Food Hydrocolloids 61 (2016) 685–694

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

# Physicochemical and rheological properties and oxidative stability of oil bodies recovered from soybean aqueous extract at different pHs

Luping Zhao, Yeming Chen, Zunhao Yan, Xiangzhen Kong, Yufei Hua*

*State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Avenue, Wuxi, Jiangsu Province 214122, PR China*

ARTICLE INFO

*Article history:*
Received 12 April 2016
Received in revised form
2 June 2016
Accepted 24 June 2016
Available online 25 June 2016

*Keywords:*
Soybean oil bodies
Thermal treatment
Particle size
ζ-Potential
Viscosity
Oxidative stability

ABSTRACT

Soybean oil bodies (OBs), naturally pre-emulsified soybean oil, have great potentials to be used in foods and cosmetics. In this study, OBs were recovered from soybean aqueous extract at pH 6.8, 8.0, 9.5, and 11.0, and recovered OBs contained decreased extrinsic protein amount and composition with increasing recovery pH. In unheated condition, particle size and viscosity decreased, whereas isoelectric point (pI) and oxidative stability increased in the order of pH 6.8-, 8.0-, 9.5-, and 11.0-OB. By heating, it was observed that 1) coalescence of OBs occurred in pH 6.8-OB emulsion, but not occurred in pH 11.0-OB emulsion; 2) pIs of all OB emulsions increased; 3) pH 9.5-OB emulsion showed the highest viscosity, followed by pH 8.0- and 6.8-OB, and pH 11.0-OB still showed the lowest viscosity; 4) gels were formed from OB emulsions with solid content of 40% except pH 11.0-OB; 5) oxidative stability was greatly improved for all OB emulsions. This study is meaningful for supplying fundamental information for selecting proper conditions for aqueous extraction of soybean OBs.



## 1. Introduction

Oil bodies (OBs), lipid-storing organelles in plant seeds, are a natural source of pre-emulsified oil. The dominant component of OBs is neutral lipid (mainly triacylglycerols, TAGs), which contributes 94.21–98.17% to the total weight of each OB (Tzen, Cao, Laurent, Ratnayake, & Huang, 1993). Two other important components of OBs are OB intrinsic proteins (0.59–3.46%) and phospholipids (0.57–1.97%) (Tzen et al., 1993), which shield TAGs from exterior and play an important role for maintaining the integrity of OBs in response to various environmental stresses, such as desiccation and freezing (Shimada & Hara-Nishimura, 2010; Shimada, Shimada, Takahashi, Fukao, & Hara-Nishimura, 2008). In addition to TAGs, intrinsic proteins, and phospholipids, OBs also contain some bioactive components (i.e., vitamin E and phytosterols) (Chen, Cao, Zhao, Kong, & Hua, 2014a, 2014b, 2014c; Fisk & Gray, 2011). Therefore, OBs are extracted from various plant seeds for potential utilisation in foods and cosmetics (Deckers, Rooijen, Boothe, Goll, & Moloney, 2001; Marcoux, Gorkiewicz-Petkow, Hirsch, & Korting, 2004).

In aqueous extraction, OB extrinsic proteins were bound to OBs, and the amount and composition of these extrinsic proteins were greatly affected by extraction conditions (i.e., pH, salt, and temperature). Fisk, White, Lad, and Gray (2008) reported that water-washed sunflower OBs, which contained more extrinsic proteins than salt-washed OBs, showed higher hydroperoxide concentration than salt-washed OBs when they were stored at 45 °C. Payne, Lad, Foster, Khosla, and Gray (2014) found that urea- and water-washed *Echium plantagineum* OBs, which contained less extrinsic proteins, had worse dispersion stability than crude OBs at pH 6.5–7.0, and isoelectric points (pIs) of these two washed OBs were higher than crude OBs. It was reported that maize germ OBs extracted by isoelectric precipitation at pH 5.0 had better physical and oxidative stability than alkali-washed OBs (Karkani, Nenadis, Nikiforidis, & Kiosseoglou, 2013; Nikiforidis & Kiosseoglou, 2010).

Soybean is an important oilseed in the world, and it is also used to extract OBs. The well-used pH conditions were pH 4.6, 7.5, and 8.6 (Fisk et al., 2008; Iwanaga et al., 2007; Kapchie, Towa, Hauck, & Murphy, 2010; Tzen & Huang, 1992; Wu et al., 2012). Chen and Ono (2010) recovered OBs from soybean aqueous extract at different pHs (6.5–11.0), and it was found that the amount of extrinsic proteins in recovered OBs decreased with increasing recovery pH; pH 11.0-OB, which mainly contained OB intrinsic proteins (24, 18, and 16 kDa oleosins; 27 and 29 kDa caleosins; 41 kDa steroleosin)

* Corresponding author.
*E-mail address:* yfhua@jiangnan.edu.cn (Y. Hua).

http://dx.doi.org/10.1016/j.foodhyd.2016.06.032
0268-005X/

(Zhao, Chen, Cao, Kong, & Hua, 2013), showed good dispersion stability against salt and thermal treatment (Chen & Ono, 2010), whereas pH 8.6 extracted OBs, which contained some extrinsic proteins, showed worse dispersion stability (Iwanaga et al., 2007). These results showed that OBs recovered at different conditions showed different properties, which should be correlated with the composition and amount of extrinsic proteins in recovered OBs. However, no researches have systematically examined the physicochemical and rheological properties of recovered OBs that contain different amounts and compositions of extrinsic proteins.

As stated above, OB recovery from soybean aqueous extract at different pHs could result in OBs that contained different amounts and compositions of extrinsic proteins. Therefore, OBs were recovered from soybean aqueous extract at pH 6.8, 8.0, 9.5, and 11.0 in this study, prepared into OB emulsions with solid contents of 1, 10, 20, and 40%, and used to examine their physicochemical and rheological properties. In addition, it was reported that thermal treatment of the pH 8.6 extracted soybean OBs immediately after extraction improved their storage stability (Chen, Mcclements, Gray, & Decker, 2012), indicating that thermal treatment was important for the shelf life of OB products. As a result, it was considered that it was necessary to examine the effects of thermal treatment on the properties of extracted OBs.

## 2. Materials and methods

### 2.1. Materials

Soybean (*Glycine max*) Heinong 64, harvested in 2014, was purchased from Northeast Soybean Research Institute (Harbin, China), packed in self-sealing bag, and stored at 4 °C and 35–40% relative humidity until use. And this study was conducted from March to December 2015. All reagents were purchased from Sigma-Aldrich Trading Co., Ltd. (Shanghai, China) or were of analytical reagent grade.

### 2.2. OBs recovered from soybean aqueous extract at pH 6.8, 8.0, 9.5, and 11.0

This was conducted with the method by Chen and Ono (2010) with the modification that the temperature was controlled at not higher than 4 °C. Soybean (100 g) was soaked in de-ionized (DI) water at 4 °C for 18 h. The soaked soybean was ground in fresh DI water (pre-cooled in a 4 °C refrigerator, seed/DI water, 1/9, w/w) with a blender (18 000 rpm, MJ-60BE01B, Midea) for 2 min. The homogenate was filtered through four layers of gauze to get soybean aqueous extract. Sucrose (200 g) was added into soybean aqueous extract (800 g) and mixed well in an ice water bath. The mixture was equally divided into four parts, and pH was respectively adjusted to 6.8, 8.0, 9.5, and 11.0 with 0.1 and 1 M NaOH solutions. They were centrifuged (25 000 *g*, 30 min, 4 °C) to obtain floating fraction, which was dispersed into 20% (w/w) sucrose solution (floating fraction/sucrose solution, 1/8, w/w) using magnetic stirrer at 4 °C, correspondingly adjusted to pH 6.8, 8.0, 9.5, and 11.0, and centrifuged (25 000 *g*, 30 min, 4 °C). The procedure above was repeated twice. Finally, floating fractions were respectively dispersed into DI water (floating fraction/DI water, 1/8, w/w), which were correspondingly adjusted to pH 6.8, 8.0, 9.5, and 11.0. By centrifugation (25 000 *g*, 30 min, 4 °C), OB pads were collected and named as pH 6.8-, 8.0-, 9.5-, and 11.0-OB.

### 2.3. Determination of moisture, protein, and lipid

Moisture content (MC) was determined by oven drying (105 °C, 5 h). Protein content was determined by amino acid analysis. Each OB sample (0.5 g, wet weight) was mixed with 3.5 mL of DI water and then the mixture was transferred into a hydrolysis tube with 4 mL of concentrated hydrochloric acid (12 M), and hydrolyzed (110 °C, 22 h). And amino acid composition was determined with an automatic amino acid analyzer (Agilent 1100, Santa Clara, CA) by pre-column online derivatization with *O*-phthaldialdehyde. The total amino acid amounts were used to calculate protein contents (Martin-Hernandez, Benet, & Obert, 2008).

The lipid content was measured by chloroform-methanol extraction. About 2 g (wet weight, $m_0$) of each OB sample was added into 150 mL Erlenmeyer flask ($w_0$), and 50 mL of chloroform/methanol (v/v, 2/1) was added. This was treated by reflux extraction in 60 °C water bath for 8 h. The procedure was repeated 2 more times, and then filtered, which was washed with 10 mL of fresh chloroform/methanol 3 times. All chloroform/methanol was collected and treated by rotary evaporator at 45 °C to evaporate organic solvent, further vacuum-drying at 60 °C to constant weight ($w_1$). The lipid could be calculated as follows:

$$\text{Lipid} = (w_1 - w_0)/[m_0 * (1-\text{MC})]$$

### 2.4. OB emulsion preparation

OB emulsion (1%, w/w) was prepared by mixing OB sample with DI water, then the pH 6.8-OB emulsion was adjusted to pH 7.0 with 0.05 M NaOH, and the pH 8.0-, 9.5-, and 11.0-OB were adjusted to pH 7.0 with 0.05 M HCl. Similarly, 10, 20, and 40% OB emulsions were prepared (pH 7.0). Further analyses were carried out immediately after emulsions prepared. To prevent microbial growth, sodium azide (3 mM) was added into the pH 6.8-, 8.0-, 9.5-, and 11.0-OB emulsions (1%, 10%, 20%, and 40%).

### 2.5. Thermal treatment

OB emulsions were heated in a boiling water bath for 15 min, and then cooled in an ice water bath. Further analyses were immediately carried out.

### 2.6. Particle size and ζ-potential measurements

The unheated and heated OB emulsions (1%, w/w) were adjusted to pH 3–8 by using 0.01 M NaOH and 0.01 M HCl, then were diluted 400 times with sodium phosphate buffer solutions (at the same pH as the emulsions). Hydrodynamic diameter ($D_h$) expressed by average diameter, size distribution by intensity and ζ-potential were analyzed via dynamic light scattering measurements using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) at 25 °C.

### 2.7. Microscope observation

Both unheated and heated pH 6.8-OB emulsion (1%, w/w; pH 7) was adjusted to pH 8 with 0.05 M NaOH. The emulsion (1 mL) was degassed in a decompression chamber for about 15 min. Then, the emulsion (10 μL) was added onto a glass slide and covered by a cover glass. It was observed at a magnification of 400 × using optical microscopy (MODEL CX31RTSF, Olympus Corporation, Tokyo, Japan).

### 2.8. Rheology measurement

The rheological properties of OB emulsions (pH 7.0) were measured using a controlled-stress rheometer AR1000 (TA