in all proportions below the boiling point of the solvent and would therefore be satisfactory solvents so far as the extraction step is concerned. It should be kept in mind that the presence in the oil of fatty acids, carbohydrates, and phosphatides may considerably alter the oil-alcohol solubility relation.

Figures 4 and 5 however show that the efficiency of the separation of the oil from the solvent at 30°C. increases as the percentage of water in the alcoholic solvent increases. Therefore from the point of view of the complete process 98.4% ethanol or 90% 2-propanol would theoretically be the optimum concentrations for use as solvent. Practically, the percentage of water in the system would be difficult to control precisely because it would depend upon the amount of moisture in the original and extracted cottonseed (2). In any case the constant boiling mixtures of ethanol (95.6%) and 2-propanol (87.9%) would present the disadvantage of requiring pressures above atmospheric during the extraction in order to attain complete miscibility with the oil.

Summary

Basic phase relation data have been obtained relative to the extraction of cottonseed oil with ethanol and 2-propanol, especially as affected by water in the solvent. Mutual solubility diagrams have been constructed for cottonseed oil with ethanol and 2-propanol of various aqueous concentrations. Tie-line data at 30° C. have been obtained for the ternary ethanol-cottonseed oil-water and 2-propanol-cottonseed oil-water systems. These combined data will be of assistance in the selection of the most desirable temperatures and moisture concentrations in the solvent extraction of cottonseed with these alcohols. Comparison with results previously published for soybean oil suggests that the mutual solubility data for cottonseed oil and aqueous ethanols are applicable to other vegetable oils over a wide range of iodine values.

In general, the results indicate that 2-propanol is the more desirable solvent since complete miscibility with the oil can be attained at temperatures below its normal boiling point even at moisture contents as high as 10% by weight whereas ethanol can tolerate only about 1.5% of water. High moisture contents result in more effective separation of the oil from the solvent when the miscella is cooled after extraction. Constant boiling aqueous ethanol and 2-propanol present the disadvantage of requiring greater than atmospheric pressure during extraction in order to attain complete miscibility with the oil.

Acknowledgments

The authors wish to thank Henry J. Portas, René L. Durr, and Robert R. Mod for their assistance in determining some of the solution temperatures, Robert Demint for the Karl Fischer moisture values, and the Analytical Section for the Wijs iodine values.

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[Received March 31, 1953]

The Compositions of Some Unhydrolyzed Naturally Occurring Waxes, Calculated Using Functional Group Analysis and Fractionation by Molecular Distillation, with a Note on the Saponification of Carnauba Wax and the Composition of the Resulting Fractions[1]

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THE biological and economic importance of the naturally occurring waxes is largely a result of their unique physical properties. In spite of much fine work in the past 140 years, resulting in the identification of numerous compounds isolated from many of the naturally occurring waxes, there is still not one of these complex mixtures whose exact chemical composition is known. Aliphatic acids, hydroxy acids, primary and secondary aliphatic alcohols, sterols, ketones, and hydrocarbons have been isolated in varying amounts from many waxes after initial saponification. But it is not enough to know the chemical composition of the hydrolysis products if we are to understand and possibly duplicate the unique physical properties of the waxes. We must also know the extent and manner of combination of the acids and alcohols.

Isolation of one or more substances from an unhydrolyzed wax may be used to establish a partial knowledge of its composition. For example, extraction (20) and adsorption separation (chromatography) (2, 17)

[1] A portion of a dissertation submitted by Thomas Wagner Findley to the Graduate School of the Ohio State University in partial fulfillment of the requirements for the Ph.D. degree.

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have been used to isolate homologous groups of compounds from unhydrolyzed waxes and similar mixtures.

For substances whose molecules have a definite orientation in the wax specimen, Kreger (14) has indicated that x-ray diffraction affords a method of identification. He was able to identify compounds or groups of compounds in 39 of the 60 waxes he examined. Most of these constituents were not esters, and some of them have never been isolated from any wax.

Warth (22) has reported approximate compositions for a great number of unhydrolyzed waxes. These were apparently arbitrary compositions consistent with saponification, acid and iodine numbers, amount of unsaponifiable material and compounds previously reported to have been isolated and identified from the wax in question. There was little or no evidence supporting the existence of many of the esters he asserted to be present in the waxes. Neither was there any evidence in conflict with his compositions.

Bertram (1) has recently made some more profound calculations on the available data about wool wax, using hydroxyl values of the wax and of the hydrolysis products in addition to the above mentioned constants. Realizing that a calculation of composition on the basis of these figures was still arbitrary, he has cryoscopically determined mean molecular weights of the wax samples and of their hydrolysis products. These point to one of the two arbitrary compositions which he first calculated as being more nearly correct than the other, hence to the presence in wool wax of a considerable amount of an hydroxy diester (two molecules of hydroxy acid plus one molecule of simple alcohol) or even of a higher polymer of the hydroxy acids. Warth, by contrast, had reported predominately simple esters and less than 1% of a lactone. Bertram's appears to be the first use of molecular weight determinations in solving the composition of a natural wax.

This paper is written to show how the results of functional group analysis and molecular weights of some unhydrolyzed waxes and fractions (simpler mixtures) from them can be applied to the calculation of their compositions. The fractionation has been carried out by molecular distillation.

Methods of Analysis for Functional Groups in Waxes

We found no procedure in the literature which could be used directly on waxes for the determination of the carbonyl group in aldehydes or ketones, and no generally acceptable procedures for ester or hydroxyl groups. With changes required by the refractory nature of high molecular weight compounds present in waxes, by their complete insolubility in solvents even partially aqueous, and by the limited amounts of some of our samples, certain existing methods for the determination of these groups in organic compounds could be adapted to give good results with small samples of a variety of naturally occurring waxes. These procedures are reported in detail.

Acid and Ester Groups

It has long been known that complete saponification of waxes requires a better solvent for the saponification products than ethanol. The studies of Koonce (11) on the saponification of carnauba wax in ethanol-toluene showed that the optimum effective alkali concentration was 0.25 N and the optimum time of refluxing 90 minutes. We have found that, for the saponification of sugar cane cuticle wax, this medium gives higher and more consistent results than the method of Knight (10), who used ethylene glycol as solvent, although both methods work equally well for carnauba wax. We have altered Koonce's method for the use of a smaller sample and for the titration of free acid before saponification of the ester.

Procedure. A sample of wax (0.1-0.5 g.) was dissolved in 5 ml. of warm pure toluene in a 250-ml. Erlenmeyer flask, and the free acid was titrated with N/2 alcoholic KOH with phenolphthalein as an indicator. With dark samples thymolphthalein was used. The alkali was added from a 10-ml. micro-burette, protected against atmospheric CO_2 with an Ascarite tube. After recording the burette reading, additional alkali was added to make a total of 5.000 ml. The sample was then refluxed under an air condenser over a hot plate for 90 min. Fifty ml. of neutral alcohol was then added and excess alkali titrated hot with N/10 HCl. Blanks were run at the same time and the size of sample was taken so that not over half of the alkali was used in the saponification.

Hydroxyl Groups

Hydroxyl groups have usually been estimated in waxes by acetylating with acetic anhydride, determining the saponification numbers of the acetylated wax and of the original wax and the necessary calculation from these data. The determination by direct acetylation has not been successful with waxes because the mixed anhydrides formed by reaction of the free wax acids and the acetic anhydride are difficult to hydrolyze quantitatively before titration. In the improved West pyridine-acetic anhydride procedure of Ogg, Porter, and Willits (16) the wax is completely precipitated by the water added to decompose the excess acid anhydride. The failure of this method to give good precision with waxes is probably due to incomplete hydrolysis of the mixed anhydrides. In our modified procedure we have added water for hydrolysis in a pyridine solution with consequent maintenance of homogeneity and better results.

Reagents. Pyridine-acetic anhydride was prepared by dissolving dry redistilled acetic anhydride in 10% solution in dry redistilled pyridine. The water-pyridine was a 10% solution of distilled water in C. P. pyridine.

Procedure. The wax (0.1-0.5 g.) was weighed into a 125-ml. Erlenmeyer flask fitted with a Ŧ stopper. Two ml. of anhydride reagent were added from an automatic pipette. The flask was stoppered and heated at 100°C. for 1 hr., after first moistening the stopper with pyridine to prevent loss of anhydride during this reaction. Ten ml. of water-pyridine reagent were added and heating continued for 10 min. Twenty-five ml. of 1-1 butanol-toluene solvent were added. After the sample was dissolved, the excess acetic acid was titrated with N/2 alcoholic KOH. The size of the sample was so taken that not more than half of the acetic anhydride was consumed in the acetylation. The titration was corrected for the amount of free acid in the sample, which must be determined separately. Table I gives the values obtained for some of the compounds analyzed by this method.

TABLE I

Determination of Hydroxyl Groups in Known Compounds

Substance	Found	Theory
	Concentration in moles/kilogram	
Carnauba wax alcohols (12), p-1	2.41	2.44[a]
Dotriacontanol (12)	2.13	2.14
Dihydroxystearic acid m.p. 94.6°C.(19)	6.24	6.32
Behenic acid (4)	0.07	0.00

[a] From molecular weight.

Carbonyl Group

A method for the quantitative determination of carbonyl group in naturally occurring waxes has not been previously reported although ketonic compounds have been isolated from several. The most common procedure for the determination of this functional group is the reaction of the ketone or aldehyde with hydroxylamine hydrochloride to form the oxime and hydrochloric acid. The acid is then titrated with standard base. The method of Bryant and Smith (3), in which pyridine is used as receiver for the acid liberated, could not be used because of interference by the free wax acids with the titration. We have used toluene to dissolve the wax sample and titrated the acid liberated directly with alcoholic alkali.

Reagents. Hydroxylamine hydrochloride reagent, N/2, was prepared by dissolving $NH_2OH \cdot HCl$ in abs. ethanol and neutralizing with alcoholic alkali to thymol blue just before use. The reagent should be prepared fresh each day.

Procedure. A sample of the wax (0.1-0.5 g.) was weighed into a 125-ml. Erlenmeyer flask and dissolved in 5 ml. of warm toluene. A few drops of thymol blue solution were added and then approximately 5 ml. of hydroxylamine hydrochloride reagent were added from a serological pipet. The flask was warmed on a hot plate for 2 minutes and the excess hydrochloric acid titrated with alcoholic alkali. The flask was replaced on the hot plate for 30 min. and again titrated. This was repeated after an hour and if necessary after 4 hours of heating. In some cases it was necessary to heat overnight to get complete reaction. Total amount of base minus the small blank was used to calculate results. Table II gives the values obtained for some of the substances analyzed.

Acetals, ketals, iron salts (18) and peroxides (21) have been reported to interfere with this determination. We have tested a number of known compounds in order to extend the known specificity of the reaction with hydroxylamine hydrochloride (see Table II). Hydroxyl groups and triple bonds do not interfere. Carboxyl groups of acids unsubstituted in the α-position do not interfere. Neither do those of α-hydroxy acids, but α-keto acids cannot be determined by this method as the carboxyl group is so strong as to interfere with the titration of HCl under these conditions. Some esters do give slight reactions with the reagent [see Table II and (21)]. This is perhaps to be expected as the reaction of esters with hydroxylamine in alkaline solution is the basis for a quantitative determination of the ester group (7).

There was little or no carbonyl group in any of the waxes studied in the experiments reported here so the fractions of the waxes were not analyzed for this functional group.

TABLE II

Determination of Carbonyl Groups in Known Substances

Substance	Found	Theory
	Concentration in moles/kilogram	
4-Ketostearic acid (6)	3.33	3.35
12-Ketostearic acid (6)	3.07	3.14[a]
2-Ketostearic acid (6)	.....[b]	.....[b]
9,10-Diketostearic acid (9)	5.29	6.31
9,10-Dihydroxystearic acid (19)	0.00	0.00
Lactic acid	0.00	0.00
Stearolic acid (9)	0.00	0.00
Cholesteryl palmitate	0.22	0.00

[a] Still contains 6.3% 12-hydroxystearic acid, as shown by OH group determination and m.p.

[b] Is too strong an acid to be determined.

Mean Molecular Weights

Molecular weights were determined by the Rast method of freezing point depression. Cholesteryl palmitate and behenic acid were used to determine the molecular depression constants.

Concentration Units

For purposes of comparison and calculations, concentrations of all functional groups had to be reported in the same units, moles per kilogram. These concentrations may readily be converted to the more conventional units. To convert concentration of acid, ester, or hydroxyl group to acid, ester, or hydroxyl number, simply multiply by 56.104. Saponification number is, of course, the sum of the acid and ester numbers. To convert concentration of unsaturation to iodine number, multiply by 25.38. Cryoscopically determined mean molecular weights were reported as total concentrations in moles per kilogram, of all compounds for comparison and calculations. These can be converted to mean molecular weights by dividing into 1,000.

Fractionation of Waxes by Molecular Distillation

There is a wide variation in molecular weights and in vapor pressures of the constituents of waxes: esters *vs.* hydrocarbons, free acids, and alcohols. Hence we expected that there would be a great difference in the chemical composition of the first and last fractions in the distillation of these complex mixtures.

Beeswax and carnauba wax have been successfully distilled in a commercial type centrifugal still (15). No analyses were run on the fractions from these distillations however, and there was no evidence proving fractionation.

Because of the solid nature of the waxes to be distilled, a pot still with removable top was used in preference to a falling film still. Agitation of the molten distilland with a magnetic stirrer together with a device for changing the distance between condenser and the bottom of the still permitted distillation of much larger quantities than would otherwise have been possible.

Apparatus

Two still pots were designed and constructed, one 7 cm. in diameter and a larger one 12 cm. in diameter (Figure 1). The condensing heads could be removed whenever a desired fraction had distilled and replaced with clean ones for further distillation while the first fraction was weighed and recovered. Brass washers were made to fit between the ground surface of the still pot and the head so that the distance between the

FIG. 1. Still pot for molecular distillation of waxes with metal head, brass washers, and magnetic stirring bar.

bottom of the pot and the condensing surface could be varied between 1.5 and 4.5 cm. at intervals of 0.5 cm.

The complete distillation apparatus is pictured in Figure 2. The still pot was heated in a Wood's metal bath in a non-magnetic stainless steel dish resting on a small electric heating coil insulated underneath with asbestos. The coil in turn rested on the base of a magnetic stirrer. The stirring bar, enclosed in glass and placed in the pot to stir the molten wax, was easily turned by the revolving magnetic field. Tap water was circulated in the condensing head of the still pot.

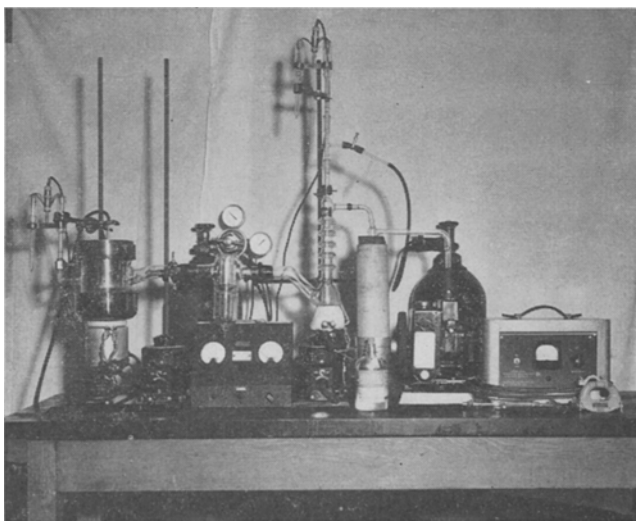

FIG. 2. Molecular distillation apparatus.

The vacuum system consisted of a one-stage, oil diffusion pump (D.P.I., G-4), using a silicone diffusion oil (Dow-Corning 702) and a Welch Duoseal mechanical forepump. Dry ice traps were placed between pumps and between still and diffusion pump. Pirani gauge filaments were placed in the system at two places, between the pumps to measure forepressure and at the pot still to measure the ultimate pressure. The joints between pot, traps, and pumps were made with beeswax-rosin sealing compound strengthened with an aluminum strip. The seal between the ground glass surface of the pot and the ground surfaces of washers was lubricated with a high vacuum silicone grease and gave little trouble with leakage. Ultimate pressure in the empty system complete with washers was as low as 0.005 microns of mercury (measured with a cold cathode ionization gauge) and the forepressure about two microns of mercury. When any decomposition of material in the pot was accompanied by evolution of gases, the forepressure rose, occasionally to as high as 30 microns.

An inlet for the introduction of nitrogen to the system, which was used whenever fractions were to be changed, was placed immediately above the diffusion pump.

Preparatory to any distillation the solid sample was introduced into the pot and the system flushed out with nitrogen. The hot (100°C.) metal bath was then raised to melt the sample. The stirrer was started and the forepump turned on intermittently to lower the pressure in the system slowly. This allowed the sample to degas slowly, preventing spattering of the wax. The temperature was then raised and the distillation begun by further lowering the pressure with the diffusion pump. Fractions were removed from the head with hot benzene. The solution was transferred to a tared beaker and concentrated to a 50% solution on the hot plate, then cooled; and the remainder of the solvent was removed in a vacuum desiccator under a gentle stream of air. This left the wax fraction in a porous condition and allowed complete removal of all solvent. The distance between the condenser and the surface of the molten wax was kept as close to 1.5 cm. as possible by removing brass washers as the liquid level in the pot went down.

Materials

The ordinary yellow beeswax of commerce was prepared for vacuum distillation by melting, mixing, and holding it molten in a vacuum desiccator until the first vigorous bubbling of escaping gases ceased. A time of about two hours was required. It melted at 50-65°C.

Caranda wax was supplied by Wallace Windus of the Imex Corporation. It was collected in the northern part of Paraguay near the Paraguay River. This sample had a green color and pleasant odor, m.p. 74-81°C.

Crude commercial candelilla wax, m.p. 60-72° C., was supplied by Innis Speiden and Company. Refined candelilla wax, m.p. 60-73°C., was supplied by Cornelius Products Company. Ouricury wax, m.p. 81-86.5° C. was supplied by J. V. Steinle of S. C. Johnson and Son Inc.

Distillation of Waxes

Beeswax. Beeswax (10.01 g.) was placed in the 7-cm. still pot and degassed at 90°C. for 20 minutes, when all ebullition had ceased. Fractions were collected as shown in Table III.

Fractions distilling at 150° C. were combined for analysis, as were fractions distilling at 250°C. Constants on the resulting three fractions and on the starting wax are shown in Table IV.

Caranda Wax. Caranda wax (10.48 g.) was degassed in the 7-cm. still pot in about 40 minutes under steadily increasing vacuum at 150°C. Appreciable quantities of water were given off. Fractions were collected as shown in Table III. Fractions distilling

at 150°C. were combined, those distilling at 250°C. were combined, and the resulting three fractions and the original wax were analyzed as shown in Table IV.

Candelilla Wax. Crude candelilla wax (10.106 g.) was degassed in the 7-cm. still pot for one hour at 100°C. An attempt was made to distil at 100°C. because of the high content of hydrocarbons previously reported in this wax (17), but the rate of distillation was so slow that the temperature was raised to 150°C. and fractions collected as shown in Table III. At 250°C. there was decomposition of the wax, as evidenced by a rise in pressure to 2 microns and a rise in the forepressure to 30 microns. There was also a

TABLE III

Molecular Distillation of Waxes

Wax	Fract.	Temp.	Press.	Time	Weight	% of charge	m.p.
		°C.	*microns of Hg.*	*hrs.*	*grams*		°C.
Beeswax	1	150±10	.....[a]	4	2.675	28.7	52-67
	2	150±10	.....[a]	1	0.197		
	3	250±10	.....[a]	4	5.305	56.5	59-67
	4	250±10	.....[a]	5	0.345		
	Res.	.....	.....	....	1.485	14.9	57-64
					10.008		
Caranda	1	150±5	3-0.2	5	1.22	13.3	76-81
	2	150±5	0.2	1	0.17		
	3	250±10	2-0.2	2	2.35	60.4	78-81
	4	250±10	0.2	4	2.37		
	5	250±10	0.2	18	1.62		
	Res.	.....	.....	....	2.49	23.7	87.5
					10.22		
Crude candelilla	1	150±10	1-0.1	2	5.34	58.1	60-67
	2	150±10	.....[a]	1	0.49		
	3	150±10	.....[a]	1	0.09		
	4	250±10	2.0	1/12	0.68	39.6	60-79
	Res.	.....	.....	....	3.32		
					9.92		
Refined candelilla	1	150±2	5-2	3	27.4	54.8	60-65.5
	2	145—50	2-0.5	3	1.5	3.0	65-80
	Res.	.....	.....	....	20.4	40.8	63-70
					49.3		
Ouricury	1	140—50	.....[a]	4	1.477	15.9	82-143
	2	150	.....[a]	1	0.112		
	3	235—50	4-2	5	2.852	28.5	80-84
	Res.	.....	.....	....	5.13	51.3	84-86
					9.57		

[a] Pressure here is less than 0.1μ.

good deal of ebullition so that most of fraction 4 was carried over mechanically. The distillation was therefore stopped and fraction 4 recombined with the residue when those distilling at 150°C. were combined for analysis. Analyses of the two fractions and the starting wax are reported in Table IV.

Refined candelilla wax (50.0 g.) was degassed in the 12-cm. still pot for 30 minutes. Distillation was then carried out at 150°C., but not at 250°C. because of the decomposition noted previously. Fractions were collected as shown in Table III. Analyses of these and of the starting wax are shown in Table IV.

Ouricury Wax. Ouricury wax (10.0 g.) was degassed in the 7-cm. still pot at 95°C. for one hour. Fractions were then distilled as shown in Table III. There was no evidence of decomposition at 150°C., but when the temperature was raised to 250°C. for the third fraction, the ultimate pressure rose to 4 microns and the forepressure rose from 8 to 40 microns. Some of fraction 3 was carried over mechanically. Fractions distilling at 150°C. were combined and the resulting three fractions and the original wax analyzed as shown in Table IV.

TABLE IV

Analyses of Fractions from Molecular Distillation of Waxes

Wax fraction	Acid group[a]	Ester group[a]	Hydroxyl group[a]	Unsaturated[b]	Total all comps.[c]	Mole % of charge[d]
	Concentrations in moles/kilogram					
Beeswax	0.35	1.32	0.27	0.48	1.89	.....
150°	0.83	0.50	0.07	0.59	2.94	43.3
250°	0.13	1.64	0.27	0.37	1.66	48.1
Residue	0.06	2.41	0.14	0.60	1.13	8.6
Weighted av.	0.32	1.43	0.19	0.47	1.95	.....
Caranda wax	0.09	1.31	0.65	0.35	1.49	.....
150°	0.21	0.27	1.81	0.75	2.42	21.6
250°	0.06	1.20	0.40	0.22	1.49	60.4
Residue	0.04	2.21	0.16	0.57	1.20	19.1
Weighted av.	0.08	1.32	0.53	0.38	1.55	.....
Crude candelilla	0.36	0.65	0.41	1.10	1.88	.....
150°	0.23	0.05	0.41	1.33	2.19	67.7
Residue	0.46	1.65	0.36	1.34	1.24	26.1
Weighted av.	0.32	0.70	0.39	1.33	1.81	.....
Ref. candelilla	0.36	0.53	0.28	0.91	1.96	.....
Frn. 1 (150°)	0.14	0.06	0.31	0.78	2.50	69.9
Frn. 2 (150°)	1.05	0.20	0.41	0.85	2.41	3.7
Residue	0.45	1.27	0.21	0.91	1.87	32.7
Weighted av.	0.30	0.56	0.27	0.84	2.11	.....
Ouricury	0.51	1.50	1.11	0.63	1.58	.....
150°	0.36	0.86	0.59	1.36	2.47	24.9
250°	0.28	1.26	1.07	0.37	1.61	28.0
Residue	0.22	1.81	0.70	0.47	0.76	24.6
Weighted av.	0.26	1.49	0.79	0.58	1.30	.....

[a] Multiply by 56.104 to convert concentration of acid, ester, or hydroxyl groups in moles/kilogram to acid number, ester number, or hydroxyl number.
[b] Multiply by 25.38 to convert to iodine number.
[c] Divide into 1,000 to obtain mean molecular weights.
[d] Does not total 100% for some waxes because of change of a few % in mean molecular weights on distillation.

Saponification of Carnauba Wax

Quantitative separation and determination of unsaponifiable material and acids in a wax usually require two quantitative transfers of dried materials, one of dried saponification residue to extractor, the other from extraction thimble prior to acidification of the soaps. We have used a procedure which eliminates the second transfer and simplifies the acidification of the soaps. This is accomplished by extracting the unsaponifiable matterial with ether in a Soxhlet extractor in the usual way and then replacing the boiling flask with one containing ether acidified with HCl. The HCl-ether complex is sufficiently volatile to distil into the extracting thimble where it completely acidifies the soaps, and the ether and HCl are readily evaporated from the acids collected. This method has the further advantage of separation of all ether-insoluble material, such as inorganic salts, which are left in the thimble. It has the disadvantage of allowing hydroxy acids, if present, to esterify in the collecting flask.

Carnauba wax was obtained from S. C. Johnson and Son Inc. It was the No. 3 chalky grade. A weighed sample (5.0 g.) of carnauba wax was saponified by refluxing with 1 ml. of 18.5 N NaOH solution, 20 ml. absolute ethanol, and 20 ml. benzene for 20 hours. The solution was concentrated, transferred to an evaporating dish with hot benzene, evaporated, and dried completely in a vacuum desiccator. The dried residue was transferred as quantitatively as possible (loss no more than 10 mg.) to a Soxhlet extractor thimble and unsaponifiable material was extracted with absolute ether for 100 hours. The solvent flask was then exchanged for one containing ether plus 2 ml. concentrated HCl and extraction continued for 48 hours. Yields and properties of the two fractions are found in Table V.

The yields of the acids and unsaponifiable material obtained from the same shipment of carnauba wax by

Koonce and Brown (12, 13) are included for comparison. The lower yield of acids reported by them is probably due to loss in transfer and acidification of the soaps.

TABLE V

Yields and Properties of Fractions from Saponification of Carnauba Wax

	Carnauba wax	Unsaponifiable material	Acids
Weight, grams	5.0	2.55	2.52
Yield, %	.....	51.0	50.0
Yield, % (12, 13)	.....	54.0	43.0
M.p., °C.	80-84.5	83.5-84.5	75-85
Acid [a]	0.07	0.00	0.98
Ester [a]	1.39	0.00	1.84
Hydroxyl [a]	0.69	2.43	0.44
Total moles [a]	1.29	2.73	1.81

[a] Conc. in moles/kg.

Calculation of Compositions

The concentrations of functional groups and mean molecular weights determined on the waxes and fractions from the molecular distillations just described yield a clearer picture of the compositions of these waxes than has hitherto been obtainable. With certain assumptions about the distillation fractions it is possible to calculate compositions for the waxes. We will go through these calculations and discuss the assumptions for beeswax in detail. For the other waxes the assumptions and methods of calculation are the same. Only the calculated compositions will be given.

Composition of Beeswax. The analytical constants for the unfractionated beeswax reported in Table IV show that the sum of concentrations of acid, ester, and hydroxyl groups (0.35 plus 1.32 plus 0.27 equals 1.94 moles per kilogram) is about equal to the total concentration of all compounds (1.89 moles per kilogram). These constants could be exhibited by a mixture of acids, alcohols, and esters, or by the same mixture plus equimolal quantities of hydrocarbons and bifunctional compounds (or smaller amounts of polyfunctional compounds). Since both hydrocarbons and an hydroxy acid (5, 8) have been isolated from beeswax by earlier investigators, the latter composition would seem more likely, even without consideration of our data on the distilled fractions.

The fraction of beeswax distilling at 150°C. has an excess of hydrocarbons, shown by the fact that the total concentration of all compounds (2.94 moles per kilogram) is much greater than the sum of the concentrations of acid, ester, and hydroxyl groups (0.83 plus 0.07 plus 0.50 equals 1.40 moles per kilogram). If we assume that this fraction contains only nonfunctional compounds (hydrocarbons) and monofunctional compounds (free acids, free alcohols, and simple esters), we can calculate its composition in percentage of each group, with results as shown below and in Table VI.

Free acids = conc. of acid group/total conc. all compounds$\times 100 = 0.83/2.94 \times 100 = 28.2$ mole %

Free alcohols=conc. of alcohol group/total conc. all compounds$\times 100 = 0.07/2.94 \times 100 = 2.4$ mole %

Esters = conc. of ester group/total conc. all compounds$\times 100 = 0.50/2.94 \times 100 = 17.0$ mole %

Hydrocarbons = 100 − (% acids + % alcohols + % esters) = 52.4 mole %.

TABLE VI

Calculated Compositions of Fractions from Waxes and of the Unhydrolyzed Waxes

Wax fraction	Hydrocarbons	Free alcohols	Free acids	Esters	Hydroxy esters	Acid esters	Diesters	Acid diesters	Hydroxy diesters
	All values in moles % [a]								
150° Beeswax	54	2	28	17	0	0	0	0	0
250° Beeswax	0	0	0	76	16	8	0	0	0
Residue beeswax [b]	0	0	0	0	0	0	67	5	12
Beeswax [c]	23	1	12	45	8	4	6	1	1
150° Caranda wax	5	75	9	11	0	0	0	0	0
250° Caranda wax	0	16	4	69	11	0	0	0	0
Residue Caranda wax	0	0	0	0	13	3	83	0	0
Caranda wax	1	26	4	44	9	1	16	0	0
150° Crude candelilla	69	19	10	2	0	0	0	0	0
Residue crude candelilla	0	0	0	1	29	37	33	0	0
Crude candelilla wax	46	13	7	2	8	10	9	0	0
Frn. 1 (150°) ref. cand	80	12	6	2	0	0	0	0	0
Frn. 2 (150°) ref. cand	31	17	44	8	0	0	0	0	0
Residue refined candelilla	0	13	6	58	23	0	0	0	0
Refined candelilla wax	57	14	7	21	8	0	0	0	0
150° Ouricury wax	27	24	15	35	0	0	0	0	0
250° Ouricury wax	0	22	0	16	45	17	0	0	0
Residue ouricury wax [d]	0	0	0	0	0	0	8	0	25
Ouricury wax	7	....	....	....	....	....	....	....	....
Unsap. carnauba wax	11	89	0	0	0	0	0	0	0
Acids carnauba wax	0	0	0	44 [e]	0	54 [f]	2 [g]	0	0
Carnauba wax	12	0	0	10	53	5	19	0	0

[a] Totals for some of the waxes are not exactly 100% because of molecular weight change of a few per cent during distillation.
[b] 13% Triester.
[c] 1% Triester.
[d] 29% Acid hydroxy diester and 38% hydroxy triester.
[e] Lactone.
[f] Includes 24% acid hydroxy ester.
[g] Lactide.

The fraction of beeswax distilling at 250°C. has an excess of bifunctional compounds, shown by the fact that the total concentration of all compounds (1.66 moles per kilogram) is less than the sum of the concentration of acid, ester, and hydroxyl groups (0.13 + 1.64 + 0.27 = 2.04 moles per kilogram). If we assume that

a) the bifunctional compounds are esters of hydroxy acids with simple acids and alcohols,
b) this fraction contains no nonfunctional compounds, trifunctional compounds, or ester made up of more than two monomeric units,
c) no free hydroxy acids are present,

we can calculate the composition in mole percentage of each group as shown below and in Table VI.

Acid esters = conc. of acid group/total conc. all compounds$\times 100 = 0.13/1.66 \times 100 = 7.9$ mole %

Hydroxy esters = conc. of hydroxyl group/total conc. all compounds$\times 100 = 0.27/1.66 \times 100 = 16.4$ mole %

Simple esters = 100 − (% acid esters + % hydroxy esters) = 75.7 mole%.

Likewise the composition of the residue can be calculated on the assumption that it contains only diesters and trifunctional compounds.

Acid diesters=conc. of acid group/total conc. all compounds$\times 100 = 0.06/1.13 \times 100 = 5.3$ mole %

Hydroxy diesters = conc. hydroxyl group/total conc. all compounds$\times 100 = 0.14/1.13 \times 100 = 12.4$ mole %

Triester = (conc. ester group − 2)/(total conc. all compounds) × 100 = (2.41 − 2)/(1.13) × 100 = 13.3 mole %

Diester = 100 − (% acid diester + % hydroxy diester + % triester) = 66.7 mole %.

The assumptions made above allow calculation of compositions of the fractions, and from them of the original wax, that cannot be far from the true picture. In such a mixture the hydrocarbons would be the first to distil, followed by free acids and alcohols, then esters, and finally by acid and hydroxy esters. It seems less than likely that any trifunctional compounds or diesters would distil at all. The presence of appreciable amounts of free hydroxy acids would result in extensive esterification during the distillation. That this esterification has not happened is demonstrated by comparison of the functional group analyses of the original wax with the weighted average of the distillation fractions. The absence of any reaction during the distillation permits us to combine the compositions of the three fractions to get the composition of the original beeswax, reported in Table VI.

These data and assumptions allow the calculation that the fatty acids of this beeswax are 25% hydroxy acids, a figure which compares favorably with the 20% reported by Ikuta (8) for Japanese beeswax.

Composition of Caranda Wax. The compositions of the fraction distilling at 150°C. and of the residue are calculated in the same manner as for beeswax. The fraction distilling at 250°C. has a lower concentration of ester group than of all compounds so it must include free acids and/or alcohols as well as esters and acid esters and/or hydroxy esters. This leads to a system of five unknowns in four equations so we have arbitrarily selected the simplest composition to report.

We can also calculate that the fatty acids of this caranda wax are 41% hydroxy acids using these data and assumptions.

Composition of Candelilla Wax. The analytical data on fractions from the two candelilla wax distillations are treated in the same manner as discussed above to obtain the compositions reported in Table VI.

Based on these data and assumptions, the fatty acids of the crude candelilla wax are 48% hydroxy acids, those of the refined candelilla wax 17%.

Composition of Ouricury Wax. The fractions from the molecular distillation of ouricury wax are treated in the same way as the waxes discussed above, but because of the change in composition during the distillation, the compositions of these fractions could not be combined to give the true composition of the original wax, except for the hydrocarbon content. We believe that this wax contains appreciable amounts of hydroxy acids, free and combined as estolides, resulting in decomposition on distillation.

It can be calculated from these data that if the bifunctional units of this wax are all hydroxy acids, the total acids are 81% hydroxy acids. This is consistent with Koonce's finding (11) that ouricury wax contains only 26.8% of unsaponifiable material. The high melting range and high unsaturation of the 150° C. fraction from this distillation may be due to the presence of the high melting (205°C.) unsaturated compound which Koonce isolated from the wax.

Composition of Carnauba Wax. The unsaponifiable fraction of carnauba wax contains 2.43 moles per kilogram of hydroxyl group (of alcohols if we assume only monohydroxy alcohols), no acid nor ester, and consequently 2.73 − 2.43 = 0.30 moles/kilo of hydrocarbons. Hence the original wax contains 0.30 × 0.51 = 0.15 moles per kilo of hydrocarbons. The wax then contains 1.29 − 0.15 = 1.14 moles/kilo of mono-, di-, or polyfunctional compounds. But it also contains 0.69 + 0.07 + 1.39 = 2.15 moles/kilo of the functional groups acid, hydroxyl, and ester. The simplest mixture that would yield these analytical results is a mixture of 0.13 moles/kilo of monofunctional compounds (acids, alcohols, esters) and 1.01 moles/kilo of bifunctional compounds (acid ester, hydroxy ester, diester). Since the amount of monofunctional compound is low and the percentage of ester group is high, we will further simplify by assuming all the monofunctional compound to be ester. This leads to the tentative composition for carnauba wax given in Table VI.

It can be further calculated that the fatty acids of this carnauba wax are 60-80% hydroxy acids, on the basis of the data and assumptions used here. This is in agreement with the work of Koonce and Brown (13) on this wax.

The composition reported here for carnauba wax is more arbitrary and hence less reliable than those for the other waxes that were fractionated by molecular distillation. Nevertheless we believe that it comes closer to the true composition of the wax than that reported by Warth (22).

Acknowledgments

We are indebted to J. V. Steinle, and the S. C. Johnson and Son Inc., Racine, Wisconsin, for the financial support which made this investigation possible, and to E. S. McLoud of this company for his counsel and advice throughout the course of the investigation.

Summary

Methods for the determination of acid, ester, hydroxyl, and ketone (or aldehyde) groups and of mean molecular weights of small samples of natural waxes are reported. Complete analyses can be made on 0.5 g. of sample. A simplified procedure for quantitative separation of acid and unsaponifiable fractions of a wax is also reported.

Molecular distillations of beeswax, caranda wax, crude and refined candelilla wax, and ouricury wax have fractionated these complex mixtures into simpler ones. Hydrocarbons and free unsubstituted alcohols and acids, if present, distil readily at 150°C. A pot still suitable for convenient molecular distillation of up to 100-g. charges of waxes or other high melting materials is described.

A method for the calculation of composition of unhydrolyzed waxes based upon function group analysis of molecular distillation fractions is described. Results of application of this method to the waxes distilled are reported and show the ubiquitousness of hydroxy acids. All of the above waxes and carnauba wax contain major proportions of esters of the hydroxy acids, and none contains as much as one-half simple esters of unsubstituted acids and alcohols.

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[Received June 20, 1952]

Factors Affecting the Stability of Crude Oils of 16 Varieties of Peanuts

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MARKED differences have been noted in the stabilities of oil of raw peanuts and roasted peanut products. Crawford and Hilditch summarized (3) information reported on composition of groundnut oils by various workers during the past 30 years. They called attention to the extreme differences from about 65% to 40% in oleic acid content and from about 18% to nearly 40% in linoleic acid content of such oils and commented that these differences might be expected to influence the relative susceptibility of the oils to oxidative rancidity. Pickett and Holley have reported (17) a greater development of peroxides in Spanish peanuts than in either Runner or Virginia peanuts on aeration and heating of the nuts at 98°C. They noted that tocopherols and other substances which affect the stability of vegetable oils have been found in peanut oil and called attention to the findings of Jamieson *et al.* (10) that oil in Spanish peanuts contained slightly less olein and more linolein than that from Virginia peanuts. Higgins *et al.* reported (8) a wide variation in the linolein and olein contents of some selected strains of Spanish and Runner peanuts. Examination of their data shows that, as a group, runner type peanuts are lower in percentage of linolein than the bunch type of peanuts.

No information has been published in which composition and stability have been determined simultaneously for crude oils from known varieties of peanuts for the purpose of relating stability to composition. In the present work the oils of 16 varieties of raw shelled peanuts, including both the bunch and runner types, have been analyzed for initial peroxide value and stability, tocopherol content, and saturated, linoleic, and oleic glyceride contents in order to determine factors that may affect the stability of crude peanut oil.

Experimental

Analysis for Moisture and Oil Content. The moisture and oil contents of the peanuts were determined by method Ab 3-49 of "The Official and Tentative Methods of the American Oil Chemists' Society" (1). Results are shown in Table I.

Extraction of Oil from Peanuts. A 600-gram sample of each variety of peanuts with the seed coat intact was sliced in a Henry slicer[2] and extracted with *ca.* 1,200 ml. of commercial pentane (Skellysolve F)[2] at room temperature. The extracted seeds were dried at room temperature, reduced to a powder and reextracted with *ca.* 1,200 ml. of pentane. The oil was freed of solvent by heating the miscella under vacuum on a steam bath and subsequently by stripping with hydrogen under vacuum at temperatures of not more than 60°C. The extracted oils were stored under hydrogen in glass-stoppered bottles at —20°C.

TABLE I

Analysis of Peanuts

Sample No.	Variety[a]	Moisture	Oil	
			As is basis	Oven-dry basis
		%	%	%
1	Spanish 146[b]	6.75	47.72	51.17
2	Spanish 205[b]	6.03	49.96	53.17
3	Spanish P. I. 121070[b]	6.20	49.74	53.03
4	Spanish 18-38[b]	6.13	50.06	53.33
5	Spanish 13-10[b]	6.08	50.26	53.89
6	Improved Spanish 2B[b]	6.17	49.72	52.99
7	Virginia-Ga. Hybrid 119-24[c]	7.10	43.22	46.52
8	Virginia-Holland Station Runner[c]	6.99	44.00	47.31
9	Virginia Bunch, Large[b]	6.79	46.76	50.17
10	Virginia Jumbo J-11-L[c]	7.14	43.69	47.05
11	Virginia P. I. 124681[c]	7.00	45.71	49.15
12	Virginia-Holland Station Jumbo[c]	6.89	43.94	47.17
13	Dixie Runner[c]	5.88	49.46	52.55
14	Runner 230-118[c]	5.86	51.09	54.27
15	N. C. Runner 56-15[c]	6.10	49.04	52.23
16	Runner-Ga. Hybrid 199-22-A-2[c]	5.81	49.95	53.03

[a] Field and handling procedures were in conformity with practices generally followed.
[b] Bunch type.
[c] Runner type.

Methods of Oil Analysis. Iodine values were determined by the American Oil Chemists' Society's modification of the Wijs method (1). Thiocyanogen values were determined by the method described by Lambou and Dollear (12, 13).

The percentages of olein, linolein, and saturated constituents expressed as glycerides were calculated

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

[2] The mention of a trade name in this article is for identification and implies no endorsement or recommendation by the Department of Agriculture for the product.