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Recovery of Phytosterols from Waste Residue of Soybean Oil Deodorizer Distillate

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1. Introduction

Phytosterols are triterpenes similar to cholesterols, both in structure, given the four-ring steroid nucleus, the 3β-hydroxyl group and often a 5, 6-double bond and in function, given their role in the stabilization of the phospholipid bilayers in cell membranes. Sterols in plants exist in the form of free alcohols, fatty-acid esters, steryl glycosides and acylated steryl glycosides (Fernandes and Cabral, 2007). Phytosterols play major roles in several areas, namely in pharmaceuticals (production of therapeutic steroids), nutrition (anti-cholesterol additives in functional foods, anti-cancer properties), and cosmetics (creams, lipstick) (Fernandes and Cabral, 2007). Phytosterols are known to have cholesterol-lowering effect. The mechanism played by phytosterols is based on the ability of plant sterol esters to reduce the intestinal absorption of diet and biliary cholesterol. Moreover, plant sterols possess anti-inflammatory and anti-atherogenicity activity and may possess anti-cancer and anti-oxidative activities. Overall, phytosterol applications are promising.

Soybean oil deodorizer distillate (SODD) is obtained as a byproduct of the deodorization step during the refining of soybean oil. In the refining of soybean oil, most bioactive compounds such as free phytosterols, fatty acid steryl esters (FASEs), tocopherols and squalenes are concentrated (Woerfel, 1995; Verleyen et al., 2001). Furthermore, SODD also contains free fatty acids (FFAs), polycyclic hydrocarbons and the bioactive compounds (Bockisch, 1998). Thus, SODD is a natural source of phytosterols (or fatty acid sterol esters, FASEs), free fatty acids (or fatty acid methyl esters, FAMEs, namely biodiesel), tocopherols and squalenes. Several strategies have been developed to recover and purify these compounds from the deodorizer distillate of vegetable oils. Many methods have been proposed especially to recover phytosterols or FASEs from the deodorizer distillate.

Torres et al. (2007) reported that a two step enzymatic procedure could be used to obtain FASEs and FAMEs from soybean oil deodorizer distillate. Two enzymatic steps were used to separate sterols esterification and ethyl esterification. Guclu-Ustundag and Temelli (2007) developed a semi-continuous column fractionation of canola oil deodorizer distillate using

supercritical CO_2 ($SCCO_2$) to determine the feasibility of value-added processing of this feed material for the recovery of bioactive components such as sterols and tocopherols and the effect of operating conditions on extract yield and separation efficiency. Gunawan et al. (2008) described a suitable method (without degradation of the FASEs) i.e. modified Soxhlet extraction, modified silica gel column chromatography and binary solvent extraction to isolate and purify natural FASEs from SODD. FASEs with a purity of 86.74% and a total recovery of 85.32% could be obtained in the final products.

Sun et al. (1998) developed a process including esterification, water-bathing, alcoholysis, cooling precipitation and distillation to extract tocopherols and sterols from by-products of refined vegetable oil. Free sterols, fatty acid methyl ester and tocopherols can be separated efficiently from DD. Although the process has no effect on extracting fatty acid methyl ester from SODD, it is still commonly used in industry. A modified process was developed by Xu et al. (2005) to recover and purify valuable compounds from SODD. In its industrial production, tocopherols and FAMEs was obtained from SODD after a process of methyl esterification by sulfuric acid catalyst, transesterification by alkaline catalyst, crystallization of sterols and molecular distillation. The waste residue of SODD (WRSODD) was obtained after the molecular distillation. WRSODD mainly contains steryl esters, acylglycerols, and hydrocarbons.

In order to be environmentally friendly for the utilization of waste resources and energy saving, the development of a suitable method to recover phytosterols from WRSODD is of great importance. Zhang et al. (1991) described a process route for the preparation of β-sitosterol from the unsaponifiable matter in tall oil. However, the average yield of β-sitosterol is low, only 1 wt%. Li and Wang (2004) reported a method to extract phytosterols from plant oil pitch or tall oil pitch, but the yield is low, only 6 wt%-10 wt%. Until now, there is no literature related to the extraction of phytosterols from WRSODD.

The purpose of the present study is to develop a catalytic and crystallization process to recover phytosterols from WRSODD. A catalyst was employed to decompose WRSODD so as to transform steryl esters into phytosterols. Several solvents were used for the crystallization of the phytosterols. The effect of different solvents on the purity and yield of recovered phytosterols was investigated. Silica gel column chromatography and FT-IR spectrum analysis were adopted to analyze the composition of WRSODD. The structure and purity of the recovered phytosterols were determined by FT-IR spectrum and gas chromatography (GC).

2. Experimental

2.1 Materials

WRSODD and standard samples of phytosterols were provided by CGOG TECH. Bioengineering Co. Ltd., Tianjin, China. Silica gel (G60) was purchased from Qingdao Haiyang Chemical Co., Ltd., Shandong, China. Cholesterols (>97 %) were from Tianjin Guanfu Fine Chemical Research Institute, China. Other chemicals, such as acetic anhydride, pyridine, methanol, ethanol, n-propanol, n-hexane, cyclohexane, petroleum ether, acetone, butanone, cyclohexanone, benzene, toluene are all analytic reagents and were used as received.

2.2 Component separation of WRSODD

In order to illustrate the compositions of WRSODD, column chromatography was used to separate WRSODD into different polarity fractions. The process was as follows: the column was packed with silica gel 60 and the sample (WRSODD) was placed on the top layer of the

column. The sample was eluted by petroleum ether, ethyl acetate and ethanol sequentially. Petroleum ether as a low polarity solvent was first used to elute the sample until the eluent became colorless. Meanwhile, TLC was used to estimate if all the low polarity compositions had been eluted. Ethyl acetate was then used to elute the sample until the eluent became colorless. In like manner, TLC was employed to determine if all the medium polarity compositions had been eluted. Lastly, ethanol was used to elute the sample until the eluent became colorless. Elution I, Elution II and Elution III were obtained after evaporating petroleum ether, ethyl acetate and ethanol, respectively.

2.3 Analysis methods

FT-IR spectra were recorded on Bruker Tensor 307 Spectrometer, BRUKER Co.

Gas chromatograph (GC) (Agilent 6890N, USA) with a HP-5 capillary column was employed to analyze the purity of sterols. All the samples of sterols were derivatized with acetic anhydride and pyridine before testing. Cholesterols were used as an internal standard. The purity of sterols can be calculated by Eq. (1).

$$Y = F\frac{A_{sample} \times m_{internal}}{A_{internal} \times m_{sample}} \times 100\% \quad (1)$$

where Y is the purity of sterols (%), F the correction factor, A_{sample} the total peak area of sterols (brassicasterols, campesterols, stigmasterols and β-sitosterols) in a sample, $A_{internal}$ the total peak area of internal standard, $m_{internal}$ the mass of the internal standard (mg), m_{sample} the mass of a sample (mg). Note: the correction factor F can be obtained from the sample with a purity of 95.30% from CGOG Tech. (Tianjin) Bioengineering Co., Ltd.

Gas chromatography-mass spectrometry (GC-MS) (HP5890-5971, USA) was further employed to confirm that the relative retention time of the peaks corresponds to certain sterols in the gas chromatogram.

Thin layer chromatography (TLC) was adopted to carry out chromogenic reactions of FASEs. Elution I and Elution II were developed by the mixture of hexane and ethyl acetate on TLC plates. Spots for FASEs and steroidal hydrocarbons were detected by spraying with a fresh solution of ferric chloride (50mg) in a mixture of water (90ml), acetic acid (5ml) and sulfuric acid (5ml). After the plates were heated at 100°C for 3-5 min, FASEs and steroidal hydrocarbons were detected by a red-violet color (Gunawan, et al., 2008).

2.4 Reaction procedure

The reaction process comprised the following steps: Firstly, the WRSODD was dissolved in ethanol in a three neck flask, and an alkali solution (40 wt%) was added into the flask as a catalyst to react at suitable temperature for several hours while stirring. Secondly, excess acid was added into the mixture to stop the reaction. The mixture was then repeatedly washed with water until pH 7 was achieved. The fatty acid steryl esters in WRSODD were then transformed to free sterols and free fatty acids.

2.5 Total content of phytosterols determination in WRSODD

Column chromatography was used to separate high polarity and fuscous components from WRSODD. The mixture of petroleum ether and ethyl acetate as a low polarity solvent was employed to elute the sample until it became colorless. The concentration of steryl esters

was obtained after removing the solvent. The concentrated feed was then saponified so as to transform the fatty acid steryl esters in WRSODD into free sterols and FFAs, and the glycerides into FFAs and glycerol as illustrated in Section 2.4. After that, free phytosterols were extracted by petroleum ether and obtained after the evaporation of the solvent. Finally, GC was employed to determine the content of sterols in the concentration of free phytosterols as illustrated in Section 2.3.

2.6 Phytosterols recovery from the feed solution after catalytic decomposing reaction

2.6.1 Selection of solvents for crystallization

The feed solution obtained from the catalytic decomposing reaction contained a large amount of free phytosterols, free fatty acids, organic phosphates, polyaclcohols, carbohydrates and other miscellaneous components. Phytosterols could be recovered by crystallization from the complex feed solution. The feed was dissolved in different solvents, such as alcohols, alkanes, ketones, benzenes and mixed solvents, and the phytosterols were then crystallized by cooling at a cooling rate of 2 °C per hour. Phytosterols crystals were finally obtained by vacuum filtration.

2.6.2 Optimization of crystallization conditions

Orthogonal experiments were carried out to optimize the crystallization conditions. Three key parameters, namely the proportion of feed solution to solvent, ripening time and ripening temperature, were optimized by orthogonal experiments.

Table 1 lists the schedule of the orthogonal test in which the key parameters including the proportion of feed solution to solvent (A), ripening time (B) and ripening temperature (C) were selected as three factors. Every factor had three levels to be optimized.

	Factor A (The proportion of feed to solvent)	Factor B (Ripening time)	Factor C (Ripening temperature)
Level 1	1:1	4	-8
Level 2	1:2.5	14	6
Level 3	1:4	24	20

Table 1. Factors and levels selected for orthogonal experiments

3. Results and discussion

3.1 Composition analysis of WRSODD

3.1.1 Separation of WRSODD by column chromatography

According to the different polarities of solvents used, three different elutions (Elution I, Elution II and Elution III) were separated sequentially from the WRSODD by column chromatography. The WRSODD was separated into four kinds of compositions. Their contents were 88.27 wt% in Elution I, 9.74 wt% in Elution II, 0.8 wt% in Elution III and 1.19 wt% left. The portion of Elution I was much larger than the others. It suggests that there were many low polarity components in the WRSODD, which might be fatty acid steryl esters and glycerides. The further structural investigation was carried out by FT-IR and TLC as follows.

3.1.2 Analysis of elutions by FT-IR

According to the IR spectrum of Elution I, the potential functional groups can be seen in Table 2. The ring vibration at 1011 cm^{-1} corresponds to the character of a steroid. The C=O and C-O-C stretching bands at 1738 cm^{-1} and 1176 cm^{-1}, and without the -OH group characteristic absorption band above 3000 cm^{-1}, indicate the presence of fatty acid steryl esters and glycerides. The band at 724 cm^{-1} indicates that there are four or more CH_2 groups in the chain. The band at 1464 cm^{-1} is the scissoring frequency of CH_2 or CH_3 groups and the band 1377 cm^{-1} shows the symmetrical bending model of a CH_3 group. The bands at 2926 cm^{-1} and 2852 cm^{-1} are inferred to be the stretching vibration of CH_3 and CH_2 groups. All the above observations imply the presence of long carbon chain groups for FASEs and glycerides.

Elution I	Adscription	Potential functional groups
2926 (s)	νCH	$-CH_3$ $-CH_2-$
2852（s）	νCH	$-CH_3$ $-CH_2-$
1738（s）	νC=O	R-CO-OR’
1464（m）	δCH	$-CH_2-$ $-CH_3$
1377（m）	δCH	$-CH_3$
1176（m）	νC-O-C	ROOR’
1011(w)	ring vibration	polycyclic compounds
724(w)	νCH	$-(CH_2)n-(n>4)$

Note: w, weak intensity; m, middle intensity; s, strong intensity; ν, stretching vibration; δ, deformation vibration

Table 2. FT-IR bands of Elution I

FT-IR spectra of Elution II and Elution III were obtained. Compared with the spectrum of Elution I, the spectrum of Elution II shows hydroxy stretching vibration at 3437 cm^{-1} and the weak ring vibration at 1011 cm^{-1}. Accordingly, the medium polar substances in Elution II may be polyaclcohols or carbohydrates with long carbon chain groups. It can be seen from the spectrum of Elution III that the wide band from 3500 cm^{-1} to 2500 cm^{-1} may be the stretching vibration of -OH from carboxylic acid. The absorption bands at 1568 cm^{-1} and 1202 cm^{-1} respond to amino group and nitryl group. This indicates that Elution III contains nitrogenous compounds. It is the reason that the color of the residue is dark brown. The absorption band at 1739 cm^{-1} corresponds to carbonyls stretching vibration, which may come from carboxylic acids and esters. The band at 3361 cm^{-1} is related to hydroxy groups. Accordingly, the Elution III has the strongest polarity among the three elutions.

3.1.3 Analysis of elution by TLC

Chromogenic reactions of FASE were carried out by using TLC. The results showed that the frontier of Elution I on the plate exhibited a red-violet color, while that of Elution II did not. This phenomenon further confirmed the existence of fatty acid steryl esters in Elution I.

3.1.4 Total content of phytosterols in WRSODD

As mentioned in Section 2.5, free phytosterols were obtained of WRSODD by purification, saponification and extraction processes. The content of phytosterols was determined by GC. The analysis results showed that the average of total sterol content in the WRSODD from

CGOG TECH Bioengineering Co. Ltd, China and Heilongjiang Jiusan Oil & Fat Co. Ltd, China was 27. 09 % and 27. 36 %, respectively.

3.2 Phytosterols recovery from the feed solution

Phytosterols can be recovered by crystallization of the complex feed solution. The precipitation of any given substance from a solution by crystallization is a direct consequence of its supersaturation. It can be achieved by solvent evaporation or by changing temperature if the solubility of the desired substance is temperature-dependent. The main process used to separate phytosterols from feed solution is to cool or chill the solution after a suitable solvent has been added. Thus the selection of a suitable solvent is the most important.

3.2.1 Selection of solvents for crystallization

In the present work, various alcohols, alkanes, ketones, aromatics, and their mixed solvents were used to explore the effect of different solvents on the crystallization of phytosterols from feed solution. The results are shown in Table 3.

The solubilities of sterols in alcohols increased with the increase in hydrocarbon chains for Test No. 1-3 as shown in Table 3. Methanol is invalid, since it could not dissolve the feed. The phytosterols product extracted by ethanol had the purity of 89.56 wt% and the yield of 21.06 wt%. The yield of phytosterols extracted by n-propanol was only 6.89 wt%, which was due to the high solubility of phytosterols in n-propanol. It can be also seen from Table 3 that the product with a high purity of above 90 wt% and a high yield of over 17 wt% was obtained by using alkanes. However, they are too expensive for commercial production.

No.	Solvent	Dissolving temperature (°C)	Ripening temperature (°C)	Ripening time(h)	Crystal color	Crystal, morphology	Purity (wt%)	Yield (wt%)
1	methanol	undissolved	/	/	/	/	/	/
2	ethanol	65	8	24	yellowish	needlelike	89.56	21.06
3	n-propanol	65	8	24	Whitish (yellowish partly)	planar	89.59	6.89
4	n-hexane	65	8	24	whitish	floc	92.90	22.04
5	cyclohexane	65	8	24	bright white	planar	92.88	17.03
6	Petroleum ether	65	8	24	whitish	floc	91.98	22.37
7	acetone	65	8	24	gray yellow	floc	89.31	19.16
8	butanone	65	8	24	white	planar	93.03	12.49
9	cyclo-hexanone	60	5	24	whitish	floc	93.34	7.89
10	benzene	65	8	24	yellowish	needlelike	89.77	9.63
11	toluene	65	8	24	white	floc	94.20	6.69
12	Acetone/ ethanol=4/1	60	5	24	yellowish	needlelike	91.82	22.95

Table 3. Experiment results of crystallization in different solvents

Furthermore, the phytosterols with a high purity were obtained by using butanone, cyclohexanone, benzene and toluene. However their yields were very low. Taking their toxicity into account, they are all not desirable solvents for phytosterol crystallization.
Furthermore, Table 3 showed that a product extracted by acetone had a purity of 89.31 wt% and a passable yield of 19.16 wt%. An interesting phenomenon can be seen was that the mixture of ethanol and acetone as a mixed solvent showed good crystallization efficiency. The purity of the product extracted by the mixed solvent (acetone/ethanol=4/1, v/v) was 91.82 wt%, and its yield was 22.95 wt%.
Taking all factors into consideration, the mixture of acetone and ethanol (volume ratio=4/1) as a mixed solvent with low cost and low toxicity could generate good crystallization of phytosterols with good crystal morphology, acceptable color, high purity and high yield.

3.2.2 Selection of crystallization conditions

Orthogonal experiments were implemented to optimize the crystallization conditions of phytosterols extracted by the mixed solvent (acetone/ethanol=4/1, v/v). The results of orthogonal experiments are listed in Table 4. The orthogonal table L9(3^4) was used to array the factors. The purity and yield of the recovered phytosterols were taken as the index points to evaluate the crystallization efficiency under different factors and levels.

Series No.	Level of A	Level of B	Level of C	Crystal color	Purity (wt%)	Yield (wt%)
1	1:1	4	-8	whitish	91.45	19.82
2	1:1	14	6	whitish	89.85	19.10
3	1:1	24	20	white	91.17	18.73
4	1:2.5	4	6	yellowish	87.85	18.74
5	1:2.5	14	20	white	90.15	18.67
6	1:2.5	24	-8	brown yellow	81.87	23.90
7	1:4	4	20	whitish	92.06	15.65
8	1:4	14	-8	yellow	84.85	20.82
9	1:4	24	6	yellowish	87.09	19.34

Table 4. Detailed schemes of orthogonal test and property results of crystallization

The range (R) analysis results of orthogonal experiments are shown in Table 5. The influence on the purity of recovered phytosterols was in the order: C>A>B, and the influence on the yield of recovered phytosterols was in the order: C>B>A. These suggested that the factor of ripening temperature (C) showed the most notable influence on whichever index points. Then the factor of ripening time (B) had the relatively remarkable influence compared with the proportion of feed solution to solvent (A) on the yield of phytosterols. Contrarily, the proportion of feed solution to solvent (A) had more influence on the purity of recovered phytosterols.
It can also be seen from Table 5 that the optimal crystallization conditions to obtain highest purity of recovered phytosterols were A1B1C3 when the proportion of feed to solvent was 1/1 (mass/mass), the ripening time was 4 hours, and the ripening temperature was 20°C (room temperature) (Table 1). By comparison, the optimal crystallization conditions in terms of yield were A2B3C1, namely, the proportion of feed solution to solvent 1/2.5 (mass/mass), the ripening time 24 hours, and the ripening temperature -8°C (chilling temperature).

k	Purity (wt%)			Yield (wt%)		
	A Feed /solvent (mass/mass)	B Ripening time (h)	C Ripening temperature (°C)	A Feed /solvent (mass/mass)	B Ripening time (h)	C Ripening temperature (°C)
k1	90.82	90.45	86.06	19.22	18.07	21.51
k2	86.62	88.28	88.26	20.43	19.53	19.06
k3	88.00	86.71	91.13	18.60	20.66	17.68
R	4.20	3.74	5.07	1.83	2.59	3.83
The order of the influence factors C>A>B				The order of the influence factors C>B>A		
The optimal scheme A1B1C3				The optimal scheme A2B3C1		

Note: ki represents the average of experimental values corresponding to level i (i=1, 2, 3). R represents the range which indicates how far it is from the lowest ki to the highest ki for a certain factor.

Table 5. Range analysis of orthogonal experimental results on purity and yield of recovered phytosterols

The effect of factors on the yield and purity of recovered phytosterols obtained in Table 5 was illustrated in Fig. 1. As shown in Fig. 1, the maximum yield of recovered phytosterols was obtained when the proportion of feed to solvent (A) was 1:2.5 (mass/mass) (Table 1). The yield increased with an increase in the ripening time (B) and decreased with an increase in the ripening temperature (C). On the contrary, the minimum purity of recovered phytosterols appeared when the proportion of feed to solvent (A) was 1:2.5 (mass/mass) (Table 1) as shown in Fig.1. The purity decreased with an increase in the ripening time (B) and increased with an increase in the ripening temperature (C).

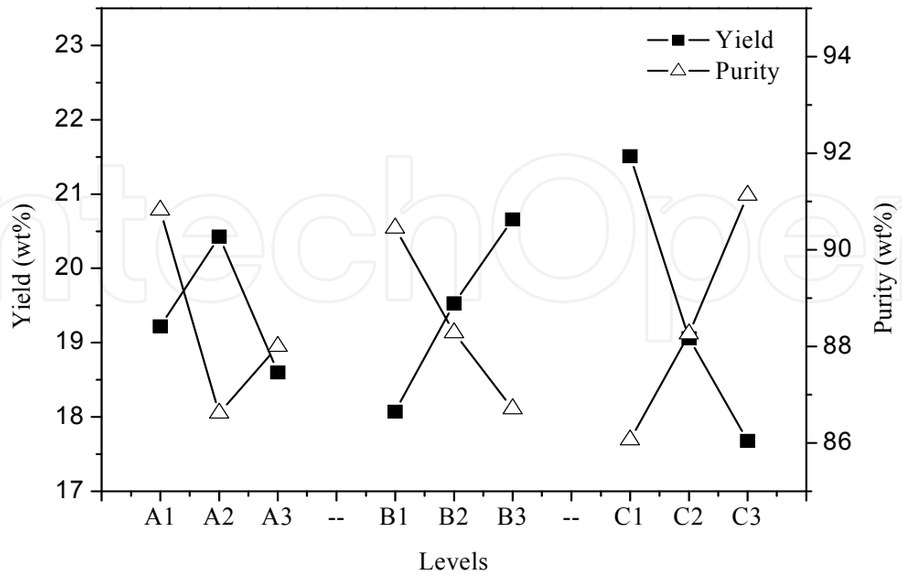


Fig. 1. Effect of factors on the yield and purity of recovered phytosterols under different levels.

In sum, the optimum crystallization conditions obtained were the proportion of feed solution to solvent 1:2.5 (mass/mass), the ripening time 24 h and the ripening temperature 6°C (cooling temperature). Following above optimized crystallization conditions, the phytosterols with a purity of 91.82 wt% and a yield of 22.95 wt% were obtained.

3.3 Purity and structure of recovered phytosterols

The structure and purity of recovered phytosterols were analyzed by FT-IR, GC and GC-MS, respectively.

3.3.1 Structure analysis of recovered phytosterols by FT-IR

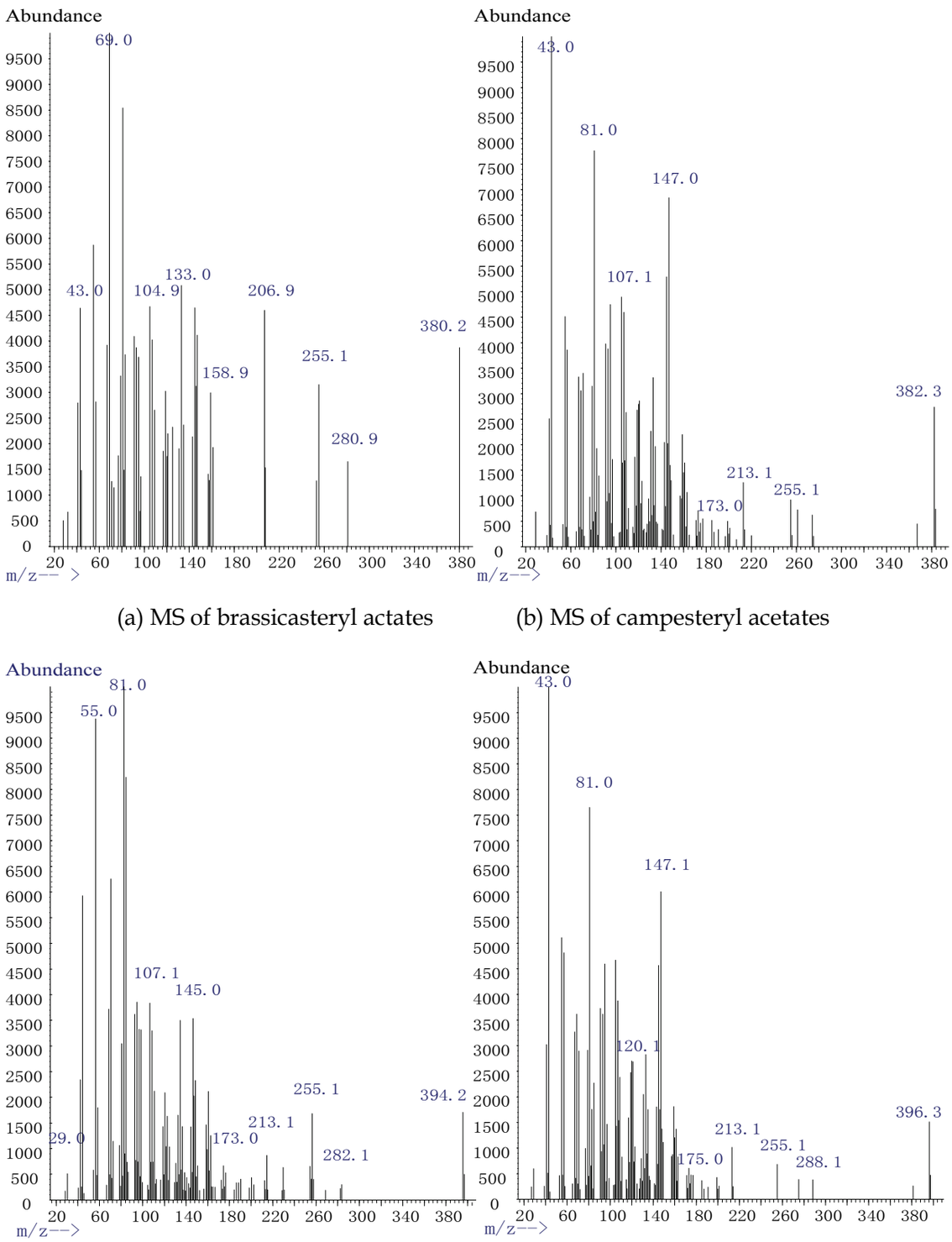
It can be seen in the FT-IR spectrum of recovered phytosterols (the 3rd crystallization) that the absorption wide band at 3428 cm^{-1} is inferred to be the stretching vibration of –OH from sterols. The absorption bands at 2936 cm^{-1} and 2867 cm^{-1} are inferred to be the stretching vibration of CH_3 and CH_2 groups, which imply the presence of long carbon side chain of sterols. No absorption bands between 1750 cm^{-1} and 1735 cm^{-1} can be observed. It indicates that no ester carbonyl group is present, which implies that esters have been hydrolyzed. The band at 961 cm^{-1} is the double band characteristic peak of phytosterols (Wang, et al., 2002).
The FT-IR spectrum of recovered phytosterols was in accordance with the standard spectrum of cholesterols, without functional groups of impurities. Further, the purity of recovered phytosterols was determined by GC and GC-MS.

3.3.2 Purity analysis of recovered phytosterols by GC and GC-MS

GC was employed to determine the purity of recovered samples of phytosterols. The results from GC of recovered samples of phytosterols obtained by the 3rd crystallization show that cholesterols (internal standard), brassicasterols, campesterols, stigmasterols and β-sitosterols were eluted with the relative retention time of 12.364, 12.888, 13.783, 14.249 and 15.223 min, respectively. According to the peak areas of sterols and internal standard and Equation (1), the purity of phytosterols sample recovered by the 3rd crystallization was calculated to be 97.17%. Following this calculation method, the purity of recovered phytosterols reached 91.82 wt% after the 1st crystallization and 92.73 wt% after the 2nd crystallization.
GC-MS was further utilized to investigate that the relative retention time of the peaks corresponds to certain sterols in the gas chromatogram. The mass spectrometry (MS) of each sterol peak is shown in Fig.2. It can be seen from Fig.2 that the maximum detectable mass of the GC-MS system was less than the molecular mass of correspondent steryl actetates by 60 mass units. Therefore, the characteristic peaks were corresponding to the loss of one molecule of acetate from molecular ions. For instance, the molecular mass of stigmasteryl acetates is about 454, and the maximum detectable mass of its MS is 394.2 (Fig. 2c). Their different value is about 60. It indicated that one molecule of acetate was lost. Furthermore, the MS of all the correspondent steryl acetates had the primary fragment peaks of m/z 255. It inferred that steryl acetates possessed steroidal nucleus (Zeng et al., 1994).

4. Conclusion

There is a large amount of combined phytosterols in the waste residue of soybean oil deodorizer distillate. This study describes a catalytic decomposition and crystallization process to recover phytosterols from WRSODD. Results showed that the total amount of



(a) MS of brassicasteryl actates (b) MS of campesteryl acetates

(c) MS of stigmasteryl actates s (d) MS ofβ-sitosteryl acetates

Fig. 2. The MS of steryl acetates

phytosterols, in the form of fatty acid steryl esters, was up to 27 wt% of WRSODD. The fatty acid steryl esters were decomposed by NaOH, and various solvents were then used for the crystallization of phytosterols. It was shown that the mixture of acetone and ethanol (volume ratio=4/1) could generate good crystallization of phytosterols with good crystal morphology, high purity and yield. Furthermore, the optimum crystallization conditions were obtained through orthogonal experiments. The phytosterols with purity of 91.82 wt% and yield of 22.95 wt% were obtained after the 1st crystallization under the optimized crystallization conditions, and the recovery rate was up to 84.7 % of total phytosterols. Moreover, the purity of recovered phytosterols obtained by GC-MS reached 92.73 wt% after the 2nd crystallization and 97.17 wt% after the 3rd crystallization.

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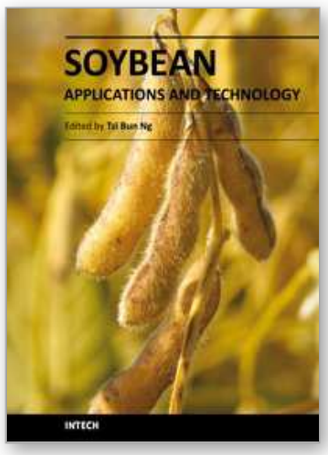

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Soybean is an agricultural crop of tremendous economic importance. Soybean and food items derived from it form dietary components of numerous people, especially those living in the Orient. The health benefits of soybean have attracted the attention of nutritionists as well as common people.

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