dized to the corresponding acids with chromic oxide in glacial acetic acid medium. The resulting crude acid mixtures were purified via the barium salt route, after which molecular weights and B spacings were determined as described above.

TABLE II

Analysis of Candelilla Wax Alcohols

Alcohols			Acids		
Fraction	Weight g.	Boiling point range (0.1 mm. Hg.) °C.	Molecular weight	B Spacing Å	Composition C_n
1	1.1	198-207	439	70.9	28+30+32
2	1.7	210-215	458	76.3	28+30+32
3	1.8	215-224	469	76.3	30+32+34
4	4.8	224-230	472	76.3	30+32+34
5	3.1	230-237	470	76.3	30+32+34
6	2.6	240-243	477	80.5	30+32+34
7	2.1	245-260	488	80.5	30+32+34
Residue	3.0	.....	486	80.5	30+32+34
Total	20.2				

Search for a Lactone. The wax was repeatedly extracted with 95% ethyl alcohol until a residue (m.p. 89-90°) was obtained. Under similar conditions Meyer and Soyka (6) obtained a product which melted at 88°C. The residue obtained in this study when saponified with an ethylene glycol solution of potassium hydroxide yielded two fractions, one of which was acidic, the other alcoholic. Both proved to be identical with the alcohol and acid fractions isolated from this wax by other methods and identified by X-ray analysis. The "lactone" therefore was in reality a mixture of wax esters and free fatty acids.

Discussion of Results

The generalizations made by the Piper-Chibnall group (7) were used to interpret the X-ray data. Pertinent to the problem in hand are the following: The B spacing of the highest melting acid in a ternary mixture will appear alone if this acid constitutes 20% or more of the whole. If however the longest chain constitutes 10% or less of the whole the spacing will be near that of the intermediate chain. The molecular weight alone shows that Fractions 1 and 2 (Table II) contain the C_{28} acid. That the C_{32} acid comprises more than 20% of these fractions is evidenced by the appearance of its spacing, which is 76.3 for the pure compound. The remaining fractions contain the C_{30}, C_{32}, and C_{34} acids, the spacing depending upon the quantity of the latter.

Summary

A method has been described for separating the constituents of candelilla wax into three fractions, viz., paraffins, acids, and alcohols. To the list of acids and alcohols reported by others as constituents of this wax may now be added the C_{28} individuals. The presence of the C_{30}, C_{32}, and C_{34} homologues of both compounds has been confirmed. All are of the *n*-type. Not confirmed however was the alleged presence of a lactone.

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Solvent Extraction of Rice Bran. Production of B-Vitamin Concentrate and Oil by Isopropanol Extraction[1,2]

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ABSTRACT

Freshly milled rice bran was extracted with hot 91 and 95% isopropanol to obtain the oil, sugars, and a considerable portion of the B-complex vitamins. After concentration of the micella a sugar-syrup phase separated from the oil. This syrup phase contained most of the extracted vitamins. Yields of oil and syrup were observed and vitamin assays made on the syrup and on the bran before and after extraction. The vitamins measured were biotin, folic acid, riboflavin, pantothenic acid, pyridoxine, thiamin, niacin, and inositol.

Introduction

RICE bran is a valuable by-product of the rice milling industry which is now poorly utilized. It contains generous quantities of most of the B-vitamins and from 12 to 20% oil. The quantity of oil available annually in the United States from this source is approximately 30 million pounds (1). This oil is quite bland and should be valuable for edible purposes.

The bran is subject to a rapid deterioration after milling, due to its finely divided state and to the great activity of the hydrolytic enzymes in splitting the fats. The oil also becomes rancid and makes the product unpalatable as a feed. Another objection to the bran is the tendency of the oil to produce soft pork when fed to swine (2, 3). These undesirable properties could be eliminated by extracting the oil from the bran immediately after milling.

Feuge *et al.* (1) have demonstrated that rice bran oil extracted by hexane can be refined to a high grade edible product. A disadvantage however is the difficulty of preventing fatty acid increase and a resulting high oil-refining loss. Colman (4) has prepared a vitamin B concentrate from rice bran by extracting the oil with 99% isopropanol and re-extracting the bran several times with 40% isopropanol. The con-

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[2] Presented at the 40th annual meeting of the American Oil Chemists' Society, New Orleans, La., May 10-12, 1949.

centrate contained about 63% of the thiamin in the original bran.

The object of the work described in this paper was to determine the feasibility of simultaneous extraction of vitamins and oil with isopropanol and the subsequent separation of a vitamin concentrate. The bran would still retain sufficient B-vitamins to be a useful ration supplement for poultry and swine, and it would no longer contain the oil which could become rancid or cause soft pork.

Extraction

The rice bran used in these experiments was obtained from the River Brands Rice Mills in Houston and was delivered to the extractor within four hours after milling.

Two extraction procedures were tested, one using 91% isopropanol and the other using 95% isopropanol as solvent. The extractions were conducted in a small, continuous, countercurrent screw extractor which consisted of three 4-inch screw sections which conveyed the bran down through the solvent, then vertically upward out of the solvent, and finally through a jacketed drying section. This extractor has been described previously by Harris (5, 6).

The miscella, after filtration to remove wax, was evaporated in a forced circulation, high velocity vacuum evaporator which maintained boiling temperatures between 57° and 59°C. The concentrate, which still contained 15 to 20% solvent, formed an oil layer, a syrup layer, and a wax-like solid upon cooling. The layers were carefully separated and the remainder of the solvent removed from the oil by water washing. The syrup was diluted with water and the solvent removed by further evaporation. The concentrated syrup was then filtered in a basket centrifuge with the use of filter acid. When 95% isopropanol was used as the solvent, solid sugars separated from the concentrate and it was necessary to add water to form a syrup which could be better separated. Data pertinent to the extraction process are given in Table I.

TABLE I

Extraction Data on Rice Bran

Isopropanol	91%	95%
Bran feed rate	7.5 lb./hr.	10 lb./hr.
Bran residence time	2 hr.	1.5 hr.
Solvent feed rate	31.9 lb./hr.	31.0 lb./hr.
Extraction temperature	71°C.	71°C.
Lipids in original bran	17.3%	20.1%
Lipids in extracted bran	.45%	1.2%
Moisture in original bran	7.3%	5.9%
Moisture in extracted bran	10.3%	12.9%
Yields per 100 lb. of original bran		
Extracted bran	77.5 lb.	81 lb.
Oil	16.6 lb.	16.2 lb.
Syrup	10.6 lb.[1]	4.0 lb.[2]

[1] 61.0% solids.
[2] 64.5% solids.

Oil

The oil extracted from rice bran by isopropanol was considerably greener in color than that extracted by hexane. After caustic refining the oil was still green. This color has been shown to be due to chlorophyll by Feuge *et al.* (1), who also indicated that acid clay was beneficial for removing it.

Browne (7) has shown that the free fatty acid in freshly milled bran increases very rapidly and may reach 60-70% within a month at 25°C. The data in Table II show that storage temperature and moisture content have considerable influence on the rate of fatty acid formation in rice bran. In this limited series of tests a low storage temperature had a greater effect on decreasing the rate of formation of free fatty acid than did a low moisture content of the rice bran. After extraction the oil is relatively more stable with respect to free fatty acid formation. Analyses made on an extracted oil indicated that the content of free fatty acids had increased from 12 to 15% after storing for four months in a clear bottle exposed to light and at room temperature.

TABLE II

Effect of Temperature and Moisture on Free Fatty Acid Formation in Rice Bran During Storage

Sample	Moisture	Storage temperature	Free fatty acid in oil, per cent		
	per cent	°C.	*4 hr.*[1]	*28 hr.*[1]	*52 hr.*[1]
S-1	5.9	35	4.5	.....	8.7
S-4	6.2	35	8.3	8.5	11.1
S-2	6.3	35	8.4	.....	11.2
S-3	8.2	35	11.8	12.0	16.4
S-5	9.0	35	10.4	14.2	17.3
S-6-C	8.4	7	2.0	2.2	2.3[2]

[1] After receipt of bran. Zero time was about 4 hours after milling.
[2] Stored for 75 hours.

TABLE III

Vitamin Assays—91% Isopropanol Extraction

Vitamin	Original bran, γ/g.	Extracted bran, γ/g.	Syrup, γ/g.	Distribution of vitamins		
				Extracted bran, per cent	Syrup, per cent	Loss, per cent
Biotin	.089	.054	.22	47.0	26.6	26.4
Folic acid	1.25	.54	.03	33.5	0.3	66.2
Riboflavin	2.2	2.3	4.03	81.0	20.0	—1.0
Pantothenic acid	18.3	6.09	113	25.8	67.9	6.3
Pyridoxine	26.9	15.5	122.5	44.6	50.2	5.2
Thiamin	30.5	11.9	105.3	30.3	38.0	31.7
Niacin	263	168	619	49.5	25.9	24.6
Inositol	8150	7150	310	68.0	0.4	31.6

Vitamin Assays

Eight of the B-vitamins were determined microbiologically in the original bran, extracted bran, and syrup concentrate samples. *Lactobacillus casei* was the test organism used for the determination of riboflavin, niacin, pantothenic acid, folic acid, and biotin, with the assay method described by Roberts and Snell (8). For thiamin the method of Sarett and Cheldelin (9) with *Lactobacillus fermentum* was employed. The procedure of Atkin *et al.* (10), using *Saccharomyces carlsbergensis* for pyridoxine and *Saccharomyces cerevisiae* for inositol, was used for these compounds. The data in Table III indicate the feasibility of removing substantial quantities of the B-vitamins along with the oil by using 91% isopropanol as the solvent. However the 95% isopropanol extracted smaller quantities of the vitamins (Table IV).

The distribution of the vitamins between the extracted bran and the syrup was calculated on the basis of the yields given in Table I, with the loss columns representing a summation of losses during the various stages of processing and probable assay errors. The assay values represent averages of at

TABLE IV

Vitamin Assays—95% Isopropanol Extraction

Vitamin	Original bran, γ/g.	Extracted bran, γ/g.	Syrup, γ/g.	Distribution of vitamins		
				Extracted bran, per cent	Syrup, per cent	Loss, per cent
Biotin	.089	.093	.294	84.6	13.2	2.2
Folic acid	1.25	1.37	.034	88.8	.1	11.1
Riboflavin	2.2	2.45	5.25	90.4	9.5	.1
Pantothenic acid	18.3	8.28	163	36.7	35.7	27.6
Pyridoxine	26.9	15.8	193	47.6	28.7	23.7
Thiamin	30.5	29.2	126.5	77.5	16.6	5.9
Niacin	263	225	797	69.4	12.1	18.5
Inositol	5500	5200	395	76.9	.3	22.8

least three separate assays on at least three different hydrolysates and are believed to be well within the limits of accuracy for the microbiological methods.

Discussion

The chief problem concerning the utilization of rice bran as a feed is the formation of free fatty acids in the oil when the bran is stored at the usual temperatures. Removal of the oil within a few hours after the rice bran is milled is proposed as a solution to the problem. The bran can be extracted with the solvent, hexane, to remove the oil, and if isopropanol is used instead, a vitamin concentrate may also be obtained. Both hexane and isopropanol are efficient solvents for the oil, but if only oil and extracted bran are to be produced, it would be more economical to use hexane. The value of the products obtained and the cost of the operation would determine the choice of the solvent. If isopropanol is to be used, the value of the vitamin concentrate should be sufficient to offset the increased cost of the solvent and additional steam required for evaporation of the isopropanol. Its latent heat of vaporization is about twice that of hexane. The choice of solvent would also influence the design of the extraction and solvent-removal equipment.

If the vitamin syrup were to be the main product, it would seem advantageous to use the present process. The vitamin content of the syrup prepared from the 91% isopropanol extraction is compared with a commercial product in Table V. The two concentrates

TABLE V

Comparison of 91% Isopropanol Syrup With Vitab II

	91% Isopropanol syrup, γ/cc.	(11) Vitab II γ/cc.
Riboflavin	5	10
Pantothenic acid	141	275
Pyridoxine	153	150
Thiamin	131	150
Niacin	775	2000

were approximately equivalent in total solids, but the commercial product contained higher concentrations of all of the vitamins listed except pyridoxine. It is possible however that the vitamin concentrate could be improved with respect to the thiamin and niacin content by decreasing the amounts of these vitamins which were reported as lost in the present process. As listed in Table III, 30% of the original thiamin remained in the extracted bran; giving an extraction of 70%. Colman (4) claimed to have extracted 63% of the thiamin. In his process the extracted thiamin should all be in the syrup whereas (Table III) 32% of the thiamin was lost in the 91% isopropanol extraction. When this loss is compared with the excellent recovery of the riboflavin and pyridoxine and the almost complete loss of inositol, it appears that something besides destruction was responsible for these losses. A logical explanation would be that the losses are due to partial solution of the vitamins in the oil phase. If this is true, then some of these could be recovered by water washing the oil phase and adding the washings to the syrup. If all of the lost thiamin were recovered, this would increase the thiamin content of the syrup above that of the commercial product. However if the lost riboflavin, pantothenic acid, and niacin were recovered, the syrup would still be low in these vitamins. Another possible way in which the vitamins could be further concentrated would be to remove some of the sugar by crystallization. The ready manner in which the sugar crystallized from the 95% isopropanol extract indicated this possibility.

The brans prepared in this study appear to have excellent storage properties and are now being used in feeding tests with chickens and swine.

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