wax containing 0.02% BHT or BHA to accelerated oxidation. The induction periods of oxidation, as determined from the oxidation curve and the refractive index curve (Table 1), proved BHT an effective antioxidant comparable to the natural antioxidant present in crude wax, while BHA was found superior even to it.

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Adulterated Butterfat: Fatty Acid Composition of Triglycerides and 2-Monoglycerides

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Beef tallow and cottonseed oil were mixed with a pure butterfat in the ratios of 2%, 4% and 6% to obtain admixtures of beef tallow with butterfat and cottonseed oil with butterfat. The hydrolysis of individual triglycerides was carried out using the lipase to obtain 2-monoglycerides. The results indicated that butterfat had a higher percentage of C14:0 and C16:0 acids than found in the triglycerides and 2-monoglycerides of beef tallow and cottonseed oil.

Beef tallow contained a higher proportion of C18:0 and C18:1 acids than butterfat and cottonseed oil triglycerides or 2-monoglycerides. Cottonseed oil had a higher percentage of C18:2 acid located in triglyceride or 2-monoglyceride than found in butterfat or beef tallow triglycerides and 2-monoglycerides.

The analysis of the samples of butterfat containing 2%, 4% and 6% beef tallow revealed that the addition of beef tallow to butterfat affected the fatty acid composition of butterfat triglycerides and 2-monoglycerides with C18:0 and C18:1 acids; the effect was increased with increasing percentages of beef tallow.

The addition of cottonseed oil to butterfat in the ratios of 2%, 4% and 6% affected the fatty acid composition of butterfat triglycerides and 2-monoglycerides. It was found that both C18:1 and C18:2 increased as the added cottonseed oil percentages increased.

Several trials were carried out to prove the purity of butterfat. The addition of beef fat less than 5% cannot be detected with certainty and may be missed. The presence of higher percentages will almost always be indicated by the melting point of the crystals deposited from ether solution (1).

TLC separation of butterfat into long and short chain triglycerides, followed by a selective lipolysis of each fraction with pancreatic lipase to determine the C16:C18 ratio in position 2 of the triglycerides of each band, could detect adulteration of butter even with low amounts (5%) of beef or foreign fats (2). The addition of small amounts of pig or buffalo fat to cow or buffalo ghee results in the appearance of an extra peak located at high temperature in the melting and crystallization curves as determined by differential scanning calorimetry. Ghee adulterations with these animal fats at levels down 5% are clearly seen in crystallization diagrams (3).

The fatty acid distribution in the triglycerides of milk and other animal fats can be determined by use of the specificity of pancreatic lipase for cleaving the fatty acids esterified in the 1 and 3 positions of the glycerol. It was found that weight percents of fatty acid composition of original triglycerides and monoglycerides formed of normal milk were C14:0, 12%, 20%; C16:0, 38%, 40%; C18:0, 14%, 8.7%; C18:1, 23%, 15%; and C18:2+3, 4.0%, 1.9%, respectively. The fatty acid compositions of original triglycerides and monoglycerides found in steer depot fat were C14:0, 4.6%, 8.8%; C16:0, 29%, 16%; C18:0, 24%, 14%; C18:1, 36%, 54%; and C18:2+3, 3.0%, 1.2%, respectively (4).

On the other hand, the distribution of fatty acids among the primary and secondary positions of the triglycerides of various species of plants demonstrated that certain fatty acids occupy specific positions on the triglyceride molecule. Palmitic and stearic acids were found to be esterified predominantly at the primary positions, and the fatty acids having a chain length of greater than 18 carbon atoms are also esterified predominantly at the primary positions (5).

Pancreatic lipase digests some classes of milk triglycerides more rapidly than others. It is concluded that milk lipase did not exhibit intramolecular specificity when glyceryl 1-palmitate 2,3 dibutyrate was the substrate (6,7). Palmitic acid showed a definite tendency to

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be preferentially esterified in the 2-position. As the molecular weight decreased, the palmitic acid shifted to random distribution and was completely reversed in the low molecular weight triglycerides, where it was preferentially esterified in the terminal position of glycerol (8).

Butyric acid was found in the 2-position of the glycerides, but was more heavily concentrated in the 1- and 3-positions. The long-chain acids C12, C14 and C16 were found in greater than random percentages in the 2-position. Less than random portions of C18 and C18:1 were esterified at this position (9).

Although oleic acid is usually associated with palmitic and stearic acids in triglycerides, the short-chain acids tend to be associated with the polyethenoid acids. Butyric and caproic acids were predominantly in the 1,3-positions, whereas lauric, myristic and palmitic tended to be in the 2-position. The C18 acids seemed to be distributed among all three positions in equal amounts (10).

On the other hand, in both plant and animal triglycerides the 2-position of the glycerol is esterified predominantly by an unsaturated acid, whereas the saturated acids occupy predominantly the 1-position.

A notable exception to this generalization is pig fat, in which the structure is reversed. Here the majority of the saturated acids are found in the 2-position and the unsaturated acids predominate in the outer position of the glyceride. Milk fat tends to resemble pig fat, but the disparity of distribution is not so wide (11–13).

The aim of this work was to use the techniques of pancreatic hydrolysis and GLC to investigate the fatty acid composition of the 2-position monoglycerides formed from triglycerides, after the addition of low amounts of adulterants such as beef tallow and cottonseed oil to the pure butterfat.

MATERIALS AND METHODS

Source of samples and sampling. Milkfat was obtained from Friesian cow's milk by extraction with chloroform/methanol (2:1, v/v). Lipids of beef tallow were obtained similarly. The solvent was evaporated using a rotary evaporator.

Local refined cottonseed oil was obtained from the market. The lipids obtained were mixed as follows: Tallow was added to butterfat at 2%, 4% and 6% w/w.

Triglycerides fractionation. A preparative thin layer chromatography (TLC) was applied to separate the triglyceride fractions of the admixtures tallow + butterfat and cottonseed oil + butterfat (14).

Procedure for lipase hydrolysis. Hydrolysis of triglyceride fractions was carried out to obtain the 2-monoglycerides (15).

Approximately 1.0 g of the sample was transferred to a tipped bottom flask, and the temperature was adjusted to 40 C ± 0.5. To the sample, 10 ml of 1.2 M ammonium chloride-ammonia (NH_4Cl-NH_4OH) buffer, adjusted to pH 8.5, 2 ml (22%, w/v) solution of calcium chloride hexahydrate and 0.1 ml of a 25%, w/v solution of bile salt (sodium taurocholate) were added. Pancreatic lipase (100 mg) was added with stirring and the pH was maintained at 8.5 during lypolysis. After 30 min, about 60 or 70% of the original neutral triglyceride was converted into 2-monoglyceride. Then the reaction was stopped by the addition of 4N hydrochloric acid to bring the pH to 1.0. The reaction mixture was extracted with ether, and the ethereal solution was dried over anhydrous sodium sulphate, filtered and evaporated under reduced pressure. The 2-monoglycerides were separated from the other lipids and free fatty acids by TLC.

Methylation and analysis of 2 monoglycerides. Samples of 2-monoglycerides were refluxed with 2% sulphuric acid in methanol for 3 hr at 60 C (16). Methyl esters of samples were subjected to a dual flame ionization detector Varian 3700, with packed column 20% diethyline glycol succenate (DEGS) on Chromosorb W (60–80 mesh). The column length was 6 ft, with internal diameter 1/8 inch. Carrier gas flow rate (He)30 ml/min, hydrogen flow rate 30 ml/min, air flow rate 300 ml/min. Column temperature 190 C, injection temperature 220 C, detector temperature 300 C.

RESULTS AND DISCUSSION

The data in Table 1 show the major fatty acid composition of cow's butterfat, tallow and cottonseed oil triglycerides, and those of 2-monoglycerides of the three fats. It is clear from these data that the fatty acid composition of triglycerides differs widely among the three fats. Butterfat is characterized by a higher percentage of C14:0 and C16:0 acids compared to tallow and cottonseed oil, which are characterized by a higher percentage of unsaturated C18:1 and C18:2, respectively.

These results are confirmed by many investigators (13,15,17,18).

Furthermore, the data obtained for the major fatty acids located at the 2-position show the prevalence of C16:0 acid in butterfat monoglyceride. This is true because C16:0 tended to be located in the 2-position of butterfat monoglyceride. The same was evident in tallow and cottonseed oil monoglycerides with respect to C18:1 and C18:2 acids. These previous observations are in accordance with many investigators (4,8,10,13,15,17,18).

From the same data (Table 1), it is obvious that the fatty acid composition of the triglycerides of 2% tallow in butterfat showed more significant variations than that of butterfat. C16:0 acid decreased, while C18:0 acid and C18:1 increased. The increase of C18:0 and C18:1 acids was expected because beef tallow triglyceride contained high amounts of these acids compared to butterfat triglyceride. The increase of tallow to 4% and 6% in butterfat led to an increase of C18:0 and C18:1 acids accompanied by a decrease in C16:0 acid. These results are supported by several trials (4,7,11,19,20,21).

On the other hand, the fatty acid composition of the 2-monoglycerides of 2%, 4% and 6% tallow in butterfat showed the same trend as the fatty acids of the triglycerides. C14:0 showed no remarkable change.

The analyses of the fatty acid composition of the 2-monoglycerides of the above-mentioned three admixtures reveal that the increase in the percentage of beef tallow added to butterfat led to an increase of certain fatty acids. This can be helpful in detecting any addition of foreign fats, even at as low a percentage as 2.0% in butterfat.

The addition of cottonseed oil at percentage of 2.0%, 4.0% and 6.0% to butterfat (Table 1) greatly affected the fatty acid composition of the resultant admixtures of triglycerides. The unsaturated fatty acids C18:1 and C18:2 increased because cottonseed oil contains high amounts of these two acids. C14:0 acid did not change

TABLE 1

Major Fatty Acid Composition of Butterfat, Tallow and Cottonseed Oil Triglycerides and 2-Monoglycerides Resulting from Lipolytic Action, and of the Admixtures of the Latter Two Fats with Butterfat

Sample[a]		C14:0	C16:0	C18:0	C18:1	C18:2
Butterfat	I	12.17	35.46	10.00	23.82	trace
		13.66	39.18	7.47	17.10	0.40
Tallow	II	1.04	31.20	18.77	45.06	3.93
		3.06	32.95	8.23	53.76	2.00
Cottonseed oil	III	1.80	24.10	1.30	24.10	47.50
		2.63	9.14	2.50	28.19	58.74
	II+ I 2%	12.17	34.72	10.27	24.47	trace
		13.66	38.26	7.47	18.02	0.40
	II+ I 4%	11.00	34.00	10.48	25.97	trace
		13.64	37.29	7.50	18.95	0.43
	II+ I 6%	11.04	33.43	10.73	26.25	trace
		13.64	36.41	7.63	20.13	0.45
	III+ I 2%	12.17	34.63	10.00	23.82	0.83
		13.33	38.66	7.47	17.35	1.00
	III+ I 4%	12.15	33.03	10.00	24.56	1.71
		13.00	37.01	7.49	18.10	2.03
	III+ I 6%	12.11	31.71	10.00	25.11	2.53
		12.00	37.00	7.44	18.72	2.65

[a]Average of 5 lipolyzed analyzed samples.

considerably from its original level in butterfat, while C16:0 acid was decreased as the added percentage of cottonseed oil increased. It can be concluded that the determination of C18:1 and C18:2 in admixture of triglycerides can be used as a practical tool to detect the adulterant in pure butterfat (1,5,18).

The results also show an increase in C18:1 at the 2-position of monoglycerides in the admixtures of butterfat because cottonseed oil contains high amounts of this acid at the 2-position. Also, C18:2 showed a pronounced increase.

The opposite trend was observed for C14:0 and C16:0 acids in the 2-monoglycerides of the admixture due to the lower amount of these acids at the 2-position of cottonseed oil monoglycerides (5,10,13,17,18,22).

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