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Behaviors of particle size and bound proteins of oil bodies in soymilk processing

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ABSTRACT

In unheated soymilk, extrinsic proteins are bound to intact oil bodies coated by one monolayer of phospholipids and oil body intrinsic oleosins. In this study, effects of heating (70–100 °C; 0–30 min) on particle size and bound proteins of oil bodies were examined in suspension (oil bodies from unheated soymilk into deionized water) and soymilk. Mass ratio of extrinsic proteins/oleosins of oil bodies in unheated suspension and soymilk was respectively 1.1 and 2.5. By heating, extrinsic proteins released from oil bodies with different rates, and Z-average size of oil bodies increased in the beginning; afterwards, residual extrinsic proteins (extrinsic proteins/oleosins: 0.31 in suspension and 0.74 in soymilk; 100 °C, 4 min) and Z-average size were affected little. The amount of residual proteins at 80–100 °C was negatively correlated with Z-average size of oil bodies in suspension (R^2 = 0.996) and soymilk (R^2 = 0.890).

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1. Introduction

Soymilk, a traditional Asian beverage derived from soybean, is turbid and stable colloidal solution that mainly contains lipids, proteins, and carbohydrates (Ono, 2008). In soybean seed cell, major storage proteins (β-conglycinin and glycinin) and minor P34 probable thiol protease (P34; also Gly m Bd 30K) are deposit in protein storage vacuoles (PSVs) (Kalinski, Melroy, Dwivedi, & Herman, 1992; Ogawa et al., 1993), and lipids (mainly triacylglycerols, TAGs) in oil bodies (OBs) (Huang, 1992), having TAGs matrix embedded with phospholipids and intrinsic oleosins (24, 18, and 16 kDa) (Zhao, Chen, Cao, Kong, & Hua, 2013). Glycinin is hexameric protein, and its subunit (A-SS-B) is composed of one acidic polypeptide (A) and one basic polypeptide (B) linked by one disulfide bond (SS). β-conglycinin is trimeric glycoprotein including α′, α, and β subunits. Wadahama et al. (2012) reported that half of α′ and α subunits of β-conglycinin were SS-linked, together or with P34, in soybean seed cell: α′/α-SS-α′/α and α′/α-SS-P34, revealing that P34 existed as its monomer as well as α′/α-SS-P34 (actually, β-conglycinin (α′/α)-SS-P34). By soaking and grinding, soybean cells and PSVs are disrupted, while OBs are not. Extrinsic proteins, including glycinin, β-conglycinin, P34, and some other proteins, are bound to intact OBs in unheated soymilk. It was reported that pH 8.0 washing could remove almost all glycinin and β-conglycinin, but part of P34 from OBs; part of P34 was still remained by pH 9.0–10.0 washing, but totally removed by pH 11.0 washing (Chen & Ono, 2010a). Zhao et al. (2013) reported that P34 monomer and β-conglycinin (α′/α)-SS-P34 interacted with 24 kDa oleosin by covalent interaction (P34–24 kDa oleosin). By heating, extrinsic proteins released from OBs (Guo, Ono, & Mikami, 1997), and Chen, Zhao, Kong, Zhang, and Hua (2014) reported that large oil droplets (>1 μm) were formed, which was also found by other researchers (Cruz et al., 2007; Toda, Chiba, & Ono, 2007).

Soymilk protein, especially in its protein particle form, is important for the network formation in tofu, yuba, touhua, and fermented soymilk. Also, OBs are considered to be important for network formation of tofu and yuba. Ono (2008) reported that each OB in tofu curd was orderly covered by protein particles and soluble proteins to form one "block", and these "blocks" interacted with each other to form network for tofu structure. Chen and Ono (2010b) found that OBs in yuba were incorporated into the network comprised of protein particles and soluble proteins.

The researches above showed that thermal treatment not only could affect particle size but also protein amount and component of OBs, indicating that different thermal treatment would produce OBs with different properties, which would affect the microstructure of soymilk and its related products. However, no research has systematically examined the behaviors of particle size and

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bound proteins of OBs during thermal treatment, and whether oleosins release from OBs is also not clear. In this study, effects of heating (70–100 °C; 0–30 min) on particle size of OBs and behaviors of extrinsic (β-conglycinin, glycinin, and P34) and intrinsic (oleosins) proteins were examined. Further, OBs extracted from unheated soymilk were dispersed into deionized water to prepare OB suspension to repeat the experiment above, and compared to the results from soymilk.

2. Materials and methods

2.1. Materials

Soybean Heinong 64, harvested in 2013, was purchased from Northeast Soybean Research Institute (Harbin, China) and stored at 4 °C until use. All reagents were purchased from Sigma–Aldrich Trading Co., Ltd. or of analytical reagent grade. Precision Plus Protein All Blue (Bio-Rad, USA) was used as protein marker.

2.2. Unheated soymilk

Soybean (20 g) was soaked in deionized (DI) water at 4 °C for 18 h. Soaking water was poured off, and swollen soybeans were washed with fresh DI water three times. Swollen soybeans were ground in fresh DI water (1 g of dry soybean per 9 mL of DI water) with a blender (18,000 rpm, MJ-60BE01B, Midea, China) for 2 min. The homogenate was filtered through four layers of gauze, and the filtrate was deemed unheated soymilk (about pH 6.6).

2.3. OB suspension

Sucrose at a final concentration of 20 (w/w)% was added into unheated soymilk (150 g) and mixed well. The mixture was treated by centrifugation (25000*g*, 30 min; 4 °C) to obtain floating, supernatant, and precipitate fractions. Floating fraction was collected and dispersed into DI water to make total weight of 150 g (OB suspension).

2.4. Thermal treatment

Unheated soymilk and OB suspension were heated in 70, 80, 90, and 100 °C water baths for 0, 1, 4, 8, 15, and 30 min, and immediately cooled in tap water bath.

2.5. Isolation of OBs from soymilk and OB suspension by two-layer ultracentrifugation

Sucrose at a final concentration of 30 (w/w)% was added into heated (unheated, control) soymilk or OB suspension and mixed well. Each sample (9.5 g) was added into ultracentrifuge tube, and to each tube, sucrose solution (3 g; 25 (w/w)%) was layered on the top of each sample. Then they were treated by a Beckman Optima L-XP ultra centrifuge (2,70,000*g*, 60 min, 4 °C; Beckman Coulter Inc., Brea, CA, USA), and solid OB pad (not contaminated with free extrinsic proteins) was floated on the top of sucrose solution.

2.6. Sample preparation for Tricine–SDS–PAGE

To each OB pad, DI water was added to the final volume of 5 mL, and mixed well to obtain OB pad suspension, which was defatted by diethyl ether (Tzen & Huang, 1992). Briefly, diethyl ether (15 mL; 4 °C) was added, thoroughly mixed for 30 min and centrifuged (15,000*g*, 5 min; 4 °C). The upper diethyl ether was removed, and the procedure above was repeated one more time. The residual diethyl ether was allowed to evaporate in a hood, and residual aqueous suspension was vortexed. Protein concentration of defatted OB pad suspension from unheated soymilk was determined by micro-Kjeldahl method, and 0.5 mL was diluted with Tricine–SDS–PAGE sample buffer to 2 mg/mL. The other defatted OB pad suspensions (0.5 mL) were diluted with the same volume of sample buffer above. This method, different from traditional method (samples were diluted with different volume of sample buffer to the same protein concentration), was beneficial for examining the protein release behaviors from OBs.

2.7. Tricine–SDS–PAGE

This was conducted according to the method by Schagger (2006). The concentrations of stacking and separating gels were 4% and 16%, respectively. Twenty microliters of 2-mercaptoethanol was added into each sample (1 mL), and heated in boiling water bath for 3 min. Then 7 μL was loaded into sample well, and electrophoresed at constant voltage of 30 mV until all samples entered into the stacking gel and then at constant voltage of 100 mV until end. The gel was stained using Coomassie Brilliant Blue G-250, and band intensities were analyzed by Image Lab Software (Bio-Rad, USA).

2.8. Sample preparation for nonreducing and reducing SDS–PAGE and diagonal electrophoresis

OB suspension was treated for 8 min in 70, 80, 90, and 100 °C water baths, and treated by two-layer ultracentrifugation as above. After collection of OB pad, supernatant (9.2 g) was collected by syringe, and residual liquid was removed. The inner face of tube was cleaned up by DI water and tissue paper without cleaning up the precipitate, and the collected supernatant was added back into the tube. The tube was vortexed till precipitate was dispersed into supernatant. The sample from OB suspension (100 °C, 8 min) was determined for protein concentration by micro-Kjeldahl method, and 0.5 mL was diluted with SDS–PAGE sample buffer to 2 mg/mL. The other samples (0.5 mL) were diluted with the same volume of sample buffer.

2.9. SDS–PAGE

SDS–PAGE was conducted with the method by Laemmli (1970) with the concentrations of stacking and separating gels being 5% and 12.5%, respectively. 2-mercaptoethanol (no 2-mercaptoethanol for non-reducing SDS–PAGE) was added into the samples above to the concentration of 2% (v/v), and heated for 3 min in boiling water bath. Then 7 μL was loaded into sample well. SDS–PAGE was performed at 15 mA until end. The gel was stained by Coomassie Brilliant Blue G-250.

2.10. Diagonal electrophoresis

Sample without 2-mercaptoethanol was loaded into a sample well and electrophoresed. After electrophoresis, the lane was excised from the gel and put into a 50 mL beaker. DI water (24.5 mL) and 2-mercaptoethanol (0.5 mL) were added and then put into boiling water bath for 3 min. The reduced lane was used for the second dimensional electrophoresis, and the gel was stained by Coomassie Brilliant Blue G-250. Here, the gel was shown as the format of false color via Image Lab Software.

2.11. Particle size analysis

Sucrose at a final concentration of 30 (w/w)% was added into soymilk and OB suspension and mixed well. The mixtures (40 g)

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