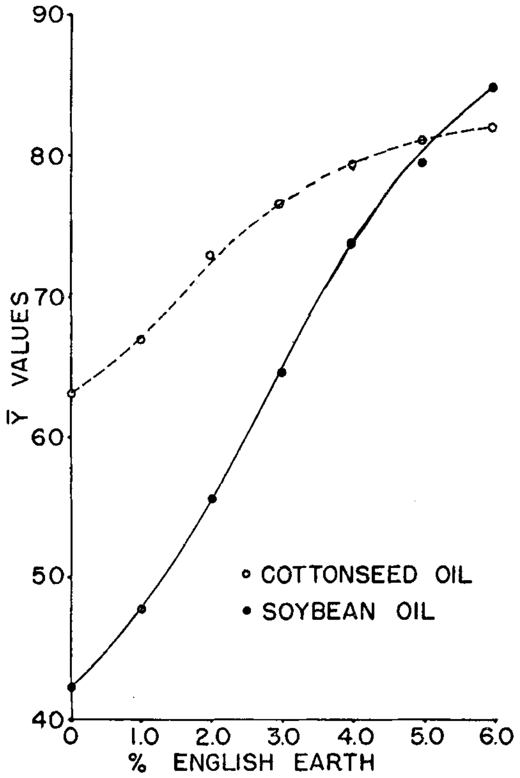

FIG. 2. Y values for bleached soybean and cottonseed oils plotted against the % of earth used.

TABLE V

Chromaticity Data

Oil	x	y	z	Y
Cottonseed Oil				
0% Bleach	.504	.490	.0066	63.12
1% Bleach	.491	497	.011	66.91
2% Bleach	.469	.493	.038	72.89
3% Bleach	.450	.485	.065	76.56
4% Bleach	.431	.471	.098	79.10
5% Bleach	.424	.465	.111	81.06
6% Bleach	.418	.460	.122	81.74
Soybean Oil				
0% Bleach	.528	.469	.0024	42.57
1% Bleach	.525	.472	.0034	47.81
2% Bleach	.517	.479	.0030	55.69
3% Bleach	.505	.492	.0030	64.42
4% Bleach	.491	.505	.0040	73.60
5% Bleach	.469	.505	.0250	79.18
6% Bleach	.433	.485	.0814	84.74

work of the Color and Bleaching Committees is directed.

General. During the past year the name of this committee has been changed from the "Oil Color Committee" to the "Color Committee." W. T. Coleman has accepted chairmanship of the subcommittee on Oil Colors and L. K. Whyte the subcommittee on Surface Colors.

R. J. Buswell
Edith A. Christensen
W. T. Coleman
M. W. Formo
E. B. Freyer
Seymore Goldwasser
D. L. Henry
Duncan Macmillan
C. L. Manning
V. C. Mehlenbacher
R. T. O'Connor
R. C. Pope
A. D. Rich
Francis Scofield
R. J. Smith
L. K. Whyte
G. G. Wilson
R. C. Stillman, chairman.

A Dye-Dilution Method for Estimating Solids Content of Plastic Fats[1]

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In a previous paper (3) we reported the separation of several plastic fats into oil and solids phases by ultracentrifugation. Extent of separation increased with the speed of centrifugation, but in no case was an oil-free solid phase obtained and ultracentrifugation alone did not permit a direct determination of the true solids content of a fat. Oil phases separated however were free from solids, and this provides an essential condition for a dye-dilution method which we have developed for estimating true solids in plastic fats. In this method a known quantity of oil-soluble dye is added to the plastic fat and thoroughly mixed to insure uniform distribution of the dye in the oil phase. A portion of the oil phase is recovered by ultracentrifugation, and the concentration of dye is determined spectrophotometrically. The quantity of oil in the sample and, by difference, the solids content can be computed from the dye concentration.

Dyes satisfactory for the dilution method should be soluble in the oil phase of the plastic fat but insoluble in the solids phase. In the present investigation a search was made for dyes suitable for use with global edible spread (6), a plastic mixture of commercial distilled monoglycerides and winterized cottonseed oil. This plastic fat was chosen for initial tests because the monoglyceride was only slightly soluble in cottonseed oil at room temperature (4); solids content of the spread thus was known from its formulation, and no independent method was required for determining solids as a check on the accuracy of the dye-dilution method.

Two dyes were found which gave satisfactory results in the determination of solids in global edible spread by the dye-dilution method. These dyes were tested further, applying the dilution method to analysis of solids content of butter, margarine, shortening, and lard. An independent estimate of the solids content for these fats was obtained by dilatometric measurements. Results presented below show the two methods to be in reasonable agreement.

Experimental

As an initial screening test for estimating the solubility of dyes in the monoglyceride comprising the solid phase of global edible spread, flakes of monoglyceride were immersed overnight in solutions of dyes in cottonseed oil. Extent of dye uptake was

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[1] Presented at the meeting of the American Oil Chemists' Society, Chicago, Ill., Nov. 2–4, 1953.

estimated visually from the depth of color of the flakes. Of 37 oil-soluble dyes tested, eight dyes which did not stain or stained the flakes only slightly, and three dyes which appeared soluble to some extent, were selected for additional investigation. Concentrated stock solutions of these dyes were made by mixing 0.05 g. of dye in 100 g. of warm cottonseed oil for 5 min. in a blendor. These solutions were centrifuged in order to remove any particles of undissolved dye.

Weighed quantities of concentrated solutions of the selected dyes were mixed with global spread, and the mixtures were centrifuged to separate a portion of the oil phase for preliminary determinations of solids. Solids content less than the amount of monoglyceride incorporated in the spread were found with the three dyes which the visual screening test had indicated to be soluble in the monoglyceride. Of the remaining eight dyes, which did not stain the solid monoglyceride, or stained it only slightly, solids contents approximating known monoglyceride contents were found with xylene-azo-α-naphtol (XAN), 1,4-bis-methyl aminoanthraquinone (MAA), and 1,4-bis-isopropyl aminoanthraquinone (IAA). Oil solutions of XAN were unstable when exposed to light, and this dye was discarded for this reason. In the experiments reported below both MAA and IAA were used.

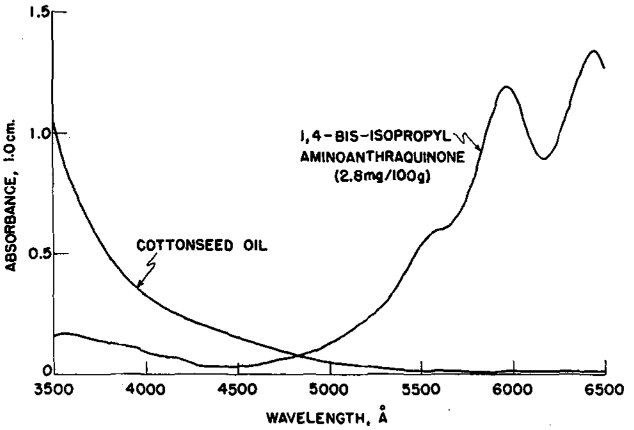

FIG. 1. Absorption spectrum for cottonseed oil and 1,4-bis-isopropyl aminoanthraquinone in cottonseed oil.

Figure 1 shows the absorption spectrum of IAA in cottonseed oil and, for comparison, the absorption spectrum of cottonseed oil. Absorption maxima of the dye at 5990 Å and 6480 Å occur in the spectral region in which cottonseed oil and the oil phase of other plastic fats are usually quite transparent. Although oils from butter and colored margarine showed appreciable absorption at wavelengths below 5300 Å, this did not interfere with absorption measurements. As shown in Figure 2, both MAA and IAA follow Beer's law over a wide absorbance range. Absorption coefficients, $E^{1\%}_{1\,cm.}$, also are given in Figure 2 for these dyes in cottonseed oil. Identical absorption coefficients were found for IAA and MAA in oils separated by ultracentrifugation from other plastic fats. The data of Figure 2 therefore are valid for IAA or MAA in oils from butter, margarine, lard, and shortening. Either of the wavelengths for absorption maxima of IAA or MAA would be satisfactory for measurement in an analytical method. In the present studies 5990 Å and 6010 Å were selected for IAA and MAA, respectively, and absorbance measurements were made on solutions containing from 0.5 to 3.5 mg. dye per 100 g. of oil. Absorbance values were, for the most part, determined with a Cary spectrophotometer,[2] but a Beckman DU spectrophotometer also was used.

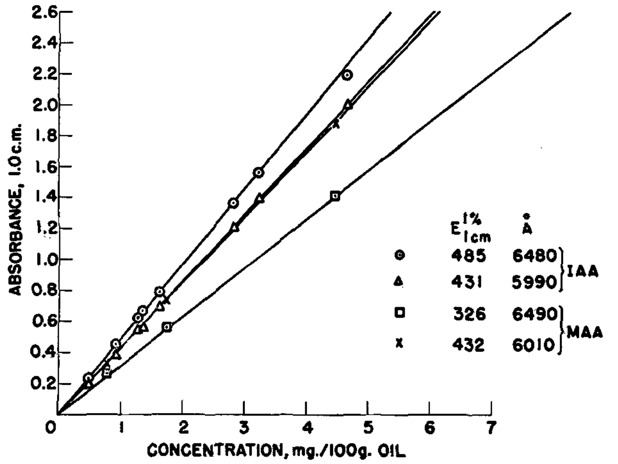

FIG. 2. Relation between absorbance and concentration of 1,4-bis-isopropyl aminoanthraquinone and 1,4-bis-methyl aminoanthraquinone in cottonseed oil.

To estimate solids in plastic fats 25 g. of fat were mixed with 2.0 g. of concentrated dye solution (15 mg. of dye per 100 g. of cottonseed oil) and then transferred to Lusteroid centrifuge tubes. Additional tubes were filled with pure fats to obtain dye-free oil samples for use as blanks in the spectrophotometric measurements. The centrifuge rotor containing tubes of dye and dye-free plastic fats was equilibrated at 25°C. before being centrifuged in a Spinco model L ultracentrifuge at 36,000 r.p.m. (85,000 times gravity) for 60 min. at 25°C. For routine work on soft fats, separation of an adequate volume of oil can be accomplished by centrifugation for 15 min.

Although operated in an evacuated chamber, the heat capacity of the ultracentrifuge rotor was insufficient to offset appreciable temperature rise resulting from heating effects, and refrigeration was required. Refrigeration controls of the ultracentrifuge were adjusted to maintain a temperature of 25°C.

The separated oil was decanted and filtered through Whatman's No. 1 filter paper prior to optical measurements. Absorbancies of the dye-oil samples from the spread were determined against a blank of the dye-free oil. Grams of oil, G, present in the fat were calculated from the relation:

$$G = [W_{ds}\,W_c\,E^{1\%}_{1\,cm.}/A_s] - W_{ds}$$

where $E^{1\%}_{1\,cm.}$ = absorption coefficient for the dye in a 1.0-cm. cell at 1% concentration
W_s = grams of spread
W_c = grams of dye in 100 g. of the concentrated dye solution
W_{ds} = grams of dyed oil added to fat
A_s = absorbance of oil separated from centrifuged fat

The percentage of solids in the fat is given by:

$$\%\ \text{solids} = \frac{W_s - G}{W_s}$$

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[2] The mention of firm names or trade products in this article does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

In the case of butter and margarine the percentage of solids was corrected for water content as determined by the hot-plate method (7).

Dilatometric solids were determined on samples of butter, margarine, lard, shortening, and global spread which had been analyzed for solids by the dye-dilution method. A volumetric dilatometer similar to that described by Fulton *et al.* (2) was used. Solids content calculations were based on approximations that the melting dilation for plastic fats is 0.100 ml. per gram and that the plots of specific volume *vs.* temperature for the solid and liquid phases of plastic fats are linear and parallel (1).

Results and Discussion

Results obtained on duplicate determinations by the dye-dilution method for a series of four tempered global spreads are presented in Table I. Based on the amount of monoglyceride incorporated in the spread, solids content ranged from 9 to 21% in this series. Solids contents determined by the dye-dilution method were found to agree with the formulated monoglyceride content within 1.0%. All experimental results reported in Table I were lower than the monoglyceride content of the spread, suggesting that the dye may have been slightly soluble in the solid phase or was adsorbed on the surface of the monoglyceride crystals. The magnitude of the difference however appears independent of the proportion of solid phase, indicating that the low experimental values arose from another cause. This is supported by results of repeated analyses on the spread formulated to contain 21% monoglyceride. As shown in Table III, the average of five determinations, 20.9%, was in good agreement with the monoglyceride content of the spread.

TABLE I

Determination of Solids in Global Spreads at 25°C. by the Dye-Dilution Method

Monoglyceride added	Solids found
%	%
9	8.8
13	11.9
17	16.4
21	20.4

Encouraged by the results on the global spreads, we applied the dye-dilution method also to the analysis of butter, margarine, lard, and shortening. Solids content of samples of these plastic fats at 25°C. as determined by the dye-dilution method are presented in Table II. To provide a check on the dye-dilution method, these samples, as well as a global spread, were analyzed for solids by the dilatometric method. Results are compared with those obtained by the dye-dilution method in Table II. Solids content as determined by the two methods are in good agreement for all plastic fats with the possible exception of lard. Better agreement than found for butter, margarine, shortening, and global spread cannot be expected, considering the precision of the methods and in view of the approximations made in calculations of solids content from the dilatometric data. Bailey (1) has discussed the reasons for making these approximations and the difficulties in obtaining an accurate analysis of solids by the dilatometric data. The principal uncertainty lies in the choice of a value for the melting dilation. Experimental values for trisaturated glycerides (1, 5) are considerably greater than 0.100 ml. per gram, the value commonly used as an average and selected for the present work, whereas melting dilations for unsaturated glycerides are less than this value. Computed values thus might be expected to be too high, particularly when the solids estimated contain a large proportion of high melting or saturated glycerides. The discrepancy between the values for lard obtained by the two methods however appears too large to be explained in this manner. It is possible therefore that the dyes tested in the present investigation were not insoluble in the solids phase of lard.

TABLE II

Comparison of Solids Contents of Plastic Fats at 25°C. as Determined by the Dye-Dilution and Dilatometric Methods

Sample	Solids Content	
	Dye-dilution	Dilatometry
Butter	11.4	11.6
Margarine	17.3	17.6
Lard	15.6	22.1
Shortening	15.9	18.5
Global Spread [a]	17.6	19.0

[a] 17.0% monoglyceride was added in formulation of spread.

An estimate of the precision of the dye-dilution method is given by the data of Table III for replicate analyses of global spread, lard, and shortening. Standard deviation of the analyses averaged about 0.6 percentage units for solids contents in the range of 15 to 20%. For comparison, the dilatometric results reported in Table II had a standard deviation of 0.3. Accurate temperature control during sample preparation and centrifugation, of course, is important to the precision of the dye-dilution method. As in the dilatometric procedure, samples were cooled to about 15–20°C., and the final equilibrium was approached from lower temperatures to hasten attainment of equilibrium solids content at the temperature of measurement.

TABLE III

Precision of Dye-Dilution Method

Sample	Solids		
	Global spread [a]	Lard	Shortening
	%	%	%
1	21.2	15.2	......
2	20.9	15.7	16.6
3	21.5	15.1	15.3
4	20.2	16.5	16.1
5	20.6	15.6	15.5
Average	20.9	15.6	15.9
Standard deviation	0.45	0.6	0.6

[a] 21.0% monoglyceride added in spread formulation.

An advantage of the dye-dilution method compared to the dilatometric method is that processed fats can be examined without melting. Since proportion of solids is a dominant factor influencing the consistency of fats, the ability to measure solids present after given processing conditions rather than after melting and resolidification in the dilatometer would aid in evaluating formulation and processing. For example, after completion of the plasticizing operation, shortening subsequently hardens or softens, depending on composition of the solids phase and its thermal treatment.

It appears too that an estimate of absolute solids content of a plastic fat may be determined more accurately 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