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Improvement of Bovine Split Hide Gelatin Quality by Addition of Soy Protein Isolate Using Transglutaminase Enzyme

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ABSTRACT

Bovine split hide is the subcutis layer with the low percentage of collagen so that the quality of the gelatin is different from that of the gelatin from cattle's skin. This study aims to improve the characteristics of bovine split hide gelatin combined with soy protein isolate (SPI) using transglutaminase (TGase) enzyme as a protein cross-linking agent. The study was conducted using a completely randomized design with 3 x 3 factorial pattern consisting of three levels of a mixture of bovine split hide gelatin : SPI at the ratios of 90 : 10, 80 : 20, 70 : 30, and three concentrations of transglutaminase enzyme, i.e. 10, 20, and 30 U. The results showed that treatments significantly affected viscosity, gel strength, the moisture, ash, fat, and protein content of gelatin. Electrophoresis of gelatin protein showed bands distribution between 60-190 kDa. The amino acid profile of the gelatin was similar to that of collagen with a high level of aspartate, glutamate, cysteine, and proline. Morphology of gelatin was observed by Scanning Electronic Microscope (SEM) and showed a compact distributed collagen crosslink. The combination of gelatin bovine split hide and SPI at the ratio of 90 : 10 provides the best physicochemical characteristic.

Keywords: quality improvement; bovine split hide gelatin; soy protein isolate; transglutaminase enzyme

INTRODUCTION

Gelatin is a proteinaceous material obtained through the acid or basic hydrolysis of collagen from animal components such as skin, bones, and tendons in the form of a polypeptide with a high molecular weight. Gelatin is commonly used as an emulsifier and stabilizer because of its ability to bind water and fat (Calvarro *et al.*, 2016). Gelatin is a potential candidate of a natural polymer for film formulations because of its excellent film forming properties, biodegradability, and low production costs for gelatin production (Liu *et al*., 2017). Recently, in Indonesia, the need for gelatin in various fields so far is heavily relied on the importation. In fact, the availability of skin and its products in Indonesia have a great prospect to be used as local raw materials for gelatin production which would further reduce the gelatin importation. A tannery is a process to produce leather from skin or hide. The tanning process produces several by-products. One of the by-products of tannery is bovine split hide. Bovine split hide is a leather that has undergone the process of splitting into two or more parts to obtain the desired thickness of the skin. However, bovine split hide mostly consists of subcutaneous layer and has low percentage of dermis so that the quality of gelatin from it is relatively lower than that of gelatin from bovine hide. In the previous research, gelatin extracted from bovine split hide showed low physicochemical properties (Wulandari *et al*., 2016; Wulandari *et al.,* 2018). Therefore, the gelatin extracted from bovine split hide needs to be improved to increase its quality.

Addition of protein can improve the quality of gelatin. Soy protein isolate (SPI) has a high protein value, i.e. about 90% (Yoo & Chang, 2016). Not only improving the protein level, the added SPI also serves as gelling and emulsification to enhance the flavor and give an elastic and soft texture. SPI is a biodegradable and nutritious material that can be used as an edible film and able to provide a polymerization process (Guo *et al.*, 2015).

Protein modification by enzyme has been applied in the food processing industry (Al-Hassan & Norziah, 2017). Erwanto *et al.* (2014) stated that transglutaminase is the most effective ingredients to improve the rheological properties of food protein. Transglutaminase (TG-ase, EC 2.3.2.12) is an enzyme that catalyzes the formation of cross-link between protein molecules. It improves food products properties such as viscosity, elas-

ticity, firmness, and water-binding capacity (Kieliszek & Misiewicz, 2014). Combination of two kinds of different proteins, bovine split hide gelatin and SPI, and the addition of TGase as a cross-linking agent, are potential to improve the quality of gelatin produced from bovine split hide. This study aims to improve the characteristics of bovine split hide gelatin combined with soy protein isolate (SPI) using transglutaminase (TGase) enzyme as a protein cross-linking agent.

MATERIALS AND METHODS

Materials

The research materials were bovine split hide gelatin, commercial Soy Protein Isolate (SPI) (Shandong Crown Soya Protein Co. LTD. Shandong, Province 252429 China), and the Transglutaminase ACTIVA TG_BW-MH Enzyme (Ajinomoto, Malaysia Berhad). The protein content of SPI is 90.1%. The composition of the Transglutaminase ACTIVA TG_BW-MH Enzyme is Sodium Caseinate, Transglutaminase, and Sodium chloride. The total protein content of transglutaminase was 91.43%. Soluble protein level was 1.17225 mg/mL with an enzyme activity of 222.51 U/g.

Method for making the combined gelatin referred to Chambi and Grosso (2006).The r atios of bovine split leather gelatin and SPI were T1= 90:10; T2= 80:20; and T3= 70:30, with the final concentration was 10% (w/v). Each gelatin from bovine split leather and SPI were diluted with distilled water and then heated in a water bath, by stirring at 55°C for 30 minutes. The mixture solution was added with 0.1 N NaOH to reach a pH=7 and stirred at 55°C for 30 minutes.

TGase enzyme as treatment (E1=10 U, E2=20 U, and E3=30 U) was diluted in 5 ml distilled water and was added into the gelatin solution so that the final concentration was 9.6%. The mixtures of TGase and gelatin solutions were heated at 50°C for 15 minutes by slow stirring. Then, the gelatin solutions were reheated at 85°C for 10 minutes to stop the enzyme activity. The final solution was poured into a tray and dried in oven at 50°C for 24-36 hours. The dried gelatin was ground and filtered. The flowchart of making gelatin was shown in Figure 1.

Chemical Compositions Analyses

Chemical compositions were analyzed for moisture, fat, protein, and ash content using the method of AOAC (2012).

Viscosity Analysis

Gelatin solution of 6.67% concentration was boiled in a water bath and continuously stirred up to 60°C. The viscosity was measured using a viscometer brook field (Zhengzhou Nanbei Instrument Equipment Co., Henan, China). A spindle was previously heated at 60°C and then installed to the viscometer brook field. The spindle position in the hot solution was set accurately, then the viscometer was turned on and the temperature of the solution was measured. When the temperature of solution reached 60°C, the viscosity value was known through the viscometer reading at scale 1-100. The reading was done after 1 minute of full rotation 2 times for spindle no. 1.

Gel-Strength Measurement

The strength of the gel was determined according to Liu *et al.* (2017), by using Universal Testing Machine (Zwick, Ulm, Germany). Gelatin samples at a concentration of 6.67% w/v were dissolved in distilled water at 60°C. The solution was stirred until the gelatin was solubilized completely. The solution in the dimensions of 5 cm in diameter and 6 cm in height was stored at 5°C for 16-18 hours. The container of gelatin sample was placed right at the bottom of the plunger (with the diameter of 13 mm) for the testing process. The measurement was conducted at a temperature of 10° C with a plunger speed of 10 mm/min and a depth of 4 mm. The calculation of the value of the gel strength was expressed in bloom= Fmax (g/mm^2 x 12.7 (a surface area of the needle).

Distribution of Molecular-Weight Determination

The distribution of molecular weight was determined according to the method of Laemmli (1970) with Sodium Sulfate Dodecyl Polyacrylamide Gel Electrophoresis (SDS PAGE) using Atto Pugeran AE 6531 (Atto, Tokyo, Japan). The sample of gelatin was di-

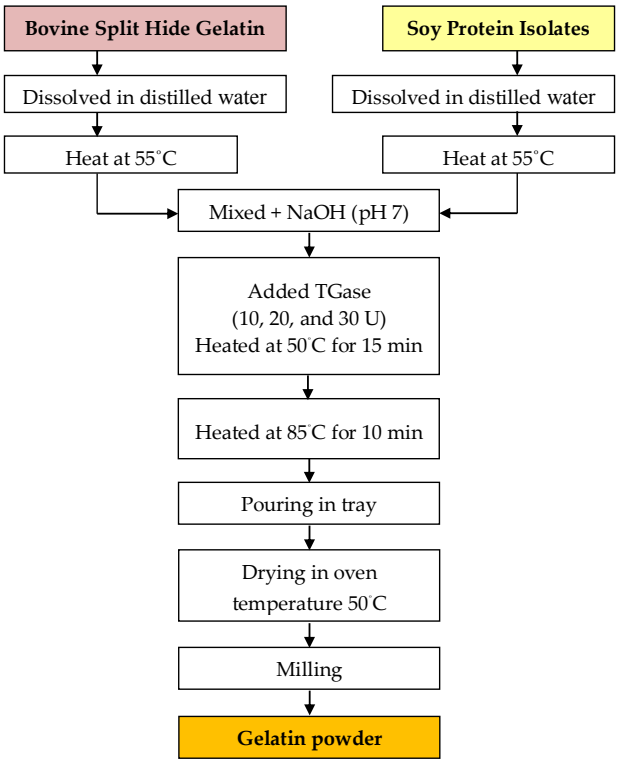

Figure 1. Flowchart of the manufacture of gelatin in combination with bovine split hide and soy protein isolate with the addition of the transglutaminase enzyme (Adapted from Chambi & Grosso, 2006).

luted with a sterile distilled water and mixed. The mixtures were centrifuged at 3000 ppm for 10 min at room temperature. Samples and supernatants were mixed with the ratio of 1:1, and the denaturation was conducted at 100°C for 5 min and cooled at room temperature. The gelatin samples at the volume of 10 µl were loaded into 12% resolving gel and a 5% stacking gel. After running, the gel was stained with Coomassie Blue.

Analysis of Amino-Acid Profile

The profile of amino acid of the samples was determined by using the High Performance Liquid Chromatography (HPLC Shimadzu LC 104, Japan).

Analysis of Gelatin Morphology

Morphology of gelatin was analyzed using a Scanning Electron Microscope (SEM). The gelatin sample was coated by gold then displayed on SEM (Model SNE 4500M SEC e-beam pioneer, SEC Co., Suwon, South Korea).

Experimental Design and Data Analysis

The study was conducted using a completely randomized design with 3x3 factorial pattern. The first factor was the ratios of gelatin from bovine split hide and SPI consisted of three levels, i.e. T1= 90:10; T2= 80:20; and T3= 70:30. The second factor was the activity of TGase consisted of 3 levels, i.e. 10 U, 20 U, 30 U. Each experimental unit was repeated 3 times. The collected data were analyzed by using the Analysis of Variance (ANOVA) and the differences between means were compared by using Duncan's Multiple Range Test. The level of statistical significance was set at $p<0.05$. The statistical software package SPSS 15.0 (SPSS, Inc., Chicago, IL, USA) was used for these data analyses. While the data of amino acid profile, molecule weight, and gelatin morphology were analyzed descriptively.

RESULTS

Chemical Compositions of Gelatin

Chemical and physical properties of gelatin produced by combining the SPI with transglutamiase enzyme were presented in Table 1. The addition of SPI and the enzyme TGase had significant effects ($p<0.05$) on the percentages of the moisture, ash, and fat. However, the addition of SPI and the enzyme TGase did not significantly affect the protein content of the gelatin. The water content of the gelatin produced in this research ranged from 11.77±0.45% to 13.47±0.07%. The dose of TGase was directly proportional to the water content of the gelatin produced. The average range of ash content was 1.17±0.40%-3.23±0.27%. The higher the SPI concentration, the higher the ash contents of the gelatin produced. The highest fat content (2.09±0.03%) was found in gelatin produced by combining the lowest SPI concentration (90:10) and the addition of the lowest TGase (10 U). The increase in SPI concentration and the addition of TGase were inversely proportional to the fat contents of the gelatin produced. All samples of gelatin produced showed high protein contents (83.75±1.03%).

Viscosity

Table 1 also showed a significant difference in viscosity ($p<0.05$) of the gelatin produced. The viscosity of the gelatin produced increased by the increasing dose of TGase. However, the increased dose of SPI or the decreased dose of bovine split leather gelatin decreased the viscosity of gelatin produced.

Gel Strength

The highest gel strength (187.94 bloom) was found in gelatin produced by a combination of bovine split leather gelatin and SPI of 90: 10 with 30 U of TGase whereas the lowest bloom (61.89 bloom) was found in gelatin produced by a combination of bovine split leather gelatin and SPI of 70: 30 with 10 U of TGase. Increased SPI content or decreased bovine split leather gelatin content resulted in the significant ($p<0.05$) decrease in gel strength, while the increased dose of the transglutaminase enzyme significantly increased the gel strength.

Distribution of Molecular Weight

Results of determination of the molecular weight using SDS PAGE were shown in Figure 2. The results showed that almost all gelatins produced by combinations of bovine split hide and soy protein isolate with the addition of transglutaminase enzyme treatments had the same patterns of molecular weight distribution, i.e. between 60 to 190 kDa.

Amino Acid Profile

Amino acid profiles of gelatins produced by different ratios of bovine split hide and SPI and concentrations of TGase were presented in Table 2. The results showed that the amino acids with the highest concentrations were proline, glycine, hydroxyproline, cysteine, and glutamic acid.

Gelatin Morphology

The morphological surface of the gelatins produced can be seen from the results of Scanning Electron Microscope (SEM) in Figure 3. The structure of gelatin from bovine split hide (T0) without the addition of SPI and TGase showed that the surface structure was not homogeneous and rude. The addition of SPI and TGase homogenized the surface of gelatin produced. This homogenization of gelatin surface was shown by the more regular and looked fibrous appearance.

DISCUSSION

The combination of non-muscle proteins with transglutaminase provides a useful approach to im-

Table 1. Physicochemical properties of gelatin combination of bovine split hide and soy protein isolate with the addition of the transglutaminase enzyme

Variables	TGase (U)	Combinated gelatin split hide + SPI			Average
		T1	T2	T3	
Moisture (%) *	E1	12.50±0.31^{d}	11.77±0.45^{b}	11.97±0.03^{b}	12.08±0.41
	E2	12.66±0.21^{d}	12.75±0.47^{d}	11.26±0.05^{a}	12.22±0.76
	E3	13.47±0.07^{e}	11.82±0.42^{b}	12.44±0.03^{c}	12.57±0.75
	Average	12.87±0.50	12.12±0.55	11.89±0.53	
Ash (%) *	E1	0.74±0.03^{a}	2.11±0.03^{b}	3.06±0.03^{c}	1.97±0.30
	E2	1.13±0.06^{a}	2.21±0.15^{c}	3.57±0.06^{c}	2.30±1.09
	E3	1.64±0.01^{b}	2.09±0.01^{b}	3.05±0.01^{c}	2.26±0.01
	Average	1.17±0.40	2.14±0.09	3.23±0.27	
Fat (%) *	E1	2.09±0.03^{d}	2.03±0.02^{d}	1.00±0.01^{c}	1.71±0.54
	E2	0.95±0.01^{c}	0.95±0.03^{c}	0.99±0.03^{c}	0.97±0.03
	E3	0.99±0.04^{c}	0.85± 0.02^{b}	0.56±0.01^{a}	0.80±0.19
	Average	1.34±0.58	1.28±0.58	0.85±0.23	
Protein (%) **	E1	85.38±0.74	82.50±0.08	81.58±0.16	83.18±1.80^{p}
	E2	85.45±0.72	83.41±0.54	82.71±0.22	83.86±1.34^{q}
	E3	85.53±0.74	83.60±0.32	83.48±0.32	84.20±1.09^{q}
	Average	85.46±0.57^{y}	83.19±0.57^{x}	82.59±0.87^{x}	
Viscosity (cP) *	E1	4.45±0.21^{b}	3.75±0.07^{a}	3.50±0.00^{a}	3.90±0.09
	E2	4.65±0.21^{c}	4.60±0.14^{c}	3.75±0.07^{a}	4.33±0.14
	E3	4.70±0.14^{c}	4.75±0.07^{c}	4.20±0.14^{b}	4.55±0.14
Average	Average	4.62±0.19	4.37±0.08	3.82±0.07	
Gel strength (Bloom) *	E1	98.25±0.74^{d}	98.37±1.87^{d}	61.89±0.53^{a}	86.17±18.83
	E2	181.83± 0.78^{g}	117.09± 2.61^{e}	76.37± 0.13^{b}	125.09±47.58
	E3	187.94±44.83^{h}	147.54± 0.72^{f}	82.82± 1.02^{c}	139.44±47.44
	Average	156.01±44.83	121.00±22.2	73.69±44.83	

Note: Means with different supercripts differ significantly (p<0.05); * = significant interaction between two treatments (p<0.05); **= means in the same row or column at average of protein data with different supercripts differ significantly (p<0.05); T1= Combinated gelatin split hide : SPI = 90:10; T2= Combinated gelatin split hide : SPI = 80 : 20; T3 = Combinated gelatin split hide : SPI = 70 : 30; E1= Transglutamiase enzim level 10 U; E2= Transglutamiase enzim level 20 U; E3= Transglutamiase enzim level 30 U.

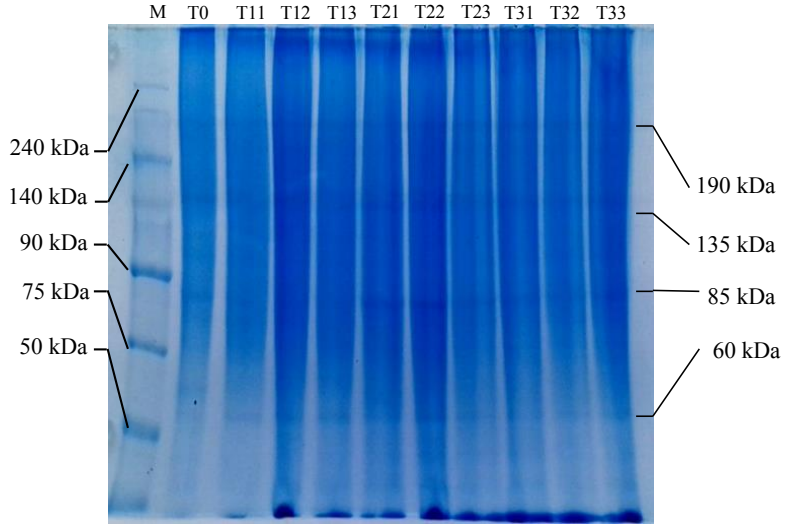

Figure 2. SDS PAGE of gelatin combination with bovine split hide and soy protein isolates with the addition of the transglutaminase enzyme. M= marker; T0= Gelatin bovine split hide + SPI; T11, T12, T13= gelatin bovine split hide : SPI = 90 : 10 + TG ase enzyme 10, 20, 30 U; T21, T22, T23= gelatin bovine split hide : SPI = 80 : 20 + TG ase enzyme 10, 20, 30 U; T31, T32, T33 = gelatin bovine split hide : SPI = 70 : 30 + TG ase enzyme 10, 20, 30 U.

Table 2. Analysis of amino acid composition in gelatin combination with bovine split hide and SPI with the addition of TGase enzyme

Amino acids (%)	Treatments										
	T0	T11	T12	T13	T21	T22	T23	T31	T32	T33	CG
Aspartic acid	3.90±0.40	3.51±0.33	3.96±0.31	4.12±0.29	4.47±0.32	4.89±0.39	5.34±0.41	6.00±0.48	6.16±0.61	6.32±0.51	4.85±0.48
Glutamic acid	7.50±0.69	8.61±0.76	8.61±0.51	10.05±0.61	8.02±0.48	8.22±0.51	9.50±0.44	8.11±0.51	8.38±0.48	11.89±0.41	10.22±0.51
Serine	2.80±0.30	1.95±0.09	2.38±0.12	2.52±0.31	1.58±0.08	1.79±0.07	2.39±0.09	1.85±0.10	2.27±0.12	2.42±0.09	2.33±0.12
Glysine	11.30±0.97	11.18±0.98	10.28±0.66	10.67±0.84	8.51±.52	8.62±0.61	9.74±0.42	7.77±0.45	8.22±0.52	8.74±0.61	15.62±0.52
Threonine	1.4±0.0.10	0.89±0.078	0.91±0.01	1.47±0.13	1.33±0.08	1.39±0.11	1.60±0.12	1.38±0.09	1.56±0.13	2.02±0.10	1.57±0.15
Arginine	6.20±0.54	4.36±0.16	4.87±0.46	5.62±0.43	4.37±0.40	5.33±0.32	5.90±0.33	4.78±0.21	5.64±0.32	5.65±0.51	6.53±0.45
Alanine	6.90±0.50	5.98±0.60	6.91±0.48	7.41±0.61	6.91±0.51	6.91±0.48	6.97±0.37	6.45±0.32	6.62±0.46	6.74±0.36	8.60±0.03
Tyrosine	1.10±0.09	0.36±0.01	0.78±0.05	1.77±0.09	0.86±0.04	1.10±0.08	1.16±0.09	1.21±0.06	1.50±0.07	3.57±0.11	0.33±0.08
Methionine	1.20±0.08	0.17±0.01	0.18±0.02	0.23±0.02	0.19±0.01	0.21±0.04	0.28±0.01	0.22±0.01	0.23±0.02	0.24±0.02	0.18±0.01
Valine	1.70±0.13	1.21±0.15	2.06±0.11	2.48±0.09	1.68±0.05	2.05±0.16	2.75±0.09	1.53±0.05	1.62±0.06	3.08±0.09	2.68±0.08
Phenylalanine	1.80±0.11	1.15±0.10	1.34±0.16	1.61±0.10	1.33±0.08	1.60±0.08	1.65±0.07	1.74±0.08	1.83±0.05	2.10±0.07	1.56±0.08
Isoleucine	1.40±0.09	0.89±0.01	1.13±0.05	0.78±0.01	1.32±0.01	1.28±0.06	0.79±0.02	1.84±0.06	0.19±0.05	1.30±0.06	0.52±0.02
Leucine	2.30±0.08	1.72±0.12	2.57±0.09	2.23±0.12	2.54±0.11	2.50±0.21	2.17±0.12	3.37±0.15	1.12±0.08	2.69±0.10	2.29±0.09
Lysine	3.00±0.12	1.91±0.11	2.83±0.19	3.64±0.13	2.30±0.12	2.83±0.31	3.28±0.28	1.95±0.11	3.36±0.18	4.34±0.21	3.12±0.15
Cysteine[a]	1.80±0.08	8.24±0.77	8.50±0.81	9.43±0.45	12.49±0.51	15.43±0.48	15.64±0.61	15.85±0.99	18.07±0.95	21.36±0.13	2.45±0.12
Proline[a]	12.30±0.96	16.33±0.64	16.43±1.05	16.59±0.48	13.57±0.61	14.43±0.51	15.78±0.45	11.75±0.61	12.56±0.54	12.57±0.71	12.54±0.91
Hydoxyproline[a]	21.48±1.25	6.19±0.37	3.62±0.15	3.02±0.08	2.88±0.09	4.30±0.13	2.93±0.23	2.84±0.09	2.75±0.05	3.71±0.06	22.95±0.98

Note: Source= HPLC analysis; a= spectrophotometric analysis; T0= Gelatin bovine split hide; CG= Commercial gelatin; T11 = Gelatin bovine split hide + soy protein isolate 10% + TG ase enzyme 10 U; T12= Gelatin bovine split hide + soy protein isolate 10% + TG ase enzyme 20 U; T13= Gelatin bovine split hide + soy protein isolate 10% + TG ase enzyme 30 U; T21= Gelatin bovine split hide + soy protein isolate 20% + TG ase enzyme 10 U; T22= Gelatin bovine split hide + soy protein isolate 20% + TG ase enzyme 20 U; T23= Gelatin bovine split hide + soy protein isolate 20% + TG ase enzyme 30 U; T31= Gelatin bovine split hide + soy protein isolate 30% + TG ase enzyme 10 U; T32= Gelatin bovine split hide + soy protein isolate 30% + TG ase enzyme 20 U; T33= Gelatin bovine split hide + soy protein isolate 30% + TG ase enzyme 30 U.

prove protein function, especially the gelation properties (Han *et al.*, 2015). The transglutaminase enzyme is a protein-polymerizing agent capable of forming iso-peptide bonds between protein-based foods, thereby increasing the protein crosslink (AL-Hassan & Norziah, 2017). Wulandari *et al.* (2018) state that protein can bind water so it can produce water molecules. Weng & Zheng (2015) also state that gelatin films present poor water resistance properties because of the highly hygroscopic property of gelatin.

The higher ash content of gelatin bovine split hide combined with SPI is caused by the high ash content of SPI (5.2%) compared to the ash content of gelatin (0.3%). Likewise, the addition of TGase enzyme significantly increases the ash contents of the gelatin produced. The increase in ash content of the gelatin produced is because the TGase enzyme contains sodium caseinate and sodium chloride which is a source of minerals. Said *et al.* (2012) producing gelatin from goat skin state that the minerals contained in the gelatin may come from: (1) feed and drinking water consumed by the goats and stored in the skin tissue, (2) water from the skin processing (soaking, unhairing, washing, and neutralization), and (3) contamination of equipment in the production process.

The fat contents of gelatin produced in the present experiment were higher than those reported by Said *et al.* (2012) which produced fat content in goat skin gelatin of around 0.55%-0.43%, but almost with similar content to food-grade commercial gelatin (0.67%) and pharmacy standard (0.52%) in Indonesia. The fat content of gelatin decreased with the addition of TGase (Table 1). TGase acts as a catalyst in the formation of crosslinks between proteins resulting water molecules. On the other hand, heating causes unsaturated fatty acid to be oxidized and decomposed into shorter carbon chains making it more soluble. During gelatin drying, the fat will evaporate along with the water (Liu *et al.*, 2009) so that the higher water contents will accelerate and increase the evaporation of fat during the gelatin drying.

Soy protein isolate (SPI) is the purest form with very high protein content, at least 95% in dry weight. Yew *et al.* (2011) said almost the same opinion that these were mainly attributed to the presence of SPI. SPI mainly comprises 85% to 90% of pure soybean protein and is usually produced from defatted soy flake. TGase is an enzyme that can improve the quality of a protein with the formation of e-(c-glutaminyl) lysine intra- and intermolecular cross-linked proteins, catalyzed by acyl-transfer reactions between c-carboxamide groups of glutamine residues and e-amino groups of lysine residues (Weng & Zheng, 2015).

The addition of TGase enzyme caused the cross-linkage between the γ-carboxamide of glutamine residues (acyl donor) and ε-amino groups of lysine residues (Liu *et al.*, 2017). Ye *et al.* (2014) state that this reaction leads to the formation of the intramolecular and/or intermolecular cross-linking of iso-peptide bonds. Therefore, the increased crosslinking causes an increase in the viscosity of gelatin from 6.81 to 35.04 cP. This result is almost similar to the results reported by Wulandari *et al.* (2016) that the viscosity of gelatin from bovine split hide is 6.48-6.88 cP.

Gel strength is the major physical property normally measured for gelatin gels (Wangtueai *et al.*, 2010). The value of gel strength has to meet the standards required by the British Standard (50-300 g bloom). The significant increase in gel strength after the addition of the TGase

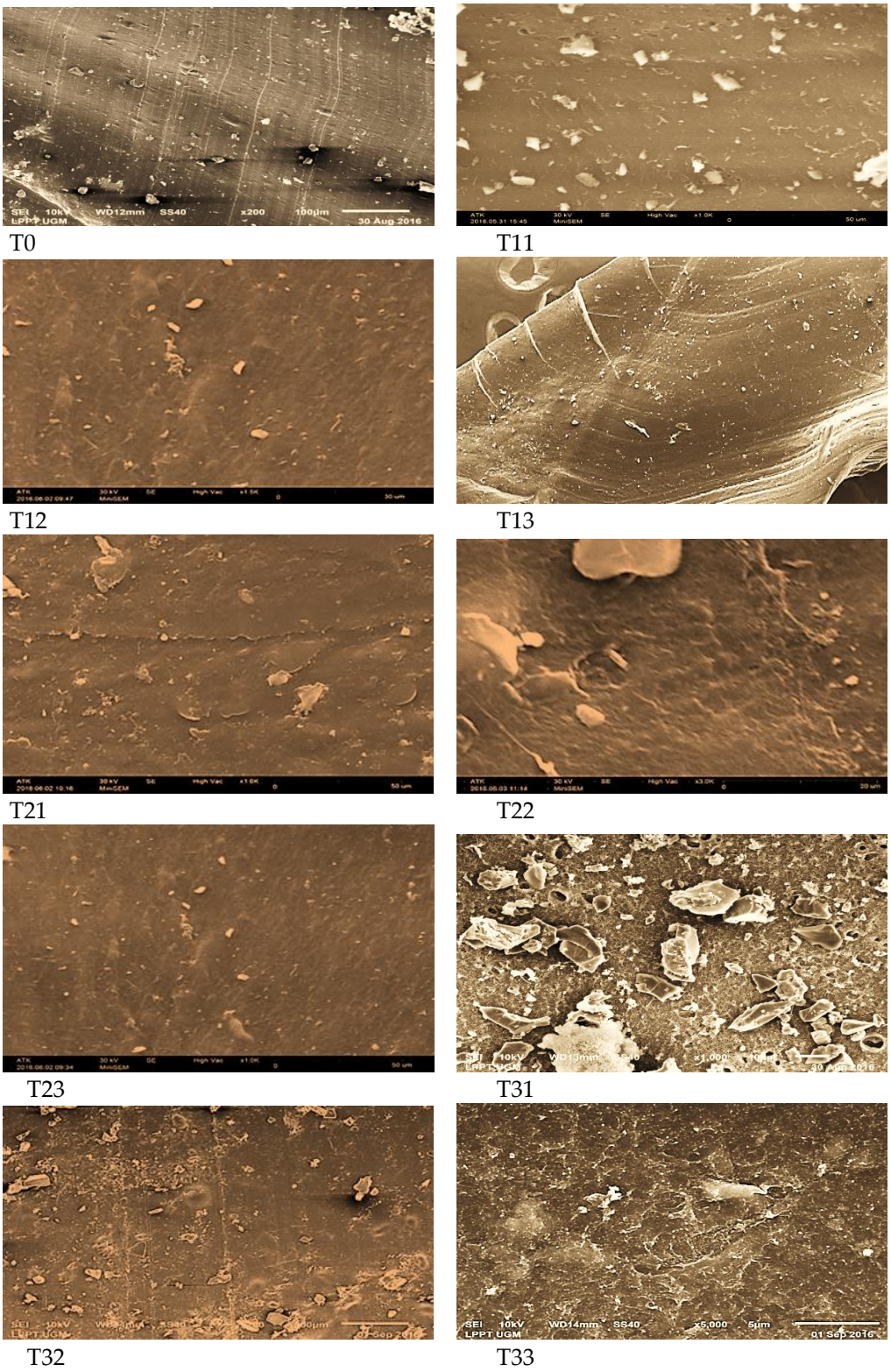

Figure 3. SEM of gelatin combination surfaces (Magnification 2000x). T0= gelatin bovine split hide; T11, T12, T13= gelatin bovine split hide : SPI = 90 : 10 + TG ase enzyme 10, 20, 30 U; T21, T22, T23= gelatin bovine split hide : SPI = 80 : 20 + TG ase enzyme 10, 20, 30 U; T31, T32, T33= gelatin bovine split hide : SPI = 70 : 30 + TG ase enzyme 10, 20, 30 U.

is due to the formation of covalent crosslinks through a non-disulfide bond. Wang *et al.* (2015) also stated that transglutaminase could induce the cross-linking between polypeptide-bound glutamine residues and multiple primary amines by generating new covalent bonds. Further, Xing *et al*. (2019) state that the increases in the amount of hydrogen electrostatic interactions between the two polymers make the plastic effect is more

absorbed in the gelatin. This statement is confirmed by Ye *et al.* (2014) that the occurrence of transglutaminase process facilitates the strong peptides intramolecular polymerization in gelatin, thus enhancing the gel strength and tensile strength.

Previous research (Wulandari *et al*., 2016) reported the higher molecular weight of gelatin were found in gelatin produced from bovine split hide, i.e. 25-40 kDa. Wangtueai *et al.* (2010) explained that production of gelatins using a high extraction temperature was discovered to contain more peptides with molecular weight less than the α-chains and greater than the β-chains. The fragmentation of major components into fragments of lower molecular weight affects the properties of gelatin such as its viscoelastic properties, melting point, and gel strength. The increase in molecular weight was due to the crosslinking between protein molecules of gelatin and SPI by enzyme transglutaminase as a crosslinking that eventually generated new structures with high molecular weights. Figure 2 shows the profile of protein molecules produced from various types of combination of bovine split hide gelatin and SPI with the transglutaminase enzyme. It appears that almost all treatments have the same pattern of molecular weight distribution, between 60 kDa and 190 kDa. This result is higher than that reported by previous study (Wulandari *et al*., 2016) that gelatin from bovine split hide has a range of molecular weight of 25-40 kDa, and higher than the molecular weight of transglutaminase enzyme from *Streptomyces* or Bacilli (≥38 kDa) (Steffen *et al*., 2017). Kyriakopoulou *et al.* (2019) stated that SPI was one of the proteins containing cystine and cysteine that formed sulfide bonds. This sulfide bond made a great contribution to the formation of crosslinks between molecules of protein of gelatin and soy.

Lawrie (2017) reported that the glycine contained in the collagen was approximately one-third of the total amino acid content, whereas hydroxyproline and proline was 23%. Glutamic acid was an amino acid commonly found in SPI (Yoo & Chang, 2016). According to Kyriakopoulou *et al.* (2019), SPI was one of the proteins containing cystine and cysteine that formed sulfide bonds. Amino acids composition of gelatin produced by combination of treatments significantly increased aspartic acid, glutamic acid, cysteine, and proline. The percentages of those four amino acids were greater than the percentages of amino acid in bovine split hide gelatin, i.e. 3.9%, 7.5%, 1.8%, and 12.5% (Wulandari *et al*., 2016). The increase in the percentage of all four amino acids is due to the crosslinks between protein molecules of gelatin and SPI. TGase was capable of adding crosslinking proteins through the formation of crosslinks α - (γ - glutamyl) lysine and improved the texture of the food product. Collagen protein was also capable of polymerizing with TG-ase enzyme and its application was possible as an additive in the manufacture of protein products to improve their qualities (Wulandari *et al*., 2016). This increase in amino acids content could have a positive effect when used as a food ingredient because free amino acids also could affect the flavor of food (Sulaiman *et al*., 2016).

Based on SEM of gelatin in Figure 3, the structure of collagen from gelatin fused with particles of SPI. This condition is due to the formation of covalent crosslinks through non-disulfide that increases the tissue structure of the gel (Kyriakopoulou *et al*., 2018). The increased in the addition of transglutaminase enzyme delivers significant results and the appearance of the microstructure coincided with the results of viscosity and gel strength. In Figure 3, T21, T22, and T23 were combination of gelatin and SPI with a ratio of 80: 20 with the addition of TGase enzyme at the levels of 10, 20, and 30 U. In this treatment, the formation of cross-linking occurred, but the homogenization between gelatin and SPI was reduced. This result indicated that the grain of SPI was not blended well. In Figure 3, the T31, T32, and T33 groups showed the appearance of gelatin with a combination treatment of gelatin and SPI 70: 30 with the addition of different levels of TGase enzyme, i.e. 10, 20, and 30 U. In this figure, it appears the improved blend between gelatin and SPI, as was indicated by the increasing number of grains or particles of SPI and the apparent absence of fibers form. Gelatin and SPI were unable to blend well that eventually decreased the viscosity and gel strength of the gelatin. Formation mixture of proteins from different sources affects the gel strength (Wangtueai *et al*., 2010).

CONCLUSION

The addition of SPI and transglutaminase enzymes in the ratio of bovine split hide gelatin and SPI 90 : 10 with transglutaminase 30 U enzyme level improve the quality of bovine split hide gelatin, not only from the physical and chemical properties but also from the molecular weight distribution, percentage of amino acids, and gelatin morphology.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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