Composition of Waxes from Crude Rice Bran Oil

S.H. YOON and J.S. RHEE*, Department of Biological Science and Engineering,
Korea Advanced Institute of Science and Technology, Seoul 131, Korea

ABSTRACT

Hard and soft waxes were separated from the tank settling of crude rice bran oil by solvent extraction and analyzed for their composition by gas liquid chromatography (GLC). The results showed that the melting points of the hard wax and the soft wax were 79.5 C and 74 C, respectively, and that the hard wax was mainly composed of saturated fatty alcohols of C24, C26 and C30, saturated fatty acids of C22, C24 and C26, and *n*-alkanes of C29 and C31. The soft wax was mainly composed of saturated fatty alcohols of C24 and C30, saturated fatty acids of C16 and C26, and *n*-alkanes of C21 and C29. In the soft wax, lauric acid was also detected.

INTRODUCTION

Waxes contained in crude rice bran oil are widely used as ingredients of various polishing agents, foodstuffs, cosmetics and industrial products (1). Rice waxes are recovered from the residues obtained from the rice bran oil refining process by a series of solvent extraction with various solvents. Depending on the types of solvent and extraction conditions, such properties as melting point, acid value, iodine value etc., vary substantially (2,3,4). The reports describing the compositions of rice waxes are found elsewhere (5,6,7). Recently, we separated hard and soft waxes from the tank settling of crude rice bran oil (8). The present paper describes the results of the studies aimed at separation and characterization of the hard and soft waxes from the locally available crude rice bran oil.

EXPERIMENTAL PROCEDURES

Materials

Rice bran oil tank settlings used for wax separation were obtained from a local rice bran oil processor. The standard fatty acids, fatty alcohols and hydrocarbons for thin layer chromatography (TLC) and gas liquid chromatography (GLC) were purchased from either Sigma Co. (St. Louis, MO) or Applied Science Lab. (State College, PA). Other reagents used were of analytical grade.

Wax Separation

Rice bran oil tank settlings were mixed with methyl ethyl ketone (MEK) to remove the oil and the methyl ethyl ketone insoluble portion (MIP) was dried in vacuo for at least 48 hr before use. MIP was, then, fractionally crystallized with isopropanol (IPA) by the method of Misonou et al. (4). MIP was boiled with IPA (1:3, w/v) in a temperature-controlled water bath with reflux for 30 min and cooled slowly to 50 C, followed by filtration under vacuum maintained at 50 C. The soluble portion at 50 C was allowed to stand at 25 C overnight to crystallize wax particles, and was then filtered. The filter cakes obtained were washed with acetone and refiltered, followed by air-drying to obtain soft wax. The insoluble portion at 50 C was boiled with IPA (1:6, w/v) with reflux for 30 min and filtered at 70 C. The soluble portion at 70 C was allowed to stand at 25 C overnight and was then filtered in vacuo and the filter cake obtained was air-dried to obtain hard wax (Fig. 1).

Determination of Physical and Chemical Properties

Wax contents were determined by the gravimetric method and melting points were determined using a capillary melting point apparatus (A.H. Thomas Co., Philadelphia, PA). Acid value, iodine value, saponification value, moisture and volatile matters, and phosphatides were determined by the AOCS methods (9).

Analysis by Column Chromatography, TLC and GLC

Components of wax such as fatty acids, fatty alcohols and hydrocarbons were fractionated by activated alumina and calcium carbonate column chromatography (10) and the purities of the fractionated components were identified by TLC (11). Kieselgel G (E. Merck AG) was used as adsorbent layer of 250-μm thickness and the mixture of benzene: chloroform (7:3, v/v) was used for developing solvent. The mixture of *n*-alkanes of C12, C16 and C30 was used as the standard for hydrocarbon; the mixture of palmitate, oleate and lignocerate for fatty acid standard; and the mixture of cetyl alcohol and lignoceryl alcohol for fatty alcohol standard. All fractions of fatty acids, hydrocarbons and fatty alcohols were analyzed by GLC and methyl esters of fatty acids were prepared by BF_3-methanol solution. In making GLC, Varian Aerograph 2800 equipped with FID was used. The column used for fatty acid analysis was a copper column (9 ft × 1/8 in. od) packed with acid washed 80-100 mesh Chromosorb W, coated with 10% DEGS. The temperatures of the column oven, detector and injector were 235 C, 295 C and 275 C, respectively. The flow rates of helium, hydrogen and air were 60, 40 and 300 mL/min, respectively. The fraction of the hydrocarbons and the fraction of the fatty alcohols which were previously reduced to the equivalent hydrocarbons were also analyzed by GLC (10,12). The column used for hydrocarbon analysis was a stainless steel

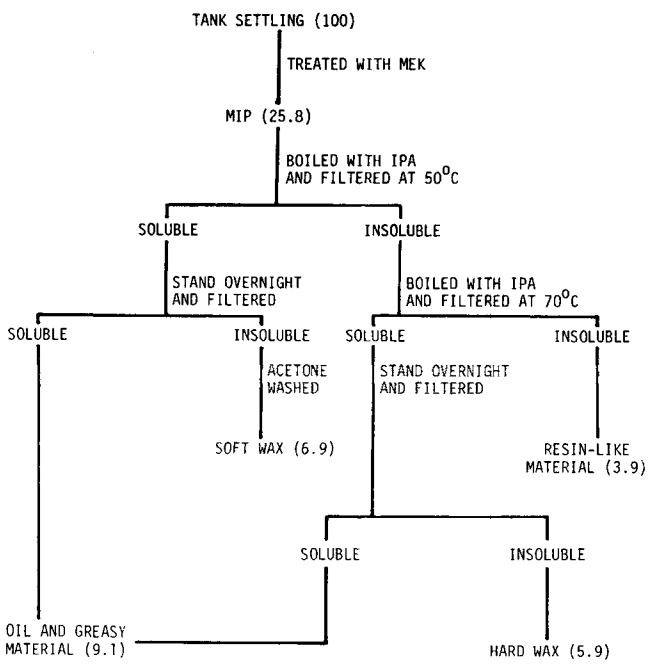

FIG. 1. Wax separation by IPA fractional crystallization from MIP of tank settling. Numbers in parentheses designate weight percentage of tank settling.

*Author to whom all correspondence should be addressed.

TABLE I

Characteristics of Tank Settling, MIP and Separated Portions

	Tank settling	MIP	Oil and greasy material	Soft wax	Hard wax	Resin-like material
Acid value	74.6	55.8	80	32	10.6	–
Iodine value	86.0	41.6	82	29.1	20.7	–
Saponfication value	165	146.3	168.1	94.8	84.3	–
Moisture and volatile matter, %	3.4	1.2	–	–	–	–
Phosphatides, %	–	0.97	–	0.02	0	–
Color	Yellowish brown	Brown	Green	Dark brown	Dark brown	Dark brown
Appearance at 25 C	Liquid	Tacky solid	Semi-solid	Solid	Solid	Solid
Melting point, C	–	67	–	74	79.5	89

column (3.3 ft × 1/8 in.od) packed with 80-100 mesh Celite 545 coated with 5% Apiezon L. The rate of temperature increase in the column oven was 8 C/min from 160 C to 280 C, and the temperature was maintained at 280 C thereafter. The temperatures of the detector and injector were 320 C and 300 C, respectively. Gas flow rates were the same as those for fatty acid analysis. The fatty alcohols, fatty acids and hydrocarbons were identified by comparing the retention times with those of reference standards. Gas chromatographic peak areas were determined by multiplying peak height by width at half-height.

RESULTS AND DISCUSSION

The yields of hard and soft wax were 5.9% and 6.9% of tank settling, respectively (Fig. 1). Based on MIP recovered, the total wax yield was 46.9%, which was almost the same as the result of Iwama et al. (7). In our experiments, more soft wax was obtained than hard wax. The resin-like material obtained was dark brown and appeared to be powdery. Oil and greasy materials melt at about 40 C, but they exist as semisolid at 25 C and showed green in color, indicating the presence of chlorophyll (1).

As shown in Table I, tank settling, MIP and oil and greasy materials contained relatively high free fatty acid: this might be due to the deterioration of raw materials such as rice bran or rice bran oil. The gummy materials, generally designated as phosphatides, were almost or completely removed from both waxes.

Melting points of the waxes varied substantially according to the extraction procedures (2,4,6,7). When compared with the literature values, the melting points of the waxes obtained were in good agreement. The common constituents of waxes were known to be fatty alcohol, fatty acid and hydrocarbon and these could be fractionated by column chromatography.

In performance of column chromatography, the column yield was about 90%. The hard wax consisted of 64.5% fatty alcohol, 33.5% fatty acid and 2% hydrocarbon; and the soft wax consisted of 51.8% fatty alcohol, 46.2% fatty acid and 2% hydrocarbon (Fig. 2). In both waxes, fatty alcohol and fatty acid were the major constituents. Results of the TLC showed that the fractionated components were pure and had the same Rf values of reference standards. The Rf values obtained were as follows: hydrocarbon, 0.85; fatty acid, 0.1; fatty alcohol, 0.2. The hydrocarbons contained in rice waxes were identified as normal alkanes of C14 to C33. The major hydrocarbons of hard wax were *n*-nonacosane and *n*-untriacontane as shown in Table II.

In soft wax, *n*-heneicosane was the most predominant one. Comparing the compositions of hydrocarbon of the two waxes, *n*-paraffins in soft wax were distributed more evenly than in hard wax, i.e., presence of the hydrocarbons of relatively low carbon number were more pronounced in soft wax than in hard wax. Even though Iwama (7) reported that hydrocarbons in rice waxes were in the range of C16 and C37, we observed the presence of *n*-tetradecane in both waxes. But long chain hydrocarbons such as C34-C37 were not detected. It has long been known that the major fatty alcohols in rice waxes are lignoceryl and myricyl alcohol (5, 7). In our experiments the hard wax was shown to be mainly composed of lignoceryl, ceryl and myricyl alcohol. On the other hand, the composition of soft wax was more simple than that of hard wax, and lignoceryl and myricyl alcohol comprised 63% of total fatty alcohols. In contrast to the cases of hydrocarbons and fatty alcohols, the fatty acids included in both waxes were shown to have only even carbon numbers. It is interesting to note that a significant amount of lauric acid was detected in this study which was not detected in previous studies (5,7). The major fatty acid components of hard wax were behenic, lignoceric and cerotic acid, whereas those of soft wax were palmitic, lignoceric and cerotic acid.

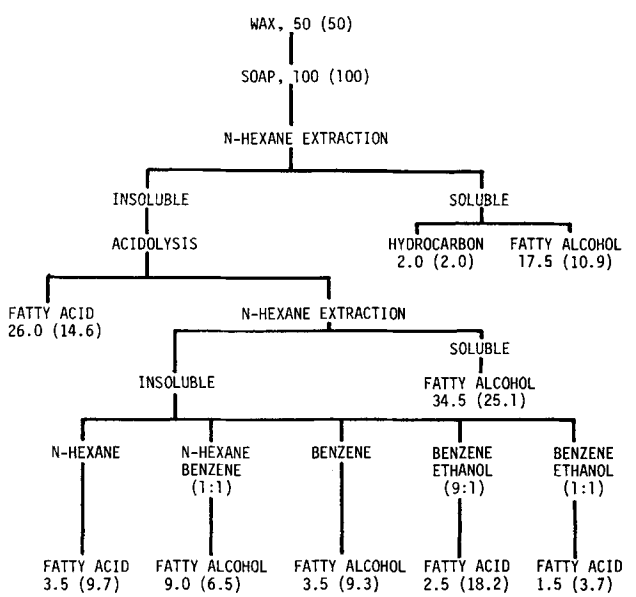

FIG. 2. Fractionation of fatty alcohol, fatty acid and hydrocarbon of wax by column chromatography. Numbers, without and with parentheses, designate weight percentage of soap for hard wax and soft wax, respectively.

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TABLE II

Percentage Composition of Hydrocarbon, Fatty Alcohol and Fatty Acid

No. of carbon	Hydrocarbon		Fatty alcohol		Fatty acid	
	Hard wax	Soft wax	Hard wax	Soft wax	Hard wax	Soft wax
12	–	–	–	–	–	4.2
14	1.2	4.5	0.9	3.2	4.7	1.1
15	0.2	0.9	3.0	2.4	–	–
16	0.3	1.2	0.5	2.2	6.3	10.6
17	0.3	1.2	1.3	2.1	–	–
18	0.2	1.9	3.3	3.9	1.4	5.2
18:1[a]	–	–	–	–	4.9	4.5
18:2	–	–	–	–	4.0	5.2
19	0.2	4.1	2.1	2.0	–	–
20	0.7	3.3	1.9	3.4	1.3	0.1
21	1.0	22.9	6.4	1.2	–	–
22	1.0	6.2	11.3	4.8	8.4	1.4
23	1.2	6.5	1.4	3.3	–	–
24	1.3	6.4	26.7	52.5	24.4	10.7
25	1.2	6.1	0.7	–	–	–
26	1.0	5.6	14.4	2.4	43.9	55.8
27	1.2	5.5	0.7	–	–	–
28	5.2	5.6	3.9	1.5	0.1	0.2
29	28.9	9.7	5.5	0.6	–	–
30	4.0	1.3	12.1	10.5	–	–
31	35.3	5.9	–	–	–	–
32	1.2	–	–	–	–	–
33	8.8	–	–	–	–	–
Unidentified	5.6	1.2	3.9	4.0	0.6	1.0

[a]Numbers following colon designate number of double bonds.

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