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ORIGINAL PAPER

Effects of Crude Rice Bran Oil Components on Alkali-Refining Loss

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Abstract The effects of minor components in crude rice bran oil (RBO) including free fatty acids (FFA), rice bran wax (RBW), γ-oryzanol, and long-chain fatty alcohols (LCFA), on alkali refining losses were determined. Refined palm oil (PO), soybean oil (SBO) and sunflower oil (SFO) were used as oil models to which minor component present in RBO were added. Refining losses of all model oils were linearly related to the amount of FFA incorporated. At 6.8% FFA, the refining losses of all the model oils were between 13.16 and 13.42%. When <1.0% of LCFA, RBW and γ-oryzanol were added to the model oils (with 6.8% FFA), the refining losses were approximately the same, however, with higher amounts of LCFA greatly increased refining losses. At 3% LCFA, the refining losses of all the model oils were as high as 69.43–78.75%, whereas the losses of oils containing 3% RBW and γ-oryzanol were 33.46–45.01% and 17.82–20.45%, respectively.

Keywords Alkali refining · Free fatty acid · Long-chain fatty alcohols · Oryzanol · Refining losses · Rice bran oil · Rice bran wax

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Introduction

Depending upon the quality of the bran, crude rice bran oil (RBO) contains from 3 to 20% FFA, ~4.8% wax, and 5–8% unsaponifiable constituents (e.g. γ-oryzanol and tocopherol), phospholipids, glycolipids and pigments which need to be removed to convert it into edible oil [1]. High-acid crude RBO tends to lose a greater amount of neutral oil in the neutralization step. A significant amount of RBO is entrapped in the soap micelles that form in this step. When the soap is removed by centrifuging, entrapped oil is also removed with the soap [2]. The losses in chemical refining are 2.5–3 times the FFA content of the oil [3]. Refining losses of high-FFA crude oils can be as much as 50% or more. Alternative methods including solvent refining [4, 5], re-esterifying combined with conventional alkali neutralizing [6], and physical refining [7] have been investigated for deacidification of RBO in order to minimize losses of neutral oil and nutraceuticals as well as eliminate soap formation, however, there are still many drawbacks to these procedures. Alkali neutralization has been the only practical method for RBO refining. Some minor constituents other than FFA, such as wax and γ-oryzanol, substantially increase refining losses in RBO while phosphatides and mono- and diglycerides have no noticeable effects [8]. Mounts [9], on the other hand, reported that higher refining losses occur when phosphatide is incorporated into the oil.

We speculated that γ-oryzanol and wax promote higher entrapment of neutral oil in the soapstock due to their distribution between the neutral oil phase and the water phase. Waxes tend to form stable emulsions during oil refining and thus reduce oil yields during processing [10]. Mishra et al. [8] showed that refining loss in RBO was much greater than for peanut oil having the same FFA content (6.8%). When γ-oryzanol or RBW were added to

peanut oil having 6.8% FFA, refining loss greatly increased. Thus, the increase in refining loss of peanut oil was ascribed to the wax or oryzanol added.

Waxes are esters of long-chain fatty acids and long-chain alcohols. In addition to wax esters, plant waxes contain a variety of very hydrophobic compounds e.g. very-long-chain hydrocarbons, non-esterified fatty alcohols, aldehydes, and FFA. Rice wax contains about 15% alkanes, 35% esters, 10% aldehydes, and 40% long chain primary alcohols [11]. Only trace amounts of FFA were found. Because the solid fraction of crude rice bran oil contains fatty alcohols (3.2%) [12], it is interesting to differentiate the effects of LCFA and wax esters on RBO refining loss.

Thus, in the present study, refined PO, SBO and SFO were used as model oil systems because these oils contain no γ-oryzanol, LCFA and wax. On the other hand, refined RBO contains both γ-oryzanol and minor amounts of wax and alcohol. Thus, the logic of using PO, SBO and SFO as the model oils for refining loss study is very similar to the study of Misha et al. [8].

Materials and Methods

Refined RBO, PO, SBO, and SFO were obtained from a local supermarket (Bangkok, Thailand). γ-Oryzanol was obtained from Tsuno Rice Fine Chemicals Co., Ltd. (Wakayama, Japan) and was greater than 99% purity (Fig. 1a). Crude RBW was the gift of Thai Edible Oil Co., Ltd. (Bangkok, Thailand). The crude wax was purified by washing two times with hot hexane and one time with isopropanol to remove triglycerides, FFA, and other lipid constituents [13] and then dried in a hot air oven (≤60 °C) before use. Purity was about 98% (Fig. 1b). FFA was prepared by saponification of refined RBO with 80% ethanolic NaOH (1 N). Neutral impurities were extracted with hexane and water. The sodium soap in the aqueous layer was acidified and the FFA was extracted with toluene and evaporated to dryness. The purity of FFA (98+%; Fig. 1c) was determined by size-exclusion HPLC [14]. Other reagents were analytical grade.

Preparation of Long-Chain Fatty Alcohol (LCFA)

LCFA was prepared from washed RBW by saponification with 90% ethanolic KOH (1 N) and solvent extraction. The purity of LCFA determined by size-exclusion HPLC [14] was 99+% (Fig. 1d).

Model Oils for Evaluating the Effect of FFA on Refining Losses

Refined PO, SBO, and SFO with endogenous FFA of <0.05% were adjusted with RBO FFA to 2, 5, 6.8, 10, 15 and 20%. The endogenous content of FFA in the oils were determined by titrating according to the standard method [15]. The oils were used to evaluate the effect of FFA on refining losses as described below.

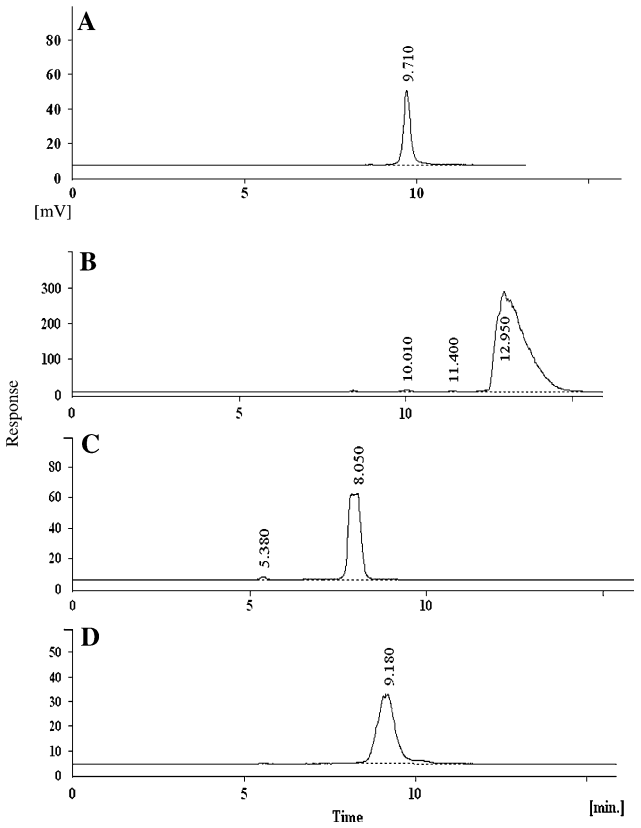

Fig. 1 Size-exclusion HPLC chromatograms of **a** γ-oryzanol, **b** RBO FFA, **c** partially extracted RBW, and **d** LCFA from RBW

Model Oils for Evaluating the Effects of Minor Components on Refining Losses

Refined PO, SBO, and SFO with <0.05% endogenous FFA were adjusted with RBO FFA to 6.8% (the same FFA percentage reported by Mishra et al. [8]). The refining losses of all the model oils with 6.8% FFA were about 13%. RBO constituents (LCFA, RBW or γ-oryzanol) of 0.1–3% were added individually to the model oils and the refining losses were evaluated as described in the next section.

Procedure for Evaluating Refining Losses

The conventional centrifuging method was used to evaluate refining loss. The procedure was followed as described by Mishra et al. [8] with slight modification. An oil sample of 5 g and the calculated quantity of 20 Be′ NaOH as described in the standard AOCS cup method [16] were mixed in a glass tube (10 × 100 mm). The mixture was

gently vortexed for 3 min at ambient temperature. The tube was then heated at 65 °C for 7 min in a water bath, cooled under running water and centrifuged at 1,409×g for 5 min. The oil was pipetted into a new vessel and weighed. The refining loss was determined from the weight of neutral oil.

When the effects of RBO constituents on refining losses were assessed, the following correction was used for the component added:

$$\text{Refining loss corrected for additives} = \frac{(\%\,\text{refining loss} - \%\,\text{additive}) \times 100}{(100 - \%\,\text{additive})}$$

High-Performance Liquid Chromatography (HPLC)

The HPLC system consisted of a Waters model 510 pump system (Waters Associate, Milford, MA01757, USA.) equipped with a Rheodyne model 7125 six-port injector (Cotati, CA, USA) coupled to an evaporative light scattering detector (ELSD) model 55 from SEDEX (Sedere, Alfortville, France). A Phenogel column (300 × 7.8 mm i.d., 100Å) purchased from Phenomenex (Phenomenex, Inc., Torrance, CA) was used to test the purity of FFA, RBW, γ-oryzanol and LCFA. Samples and references were prepared in toluene and analyzed on the Phenogel column with 0.25% acetic acid in toluene (v/v) as the mobile phase at 1 ml/min flow rate [14]. The ELSD drift tube was set at 30 °C and the N_2 flow through the nebulizer was set at two bar. Peaks were identified by comparison with reference standards.

Statistical Analysis

All experiments were carried out in triplicate. The experimental data were analyzed by Microsoft Excel Version 8.0 and one-way ANOVA on data analysis and graphing software of Origin Version 8.0 (Origin Lab Corporation, MA, USA). A significance level of 5% was used.

Results and Discussion

Effect of FFA Contents on Refining Loss

Higher contents of FFA in crude oil cause higher neutral oil losses in the deacidification step of refinery [8]. Relationships between FFA contents and refining losses of different oil models were relatively linear as shown in Fig. 2. The coefficient of variance (R^2) between FFA contents and % refining losses of PO, SBO, and SFO were 0.9754, 0.9889, and 0.9769, respectively (Table 1). Similar trends of refining losses were observed in all of the model oils. FFA exerted a similar effect on the refining losses of the oil ($p > 0.05$) and had nothing to do with the different types of

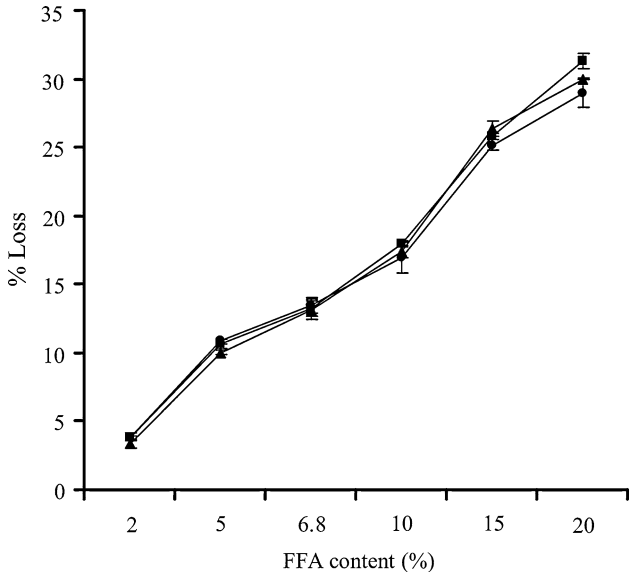

Fig. 2 Effects of RBO FFA on the refining loss of PO (*filled circle*), SBO (*filled square*), and SFO (*filled triangle*). Refining loss (%) = mean ± SD ($n = 3$)

the oils. Refining losses were increased from 3.31–3.87 to 28.94–31.30% by increasing FFA in the oils from 2 to 20%. The effect of FFA on refining losses may be ascribed to the increase in anionic micellar aggregates of sodium salt of FFA in aqueous medium [2]. Thus, the amount of entrapped oil increased as the concentration of the micelle increased. The average refining losses for the three model oils incorporated with 6.8 and 10% FFA were slightly lower (13.2–13.4%) and (16.9–17.9%) than those reported by Mishra et al. [8] who used peanut oil as the model oil (15.5 and 19.2%, respectively).

Effect of Wax on Refining Loss

Model oils containing 6.8% FFA (initial losses 13.16–13.42%) were used in this study. Below 1.0% of RBW, refining loss increased slowly as the amount of RBW increased (Fig. 3) and similar effects were observed for the refining losses of all model oils. At higher RBW content (>1.0%), the losses in the PO model deviate from the other two oils. The losses in the PO model sharply increased ($p < 0.05$) to 35 and 45% when 2.0 and 3.0% RBW were added. The losses were 3.00 and 3.35 times the initial loss (no wax added). The losses in SBO and SFO increased much slower than in PO at higher RBW concentration. Only 2.5 and 2.7 times of the initial losses were observed at 3% wax for SBO and SFO, respectively. However, the losses were slightly higher than those reported by Mishra et al. [8] for peanut oil where the loss was only twice as much at 3% wax content. Wax tends to form o/w emulsions and reduce process yield [10]. The emulsion formed from different oils

Table 1 Comparison of refining losses for different oil models with different RBO FFA contents

FFA added (%)	Refining losses (%)[a]		
	Palm oil	Soybean oil	Sunflower oil
2.0	3.81 ± 0.10	3.87 ± 0.28	3.31 ± 0.29
5.0	10.83 ± 0.15	10.64 ± 0.34	10.03 ± 0.20
6.8	13.42 ± 0.57	13.26 ± 0.59	13.16 ± 0.75
10.0	16.94 ± 1.12	17.94 ± 0.27	17.39 ± 0.42
15.0	25.17 ± 0.39	25.82 ± 0.26	26.38 ± 0.58
20.0	28.94 ± 1.02	31.30 ± 0.57	30.00 ± 0.01
Relationship between refining losses and FFA contents	$y = 1.3728x + 3.0653$	$y = 1.5003x + 2.4351$	$y = 1.4876x + 2.1336$
R^2	0.9754	0.9889	0.9769

[a] Refining loss (%) = mean ± SD ($n = 3$)

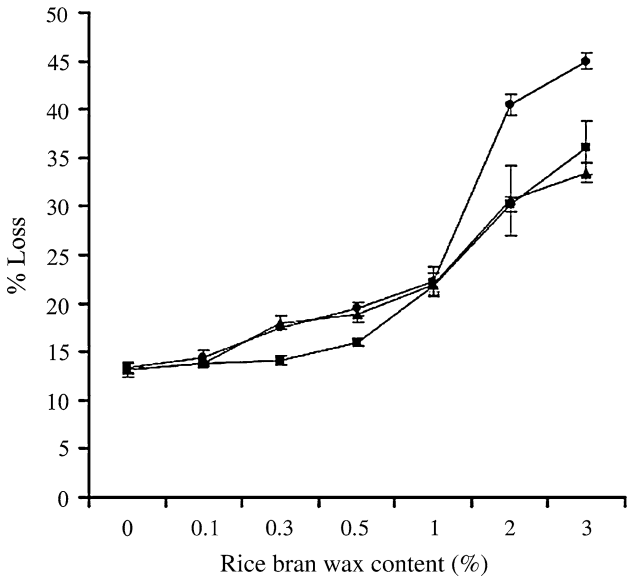

Fig. 3 Effects of RBW on refining losses of PO (*filled circles*), SBO (*filled squares*), and SFO (*filled triangles*). Refining loss (%) = mean ± SD ($n = 3$)

might be different. Therefore, process yield would be different for different oils. Also, Gopala-Krishna et al. [17] reported that wax significantly increases the viscosity of RBO, and refining loss was directly dependent on the viscosity of the oil. Thus, the differences in refining losses between PO and other model oils at high wax contents may be due to differences in the oil viscosities and ability to form emulsions. Viscosity at 40 °C of refined PO was 40 mm^2/s, which was higher than SBO (31 mm^2/s) and SFO (33 mm^2/s) [18]. In addition, the viscosity of RBO (42.2 mm^2/s) [19] was higher than for PO. These results confirm the adverse effect of wax on refining losses and indicate the importance of dewaxing step prior to neutralization step. Mezouari et al. [12] reported that pre-dewaxing reduced the FFA content of the oil by ~45% and significantly lowered refining losses due to FFA and wax. However, pre-dewaxing reduced the minor components, resulting in decreased oxidative and thermo-oxidative stabilities of RBO.

Effect of γ-Oryzanol on Refining Loss

Normally the γ-oryzanol content of RBO varies within the range of 1.1–2.6% [2]. Large amounts (~78%) of γ-oryzanol were removed in the neutralization step of RBO refining [20]. This compound is hydrophobic and can associate with the anionic micellar aggregation, that occurs from the sodium salts of fatty acids (anionic surfactants) in aqueous medium [2]. Figure 4 shows that the refining loss of PO was significantly higher than other oils ($p < 0.05$) at <2% γ-oryzanol. The refining loss of PO approached those of SBO and SFO at 2 and 3% γ-oryzanol, respectively. Gopala-Krishna [17] reported that γ-oryzanol did not affect the viscosity of RBO but monoglyceride and RBW had a synergistic effect on RBO viscosity. γ-Oryzanol, on the other hand, reduced the synergistic effect of RBO containing RBW and monoglyceride [17].

Effect of Long-Chain Fatty Alcohol on Refining Loss

RBW caused substantial loss in RBO refining and RBW contained a high content of fatty alcohols of C_{24}–C_{38} carbon chain length [21]. Although, the effect of LCFA on refining loss has not been reported, it is necessary to carefully differentiate the effect RBW on refining loss, whether the loss arises from the esters or the free alcohols. Figure 5 shows that refining losses substantially increased in oil to which LCFA were incorporated and losses of different model oils were significant different ($p < 0.05$). Greater losses were observed for oils containing LCFA than oils containing RBW. The losses in oils with 3% LCFA were 69.43–78.75%, whereas the oils containing 3% RBW lost up to only 33.46–45.01%. The differences may be the results of two important properties of LCFA: (1) its good stabilization property [22] and (2) its high viscosity. Gopala-Krishna et al. [17] reported that wax was the only

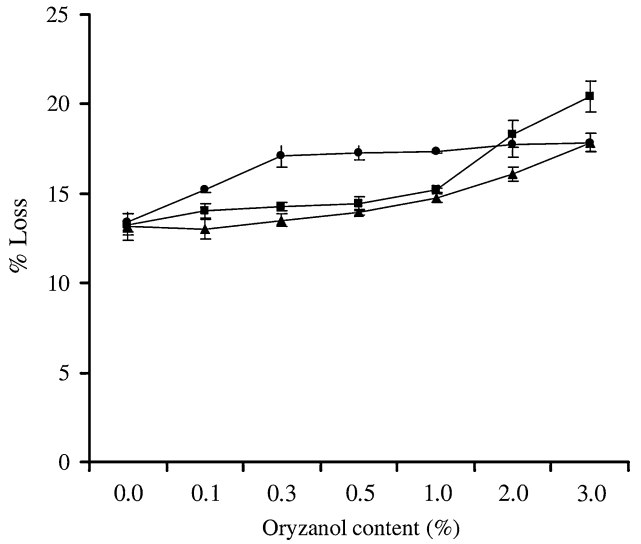

Fig. 4 Effects of γ-oryzanol on refining losses of PO (*filled circles*), SBO (*filled squares*), and SFO (*filled triangles*). Refining loss (%) = mean ± SD ($n = 3$)

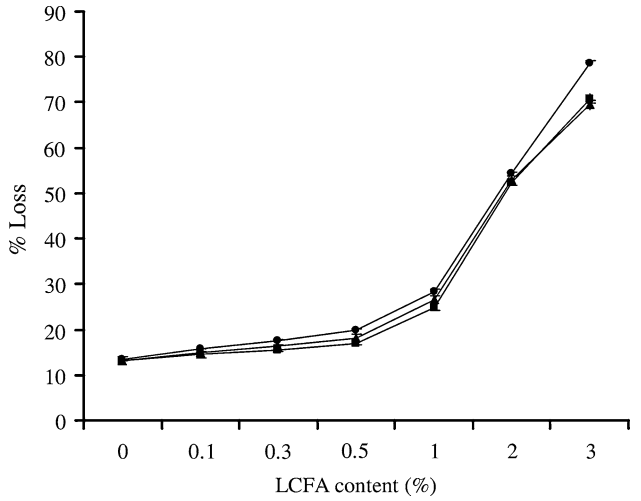

Fig. 5 Effects of LCFA on refining losses of PO (*filled circle*), SBO (*filled square*), and SFO (*filled triangle*). Refining loss (%) = mean ± SD ($n = 3$)

constituent of RBO that significantly increased the viscosity (81.5%) of the oil, however, LCFA was not taken into account. We observed that medium-chain fatty alcohols (C_{14}–C_{20}) did not affect the refining loss of the PO model (data not shown). In general, viscosity increased by increasing the number of carbon atoms in the molecule and decreased with increasing temperature.

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