Deacidification of rice bran oil using a deep eutectic solvent

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Abstract. The liquid-liquid extraction (LLE) will be applied in this study using a deep eutectic solvent (DES) of choline chloride and ethylene glycol with a 1:2 molar ratio. The LLE process will compare the results of removing free fatty acids (FFAs) between single and multiple extractions. Rice bran oil (RBO) with various initial FFAs contents (5%, 10%, 20%, 30%, 40%, and 60%, w/w) and γ-oryzanol (2%, w/w) are used in this study. Deacidification is carried out by mixing RBO with a certain level of FFAs and γ-oryzanol, a volume ratio of RBO: solvent (DES) = 1:2, and total extraction time in multiple LLE was 1200 min. (240 min./stage x 5 stage). It was obtained that the removal of FFAs in the single LLE are 10.04%, 23.28%, 13.43%, 17.55%, and 17.49%, respectively, while in the multiple LLE are 48.64%, 60.37%, 63.15%, 41.79%, respectively, for RBO with an initial FFAs content of 5%, 10%, 20%, 30%, 40% and 60%, respectively. The losses of γ-oryzanol in the single LLE are 48.32%, 61.26%, 73.32%, 74.22%, and 89.59%, respectively, while in the multiple LLE are 87.16%, 95.58%, 99.14%, 99.77%, and 99.77%, respectively, for RBO with an initial FFAs content of 5%, 10%, 20%, 30%, 40% and 60%, respectively. Deacidification of RBO using DES in the multiple LLE removed FFAs 3.63 times higher than that in the single LLE. However, the losses of γ-oryzanol increased in the multiple LLE which is 1.43 times higher than that in the single LLE.

1. Introduction

Rice bran is a by-product of the rice milling process and has not been used optimally. Currently, the use of rice bran is only as material for animal feed. However, rice bran can be processed into rice bran oil (RBO) that has many health benefits for humans, since it contains large amounts of varying nutraceuticals [1,2,3]. In general, crude RBO obtained by solvent extraction using hexane, and it has several impurities that are required to be removed before human consumption [3]. Further refining of the RBO involves degumming, neutralization, bleaching, dewaxing and deodorizing to produce quality cooking oil [1,2,3,4]. Degumming involves the removal of phospholipids and lipoprotein through hydration by acidic water. Neutralization is applied to remove free fatty acids (FFAs) through the saponification process of FFAs and caustic soda (NaOH). The bleaching step using activated carbon/bleaching earth can be adsorbed the pigments including chlorophylls and carotenoids. A dewaxing and winterization steps carried out by maintaining the oil at low and very low temperature respectively to cause the solidified waxes and other high melting point substances as well as high melting...
point wax. Deodorization is applied to vaporize the remaining FFAs and odoriferous compounds and carry this volatiles away from the feedstock using steam distillation in the process of high temperature and high vacuum conditions [3].

The increasing of RBO utilization due to it contains large amount of antioxidant compounds such as vitamin E (tocopherols and tocotrienols) and γ-oryzanol. However, crude RBO available in most Asian countries contains 40-50% of FFAs, which is not feasible for oil production [5,6]. Besides, the high value of FFAs can have a detrimental effect including a decrease in oil quality (taste, colour, and durability), and it causes refining loss when using inappropriate deacidification techniques. Therefore, to reduce the level of FFAs in crude RBO through appropriate deacidification process is an important step [7,8].

Deacidification of oils is industrially performed by chemical, physical or miscella methods. However, for oils with high acidity, chemical refining causes high losses of neutral oil due to saponification and emulsification. Crude oil with 5% of FFAs is processed by neutralization causes 12-40% losses of neutral oil [2,7]. The physical method is also a feasible process for deacidification of highly acidic oils since it results in a loss of neutral oil that is lower than the chemical method, but more energy is consumed [2,3]. Moreover, in some cases, the refined oil is subject to undesirable alteration in colour and a reduction of stability to oxidation. The miscella method has been applied to a variety of oils but commercially it is used almost exclusively for the refining of cottonseed oil. However, it requires high initial investments with explosion-proof equipment’s that limit the application of this method [3].

Other approaches for deacidification of oils have been proposed in kinds of literature are biological deacidification, chemical reesterification, supercritical fluid extraction, membrane processing, and solvent (or liquid-liquid) extraction. However, biological deacidification requires the high cost of the enzymes [7,9]. The high temperatures used in chemical reesterification, make chemical reesterification a costly process. The supercritical fluid extraction is an expensive process, and its use is only justified for deacidifying special oils and fats with high initial acidity, in which the quality and purity of the extracted components are of great importance. The membrane processing has some limitation due to the small difference between triacylglycerols and FFAs molecular weight that makes the separation process difficult and the non-availability of a membrane with high selectivity [3].

The liquid-liquid extraction (LLE) process has been receiving attention due to its advantages in comparison to the physical and chemical refining. As the process is carried out at room temperature and atmospheric pressure, less energy is consumed, the oil submitted to a softer treatment, and the loss of neutral oil can be minimized [1,2,4]. Moreover, the solvent used such as ionic liquid [10], deep eutectic solvent (DES) [8], and natural deep eutectic solvent [11,12] are some types of green solvent that has been characterized as safe, non-toxic, biodegradable, and non-flammable [13]. Besides, those solvents showed high deacidification efficiency and can be preserved nutraceuticals compounds in the oils. In the previous work, DES which results in high removal of FFAs is from a mixture of choline chloride and ethylene glycol with 1:2 molar ratios. However, FFAs removal was still low at only 19.03% using once-through LLE of DES from choline chloride-ethylene glycol with a molar ratio of 1:2 [8]. Therefore, in this study, the improvement of deacidification RBO using DES of choline chloride and ethylene glycol with a molar ratio of 1:2 at different initial FFAs level was carried out using single and multiple LLE.

2. Materials and Methods

2.1 Materials

Commercial rice bran oil (RBO), Oryza grace, was obtained from Kasisuri Co. Ltd., Thailand. γ-oryzanol was obtained from Wako Pure Chemicals Corporation, USA, and oleic acid (purity ≥ 98%) was obtained from Showa, Japan. Choline chloride and ethylene glycol were obtained from Sigma Aldrich, USA. The initial FFAs and γ-oryzanol contents in the commercial RBO are 0.23 ± 0.01% and 0.41%, respectively.
2.2 Liquid-Liquid Extraction Process Using DES
Oleic acid and γ-oryzanol are added into commercial RBO to make an initial FFAs and γ-oryzanol contents in the RBO at a certain value. RBO was placed in a stoppered glass and mixed with a deep eutectic solvent (DES) composed of chlorine chloride and ethylene glycol with a molar ratio of 1:2. DES was prepared according to [14]. In the multiple LLE, the ratio of DES and sample was 1:2 (v/v). Total DES used in the multiple LLE is the same used in the single LLE for the same amount of sample maintained at similar initial FFAs and γ-oryzanol contents. LLE was performed at 50°C and 300 rpm for 240 min./stage for multiple LLE and 1200 min. for single LLE. The oil layer was separated from the DES layer by mean a centrifuge with a speed of 3000 rpm for 10 minutes. The oil layer was analysed for their FFAs and γ-oryzanol contents. In the multiple LLE, the oil layer obtained from the previous stage was separated from the DES layer and added with fresh of DES using the same volume ratio of the oil and DES of 1:2.

2.3 Free Fatty Acids(FFAs) and γ-Oryzanol Analysis
FFAs were analysed using the titration method according to Rukunudin et al [15]. In general, samples were weighed according to the data on Table 1 and dissolved in ethanol solution. The mixture was heated and stirred to make it homogeneous, and added 3 drops of phenolphthalein indicator and titrated using sodium hydroxide (NaOH) solution until reached the endpoint. FFAs content, removal of FFAs and deacidification efficiency were calculated according to equation 1, 2, and 3, respectively.

\[
FFAs\ content\ (%) = \frac{\text{Vol} \text{ of NaOH (mL)} \times \text{Normality of NaOH} \times 28.2}{\text{weight of sample (g)}} \times 100\% \tag{1}
\]

\[
\text{Removal of FFAs}\ (%) = \frac{\text{initial weight of FFAs (g)} - \text{final weight of FFAs (g)}}{\text{initial weight of FFAs (g)}} \times 100\% \tag{2}
\]

\[
\text{Deacidification efficiency}\ (%) = \frac{\text{initial FFAs content (%) - final FFAs content (%)}}{\text{initial FFAs content (%)}} \times 100\% \tag{3}
\]

| FFA Range (%) | Weight of sample (g) | The volume of ethyl alcohol (mL) | Normality of NaOH (N) |
|---------------|----------------------|-------------------------------|-----------------------|
| 0.01 - 0.2    | 5.64                 | 5                             | 0.013                 |
| 0.2 - 1       | 2.82                 | 5                             | 0.013                 |
| 1 - 30        | 0.7                  | 7.5                           | 0.031                 |
| 30 - 50       | 0.7                  | 10                            | 0.13                  |
| 50 - 100      | 0.35                 | 10                            | 0.125                 |

γ-oryzanol content was analysed quantitatively using a UV-Vis Spectrophotometer Genesys type 10uv scanning according to [16]. The calibration curve was obtained from a standard solution of γ-oryzanol with a concentration of 1-30 ppm at a wavelength of 311 nm. In general, the sample was dissolved in n-hexane and a homogeneous solution was put in a 1-cm quartz cell cuvette and operated in bandwidth = 1 nm and data pitch = 1 nm. The γ-oryzanol content in the sample, losses of γ-oryzanol, recovery of γ-oryzanol, and deoryzanolification efficiency were calculated according to eq. 4, 5, 6, and 7, respectively.

\[
\text{Oryzanol content}\ (%) = \frac{\text{weight of oryzanol in sample (g)}}{\text{weight of sample (g)}} \times 100\% \tag{4}
\]

\[
\text{Losses of oryzanol}\ (%) = \frac{\text{initial weight of oryzanol (g)} - \text{final weight of oryzanol (g)}}{\text{initial weight of oryzanol (g)}} \times 100\% \tag{5}
\]
Recovery of oryzanol (%) = \frac{\text{final weight of oryzanol (g)}}{\text{initial weight of oryzanol (g)}} \times 100 \% \quad (6)

Deoryzanolification efficiency (%) = \frac{\text{initial oryzanol content (%)} - \text{final oryzanol content (%)}}{\text{initial oryzanol content (%)}} \times 100 \% \quad (7)

3. Results and Discussions

3.1 Effect of Initial FFAs Content in Multiple Liquid-Liquid Extractions (LLE)
Various refining methods have been investigated for preserving the \( \gamma \)-oryzanol content in rice bran oil (RBO) while deacidifying it. The use of liquid-liquid extraction (LLE) for deacidification is a feasible alternative to achieve this goal because LLE can be performed at ambient temperature and atmospheric pressure, and probably minimized the losses of \( \gamma \)-oryzanol. The process of LLE, when carried with the total amount of the single solvent in a single operation, is referred to as simple/single LLE. To recover the maximum amount of the substance from a solution, the extraction is made in two or more successive operation using small portions of the solvent provided. This is called multiple LLE or multi-step LLE.

**Figure 1.** The removal of FFAs and the losses of \( \gamma \)-oryzanol in multiple liquid-liquid extractions (LLE) with initial FFAs content of 5%.

**Figure 2.** The removal of FFAs and the losses of \( \gamma \)-oryzanol in multiple liquid-liquid extractions (LLE) with initial FFAs content of 10%.

**Figure 3.** The removal of FFAs and the losses of \( \gamma \)-oryzanol in multiple liquid-liquid extractions (LLE) with initial FFAs content of 30%.

**Figure 4.** The removal of FFAs and the losses of \( \gamma \)-oryzanol in multiple liquid-liquid extractions (LLE) with initial FFAs content of 40%.
Effect of different initial free fatty acids (FFAs) content in rice bran oil (RBO) with a fixed amount of γ-oryzanol on the removal of FFAs and the losses of γ-oryzanol showed in Figures 1 - 5. In general, the removal of FFAs is affected by initial FFAs contents, since the average of the distribution coefficient of FFAs (concentration of FFAs in DES layer/concentration of FFAs in oil layer) at low (5%) and very high (60%), initial FFAs contents are half than those with medium initial FFAs contents (10% -40%). It means that the removals of FFAs in RBO with low and very high FFAs contents are more difficult (the removal of FFAs by 10% for each stage) compare to the removal of FFAs in RBO with medium FFAs contents (the removal of FFAs by 17% for each stage).

The loss of γ-oryzanol is unavoidable and it was affected by initial FFAs contents. The average of the distribution coefficient of γ-oryzanol (concentration of γ-oryzanol in DES layer/concentration of γ-oryzanol in oil layer) increased with increasing of FFAs content in RBO. Therefore, the losses of γ-oryzanol increased with increasing FFAs content in RBO. Even though this solvent cannot preserve γ-oryzanol in the refined RBO, separation of γ-oryzanol from this solvent was quite easy.

### 3.2 The Comparison between Single and Multiple Liquid-Liquid Extractions (LLE)

The comparison between single and multiple liquid-liquid extractions (LLE) on deacidification and deoryzanolification efficiencies can be seen in Figures 6-8. In the single and multiple LLE, deacidification and deoryzanolification efficiencies were affected by initial FFAs contents in RBO. In general, deacidification efficiency was effective in multiple LLE compared to single LLE. However, deoryzanolification efficiency was also higher in multiple LLE.
4. Conclusions

Excessive losses of neutral oil occur in the alkali refining especially for oil with high acidity like rice bran oil, can be minimized by multiple LLE using choline chloride-ethylene glycol based deep eutectic solvent. Deacidification efficiency can be increased almost 4 times higher compare to single LLE. However, choline chloride-ethylene glycol based deep eutectic solvent more selective to γ-oryzanol than that of oleic acid.

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