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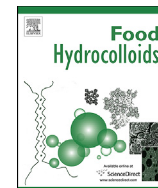

Characterization of particles in soymilks prepared by blanching soybeans and traditional method: A comparative study focusing on lipid-protein interaction

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Bromophenol blue (PubChemCID: 8272)
Coomassie brilliant blue G-250 (PubChemCID:61364)
Glycine (PubChemCID:750)
Hexane (PubChemCID:8058)
Methanol (PubChemCID:887)
2-mercaptoethanol (PubChemCID:1567)
Sodium azide (PubChemCID:33557)
Sodium bicarbonate (PubChemCID:516892)
Sodium dodecyl sulfate (PubChemCID:3423265)
Urea (PubChemCID:1176)

A B S T R A C T

The beany flavor of soymilk can be eliminated by blanching soybeans at high temperatures. However, compared with the traditional preparation methods, this pre-extraction heating process may have additional effects on protein aggregation. In this study, the distribution of lipid and protein was analyzed by an ultracentrifugation method that separates different fractions of soymilk on the basis of the size and density of particles. For traditional soymilk, results showed that ~95% of total lipids were enriched in the floating fraction. For the soymilk prepared by blanching soybeans (blanched soymilk), only ~ 55% of total lipids were found in the floating fraction, in accordance with ~40% of total lipids presenting in the particulate fraction. SDS-PAGE also showed that the oleosin band existed in the particulate fraction of the blanched soymilk. Moreover, compared with traditional soymilk, blanched soymilk exhibited an obvious red shift in the intrinsic fluorescence spectrum and was composed of particles with significantly high surface hydrophobicity. In conclusion, blanching process alters the denaturation and aggregation mechanism of soymilk particles, thus trapping parts of oil bodies into particulate proteins.

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1. Introduction

Soymilk is a nutritious protein beverage that comprises ~3% proteins, ~2% fats and ~2% non-lactose carbohydrates (i.e. sucrose, stachyose, rhamnose) (Giri & Mangaraj, 2012). Traditional production of soymilk uses water to extract imbibed soybean seeds at room temperature with mechanical grinding. After the removal of okara (the insoluble extraction), raw soymilk is heated to boiling and then cooled to obtain the final product. During soymilk production, the soybean protein bodies are firstly ground into fragments and suspended in an aqueous extract. Upon cooking on the raw soymilk, protein molecules with different thermal stability values are denatured, dissociated from the fine protein body fragments, and polymerized to form particulate proteins that vary in size, composition and morphology (Ono, Choi, Ikeda, & Odagiri, 1991). The particles formed in this process can influence the stability and processability of soymilk products (Guo et al., 2002; MalakiNik, Tosh, Poysa, Woodrow, & Corredig, 2008). Fat globules or soybean oil bodies are the other important colloidal particles found in soymilk. Natural soybean oil bodies, which have an average size of 300 nm–400 nm, possess a micelle structure that is made up of oleosins, phospholipid membranes, and triglyceride (Ono, 2008). After being cooked, the oil bodies exist in the form of

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either monomer (0.3 μm) or aggregate (1 μm–30 μm).

Guo, Ono, and Mikami (1997) described the forming mechanism of particulate protein (>40 nm) and soluble protein (<40 nm) in the preparation of traditional soymilk. In raw soymilk, oil bodies are incorporated within the structure of protein bodies consisting of β-conglycinin (7S) and glycinin (11S). When raw soymilk is heated to 75 °C, the 7S dissociates with the release of the oil body at the same time. When the temperature reaches 85 °C, the 11S begins to denature, and the oil body is completely released. The dissociated 11S and 7S subunits then form a new particulate protein by aggregation and rearrangement. On the basis of the previous works, Ren, Tang, Zhang, and Guo (2009a) revealed the structure of this heat-induced particulate protein. The basic (B) subunit of the 11S and the β subunit of the 7S form the core of the particulate protein, whereas the hydrophilic subunits, such as acidic (A), α, and α′, are located around the core and stabilize the formed particle. In addition to 7S and 11S, whey proteins consisting of ~10% of total soymilk protein also form large particulate proteins in this heat-induced aggregation process. However, the α and α′ subunits of 7S can hinder the growth of the large particulate protein (Ren, Tang, Zhang, & Guo, 2009b).

The *lipoxygenase* in soybean is responsible for the beany flavor produced by the enzymatic oxidation of unsaturated fatty acids, which is unacceptable for western customers (Axelrod, 1981). Besides the traditional method for soymilk production, manufacturers also widely apply a blanching process to inactivate lipoxygenases before extraction to inhibit the formation of beany flavors (Lopez & Burgos, 1995). The blanching method for soymilk production normally includes immersing soaked soybeans in hot water (≥80 °C) for the extended time (≥5 min), hot water grinding and extraction, the removal of okara and cooking process. Aside from the denaturation of lipoxygenase and the elimination of beany flavors, blanching process also brings about other significant effects. Peng and Guo (2015) reported that the 7S was denatured within the soybean seed in the non-hydrated folded state, when blanching was applied. It also changed the textural property of soymilk gel. The soymilk prepared by blanching soybeans (blanched soymilk) exhibits a smaller average particle size but higher tendency to form precipitates than traditional soymilk (Shimoyamada, Mogami, Tsuzuki, & Honda, 2014); therefore, the former has a less stable shelf-life than the latter. According to the above studies, traditional soymilk and blanched soymilk have different stability and gel properties, but the reason still remains unclear.

This study compared the surface characteristics, chemical composition, morphology, and size distribution of the particles of blanched and traditional soymilks. Furthermore, the distributions of lipid and protein in different soymilk fractions were determined. The results contribute to the production of high-quality soymilk products.

2. Materials and methods

2.1. Materials

Soybeans harvested from northeast China were obtained from a local market and stored at room temperature. Coomassie brilliant blue G-250 was purchased from Sigma Chemical Co., Ltd. (St. Louis, Mo., USA). 2-mercaptoethanol was purchased from Amresco Co., Ltd. (USA). Urea was purchased from Beijing Beihua Fine Chemicals CO., Ltd (Beijing, China). All reagents were of analytical purity and no further purification was needed before use.

2.2. Soymilk preparation

Soymilk was prepared according to the following procedures. Soybeans (50 g) were washed and soaked in 150 mL distilled water for 10–12 h at 4 °C. After removing the excessive water, the hydrated soybeans were mixed with 150 mL of 3‰ (w/v) $NaHCO_3$ solution in a 400 mL beaker. The beaker was placed into a thermostat water bath and the mixtures were maintained at 80 ± 2 °C for 5 min. After blanching, soybeans were drained and then ground with 350 mL of distilled water at 80 °C using a soymilk grinder (JYL – C020, Shandong Joyoung Household Electrical Appliances Co., Ltd., China) for 2 min. The resulting slurry was filtered through a defatted cotton sheet and raw soymilk was obtained. This raw soymilk was kept in a water bath at a temperature above 95 °C for 5 min, and then quickly cooled to 40 °C in an iced water bath. The soymilk obtained through the above steps was designated as the soymilk prepared by blanching soybeans (or the blanched soymilk), and coded as BT-80. Traditional soymilk was also prepared according to the above method, except without the aforementioned blanching process. The obtained traditional soymilk was used as the control (coded as CN-20).

2.3. Preparation of soymilk ultrafiltrate

The ultrafiltrate of soymilk was prepared by using a stirred ultrafiltration cell (Micon-8200, Millipore Corporation, Bedford, MA 01730 USA) equipped with the ultrafiltration membrane (MWCO of 10 kDa). The ultrafiltration process was operated at 4 °C in a refrigerator. The ultrafiltrate of soymilk was used to simulate soymilk dispersion.

2.4. Preparation of particle, soluble, and floating fractions

According to the method of Guo, Ono, and Mikami (1999), p. 11 mL of soymilk was separated into floating fraction, soluble fraction, and particulate fraction by centrifugation at 156, 000 × g for 30 min at 20 °C. The floating fraction in the top layer and particle fraction in the bottom layer were carefully collected and washed with distilled water for three times and dispersed with 11 mL ultrafiltrate by a tissue grinder (Wheaton, NJ, USA) for 10 min. The soluble fraction was directly adjusted to a volume of 11 mL by using ultrafiltrate. The particulate, soluble, and floating fractions were lyophilized and stored in the refrigerator at 4 °C before use.

2.5. Analysis of protein particle size distribution

According to the method of Chen and Ono (2014), the distribution of the protein particle size was measured by laser light scattering using a series particle size analyzer (Beckman Coulter LS 230) with a small volume module sample platform. The refractive index used for the dispersed phase was 1.570, and the correspondent to water was 1.333. Soymilk was diluted until the protein content was 0.1–0.3 mg/mL. Each sample was measured three times. The particle size distribution was shown by the volume ratio of particles of different diameters.

2.6. Fluorescence emission spectroscopy

Fluorescence emission spectroscopy was determined by using an LS-55 luminescence spectrometer fluorescence spectrometer (Perkin Elmer, Waltham, MA, USA), according to the method of Shen and Tang (2012) with slight modification. Protein dispersions (0.1–0.2 mg/mL) were prepared with 0.1 M phosphate buffer (pH 6.80). Protein solution was excited at 290 nm, and the emission spectra were recorded from 300 nm to 420 nm at a constant slit of 10 nm. The scan rate was 200 nm/min.

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