Chemical Examination of Sugar Cane Oil[1]

DONALD EDWARD WHYTE and BETTY HENGEVELD, S. C. Johnson & Son Inc., Racine, Wisconsin

SUGAR cane oil is obtained by solvent fractionation of crude sugar cane wax which has been extracted from the filter press muds of a cane crushing plant. Suitable solvents such as acetone will separate the crude wax, which is a dark green to brown tacky solid, into a hard wax and an oil. It is the composition of this sugar cane oil that we have investigated.

The oil is probably present in specialized cells throughout the cellular structure of the cane while the hard wax occurs principally on the surface of the cane stalk. In the milling of the cane approximately 40% of the total waxes and oils (1) are separated from the bagasse and are carried off with the crude sugar solution. These waxes and oils are removed from the juice during the clarification and appear in the press muds or "cachaza" from which they can be solvent extracted to produce the crude cane wax. The amount and type of extractable material obtained from the press muds will depend on the extraction solvent and the conditions of the extraction. A variety of solvents such as benzene (2), toluene (2), naphtha (2), and liquid sulfur dioxide (3) have been used on both the dried and wet press cake. The crude cane wax used as our source of sugar cane oil was obtained by hot heptane extraction of the aqueous muds (4).

The commercial extraction plant for the crude wax is at the Cuban American Sugar Company Refinery at Chaparra, Cuba, where the wet muds as they come directly from the cane crushing plant are extracted in a continuous counter current unit. The crude cane wax had the average constants given in Table I, which may vary to some extent depending on the type of cane, the condition of the crop, and processing requirements. The constants for crude cane waxes from other sources are included in Table I for comparative purposes.

The sugar cane oil was obtained by acetone extraction (8) of the crude cane wax in the plant of S. C. Johnson and Son Inc. and the Cuban American Sugar Company at Gramercy, La. The hard wax was insoluble and was filtered off leaving an acetone solution of the sugar cane oil. This was then distilled to recover the acetone, leaving the sugar cane oil as a viscous dark green fluid. The following work on the composition of sugar cane oil was carried out because no data had been reported for sugar cane oil from crude Cuban cane wax. Balch (9) has reported on the approximate composition of sugar cane oil from Louisiana crude cane wax.

Experimental

The sample of sugar cane oil used in this work had a saponification number of 156.0, acid number 24.6, iodine number (Wijs) 85.8, unsaponifiable matter 23%, and moisture and volatile matter 0.8%. The sugar cane oil (500 g.) was hydrolyzed by refluxing it in a solution of 70 g. sodium hydroxide and 87 g. of isopropanol in 2,250 g. of water for four hours. The unsaponifiable material was then isolated, following the extraction procedure described below, for it was found that if the initial extraction were accompanied by vigorous shaking, a stable emulsion formed that resisted all attempts at breaking.

The hydrolyzed product was placed in a separatory funnel and 600 ml. of ethyl ether added. The funnel was gently swirled but not shaken. The two layers were separated and the aqueous layer extracted twice more with fresh ethyl ether in the same manner. The

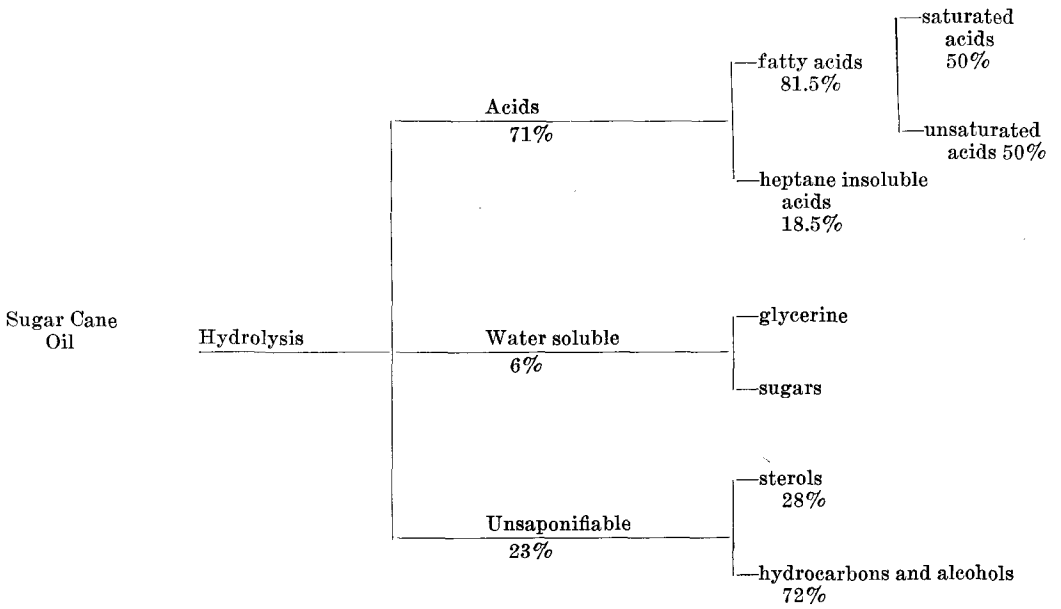

FIG. 1. Approximate composition of Cuban sugar cane oil.

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TABLE I

Crude Sugar Cane Wax Constants

Source of Crude Wax	Extraction Solvent	Melting Point, °C.	Sap. No.	Acid No.	Iodine No.	Acetyl No.	Reference
Cuba	Warm Heptane	79	120	27	57	95	....
Louisiana	Naphtha	78	91	23	38	....	....
South Africa	Benzene	55	168	39	60	73	(5)
India	Benzene	56	128	24	32	89	(6)
Brazil	Benzene	68	83	6	34	....	(7)

fourth and fifth extractions can then be carried out with vigorous shaking. The ether layers were combined, dried, and the solvent evaporated to give a 23% yield of unsaponifiable. The aqueous solution was acidified with dilute HCl and the freed acids ether extracted and isolated in a 71% yield.

The approximate composition of Cuban sugar cane oil is set forth in Fig. 1, which is similar to that exhibited by sugar cane oils from Louisiana (9), India (6), and South Africa (5).

Preliminary work on the separation of the acid fraction into its constituents indicated that it was not a simple mixture of normal fatty acids but contained acids which were susceptible to heat polymerization. The presence of abnormal fatty acids had been previously suggested by Balch (10) in order to account for his inability to distill more than about 55% of the acid fraction. A fraction of the acids was found to be insoluble in heptane and could be separated from the fatty acids in this manner.

The acids (300 g.) were heated in 4 liters of heptane on a steam bath for 2 hours. The solution was decanted from the insoluble acids which settled out. Further heating on the steam bath followed by standing overnight caused smaller amounts of the insoluble acids to settle out. These insoluble acids, which constituted 18.5% of the acid fraction, were obtained as a viscous tacky black liquid that was insoluble in heptane and soluble in acetone. A major portion of the color bodies present in the sugar cane oil were carried along with the heptane insoluble acids.

The fatty acids which were soluble in heptane were recovered and separated into saturated and unsaturated fractions by the Twitchell lead salt alcohol process (11). The results of this analysis indicated the presence of considerable amounts of unsaturated acids so that it was decided to separate the acids by low temperature crystallization, following the procedure of Brown (12), and then analyze these fractions by alkali isomerization and ultraviolet absorption, using the most recent constants of Bryce (13).

The fatty acids were first crystallized from acetone (10 cc./g.) at −30°C., the precipitate being then recrystallized from acetone (15 cc./g.) at −30°C., giving precipitate A. The two filtrates were combined and crystallized from acetone (10 cc./g.) at −65°C. giving precipitate B and filtrate C. The acids (170 g.) gave the fractions which are listed in Table II.

The three fractions of the acids were analyzed spectrographically after alkali isomerization by the method of Bryce and Swain (14), from which the proportions of linoleic and linolenic acids were determined. The results are given in Table II.

A separate sample of the fatty acids (99 g.) was esterified with methanol, using the procedure of Bjarnason and Meara (15). The esters were hydrogenated and then fractionally distilled through an electrically heated 12″ Widmer column. The fractions were analyzed for saponification number and iodine number and the composition of the fractions calculated in the manner described by Hilditch (16).

TABLE II

Spectrographic Analysis of Acids A, B, and C From Low Temperature Crystallization of Sugar Cane Oil Fatty Acids

Fraction	%	Acid No.	Iodine No.	232 mμ.	268 mμ.	% Linoleic	% Linolenic
A	36.5	193	25.0	7.42	0.77	7.0	1.6
B	35.3	200	121.0	46.60	2.48	47.1	5.1
C	28.2	191	124.3	43.70	5.90	39.5	12.1
Total						30.3	5.8

Calculation of the residue from this distillation in the usual way gave results which would indicate the presence of C_{28} and C_{30} acids; however the residue was found to be a tacky semifluid, which was believed to result from the polymerization of the heptane insoluble acid precursors that were not removed by the heptane extraction. It has been shown (17) that if the sugar cane oil were hydrolyzed by an acid hydrolysis such as a Twitchell hydrolysis, no heptane insoluble acids could be separated from the acid fraction. This pointed to the presence of a heptane insoluble acid precursor, which on treatment with alkali was converted into the heptane insoluble acid. It seemed probable that not all of the heptane insoluble acid precursors were converted into heptane insoluble acids and that this material polymerized during the distillation.

The final composition of sugar cane oil fatty acids was then arrived at by calculating the percentage of stearic acid present due to the hydrogenation of the unsaturated C_{18} acids. The stearic acid derived from linolenic acid was 5.9%, that due to linoleic acid was 30.8%, and the stearic acid originally present was 3.9%,[2] giving a total of 40.6% stearic acid. The percentage of stearic acid found in the hydrogenated acids was 49.2%. This difference of 8.6% stearic acid must be due to oleic acid. Therefore the percentage of oleic acid present was 8.6%. The final composition of fatty acids of sugar cane oil is given in Table III.

The methyl esters of fatty acids from sugar cane oil had the following analytical characteristics: saponification number 185.0 and iodine number 87.2. The accuracy of the procedure may be checked by comparing the observed values with the saponification number (186.0) and iodine number (88.5) calculated from the composition data given in Table III. It was necessary to assume a saponification number of 98.0 and an iodine number of 61.8 for the non-fatty acid fraction because its structure was unknown. The saponification number of 98.0 was the actual value observed for this fraction while the

[2] The percentage of stearic acid originally present was found by a separation of the fatty acids into soluble and insoluble portions by means of the Twitchell lead salt alcohol method, followed by fractional distillation of their methyl esters.

iodine number of 61.8 was the value for the heptane insoluble acids which are believed to be closely related to this fraction. This check on the accuracy of the procedure was only partially satisfactory because of the assumptions which were necessary.

The heptane insoluble acids are a dark viscous tacky liquid, which had the following constants: acid number 176, saponification number 198, iodine number 61.8, and n_D^{25} 1.493. Distillation of the heptane insoluble acids resulted in polymerization of the acids in the still pot to a rubbery polymer at a temperature of 240-250°C. The insolubility of these acids in heptane as well as the high refractive index would indicate the presence of a hydroxy acid such as ricinoleic. However this hydroxy acid is much more unstable than ricinoleic since distillation of the methyl esters resulted in polymerization. The methyl esters of this acid were prepared by the procedure of

TABLE III

Calculated Composition of Fatty Acids of Sugar Cane Oil

Acids	% Acids	% Weight Fatty Acids, excluding non-fatty acids	% Mol. Fatty Acids, excluding non-fatty acids
Caprylic	1.7	2.0	3.7
Capric	1.0	1.2	1.8
Lauric	2.8	3.3	4.4
Myristic	2.6	3.1	3.7
Palmitic	21.1	25.0	25.7
Stearic	3.9	4.6	4.3
Arachidic	6.4	7.6	6.4
Oleic	8.6	10.2	9.5
Linoleic	30.5	36.1	34.0
Linolenic	5.8	6.9	6.5
Non-fatty acids	15.6	.....	.....
Total	100.0	100.0	100.0

Bjarnason and Meara (15). The ester had a saponification number of 199.9 and hydroxyl number of 102.2. Considerable work on the identification of this acid has not resulted in any acceptable structure.

The unsaponifiable portion of sugar cane oil was a dark red viscous oil, from which a sterol fraction can be crystallized using acetone as the solvent. It was later found that a combination of first crystallizing from acetonitrile and then from acetone or ethyl acetate gave a cleaner separation of the sterols. The unsaponifiable fraction (92 g.) was repeatedly extracted with 1-liter portions of boiling acetonitrile in which the sterols are soluble but precipitate out rapidly on cooling. The crude sterols (26 g. or 28%) were then recrystallized from acetone or ethyl acetate, giving an 18% yield of sterols as flat plates melting at 135-138° C. and optical activity of α_{30}^{D} −31.5°. These results seem to indicate the presence of a mixture of sitosterols and stigmasterol.

The non-sterol portion of the unsaponifiable was a clear resinous fraction of hydrocarbons and alcohols. No "ceryl" alcohol was found in contrast to Balch (18), who reported 3% "ceryl" alcohol in Louisiana sugar cane oil. This difference may be due to the difference in the methods of isolation of the sugar cane oil rather than to any specific variation in the constituents of the sugar cane.

Non-sterol portion of the unsaponifiable amounted to 72% and had the following properties: iodine number 73.7, hydroxyl number 46.5, and n_D^{35} 1.5098. This material (47 g.) was fractionally distilled through a Vigreux column at a pressure of 0.7 mm. The distillation results and analyses are given in Table IV.

TABLE IV

Distillation of Unsaponifiable

Cut	Temperature °C.	Weight	n_D^{35}	Hydroxyl No.	Iodine No.
1	112-150	4.66	1.4869	15.8	91.8
2	150-252	10.84	1.5129	12.2	91.1
3	247-250	13.04	1.5193	12.9	90.2
4	250-252	5.24	1.5198	21.9	70.0
5	252-255	5.57	1.5155	27.2	73.1
6	255-falling	1.68	1.5128	25.7	74.2
Residue, holdup, and trap		5.41			

The unsaturated alcohols and unsaturated hydrocarbons apparently distilled off together with no evidence of any separation. Considerable work probably would be necessary to elucidate the structure of both the alcohols and hydrocarbons.

The water soluble fraction of sugar cane oil was found to be approximately 6%. This fraction was recovered by neutralizing and evaporating to dryness the aqueous solutions from which the free acids had been extracted after hydrolysis of the oil. The residue from the evaporation was extracted several times with a mixture of ether-alcohol (1:2). The solvents were removed, leaving a viscous sweet liquid which gave a positive acrolein test as well as a tribenzoate with a melting point and mixed melting point with a known glycerol tribenzoate of 71-72°C. These results confirm the presence of glycerol.

It can be assumed that about 69% of the acids in sugar cane oil are present as glycerides because the combined hydroxyl values of the sterols, resinous alcohols, and heptane insoluble acids are only about 31% of the hydroxyl values required for the acids present. The evaporation residue gave a positive Fehling's test which indicated the presence of a reducing sugar.

Sugar cane oil is a by-product in the production of sugar cane wax from the muds of a cane crushing plant. The magnitude of the sugar industry and the increasing use of sugar cane wax indicate that sugar cane oil should soon be available in quantity. It is expected to become a stable source of a variety of materials such as fatty acids, sterols, and unsaturated hydrocarbons.

Two practical methods (17) for the separation of the fatty acids have been developed and appear to show promise as commercial methods. The first method involves methanolysis of the sugar cane oil, followed by vacuum distillation of the methyl esters of the fatty acids. The second method is the use of a Twitchell hydrolysis followed by vacuum distillation of the free acids. The sterols can be isolated before or after removal of the fatty acids.

Summary

Sugar cane oil from Cuban cane has been shown to contain fatty acids, heptane insoluble acids, sterols, resinous hydrocarbons and alcohols, glycerine, and sugars. The acids from sugar cane oil can be separated into two major fractions, fatty acids and heptane insoluble acids. The latter (which are probably hydroxy acids) are relatively unstable to heat and polymerize during distillation. The fatty acids were found to consist mainly of linoleic (36.1%) and palmitic (25.0%) acids with lesser amounts of oleic (10.2%), linolenic (6.9%), and arachidic (7.6%) acids. Glycerine is present, and it would appear that the greater part of the acids occur as glycerides

in the sugar cane oil. The unsaponifiable fraction is mainly unsaturated hydrocarbons with smaller amounts of sterols and other alcohols.

Acknowledgment

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The Application of Infrared Spectroscopy to Fat and Oil Chemistry[1]

E. F. BINKERD and H. J. HARWOOD, Armour and Company, Chicago, Illinois

THE application of spectroscopy to the field of organic chemistry during the past 10 years has been most spectacular. Most oil chemists are familiar with the analytical uses of ultraviolet spectrophotometry and with the proposed change in the approved method for the determination of oil color employing visible spectrophotometry. Another section of the electromagnetic spectrum, the infrared, is now available to the chemist in his research. It is with this that this paper is concerned.

Knowledge of the infrared region of the spectrum and of its potentialities as a powerful analytical tool goes back about 50 years, but practical application on a routine basis is not yet 10 years old. It was during the war, in fact, that the infrared spectrophotometer came of age. The analyses of synthetic rubber and high-octane gasolines as well as the characterization of penicillin are but examples of the many fields in which the infrared spectrophotometer performed functions which would have been impossible by any other available means in the time allowed. Since the war infrared spectrophotometry has found its way into almost every phase of organic chemistry, and the tremendous potential it possesses should not be overlooked by workers in fat and oil chemistry.

The applicability of the infrared and related regions of the spectrum to the analysis of organic molecules is based on the absorption of light energy by these molecules and the atoms of which they are composed. Every combination of atoms has the ability to vibrate in response to energy of a certain frequency (or wave length), and the energy thus absorbed may be determined photometrically. When organic substances are exposed to energy of appropriate wave length, absorption occurs with accompanying conversion of the energy into one of three kinds of molecular or atomic motion, depending upon the wave length of the light. This results in three types of spectra, rotational, electronic, and vibrational. Rotational spectra occur in the far infrared and microwave regions and to date have had little application to organic chemistry. Electronic spectra are those with which research workers are more familiar, forming the basis for visible and ultraviolet spectroscopy. The third type occurs when the absorption of radiant energy gives rise to changes in the vibration of the molecule, and it is the measurement of this absorption which constitutes practical infrared spectroscopy.

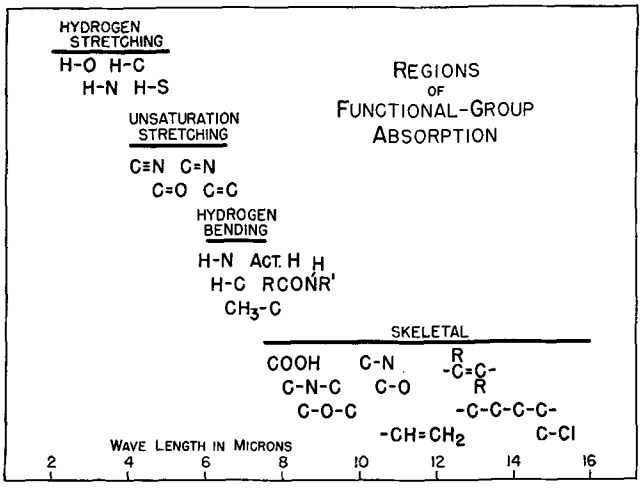

FIG. 1.

It has been established that a molecule having n atoms has 3 n kinds of motion. Of these, five or six, depending on the molecular symmetry, are motions of the molecule as a rigid unit and do not result in vibrational spectra. The remaining kinds of motion are vibrational in nature, and each has a characteristic frequency. It is possible to derive mathematically the frequencies characteristic of certain functional groups in a simple molecule, and this derivation forms a means of identification for absorption bands in the infrared (1). In practice most absorption bands have been established by empirical means using known compounds.

THE region of the infrared spectrum which is most commonly used at present extends from 2 to 16 microns and is known as the "rock-salt region," since it is the region of high transmission of sodium chlo-

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