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Interfacial and emulsifying properties of crude and purified soybean oil bodies

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ABSTRACT

Soybean oil bodies are natural and environment-friendly emulsifying agents from plant resources required as a substitute for emulsifiers such as low-molecular-weight one and animal proteins. The aim of this study was to reveal the interfacial properties and the emulsifying properties of two types of oil bodies extracted from soybean seeds at different pH conditions, that is purified oil bodies (OB) and crude oil bodies (OBC) including storage and other minor proteins. Particle size and zeta-potential measurements and SDS-PAGE revealed that the presence of the involved storage and other minor proteins promoted oil body aggregations and imparted the high zeta-potential to the oil bodies. OB adsorbed onto the oil-water (O/W) interface in closely packed state and formed more elastic adsorbed layer than OBC according to the results from tensiometry. Emulsions stabilized by OB (OB-E) were more stable against oil droplet coalescence than those by OBC (OBC-E) due to the absence of the involved proteins at the emulsion droplet surfaces. Cryo-SEM observation suggests that during emulsification most of the OB and OBC particles adsorbed onto the newly-created O/W interface and then rapidly ruptured.

1. Introduction

Many food products such as ice creams, dressings, and mayonnaises exist as an oil-in-water (O/W) emulsion where an oil phase is dispersed in a continuous aqueous phase. The emulsion system is usually stabilized by synthesized low-molecular-weight emulsifiers often in combination with natural resources, whereas consumers nowadays tend to avoid the emulsifiers because of an increasing demand for natural materials. High-molecular-weight functional animal proteins like milk proteins are widely used in the food industry, while such animal proteins often need more energy for the production, compared to that of plant materials (Pimentel & Pimentel, 2003; Pimentel et al., 2008). Plant materials also attract the consumers' attention due to their health benefits based on various secondary metabolites. Natural and environment-friendly emulsifying agents from plant resources are highly required as a substitute for functional animal proteins (Rodrigues, Coelho, & Carvalho, 2012; Karaka, Low, & Nickerson, 2015; McClements & Gumus, 2016).

Since the energy cost of soybean production is relatively lower than other crops mainly based on their high nitrogen fixation ability (Liu, 1997), soybean proteins as a natural-based emulsifying agent are enthusiastically investigated in the previous researches (Palazolo, Mitidieri, & Wagner, 2003; Palazolo, Sorgentini, & Wagner, 2005; Keerati-u-rai & Corredig, 2009; Palazolo, Sobral, & Wagner, 2011). Soybean proteins are usually isolated from defatted soybeans in the food industry and mainly consist of storage proteins, e.g. β-conglycinin (7S) and glycinin (11S). They are hydrophilic globular proteins that have good emulsifying properties (Chove, Grandison, & Lewis, 2001; Keerati-u-rai & Corredig, 2010; Molina, Papadopoulou, & Ledward, 2001), but the properties are not necessarily better than those of animal proteins (Hu, McClements, & Decker, 2003; Amine, Dreher, Helgason, & Tadros, 2014). In this context, recently, there have been an increasing interest in lipophilic membrane proteins called oleosin, another component of soybean seeds that can be also seen in various crop seeds (Rayner, 2015). Oleosins are composed of hydrophilic and hydrophobic domains (Huang, 1992), related to the surface activity at the air-water (A/W) or oil-water (O/W) interface. Nikiforidis

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et al. (2013) indicated that the purified oleosins from maize form strong elastic films at the A/W interface. Wijesundera et al. (2013) and Deleu et al. (2010) reported that oleosins from canola and rapeseed can stabilize O/W emulsions for several weeks. On the other hand, even though Samoto et al. (2007) successfully collected an oleosin-rich fraction from defatted soybean meals, almost pure oleosin fractions cannot be easily isolated from the meals due to strong tendency of hydrophobic oleosins to form aggregates without covering oil droplets and due to abundance of other seed proteins such as 7S and 11S. This situation is different from the other crops such as maize, sesame, and rapeseed.

Soybean oleosins are originally present in an intracellular organelle, so-called oil body, oleosome or lipid body depending on the literature. They are known to cover neutral lipids globules containing phytosterols and tocopherols together with phospholipids (Fisk & Gray, 2011; Tzen & Huang, 1992; Chen, Cao, Zhao, Kong, & Hua, 2014). The hydrophobic domain of oleosins is embedded into the lipids globules across the phospholipids monolayer, while the hydrophilic domains cover the surface of the lipids globules (Huang, 1992; Tzen & Huang, 1992). Since the oleosins and phospholipids form a strong membrane, oil bodies are stable against coalescence and lipid oxidation during seed dehydration (Huang, 1992); therefore, several researchers expected oil bodies from sunflower seed, maize germ, and soybean seed as pre-emulsified capsules carrying hydrophobic bioactive materials (White et al., 2008; Nikiforidis & Kiosseoglou, 2009; Chen, McClements, Gray, & Decker, 2012; Wu et al., 2011; Wu et al., 2012). In these researches, oil bodies are often extracted from soybean seeds via an aqueous extraction, in which soybeans soaked in water are homogenized by a high speed mixer (Chen et al., 2012). During the homogenization process, oil bodies inevitably involve other seed proteins, i.e., 7S, 11S, allergen proteins (Gly m Bd 30 k etc.), and trypsin inhibitor (Chen & Ono, 2010). Recently Chen and Ono (2010) reported that these involved proteins can be removed from oil bodies under alkaline conditions to prepare intact oil bodies.

Several researches have been carried out to characterize interfacial properties of intact oil bodies. The intact oil bodies extracted under the alkaline condition above pH 9.0 adsorb to the A/W interface and seem to rupture and spread onto the interface (Maurer et al., 2013; Waschatko, Junghans, & Vilgis, 2012a). Waschatko, Schiedt, Vilgis, & Junghans (2012b) also reported in another study that purified oil bodies adsorbed to the A/W interface to form elastic films. Maurer et al. (2013) investigated fundamental roles of soybean oleosin for stabilizing oil bodies and directly compared O/W emulsions stabilized by the intact and enzymatically-modified oil bodies to prove the function of oleosins themselves. These results imply that isolated and non-modified oil bodies play an important role in stabilizing the interfaces. In fact, egg yolk low density lipoproteins (LDL) as a complex globule structured by neutral lipids, phospholipids, and lipophilic proteins are considered to be responsible for emulsifying ability of egg yolk (Kiosseoglou & Sherman, 1983), probably due to its complex structure to allow the lipophilic proteins to easily access the O/W interface without possible aggregation (Mine, 1998; Martinet, Saulnier, Beaumal, Courthaudon, & Anton, 2003; Jolivet, Boulard, Beaumal, Chardot, & Anton, 2006). Considering practical use of the intact oil bodies in the food industry, however, sufficient fundamental information of the intact oil bodies such as interfacial properties at the O/W interface and emulsifying properties is still unavailable.

The objective of this study is to reveal interfacial and emulsifying properties of the intact oil bodies (OB) from soybeans via the aqueous extraction under a condition of pH 11.0 in comparison with crude oil bodies that involve the other proteins (OBC) prepared in the more simplified way without pH adjustments. Colloidal and structural properties of OB and OBC were analyzed by particle size and zeta-potential measurements, SDS-PAGE, and pendant drop tensiometry. Subsequently, emulsifying properties of OB and OBC were evaluated by particle size measurement, visual observation, microscopy, and compositional analysis of oil droplet surfaces.

2. Materials and methods

2.1. Materials

Commercial dried soybeans (*Glycine max* (L.) Merr. 'Tamahomare') for OB and OBC extraction were purchased from a local market. Soybean oil, sucrose, and sodium dodecyl sulphate (SDS) were bought from Wako Pure Chemical Industries Ltd., Japan. A precast polyacrylamide gel (e-PAGEL mini gel E-T1020L, 10–20%) was obtained from Atto Co., Ltd., Japan. Florisil was bought from Sigma Aldrich, USA. BCA protein assay kit was purchased from Takara Bio Inc., Japan. Deionized water was used for preparation of all reagents and samples.

2.2. Preparation of OBC and OB dispersions

2.2.1. Extraction of OBC and OB

OB and OBC were extracted according to Chen and Ono (2010). Soybeans (50 g) were soaked overnight in deionized water at 4 °C, and then the excessive unabsorbed water was discarded. The remained soaked soybeans were rinsed twice with deionized water, and subsequently ground with 200 ml of additional deionized water in a mixer (BM-RE08, Zojirushi Co., Japan) for 1 min. The homogenate was filtrated through three layers of gauze to obtain approximately 200 g of raw soymilk, followed by the addition of sucrose to achieve a final concentration of 20 wt%.

For OBC preparation, the mixture was centrifuged using a centrifuge (Kubota 3740, Kubota Co., Japan) at 4 °C for 40 min at 22,140*g*. The top cream layer was collected and dispersed into another 70 ml of 20 wt% sucrose solution, and then the dispersion was centrifuged at the same condition described above; this washing process was repeated again. For OB preparation, the pH value of raw soymilk containing 20 wt% of sucrose was adjusted to 11.0 with 1 M NaOH, followed by a centrifugation under the same condition stated above. Except for adjusting the pH value of sucrose solution to 11.0, the top layer was collected and washed in the same way as OBC preparation.

In order to remove sucrose, the OB and OBC were dispersed into 70 ml of 10 mM sodium phosphate buffer solution (pH 7.0). Subsequently, these dispersions were centrifuged under the same condition described above, and then the top layer was collected and dispersed into another phosphate buffer solution. Finally, the OB and OBC were collected from the dispersion via the centrifugation and mixed with 40 ml of phosphate buffer solution again. The mixtures were stirred on a magnetic stirrer overnight to ensure the complete dispersion. These dispersions were stored at 4 °C with 0.02% (w/v) of sodium azide as an antimicrobial agent.

2.2.2. Standardization of the concentration of OB and OBC dispersion

In order to standardize the concentration of OB and OBC, a small aliquot (3 ml) of OB and OBC dispersion was poured into an aluminum cup to measure the total weight of the samples, and then heat-dried at 130 °C for 3 h (AOAC Method 925.10) to measure the dry weight (Kapchie, Towa, Hauck, & Murphy, 2010). Based on the results, the solid content of original OB and OBC dispersion was determined, and OB and OBC were appropriately diluted with 10 mM sodium phosphate buffer solution (pH 7.0) to obtain 1 wt% of OB or OBC dispersion.