TABLE IV
Biodegradation of an Isomeric Mixture of Straight Chain Sulfonates Calculated from GLC Data

Sulfonate	% Degradation in 24 hr					
	Isomer					
	Total	2-Phenyl	3-Phenyl	4-Phenyl	5-Phenyl	5- and 6-Phenyl
Dodecylbenzene	45	80	55	30		15
Decylbenzene	20	45	15	0	0	....
Octylbenzene	5	10	0	0	....	....
Hexylbenzene	0	0	0	....	....	....

bility of an alkylbenzene sulfonate to bacterial degradation. When this number becomes less than ca. 5 carbon atoms as in 4-phenyl octane and 2 and 3 phenyl hexane sodium sulfonates, biodegradation becomes slower.

Data shown in Table IV summarize the chromatographic data in Figure 4, and indicate the relationship of molecular weight and side chain structure to ease of biodegradation. The governing factor in this relationship seems to be the length of the alkyl carbon chain from the benzene sulfonate group to the terminal methyl group.

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Triglyceride Gas Chromatography as a Means of Detecting Butterfat Adulteration[1]

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Abstract

Gas chromatography was used for the separation and quantitative estimation of triglycerides by carbon number from native and adulterated butterfats. When the triglyceride type composition of the native butter was known, adulteration with vegetable fat could be detected at the 1% level, and with lard at the 3% level, as these adulterants added significantly to only a few of the butterfat triglyceride peaks resulting in a noticeable distortion of the chromatographic elution pattern of this fat. Due to considerable variations observed in the elution patterns with butterfats of different origin, the lower limit for the detection of adulteration of an unknown butter sample with lard or vegetable fat was of the order of 5–10%. The ease of detection and identification of the adulterant varied with the type of fat added. It was demonstrated that mixtures of coconut oil and lard could be made which matched the gas chromatograms of butterfat closely and remained undetected even when added in relatively large amounts. Despite this, the simplicity of the technique and its rapid and relatively reliable applicability to the widest variety of problems of natural fat characterization recommends it as one of the methods in any fat test or control laboratory.

Introduction

DURING QUANTITATIVE studies (1,2) on gas chromatographic fractionation of natural triglyceride mixtures it became necessary to determine the extent to which the individual components were recovered. Although under the chosen experimental conditions the estimated proportional contributions of the recovered triglyceride peaks remained constant, it was possible that constant losses might have occurred. In order to guard against such a possibility, known weights of selected synthetic triglycerides were added to weighed quantities of various natural fats and the proportional recoveries of the components measured. The results obtained were sufficiently encouraging to extend these studies to mixtures of natural fats, and during the course of this work it occurred to us that triglyceride gas chromatography offered great promise as a technique for the detection of adulteration of butterfat. This possibility was explored by examining the quantitative variation among the various triglyceride types observed in butterfats of different origin and the levels at which adulteration with lard and vegetable fats could be reliably detected by means of triglyceride gas chromatography.

Experimental

The samples of butter, lard, vegetable fat, and their mixtures used in these studies were obtained through the courtesy of Madhu Sahasrabudhe, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada. The butterfats were authentic samples and their physical and chemical properties represented the range commonly found in this food. The code and certain chemical constants for these four butter samples are given in Table I. The vegetable fat was partially hydrogenated cottonseed oil. The fatty acid composition of the butterfats and the lard and vegetable fat samples are given in Table II. The fatty acid composition was determined by gas chromatography as previously described (2). The values give approximate weight composition. The triglyceride gas chromatography was performed as described by Kuksis and McCarthy (3) except

TABLE I
Selected Chemical Characteristics of Some Commercial Butterfats[a]

Code	Iodine no.	Reichert-Meissl no.	Polenske no.
Butterfat K	36.9	26.2	1.75
Butterfat L	39.1	26.8	1.95
Butterfat M	38.3	29.7	2.49
Butterfat N	34.8	30.1	2.70

[a] Constants supplied by Madhu Sahasrabudhe, Department of National health and Welfare, Ottawa, Canada.

[1] Work supported by the Medical Research Council of Canada and the Ontario Research Foundation, Canada.

TABLE II

Fatty Acid Composition of Some Butterfats, Lard, and Vegetable Fat [a]

	Fatty acid: Weight (%) [b]															
	4:0 [c]	6:0	8:0	10:0	12:0	14:0	14:1	15:0	16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3	19:0	20:0	Total
Butterfat K	3.5 [d]	1.8	1.0	2.3	2.4	10.1	1.0	1.0	26.8	1.2	0.5	0.2	14.5	30.7	1.8	0.5	0.3	0.5	100.0
Butterfat L	3.5	1.8	1.0	2.4	2.4	10.5	0.8	0.9	27.0	0.5	0.5	0.5	15.1	31.1	1.5	0.0	0.0	0.5	100.0
Butterfat M	3.5	2.5	1.5	3.0	3.0	11.5	1.6	1.0	26.3	1.5	0.5	0.5	14.2	26.1	2.0	0.5	0.2	0.5	100.0
Butterfat N	3.5	2.3	1.6	3.0	3.0	10.9	2.0	1.4	25.5	2.0	0.5	0.5	13.7	26.1	2.5	1.0	0.0	0.5	100.0
Lard	....	....	....	....	....	3.0	0.4	0.4	27.4	3.8	0.4	0.3	13.5	43.0	7.9	....	....	....	100.0
Vegetable fat	....	....	....	....	....	.....	.....	....	14.4	....	....	....	6.5	68.4	10.6	....	....	....	100.0

[a] Averages of three or four individual estimates.
[b] The % area distributions approximate those for the weight distribution of the fatty acid methyl esters in the filament cell detector.
[c] First figure refers to number of carbons; second to number of double bonds.
[d] Not determined; assigned on the basis of reference 4.

that lower operating temperatures (indicated in the figures) were used. The relative proportions of the peaks were estimated from their areas under the curves by triangulation and mechanical integration. Three to four replicate determinations were made on each sample. A variation of 5–10% in the computed proportions of the peak areas was observed on repeated injection of the same material.

Results and Discussion

Examination of the Butterfat Samples. Figure 1 shows the gas liquid chromatographic elution patterns obtained for two of the four butterfat samples examined. To calculate the quantitative contributions of the individual triglyceride types, three or four separate chromatograms of each butterfat sample were made, and the peak areas estimated. Averages of these estimates are presented in Table III. From this table it is possible to appraise the natural variation encountered in native butter samples of differing origin. It may be noted that butterfats K and L contain significantly greater proportions of the long chain triglycerides than butterfats M and N. These differences can be correlated with the fatty acid compositions presented in Table II. Both K and L contain proportionally more of the 18:0 and 18:1 fatty acids. (First figure refers to number of carbons; second to number of double bonds.) This variation is to be kept in mind when dealing with comparisons between summer and winter butters, as the former have been reported (4) to contain significantly greater proportions of the longer chain fatty acids. The presence of greater amounts of longer chain fatty acids in butterfats K and L is also reflected in lower Reichert-Meissl and Polenske numbers (Table I), which measure the steam volatile water soluble and the steam volatile water insoluble fatty acids, respectively. The chemical constants recorded in Table I for all four of these butterfats are in the range accepted for most legal and regulatory purposes. Thus the Reichert-Meissl values are close to the prescribed range of 27–30, and the Polenski values do not exceed 10% of the Reichert-Meissl number (5).

Table III also includes the average values calculated for the individual triglyceride types for the four butter samples. The values for the various triglyceride peaks in the individual butterfats may differ considerably from the average, and may exceed 25% for some of the minor ones. The major peaks, however, do not appear to vary by much more than 10% from the overall average. In these studies only the even carbon number triglycerides have been considered, since at present reproducible estimates for the odd carbon number triglycerides are difficult to obtain. The error introduced in neglecting these contributions is small, since they add a maximum of only about 5% to the adjacent major peaks.

Examination of Selected Samples of Adulterated Butterfat. Mixtures of butterfat K with 1, 3, 5, 10, and 25% (w/w) quantities of vegetable oil (partially hydrogenated) served as examples of butterfat adulteration with plant fats. Figure 1 illustrates the gas chromatographic elution patterns obtained with vegetable fat and lard, and compares them to the butterfats employed in the adulteration mixtures. The contributions of the individual triglyceride types to the total triglyceride mixture in both lard and vegetable fat were calculated. These data, averages of

TABLE III
Distribution of Triglyceride Types in Some Butterfats and Lard and Vegetable Fat [a]

Triglyceride type	Butterfats and adulterants: Weight (%) [b]						
	Butterfat K	Butterfat L	Butterfat M	Butterfat N	Average	Lard	Vegetable fat
C_{56} [c]	0.9	0.9	0.6	0.8	0.8	3.2	3.2
C_{54}	7.8	9.4	6.6	6.6	7.5	23.6	61.2
C_{52}	13.9	15.3	11.5	11.7	13.3	52.0	30.9
C_{50}	13.4	13.3	10.9	11.4	12.2	14.5	4.3
C_{48}	8.9	8.2	8.5	8.8	8.6	5.1	0.3
C_{46}	6.5	5.6	6.4	7.0	6.4	1.4	0.1
C_{44}	5.5	4.6	5.6	6.4	5.5	0.2	.....
C_{42}	6.1	6.2	6.8	6.9	6.5	0.1	.....
C_{40}	9.6	10.9	12.0	11.0	10.9	.....	.....
C_{38}	12.6	12.3	13.1	12.2	12.5	.....	.....
C_{36}	8.4	7.9	9.6	9.2	8.7	.....	.....
C_{34}	3.9	3.2	4.9	4.5	4.1	.....	.....
C_{32}	1.4	1.3	2.1	2.0	1.7	.....	.....
C_{30}	0.6	0.6	0.7	0.9	0.7	.....	.....
C_{28}	0.3	0.4	0.4	0.4	0.3	.....	.....
C_{26}	0.1	0.1	0.2	0.1	0.1	.....	.....
C_{24}	0.1	0.2	0.2	0.1	0.2	.....	.....

[a] Averages of three or four individual estimates.
[b] The % area distributions approximate those for the weight distribution of simple triglycerides in the hydrogen flame ionization detector.
[c] The triglyceride type is indicated by the total number of fatty acid carbons.

four separate determinations, are presented in the last two columns of Table III and show that when either lard or vegetable fat is added to butter, contributions are made to only a few triglyceride types. The percentage change may be calculated from the chromatograms for the adulteration mixture. The upper part of Figure 2 illustrates the distortions in the elution patterns obtained when butterfat K was supplemented with lard at the 5 and 25% level. The lower part of Figure 2 shows the effect of adding similar amounts of vegetable fat to butterfat M. The proportional contributions of the individual triglyceride types to the total mixture derived from three or four replicate determinations at the various levels of supplementation are presented in Tables IV and V, together with the anticipated proportional recoveries calculated from the gas chromatographic data obtained on the butterfats and the adulterant fats separately.

Tables IV and V reveal that the presence of the adulterating fats already can be detected at the 1% level. This is because, although the total contribution of the adulterant is only 1%, in the case of the vegetable fat, e.g., 60% of this contribution goes to the C_{54} peak which makes up only 5.88% of the total. This results in an increase of almost 10% in the C_{54} peak of the mixture. The case of lard is similar. Here, however, 52% of the added fat goes to the C_{52} peak which makes up 13.9% of the total mixture, resulting in a 5% increase when the overall supplementation is at the 1% level. This is actually below the level of reproducibility usually observed with this technique, and the fact that differences were detected in the elution pattern following this low level of supplementation with lard may have been only a coincidence. When more than 1% of these fats were added, however, the contributions to the C_{52} and C_{54}, or both peaks, were readily detectable.

The detection of adulteration of butterfat by lard or vegetable fat at the 1% level, however, may be possible only when the triglyceride composition of the native butterfat is known. Since in practice this would be unlikely, some other reference standard would be necessary. It is probable that dependence

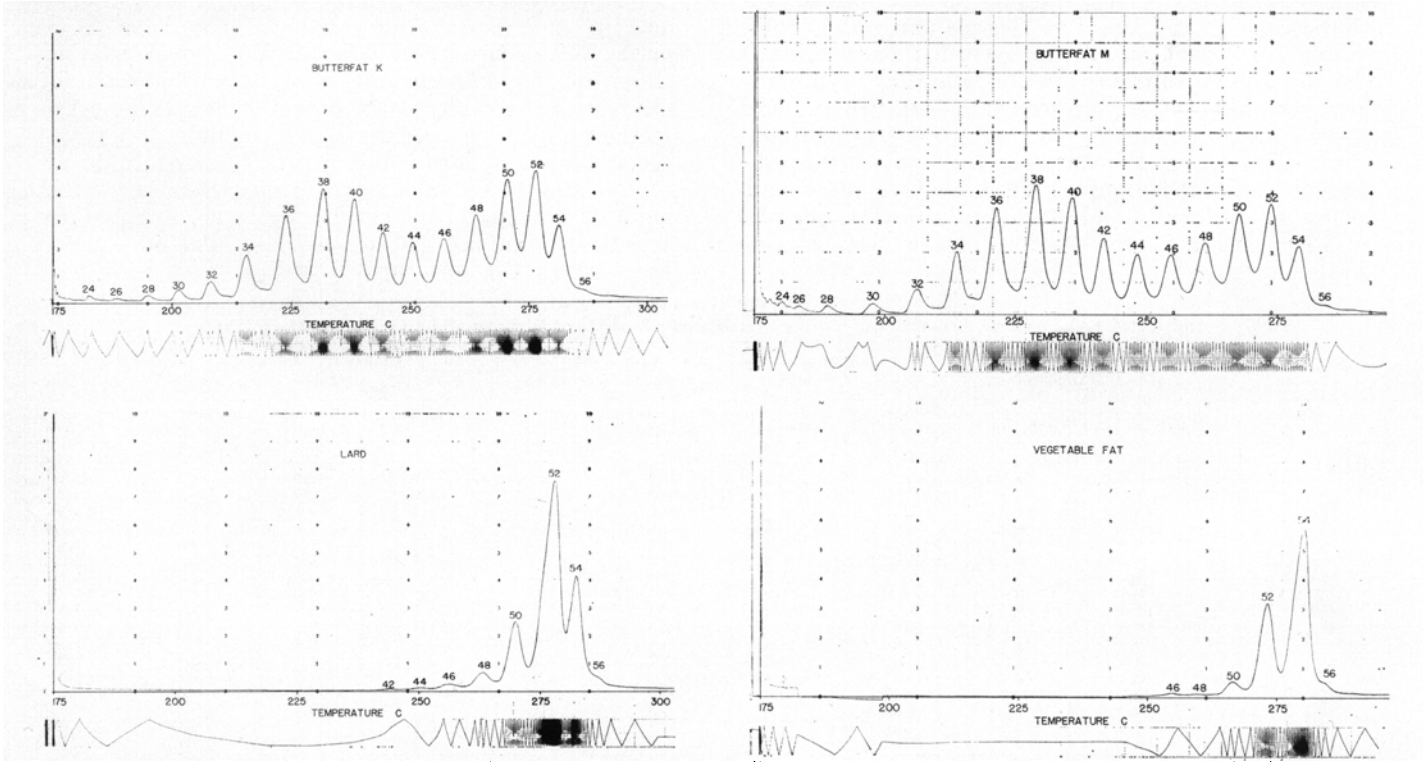

FIG. 1. Gas-liquid chromatographic elution patterns recorded for the butter, lard, and vegetable fat samples employed in the adulteration studies.

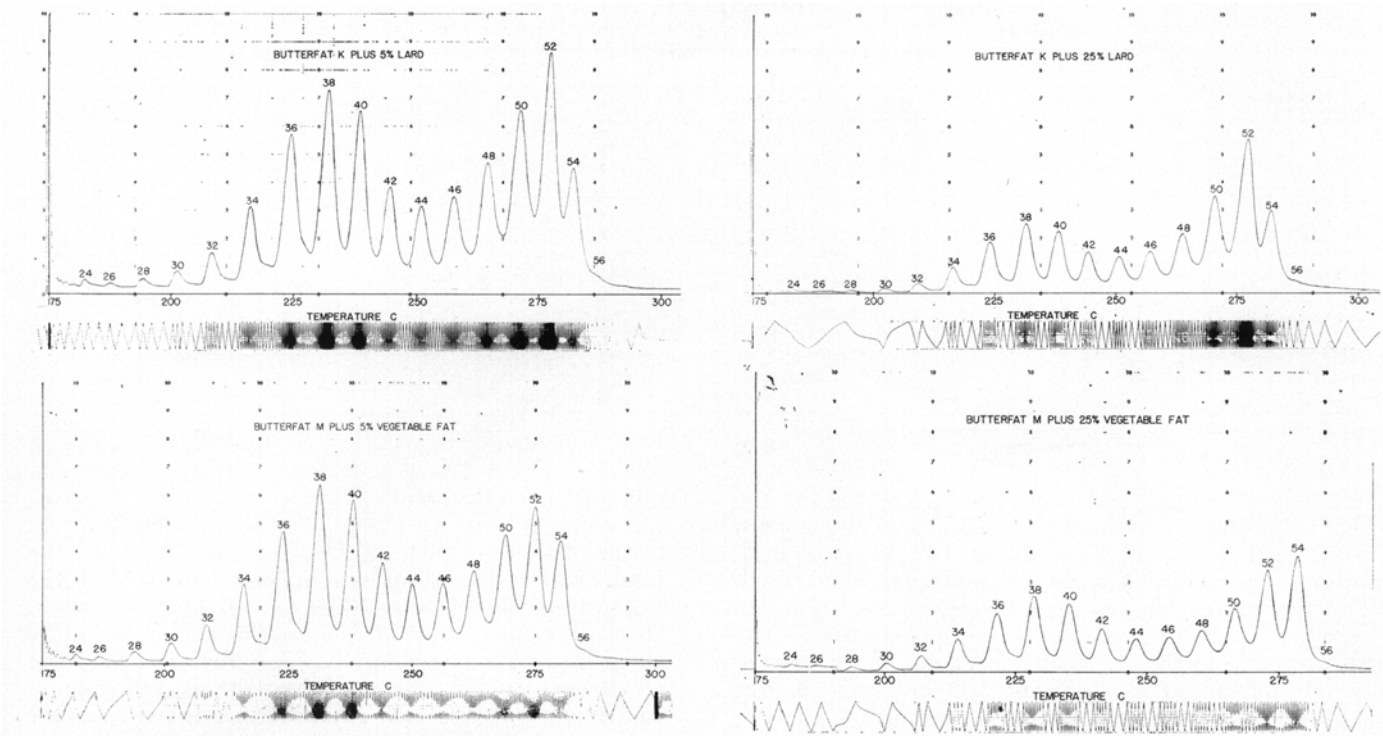

FIG. 2. Gas-liquid chromatographic elution patterns recorded for mixtures of butter with lard and vegetable fat.

on certain ranges of natural variation for all or selected triglyceride peaks might provide a suitable baseline. If the average contributions of the various triglyceride types in the four butterfat samples described here is taken as the baseline, it may be seen that adulteration of an unknown butterfat sample with lard or vegetable fat may be reliably spotted only above the 5% level.

From the above discussion it is obvious that artificial triglyceride mixtures might be devised that would match the gas chromatographic elution pattern of butterfat perfectly. Simple calculations of the type used here and based on the gas chromatographic elution pattern of coconut oil recorded elsewhere (3) and the present data for lard, indicate that relatively large amounts of an appropriate mixture of lard and coconut oil could be added to butterfat without being detected by this means. In view of this, the present technique would always require an examination of suspected adulterated butterfat samples by other methods whenever negative results were obtained.

The ease of detection of butterfat adulteration will vary with the type of adulterant. Any foreign fat that will contribute significantly any fatty elements not usually found in butter should be readily detected at the 1% or lower level. In practice this method should permit an easy spotting of butterfat supplementation with most common fat products at the 10% level, a quantity which is often quoted to permit marginal profits. When dealing with sophisticated offenders using carefully balanced proportions of lard and coconut or palm kernel oil, the limit of detection by this method may be considerably higher, and in such cases other methods may have to be used. With experience, however, additional relationships for the characterization of native butterfat by gas chromatography may become obvious and this method may then be extended to include also mixed coconut oil and lard adulteration. Among these potential and as yet unexplored approaches, one might mention the glyceride elution patterns obtained for selectively oxidized (6) oil samples, and oil samples from which the unsaturated triglycerides have been

TABLE IV

Anticipated and Observed Recoveries of Various Triglyceride Types from Mixtures of Butter and Vegetable Fat [a]

Triglyceride type	Butterfat M plus 1% vegetable fat		Butterfat M plus 3% vegetable fat		Butterfat M plus 5% vegetable fat		Butterfat M plus 10% vegetable fat		Butterfat M plus 25% vegetable fat	
	Anticipated [b] % [c]	Found %	Anticipated %	Found %	Anticipated %	Found %	Anticipated %	Found %	Anticipated %	Found %
C_{56}	0.6	0.5	0.7	0.9	0.8	0.8	0.9	1.0	1.3	1.1
C_{54}	7.1	7.0	8.2	8.9	9.3	9.3	12.0	11.0	20.2	18.2
C_{52}	11.7	11.7	12.1	12.3	12.5	11.6	13.4	13.1	16.3	15.8
C_{50}	10.8	10.2	10.7	11.6	10.6	10.8	10.2	10.2	9.2	9.1
C_{48}	8.4	7.8	8.2	8.1	8.1	7.8	7.7	7.2	6.4	6.4
C_{46}	6.3	6.1	6.2	6.6	6.0	6.0	5.7	6.0	4.8	5.2
C_{44}	5.6	5.8	5.4	5.6	5.3	5.6	5.1	5.2	4.2	4.7
C_{42}	6.7	6.8	6.6	7.2	6.5	7.0	6.1	6.9	5.1	5.7
C_{40}	11.9	12.2	11.6	11.0	11.4	11.3	10.8	10.9	9.0	9.5
C_{38}	13.0	13.1	12.7	12.0	12.5	12.3	11.8	12.2	9.8	10.3
C_{36}	9.5	9.4	9.3	8.8	9.1	9.4	8.6	8.8	7.2	7.9
C_{34}	4.9	5.3	4.8	4.0	4.7	4.5	4.5	4.1	3.7	3.8
C_{32}	2.1	2.4	2.1	1.7	2.0	2.1	1.9	2.0	1.6	1.5
C_{30}	0.7	0.9	0.7	0.8	0.7	1.0	0.7	0.8	0.6	0.7
C_{28}	0.4	0.5	0.4	0.3	0.3	0.5	0.3	0.4	0.3	0.2
C_{26}	0.2	0.2	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.0
C_{24}	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0

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

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