of the supernatant were titrated with 0.5 N HCl to various pH values, ranging from 2.0 to 6.5. The precipitate formed was separated by centrifugation and resolubilized with 0.2% NaOH prior to Biuret test (Robinson & Hodgen, 1940). Total protein was determined using SPI/C/S(2) at pH 12 since no precipitation was found after the first centrifugation. Percentage of solubility was calculated as follows:

$$\%\ \text{solubility} = 100 - \left(\frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{total}}} \times 100\right), \tag{4}$$

where $\text{Abs}_{\text{sample}}$ defined as absorbance obtained by the precipitated protein of sample at given pH whereas $\text{Abs}_{\text{total}}$ defined as absorbance obtained by total protein of the sample.

Table 1
Variation in mechanical properties and stress relaxation parameters of SPI/C/S(2), SPI/MTG(24)/S(2), SPI/C/R(2) and SPI/MTG(24)/R(2) gels obtained

Sample*	% Deformation	Compressive gel strength (kPa)	K_1 (s)	K_2	E_a (kPa)
SPI/C/S(2)	27.6 ± 2.6^{c}	1.03 ± 0.08^{a}	4.35 ± 0.45^{a}	1.26 ± 0.03^{a}	0.56 ± 0.05^{a}
SPI/MTG(24)/S(2)	15.8 ± 0.1^{a}	2.68 ± 0.83^{c}	5.41 ± 0.81^{ab}	1.58 ± 0.08^{b}	2.91 ± 0.34^{c}
SPI/C/R(2)	22.7 ± 1.5^{b}	1.57 ± 0.28^{ab}	4.90 ± 0.37^{a}	1.45 ± 0.08^{ab}	1.71 ± 0.10^{b}
SPI/MTG(24)/R(2)	14.9 ± 1.5^{a}	3.06 ± 0.94^{cd}	6.22 ± 0.81^{b}	1.61 ± 0.21^{b}	3.42 ± 0.22^{cd}

Note: Comparison within the columns was shown in the table with the data written as mean ± standard deviation ($n = 3$). Means within the same column not followed by the same letter are significant different at $p < 0.05$ level of significance, according to Duncan's Multiple-Range Test.

* *Sample definitions and descriptions:*
SPI/C/S(2): non-MTGase incubated sample heated with 2% (w/v) sucrose solution for 2 h.
SPI/MTG(24)/S(2): MTGase incubated sample (incubated for 24 h) heated with 2% (w/v) sucrose solution for 2 h.
SPI/C/R(2): non-MTGase incubated sample heated with 2% (w/v) ribose solution for 2 h.
SPI/MTG(24)/R(2): MTGase incubated sample (incubated for 24 h) heated with 2% (w/v) ribose solution for 2 h.

2.3.3. Net titratable charge

pH-titration of different samples was carried out on 1% (w/v) protein solutions in 0.1 M NaCl (Ma, Oomah, & Holme, 1986). The pH of the protein solution was adjusted to 10.0 and 0.1 N HCl was added in a small increment until an arbitrary end point of pH 3.0 was reached. Net titratable charge was determined as mmol H^+/g protein.

2.3.4. Microstructure analysis of gels

Lyophilized gels were carefully cut using a razor blade and mounted onto a SEM specimen stub with a double-sided sticky tape prior coating. Specimens were coated with gold using Polaron SC 515 Sputter Coater (Fisons Instruments, VG Microtech, Sussex, UK). The cross-section of specimens were subsequently photographed using Leo Supra 50VP Field Emission Scanning Electron Microscope (FESEM) equipped with Oxford INCA 400 energy dispersive X-ray microanalysis system (Oxford Instruments Analytical, Bucks, UK).

2.4. Statistical analysis

An analysis of variance (ANOVA) of the data was performed and Duncan's Multiple-Range Test with a confidence interval of 95% was used to compare the means.

3. Results and discussion

In this study, MTGase was first used to cross-link SPI to form a gel that represents a model high protein food product. The gel containing cross-linked protein aggregates was then freeze-dried into powder that was then heated in solutions containing ribose. Ribose was not incorporated together with MTGase so as to mimic industrial practice of production of MTGase cross-linked protein products. The incubation treatment was aimed at maximizing the initial development of network via ε-(γ-glutamyl)lysine cross-links before adding in ribose. As a result of this procedure, it may be envisaged that some level of gel network formed via MTGase incubation was disturbed during freeze-drying and ribose incorpora-

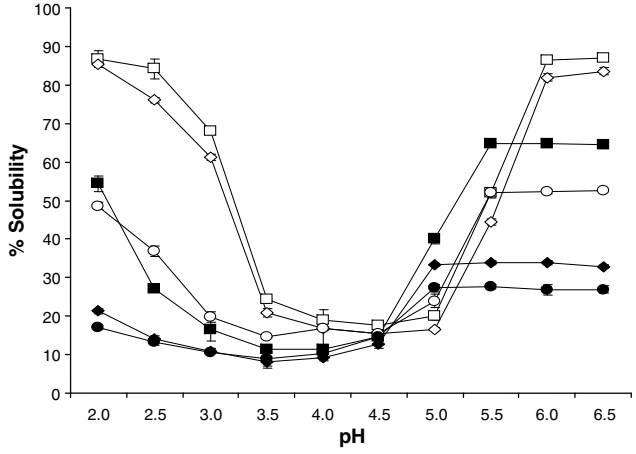

Fig. 2. Effect of pH on gel solubility profiles of (□) SPI/C/S(2), (■) SPI/C/R(2), (♦) SPI/MTG(5)/S(2), (◇) SPI/MTG(5)/R(2), (○) SPI/MTG(24)/S(2) and (●) SPI/MTG(24)/R(2). Data points are mean ± standard deviation. $n = 3$.

Sample definitions and descriptions:
SPI/C/S(2): non-MTGase incubated sample heated with 2% (w/v) sucrose solution for 2 h.
SPI/MTG(5)/S(2): MTGase incubated sample (incubated for 5 h) heated with 2% (w/v) sucrose solution for 2 h.
SPI/MTG(24)/S(2): MTGase incubated sample (incubated for 24 h) heated with 2% (w/v) sucrose solution for 2 h.
SPI/C/R(2): non-MTGase incubated sample heated with 2% (w/v) ribose solution for 2 h.
SPI/MTG(5)/R(2): MTGase incubated sample (incubated for 5 h) heated with 2% (w/v) ribose solution for 2 h.
SPI/MTG(24)/R(2): MTGase incubated sample (incubated for 24 h) heated with 2% (w/v) ribose solution for 2 h.

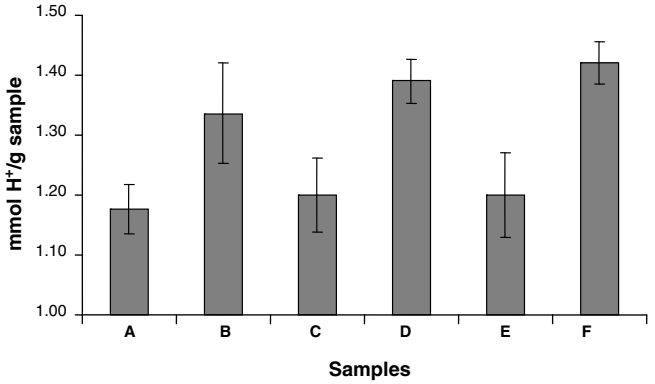

Fig. 3. Variation of net titratable charge in (A) SPI/C/S(2), (B) SPI/C/R(2), (C) SPI/MTG(5)/S(2), (D) SPI/MTG(5)/R(2), (E) SPI/MTG(24)/S(2) and (F) SPI/MTG(24)/R(2) gels at a concentration of 15% (w/v). Bars are mean ± standard deviation. $n = 3$. *Note*: pH-titration was performed from pH 10.0 to 3.0.

Sample definitions and descriptions:
SPI/C/S(2): non-MTGase incubated sample heated with 2% (w/v) sucrose solution for 2 h.
SPI/MTG(5)/S(2): MTGase incubated sample (incubated for 5 h) heated with 2% (w/v) sucrose solution for 2 h.
SPI/MTG(24)/S(2): MTGase incubated sample (incubated for 24 h) heated with 2% (w/v) sucrose solution for 2 h.
SPI/C/R(2): non-MTGase incubated sample heated with 2% (w/v) ribose solution for 2 h.
SPI/MTG(5)/R(2): MTGase incubated sample (incubated for 5 h) heated with 2% (w/v) ribose solution for 2 h.
SPI/MTG(24)/R(2): MTGase incubated sample (incubated for 24 h) heated with 2% (w/v) ribose solution for 2 h.

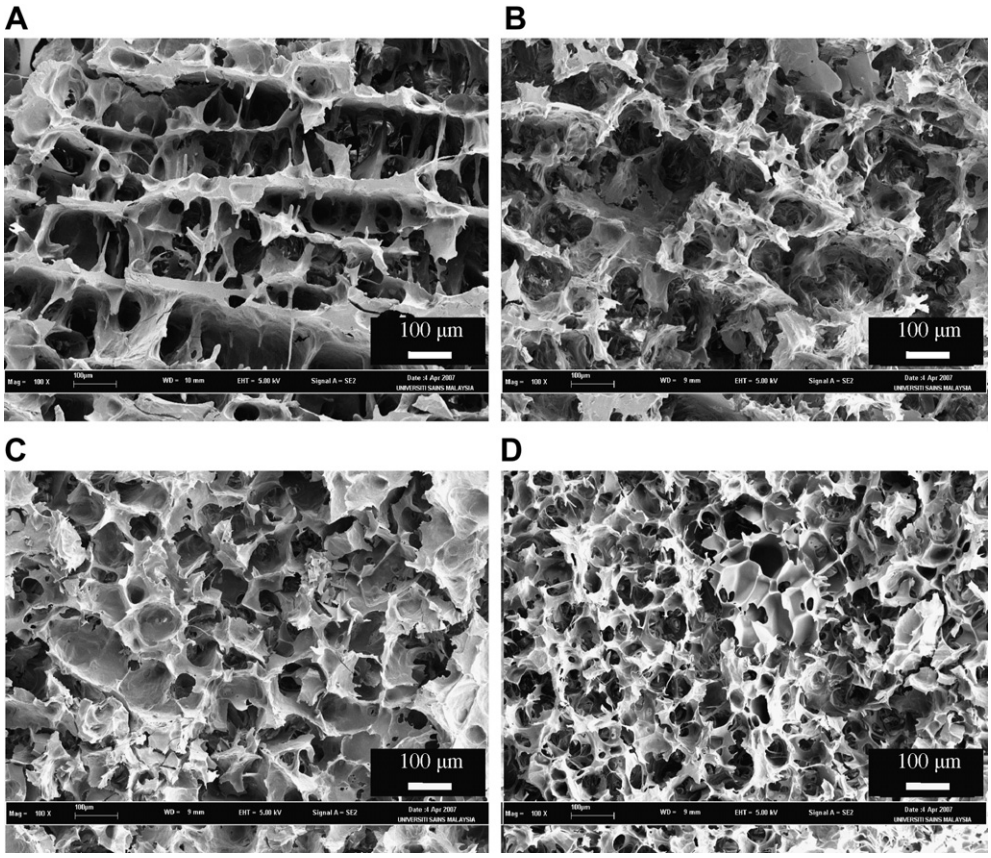

Fig. 4. Scanning electron micrographs of various gels after heated at 95 °C for 2 h. Panel A: SPI/C/S(2), Panel B: SPI/MTG(5)/S(2), Panel C: SPI/C/R(2) and Panel D: SPI/MTG(5)/R(2) at magnification ×100.

Sample definitions and descriptions:

SPI/C/S(2): non-MTGase incubated sample heated with 2% (w/v) sucrose solution for 2 h.
SPI/MTG(5)/S(2): MTGase incubated sample (incubated for 5 h) heated with 2% (w/v) sucrose solution for 2 h.
SPI/MTG(24)/S(2): MTGase incubated sample (incubated for 24 h) heated with 2% (w/v) sucrose solution for 2 h.
SPI/C/R(2): non-MTGase incubated sample heated with 2% (w/v) ribose solution for 2 h.
SPI/MTG(5)/R(2): MTGase incubated sample (incubated for 5 h) heated with 2% (w/v) ribose solution for 2 h.
SPI/MTG(24)/R(2): MTGase incubated sample (incubated for 24 h) heated with 2% (w/v) ribose solution for 2 h.

tion. From visual observation however this effect was not as serious, in fact smooth, self-standing gels were formed during heating of solutions containing "pre-gelled" SPI.

To physically compare the effect of different cross-linking treatments on gel mechanical properties, four types of gel samples at SPI concentration of 15% (w/v) were selected and assessed in a stress relaxation experiment. The samples used were: SPI/C/S(2), SPI/MTG(24)/S(2), SPI/C/R(2) and SPI/MTG(24)/R(2) gels. The mechanical properties and relaxation parameters of the gels are given in Table 1. The gels differed considerably with respect to deformation, strength, viscoelasticity (K_1 and K_2) and gel solidity (E_A). As the values of K_1 and K_2 increase, the closer the behavior of the sample to that of an ideal solid when K_1 and $K_2 \to \infty$ (Peleg, 1979). With the combinations of cross-linking treatment, SPI/MTG(24)/R(2) gel showed the highest values of compressive gel strength, viscoelasticity and solidity, and the lowest value of % deformation as compared to other gels tested. The covalent cross-links induced within SPI gels of the combined-treated sample increased the cross-link density that resulted in higher elasticity of the gel.

The established isoelectric point of soy protein is ~pH 5.0 (Wolf & Cowan, 1971). Generally, gels produced using ribose through the Maillard reaction had lower pHs (Beck, Ledl, & Severin, 1990; Yaylayan, 1997). Therefore, the solubility of SPI gels at different pH values was performed in order to investigate the effect of combined cross-linking treatments on alteration of functional groups on the protein molecules. The solubility of the selected SPI gels at different pH values is depicted in Fig. 2. A high solubility was obtained in the control gel of SPI/C/S(2) and single-treated gel SPI/MTG(5)/S(2) at pHs lower than 3.0 or pHs higher than 6.0, and these gels were least soluble between pH 3.5 and 5.0. The % solubility of other gel samples showed similar pattern in solubility as a function of pH. Almost at any pH the % solubility of single-treated gels of SPI/C/R(2) and SPI/MTG(24)/S(2) gels were higher that those of combined-treated gels of SPI/MTG(5)/R(2) and SPI/MTG(24)/R(2). The pH ranges at which the gels became least soluble were wider in the combined-treated gels than those of single-treated. This suggests that the MTGase and/or ribose-induced "Maillard cross-linking" resulted in a significant ($p < 0.05$) decrease in the overall solubility in pH range of 2.0–6.5. Such a variation in solubility at different pHs were probably due to the changes in molecular mass, overall net charge and the content of exposed hydrophobic group (Nielsen, 1997) as a result of cross-linking treatments.

In combined-treated gels, a shift of the region for minimum solubility occurred over a wide range of pH. This result suggests an increase of overall net charge (i.e. more negatively charged) had occurred on the molecules of the cross-linked proteins. This might have been attributed to the reduction of ε-amino group of lysine

residues and γ-carboxyamide group in glutamine residues during the prolonged MTGase incubation (Motoki & Seguro, 1998). In addition, the loss of positively charged side groups from lysine or arginine via the Maillard reaction could has also accounted for this change that resulted in protein gel with more acidic isoelectric points (Beck et al., 1990; Easa, 1996; Yaylayan, 1997). The suggestion of the increase of net negative charge could be confirmed using pH-titration test depicted in Fig. 3. A higher value of net titratable charge on a gel would indicate a higher content of acidic groups within the gels (Choi & Ma, 2006). The result indicates that the ribose-induced "Maillard cross-linking" resulted in a more pronounced charge increase than that of MTGase incubation. The ribose-induced "Maillard cross-linking" caused a progressive increase in the bound H^+ with increasing level of modification (Beck et al., 1990; Easa, 1996; Yaylayan, 1997). Therefore, a significant ($p < 0.05$) increase in negative charges was only found in single-treated SPI/C/R(2) gel, and combined-treated SPI/MTG(5)/R(2) and SPI/MTG(24)/R(2) gels. The variation of solubility of the gels with pH can have some implications in products where solubility contributes greatly to product quality.

The physical properties of a gel can be linked to its network structure. The microstructures of selected gels investigated using field emission scanning electron microscope (FESEM) are depicted in Fig. 4. The FESEM images of the gels prepared from different gel treatments differed. The network of SPI/C/S(2) control gel revealed a typical protein network, explicitly sheet-like structure with thick strands and large pores (Fig. 4A). This formation of three-dimensional protein network was probably due to the filamentous nature of soy protein (Ornebro, Wahlgren, Eliasson, Fido, & Tatham, 1999). Soy protein gels can be categorized in different dimensions, from an ordered network strands to phase separated or aggregated structures, to an extreme where the protein is insoluble and a gel is formed (Hermansson, 1986). Based on this, the control SPI/C/S(2) gels may be categorized as an ordered network strands.

The network of single-treated SPI/MTG(5)/S(2) gels seemed to exhibit a thin film-like structure with smaller pores (Fig. 4B) with MTGase cross-linking compared to SPI/C/S(2), whereas single-treated "Maillard cross-linked" gel (SPI/C/R(2)) appeared in a structure that has small and spherical pores (Fig. 4C). A denser and finer network was obtained in the combined-treated SPI/MTG(5)R(2) gels (Fig. 4D). A decrease of pH from the initial pH of 6.8 to ~5.8 (result not shown) in SPI/C/R(2), SPI/MTG(5)/R(2) and SPI/MTG(24)/R(2) gels was observed as the Maillard reaction occurred during heating. As a result of the pH approaching the isoelectric point of protein one may predict that the network structures to coarsen (Doi, 1993). However, the change in the net charge (Fig. 3) during heating also influenced the nature of protein aggregation and thus influenced the type of network structure formed. Increasing net charge as a result of the Maillard reaction suppressed random aggregation of protein that resulted in the production of an ordered strand structure (Easa, 1996; Hermansson, 1986). As a result of charge modification, the networks of the combined-treated gels were made up of highly cross-linked protein structures with virtually no clumps. As gels were lyophilized prior to SEM analysis, the dried gels can sometimes turn too "brittle" to handle. Therefore the FESEM results of single-treated gels of SPI/MTG(24)/S(2) and combined-treated gels of SPI/MTG(24)/R(2) that were too brittle to be cut using razor blade were omitted from analyses.

4. Conclusion

This paper evaluates combined-treated gels produced by cross-linking treatments of SPI via MTGase mediated ε-(γ-glutamyl)lysine bonds followed by ribose-induced "Maillard cross-linking". The combined-treated gel was self-standing, with an improved mechanical and microstructure properties. This improvement was achieved probably via cross-linking density enhancement that was attained via the combined cross-linking techniques.

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References

Armstrong, H. J., Hill, S. E., Schrooyen, P., & Mitchell, J. R. (1994). A comparison of the viscoelastic properties of conventional and Maillard protein gels. *Journal of Texture Studies, 25*, 285–298.
Ashoor, S. H., & Zent, J. B. (1984). Maillard browning of common amino acids and sugars. *Journal of Food Science, 49*, 1206–1207.
Beck, J., Ledl, F., & Severin, T. (1990). Formation of acids, lactones and ester through the Maillard reaction. *Zeitschrift Fur Lebensmittel-Untersuchung Und- Forschung, 90*, 212–216.
Caballero, P. A., Gómez, M., & Rosell, C. M. (2007). Improvement of dough rheology, bread quality and bread shelf-life by enzymes combination. *Journal of Food Engineering, 81*, 42–53.
Choi, S. M., & Ma, C. Y. (2006). Extraction, purification and characterization of globulin from common buckwheat (*Fagopyrum esculentum Moench*) seeds. *Food Research International, 39*, 974–981.
Doi, E. (1993). Gels and gelling of globular proteins. *Trends in Food Science and Technology, 4*, 1–5.
Easa, A. M. (1996). Factors affecting Maillard induced gelation of protein-sugar systems. PhD thesis. University of Nottingham, United Kingdom. p. 199.
Gerrard, J. A., & Brown, P. K. (2002). Protein cross-linking in food: Mechanisms, consequences, applications. *International Congress Series, 1245*, 211–215.
Graham, L. (1996). A comprehensive survey of the acid-stable fluorescent cross-links formed by ribose with basic amino acids, and partial characterization of a novel Maillard cross-link. *Biochimica Biophysica Acta, 1297*, 9–16.
Hellsten, Y., Skadhauge, L., & Bangsbo, J. (2004). Effect of ribose supplementation on resynthesis of adenine nucleotides after intermittent training in humans. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 286*, R182–R188.
Hermansson, A. M. (1986). Soy protein gelation. *Journal of the American Oil Chemists' Society, 63*, 658–666.
Hill, S., & Easa, A. M. (1998). *The Maillard reaction in foods and medicine*. United Kingdom: The Royal Society of Chemistry.
Jongjareonrak, A., Benjakul, S., Visessanguan, W., & Tanaka, M. (2006). Skin gelatin from bigeye snapper and brownstripe red snapper: Chemical compositions and effect of microbial transglutaminase on gel properties. *Food Hydrocolloids, 20*, 1216–1222.
Lorenzen, P. C. (2007). Effects of varying time/temperature-conditions of pre-heating and enzymatic cross-linking on techno-functional properties of reconstituted dairy ingredients. *Food Research International, 40*, 700–708.
Lqari, H., Vioque, J., Pedroche, J., & Millán, F. (2002). *Lupinus angustifolius* protein isolates: Chemical composition, functional properties and protein characterization. *Food Chemistry, 76*, 349–356.
Ma, C. Y., Oomah, B. D., & Holme, J. (1986). Effect of deamidation and succinylation on some physicochemical and baking properties of gluten. *Journal of Food Science, 51*, 99–103.
Md Yasir, S. B., Sutton, K. H., Newberry, M. P., Andrews, N. R., & Gerrard, J. A. (2007a). The impact of transglutaminase on soy proteins and tofu texture. *Food Chemistry, 104*, 1491–1501.
Md Yasir, S. B., Sutton, K. H., Newberry, M. P., Andrews, N. R., & Gerrard, J. A. (2007b). The impact of Maillard cross-linking on soy proteins and tofu texture. *Food Chemistry, 104*, 1502–1508.
Motoki, M., & Seguro, K. (1998). Transglutaminase and its use for food processing. *Trends in Food Science and Technology, 9*, 204–210.
Nielsen, P. M. (1997). *Food proteins and their applications*. New York: Marcel Decker.
Nussinovitch, A., Kaletunc, G., Normand, M. D., & Peleg, M. (1990). Recoverable work versus asymptotic relaxation modulus in agar, carageenan and gellan gels. *Journal of Texture Studies, 21*, 427–438.
Oliver, C. M., Melton, L. D., & Stanley, R. A. (2006). Creating proteins with novel functionality via the Maillard reaction: A review. *Critical Reviews in Food Science and Nutrition, 46*, 337–350.
Ornebro, J., Wahlgren, M., Eliasson, A. C., Fido, R. J., & Tatham, A. S. (1999). Adsorption of α-, β-, γ-, and ω-gliadins onto hydrophobic surfaces. *Journal of Cereal Science, 30*, 105–114.
Peleg, M. (1979). Characterization of the stress relaxation curves of solid foods. *Journal of Food Science, 44*, 277–281.
Puppo, M. C., & Añón, M. C. (1999). Rheological properties of acidic soybean protein gels: Salt addition effect. *Food Hydrocolloids, 13*, 167–176.
Renkema, J. M. S., & van Vliet, T. (2002). Heat-induced gel formation by soy proteins at neutral pH. *Journal of Agricultural and Food Chemistry, 50*, 1569–1573.
Robinson, H. W., & Hodgen, C. G. (1940). The biuret reaction in determination of serum protein. I. A study of the condition necessary for the production of the

stable color which bears a quantitative relationship to the protein concentration. *Journal of Biological Chemistry, 135*, 707–725.

Tang, C. H. (2007). Effect pf thermal pretreatment of raw soymilk on the gel strength and microstructure of tofu by microbial transglutaminase. *LWT-Food Science and Technology, 40*, 1403–1409.

Tang, C. H., Li, L., Wang, J. L., & Yang, X. Q. (2007). Formation and rheological properties of 'cold-set' tofu induced by microbial transglutaminase. *LWT-Food Science and Technology, 40*, 579–586.

Tay, S. K., Xu, G. Q., & Peter, C. O. (2005). Aggregation profile of 11S, 7S and 2S coagulated with GDL. *Food Chemistry, 91*, 457–462.

Torrezan, R., Tham, W. P., Bell, A. E., Frazier, R. A., & Cristianini, M. (2007). Effects of high pressure on functional properties of soy protein. *Food Chemistry, 104*, 140–147.

Trespalacios, P., & Pla, R. (2007). Simultaneous application of transglutaminase and high pressure to improve functional properties of chicken meat gels. *Food Chemistry, 100*, 264–272.

Wolf, W. J., & Cowan, J. C. (1971). Soybean as a food source. *CRC Critical Reviews in Food Technology, 2*, 81–158.

Yaylayan, V. A. (1997). Classification of the Maillard reaction: A conceptual approach. *Trends in Food Science and Technology, 8*, 13–17.