sec. According to their measurements, T_d for bis(2-ethylhexyl)sebacate and dibenzyl sebacate was 284C and 296C, respectively. Our results are approximately 5 degrees higher.

The stability of the ester appears to depend upon the stability of the free radical formed in the intermediate state. The greater the stability of that radical the greater the chance for reaction. Since the radical formed by the unsubstituted benzyl ester would probably be the least stable (or the most difficult to produce), its ester is expected to be the most stable. In contrast, bis(p-nitrobenzyl)dilinoleate should produce the most stable radical; therefore, it should be the least stable ester. The stabilities of the other esters are in between these extremes, as expected. All of the benzyl dilinoleate esters are more stable than the control compounds except bis(p-nitrobenzyl)dilinoleate. Partial hydrogenation gives added stability to the benzyl esters, showing the effect of unsaturation far away from the active site of the molecule.

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The Chemical and Physical Properties of Interesterified Milk Fat Fractions[1]

MOORE, J. L., T. RICHARDSON, and C. H. AMUNDSON, Department of Dairy and Food Industries, University of Wisconsin, Madison

Abstract

Five fractions of anhydrous milk fat were obtained by fractional crystallization in the absence of solvent. The fractions were characterized, physically and chemically, before and after interesterification.

Cholesterol tended to fractionate into the lowest-melting fraction.

Slipping points of 38.5C, 32C, 28C, 22.5C, and 17C, respectively, were reflected in widely varying micropenetration curves, while differences in fatty acid composition were relatively small. The three higher-melting fractions, with very similar fatty acid compositions, had very similar physical properties after interesterification. Slipping points of 38C, 37C, 37C, 33C, and 32C, respectively, indicated that interesterification generally hardened the fractions. Interesterification altered the physical properties of lower-melting fractions more than the properties of higher-melting fractions.

Interesterification caused some triglyceride degradation and some butyrate loss, but these factors could not fully explain the unequal physical property changes induced in the different fractions.

Fractionation possibly tends to separate symmetrical triglycerides from their lower-melting, unsymmetrical isomers, yielding fractions with apparent differences in the degree of randomness of their triglyceride structure.

Positioning of fatty acids within the triglyceride molecule seems to be a principal determinant of the physical properties of milk fat fractions.

Introduction

FULL UTILIZATION of milk fat in the future may depend upon the development of fractions having greatly modified properties, perhaps even "tailor-made" to specifications.

Fractional crystallization in the absence of solvent, interesterification, or combination of the two, might be used to produce milk fat fractions with widely varying physical properties. Interesterification is already industrially utilized and a recent patent (1) indicates that centrifugal fractionation of fat may also become commercially practical. Weihe (2) found that milk fat was hardened by interesterification. de Man (3) observed that the hardening of milk fat after interesterification was accompanied by an increased content of high-melting glycerides. Mickle, et al. (4) attributed the softening of butter-like products made from interesterified milk fat to triglyceride degradation.

Jack et al. (5,6) studied the composition of milk fat fractions derived by solvent fractionation. Physical properties of their fractions were not measured nor correlated with composition, and the fractions were not interesterified.

This study combined fractionation of milk fat with interesterification of the fractions to create physically modified fats. This paper attempts to correlate the differences in physical properties with composi-

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tion and with positional effects of fatty acids within the triglyceride molecule.

The distribution of cholesterol with fractionation was determined. The fractions were also characterized before and after interesterification by slipping points, by micropenetration curves, by fatty acid composition, by high-melting glyceride content, and by mono-, di-, and triglyceride composition.

Experimental Procedure

Sample Preparation

Anhydrous milk fat, prepared by Rice Lake Creamery Company, Rice Lake, Wisconsin, using the Creamery Package Process, was representative of 140,000 pounds of milk from mixed herds on winter rations. A 1 kg sample was fractionally crystallized in the absence of solvent, under nitrogen, using 72-hour incubation periods at 5C intervals from 30C to 15C. Crystals were packed by centrifuging, and liquid fat was decanted and placed at the next incubation temperature. Solid fractions at 30C, 25C, 20C, and 15C, and a liquid fraction at 15C were obtained and stored under nitrogen at −20C.

Part of each fraction and an unfractionated sample were interesterified by sealing the samples under nitrogen with 0.5% sodium methylate and heating at 100C for 1 hour. Catalyst was inactivated with acetic acid, samples were washed three times, dried, and stored under nitrogen at −20C.

Cholesterol

Cholesterol, assayed spectrophotometrically according to Mann (7), was determined by a modification of the method of Kritchevsky and Tepper (8). Total cholesterol was determined for a sample of the dry milk fat. Ester cholesterol was determined on a portion of the same sample from which free cholesterol had been precipitated as the digitonide, and free cholesterol was calculated by difference. Complete removal of free cholesterol required at least three washings with the alcoholic digitonin solution.

Physical Properties

Slipping points (open-capillary melting points, slip points or softening points) were determined according to AOCS method (Cc 3-25) described by Boekenoogen (9). Closed-capillary melting points yielded less reproducible results.

Micropenetration curves were determined accordto Feuge and Bailey (10).

Composition

Fatty acid composition of each fraction was determined by gas-liquid chromatography (GLC) according to Smith (11). Ethyl chloride extracts of the methyl esters were evaporated under standardized conditions and 0.16–0.20 μl aliquots were chromatographed. Esters from each fraction, hydrogenated by the method of Farquhar et al. (12), were also chromatographed.

An Aerograph A-600B Gas Chromatograph equipped with a hydrogen flame ionization detector was used. The column was 5 ft x 1/8 in. stainless steel packed with 10% ethyleneglycol succinate on 60/80 mesh Chromosorb W which had been treated with hexamethyldisilizane. Hydrogen and nitrogen (carrier gas) flow rates were 30 and 25 ml/min, respectively. Column and injector block temperatures were 185C and 250C, respectively. Recorder chart speeds of 240 in./hr for butyrate through laurate and 24 in./hr for remaining esters facilitated peak quantification.

TABLE I

Free, Ester, and Total Cholesterol Concentrations in Milk Fat Fractions (Data Are Averages of Two Observations)

Fraction		Cholesterol concentrations		
Number	Description	Free	Ester	Total
		(mg/100 g fat)		
1	Solid at 30C	216	20	236
2	Solid at 25C	258	19	277
3	Solid at 20C	226	17	243
4	Solid at 15C	234	37	271
5	Liquid at 15C	310	32	342
(Unfractionated) (Control)		230	30	260

Correction factors were necessary since peak area per cent response of the flame ionization detector is not equivalent to weight per cent when short chain methyl esters are present (13).

Peak areas were determined by triangulation, and area per cents were converted to weight per cents using factors to correct for the area per cent—weight per cent disparity and for volatility losses of short chain esters. Correction factors were developed by chromatographing standard mixtures of the major methyl esters after subjection to the extraction and evaporation procedure used for the samples. All significant peaks were quantified and identified using retention times of standards relative to methyl stearate, semi-log plots of retention time versus carbon number, chromatograms before and after hydrogenation, and comparison with published results (11,14–16).

All fractions were analyzed for high-melting glyceride content according to the solvent fractionation method of de Man (3). The resulting crystals were washed, dried, and weighed. Their glyceride compositions were observed by thin-layer chromatography (TLC) using the solvent system of Privett and Blank (17), and their fatty acid compositions were determined by GLC.

The Florisil column chromatography technique of Carroll (18) was used to characterize the changes in mono-, di-, and triglyceride concentrations resulting from interesterification.

Results and Discussion

Cholesterol

Cholesterol, as shown in Table I, tended to concentrate in the lowest-melting fraction. Other fractions were similar in cholesterol concentration. No large differences occurred in the relative proportions of free and ester cholesterol with fractionation.

Total cholesterol concentration in the unfractionated sample is comparable to that reported by Kritchevsky and Tepper (8) for butter and creams, but our values for the proportion of ester cholesterol are less. This may reflect biological variation, or our rigorous precipitation of free cholesterol.

Physical Properties

From Table II, fractionated milk fat yielded 40.3% of the highest-melting fraction and fairly equal amounts of the four lower-melting fractions. The slipping points of unaltered fractions had a wide range of values, with slipping points of successive fractions differing by 4C to 6C, and with a 21C total range from the highest- to the lowest-melting fraction. In contrast, slipping points of successive interesterified samples differed by 0C to 3C and the

TABLE II

Yields of Milk Fat Fractions and Slipping Points of Unaltered and Interesterified Milk Fat Fractions

Fraction		Yield[a]	Slipping points	
Number	Description		Before interesterification	After interesterification[b]
		(%)	(degrees C)	
1	Solid at 30C	40.3	38.5	38
2	Solid at 25C	14.9	32	37
3	Solid at 20C	11.9	28	37
4	Solid at 15C	15.8	22.5	33
5	Liquid at 15C	17.1	17	32
(Unfractionated)	(Control)	.....	33.5	35.5

[a] Percent of total yield; total yield 97.3%.
[b] Average of five interesterifications.

total range was reduced to only 6C. Interesterification hardened all samples except the highest-melting fraction.

The consistency characteristics of the unaltered fractions varied widely as shown in Figure 1. The micropenetration curves of the interesterified fractions converged toward a high value, approaching that of the highest-melting, unaltered fraction (Fig. 2). These data support the slipping point observations by confirming the general increase in hardness of each fraction and the decrease in range of hardness values of the fractions after interesterification.

The physical properties of all fractions except the highest-melting were changed significantly by interesterification. These induced changes were not unusual, considering the evidence that milk fat has a nonrandom triglyceride structure (19–27). That identical randomization conditions should cause successively larger changes in slipping points from the highest- to the lowest-melting fractions (Table II) was interesting in view of the fatty acid patterns.

As seen in Table III, small differences were noted in fatty acid composition of the fractions before interesterification. Trends were evident but not continuous through all fractions. The 4:0 through 12:0 saturates and all unsaturates tended to increase from fraction 1 to fraction 5, while 14:0 through 18:0 saturates tended to decrease. Interesterification generally made little difference in the fatty acid composition of the fractions. Butyrate was, however, significantly decreased, 8% to 25% in various fractions, with fraction 3 having the greater loss. Other volatile fatty acids were retained to a much greater degree.

The fatty acid loss implied that some degradation had occurred, and thin-layer chromatography confirmed this by indicating an increased diglyceride content for the interesterified fractions. Thin-layer patterns indicated equal degradation for all fractions. Separation of the glycerides of fraction 1, by Florisil column chromatography, showed 1.4%, 2.4%, and 94.4% mono-, di-, and triglycerides, respectively, be-

TABLE III

Fatty Acid Composition of Unaltered Milk Fat Fractions (Data Are Averages of Triplicate Analyses)

Fatty acid[a] methyl ester	Fraction					Unfractionated
	1	2	3	4	5	
	(Weight % of total)					
4:0	3.24	4.27	5.31	5.27	5.34	3.98
6:0	2.01	2.47	2.92	2.66	2.83	2.36
8:0	1.19	1.34	1.49	1.39	1.66	1.36
10:0	2.66	2.78	3.07	2.70	3.17	2.76
10:1	0.27	0.31	0.32	0.33	0.40	0.32
12:0	2.93	3.25	3.25	2.96	3.50	3.14
13:0 (12:1)	0.08	0.13	0.08	0.12	0.10	0.14
14br	0.05	0.16	0.06	0.14	0.10	0.12
14:0	9.72	9.36	8.98	9.58	9.37	8.39
14:1 (15br)	1.89	1.64	1.55	1.80	1.86	1.84
15:0	1.20	1.40	1.19	1.28	0.98	1.34
16br	0.27	0.35	0.24	0.35	0.35	0.35
16:0	32.08	29.57	30.60	29.25	24.34	30.05
16:1 (17br)	2.78	2.79	2.49	2.65	3.11	2.80
17:0	1.08	1.02	0.82	1.04	0.77	1.00
17:1 (18br)	0.46	0.51	0.29	0.43	0.40	0.37
18:0	12.93	12.00	11.77	10.59	8.71	11.74
18:1	22.42	23.85	23.27	24.32	29.15	24.93
18:2	1.55	1.66	1.36	1.97	2.21	1.78
18:3 (20:0)	1.19	1.14	0.94	1.19	1.65	1.23

[a] Carbon number: Number of double bonds. br = branched.

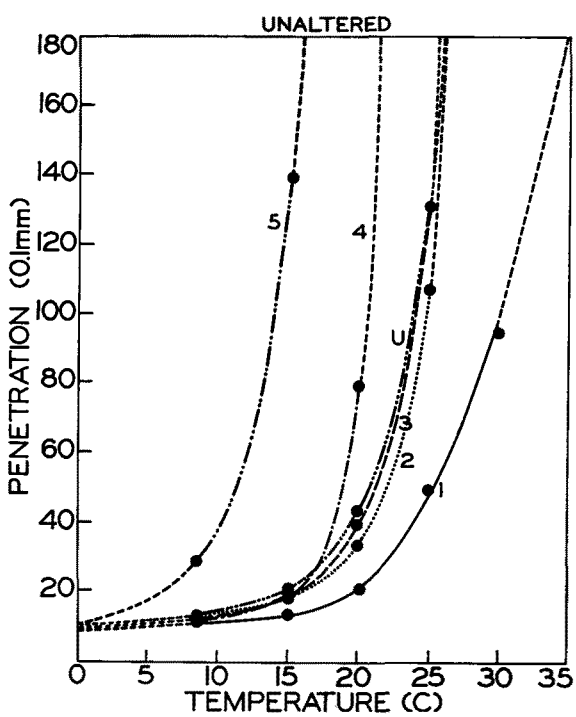

FIG. 1. Micropenetration curves of milk fat fractions before interesterification. Fraction 1—solid at 30C. Fraction 2—solid at 25C. Fraction 3—solid at 20C. Fraction 4—solid at 15C. Sample U—unfractionated control. Fraction 5—liquid at 15C.

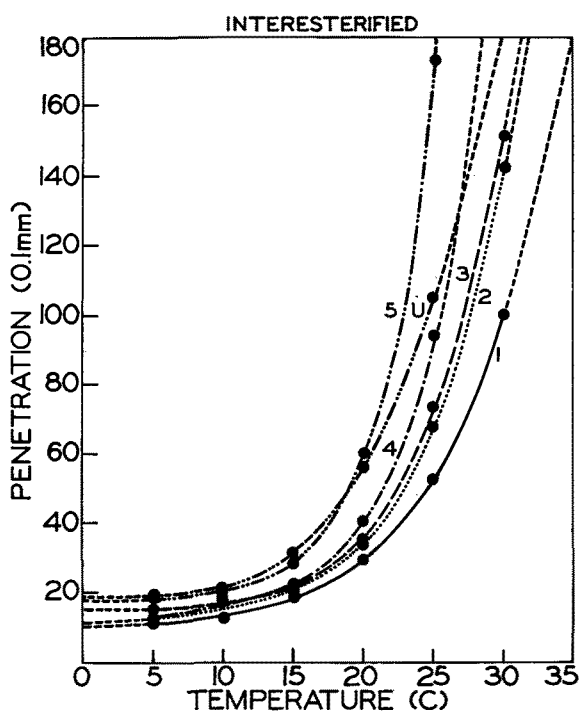

FIG. 2. Micropenetration curves of milk fat fractions after interesterification. Fraction 1—solid at 30C. Fraction 2—solid at 25C. Fraction 3—solid at 20C. Fraction 4—solid at 15C. Sample U—unfractionated control. Fraction 5—liquid at 15C.

TABLE IV

High-Melting Glyceride Content of Milk Fat Fractions Before and After Interesterification

Fraction		High-melting glyceride content	
Number	Description	Before interesterification	After interesterification
		(% of total)	
1	Solid at 30C	19.0	20.4
2	Solid at 25C	Tr.	18.2
3	Solid at 20C	0	17.4
4	Solid at 15C	0	4.1
5	Liquid at 15C	0	1.6
Unfractionated	Control	1.8	27.7

fore interesterification. The values after interesterification were 1.9%, 8.6%, and 89.5% mono-, di-, and triglycerides, respectively. Evidently the interesterification conditions resulted in some triglyceride degradation.

The decrease in butyrate may tend to explain the increased hardness of the fractions. The effects of an increased diglyceride content on the physical properties of the individual fractions are not known. Since the increase in diglycerides was apparently constant for all fractions, and butyrate loss was not directly correlated to increased hardness, these factors could not satisfactorily explain the hardening of the fractions. They certainly could not explain why the same interesterification conditions produced successively larger changes in physical properties from the highest- to the lowest-melting fraction.

As shown in Table IV, interesterification increased the HMG content of each fraction. There appeared to be a correlation between HMG content and physical properties or slipping points. Fractions with nearly equal slipping points (Table II) displayed nearly equal HMG contents with HMG varying in all fractions generally in proportion to physical properties. The single exception was the HMG fraction isolated from the interesterified unfractionated sample, which was in unusually high yield and exhibited a low slipping point of 39C, compared to 51C for the HMG from unaltered fraction 1. This HMG fraction was apparently contaminated with lower-melting glycerides.

Thin-layer chromatography showed that all HMG fractions consisted of triglycerides only. Fatty acid analyses, given in Table V, showed that all HMG fractions had a predominance of 14:0, 16:0, 18:0, and 18:1, and HMG fractions from interesterified fractions 1, 2, and 3 showed very similar composition in these fatty acids. The HMG fractions from fraction 1 before and after interesterification had nearly equal slipping points, 50C and 51C, despite differences in 16:0 and 18:1 concentrations.

Relatively small differences in fatty acid composition and small to moderate differences in total unsaturation contrast with the gross differences in physical properties of the fractions before interesterification. Differences in triglyceride structure could help account for an apparent lack of correlation between fatty acid composition and physical properties.

Interesterification of fraction 1 caused little change in fatty acid composition, HMG yield, and physical properties indicating a nearly random structure for this fraction. Unaltered fractions 2 and 3 were very similar to fraction 1 in fatty acid composition, but significantly different in HMG yields and physical properties. After interesterification, both had HMG

TABLE V

Fatty Acid Composition of High-Melting Glyceride Fractions Isolated from Milk Fat Fractions Before and After Interesterification (Data Are Averages of Triplicate Analyses)

Fatty acid [a] methyl ester	Fraction from which HMG isolated [b,c]				
	1 Before	1 After	2 After	3 After	Unfractionated After
	(Weight % of total)				
4:0	1.27	0.51	1.87	0.82	2.95
6:0	0.70	0.27	0.28	0.42	1.16
8:0	0.49	0.20	0.18	0.24	0.68
10:0	1.35	0.80	0.65	0.83	1.62
10:1	0.12	0.06	0.05	0.06	0.13
12:0	2.25	1.79	1.55	1.72	2.16
13:0 (12:1)	0.09	0.05	0.07	0.08	0.20
14br	0.05	0.02	0.04	0.04	0.02
14:0	10.10	10.29	11.37	9.29	10.48
14:1 (15br)	0.88	0.65	0.56	0.55	0.96
15:0	1.70	1.83	1.77	1.91	1.56
16br	0.44	0.38	0.20	0.16	0.22
16:0	41.97	48.16	49.51	50.07	39.84
16:1 (17br)	2.07	1.58	1.48	1.53	1.96
17:0	1.44	1.76	1.62	1.67	1.25
17:1 (18br)	0.18	0.18	0.08	0.18	0.02
18:0	21.85	23.76	21.66	22.09	17.33
18:1	11.26	6.17	5.87	6.70	15.49
18:2	0.92	0.84	0.67	0.96	1.08
18:3 (20:0)	0.87	0.70	0.52	0.68	0.90

[a] Carbon number: Number of double bonds. br = branched.
[b] Before or after interesterification.
[c] Insufficient amount of HMG for analysis isolated from unlisted fractions.

yields and physical properties similar to fraction 1. Presumably, fractions 2 and 3 possessed less random triglyceride structures. Fractions 4 and 5 appeared to have progressively less random structures. Apparently, fatty acid positioning plays a major role in determining physical properties of milk fat fractions, overshadowing moderate differences in fatty acid composition and total unsaturation. Possibly, fractional crystallization tends to separate symmetrical triglycerides from their lower-melting unsymmetrical isomers, yielding fractions with apparent differences in randomness of triglyceride structure.

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