



Analytical Methods

Solubilization of proteins in extracted oil bodies by SDS: A simple and efficient protein sample preparation method for Tricine–SDS–PAGE



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ABSTRACT

A simple and efficient method for preparing Tricine–SDS–PAGE protein sample of extracted oil bodies (OBs) was supplied: OB suspension was vortexed with SDS buffer (pH 6.8) for 2 min at room temperature with SDS/protein of 1.52/1 (w/w), which could be analyzed by Tricine–SDS–PAGE after simple treatments (dilution and 2-mercaptoethanol). At SDS/protein of 1.52/1, about 95% of proteins in soybean OB suspension were solubilized, whereas residual 5% of proteins were weakly bound to SDS-destroyed OBs; proteins in destroyed OBs might be further solubilized by SDS in the gel and cathode buffer of Tricine–SDS–PAGE, causing about 99% of proteins in soybean OB suspension recover on Tricine–SDS–PAGE gel, which was better than acetone (89%) and diethyl ether (96%) harvested protein samples. Higher or lower SDS/protein was unbeneficial for protein solubilization from OBs. Additionally, the above method was also better than organic solvent method for peanut, sesame, and rapeseed OB suspensions.

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1. Introduction

Oil bodies (OBs) are important organelles in plant seeds, which have triacylglycerols (TAGs) matrix core, and coated by one monolayer of phospholipids embedded with OB intrinsic proteins (mainly oleosins) (Huang, 1992; Lin, Liao, Yang, & Tzen, 2005). On one hand, OBs are mobilized to supply energy for seed germination and seedling growth, and it is considered that many enzymes (i.e., protease, phospholipase, and lipase) should be bound to the OB surface to start mobilization (Matsui, Hijiya, Tabuchi, & Kajiwara, 1999; Rudolph et al., 2011; Vandana & Bhatla, 2006). One good method for examining the interactions between OB and these enzymes is to extract the OBs with physiological pH buffer followed by SDS–PAGE (or Tricine–SDS–PAGE) analysis (Chen, Zhao, Cao, Kong, & Hua, 2014; Zhao, Chen, Cao, Kong, & Hua, 2013). On the other hand, OBs are extracted from various plant seeds for food, cosmetics, and pharmaceutical applications due to the fact that they can act as highly stable oil-in-water emulsions (Adams et al., 2012; Iwanaga et al., 2007; Kapchie, Wei, Hauck, & Murphy, 2008). In the extraction, many extrinsic proteins are bound to the OB surface, and protein amount and composition of extracted OBs are greatly affected by the extraction conditions

(such as temperature and pH) (Chen & Ono, 2010; Guo, Ono, & Mikami, 1997), which results in OB emulsions with different properties. Three maize OB emulsions obtained by different recovery methods, differing in their protein composition, differ both in their oxidative and physical stability (Karkani, Nenadis, Nikiforidis, & Kiosseoglou, 2013). Soybean OBs isolated from raw and heated soymilk, also differing in protein composition, reveal different dispersion stabilities as a function of pH (Chen, Zhao, Kong, Zhang, & Hua, 2014), and thermal treatment of soybean OBs immediately after extraction from raw soymilk improves their storage stability (Chen, McClements, Gray, & Decker, 2012). Also, it is considered that the analysis for the protein composition of extracted OBs is very important for selecting a proper condition for OB extraction and utilization.

Traditionally, organic solvents, including diethyl ether, chloroform/methanol (v/v, 2/1), and acetone, are used to harvest the proteins of extracted OBs, which are dissolved into sample buffer for SDS–PAGE (or Tricine–SDS–PAGE) analysis (Jolivet et al., 2009; Tzen & Huang, 1992). However, the defatting procedure is time-consuming (defatted several times), not environment-friendly, and can cause protein loss (Martin-Hernandez, Benet, & Obert, 2008). In addition, OB suspension is mixed with Tris–HCl buffer (pH 8.0; containing urea, sodium dodecyl sulfate (SDS), glycerol, and 2-mercaptoethanol) to obtain SDS–PAGE sample (SDS/protein, 13.33/1, w/w) without defatting by organic solvents, but the bands on SDS–PAGE gel are very vague

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(Nikiforidis, Biliaderis, & Kiosseoglou, 2012; Nikiforidis & Kiosseoglou, 2011).

It is known that several kinds of detergents are used to solubilize membrane proteins (le Maire, Champeil, & Moller, 2000; Seddon, Curnow, & Booth, 2004), and the mechanism of which can be expressed as follows: at low concentrations, the detergent monomers merely bind to membrane by penetrating into the membrane; with increasing detergent concentration, the membrane is saturated with detergents to cause the membrane disintegrate to form mixed micelles with the detergents, and the hydrophobic regions of the membrane proteins are surrounded by the hydrophobic chains of micelles; at last, the solubilization of membrane leads to the formation of lipid-detergent and protein-detergent (usually one protein molecule per micelle) micelles. According to Huang's OB model, OB surface, mainly comprised of phospholipids and proteins, can be considered as half-unit membrane (Huang, 1992), which may show similar behaviors when exposed to detergents. It was reported that some proteins (including oleosins) of extracted OBs could be replaced by the surfactant of Tween 80 (Nikiforidis & Kiosseoglou, 2011), and the integrity of OBs could be destroyed by SDS at a concentration higher than 0.05% (Tzen, Peng, Cheng, Chen, & Chiu, 1997). In SDS–PAGE (or Tricine–SDS–PAGE), SDS, an anionic detergent, is existed in sample buffer with high concentration: protein sample for SDS–PAGE analysis has SDS concentration of 2 (w/v)% after sample buffer is mixed with sample (Laemmli, 1970), while that for Tricine–SDS–PAGE analysis has SDS concentration of 3 (w/v)% (Schägger, 2006). In addition, it was reported that SDS was extremely effective in the solubilization of membrane proteins (Seddon et al., 2004). As a result, it is realized that SDS buffer may be directly used to solubilize OB intrinsic and extrinsic proteins to form protein–SDS micelles for SDS–PAGE (or Tricine–SDS–PAGE) analysis.

It was reported that 1 g of protein could be expected to bind as much as 1.4 g of SDS (Reynolds & Tanford, 1970), so the SDS/protein mass ratio should be one important parameter in the solubilization of proteins in extracted OBs. It was considered that the high SDS/protein mass ratio (13.33/1) might be one reason for the vague bands on SDS–PAGE gel (Nikiforidis & Kiosseoglou, 2011; Nikiforidis et al., 2012). In this study, the SDS-induced solubilization behavior of proteins in soybean OB suspension was systematically examined when SDS/protein mass ratios were 0/1, 0.5/1, 1/1, 1.52/1, and 2/1, respectively. Then protein sample was prepared by using the optimal SDS/protein mass ratio for Tricine–SDS–PAGE analysis, and compared to the protein sample from traditional method by using organic solvents. At last, the method was also used to prepare protein samples of extracted OBs from some other plant seeds (peanut, sesame, and rapeseed) for Tricine–SDS–PAGE analysis.

2. Materials and methods

2.1. Material

Soybean Nannong 88–31, harvested in 2012, was bought from Soybean Research Institute, Nanjing Agricultural University (Nanjing, China), and stored at 4 °C until use. Some other plant seeds, including sesame (*Sesamum indicum* L., Zhongzhi 13), peanut (*Arachis hypogaea* L., Huayu 33), and rapeseed (*Brassica napus* L., Zheyou 50), were purchased from local market (Wuxi, China). All seeds were stored at 4 °C until use. The chemicals and reagents were all of analytical grade.

2.2. Preparation of buffers

Buffer A: 500 mM Tris–HCl buffer (pH 6.8); buffer B: to 100 mL of buffer A, glycerin (100 g) was added and mixed well; SDS

buffers: to 20 mL of buffer A, glycerin (20 g) and different amounts of SDS were added and mixed well, and these buffers had SDS concentrations of 0.73, 1.23, 1.45, 1.64, 2.20, 2.31, and 2.90 (w/v)%, respectively.

2.3. Preparation of OB suspensions

Twenty grams of soybean (or peanut) was soaked in de-ionized (DI) water at 4 °C for 18 h before 2 min grinding with seed/DI water (4 °C) mass ratio of 1/9. For sesame (or rapeseed), twenty grams of seeds was added into DI water (4 °C) and directly ground for 2 min with seed/DI water mass ratio of 1/9. The homogenate was filtered through four layers of cheesecloth to obtain filtrate, into which sucrose was added to make the concentration of 25% (w/w) and mixed well. The mixture was separated into floating, supernatant, and precipitate fractions by centrifugation (25,000g, 45 min; 4 °C). The floating fraction was collected, and filter paper was used to absorb the remaining supernatant to obtain crude OBs (6.9 g/20 g of soybean; 8.9 g/20 g of peanut; 7.0 g/20 g of sesame; 2.6 g/20 g of rapeseed). Soybean crude OBs (6.9 g) were dispersed into 50 mL of buffer A by magnetic stirring at 4 °C to obtain soybean OB suspension, and the crude OBs of peanut, sesame, and rapeseed (1.4 g) were respectively dispersed into 10 mL of buffer A by magnetic stirring at 4 °C to obtain their OB suspensions. These OB suspensions all had similar densities to DI water.

2.4. Protein concentration determination by amino acid analysis

Each OB suspension sample (4.00 g) was transferred into a hydrolysis tube with 4 mL of concentrated hydrochloric acid (12 M), and hydrolyzed at 110 °C for 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*-phthalaldehyde. The total amino acid amounts were used to calculate protein concentrations in samples (Martin-Hernandez et al., 2008). The protein concentrations of soybean, peanut, sesame, and rapeseed OB suspensions were determined as 1.45, 0.81, 1.08, and 1.52 (w/v)%, respectively.

2.5. Effects of SDS on the solubilization of proteins in soybean OB suspension

Soybean OB suspension was added into five centrifuge tubes (8.00 g in each tube), and 0, 0.058, 0.116, 0.176, and 0.232 g of SDS was added into the five tubes to make the SDS/protein mass ratio of 0/1, 0.5/1, 1/1, 1.52/1, and 2/1, respectively. Then 8.00 g of glycerin was added into the five tubes. These mixtures were magnetically stirred (180 rpm) at room temperature for 30 min, and their volumes (smaller than 16 mL) were recorded (V_n : V_0 , V_1 , V_2 , V_3 , and V_4). DI water (8.00 g) was layered on the top of each mixture. These were treated by centrifugation (25,000g, 45 min; room temperature), and proteins in mixture were separated into three parts: floating fraction (SDS-destroyed OBs), DI water layer, and SDS–glycerin solution layer. The three parts (floating fraction and DI water layer were combined) were treated as shown in Fig. 1A to get samples for Tricine–SDS–PAGE analysis. Assuming all proteins were distributed in SDS–glycerin solution layer or in combined floating fraction and DI water layer, then their samples for Tricine–SDS–PAGE analysis would have protein concentrations of 2 mg/mL by using the dilution method in Fig. 1A (actually, their sum was 2 mg/mL). The equation below was used to calculate how much DI water was needed to dilute 2 mL of SDS–glycerin solution.

$$V_{\text{DI water}} = 2 \text{ mL} \times (16 \text{ mL} - V_n) / V_n \quad (1)$$



Fig. 1. (A) Sample preparation of SDS-glycerin solution layers and destroyed OB suspensions from the mixtures of soybean OB suspension (8.00 g) + SDS (0, 0.058, 0.116, 0.176, and 0.232 g) + glycerin (8.00 g), OB suspension, and defatted OB suspension. (B) Sample preparation of SDS-glycerin solution layer, DI water layer, and destroyed OB suspension from the mixture of soybean OB suspension (8.00 g) + SDS (0.176 g) + glycerin (8.00 g), and one SDS-glycerin solution layer and two glycerin solution layers from the washing procedure of destroyed OB suspension.

where 2 mL is 2 mL of SDS-glycerin solution; 16 mL, the protein concentration in 8.00 g of OB suspension will be half when 8.00 g of OB suspension is diluted to 16 mL; V_n ($n = 0, 1, 2, 3$, and 4) is the volume of mixture of soybean OB suspension (8.00 g), SDS (0, 0.058, 0.116, 0.176, and 0.232 g), and glycerin (8.00 g).

2.6. Preparation of DI water layer and destroyed OB suspension

Soybean OB suspension was added into two centrifuge tubes (8.00 g in each tube). SDS (0.176 g) and glycerin (8.00 g) were added into each tube and magnetically stirred (180 rpm) at room temperature for 30 min, and then 8.00 g of DI water was layered

on the top of each mixture. These were centrifuged (25,000g, 45 min; room temperature) to obtain floating fraction, DI water layer, and SDS-glycerin solution layer, and they were treated as shown in Fig. 1B to get Tricine-SDS-PAGE samples.

2.7. Effect of washing on proteins in destroyed OB suspension

Soybean crude OBs (4.14 g) were dispersed into 30 mL of buffer A to obtain soybean OB suspension, and another 1.38 g was dispersed into 10 mL of DI water to obtain soybean aqueous OB suspension, whose nitrogen content was determined by micro-Kjeldahl method. Soybean OB suspension (8.00 g) was added into

a centrifuge tube, followed by 0.176 g of SDS and 8.00 g of glycerin. The mixture was magnetically stirred (180 rpm) at room temperature for 30 min, and 8.00 g of DI water was layered on the top of the mixture. This was treated as shown in Fig. 1B to obtain one SDS–glycerin solution (and its Tricine–SDS–PAGE sample), two glycerin solutions (and their Tricine–SDS–PAGE samples), and 8.00 g of washed SDS-destroyed OB suspension, whose nitrogen content was determined by micro-Kjeldahl method.

2.8. Protein samples with different SDS/protein mass ratios

Soybean OB suspension (0.5 mL) was mixed with 0.5 mL of buffer B, 0.73, 1.45, 2.20, and 2.90 (w/v)% SDS buffers to make the SDS/protein mass ratio of 0/1, 0.5/1, 1/1, 1.52/1, and 2/1, respectively. They were vortexed for 2 min at room temperature, and diluted with 2.63 mL of buffer B to protein concentrations of 2 mg/mL.

2.9. Proteins harvested by organic solvents

Proteins in soybean OB suspension were harvested by using pre-cooled diethyl ether (4 °C) and acetone (4 °C), respectively. Briefly, to 5.00 g of soybean OB suspension, fifteen milliliters of acetone (or diethyl ether) was added. The mixtures were vortexed and centrifuged at 5000g for 10 min, and the upper organic solvent was removed. The procedure above was repeated two more times to obtain protein aqueous suspension, and the residual organic solvent was evaporated under nitrogen. At last, DI water was added to make the total weight of m_0 (centrifuge tube) + 5.00 g, and this suspension was thoroughly mixed and deemed defatted OB suspension. Proteins in peanut, sesame, and rapeseed OB suspensions were harvested by diethyl ether with the same procedure as above. Soybean OB suspension or defatted OB suspension was treated as shown in Fig. 1A to get their Tricine–SDS–PAGE samples. This experiment was conducted three times, and the results showed the same trend.

Peanut, sesame, and rapeseed OB suspensions (0.5 mL) were respectively mixed with 0.5 mL of 1.23, 1.64, and 2.31 (w/v)% SDS buffers to make SDS/protein mass ratio of 1.52/1. Their corresponding defatted OB suspensions (0.5 mL) were also respectively mixed with 0.5 mL of 1.23, 1.64, and 2.31 (w/v)% SDS buffers. The six mixtures were all diluted with 2.8 mL of buffer B to get Tricine–SDS–PAGE samples. In this condition, rapeseed OB sample had protein concentration of 2 mg/mL, and the other samples had smaller protein concentrations.

2.10. Tricine–SDS–PAGE

Tricine–SDS–PAGE was conducted according to the method by Schagger (2006). To 1 mL of Tricine–SDS–PAGE sample, twenty microliters of 2-mercaptoethanol was added and heated in boiling water bath (100 °C) for 3 min. The concentrations of stacking and separating gels were 4% and 16%, respectively. Each sample (8 or 16 μ L) was loaded into a sample well by a microsyringe, and the samples were 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. After electrophoresis, the gel was fixed with a solution of 100 mM ammonium acetate dissolved in methyl alcohol/acetic acid (5/1, v/v) for 2 h. After fixing, gel was stained with 0.025% (w/v) Coomassie Blue G-250 in 10% (v/v) acetic acid for 2 h, and destained by 10% (v/v) acetic acid. The band intensities on gel were analyzed by Bio-Rad Image Lab Software.

3. Results and discussion

3.1. Effects of SDS on the solubilization of proteins in soybean OB suspension

It was reported that detergent started to solubilize membrane at the concentration larger than its critical micelle concentration (CMC) (le Maire et al., 2000; Seddon et al., 2004), and SDS had CMC of 0.23–0.29 (w/v)% at 20–40 °C (Shah, Jamroz, & Sharif, 2001). After 0 (blank), 0.058, 0.116, 0.176, and 0.232 g of SDS was respectively added into 8.00 g of soybean OB suspension (1.45 (w/v)%), the SDS/protein mass ratios would be 0/1, 0.5/1, 1/1, 1.52/1, and 2/1 (w/w), and the SDS concentrations were all larger than the CMC of SDS. At SDS/protein (0/1), the glycerin solution layer (lane 1 in Fig. 2) mainly contained lipoxygenase, β -conglycinin, γ -conglycinin, and glycinin (OB extrinsic proteins), but no oleosins (OB intrinsic proteins). With increasing SDS/protein, both OB extrinsic and intrinsic proteins (24, 18, and 16 kDa oleosins) increased in SDS–glycerin solution layer (lanes 1, 3, 5, and 7), and the SDS–glycerin solution layer (lane 7) contained the most proteins at SDS/protein (1.52/1). At SDS/protein (2/1), the protein amount (lane 9) was less than that (lane 7) at SDS/protein (1.52/1) (data not shown). For destroyed OB suspension, lane 8 possessed the least protein. The results above might be explained as follows: it was reported that 1 g of protein could be expected to bind as much as 1.4 g of SDS (Reynolds & Tanford, 1970); at the ratio smaller than 1.4/1, SDS was not enough to bind to proteins, and at the ratio larger than 2/1, large amount of SDS was existed in the system, which might induce electrostatic repulsion to inhibit protein solubilization from OBs. The results revealed that SDS could solubilize the most proteins from OBs at SDS/protein (1.52/1).

3.2. Protein distribution in SDS–glycerin solution layer, DI water layer, and floating fraction (SDS-destroyed OBs)

Fig. 3A shows that the protein bands of SDS–glycerin solution layer (lane 1) were denser than those of DI water layer (lane 2) and destroyed OB suspension (lane 3), and it was found that DI water layer had the similar protein composition to SDS–glycerin solution layer. In this study, the sample for centrifugation had two layers (Fig. 1A): DI water layer (containing no proteins) and SDS–glycerin layer (containing proteins). As a result, the proteins in SDS–glycerin layer tended to diffuse into DI water layer, and this should be the origin of proteins in DI water layer after centrifugation. As stated above, destroyed OB suspension not only contained destroyed OBs (floating fraction), but also DI water layer. It was found that the band intensities (α' , α , and β subunits of β -conglycinin, acidic and basic polypeptides of glycinin, Gly m Bd 30 K (Bd 30 K), and 24 kDa oleosin) of destroyed OB suspension (lane 3) were larger than the corresponding band intensities of DI water layer (lane 2), revealing that some proteins remained in destroyed OBs, and all (or part) of them could be resolved into Tricine–SDS–PAGE gel by electrophoresis. In addition, the results showed that the proteins in destroyed OBs were α' , α , and β subunits of β -conglycinin, acidic and basic polypeptides of glycinin, Bd 30 K, and 24 kDa oleosin (Figs. 2 and 3A). Oleosin could be divided into N-terminal, hydrophobic central domain, and C-terminal with hydrophobic central domain buried in the TAGs matrix of OBs and N- and C-terminal exposed to exterior, so it was acceptable that some 24 kDa oleosins remained in destroyed OBs. It was reported that half of α' and α subunits of β -conglycinin were disulfide (SS) linked, together or with Bd 30 K, in the soybean seed cotyledon: α'/α -SS- α'/α and α'/α -SS-Bd 30 K (Wadahama et al., 2012), and the latter one could be further bound to 24 kDa oleosin

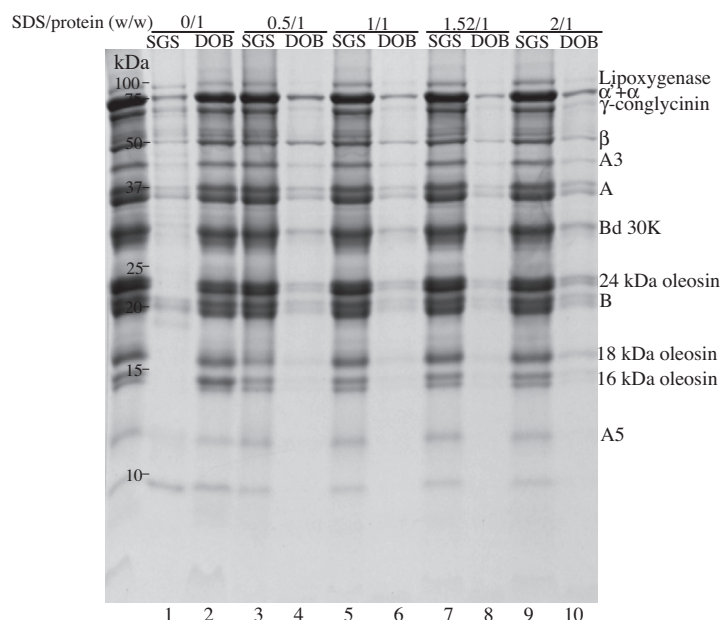


Fig. 2. Tricine-SDS-PAGE profiles of SDS-glycerin solution layers and destroyed OB suspensions. Their samples were prepared as shown in Fig. 1A. Lanes 1, 3, 5, 7, and 9, SDS-glycerin solution layers (SGS) from the mixtures with SDS/protein of 0/1, 0.5/1, 1/1, 1.52/1, and 2/1, respectively; lanes 2, 4, 6, 8 and 10, destroyed OB suspensions (DOB) from the mixtures with SDS/protein of 0/1, 0.5/1, 1/1, 1.52/1, and 2/1, respectively. Each sample (8 μ L) was used. α' , α , and β are three subunits of β -conglycinin, and A3, A, and A5 are acidic polypeptides of glycinin, while B is basic polypeptides of glycinin; Bd 30 K, Gly m Bd 30 K; oleosin (16 kDa) has two isoforms.

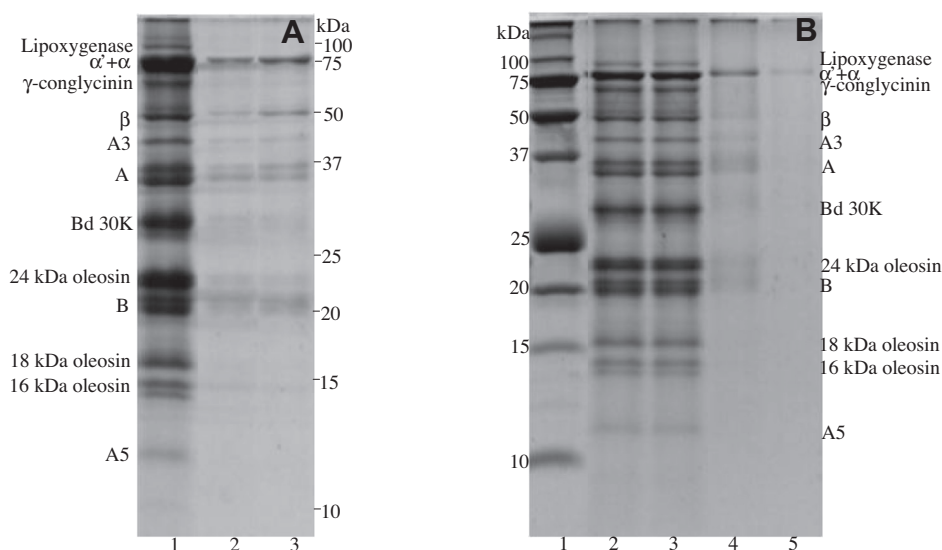


Fig. 3. (A) Tricine-SDS-PAGE profiles of SDS-glycerin solution layer (lane 1), DI water layer (lane 2), and destroyed OB suspension (lane 3) from the mixture of soybean OB suspension (8.00 g) + SDS (0.176 g) + glycerin (8.00 g). Their samples were prepared as shown in Fig. 1B, and each sample (16 μ L) was used. (B) Tricine-SDS-PAGE profiles of one SDS-glycerin solution layer (lane 3) and two glycerin solution layers (lanes 4 and 5) from the mixture of soybean OB suspension (8.00 g) + SDS (0.176 g) + glycerin (8.00 g). Their samples were prepared as shown in Fig. 1B, and each sample (8 μ L) was used. Lane 1, marker; lane 2, soybean OB suspension (Fig. 1A).

by SS (Chen et al., 2014; Zhao et al., 2013), which might be the reason that some β -conglycinin and Bd 30 K remained in destroyed OBs. The remained glycinin in destroyed OBs might be due to the reason which was similar to β -conglycinin (Zhao et al., 2013).

In order to further examine the proteins in destroyed OB suspension, soybean OB suspension (8.00 g) was treated as shown in Fig. 1B to obtain one SDS-glycerin solution, two glycerin solutions, and washed SDS-destroyed OB suspension (8.00 g). The nitrogen contents of soybean aqueous OB suspension (1.38 g of soybean crude OBs into 10 mL of DI water) and washed SDS-destroyed OB suspension were determined, and it was found that the latter nitrogen content was about 0.06% of the former one, revealing that

nearly 100% of proteins in soybean OB suspension were distributed in the SDS-glycerin solution layer and the following two glycerin solution layers. Their samples were treated by Tricine-SDS-PAGE (lanes 3, 4, and 5 in Fig. 3B), and it was calculated that 87%, 12%, and 1% of proteins in soybean OB suspension were distributed in the SDS-glycerin solution layer and the following two glycerin solution layers, respectively.

According to the results above, it was suggested that the proteins in destroyed OBs could release from destroyed OBs by aqueous washing, indicating that they had weak interactions with destroyed OBs. Therefore, the proteins in destroyed OBs should be solubilized after the destroyed OB suspension was mixed with

SDS (2.20 (w/v)%) buffer (lane 8 in Fig. 2 and lane 3 in Fig. 3A). It was calculated that 85%, 10%, and 5% of proteins in soybean OB suspension were distributed in the SDS–glycerin solution layer, DI water layer, and floating fraction (destroyed OBs) (Fig. 3A), which was in good agreement with the results in Fig. 3B.

The results above revealed that 95% of proteins in soybean OB suspension were solubilized and residual 5% of proteins weakly interacted with destroyed OBs in the condition of SDS/protein (1.52/1, w/w). This made us realize that mixing soybean OB suspension with SDS buffer (SDS/protein, 1.52/1) might be an efficient method (deemed as SDS method in this study) for preparing protein sample for Tricine–SDS–PAGE analysis. Lane 2 in Fig. 3B was from the sample prepared as just above, and it was calculated that its total band intensity was 99% of the sum of the total band intensities on lanes 3, 4, and 5 (Fig. 3B). It was considered that the additional 4% might be induced by the SDS in the gel and cathode buffer of Tricine–SDS–PAGE (Schägger, 2006).

3.3. Proteins harvested from OB suspension by organic solvents

Traditionally, the proteins in OB suspension were harvested by organic solvents for SDS–PAGE analysis (deemed as traditional method). In this study, acetone and diethyl ether were selected to harvest proteins from soybean OB suspension. Fig. 4 shows the Tricine–SDS–PAGE profiles of acetone-defatted OB suspension (lane 3) and diethyl ether-defatted OB suspension (lane 4), and it was calculated that their total band intensities were respectively 89 and 96% of the total band intensity of sample prepared by SDS method (lane 2), revealing that SDS method was better than traditional method for Tricine–SDS–PAGE sample preparation. This was reasonable because some proteins could be dispersed into organic solvents during defatting.

3.4. Protein samples with different SDS/protein mass ratios

Fig. 5 shows the Tricine–SDS–PAGE profiles of protein samples (mixing soybean OB suspension with different SDS buffers) with SDS/protein mass ratios of 0/1 (lane 2), 0.5/1 (lane 3), 1/1 (lane 4), 1.52/1 (lane 5), and 2/1 (lane 6), respectively. Unexpectedly,

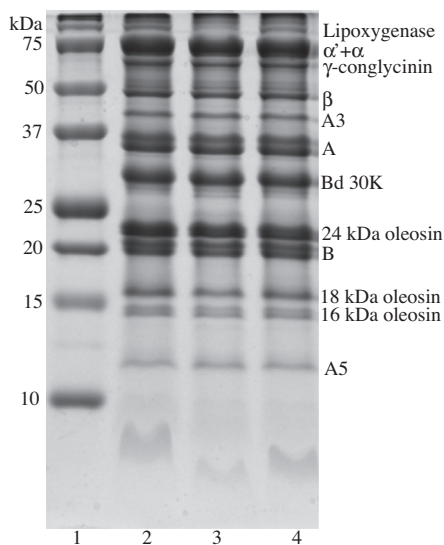


Fig. 4. Tricine–SDS–PAGE profiles of proteins harvested from soybean OB suspension by organic solvents. Lane 1, marker; lane 2, soybean OB suspension; lane 3, acetone-defatted OB suspension; lane 4, diethyl ether-defatted OB suspension. Their samples were prepared as shown in Fig. 1A, and each sample (16 μ L) was used.

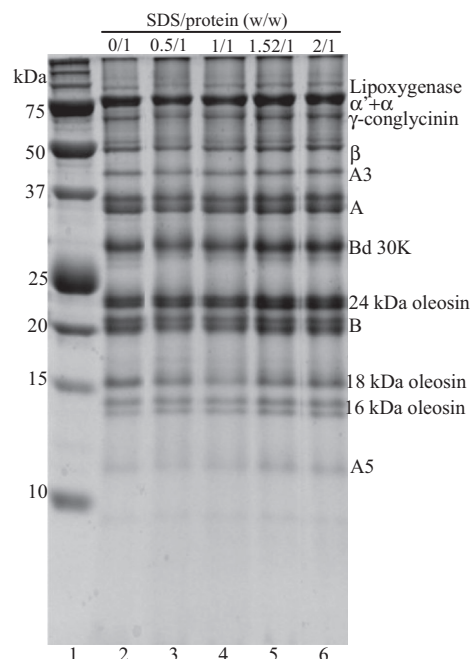


Fig. 5. Tricine–SDS–PAGE profiles of protein samples with different SDS/protein ratios. Lane 1, marker; lanes 2–6, soybean OB suspension (0.5 mL) was mixed with 0.5 mL of buffer B, 0.73, 1.45, 2.20, and 2.90 (w/v)% SDS buffers to make the SDS/protein of 0/1, 0.5/1, 1/1, 1.52/1, and 2/1, respectively. They were vortexed for 2 min at room temperature, and diluted with 2.63 mL of buffer B to protein concentrations of 2 mg/mL. Each sample (8 μ L) was used.

the band intensities (except 24 and 16 kDa oleosins) on lane 2 (SDS/protein, 0/1) were larger than those on lane 3 (SDS/protein mass ratio, 0.5/1). This might be induced by the SDS in the gel and cathode buffer of Tricine–SDS–PAGE (Schägger, 2006), which could partly solubilize the proteins (8 μ L \times 2 mg/mL = 16 μ g) in OBs. Fig. 5 shows that all band intensities increased from SDS/protein (0.5/1) to SDS/protein (1.52/1), and all band intensities at SDS/protein (2/1) were smaller than those at SDS/protein (1.52/1), revealing that the proper SDS/protein mass ratio (1.52/1) was very important for the protein sample preparation for Tricine–SDS–PAGE. This might be the reason for the vague bands on SDS–PAGE gel reported by two researches (SDS/protein mass ratio, 13.33/1) (Nikiforidis & Kiosseoglou, 2011; Nikiforidis et al., 2012).

3.5. OB suspensions from peanut, sesame, and rapeseed

A simple and efficient method (SDS method) for preparing protein sample of soybean OB suspension for Tricine–SDS–PAGE was stated above. Whether SDS method was also proper for other plant seed OB suspensions was not clear. Therefore, peanut, sesame, and rapeseed OB suspensions were prepared, and their protein concentrations were determined as 0.81, 1.08, and 1.52 (w/v)%, respectively. They (0.5 mL) were respectively mixed with 0.5 mL of 1.23, 1.64, and 2.31 (w/v)% SDS buffers, and further diluted with 2.8 mL of buffer B. In this condition, the SDS/protein mass ratios of the three mixtures were all 1.52/1. In order to examine the efficiency of SDS method, the three OB suspensions were defatted by diethyl ether, and they (0.5 mL) were respectively mixed with 0.5 mL of 1.23, 1.64, and 2.31 (w/v)% SDS buffers, and further diluted with 2.8 mL of buffer B. Fig. 6 shows that all bands on lanes 3, 5, and 7 (SDS method) were denser than the corresponding bands on lanes 2, 4, and 6 (traditional method). By Bio-Rad Image Lab Software (data not shown), it was calculated that the total band intensities on lanes 2, 4, and 6 were 88%, 91%, and 94% of the total band

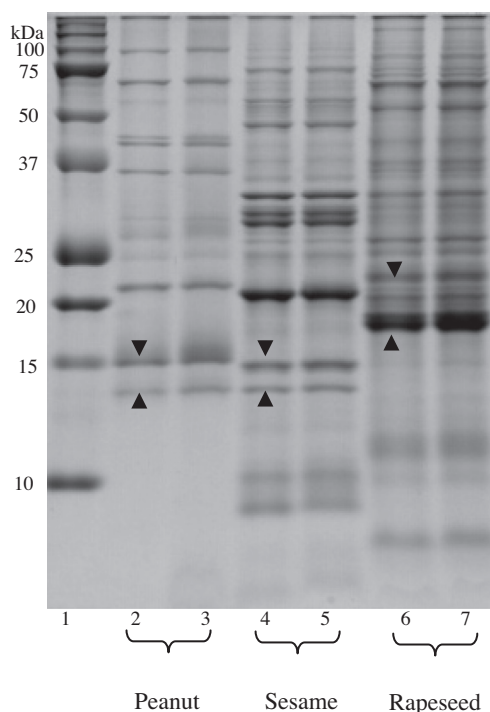


Fig. 6. Tricine-SDS-PAGE profiles of peanut, sesame, and rapeseed OB suspensions and defatted OB suspensions. Lane 1, marker; lanes 2, 4, and 6, diethyl ether-defatted peanut, sesame, and rapeseed OB suspensions (0.5 mL), which were respectively mixed with 0.5 mL of 1.23, 1.64, and 2.31 (w/v)% SDS buffers, and further diluted with 2.8 mL of buffer B; lanes 3, 5, and 7, peanut, sesame, and rapeseed OB suspensions (0.5 mL), which were respectively mixed with 0.5 mL of 1.23, 1.64, and 2.31 (w/v)% SDS buffers, and further diluted with 2.8 mL of buffer B. Each sample (8 μ L) was used. Bands indicated by arrows are oleosins (Jolivet et al., 2009; Tzen, Cao, Laurent, Ratnayake, & Huang, 1993).

intensities on lanes 3, 5, and 7, respectively. These results revealed that SDS method was more efficient than traditional method. However, the optimal SDS/protein mass ratios for peanut, sesame, and rapeseed OB suspensions might need further researches.

4. Conclusions

In this study, one simple and efficient method (SDS method) for preparing protein sample of OB suspension for Tricine-SDS-PAGE was supplied: OB suspension was directly vortexed with SDS buffer (pH 6.8) for 2 min at room temperature with SDS/protein mass ratio of 1.52/1. SDS method was more efficient than traditional method (organic solvent defatting) when soybean, peanut, sesame, rapeseed OB suspensions were used. When soybean OB suspension (protein concentration, 1.45 (w/v)%) was mixed with the same volume of SDS (2.20 (w/v)%) buffer, about 95% of proteins in soybean OB suspension were solubilized, and residual 5% of proteins weakly interacted with destroyed OBs. By electrophoresis, about 99% of proteins could be recovered in the Tricine-SDS-PAGE gel.

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References

- Adams, G. G., Imran, S., Wang, S., Mohammad, A., Kok, M. S., Gray, D. A., et al. (2012). Extraction, isolation and characterization of oil bodies from pumpkin seeds for therapeutic use. *Food Chemistry*, 134, 1919–1925.
- Chen, B., McClements, D. J., Gray, D. A., & Decker, E. A. (2012). Physical and oxidative stability of pre-emulsified oil bodies extracted from soybeans. *Food Chemistry*, 132, 1514–1520.
- Chen, Y., & Ono, T. (2010). Simple extraction method of non-allergenic intact soybean oil bodies that are thermally stable in an aqueous medium. *Journal of Agricultural and Food Chemistry*, 58, 7402–7407.
- Chen, Y., Zhao, L., Cao, Y., Kong, X., & Hua, Y. (2014). Oleosins (24 and 18 kDa) are hydrolyzed not only in extracted soybean oil bodies but also in soybean germination. *Journal of Agricultural and Food Chemistry*, 62(4), 956–965.
- Chen, Y., Zhao, L., Kong, X., Zhang, C., & Hua, Y. (2014). The properties and the related protein behaviors of oil bodies in soymilk preparation. *European Food Research and Technology*, 239, 463–471.
- Guo, S., Ono, T., & Mikami, M. (1997). Interaction between protein and lipid in soybean milk at elevated temperature. *Journal of Agricultural and Food Chemistry*, 45, 4601–4605.
- Huang, A. H. C. (1992). Oil bodies and oleosins in seeds. *Annual Review of Plant Physiology and Plant Molecular Biology*, 43, 177–200.
- Iwanaga, D., Gray, D. A., Fisk, I. D., Decker, E. A., Weiss, L., & McClements, D. J. (2007). Extraction and characterization of oil bodies from soy beans: A natural source of pre-emulsified soybean oil. *Journal of Agricultural and Food Chemistry*, 55, 8711–8716.
- Jolivet, P., Boulard, C., Bellamy, A., Larre, M., Rogniaux, H., Andrea, S., et al. (2009). Protein composition of oil bodies from mature *Brassica napus* seeds. *Proteomics*, 9, 3268–3284.
- Kapchie, V. N., Wei, D., Hauck, C., & Murphy, P. (2008). Enzyme-assisted aqueous extraction of oleosomes from soybeans (*Glycine max*). *Journal of Agricultural and Food Chemistry*, 56, 1766–1771.
- Karkani, O. A., Nenadis, N., Nikiforidis, C. V., & Kiosseoglou, V. (2013). Effect of recovery methods on the oxidative and physical stability of oil body emulsions. *Food Chemistry*, 139, 640–648.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680–685.
- le Maire, M., Champeil, P., & Moller, J. V. (2000). Interaction of membrane proteins and lipids with solubilizing detergents. *Biochimica et Biophysica Acta*, 1508, 86–111.
- Lin, L. J., Liao, P. C., Yang, H. H., & Tzen, J. T. C. (2005). Determination and analyses of the N-termini of oil body proteins, steroleosin, caleosin and oleosin. *Plant Physiology and Biochemistry*, 43, 770–776.
- Martin-Hernandez, C., Benet, S., & Obert, L. (2008). Determination of proteins in refined and nonrefined oils. *Journal of Agricultural and Food Chemistry*, 56, 4348–4351.
- Matsui, K., Hijiya, K., Tabuchi, Y., & Kajiura, T. (1999). Cucumber cotyledon lipoxygenase during postgerminative growth. Its expression and action on lipid bodies. *Plant Physiology*, 119, 1279–1287.
- Nikiforidis, C. V., Biliaderis, C. G., & Kiosseoglou, V. (2012). Rheological characteristics and physicochemical stability of dressing-type emulsions made of oil bodies-egg yolk blends. *Food Chemistry*, 134, 64–73.
- Nikiforidis, C. V., & Kiosseoglou, V. (2011). Competitive displacement of oil body surface proteins by Tween 80 – Effect on physical stability. *Food Hydrocolloids*, 25, 1063–1068.
- Reynolds, J. A., & Tanford, C. (1970). Binding of dodecyl sulfate to proteins at high binding ratios. Possible implications for the state of proteins in biological membranes. *Proceedings of the National Academy of Science of the United States of America*, 66(3), 1002–1007.
- Rudolph, M., Feussner, I., Korner, M., Feussner, K., Berndt, E., Meler, M., et al. (2011). The lipoxygenase-dependent oxygenation of lipid body membranes is promoted by a patatin-type phospholipase in cucumber cotyledons. *Journal of Experimental Botany*, 62, 749–760.
- Schägger, H. (2006). Tricine-SDS-PAGE. *Nature Protocols*, 1, 16–22.
- Seddon, A. M., Curnow, P., & Booth, P. J. (2004). Membrane proteins, lipids and detergents: Not just a soap opera. *Biochimica et Biophysica Acta*, 1666, 105–117.
- Shah, S. S., Jamroz, N. U., & Sharif, Q. M. (2001). Micellization parameters and electrostatic interactions in micellar solution of sodium dodecyl sulfate (SDS) at different temperatures. *Colloids and Surfaces A*, 178, 199–206.
- Tzen, J. T. C., Cao, Y. Z., Laurent, P., Ratnayake, C., & Huang, A. H. C. (1993). Lipids, proteins and structure of seed oil bodies from diverse species. *Plant Physiology*, 101, 267–276.
- Tzen, J. T. C., & Huang, A. H. C. (1992). Surface structure and properties of plant seed oil bodies. *Journal of Cell Biology*, 117, 327–335.
- Tzen, J. T. C., Peng, C. C., Cheng, D. J., Chen, E. C. F., & Chiu, J. M. H. (1997). A new method for seed oil body purification and examination of oil body integrity following germination. *Journal of Biochemistry*, 121, 762–768.
- Vandana, S., & Bhatla, S. C. (2006). Evidence for the probable oil body association of a thiol-protease, leading to oleosin degradation in sunflower seedling cotyledons. *Plant Physiology and Biochemistry*, 44, 714–723.
- Wadahama, H., Iwasaki, K., Matsusaki, M., Nishizawa, N., Ishimoto, M., Arisaka, F., et al. (2012). Accumulation of β -conglycinin in soybean cotyledon through the formation of disulfide bonds between α' - and α -subunits. *Plant Physiology*, 158, 1395–1405.
- Zhao, L., Chen, Y., Cao, Y., Kong, X., & Hua, Y. (2013). The integral and extrinsic bioactive proteins in the aqueous extracted soybean oil bodies. *Journal of Agricultural and Food Chemistry*, 61, 9727–9733.