Food Chemistry 167 (2015) 168–174

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Preparation of organic tofu using organic compatible magnesium chloride incorporated with polysaccharide coagulants

Meng Li [a], Fusheng Chen [a], Bao Yang [b], Shaojuan Lai [a], Hongshun Yang [c,d,*], Kunlun Liu [a], Guanhao Bu [a], Caili Fu [c,d], Yun Deng [e]

[a] College of Food Science and Technology, Henan University of Technology, Zhengzhou, Henan 450052, China
[b] Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China
[c] Food Science and Technology Programme, Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore
[d] National University of Singapore (Suzhou) Research Institute, 377 Lin Quan Street, Suzhou Industrial Park, Suzhou, Jiangsu 215123, China
[e] Department of Food Science and Technology, College of Agriculture & Biology, Shanghai Jiao Tong University, 800 Dongchuan Rd., Minhang, Shanghai 200030, China

ARTICLE INFO

Article history:
Received 5 February 2014
Received in revised form 30 April 2014
Accepted 25 June 2014
Available online 4 July 2014

Keywords:
Organic tofu
Gypsum
Coagulant
Magnesium chloride
Guar gum

ABSTRACT

Organic tofu using organic compatible coagulants of magnesium chloride and three polysaccharides including carrageenan, guar gum and gum Arabic were generated. For $MgCl_2$ coagulated tofu, carrageenan significantly increased the hardness from 969.5 g to 1210.5 g whereas guar gum (0.6 g) decreased the hardness to 505.5 g. Interestingly, gypsum and guar gum (0.6 g) increased the yield of tofu significantly. These organic compatible coagulants didn't affect most of 7S and 11S protein subunits. Importantly, the overall-acceptability of organic tofu prepared with $MgCl_2$ combined with guar gum or gypsum was almost the same as conventional tofu made with gymsum while having more beany-flavour. Among these organic coagulants, tofu made from 0.6 g guar gum and $MgCl_2$ mixture was the most similar to that coagulated by conventional gypsum. Thus this mixture is promising as coagulant for making organic tofu.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Organic food is "food guaranteed to have been produced, stored and processed without the addition of synthetically produced fertilisers and chemicals" (Lockie, Lyons, Lawrence, & Mummery, 2002). Recently, consumption of organic food has increased rapidly worldwide, especially in developed countries. For instance, organic sales in the U.K. is more than ten times that of ten years ago (Jiwan, Duane, O'Sullivan, O'Brien, & Aherne, 2010). In the U.S. an increased number of consumers choose to consume organic foods instead of conventional foods. In 2009, approximately 4% of food retail sales in the U.S. were organic foods which accounted for nearly half of organic food sales globally (Nie & Zepeda, 2011). In 2011, worldwide organic food sales was approximately $\$6.3 \times 10^{10}$ while U.S. alone reached $\$2.9 \times 10^{10}$ (Weedsnetwork. Global organic food, 2013).

The current trend shows more and more organic foods are demanded; however, the rigid organic food regulations and the strict certification restrict many products being considered as "organic food". Take the U.S. for instance, the National List of Allowed and Prohibited Substances excludes many popular foods being considered as organic once the foods are made with some additives which are not on the allowed list.

Tofu, originated from China, is gaining increasing popularity throughout the world. Being a valuable protein source compared to meat, fish and cheese, especially important to Asian people and vegetarian people. Furthermore, tofu is cholesterol-free and has a less amount of saturated fat compared to animal source proteins like meat and milk (Liu, 1997). It even has health-promoting functions such as lowering the incidence of many types of cancers (Messina, Persky, Setchell, & Barnes, 1994). Recently, application of $MgCl_2$ as a coagulant in tofu attracted much attention (Hsieh, Yu, & Tsai, 2012; Li, Cheng, Tatsumi, Saito, & Yin, 2014; Nagano & Tokita, 2011; Toda, Nakamura, Takahasi, & Komaki, 2009; Toda et al., 2003). Findings indicate that $MgCl_2$ creates a more natural flavour for tofu and allows the taste of soybean to be retained, which makes $MgCl_2$ one of the most popular tofu coagulants. Moreover, consumers prefer $MgCl_2$ coagulated tofu because it retains the original sweet taste of the soybeans, and maintains the quality of soybean oil (Li et al., 2014). However, $MgCl_2$ is a quick-acting coagulant. Tofu coagulated by $MgCl_2$ solidifies more rapidly and has a lower yield, resulting in a harder and non-uniform tofu (Watanabe, 1997). The National List of Allowed and Prohibited

---

* Corresponding author. Tel.: +65 65164695; fax: +65 67757895.
E-mail address: chmynghs@nus.edu.sg (H. Yang).

http://dx.doi.org/10.1016/j.foodchem.2014.06.102
0308-8146/© 2014 Elsevier Ltd. All rights reserved.

Substances (U.S.) contains $MgCl_2$ (derived from sea water) and $CaSO_4$ (mined) as organic compatible components. The use of $MgCl_2$ in generating organic tofu has not been reported due to the above described disadvantages. While $CaSO_4$ has been used in the production of organic tofu in the U.S., tofu coagulated by $CaSO_4$ is smoother and of higher yield. Other potential organic allowed coagulants include polysaccharides like guar gum. In a protein based food system, polysaccharides play an important role in affecting the textural properties. For instance, polysaccharide carrageenan and coagulants including calcium acetate, calcium sulphate and glucono-delta-lactone (GDL) affected the yield and some other physicochemical properties of tofu (Abd Karim, Sulebele, Azhar, & Ping, 1999; Hua, Cui, & Wang, 2003). Moreover, guar gum and gum Arabic impacted on the protein gelling property via segregative interactions and electrostatic attractions (Fitzsimons, Mulvihill, & Morris, 2008; Harrington & Morris, 2009; Yang, Anvari, Pan, & Chung, 2012).

Here we investigated the possibility of producing organic tofu using organic compatible polysaccharides and $MgCl_2$ as coagulants and organic practises. Our goal is to identify a novel organic coagulant including $MgCl_2$ from the National List of Allowed and Prohibited Substances for organic foods suggested by U.S. National Organic Program. Effects of just $MgCl_2$ or $MgCl_2$ combined with three different polysaccharides (carrageenan, guar gum and gum Arabic) as tofu coagulant agents were investigated and compared with gypsum, a traditional tofu coagulant.

2. Materials and methods

2.1. Materials

Organic soybean was obtained from Beidahuang Yikang Organic Foods Co., Ltd. (Beian, Heilongjiang, China). Carrageenan was bought from Hainan Mengyuan Food Co., Ltd. (Qionghai, Hainan, China). Guar gum was obtained from Qingdao Liuhe Chemical Co., Ltd. (Qingdao, Shandong, China), and gum Arabic was from Tianjin Arthurbranwell Co., Ltd. (Tianjin, China). Magnesium chloride was from Tianjin Jinlun Salt Industry Co., Ltd. (Tianjin, China) and gypsum was from a local market (Zhengzhou, Henan, China). All these reagents were food grade or approved food grade additives in China.

2.2. Preparation of tofu

Tofu was prepared following previous methods with some modifications (Chang, Lin, & Chen, 2003; Lee & Kuo, 2011). Soybeans (100 g) were soaked in distilled water at a soybean water ratio of 1:3 (w/w) for 12 h at 25 °C. The swollen soybeans were drained and then ground with distilled water (1:8, w/w) in a soymilk grinder (Tangshan Tieshi grinder Co., Ltd., Tangshan, Hebei, China), and later filtered through 120 mesh (125 μm) gauze to collect raw soymilk (approximately 800 ml). The raw soymilk in a beaker was incubated in water bath at 95 °C for 5 min, then cooled to 80 °C, added with respective coagulant (2.93 g $MgCl_2$ only, 2.93 g $MgCl_2$ combined with one of the above-mentioned three polysaccharides (0.2 g or 0.6 g) or 2.75 g gypsum), stirred and incubated for 20 min in an 80 °C water bath. The curd was transferred to a cheesecloth placed in a 7 cm × 7 cm × 7 cm mold. The whey in the curd was removed via sequentially pressing at 10 g/cm^2 for 10 min and then 20 g/cm^2 for 40 min. The resulting tofu was stored in a refrigerator at 4 °C until the next day for analysis.

2.3. Proximate composition analyses

Proximate composition analyses were conducted using standard methods (AOAC, 2000). Total protein was assayed by the microKjeldahl method and crude fat by the Soxhlet method. Moisture of tofu was obtained by drying a certain amount of tofu sample to a constant weight at 105 °C for 24 h in an oven. Ash was assayed by AOAC method 14.006. The results were reported on a wet basis.

2.4. Yield and syneresis

Yield of tofu was recorded and expressed as weight of tofu obtained from 100 g dry soybeans. The syneresis of tofu was measured according to a previous method (Lee & Kuo, 2011). Specifically, tofu was cut into slices 15 mm each in length and width, while 5 mm in thickness. Slices (6 pieces) were weighed and placed on a stainless steel mesh which was placed in a plastic box. Small sticks were applied to lift the mesh so that the exuded liquid could be separated from the tofu samples. The box was sealed with parafilm to minimise the loss of moisture content. The sample was then stored in the box at 4 °C for 24 h. The total liquid exuded during this 24 h time period was measured. Syneresis was calculated as the weight of exuded liquid as a percentage of weight of sliced tofu sample.

2.5. Texture measurement

Texture of tofu were analysed with a TA. XT. Plus Texture Analyser (Stable Micro Systems, Goldaming, Surrey, U.K.) using a probe P35. Cylindrical samples, 2.0 cm in diameter and 2.0 cm in height were prepared and compressed to 50% deformation. The test settings were set as Pretest speed: 5.0 mm/s; Test speed: 1 mm/s; Posttest speed: 1 mm/s. Hardness was expressed as the height of the peak force on first bite, which was the force used for achieving a certain deformation. Hardness, springiness, cohesiveness and gumminess of each tofu sample was determined from the texture profile analysis (TPA) curve as described previously (Bourne, 2002; Yang et al., 2007).

2.6. Colour measurement

The colour of tofu, expressed in Hunter *L* (lightness), red–green (+a or −a) and yellow–blue (+b or −b) values according to the CIE definition, was measured using a CR-400 chromametre (KonikaMinolta, Tokyo, Japan). A standard white plate with $L = 88.33$, $a = 3.95$, $b = 0.67$ was applied for calibration. The colour differences among the samples were calculated as $\Delta E = [(\Delta L)^2+(\Delta a)^2+(\Delta b)^2]^{0.5}$ (Rufián-Henares, Guerra-Hernandez, & García-Villanova, 2006).

2.7. Protein subunit analysis with sodium dodecyl suphate-polyacrylamide gel electrophoresis (SDS–PAGE)

Extraction of crude protein from tofu was performed according to a previous method (Cai & Chang, 1999). After protein content was analysed using the Bradford method (Bradford, 1976), protein extract was modified to 2 mg/mL using distilled water. SDS–PAGE was carried out in a vertical electrophoresis unit (DYY-6D, Beijing Liuyi Instrument Factory, Beijing, China). Concentrations of stacking gel and separating gel were 4% (w/v) and 12% (w/v), respectively. A broad range standard molecular weight marker was applied in the gel which included rabbit phosphorylase B (97.4 kDa), bovine serum albumin (66.2 kDa), rabbit actin (43 kDa), bovine carbonic anhydrase (31 kDa), human growth hormone (22 kDa) and hen egg white lysozyme (14.4 kDa). Protein bands in the gel were stained using coomassie brilliant blue G-250 and were recorded by UNIS scanner (UNIS A688, Beijing, China). Bandscan 5.0 (Glyko, Hayward, CA, USA) was applied to quantitatively analyse the relative ratio of protein ingredients in the protein samples.

2.8. Amino acid profile

The amino acid profile of the tofu samples was tested in the Agricultural Products Quality Supervision and Testing centre, Ministry of Agriculture (Zhengzhou, Henan, China) using an Amino acid analyser (L-8800 system, Hitachi, Tokyo, Japan). Analytical 2622# (4.6 mm × 60 mm) and guard 2650# (4.6 mm × 40 mm) columns were utilised for determining free amino acids. Right after injection into the columns, an auto-sampler was used for the inline-derivatisation by Ninhydrin (NIN) post-column derivatisation. The NIN-derivatised amino acids were assayed at 570 nm and 440 nm.

2.9. Fatty acid composition

Fatty acid compositions as methyl esters were analysed by gas chromatography (GC). The method described by Fontecha, Rıos, Lozada, Fraga, and Juárez (2000) was used for the determination of the methyl esters. Tofu fat (0.1 g) was added into the mixture of 1 ml hexane and 0.05 ml methanol containing of 2 M potassium hydroxide. A volume of 0.5 µl was injected into a GC apparatus (GC-2010, Shimadzu, Kyoto, Japan). The various compounds were separated by the carbon number and compared with the methyl ester of standard fatty acids.

2.10. Sensory evaluation

Twenty panellists (10 male and 10 female, aged between 20 and 35) were trained and asked to recognise and score the sensory attributes of the tofu samples. The attributes selected as appropriate indexes for indicating the tofu quality were mouth-feel, firmness, beany-flavour, colour and overall acceptability. The results were recorded using a 5-point scale (1 point indicates very poor (weak) while 5 point denotes very good (strong)) for each attribute. Fresh and steamed tofu samples were included in the evaluation. The steamed tofu was made from fresh tofu steamed for 10 min in a pan (Adams, 2012). All samples were cut into cubes (1.5 cm × 1.5 cm × 1.5 cm), coded and evaluated using a randomised design.

2.11. Data analysis

All experiments were performed in triplicate. Statistical analyses using analysis of variance (ANOVA) and Duncan's multiple range test for differences among different tofu groups were performed with SAS software (Version 9.1.3, SAS, Cary, NC, USA). Comparisons that yielded $P < 0.05$ were considered significant.

3. Results and discussion

3.1. Proximate composition and yield

The proximate composition, yield, syneresis and colour of tofu made with different coagulants are shown in Table 1. When tofu was made with carrageenan and gum Arabic, it had a protein content of 13% and a moisture content of approximately 77%, which were comparable to those of $MgCl_2$ coagulated tofu. However, with the addition of 0.6 g guar gum, the protein content of tofu decreased to 12.33%, which was significantly lower than $MgCl_2$ coagulated tofu, although the moisture content remained similar. Interestingly, among the different tofu types, gypsum coagulated tofu had the lowest amount of protein content, crude fat and ash, while the highest moisture content.

Gypsum yielded more tofu than $MgCl_2$ (211.27 g vs. 173.47 g) (Table 1). The discrepancy may be due to the different coagulation rates of the two coagulants. It was reported that chloride

Table 1
Proximate composition, yield, syneresis, colour and texture of tofu made with different coagulants.

	Coagulant							
	2.93 g $MgCl_2$	2.93 g $MgCl_2$		2.93 g $MgCl_2$		2.93 g $MgCl_2$		2.75 g Gypsum
		0.2 g Carrageenan	0.6 g Carrageenan	0.2 g Guar gum	0.6 g Guar gum	0.2 g Gum Arabic	0.6 g Gum Arabic	
Protein (%)	13.72 ± 0.29^{a}	13.40 ± 0.55^{a}	13.32 ± 0.43^{a}	13.16 ± 0.24^{a}	12.33 ± 0.54^{b}	13.33 ± 0.41^{a}	13.39 ± 0.40^{a}	11.97 ± 0.42^{b}
Crude fat (%)	4.66 ± 0.24^{a}	4.76 ± 0.12^{a}	4.87 ± 0.23^{a}	4.50 ± 0.21^{ab}	3.59 ± 0.33^{c}	4.76 ± 0.15^{a}	4.12 ± 0.18^{b}	2.92 ± 0.22^{d}
Ash (%)	1.85 ± 0.21^{a}	1.86 ± 0.23^{a}	2.08 ± 0.17^{a}	1.89 ± 0.10^{a}	1.88 ± 0.19^{a}	1.76 ± 0.20^{a}	1.93 ± 0.11^{a}	1.12 ± 0.16^{b}
Moisture (%)	77.16 ± 1.65^{bc}	77.55 ± 1.37^{bc}	77.48 ± 1.46^{bc}	78.88 ± 0.76^{b}	79.19 ± 1.04^{b}	76.10 ± 0.67^{c}	76.91 ± 1.0b^{c}	82.07 ± 1.47^{a}
Yield	173.47 ± 9.74^{b}	174.4 ± 7.76^{b}	181.67 ± 6.88^{b}	173.73 ± 5.71^{b}	199.43 ± 12.05^{a}	174.03 ± 4.68^{b}	173.83 ± 6.73^{b}	211.27 ± 11.51^{a}
Syneresis (%)	2.48 ± 0.14^{c}	1.23 ± 0.15^{e}	1.69 ± 0.17^{d}	2.50 ± 0.18^{c}	5.43 ± 0.27^{a}	3.18 ± 0.29^{b}	3.41 ± 0.28^{b}	3.27 ± 0.29^{b}
L	77.70 ± 1.02^{a}	79.99 ± 2.47^{a}	79.03 ± 2.31^{a}	77.71 ± 0.73^{a}	77.91 ± 0.83^{a}	78.83 ± 1.85^{a}	79.59 ± 2.40^{a}	78.2 ± 1.47^{a}
a	2.01 ± 0.05^{a}	1.88 ± 0.05^{b}	2.07 ± 0.04^{a}	1.54 ± 0.07^{c}	1.55 ± 0.06^{c}	1.50 ± 0.06^{c}	1.55 ± 0.09^{c}	2.00 ± 0.05^{a}
b	9.95 ± 0.14^{c}	11.99 ± 0.74^{a}	11.92 ± 0.68^{a}	10.46 ± 0.63^{bc}	10.23 ± 0.59^{bc}	10.45 ± 0.76^{bc}	11.25 ± 0.64^{ab}	10.47 ± 0.34^{bc}
Hue angel(°)	1.37 ± 0.006^{a}	1.42 ± 0.009^{a}	1.39 ± 0.011^{a}	1.42 ± 0.012^{a}	1.42 ± 0.003^{a}	1.43 ± 0.014^{a}	1.44 ± 0.013^{a}	1.38 ± 0.007^{a}
Hardness (g)	917.7 ± 42.9^{b}	969.5 ± 26.2^{b}	1210.5 ± 67.1^{a}	740.1 ± 33.1^{c}	505.8 ± 29.8^{d}	911.0 ± 43.8^{b}	899.7 ± 40.3^{b}	516.8 ± 35.5^{d}
Springiness (mm)	0.86 ± 0.02^{a}	0.85 ± 0.01^{a}	0.85 ± 0.01^{a}	0.85 ± 0.02^{a}	0.81 ± 0.01^{a}	0.84 ± 0.01^{a}	0.86 ± 0.02^{a}	0.82 ± 0.03^{a}
Cohesiveness	0.48 ± 0.02^{ab}	0.43 ± 0.04^{bc}	0.49 ± 0.02^{a}	0.46 ± 0.03^{ab}	0.43 ± 0.04^{bc}	0.47 ± 0.03^{ab}	0.49 ± 0.01^{a}	0.40 ± 0.03^{c}
Gumminess (g)	436.9 ± 39.9^{b}	439.6 ± 31.7^{b}	614.4 ± 28.9^{a}	336.9 ± 22.1^{c}	220.7 ± 19.7^{d}	436.3 ± 31.3^{d}	475.5 ± 36.2^{b}	217.3 ± 18.7^{d}
Chewiness (g mm)	365.9 ± 30.8^{bc}	375.3 ± 27.6^{bc}	538.8 ± 29.9^{a}	287.4 ± 23.5^{d}	173.7 ± 18.3^{e}	333.9 ± 16.28^{c}	382.5 ± 34.4^{b}	190.0 ± 21.1^{e}

Means in a same line with different letters are significantly different ($P < 0.05$). Variation of the mean represents standard deviation of triplicate for each measurement.

Table 2
Fatty acid composition of tofu made with different coagulants.

Coagulant	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
2.93 g $MgCl_2$	12.64 ± 0.07^a	5.48 ± 0.10^a	23.29 ± 0.06^{ab}	49.62 ± 0.59^a	8.16 ± 0.04^a
2.93 g $MgCl_2$ + 0.2 g Carrageenan	12.65 ± 0.05^a	5.50 ± 0.17^a	23.33 ± 0.18^{ab}	49.64 ± 0.56^a	8.15 ± 0.09^a
2.93 g $MgCl_2$ + 0.6 g Carrageenan	12.64 ± 0.06^a	5.50 ± 0.20^a	23.40 ± 0.17^{ab}	49.53 ± 0.37^a	8.12 ± 0.11^a
2.93 g $MgCl_2$ + 0.2 g Guar gum	12.93 ± 0.10^a	5.43 ± 0.14^a	23.15 ± 0.15^b	49.42 ± 0.36^a	8.14 ± 0.05^a
2.93 g $MgCl_2$ + 0.6 g Guar gum	12.77 ± 0.30^a	5.50 ± 0.19^a	23.42 ± 0.02^{ab}	49.44 ± 0.28^a	8.08 ± 0.02^a
2.93 g $MgCl_2$ + 0.2 g Gum Arabic	12.75 ± 0.28^a	5.58 ± 0.09^a	23.43 ± 0.15^{ab}	49.36 ± 0.33^a	8.06 ± 0.06^a
2.93 g $MgCl_2$ + 0.6 g Gum Arabic	12.72 ± 0.16^a	5.32 ± 0.34^a	23.48 ± 0.12^{ab}	49.48 ± 0.45^a	8.10 ± 0.07^a
2.75 g Gypsum	13.51 ± 0.13^a	5.59 ± 0.07^a	23.60 ± 0.35^a	49.54 ± 0.51^a	8.13 ± 0.12^a

Means in a same column with different letters are significantly different ($P < 0.05$). Variation of the mean represents standard deviation of triplicate for each measurement.

salt ($MgCl_2$) had a faster rate in coagulating soybean proteins than sulphate salt. Gypsum is a slow acting coagulant, which may explain why gypsum yielded more tofu (Prabhakaran, Perera, & Valiyaveettil, 2006). Previous reports indicated that no significant correlations were found between the content of tofu protein and tofu yield when only one coagulant was applied (Cai, Chang, Shih, Hou, & Ji, 1997). In the current study, soy protein was coagulated with different coagulants or more than one coagulant. The resulted tofu was of low protein content but high yield, suggesting that different coagulants had different coagulation rates. Slower aggregating rates may lead to the formation of a more homogeneous structure of soy protein and more water trapped in tofu (Prabhakaran et al., 2006), which is supported by the high amount of moisture content in gypsum group (slow coagulant group). Low protein content of tofu indicated a slow coagulating process, which made soy protein more efficient to aggregate into tofu, and increased tofu yield. On the contrast, the tofu protein content and tofu yield made by $MgCl_2$ and 0.6 g guar gum was similar to those made by gypsum. The reason could be that guar gum is highly viscous even at low concentrations. With the addition of guar gum, the soymilk became highly viscous, thus having a reduced coagulation rate. Although addition of 0.6 g gum Arabic decreased the content of crude fat significantly, fatty acid composition of crud fat in tofu was pretty stable. The fatty acid composition in different tofu samples is shown in Table 2.

3.2. Syneresis and colour

Generally, syneresis is pertinent to cross-linking among protein molecules (Abd Karim et al., 1999; Lee & Kuo, 2011; Noh, Park, Pak, Hong, & Yun, 2005). Here, the results demonstrated that all of the three polysaccharides affected syneresis in tofu in a greater extent than tofu coagulated by $MgCl_2$. With the addition of 0.2 g carrageenan, syneresis was reduced by 1.25%, which could be caused by the extensive network structure developed by carrageenan. As a consequence more water was trapped in interstitial parts of the tofu gel (Abd Karim et al., 1999). Interestingly, with 0.6 g carrageen, tofu syneresis increased (Table 1). It was postulated that as the concentration of carrageenan increased, quicker gel formation was initiated, leading to a coarse net structure and less water being retained (Harrington, Foegeding, Mulvihill, & Morris, 2009). For polysaccharides which have no gelling effects, guar gum and gum Arabic only affected the coagulation rate of tofu; however, both of them increased the syneresis. The increased syneresis of protein gel was suggested to be caused by the increased cross-linking among protein molecules through various interactions, which allowed the exuded water to be trapped within the gel (Lee & Kuo, 2011). Increase of guar gum to 0.6 g also increased the syneresis to 5.43%. While this effect may be due to the increased protein aggregation by guar gum (Fitzsimons et al., 2008), the effect of gum Arabic on tofu may be related to the fact that gum Arabic molecules aggregate themselves and become compact by means of a draining effect (Li et al., 2009).

In this current study, all of the tofu products were light yellow (Table 1), suggesting the quality of the tofu was good, as good quality tofu is supposed to be light yellow or white in colour. The addition of carrageenan increased the value of *b* (yellowish) significantly, suggesting it may improve the quality of tofu.

3.3. Texture analyses

To understand the textural properties of tofu made by the above described coagulants, the TPA including hardness, springiness, cohesiveness, gumminess and chewiness was performed and results are presented in Table 1. As shown, the addition of 0.6 g carrageenan significantly increased the hardness to 1210.5 g, whereas guar gum decreased the hardness to 505.8 g. Increased hardness by carrageenan was suggested to be induced by carrageenan interacting with protein, resulting in increased gel strength due to the effect of segregative interactions (Arda, Kara, & Pekcan, 2009; Harrington et al., 2009). In addition, 0.6 g guar gum increased the tofu moisture content to 79.19%. Although the result was not significantly different from $MgCl_2$ only coagulated tofu, indicating that the structure of tofu with guar gum addition was less dense than $MgCl_2$ only treated tofu. Tofu coagulated with 0.6 g guar gum had a similar texture profile to tofu coagulated with gypsum which also had a slower coagulation rate than $MgCl_2$. Our results are consistent with a previous finding suggesting that it may be due to the slow coagulation rate caused guar gum. Tofu's protein network was pertinent to textural properties, the more orderly and denser the structure, the higher the values of textural properties (Noh et al., 2005).

3.4. SDS–PAGE and amino acid composition

7S and 11S are two major protein components of soybean (soymilk) protein. The 7S globulin conglycinin has three subunits: α, α′ and β which form a trimeric glycoprotein. Glycinin is an 11S haxamer globulin which contains six subunits (Natarajan, Xu, Bae, Caperna, & Garrett, 2006). Based on SDS–PAGE results, soybean seed composition is considered to be related to the yield and quality of tofu (Poysa & Woodrow, 2002; Poysa, Woodrow, & Yu, 2006). In addition, processing and concentration of coagulants also affected tofu quality (Cai & Chang, 1999; Hsieh et al., 2012; Puppo & Añón, 1998; Yasir, Sutton, Newberry, Andrews, & Gerrard, 2007a; Yasir, Sutton, Newberry, Andrews, & Gerrard, 2007b). It was suggested that the process of tofu production involves a complex interaction of multiple factors such as chemical composition, processing techniques and conditions, and physical attributes of soybean (Watanabe, 1997). In the current report, the subunits of 11S and 7S of tofu were separated on SDS–PAGE and the results are presented in Fig. 1 and Table 3. The content of β was approximately 14% while the value of the A_3 content

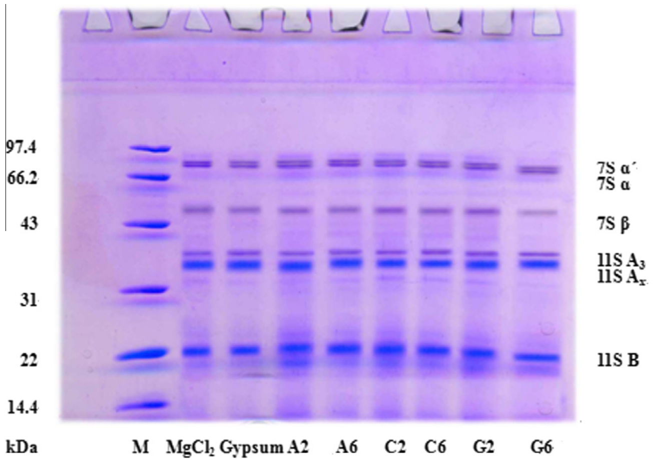

Fig. 1. SDS–PAGE electrophoresis of tofu proteins with different coagulants. M – Marker, A2 – $MgCl_2$ + 0.2 g Gum Arabic; A6 – $MgCl_2$ + 0.6 g Gum Arabic; C2 – $MgCl_2$ + 0.2 g Carrageenan; C6 – $MgCl_2$ + 0.6 g Carrageenan; G2 – $MgCl_2$ + 0.2 g Guar gum; G6 – $MgCl_2$ + 0.6 g Guar gum; Ax – A1a, A1b, A2, A4.

ranged from 9% to 13.5%, and the content of B varied from 30.95% to 42.85%. The results among different tofu groups were not significantly different indicating a variety of coagulants did not affect the composition of the 7S and 11S in tofu protein, but changed the protein network formation. Amino acid profiles of tofu using the two coagulants ($MgCl_2$ vs. $MgCl_2$ plus 0.6 g guar gum) were compared (Table 4). The results demonstrated that the basic amino acids, including Lys (0.91% vs. 0.75%), His (0.4% vs. 0.32%) and Arg (1.13% vs. 0.92%), were decreased by adding 0.6 guar gum together with $MgCl_2$, compared to $MgCl_2$ alone. The essential amino acids (Lys, Met, Leu, Ile, Val) had a similar tendency of change between the two groups. On the contrast, there were no significant differences between tofu coagulated by gypsum and that coagulated by $MgCl_2$.

3.5. Sensory characteristics

Tofu made with $MgCl_2$ plus 0.6 g guar gum had a similar texture profile to tofu prepared with gypsum, but had an increased yield compared with that produced by $MgCl_2$ only. This result made us focus on the coagulant of $MgCl_2$ for sensory analysis (Fig. 2). In the fresh tofu group, $MgCl_2$ plus 0.6 g guar gum retained a more beany-flavour for tofu than gypsum, suggesting the benefit of $MgCl_2$ remained. Firmness was decreased to a value close to that of tofu prepared with gypsum when guar was added, which was in compliance with TPA result. Colour difference and overall acceptability was similar among tofu groups. The overall acceptability was even similar among steamed tofu, tofu prepared with

Table 3
7S and 11S contents of tofu made with different coagulants.

Coagulant	7S (%)		11S (%)		
	α′ + α	β	A_3	A_x	B
2.93 g $MgCl_2$	6.6 ± 2.26^{ab}	16.3 ± 5.66^{a}	13.2 ± 3.25^{a}	32.6 ± 4.1^{a}	31.3 ± 0.57^{a}
2.93 g $MgCl_2$ + 0.2 g Carrageenan	10.2 ± 0.99^{a}	16.7 ± 1.77^{a}	11.7 ± 2.33^{a}	30.6 ± 4.38^{a}	31.0 ± 4.74^{a}
2.93 g $MgCl_2$ + 0.6 g Carrageenan	7.6 ± 1.34^{ab}	14.7 ± 0.42^{a}	14.6 ± 7.64^{a}	24.3 ± 9.05^{a}	38.8 ± 2.33^{a}
2.93 g $MgCl_2$ + 0.2 g Guar gum	6.2 ± 0.14^{ab}	14.5 ± 7.78^{a}	9.0 ± 0.14^{a}	27.4 ± 0.92^{a}	42.9 ± 6.86^{a}
2.93 g $MgCl_2$ + 0.6 g Guar gum	6.1 ± 0.21^{ab}	14.2 ± 7.14^{a}	11.9 ± 0.35^{a}	28.5 ± 3.75^{a}	39.5 ± 2.78^{a}
2.93 g $MgCl_2$ + 0.2 g Gum Arabic	7.0 ± 1.06^{ab}	16.3 ± 7.92^{a}	10.9 ± 0.49^{a}	28.3 ± 1.41^{a}	37.6 ± 10.89^{a}
2.93 g $MgCl_2$ + 0.6 g Gum Arabic	9.2 ± 1.91^{ab}	14.2 ± 3.54^{a}	13.2 ± 0.07^{a}	33.8 ± 6.65^{a}	32.2 ± 8.34^{a}
2.75 g Gypsum	5.6 ± 3.39^{b}	12.1 ± 0.57^{a}	13.5 ± 0.49^{a}	34.4 ± 1.98^{a}	34.5 ± 6.58^{a}

Ax – *A*1a, *A*1b, *A*2, *A*4.
Means in a same column with different letters are significantly different ($P < 0.05$). Variation of the mean represents standard deviation of triplicate for each measurement.

Table 4
Amino acid profile of tofu made with different coagulants.

	Coagulant							
	2.93 g $MgCl_2$	2.93 g $MgCl_2$		2.93 g $MgCl_2$		2.93 g $MgCl_2$		2.75 g Gypsum
		0.2 g Carrageenan	0.6 g Carrageenan	0.2 g guar gum	0.6 g guar gum	0.2 g Gum Arabic	0.6 g Gum Arabic	
Asp	1.58 ± 0.06^{a}	1.49 ± 0.15^{ab}	1.47 ± 0.12^{ab}	1.30 ± 0.16^{b}	1.31 ± 0.15^{b}	1.51 ± 0.08^{ab}	1.49 ± 0.19^{ab}	1.37 ± 0.05^{ab}
Thr	0.50 ± 0.02^{a}	0.48 ± 0.05^{a}	0.47 ± 0.04^{a}	0.42 ± 0.05^{a}	0.42 ± 0.05^{a}	0.49 ± 0.02^{a}	0.48 ± 0.06^{a}	0.44 ± 0.02^{a}
Ser	0.67 ± 0.03^{a}	0.62 ± 0.06^{ab}	0.62 ± 0.06^{ab}	0.54 ± 0.06^{b}	0.55 ± 0.06^{b}	0.64 ± 0.03^{ab}	0.63 ± 0.08^{ab}	0.57 ± 0.02^{ab}
Glu	2.69 ± 0.11^{a}	2.53 ± 0.25^{ab}	2.49 ± 0.21^{ab}	2.23 ± 0.27^{b}	2.22 ± 0.25^{b}	2.53 ± 0.12^{ab}	2.53 ± 0.30^{ab}	2.34 ± 0.09^{ab}
Gly	0.56 ± 0.03^{a}	0.52 ± 0.06^{ab}	0.52 ± 0.04^{ab}	0.47 ± 0.05^{b}	0.47 ± 0.06^{b}	0.54 ± 0.03^{ab}	0.53 ± 0.06^{ab}	0.48 ± 0.02^{ab}
Ala	0.58 ± 0.02^{a}	0.54 ± 0.06^{ab}	0.54 ± 0.04^{ab}	0.48 ± 0.06^{b}	0.48 ± 0.06^{b}	0.55 ± 0.02^{ab}	0.54 ± 0.07^{ab}	0.50 ± 0.02^{ab}
*Cys	0.14 ± 0.00^{ab}	0.13 ± 0.01^{ab}	0.13 ± 0.02^{ab}	0.14 ± 0.01^{ab}	0.13 ± 0.01^{b}	0.14 ± 0.00^{ab}	0.15 ± 0.01^{a}	0.13 ± 0.01^{ab}
Val	0.72 ± 0.06^{a}	0.64 ± 0.06^{ab}	0.63 ± 0.05^{ab}	0.57 ± 0.07^{b}	0.57 ± 0.06^{b}	0.64 ± 0.04^{ab}	0.63 ± 0.08^{ab}	0.60 ± 0.03^{b}
Met	0.17 ± 0.02^{a}	0.15 ± 0.02^{ab}	0.14 ± 0.01^{b}	0.13 ± 0.02^{b}	0.13 ± 0.02^{b}	0.15 ± 0.01^{ab}	0.15 ± 0.01^{ab}	0.13 ± 0.01^{b}
Ile	0.65 ± 0.03^{a}	0.61 ± 0.06^{ab}	0.61 ± 0.05^{ab}	0.54 ± 0.07^{b}	0.54 ± 0.06^{b}	0.60 ± 0.03^{ab}	0.61 ± 0.06^{ab}	0.57 ± 0.03^{ab}
Leu	1.09 ± 0.05^{a}	1.02 ± 0.11^{ab}	1.01 ± 0.08^{ab}	0.90 ± 0.11^{b}	0.90 ± 0.10^{b}	1.03 ± 0.04^{ab}	1.02 ± 0.12^{ab}	0.94 ± 0.04^{ab}
Tyr	0.53 ± 0.02^{a}	0.47 ± 0.05^{ab}	0.47 ± 0.05^{ab}	0.41 ± 0.04^{b}	0.40 ± 0.04^{b}	0.44 ± 0.02^{b}	0.46 ± 0.05^{ab}	0.45 ± 0.03^{b}
Phe	0.75 ± 0.02^{a}	0.70 ± 0.07^{a}	0.70 ± 0.05^{a}	0.64 ± 0.07^{a}	0.64 ± 0.08^{a}	0.75 ± 0.04^{a}	0.73 ± 0.11^{a}	0.66 ± 0.04^{a}
Lys	0.91 ± 0.04^{a}	0.85 ± 0.09^{ab}	0.84 ± 0.07^{ab}	0.75 ± 0.09^{b}	0.75 ± 0.08^{b}	0.86 ± 0.04^{ab}	0.85 ± 0.10^{ab}	0.79 ± 0.03^{a}
His	0.40 ± 0.02^{a}	0.37 ± 0.04^{ab}	0.37 ± 0.03^{ab}	0.33 ± 0.04^{ab}	0.32 ± 0.04^{b}	0.37 ± 0.02^{ab}	0.37 ± 0.05^{ab}	0.34 ± 0.02^{ab}
Arg	1.13 ± 0.05^{a}	1.06 ± 0.10^{ab}	1.04 ± 0.09^{ab}	0.93 ± 0.16^{b}	0.92 ± 0.11^{b}	1.06 ± 0.05^{ab}	1.06 ± 0.12^{ab}	0.97 ± 0.04^{ab}
Pro	0.69 ± 0.04^{a}	0.66 ± 0.07^{ab}	0.64 ± 0.06^{ab}	0.57 ± 0.08^{ab}	0.57 ± 0.07^{b}	0.67 ± 0.04^{ab}	0.65 ± 0.09^{ab}	0.61 ± 0.03^{ab}
Try	0.13 ± 0.01^{a}	0.12 ± 0.01^{a}	0.12 ± 0.02^{a}	0.13 ± 0.04^{a}	0.12 ± 0.02^{a}	0.13 ± 0.01^{a}	0.13 ± 0.03^{a}	0.12 ± 0.01^{a}

Means in a same line with different letters are significantly different ($P < 0.05$). Variation of the mean represents standard deviation of triplicate for each measurement. Symbol ‘*’ indicates that the results are for reference only.

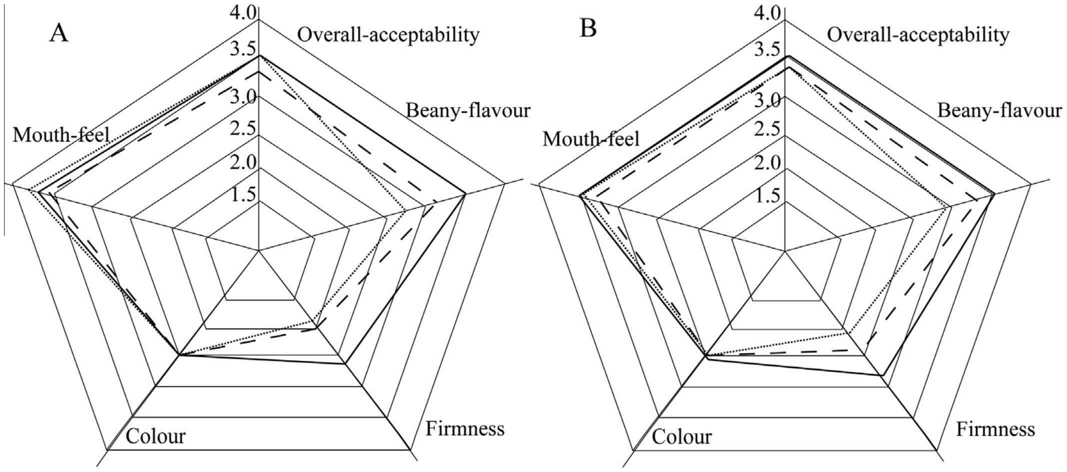

Fig. 2. Sensory evaluation of tofu (A fresh, B steamed, 1 = very weak, 5 = very strong). –$MgCl_2$; --$MgCl_2$ + 0.6 g guar gum; . . .gypsum.

$MgCl_2$ and gypsum and commercial products purchased from the market. These sensory evaluation results indicate the coagulant $MgCl_2$ plus 0.6 g guar gum is an appropriate coagulant in making tofu especially organic tofu.

4. Conclusion

All the three polysaccharides including carrageenan, guar gum and gum Arabic affected the properties of tofu, mainly its yield, syneresis and proximate composition. Guar gum also played a critical role in the quality of tofu. As an organic compatible coagulant, when combined with 2.93 g $MgCl_2$, guar gum (0.6 g) and gypsum affected the yield and textural properties of tofu in a similar way. In addition, tofu made with $MgCl_2$ combined with 0.6 g guar gum had sensory acceptability comparable to commercial tofu products made with traditional gypsum, suggesting consumers will accept the organic tofu because they can't discriminate it from conventional one. Although the organic compatible coagulants increased syneresis of tofu, this disadvantage was counterbalanced by the increased yield of tofu. Moreover, the retained beany-flavour by the organic compatible coagulants would please the consumers and benefit the marketing of tofu. In summary, the organic compatible coagulant (2.93 g $MgCl_2$ plus 0.6 g guar gum) is promising as a coagulant in making quality tofu especially for organic sales.

Acknowledgements

H.Y. thanks the start-up Grant (R-143-000-561-133), Singapore Ministry of Education Academic Research Fund Tier 1 (R-143-000-583-112), teaching enhancement grant (C-143-000-041-001) and learning innovation fund-technology (LIFT) (C-143-100-002-511) provided by the National University of Singapore for supporting this work. Projects 31371851, 31071617 and 31200801 supported by NSFC contributed to this research as well.

References

Abd Karim, A., Sulebele, G. A., Azhar, M. E., & Ping, C. Y. (1999). Effect of carrageenan on yield and properties of tofu. *Food Chemistry, 66*, 159–165.

Adams, A. Top 10 ways to cook tofu, 2012. URL http://m.anchoragepress.com/arts_and_entertainment/crash_test_dummy/top-ways-to-cook-tofu/article_da08c4cc-6f02-11e1-a2f3-001871e3ce6c.html?mode=jqm. Accessed 29 Apr 2014.

AOAC (2000). *Official Methods of Analysis*. Arlington: Association of Official Analytical Chemists.

Arda, E., Kara, S., & Pekcan, Ö. (2009). Synergistic effect of the locust bean gum on the thermal phase transitions of κ-carrageenan gels. *Food Hydrocolloids, 23*, 451–459.

Bourne, M. (2002). Food Texture and Viscosity: Concept and Measurement: Access Online via Elsevier.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Cai, T., & Chang, K. C. (1999). Processing effect on soybean storage proteins and their relationship with tofu quality. *Journal of Agricultural and Food chemistry, 47*, 720–727.

Cai, T. D., Chang, K. C., Shih, M. C., Hou, H. J., & Ji, M. (1997). Comparison of bench and production scale methods for making soymilk and tofu from 13 soybean varieties. *Food Research International, 30*, 659–668.

Chang, K., Lin, Y.-S., & Chen, R. (2003). The effect of chitosan on the gel properties of tofu (soybean curd). *Journal of Food Engineering, 57*, 315–319.

Fitzsimons, S. M., Mulvihill, D. M., & Morris, E. R. (2008). Large enhancements in thermogelation of whey protein isolate by incorporation of very low concentrations of guar gum. *Food Hydrocolloids, 22*, 576–586.

Fontecha, J., Ríos, J., Lozada, L., Fraga, M., & Juárez, M. (2000). Composition of goat's milk fat triglycerides analysed by silver ion adsorption-TLC and GC–MS. *International Dairy Journal, 10*, 119–128.

Harrington, J. C., Foegeding, E. A., Mulvihill, D. M., & Morris, E. R. (2009). Segregative interactions and competitive binding of Ca^{2+} in gelling mixtures of whey protein isolate with Na^+ κ-carrageenan. *Food Hydrocolloids, 23*, 468–489.

Harrington, J. C., & Morris, E. R. (2009). Conformational ordering and gelation of gelatin in mixtures with soluble polysaccharides. *Food Hydrocolloids, 23*, 327–336.

Hsieh, J.-F., Yu, C.-J., & Tsai, T.-Y. (2012). Proteomic profiling of the coagulation of soymilk proteins induced by magnesium chloride. *Food Hydrocolloids, 29*, 219–225.

Hua, Y., Cui, S. W., & Wang, Q. (2003). Gelling property of soy protein–gum mixtures. *Food Hydrocolloids, 17*, 889–894.

Jiwan, M. A., Duane, P., O'Sullivan, L., O'Brien, N. M., & Aherne, S. A. (2010). Content and bioaccessibility of carotenoids from organic and non-organic baby foods. *Journal of Food Composition and Analysis, 23*, 346–352.

Lee, C.-Y., & Kuo, M.-I. (2011). Effect of γ-polyglutamate on the rheological properties and microstructure of tofu. *Food Hydrocolloids, 25*, 1034–1040.

Li, J., Cheng, Y., Tatsumi, E., Saito, M., & Yin, L. (2014). The use of W/O/W controlled-release coagulants to improve the quality of bittern-solidified tofu. *Food Hydrocolloids, 35*, 627–635.

Li, X., Fang, Y., Al-Assaf, S., Phillips, G. O., Nishinari, K., & Zhang, H. (2009). Rheological study of gum Arabic solutions: Interpretation based on molecular self-association. *Food Hydrocolloids, 23*, 2394–2402.

Liu, K. (1997). Nonfermented oriental soyfoods. In E. Daniel (Ed.), *Soybeans*. Springer.

Lockie, S., Lyons, K., Lawrence, G., & Mummery, K. (2002). Eating 'Green': motivations behind organic food consumption in Australia. *Sociologia Ruralis, 42*, 23–40.

Messina, M. J., Persky, V., Setchell, K. D., & Barnes, S. (1994). Soy intake and cancer risk: A review of the in vitro and in vivo data. *Nutrition and Cancer, 21*, 113–131.

Nagano, T., & Tokita, M. (2011). Viscoelastic properties and microstructures of 11S globulin and soybean protein isolate gels: Magnesium chloride-induced gels. *Food Hydrocolloids, 25*, 1647–1654.

Natarajan, S. S., Xu, C., Bae, H., Caperna, T. J., & Garrett, W. M. (2006). Characterization of storage proteins in wild (Glycine soja) and cultivated (Glycine max) soybean seeds using proteomic analysis. *Journal of Agricultural and Food Chemistry, 54*, 3114–3120.

Nie, C., & Zepeda, L. (2011). Lifestyle segmentation of US food shoppers to examine organic and local food consumption. *Appetite, 57*, 28–37.

Noh, E. J., Park, S. Y., Pak, J. I., Hong, S. T., & Yun, S. E. (2005). Coagulation of soymilk and quality of tofu as affected by freeze treatment of soybeans. *Food Chemistry, 91*, 715–721.

Poysa, V., & Woodrow, L. (2002). Stability of soybean seed composition and its effect on soymilk and tofu yield and quality. *Food Research International, 35*, 337–345.

Poysa, V., Woodrow, L., & Yu, K. (2006). Effect of soy protein subunit composition on tofu quality. *Food Research International, 39*, 309–317.

Prabhakaran, M. P., Perera, C. O., & Valiyaveettil, S. (2006). Effect of different coagulants on the isoflavone levels and physical properties of prepared firm tofu. *Food Chemistry, 99*, 492–499.

Puppo, M. C., & Añón, M. C. (1998). Structural properties of heat-induced soy protein gels as affected by ionic strength and pH. *Journal of Agricultural and Food Chemistry, 46*, 3583–3589.

Rufián-Henares, J. Á., Guerra-Hernandez, E., & García-Villanova, B. (2006). Colour measurement as indicator for controlling the manufacture and storage of enteral formulas. *Food Control, 17*, 489–493.

Toda, K., Nakamura, Y., Takahasi, K., & Komaki, K. (2009). Components in soybean seeds affecting the consistency of tofu. *Japan Agricultural Research Quarterly, 43*, 295–300.

Toda, K., Ono, T., Kitamura, K., Hajika, M., Takahashi, K., & Nakamura, Y. (2003). Seed protein content and consistency of tofu prepared with different magnesium chloride concentrations in six Japanese soybean varieties. *Breeding Science, 53*, 217–223.

Watanabe, T. (1997). *Science of tofu*. Kyoto, Japan: Food Journal Co. 14–54.

Weedsnetwork. Global organic food and beverage sales approach $US63 billion, 2013. URL <http://weedsnetwork.com/traction/permalink/WeedsNews4324>. Accessed 25.06.14.

Yang, H., Wang, Y., Jiang, M., Oh, J. H., Herring, J., & Zhou, P. (2007). 2-Step optimization of the extraction and subsequent physical properties of channel catfish (*Ictalurus punctatus*) Skin Gelatin. *Journal of Food Science, 72*, C188–C195.

Yang, Y., Anvari, M., Pan, C.-H., & Chung, D. (2012). Characterisation of interactions between fish gelatin and gum Arabic in aqueous solutions. *Food Chemistry, 135*, 555–561.

Yasir, S., Sutton, K., Newberry, M., Andrews, N., & Gerrard, J. (2007a). The impact of Maillard cross-linking on soy proteins and tofu texture. *Food Chemistry, 104*, 1502–1508.

Yasir, S., Sutton, K., Newberry, M., Andrews, N., & Gerrard, J. (2007b). The impact of transglutaminase on soy proteins and tofu texture. *Food Chemistry, 104*, 1491–1501.