African Journal of Biotechnology Vol. 11(28), pp. 7247-7257, 5 April, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.202
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Effects of different factors on the forward extraction of soy protein in reverse micelle systems

Guanhao Bu, Haiyuan Liu, Fusheng Chen*, Kunlun Liu, Yingying Yang and Yanxiu Gao

College of Food Science and Technology, Henan University of Technology, Zhengzhou 450001, China.

Accepted 14 March, 2012

Reverse micelle extraction is a new technology for the extraction of protein. In this research, three kinds of reverse micelle systems, anionic surfactant sodium bis(2-ethylhexyl) sulfosuccinate (AOT) reverse micelle system, sodium dodecyl sulfate (SDS) reverse micelle system, and cationic surfactant cetyltrimethyl ammonium bromide (CTAB) reverse micelle system, were used to extract soy protein respectively. Effects of soy flour concentration, Wo ([H_2O]/[AOT]), temperature, time, pH, ionic strength and ultrasonic power on forward extraction efficiency of soy protein were investigated. The effect of AOT reverse micelle diameter was studied as well. AOT reverse micelle system had higher extraction efficiency than SDS and CTAB systems. The main factors that affected the forward extraction were soy flour concentration, temperature and pH. The optimal conditions in AOT system were soy flour concentration being 0.007 g/ml, Wo 16, pH 6.5, temperature 34°C, time 20 min, KCl concentration of 0.1 mol/L and ultrasound power of 240 W. Under these conditions, the extraction efficiency of soy protein was 85.5%. The forward extraction efficiency of soy protein in AOT reverse micelle system increased with the increase of the reverse micelle diameter. Reverse micelle extraction is an effective way to extract soy protein.

Key words: Soy protein, reverse micelle, forward extraction, sodium bis(2-ethylhexyl) sulfosuccinate (AOT), sodium dodecyl sulfate (SDS), cetyltrimethyl ammonium bromide (CTAB).

INTRODUCTION

Reverse micelle extraction is a novel technology for liquid-liquid extraction, which has recently received immense attention for the isolation and purification of proteins. The reverse micelles are nanometersized aggregates of surfactant molecules in non-polar solvents which are thermodynamically stable and optically transparent (Nandini and Rastogi, 2009). This technique offers several advantages such as low interfacial tension, ease of scale-up and continuous mode of operation. Reverse micellar extraction of lipase was carried out by many research groups using AOT or CTAB as surfactants (Naoe et al., 2007; Shen et al., 2005; Wu et al., 2006; Yu et al., 2003). Isooctane, hexane and carbon tetrachloride were used as solvents, whereas, hexanol, isopropanol and butanol were used as co-surfactant/co-solvent. Shen et al. (2005) demonstrated that mixed reverse micelles consisting of CTAB for the extraction of industrial lipase resulted in maximum activity recovery of 70%. Reverse micelle can also preserve the properties of proteins and other bioactive molecules. The biotechnological relevance of these structures arises from their ability to solubilize water and hydrophilic molecules, such as proteins in their polar cores (Lye et al., 1994).

Soy proteins are widely used in many kinds of foods as functional ingredients due to their high nutritional value, functional properties, and low cost. Soy proteins are used in a variety of foods such as salad dressings, soups, imitation meats, beverage powders, cheeses, non-dairy creamer, frozen desserts, whipped topping, infant formulas, breads, breakfast cereals, pastas, and pet foods. The Alkali dissolving acid sedimentation approach will reduce the activity of soy protein and as such new

*Corresponding author. E-mail: fushengc@yahoo.com.cn. Tel: +86-371-67756166. Fax: +86-371-67756667.

Abbreviations: SDS, Sodium dodecyl sulfate; **AOT,** sodium bis(2-ethylhexyl) sulfosuccinate; **CATB,** cetyltrimethyl ammonium bromide.

methods should be researched. Several research groups have engaged in the studying of the extraction of proteins by reverse micelles (Matzke et al., 1992; Zhao et al., 2011). Zhao et al. (2008a, b) investigated the extraction of soy 7S and 11S globulins from AOT reverse micelle. There are many factors that can affect the efficiency of extraction. The distribution of proteins between the micellar phase and the aqueous phase is largely determined by the environments of bulk aqueous phase, such as pH, ionic strength, and type of salts. Parameters related to the organic phase also affect the partition of protein, such as the concentration and type of surfactant, presence of co-surfactant, and type of solvent (Pires et al., 1996). Reverse micelle can be formed by anionic surfactant (such as AOT and SDS), cation surfactant (such as CTAB), and non-ionic surfactants (such as Spans and Tweens) in different systems where the factors that affect the extraction efficiency are different.

The process of reverse micelle extraction, in general, consists of the forward extraction of protein from the feed aqueous phase to the reverse micelle organic phase and the backward extraction of protein from the reverse micelle organic phase to the recovery aqueous phase (Noritomi et al., 2006a). The objectives of this research were to investigate the forward extraction efficiency of soy protein in three reverse micelle systems, namely, anionic surfactant AOT and SDS systems, and cation surfactant CTAB system, and to study the effects of various factors such as soy flour concentration, Wo, temperature, time, pH, ionic strength, and ultrasonic power on forward extraction efficiency of soy protein, and to optimize the extraction conditions as well.

MATERIALS AND METHODS

Materials and chemicals

Soy flour (100 mesh) was obtained from Anyang Mantianxue Food Manufacturing Co. Ltd (Anyang, China). It contained 377.5 g total protein, 223.7 g crude fat, and 68.5 g humidity per kilogram. Spectrophotometric-grade isooctane and Karl-Fischer titrant were obtained from Tianjin Kemiou Chemical Reagent Company (Tianjin, China). All other chemicals used in the experiment were of analytical grade.

Chemical analysis

Crude protein of soy flour was determined using the micro-Kjeldahl method (Concon and Soltess, 1973). Crude fat was measured by Soxhlet extraction (AOAC, 1984). The moisture content was determined by drying in an oven at 105°C until a constant weight was obtained.

Preparation of three reverse micelle systems

The first reverse micelle system was formed by sodium bis(2-ethylhexyl) sulfosuccinate (AOT), isooctane and KCl solution. Various amounts of AOT were mixed with 50 ml isooctane and stirred at room temperature. When AOT dissolved completely, KCl solution was added with various concentrations at (0.025, 0.05, 0.10, 0.15, 0.30, 0.40 mol/L) and pH at (5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5). Wo (6, 8, 10, 12, 14, 16) was the molar ratio of water to surfactant (Wo = $[H_2O]/[AOT]$) and it was determined by the Karl-Fischer method. It was reverse micelle if the solution was transparent, and when otherwise, it was not reverse micelle.

The second reverse micelle system was formed by sodium dodecyl sulfate (SDS)/isooctane/n-octyl alcohol and KCl solution. The third reverse micelle system was formed by cetyltrimethyl ammonium bromide (CTAB)/isooctane/n-octyl alcohol and KCl buffer solution. They were both prepared with the same procedure as that for the first reverse micelle system.

Forward extraction of soy protein

All extraction experiments were carried out in 250 ml Erlenmeyer flask with stoppers. Various concentrations of soy flour (0.01, 0.015, 0.02, 0.025, 0.03, 0.035, 0.04 g/ml) and reverse micellar systems were mixed together in Erlenmeyer flasks and extracted with sonication at 150, 180, 210, 240, 270, 300 W for 10, 20, 30, 40, 50, 60 min at different temperatures (25, 30, 35, 40, 45, 50, 55, 60°C), respectively. The undissolved residue was separated by centrifugation at 5000 rpm for 10 min and the volume of supernatant measured.

Analysis of soy protein concentration

Soy protein concentrations in organic of forward extractions were determined by spectrophotometer (UV-160A, Shimadzu, USA) at 280 nm (Zhao et al., 2010). Efficiency of forward extraction was estimated using the given equation:

$$\text{Forward extraction efficiency (\%)} = \frac{\text{total protein in the reverse micelle system}}{\text{total protein in soy flour}} \times 100 \quad (1)$$

Determination of reverse micelle diameter

Diameter of reverse micelle (The Wo of reverse micelle was different) was determined directly by ZetaPlus instrument (Brookhaven Instrument Co., USA).

Experimental design and statistical analysis

All analysis was carried out in triplicate. Efficiencies of extraction were expressed as means ± SD. On the basis of single factor experiments, further study was designed with Response Surface Analysis in the SAS 8.1 software (SAS Institute Inc., Cary, NC, USA). SAS statistical package was also used for regression analysis of the data and estimation of the coefficients of the regression equation. The statistical significance of the model was determined by the application of Fisher's *F*-test. The significance of each coefficient was determined using the Fisher's *F*-test and *P* value.

RESULTS AND DISCUSSION

Effects of various factors on forward extraction efficiency of soy protein

Figure 1 shows the forward extraction efficiencies of soy

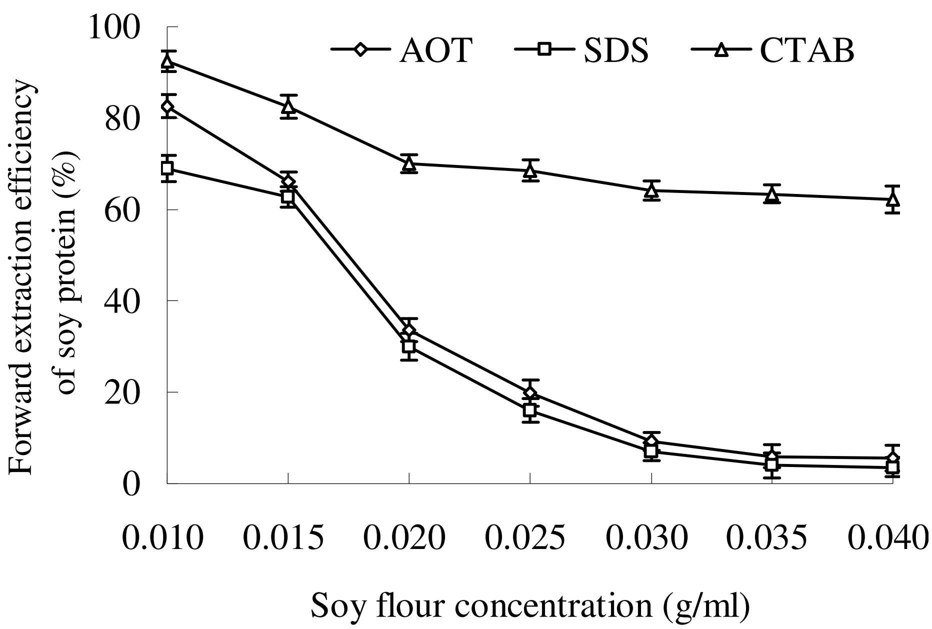

Figure 1. Effects of soy flour concentrations on the forward extraction efficiency of soy protein using different reverse micelle systems. Extraction conditions were as follows: Wo 16, pH 6.5 for AOT and SDS, and pH 10 for CTAB, temperature 35°C, extraction time 20 min, KCl concentration 0.1 mol/L, ultrasound power 240 W for AOT, 210 W for SDS and 270 W for CTAB.

protein at various concentrations of soy flour using different reverse micelle systems (AOT, SDS and CTAB). Results indicate that when soy flour concentration varied from 0.01 to 0.04 g/ml, the forward extraction efficiency decreased gradually in these three systems. It was due to this concentration that the reverse micelle was constant, and soy protein which could enter the micelle was limited. These results are consistent with the conclusion of Sun et al. (2008). In addition, the extraction efficiency decreased more slowly in CTAB system than in AOT and SDS systems, this may be explained by the fact that CTAB held more water in the micelle, and the size of CTAB reverse micelle was bigger than AOT and SDS systems, therefore, it could take more soy protein (Ekwall et al., 1971; Fang and Yang, 1999; Li et al., 2006).

As shown in Figure 2, the forward extraction efficiency increased with the increase of Wo. When Wo reached up to 16, the forward extraction efficiency reached a maximum in the three systems. The increase of Wo caused an increase of the reverse micelle size, meanwhile, the protein solubilization strongly depended on the reverse micelle size. The size of micelle relative to the size of a protein was critical to the ability of the micelle to solubilize protein (Sechler et al., 2010). The addition of protein to reverse micelles did not appreciably solubilize the protein until the diameter of the reverse micelle was similar to that of the protein (Matzke et al., 1992). With the increasing of Wo, some larger reverse micelles were formed and that were able to include plural protein molecules. This result is consistent with the report from Leser et al. (2008).

It could be observed in Figure 3 that the forward extraction efficiency increased slowly after 20 min. Zhao (2001) reported that the mass transfer rate of protein decreased with elongation of extraction time and the forward extraction efficiency slowly increased. Therefore, in order to save extraction time and obtain high extraction efficiency, the optimal extraction time was selected at 20 min. From Figure 4, it could be seen that the forward extraction efficiency reached the maximum when temperature was at 35°C in SDS and CTAB systems, and 40°C in AOT system. However, after that the forward extraction efficiency significantly decreased with temperature increased in the three systems. This may be explained by two reasons. Firstly, the water solubility in the reverse micelles reduced by decreased temperature. The decrease of the water solubility resulted in the decrease of solubilization of protein into the reverse micelle because the protein was entrapped into the reverse micelle by accompanying the water (Noritomi et al., 2006b). In addition, Kommareddi et al. (1993) found that the effect of increasing temperature on the microstructure of AOT reverse micelles included: faster tumbling of the reversed micelles, and increased lateral diffusion of the surfactant molecules due to the increase of thermal energy. This accounted for the forward extraction efficiency increased with the increasing temperature. However, higher temperature led to the expulsion of water from reverse micelles and the reduction of protein solubilization, thus the forward extraction efficiency

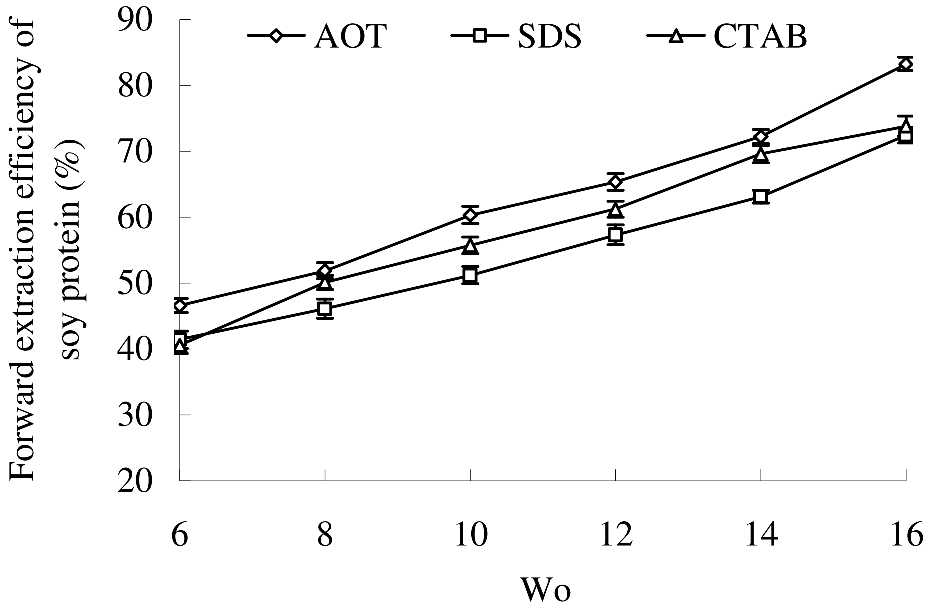

Figure 2. Effects of Wo on forward extraction efficiency of soy protein using different reverse micelle systems. Extraction conditions were as follows: soy flour concentration 0.01 g/ml, pH 6.5 for AOT and SDS, and pH 10 for CTAB, temperature 35°C, extraction time 20 min, KCl concentration 0.1 mol/L; ultrasound power 240 W for AOT, 210 W for SDS and 270 W for CTAB.

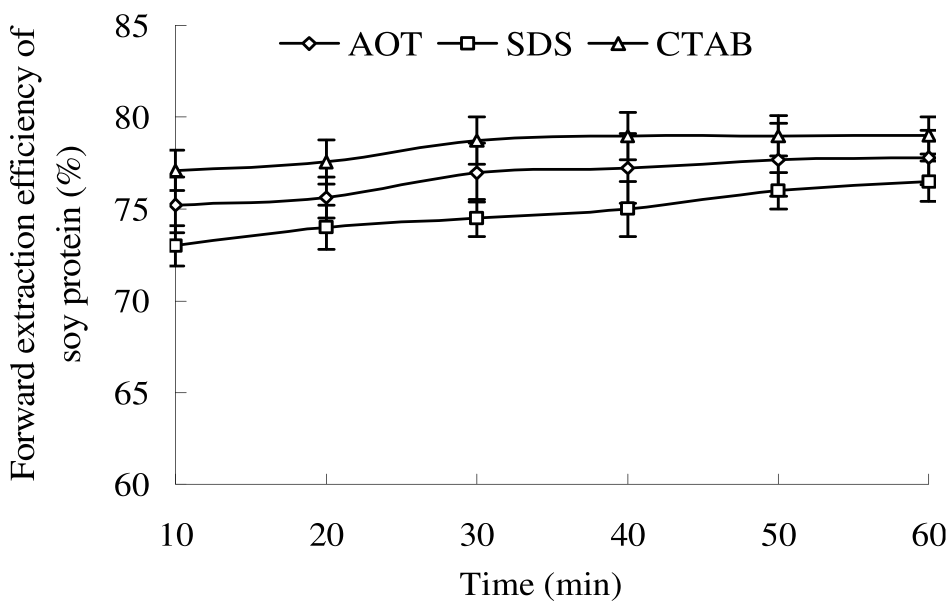

Figure 3. Effects of extraction time on forward extraction efficiency of soy protein using different reverse micelle systems. Extraction conditions were as follows: soy flour concentration 0.01 g/ml, Wo 16, pH 6.5 for AOT and SDS, and pH 10 for CTAB, temperature 35°C, KCl concentration 0.1 mol/L; ultrasound power 240 W for AOT, 210 W for SDS and 270 W for CTAB.

reduced (Hilhorst et al., 1992).

As shown in Figure 5, with the increase of the KCl concentration from 0.025 to 0.4 mol/L, the forward extraction efficiency increased at the beginning, while it considerably decreased when KCl was higher than 0.1 mol/L in the three reverse micelle systems. KCl concentration

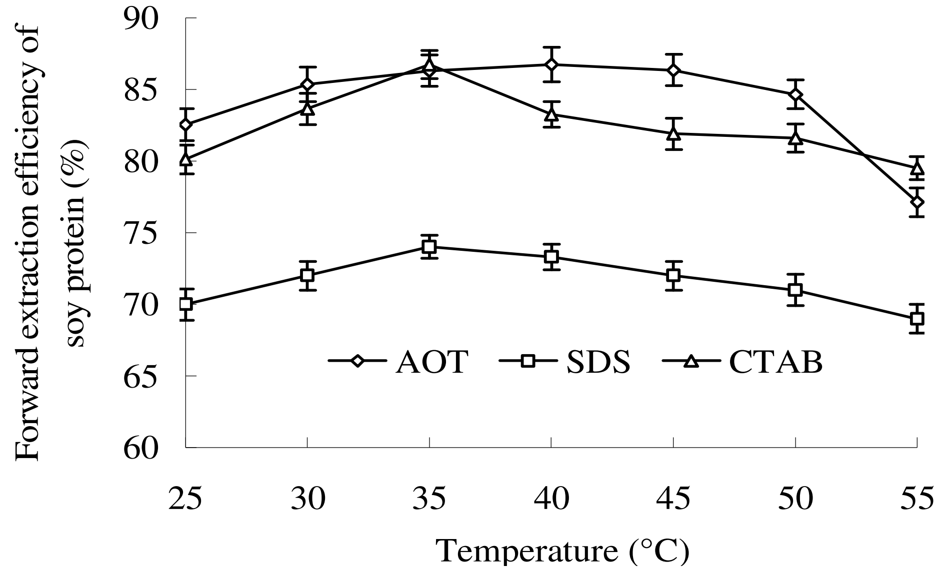

Figure 4. Effects of temperature on forward extraction efficiency of soy protein using different reverse micelle systems. Extraction conditions were as follows: soy flour concentration 0.01 g/ml, Wo 16, pH 6.5 for AOT and SDS, and pH 10 for CTAB, extraction time 20 min, KCl concentration 0.1 mol/L; ultrasound power 240 W for AOT, 210 W for SDS and 270 W for CTAB.

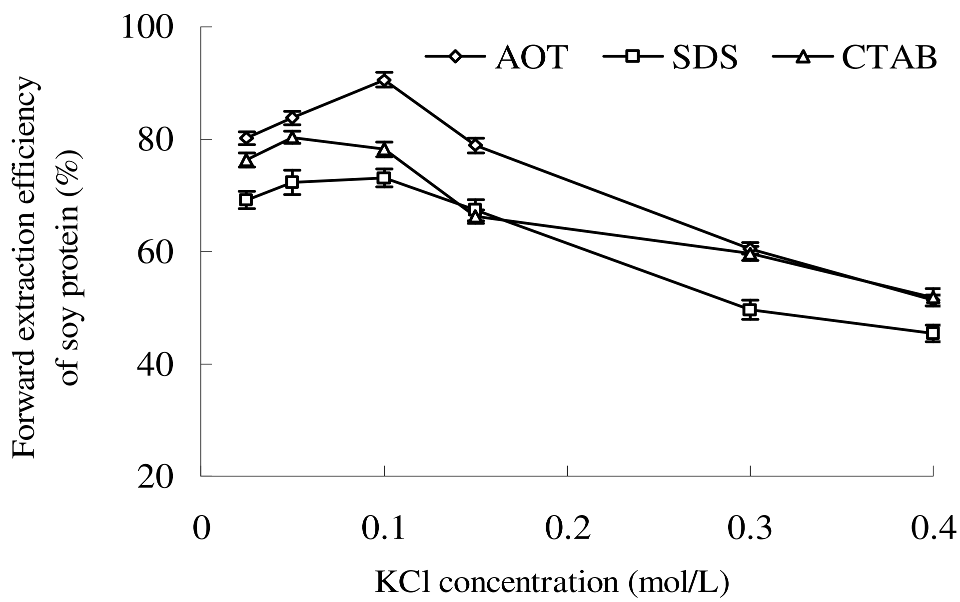

Figure 5. Effects of KCl concentration on forward extraction efficiency of soy protein using different reverse micelle systems. Extraction conditions were as follows: Soy flour concentration 0.01 g/ml, Wo 16, pH 6.5 for AOT and SDS, and pH 10 for CTAB, temperature 35°C, extraction time 20 min, ultrasound power 240 W for AOT, 210 W for SDS and 270 W for CTAB.

affected the transfer behavior of protein due to micelle size changes or screening of electrostatic interactions between the protein and the micelle wall (Goklen and Hatton, 1985). When concentration of salt ions was higher, the electrostatic repulsion of the surfactant polar head could be reduced and the reverse micelles became smaller, thus, solubilization of water and biological molecules in reverse micelle decreased (Dekker et al., 1989).

Figure 6 shows that the forward extraction efficiency

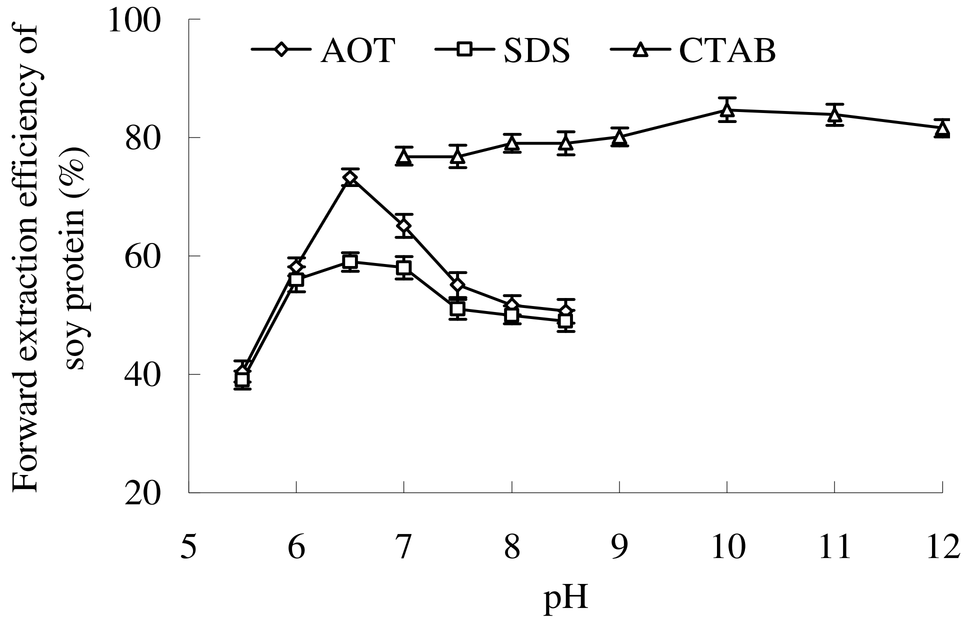

Figure 6. Effects of pH on forward extraction efficiency of soy protein using different reverse micelle systems. Extraction conditions were as follows: soy flour concentration 0.01 g/ml, Wo 16, temperature 35°C; extraction time 20 min; KCl concentration 0.1 mol/L; ultrasound power 240 W for AOT; 210W for SDS and 270 W for CTAB

increased and reached the maximum at pH 6.5 in AOT and SDS systems, and then decreased. For CTAB system, the forward extraction efficiency increased and reached the maximum at pH 10 while it decreased from pH 10 to 12.

The pH of aqueous phase plays a major role in controlling electrostatic interaction between enzyme and surfactant (Bansal-Mutalik and Gaikar, 2006). In the ionic reverse micellar systems, protein solubilization was regulated mainly by electrostatic interaction between the protein and the polar head of the surfactants (Zhao et al., 2010). AOT and SDS are anionic surfactant, so the reverse micelle polar heads of surfactant are negative electricity. The average isoelectric point of soy protein was about 4.5 and the protein was negatively charged above the isoelectric point. Therefore, it is deduced that electrostatic interaction cannot be a single driving force causing protein transfer at this time. Through the investigation, when the protein was negatively charged above the isoelectric point, it was inferred that the hydrophobic interaction between protein and surfactant led to protein solubilization (Paradkar and Dordick, 1994). However, the confirmatory mechanism of protein solubilization above the isoelectric point needed to be further researched.

CTAB is a cationic surfactant with positive electricity polar head. Thus, pH should be above the average isoelectric point of soy protein. From the research, the extraction efficiency reached the maximum at pH 10.

The conclusion could be drawn from the aforementioned research that reverse micelle formed by anionic and cationic surfactants had different extraction pH and electrostatic interaction between the protein and the polar head of the surfactants was one driving force in extraction, while there may be other driving forces during the extraction.

Figure 7 presents the effect of ultrasonic power on the forward extraction efficiency. In AOT and SDS reverse micelle systems, the extraction efficiency reached the maximum at 240 W, while in CTAB reverse micelle system, it was maximal at 270 W.

Assessment of model and effects of independent variables on response

AOT system had the highest efficiency based on the independent factor experiments. Therefore, further study was designed with Response Surface Analysis in the SAS software to gain the optimal conditions of forward extraction of soy protein in AOT reverse micelle system. The independent variables and their levels were shown in Table 1. The experimental conditions and the corresponding response value from the experimental design were presented in Table 2. The regression equation for the forward extraction efficiency of soy protein (Y) is thus, presented as:

$$Y = 41.04649 - 223.7724X_1 - 0.407296X_2 + 16.91792X_3 - 6337.437X_1X_1 + 9.61332X_1X_2 - 2.952147X_1X_3 - 0.020037X_2X_2 + 0.256886X_2X_3 - 1.989303X_3X_3 \quad (2)$$

The effects of independent variables on response (Y) and

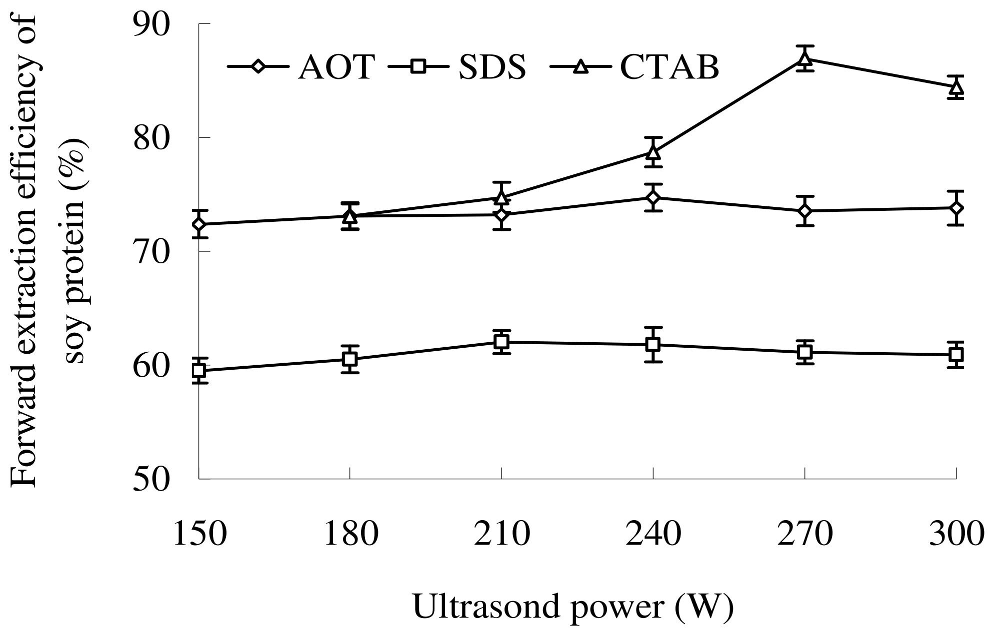

Figure 7. Effects of ultrasound power on forward extraction efficiency of soy protein using different reverse micelle systems. Extraction conditions were as follows: soy flour concentration 0.01 g/ml, Wo 16; pH 6.5 for AOT and SDS, and pH 10 for CTAB; temperature 35°C; extraction time 20 min; KCl concentration 0.1 mol/L.

Table 1. Independent variables and their levels employed in Response Surface Analysis experimental design.

Coded level	Independent variables		
	Soy flour concentration (g/ml)	Temperature (°C)	pH
	X_1	X_2	X_3
-1.414	0.0037	33	4.9
-1	0.010	35	5.5
0	0.025	40	7
1	0.040	45	8.5
1.414	0.046	47	9.1

analysis of variance (ANOVA) of regression model are presented in Table 3. For model fitted, the coefficient of determination (R^2), which could check the goodness of a model was 0.9976. This implied that the sample variation of 99.76% for the forward extraction efficiency of soy protein was attributed to the independent variables and only 0.24% of total variation cannot be explained by the model. The data proved that the developed model was adequate to represent the actual relationship among the parameters chosen. It can be easily seen that the model was highly significant and the effect order of independent factor was soy flour concentration > pH > temperature (Table 3). The independent variable *P*-values suggested that X_1 (soy flour concentration) and X_3 (pH) significantly affected Y (forward extraction efficiency of soy protein) ($P < 0.01$). All of the quadratic terms also had significant effects on Y ($P < 0.01$). The response surface plot and their corresponding counter plot for the forward extraction efficiency of soy protein by the fitted second-order polynomial model are shown in Figures 8 to 10.

Validation of the model

For validation of the model, soy protein in the reverse micellar solution was extracted under the optimal conditions and the forward extraction efficiency was determined. The experimental value was compared with the predicted one in order to determine the validity of the model. The stationary point giving a maximum forward extraction efficiency of soy protein had the following critical values: soy flour concentration 0.007 g/ml, temperature 34°C, and pH 6.5. The predicted forward extraction efficiency for these conditions by SAS statistical package

Table 2. Response Surface Analysis experimental design matrix with experimental values of the forward extraction efficiency of soy protein.

Assay	Independent variables[a]	Dependent variable		
	Soy flour concentration (g/ml)	Temperature (°C)	pH	Extraction efficiency (%)
1	0.010 (-1)	35 (-1)	8.5 (1)	79.2
2	0.010 (-1)	45 (1)	5.5 (-1)	80.1
3	0.040 (1)	35 (-1)	5.5 (-1)	78.5
4	0.040 (1)	45 (1)	8.5 (1)	77.9
5	0.0037 (-1.414)	40 (0)	7 (0)	85.8
6	0.046 (1.414)	40 (0)	7 (0)	78.3
7	0.025 (0)	33 (-1.414)	7 (0)	83.7
8	0.025 (0)	47 (1.414)	7 (0)	84.1
9	0.025 (0)	40 (0)	4.9 (-1.414)	77.5
10	0.025 (0)	40 (0)	9.1 (1.414)	74.4
11	0.025 (0)	40 (0)	7 (0)	85.2
12	0.025 (0)	40 (0)	7 (0)	85.2
13	0.025 (0)	40 (0)	7 (0)	85.2
14	0.025 (0)	40 (0)	7 (0)	85.2
15	0.025 (0)	40 (0)	7 (0)	85.2

[a] Values in parentheses are coded levels of independent variables.

Table 3. Significance of regression coefficients for response (Y) and analysis of variance (ANOVA) for model.

Source of variation	Degrees of freedom	Sum of squares	Mean square	*F* value	*Pr* > *F*	Significance
X_1	1	28.125	28.125	287.087	0.0001	**
X_2	1	0.080	0.080	0.817	0.408	ns
X_3	1	4.805	4.805	49.046	0.0009	**
X_1* X_1	1	15.685	15.685	160.107	0.0001	**
X_1* X_2	1	1.040	1.040	10.613	0.023	*
X_1* X_3	1	0.009	0.009	0.090	0.776	ns
X_2* X_2	1	1.936	1.936	19.759	0.007	**
X_2* X_3	1	7.424	7.424	75.780	0.0003	**
X_3* X_3	1	154.547	154.547	1577.551	0.0001	**
Model	9	203.310	22.590	230.589	0.0001	**
Error	5	0.490	0.098			
Total	14	203.8				

*Significant at the 0.05 level; ** Significant at the 0.01 level; ns, not significant at the 0.05 level.

was 86.2%, while the experimental value for these conditions was 85.5%. The results indicate that the experimental value (85.5%) was in agreement with the predicted one (86.2%).

The effect of reverse micelle diameter on forward extraction efficiency

The diameter of AOT reverse micelle was detected by ZetaPlus instrument and the extraction efficiency was studied at different diameter. Figure 11 shows the forward extraction efficiencies of soy protein at various diameters of AOT reverse micelle. The results indicate that with the increased reverse micelle diameter, the forward extraction efficiency increased. Previous reports showed that large micelles afford more space to the enzyme and presumably permit greater conformational flexibility (Spreti et al., 1999; Zhao et al., 2004). Hieda et al. (2008) reported that the size of gold nanoparticles formed inside the water droplets was regulated by the size of reverse micelles.

Conclusions

The research demonstrated the feasibility of the forward extraction of soy protein from soy flour by three different

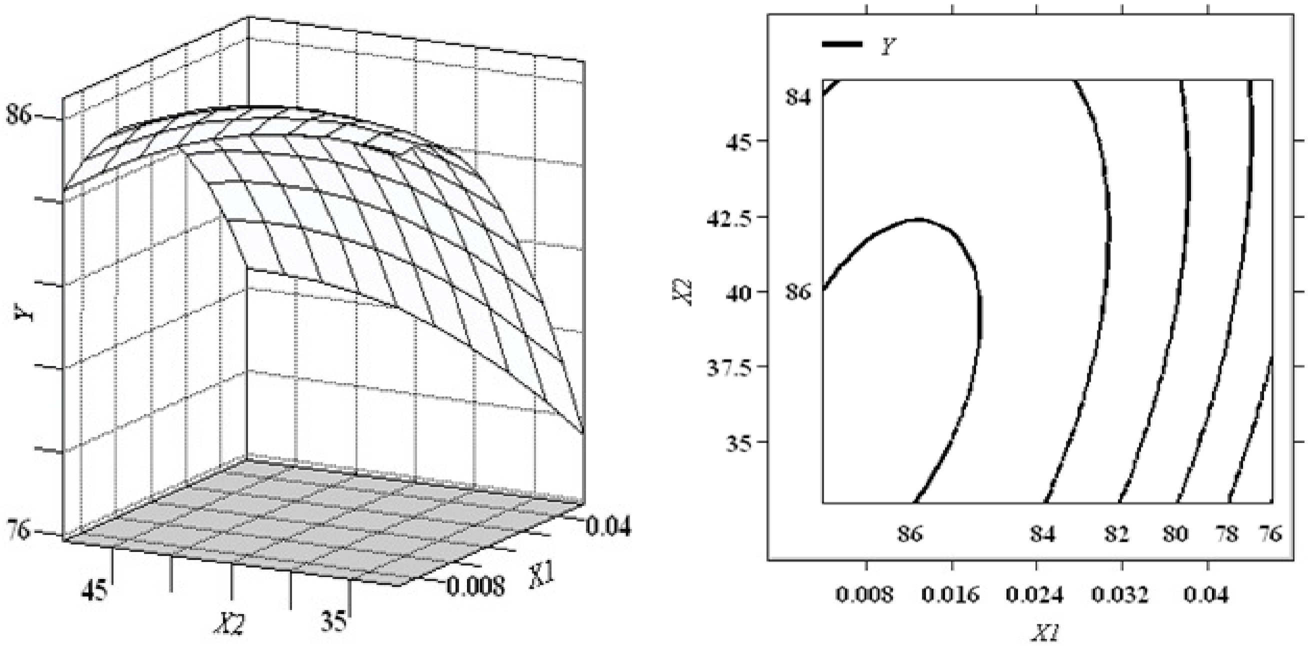

Figure 8. Response surface and contour plots for the effects of soy flour concentration (X_1) and temperature (X_2) on forward extraction efficiency of soy protein (Y) at pH 7.

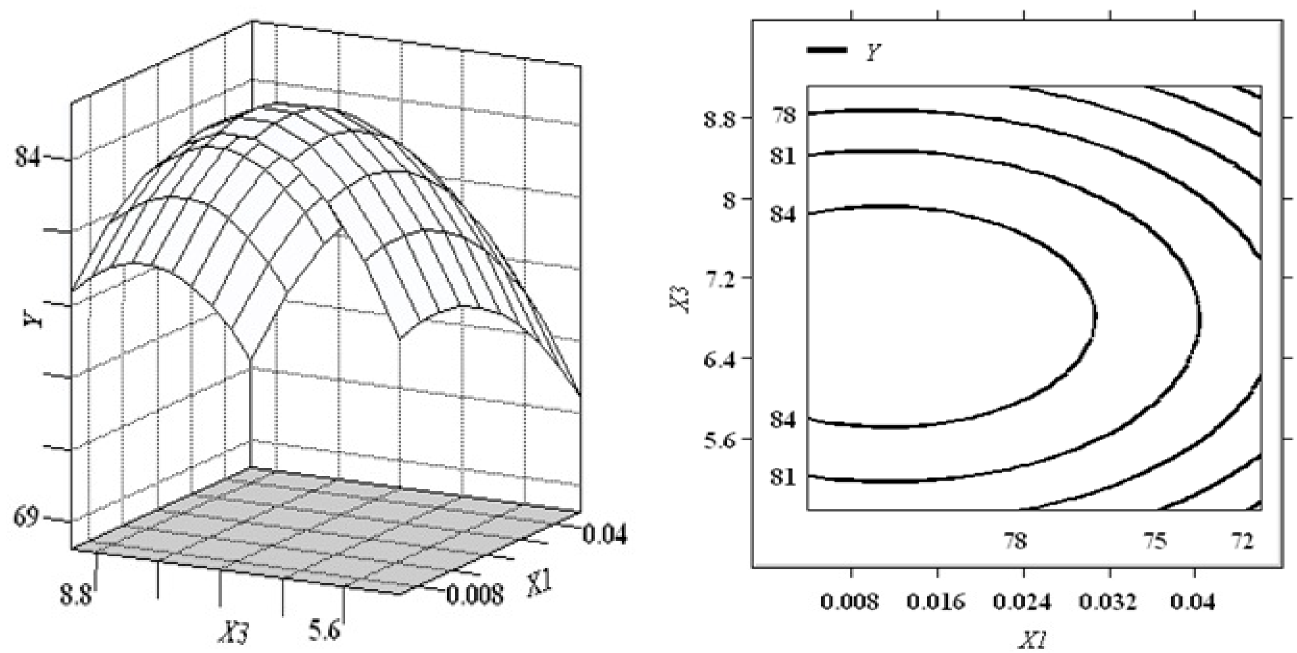

Figure 9. Response surface and contour plots for the effects of soy flour concentration (X_1) and pH (X_3) on forward extraction efficiency of soy protein (Y) at a temperature of 40°C.

reverse micelles. AOT reverse micelle system had higher extraction efficiency than SDS and CTAB systems. The main factors that affected the forward extraction were soy flour concentration, temperature and pH. The optimal conditions of forward extraction of soy protein in AOT reverse micelle system were soy flour 0.007 g/ml, Wo 16, pH 6.5, 34°C, extraction time 20 min, KCl 0.1 mol/L, ultrasound power 240 W. Size of reverse micelle affected the forward extraction of soy protein. Electrostatic interaction between the protein and the polar head of the

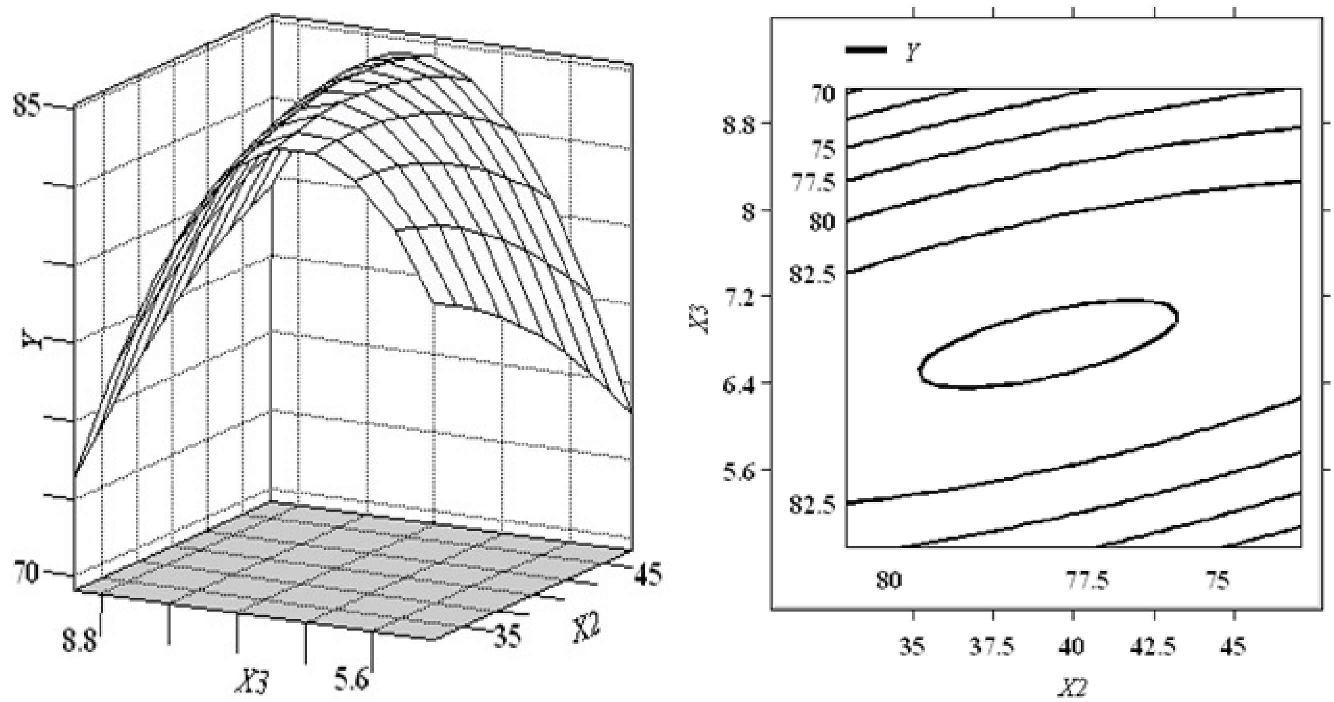

Figure 10. Response surface and contour plots for the effects of temperature (X_2) and pH (X_3) on forward extraction efficiency of soy protein (Y) at soy flour concentration 0.025 g/ml.

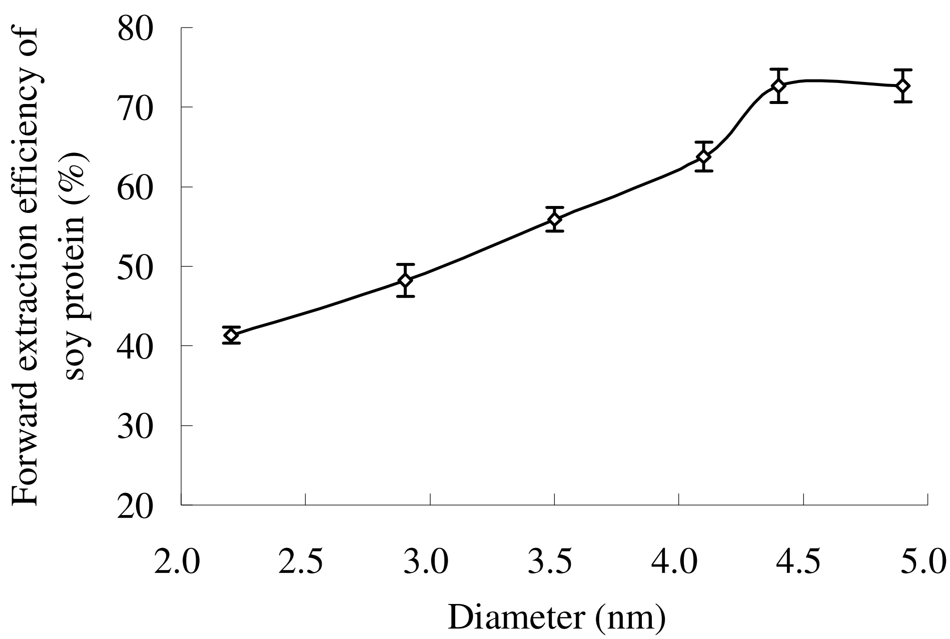

Figure 11. Effects of diameter of AOT reverse micelle on forward extraction efficiency of soy protein.

surfactants was one main driving force in extraction. The extraction mechanism of protein transferred from soy flour to reverse micelle should be also studied in the future.

ACKNOWLEDGEMENTS

This study was supported by the National Natural Science Foundation of China (No. 20976037), the Doctor

Foundation of Henan University of Technology (No. 2010BS014) and the Innovation Scientists and Technicians Troop Construction Projects of Zhengzhou City.

REFERENCES

AOAC (1984). Official Methods of Analysis, 14th ed. Association of Official Analytical Chemists, Washington, DC.

Bansal-Mutalik R, Gaikar VG (2006). Reverse micellar solutions aided permeabilization of baker's yeast. Process Biochem. 41(1): 133-141.

Concon JM, Soltess D (1973). Rapid micro Kjeldahl digestion of cereal grains and other biological materials. Anal. Biochem. 53(1): 35-41.

Dekker M, Hilhorst R, Laane C (1989). Isolating enzymes by reversed micelles. Ann. Biochem. 178(2): 217-226.

Ekwall P, Mandell L, Solyom P (1971). The solution phase with reversed micelles in the cetyl trimethylammonium bromide-hexanol-water system. J. Colloid Interface Sci. 35(2): 266-272.

Fang XL, Yang CF (1999). An Experimental Study on the Relationship between the Physical Properties of CTAB/Hexanol/Water Reverse Micelles and ZrO_2–Y_2O_3 Nanoparticles Prepared. J. Colloid Interface Sci. 212(2): 242-251.

Goklen KE, Hatton TA (1985). Protein extraction using reverse micelles. Biotechnol. Progr. 1(1): 69-74.

Hieda J, Saito N, Takai O (2008). Size-regulated gold nanoparticles fabricated by a discharge in reverse micelle solutions. Surf. Coat. Tech. 202(22-23): 5343-5346.

Hilhorst R, Fijneman P, Heering D, Wolbert RBG, Dekker M, Riet KV, Bijsterbosch BH (1992). Protein extraction using reversed micelles. Pure Appl. Chem. 64: 1765-1770.

Kommareddi NS, John VT, Waguespack YY, McPherson GL (1993). Temperature and gas pressure induced microstructural changes in AOT water-in-oil microemulsions: characterization through electron paramagnetic resonance spectroscopy. J. Phys. Chem. 97(21): 5752-5761.

Leser ME, Luisi PL, Palmieri S (1989). The use of reverse micelles for the simultaneous extraction of oil and proteins from vegetable meal. Biotechnol. Bioeng. 34: 1140-1146.

Li JC, Zhang JL, Han BX, Gao YN, Shen D, Wu ZH (2006). Effect of ionic liquid on the polarity and size of the reverse micelles in supercritical CO_2. Colloid Surface A. 279(1-3): 208-212.

Lye GJ, Asenjo JA, Pyle DL (1994). Protein extraction using reverse micelles: kinetics of protein partitioning. Chem. Eng. Sci. 49(19): 3195-3204.

Matzke SF, Creagh AL, Haynes CA, Prausnitz JM, Blanch HW (1992). Mechanisms of protein solubilization in reverse micelles. Biotechnol. Bioeng., 40(1): 91–102.

Nandini KE, Rastogi NK (2009). Reverse micellar extraction for downstream processing of lipase: Effect of various parameters on extraction. Process Biochem. 44(10): 1172-1178.

Naoe K, Takeuchi C, Kawagoe M, Nagayama K, Imai M (2007). Higher order structure of Mucor miehei lipase and micelle size in cetyltrimethyl ammonium bromide reverse micellar system. J. Chromatogr. B. 850(1-2): 277-284.

Noritomi H, Ito S, Kojima N, Kato S, Nagahama K (2006a). Forward and backward extractions of cytochrome c using reverse micellar system of sucrose fatty acid ester. Colloid Polym. Sci. 284(10): 604-610.

Noritomi H, Kojima N, Kato S, Nagahama K (2006b). How can temperature affect reverse micellar extraction using sucrose fatty acid ester. Colloid Polym. Sci. 284(6): 683-687.

Paradkar VM, Dordick JS (1994). Mechanism of extraction of chymotrypsin into isooctane at very low concentrations of aerosol OT in the absence of reverse micelles. Biotechnol. Bioeng. 43(6): 529-540.

Pires MJ, Aires-Barros MR, Cabra JMS (1996). Liquid-liquid extraction of proteins with reversed micelles. Biotechnol. Progr. 12(3): 290-301.

Sechler TD, DelSole EM, Deak JC (2010). Measuring properties of interfacial and bulk water regions in a reverse micelle with IR spectroscopy: a volumetric analysis of the inhomogeneously broadened OH band. J. Colloid Interf. Sci. 346(2): 391-397.

Shen R, Liu JG, Xing JM, Liu HZ (2005). Extraction of industrial lipase with CTAB-based mixed reverse micelles. Chin. J. Process Eng. 5(3): 255-259.

Spreti N, Alfani F, Cantarella M, D'Amico F, Germani R, Savelli G (1999). alpha-chymotrypsin superactivity in aqueous solutions of cationic surfactants. J. Mol. Catal. B: Enzyme, 6(1): 99-110.

Sun XH, Zhu KX, Zhou HM (2008). Protein extraction from defatted wheat germ by reverse micelles: Optimization of the forward extraction. J. Cereal Sci. 48(3): 829-835.

Wu JC, Chau PS, Chow Y, Li R, Carpenter K, Yu H (2006). Extraction of Candida rugosa lipase from aqueous solutions into organic solvents by forming an ion-paired complex with AOT. J. Chem. Technol. Biot. 81(6): 1003-1008.

Yu YC, Chu Y, Ji JY (2003). Study of the factors affecting the forward and back extraction of yeast-lipase and its activity by reverse micelles. J. Colloid Interf. Sci. 267(1): 60-64.

Zhao JT (2001). Study on the use of reverse micelle for the simultaneous extraction oil and protein. J. Zhengzhou Univ. Tech. 22: 54-56.

Zhao L, Pal SK, Xia T, Zewail AH (2004). Dynamics of ordered water in interfacial enzyme recognition: bovine pancreatic phospholipase A2. Angew. Chem. Int. Edit. 43(1): 60-63.

Zhao XH, Li YM, He XW, Zhong NJ, Xu ZB, Yang LS (2010). Study of the factors affecting the extraction of soybean protein by reverse micelles. Mol. Biol. Rep. 37(2): 669-675.

Zhao XY, Chen FS, Chen JQ, Gai GS, Xue WT, Lee LT (2008a). Effects of AOT reverse micelle on properties of soy globulins. Food Chem. 111(3): 599-605.

Zhao XY, Chen FS, Gai GS, Chen JQ, Xue WT, Lee LT (2008b). Effects of extraction temperature, ionic strength and contact time on efficiency of bis(2-ethylhexyl) sodium sulfosuccinate (AOT) reverse micellar backward extraction of soy protein and isoflavones from soy flour. J. Sci. Food Agric. 88(4): 590-596.

Zhao XY, Chen J, Zhu QJ, Du FL, Ao Q, Liu J (2011). Surface characterization of 7S and 11S globulin powders from soy protein examined by X-ray photoelectron spectroscopy and scanning electron microscopy. Colloid Surface B. 86(2): 260-266.