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Research Article

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Ultrasound-assisted extraction of dietary fiber from *Citrus Changshan-huyou* peels

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ABSTRACT

Response surface methodology (RSM) was applied to optimize the ultrasound-assisted extraction (UAE) of dietary fiber (polysaccharides) from Citrus Changshan-huyou peels. Box-Benhnken experimental design (BBD) was used for experimental design and data analysis was conducted to obtain the optimum extraction conditions. Results: The obtained optimum conditions were material/water ratio 1:37 (g/mL), extraction time 20 min, extraction power 60 W, extraction temperature of 70 ℃ and sodium hexametaphosphate 2 wt%. Under the optimized conditions, the yield of dietary was 6.62%, in close agreement with values predicted by the mathematical model. The content of polysaccharide in the product is 29.3 g/L. Compared with Conventional solvent extraction (CSE), UAE performed better to extract dietary fiber.

Keywords: Ultrasound-assisted extraction, Dietary fiber, Response surface methodology

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INTRODUCTION

Pomelo, known as one of the principal fruits in China, is easy to grow and has high production and good economic returns. *Citrus Changshan-huyou*, which is a cross between the *Citrus-grandis (L.) Osbeck* and the *Citrus-sinensis (L.) Osbeck*, has a history of more than one hundred years[1]. In recent years, more attentions have been paid on *Changshan-huyou* and it is a local citrus species native to *Changshan County, Zhejiang Province*. *Citrus changshan-huyou* has excellent tolerance of cold, leanness and storage[2]. Besides, *Hu pomelo* peels, about 20%-25% of the whole pomelo weight, are abundant of dietary fiber and other significant characteristics such as bioactive substances[3].

Conventional solvent extraction (CSE) is the most commonly used method to extract polysaccharides. CSE is simple and safe, but high temperature and long extraction time of CSE lead to the degradation of polysaccharides and the decrease of the pharmacological activity of polysaccharides[4]. Recently, some new extraction methods have been developed to improve the extraction process, especially the ultrasound-assisted extraction (UAE)[5]. As reported[6], the mechanical effect of ultrasound is able to accelerate the extraction of the organic compounds which contained within the body of plants, due to disruption of the cell walls and enhanced mass transfer of cell contents. Ultrasonic treatment is widely used in the processing of plant materials, particularly, for extracting molecular substances[7,8]. Besides, research has shown that, ultrasound-assisted has obtained a satisfactory extraction result in extraction polysaccharides [4,9-11].

As we all know, the insoluble plant residue, a waste material formed during the production of herbal tinctures, represents a rich source of polysaccharides which might possess biological activities, similar to those obtained with other plants[12]. The aim of this study was to determine the effects of extraction power, extraction temperature and extraction time in UAE on the yield of polysaccharides (soluble dietary fibre) from *Citrus Changshan-huyou* peels.

EXPERIMENTAL SECTION

2.1 Materials

Fresh *Citrus Changshan-huyou* peels (*Changshan County*, *Zhejiang Province*, China). The pomelo peel's moisture content was determined by drying at 80℃ to constant weight. Then the pomelo peels were ground into fine power (about size of 0.2mm). D-Glucose was obtained from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade.

2.2 Extraction methods

2.2.1 Conventional solvent extraction (CSE) of polysaccharides from pomelo peels

2.0 g of the ground power was mixed with sodium hexametaphosphate buffers in a 500 mL round bottomed flask. The round bottomed flask is immersed in a constant temperature bath. The filtration experiment equipment adopts vacuum pump and filler. Filtered supernatant vacuum concentration to a quarter of the original volume, and then add three times the volume of 95% ethanol. The solution was then centrifuged (3500 r/min, 10 min), and the precipitate was dried under vacuum. The result was coarse polysaccharide.

2.2.2 Ultrasound-assisted extraction (UAE) of dietary fiber from pomelo peels

2.0 g of the ground power was mixed with sodium hexametaphosphate buffers in a 500 mL round bottomed flask. The round bottomed flask for ultrasonic processing. The same process goes for other operation method as above.

2.3 Purification of dietary fiber

The experiment processes of dietary fiber purified see the literature[13].

2.4 Determination of the total polysaccharide

The content of total polysaccharide in the coarse polysaccharide extracted from pomelo peels was determined with phenol-sulfuric acid method[14].

RESULTS AND DISCUSSION

3.1 Effects of operation parameters of CSE on the yield of soluble dietary fibre

There are many factors which can influence the yield of soluble dietary fibre, such as ratio of water to raw material, extraction time, extraction temperature and mass fraction of extraction liquid, etc. In this study, the effect of ratio of water to raw material, extraction temperature and mass fraction of extraction liquid (sodium hexametaphosphate) were studied.

3.1.1 Effects of the single factor of CSE on the yield of soluble dietary fibre

The influence of ratio of water to raw material on the yield of soluble dietary fibre was shown in Fig. 1a. As ratio of water to raw material increased, the contact area between material and solvent also increased, making dietary fibre more fully dissolved out from pomelo peels and the yield of soluble dietary fibre increased. When water to raw material was 35:1 (mL/g), 5.7% of yield of soluble dietary fibre was obtained. However, the extraction efficiency of soluble dietary fibre declined slightly when the water to raw material was over 35:1 (mL/g). It was probably due to the increasing water content accelerated the dissolution of impurities so as to hinder the dissolution of polysaccharides[15]. Considering yield of soluble dietary fibre and energy consumption, less cost effective, the optimum ratio of water to raw material was 35:1 (mL/g).

Fig. 1b listed different extraction temperature on the yield of soluble dietary fibre when other extraction conditions were kept constant. From Fig. 1b, we found that the yield of dietary fibre increased with increasing extraction temperature. The maximum extraction yield was obtained at 75℃. The yield of dietary fibre decreased at the temperature over 75℃. The heat resistance of the soluble dietary fiber is poorer. So the optimum extraction temperature should be around 75℃.

Fig. 1c listed different mass fraction of extraction liquid on the yield of soluble dietary fibre when other extraction conditions were fixed as follows: extraction temperature of 70℃ and ratio of water to raw material of 35:1 (mL/g). The results showed that the maximum extraction yield of soluble dietary fibre was obtained with 2% of mass fraction of extraction liquid. With increasing mass fraction of sodium hexametaphosphate, the yield soluble dietary fibre decreased slighthly, so the mass fraction of sodium hexametaphosphate should be 2%.

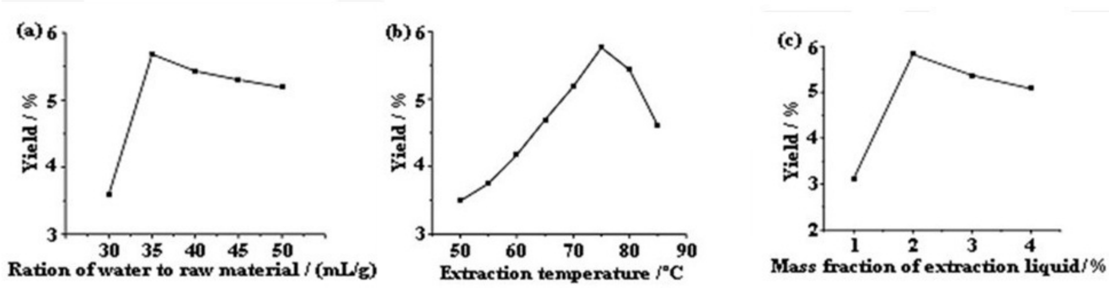

Fig. 1 Effects of three factors on the yield of polysaccharides: (a) ratio of water to raw material, (b) extraction temperature, and (c) mass fraction of extraction liquid

3.1.2 The Response Surface Analysis of CSE

According to the central composite experimental design principles of Box-Benhnken, the effects of ratio of water to raw material, extraction temperature and mass fraction of extraction liquid on the extraction yield of pomelo soluble dietary fibre were optimized with response surface methodology. Specific experimental scheme are shown in Table 1.

Table 1 The results of Response Surface Analysis of CSE

Experiments	Temperature/℃	Ratio of water to raw material/(mL/g)	Mass fraction of extraction liquid/%	Yield/%
	x_1	x_2	x_3	
1	80	30:1	1	2.1
2	80	40:1	1	3.8
3	70	30:1	2	2.8
4	70	40:1	2	4.2
5	80	30:1	3	3.9
6	80	40:1	3	6.7
7	80	35:1	2	5.5
8	80	35:1	2	6.1
9	90	35:1	3	3.4
10	90	40:1	2	4.8
11	90	30:1	2	5.5
12	70	35:1	3	3.4
13	80	35:1	2	6.6
14	90	35:1	1	5.0
15	80	35:1	2	5.6
16	80	35:1	2	5.9
17	70	35:1	1	4.1

3.1.3 Interaction between the factors of CSE

The Design-Expert 7.1 analysis software is used to establish mathematical model and obtain the optimum conditions of CSE, according to the regression equation to draw maps[16]. The results of experiments were summarized in Fig. 2. Among which, Figs.2a and 2d represented the extraction yield of pomelo soluble dietary fibre with extraction temperature and ratio of water to raw material. We could see that ratio of water to raw material showed a more significant influence in comparison to extraction temperature. The relationship between extraction temperature and mass fraction of extraction liquid were showed in Figs.2b and 2e. When mass fraction of extraction liquid was kept constant, the yield increased with increasing extraction temperature, passing through a maximum and then decreased. The trend was similar to the effect of ratio of water to raw material and mass fraction of extraction liquid as shown in Figs. 2c and 2f.

3.1.4 The determination of the extraction process of CSE

Combined with the mathematical analysis of the regression model, the best technology parameter of CSE as follows: ratio of water to raw material of 37.21:1 (mL/g), extraction temperature of 81.34℃ and mass fraction of extraction liquid of 2.19%; The yield of soluble dietary fibre of the theoretical value is 6.105% under the optimal extraction conditions. To test the reliability of the method of RSA, using the optimal extraction conditions of soluble dietary fibre extraction experiment was carried out. At the same time, considering the convenience of the actual operation, the optimal extraction conditions were modified: ratio of water to raw material of 37:1 (mL/g), extraction temperature of 81℃ and mass fraction of extraction liquid of 2%. The actual yield of soluble dietary fibre was 6.03%. Compared with the theoretical prediction, the relative error is smaller than 1%. Therefore, the result of the optimal extraction conditions of polysaccharide extraction by RSA can meet the requirement of reliability, and the method is practical.

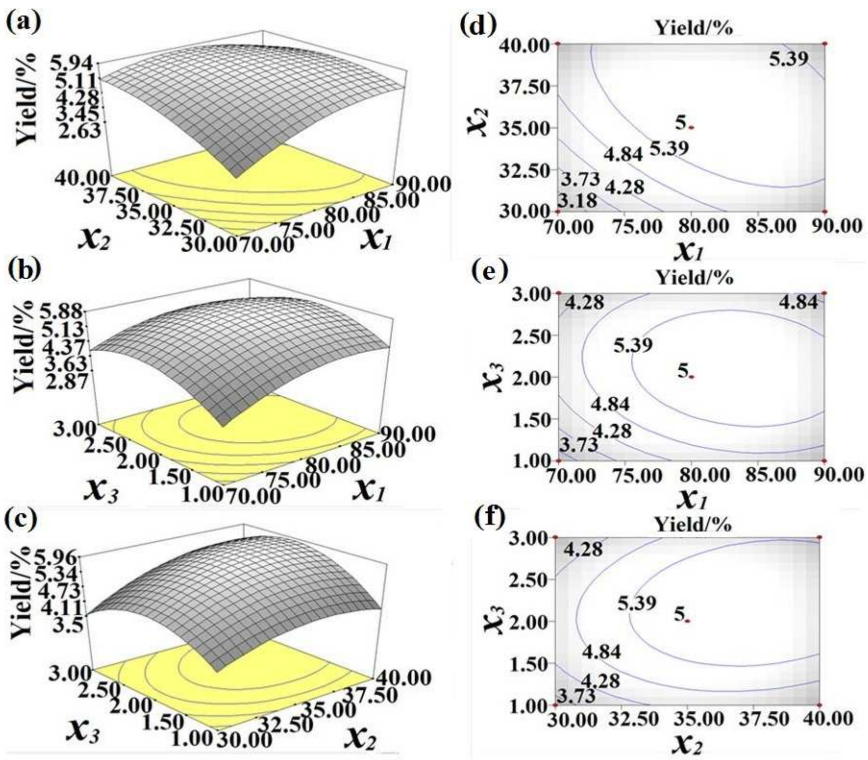

Fig.2 (a-c) 3D response surface and (d-f) contour plots showing variations between a pair of experimental variables on predicted values of polysaccharides yield while keeping other variables at a constant level: (a) and (d) ratio of water to raw material vs extraction temperature; (b) and (e) ratio of water to raw material vs mass fraction of extraction liquid; (c) and (f) extraction temperature vs mass fraction of extraction liquid.

3.2 Effects of operation parameters of UAE on the yield of soluble dietary fibre

3.2.1 Effects of single factor of UAE on the yield of soluble dietary fibre

The extraction efficiency of UAE was influenced by various factors. Fig.3a listed the effect of extraction time on the yield of soluble dietary fibre, and other extraction conditions were fixed as follows: extraction temperature of 70℃, extraction power 60 W, ratio of water to raw material of 37:1 (mL/g). The results showed that The optimum yield of soluble dietary fibre was obtained when extraction time was 20 min. Ultrasound facilitated the soluble dietary fibre inside the cells release to the exterior solvent. Most of the soluble dietary fibre in broken cells released at the early period of extraction, and the yield of soluble dietary fibre increased in the first 20 min. However, long extraction time induced the degradation of soluble dietary fibre, and the yield of soluble dietary fibre decreased. Thus, the optimum extraction time was 20 min.

Fig. 3b listed different ultrasound temperature on the yield of soluble dietary fibre when other extraction conditions were fixed. From the results, we can see that the yield of soluble dietary fibre increased with the increasing ultrasound temperature from 50℃ to 70℃. High temperature can improve molecular movement, so, high-temperature solution will be conducive to make soluble dietary fibre dissolved from plant cells[16]. However, high temperature led to the decrease of surface tension and the increase of vapor pressure with micro bubbles, causing the damping of the ultrasonic wave[17]. Thus, the yield of soluble dietary fibre decreased when extraction temperature was over 70℃. Based on these results, the optimum extraction temperature was 70℃. The effect of extraction power on the yield of soluble dietary fibre in Fig.3c was studied with extraction temperature of 70℃ and other conditions fixed. The results showed that the yield of soluble dietary fibre increased significantly with increasing extraction power, and then decreased when extraction power was over 60 W. It was well known that extraction power facilitated the disruption of cell walls. Stronger extraction power improved the yield of soluble dietary fibre at the early period. However, the more chemical decompositions were generated with stronger extraction power[11].Thus, the optimum extraction power was 60 W.

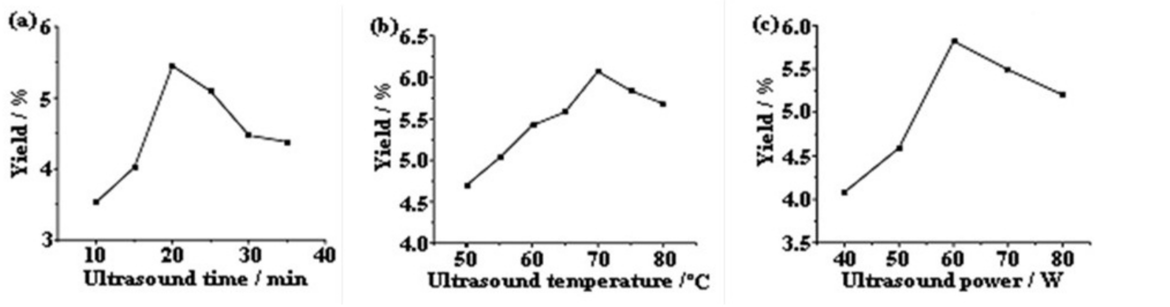

Fig. 3 Effects of three factors on the yield of polysaccharides: (a) ultrasound time, (b) ultrasound temperature, and (c) ultrasound power

3.2.2 The Response Surface Analysis of UAE

According to the central composite experimental design principles of Box-Benhnken, the effects of ultrasound time, ultrasound temperature and ultrasound power on the extraction yield of pomelo soluble dietary fibre were optimized with Response Surface methodology. Specific experimental scheme are shown in Table 2.

Table 2 The results of Response Surface Analysis of USE

Experiments	Temperature/℃	Ultrasound time/ min	Ultrasound power/W	Yield/%
	x_1	x_2	x_3	
1	70	20	60	6.3
2	70	20	60	7.1
3	80	20	70	5.8
4	70	25	50	5.8
5	70	25	70	5.9
6	80	15	60	5.2
7	60	15	60	4.5
8	70	15	50	5.5
9	70	15	70	6.0
10	60	20	70	3.7
11	60	20	50	3.5
12	70	20	60	6.5
13	80	20	50	6.2
14	80	25	60	4.8
15	70	20	60	6.5
16	70	20	60	5.9
17	60	25	60	5.6

3.2.3 Interaction between the factors of UAE

The three dimensional (3-D) response plots and contour plots of yield of pomelo soluble dietary fibre with ultrasound temperature, ultrasound time and ultrasound power were given in Fig.4 respectively. As could be seen in Figs.4d and 4e, the two-dimensional contour lines were both saddle and it indicated that ultrasound temperature was likely to affect the yield of pomelo soluble dietary fibre than ultrasound time and ultrasound power, respectively. From Figs.4c and 4f, we could see that the effect of ultrasound power on yield of pomelo soluble dietary fibre was stronger than that of ultrasound time, yield of soluble dietary fibre changed more at the power range than at the time range.

3.2.4 The determination of the extraction process of UAE

Combined with the mathematical analysis of the regression model, the best technology parameter of UAE as follows: ultrasound temperature of 72.27℃, ultrasound time of 20.10 min and ultrasound power of 60.28 W. The theoretical value of the yield of soluble dietary fibre of is 6.68 % under the optimal extraction conditions. To test the reliability of the method of RSA, using the optimal extraction conditions of soluble dietary fibre extraction experiment was carried out. At the same time, considering the convenience of the actual operation, the optimal extraction conditions were modified: ultrasound temperature of 70℃, ultrasound time of 20 min and ultrasound power of 60 W. The actual yield of soluble dietary fibre was 6.62%. Compared with the theoretical prediction, the relative error is smaller than 1%. Therefore, the result of the optimal extraction conditions of soluble dietary fibre extraction by RSA can meet the requirement of reliability, and the method is practical.

3.3 The content of total sugar

Water-soluble non-starch polysaccharides are the main active components of dietary fiber. Phenol-sulfuric acid method was employed to determine polysaccharide contents.

Results: The standard curve equation is C=140.17A-9.3862, R^2= 0.9963. The absorbance values of the samples is A=0.276, so the content of polysaccharide in the product is 29.3 g/L.

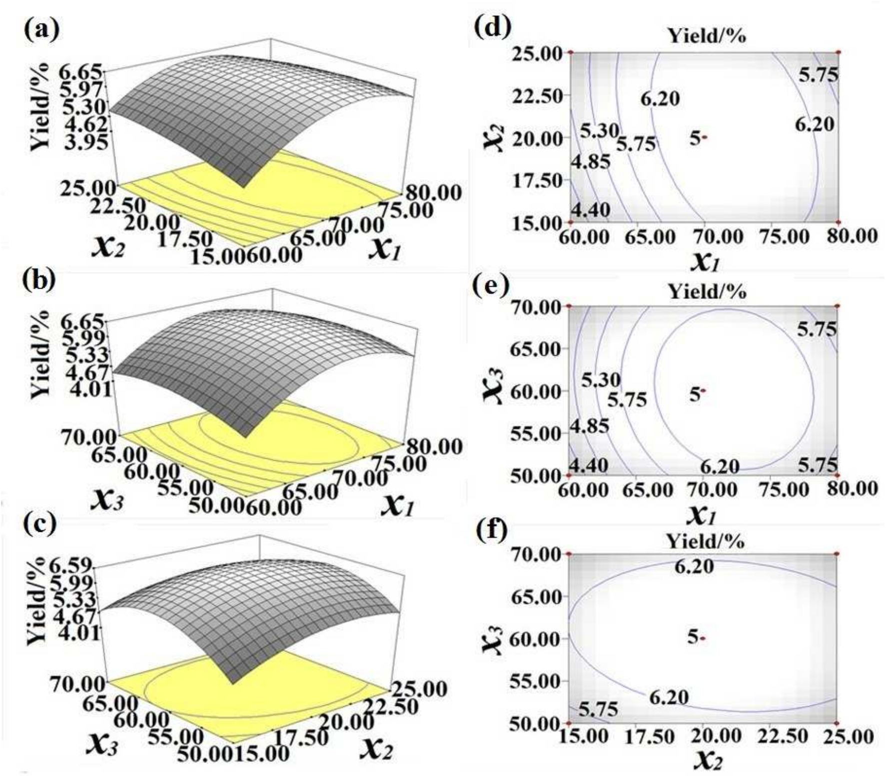

Fig.4 (a-c) 3D response surface and (d-f) contour plots showing variations between a pair of experimental variables on predicted values of polysaccharides yield while keeping other variables at a constant level: (a) and (d) ultrasound temperature vs ultrasound time; (b) and (e) ultrasound temperature vs ultrasound power; (c) and (f) ultrasound time vs ultrasound power

CONCLUSION

The factors of CSE and UAE on the yield of soluble dietary fibre from the *Citrus Changshan- huyou* peels were studied. On the basis of the response surface method, we obtained the optimum technological conditions for soluble dietary fibre extraction. The CSE: ratio of water to raw material of 37:1 (mL/g), extraction temperature of 81℃ and mass fraction of extraction liquid of 2%, the actual yield of soluble dietary fibre was 6.03%. The UAE: ultrasound temperature of 70℃, ultrasound time of 20 min and ultrasound power of 60 W, the actual yield of soluble dietary fibre was 6.62%. After treated by ultrasonic extraction the yield of soluble dietary fibre from the *Citrus Changshan-huyou* peels increased to 6.62%, from 6.03% of CSE. The application of ultrasound during the extraction of polysaccharides from the *Citrus Changshan-huyou* peels had a positive effect on the extraction yield.

REFERENCES

[1] Z Wei; QQ He. Flora of Zhejiang, Zhejiang Science and Technology Press, Hangzhou, **1986**, 438-439.
[2] JR Xing. Research on Processing Key Technology of Citrus *Changshanhuyou* Fruit Drinks. College Of Postgraduation, Bei Jing, **2012**.
[3] XM Zhao; XQ Ye; YF Xi, *Journal of Chinese Institute of Food Sci. and Technol.*, **2004**, 4(2), 19-24.
[4] Z Hromádková; A Ebringerová; P Valachovic, *Ultrason. Sonochem*., **2002**, 9(1), 37-44.
[5] IE El'Piner. Ultrasound: Physical, Chemical and Biological Effects. Consultants Bureau, New York, **1964**, 98-99.
[6] XB Zhang. Extraction of Water-soluble Soybean Polysaccharides and Their Application in Acidic Milk Drinks. Zhejiang GongShang University, Hang Zhou, **2009**.
[7] TJ Mason; L Paniwnyk; JP Lorimer, *Ultrason. Sonochem*., **1996**, 3(3), 253-260.
[8] M Salisová; S Toma; TJ Mason, *Ultrason. Sonochem*., **1997**, 4(2), 131-134.
[9] EL Zhao; XH Liang; HR Zhang, *Food Sci. and Technol*., **2006**, 27(6), 65-82.
[10] YY Chen; HY Luo; AP Gao; M Zhu, *Innovative Food Sci. and Emerging Technol*., **2011**, 12(3), 305-309.
[11] Z Ying; XX Han; JR Li, *Food Chem.*, **2011**, 127(3), 1273-1279.
[12] R Srivastava; DK Kulshreshtha, *Phytochem*., **1989**, 28(11), 2877-2883.

[13] XL Li; ZH Wang; YX Zhao; SJ Luo; DW Zhang; SX Xiao; ZH Peng, *Carbohyd. Polym*., **2012**, 89(4), 1232-1237.
[14] G Cuesta; N Suarez; MI Bessio; F Ferreira; H Massaldi, *J. Microbiol. Meth*., **2003**, 52(1), 69-73.
[15] YH Wu; GH Huang; ZY Gu; J Dong; F Yu, *Food Sci*., **2008**, 29(11), 196-200.
[16] L Yang; YL Cao; JG Jiang; QS Lin; J Chen; L Zhu, *J. Sep. Sci*., **2010**, 33(9), 1349-1355.
[17] S Hemwimol; P Pavasant; A Shotipruk, *Ultrason. Sonochem*., **2006**, 13(6), 543-548.