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Particle formation and gelation of soymilk: Effect of heat

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Backgrounds: Soymilk is a complex colloidal system that mainly comprises protein and lipid particles and other minor components. Soymilk is consumed worldwide as a nutritious protein beverage and used as raw material to produce soy gel foods, such as tofu and soy yogurt. Recently, soymilk has been applied in dairy industry as a functional ingredient to improve the texture, flavor, and nutritional value of various products. To fulfill these traditional and innovative applications, researchers must elucidate the structure—function relationship of the major components of soymilk.

Scope and approach: This review aims to provide a fundamental picture of the microstructural nature of soymilk and its gel. The behavior of particles during soymilk gelation is also discussed. This study emphasizes the influence of heat on formation of particles and gelation of soymilk.

Key findings and conclusions: When heating raw soymilk, the subunits of soy protein are denatured, dissociated, and subsequently aggregated to form particles with heterogeneous sizes, composition, and molecular structure. The ion- or acid-induced coagulation between denatured soy protein and lipid particles results in soymilk gelation. Models based on published data are proposed to illustrate protein—lipid interaction during soymilk production and gelation.

1. Introduction

Soymilk is widely consumed in East Asian countries as a traditional protein beverage and as raw material for tofu production. Imbibed soybean seeds are ground in water to produce a viscous slurry. After removal of insoluble residuals (okara) from the slurry, the aqueous dispersion (raw soymilk) is boiled for several minutes to obtain soymilk. Soymilk is a colloidal system that comprises ~3% proteins, ~2% fats, ~2% non-lactose carbohydrates, and minerals (Giri & Mangaraj, 2012). Aside from having these macronutrients, soymilk is rich in bioactive compounds such as isoflavone and saponin. These components provide multiple health benefits, including the ability of soymilk to lower blood cholesterol level and to reduce the risk of cardiovascular diseases (Ang, Kwik, & Theng, 1985; Friedman & Brandon, 2001). Soymilk has recently become popular in Western countries because of its high nutritional value and health benefits.

Soy protein and lipid interact with each other and form complex "particles" that differ in size and structure. The word "particle" is frequently used to describe the oligomeric or polymeric aggregates in soymilk. Depending on the chemical nature and research objectives, the meaning of "particle" varies in different studies. Therefore, all relevant terms used in this paper are defined in Table 1. The quality and processability of soymilk are closely associated with the nature of soymilk particles (e.g., particle composition, size distribution, and physicochemical characteristics) (Guo et al., 2002; Nik, Tosh, Poysa, Woodrow, & Corredig, 2008; Ono, Choi, Ikeda, & Odagiri, 1991). The nature of soymilk particles are determined during thermal processing of raw soymilk (Guo, Ono, & Mikami, 1997) accompanied by denaturation of soy globular proteins (glycinin, 11S, and β-conglycinin, 7S) (Molina, Papadopoulou, & Ledward, 2001; Nagano, Hirotsuka, Mori, Kohyama, & Nishinari, 1992).

Since the 1980s, researchers have exerted efforts to establish the mechanism illustrating the formation of protein particles and their gel-forming properties (Grygorczyk & Corredig, 2013; Guo & Ono, 2005; Guo et al., 1997; Ono et al., 1991). Models that elaborate Ca^{2+}- or δ-glucolactone (GDL)-induced soymilk gelation are gradually proposed and discussed (Guo et al., 2002; Kohyama, Sano, & Doi, 1995). Many studies have focused on the effect of heat and demonstrated the importance of optimized heat treatment to

Abbreviations: 11S, Glycinin; 7S, β-conglycinin; GDL, δ-glucolactone; OB, oil body; KTI, Kunitz trypsin inhibitor; LENP, lumry-eyring nucleated polymerization model; SSP, storage soy protein; TAG, triglycerides; WSP, soybean whey proteins.

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Table 1
Terms and definitions.

Term	Definition
Soymilk particle	All the proteins and lipids existing as monomer, oligomer or polymer in soymilk.
Protein particle	Also named particulate protein, The polymeric aggregates (diameter > 40 nm) formed from soy protein subunits.
Soluble protein	Monomer or oligomer of soy protein subunits (diameter < 40 nm).
Lipid particle	Monomer or aggregate of soybean oil body or coalesced soybean oil droplet.
Particulate fraction	Precipitates separated by ultracentrifuge.
Soluble fraction	Supernatants separated by ultracentrifuge.
Floating fraction	Buoyant layer separated by ultracentrifuge.

improve the qualities of soymilk and soymilk gel. This review presents the microstructural nature of soymilk and its gel. In addition, this paper focuses on the effects of heat on the formation of particles and gelation of soymilk. This study may contribute to the theory and practice on the production of soymilk and soy gel foods (e.g., tofu and soy yogurt).

2. Soymilk particles

Soybean seeds contain ~40% protein and 20% fat on dry weight basis (Kwok & Niranjan, 1995). Soymilk is obtained by extracting ground soybean with water. Soymilk generally contains 4%–6% protein (Cruz et al., 2007; Iwuoha & Umunnakwe, 1997), and nearly 50% of the protein exist in particles with a diameter of >40 nm (Ono et al., 1991). Similar to proteins, soy lipids are found in soymilk in the form of oil bodies (OBs) (Ono, 2008). Many studies have found that the quality and processability of soymilk are influenced by soymilk particles formed through the interaction between proteins and lipids (Guo & Ono, 2005; Ono, Katho, & Mothizuki, 1993; Tezuka, Taira, Igarashi, Yagasaki, & Ono, 2000).

2.1. Composition of soymilk particles

The chemical components of soymilk particles mainly include proteins and lipids. Soymilk can be easily fractionated into three parts (floating, soluble, and particulate fractions) through ultracentrifugation (156,000 $\times$ g, 30 min) (Guo et al., 1997; Ono et al., 1991). The precipitated fraction is composed of particulate proteins (with particle size of >40 nm). The supernatant fraction contains soluble proteins (with particle size of <40 nm), whereas all lipids exist in the floating fraction.

Soymilk protein subunits are unevenly distributed among soluble and particulate proteins. Soluble proteins are hydrophilic. The α and α' subunits of 7S, the acidic (A) subunit of 11S, and the Bowman–Birk inhibitor of whey soybean proteins (WSPs) are commonly found in soluble proteins. By contrast, other WSPs, the β subunit of 7S, and the basic subunit (B) of 11S are abundant in particulate proteins (Ren, Tang, Zhang, & Guo, 2009a). In addition to protein components, some polar lipids, mainly phosphatidylcholine, interact with the hydrophobic regions of particulate proteins, resulting in the presence of trace amounts of lipids (Ohtsuru, Ymashita, Kanamoto, & Kito, 1979).

The distribution of lipid in the three soymilk fractions is quite different from that of proteins because triglycerides (TAG) are prone to form buoyant fraction after centrifugation. In contrast to that of soluble and particulate fractions, the major component (>90%) of the floating fraction is TAG, which exists as OBs and accounts for 85% of the total lipids in soymilk. In addition, over 50% of the total protein present in the floating fraction are OB intrinsic oleosins (18 and 24 kDa) (Guo et al., 1997).

2.2. Size of soymilk particles

2.2.1. Whole soymilk

Dynamic laser scattering (DLS) is a useful method to analyze particle size distribution. The result of DLS analysis has been applied to evaluate the stability and processability of soymilk. Two typical peaks (0.1–1 μm and 2–10 μm) are characterized from the result of DLS analysis of soymilk (Nik, Tosh, Woodrow, Poysa, & Corredig, 2009). The smaller peak represents OB monomers (~400 nm) (Toda, Chiba, & Ono, 2007). However, the identity of the particles associated with the larger peak remains controversial. Toda et al. (2007) suggested that the larger peak originates from OB–protein aggregates. Moreover, Chen, Zhao, Kong, Zhang, and Hua (2014) combined the results of light microscopy and particle distribution and concluded that the large particles (>1 μm) of heated soymilk are oil droplets that originated from coalesced OBs.

2.2.2. Protein particle

Protein particles or particulate proteins are formed from aggregation of dissociated soy protein subunits during heating. Ono et al. (1991) analyzed the protein particles through ultracentrifugation and found that their diameter is > 40 nm. Thus, the protein particles are smaller than OBs and they possess considerably less volume than the lipid particles in soymilk. Ren et al. (2009a) examined the size distribution of protein particles by preparing non-fat soymilk from defatted low-heat soybean meal, and they confirmed that the size of the protein particles ranges between 40 nm and 200 nm.

2.2.3. Soluble protein

In contrast to the protein particles, the proteins with a diameter of <40 nm are defined by Ono et al. (1991) as soluble proteins, which are speculated to be different monomers of soy protein subunits. Subsequent studies have demonstrated through SDS-PAGE analysis that the soluble proteins are also composed of oligomer subunits, and these oligomers (e.g., small stable protein (20–40 nm) fractionated by ultracentrifugation) (Chen & Ono, 2014) are disulfide-linked aggregates of the A subunits of 11S (Chen & Ono, 2010; Kuipers & Gruppen, 2008; Sun et al., 2008).

2.3. Structure of soymilk particles

2.3.1. Protein particle

Protein subunits are heterogeneously distributed in different protein particles. The B subunit of 11S and β subunit of 7S tend to form particulate protein, whereas the A subunit of 11S and the α and α' subunits of 7S exist as soluble protein (Ono et al., 1991; Ren et al., 2009a; Tezuka, Yagasaki, & Ono, 2004). The 7S/11S ratio is negatively correlated with protein particle content; that is, the amount of particulate protein increases with 11S content in soymilk (Guo & Ono, 2005). By contrast, 7S exerts inhibitory effects on the formation of large protein particles (Guo et al., 2012).

Ren et al. (2009a) proposed a model based on the abundance of