TABLE V

Anticipated and Observed Recoveries of Various Triglyceride Types from Mixtures of Butterfat and Lard [a]

Triglyceride type	Butterfat K plus 1% lard		Butterfat K plus 3% lard		Butterfat K plus 5% lard		Butterfat K plus 10% lard		Butterfat K plus 25% lard	
	Anticipated [b] %[c]	Found %	Anticipated %	Found %	Anticipated %	Found %	Anticipated %	Found %	Anticipated %	Found %
C_{56}	0.9	0.9	1.0	0.6	1.0	0.9	1.1	0.8	1.5	1.5
C_{54}	7.9	8.4	8.3	7.6	8.6	8.3	9.4	8.7	11.7	11.1
C_{52}	14.3	14.6	15.0	14.7	15.8	15.5	17.7	17.7	23.4	22.4
C_{50}	13.4	13.7	13.4	13.4	13.5	13.0	13.5	13.4	13.7	14.0
C_{48}	8.9	9.6	8.8	9.2	8.7	8.8	8.5	8.3	7.9	8.8
C_{46}	6.4	6.8	6.3	6.7	6.2	7.0	6.0	5.8	5.2	6.0
C_{44}	5.5	5.3	5.4	5.6	5.3	6.1	5.0	4.7	4.2	4.8
C_{42}	6.1	6.0	5.9	5.9	5.8	6.2	5.5	5.5	4.6	5.4
C_{40}	9.5	9.5	9.4	9.9	9.2	8.9	8.7	9.3	7.2	7.5
C_{38}	12.5	11.7	12.2	11.8	12.0	11.6	11.4	11.4	9.5	9.0
C_{36}	8.3	8.1	8.2	8.6	8.0	9.0	7.6	8.7	6.3	5.5
C_{34}	3.8	3.4	3.7	3.8	3.7	3.4	3.5	4.0	2.9	2.8
C_{32}	1.4	1.3	1.4	1.5	1.4	1.0	1.3	1.3	1.1	0.8
C_{30}	0.6	0.5	0.6	0.6	0.6	0.4	0.6	0.5	0.5	0.4
C_{28}	0.3	0.2	0.3	0.1	0.3	0.1	0.2	0.2	0.2	0.9
C_{26}	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0
C_{24}	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0

[a] Averages of three or four individual estimates.
[b] Calculations based on the values for lard and butterfat K recorded in Table III.
[c] The % area distributions approximate those for the weight distribution.

removed (as far as gas chromatography is concerned) by some such means as bromination, already successfully exploited in the gas chromatography of fatty acid methyl esters (7).

Whatever the present commercial significance of butterfat adulteration, the applicability of this technique to the characterization of most triglyceride mixtures is obvious from the above examples and needs no further discussion. Collections of reference gas chromatographic elution patterns may be readily made for any well defined natural triglyceride mixture. Though difficulties might arise in the assignment of carbon numbers to the triglyceride peaks obtained when dealing with fats containing significant amounts of branched or epoxy and hydroxy fatty acids, there seems to be no reason why reproducible elution patterns should not be obtainable. The simplicity of the technique and its ready applicability to the widest variety of problems of natural oil characterization recommends it as one of the methods of choice in any fat test or control laboratory.

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A Colorimetric Method for Determining Free Fatty Acids in Vegetable Oils

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Abstract

A rapid method is presented for determining free fatty acids in vegetable oils. The method utilizes the reaction of fatty acids with cupric acetate to form salts whose blue color in benzene solution may be measured colorimetrically. When 65 samples of crude soybean oil were tested by this colorimetric method and the results compared with the results of titration with standard alkali, the correlation was 0.992 with a standard deviation of 0.08% free fatty acid (F.F.A.).

Introduction

FREE FATTY ACID CONCENTRATION is a factor in determining the condition of crude vegetable oils and this concentration is generally determined by titration with standard alkali. The amount of F.F.A. in benzene solution may be determined colorimetrically by reaction with an aqueous solution of cupric acetate. When the two solutions are shaken together, copper salts of the fatty acids are formed which are soluble in the benzene layer and impart a blue color to the solution. The intensity of the color is relative to the concentration of the F.F.A. and may be measured by a colorimeter or spectrophotometer. This procedure has been adapted to the determination of fat acidity in grain (2) and may also be used to determine the amount of F.F.A. in crude vegetable oils.

Apparatus and Reagents

Ordinary laboratory apparatus is used, including a colorimeter or spectrophotometer for measuring per cent transmittance at 640 mμ. Reagents are benzene and 5% cupric acetate solution. A standard solution of 0.0188N oleic acid in benzene is used to prepare the standard curve for the colorimeter.

Procedure

Weigh 8 g of the crude oil into a flask and dissolve in 50 ml benzene, mixing thoroughly. Measure 10 ml of the benzene-oil solution into a test tube containing 2 ml 5% cupric acetate solution. Stopper the tube

TABLE I
Ml of 0.0188N Oleic Acid, Diluted to 10 ml, Representing Chosen Percentages of Free Fatty Acid

Ml 0.0188N oleic acid	Ml benzene	% F.F.A. represented
0.5	9.5	0.2
1.5	8.5	0.6
2.5	7.5	1.0
3.5	6.5	1.4
5.0	5.0	2.0
5.5	4.5	2.2
6.5	3.5	2.6
7.5	2.5	3.0
8.5	1.5	3.4
9.5	0.5	3.8
10.0	0.0	4.0

and shake vigorously by inverting the tube at least 50 times. After the mixture has settled into two layers, decant the benzene layer through a fluted filter paper into a colorimeter tube and read the per cent transmittance at 640 mμ. Use benzene as the blank for adjusting the instrument to 100% transmittance.

A standard curve is prepared for the instrument from cupric oleate solutions of known concentration. Table I gives the amount of 0.0188N oleic acid in benzene, to be diluted to 10 ml, which represent chosen percentages of F.F.A. Pipet the standard oleic acid and the benzene into a test tube and add 2 ml 5% cupric acetate solution. Stopper and shake as above. Decant the benzene layer through a fluted filter paper into a colorimeter tube and read the per cent transmittance at 640 mμ. Plot per cent transmittance (Y) against per cent F.F.A. (X) and construct the standard curve. This curve is used for converting transmittance readings of unknown samples to per cent F.F.A.

Results and Discussion

Sixty-five samples of crude soybean oil, ranging in F.F.A. concentration from 0.13% to 3.28%, were tested by the colorimetric method and by titration with standard alkali according to the AOCS Official Method Ca 5a-40 (1). A comparison of the two methods (Fig. 1) shows a correlation of +0.992 with sample standard deviation from regression of 0.08% F.F.A. Figure 2 shows the relationship between per cent transmittance of the fatty acid copper salt solutions and the per cent F.F.A. determined by titration with standard alkali. The precision of the method was checked by making 24 determinations on one sample of soybean oil containing 1.49% F.F.A. The standard deviation was 0.025% F.F.A.

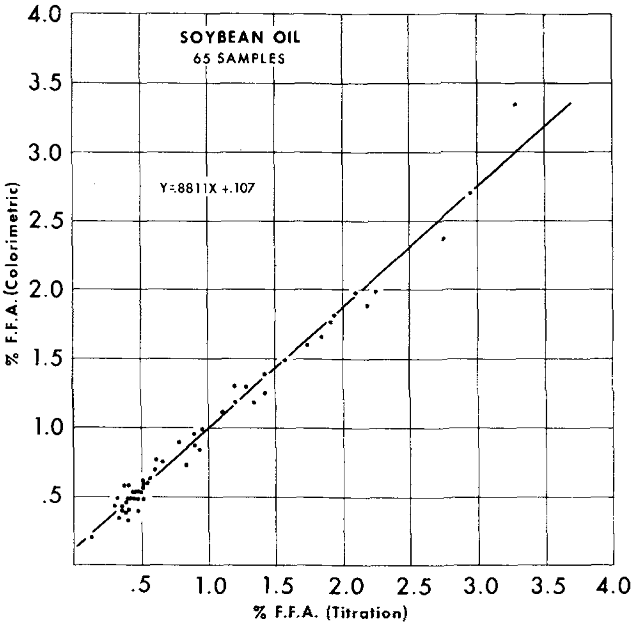

FIG. 1. Comparisons of F.F.A. values determined by titration and by colorimetric method.

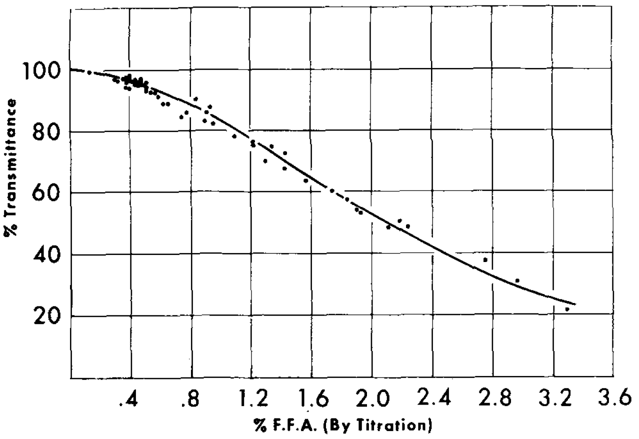

FIG. 2. Relationship of per cent transmittance to per cent F.F.A.

Oleic acid solutions in benzene containing various known percentages of oleic acid were reacted with cupric acetate and the transmittance curves from 350 to 750 mμ were made with a Bausch and Lomb Spectronic 20 spectrophotometer. From these curves the wavelength of 640 mμ was chosen for measuring the per cent transmittance of the copper salts of the fatty acids in the soybean oil samples. The color of the soybean oil samples ranged from light yellow to a dark color which was almost brown. This dark color, found in some of the soybean oil samples, did not interfere with the transmittance measurement at 640 mμ. At this wavelength, the transmittance of the soybean oil solutions in benzene is 98–100%.

The copper salts of lauric, oleic, linoleic and linolenic acids, which are the predominating fatty acids found in vegetable oils, are soluble in benzene and are the main contributors to the blue color. The copper salts of palmitic and stearic acids were found to be insoluble in benzene. The method is sufficiently sensitive to determine the F.F.A. concentration in crude vegetable oils, but some modification would be necessary to adapt the procedure to refined oils whose F.F.A. concentration is usually below 0.1%.

The method has been successfully tested with oil samples from wheat, corn, soybeans, and coconuts. The only trouble encountered was in cottonseed oil where the dark red color of some samples masked the blue color of the copper salts.

The method has the following advantages as a rapid, simple method. It eliminates the titration of the sample and no visual endpoint need be detected. The only standard needed is the fatty acid solution for preparing the standard curve for the colorimeter. Once this curve has been prepared, it can be used for an unlimited number of determinations. A mechanical shaking device could be substituted for the hand shaking of the reaction tubes.

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