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The suitable conditions were determined for the recovery of high-value antioxidative compound, γ-oryzanol, from low cost rice bran soapstock by-product of the rice bran oil industry. First, soapstock was saponified and was then dehydrated and extracted with ethyl acetate. The extract was further purified by crystallization twice in appropriate solvent systems. At the most suitable conditions, using 20% v/v of ethyl acetate in methanol, and at 30°C and 1 h for the first crystallization step, and 5°C and 24 h for the second, the yield and purity of γ-oryzanol were 55.17 ± 0.59 wt% and 74.60 ± 4.12 wt%, respectively.

Keywords γ-oryzanol; crystallization; rice bran oil; soapstock

INTRODUCTION

Today, cooking oil produced from rice bran, a major by-product of the rice milling industry, is gaining considerable popularity since the oil contains a high content of nutraceutical substances such as vitamin E and γ-oryzanol. Of particular interest, γ-oryzanol, which is a characteristic compound found only in rice bran oil and not in other vegetable oils exhibits several health benefits such as being an anti-aging agent and reducing blood cholesterol (1–4). Moreover, it is non-genotoxic and non-inhibitory of cellular communication, and is therefore safe for use as supplement, food additive, or cosmetics (5). For these reasons, the recovery of γ-oryzanol from rice bran or by-products of rice bran oil industries has become a subject of great interest as indicated by a significant number of research studies on the subject (6–12).

Normally, in the rice bran oil refining process, alkali such as sodium hydroxide is first used to remove free fatty acids, and then other impurities such as phosphatides, proteinaceous, and mucilaginous substances. The deacidification treatment with alkali gives rise to *soapstock* which can then be easily separated from the oil (triglycerides). At this step, a large amount of γ-oryzanol (83–95% of the amount contained in crude oil) is lost into the soapstock (6) making it the most interesting of by-products from the rice bran oil refining process.

To obtain γ-oryzanol enriched fraction from soapstock, Indira et al. (2005) proposed a simple process starting with saponification of the soapstock, followed by extraction of γ-oryzanol from the dried matter with organic solvent. The extract (γ-oryzanol enriched fraction) could then be refined further to achieve higher purity. Processes for γ-oryzanol purification previously proposed involve dissolution of γ-oryzanol enriched fraction in a suitable mixture, followed by fractional precipitation of mucilaginous impurities and crystallization of γ-oryzanol from supernatant (7). Of the solvent systems for extraction and purification suggested in previous research (i.e., hexane, methanol, and diethyl ether), our preliminary investigation suggests that ethyl acetate is the most suitable extraction solvent and the acetone: methanol mixture appears to be the most suitable crystallization solvent system. In this study, ethyl acetate was chosen as extraction solvent due to its solvent property as well as the acceptability for use in food and pharmaceutical industries. Moreover, the possibility of using ethyl acetate: methanol mixtures were investigated here. The advantage of this solvent system is that the same solvent (ethyl acetate) could be employed for both extraction and crystallization processes, eliminating the need for solvent evaporation, and redissolution prior to crystallization. Here, the appropriate NaOH concentration and time of saponification were first determined. Then, the suitable extraction condition, the compositions of crystallization solvents, and the crystallization conditions (temperature and time) were determined to obtain the optimum yield and purity of γ-oryzanol from local rice bran oil soapstock.

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MATERIALS AND METHODS

Materials and Chemicals

Rice bran oil soapstock was obtained from Thai Edible Oil Co., Ltd., Samutprakarn, Thailand. γ-oryzanol

standard (98% purity) was purchased from Wako, Japan. Ethyl acetate (AR grade 99.98%), sodium hydroxide (AR grade 98%), and sodium bicarbonate (AR grade 99.9%) were purchased from APS fine chem, NSW, Australia. Acetone was purchased from VWR, UK. Methanol (HPLC grade 99.99%) was purchased from Fisher Scienctific, UK.

Determination of Soapstock Sample Properties

Prior to the recovery of γ-oryzanol, the pH, water content, and γ-oryzanol content of the soapstock sample were determined. For the pH measurement, 5 g of soapstock was dissolved in 20 ml water and the mixture was then heated at 40°C until the soapstock was completely suspended. The pH of the mixture was measured using a pH meter (Hanna HI9813, Japan). The water content of the soapstock was determined by drying the sample to constant weight in a vacuum oven at 105 ± 2°C. The sample was allowed to cool to room temperature in a desiccator and weighed. In addition, the γ-oryzanol contents in the starting soapstock sample and the saponified sample were measured and used as a basis for the calculation of the yield and purity at each step of the separation process. For the determination of the γ-oryzanol content, the soapstock sample (original or saponified) was first dehydrated in an oven at 105 ± 2°C, after which 3 g of the dehydrated soapstock was extracted with 200 ml ethyl acetate for 4 h in a soxhlet apparatus. The sample residue was then extracted with 3 volumes of 50 ml of ethyl acetate under sonication at 40°C. The extracts were combined and analyzed for the amount of γ-oryzanol by a UV-visible spectrophotometer (Spectronic 20 Genesys, UV-2450, Japan). This procedure gave about 99% recovery of γ-oryzanol from the original soapstock.

Separation of γ-Oryzanol

The procedures for the separation of γ-oryzanol from soapstock consisted of three main steps: saponification, extraction and crystallization.

Saponification of Soapstock

The saponification process began with drying the soapstock in order to reduce the volume of the sample being processed. Then, 100 g of partially dried soapstock was weighed and transferred into a 600 ml beaker, into which a calculated quantity of NaOH in aqueous solution that was added. This quantity of NaOH could be determined based on the *saponification value*, following AOCS official method, Cd 3b-76, 1989, using potassium hydroxide (KOH). The saponification value could then be calculated from Eq. (1).

$$\text{Saponification value (g KOH/g sample)} = \frac{(a-b) \times 28.05}{\text{weight of sample (g)}} \quad (1)$$

in which a is the volume (ml) of 0.5 mol/l hydrochloric acid consumed in the blank test and b is the volume (ml) of 0.5 mol/l hydrochloric acid consumed in the soapstock sample test. The equimolar quantity of NaOH was used for saponfication of the soapstock in this study. The reaction was carried out at 100°C with constant stirring over a period of 2 h. After the reaction was completed, the excess alkali was neutralized with sodium bicarbonate. The saponified soapstock was then dehydrated in a vacuum oven at 90°C for 2–3 h.

Extraction of Saponified Soapstock

The dehydrated saponified soapstock was ground, weighed, and transferred into a thimble (33×100 mm), which was then placed in a holder of a Soxhlet extractor. The extraction was carried out using 100 ml of ethyl acetate over a period of 4 h at various ratios of sample to ethyl acetate: 3 g/200 ml, 6 g/200 ml, 10 g/200 ml, and 15 g/200 ml. After the extraction process, the majority of ethyl acetate was evaporated from the extract using a rotary vacuum evaporator (EYELA rotary evaporator N-1000, Japan). The remaining ethyl acetate was then dried under nitrogen flow to obtain γ-oryzanol enriched fraction. The γ-oryzanol enriched fraction was then weighed and the γ-oryzanol content (purity) in this dried extract was quantified by first re-dissolving 0.01 g of the γ-oryzanol enriched sample in 5 ml of ethyl acetate. The solution was then diluted 100–200 times with ethyl acetate prior to analysis. The absorbance of the resulted solution was then measured using a UV-visible spectrophotometer. The purity and yield of γ-oryzanol were calculated from the following equations:

$$\%\text{ purity of } \gamma\text{-oryzanol} = \frac{\text{Amount of } \gamma\text{-oryzanol in sample}}{\text{Weight of sample}} \times 100 \quad (2)$$

$$\%\text{ yiled of } \gamma\text{-oryzanol} = \frac{\text{Amount of } \gamma\text{-oryzanol in sample}}{\text{Amount of } \gamma\text{-oryzanol in dehydrated saponified soapstock}} \times 100 \quad (3)$$

in which the amount of γ-oryzanol in the dehydrated saponified soapstock was determined from the sum of the amount of γ-oryzanol extracted and that recovered from the sample residue (after each extraction) by repeatedly extracting it 3 times with 50 ml of fresh ethyl acetate at 40°C under sonication.

Crystallization of γ-Oryzanol

After evaporation of ethyl acetate, γ-oryzanol enriched fraction was obtained, which was then purified further through two-step crystallization. The first crystallization step was to precipitate the mucilaginous impurities

including waxes and gums, that would otherwise, by disrupting the crystal growth, interfere with the crystallization of γ-oryzanol in the second step. The supernatant was crystallized again in the second step to obtain γ-oryzanol crystals with increased purity.

In the first crystallization step, 1 g of γ-oryzanol enriched fraction obtained from the previous extraction step was added to the 5 ml of a solvent mixture in a 250 ml flask. The mixture was refluxed at elevated temperature to facilitate the complete dissolution of γ-oryzanol enriched fraction. The temperature of the hot solution was then gradually lowered and maintained at a set crystallization temperature for a desired period of time, during which the mucilaginous impurities were allowed to precipitate. The suitability of two crystallization solvent mixture systems (acetone:methanol at 15, 25, and 35% v/v and ethyl acetate:methanol at 10, 20 and 30% v/v), crystallization temperature (25 or 30°C), and time (1 or 2 h) were examined, in view of the γ-oryzanol loss and the yield. Here, the yield was defined in the similar fashion as in Eq. (3), where the amount of γ-oryzanol in the sample was taken to be that in the supernatant, whereas the γ-oryzanol loss was defined by Eq. (4) as the percentage of the amount γ-oryzanol in the extract that was lost into the precipitated mucilaginous impurities.

$$\%\ \gamma\text{-oryzanol loss} = \frac{\text{Amount of } \gamma\text{-oryzanol in mucilaginous impurities}}{\text{Amount of } \gamma\text{-oryzanol in extract}} \times 100 \quad (4)$$

After the first crystallization step, the clarified supernatant from the first crystallization step was carefully decanted into a 50 ml test tube and was allowed to crystallize again at low temperature for a period of time in a cooling water bath (EYELA cool ace CA-1111, Japan). The resulted yellowish crystal from this second crystallization step was filtered using a filter paper (Whatman No. 1) and quantified for the γ-oryzanol content using a UV-spectrophotometer and HPLC, from which the purity and yield of γ-oryzanol in the final product could be calculated (Eqs. (2) and (3)). At this step, the effect of the crystallization temperature (2–10°C) and time (8–24 h) were determined on the yield and the purity of the resulted γ-oryzanol crystals.

Analysis of γ-Oryzanol

UV-Spectrophotometric Analysis

For the quantification of γ-oryzanol content in the sample with UV-spectrophotometric analysis, the absorbance of the sample solution in ethyl acetate was measured at the wavelength of 320 nm using UV-visible spectrophotometer (UV-2450 UV-Visible Spectrophotometer, Japan). γ-oryzanol (98% purity) was used as a standard. The standard calibration equation was found to be $y = 24.9x$ for the range of standard concentration between 0–25 μg/mL, in which y is the concentration of γ-oryzanol and x is the absorbance at 320 nm.

HPLC Analysis

The analysis for the quantification of γ-oryzanol in the sample could also be carried out with a reverse phase HPLC. The HPLC apparatus consisted of a pump (All tech model 626, USA), equipped with an ELSD detector (All tech ELSD 2000ES, USA). The detector condition was set at the tube temperature of 60°C, and nitrogen gas flow of 1.7 L/min and the impactor was off. The analysis was carried out at room temperature in which 5 μl of the sample solution was injected onto an Altima C18, 250 mm × 4.60 mm I.D. column. The mobile phase consisted of methanol:isopropanol (70:30 v/v), and the flow rate was controlled at 1.2 ml/min. Here, the standard calibration curve was determined for the standard concentration range of 0–6 mg/mL to be $y = 8 \times 10^{-5}x$, in which y is the γ-oryzanol concentration and x is the peak area. The percent γ-oryzanol loss, purity, and yield of γ-oryzanol were calculated using Eqs. (2) and (3) as defined previously.

RESULTS AND DISCUSSION

Properties of Rice Bran Oil Soapstock

The pH of the rice bran oil soapstock sample used in this study was measured to be 10.5. The moisture content was found to be approximately 56.96 wt%, thus the other 43.04 wt% was therefore soap, glycerides, and unsaponifiable matters. The spectroscopic scan of the ethyl acetate extract of the soapstock is shown in Fig. 1 which was found to be similar to that of the γ-oryzanol standard solution. From the spectroscopic measurement at 320 nm, the γ-oryzanol content in the soapstock was found to be approximately 4.9 wt% on a wet basis (26.46 g of γ-oryzanol in 540 g soapstock), or 7.7 wt% on a dry basis, whereas the literature values vary from 1.5–6.5 wt% on the wet basis (8–10).

Saponification of Soapstock

To make the extraction process of γ-oryzanol easier, any remaining glycerides in the soapstock was converted into insoluble soap by saponification. The suitable quantity of NaOH could be estimated based on the saponification value, using a standard method (AOCS Cd 3b-76, 1989), which was found to be 24 mg NaOH/g soapstock (or 2.4 wt%). The high performance liquid chromatography (HPLC) analysis of the extract showed that the soapstock that was saponified with 2.4 wt% of NaOH no longer contained glycerides, indicating that the complete conversion of triglyceride could be achieved (Fig. 2). Nevertheless,

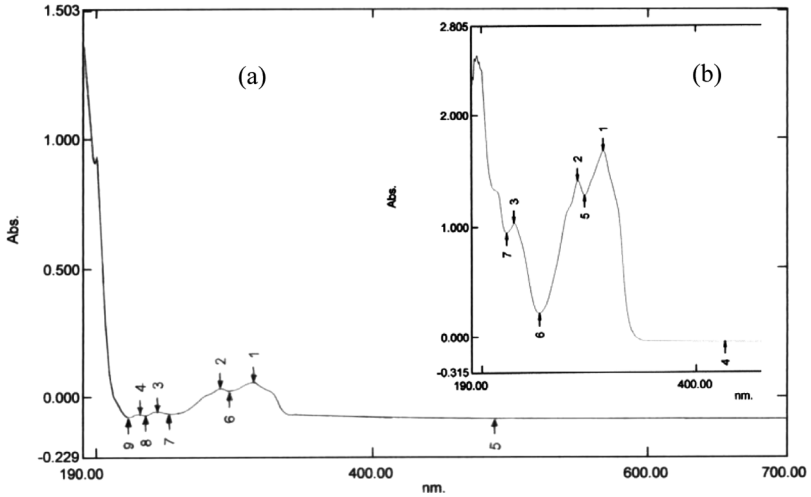

FIG. 1. Spectroscopic scan of ethyl acetate extract of (a) saponified soapstock sample and (b) standard γ-oryzanol.

some amount of γ-oryzanol was lost during the saponification process. That is, of the 540 g soapstock (containing 26.46 g of γ-oryzanol), 240 g dehydrated saponified soapstock (containing 15.74 g of γ-oryzanol) was recovered. This was a 40.5% loss of γ-oryzanol (26.46–15.74 g loss/26.46 g in the original soapstock), which could be due to the fact that the compound had undergone saponification, despite the fact that γ-oryzanol is often classified as an unsaponifiable matter (13). Besides, some amount of this viscous saponified material was lost during the transfer of the materials between the stirred reactors and the different vessels. Despite this loss, 2.4 wt.% NaOH was found to be suitable for saponification of the soapstock and would be used for the preparation of saponfied soapstock for subsequent crystallization study. For the preparation of the materials for the subsequent step, the saponified reaction product was dehydrated and ground into fine particles. The amount of γ-oryzanol contained in the dried saponified material (5.83% wt by UV-spectrophotometer and 3.46% wt by HPLC analysis) was taken to be 100% and the yield and purity of the γ-oryzanol obtained during each separation step would be determined based on this value.

Extraction of Dehydrated Saponified Soapstock

The effect of the ratio of dehydrated saponified soapstock to ethyl acetate was investigated on the yield of γ-oryzanol extracted. Extraction was carried out in a soxhlet apparatus. The extract was evaporated under vacuum and analyzed for the content of γ-oryzanol. As shown in Fig. 3, the yields of γ-oryzanol in the extracts obtained with various sample to solvent ratios (3 g/200 ml, 6 g/200 ml, 10 g/200 ml and 15 g/200 ml) were not significantly different, and in all cases, as high as 97–98% yields were achieved within the extraction time of 4 h. The purity of γ-oryzanol in the dried extract was determined spectrophotometrically to be about 30–38% wt, which was much higher than the original content in the saponified soapstock (about 5.83% dry wt). Based on these results, the suitable ratio of saponified and dehydrated soapstock to ethyl acetate for extraction was 15 g/200 ml (98% yield and 38% purity) and this ratio was therefore used for the preparation of the γ-oryzanol enriched fraction for the subsequent crystallization study.

Crystallization of γ-Oryzanol

First Step Crystallization

Effect of Solvent Mixture Composition. To carry out crystallization, the sample needs to solubilize first in a solvent. Then, it is necessary for the system to be at the state of supersaturation in order for crystallization to take place. The use of solvent mixtures, rather than pure solvent, therefore allows greater controllability or adjustability of the solvent polarity, thus providing more effective crystallization. In the first crystallization step in this study, the γ-oryzanol enriched fraction was dissolved in the crystallization solvent under reflux to ensure complete solubilization. Two systems of solvent mixture at different composition were selected for the crystallization study, acetone:methanol in the range of 15–35% v/v of acetone and ethyl acetate:methanol in the range of 10–30% v/v of ethyl acetate. The effects of the different composition of the solvent mixtures on the yield and the percentage of γ-oryzanol loss are shown in Fig. 4. At low acetone or ethyl acetate contents (15% of acetone and 10% of ethyl acetate), the poor solubility of γ-oryzanol in these solvent mixtures caused it to crystallize along with other impurities, resulting in high γ-oryzanol loss. At higher percentages of acetone (35%) and ethyl acetate (30%) in the methanol mixtures on the other hand, the solubility of γ-oryzanol increased and the compound remained soluble in the

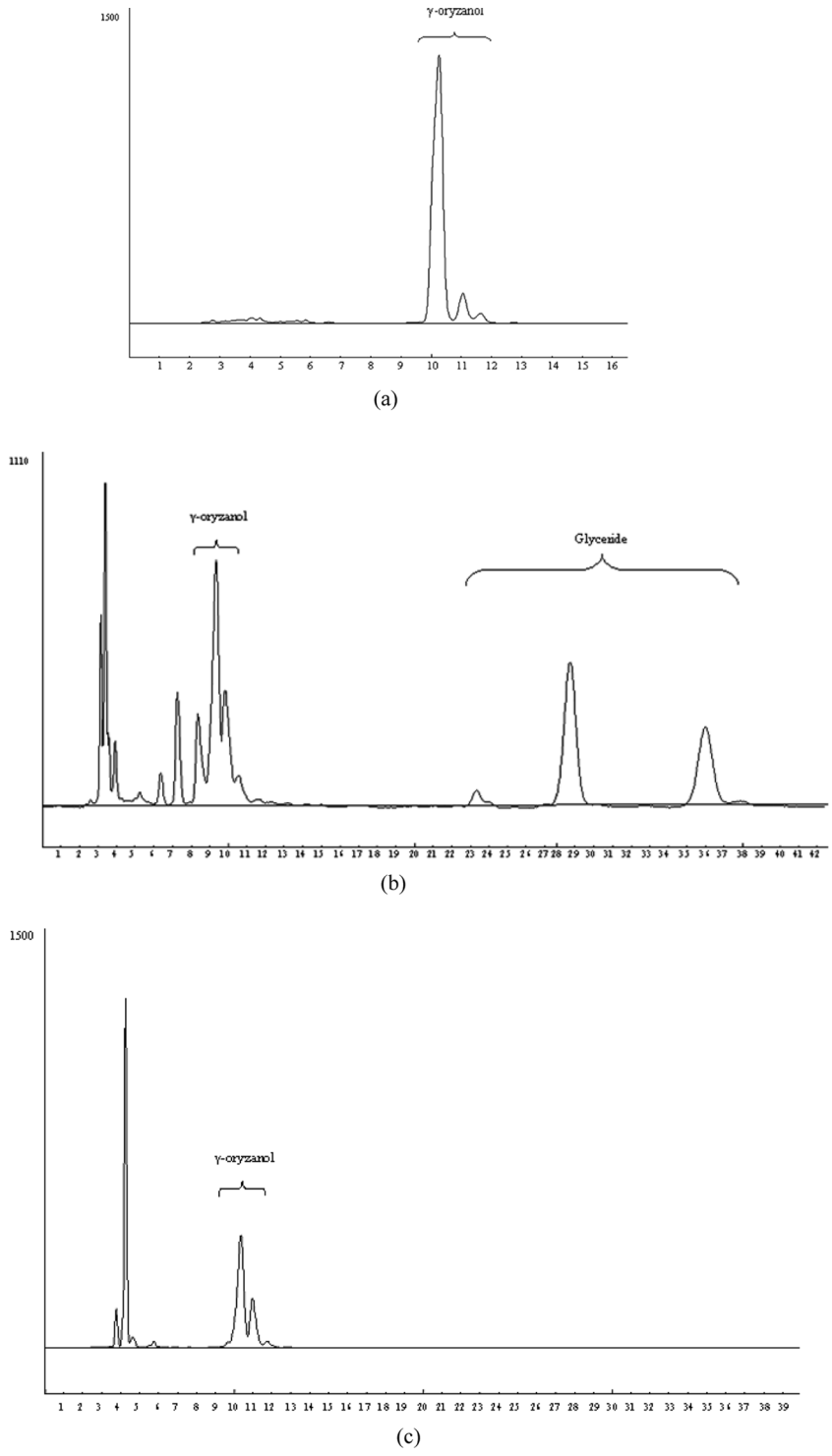

FIG. 2. Chromatogram of (a) standard γ-oryzanol, (b) dehydrated soapstock extract, and (c) dehydrated saponified soapstock extract, in ethyl acetate.

supernatant, and thus the smaller γ-oryzanol loss. Since a high amount of the compound remained in the supernatant, the γ-oryzanol yield as shown in Fig. 5 was therefore higher for crystallization in the solvents with a high percentage of acetone or ethyl acetate.

In addition to the above results, the supernatant obtained after the first crystallization step was then allowed to crystallize again in the second step at $5 \pm 1^{\circ}C$ for 16 h. The chromatograms of the γ-oryzanol sample after the first and the second step crystallization are shown in Fig. 4.

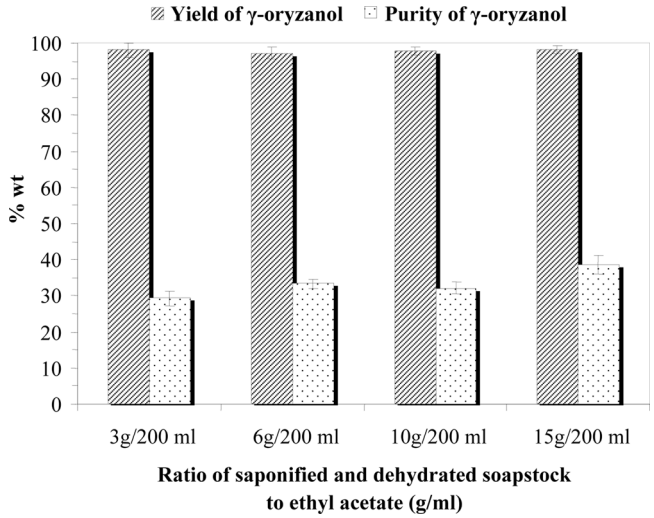

FIG. 3. Percent purity and yield of γ-oryzanol in extracts obtained at different sample to ethyl acetate ratios.

The yield and the purity of the γ-oryzanol crystals were determined. The quantification of γ-oryzanol yield and purity of the crystallized samples obtained in the second step was carried out both by using UV-visible spectrophotometer and HPLC and the results are shown in Figs. 6(a) and (b).

For UV-visible spectrophotometric analysis (Fig. 6(a)) of the crystallization system of acetone:methanol solvent mixture, it can be observed that at 15% v/v of acetone, although high purity of γ-oryzanol could be obtained, low yield was resulted as a large amount of γ-oryzanol was lost with the impurities in the first crystallization step. At 35% v/v of acetone, both the yield and the purity were

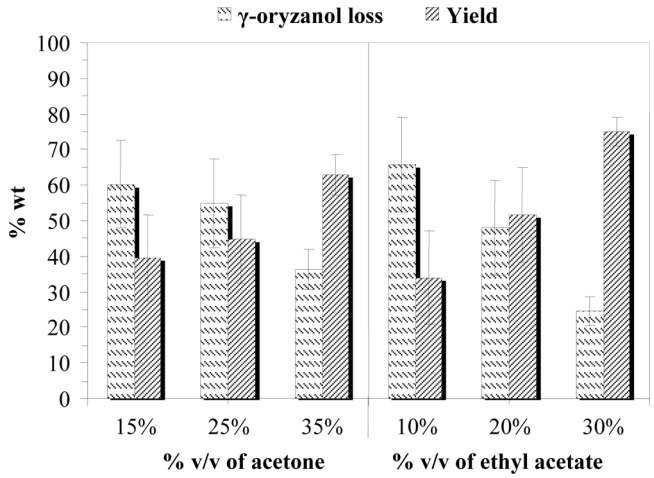

FIG. 4. Effect of solvent mixture composition on percent γ-oryzanol loss and yield of γ-oryzanol after first crystallization step at 25°C for 1 h by using solvent mixtures of various ratios; UV-visible spectrophotometric measurement was employed for γ-oryzanol analysis.

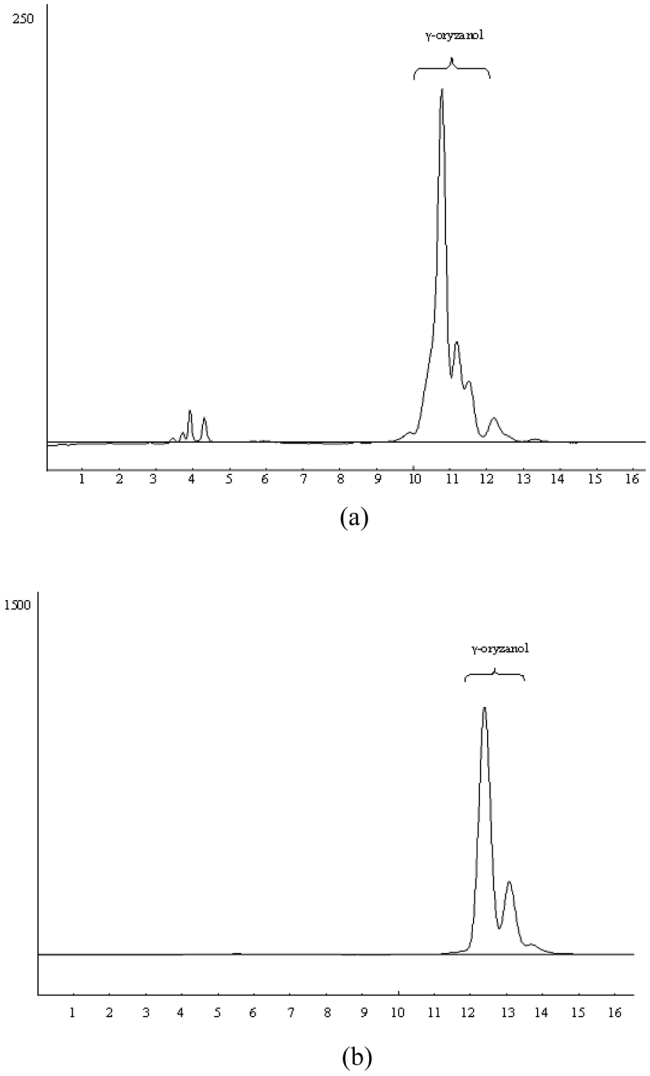

FIG. 5. Chromatogram of (a) mucilaginous impurities sample from first step crystallization and (b) crystallized sample from second step crystallization.

low. The low yield could be a result of crystallization γ-oryzanol with the impurities in the first step. Moreover, the mucilaginous impurities retained in supernatant after the first crystallization step could also crystallize in this step when the temperature was reduced to 5 ± 1°C and thus resulted in low purity. The mixture of 25% v/v of acetone in methanol was found to be the most suitable and this result is consistent with that reported by Narayan et al. (2004).

For the crystallization with ethyl acetate:methanol system, the yield of γ-oryzanol after the second crystallization step was found to increase with increasing percentage of ethyl acetate, while the highest purity obtained from this system was found with the 20% of ethyl acetate in methanol (Fig. 6(a)). The yield of γ-oryzanol from this system

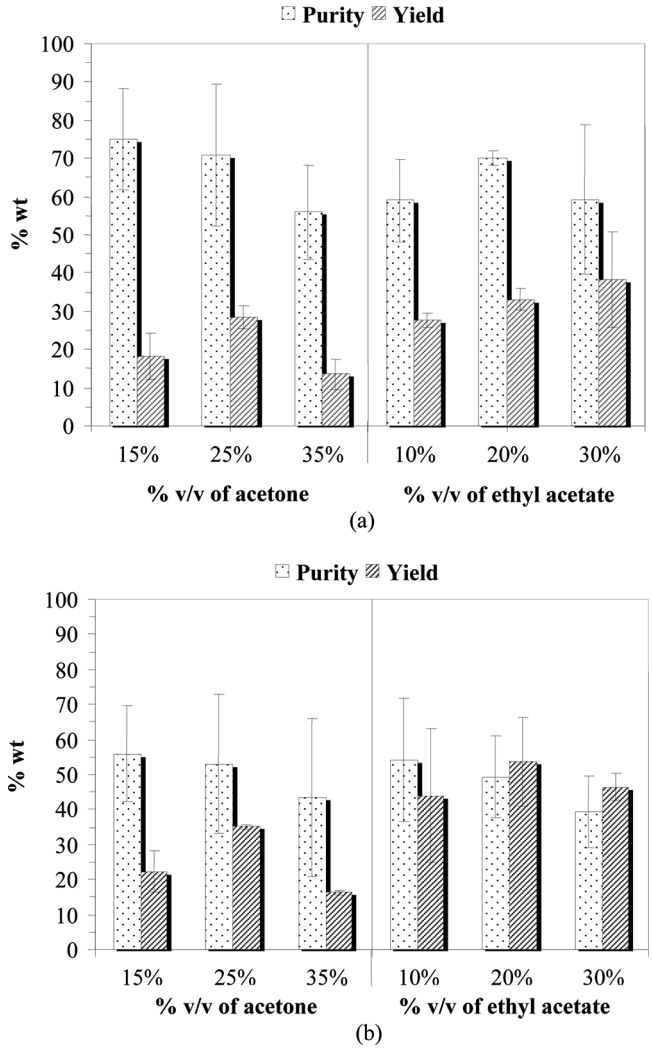

FIG. 6. Effect of solvent mixture composition on percent purity and yield of γ-oryzanol after second crystallization step at temperature $5 \pm 1°C$ for 16 h; (a) analyzed by UV-visible spectrophotometer and (b) analyzed by HPLC.

was slightly higher than that obtained at 25% of acetone, which was the most suitable composition for acetone: methanol system, while the purities obtained by the two systems were comparable.

To confirm these results, the yield and purity of the crystallized products were analyzed using HPLC as shown in Fig. 5(b). For the acetone:methanol solvent mixture system, HPLC analysis show rather similar behavior, that is, the purity slightly decreased with the increasing percentage of acetone and the highest yield was found at 25% of acetone. For ethyl acetate:methanol system nevertheless, a rather different behavior was observed. Based on UV-visible spectrophotometric analysis, the purity was the highest at 20% ethyl acetate and the yield increased with increasing percentage of ethyl acetate. The purity determined by HPLC analysis slightly decreased with increasing percentage of ethyl acetate while the highest yield was found at 20% of ethyl acetate. It should be noted that the spectroscopic analysis and the HPLC analysis of γ-oryzanol in the samples were rather different due to the different underlying principles of measurement. Unlike the spectroscopic measurement, in the chromatographic analysis, the amount of γ-oryzanol is detected and measured after it is separated from other impurities, it is expected to give more accurate results. Nevertheless, it can be drawn from both analyses considering both the yield and the purity of γ-oryzanol that 20% of ethyl acetate was the most suitable of all the solvent mixtures tested. It was therefore used in the preparation of the supernatant used for subsequent studies.

Effect of Crystallization Temperature. The effect of temperature for the first crystallization was determined using 20% v/v of ethyl acetate as a crystallization solvent mixture and the crystallization time was 1 h. The temperatures in which the process was compared were 25 and 30°C. It was found that the two temperatures gave no significant difference in the percentage of γ-oryzanol loss (25°C: $47.98 \pm 13.35\%$ and 30°C: $46.55 \pm 0.92\%$) and the percentage of γ-oryzanol in the supernatant after the first crystallization step (25°C: $51.62 \pm 13.25\%$ and 30°C: $53.04 \pm 0.91\%$).

When the supernatants taken from the first step, both at 25 and 30°C, were further crystallized in the second step at $5 \pm 1°C$ for 16 h, the resulting crystal obtained from this step was quantified for the γ-oryzanol content by using both UV-visible spectrophotometer and HPLC. The yield and the purity were obtained and are shown in Figs. 7(a) and (b). The results obtained by both methods of analysis showed no significant differences in the yields and purities at both temperatures. Therefore, the suitable temperature for the first crystallization step was 30°C and this condition was used in subsequent study.

It should be noted from the results in Figs. 6(a) and (b) that the yields quantified by HPLC were higher than those obtained by UV-visible spectrophotometer. This was because the yields were calculated from the ratio of the content of γ-oryzanol in crystallized product to that in the starting saponified soapstock. In a rather purified crystallized product, the compound content measured by both methods were comparable; however, when measuring the content of γ-oryzanol in the starting saponified soapstock, the presence of the impurities within the sample made the determination of the amount of γ-oryzanol contents by UV-spectrophotometer a slight over estimation. The γ-oryzanol content of 5.83 wt% was obtained by UV-Spectrophotometric analysis while the HPLC analysis gave the γ-oryzanol content of 3.46 wt%, thus

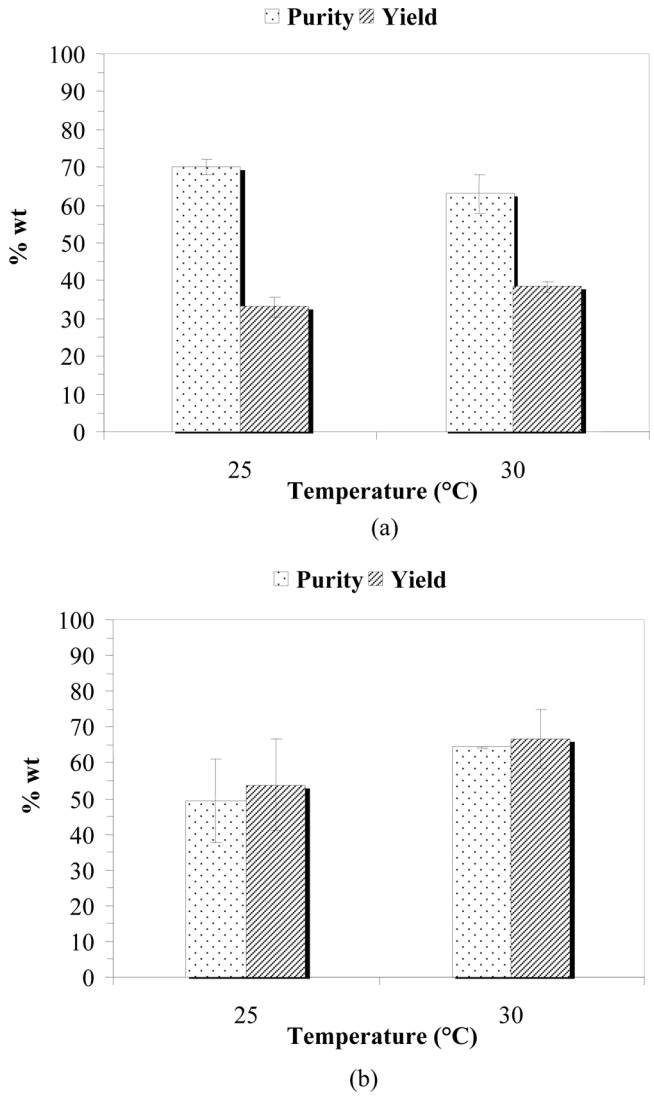

FIG. 7. Effect of temperature for first crystallization on percent purity and yield of γ-oryzanol after second crystallization step at 5 ± 1°C for 16 h; (a) analyzed by UV-visible spectrophotometer and (b) analyzed by HPLC.

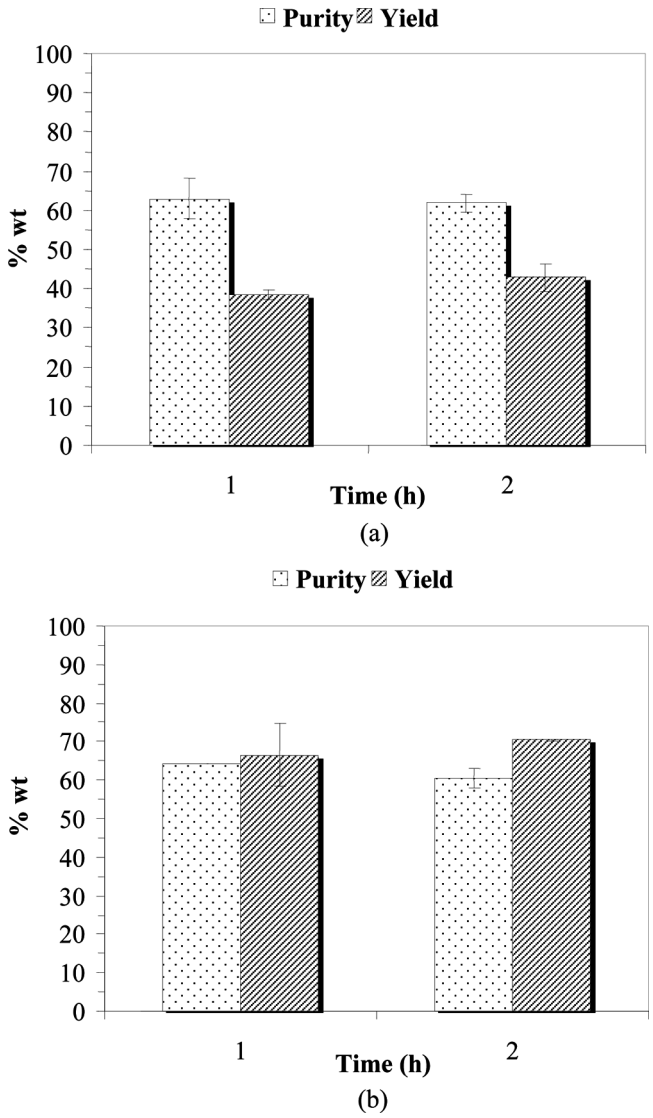

FIG. 8. Effect of time for first crystallization on percent purity and yield of γ-oryzanol after second crystallization step at 5 ± 1°C for 16 h; (a) analyzed by UV-visible spectrophotometer and (b) analyzed by HPLC.

the overall yields determined from both methods were therefore different.

Effect of Crystallization Time. The effect of the first step crystallization time (1 and 2 h) was investigated at 30°C for the crystallization system of 20% v/v of ethyl acetate in methanol. The results showed no significant differences for the γ-oryzanol loss (1 h: 46.55 ± 0.92%, 2 h: 44.12 ± 0.77%) and the yield (1 h: 53.04 ± 0.91%, 2 h: 55.45 ± 0.76%).

The supernatant from the first step was then separated and allowed to crystallize in the second step at 5 ± 1°C for 16 h, the purity and yield of γ-oryzanol are shown in Figs. 8(a) and (b) obtained by UV-visible spectrophotometric and HPLC analysis, respectively. The results from these figures indicated that crystallization time in the range of this study did not affect the yield and the purity of γ-oryzanol. Therefore, crystallization time for 1 h was sufficient to separate mucilaginous impurities and this was used for the subsequent study for the second crystallization step. The results here again showed that the yield obtained by HPLC analysis was higher than that obtained by UV-visible spectrophotometric analysis, while the purities were comparable.

Second Step Crystallization

Effect of Temperature. The supernatant from the most suitable conditions for the first crystallization step was allowed to crystallize in the second step at different

temperatures of 2, 5, and 10°C for up to 16 h in the cooling water bath. The yield of γ-oryzanol slightly decreased with increasing temperature (2°C: 44.07 ± 9.82%, 5°C: 38.43 ± 1.43% and 10°C: 33.04 ± 8.29%). The purity obtained at 2 and 5°C were not significantly different (2°C: 65.97 ± 7.29% and 5°C: 62.96 ± 5.17%) but at 10°C, the purity was found to be lower (47.47 ± 9.92%) when analyzed by a UV-visible spectrophotometer. These results were also consistent with that obtained from HPLC analysis in which the yield was 65.25 ± 9.20%, 66.57 ± 8.17% and 59.58 ± 2.05% and the purity was 58.26 ± 1.6, 64.31 ± 0.12 and 51.12 ± 3.96% at 2, 5, and 10°C, respectively. The reason for low yield and purity of γ-oryzanol at higher temperature of 10°C was probably because both γ-oryzanol and other impurities are soluble in crystallization solvents, thus the supernatant from the first crystallization step contain high amount of impurities, making it more difficult to achieve high yield and purity in the subsequent step.

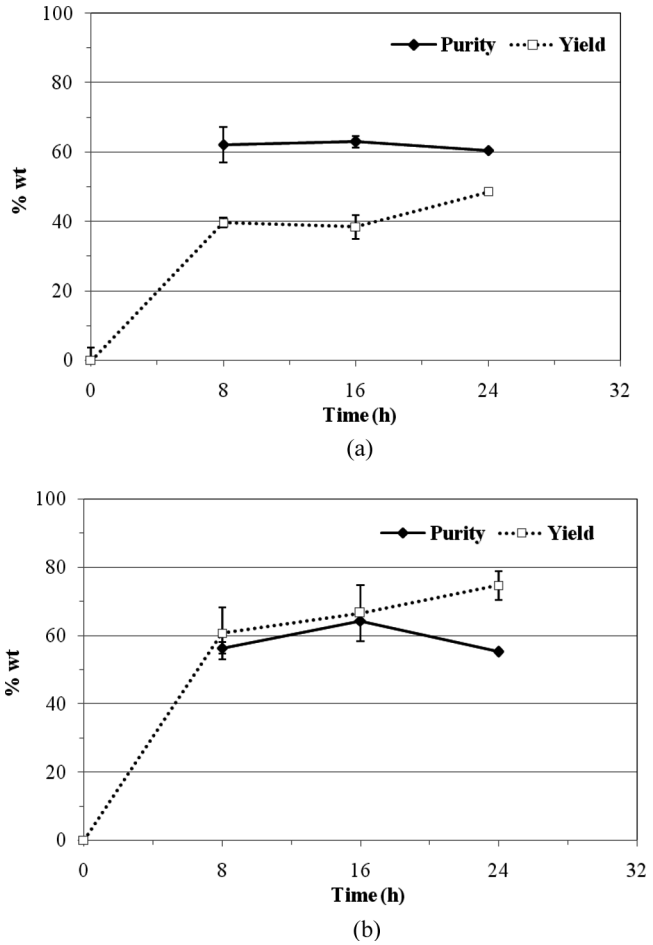

FIG. 9. Effect of time for second crystallization on percent purity and yield of γ-oryzanol after second crystallization step at 5 ± 1°C for 8, 16 and 24 h; (a) analyzed by UV-visible spectrophotometer and (b) analyzed by HPLC.

From these results, 5°C was considered the suitable temperature, which gave similar results compared with those achieved at 2°C. Thus, in the subsequent experiment to determine the suitable second step crystallization time, the temperature of 5°C was used.

Effect of Crystallization Time

After the first crystallization step in 20% v/v of ethyl acetate in methanol, at 30°C for 1 h, the supernatant was allowed to crystallize at 5°C for 8, 16, and 24 h. The quantification of γ-oryzanol in the crystal samples were carried out both by using UV-visible spectrophotometer and HPLC and the results are shown in Figs. 9(a) and (b). From Fig. 9(a), it was observed that the percent purity was not significantly different for the range of crystallization time employed in this study (62.11 ± 0.09%, 62.96 ± 5.17% and 60.51 ± 1.64%, respectively). The yield of γ-oryzanol was found not to be significantly different when the time was increased from 8 to 16 h. Nevertheless, the γ-oryzanol yield increased as the time increased from 16 to 24 h.

When HPLC analysis was used to quantify the γ-oryzanol content in the crystal sample, the results of γ-oryzanol purity and yield in Fig. 9(b) showed the same trends as those obtained with the UV-visble spectrophometric analysis in Fig. 9(a). In other words, the purity was comparable for different crystallization time while the yield increased with increasing time from 8 to 24 h from 60.61 ± 7.52% to 74.60 ± 4.12%. The yield and purity obtained in this study were comparable with those previously obtained (75.7% yield and 51.4% purity), using methanol and a mixture of methanol/acetone (2:1 v/v) for the first and the second crystallization steps (2).

CONCLUSIONS

In this study, the suitable conditions for the separation of γ-oryzanol from rice bran oil soapstock were examined. For the soapstock containing 4.9% wt of γ-oryzanol used, the most suitable saponification process required 2.4 wt% NaOH to convert the remaining glycerides into soap. After saponification, dried saponified material was extracted by ethyl acetate, and the suitable ratio of the saponified soapstock to ethyl acetate was found to be 15 g/200 ml. The mucilaginous impurities in the extract could be suitably separated in the first 1 h crystallization step using 20% v/v of ethyl acetate mixture in methanol at 30°C. Subsequently, the supernatant obtained was allowed to crystallize in the second step to obtain γ-oryzanol crystals. The use of ethyl acetate: methanol crystallization solvent system would simplify the overall purification process as the step for the evaporation of the ethyl acetate extraction solvent could be eliminated and the solvents could be recovered and reused.

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