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Comparison of Various Extraction Methods for Policosanol from Rice Bran Wax and Establishment of Chromatographic Fingerprint of Policosanol

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A capillary gas chromatographic (GC) method has been developed for the separation and determination of policosanol components extracted from rice bran wax. A Varian CP-sil 8 CB column was employed, and an oven temperature was programmed. Gas chromatography−mass spectrometry (GC-MS) was used to identify the composition of policosanol. Quantitative analysis was carried out by means of hydrogen flame ionization detector (FID) with dinonyl phthalate (DNP) as internal standard. The results indicated that the extract obtained by dry saponification has the highest contents of octacosanol and triacontanol among extracts by all used extraction methods including dry saponification, saponification in alcohol, saponification in water (neutralized and non-neutralized), and transesterification. Meanwhile, the GC-MS fingerprint of policosanol extracted by dry saponification has been established. Euclidean distance similarity calculation showed remarkable consistency of compositions and contents among 12 batches of policosanol from a rice bran wax variety. This protocol provided a rapid and feasible method for quality control of policosanol products.

KEYWORDS: Policosanol; rice bran wax; gas chromatography; gas chromatography−mass spectrometry; fingerprint; Euclidean distance similarity

INTRODUCTION

In plants, the surfaces that are exposed to the atmosphere usually have a layer that contains wax. Stems, fruits, petals, and leaves may all be covered with wax. The wax preserves the water balance of the plant, minimizes mechanical damage to the cells, and inhibits fungal and insect attacks (*1*). Rice bran wax, a kind of natural lipid biochemically synthesized during rice growth, lies in the rice cortex. It is involved in bran when rice is processed and is isolated along with the lipid during the squeezing of bran oil. The average molecular weight of rice bran wax is 780. It exists in the form of a mixture of esters composed of high molecular weight (HMW) saturated fatty acids (SFAs) and HMW aliphatic primary alcohols (APAs). The dominant components are those esters made from SFAs with 22−24 carbons and APAs with 28−32 carbons (*2*). The existence of wax usually has adverse effects on the quality of rice bran oil. As a matter of fact, rice bran wax used to be rejected as a side product during oil refinement. Even though it could be used, it acted only as a polishing material such as floor polish, coating material, antistaling agent, insulated coating, impregnanting agent, and the like. Recently, studies on the development of products of highly processed rice bran wax, policosanol (PC) including octacosanol (OC) and triacontanol (TC), have been carried out extensively, especially in China because of the large rice output.

PC is the common name for a mixture of HMWAPAs (20−36 carbons), which has been reported to improve human physical fitness, and this effect is attributed to its high PC, specifically high OC, content (*1*). It is well-known that OC is a substance of antifatigue. OC can increase athletic performance, lower low-density lipoprotein levels, decrease the incidence of coronary heart disease, resist the coagulation of hematoblasts, cure blood vessel diseases of the heart such as thrombus, and enhance men's sexual ability, etc. (*3*, *4*). On the other hand, TC is used as a plant growth regulator. It exerts effects on photosynthesis, enzymic activity and respiration, antidisease ability, plant growth, and yield (*5*). Nowadays, the international market is demanding large amounts of PC products with high OC content. In addition, PC products with high TC content are also welcome. China has become an important exporter of rice bran wax PC products in the world. Necessarily, PC products have to go through strict quality control to enter the international market for downstream applications.

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Recently, we have put forward the concept of fine chemical chromatographic fingerprinting based on the experience of traditional Chinese medicines. This strategy has been playing a more and more important role in fine chemical quality control (*6*, *7*). Preparation of PC from raw rice bran wax requires two steps including extraction and refinement according to the product purposes. The PC product, mixture of HMWAPAs, is generally obtained just in the fist step. The APA distribution, that is, fingerprint, is dependent on the species and resource of the natural rice processed, whereas the extraction procedure affects only the total PC yield. If a single APA such as OC or TC is the target compound, unavoidably, the second step, such as high-vacuum distillation, has to be conducted. Even in refining, it is very hard to obtain absolutely pure OC or TC due to other inherent APAs in the raw material. As a result, some HMWAPAs, especially those that possess carbon numbers close to those of OC or TC, exist in the final refined products more or less. Therefore, it is essential to separate and determine the PC components in each step and to create their fingerprints for informing the selection of techniques and controlling the product quality.

Up to now, gas chromatography (GC) has been most frequently used to analyze HMWAPAs in PC with and without derivatization. Most of the derivatization methods employed *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) as silylation reagent (*1*, *8*). Acetic anhydride was also used once as acetylation reagent for GC analysis of PC (*9*). Derivatization lowers the boiling point of HWMAPA and makes the GC analysis doable, but the procedures are usually laborious and time-consuming. Too many factors affect the reaction yield. Especially acetylation revealed poor yield in our experiments. This may be due to weak reaction activity of HWMAPAs with acetic anhydride. It is so difficult to achieve identical yields that quantitative analysis is limited in both precision and accuracy. Moreover, the final sample solution for GC analysis becomes quite complicated, and the chromatograms are unable to be used to draw the fingerprints.

Some researchers developed nonderivatization GC methods for PC analysis (*10*−*14*). However, the internal standard method was less widely used than the external standard method for quantitation (*12*−*14*). Considering the demands on trace determination, a homologous compound of HMWAPAs was often chosen as internal standard (*12*). However, the objective is a mixture of APAs in PC, so the optional range of internal standards available is reduced. For the purpose of establishing fingerprints, the homologous compounds are unsuitable to be used as the internal standards. 1,3,5-Triphenylbenzene (TPB) (*13*) and dinonyl phthalate (DNP) (*14*) have been employed as the internal standards, respectively, for measuring OC and TC and for TC in PC products. In the present work, DNP was chosen as the internal standard for determining PC components extracted from rice bran wax. The reliability and adaptability of the proposed method were verified by linear range of internal standard curve, recovery, and reproducibility of real sample determination. On the basis of satisfying the analytical method, the extraction performance of PC from rice bran wax was compared by different extraction methods including saponification in alcohol, saponification in water (neutralized and nonneutralized), and dry saponification and transesterification. Furthermore, all components in PC extracted from rice bran wax were identified by use of hybrid gas chromatography−mass spectrometry (GC-MS). The chromatographic fingerprint of PC was established for the first time.

MATERIALS AND METHODS

Chemicals and Materials. Reference substances (RS) of OC (99%) and TC (96%) were purchased from Sigma-Aldrich (St. Louis, MO). Dinonyl phthalate RS (for GC stationary phase) was obtained from Shanghai First Reagent Factory (Shanghai, China). Sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide [$Ca(OH)_2$], calcium chloride anhydrous ($CaCl_2$), hydrogen chloride (HCl), ethanol (95%), *n*-butanol, acetone, and chloroform (all of analytical-reagent grade) were purchased from Nanjing Chemical Reagent Factory (Nanjing, Jiangsu, China), Tianjing Second Reagent Factory (Tianjing, China), or Shanghai First Reagent Factory (Shanghai, China). Raw rice bran wax treated with ethanol (*9*) was a gift from manufacturer X (China). An additional PC product extracted from another rice bran wax by dry saponification, as well as refined OC and refined TC from this PC product, was presented by manufacturer Y (China).

Extraction of PC from Rice Bran Wax. To investigate the effectiveness of different extraction methods for extracting PC, especially OC and TC, five extraction methods (A−E) were applied to the raw rice bran wax from manufacturer X. Each extraction was run in duplicate.

Method A. Saponification in alcohol was performed on the basis of the previously described procedure (*15*) with some minor modifications. Briefly, 5 g of rice bran wax was mixed with 25 mL of 95% ethanol and 0.5 g of NaOH in a 100 mL four-hole flask and subsequently hydrolyzed by refluxing in a water bath for 8 h with continuous stirring. Ten milliliters of alcoholic $CaCl_2$ solution (12 g of $CaCl_2$ plus 200 mL of 95% ethanol) was added, and the mixture was filtered while it was hot. After two washings with 95% ethanol, the mud cake was discarded. The collected filtrates were combined, cooled, and filtered again. The mud cake obtained in this step was dissolved with 3 times the volume of acetone preheated at about 50 °C. The solution in acetone was filtered after cooling. The resultant white PC product was naturally dried at ambient temperature.

Method B. Saponification in water (non-neutralized) was performed on the basis of a previously described procedure (*16*, *17*) with some minor modifications. Briefly, 5 g of rice bran wax and 3 times the volume of water were placed in a 100 mL four-hole flask. After the wax was melted at 85 °C in a water bath, 0.5 g of NaOH was added. The mixture was boiled for 12 h followed by heat preservation for 36 h. One milliliter of saturated $CaCl_2$ in water was added, and the reaction was continued for an additional 3 h at 80 °C. After filtration, the mud cake was washed to neutrality with hot water (∼80 °C) and dried at 65 °C. Subsequently, the solid was loaded to a Soxhlet apparatus and was extracted with 6−8 times the volume of acetone as solvent for 16 h. Once the extract solution had cooled, the PC product was crystallized. Then it was filtered and dried at 65 °C.

Method C. Saponification in water (neutralized) was performed on the basis of the previously described procedure (*18*) with some minor modifications. Briefly, 5 g of rice bran wax, 35 g of water, and 0.75 g of NaOH were placed into a 100 mL four-hole flask followed by continuous stirring for 20 h at 98 °C in a water bath. Ten grams of 10% HCl solution was subsequently added to neutralize the resultant. After 10 g of 8% $CaCl_2$ solution had been added, the reaction was kept for an additional 3 h. The resultant was cooled and filtered. After drying, the solid was refluxed with 10 times the volume of acetone for 12 h. The extract solution was cooled and filtered again. PC product was obtained after drying.

Method D. Dry saponification was performed on the basis of the previously described procedure (*19*) with some minor modifications. Briefly, 10 g of rice bran wax and 3 g of 50% $Ca(OH)_2$ soliquoid in water were added to a 100 mL four-hole flask and heated at around 100 °C in a water bath with continuous stirring for 5 h. The brown resultant was solidified once cooled. After the solid, which is the mixture of APAs and Ca-SFAs was weighed and ground, 5 g of the powder and 75 g of 95% ethanol were transferred into another flask and refluxed for 2 h under stirring. The mixture was filtered while it was hot. After the filtrate had cooled, the PC product was crystallized from the filtrate and was filtered and dried at 65 °C.

Method E. Transesterification was performed on the basis of the previously described procedure (*20*) with some minor modifications.

Briefly, 5 g of rice bran wax and 50 mL of 0.1% KOH solution in *n*-butanol were placed into a 100 mL four-hole flask. After 8 h of refluxing with continuous stirring, the reactant was cooled and substantially filtered. The mud cake was washed with hot water until it was neutral. After drying, the mud cake was loaded into a Soxhlet apparatus and extracted with acetone as the solvent for 12 h. The extract solution was cooled and filtered. The PC product was obtained after drying.

GC-FID and GC-MS Analyses. A Varian CP-3800 gas chromatograph (Varian, Walnut Creek, CA) equipped with a split/splitless injection port (operating in the split mode) and a flame ionization detector (FID) was used for quantitative analysis. A Thermo Finnigan Trace DSQ gas chromatograph−mass spectrometer with an AS3000 autosampler (Thermo Electron Corp., San Jose, CA) was used for identification of PC components. A GH-300C hydrogen generator and a GA2000A low-noise air pump (both from Zhongxing Huili Tech. Dev. Co., Ltd., Beijing, China) were used to generate hydrogen and air, respectively. Nitrogen (≥99.999%) was purchased from Nanjing Mucop Nanfen Special Gas Co. Ltd. (Nanjing, Jiangsu, China). GC separations were performed using a Varian CP-sil 8 CB capillary column (30 m × 0.25 mm i.d., film thickness = 0.25 μm) (Varian). The oven temperature was programmed as follows: the initial temperature was set at 280 °C for 18 min, raised to 295 °C at 5 °C/min, and held at 295 °C for 20 min. The injector temperature was set at 280 °C. For flame ionization detection, the carrier gas was nitrogen with a flow rate of 1 mL/min and the detector temperature was 310 °C. The pressures of hydrogen and air were 0.3 and 0.4 MPa, respectively. The injection volume was 1 μL and the split ratio 1:20. For MS detection, the carrier gas was helium and the injection volume was 1 μL, with the split ratio of 1:10. The EI ion source was operated at 200 °C with an ionization energy of 70 eV. The transfer line temperature was 250 °C. The emission current was 100 μA, and the voltage of the electron magnifier was 1160 V. Mass scan ranged from 20 to 500 amu.

A mixed stock solution of OC and TC was prepared by accurately weighing 5.17 mg of OC RS and 5.07 mg of TC RS into a 10 mL volumetric flask and adding chloroform to make up to the mark. The stock concentrations of OC and TC were 0.5118 and 0.4867 mg/mL, respectively. DNP stock solution was prepared by accurately weighing 58.74 mg of DNP RS into a 10 mL volumetric flask and adding chloroform to make up to the mark. Exactly 1.00 mL of DNP stock solution was transferred into a 10 mL volumetric flask, and chloroform was added to make up the volume. Thus, the concentration of the DNP internal standard working solution was 0.5874 mg/mL. Mixed standard solutions were prepared by serial dilution of the above mixed stock solution of OC and TC with chloroform. DNP internal standard working solution was added in each mixed standard solution to reach a final DNP concentration of 0.0587 mg/mL. Sample solutions were prepared by accurately weighing the proper amount of PC samples obtained as described under Extraction of PC from Rice Bran Wax or provided directly by manufacturer Y into a 10 mL volumetric flask, adding 1.00 mL of DNP internal standard working solution, and then adding chloroform to the mark. The final DNP concentration was 0.0587 mg/mL as in internal standard solutions.

RESULTS AND DISCUSSION

Chromatograms and Mass Spectra. The total ion current (TIC) chromatogram of a typical PC sample prepared from dry saponification (method D) is shown in **Figure 1**. Usually, it is difficult to obtain the molecular ions ($[M]^+$) of APAs by EI ionization, especially those with HMW, because these compounds are too active to give $[M]^+$ in EI mode. However, as observed from their mass spectra in **Figure 2a**,**c**−**g**, they produce some characteristic fragments such as $[M - 18]^+$ by loss of a water molecule and $[M - 46]^+$ by loss of an ethene molecule and a water molecule through rearrangement. Meanwhile, there are serial fragment ions of *m*/*z* 181, 153, 139, 125, 111, 97, 83, 69, 57, 43, and 29 in their mass spectra, which feature the long-chain hydrocarbon compound. Moreover, the abundance of these fragment ions decreases rapidly with the increase of ion mass, which indicates that there are no branched chains in these compounds. The base peak of *m*/*z* 88 (**Figure 2b**) from the peak at 5.17 min in the TIC is a characteristic fragment of an ethyl ester of fatty acid with no fewer than four carbons in the acid through McLaffety rearrangement. Also, there is an obvious fragment of $[M]^+$ of ethyl tetracosanoate (*m*/*z* 396). The existence of this compound in PC product may be attributed to the reaction of tetracosanoic acid and ethanol during the course of extraction. Although calcification was performed previously, tetracosanoic acid could not be thoroughly eliminated through reaction with Ca^{2+}, presumably due to the slight solubility of calcium tetracosanoate in alcohol. The peaks at 25.79, 35.37, and 36.38 min came from column bleed. The assignment of the main peaks is listed in **Table 1**.

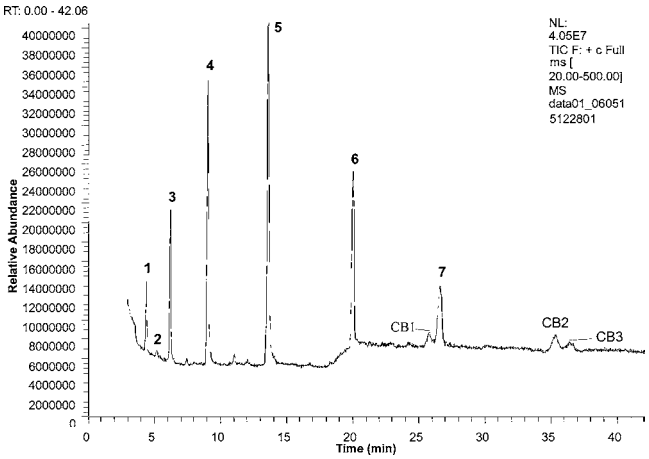

Figure 1. TIC chromatogram of a typical PC product. Peaks: 1, tetracosanol; 2, ethyl tetracosanoate; 3, hexacosanol; 4, octacosanol; 5, triacontanol; 6, duotriacontanol; 7, tetratriacontanol; CB 1, CB 2, CB 3, column bleeding.

Linear Range and Determination Limit. The GC-FID chromatogram of a mixed standard solution is given in **Figure 3**. Peaks 1, 2, and 3 are internal standard DNP, OC, and TC, respectively. Every standard solution was injected thee times. Good linearity is obtained within the concentration range of 0.0100−0.250 mg/mL for both OC and TC. The linear regression equations of OC and TC are $y_{OC} = 15.86x_{OC} - 0.0284$ and $y_{TC} = 12.90x_{TC} - 0.0457$, respectively, with relative coefficients *r* of 0.99959 and 0.99948, where *x* represents the concentration of OC or TC and *y* the average peak area ratio of OC or TC to internal standard DNP. The detection limits for OC and TC are 0.005 mg/mL at a signal-to-noise ratio of 3 (S/N = 3).

Analysis of PC Samples. The GC chromatograms of extracts obtained from the five different methods were compared (**Figure 4**) and displayed similar profiles. The qualitative analysis of PC components was carried out by mass spectra collected in GC-MS (not shown). OC and TC are the two substances of greatest concern in PC products from rice bran wax; therefore, they were determined by using their individual RSs. An internal standard curve method was adopted to guarantee a reliable result. In all chromatograms of real samples, there are no peaks before or behind DNP, so DNP is the proper internal standard. All of the samples prepared by the different extraction methods (A−E) under Extraction of PC from Rice Bran Wax were analyzed in triplicate, respectively, and the results are summarized in **Table 2**. The data in **Table 2** show that in PCs from raw rice bran wax of manufacturer X, the content of OC is lower than that of TC regardless of the employed extraction method. Compared with method A (saponification in alcohol) and

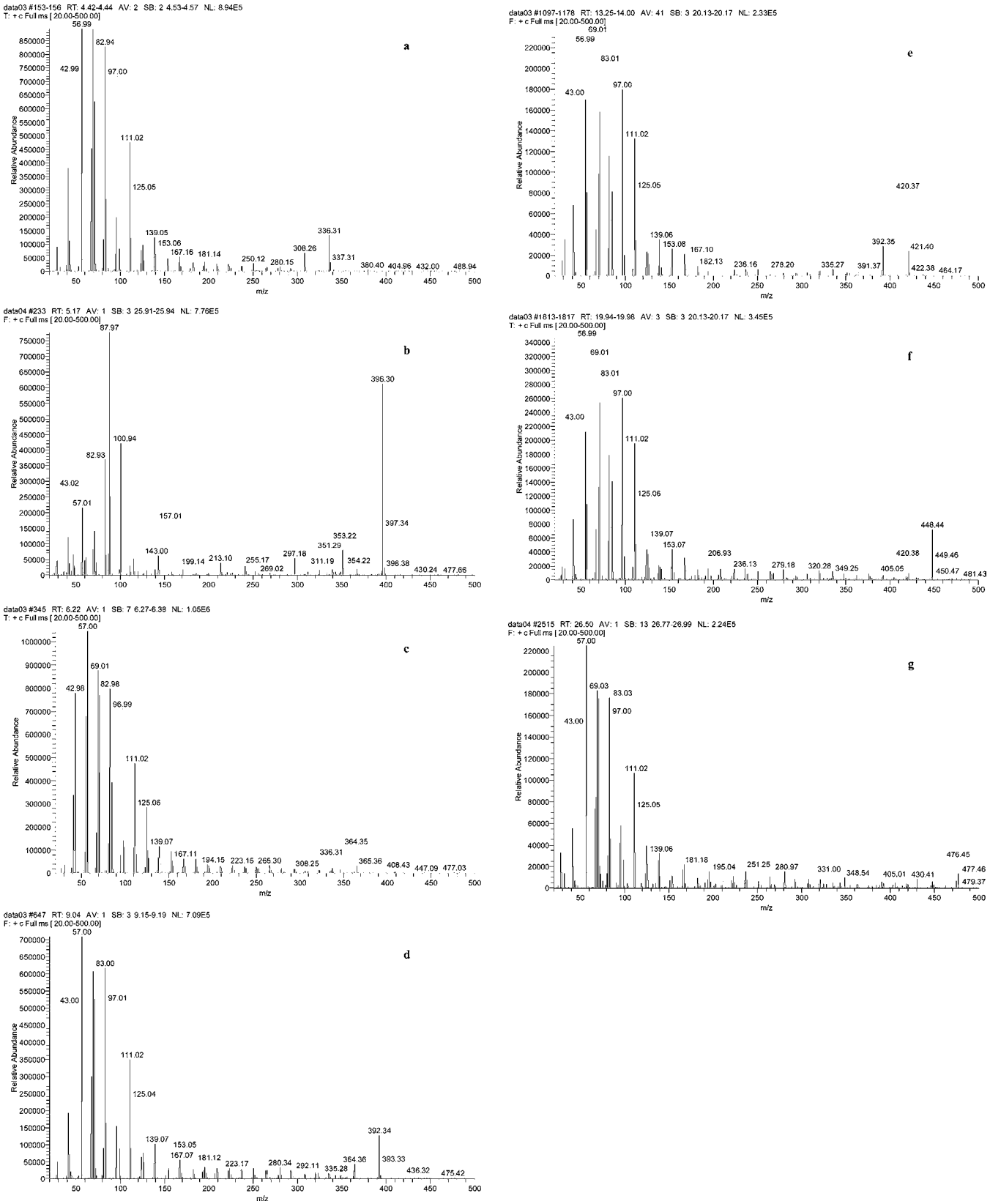

Figure 2. Mass spectra of PC components at retention times of 4.4 min (**a**, peak 1), 5.2 min (**b**, peak 2), 6.2 min (**c**, peak 3), 9.0 min (**d**, peak 4), 13.7 min (**e**, peak 5), 20.0 min (**f**, peak 6), and 26.5 min (**g**, peak 7) in **Figure 1**.

method D (dry saponification), methods B and C (saponification in water) give obviously less OC and TC, which implies that rice bran wax has a lower hydrolyzation efficiency by saponification in water. The total contents of OC and TC from method E (transesterification) are much lower than those from other methods despite the highest PC yield, probably owing to incomplete replacement of long-chain alkyls in rice bran wax by *n*-butyl in *n*-butanol. Although a slightly lower PC yield is given in dry saponification, the highest contents of both OC and TC are given among all extraction methods. Therefore, PC yield is not an appropriate criterion of quality estimate. Furthermore, the content ratio of OC to TC is very close (0.62−

Table 1. Assignment of Peaks in TIC Chromatogram of PC

peak	retention time (min)	*m*/*z*	compound	mol wt
1	4.44	336, 208	tetracosanol	354
2	5.17	396, 88	ethyl tetracosanoate	396
3	6.22	364, 336	hexacosanol	382
4	9.08	392, 364	octacosanol	410
5	13.68	420, 392	triacontanol	438
6	20.03	448, 420	duotriacontanol	466
7	26.62	476, 448	tetratriacontanol	494

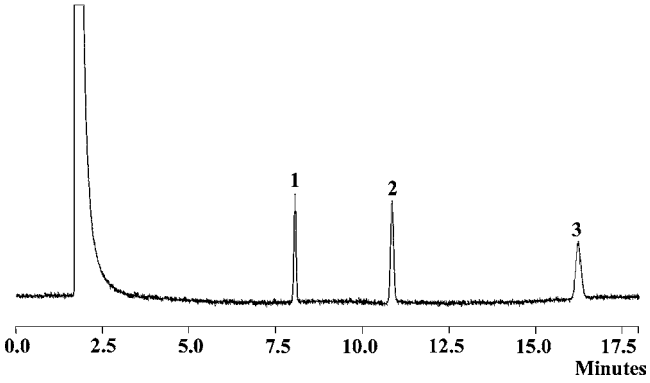

Figure 3. GC chromatogram of mixed standard solution with FID detection. Peaks: 1, DNP (IS); 2, OC; 3, TC.

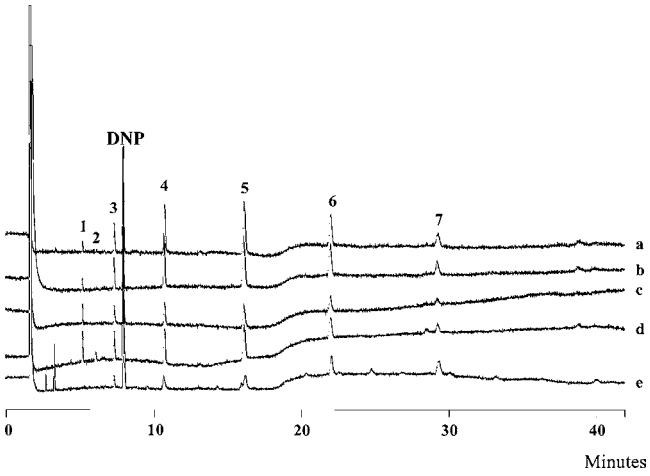

Figure 4. GC chromatograms of PC products from different extraction methods: a, saponification in alcohol; b, saponification in water (non-neutralized); c, saponification in water (neutralized); d, dry saponification; e, transesterification. Peaks: 1, tetracosanol; 2, ethyl tetracosanoate; 3, hexacosanol; 4, octacosanol; 5, triacontanol; 6, duotriacontanol; 7, tetratriacontanol.

0.67) regardless of method, which indicates that the extraction efficiencies of OC and TC are equal. Consequently, different extraction methods have no significant influence on the distribution of HMWAPAs extracted from a rice bran wax. That is to say, the fingerprint of PC from rice bran wax can be maintained during extraction. In addition, from the appearance of these PC products it is found that methods A and D, with ethanol as extracting solvent throughout the procedure, gave white products, whereas methods B, C, and E, with acetone as extracting solvent, gave yellowish products. Considering the yield of each extraction method, it is concluded that it arises from the more lipophilic property of acetone that dissolves some colored lipophilic substances in rice bran wax. Therefore, ethanol is a more suitable extracting solvent for raw rice bran wax.

Table 2. Efficiency Comparison of Various Extraction Methods for PCs from Rice Bran Wax

extraction method	PC yield[a] (%)	OC content[b] (%)	TC content[b] (%)	OC/TC
A	20.2	9.72 ± 1.36	15.16 ± 2.64	0.64
B	21.0	4.95 ± 0.37	7.94 ± 0.58	0.62
C	28.0	3.85 ± 0.45	5.79 ± 0.92	0.66
D	18.1	10.53 ± 0.72	15.94 ± 1.51	0.66
E	21.0	0.22 ± 0.02	0.33 ± 0.09	0.67

[a] In this current experiment, the yields of total PC for method D (dry saponification) were calculated through the formula (*19*) PC (%) = $[m/(m_1 \times m_2)/M] \times 100$, where m is the weight of the final PC product (g), m_1 is the weight of APAs and Ca-AFAs mixture used for refluxing (g), m_2 is the total weight of APAs and Ca-AFAs mixture obtained from the saponification reaction (g), and M is the weight of rice bran wax (g). For methods A (saponification in alcohol), B and C (saponification in water), and E (transesterification), the yields of total PC were directly expressed as PC (%) = $(m/M) \times 100$. The data in this column are the averages of two runs. [b] $n = 6$.

Recovery and Precision. Into each of four 10 mL volumetric flasks was accurately weighed 5.00 mg of PC extracted from rice bran wax of manufacturer X by method A (saponification in alcohol), and 1.00 mL of DNP internal standard working solution was added. Then, 0.00, 0.60, 1.20, and 2.40 mL of mixed stock solution of OC and TC were spiked, respectively. After chloroform had been added to the mark, each of the above solutions was injected in triplicate. The recoveries were 100.3−106.8 and 101.9−107.4% for OC and TC, respectively, with relative standard deviations (RSD, $n = 3$) of 3.15 and 2.98%, displaying a satisfactory accuracy of the present method.

Into each of five 10 mL volumetric flasks were placed 5.00 mg of accurately weighed PC from rice bran wax of manufacturer X by method A (saponification in alcohol) and 1.00 mL of DNP internal standard working solution. Then chloroform was added to the mark. Each of the above solutions was injected three times. The contents of OC and TC were 10.70 and 16.92%, respectively, with intraday RSDs ($n = 5$) of 0.93 and 1.01%. In addition, a solution prepared according to the same procedure every day was analyzed by the same method for 5 days. The contents of OC and TC were 10.74 and 16.96%, respectively, with interday RSDs ($n = 5$) of 1.35 and 2.11%. Both intra- and interday precision showed very good measurement reproducibility.

GC-MS Fingerprint of PC and Its Application. It has been mentioned that HMWAPA distribution of PC in a rice bran wax, that is, the chromatographic fingerprint, was maintained after extraction. The fingerprint should comprise the following principles: lots of information, good stability, and characteristic (*21*). From the gas chromatograms (**Figure 5**) of 12 batches of PCs extracted from rice bran wax of manufacturer X by dry saponification (method D) we could find that the profiles of these products are very similar, which reflect the fingerprint feature. In addition to OC and TC, other HMWAPAs were determined to obtain similarity of fingerprints. The mass spectra of individual components were recorded by GC-MS for identification prior to quantitative analysis. Because only two reference substances were available in our laboratory, tetracosanol, ethyl tetracosanoate, and hexacosanol were quantitated with OC as RS, whereas duotriacontanol and tetratriacontanol were quantitated with TC by internal standard curves. Results are listed in **Table 3**. Euclidean distance similarity was used to measure the consistency of PC products. Although those products were processed according to the same procedure, the contents of components were a little different. Euclidean distance

Table 3. Similarity of 12 Batches of PC Products by Dry Saponification from Rice Bran Wax

	PC composition							
batch	1	2	3	4	5	6	7	similarity
a	1.74 (1.70)[a]	3.01 (0.63)	4.01 (1.67)	9.42 (0.72)	13.98 (0.97)	7.50 (3.43)	4.14 (2.02)	0.9882
b	0.18 (2.02)	1.16 (2.77)	1.30 (3.87)	5.06 (1.93)	8.49 (0.55)	4.76 (2.14)	2.72 (0.75)	0.9876
c	0.40 (3.59)	2.59 (1.94)	1.63 (2.45)	4.50 (1.11)	7.43 (1.33)	4.33 (1.04)	2.23 (4.07)	0.9793
d	nd	0.69 (4.16)	0.93 (4.04)	4.57 (0.62)	8.79 (0.74)	5.33 (0.70)	3.14 (2.28)	0.9880
e	nd	0.15 (3.01)	0.53 (4.18)	3.60 (1.46)	7.62 (1.09)	4.70 (3.30)	3.04 (3.40)	0.9768
f	0.18 (0.14)	1.05 (4.43)	1.46 (3.06)	6.67 (1.20)	11.96 (1.78)	7.36 (0.36)	4.31 (3.88)	0.9984
g	0.72 (2.78)	1.86 (1.89)	2.76 (1.99)	8.81 (0.90)	13.90 (0.48)	8.29 (1.92)	5.14 (2.61)	0.9913
h	nd	0.59 (3.80)	1.10 (5.33)	5.62 (2.47)	9.80 (2.99)	6.01 (5.34)	3.57 (7.86)	0.9947
i	nd	0.60 (3.49)	0.90 (5.76)	5.09 (3.49)	10.25 (1.29)	6.45 (0.59)	4.13 (3.69)	0.9943
j	0.82 (4.24)	2.01 (0.78)	2.37 (3.03)	8.04 (1.19)	13.78 (1.73)	8.42 (4.69)	4.83 (1.18)	0.9933
k	4.84 (0.98)	1.66 (2.53)	5.43 (0.90)	10.92 (0.48)	15.60 (0.55)	8.43 (0.70)	4.60 (0.35)	0.9626
l	3.77 (0.87)	2.21 (1.12)	5.30 (1.23)	11.16 (1.15)	16.28 (1.01)	8.80 (2.39)	4.99 (2.81)	0.9608

[a] The data in parentheses are RSD (%, $n = 3$). The peak numbers are the same as in **Figure 4**.

Table 4. Content (Percent) of Policosanol Constituents in PC and Refined OC and TC

	PC composition						
sample	1	2	3	4	5	6	7
PC	5.59 (1.03)[a]	0.12 (4.42)	7.04 (0.69)	11.19 (0.41)	16.83 (1.17)	9.97 (2.26)	5.34 (1.10)
refined OC	nd	nd	3.91 (5.44)	83.17 (0.32)	7.04 (1.37)	nd	nd
refined TC	nd	nd	nd	20.42 (1.55)	74.46 (0.96)	nd	nd

[a] The data in parentheses are RSD (%, $n = 3$). The peak numbers are the same as in **Figure 4**.

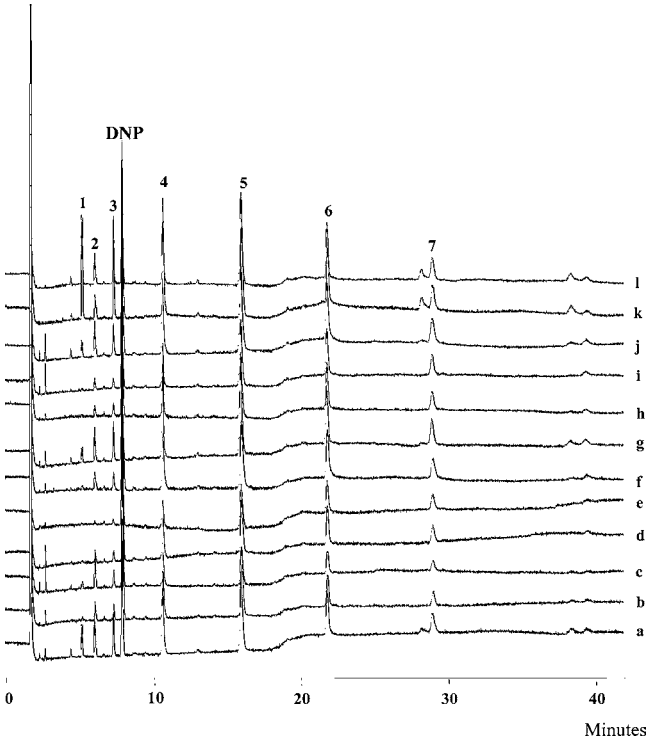

Figure 5. Chromatographic fingerprints of 12 batches of policosanol from dry saponification (a-l, batch no.). Peak numbers are the same as in **Figure 4**.

similarity is the very reflection of this fluctuation. Therefore, average contents of components were considered to be the standard average parameter of the chromatographic fingerprint.

Euclidean distance similarity was adopted in this work for quality evaluation of PC products. Whether a product is qualified or not can be intuitively estimated by analyzing its chromatogram with comparison to the fingerprint. If the chromatogram of a PC product is similar to its fingerprint, we can rapidly estimate that it is primarily eligible. Then, the product quality

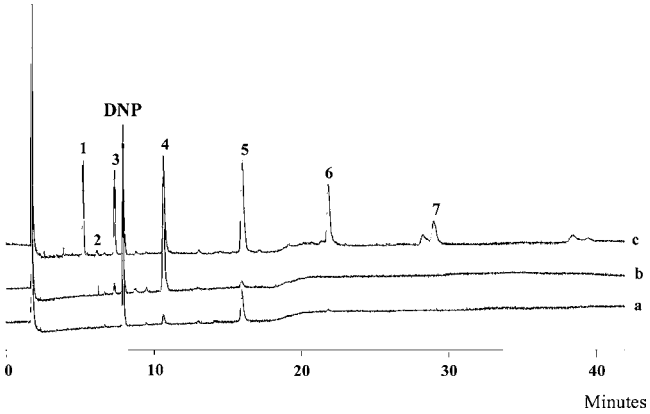

Figure 6. GC chromatograms of refined TC (a), refined OC (b), and PC (c). Peak numbers are the same as in **Figure 4**.

was measured by similarity between the chromatogram of sample and the standard average parameter of the chromatographic fingerprint. Results of Euclidean distance similarity indicate that different batches of PCs have remarkable consistency of composition and content. Good similarity can ensure stability and consistency of product quality (*22*). Contrarily, abnormal chromatograms imply failed products. For these failed products, it is not necessary to carry out further analysis and testing, because a whole set of multi-items and multistep analytical procedure for a PC quality inspection normally is laborious, time-consuming, and costly (*6*, *7*).

In addition, GC chromatograms of refined TC and refined OC, processed by high-vacuum distillation of the PC provided by manufacturer Y, are compared in **Figure 6**. Changes of contents of the fingerprint constituents from the PC to refined OC and TC are listed in **Table 4**. **Figure 6** and **Table 4** indicate that high-vacuum distillation can affect the refining of PC and remarkably change its fingerprint. Duotriacontanol and tetratriacontanol have much higher, and tetracosanol and ethyl tetracosanoate much lower, boiling points than OC. These four substances could be eliminated from the PC extracted from rice

bran wax. Hexacosanol and TC have molecular weights, molecular structures, and boiling points very close to those of OC, so there is some hexacosanol and TC in refined OC product. Similarly, there is some OC in refined TC product.

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