



# Effects of high-temperature pressure cooking and traditional cooking on soymilk: Protein particles formation and sensory quality



Zuo Feng<sup>a,b</sup>, Peng Xingyun<sup>a</sup>, Shi Xiaodi<sup>a</sup>, Guo Shuntang<sup>a,\*</sup>

<sup>a</sup> College of Food Science and Nutritional Engineering, China Agricultural University, No. 17, Qinghua Donglu, Haidian District, Beijing 100083, China

<sup>b</sup> College of Food Science, Heilongjiang Bayi Agricultural University, Daqing, Heilongjiang 163319, China

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Glycine (PubChem CID: 750)

Ethylenediaminetetraacetic acid (PubChem CID: 6049)

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## ABSTRACT

This study focused on the effect of high-temperature pressure cooking on the sensory quality of soymilk. Soymilk was prepared by high-temperature pressure cooking (105–125 °C and 0.12–0.235 MPa) and traditional cooking (97 °C and 0.1 MPa). The size distribution and composition of protein particles and the rheological properties of soymilk were compared. Results showed that the content of protein particles and the average size of soymilk particles were higher in high-temperature pressure cooking than in traditional cooking ( $p < 0.05$ ). High-temperature pressure cooking affected soymilk protein denaturation and favored protein aggregation. Similar to traditional soymilk, soymilk cooked at 115 °C was categorized as a Newtonian fluid but was found with increased viscosity in the rheological test. Soymilk cooked at 115 °C for 10 min exhibited a homogeneous, smooth, and creamy texture with a high acceptability in the sensory test.

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## 1. Introduction

Soymilk is a homogeneous protein colloid obtained from a series of processing technologies, such as soaking, grinding, soy pulp removing, and cooking (Guo, Ono, & Mikami, 1997). Raw soymilk should be heated and kept boiling for 5–15 min to kill food pathogens and denature lipoxigenase and other anti-nutrition factors, such as trypsin inhibitors and lectin. The heating of raw soymilk assures edible safety, improves nutritional value and sensory quality, and extends product shelf-life (Kumar, Rani, Tindwani, & Jain, 2003; Kwok & Niranjani, 1995).

Soymilk protein subunits dissociate and reassociate to form aggregates accompanying denaturation process when soymilk is boiled under atmospheric pressure. As a result, nearly 50% of soymilk protein exists as particulate aggregates with diameters of >40 nm (Guo et al., 1997; Wang, Johnson, & Wilson, 2003). Protein subunits form particles through heat-induced protein aggregation and assemble into consistent spatial structures. The basic (B) subunit of globulin (11S) and the  $\beta$  subunit of  $\beta$ -conglycinin (7S) are present in the core of protein particles; the  $\alpha$  and  $\alpha'$  subunits of 7S and the acidic (A) subunit of 11S are found at the outer shell of protein particles (Ren, Tang, Zhang, & Guo, 2009b). Moreover, soy whey proteins interact with 7S and form protein particles at relatively low denaturation temperatures (Ren, Tang, Zhang, & Guo, 2009a). The content of heat-induced protein particles differs among soybean varieties and processing technologies but

\* Corresponding author.

E-mail address: [shuntang@cau.edu.cn](mailto:shuntang@cau.edu.cn) (S. Guo).

generally ranges from 35% to 75% (Guo & Ono, 2005; Guo et al., 2002; Nik, Tosh, Woodrow, Poysa, & Corredig, 2009). Previous studies reported that the content and physicochemical properties of the formed protein particles greatly influence the sensory quality and processability of soymilk (Nik, Tosh, Poysa, Woodrow, & Corredig, 2008; Ono, Katho, & Mothizuki, 1993).

In traditional soy food production, cooking increases the temperature of soymilk through convective heat transfer under atmospheric pressure. In industrial-scale production, traditional cooking normally results in the non-uniform heating of soymilk. High-temperature pressure cooking (HTPC) enhances heat transfer efficiency by applying pressure cooking and by increasing the cooking temperature above the boiling point of water. However, studies on the effects of HTPC on the quality and processability of soymilk have been rarely performed. This study focused on soymilk protein particles and evaluated the effects of HTPC and traditional cooking on the formation, size, and composition of protein particles. This study also aimed to clarify the effects of HTPC on the rheological property and sensory quality of foods and thus apply HTPC to industrial production.

## 2. Materials and methods

### 2.1. Materials

Soybean was purchased from a market. Coomassie brilliant blue G-250 was purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, USA). All chemical reagents used in this study were of reagent grade.

### 2.2. Preparation of soymilk

Soybeans were washed and steeped with a threefold weight of water for 12 h at room temperature. The excess water was discarded. The hydrated soybeans were ground with a sevenfold weight of water by a pilot scale soymilk blender equipped with a 0.15 mm screen (Kangdeli Mechanical Company, Beijing, China). After soy pulp was removed, the obtained raw soymilk (protein content,  $3.05 \pm 0.17$  g per 100 g soymilk; dry matter,  $6.07 \pm 0.24$  g per 100 g soymilk; pH =  $6.80 \pm 0.05$ ) was divided into two parts and used for traditional cooking or HTPC.

One part of the raw soymilk was transferred into the cooking equipment (DDJ-80, Kangdeli Mechanical Company, Beijing, China). The raw soymilk was heated at rates of  $8^\circ\text{C}/\text{min}$  and kept at boiling temperature for 10 min (unless otherwise specified) with continuous stirring under atmospheric pressure. The heating rate was controlled by adjusting the injection rate of steam. Heated soymilk was quickly cooled in an iced water bath to room temperature. The obtained soymilk was designated as traditional soymilk. The other part of the raw soymilk was cooked with the same equipment in the pressure-cooking mode. Raw soymilk was heated until boiling at heating rates of  $8^\circ\text{C}/\text{min}$ . When exhaust the excess air in the equipment, the pressure-cooking mode was subsequently applied to raise the temperature to 105, 110, 115, 120, and  $125^\circ\text{C}$  respectively for 10 min (unless otherwise specified). The obtained product was cooled in an iced water bath and designated as HTPC soymilk.

### 2.3. Determination of the content of soymilk protein particle

According to the method reported by Guo et al. (1997), soymilk was centrifuged at  $156,000\times g$  for 30 min at  $20^\circ\text{C}$ . The protein concentrations of the whole soymilk before and after centrifugation were measured by the Bradford method (Ono, Takeda, & Guo,

1996). The protein particle content of the samples were calculated using the following formula:

$$\begin{aligned} \text{Protein particle content (\%)} &= 100\% \\ &\times (\text{Protein concentration of the sample} \\ &- \text{Protein concentration of the supernatant}) \\ &/ (\text{Protein concentration of the sample}) \end{aligned}$$

### 2.4. Distribution of lipids in soymilk

The soymilk was separated into the precipitate, supernatant, and floating fractions by centrifugation at  $156,000\times g$  for 30 min at  $20^\circ\text{C}$ . Each fraction was frozen in liquid nitrogen and lyophilized with a freeze dryer FD-1 (Beijing Boyikang Co., Ltd., Beijing, China) at  $-50^\circ\text{C}$ . The freeze-dried samples contained little moisture (about 0.01%). The lipid content of each fraction was estimated by determining the weight on a dry basis before and after defatting with *n*-hexane.

### 2.5. Analysis of particle size distribution

The distribution of particle sizes was measured by laser light-scattering with a Series Particle Size analyzer (Beckman Coulter LS 230; California, USA) equipped with a small volume module sample platform. The refractive indexes used for the dispersed phase and water were 1.570 and 1.333, respectively. Each sample was measured in triplicate and expressed as the percentage of volumetric particle size distributions by the Beckman Coulter LS Version 3.29 analytical software.

### 2.6. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE in the presence of 2-ME was performed on a vertical gel of 1 mm thickness (BIO CRAFT model BE-210N, Japan) in an alkaline discontinuous buffer. The concentrations of the stacking gel and the separating gel were 4% and 12.5%, respectively. Subsequently, 1 mg protein was carefully weighed and dispersed in the sample buffer. The sample buffer contained 0.125 M Tris–HCl (pH 6.8), 1% SDS, 6 M urea, 20% glycerol, 2% 2-mercaptoethanol, and 1% bromophenol blue. The solution was heated at  $95^\circ\text{C}$  for 5 min. Aliquots (5  $\mu\text{L}$ ) were loaded onto the gel. After the run was completed, the protein bands in the gel were stained with Coomassie brilliant blue G-250. The SDS–PAGE images were captured by an HP scanner (HP 1000).

Densitometric analysis of the stained gels was carried out with a BIO-RAD Multi-Analyst instrument (BIO-RAD, Hercules, CA).

### 2.7. Measurement of sulfhydryl (SH) group content

The SH group content on the protein surface was estimated according to the method described by Ou, Kwok, Wang, and Bao (2004). In brief, 1 mL of soymilk and 9 mL of anhydrous acetone were mixed and stirred, then held for 10 min before centrifugation at  $3000\times g$  for 15 min. The precipitate was washed with 5 mL of acetone and centrifuged. The wash procedure was performed thrice. The residual acetone in precipitate was removed by evaporation with streams of cold air. The precipitate was dissolved in 5 mL of buffer, which contained 10.4 g of trihydroxymethyl aminomethane (Tris), 6.9 g of glycine, and 1.2 g of ethylenediaminetetraacetic acid (EDTA) in 1 L of deionized water at pH 8.0. A mixture of 1 mL of pretreated soymilk (as described above), 2.0 mL of buffer, and 0.02 mL of Ellman's reagent (DNTB) was prepared; the reaction was incubated at  $25^\circ\text{C}$  for 5 min. The absorbance at 412 nm was obtained with a Shimadzu UV-1800

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