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Effects of endogenous small molecular compounds on the rheological properties, texture and microstructure of soymilk coagulum: Removal of phytate using ultrafiltration

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ABSTRACT

This study aims to clarify the roles played by endogenous small molecular components in soymilk coagulation process and the properties of gels. Soymilk samples with decreasing levels of small molecules were prepared by ultrafiltration, to reduce the amount of phytate and salts. $CaSO_4$-induced coagulation process was analyzed using rheological methods. Results showed that removal of free small molecules decreased the activation energy of protein coagulation, resulting in accelerated reaction and increased gel strength. However, too fast a reaction led to the drop in storage modulus (G′). Microscopic observation suggested that accelerated coagulation generated a coarse and non-uniform gel network with large pores. This network could not hold much water, leading to serious syneresis. Endogenous small molecules in soymilk were vital in the fine gel structure. Coagulation rate could be controlled by adjusting the amount of small molecules to obtain tofu products with the optimal texture.

1. Introduction

Tofu is a traditional food that has been widely consumed in the East and Southeast Asia since the ancient times. Although tofu can be prepared using various coagulants (salts and acids) and processing techniques, this product is essentially a salt- or acid-coagulated soy protein gel, with lipids and other constituents trapped in its network (Saowapark, Apichartsrangkoon, & Bell, 2008). Tofu coagulation comprises two steps, namely, 1) protein denaturation by heat and 2) hydrophobic coagulation promoted by protons from glucono-δ-lactone (GDL) or calcium ions. Regardless of the type of coagulant, it promotes protein aggregation mainly via hydrophobic interactions. Charge-charge interactions

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may also be subordinately involved (Kohyama & Nishinari, 1992; Kohyama, Sano, & Doi, 1995).

Protein content and composition as well as mineral content vary because of differences in varieties and cultivating conditions (Ishiguro, Ono, Wada, Tsukamoto, & Kono, 2006; Kong & Chang, 2012; Poysa & Woodrow, 2002). Coagulation induced by a certain coagulant is largely affected by protein composition and small molecules in soymilk. Coagulant requirement of particulate protein ($d > 40$ nm) is lower than that of non-particulate or soluble protein fractions ($d < 40$ nm) (Ono, Katho, & Mothizuki, 1993). However, the non-particulate fraction preferentially aggregates to form new protein particles in soymilk, wherein particulate protein was surrounded by small non-particulate protein molecules. Gel networks are formed by the aggregation of newly formed and original particles, with lipid droplets conjugated into the coagulum (Guo, Ono, & Mikami, 1999). In contrast to soybean with high levels of β-conglycinin (7S), soybean varieties with high glycinin (11S) content require low amounts of coagulant to form tofu gels because of the relatively high level of particulate protein in soymilk; the resulting tofu samples exhibit increased hardness (Guo & Ono, 2005). However, the mechanism proposed by Guo et al. (1999) did not consider other non-protein constituents in soymilk.

Our previous study verified that small molecular compounds such as polyacid ions in soymilk could reduce the odds of direct interaction between Ca^{2+} and protein by preferential consumption of Ca^{2+} and formation of unionized compounds (Wang, Xie, & Guo, 2015). Phytic acid is an important small molecular component in soybean. Several studies revealed the effect of phytic acid or phytate on tofu quality. Saio, Koyama, Yamazaki, and Watanabe (1969) reported that an increase in phytic acid content in soymilk resulted in slow coagulation reaction between soybean protein and calcium. However, this result has not been confirmed by any kinetic models. Phytic acid contributes to the soft texture and high yield of tofu because this compound consumes calcium ions (Ishiguro et al., 2006; Saio et al., 1969; Toda, Takahashi, Ono, Kitamura, & Nakamura, 2006; Toda et al., 2003). However, these studies did not clearly reveal the effects of phytate on tofu structure and protein coagulation kinetics. As such, the mechanism through which endogenous phytate in soymilk affects the coagulation and microstructure of tofu must be further investigated. Moreover, the interaction of phytate and protein with calcium induces a restricting effect on phytate (Toda, Nakamura, Takahashi, & Komaki, 2009).

In this study, endogenous free small molecular compounds (molecular weight of <10 kDa) were removed using an ultrafiltration membrane. Soymilk samples with different amounts of small molecules were then prepared. Rheological properties during coagulation process, texture profile and microstructure of tofu were assessed. In addition, the mechanism through which coagulation rate affects the formation of gel structure and texture was discussed.

2. Materials and methods

2.1. Materials

Soybean [*Glycine max* (L.) Merr.] was harvested from Heilongjiang Province, China in 2015. Seeds were stored at 4 °C and used within 1 year. All reagents were of analytical purity and no further purification was needed.

2.2. Preparation of soymilk, coagulum and squeezed firm tofu

Briefly, 100 g of soybean seeds were washed and soaked in distilled water overnight at 4 °C. The swelled beans were drained and ground with 700 mL of distilled water in a blender (Joyoung C-020, Shandong, China) for 2 min. The homogenate was filtered through a defatted cotton sheet to remove okara. The filtrate was heated in a water bath at 95 °C for 5 min and rapidly cooled to 25 °C in an ice water bath. The heated filtrate was designated as soymilk.

To prepare soymilk coagulum (coagulated gels) samples for texture analysis, soymilk (100 mL) was poured into a beaker. The soymilk was added with 10% freshly prepared $CaSO_4$ suspension at 25 °C to reach a final concentration of 0.2%. The mixture of soymilk and $CaSO_4$ was quickly stirred for 10 s and immediately distributed into five plastic syringes (20 mL in volume, 20 mm in diameter and 95 mm in length). The filled syringes were placed in a water bath at 70 °C for 1 h, cooled to room temperature, and stored in a refrigerator at 4 °C overnight for aging. For simple morphological observation, 20 mL of soymilk was coagulated in a beaker.

Briefly, to preparing squeezed firm tofu, 150 mL of soymilk was coagulated, using similar steps. After coagulation at 70 °C for 1 h, the curd was evenly cut five times both horizontally and vertically with a knife. Cracked curd was poured into a cubic plastic mold (75 mm long, 55 mm wide and 35 mm deep) and then wrapped with gauze. The curd was pressed at 5.56 g/cm^2 for 15 min and 11.60 g/cm^2 for another 15 min. Then the tofu samples were carefully removed and weighed. Tofu yield was determined as fresh weight of tofu preparing using 150 mL of soymilk. The samples were then stored at 4 °C overnight. Furthermore, the samples were placed at room temperature for 1 h prior to texture analysis.

2.3. Removal of small molecules in soymilk through ultrafiltration

Ultrafiltration was conducted in accordance with the method of Wang et al. (2015) using a 10 kDa ultrafiltration membrane (Mosu, Shanghai, China) to remove small molecules from soymilk. Soymilk circulated through the membrane and a flow solution consisting of only small molecular compounds (molecular weight <10 kDa) was separated from an outlet, to continuously concentrate the soymilk. As the ultrafiltration proceeded, a set volume of concentrated soymilk samples were obtained at different times, and named as CSM1, CSM2 and CSM3 (with increased protein concentration). The original soymilk sample was named as OSM. Protein contents of OSM and CSM samples were determined using the method of Bradford (1976). The CSM samples were diluted separately with distilled water to reach the same level of soluble protein as OSM. The diluted soymilk samples were designated as DSM1, DSM2, and DSM3, with decreasing levels of small molecules.

2.4. Determination of chemical composition, particle size and zeta potential of soymilk samples

Soluble protein content of soymilk was quantified using Bradford (1976) method. Conductivity was measured with a DDSJ-308A conductivity meter (INESA, Shanghai, China). Mineral content was determined using ICP-AES method according to ISO 27085-2009 with an ICP 6300 atomic emission spectrometer (Thermo Fisher Scientific, German). Phytic acid content of soymilk was measured through FTIR analysis according to the method proposed by Isiguro, Ono, Nakasato, and Tsukamoto (2005). Particle size distribution and zeta potential were measured at 25 °C with a Nano ZS90 zetasizer (Malvern, Worcestershire, UK).

2.5. Dynamic rheological measurement during coagulation

The coagulation process was observed using a dynamic rheometer (DHR-1, TA Instruments, Delaware) operated with a 40 mm parallel plate geometry following the method of Bi, Li, Wang, and Adhikari (2013). Measurements were performed at a constant strain of 1% within the linear viscoelastic region (0.3–2% was sufficient for