International Journal of
Food Science & Technology
International Journal of Food Science and Technology 2014, **49**, 1079–1089

Original article

Extraction and physicochemical properties of soya bean protein and oil by a new reverse micelle system compared with other extraction methods

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(Received 22 April 2013; Accepted in revised form 25 September 2013)

Summary Reverse micelle extraction is a novel technology for the separation of plant components such as proteins and oil. In this study, sodium bis(2-ethylhexyl) sulphosuccinate (AOT) reverse micelle system and AOT/Tween 85 reverse micelle system were used to extract soya bean protein and oil from soya bean flour. The physicochemical properties of the protein and oil extracted were investigated and compared with traditional extraction methods. The results showed that the efficiency of forward extraction of soya bean protein using an AOT/Tween 85 reverse micelle system was superior to that using an AOT reverse micelle system at the optimal extraction conditions. In addition, soya bean proteins extracted using reverse micelle extraction had no unordered structure under Fourier transform infrared spectroscopy. The acid and peroxide values of oil products from two reverse micelle extractions were lower than that from immersion. The results indicated that AOT/Tween 85 reverse micelle system is effective in extracting soya bean protein and oil.

Keywords AOT, reverse micelle extraction, soya bean oil, soya bean protein, Tween 85.

Introduction

Reverse micelle extraction, a relatively new technology for liquid–liquid extraction, especially for the isolation and purification of proteins, has recently received due attention (Luisi *et al*., 1979; Harikrishna *et al*., 2002). Reverse micelles are formed by surfactants in nonpolar organic solvents, while in their polar cores, nanometre-sized water pools are formed by the solubilisation in water (Luisi *et al*., 1979; Naoea *et al*., 2011). With self-assembly capacity, reverse micelle systems are not only dynamic balancing systems but also stable and transparent thermodynamic systems (Liu *et al*., 2006). The overall mechanism of reverse micelle extraction consists of two fundamental steps: forward extraction (protein is transferred into polar water pools and oil is transferred into the organic phase) and backward extraction (protein is released from polar water pools and transferred into an aqueous phase to be recovered, resulting in separation of aqueous phase and oil phase) (Zhao *et al*., 2010a).

The polar water pools inside reverse micelles can solubilise hydrophilic biomolecules, such as proteins, enzymes, DNA, nucleic acids, short peptides and amino acids. The size of water pools plays a significant role in the solubility of biomolecules in the micelle core. W_0, the determining factor of the size of water pools and the molar ratio, is the ratio of water and surfactant, which can be adjusted by an aqueous buffer containing an appropriate amount of salt (Ghazi *et al*., 2006). Reverse micelles form a three-phase system including water–surfactant–organic solvent (i.e. the form of W/O (water/oil) with various shapes, such as spherical, oval and rod); thus in this system, the biomolecules inside the polar water pools are protected from denaturation by organic solvents.

Reverse micelles are more suitable for separating proteins than regular liquid–liquid extraction or other separation methods because in those methods, the trans-

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doi:10.1111/ijfs.12403

fer of proteins into solvents often results in irreversible denaturation and loss of biological activity (Guo *et al.*, 2008). Luisi *et al.* (1979) first pointed out the potential of reverse micelle solubilisation of proteins for protein separation. Dekker *et al.* (1986) and Goklen & Hatton (1985) developed the process systematically for practical use of reverse micelle systems. Later on, many researchers demonstrated the factors including molar ratio W_0, aqueous phase pH and ionic strength (Rho & Kang, 2004), surfactant type and concentration (Shin & Vera, 2002), and co-surfactant (Lee *et al.*, 2004) affecting protein solubilisation based on the interactions between reverse micelles and proteins. Among these parameters, surfactant is well known as playing an important role in stabilising protein solubilisation in reverse micelles. Most studies to date have focused on the extraction of proteins by reverse micelle systems, whereas studies on the extraction of oil have been rarely reported.

With the development of protein extraction by ionic reverse micelles, protein sectional deactivation caused by the strong electrostatic interaction occasionally occurs in the extraction process (Harikrishna *et al.*, 2002). Therefore, some nonionic surfactants are used to form nonionic reverse micelles for protein extraction to avoid protein deactivation. Tween 85 is usually used for reverse micelle extractions, and it does not has detrimental effects on the structure, function and stability of proteins solubilised in reverse micelles (Sawada *et al.*, 2004). However, the extraction efficiency by Tween 85 itself was very low.

In this study, the efficiency of two reverse micelle systems, an anionic surfactant–nonionic surfactant AOT/Tween 85 mixed system and an anionic surfactant AOT system in extracting protein and oil from soya bean flour, was investigated. Effects of various factors including soya bean flour concentration, W_0, temperature, time, pH, ionic strength and ultrasonic power on the efficiency of forward extraction of soya bean protein were examined. The physical and chemical properties of oil extracted by reverse micelle were compared with oil immersed, such as acid value (AV), peroxide value (POV) and fat acid (FA) composition.

Materials and methods

Materials

Chemically pure AOT and Tween 85 were purchased from Shanghai Haiqu Chemical Co. Ltd. (Shanghai, China) and Shanghai Lichen Limited Company (Shanghai, China), respectively. All other reagents used were of analytical grade. Full-fat soya bean flour sieved through a 100-mesh screen and containing 42.2% protein, 4.3% moisture and 24.7% oil was obtained from Anyang Mantianxue Food Manufacturing Co. Ltd (Anyang, Henan, China).

Proximate composition analysis

Crude protein of soya bean flour was determined using the micro-Kjeldahl method (Concon & Soltess, 1973), whereas crude fat was determined by Soxhlet extraction (AOAC, 1984). The moisture content was measured by drying in an oven at 105 °C until constant weight was obtained. AV, POV and FA composition were determined according to standard methods of the International Union of Pure and Applied Chemistry for analysis of oil and fats.

Preparation of two reverse micelle systems

An AOT reverse micelle was obtained by dissolving AOT in isooctane and then injecting the mixture with KCl–phosphate buffer solution. A stock solution of 0.08 g mL^{-1} AOT reverse micelle system was obtained by mixing AOT with isooctane and then stirring the mixture using a magnetic stirrer at room temperature. When AOT was dissolved completely, KCl–phosphate buffer solutions with certain pH and different concentrations were added for creating water content. The water content in reverse micelles is expressed as W_0, which is the molar ratio of water to surfactant. The water content was determined by the Karl–Fischer method (Bu *et al.*, 2012).

An AOT/Tween 85 reverse micelle system was achieved by dissolving AOT and Tween 85 (mass ratio, 4:1) in isooctane and n-octyl alcohol (volume ratio, 4:1) and the same procedure was used for the AOT reverse micelle system (Hemavathi *et al.*, 2010).

Extraction of soya bean protein and oil by reverse micelle systems

Soya bean flour was added to the different reverse micelle systems and then extracted for a certain period by an ultrasonic-assisted method. The resulting mixture with protein dissolved in polar core and oil contained in organic solvent phase was centrifuged at 5000 × ***g*** for 15 min, and clear supernatant was used for the next step of extraction. The above-described procedure is designated as the forward extraction.

The same volume of clear supernatant and buffer solution was mixed and extracted in an ultrasonic cleaning machine. The resulting mixture was centrifuged at 5000 × ***g*** for 10 min. The oil and aqueous phases were then separated and stored. This step is designated as the backward extraction. The protein content in the aqueous phase was measured by a spectrophotometer (UV-160A; Shimadzu, Kyoto, Japan) at 280 nm. NaCl solution (0.1 M in 70% ethanol solution, with pH adjusted to 4 with 0.1 M HCl and 0.1 M NaOH) and the upper oil phase were mixed at the proportion of 1:2.5 (v:v), stirred at 30 °C for 1 h, left

to stand and stratified. Isooctane was subsequently removed from the upper organic phase using a rotary vacuum evaporator at 90 °C. The oil product was obtained following the above-described steps.

Immersion of soya bean oil in petroleum ether

Full-fat soya bean flour in petroleum ether was defatted for 12 h. Next, the petroleum ether was completely recovered, with the remaining material being the oil product (Ramadan *et al*., 2010).

The preparation of protein samples

Around 1.000–2.000 g protein of soya bean flour was added into the individual two reverse micelle solutions (AOT/Tween 85 reverse micelle system and AOT reverse micelle system), respectively. After forward extraction and backward extraction, two kinds of extraction solutions were added to the treated dialysis bag. Dialysis process lasted for 48–72 h. During this process, water was changed regularly about every 8 h. Finally, solutions were freeze-dried for protein sample collection.

Scanning electron microscopy analysis

A low amount of each soya bean protein product was glued on a double-sided adhesive tape, which was covered by the glass slide. The sample was then coated with platinum of 10 nm thickness to make them conductive and examined with a JMS-5610 scanning electron microscope (SEM) at 20 kv to observe the microscopic structure of the soya bean protein. Pictures were taken under a certain magnification (400 ×). The morphological characteristics of protein samples were analysed on images acquired using a JMS-5610 scanning electron microscope at an accelerated voltage of 150 kv and a working distance of 10–15 mm (Zhao *et al*., 2010c).

Infrared spectra measurement

The protein samples were prepared using the potassium bromide pellet method (Zhao *et al*., 2008c). Infrared spectra of soya bean protein were measured with a Perkin–Elmer Model GX Fourier transform infrared spectrophotometer at 20 °C. Reference spectra were recorded under identical conditions, but with potassium bromide containing no protein. Scanning range of Fourier transform infrared spectroscopy was 4000–400 cm^{-1}, and resolution ratio was 4 cm^{-1}. Reactions inside the sample could be deducted based on the changes in protein structure from infrared spectra. The second derivative and Fourier deconvolution technique were used to improve the resolution ratio, and peak fit software was utilised to determine the limit of amide belt I for gaining secondary deconvolution spectra, which could analyse protein secondary structure changes.

Statistical analysis

All experimental data in this study were an average of triplicate observations and subject to one-way analysis of variance (ANOVA) using SAS 9.0 software (SAS Institute Inc., Cary, NC, USA).

Results and discussion

The yield and purity of soya bean protein products

Using AOT/Tween 85 reverse micelle system, 0.29 g protein products was extracted from 1 g soya bean flour, while using AOT reverse micelle system, 0.28 g protein was extracted from 1 g soya bean flour. The purity of protein extracted by AOT reverse micelle extraction was 80.2%, and by AOT/Tween 85 reverse micelle extraction, it was 79.1%. Thus, the yield and purity of protein products by these two systems were similar. The content of surfactant in soya bean protein separated by the reverse micelles is very low and most of the surfactant retained in oil phase. If further purification is needed, soya bean protein could be washed with 65% ethanol solution to remove any residual surfactant (Zhu *et al*., 2010).

Effects of various factors on the efficiency of forward extraction of soya bean protein

Aqueous phase pH

The aqueous phase pH in the reverse micelle systems varied from 6 to 10 as shown in Fig. 1a. Soya bean protein extraction efficiency increased with increasing aqueous phase pH, reaching a maximum at pH 7.0 and decreased with a further increase in pH. At pH 7.0, the efficiency of protein extraction in the AOT/Tween 85 system (92.5%) was higher than that in the AOT system (88.4%).

Electrostatic interactions between the ionic surfactant molecules and the counter charge of protein molecules are considered the main driving force in the forward extraction process (Dekker *et al*., 1986). pH that mainly affects the charge numbers of proteins is a dominant factor for the extraction process. Proteins in solution exhibit an amphoteric ionisation phenomenon. When the net charge and electric field mobility are equal to zero, the pH value stands for pI (protein isoelectric point). When pH is higher than pI, the proteins are charged with negative electricity; otherwise, the proteins are charged with positive electricity. In our two reverse micelle systems, surfactant molecules

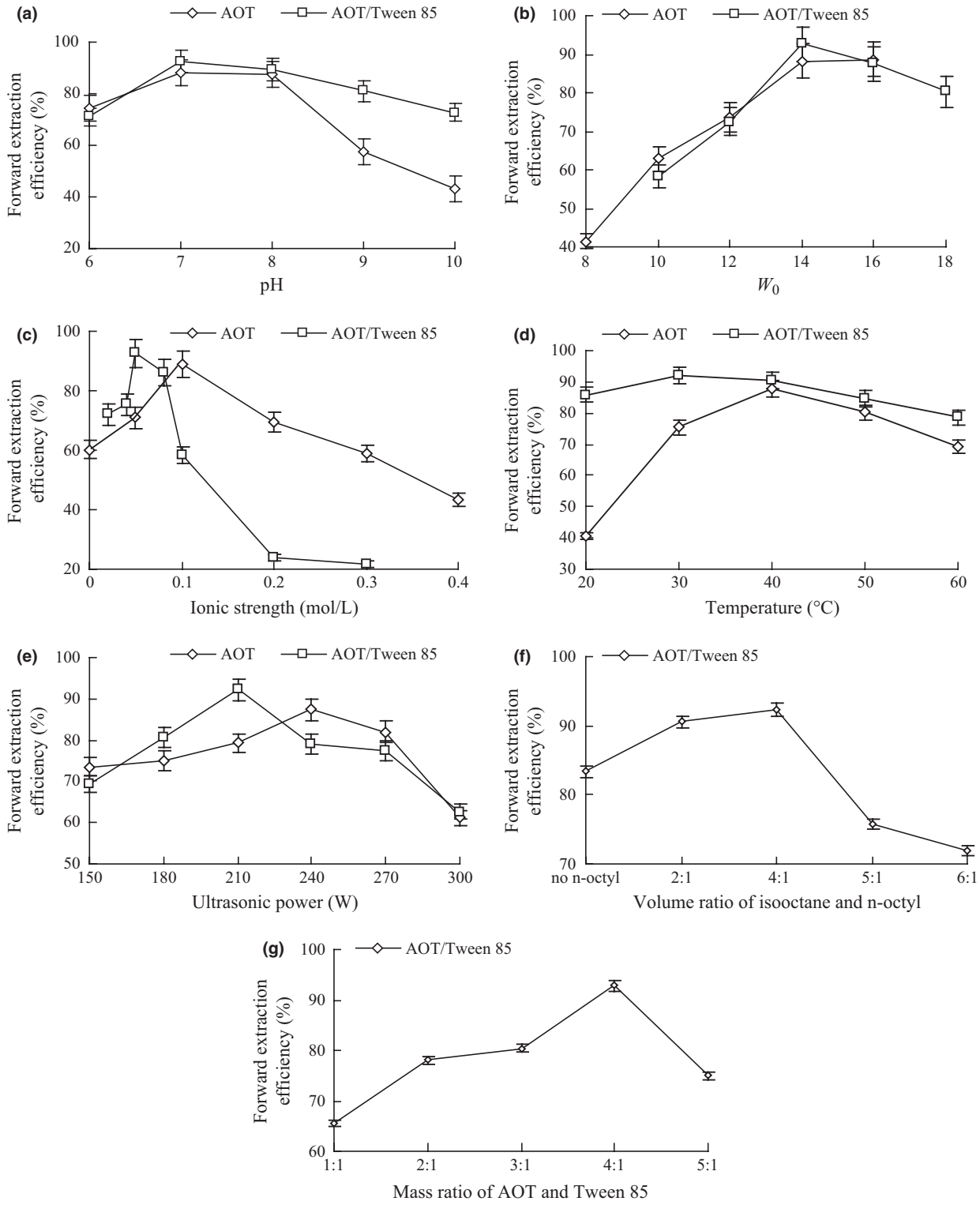

Figure 1 Effects of aqueous phase pH (a), W_0 (b), ionic strength (c), ultrasonic temperature (d), ultrasonic power (e), volume ratio of isooctane and n-octyl (f) and mass ratio of AOT and Tween 85 (g) on the efficiency of forward extraction.

were charged with negative electricity because AOT is an anionic surfactant and Tween 85 is a nonionic surfactant. In the forward extraction, the optimum extraction condition occurred at pH 7; thus, the protein was negatively charged because the pI of soya bean protein is about 4.5. These results indicate that electrostatic interactions should not be the sole driving force behind protein solubilisation (Barbosa *et al*., 2006). When the protein was negatively charged and solution pH is above its pI, hydrophobic interactions between proteins and surfactants may have led to protein solubilisation.

W_0

As shown in Fig. 1b, W_0 strongly affected the extraction efficiency, and the optimum W_0 of the two reverse micelle systems was 14. W_0 is an important physical factor determining the structural and physical properties of micelles. The relationship between micelle sizes and W_0 was described by the water shell model (Luisi *et al*., 1979). In addition, the water pool radius is proportional to water content, and W_0 itself thus reflects the size of the reverse micelle (Liu *et al*., 2006). The pool radius increased with increasing W_0, indicating that not only small molecule proteins but also macromolecular proteins can enter the micelle. These findings also suggest that protein extraction efficiency increases with increasing W_0 (Harikrishna *et al*., 2002). However, when W_0 is excessively high, surfactant molecules would release from reverse micelles into the organic phase owing to the hydrophobic groups, thereby reducing the number of micelle aggregates and the efficiency of protein extraction (Bu *et al*., 2012).

Ionic strength

The salts added to the aqueous solution to adjust ionic strength largely influence protein extraction. In protein solution, KCl ions can increase the protein surface charge as well as enhance the reaction between protein and water molecules, indicating that protein solubility increases in aqueous solution and KCl plays a role as salt dissolver (Zhao *et al*., 2008b). When salt ions are at high concentrations, the electrostatic repulsion of the surfactant polar head reduces and reverse micelles become smaller, thus diminishing the solubilisation of water and biological molecules in reverse micelles. As shown in Fig. 1c, the optimum KCl concentration in the AOT/Tween 85 system was significantly lower than that in the AOT system, which can be attributed to the fact that AOT is an anionic surfactant, whereas Tween 85 is a nonionic surfactant.

Ultrasonic temperature

As shown in Fig. 1d, the aqueous ultrasonic temperature varied from 20 to 60 °C. The efficiency of soya bean protein extraction increased with increasing ultrasonic temperature, reaching a maximum of 87.6% at 40 °C for AOT system and 92.2% at 30 °C for AOT/Tween 85 system. The efficiency then decreased with a further increase in ultrasonic temperature. The phenomenon occurred because within a certain range, molecular thermal motion increases with increasing temperature, which influences the aggregate number of surfactant molecules (Bu *et al*., 2012). In addition, temperature has a critical impact on micelle concentration. When temperature increases, the critical micelle concentration increases to a certain degree, indicating that the amount of protein dissolved in polar core increases with an increase in micelle aggregate numbers (Sun *et al*., 2008). However, excessively higher temperature causes not only protein denaturation and reduced particle diameter but also reduced affinity between surfactant and water, thus leading to reduced water in reverse micelles, unstable reverse micelle systems and diminished protein extraction efficiency.

Ultrasonic power

As shown in Fig. 1e, the efficiency of soya bean protein extraction increased with an increased ultrasonic power, reaching its maximum of 92.2% at 210 W for AOT/Tween 85 system and 87.4% at 240 W for AOT system, and then decreased with a further increase in ultrasonic power. In the study of Zhu *et al*. (2009), response surface methodology was used to optimise the ultrasound-assisted extraction parameters for enhancing the forward extraction efficiency of defatted wheat germ proteins by reverse micelles. Their results showed that ultrasound-assisted extraction can not only shorten extraction time, but also increase the forward extraction efficiency of defatted wheat germ proteins from 37% to 57%. These suggested that the combination of ultrasound-assisted extraction and reverse micelles could improve the forward extraction efficiency of proteins from plant raw materials.

Ultrasonic power can provide a certain impetus for reverse micelle extraction of protein, and it modifies the spatial structure of proteins, indicating that proper levels of ultrasonic power can make the structure of proteins more conducive to enter reverse micelle systems (Zhu *et al*., 2009). Ultrasonic cavitation and mechanical effects strengthen the solid–liquid interface turbulence, and the microjet generated by cavitations promotes the diffusion of reverse micelle solvent onto the soya bean flour surface (Wheat *et al*., 1996) as well as protein leaching and transferring. These indicate that ultrasonic power improves the efficiency of protein extraction. When ultrasonic power is not strong enough, extraction is not optimal. On contrast, when ultrasonic power is extremely strong, other substances enter reverse micelles and affect protein extraction. Therefore, suitable ultrasonic power levels should be chosen for effective extraction of proteins.

Volume ratio of isooctane and n-octyl

As shown in Fig. 1f, the extraction efficiency in the AOT/Tween 85 reverse micelle system increased with an increased volume ratio of isooctane to n-octyl, reaching a maximum of 92.4%, and then decreased. With increased n-octyl, the aggregation numbers of reverse micelle groups increase and pool diameters become larger, which result in an increased efficiency of protein molecule extraction (Zhao *et al*., 2010b; Uda *et al*., 2011). Conversely, under excessive co-solvent, the aggregation numbers of reverse micelle groups decreased (Bansal-Mutalik & Gaikar, 2006).

Mass ratio of AOT and Tween 85

As shown in Fig. 1g, the extraction efficiency in the AOT/Tween 85 reverse micelle system achieved its maximum when the mass ratio of AOT and Tween 85 was 4:1. An appropriate combination of anionic surfactant and nonionic surfactant could form a stable reverse micelle solution. The addition of a nonionic surfactant was beneficial to the enlargement of the pool diameter, which solubilised more proteins (Dekker *et al*., 1986). However, further increase in co-surfactant Tween 85 led to an instable reverse micelle system, thereby compromised extraction efficiency.

Microstructure analysis of soya bean protein isolate (SPI) extracted

Microstructures of three kinds of soya bean proteins analysed by SEM are shown in Fig. 2. SPI1, SP2 and SP3 were SPIs obtained using the alkali solution and acid isolation method, AOT reverse micelle extraction and AOT/Tween 85 reverse micelle extraction, respectively. As shown by the SEM micrographs, the microstructures of soya bean proteins differed. SPI1 was mainly spherical in structure, whereas SPI2 and SPI3 exhibited a mostly lamellar structure. The structures of soya bean proteins obtained using the two reverse micelle systems were similar, but particle sizes in the AOT/Tween 85 system were larger. This phenomenon may be related to solution pH, pool size and surfactants. For instance, hydrophilic and hydrophobic interactions produced between reverse micelles and proteins altered the protein structure and then resulted in varying protein particle sizes. Pool size also influenced the size of proteins. Under special microenvironmental conditions, some factors induced changes in protein structure, such as electrostatic interactions that occur by molecule charges as well as the number and size of reverse micelles affected by surfactants in organic phase (Hua *et al*., 2005; Zhao *et al*., 2008a).

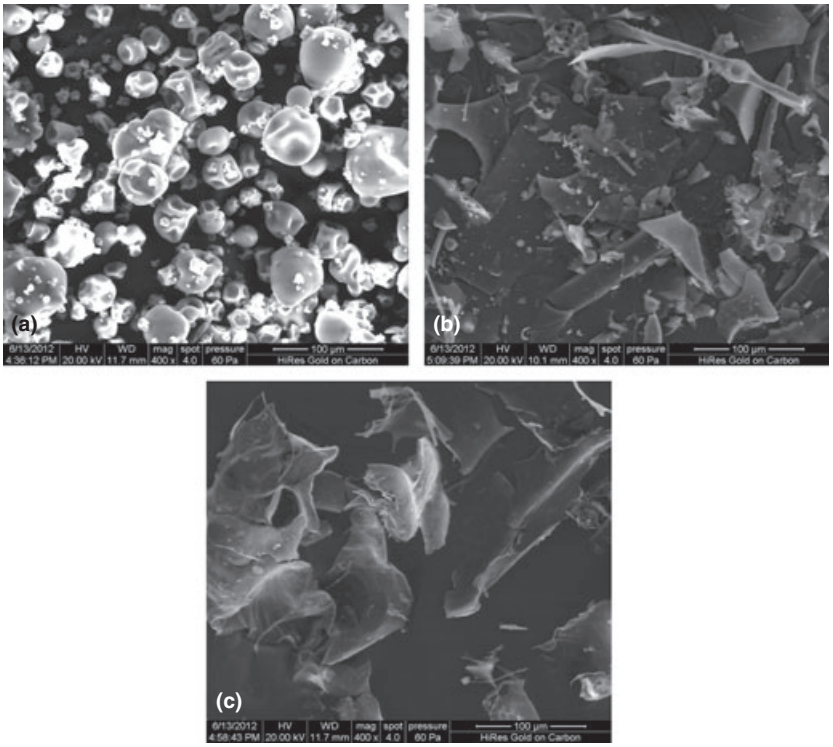

Figure 2 SEM micrographs of soybean protein isolate (SPI): (a) SPI1: SPI obtained using alkali solution and acid isolation method; (b) SPI2: SPI obtained using AOT reverse micelle extraction; (c) SPI3: SPI obtained using AOT/Tween 85 reverse micelle extraction.

Secondary structure of SPI

As shown in Fig. 3, the identified spectrum peak of amide belt I was somewhat different for three kinds of soya bean proteins (SPI1, SP2 and SP3). Through the calculation of test results and based on literature review, the following parameters were used: α-helix, 1660–1650 cm^{-1}; β-sheet, 1640–1600 cm^{-1}; β-turn, 1700–1660 cm^{-1}; and unordered structure, 1650–1640 cm^{-1}. As shown in Tables 1 and 2, the primary difference between soya bean proteins using reverse micelle extraction and SPI1 is that the former had no unordered structure, which may be due to changes in the hydrogen bond. The protective role of the reverse micelle pool led to a strong interaction between the charged amino acids on the protein surface and to a reduction in the frequency of amide belt I, which can account for the disappearance of the unordered structure (Zhao *et al.*, 2009).

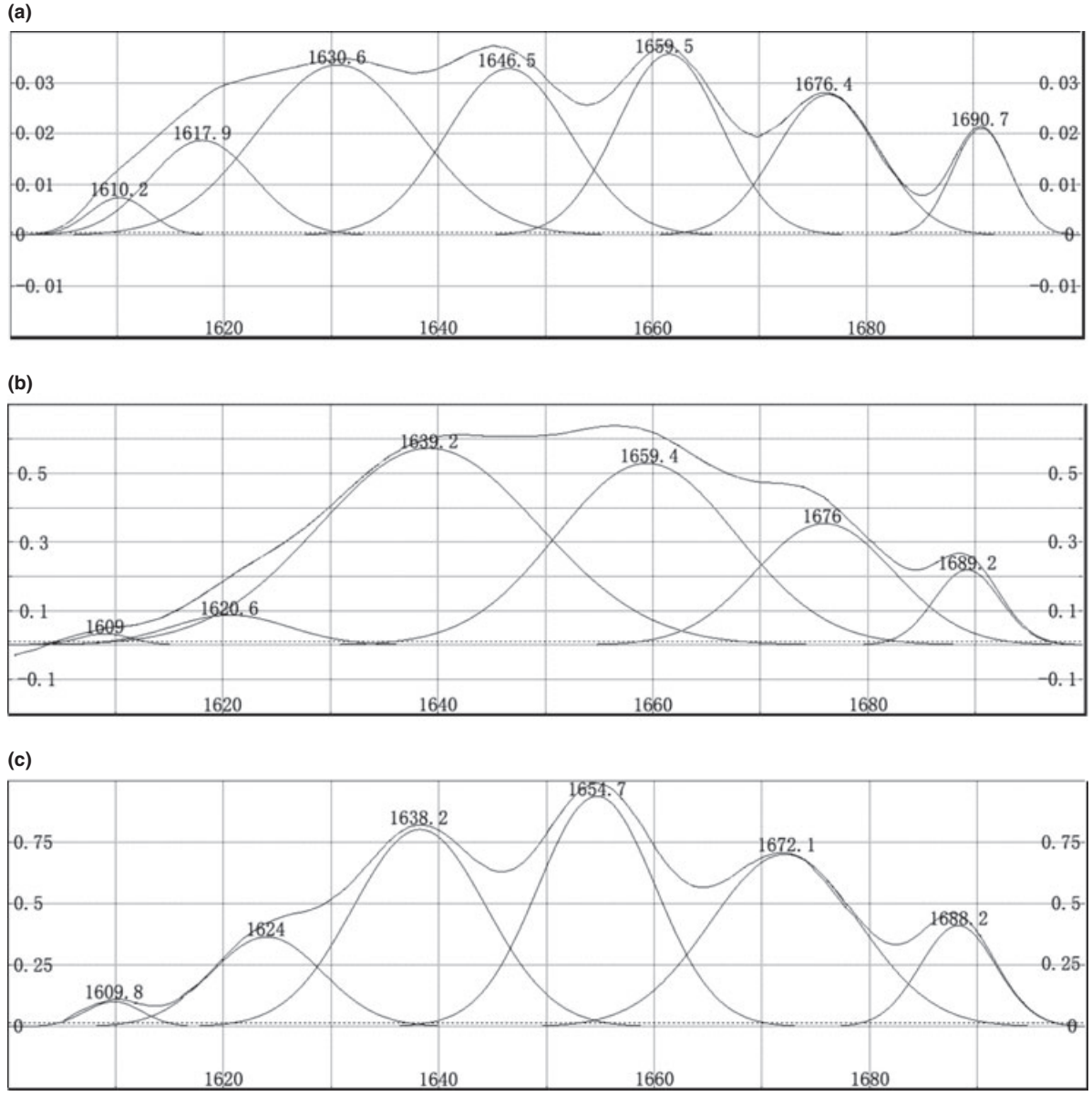

Figure 3 Second order reciprocal spectrum and fitting calculated spectrum of SPI amide belt I: (a) SPI1: Soybean protein isolate obtained using alkali-solution and acid-isolation method (b) SPI2: Soybean protein isolate obtained using AOT reverse micellar extraction (c) SPI3: Soybean protein isolate obtained using AOT-Tween 85 reverse micellar extraction.

Table 1 Frequency of amide belt I (cm^{-1}) of SPI

Protein	β-sheet	Unordered structure	α-helix	β-turn
SPI1	1610.2, 1617.9, 1630.6	1646.5	1659.5	1676.4, 1690.7
SPI2	1609.0, 1620.6, 1639.1	–	1659.4	1676.0, 1689.2
SPI3	1609.8, 1624.0, 1638.2	–	1654.7	1672.1, 1688.2

Table 2 The secondary structure of different SPIs by determination with FTIR

	Secondary structure (%)			
Protein	β-sheet	Unordered structure	α-helix	β-turn
SPI1	39.5	20.8	19.4	20.3
SPI2	46.5	–	32.1	21.4
SPI3	38.4	–	27.7	33.9

The spectra of soya bean proteins extracted by the two reverse micelle systems were also different, which may be due to the difference in surfactant that influenced the shift of the spectrum belt and the strength of the peak.

In this study, soya bean proteins extracted by AOT system have more α-helix, β-sheet and β-turn, but no unordered structure than by aqueous solution. Another research showed that the soya bean proteins separated by AOT reverse micelles contained less turn and more α-helix, β-sheet and random coil than by aqueous solution by the determination of FTIR spectroscopy (Chen *et al*., 2013). The discrepancy in the secondary structures of soya bean proteins in above two studies may be due to the differences in extraction conditions and mode by reverse micelle, such as the ultrasound-assisted extraction employed in our study. Zhu *et al*. (2010) determined the secondary structures of defatted wheat germ protein separated by reverse micelles (DWGRMPI) and defatted wheat germ protein separated by alkaline extraction and isoelectric precipitation (DWGPI) using CD spectra. They found that DWGRMPI contained less random coil and more α-helix than DWGPI, indicating that DWGRMPI would have a compact and ordered conformation compared with DWGPI. Reverse micelle extraction had the least influence on the secondary structures of proteins, and the proteins could remain in its native conformation (Zhu *et al*., 2010; Chen *et al*., 2013).

Comparison of oil extracted by reverse micelle and immersion

Extraction efficiency of oil products

As shown in Table 3, the efficiency of soya bean oil extracted by immersion was higher than that of the reverse micelle systems. The efficiency of soya bean oil extraction in the AOT/Tween 85 reverse micelle system (77.2%) was higher than that in the AOT reverse micelle system (72.5%; $P < 0.05$). The surfactant Tween 85 influenced the extraction efficiency probably because the viscosity of Tween 85 is lower than that of AOT, which resulted in greater interface resistance in the AOT reverse micelle system compared with the AOT/Tween 85 system. Thus, oil easily entered the isooctane of the AOT/Tween 85 reverse micelle system.

Properties of oil products

Two main indexes were adopted to evaluate oil characteristics, POV and AV. The higher the POV (an index of oil fat rancidity degree) is, the more severe the oil fat rancidity becomes (Liu *et al*., 2011). The oxidative rancidity of oil produces some small molecules and has adverse effects on the human body, such as the generation of free radicals, rendering oil with high POV. Acid value represents free FA content (Terigar *et al*., 2010). In the process of oil manufacturing, the acid value can be used as an indicator of the degree of hydrolysis and can indicate rancidity during the storage of oil. The smaller the acid value is, the better the oil quality (Suja *et al*., 2004). As shown in Table 4, the acid value and POV of the oil extracted by reverse micelle were lower than those of the oil immersed ($P < 0.05$); therefore, the quality of oil from reverse micelle extraction is better than that from immersion.

Table 3 Extraction efficiency of soya bean oil obtained by different extraction methods

Extraction method	Extraction efficiency (%)
Immersion	80.0 ± 0.2^{a}
AOT reverse micelle	72.5 ± 0.1^{c}
AOT/Tween 85 reverse micelle	77.2 ± 0.1^{b}

Means ± SD (standard deviation) for three experiments within a column sharing common lower-case letters were not significantly different ($P > 0.05$).

Table 4 Properties of soya bean oil obtained by different extraction methods

Extraction method	Acid value (mgKOH g^{-1})	Peroxide value (meq kg^{-1})
Immersion	1.76 ± 0.05^{a}	23.7 ± 0.6^{a}
AOT reverse micelle	1.25 ± 0.03^{c}	17.5 ± 0.3^{b}
AOT/Tween 85 reverse micelle	1.41 ± 0.02^{b}	12.2 ± 0.3^{c}

Means ± SD (standard deviation) for three experiments within a column sharing common lower-case letters were not significantly different ($P > 0.05$).

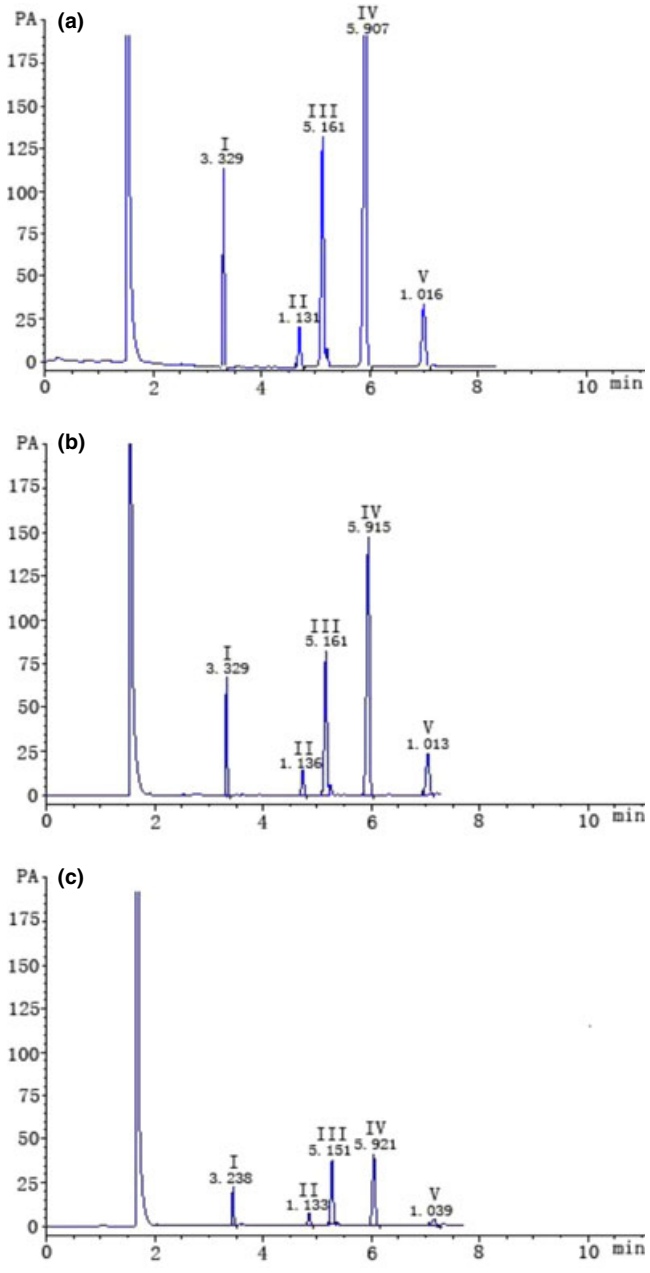

Figure 4 Gas chromatogram of soybean oil: (a) soybean oil obtained using immersion; (b) soybean oil obtained using AOT reverse micelle extraction; (c) soybean oil obtained using AOT/Tween 85 reverse micelle extraction. I: palmitic acid, II: stearic acid, III: oleic acid, IV: linoleic acid, V: linolenic acid.

FA composition of oil products

Gas chromatograms of oil products obtained by immersion, AOT reverse micelle system extraction and AOT/Tween 85 reverse micelle system extraction are presented in Fig. 4. The FA composition obtained from gas chromatography is shown in Table 5. The FA content is not the same in these three extraction methods. Polyunsaturated FA and unsaturated FA in oil from AOT/Tween 85 extraction were lower and higher, respectively, than those from the other methods. In general, the FA composition of the oil obtained by the three extraction methods was at a reasonable level and good quality because it is rich in linoleic acid, an essential FA for human body possessing vital physiological functions such as significantly reducing serum cholesterol and preventing cardiovascular disease (Ramadan *et al*., 2012).

Table 5 Fatty acid composition of soya bean oil obtained by different extraction methods

	Fatty acid composition (%)				
Extraction method	**Palmitic acid**	**Stearic acid**	**Oleic acid**	**Linoleic acid**	**Linolenic acid**
Immersion	11.6	3.7	23.9	52.3	8.5
AOT reverse micelle	11.7	3.9	24.4	50.7	9.3
AOT/Tween 85 reverse micelle	12.1	6.1	33.9	43.6	4.3

Conclusions

Reverse micelle systems can be applied for the extraction of protein and oil from soya bean flour. The research revealed that the efficiency of forward extraction of soya bean protein using an AOT/Tween 85 reverse micelle system was superior to that using an AOT reverse micelle system when both extractions occurred under their optimal extraction conditions. SEM micrographs showed that soya bean proteins obtained using alkali solution and acid isolation extraction were mainly spherical in structure, while those from reverse micelle extraction exhibited a majority of lamellar structure. The microstructures of soya bean proteins using the two reverse micelle extraction systems were similar; however, particle sizes in the AOT/Tween 85 system were greater than those in the AOT system. In addition, Fourier transform infrared spectroscopy showed that soya bean proteins obtained using reverse micelle extraction had no unordered structure. The extraction efficiency of soya bean oil using immersion, AOT reverse micelle system and AOT/Tween 85 reverse micelle system was 80.0%, 72.5% and 77.2%, respectively. The acid and peroxide values of oil products from two reverse micelle extractions were lower than those from immersion. Overall, the results indicate that AOT/Tween 85 reverse micelle system is effective in extracting soya bean protein and oil.

For further improving the efficiency of protein extraction, other reverse micelle systems will be applied in the future, including ionic surfactant-based reverse micelles, nonionic surfactant-based reverse micelles, mixed reverse micelles and reverse micelles with inner solubilisation of enzymes. Notably, the solubilisation of enzymes in the micelles can be used as a model system. First, the confinement of enzymes in the water pool of reverse micelle could simulate the enzyme action in living systems. Second, most of the enzymes will retain their catalytic activity and stability and even represent hyperactivity in the reverse micellar system (Orlich & Schomäcker, 2002). Thus, proteins will be efficiently extracted and simultaneously hydrolysed in the reverse micelle system. In our next work, to further improve the extraction efficiency and properties of soya bean protein, it is essential to carry out careful studies using reverse micellar system accompanied by the solubilisation of enzymes.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (No. 20976037, 31201293, 21176058, 31171790), the Doctor Foundation of Henan University of Technology (No. 2010BS014), the Innovation Education Foundation of Henan University of Technology (No. 11YJCX25) and the Innovation Scientists and Technicians Troop Construction Projects of Zhengzhou City.

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