curately by the dye-dilution method than by dilatometry. Accuracy of the former method depends basically on the selection of a dye insoluble in the solids phase whereas accurate interpretation of dilation data depends on the ability to assign appropriate dilation values to each portion of melting curve. Inherent difficulties of the latter problem for a multicomponent plastic fat have been discussed by Bailey (1).

Essential requirements for application of the dye-dilution method to a plastic fat are a technique for separating a portion of the oil phase free from solids and a dye which is soluble only in the oil phase. Separation of oil phase might be accomplished by several methods other than ultracentrifugation. We have investigated several alternative possibilities but have not found any generally satisfactory. Temperature rise during centrifugation limited the application of high-speed, angle-head centrifuges operating in air at atmospheric pressure to global edible spreads. Temperature rise in these and other centrifuges, having rotors operating in air, might be minimized by cooling the air, but it appears simpler to use a centrifuge with a rotor operating in a vacuum chamber to avoid air friction.

Methods also were tested which depended on application of vacuum or pressure to separate the oil phase from plastic fats. Temperature control problems were simplified, but oil separation was slow and the methods were effective only for soft lard, shortening, and global edible spread. At temperatures near 30°C. sufficient oil was obtained for measurements of dye concentration by supporting the fat on a sintered-glass filter and applying vacuum to the filter. In order to prevent clogging pores in the filter and to insure free passage of the oil, a disk of filter paper was placed between the fat and the supporting septum. A Carver hydraulic press also was used to express the oil phase from a plastic fat by applying pressure to a piston acting against a fat contained in a cylinder. The expressed oil which passed between the cylinder wall and the piston was collected and filtered prior to optical measurements. To prevent contamination of the oil with solids the cylinder was loaded from the bottom, and the fat was separated from the surface of the piston by several layers of filter paper.

Perhaps the simplest method of separating oil from a plastic fat is by absorption in filter paper. Oil is removed from the solids phase by capillarity. This process proceeds slowly; moreover the oil must be subsequently extracted from the filter paper with an appropriate solvent. Greatest disadvantage of the method is the small quantity of oil absorbed per unit volume of filter paper, necessitating use of special cells of low volume for the spectrophotometric measurements.

Summary

Solids content of a plastic fat can be estimated by adding a weighed quantity of oil-soluble dye to a known weight of fat, separating a portion of the dye-oil phase in the ultracentrifuge, and determining the concentration of the dye in the separated oil by absorbance measurements. Since the dye is distributed throughout the oil phase, this yields the amount of oil in the spread; solids represent the difference between the amount of spread taken and the amount of oil determined. Of 37 dyes tested, 1,4-bis-methyl aminoanthraquinone and 1,4-bis-isopropyl aminoanthraquinone gave satisfactory results for global edible spreads in which solids contents were known from the formulation. The method also was applied to the estimation of solids in butter, margarine, lard, and shortening. Except for lard, results were in good agreement with solids content determined by dilatometric measurements. The measure of true solids which is obtained by this technique, without prior melting of the fat sample, would be valuable in the study of consistency changes arising from diverse processing conditions.

Separation of oil phase from a plastic fat by pressure filtration or by capillary absorption in filter paper was less satisfactory than centrifugation in the dye-dilution method.

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Studies of the Refractive Indices of Binary Wax Mixtures

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DATA on the physical chemistry of waxes are meager and difficult to obtain. Nevertheless there have been workers who have accepted this challenge, and their findings point to vast possibilities that could be developed. The unique physical properties of the natural waxes account largely for their biological and economic usefulness. Additional information on the nature of these properties is needed because better understanding of the effect of mixing with possible chemical interaction on these properties would increase the range of usefulness of these waxes and wax mixtures.

In this paper the physical property of the refractive index of waxes used for pharmaceuticals, cosmetics, dental waxes, and industrial products is related to the composition and structure of the molecular species involved. Since the waxes used in this study are combinations of hydrocarbons, lactones, higher alcohols and their esters, fatty acids and their glycerides, resins and smaller amounts of inorganic matter, some combinations would be expected to deviate from ideal behavior. This investigation examines solubility effects on the refractive indices of wax mixtures and compares the results with changes observed in the study of other physical properties.

Experimental

The effect of mixing natural waxes was studied by determining the refractive indices at 75°C. and 85°C. The values were read from an Abbe-56 refractometer. Temperature control was maintained by a Precision constant temperature bath, ±0.03°C., and a series of binary mixtures was prepared, ranging in composition from 100% of the first component to 100% of the second component. The mixtures were spaced at 10% intervals and, in this study, were volume percentage.

The refractive indices of the waxes and wax mixtures were taken at their melting points according to the method described by Dobran, Ackar, and Frediani (2), who used a Fisher refractometer. With an Abbe-56 refractometer thin slabs of the soft, low-melting waxes or layers of small crystals of the hard waxes were placed on the lower prism. When the sample became warm, the prism box was firmly closed and the excess wax was extruded, leaving a layer of uniform thickness of approximately 0.4 mm. between the prisms. The field was opaque. The temperature was gradually raised. When the melting point was reached, the eyepiece was sharply focussed on the cross-hairs, and the dividing line was brought upon their intersection by means of the handwheel. The index of refraction and the temperature of the melting point were read almost simultaneously.

The precision of the refractometric method is very good. Three determinations on a paraffin wax sample gave an average refractive index value of 1.4377 ±0.0002 with a melting point of 51.0°C. in each trial. Three determinations on a beeswax sample gave a refractive index value of 1.4460 each time with an average melting point of 65.0 ±0.3°C. Three determinations on a candelilla wax sample gave an average refractive index value of 1.4612 ±0.0004 with an average melting point of 69.7 ±0.2°C.

The iodine number was determined by the Hanus method.

The refined beeswax, yellow beeswax, candelilla wax, carnauba wax (Brazil), and the paraffin wax (special filtered) were purchased from the E. H. Sargent and Company. The Japan wax, Chinese insect wax, ozocerite wax, and ouricury wax were supplied through the courtesy of the S. C. Johnson and Son Inc., Racine, Wis. The waxes were used without further purification.

Results and Discussion

According to the Gladstone-Dale equation (4) for binary solutions, the refractive index of either component can be calculated as follows:

$$\frac{n_c-1}{d_c}=\frac{n_a-1}{d_a}\left[\frac{p}{100}\right]+\frac{n_b-1}{d_b}\left[\frac{100-p}{100}\right]$$

where

n_a and n_b are the refractive indices of the respective components, a and b.

n_c is the refractive index of the solution.

d_a and d_b are the densities of the respective components, a and b.

d_c is the density of the solution.

p is percentage of component a present.

100 — p is percentage of component b present.

Using this equation, the calculated values plot out linearly for ideal mixtures. These calculated values are not shown on the graphs. The refractive indices of the ouricury wax mixtures (Figure 1) with the animal waxes (refined beeswax and Chinese insect wax) show that the experimental values for these mixtures are examples of ideal binary mixtures since they plot as a straight line. The values for the candelilla wax-ouricury wax mixture and the Japan wax-ouricury wax mixture cannot be calculated with the above equation and are examples of non-ideal behavior. The waxes with low iodine numbers give ideal behavior. Deviation from ideal behavior increases with the unsaturation of the waxes.

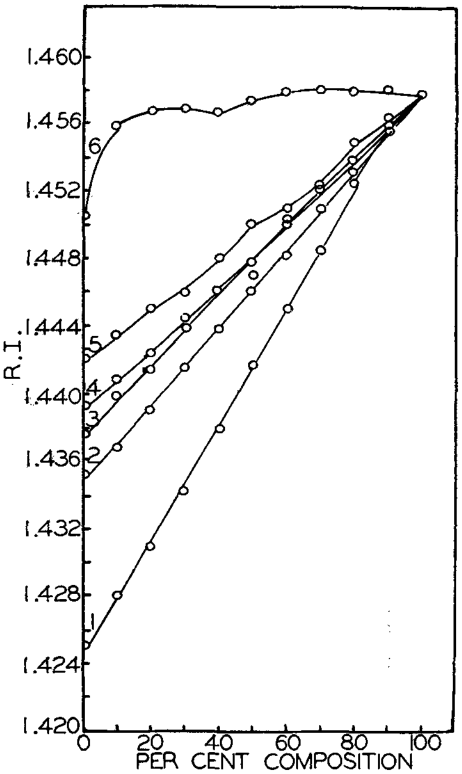

FIG. 1. Refractive indices of ouricury wax mixtures, at 85°C. with 1, paraffin; 2, ozocerite; 3, refined beeswax; 4, Chinese insect wax; 5, Japan wax; 6, candelilla wax.

The paraffin wax mixtures (Figure 2) with candelilla wax, refined beeswax, Chinese insect wax, and ouricury wax again form relatively ideal solutions. Irregular curves are obtained with ozocerite wax, Japan wax, and carnauba wax. This irregularity might be accounted for by the dissimilarity of the chemical composition of the components.

The carnauba wax mixtures plot out as sigmoid curves with the greatest change appearing between 70% and 80% carnauba wax.

The candelilla wax mixtures (Figure 3) show slight deviation from the expected values with the exception of the mixtures with carnauba wax and ouricury wax, which show greater deviations. These two waxes are of vegetable origin as is also candelilla wax. These wax mixtures bring together waxes containing relatively high percentages of hydroxy esters and a greater degree of unsaturation.

It is difficult to analyze refractometric data adequately because of the incomplete knowledge concerning the exact composition of the waxes. The analysis reported by Findley and Brown (3) has suggested a partial explanation of the non-ideal behavior of some of the mixtures. For example, mixtures consisting of waxes in combination with higher percentages of carnauba wax, a wax containing large amounts of hydroxy esters (3), exhibit optical exaltation.

Comparing the degree of unsaturation as given by the iodine numbers with the refractive indices of

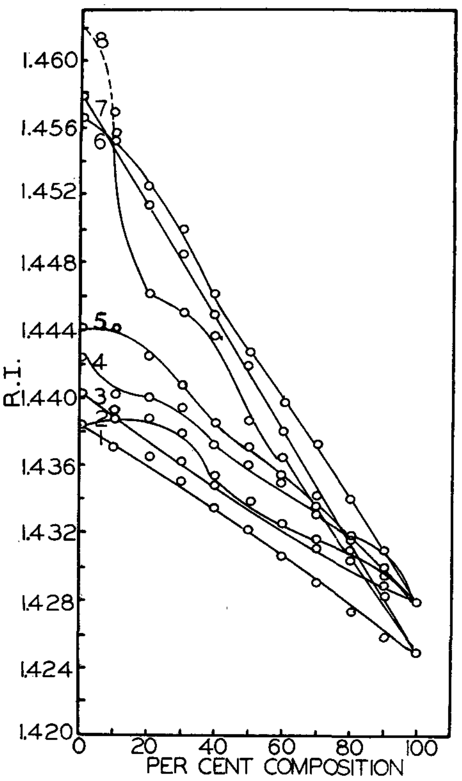

Fig. 2. Refractive indices of paraffin wax mixtures. Group I at 85°C. with 1, Chinese insect wax; 8, carnauba wax; 7, ouricury wax; Group II at 75°C. with 2, ozocerite; 3, refined beeswax; 4, yellow beeswax; 5, Japan wax; 6, candelilla wax.

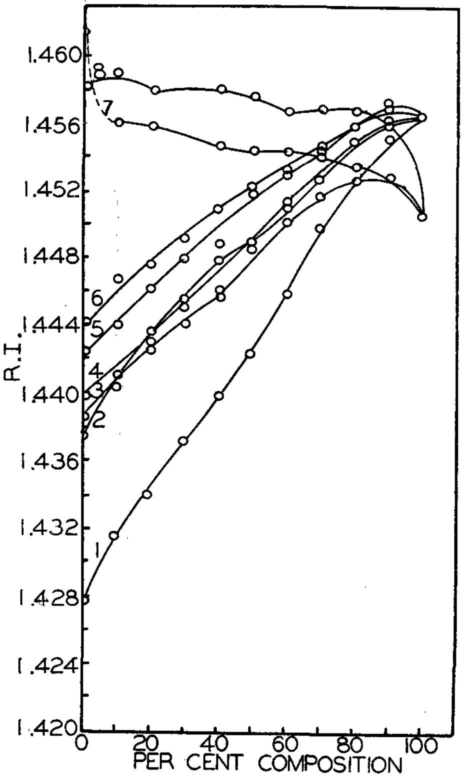

Fig. 3. Refractive indices of candelilla wax mixtures. Group I at 85°C. with 3, Chinese insect wax; 7, carnauba wax; 8, ouricury wax; Group II at 75°C. with 1, paraffin wax; 2, ozocerite; 4, refined beeswax; 5, yellow beeswax; 6, Japan wax.

several of the pure waxes, the waxes with the higher iodine numbers (the vegetable waxes) give greater increases in the refractive index values (Table I). Carnauba wax having a relatively high degree of unsaturation and a very high percentage of hydroxy esters has a very high refractive index. Figure 4 illustrates this same relationship in a series of binary mixtures consisting of varying percentages of a saturated and an unsaturated wax. Japan wax, a wax composed of palmitic and other high molecular weight fatty acids and their glycerides, gives the most irregular curves of all of the mixtures studied.

TABLE I

Relation of Iodine Number to Refractive Index

Wax	Iodine No. (1)	Refractive Index at 75°C.
Paraffin	0	1.4277
Ozocerite	7.8– 9.2	1.4372
Beeswax	8.3–11.0	1.4409
Japan Wax	4.0–15.0	1.4441
Candelilla Wax	14.0–36.8	1.4566

The determination of the melting points, using the Abbe-56 refractometer, agreed to 1.5°C. with those determined by thermal analysis (the A.S.T.M. melting point method: D87-37). These melting point determinations are more quickly and easily determined than those by the cooling curve method. Only a small amount of the sample is required, and two physical constants can be determined simultaneously. Table II shows how closely the values agree as determined by the two methods. The melting points given in the

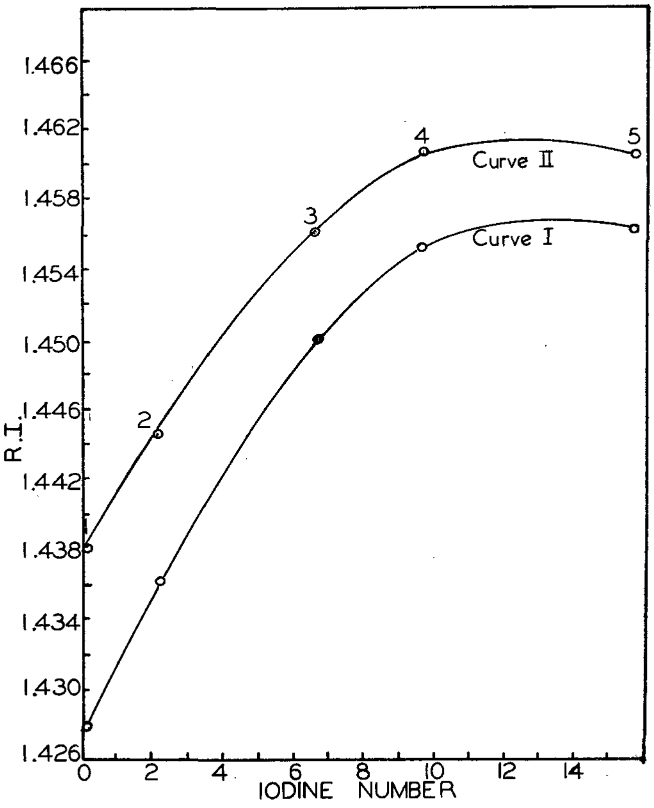

Fig. 4. Influence of unsaturation on refractive indices of wax mixtures. Curve I, R. I. at 75°C.; Curve II, R. I. at the melting point of the mixtures. Mixtures: 1, 100% paraffin; 2, 70% paraffin—30% candelilla; 3, 30% paraffin—70% candelilla; 4, 10% paraffin—90% candelilla; 5, 100% candelilla.

TABLE II
Melting Points

Wax Sample	Melting Point, °C.	
	Determined with Refractive Index	Determined from Cooling Curve[a]
Refined beeswax	65.0	65.5
Candelilla wax	69.7	69.5
Paraffin wax	51.0	51.5
10% Beeswax—90% Paraffin Wax	51.2	51.0
30% Beeswax—70% Paraffin Wax	53.0	52.8
50% Beeswax—50% Paraffin Wax	59.0	58.5
70% Beeswax—30% Paraffin Wax	61.0	61.0
90% Beeswax—10% Paraffin Wax	63.0	62.5
10% Beeswax—90% Candelilla Wax	68.0	67.5
30% Beeswax—70% Candelilla Wax	67.5	66.0
50% Beeswax—50% Candelilla Wax	67.0	66.0
70% Beeswax—30% Candelilla Wax	66.0	65.5
90% Beeswax—10% Candelilla Wax	66.0	65.0
10% Paraffin—90% Candelilla Wax	69.5	68.0
30% Paraffin—70% Candelilla Wax	67.0	66.0
50% Paraffin—50% Candelilla Wax	60.5	60.0
70% Paraffin—30% Candelilla Wax	56.0	55.0
90% Paraffin—10% Candelilla Wax	52.8	52.2

[a] Method D 87-37 for A.S.T.M. melting point.

literature are: beeswax, 61.0–65.0°C.; paraffin, 50.0–51.3°C.; candelilla, 65–69.0°C.

The melting points of the wax mixtures showing ideal behavior with respect to refractive index values lie between the melting points of the individual components. The non-ideal solutions studied do not show this linear relationship.

Studies of possible chemical interaction of these binary wax mixtures in terms of changes in physical properties are being extended to include the effect of mixing on surface tension, viscosity, freezing point lowering, light absorption, and fluorescence.

Summary

The greatest deviations in refractive index from ideal behavior of the binary wax mixtures are obtained with mixtures of vegetable waxes. These are waxes with higher unsaturation, waxes containing a higher percentage of hydroxy esters, and waxes containing glycerides or combinations of these.

Melting points and refractive indices at the melting points can be determined simultaneously with the Abbe-56 refractometer.

Acknowledgment

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The Estimation of Peanut Meal in a Premix for Bread Enrichment

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ONE OF SOUTH AFRICA'S FOOD PROBLEMS is a deficiency in the diet of good quality protein, with its associated nutrients. In a national food enrichment program it was therefore decided to enrich bread made of 90% extraction wheat flour with skim milk powder (70 mesh), defatted peanut meal (34 mesh), and calcium carbonate (100 mesh). These supplements are previously mixed, and the "premix" is supplied to bakers for addition to 90% extraction wheat flour.

The addition of this premix to flour is controlled by the South African Wheat Industry Control Board through analysis of the enriched bread, but control of the composition of the premix is a function of this Institute.

Total protein content is determined by the improved Kjeldahl method (1, 2) whereas the contribution of skim milk powder is satisfactorily deduced from lactose content (3). Similarly the estimation of peanut meal is done indirectly. Stansbury, Field, and Guthrie (4) observed that the tannin and leucoanthocyanin of peanut skins can be converted into red products by heating in alcoholic hydrochloric acid solution. This fact was applied to estimate the skin content of peanut meals according to an elaboration by Stansbury and Hoffpauir (5). A modification of the latter method was introduced at this laboratory to estimate peanut flour content of the above-mentioned premix (3).

It was found however that the de Lange method gave inconsistent results. Very little was known about the reliability of the method and the reproducibility of results. The investigations described in this paper led to a few alterations in the method of de Lange, and it was established that the modified procedure described below gives excellent results, which are satisfactorily reproducible.

Variation in the pigment content of peanut skins with variety and geographical origin (6) may present an obstacle. Preliminary investigations on available samples suggest that the pigment content of peanut varieties used in this country for the manufacture of premix does not vary appreciably and will not seriously affect the estimation of peanut meal by a method based on the incidence of this component. This matter however is being further investigated.

Reagents

1. Ethanol 95%.
2. Sulphuric acid, 5% solution. Add 5.6 ml. of conc. sulphuric acid (C.P., S.G. 1.84) to 190 ml. of water.
3. Neutral lead acetate, 10% solution.
4. Hydrochloric acid, A.R., S.G. 1.18.
5. Tungstate-phosphate solution. Dissolve 50 g. of sodium tungstate (C.P.) and 6 g. of sodium mono-acid phosphate (C.P.) in 200 ml. of water,