A Study of Rice Bran Oil Refining

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ABSTRACT

Examination of a number of rice bran oils revealed the presence of monoglycerides (0.5-1.4%) and other hydroxylated compounds such as diglycerides and glucosides. The hydroxyl numbers of the samples ranged from 8.5 to 27, depending on their acidity. On the assumption that the inordinately high refining losses of rice bran oil are due, along with the acidity, to the presence of hydroxylated compounds, the hydroxyl numbers of several samples of that oil were reduced by progressive acetylation with acetic anhydride. This was accompanied by gradual reduction of the refining losses, which seems to support the above mentioned assumption.

INTRODUCTION

The refining of rice bran oil with aqueous sodium hydroxide solutions is accompanied by losses considerably greater than those encountered in many other vegetable oils with similar free fatty acid (FFA) content. This behavior has been the subject of numerous investigations (1-4), but in a recent monograph (5) one can still find the statement that the reason for the inordinately high refining losses of rice bran oil is not completely understood. An earlier article by Cousins et al. (6) contains the following observation:

> When normal refining procedures are employed, the foots formed from crude rice oils are unusual in the inability to cohere and settle out of the oil clearly.... The peculiar behavior of rice oil foots has been attributed to some unknown material in the crude oil that tends to emulsify the oil under the conditions of refining. Light non-settling foots are thus formed.

The same authors stated that the work in their laboratory, the Southern Regional Research Center, was directed towards isolating and defining the unknown material in question and announced a forthcoming report on this subject. However, a search of the literature did not reveal the existence of such a report. In a more recent publication by Ramos (7), the high refining losses of rice bran oil were attributed to the presence of saponins but without any experimental evidence to support this statement.

Many suggestions have been made in the past to reduce refining losses, but these studies, performed mainly in the U.S. and Japan, have been of an empirical character without entering into the reasons for the losses. The present investigation has been undertaken to characterize the compounds responsible for the difficulties, in the belief that this would help to overcome at least some of them.

In planning this work, we assumed that the compounds responsible for the high refining losses could be inherent components of brown rice but could also develop during the milling and storing of rice bran. The pronounced enzymatic activity of the rice bran, which causes a rapid lipolysis of the oil in the first few hr following the rice polishing process, suggested the formation of partial glycerides. It therefore appeared advisable to test the rice bran oil for the presence of monoglycerides (MGs) whose emulsifying properties are well known and to look further for glycosides and other potential emulsifiers.

MATERIALS AND METHODS

Samples of crude rice bran oils were obtained from local manufacturers and denoted C.1-C.4. Several lots of brown rice and rice bran were extracted in the laboratory after various storage times to obtain oils with FFA contents ranging from <2% to 76% and denoted L.1–L.8.

Determination of the characteristics of the oils, such as acidity, hydroxyl number, and unsaponifiable matter, were made following official AOCS methods (8-10). The results for 1-monoglycerides obtained by AOCS Method Cd 11-57(11) appeared exceedingly high; consequently we applied a recently published method for determining MGs present at low levels (enrichment of MGs, silylation, and gas chromatography) (12) modified by the purification of the silylated compounds (13).

The glyceride composition was investigated by thin layer chromatography (TLC) using silica gel G and developing the chromatograms with an ethyl ether:hexane mixture 30:70 (14).

Testing for glycosides was done by refluxing 40 g oil samples with 40 ml of 2 n HCl with stirring for 1 hr. The presence of reducing sugars in the aqueous layer was verified by a positive Molisch test and their amount determined by the Fehling method (15).

Refining losses were determined by adding to 30 g of oil a 17% (w/w) aqueous solution of NaOH in 20% excess over the theoretical amount and heating to 65 C with stirring. The mixture was centrifuged 5 min in a Damon IEC UV centrifuge at 3000 rpm and the neutralized oil decanted at 65 C and weighed.

RESULTS AND DISCUSSION

Mono- and Diglycerides

The presence of partial glycerides in the rice bran oils was established by TLC. Figure 1 shows a typical chromatogram in which the location of the components was verified by standard compounds such as pure palmitic acid and its mono-, di-, and triglycerides.

The quantitative estimation of MGs presented some difficulty owing to the small amounts involved. Their determination by periodic acid proved impractical inasmuch as periodic acid may react with hydroxylated compounds other than MGs (16) and even with unsaturated acids (17). When the quantity of MGs present is small, the relative

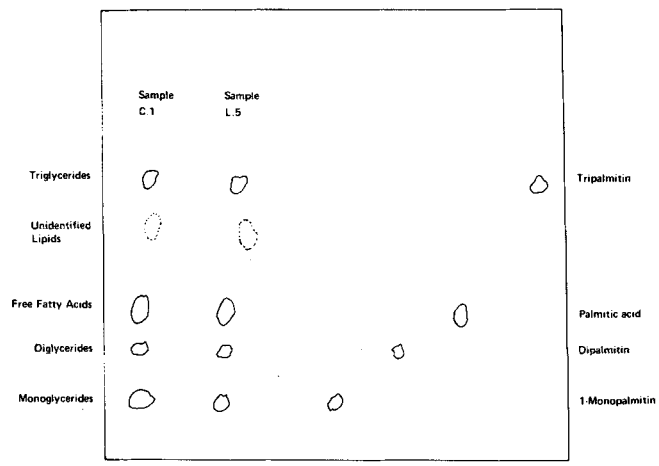

FIG. 1. Thin layer chromatogram of rice oils and of palmitic acid and its glycerides. Sample C.1 is commercial oil; sample L.5 was extracted from rice bran 2 days after milling.

TABLE I

Free Fatty Acid, Monoglycerides, and Hydroxyl Numbers of Various Rice Oils

Sample	FFA as oleic (%)	Monoglycerides (%)	Hydroxyl number (mg KOH/g)
L.1 (extracted from fresh, ground brown rice)	1.73	0.46	8.44
L.2 (extracted from rice bran 1 hr after milling)	3.65	0.62	12.32
L.3 (extracted from rice bran 2 hr after milling)	4.41	0.71	13.66
L.4 (extracted from ground brown rice)	5.64	0.60	10.17
L.5 (extracted from rice bran 2 days after milling)	7.89	0.84	16.40
C.1 (commercial oil)	8.63	1.14	21.12
C.2 (commercial oil)	11.02	1.28	23.27
C.3 (commercial oil)	12.84	1.41	24.22
C.4 (commercial oil)	18.16	1.35	27.15
L.6 (extracted from rice bran 10 days after milling)	21.74	1.38	23.41
L.7 (extracted from rice bran 22 days after milling)	35.36	1.24	22.73
L.8 (extracted from rice bran 90 days after milling)	76.35	1.05	15.92

TABLE II

Reducing Sugars Found After Acid Treatment of Rice Bran Oils

Sample	Reducing sugars as glucose (%)
C.1 (commercial oil)	0.031
C.2 (commercial oil)	0.019
C.3 (commercial oil)	0.025
L.2 (extracted from rice bran 1 hr after milling)	0.034
L.3 (extracted from rice bran 2 hr after milling)	0.044
L.5 (extracted from rice bran 2 days after milling)	0.038

error may be considerable. Accordingly, we resorted to enrichment of MGs by extraction with acetonitrile, followed by silylation and gas chromatographic analysis (12,13). The amount of diglycerides could be calculated with some approximation from hydroxyl numbers, but, in view of the small emulsifying power of these compounds, their exact determination appeared of secondary importance. On the other hand, the hydroxyl numbers proved very useful, as will be shown subsequently.

Table I shows the MG contents and hydroxyl numbers of oils arranged according to their acidity. Practically all samples examined showed a presence of MGs in quantities which could have a bearing on the refining losses. It may be seen that even the sample L.1 with 1.73% of FFA, whose preparation required meticulous precautions, shows 0.46% MG. This table also demonstrates that the MG contents and hydroxyl numbers increase with rising acidity, but only to a certain point, and subsequently diminish. Thus, an oil with 76% FFA shows a lower MG content and hydroxyl number than an oil with 35% FFA. This should have been accompanied by the liberation of glycerol, but no free glycerol could be detected in any of the rice bran samples stored, probably because of the intense enzymatic activity which leads to the formation of volatile compounds, including CO_2, from the liberated glycerol.

Glycosides and Other Hydroxylated Compounds

The presence of glycosides was established by acid hydrolysis of several oils samples and determination of reducing sugars combined with a positive Molisch test.

Table II shows the quantities of the sugars calculated as glucose found in various commercial and laboratory prepared oils.

Free fatty alcohols (7) may also contribute to the hydroxyl number of rice bran oil. The unsaponifiable matter of this oil contains, besides fatty alcohols, other hydroxylated compounds whose identification has not been attempted in the present work.

Effect of Hydroxylated Compounds on Refining Losses

The presence of MGs in crude rice bran oils and the

TABLE III

Extraction of Rice Bran with 1:1 Mixture of Hexane: 90% Aqueous Ethanol

Sample	Hexane phase[a]				Ethanol phase	
	Amount of oil (g)	FFA[b] (%)	Refining loss (%)	Total[c] loss	Amount of oil (g)	FFA (%)
Rice bran Extraction with hexane: ethanol 1:1	55.4	9.73	22.6	26.0	3.6	55.61
Extraction with hexane only	61.5	12.95	28.6			
Crude oil C.2[d] partitioned between hexane and 90% ethanol	55.0	7.54	19.0	22.1	4.5	41.31

[a]Concentration of oil ca. 25% v/v.

[b]FFA = free fatty acid.

[c]Total loss = refining loss + acid oil in the ethanol phase.

[d]FFA of the original oil = 11.2; refining loss = 23.6%; C.2 is commercial oil.

TABLE IV

Refining Losses of Oils Treated with Various Amounts of Acetic Anhydride

Sample[b]	Addition of acetic anhydride (%)	FFA[a] (%)	Hydroxyl number	Refining loss (%)
C.2	0	11.02	23.27	23.6
C.2	1	9.12	22.82	22.0
C.2	2	8.90	17.63	18.0
C.2	3	7.50	10.16	16.3
C.2	4	7.12	7.25	14.8
C.2	5	6.75	2.99	12.3
L.2	0	3.65	12.32	16.8
L.2	1	3.48	6.24	12.3
L.3	2	3.36	1.77	8.2
L.3	0	4.41	13.66	18.5
L.3	2	3.84	1.98	8.3

[a]FFA = free fatty acid.

[b]C.2 is commercial oil; L.2 and L.3 were extracted from rice bran 1 hr and 2 hr, respectively, after milling.

comparatively high hydroxyl numbers of these oils suggested that hydroxylated compounds, along with FFAs, can be responsible for the elevated refining losses. To test this assumption, experiments were conducted to reduce the amount of MGs and other hydroxylated compounds and to examine the effect of such treatment. Initial experiments consisted of (a) partition of crude oils between hexane and 90% aqueous ethanol, and (b) heating of bleached oils at 180-220 C in vacuo for 2-4 hr in the presence of catalysts such as stannous chloride to induce re-esterification of FFAs by the partial glycerides present. The treated and untreated oils were subsequently refined using the procedure described in "Materials and Methods," whose conditions–such as concentration of the NaOH solution, temperature, and the means of separating the soapstock–were chosen somewhat arbitrarily. Reproducibility of results was ± 1% and thus inferior to that of the AOCS Cup refining method (11) but the procedure required 30 g instead of 500 g and the centrifugal separation of the soapstock was more in line with industrial practice. The refining losses thus obtained were much lower than those of the Cup method and probably lower than industrial losses but made it possible to draw conclusions about the treatments employed. Partition between hexane and aqueous ethanol and re-esterification both led to a substantial reduction of FFAs. However, the difference between the refining losses of treated and untreated oils was rather small, as may be seen in Table III, which shows some results obtained with the partition between the two solvents mentioned. Although the refining losses of oils after partitioning were lower than those of the untreated oils, the additional loss represented by the acid oil in the ethanolic phase reduced considerably the margin between the two yields. Results of re-esterification were equally disappointing. Thus, the refining loss of the oil sample C.2 of 23.6% FFA was reduced after re-esterification by only 1.1%.

Much more effective was the acetylation of oils with acetic anhydride. The treatment consisted of refluxing the oils with various quantities of acetic anhydride at 120-125 C with stirring during 1 hr, followed by heating for 1 more hr in vacuo to remove the residual anhydride and the acetic acid formed. The original and acetylated oils were neutralized with aqueous NaOH as previously described. Results appear in Table IV and show that the progressive reduction of hydroxyl numbers was accompanied by gradual reduction of refining losses to a level comparable to that observed in other vegetable oils. The treatment with acetic anhydride resulted in all cases in decreased acidity. This can be explained by the formation of a certain amount of fatty acid anhydrides during the treatment, which reacted with mono- and diglycerides present. However, as can be seen from the oils L.2 and L.3, it was the reduction of the hydroxyl number and not the decrease of acidity which contributed to the diminishing of the refining loss. This seems to confirm the supposition that the hydroxylated compounds are mainly responsible for the exorbitant refining losses of the rice bran oils.

Apparently, therefore, a means of diminishing the refining losses of rice bran oil, besides keeping its acidity as low as possible, would be a substantial reduction of its hydroxyl number. The results of the present work show that treatment with acetic anhydride could be one of the methods to achieve this aim. Incidentally, the use of acetic anhydride is not unknown in the edible oil industry. It has been applied with good results in the degumming of soybean oil (18). However, the price of acetic anhydride would limit its use to oils with a low hydroxyl number, which would reduce the amount of the reagent needed to 1-2%.

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