Effect of magnesium salt concentration in water-in-oil emulsions on the physical properties and microstructure of tofu

Qiaomei Zhu^a, Feifei Wu^a, Masayoshi Saito^b, Eizo Tatsumi^b, Lijun Yin^{a,c*}

^a Beijing Key Laboratory of Functional Food from Plant Resources, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing, China.

^b Biological Resources and Post-harvest Division, Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki, Japan.

^c College of Food Science and Technology, Henan University of Technology, Zhengzhou, China.

*Corresponding author (✉)

(Lijun Yin) Tel. /Fax: +86-10-62737424, E-mail: ljyin@cau.edu.cn

Affiliation addresses: 17, Qinghua East Road, Haidian, Beijing, 100083, P.R. China

Post addresses: P.O. Box 40, 17, Qinghua East Road, Haidian, Beijing, 100083, P.R. China

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Abstract

The aim of this research was to prepare water-in-oil (W/O) emulsions encapsulating different concentrations of magnesium chloride (MgCl₂) and to investigate the effect of W/O emulsions on the physical properties and microstructure of tofu. The results showed that the stability of W/O emulsions improved as the concentrations of polyglycerol polyricinoleate (PGPR) and MgCl₂ increased.

Dynamic viscoelastic measurements indicated that gelation time decreased with increasing MgCl_2 concentration in W/O emulsions, suggesting a more rapid reaction between magnesium ions and protein molecules. As the concentration of MgCl_2 in W/O emulsions increased, the yield and water content of tofu decreased, while the protein and crude fat contents and hardness values increased. At a concentration of 2.0 M MgCl_2 in W/O emulsion, the WHC and microstructure of the tofu samples were optimal. The variations in the physical properties of tofu were attributed to the concentration of magnesium ions and the coagulation rate.

Keywords: W/O emulsions; magnesium chloride; stability; tofu; texture; microstructure.

1. Introduction

Tofu, originating in China 2000 years ago, is one of the most important traditional soybean foods. Tofu is now increasing in popularity all over the world as it is cholesterol free and contains many nutrients, such as minerals, vitamins, omega-3 fatty acids and isoflavone (Hou, Chang, & Shih, 1997). The main processes of tofu manufacturing are heat treatment, coagulation, moulding and pressing. Of these, the coagulation process, which is affected by coagulant type and coagulant concentration, is the key step in determining the yield and quality of tofu (Prabhakaran, Perera, & Valiyaveetil, 2006).

Various coagulants have been used in tofu manufacture with each coagulant resulting in tofu with different textural properties, varying from soft to firm (Karim, Sulebele, Azhar, & Ping, 1999). The major coagulants used in tofu production are

calcium sulfate, magnesium chloride (MgCl_2), and glucono- δ -lactone. Of these coagulants, tofu coagulated with MgCl_2 is preferred by consumers because MgCl_2 creates a more natural flavour and preserves the sweet taste of the original soybean. However, the ability of magnesium ions to quickly dissolve in soymilk results in a rapid solidification of the gel and therefore produces tofu with a low yield, a low water-holding capacity (WHC), and a coarse structure (Watanabe, 2000). Several recent studies have focused on exploring methods that may improve the yield and texture of tofu. Li et al. (2015) used a mixture of polysaccharide and MgCl_2 as coagulant to improve the yield and texture of tofu. They suggested that adding polysaccharide to heated soymilk could reduce the coagulation rate of MgCl_2 as it increased the viscosity of soymilk, thus improving the yield and textural characteristics of tofu. In addition, the segregating interaction between polysaccharides and protein molecules might also contribute to a reduction in the coagulation rate. Previously, our laboratory has developed water-in-oil (W/O) emulsions specifically designed to encapsulate bittern solution (mainly containing MgCl_2) and to control the release of magnesium ions from the water phase by optimizing the preparation conditions (Zhu et al., 2015; Li et al., 2014). Compared with traditional MgCl_2 coagulant, the use of a W/O emulsion could significantly slow down the gelation rate of tofu and thereby increase its yield and improve its texture. The release rate of magnesium ions from W/O emulsions could be regulated by the emulsifier type, emulsifier concentration, phase ratio, and homogenization conditions. In addition, compared to W/O emulsion with no added salt, magnesium salt added to the aqueous phase of such emulsions was found to be beneficial for its stability (Zhu, Han, Li & Yin, 2015b). Therefore, it can be expected that the concentration of MgCl_2 in the aqueous phase affects the stability of W/O emulsions and the subsequent quality

of tofu. However, little information is available on modifying the yield and textural characteristics of tofu by adjusting the concentration of magnesium in W/O emulsions.

Several studies have investigated the effect of inorganic salts on the stability of W/O emulsions. Increasing the calcium chloride concentration enhanced the stability of W/O emulsion against coalescence because of the reduction in water droplet size and the increased adsorption rate of surfactant at the oil-water interface (Kent & Saunders, 2001; Márquez, Medrano, Panizzolo, & Wagner, 2010). Koroleva and Yurtov (2003) found that adding inorganic salts to the dispersed phase could reduce the Ostwald ripening rate of W/O emulsions. Nadin, Rousseau, and Ghosh (2014) found that the salt concentration-dependent osmotic pressure gradients also influenced the stability of the emulsion and the release rate of ions in the internal aqueous phase. Melnik, Spyropoulos, and Norton (2010) suggested that a large osmotic pressure gradient, caused by the variation in salt concentrations between the two aqueous phases, could promote the rapid release of encapsulated solutes in primary W/O emulsion to the outer aqueous solution. In addition, coagulant concentration also affects the yield, textural properties, and microstructure of tofu. Saio (1979) found that with an increasing concentration of coagulant, the bulk yield of tofu decreased and the firmness increased. The optimal coagulant concentration for obtaining a finer tofu network is important for tofu preparation. Arii and Takenaka (2013) claimed that a high concentration of magnesium salt induced tofu-like precipitates with a rough surface, while a low concentration produced a smooth surface. Based on these research reports, the quality of tofu might be adjusted to meet different consumers' preferences in the final products by controlling the concentration of magnesium salt in W/O emulsions.

Therefore, the objective of the present study was to determine the effect of varying the concentrations of MgCl_2 and polyglycerol polyricinoleate (PGPR) on the stability of W/O emulsions to obtain suitable emulsion coagulants. Additionally, rheological measurements and scanning electron microscopy (SEM) were used to study the gelation process and microstructure of tofu gel to clarify the effect of W/O emulsions on the physical and textural properties of tofu.

2. Materials and methods

2.1. Materials

PGPR (818SK) was supplied by Taiyo Kagaku Co., Ltd (Tokyo, Japan). Soybean oil was purchased from a local supermarket and used without further purification. Commercial soybeans (ZhongHuang 13, approval No.2001009) were purchased from the Chinese Academy of Agricultural Sciences (Beijing, China). Magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) was obtained from Lanyi Co., Ltd. (Beijing, China). All other reagents were of analytical grade.

2.2. Emulsion preparation

W/O emulsions were prepared as in previous methods with some modifications (Zhu et al., 2015). The oil phase was prepared by mixing 0.6 or 1.0 g PGPR with 60% (w/w) soy oil for 100 g of emulsion. The aqueous phase, with concentrations of MgCl_2 varying from 0.2 to 2.6 M, was dispersed in the PGPR-soy oil blend and the mixture stirred for 15 min at 65°C. A coarse emulsion was made using a high-speed shear machine (Ultra Turrax, model T25 basic, IKA Labortechnik, Staufen, Germany) at 13,000 rpm for 2 min at room temperature. The second step of emulsification used a high-pressure homogenizer (NS1001L Panda2K, GEA Niro Soavi, Parma, Italy) at 60 MPa to obtain the final emulsions.

2.3. Evaluation of W/O emulsions stability

2.3.1. Particle size measurement

The mean particle sizes of the W/O emulsions were determined as described by Choi, Decker, and McClements (2009) with small modifications. All measurements were performed in triplicate by dynamic light scattering using a Zetasizer Nano ZS90 particle size analyzer (Malvern Instruments, Worcestershire, UK) at a fixed angle of 90 °. The emulsions were appropriately diluted with dodecane (refractive index=1.422, viscosity=1.34 mPa.s at 25°C), measured by transferring the diluted emulsions into 3 ml plastic cuvettes. Particle size was reported as the z-average and the polydispersity index (PDI).

2.3.2. Emulsion stability measurement

The stability of the emulsions was studied by multiple light scattering using a Turbiscan Lab Exper Stability Analyzer (Formulaction, L'Union, France). The transmittance detector received the light that passed through the dispersion at an angle of 180° with respect to the source, while the back scattering detector received the light scattered backwards by the dispersion at an angle of 45°. The analysis of stability was performed as a variation of backscattering (BS) profiles and then exported as Δ BS profiles by the Turbiscan EasySoft Converter software. Then 20-ml W/O emulsion samples were put into glass tubes and loaded into the Turbiscan Lab Expert Stability Analyzer. Two detectors scanned the entire height of the emulsions at 65 °C for 24 h.

The stability of emulsions can be evaluated using the TSI (Turbiscan Stability Index) parameter. The following formulae were applied:

$$BS \approx \frac{1}{\sqrt{\lambda^*}} \quad (1)$$

148

$$\lambda^*(\varphi, d) = \frac{2d}{3\varphi(1-g)Q_s} \quad (2)$$

150

$$TSI = \sqrt{\frac{\sum_{i=1}^n (\chi_i - \chi_{BS})^2}{n-1}} \quad (3)$$

152

153 where λ^* is the photon transport mean free path in the analyzed dispersion, φ is
 154 the volume fraction of particles, d is the mean diameter of particles, g and Q_s are the
 155 optical parameters given by the Mie theory. χ_i is the average backscattering for each
 156 minute of measurement, χ_{BS} is the average χ_i , and n is the number of scans.

157 2.3.3. Oil-water interfacial tension measurement

158 The interfacial tension at the soy oil-water interface was determined using a
 159 spinning drop video tensiometer (SVT 20N, Data Physics, Filderstadt, Germany) at
 160 25°C. A tube was filled with the aqueous phase containing the different concentrations
 161 of $MgCl_2$ and closed using a Teflon cap with a rubber septum. The oil phase was then
 162 injected into the tube using a syringe after which the capped tube was inserted into the
 163 tensiometer slot. A suitable rotation speed was selected to elongate the oil droplet and
 164 the final interfacial tension was calculated by the built-in software. The measurements
 165 were performed three times for each sample.

166 2.4. Tofu preparation

167 The tofu was prepared as in previous methods with some modifications (Zhu et
 168 al., 2015). The soybeans were soaked in distilled water at room temperature for at
 169 least 10 h. The soaked beans were washed, then ground to produce soymilk. The
 170 soymilk was heated to 95 °C and boiled for 10 min using an electromagnetic oven

(Media Co. Ltd., Foshan, China). After heating, 400 ml of soymilk was removed from the heating kettle and cooled to 80 °C with 12 g W/O emulsion coagulants or 4 g, 2 M MgCl₂ solution being added to the soymilk. A high-speed shearing machine at 11,000 rpm diffused the W/O emulsion coagulants for 90 s or the MgCl₂ solution for 10 s. The coagulated tofu jellies were placed in a hot water bath at 85 °C for 30 min and then broken into pieces. The tofu jelly pieces were placed in a container (10 cm×7 cm× 8 cm, with 16 holes in the bottom) and pressed using a weight of 3000 g for 30 min to produce the tofu.

2.5. Evaluation of physical, textural, and microstructural properties of tofu

2.5.1 Yield and proximate composition

The yield of tofu was expressed as the weight of tofu obtained from 400 ml of soymilk. The proximate composition was analyzed using standard methods (AOAC, 2000). The moisture content was determined by drying 1.0 g of fresh tofu in a drying oven at 105°C to a constant weight. The total protein was assayed using the microKjeldahl method and crude fat using the Soxhlet method.

2.5.2. Water-holding capacity (WHC)

Tofu samples (6 g) were removed from the central portion of the prepared tofu using a stainless steel knife then centrifuged at 7000 × g for 20 min at 4 °C. The weight of released water was subsequently measured. WHC was expressed as the ratio of the weight of the released water to the initial weight of water in the gel samples:

$$WHC = \frac{W_t - W_r}{W_t} \times 100 \quad (4)$$

where W_t is the total weight of water in the tofu and W_r is the weight of water released from the tofu gels. Analyses were performed at least in duplicate.

2.5.3. Texture measurement

The textural characteristics of the tofu samples were determined using a

TMS-Pro Texture Analyzer (Food Technology Corp., Sterling, VA, USA). Samples (1.5 cm×1.5 cm×1.5 cm) were compressed by a plunger (D: 44 mm) to 30% deformation at a speed of 20 mm/min. From the texture profile analysis curves, the textural parameters, hardness, springiness and chewiness, were obtained. The textural parameters of tofu were evaluated according to the definitions given by Bourne (2000).

2.5.4. Colour measurement

The colour of the fresh tofu samples was evaluated using a CR 300 Colorimeter (Konica Minolta Company, Tokyo, Japan). A standard white plate with CIE values of L= 92.7, a = 0.3136, and b = 0.3193 was used for calibration. According to the CIE definition, the values of L (0.00 = dark black, 100.0 = white), a (+ = red, – = green), and b (+ = yellow, – = blue) were determined for the samples. Each measurement was repeated four times and the mean value was calculated.

2.5.5. Dynamic viscoelastic measurement

Small-deformation oscillatory measurements of samples were performed using a TA 1500ex rheometer (TA Instruments Ltd., Crawley, UK). An aluminum parallel plate (40-mm diameter, 1-mm gap) was chosen to monitor the gelling process and measure the gel strength. The temperature was controlled by a Peltier unit attached to the water circulation system. Different W/O emulsions and MgCl₂ solutions were added to pre-heated soymilk separately and homogenized using the Ultra Turrax T25 (IKA Labortechnik) at 11,000 rpm for 20 s to simulate the tofu coagulation process. Samples were transferred to the parallel plates, then gels were allowed to form *in situ* at 80 °C. A thin layer of silicone oil was placed on the surface of the samples to prevent water loss due to evaporation. The storage and loss moduli were recorded during the gel formation process using a constant frequency of 1 Hz and a strain of

1%. The line viscoelastic region was measured for each sample using the strain sweep test, where the strain varied from 0.001 to 0.1, at 1 Hz (data not shown).

2.5.6. Scanning electron microscopic observation

Samples were prepared as described by Zhu, Li, Fan, and Yin (2015a) with some modifications. The soy protein gels were cut into pieces (2 mm × 2 mm × 1 mm) with a razor blade then fixed in freshly prepared 2.5% glutaraldehyde solution (prepared by 0.1 M phosphate buffer, pH7.4) for 14 h at 4°C. The tofu samples were then dehydrated using increasing concentrations of ethanol from 60 to 100% (v/v) in 10% increments (15 min at each concentration). After dehydration, pure isoamyl acetate was used to completely replace the ethanol. The samples were critical-point dried and then the surface was coated by spraying with powdered gold. A scanning electron microscope SU8010 (Hitachi, Co. Ltd., Tokyo, Japan) was used to obtain the micrographs of the sample microstructure.

2.6. Data analysis

Statistical analyses were performed using SPSS (ver. 20.0, IBM software, Chicago, USA). One-way analysis of variance and Duncan's multiple range test were used to evaluate the significance of the differences between tofu sample groups at a level of $p \leq 0.05$

3. Results and discussions

3.1. Particle size distribution of W/O emulsions

The effect of different concentrations of PGPR and magnesium salt on the average droplet size and droplet PDI of W/O emulsions are shown in Fig. 1. All PGPR and MgCl₂ at the concentrations tested resulted in nanoemulsions because the average droplet size was less than 500 nm. The droplet size tended to decrease as the surfactant concentration increased. At a PGPR concentration of 0.6% (w/w), the

average particle size increased from 457 to 472 nm as the concentration of magnesium salt increased from 0.4 to 2.6 M. When the concentration of PGPR increased to 1.0% (w/w), the average droplet size decreased to around 390 nm. Lower concentration of PGPR also resulted in W/O emulsions with much larger PDI values. The average particle size can be regarded as the most important characteristic for evaluating the stability of emulsion systems. McClements (1999) suggested that a smaller droplet size could improve the stability of emulsion against coalescence because of a lower collision efficiency owing to the higher emulsion viscosity. A higher concentration of PGPR could also rapidly coat and rigidify the interfacial film of a newly-formed emulsion and therefore prevent the occurrence of coalescence (Niknafs, Spyropoulos, & Norton, 2011). The mean droplet diameter of W/O emulsion droplets remained relatively constant at all concentrations of magnesium salt.

3.2. Stability of W/O emulsions prepared with PGPR and $MgCl_2$

The TSI values of all W/O emulsions increased after 1 day, indicating that they tended to be unstable. As the concentration of $MgCl_2$ increased, the TSI values decreased after 1 day of storage at 25 °C, indicating that adding magnesium salt improved the stability of W/O emulsions and that it was possible to prepare emulsions encapsulating high concentrations of $MgCl_2$ (up to 2.6 M) successfully. Detailed results can be found in supplementary Fig. 1 [and Fig.2](#).

For a better comparison of the stability of these emulsions over 14 days of storage, the delta-backscattering (ΔBS) profiles in reference mode of W/O emulsions for different concentrations of PGPR and magnesium salt were obtained (Fig. 2). A slight increase in ΔBS in the middle of the tube was observed, which indicated an incremental increase in the particle size caused by flocculation or coalescence (Kang

et al., 2011). The Δ BS profiles at the bottom of the emulsion samples continuously decreased to -41, or -20 for emulsions stabilized with 0.6% (w/w) PGPR and 0.4 or 2.6 M MgCl_2 , respectively. This indicated that the phases had separated for emulsions stabilized with 0.6% (w/w) PGPR and 0.4 M MgCl_2 . Thus, adding more magnesium salt can retard phase separation. Meanwhile, the reduction in Δ BS at the top of the tube indicated an oil-off phenomenon, resulting in the formation of a more transparent upper phase. These phenomena could also be visually seen in the W/O emulsions after storage at room temperature for 2 weeks, as shown in supplementary Fig. 2. These pictures show that the emulsion with 0.6% (w/w) PGPR and 0.4 M MgCl_2 not only exhibited a creaming effect but also the appearance of a transparent layer at the bottom of the tube. Increasing the concentration of magnesium salt to 2.6 M could thus effectively improve the stability of the emulsion against both creaming and coalescence. The W/O emulsions, prepared with 1.0% (w/w) PGPR and different concentrations of magnesium salt, were also relatively stable during 14 days of storage. Therefore, W/O emulsions containing 1.0% (w/w) PGPR are more suitable for use as coagulants for tofu production.

3.3. Determination of interfacial properties

To better understand the mechanism of the effect exhibited by MgCl_2 on PGPR-stabilized W/O emulsions, the interfacial tension was studied. Table 1 shows the interfacial tension between soy oil and water at different concentrations of MgCl_2 . The interfacial tension decreased as the concentration of MgCl_2 increased. At a PGPR concentration of 0.6% (w/w), the interfacial tension with 0.4 M MgCl_2 was 6.25 mN.m^{-1} , with 1.2 M MgCl_2 this decreased to 1.2 mN.m^{-1} and with 2.6 M MgCl_2 it further decreased to 0.34 mN.m^{-1} . Increasing the concentration of PGPR to 1.0% (w/w) also reduced the interfacial tension. Interfacial tension between oil and water plays an

important role during emulsification with a lower interfacial tension producing smaller droplet sizes. Therefore, many nano-scale droplets were formed after high-pressure homogenization in the present study. This decrease in interfacial tension indicated a higher adsorption density of PGPR at the interface with several studies also reporting a reduction in interfacial tension with increased concentrations of inorganic salts (Márquez et al., 2010, Lee, Hancocks, Noble, & Norton, 2014). These studies suggested that adding electrolytes could compress the electrical double layer and thereby reduce the interfacial tension. The salt could also bind free fatty acids in the oil to reduce their interfacial activity. In the present study, the soybean oil may also have contributed free fatty acid such as linoleic acid and linolenic acid. Magnesium salts might bind these free fatty acids and thereby increase the adsorption of PGPR at the interface, strengthening the interface against breakdown. It has also been reported that the presence of inorganic salt can strengthen the interfacial film and enhance the stability of emulsions by counter balancing the Ostwald ripening caused by Laplace pressure between emulsion droplets (Rosano, Gandolfo, & Hidrot, 1998). Further investigations are needed to determine how electrolytes act with PGPR at the water-oil interface to stabilize W/O emulsions.

3.4. Coagulation process of soymilk gel prepared with different W/O emulsion coagulants

Rheological measurements were performed to simulate the gelation process of tofu prepared from MgCl_2 alone and MgCl_2 -containing W/O emulsion as coagulants. The pattern of changes in the G' and G'' moduli could reflect the gelation process and the structure of the gels formed (Maltais, Remondetto, & Subirade, 2008). Figure 3 shows the changes in G' and G'' over 30 min at 80 °C for soy milk protein with different coagulants. The G' modulus increased rapidly in all gel samples during the

first 5 min, reflecting the process of gelation in tofu. Although the values of G' and G'' both increased with time, the G' values reached higher values than the G'' values, indicating that the gelation point (the point at which G' was higher than G'') was reached once the coagulants had been added to the pre-heated soymilk. The initial values of G' for tofu prepared from W/O emulsions containing 1.2 and 1.6 M $MgCl_2$ were 1.7 and 8.1 Pa, respectively, which was lower than for W/O emulsions containing higher concentrations of $MgCl_2$. This illustrates that high concentrations of $MgCl_2$ in W/O emulsions can accelerate the solidification reaction in soymilk. The initial G' values of tofu prepared from $MgCl_2$ alone reached up to 68.2 Pa, with the viscoelastic curve showing few changes during the whole gelation process. This demonstrated that without the use of emulsion coagulant, $MgCl_2$ alone reacted much more rapidly with protein molecules and using W/O emulsions could thus retard the release of magnesium ions from the internal phase to soymilk. After 30 min, the values of G' in all samples seemed to reach a plateau, indicating the formation of a soy protein gel. For $MgCl_2$ -induced soy protein gel, the gelation time was short and the G' value of the final soy protein gel was 122.7 Pa. While the gelation time for soy protein gels coagulated with W/O emulsion was much longer and the gelation time decreased as more $MgCl_2$ was added to the aqueous phase, indicating that more magnesium ions were available to react with the protein molecules and thereby causing a more rapid reaction.

Kohyama et al. (1995) reported that denatured soy protein molecules were negatively charged and that the net charge of protein could be neutralized by divalent salts. In the present study, adding higher concentrations of $MgCl_2$ to pre-heated soymilk seemed to neutralize the surface charge of soluble aggregates more rapidly, thus leading to a more rapid gel formation. Nagano and Tokita (2011) also found that

the G' value of soy protein gels increased as the concentration of MgCl₂ increased. A higher concentration of MgCl₂ led to the formation of a coarse protein gel structure. Therefore, the gelation time and rate could be controlled by regulating the concentrations of MgCl₂ in W/O emulsions, which can also modify the microstructure of tofu.

3.5. Effect of MgCl₂ concentrations contained in W/O emulsions on the microstructure of tofu

The scanning electron microscopic (SEM) observations explored the structure of tofu gel prepared with different coagulants. All samples exhibited a particulate structure with protein aggregates held together (Fig. 4). However, the size of the aggregates and their density and homogeneity differed. The control tofu sample, using MgCl₂ alone as a coagulant, formed thicker aggregates with a more compact structure and no obvious pores in comparison to the other tofu samples. For W/O emulsion-coagulated tofu gel, many discontinuous fragments and large holes were visible in tofu prepared with 1.2 and 1.6 M MgCl₂. When the concentrations of MgCl₂ increased to 2.0 M, the tofu gel network became uniform and continuous. A further increase to 2.6 M, reduced the uniformity of the gel structure with large aggregates forming on the surface of the gel. The W/O emulsion containing 2.0 M MgCl₂ formed the most homogeneous and ordered microstructure compared to the other MgCl₂ concentrations.

3.6. Proximate composition, yield and WHC of tofu

The proximate composition, yield, and WHC of tofu prepared with different coagulants are presented in Table 2. The yield of tofu prepared with MgCl₂ alone was 116.1 g, which was much lower than that those prepared with W/O emulsions. The yield of tofu prepared with W/O emulsions was also found to be strongly dependent

on the concentration of MgCl_2 . When the concentration decreased from 2.6 to 1.2 M in W/O emulsions, the tofu yield increased significantly from 169.2 to 260.4 g. This increase in tofu yield coincided with an increase in moisture content from 78.2 to 85.9%, suggesting that the yield and moisture contents of tofu were highly correlated, as expected (Cai et al., 1997). A higher magnesium concentration and quicker coagulation rate would result in a denser and more compact structure. Such a structure allows water to be released from the protein gel structure more easily during the coagulation process (Noh et al., 2005).

The protein content (wet basis) of tofu prepared with MgCl_2 was found to be significantly higher than that prepared with W/O emulsions: 12.1% for MgCl_2 alone, 9.7% for W/O emulsion containing 2.6 M MgCl_2 and 6.9% for those containing 1.2 M MgCl_2 . The tofu samples prepared with W/O emulsion containing low concentrations of MgCl_2 had a low protein content, suggesting that a slow coagulation process could make soy protein more efficient at aggregating into tofu (Li et al., 2015). Crude fat may be important for tofu production as it provides characteristic flavours and texture as integral diet components. The crude fat content (dry basis) of the tofu samples increased from 4.3 to 6.0% when the magnesium salt concentration in W/O emulsions increased from 1.2 to 2.6 M.

WHC indicates the amount of water retained within the protein gel network, which could reflect the spatial structure of the protein gel (Wu, Hua, & Lin, 2014). The results showed that the WHC values of tofu gel prepared with W/O emulsions containing 1.2 or 1.6 M MgCl_2 were lower than those of the other coagulants. The highest WHC value of 84.9% was achieved with a W/O emulsion containing 2.0 M MgCl_2 . At a lower magnesium salt concentration, the interaction of protein molecules with magnesium ions was not sufficient to form a firm tofu gel and so the water was

easily released from the gel structure. Owing to the incomplete precipitation of soy proteins, weaker gels were formed, which resulted in a decrease in the WHC of tofu (Xia, Abdalhai, Emmanuel, & Qian, 2015). When the magnesium content increased further, too many cross-linkages formed, causing the gel structure to become inhomogeneous (Fig. 4). This structure resulted in a severe reduction in water content but increased the WHC, as was also shown previously (Kao, Su, & Lee, 2003). The changes in WHC were reflected in the observed microstructure of the tofu samples (Fig. 4).

3.7. Textural properties of tofu

The textural characteristics of tofu contribute to both the sensation and feelings perceived when it is tasted and consumed. The textural properties of tofu prepared with MgCl_2 alone and the W/O emulsions varying in MgCl_2 concentration are shown in Table 2. Compared with MgCl_2 , the tofu samples coagulated with W/O emulsions had completely different textural properties. The values of hardness, springiness, and chewiness of W/O emulsion-coagulated tofu samples were lower than in tofu samples coagulated with MgCl_2 alone. After adding MgCl_2 to pre-heated soy milk, the hardness of tofu reached 5.4 g (Table 2). The higher hardness value for MgCl_2 -coagulated tofu samples was probably due to the quicker coagulation, which induced an intense interaction between soy protein and magnesium ions, thus creating a more compact tofu structure (Prabhakaran, Perera, & Valiyaveetil, 2006). For W/O emulsion-coagulated tofu, as the concentration of MgCl_2 increased, the hardness, springiness, gumminess, and chewiness also tended to increase. Li et al. (2015) reported that the texture of tofu was relevant to its microstructure and was indirectly reflected by its moisture content. Increasing the amount of MgCl_2 in W/O emulsions led to tofu with a lower moisture content and a more compact gel structure, which

therefore also had increased hardness. Saio (1979) also investigated the relationship between tofu gel structure and texture, concluding that the density of the gel network and size of the protein aggregates affected the gel texture. The SEM results (Fig. 4) of the present study showed that as the concentration of MgCl_2 in W/O emulsions increased, the tofu gel structure became denser with larger protein aggregates, which matches the textural results (Table 2) that showed an increased hardness.

Conclusions

Stable W/O emulsions containing high concentrations of MgCl_2 can be successfully prepared with 1.0% (w/w) PGPR-and the stability of W/O emulsions was enhanced by increasing the amount of added MgCl_2 . For tofu preparation, the concentration of MgCl_2 in W/O emulsions had a great influence on the yield, WHC, proximate composition, and microstructure of the tofu. The yield decreased as the concentration of MgCl_2 in W/O emulsions increased.-With a concentration of 2.0 M MgCl_2 in W/O emulsion, the WHC and microstructure of the tofu samples were optimal.-This work brings understanding on the controlled release of MgCl_2 in W/O emulsion using PGPR as a hydrophobic emulsifier determining yield and quality of tofu, which could be exploited in the tofu industry. According to the report from the commission on Dietary Food Additive Intake in the European Union, the acceptable daily intake amount of PGPR is 7.5 mg kg^{-1} . Although the usage amount of PGPR in this study was suitable, as a synthetic emulsifier, reducing the concentration of PGPR in food systems may be more desirable for consumers' acceptance. Therefore, partial replacement of PGPR by natural macromolecules without affecting the stability of W/O emulsions will be considered in our future studies.

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Figure captions

Fig. 1. Effect of PGPR and MgCl_2 concentrations on: (a) mean droplet size, (b) PDI of the W/O emulsions. Error bars represent standard deviations ($n=3$). Columns headed by different letters have significantly different values ($p \leq 0.05$).

Fig. 2. Backscattering profiles of W/O emulsions stabilized using different concentrations of PGPR and MgCl_2 : (a) 0.6% (w/w) PGPR and 0.4 M MgCl_2 , (b) 0.6% (w/w) PGPR and 2.6 M MgCl_2 , (c) 1.0% (w/w) PGPR and 0.4 M MgCl_2 , (d) 1.0% (w/w) PGPR and 2.6 M MgCl_2 .

Fig. 3. Gelation profiles of soymilk gel samples prepared with different coagulants.

Fig. 4. SEM micrographs of tofu prepared with different coagulants. A: W/O emulsion containing 1.2 M MgCl_2 , B: W/O emulsion containing 1.6 M MgCl_2 , C: W/O emulsion containing 2.0 M MgCl_2 , D: W/O emulsion containing 2.2 M MgCl_2 , E: W/O emulsion containing 2.6 M MgCl_2 , F: 2.0 M MgCl_2 alone. Scale bar = 3 μm .

Table 1

Effect of MgCl_2 and PGPR concentration on the interfacial tension between soy oil and water.

PGPR concentration (%)	Magnesium concentrations(M)	Interfacial tension (mN.m^{-1})
0.6	0.4	6.25 ± 0.03^a
	1.2	1.2 ± 0.03^d
	2.0	0.41 ± 0.01^f
	2.6	0.34 ± 0.01^g
	0.4	5.44 ± 0.10^b
0.8	1.2	1.09 ± 0.03^e
	2.0	0.36^{fg}
	2.6	0.27^h
1.0	0.4	4.53 ± 0.02^c
	1.2	1.02 ± 0.03^e
	2.0	0.27^h
	2.6	0.24^h

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 2.

Proximate composition, yield, WHC and texture of tofu prepared with different coagulants

Coagulant	W/O-1.2M MgCl ₂	W/O-1.6M MgCl ₂	W/O-2.0M MgCl ₂	W/O-2.2M MgCl ₂	W/O-2.6M MgCl ₂	2.0M MgCl ₂
Yield(g)	260.4±10.04 ^d	250.75±4.60 ^{cd}	250.05±10.70 ^{cd}	232.4±3.11 ^c	169.15±9.12 ^b	116.05±1.06 ^a
Moisture (%)	85.9±0.01 ^c	85.7±0.02 ^c	85.5±0.02 ^c	83.2±0.01 ^c	78.2±0.02 ^b	73.03±0.02 ^a
WHC(%)	78.88±0.02 ^a	79.33±0.02 ^a	84.93±0.01 ^b	82.98±0.01 ^{ab}	80.77±0.01 ^{ab}	82.07±0.03 ^{ab}
Protein (%)	6.93±1.04 ^a	7.64±1.03 ^{ab}	7.89±1.54 ^{ab}	7.46±0.48 ^{ab}	9.71±0.56 ^{bc}	12.07±1.25 ^c
Crude fat(%)	4.27±0.01 ^b	4.35±0.01 ^{bc}	5.22±0.01 ^c	5.32±0.01 ^{bc}	5.99±0.01 ^b	6.77±0.01 ^a
Hardness (N)	1.34±0.02 ^a	1.78±0.33 ^{ab}	1.83±0.02 ^{ab}	1.99±0.22 ^b	2.77±0.02 ^c	5.40±0.4 ^d
Cohesiveness	0.39±0.04 ^a	0.54±0.15 ^{ab}	0.56±0.03 ^{ab}	0.57±0.01 ^b	0.62±0.01 ^b	0.68±0.02 ^b
Springiness(mm)	0.81±0.18 ^a	1.56±0.66 ^{ab}	1.68±0.06 ^b	1.66±0.3 ^b	2.51±0.13 ^c	3.84±0.02 ^d
Gumminess (N)	0.51±0.03 ^a	1.0±0.39 ^b	1.03±0.04 ^b	1.13±0.1 ^b	1.71±0.05 ^c	3.66±0.2 ^d
Chewiness (mJ)	0.42±0.01 ^a	1.76±0.98 ^{ab}	1.71±0.01 ^{ab}	1.89±0.5 ^b	4.29±0.33 ^c	14.04±0.74 ^d

Means in a same line with different letters are significantly different (P<0.5). Variation of the mean represents standard deviation of triplicate for each measurement.

Figure(s)

Fig.1

Figure(s)

Fig.2

Figure(s)

Fig.3

Figure(s)

Fig.4