Technical

An Investigation of Rice Bran Oil Tank Settling

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A wax-like settling is observed in tanks in which rice bran oil is stored. "Soft" and "hard" wax fractions have been isolated from this settling by solvent crystallization. Previous investigation has shown that the settling consists mainly of wax esters of long chain alcohols and long chain fatty acids. The present work describes the column chromatographic analysis of unhydrolyzed tank settling. The presence of an aromatic moiety is indicated in the infra red spectrum. Comparison of data obtained by analysis of the tank settling before and after hydrolysis shows that it contains only 33% of monomeric esters; the remainder may be present as a polymeric ester, as found in carnauba wax. Investigation of different samples of rice bran oil has shown that the ratio of monomeric to polymeric fraction varies with the history of the bran.

One of the characteristic features of rice bran oil is the settling of a wax-like material when the oil is stored in tanks. These settlings usually settle during transport and storage periods, which may vary from one week to two months. The solids that settle out form a hard layer and need heat and manual scraping for their removal. At present, this settling has only a nuisance value to the oil processor.

Earlier investigators (1,2) based their findings on the data obtained after saponification. No detailed studies are available on the unhydrolyzed product. Lack of quantitative data has led to the simple assumption that the settling is made up of two fractions, "hard wax" and "soft wax." It has been reported (2) that both these fractions are simple wax esters of long chain alcohols and long chain fatty acids.

In the present investigation two approaches were followed. The tank settling was saponified and quantitative data of various fractions obtained. The settling was also subjected as such to quantitative column chromatographic analysis. The results show that these data do not agree with each other if a simple wax ester structure is assumed for the settling. The infra red spectrum and the column chromatographic behavior of the unhydrolyzed settling indicate that a considerable amount of tank settling has a polymeric ester linkage in which the aromatic group acts as a link similar to the one in carnauba wax (3,4).

EXPERIMENTAL PROCEDURES

Materials. Petroleum ether with a boiling range of 40–60 C was used unless otherwise stated. All the solvents were purified by distillation before use. Gas liquid chromatographic (GLC) analysis were performed on a Perkin-Elmer Sigma-300 gas chromatograph equipped with a flame ionization detector (FID) and LC-100 computing integrator. In all the analyses nitrogen was used as a carrier gas, and a temperature of 375 C was maintained for both injector and detector. All the GLC compositions are expressed in weight percent. Fatty alcohols and sterols were analyzed as their trimethylsilyl (TMS) derivatives prepared according to the standard procedure (5). Fatty alcohols were characterized against standard C_{26}, C_{27}, C_{28} and C_{30} (Analabs, North Haven, Connecticut) and sterols against standard stigmasterol and β-Sitosterol (Fluka, AG, Bucks, Switzerland). The analysis conditions were as follows: column 0.55 m stainless steel column of 0.25 mm i.d. packed with 1% OV-101 coated on 80–100 mesh chromosorb WHP; operating temperature programmed from 180 C to 300 C at the rate of 4 C/min, and carrier gas flow, 70 ml/min.

Fatty acids were converted to methyl esters by diazomethane and analyzed on a 2 m × 3 mm stainless steel column packed with 20% DEGS on chromosorb; operating temperature, 200 C, at a carrier gas flow of 40 ml/min. Fatty acids were characterized against standard C_{12}, C_{14}, C_{16}, C_{18}, C_{20} and C_{22} fatty acids (Godrej).

Thin layer chromatography (TLC) was run on plates coated with silica gel (0.1 mm thickness) and activated at 110 C before use. The TLC chamber was presaturated with the solvent before use. Spots were visualized by spraying 50% sulfuric acid in absolute alcohol and charring the plates at 130 C. Column chromatography was performed using chromatographic grade silicic acid (Loba Chemie, for preparation of lipids) activated at 110 C for one hr before use. Column dimensions were as follows: 300 mm long and 22 mm i.d. Columns were prepared by using a slurry of silicic acid in petroleum ether and the compounds loaded on the column as a benzene solution. IR spectra were recorded in nujol on a Model No. 581, Perkin Elmer Spectrophotometer.

Rice bran oil was obtained by extraction of rice bran (from solvent extraction plant) with petroleum ether (60–80 C) at 45 C for 12 hr.

Isolation of the isopropanol insoluble fraction (IIF) from rice bran oil settling. Rice bran oil (200 g) was allowed to stand at 27 C for 10 days. The supernatant oil was carefully decanted off. The settling was refluxed with isopropanol (200 ml) for one hr and allowed to stand overnight at 27 C. The insoluble portion was filtered, boiled again with isopropanol (200 ml) and filtered. The insoluble portion, on drying in vacuum, gave a dark brown material (1.66 g, 0.83%). TLC did not show the presence of free fatty acids or glycerides. The isopropanol-soluble portion was added back to the original oil after removal of the solvent.

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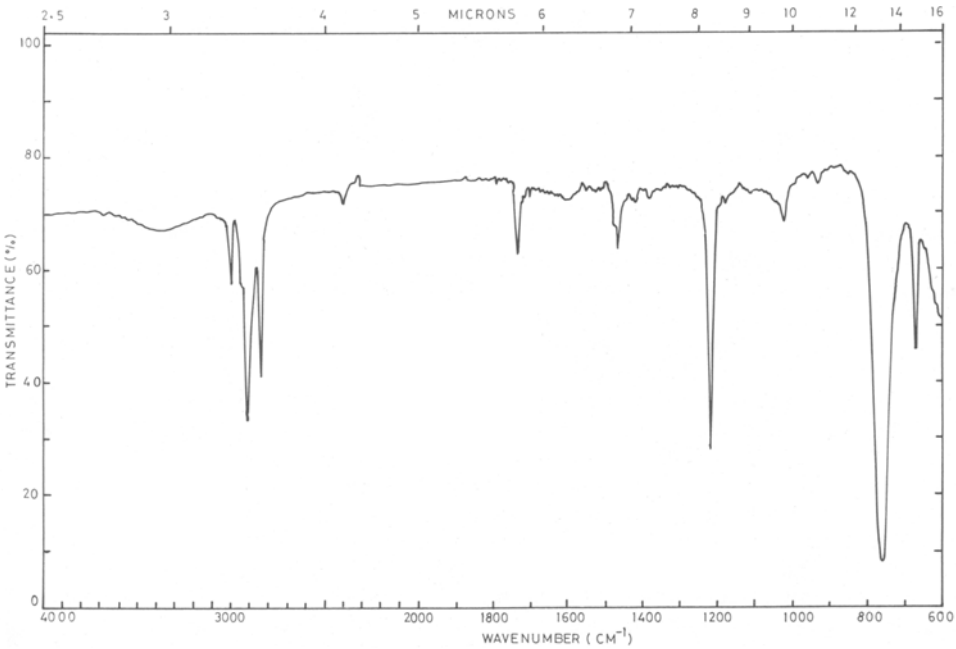

FIG. 1. In spectrum of IPA insoluble fraction from rice bran oil settling.

The IIF had the following characteristics: mp 66.70 C, IR (Fig. 1) bands at 670(s), 760(vs), 930(w), 1020(w), 1220(s), 1460(ms), 1600(w, broad), 1740(ms), 2840(s), 2920(s), 3010(s), 3150 to 3600 cm^{-1}, unsap-40.4%, GLC composition of the unsaponifiable, C_{24}, 13.53%; C_{26}, 14.17; C_{28}, 15.86; C_{30}, 22.49; C_{32}, 13.65; C_{34}, 12.19; C_{36}, 4.88; C_{36}, 4.88; C_{38}, 1.24, and unknown, 2.

Column chromatography of IIF. IIF (1 g) was dissolved in hot benzene (20 ml) and loaded on a column of silicic acid (30 g). The details of the column chromatography results are summarized in Table 1.

Alkaline hydrolysis of IIF and quantitative column chromatography of the saponifiable fraction. IIF (1.8331 g) was refluxed with isopropanol (50 ml) containing KOH pellets (5 g) on a steam bath for four hr. Most of the solvent was removed under vacuum and the residue extracted with Petroleum ether (4 × 250 ml). Addition of a small amount of methanol was necessary to break the emulsion. The combined petroleum ether layer was washed with 10% aqueous ethanol (4 × 25 ml) and dried over anhydrous sodium sulfate. Removal of the solvent gave unsaponifiable matter (0.7408 g, 40.41%). The unsaponifiable matter was shown by TLC and IR spectrum to contain only alcohols. The average molecular weight of alcohols by GLC was 421.6.

The aqueous layer from above was acidified with 1:1 sulfuric acid and extracted with ether (4 × 250 ml). Some ether-insoluble material remains in the interphase. The ether layer was washed free of acid, dried and evaporated to get ether-soluble material (0.5417 g, 29.55%). The aqueous layer contained insoluble lumps. This was allowed to stand overnight and the solids filtered off, followed by wasing with chilled acetone (25 ml). The material was dried and finally weighed (0.3019 g, 16.47%), mp 78–80 C.

A part (0.3673 g) of the ether-soluble portion was chromatographed on silicic acid (5 g) and eluted with 100 ml petroleum ether:ether (85:15) to get 0.3181 g (86.60%) of the material. This was found by TLC and IR spectrum to be fatty acids. The GLC composition of the fatty acids was as follows: C_{16}, 6.79; $C_{18:0}$, 1.14; $C_{18:1}$, 2.18; $C_{18:2}$, 1.10; C_{20}, 1.69; C_{22}, 28.38; C_{24}, 55.18; and C_{26}, 3.53. The average molecular weight of fatty acids was 349.

Elution of the column was continued with methanol (100 ml) to get 0.0415 g (11.30%) of methanol eluate which was not investigated in the present work.

Saponification of the dewaxed rice bran oil. The oil obtained after removal of the settling was saponified by standard AOCS procedure. The unsaponifiable fraction (4.4%) showed predominantly sterols and a negligible amount of alcohols by TLC and GLC.

RESULTS AND DISCUSSION

It has been reported (6) that rice bran wax can be used as a replacement for carnauba wax. This is based on the close resemblance of rice bran wax to carnauba wax in hardness and high melting point. In carnauba wax, these properties are attributed to the existence of polymerized diesters of cinnamic acid formed during aging of the wax (3,4). The present work was prompted by these finding. Literature available (1,2) on rice bran oil settling indicated varying amounts of "hard" and "soft" waxes and a resin-like material which has not been investigated. Several experiments during the present investigation (unpublished results) showed that the amount and nature of these fractions from different samples of rice bran oil were not consistent and varied with both the history of the bran and the efficiency of extraction of the oil. The findings in the present study should therefore be applied with caution until a detailed investigation is made relating the history of the bran and the chemical composition of the settling.

TABLE 1

Column Chromatography of Isopropanol Insoluble Fraction from Rice Bran Settling

Fraction/ volume collected (ml)	Weight percent	Melting (C)	Unsaponifiables (%)	Fatty alcohol composition (%)								
				C_{24}	C_{26}	C_{28}	C_{30}	C_{32}	C_{34}	C_{36}	C_{38}	Unknown
Petroleum ether:benzene 1:1 (300)	33.9	70.74	48.3	19.24	13.11	10.99	16.50	13.90	18.79	7.08	0.34	3.72
Ethyl acetate (200)	13.3	62.64	40	10.61	9.61	11.19	16.73	12.78	17.30	9.84	3.02	8.87
Uneluted fraction	52.9	Does not melt even at 300 C	–	6.96	9.96	12.83	19.77	14.27	17.39	11.49	3.0	4.88

Column chromatography is a very useful technique for the separation of classes of compounds. The application of this technique to the analysis of saponified products of waxes is described adequately in the literature (7). The analysis of unhydrolyzed waxes by column chromatography gives a better insight into the nature of the combination of their constituents. In the case of carnauba, for example, the unusual behavior of the wax on the column led to its further investigation (3,4). The findings in the present investigation show that development of analytical techniques for the analysis of unhydrolyzed waxes is essential to understanding the true composition of waxes. GLC has been used for the analysis of unhydrolyzed wax esters, but presently it is a highly specialized technique (8). Column chromatography is simple and convenient and has the added advantage of being non-destructive and quantitative.

Rice bran oil is characterized by the presence of a fairly large amount of sterol esters which markedly influence the refining losses of the oil (Belavadi, V.K., unpublished work). The separation of sterol esters and wax esters from the oil by column chromatography is well documented (9). Attempts to isolate all the simple esters of sterol/alcohol with fatty acids from rice bran oil by column chromatography yielded mainly sterol esters and only a small fraction of wax esters. This is not consistent with the quantity of alcohols obtained from the unsaponifiables of the whole oil. This indicated that the wax esters were behaving in an unusual way on silicic acid columns. Further, attempts to elute wax esters isolated from oil settling by TLC using a conventional solvent system for wax analysis also met with little success. Initially this failure was attributed to the poor solubility of wax esters in the eluting sol-

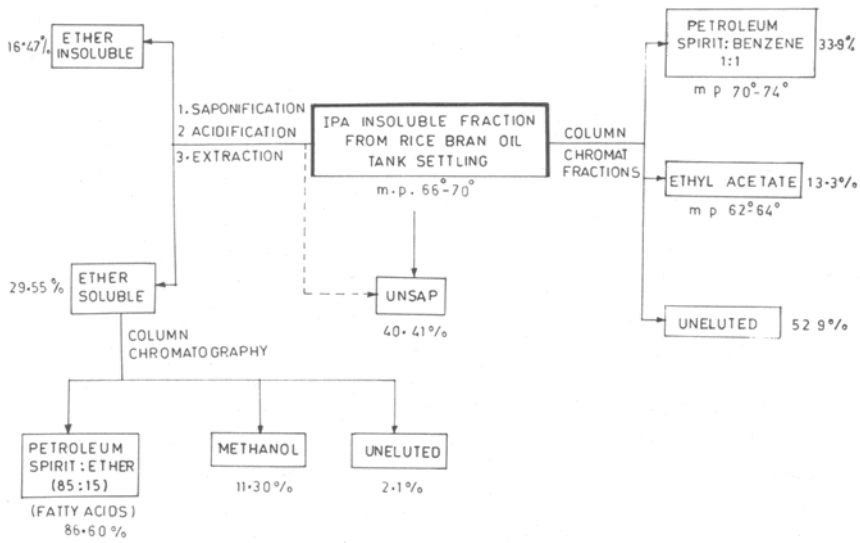

FIG. 2. Quantitative data of analysis of saponified fraction of IIF.

vent (10), but the settling was taken up for further investigation.

Earlier reports (1,2) on the analysis of tank settling were based on analyses of the oil obtained from local rice bran mills. Rhee (2) reported, for the first time, the occurrence of lauric acid as one of the fatty acid constituents in the wax. This finding could not be substantiated in the present work. Such discrepancies may be due to the contamination of rice bran oil with other oils in the storage tank. It was, therefore, decided to base the present findings on rice bran oil extracted in the laboratory. The amount and composition of the wax in rice bran oil may vary with the efficiency of extraction of the bran (1). Therefore, the extraction of bran was carried out at 45 C for 12 hr to extract out all the waxy material. Isopropanol was used as the solvent for the separation of settling, as it is known to give pure wax almost quantitatively (1). The unsaponifiable fraction of the oil from which the settling was removed as isopropanol-insoluble material showed, by TLC and GLC, the presence of very small amounts of fatty alcohols, indicating that almost all the wax esters of rice bran had remained as isopropanol-insoluble material.

Figure 2 shows the quantitative data of the analysis of the saponified fraction of IIF and compares the column chromatography results of the unhydrolyzed material. On the basis of GLC analysis, the average molecular weights of fatty alcohols (421.6) and fatty acids (349) were calculated. Theoretically, a wax ester made up of the above constituents should give, on saponification, 46.37% fatty acids and 53.63% fatty alcohols, whereas in the present work 25.5% and 40.41% of fatty acids and fatty alcohols were obtained, respectively. Apparently, from the above data, it can be calculated that about 65% of the esters have a simple wax ester structure.

Column chromatography of IIF, however, did not substantiate the above results. Only 33.9% wax esters from the column could be eluted using a fairly large volume of petroleum ether:ether (1:1), a solvent which elutes most of the wax esters. This implied that the alcohols and acids are not combined as simple esters but may occur as a polymer, as in the case of carnauba wax which shows similar behavior on silicic acid columns (3,4). The IR spectrum of IIF substantiated this assumption as it showed fairly strong absorption in the fingerprint region characteristic of aromatic compounds (11). The wax esters of IIF eluted from the column by petroleum ether:benzene (1:1) did not show any aromatic group absorption in IR (Fig. 3). It is, therefore, clear that the molecules containing aromatic group in the settling do not elute with solvents, normally used for wax esters. It may be argued that the uneluted fraction from the column may be monomeric esters of fatty alcohols and aromatic acids which might show a chromatographic behavior different from that of the wax esters of fatty alcohols and fatty acids. This possibility was discounted on the basis of the chromatographic behavior of such esters prepared synthetically from long chain alcohols and hydroxy benzoic acids (unpublished results). These monomeric esters show the same behavior as wax esters and are quantitatively eluted with petroleum ether:benzene (1:1) on a silicic acid column. It therefore appears that the aromatic ring acts in some way as a link in the polymeric structure holding fatty acids and alcohols. The report in this work of occurrence of ferulic acid (12) in rice bran oil and the presence of a strong peak at 1215 cm^{-1}, characteristic of the aromatic methoxy group, lends strength to the assumption that a significant amount of esters present in the settling have a structure with an aromatic group in them.

Preliminary investigation of wax which settles on storage of sunflower oil has shown a similar type of composition (unpublished results), the details of which will form the contents of a different communication.

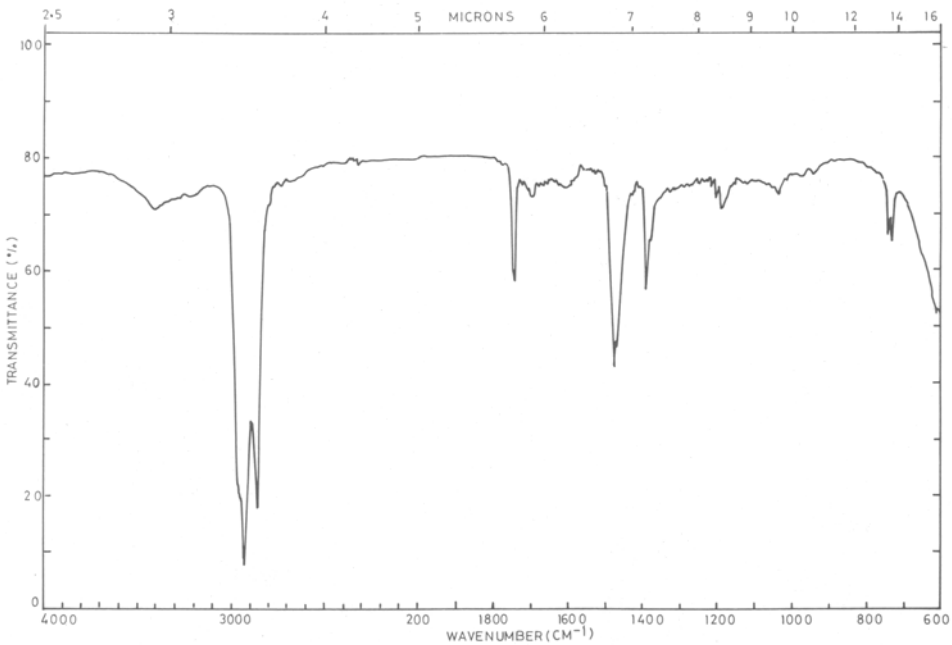

FIG. 3. IR spectrum of petroleum spirit:benzene (1:1) fraction of rice bran oil settling.

The IR spectra of other waxes like Hospita, Caranda, *Ceroxylin andicola*, Cork, Ouricuri, Salo Palmetto and Spanish moss show the presence of similar aromatic moieties (4). It therefore appears that waxes with an aromatic molecule in them have a special role to play in plant organs exposed to sunlight, probably as sunscreens. The variation in amount and composition that is characteristic of such waxes seems to depend on the degree of polymerization induced by climatic conditions. Studies toward the elucidation of structure of different fractions of waxes from rice bran, sugar cane and sunflower are in progress.

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