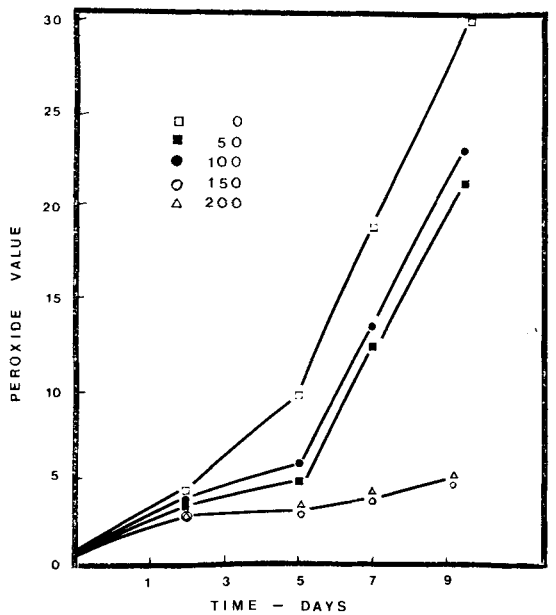

FIG. 5. Peroxide values of potato chips produced in oils containing different levels of TBHQ during storage at 55 C.

potato chips.

Although thermal destruction and loss through steam distillation of some phenolic antioxidants during frying of oils have been reported by Stuckey (17), the results in Figures 4 and 5 indicated that TBHQ added to the frying oil increased the oxidation stability of the potato chips. Since the higher the added TBHQ, the lower the pentane and peroxide values of potato chips, the total TBHQ content added in the potato chips could be up to 150 or 200 ppm which is the maximum legal limit of TBHQ based upon the oil content of potato chips (18).

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Fractional Crystallization and Gas Chromatographic Analysis of Fatty Acids as a Means of Detecting Butterfat Adulteration

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ABSTRACT

A method has been devised which gives the distribution of saturated and unsaturated fatty acids of pure and adulterated cow and buffalo ghee with lard or margarine. It involves fractionation of pure and adulterated butterfat into fractions by fractional crystallization. The composition of the fatty acids liberated by the hydrolysis of each of the fractions was determined by gas chromatography. Adulteration of cow and buffalo ghee with various levels of lard or margarine caused significant changes in certain fatty acids, i.e., 22:0, 18:1, 18:0 and 16:0. It is possible to determine the extent of admixture of lard or margarine to either cow or buffalo ghee by applying a simple regression equation for certain fatty acids. This technique provides a basis for the detection of lipid adulteration.

INTRODUCTION

Butterfat is much higher in price in comparison with other fat sources. Unethical suppliers used to adulterate butterfat with manufactured and other fats which are quite similar in chemical composition and less expensive. Adulteration of butterfat is a continuing problem for food law enforcement and commercial quality control laboratories. Substantial endeavors among scientists were made to find ways to detect butterfat adulteration. Consequently, several methods have been proposed in this respect such as differential thermal analysis (1) and various chromatographic techniques (2-4). The latter methods dealt with fatty acids, unsaponifiables and ratios of some compounds belonging to each lipid class and seem to be superior to the other methods in detecting lipid adulteration. Continuing efforts to achieve decisive techniques to check lipid adulteration are being made. The present investigation describes the fractional crystallization process at different temperatures in conjunction with gas chromatography as a satisfactory tool for characterization of lipid adulteration.

MATERIALS AND METHODS

Sources of Samples

Pure cow and buffalo ghee were obtained from the Food Science and Technology Department, Dairy Science Division, Faculty of Agriculture, Cairo University. Margarine and crude lard were purchased from the Abu-Zaabal Company and the local market, respectively. The dissected lard was heated at 70 C and the melted fat was filtered while warm through Whatman no. 1 filter paper to obtain tissue-free and water-free lard lipids. These lipid samples can be considered as authentic materials. Mixtures were prepared containing 5, 10, 15, 20, 25 and 30% (w/w) for margarine or lard in cow or buffalo ghee.

Sources of Authentic Fatty Acids

A set of standard fatty acids 10:0, 11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 16:1, 17:0, 18:0, 18:1, 18:2, 18:3, 20:0, 21:0 and 22:0 with stated purity of 99% by gas liquid chromatography (GLC) was purchased from Nu-Chek-Prep. The purity of each standard compound was checked by GLC and gave one peak.

Fractionation of Lipid Materials

A series of crystallizations were conducted on lipid samples from silver ion-containing solvents. The lipid materials to be fractionated were dissolved in the silver nitrate-saturated methanol/acetone mixture (70:30, v/v) (5). The ratio of lipid to silver nitrate-saturated solvent was 1:10 (w/v). The crystallization process was carried out in a constant temperature cabinet and crystals were separated at the same temperature at which crystallization had taken place. The lipid solution was held at 22 ± 1 C for 24 hr before filtering off the precipitate. The crystals were washed twice with fresh solvent mixture (5 mL each time) precooled to the temperature of the crystallization cabinet. The mother liquor was used again and the same process was repeated at 7 ± 1 C and -8 ± 1 C. Hence, each lipid sample was fractionated by fractional crystallization into 3 different fractions.

Methylation of Lipid Materials

The conversion of lipid samples and standard fatty acids to fatty acid methyl esters suitable for GLC analysis was done by methanolysis using methanolic potassium hydroxide (6).

Separation, Identification and Determination of Fatty Acid Methyl Esters

The methyl esters of the fatty acids and authentic compounds were analyzed with a GCV Pye Unicam gas chromatograph equipped with dual flame ionization detectors and dual channel recorder. The chromatographic conditions were identical to those described by Farag et al. (7). Standard methyl esters of fatty acids were used as authentic materials for the characterization of the unknown fatty acids, by comparing relative retention times. The retention time for palmitic acid (16:0) was given a value of 1.00. The peak area was measured by triangulation and the corrected peak areas were obtained by dividing the areas by GLC response factors for each fatty acid. The percentage of the fatty acid was calculated as the ratio of the partial areas to the total area. All samples were analyzed in duplicate and average values are presented.

Statistical Analyses

The fatty acids which exhibit major changes in their amounts due to admixture with lard or margarine were subjected to various statistical analyses (t-test, correlation coefficient (r), coefficient of determination [R^2%], regression coefficient and multiple regression). The fatty acid percentages were transformed using arcsine transformation for correlation analysis (8).

RESULTS AND DISCUSSION

There is a growing need to be able to predict the presence of foreign fats in dairy products before they appear on the market. Hence, a series of crystallizations at different temperatures (22, 7 and -8 C) were conducted on pure and adulterated lipid materials to find a satisfactory method of detecting lipid adulteration. GLC techniques were employed in the present study for the qualitative and quantitative determination of various fatty acid methyl esters of lard and margarine admixture to buffalo and cow ghee. The chromatographic separation conditions were selected in order to determine only the medium- and long-chain fatty acids (C_{10}–C_{22}). The fatty acids 11:0, 13:0, 15:0, 15:1, 16:1, 17:0, 18:2 and 18:3 were found in amounts less than 0.2%, while the values of 10:0 and 12:0 ranged between 1% and 2%. None of these acids are listed in the tables given here. In general, the amounts of various fatty acids were divided into three categories, i.e., trace (<1%), minor (>1% –<10%) and major (>10%) components.

Influence of Fractional Crystallization on Fatty Acid Composition of Pure Lipids

Data of relative percentage distribution of the various fatty acid components obtained from buffalo ghee crystallized at 22 C are summarized in Table I. The major fatty acids were 14:0, 16:0, 18:1 and 22:0, with 16:0 being the predominant one. A comparison of the fatty acids separated from buffalo ghee crystallized at 22, 7 and -8 C indicated a gradual decrease and increase in the amounts of 14:0, 16:0, 18:0, 18:1, 20:0 and 22:0, respectively.

The fatty acid composition of cow ghee separated at 22 C indicated the presence of 10:0 as a trace substance; 12:0, 14:0, 18:0, 18:1 as minor materials; and 16:0 and 22:0 as major constituents, with 22:0 being the predominant acid (Table II). The fatty acid profile of cow ghee crystallized at 7 C was quite different from the fraction crystallized at 22 C. For instance, the amount of 22:0 was 1.4 times greater than that in the fraction obtained at 22 C, while the amounts of 18:1, 18:0, 16:0 and 14:0 were respectively, 1.3, 2.6, 1.5 and 1.6 times lower than that present in the first fraction. The same changes were found in fatty acids of the fraction obtained at -8 C. In general, the greatest changes had taken place with 22:0, 18:1 and 16:0 acids.

Table I shows the fatty acid composition of lard fat crystallized at 22 C. The prevalent acids were 16:0, 18:0, 18:1 and 22:0. The fractional crystallization at 7 and -8 C caused an enormous decrease in the quantities of 16:0, 18:0, 18:1; a decrease in the amount of 20:0 and a remarkable increase in the 22:0 content.

The analysis of margarine fraction separated at 22 C indicated the presence of 16:0, 18:1 and 22:0 as major constituents, with 18:1 as the most abundant fatty acid (Table II). Cooling the margarine mother liquor at 7 and -8 C caused a remarkable decrease and increase in the amounts of 16:0, 18:0, 18:1 and 20:0, 22:0 fatty acids, respectively. The ratio of total saturated to total unsaturated acids (TS/TU) for margarine was the most different from the other lipids.

In all pure lipid substances (cow, buffalo, lard and margarine) the TS/TU and 22:0/16:0 ratios were increased by repeating the crystallization process at different temperatures (7 and -8 C). In other words, fractional crystallization tended to concentrate the saturated even-numbered

TABLE I

Changes in the Most Abundant Fatty Acids Obtained from Buffalo, Lard, Margarine and Adulterated Samples by Fractional Crystallization

Extent of admixture		14:0	16:0	18:0	18:1	20:0	22:0	14:0	16:0	18:0	18:1	20:0	22:0	14:0	16:0	18:0	18:1	20:0	22:0
Buffalo	Lard			Fraction 1						Fraction 2						Fraction 3			
100	0	10.7	30.1	6.9	18.7	8.5	22.2	2.7	12.1	1.9	1.5	12.4	67.1	2.1	5.8	1.7	1.4	17.5	68.4
95	5	10.7	32.5[a]	7.4	19.3	7.3	20.0	1.1	12.5	2.8	1.3	11.6	67.2	2.0	4.9	2.2	1.0	17.0	69.2
90	10	8.7	32.5[a]	9.0[b]	22.3[b]	5.1	19.9	1.8	12.8	2.8	1.3	12.6	65.5	1.9	4.7	1.5	0.9	17.0	70.1
85	15	8.0	32.5[a]	9.1[b]	22.9[b]	5.1	20.0	1.7	12.0	2.1	1.3	13.0	68.0	1.0	3.1	1.9	0.7	17.3	74.0
80	20	7.7	33.5[b]	9.1[b]	23.5[b]	5.4	18.1	1.7	12.4	2.0	1.3	12.4	67.6	1.2	3.2	2.0	0.5	17.0	72.3
75	25	7.1	33.3[b]	9.6[b]	25.1[b]	4.9	17.8	0.9	12.1	1.9	1.4	11.1	70.8	1.0	3.0	1.3	1.0	15.3	75.4[a]
70	30	5.9	34.2[b]	8.9[b]	27.5[b]	4.4	17.3	0.8	12.3	1.4	1.3	10.3	72.1	1.3	2.4	1.5	1.1	13.9	77.2[a]
0	100	2.2	36.2	12.8	26.3	4.0	18.3	0.1	2.2	1.1	1.6	6.7	88.1	0.2	1.7	0.5	0.9	6.2	90.0
r			0.961[c]	0.971[c]	0.582														0.984[c]
Buffalo	Margarine																		
100	0	10.7	30.1	6.9	18.7	8.5	22.2	2.7	12.1	1.9	1.2	12.7	67.1	2.1	5.8	1.7	1.4	17.5	68.4
95	5	11.2	31.9	5.9	21.7	7.7	18.4	1.8	11.5	2.0	1.1	14.6	66.1	1.9	6.0	1.9	1.5	16.4	71.3
90	10	7.8	32.5	5.6	24.4	7.0	19.9	1.7	12.0	2.4	1.1	12.2	67.6	1.5	4.7	1.3	1.5	16.0	71.2
85	15	9.0	31.2	5.1	26.5	5.3	20.3	1.0	12.1	2.5	1.3	11.5	69.0	2.4	4.8	2.1	1.6	12.0	73.1
80	20	7.3	30.8	4.6	34.8[b]	4.8	14.9	1.5	12.2	2.2	1.1	12.2	68.5	1.6	5.9	1.9	1.6	11.1	74.4[a]
75	25	8.3	30.5	3.2	35.9[b]	3.7	16.2	1.4	12.1	2.5	1.5	10.5	69.3	1.2	4.8	1.8	1.7	10.2	76.8[b]
70	30	7.9	31.1	3.3	35.8[b]	3.8	16.5	1.2	12.1	2.5	2.6	10.8	69.0	1.4	5.4	2.0	1.8	9.9	77.0[b]
0	100	1.0	28.5	1.0	55.1	1.0	13.2	0.3	3.6	0.1	8.4	1.7	85.7	0.2	2.8	0.1	6.8	4.3	85.6
r					0.997[c]														0.956[c]

[a,b]Mixtures are significantly different from the pure ghee at 5% and 1% levels, respectively.
[c]Highly significant at 1% level.
r indicates correlation coefficient.

TABLE II

Changes in the Most Abundant Fatty Acids Obtained from Cow, Lard, Margarine and Adulterated Samples by Fractional Crystallization

Extent of admixture		14:0	16:0	18:0	18:1	20:0	22:0	14:0	16:0	18:0	18:1	20:0	22:0	14:0	16:0	18:0	18:1	20:0	22:0
Cow	Lard				Fraction 1						Fraction 2						Fraction 3		
100	0	7.7	24.7	7.0	6.9	10.8	40.0	4.8	16.9	2.7	5.4	11.3	56.5	6.0	16.0	2.3	5.0	12.1	56.5
95	5	11.3	29.4[a]	7.5	17.5[b]	7.8	23.4	2.6	16.3	2.6	1.2	13.3	61.0	5.0	16.3	2.2	6.2	12.0	56.0
90	10	11.3	29.6[a]	9.2[b]	19.4[b]	4.8	22.6	2.5	14.8	3.1	1.3	13.2	62.0	4.7	15.9	2.5	5.2	12.3	56.6
85	15	10.8	29.5[a]	9.8[b]	20.7[b]	4.5	21.1	2.1	14.1	2.5	1.3	13.2	65.0	2.2	10.4	2.1	2.4	12.6	67.8[a]
80	20	9.1	35.0[b]	9.2[b]	24.3[b]	4.4	15.1	2.2	13.8	2.4	1.4	12.5	65.4	2.8	8.8	2.6	1.8	12.6	68.6[a]
75	25	8.7	36.4[b]	10.2[b]	24.7[b]	3.2	15.3	2.2	12.9	1.8	1.4	11.5	67.8	2.1	7.6	2.0	1.0	12.0	73.0[b]
70	30	8.6	36.7[b]	9.6[b]	23.5	2.8	17.1	2.1	12.8	1.8	1.4	11.3	69.2	2.0	6.6	1.3	1.0	12.4	74.0[b]
0	100	2.2	36.2	12.8	22.3	8.0	18.3	0.1	2.2	1.1	1.6	6.7	88.1	0.2	1.7	0.5	0.9	6.2	90.0
r			0.578	0.965[c]	0.321														0.988[c]
Cow	Margarine																		
100	0	7.7	24.7	7.0	6.9	10.8	40.0	4.8	16.9	2.7	5.4	11.3	56.5	6.0	16.0	2.3	5.0	12.1	56.5
95	5	8.5	23.9	6.1	8.3	10.5	40.0	3.7	16.7	2.9	3.1	11.5	59.7	4.9	15.4	2.9	4.4	12.1	58.0
90	10	8.2	22.7[a]	6.2	15.3	6.9	37.8	4.6	15.3	2.3	3.2	18.7	59.2	3.9	13.7	2.9	4.4	10.9	60.9
85	15	8.0	27.2[a]	5.4	24.5[b]	4.9	27.7	2.2	14.9	2.2	1.2[b]	13.7	64.0	3.9	10.6	2.0	4.0	9.0	67.4[a]
80	20	8.2	27.3[a]	5.7	25.0[b]	4.9	26.3	2.0	15.0	3.0	1.2[b]	13.9	62.7	3.4	10.5	2.8	4.4	8.6	66.2[a]
75	25	7.0	27.1[a]	5.0	29.0[b]	5.0	24.2	1.7	12.7	3.2	1.2[b]	12.2	64.0	2.6	10.4	2.6	4.3	7.8	69.2[b]
70	30	6.2	28.2[b]	4.2	37.5[b]	4.2	17.7	1.8	12.6	3.0	1.1[b]	12.0	67.1[a]	2.6	8.7	2.2	4.2	7.8	71.4[b]
0	100	1.0	28.5	1.0	55.1	1.0	13.2	0.3	3.6	0.1	8.4	1.7	85.7	0.2	2.8	0.1	6.8	4.3	85.6
r			0.534		0.961[c]						0.985[c]		0.992[c]						0.990[c]

[a,b] Mixtures are significantly different from the pure ghee at 5% and 1% levels, respectively.
[c]Highly significant at 1% level.
r indicates correlation coefficient.

TABLE III

Linear Regression Equations for the Adulteration Ratios (X) of Adulterants Mixed with Pure Ghee and Certain Fatty Acids (Y)

Fatty acid	Mixing with lard	Fatty acid	Mixing with margarine
	Cow		
18:0	$Y = 8.824 + 0.039\ X$ $R^2\% = 93.1$	18:1	$Y = 20.918 + 0.350\ X$ $R^2\% = 92.4$
	Buffalo		
16:0 (Y_1)	$Y = 32.36 + 0.04\ X$ $R^2\% = 92.4$	18:1	$Y = 29.049 + 0.259\ X$ $R^2\% = 99.4$
18:0 (Y_2)	$Y = 8.284 + 0.044\ X$ $R^2\% = 94.3$ $X = -463.917 + 12.645Y_1 + 11.097Y_2$[a] $R^2\% = 99.5$		

[a]Represents the multiple regression equation between adulteration ratio and the percentage of 16:0 and 18:0 fatty acids in buffalo ghee.

fatty acids in the semisolid fraction by crystallization sequence.

Influence of Fractional Crystallization on the Adulterated Lipid Fatty Acids.

Identification of fatty acids is of practical interest because when taken as a group, they furnish a fingerprint for detection of butterfat adulteration. Adulteration in the current study was determined by using the fatty acids occurring in the adulterants in amounts higher than that in pure lipids. In contrast, the presence of compounds in higher quantities in pure lipids than in adulterants was not used as an indicator of admixture.

Statistical analyses indicated that the amounts of 16:0, 18:0 and 18:1 acids were significantly changed with different adulteration levels and can be used as a marker to detect admixture. The significant differences were found in these acids of the first fraction. Consequently, statistical information illustrated that there is no need to carry on further crystallizations at different temperatures in routine analysis and one should rely on the acids of the first crystallization process to check lipid adulteration. It is worth mentioning that 18:0 and 18:1 obtained from the first fraction were the momentous acids when lard and margarine mixed with pure lipids, respectively, to detect adulteration. However, 22:0 of the second and third fractions was the important acid in this respect, although it did not increase significantly up to a level of 20%.

In both cow and buffalo ghee, 18:0 had a highly significant correlation coefficient with admixture by lard. This acid showed significant difference when lard was added to pure cow or buffalo ghee at a 10% level. However, the correlation coefficient with various adulteration levels using 18:1 was insignificant. Therefore, this acid cannot be used to characterize adulteration of cow or buffalo ghee with lard. In contrast, this acid became the most important acid since it showed a highly significent (1%) correlation with various margarine adulteration levels. The amount of 18:1 increased significantly when it was present at 15% and 20% levels in cow and buffalo ghee, respectively.

It is possible to determine the extent of admixture of lard or margarine to either cow or buffalo ghee by applying a simple regression equation for certain fatty acids (Table III). By analyzing the fatty acids of lipid samples and according to the following equation, the adulteration levels can be determined:

$$Y = A + BX$$

where Y = the concentration of a particular fatty acid; A = constant value, the intercept of the regression line; B = regression coefficient; and X = adulteration ratio.

The multiple regression equation which had the highest (R^2) value will be the predominant equation to detect the adulteration of buffalo ghee. Generally, when the percentages of 16:0, 18:0 and 18:1 acids of fraction 1 for the pure buffalo ghee were known, adulteration with lard could be detected at the 10% level and with margarine at the 20% level. Concerning cow ghee, adulteration with lard could be distinguished at the 5% level and with margarine at the 15% level. This approach provides a basis for the detection of lipid adulteration. Further work is needed using ghees, lard and margarine produced under a variety of conditions, e.g., season of the year, oils used by different countries in making margarine and the effect of hydrogenation in order to generalize the present technique.

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