Effect of pH on physicochemical properties of oil bodies from different oil crops

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Revised: 3 July 2018 / Accepted: 2 October 2018 / Published online: 27 November 2018
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Abstract The objective of this study was to determine the effects of pH on the physicochemical properties of soybean oil bodies (SBOBs), peanut oil bodies (PNOBs) and sunflower oil bodies (SFOBs). The mean particle diameter ($D_{[4,3]}$) of oil bodies (OBs) changed to a stationary trend with increased pH. The surface hydrophobicity (H_0) of SBOBs, PNOBs and SFOBs significantly decreased with increasing pH 2–12. The emulsifying activity index of SBOBs, PNOBs and SFOBs decreased with increased pH from 2 to 10. The viscosity modulus (G'') value of SBOBs at pH 4 was significantly higher than at pH 7 and pH 9. The initial elastic modulus (G') and G'' values of PNOBs at pH 9 were significantly higher than at pH 4 and pH 7. The G'' values of SFOBs at pH 4 and pH 9 were significantly lower than at pH 7. The steroleosin protein bands of SBOBs significantly decreased at pH 12. The protein bands of PNOBs were reduced at pH 2–4 and pH 10–12, and protein bands decreased most obviously at pH 2. The enthalpy of denaturation (ΔH) values of the oil body (OB) protein at pH 9 were significantly higher than at pH 4 and pH 7. The results showed that the ζ -potential, $D_{[4,3]}$, emulsifying property and H_0 of SBOBs, PNOBs and SFOBs were similar to the change of pH value. The three types of OBs have better stability away from the isoelectric point.

Keywords Oil body · pH · Emulsifying property · Rheological · Property

Introduction

Soybean, peanut and sunflower seeds are the most important oil crops in the world. Within the rich content of lipids in their seeds, the triacylglycerols (TAGs) are the main constituents to supply seed germination. Instead of polymerizing with each other, the TAGs consist of many particles of good stability. (Huang 1996). The negative electronegativity and steric hindrance among particles lead to their existence as independent particles. These relatively stable particles are named oil bodies (OBs) (Tai et al. 2002). The OBs are composed of an exterior layer of phospholipid molecules and TAGs wrapped up by integral membrane proteins (Tzen et al. 1992). The steric hindrance of the oil body proteins on the surface of the OBs may play an important role in preventing the fusion of OBs (Boulard et al. 2015). Apart from TAGs, proteins, and phospholipids, the OBs also contain bioactive substances (i.e., vitamin E and phytosterols) (White et al. 2006). Due to the small particles and high permeability, the bioactive substances of OBs are more easily absorbed by the body (Pal et al. 2015). Therefore, OBs extracted from various plant seeds are widely applied in of fields such as foods, personal care products, pharmaceuticals, cosmetics and so on (Sukhotu et al. 2016; White et al. 2008). In the production of chocolates, OBs, such as natural emulsion, may demonstrate remarkable stability and high nutritive value for it is immune to refining treatments (Nikiforidis et al. 2014). Therefore, OBs could be applied in the research and development of artificial dairy beverages. For instance, soymilk can be produced by mixing a thermally-treated

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s13197-018-3453-y>) contains supplementary material, which is available to authorized users.

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aqueous extract of soybeans. OBs in soybeans have been added to soymilk as a natural emulsifier (Gallier et al. 2012).

Over the years, many scholars have devoted themselves to the study of the OBs from oil crops, which have excluded the extraction process and mainly studied for their characteristics and application value (Sukhotu et al. 2016; Wang et al. 2017). There are several topics centering on the oxidative stability of SFOBs, the characterization of SBOBs, as well as the physiochemical properties and stability of PNOBs (Fisk et al. 2010; Sukhotu et al. 2016; Wang et al. 2017). However, researches on the physicochemical properties of three types of OBs still require further explorations. Currently, most studies concern the surface hydrophobicity, rheology and stability under different temperatures or ionic strength conditions (Fisk and Gray 2011; White et al. 2008). However, not only that the temperature and ionic concentration can affect the properties of emulsifier, pH is also an important factor that affects the stability (Zajic et al. 1976). Mohamed et al. (2005) has proved direct relations between the shear storage modulus of the lupin meal suspension and both pH and temperature treatment. In addition, according to Zhao et al. (2016), pH value could significantly impact the rheological properties and oxidative stability of OBs recovered from soybean aqueous extract. To study the topic of this paper, we have extracted OBs from soybeans, peanuts and sunflowers with identical aqueous extraction procedures. We then analyze the influence of pH on the composition, physicochemical properties, and thermal properties of SBOBs, PNOBs and SFOBs.

Materials and methods

Materials

Soybean, peanut and sunflower seeds were purchased from local seed stores (Harbin, China) and stored at 4 °C until be used. All chemicals and solvents used were of analytical grade and were purchased from the Tianjin Tianli Chemical Reagent Co., Ltd. (Harbin, China) and the Sigma Chemical Company (Shanghai, China).

Preparation of OBs

SBOBs, PNOBs and SFOBs were extracted according to the method of Tzen et al. (1997), with slight modifications. Mature seeds were soaked in distilled water (1:5 w v⁻¹) at 4–6 °C for 16–20 h. The soaked seeds were suspended in a fivefold volume of grinding medium (50 mmol L⁻¹ Tris-HCl, pH 7.5, 0.4 mol L⁻¹ sucrose, 0.5 mol L⁻¹ NaCl) and ground with a tissue grinder (HK-02; Guangzhou

HongXing Machinery Co., China). The homogenate was filtered through 3 layers of cheesecloth and centrifuged at 10,000 rpm min⁻¹ for 30 min at 4 °C in a high-speed refrigerated centrifuge (model GL-21 M; Hunan Instrument Co., China). The resuspension was collected and resuspended in 0.1% Tween-20, dispersed an equal volume of buffer solution (50 mmol L⁻¹ Tris-HCl, pH 7.5) and centrifuged according to the above method. The resuspension was collected and resuspended uniformly in 9 mol L⁻¹ urea. The resuspension was dispersed in buffer (50 mmol L⁻¹ Tris-HCl, pH 7.5) and centrifuged as above. The above process was repeated thrice to obtain the upper emulsions, which was the seed OBs. Fresh OBs were stored at 4 °C.

Confocal laser scanning microscopy (CLSM)

The structure of OBs in vitro conditions was observed using CLSM (TCS sp2 AOBS; LEICA Co., Germany) (Nantiyakul et al. 2013). The oil samples were diluted with deionized water to the appropriate concentration of the emulsion; 2 mL of the sample emulsions were added to 80 µL of uniformly mixed mixture containing Nile Red (0.02%) and Nile Blue A (0.1%). And a drop of the stained sample was placed on a slide and covered with a cover slip (filter paper absorbed excess emulsion). Using the Ar/K and He/Ne dual channel laser modes, CLSM observation was performed at 488 nm and 633 nm excitation wavelengths. The image was collected with a 20 × HCPL APO/0.70NA oil mirror at a resolution of 1024 × 1024 using LEICA TCS sp2 Analysis software (version sp2, LEICA Institute Inc, Germany) for image analysis.

Composition of OBs

The protein, fat and water contents of the OBs were determined following the standard procedures specified by the Association of Official Analytical Chemists (Feldsine et al. 2002). Protein content was determination with the Kjeldahl method using a conversion factor of 6.25. Fat content was assayed using the Soxhlet method. Moisture content was measured by drying the OB samples at 110 °C to constant weight under forced convection in the oven (DHG-9140A, Shanghai YiHeng Co., China).

ζ—potential and mean particle diameter of OBs

The determination of ζ-potential and mean particle diameter was based on the method of Iwanaga et al. (2008). One gram of fresh OBs were uniformly dispersed in 9 g of Tris-HCl buffer (10 mmol L⁻¹, pH 7.0). The oil emulsions were adjusted to pH 2, 4, 6, 8, 10 and 12 with NaOH or HCl (0.1 mol L⁻¹), and stored at room temperature for 24 h.

The ζ -potential was measured by the Zeta potential analyzer (Zetasizer Nano ZS, Malvin Co. UK), and each sample was measured three times. The $D_{[4,3]}$ of the OB suspensions were analyzed using a particle size analyzer (Nano-ZS90, Malvin Co. UK), and each sample was measured three times. The refractive index was 1.08, and the mean particle diameter was calculated using the formula ($D_{[4,3]} = \sum n_i d_i^4 / \sum n_i d_i^3$), where n_i is the number of droplets of diameter d_i .

Surface hydrophobicity of OBs

The H_0 of OBs was measured by ANS fluorescence according to the method of Kato and Nakai (1980), with improvements. One gram of fresh OBs were uniformly dispersed in 9 g of Tris–HCl buffer (10 mmol L⁻¹, pH 7.0). The oil emulsions were adjusted to pH 2, 4, 6, 8, 10 and 12 with NaOH or HCl (0.1 mol L⁻¹), and stored at room temperature for 24 h. The OB emulsions were diluted to 0.25, 0.50, 1.00, 1.50 and 2.00% (w v⁻¹) with PBS (10 mmol L⁻¹, pH 7). Each dilution was 20 mL, and 200 μ L ANS fluorescent probe (8 mmol/L) was added to it. The solution was mixed evenly and left to stand avoiding light for 15 min, and the fluorescence was colorimetrically determined by fluorescence spectrophotometer (F-4500, Hitachi Co., Japan). The test conditions were as follows: 390 nm of excitation wavelength, 470 nm of emission wavelength, 5 nm of excitation unit slit, 5 nm of emission unit slit, and 600 V of photocell negative high pressure. The initial slope was the H_0 value of the sample. The fluorescence intensity was the ordinate, and the sample protein concentration was plotted on the abscissa.

Emulsification of OBs

The emulsifying properties of OBs were determined in accordance with the method of Pearce and Kinsella (1978), with improvements. One gram of fresh OBs were uniformly dispersed in 9 g of Tris–HCl buffer (10 mmol L⁻¹, pH 7.0). The oil emulsions were adjusted to pH 2, 4, 6, 8, 10 and 12 with NaOH or HCl (0.1 mol L⁻¹), and stored at room temperature for 24 h. 8 mL of the sample dilutions were mixed evenly with 2 mL of soybean oil, homogenized at 10,000 rpm min⁻¹ for 1 min, and allowed to stand for 10 min. 200 μ L of the bottom emulsion which was at static 0 min and 10 min was added to 10 mL of 1% SDS solution, and were mixed thoroughly. The absorbance was measured at 500 nm using a spectrophotometer (UV-6100, Shimadzu Co., Japan).

Rheological properties of OBs

The rheological properties of OBs were analyzed according to the methods of Nikiforidis et al. (2016) using the rotational rheometer (Gemini II, Malvin Co., UK). The fresh OBs were evenly distributed in the Tris–HCl buffer (10 mmol L⁻¹, pH 7), which was prepared to contain 50% OBs lotion. The pH of OBs emulsions were adjusted to 4, 7 and 9 with NaOH or HCl (0.1 mol L⁻¹), and stored at room temperature for 4 h. 1 mL of emulsion samples were placed between the plates whose clip distance was CP4/40 mm and then remove the excess sample and sealed. After equilibration, keeping the temperature to 25 °C and the frequency was 0.1–10 Hz. The trend of G' scanning frequency was recorded. 1 mL of emulsion samples were placed between the plates, and the equilibrium treatment was performed. The temperature was kept constant at 25 °C, and was cut by 0.001–100 s⁻¹ lasting 120 s. The trend of G'' with shear rate is recorded.

SDS-PAGE gel electrophoresis

SDS-PAGE gel electrophoresis was analyzed using the methods of Nikiforidis and Kiosseoglou (2009) with slight modification. One gram of fresh OBs were uniformly dispersed in 9 g of Tris–HCl buffer (10 mmol L⁻¹, pH 7.0). The oil emulsions were adjusted to pH 2, 4, 6, 8, 10 and 12 with NaOH or HCl (0.1 mol L⁻¹), and stored at room temperature for 24 h. The SDS-PAGE gel was 13%, and the concentrated gel was 5%. Oil emulsions (1 mg mL⁻¹) were treated with a 6.25 mmol L⁻¹ Tris–HCl buffer solution (2% SDS, 10% glycerol, 0.1% bromophenol blue, 5% β -mercaptoethanol), boiled for 5 min and subjected to two cycles of freezing and thawing. The supernatant clear liquid containing the OB protein (3–4 mg mL⁻¹) was obtained by centrifugation. And the solution was subjected to SDS-PAGE electrophoresis (Powerpac™ Basic, Bio-Rad Co., USA). The electrophoretic gel was stained with 0.25% Coomassie Brilliant Blue (R250), and decolorized with the solution of CH₃OH/CH₃COOH.

Thermal properties of oil body proteins

The thermal properties of oil body proteins were determined according to the methods of Tang et al. (2007). 1 mg of oil samples were added to the liquid aluminum box, and dissolved with 5 μ L of PBS (50 mol L⁻¹) at pH 4, 7 and 9, respectively. The aluminum plate was compressed and sealed at room temperature for 12 h, and then used a DSC analyzer (NET2s CH STA449C, Netzsh Co., Germany). The temperature was raised and cooled at 25–120 °C at a rate of 5 °C min⁻¹.

Statistical analysis

All experiments were repeated at least 3 times using prepared samples. Average and standard deviations were calculated from 3 measurements. All data were analyzed by SPSS Statistix (version 17.0, SPSS Institute Inc, USA). The images were processed in Origin (version 8.5, OriginLab Institute Inc, USA). The significant difference was analyzed at $P < 0.05$ (LSD_{0.05}).

Results and discussions

Stereoscopic structure of various OBs

Microstructures of SBOBs, PNOBs and SFOBs are shown in Figure 1S. After being extracted by the buffer solution, the OBs can disperse in aqueous medium, and the structure of which remain independent and integrity. The sizes of OBs differ in different oil crops and within the same oil source (Figure. 1S), in agreement with Tzen et al. (1993), who has discovered that the distribution, size and morphology of OBs are closely related to its source. This is because that the fat content of PNOBs was significantly higher than that of SBOBs (Table 1). It can be seen in the CLSM image that the volume of the PNOBs is the largest and that of SBOBs is the smallest, which is similar to the results documented by Shimada and Hara-Nishimura (2010). The contents of fat and protein in plant seeds can affect the size of OBs. OBs can maintain structural integrity and independence even in the absence of the body, according to the findings of Tzen et al. (1992). The three types of oil crops have shown similar structural compositions, consistent with those observed by Nantiyakul et al. (2013), who have researched the CLSM images of the maize germ and pine nut female gametophyte OBs.

Table 1 Major compositions of soybean oil bodies (SBOBs), peanut oil bodies (PNOBs), and sunflower oil bodies (SFOBs) (% w w⁻¹)

	Protein content	Fat content	Moisture content
SBOBs	3.92 ± 0.05 ^a	45.35 ± 1.10 ^c	50.18 ± 0.64 ^a
PNOBs	1.51 ± 0.03 ^c	85.48 ± 1.16 ^a	12.57 ± 0.81 ^c
SFOBs	2.00 ± 0.06 ^b	70.77 ± 0.05 ^b	26.59 ± 0.20 ^b

Results are expressed as the mean ± standard error of means; n = 3 set of data analyzed in duplicate. Values in the same column are significantly different if there are no identical letters in their superscript ($P < 0.05$)

Major composition of OBs

As is shown in Table 1, when the same extraction method is applied, there is a difference in the basic composition of OBs from different sources of oil crops. The protein content of SBOBs (3.92 ± 0.05%) was significantly higher than PNOBs (1.51 ± 0.03%) and SFOBs (2.00 ± 0.06%) ($P < 0.05$). However, the fat content of SBOBs (45.35 ± 1.10%) was significantly lower than PNOBs (85.48 ± 1.16%) and SFOBs (70.77 ± 0.05%) ($P < 0.05$). These findings are in accord with Hu et al. (2009), whose research has demonstrated that the fat and protein contents of OBs were inversely proportional. The proportions of protein and fat in the three types of OBs were 0.086:1, 0.018:1 and 0.028:1, respectively. However, the proportions of protein and fat in the three types of oil seeds are 1.45:1, 0.47:1 and 0.41:1, lower than the ones in the seed (Tzen et al. 1993). The OBs retain most of the fat in the seed during the extraction process, but the vast majority of the exogenous protein is removed (Payne et al. 2014).

Effect of pH on ζ-potential and mean particle diameters of SBOBs, PNOBs, and SFOBs

ζ-potential is a scientific term for electrokinetic potential in colloidal dispersions, and is widely used for quantification of the magnitude of the charge in food analysis (White et al. 2008). Figure 1a shows that the ζ-potentials of SBOBs, PNOBs, and SFOBs ranged from + 20.03 to − 32.97 mV, + 21.57 to − 29.70 mV, and + 23.43 to − 32.30 mV respectively, with their pH ranging from 2 to 12. The ζ-potential of the three types of OBs were positive when the pH was below 4, and reduced to 0 mV between pH 4–6, which reached the isoelectric point of the OBs. The ζ-potentials of the three types of OBs would be decreased and negative, and then increased gradually as the pH rised, similar to the results recorded by Iwanaga et al. (2007). The results show that the surface charge of the OBs decreases and aggregation occurs when the pH is near the isoelectric point, which can directly affect the emulsifying properties of the OBs (Sukhotu et al. 2016). The isoelectric points of SBOBs, PNOBs and SFOBs were pH 4.3, 4.7 and approximately 5, respectively (Fig. 1). However, Tzen et al. (1993) have argued that the isoelectric points of the OBs from different plant sources are approximately pH 5–6. This can be due to the differences in extraction method and seeds sources, which lead to different isoelectric point.

The effect of pH on the $D_{[4,3]}$ of the three types of OBs are shown in Fig. 1b (Fig. 1b). The $D_{[4,3]}$ of the three OBs increased significantly ($P < 0.05$) at pH 4–6, and significantly decreased at pH 6 ($P < 0.05$) and then gradually became stable. The $D_{[4,3]}$ of the SBOBs was approximately

Fig. 1 Effect of pH on ζ -potential and mean particle diameter of soybean oil bodies (SBOBs), peanut oil bodies (PNOBs), and sunflower oil bodies (SFOBs)

0.93 μm at pH 2 and ranged from pH 8–10. Nevertheless, it reached $12.73 \pm 0.39 \mu\text{m}$ at pH 4. The $D_{[4,3]}$ of PNOBs was 2.45 μm at pH 2 and pH 8–10. It was about 2.16 μm at pH 4–6. The $D_{[4,3]}$ of SFOBs was 1.49 μm at pH 2 and pH 8–10. The results are in line with those reported by Papalamprou et al. (2010). In addition, previous studies have demonstrated that, when pH values of OBs are far away from the isoelectric point, it would result in electrostatic repulsion much larger than that at isoelectric point (Sukhotu et al. 2016). The increase of electrostatic repulsion leads to the decrease of $D_{[4,3]}$. There were significant differences among the $D_{[4,3]}$ of the three OBs at the isoelectric point ($P < 0.05$).

Effect of pH value on surface hydrophobicity of different types OBs

The H_0 of SBOBs, PNOBs and SFOBs ranged from 676.36 ± 1.9 , 75.00 ± 2.79 and 1107 ± 3.23 to 26.50 ± 1.0 , 49.31 ± 0.41 and 8.18 ± 0.69 , respectively, as pH increased ($P < 0.05$) (Fig. 2). This can primarily be attributed to that the ANS probe carries a negative charge (ANS⁻). Under acidic conditions, the surface of the OBs carries a positive charge. ANS⁻ combining with the positive surface charges of OBs and the hydrophobic regions, lead to an increase in the fluorescence intensity and the H_0 . At the same time, the pH value decreases, and the amino acid residues buried in the non-polar region of the oil molecule are exposed to the surface, resulting in an increase in the hydrophobicity of OBs (Mohamed et al. 2005). The rate of decreases in PNOBs and SFOBs from pH 2–8 were significantly higher than the one from pH 8–12 ($P < 0.05$). At pH 2–6, the H_0 of SFOBs was significantly higher than that of SBOBs and PNOBs. Gowder et al. (2014) have shown

Fig. 2 Effect of pH on H_0 of soybean oil bodies (SBOBs), peanut oil bodies (PNOBs), and sunflower oil bodies (SFOBs)

that the H_0 was related to the secondary and tertiary structure of the OB protein. The change of pH can cause denaturation and changes in the microstructure of the protein (Zhao et al. 2016). The differences in the OB proteins from SFOBs, SBOBs and PNOBs may bring about prominent differences in the H_0 among OBs at the same pH value. But there was no significant differences among the three types of OBs at pH 8–12 ($P > 0.05$).

Effect of pH on emulsifying properties of various OBs

At pH 2–4, the Emulsifying activity index (EAI) of SBOBs, PNOBs and SFOBs would significantly reduced from 49.40 ± 0.41 , 51.82 ± 0.57 and $52.28 \pm 0.49 \text{ m}^2 \text{ g}^{-1}$ to $35.29 \pm 1.14 \text{ m}^2 \text{ g}^{-1}$, $39.73 \pm 2.13 \text{ m}^2 \text{ g}^{-1}$ and $7.24 \pm 1.02 \text{ m}^2 \text{ g}^{-1}$ ($P < 0.05$)

respectively (Fig. 3a). With increased pH, the EAI of the three types of OBs increase correspondingly. The EAI of SBOBs and SFOBs were $50.52 \pm 1.75 \text{ m}^2 \text{ g}^{-1}$ and $65.29 \pm 0.90 \text{ m}^2 \text{ g}^{-1}$ at pH 10 and fell to $45.94 \pm 0.08 \text{ m}^2 \text{ g}^{-1}$ and $56.05 \pm 0.04 \text{ m}^2 \text{ g}^{-1}$ ($P < 0.05$) at pH 12. The maximum value of EAI for PNOBs was $54.95 \pm 2.46 \text{ m}^2 \text{ g}^{-1}$ at pH 8. Accordingly, as pH decreases, the EAI of PNOBs also lower gradually. Compared with the EAI of SBOBs and PNOBs, the EAI of SFOBs reached the highest at pH 4–10.

According to Fig. 3b, the emulsion stability index (ESI) of three types of OBs all had minimum values at pH 4–6. The ESI of SBOBs was $10.40 \pm 0.02 \text{ m}^2 \text{ g}^{-1}$ and hit the lowest at pH 4. With increased pH, the ESI of the SBOBs gradually increase to approximately $21.89 \pm 0.60 \text{ m}^2 \text{ g}^{-1}$ at pH 12. The ESI of PNOBs and SFOBs were the lowest at pH 6 (15.43 ± 0.02 and $15.68 \pm 0.20 \text{ m}^2 \text{ g}^{-1}$), and then increase as the pH rised. The ESI of PNOBs was maximal ($31.76 \pm 1.20 \text{ m}^2 \text{ g}^{-1}$) at pH 12, while the ESI of SFOBs was maximal ($46.30 \pm 0.70 \text{ m}^2 \text{ g}^{-1}$) at pH 10, but significantly declined to $23.02 \pm 0.37 \text{ m}^2 \text{ g}^{-1}$ at pH 12. The ESI of SFOBs was significantly higher than that of SBOBs and PNOBs ($P < 0.05$) at pH 2, 8, and 10, respectively.

Near pH 4, EAI and ESI of the three types of OBs reach the lowest value (Fig. 3). This can be attributed to the fact that pH 4 is near the isoelectric point of the three types of OBs, and the surface charge of OBs is almost zero. OBs are associated with each other, preventing the surface protein of oil hydrophilic groups from completely adsorbed water phase and oil phase, which results in the decreased emulsifying activity and stability (Zhao et al. 2016). With the increased surface charge of the OBs, both the electrostatic repulsion among the molecules and the thickness of the hydration layer witness an increase. In addition, the

emulsifying property of the OBs can be improved when the pH exceeds the isoelectric point and the stability against droplets aggregation will be enhanced (Iwanaga et al. 2007; Sukhotu et al. 2016). However, the ability of oil protein to bind water is weakened and the emulsifying activity decreases due to the influence of the strong alkaline environment on the surface protein and phospholipid structure at a pH above 10 (Mohamed et al. 2005). In practical applications of OBs as emulsifier, the neutral and weak alkali treatment of OBs were carried out to achieve the maximization of emulsification.

Effect of pH on rheological properties of different OBs

With treatment at various pH values, the elastic modulus (G') and viscosity modulus (G'') of three types of OBs have shown similar frequency dependences, and the vibration in the frequency range of G' is always greater than G'' (Fig. 4a–c). The result agrees with White et al. (2006), whose results demonstrate that the three types of OBs were typical weak gel emulsion systems that rely on gravity flow. They exhibit liquid properties at low frequencies, but were semi-solid at higher frequencies. The initial G' and G'' of SBOBs and SFOBs were significantly reduced ($P < 0.05$) at pH 4 and pH 9. When the frequency was in the range of 1–10 Hz, the G'' of SBOBs at pH 4 was significantly higher than that at pH 7 and pH 9 ($P < 0.05$). The initial G' and G'' of PNOBs at pH 9 were significantly higher than that at pH 4 and pH 7 ($P < 0.05$) and reached the lowest at pH 4. The effect of pH on G'' was not prominent ($P > 0.05$). The G'' of the three kinds of OBs showed suggests a similar trend with the change of shear force, although it was under different pH values. Because

Fig. 3 Effect of pH on emulsifying activity index (EAI) and emulsion stability index (ESI) of soybean oil bodies (SBOBs), peanut oil bodies (PNOBs), and sunflower oil bodies (SFOBs). “a” versus

“a” meaning there is no significant difference ($P > 0.05$); “a”, “b” versus “c” meaning there is significant difference ($P < 0.05$). Note a SBOBs; b PNOBs; c SFOBs

Fig. 4 Effect of pH on elastic modulus (G') and viscosity modulus (G'') of soybean oil bodies (SBOBs), peanut oil bodies (PNOBs), and sunflower oil bodies (SFOBs). Note **a** SBOBs; **b** PNOBs; **c** SFOBs

Fig. 5 Effect of pH on thermal behavior of proteins in soybean oil bodies (SBOBs), peanut oil bodies (PNOBs), and sunflower oil bodies (SFOBs)

with lower shear rate, the weak shear force cannot break the interaction among OBs, and the floccus act as large aggregates of OBs. But if as the shear rate increase, the floccus was broken down and therefore produced a decrease in viscosity (Zhao et al. 2016).

The G'' of OBs varies with pH values (Fig. 4a'–c'), but pH values do not have noteworthy impact on the G'' of PNOBs. When the shear rate was less than 0.1 s^{-1} , the G'' of SBOBs at pH 4 and pH 9 was significantly lower than that at pH 7 ($P < 0.05$). The G'' of SFOBs at pH 4 was significantly higher than that at pH 7 and pH 9 ($P < 0.05$), and the initial G'' was the lowest at pH 9. The surface proteins of SBOBs may undergo serious degeneration at extreme pH values, leading to an increasingly disordered crimp and loose structure. An alkaline environment also contributes to the destruction of the phospholipid molecular structure (Zhao et al. 2010), which leads to the decrease in G'' of the OBs. The G' and G'' values of three types of OBs at pH 4 were lower than that under the neutral condition, just as the Jms (2004) suggested. Because pH 4 approximates to the isoelectric point of the three types of OBs, in which the surface charge is low, the interaction force is weak, and the network structure is loose, resulting in a decrease in viscoelasticity (Mohamed et al. 2005). The G' and G'' values of SBOBs and SFOBs at pH 9 were lower than that under the acidic and neutral conditions. The G' and G'' values of PNOBs at pH 9 were higher than that at neutral conditions, in agreement with Langton and Hermansson (1992). This is because that a certain alkaline environment can results in cross-linking of PNOB protein peptide chains and the formation of a gel network.

SDS-PAGE electrophoresis analysis

The pH values did not have a significant impact on SBOBs proteins (Figure 2S), but the steroleosin protein bands of the SBOBs decreased significantly at pH 12, indicating that

a strong alkaline environment may lead to the change in SBOBs teroleosin protein. The protein bands of PNOBs weakened at pH 2–4 and pH 10–12, and the protein bands witness the most prominent reduction at pH 2 (Figure 2S b). Acidic and alkaline environments can all lead to the reduction of some oil mosaic proteins and storage proteins which is in agreement with Zhao et al. (2010). Since the pH can affect the surface charge properties of OBs and the interaction between phospholipid and protein, it may lead to the rearrangement of the surface proteins of OBs (Mohamed et al. 2005).

Effect of pH on thermal properties of different OBs

There were two exothermic peaks in SBOB and PNOB proteins at pH 4 and pH 9 (Fig. 5a, b). The T_d value of each OB protein appeared the same as for pH 9, approximately 79.6 and 84.9 °C, 72.0 and 79.4 °C respectively. There were no differences between the two types of OB proteins at pH 7. The ΔH value of SBOBs was 0.049 and 0.471 J g^{-1} at pH 4. The ΔH value of PNOBs was 0.095 and 0.764 J g^{-1} . The ΔH values of SBOBs and PNOBs were 0.085 and 0.535 J g^{-1} , 0.110 and 0.823 J g^{-1} at pH 9. There were two exothermic peaks in SFOB protein at pH 4. The T_d values were between 73.4 to 78.9 °C and 79.6 to 81.3 °C. The ΔH values read 0.593 and 0.064 J g^{-1} . There was a single exothermic peak for SFOBs observed between 73.7 to 79.4 °C. The ΔH value was 0.752 J g^{-1} (Fig. 5c). The ΔH values of three types of OB proteins rised significantly at pH 9 than at pH 7 ($p < 0.05$), which is in agreement with the results reported by Mohamed et al. (2005). The total ΔH of the same source oil protein at pH 4 and pH 9 was significantly higher than that of pH 7 ($P < 0.05$), in line with Petrucci and Anon (1996). This is due to the increases of pH value, protein surface charge, the enhancement of intermolecular interaction, and strong

aggregation of protein residues, which enhance the heat required for denaturation.

Conclusion

The OBs from different crops exhibit different composition in terms of fat and proteins as well as in their physical and chemical stability. SBOBs had better thermal stability compared to PNOBs and SFOBs. The emulsifying property increased gradually when the pH value was far away from the isoelectric point. But the protein contents of SBOBs, PNOBs and SFOBs were reduced under strong acid and strong alkali treatment. Therefore, due to its emulsification and stability, OBs can be applied in the chocolate, cream and other emulsified foods technology field.

Acknowledgements This research was supported by the National Natural Science Foundation of China (Nos. 31871727, 31371784, 31430067) and the Academic Backbone Project of Northeast Agricultural University (15XG23).

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