Application of transglutaminase for quality improvement of whole soybean curd

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Revised: 21 October 2018 / Accepted: 29 October 2018 / Published online: 1 January 2019
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Abstract To improve the quality of whole soybean curd (WSC), effects of compound coagulants consisting of calcium chloride (CaCl₂) and transglutaminase (TGase) were investigated. The results showed that TGase modified the water distribution and reduced the cooking loss of WSC, accompanying with increased water holding capacity. The bloom value of WSC was upgraded as the TGase concentration increased. Although certain sensory parameters showed different scores in groups, the overall acceptability of 1500 ppm group was the highest. TPA test suggested that hardness, springiness and chewiness were promoted by TGase significantly. Smooth appearance of WSC was observed, resulting from the transformation of microstructure. Protein subunits of 7S α ', 7S α , 7S β , 11SA3, 11S acidic, and 11S basic proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, band intensity of protein subunits declined at storage and cooking phase, indicating that crosslinking of proteins was still in progress. In conclusion, the addition of TGase improved the quality of CaCl₂-induced WSC and could be used to facilitate the production of this new type of soybean product.

Keywords Whole soybean curd · Transglutaminase · Textural properties · Cooking loss · Microstructure · Protein subunit composition

Introduction

Soybean curd, also known as tofu, is a popular soy-based food item in oriental diet. Consumption of soybean curd has been growing rapidly in western countries because of its nutritional and healthful characteristics (Adams et al. 2005; Hou et al. 1997; Andrade et al. 2010). It has previously been observed that consumption of soybean curd could reduce the risk of some diseases, such as diabetes, hypertension, arteriosclerosis, obesity, and breast cancer (Friedman and Brandon 2001).

According to the traditional preparation process of soybean curd, soybean seeds are soaked, ground, and then filtered to produce raw soymilk, which is then heated to 95 °C for several min to denature soybean proteins. Coagulant is added and mixed when the soymilk is cooled to about 65 °C and then together heated to 80 °C to form soybean curd (Liu et al. 2013). During the filtration step of soymilk, most of raw materials are filtered and discarded and about 47% of raw materials still remain in okara (Liu 1997). The waste materials of filtration cannot be used for human nutrition directly due to its undesirable sensory quality and they are commonly processed into fodder. Actually, okara is an essential food ingredient and rich in dietary fiber (Jiménez-Escrig et al. 2008). Apart from the health benefits, utilization of proteins contained in okara is more efficient (O'Toole 2016), for example, the protein efficiency ratio (PER: average weight gain per 1 g protein intake) of okara is 2.71 compared with 2.11 in soymilk. Therefore, to improve the utilization of soybean materials,

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research on the value-added utilization of okara become a core issue.

Processing of whole soybean curd (WSC), which eliminates the filtering step and retains okara in soybean curd, has attracted extensive attention of scholars (Zhang et al. 2018; Liu et al. 2013). However, in comparison with traditional soybean curd, formation of WSC is more difficult due to the presence of okara. To solve this problem, great efforts in physical and biological processes have been applied to produce WSC with desired properties. For example, ultra-high pressure homogenization (UHPH) technique was applied as an alternative to traditional thermal processing. This process requires the liquid to pass through a gap valve, resulting in a great pressure of 300–350 MPa and high shear stress. Recently, UHPH was applied to modify particle size of soy flour suspension as well (Liu et al. 2013). As a result, particle size of soy flour suspension was dramatically reduced and the prepared WSC showed similar textural properties compared to traditional soybean curd. However, this method has a drawback of high energy consumption, which is the main barrier for its practical production.

Being a calcium ion dependent enzyme, transglutaminase (TGase, EC 2.3.2.13) catalyzes acyltransfer reaction, intra- and intermolecular crosslinking (polymerisation) (Kang et al. 1994; Chang et al. 2011). These reactions brought great changes in protein structure, resulting in upgraded texture in terms of gelation, water-binding capacity and syneresis. Furthermore, TGase stays active state in wide ranges of pH and temperature (pH 5.0–8.0, temperature 40–70 °C), and the activity of TGase doesn't require special factors. Based on these advantages, TGase was widely used in dairy products (Lorenzen 2007) and wheat products (Caballero et al. 2007). TGase was also applied to promote the formation of soybean curd and enhance its rheological properties (Joo et al. 2011). Soybean curd prepared with enzyme exhibited excellent textural properties and retained most of the nutrients in soybean seeds; therefore, the addition of TGase was considered a cost-effective method to produce WSC. However, if TGase was solely added as a coagulant to produce soybean curd, it would take too much time to form a gel. Tang's (2007) research revealed that the heated soymilk needed to be kept at 37–40 °C for 16–20 h to form a gel, resulting in microbial contamination. Moreover, firmer curd could be prepared by coagulating together with calcium sulfate or GDL (Kwan and Easa 2003). Consequently, to shorten the time to form soybean curd and improve the firmness of WSC, research of compound coagulants became a focus.

The factors that affect the ultimate quality of WSC are various, include not only the particle size of soymilk, coagulant composition, and operation conditions, but also

the most crucial factor of coagulation (Zhu et al. 2016). In this study, a standard WSC making model was set as the control group, compound coagulants consisting of CaCl_2 and TGase were selected to investigate the effect of TGase on moisture distributions, textural, and cooking properties, and analyze the correlations between the composition of protein subunits and quality of WSC.

Materials and method

Materials

Soybean seeds (species: Nandou 12, consisted of 46% crude protein, 11% moisture and 14% crude fat) were obtained from Nanchong Academy of Agricultural Science (Nanchong, Sichuan, China) and stored at 15 °C. TGase (EC 2.3.2.13, enzyme activity: 0.1 KU/g) was purchased from Bomei Biotechnology Company Ltd. (Hebei, China). All chemicals and reagents were analytical grade and obtained from Titan Chemical Company Ltd. (Shanghai, China) and Macklin Chemical Company Ltd. (Shanghai, China). Bromo phenol blue and standard protein for SDS-PAGE were purchased from Tiangen Biotechnology Company Ltd. (Beijing, China).

Preparation of standard WSC and traditional soybean curd

Standard WSC was prepared with the combination of methods reported by Yasir et al. (2007) and Joo et al. (2011) with little modification. Briefly, cleared soybean seeds were ground into powder using a high-speed cyclone mill (Model HK-20B, Xulang Machinery Factory, Guangzhou, China). Soy powder suspension (concentration 14%, w/v) was prepared by mixing 100 g powder into 700 mL water (2530 °C) with a magnetic stirrer (Model HSC-19, Joan Laboratory, Zhejiang, China) at a speed of 1400 rpm for 2 min. Glyceryl monostearate (0.3%, w/v, in dry weight) was added into soymilk as defoaming agent. The suspension was first pre-heated at 50 °C for 8 min and then heated to 95 °C and kept for 5 min. The heated soymilk was poured into a beaker and cooled to 50 °C under ice bath condition. Freshly prepared coagulant (0.94 g CaCl_2 dissolved in 50 mL water) was added to the suspension, the mixture was incubated at 50 °C for 5 min to form a gel. This gel was broke and poured into a wooden mold (15L × 17 W × 9H cm) and then pressed at 16 g/cm² for 30 min. After pressing, the curd was cooled and stored at 8 °C for 12 h prior for further analysis.

As for traditional soybean curd, briefly, in the case of no change in other steps, the heated soymilk was filtered to remove okara.

Preparation of CaCl₂-induced WSC by adding TGase

TGase-added WSC was prepared based on the method of standard WSC with following modifications. (1) Certain amount of TGase was dissolved in the freshly prepared CaCl₂ aqueous solution (0.94 g CaCl₂ in 50 mL water) to prepare the compound coagulant. (2) The compound coagulant was added to soymilk at 50 °C and incubated for 8 min before the soymilk was heated at 95 °C for 5 min. (3) 0 mg (control), 23 mg (500 ppm, mass ratio between TGase and soybean protein), 46 mg (1000 ppm), 69 mg (1500 ppm), 138 mg (3000 ppm) and 276 mg (6000 ppm) of TGase were added for the comparative study.

Water distribution and syneresis

Previous study demonstrated that the distribution state of water in curd influenced its texture and stability (Liu et al. 2013). Thus, the contents of expressible water and entrapped water were measured. After storing at 8 °C for 24 h, 10 g of the curd was placed into a centrifuge tube with absorbent cotton at the bottom. After centrifuging at 2500× *g* for 75 min at 4 °C, the curd was taken out and weighted. The expressible water was defined as the percentage of expressed water in the original curd by weight. Entrapped water was calculated by deducting expressed water from the total water content.

Measurement of syneresis was modified from the method of Armstrong et al. (1994). Briefly, standard cuboids samples (2L × 2 W × 1H cm) were placed in a container equipped with wire netting. After that, a sheet of polythene was covered on the container to prevent moisture evaporation. After storing at 4 °C for 24 h, the samples were weighted and the decreased weight was defined as effluent water. Syneresis was defined as the weight percentage of effluent water in original curd.

WHC and cooking loss

Calculation of water holding capacity (WHC) was based on the method proposed by Puppo and Anon (1998). Approximately 5 g of curd sample was placed in a 50 mL centrifuge tube with absorbent cotton at the bottom. Centrifuged at 800 × *g* for 10 min, the sample was weighed as *W*₁ and then heated to a constant weight (*W*₂) at 103 °C. WHC was calculated by following formula.

$$\text{WHC (\%)} = (W_1 - W_2)/W_1 \times 100 \quad (1)$$

Cooking loss was an essential index for the evaluation of reprocessing performance of soybean curd. Approximately 10 g of sample were cut into standard cuboids (2L × 2 W × 1H cm) and boiled in 800 mL moisture for

8 min (Chang et al. 2011), cooking loss was calculated as follows.

$$\text{Cooking loss (\%)} = (W_1 - W_2)/W_1 \times 100 \quad (2)$$

*W*₁ and *W*₂ were the weights of sample before and after boiling, respectively.

Bloom test and texture profile analysis (TPA)

Determination of bloom value (also known as gel strength) of WSC was according to the standard of Gelatin Manufacturers Institute of America (GMIA 2013) using a TA-XT Plus Texture Analyzer (Stable Micro System Ltd. Haslemere, Surrey, UK). WSC was cut into standard cuboids (2L × 2 W × 1H cm) and then compressed by a P/0.5 probe to a target distance (10 mm). The force–time–distance data were gathered to calculate bloom value, rupture strength, and rupture distance.

TPA test was carried out as described by Aly and Seleem (2015). Briefly, curd samples were cut into same cuboids (4L × 3 W × 3H cm) and then compressed twice by a P/36R probe with constant moving speed 1.0 mm/s. It was worth mentioning that the deformation percentage was set as 30% because higher deformation percentage would destroy the structure of WSC, making the results of TPA test senseless.

Appearance and scanning electron microscopy (SEM) of WSC

WSC was sliced to a cuboid (5L × 5 W × 3H cm), A SLR camera (EOS 1100D, Canon Inc., Tokyo, Japan) equipped with an EF-S 18–55 mm f/4–5.6 lens was used to catch appearance changes of WSC.

To examine the microstructure of WSC, SEM was employed with the method reported by Noh et al. (2005). Briefly, WSC was sliced into cubes (ca. 3 mm × 3 mm × 3 mm) that were added in 2.5% glutaraldehyde in 0.1 M phosphate, pre-fixed at room temperature for 10 min and then cooled to 4 °C for 1 h. The samples were washed twice with 0.1 M phosphate buffer and then post-fixed in 1% osmium tetroxide solution. The samples were dehydrated in series ethanol (60%, 70%, 80%, 90%, 95% and 99.5%) for 15 min individually and then freeze-dried with a freeze-dryer (Model 18 N, Scientz scientific, China). Broken sides of dried samples were positioned up and then coated with gold at 1.2 kV and 15 MPa for 180 s in an ion beam sputtering system (Model Supra55; ZEISS; Germany). The final specimens were examined using a scanning electron microscope (Model Supra55; ZEISS; Germany).

Sensory

Sensory evaluation of soybean curd was conducted using seven-point hedonic scoring test as described by Yeoh et al. (2011) with little modification. Sensory assessment was carried out under white light, at room temperature, by a panel of 15 panelists. Before analysis, samples were sliced into equal size, coded with three-digit numbers and then randomly served. Soybean curd were evaluated on the basis of acceptance of their color, appearance, taste, hardness, and springiness. Overall acceptability score was calculated as an average of whole sensory parameters.

Protein subunit composition

Briefly, 1 g of defatted WSC sample was freeze-dried by a freeze-dryer (Model 18 N, Scientz scientific, China). SDS-PAGE was carried out to analyze the mode of protein polymerization with a 12.5% acrylamide separating gel. Dried samples (5 mg) were dissolved in 1 mL of buffer (0.15 mol/L Tris-HCl, 4% SDS, 5% β -mercaptoethanol) and mixed for 1 h. Centrifugated at $8000 \times g$ for 5 min, the supernatant was extracted and mixed with sample buffer followed by heating at 100 °C for 3 min. SDS-PAGE was conducted with 120 V for stacking gel and separating gel. A pre-stained molecular marker (11–245 kDa, Tiangen Biotech Ltd., Beijing, China) was used. After electrophoresis, gel image was scanned with an image scanner (Smart Gel 130, Saizhi scientific Co., Beijing, China) and analyzed by Quantity one software, (version 4.6.2, Bio-Rad Laboratories, Inc., Hercules, CA, USA) for protein subunits.

Data treatment

All experiments were conducted in triplicated. Analysis of variance (ANOVA) was performed using SPSS statistical package (Minitab Inc.), and significant difference between groups were analyzed by the Duncan multiple range test ($p < 0.05$).

Results and discussion

Changes in moisture content, water distribution state and syneresis

During the formation of soybean curd, water was abundant and directly determined the final quality of curd, which reflected texture and stability. According to current study, the denatured soy proteins aggregated and held water in pores (Peng et al. 2016). Thus, the addition of TGase might affect the aggregation of proteins and distribution state of

water consequently. The moisture content and distribution state of WSC added with various concentration of TGase were shown in Table 1. Obviously, moisture content of WSC was significantly changed when TGase was added. Moisture content of the control group was 73.62% while the value increased to 75.87%, 77.54% and 78.45% (range 500–1500 ppm) and then decreased to 75.11% and 76.63% (range 3000–6000 ppm), respectively. First, as a calcium ion dependent enzyme, TGase could catalyze the formation of intra- and intermolecular crosslinking by γ -glutamyl- ϵ -lysine chain bridge, and a more developed protein structure promoted the water-binding capacity and improved moisture content is consequence (Nonaka et al. 1996). Secondly, decline of moisture content was detected within TGase concentration of 3000–6000 ppm, it was likely that hydrolysis of glutamine residues was promoted by TGase, Fig. 1 revealed the mechanism that either ϵ -(γ -glutamyl) lysyl hydrolysed into glutamate and lysine, or the glutamyl residue hydrolysed directly into glutamate and consumed the water, these results were in agreement with the research report by Yasir et al. (2007). Moreover, compared with traditional soybean curd, WSC samples exhibited a lower moisture contents. This result was hard to explain because okara has high water retention capacities (Achouri et al. 2008), which might be due to the different formation mechanism of WSC.

In a curd system, water distribution state could be typically divided into two categories, expressible state (water held in the hole of network) or in another case entrapped state (water bound to a functional group). As shown in Table 1, content of expressible water for the control was 36.56% and it was significantly decreased to 30.45% at 1000 ppm concentration and then dropped to a minimum (24.94%) at 6000 ppm group. Correspondingly, content of entrapped water presented an opposite trend as the TGase concentration increased, and the peak value was 51.69% at 6000 ppm group. Obviously, TGase modified the distribution state of water; moreover, percentage of entrapped water of traditional soybean curd was higher than that of WSC. It was likely that the presence of fiber in WSC weakened the structure of protein network (Liu et al. 2013; Cruz et al. 2009), thus, as for WSC, more moisture could be separated by centrifugation.

Generally, syneresis was defined as the water expelled or excreted from soybean curd during the storage process. As shown in Table 1, syneresis of the control group was 3.70% and the value sharply risen to 5.45% at 1000 ppm concentration. This was attributed to the formation of chain bridge, which was catalyzed by crosslinking effect of TGase. It was reported that the occurrence of bridging reaction led to the increase of syneresis and loss of soluble substance (Sun and Breene 1991). Moreover, compared with traditional soybean curd, without filtration step, solid

Table 1 The moisture content, water distribution state, syneresis, cooking loss, and WHC of soybean curd prepared with varied concentrations of TGase**

Concentration (ppm)	Moisture content (%) [*]	Expressible water (%) [*]	Entrapped water (%) [*]	Syneresis (%) [*]	Cooking loss (%) [*]	WHC (%) [*]
Traditional	83.48 ± 0.97 ^g	28.37 ± 0.88 ^b	55.11 ± 0.87 ^f	3.96 ± 0.12 ^{ab}	14.17 ± 0.71 ^d	72.79 ± 0.88 ^b
Control	73.62 ± 0.62 ^a	36.56 ± 0.67 ^f	37.06 ± 1.28 ^a	3.70 ± 0.07 ^a	18.56 ± 0.44 ^e	67.98 ± 0.61 ^a
500	75.87 ± 0.21 ^c	34.28 ± 0.55 ^e	41.59 ± 0.68 ^b	3.61 ± 0.03 ^a	14.82 ± 0.86 ^d	72.30 ± 0.94 ^b
1000	77.54 ± 0.41 ^c	30.45 ± 0.60 ^c	47.10 ± 0.35 ^d	5.45 ± 0.25 ^b	13.55 ± 0.37 ^c	73.49 ± 0.90 ^c
1500	78.45 ± 0.40 ^f	31.65 ± 0.59 ^d	46.81 ± 0.99 ^d	5.28 ± 0.23 ^b	10.54 ± 0.41 ^b	72.41 ± 0.51 ^b
3000	75.11 ± 0.20 ^b	31.52 ± 0.59 ^{cd}	43.59 ± 0.39 ^c	5.80 ± 0.08 ^c	11.40 ± 0.43 ^b	72.64 ± 0.38 ^b
6000	76.63 ± 0.41 ^d	24.94 ± 0.76 ^a	51.69 ± 1.16 ^e	5.15 ± 0.22 ^b	8.54 ± 0.37 ^a	76.38 ± 0.39 ^d

*Values are expressed in the form of mean ± standard deviation (SD) (n = 3)

**WHC, water holding capacity; TGase, transglutaminase

^{a–g}Letters within same list represent significant difference between groups by Duncan's multiple range test ($p < 0.05$)

Fig. 1 Hydrolysis mechanism of glutamyl residue of the protein by TGase (Motoki et al. 1986) (TGase, transglutaminase)

components from okara added to soy flour suspension might contribute to reduce syneresis of soybean curd as well (Liu et al. 2013).

Analysis of WHC and cooking loss of TGase-treated WSC

During conventional cooking process, soybean curd is not consumed directly, but will undergo cooking, such as boiling and frying. Cooking loss reflects the weight loss during cooking process, which is an essential indicator to evaluate the reprocessing ability of curd. WHC reflected the water-binding capacity of curd sample. Thus, these two indicators were discussed together because cooking loss mainly consisted of water and soluble components that was relevant to WHC.

The cooking loss of traditional soybean curd and WSC incubated with 0–6000 ppm TGase was investigated. As shown in Table 1, cooking loss of traditional soybean curd and the control group were 14.17% and 18.56%, respectively. With okara and absence of TGase, the structure of

control group was more likely to be destroyed and the curd would melt in boiling water partly. Addition of TGase effectively reduced cooking loss and improved WHC of WSC prepared with $CaCl_2$. Firstly, TGase promoted the formation of intermolecular crosslinks between soy protein molecules. Secondly, it could be related to the change of water distribution as we discussed above. Entrapped state water had better water binding capacity, which was less likely to drain during cooking.

Previous study reported that the addition of TGase could suppress retorted-induced water release of soybean curd incubated with glucono- δ -lactone (Kwan and Easa 2003). Moreover, Nonaka et al. (1996) found that the content of ϵ -(γ -glutamyl)-lysine in tofu incubated with TGase increased, accompanying with the polymerization of soybean proteins.

Textural properties of soybean curd

As a critical indicator of tofu quality, textural analysis possesses an important role in consumer acceptability and

quality evaluation (Mujoo et al. 2003). To investigate the effect of addition of TGase on textural properties of WSC, we designed two parts of textural experiments, which simulated texture changes of curd during chewing process. The first part was a bloom value test, according to the standard of Gelatin Manufacturers Institute of America (GMIA 2013). Bloom value represents the force value of the probe at 4 mm extrusion distance. Rupture strength reflects the force when biting and rupture distance indicates the distance when the sample breaks.

The bloom values of WSC prepared with CaCl_2 and TGase are summarized in Table 2. Obviously, as the presence of okara fibers, the formation of the control group's structure was weakened, reflecting in decreased bloom value, rupture strength, and rupture distance. Compared with the control group, WSC exhibited a positively concentration-dependent bloom value with TGase and presented two stages of growth. In the first stage, the increase of bloom value mainly concentrated within the range of 500–1000 ppm, which was attributed to the polymerization promoted by TGase, since a curd's firmness was connected with crosslinking degree. However, bloom value declined to 206.14 g at 1500 ppm group. Excessive crosslinking degree inhibited the formation of curd network, and similar results were reported in gel system (Guo

et al. 2013). In the second stage (range within 1500–6000 ppm), bloom value increased gradually and peaked in 6000 ppm group (409.41 g). While WSC become tough and inelastic, which might be caused by the aggregation of aggregated groups, resulting in the formation of a group with larger particle. Similar two stages of growth was presented in rupture strength, but rupture distance exhibited irregular change. During cooking process, loss of water and soluble ingredients was often accompanied by changes in color, sensory, as well as texture. In order to investigate the reprocessing performance of curd after cooking, bloom value test was carried out. For WSC sample added certain concentration of TGase, without exception, bloom value and rupture strength increased significantly.

TPA test, a twice-compression experiment, which simulated textural changes of curd during chewing process. The hardness of curd was an indicator for its resistance to destructive force. As shown in Table 3, hardness, the force value necessary to attain a given distance, increased from 304.21 g in the control to 348.92 g in 500 ppm, 355.55 g in 1000 ppm, 372.79 g in 1500 ppm, 390.11 g in 3000 ppm, and a sharp increase of hardness was presented as the amount increased to 6000 ppm (687.78 g). The texture of curd became tough and it was unacceptable as

Table 2 Changes of bloom value, rupture strength, and rupture distance of soybean curd prepared with varied concentrations of TGase before and after cooking process

Treatment	Concentration (ppm)	Bloom value (g)	Rupture strength (g)	Rupture distance (mm)
Before cooking	Traditional	*237.46 ± 3.28 ^c	*258.95 ± 3.12 ^c	**4.28 ± 0.08 ^b
	Control	*198.40 ± 5.00 ^a	*202.98 ± 3.51 ^a	**4.01 ± 0.11 ^a
	500	*228.10 ± 2.56 ^b	*247.53 ± 4.12 ^b	**4.39 ± 0.16 ^c
	1000	*248.40 ± 1.62 ^d	*268.22 ± 2.55 ^d	*4.03 ± 0.06 ^a
	1500	*206.14 ± 5.36 ^a	*203.90 ± 1.85 ^a	*4.15 ± 0.06 ^{ab}
	3000	*258.75 ± 6.76 ^c	*275.55 ± 5.30 ^c	*4.47 ± 0.05 ^c
	6000	*409.41 ± 2.81 ^f	*436.68 ± 11.81 ^f	*4.20 ± 0.02 ^b
After cooking	Traditional	**391.12 ± 4.96 ^c	**424.19 ± 3.19 ^c	*4.03 ± 0.16 ^b
	Control	**303.18 ± 6.69 ^a	**317.14 ± 4.09 ^a	*3.64 ± 0.09 ^a
	500	**378.13 ± 4.01 ^b	**406.18 ± 6.10 ^b	*3.96 ± 0.08 ^b
	1000	**420.57 ± 5.12 ^d	**444.21 ± 7.52 ^d	**4.32 ± 0.07 ^d
	1500	**436.03 ± 9.94 ^c	**470.44 ± 7.41 ^c	**4.92 ± 0.07 ^c
	3000	**524.39 ± 5.17 ^f	**520.48 ± 5.26 ^f	*4.41 ± 0.05 ^d
	6000	**753.16 ± 5.60 ^g	**768.85 ± 2.57 ^g	*4.16 ± 0.05 ^c

*Values are expressed in the form of mean ± standard deviation (SD) (n = 3)

**Asterisk represents significant difference by Duncan's multiple range test ($p < 0.05$) between the group added same concentrations of TGase before and after cooking process

^{a–g}Letters within same list represent significant difference between groups by Duncan's multiple range test ($p < 0.05$)

Table 3 The TPA test of soybean curd prepared with varied concentrations of TGase before and after cooking process

Treatment	Concentration (ppm)	Hardness (g)	Springiness (g)	Chewiness (g × mm)	Cohesiveness (–)
Before cooking	Traditional	*356.77 ± 5.12 ^{bc}	**1.13 ± 0.01 ^d	*291.38 ± 4.38 ^c	**0.98 ± 0.01 ^e
	Control	*304.21 ± 6.03 ^a	**0.85 ± 0.01 ^a	*194.26 ± 4.45 ^a	*0.74 ± 0.01 ^a
	500	*348.92 ± 4.39 ^b	**0.99 ± 0.03 ^c	*286.19 ± 5.39 ^c	*0.83 ± 0.01 ^{bc}
	1000	*355.55 ± 6.42 ^b	**0.97 ± 0.01 ^{bc}	*287.20 ± 5.33 ^c	*0.84 ± 0.01 ^c
	1500	*372.79 ± 3.36 ^c	**0.87 ± 0.01 ^a	*269.34 ± 3.27 ^b	*0.82 ± 0.01 ^b
	3000	*390.11 ± 1.77 ^d	**0.96 ± 0.01 ^b	*316.16 ± 10.45 ^d	**0.83 ± 0.01 ^{bc}
	6000	*687.78 ± 5.24 ^e	**0.96 ± 0.01 ^b	*576.34 ± 3.34 ^e	**0.88 ± 0.01 ^d
After cooking	Traditional	**813.46 ± 1.57 ^d	*0.92 ± 0.01 ^d	**518.45 ± 3.57 ^c	*0.86 ± 0.01 ^c
	Control	**776.21 ± 3.57 ^b	*0.80 ± 0.02 ^a	**444.59 ± 6.10 ^a	*0.73 ± 0.01 ^a
	500	**792.55 ± 2.37 ^c	*0.85 ± 0.01 ^b	**549.80 ± 3.73 ^d	*0.81 ± 0.02 ^b
	1000	**829.40 ± 3.92 ^c	*0.85 ± 0.02 ^b	**506.67 ± 4.61 ^b	*0.73 ± 0.01 ^a
	1500	**839.75 ± 2.67 ^f	*0.85 ± 0.02 ^b	**568.37 ± 5.04 ^e	*0.82 ± 0.02 ^b
	3000	**759.07 ± 3.87 ^a	*0.86 ± 0.01 ^{bc}	**516.31 ± 3.97 ^c	*0.80 ± 0.02 ^b
	6000	**868.27 ± 4.47 ^g	*0.88 ± 0.01 ^c	**601.70 ± 4.22 ^f	*0.79 ± 0.03 ^b

*Values are expressed in the form of mean ± standard deviation (SD) (n = 3)

**Asterisk represents significant difference by Duncan's multiple range test ($p < 0.05$) between the group added same concentrations of TGase before and after cooking process

^{a–g}Letters within same list represent significant difference between groups by Duncan's multiple range test ($p < 0.05$)

curd to some extent. The high amounts of entrapped water and improved bind intensity, loss of ingredients in cooking process were the main reasons for changes of hardness. Besides, Springiness, defining as the distance where the sample bounces at the first compression, was closely connected with chewiness, because higher the value of springiness, more energy would be needed when chewing. By comparison with the control group, springiness and chewiness in the experimental group were upgraded in different degrees. But after cooking, springiness decreased while changes of chewiness exhibited opposite trends. Cohesiveness was an indicator of binding intensity between groups inside the curd. The internal gel bonds of WSC was generally weakened compared with traditional soybean curd. In TPA model, cohesiveness represented the ability to resist deformation at a second compression. The value of cohesiveness in control group was 0.74 and it was significantly increased to 0.83, 0.84, 0.82, 0.83 and 0.88 in experimental groups (within range of 500–6000 ppm), respectively. Moreover, after cooking, with the weakening of binding intensity between groups in curd system, the value of cohesiveness kept declining. Overall, addition of TGase increased hardness, springiness, chewiness, and cohesiveness, but excessive amount of TGase led to tough texture.

Fig. 2 Evaluation of the sensory attributes of soybean curd (Traditional: traditional soybean curd; Control: whole soybean curd, without addition of TGase; 500–6000 ppm: whole soybean curd with addition of TGase)

Sensory evaluation

Sensory evaluation of traditional soybean curd and TGase-treated WSC were compared and the scores are exhibited in

Fig. 2. Overall, some sensory attributes including color and appearance showed highest values for traditional soybean curd. The lowest score of color, appearance, taste, hardness, and springiness in control group might be due to the original structure that was weakened by okara fibers and not cross-linked by TGase. With addition of TGase, the springiness score of WSC was improved, which was in good coincidence with the results of bloom value and springiness (Table 3). The network structure of TGase-treated soybean curd was filled with cross-linked proteins and exhibited greater gel strength. However, excessive amount (6000 ppm) of TGase increased the hardness but reduced the taste and springiness of WSC, which made WSC tough and it was unacceptable as curd to some extent. For overall acceptability, the score of 1500 ppm group was highest. Therefore, from the view of sensory evaluation, 1500 ppm addition of TGase was most acceptable for WSC.

Appearance and microstructure of soybean curd

The surface appearance and scanning electron micrographs of WSC are shown in Fig. 3A, the control group (a) showed a rough and rigid appearance. With the increase of TGase level, the appearance transformed to a denser and more homogeneously state and best appearance was detected in 6000 ppm group. Changes of surface

appearance after cooking were presented in Fig. 3B, with the loss of water and soluble ingredients, the surface of the control group has an apparent shrinkage. In the experimental group, obviously, the appearances became smoother and more compact.

From SEM analysis in Fig. 3C, unlike the honey-comb structure of okara-filtered curd (Guo et al. 2018), the microstructure of the control group was irregular and a porous structure was detected in control group. This was likely that okara fibers affected the formation of a fine network, thus making it difficult to trap water and other soluble materials into the gel network and possibly explaining the inferior texture and WHC of soybean curd untreated with TGase (Guo et al. 2018). With the proceeding of heating, binding intensity among groups in WSC was weakened by cooking process, resulting in the weight loss and shrinkage of soybean curd, as shown in Fig. 3C-(n), the porous structure disappeared and the network became tight. Correspondingly, with addition of TGase in the group of 500 ppm [Fig. 3C-(o)], binding intensity among groups was strengthened and the curd network became uniformly formed by okara and soybean protein, no significant changes in microstructure were detected after cooking. There were no significant differences observed in other groups and electron micrographs were placed in the “Appendix”.

Fig. 3 The appearance and scanning electron micrographs of WSC incubated with varied amount of TGase [A freshly-prepared WSC, a control group, b 500 ppm, c 1000 ppm, d 1500 ppm, e 3000 ppm, f 6000 ppm. B cooked WSC, g control group, h 500 ppm, i 1000 ppm, j 1500 ppm, k 3000 ppm, l 6000 ppm. C scanning

electron micrographs of WSC, (m) freshly-prepared WSC, control group, (n) cooked WSC, control group, (o) freshly-prepared WSC, 500 ppm, (p) cooked WSC, 500 ppm; TGase, transglutaminase; WSC, whole soybean curd]

Fig. 4 SDS-PAGE profiles and band intensity of protein subunits extracted from freshly-prepared WSC (a) refrigerated WSC (b) and boiled WSC (c) incubated with varying amount of TGase (M: marker, S: protein extracted soy powder, 0 control group, 1 500 ppm, 2

1000 ppm, 3 1500 ppm, 4 3000 ppm, 5 6000 ppm; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TGase, transglutaminase; WSC, whole soybean curd)

SDS-PAGE analysis of proteins extracted from fresh-prepared, stored, and boiled WSC

Since the impact of TGase on quality of WSC was concentration-dependent, we expected to figure out that whether these conditions were accompanied with composition changes in protein subunits. Proteins extracted from fresh-

prepared WSC incubated with various amounts of TGase were analyzed by SDS-PAGE. As shown in Fig. 4a, 7S α' , 7S α , 7S β , 11SA3, 11S acid, and basic protein subunits were separated. Band intensities of 7S α' , 7S α , 7S β , 11SA3, and 11S basic protein subunits of the control group (0 ppm) was 123.52 A.U, 142.07A.U, 157.39A.U, and 252.93 A.U, which increased to 240.13 A.U to 141.36 A.U, 179.78 A.U,

180.79 A.U, 311.96 A.U, and 262.71 A.U at 500 ppm, respectively. These changes indicated that 500 ppm of TGase might retard the crosslinking rate of above protein subunit and improved their content. Moreover, band intensities of 7S α' , 7S α , 7S β , 11SA3, 11S acid, and basic protein subunits declined as the concentration of TGase increased, which was attributed to the reason that these subunits were suitable substrate of TGase (Hsieh et al. 2014). Polymerization of 7S and 11S by TGase into higher molecular weight proteins was observed as well, which was demonstrated by increased protein intensity on the top of 3000 ppm and 6000 ppm SDS-PAGE profiles. Similar results were reported by Hsieh et al. (2014). Furthermore, band intensity of 7S β subunits significantly reduced at 3000 ppm group while the sharp decline of 11SA3 subunit was detected at 1500 ppm. This result was related to crosslinking reaction sequence, which has been suggested that the TGase-mediated polymerization in soymilk was a three-step reaction. TGase catalyzed crosslinking of 11SA3 was at the first step, since 11SA3 was more reactive in comparison with 7S subunits and other 11S subunits. The second step involved the crosslinking of 7S α' , 7S α , and 7S β and the last step involved intermolecular crosslinking of 7S α' , 7S α , and 7S β subunits as well as the 11S A1a, 11S A1b, 11SA2, 11SB1a, 11SB1b, and 11SB3 subunits.

Generally, TGase stayed active within the temperature range of 40–70 °C. To study whether the enzyme was active and catalyzed crosslinking during refrigerated (8 °C) condition and cooking progress, proteins extracted from refrigerated and cooked WSC incubated with various amount of TGase were analyzed. As shown in Fig. 4b, after 12 h of storage at 8 °C, band intensities of 7S α' , 7S α , 7S β , 11SA3, 11S acid, and basic protein subunits in 500 ppm declined to 118.92 A.U, 113.61 A.U, 164.93 A.U, 252.89 A.U, and 154.56 A.U. Similar results were detected in other experimental groups. Therefore, it was obvious that the crosslinking reaction was still in progress during storage at 8 °C. After cooking, as shown in Fig. 4c, band intensities of above subunits kept falling.

Conclusion

Compound coagulants consisting of CaCl₂ and TGase were utilized to investigate the effect of TGase on moisture distributions, textural and cooking properties of WSC.

As a result, addition of TGase promoted the water-binding capacity and improved moisture content. In addition, water distribution state in WSC was modified from expressible state to entrapped state as the concentration of TGase increased. Moreover, addition of TGase also elevated the reprocessing ability of WSC, as indicated by reduced cooking loss and improved WHC. TGase amount affected textural properties of WSC as well. Bloom value of TGase-treated WSC was more comparable; however, excessive mount (6000 ppm) of TGase made WSC tough. TPA test suggested that hardness, springiness, and chewiness were significantly strengthened by TGase. Appearance changes were detected as well, with the increase of TGase amount, the appearance transformed to a dense and homogeneously state. Furthermore, TGase contributed to form a smoother appearance after cooking. The results of sensory evaluation test indicated that WSC untreated with TGase got lowest score, which might be due to the original structure that was weakened by okara fibers and not cross-linked by TGase. With addition of TGase, the springiness score of WSC was improved and in good agreement with textural results, from overall acceptability, 1500 ppm addition of TGase was most acceptable for WSC. The microstructure of WSC became denser and more homogeneously compared with the control group. Finally, SDS-PAGE analysis revealed that cross linking was still in progress in refrigerated condition and cooking process, 7S α' , 7S α , 7S β , 11SA3, 11S acid, and basic protein subunits were suitable substrates of TGase and 500 ppm of TGase might retard the cross-link rate of protein subunits.

In summary, addition of TGase was an effective way to improve the textural and sensory properties of WSC, reflecting in reduced cooking loss and improved WHC. Quality changes of WSC was accompanied by composition changes of protein subunits and microstructure as well.

Acknowledgements We are grateful for the soybean material assistant from Nanchong Academy of Agricultural Science. We also thank Masters Menglu Yang and Yuan Qu for guidance on SDS-PAGE analysis.

Appendix

See Fig. 5.

Fig. 5 The scanning electron micrographs of WSC samples made from varied amounts of TGase with cooking process at 500 × magnification (a 1000 ppm, b 1000 ppm, after cooking, c 1500 ppm, d 1500 ppm, after cooking, e 3000 ppm, f 3000 ppm, after cooking, g 6000 ppm, h 6000 ppm, after cooking)

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