

# Effect of $\gamma$ -polyglutamate on the rheological properties and microstructure of tofu

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## ABSTRACT

The effects of  $\gamma$ -polyglutamates with different concentrations (0.1%, 0.15%, 0.2%) and molecular weights (high, medium, low) addition on the rheological properties, microstructure, and syneresis of tofu were studied. The addition of  $\gamma$ -polyglutamate increased the gelation time, and decreased the storage modulus ( $G'$ ) and the loss modulus ( $G''$ ) of tofu. The molecular weight and concentration of  $\gamma$ -polyglutamate effectively changed the rheological properties of tofu. The network of tofu without  $\gamma$ -polyglutamate addition was constructed by fine strands in a dense arrangement as seen by using scanning electron microscope. However, the addition of  $\gamma$ -polyglutamate reduced the thickness of the strands in tofu network. Tofu syneresis was also reduced by the addition of  $\gamma$ -polyglutamate. Increase the concentration of  $\gamma$ -polyglutamate significantly decreased the syneresis of tofu. This trend was more evident on the tofu with high molecular weight  $\gamma$ -polyglutamate.

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## 1. Introduction

Soybeans contain 35–40% of protein (on a dry wet basis) and have been processed to make various forms of soy foods. About 90% of soybean proteins are consumed in the form of tofu in Asia. Tofu is usually recognized as a salt- or acid-coagulated soy protein gel containing water, soy lipid, and other constituents entrapped inside its network. Glycinin (11S globulin) and  $\beta$ -conglycinin (7S globulin) are two major proteins in soybeans, which account for about 65–80% (by weight) of the total seed proteins present (Liu, 1999). In commercial tofu production, the 11S and 7S globulins can be induced to form gels by heat and coagulant addition. According to Kohyama, Sano, and Doi (1995), heat-induced denaturation resulted in the exposure of the hydrophobic regions of the soy proteins. These denatured soy proteins were negatively charged (Kohyama & Nishinari, 1993). Coagulant addition neutralized the net charge of denatured soy proteins. Consequently, the hydrophobic interaction induced the random aggregation of denatured soy proteins, leading to the gel formation of tofu (deMan, deMan, & Gupta, 1986). Rearrangement of the gel network during storage was still in progress, resulting in the increase of syneresis in tofu (Lee, 2007; Shen, 2008). Several macromolecules have been applied as food additives in tofu preparation to modify its texture, or to extend its shelf-life (Chang, Lin, & Chen, 2003; Karim, Sulebele, Azhar, & Ping, 1999; Kim & Han, 2003).

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The  $\gamma$ -polyglutamic acid ( $\gamma$ -polyglutamate, H form) and the  $\gamma$ -polyglutamates ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{NH}_4^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$  forms) are non-toxic polypeptides produced by *Bacillus subtilis* through a fermentation process (Ho, Yang, & Yang, 2006). They are consisted of numerous glutamic acid units connected by  $\alpha$ -amino and  $\gamma$ -carboxyl groups. These polypeptides usually have a molecular weight ranging from 5000 up to 900,000. The  $\gamma$ -polyglutamates not only possess excellent water absorption property but also have good capacity in coordination of metal ions (Ho et al., 2006). Those properties allow it to have a broad range of industrial applications, including the use of nutrition supplements and food additives. Although  $\gamma$ -polyglutamate has been reported to be used as an antifreeze agent, or the carrier for encapsulation (Chiu et al., 2007; Mitsuiki, Mizuno, Tanimoto, & Motoki, 1998), its water adsorption capacity have not been widely studied in the food systems.

Understanding the mechanism involving in the interactions between soy proteins and macromolecules is important for exploring its potential in developing a novel gel texture. Dynamic rheological analysis along with small amplitude oscillatory tests has been used to give a general indication of the structure change in soy protein gel under non-destructive condition (Apichartsrangkoon, 2003; Kohyama et al., 1995; Kohyama, Yoshida, & Nishinari, 1992; Maltais, Remondetto, & Subirade, 2008). Scanning electron microscope (SEM) has been used to provide a stereoscopic image of the fine structure of tofu (Kao, Su, & Lee, 2003; Shen, 2008). The objective of this study was to investigate the effect of  $\gamma$ -polyglutamate addition on the microstructure and the rheological properties of tofu.

## 2. Materials and methods

### 2.1. Materials

Non-GMO soybeans were purchased from a local supplier. The  $\gamma$ -polyglutamates in  $\text{Na}^+$  form of low molecular weight (low MW, 200–400 kDa), medium molecular weight (medium MW, 600–800 kDa), and high molecular weight (high MW, 1000–1500 kDa) were provided by Vedan Enterprise Co (Taichung, Taiwan). Glucono- $\delta$ -lactone (GDL) was obtained from Sigma–Aldrich Co (St. Louis, USA).

### 2.2. Preparation of tofu

Tofu was prepared according to the method as in Liu and Chang (2003) with a few modifications. Soybeans (1000 g) were washed and soaked in a tank of 3000 mL distilled water at 25 °C. After 8 h of soaking, another 3000 mL of distilled water was added and soybeans were ground with water in a food grinder (CL-010, Great Yen Electric Food Grinder Co. Ltd., Taoyuan, Taiwan). Subsequently, soymilk was strained through a 120 mesh sieve, and was heated to 95 °C for 5 min with regular stirring. The cooked soymilk was cooled in the refrigerator to 10 °C and mixed with 0.3% w/w GDL alone, or together with  $\gamma$ -polyglutamate at 0.1%, 0.15%, and 0.2% concentrations. The mixture was then transferred to a container (10 × 8 cm) and was heated in the water bath at 80 °C for 30 min. The pH of mixture was recorded during heating. After the tofu was cooled down to room temperature, it was stored for 1 day at 4 °C for further analysis. Tofu that was prepared with GDL alone was used as control.

### 2.3. Rheological measurement

A controlled stress dynamic rheometer (AR2000 ex, TA Instruments, Inc., New Castle, USA) was used to investigate the dynamic viscoelastic properties of samples. Stress amplitude sweeps were performed firstly to ensure that all measurements were carried out within the linear viscoelastic region (data not shown). According to the results, the stress amplitude of 1 Pa was chosen for further analysis.

The gelation process of tofu was observed based on the method of Maltais et al. (2008) with a few modifications. After the addition of GDL or  $\gamma$ -polyglutamate, cooked soymilk was immediately loaded onto the rheometer. A cone and plate geometry (40 mm diameter) with a cone angle of 2° was used. An oscillating stress was applied at the frequency of 1 Hz. Temperature sweep of sample was performed from 25 °C to 80 °C at the heating rate of 5 °C/min. Time sweep of sample was then carried out at 80 °C for 30 min. Storage modulus ( $G'$ ) and loss modulus ( $G''$ ) were recorded as a function of time.

The rheological properties of tofu were measured according to the method of Molina, Puppo, and Wagner (2004). Tofu was cut into cylinders with 40 mm in diameter and 2 mm in thickness. The parallel plate geometry (40 mm diameter) with a gap of 2 mm was used. Frequency sweep was conducted on the sample at 4 °C from 0.01 to 10 Hz. All the rheological measurements were carried out in triplicates.

### 2.4. Syneresis analysis

The syneresis of tofu was measured according to the method proposed by Armstrong, Hill, Schrooyen, and Mitchell (1994) with modifications. Tofu was cut into slices with 15 mm in diameter and 5 mm in thickness. A group of six pieces of sliced sample were weighted and put on the stainless steel mesh inside a plastic box. The mesh was lifted by small sticks. Thus, the exuded liquid could drip away from the sliced tofu samples. The box was sealed with parafilm to prevent the evaporation of free water. The sample was stored in

the box at 4 °C for 24 h. The total liquid exuded during the 24 h storage period was weighted. Syneresis was then expressed as the percentage of exuded liquid weight to the sliced tofu sample weight.

### 2.5. Microstructure analysis

Scanning electron microscope (S-3000N, Hitachi High-Tech-nologies Co., Japan) was used to examine the microstructure of tofu. Tofu was cut into 5 × 5 × 5 mm cubes. Samples were frozen in liquid nitrogen and freeze-dried. Dried samples were placed on the aluminum stub and were fixed by using double-sided adhesive carbon-tapes. The nature-broken side of each cubic sample was faced on the top. Samples were then sputter-coated with gold. The voltage of microscope was operated at 15 kV for observation.

### 2.6. Statistical analysis

The Statistical Analysis System (SAS Institute Inc., Cary North, USA) software was used for data analysis. Effects of  $\gamma$ -polyglutamates addition were determined by using the Analysis of Variance (ANOVA), whereas Duncan's multiple range test was used for multiple comparison among the means of the samples.

## 3. Results and discussion

### 3.1. Gelation

Fig. 1 shows the pH of soymilk containing 0.3% GDL and 0.2%  $\gamma$ -polyglutamates in different molecular weights during heating at 80 °C for 30 min. The initial pH of soymilks was between 6.0 and 6.5. The pH of soymilk did not change significantly during 30 min heating. However, the pH of control soymilk containing GDL decreased gradually during heating. Similar changes in pH of soymilks containing GDL and 0.2%  $\gamma$ -polyglutamates in different molecular weights were observed. The final pH of soymilks was between 4.5 and 5.0. These results indicated that the addition of  $\gamma$ -polyglutamates did not affect the pH change of soymilk containing GDL during heating.

Fig. 2 illustrates the viscoelastic behavior of soymilk with 0.3% GDL during non-isothermal heating from 25 °C to 80 °C at 5 °C/min followed by isothermal heating at 80 °C for 30 min. Fig. 2A shows the gelation curve of soymilk as a function of temperature. The values of  $G'$  were not stable around 0.01 Pa, and the values of  $G''$  decreased

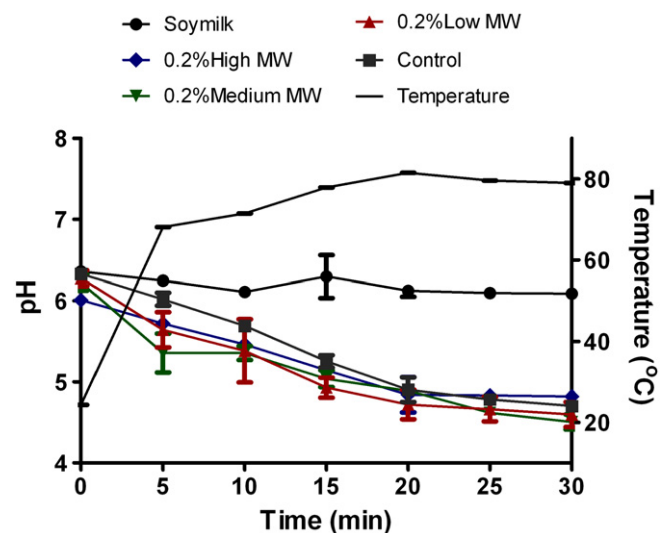


Fig. 1. The pH of soymilk containing 0.3% (w/w) GDL and 0.2%  $\gamma$ -polyglutamates in different molecular weights during heating at 80 °C for 30 min.

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