be obtained by pure chance are computed as 1 in 813. If each oil in this presentation (column IV, Table IV) were considered as being presented individually (probability 1/2), the first and second samples would exhibit significant results only (5% level), and the third sample would be nonsignificant. These results show that when a small number of tasters are employed, it is more difficult to establish significant differences in samples with a chance probability of only 1/2.

The binomial expansion is of limited value in applied statistics for two reasons (6). It is not a continuous curve, and when n is large, the size of the numbers involved in the calculations is too great to be handled. However, for small samples and for probabilities of success appreciably smaller than 1/2, the normal frequency curve fails to give the exact probabilities in which we are interested. We must rely therefore upon the binomial expansion.

Summary

In the evaluation or identification of an edible oil it is shown how the probabilities of chance selection for a single taster are dependent upon the manner in which the samples are presented. The probabilities of 1/2, 1/3, 1/4, 1/6, and 1/8 are all possible, depending upon the method of sample presentation, i.e., as pairs, multiple pairs, odd sample tests, or other combinations.

The expansion of the binomial expression for the probabilities of success and failure for a single taster make it possible to calculate the compound probabilities for taste panels up to 20 members. By knowing the compound probabilities, significance at any level can be easily calculated. Tables showing the number of errors permissible have been calculated for the significant levels of 1% and 5% for panels of 1 to 15 members.

Application and use of the tables, based on data obtained in the identification of simulated soybean oil by a taste panel of 10 members, are presented and the data discussed.

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A Comparison of Several Methods for the Separation of Unsaponifiable Material From Carnauba and Sorghum Grain Waxes[1,2]

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A WHITE crystalline solid isolated (1) from the lipid fraction of sorghum grain subsequently was shown to be a wax which had properties similar to those of carnauba wax (2). In the present investigation several different methods of separating the components of waxes were applied to carnauba and sorghum grain waxes. The results obtained were correlated with the constants for carnauba wax and four different varieties of sorghum grain wax.

Experimental

Purification of solvents. The Skellysolve B, isopropyl ether, benzene, ethyl ether, and acetone used in these experiments were dried over $CaCl_2$ and distilled in an all-glass apparatus.

Extraction and purification of sorghum grain wax. The wax was extracted from either the whole grain or bran. The whole grain was extracted in 5-lb. batches by refluxing on a steam cone for 1 hr. with 2 l. of Skellysolve B. The hot extract was filtered, cooled to 0°C., and the resulting precipitate removed with the aid of a Buchner funnel. Yields of about 0.25% wax were obtained. The sorghum bran was extracted in a large Soxhlet extractor with Skellysolve B for 20 hrs. Bran from the Cody variety yielded 6% of crude wax based on the weight of the bran.

The crude wax was purified by recrystallization from a mixed solvent. A solution of 50 gm. of crude wax in 2 l. of Skellysolve B was filtered through a water-jacketed filter at 70°C., one liter of solvent removed by distillation, 2 l. of acetone added, and the solution placed in a refrigerator at 0°C. for 24 hrs. The purified wax was recovered by filtration. The yield was in the range of 80 to 85% for the varieties tested.

Preparation of calcium stearate. Stearic acid was synthesized from U.S.P. castor oil by the method of Schuette and Roth (3) and converted to the calcium salt.

Determination of wax constants. Acid values were determined by titration of a hot solution of 0.5 gm. of wax in 50 ml. of ethanol and 10 ml. of toluene with 0.05 N KOH in alcohol. The titrations were carried out in an Erlenmeyer flask equipped with a side arm condenser and stirred with a magnetic paddle. Acetyl values were determined by the alkalimetric method of Roberts and Schuette (4), modified to employ a smaller sample. This method was checked by the iodimetric method of Elek and Harte (5). When sufficient sample was available, quintuplicate deter-

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TABLE I

Chemical Properties of Sorghum and Carnauba Waxes

Property	Standard Blackhull	Westland	Cody	Sunrise	Carnauba
Acetyl number	53.3	55.1 ±2.8	44.3 ±3.5	62.9 ±4.4	39.4 ±5.4
Acid number	10.05±0.61	13.80±0.83	16.22±1.85	13.51±0.55	7.18±1.13
Sap. number[a]	16	20	44	25	78
Ester number	6	6	28	11	71
Iodine number[b]	19.35±0.87	20.88±0.40	15.66±0.87	15.69±1.63	9.87±0.54
Melting range (°C.)[c]	78-80	77-82	77-79	79-82	80-84

[a] Determined by the method of Knight (10).
[b] Determined by the Wijs method (11).
[c] Determined by the closed capillary tube method.

minations were made, and the result was expressed as an average value bounded by certain limits. These limits are those within which the true average may be expected to lie 99 times in 100 (6). The results are shown in Table I.

Saponification methods. Four different saponification methods were used. The first procedure, described by Zweig and Taub (7), was used to saponify Westland sorghum grain wax and fatty grey carnauba wax. The waxes were extracted with ethyl alcohol at 25° C., the alcohol-insoluble portions saponified, and the unsaponifiable matter removed by benzene extraction of the potassium soaps. Further separation of alcohols from hydrocarbons was based upon the insolubility of the latter in a mixture of hot amyl alcohol and hydrochloric acid. Results are shown in Table II.

TABLE II

Zweig-Taub Fractions

Fraction	Composition (% of entire wax)		Melting range (°C.)	
	Carnauba	Sorghum	Carnauba	Sorghum
Entire wax	....	....	80-84	77-82
Alcohol soluble fraction	2	11	.....	.....
Alcohol insoluble fraction	98	89	81-84	80-84
Acids	40	16	70-74	71-77
Unsaponifiables	54	67	81-86	76-81
Hydrocarbons	0	48	.....	75-83
Alcohols	54	19	81-86	80-83

The second saponification method, which has been employed by Koonce and Brown (8) for the analysis of carnauba wax, was applied to samples of carnauba wax [5] and Western Blackhull sorghum grain wax. In this procedure the unsaponifiable substances were separated from the potassium soaps by extraction with ethyl ether in a Soxhlet extractor.

The third method was based on the procedure developed by Chibnall *et al.* (9). It was applied to Sunrise sorghum wax and carnauba wax. In this method the waxes were analyzed by extraction of their calcium soaps with hot benzene.

The fourth method was similar to the one developed by Koonce and Brown except that di-isopropyl ether instead of di-ethyl ether was used to extract the calcium soaps. Ten gms. of Westland sorghum wax were dissolved in 250 ml. of hot benzene, 150 ml. of 10% KOH in alcohol added, and the solution refluxed for 12 hrs. A solution of 20 gms. of $CaCl_2$ in 400 ml. of 95% ethyl alcohol then was added, and refluxing continued for three hrs. The solvent was removed by distillation, then the residue ground with water in a mortar and filtered on a Buchner filter to remove inorganic salts. The powdered solid was washed until the washings were no longer alkaline to litmus. The solid then was dried in an oven at 60°C. and extracted in a Soxhlet extractor for eight hrs. with isopropyl ether. This procedure was repeated for fatty grey carnauba wax. The results obtained by the various saponification methods are summarized in Table III.

[5] Supplied by the Johnson Wax Co., Racine, Wis.

Results and Discussion

The results indicated that the four varieties of sorghum grain wax had a higher acetyl value than carnauba wax. In the case of the Cody variety the significance of the difference is doubtful, but the difference is real between the three remaining varieties of sorghum grain wax and carnauba wax. The acid number was significantly higher in the sorghum waxes than in carnauba wax. Calculated on the basis of a molecular weight of 368 (tetracosanoic acid), the amount of free acid was 9% for Westland sorghum wax and 5% for carnauba wax.

Saponification numbers presented the most striking difference between the sorghum grain waxes and carnauba wax. The average value for all the sorghum waxes tested was 26, just one-third of the saponification number of carnauba wax. In order to economize on samples the determinations were made in duplicate, and the usual statistical treatment could not be carried out. However the precision of these determinations is indicated from the average deviation of the duplicate determinations from their mean value. This deviation was 1.7. From the saponification numbers of the sorghum waxes it is evident that either the sorghums contained much less total fatty acid than carnauba wax, or the molecular weight of the sorghum fatty acids was about three times greater than that of the carnauba wax acids. The latter conclusion appeared unlikely because there was not enough difference between the melting points of the acids to

TABLE III

Comparison of Results Obtained From Saponification by Different Methods

Method	First Method		Second Method		Third Method		Fourth Method		Calculated from Sap. No.	
Wax	C	S_1	C	S_2	C	S_3	C	S_1	C	S_1
% Acids in Hydrolyzate	40	16	45	67	42	36	40	22	51	13
% Unsap. in Hydrolyzate	54	67	55	33	56	64	60	78	49	87

C: carnauba wax. S_1: sorghum wax (Westland). S_2: sorghum wax (Western Blackhull). S_3: sorghum wax (Sunrise).

indicate such a large difference between their molecular weights. The melting range for the carnauba wax acids was 70 to 74°C. while that of the sorghum wax acids was 71 to 77°C.

The amount of ester was estimated by assuming that the average value for the molecular weight of the esters of natural waxes is 620, making the percentage of ester in the wax roughly equal to 1.1 times the ester value (11). On the basis of this calculation Westland wax would contain 7% while carnauba wax would contain 78% of ester.

The fractions obtained by the Zweig-Taub system of analysis are in agreement with the wax constants for carnauba and Westland sorghum waxes (Table II). The large ester number obtained for carnauba wax is consistent with the high percentages of acids and alcohols in the Zweig-Taub separations. Westland sorghum wax had a small ester number consistent with the low percentage of acids and alcohols and the large "hydrocarbon" fraction. Carbon and hydrogen analysis of this fraction gave C 83.8%, H 13.8%. It is possible therefore that some oxygen-bearing substance, e.g., a ketone having a molecular weight of about 670, may be the principal constituent of the unsaponifiable fraction. This point is being investigated further.

The method of Koonce and Brown, while satisfactory for carnauba wax, gave erratic results when applied to sorghum wax. Carnauba wax yielded 55% while sorghum wax yielded only 33% of unsaponifiable material (Table III). The apparently large acid fraction thus obtained is inconsistent with both the saponification number and the Zweig-Taub separations. This acid fraction had a molecular weight (Rast) of 600, but its neutral equivalent was 1,800. A small sample of unsaponified sorghum wax was extracted with di-ethyl ether in a Soxhlet extractor for 27 days. Approximately 30% of the sample remained undissolved. It is possible that in the Koonce-Brown procedure some non-acid material may remain insoluble even after saponification and serve as a diluent of the acid fraction. The separation of unsaponifiable substances of sorghum grain wax from Ca salts by extraction with hot benzene gave an unsaponifiable fraction of 64%, agreeing with the value of 67% obtained by the Zweig-Taub procedure.

The most satisfactory method found for separating the acids from the unsaponifiable substances of sorghum wax involved some features of both the Chibnall and the Koonce-Brown procedures. Conversion of potassium soaps to calcium soaps eliminated the necessity of carrying out the extraction under anhydrous conditions. The length of this extraction period was shortened from one month to eight hours by substituting a higher-boiling solvent, di-isopropyl ether, for di-ethyl ether. A test extraction (Soxhlet) of unsaponified sorghum wax with isopropyl ether showed it to be completely soluble within eight hours. Calcium stearate was extracted for 11 hours with this solvent and found to be insoluble.

Summary

The characteristics of carnauba and four varieties of sorghum grain waxes were compared. The results indicated that sorghum grain wax had higher acetyl, acid, and iodine numbers and a lower saponification number than carnauba wax. Sorghum grain wax also contained a lower percentage of esters and nonsaponifiable material than carnauba wax, and a hydrocarbon fraction which was absent in the latter.

Of four methods tested for the quantitative separation of saponifiable and unsaponifiable components, the best separation was obtained by an extraction of the calcium soaps with di-isopropyl ether.

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ABSTRACTS . . . . . Don Whyte, Editor

• Oils and Fats

R. A. Reiners, Abstractor

Unsaturated fat acid content of the serum of normal human adults. A. Chevallier, S. Manuel, C. Burg and J. Rouillard (Univ., Strasbourg, France). *Compt. rend. soc. biol.* **144**, 577-8(1950). Fat extracted from the serum of 40 fasting subjects was examined by a modification of the method of Beadle and Kraybill. It contained 250-950 mg./g. of oleic acid, 15-115 mg./g. of diene acids, 5-35 mg./g. of triene acids, and 35-65 mg./g. of tetraene acids. (*Chem. Abs.* **45**, 238)

Separation of fat acids by selective adsorption. II. Use of silica gel as the adsorbent. J. Gracian and A. Viogue. *Anales fis y quim. (Madrid)* **46**, 375-384(1950). A systematic study of the separation of fat acids using silica gel was made. The effect of chain length, double bonds and the selective capacity of the column used was investigated.

Investigation of the ability of some esters of gallic acid and of a few other substances to prevent rancidification of different fats. P. Kring (Ferrosan Lab., Malmo, Sweden). *Dansk Tids. Farm.* **24**, 211-27(1950). Investigations have been made on the antioxidizing effect of the following phenolic antioxidants: esters of gallic acid, hydroquinone, N.D.G.A., 2,6-di-*tert*-butyl-*p*-cresol and butylated hydroxyanisole (3-*tert*-butyl-4-hydroxyanisole). Experiments were carried out with the antioxidants in lard, peanut oil, soybean oil, cod-liver oil, and peanut oil fortified with vitamin A concentrate. The products were stored in open containers at 35° and the effect of the antioxidants was measured by their ability to prevent formation of peroxide. The effect of the lower esters of gallic acid seems to be somewhat superior to that of the other compounds tested. The effect was enhanced by addition of citric acid and phosphoric acid, which acids also proved to possess a slight effect when used alone. (*Chem. Abs.* **45**, 369)

Sulfurous acid as a catalyst in the fat industry. E. A. M. F. Dahmen, H. I. Waterman and P. M. Heertjes. *Chimie & Industrie* **64**, 557(1950). The advantages of hardening edible fats and oils by elaidinizing them with a sulfur dioxide catalyst instead of hydrogenation is discussed.