The Modification and Analysis of Vegetable Oil for Cheese Making

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ABSTRACT: Objectives of this study were (i) to incorporate short-chain fatty acids (SCFA) in vegetable oils to obtain a bland product that could be used as a milk fat substitute in cheese making and (ii) to improve the methods for fatty acid analysis of vegetable oils modified with SCFA. Short-chain triglycerides (SCTG) were synthesized by esterifying SCFA with glycerol, and using a toluene azeotrope to remove the water of esterification. SCFA from two sources were used: (i) commercial acids and (ii) acids isolated by double distillation of milk fat methyl esters. The SCTG had a bitter, unacceptable flavor, but after interesterification with high-oleic sunflower oil (HOSO) and deodorization, the flavor was quite acceptable. SCTG were incorporated into HOSO at 100 and 120% of the levels found in the milk fat, by sodium methoxide-catalyzed interesterification. For fatty acid analysis, milk fat and simulated milk fat were converted to their decyl ester derivatives and analyzed by gas chromatography without further purification. The method was accurate and rapid for fatty acid analysis of fats containing a wide range of fatty acid chain lengths. All modified HOSO gave bland and acceptable flavors and had a SCFA composition close to that of milk fat. Results from using the modified HOSO in cheese making are reported in a later paper.

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KEY WORDS: High-oleic sunflower oil, interesterification, milk fat, oil modification, short-chain triglyceride, vegetable oil.

Milk fat is a major component in most cheese varieties, and many consumers limit their consumption of cheese because of concern about milk fat's saturated fatty acid and cholesterol content. Milk fat contains over 70% saturated acyl groups, and of these, laurate, myristate, and palmitate are considered particularly atherogenic (1). It has been suggested that from a nutritional standpoint, ideal milk fat would contain 10% polyunsaturated, 8% saturated, and 82% monounsaturated acyl groups (2). Milk fat can be made more nutritionally desirable by altering the fat in the cow's feed (3–5), but current markets give producers little incentive to feed such diets.

Vegetable oils such as high-oleic sunflower oil (HOSO) with fatty acid compositions resembling "ideal milk fat" are commercially available. HOSO typically contains 9% linoleate, 10% saturates, and 81% oleate, but no short-chain fatty acids (SCFA), and the milk fat fatty acids with chain lengths 4 to 10 are believed to be an important source of cheese flavor (6,7). Because acyl groups with chain lengths up to 10 are digested differently from longer chains, they have no effect on the plasma cholesterol (8,9). Thus, incorporation of SCFA into an oil such as HOSO could improve its performance as a substitute for milk fat in cheese without entailing nutritional disadvantages.

Whitehouse (10) and Johnson (11) incorporated SCFA into long-chain vegetable oil by synthesizing short-chain triglycerides (SCTG) from SCFA and interesterifying the SCTG into corn oil or HOSO with a sodium methoxide catalyst. To drive the synthesis of SCTG, they used a benzene azeotrope to remove the water of esterification. This process is not acceptable as a commercial food process because benzene is considered a carcinogen (12). In this study, we explored the possibility of using a toluene azeotrope instead of benzene since toluene is considered less toxic (13–16).

The analysis of fats containing SCFA by gas chromatography (GC) is complicated because of the potential loss of short-chain esters during analysis. This problem was overcome by Vangtal (7) who converted the acyl groups to their decyl esters, and we have used a variation of his method for our analysis.

Here we report the successful incorporation of SCFA into HOSO to produce a bland product suitable for cheese production and a method for analyzing its fatty acid composition. The application of the modified HOSO in cheese making is given in a later paper (17).

EXPERIMENTAL PROCEDURES:

Preparation of SCFA from milk fat. U.S. Department of Agriculture Grade AA sweet cream butter, purchased locally, was melted, centrifuged for 20 min at 1900 × *g*, and the clear oil phase was recovered. Milk fat was converted to methyl esters by reacting milk fat with 130% of the theoretical amount of methanol and sodium methoxide (5.4 M sodium methoxide in methanol; Fluka Chemical Co., Ronkonkoma, NY) at 1% of the fat weight and 40°C with stirring for 12 h. The methyl esters were washed with water and dried over anhydrous sodium sulfate (Fisher Scientific, Fair Lawn, NJ).

SCFA methyl esters (chain length 4–10) were isolated

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*To whom correspondence should be addressed at Department of Food Science and Human Nutrition, 2313 Food Sciences Building, Ames, IA 50011. E-mail: hammond@iastate.edu from milk fat methyl esters by two successive vacuum distillations using a 30-cm Widmer column. A Cartesian manostat was used to control the pressure. During the first distillation, the pressure was slowly decreased from 140 to 1.0 Torr as the column head temperature was increased from 65 to 120°C. The first distillate was redistilled as before except the column head temperature was only increased to 78°C.

Distilled methyl esters were saponified as follows (18): 1 L of methyl esters was added with vigorous stirring to a solution of 380 g of potassium hydroxide in 360 mL of water and 10 mL of ethanol was added as a catalyst. The mixture was heated to boiling momentarily and cooled. Methanol and ethanol were removed from the saponified fat with a rotary evaporator (Fisher Scientific) at 45°C, and 600 mL concentrated hydrochloric acid (Fisher Scientific) was added slowly to the soaps while keeping the reaction cool with ice. The upper phase was recovered and dried over anhydrous sodium sulfate.

SCTG preparation. SCTG were synthesized from both commercial fatty acids and those isolated from milk fat using a modification of the method described by Whitehouse (10). For example, a mixture of butyric, caproic, caprylic, and capric acids (Sigma Chemical Co., St. Louis, MO) at the same ratios found for a milk fat sample and totaling 7.26 mol, 21.42 g of *p*-toluenesulfonic acid (Sigma Chemical Co.), 2.305 mol of glycerol (Sigma Chemical Co.), and 458 mL of toluene (Fisher Scientific) was refluxed with a Dean-Stark water trap for 6 h. The reaction was considered complete when no more water dripped into the trap. The SCTG were washed once with 5% sodium carbonate solution and several times with water. Then, the SCTG were heated at 85°C in a rotary evaporator to remove water and toluene. The SCTG synthesized from commercial acids was decolorized by stirring with 3% bleaching earth (AOCS Official Natural Bleaching Earth, Champaign, IL) at 120°C for 5 min, and filtered through P5 filter paper (Fisher Scientific, Pittsburgh, PA) under reduced pressure (AOCS Official Method Cc 8b-52) (19).

Interesterification of SCTG and HOSO. SCTG from both commercial and natural sources were interesterified with HOSO (Trisun 80, RBD; AC Humko, Memphis, TN) at a SCTG/HOSO ratio of 1:8.82 in order to produce a fat that had the same percentage of SCFA as that of milk fat. SCTG from the commercial source were also interesterified at a SCTG/HOSO ratio of 1:7.19 to produce a fat that had a level of SCFA equal to 120% of that in milk fat. Sodium methoxide (Aldrich Chemical Company, St. Louis, MO) was used as a catalyst at 0.5% of total oil weight. The reaction was carried out at 65°C under nitrogen with stirring for 6 h. Next, 5% acetic acid (Fisher Scientific) was added to neutralize the catalyst, and the oil was then washed several times with distilled water and dried on a rotary evaporator for 30 min at 90°C.

Deodorization of modified oils. A pilot-scale continuous deodorizer similar to the one described by Smouse (20) was used to deodorize the interesterified oils. The oil flow rate was 600 mL/h, the column temperature was 180°C, pressure was 0.5 Torr, and the steam rate 12.6 mL/h. Each batch of deodorized oil was tasted by two experienced observers to ensure that the flavor was good. The deodorized oil was stored at 4°C until used for cheese making.

Determination of residual toluene by direct GC. The residual toluene in modified and deodorized oil was checked by direct injection on a HP 5890 Series II Gas Chromatograph (Hewlett-Packer Company, Avondale, PA) equipped with a flame-ionization detector (FID) (21,22). About 30 mg of oil sample was weighed onto the glasswool packed in the inlet liner. The septum, nut septum, and retainer nut of the GC were removed, the liner with sample was inserted into the injection port of the GC, and the inlet system was closed rapidly. The oven was held at 40°C for 5 min and programmed to 100°C at 5°C/min. The injector temperature was 100°C, and the detector temperature was 200°C.

Fatty acid analysis by GC. A modification of the method of Vangtal and Hammond (23) was used for fatty acid analyses of milk fat and modified vegetable oils. A several-fold molar excess of decanol (Aldrich Chemical Company) containing 2% sulfuric acid (Fisher Scientific) and measured amounts of pentanoic, tridecanoic, and heptadecanoic acids as internal standards were heated with the fat at 56°C overnight in a sealed vial to convert the acyl groups to decyl esters. After the reaction, 1 mL hexane was added, and the hexane layer was recovered. Decyl esters containing only chain lengths equal to or greater than decyl butyrate could be injected into the GC without further purification. Samples containing decyl acetate and propionate, for example cheese fats, could be freed of interfering decanol by dilution of 0.1 mL of hexane extract with 0.5 mL additional hexane and passage through a 900-mg silica cartridge (Alltech Associates, Inc., Deerfield, IL), which had been preconditioned with 5 mL hexane. Six milliliters of 5% diethyl ether in hexane was used to elute the decyl esters from the cartridge. The eluate was concentrated under nitrogen and analyzed by GC.

An HP 5890 Series II gas chromatograph (Hewlett-Packer Company) with a fused-silica capillary column SPB-1 (30 m × 0.25 mm i.d., 0.25 μm) (Supelco, Inc., Bellefonte, PA) was used. The oven temperature was held at 140°C for 4 min, programmed to 300°C at 5°C/min, and held at 300°C for 6 min. The injector temperature was 280°C, and the detector temperature was 300°C. The carrier gas (helium) flow rate was 1.73 mL/min, and the split ratio was 4.6. The hydrogen flow rate was 30 mL/min, and the air flow rate was 380 mL/min. To quantify individual fatty acids, the peak areas for the decyl esters were divided into three chain-length groups, namely, 2–8, 10–14, and 16–22; and 5:0, 13:0, and 17:0 fatty acids were used as internal standards for each group, respectively. The relative FID-response factors of each individual fatty acid to the internal standards were determined.

The fatty acid composition of the distilled milk fat methyl esters was determined using the same GC column as described above. The oven temperature was programmed from 30°C for 5 min to 250°C at a rate of 8°C/min, and held at 250°C for 5 min. The injector temperature was 220°C, and the detector temperature was 250°C. To determine the fatty acid composition of HOSO, HOSO was converted to methyl esters by using sodium methoxide as a catalyst. The HOSO methyl esters were separated on an H-P Model 5890 gas chromatograph equipped with a J & W Scientific (Deerfield, IL) DB-23 fused-silica column (15 m, 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was 220°C, the injector temperature was 250°C, and the detector temperature was 250°C.

Thin-layer chromatography (TLC). Thin-layer chromatograms of the SCTG on silica gel plates were developed with hexane/ethyl ether/acetic acid (50:50:1, vol/vol/vol), visualized by spraying with 0.1% dichlorofluorescein, and viewed under ultraviolet light to check for the presence of mono- and diglycerides.

RESULTS AND DISCUSSION

By assigning the fatty acids to the three chain-length groups, relative FID-response factors close to 1.00 were obtained for each decyl ester. The fatty acid composition of the milk fat used in this study is presented in Table 1 and is similar to other published data (24). Results from two sample preparation methods are listed. One method included a silica cartridge purification step to remove decanol; the other, direct GC injection of the unfractionated transesterification mixture. The results were quite similar. Under the GC conditions used in this study, decanol emerged from the GC column before any of the decyl esters; therefore, the cartridge purification step was not necessary for analysis of milk fat or simulated milk fat.

To isolate SCFA from milk fat for incorporation into HOSO, milk fat was first converted to methyl esters. Distillation with a Widmer column gave a product with a very small amount of methyl laurate and only traces of methyl myristate in the distillate as shown in Table 2. No methyl esters with chain lengths shorter than laurate were found in the distillation pot residue by GC. The distillate composition expected from the milk fat composition (Table 1) is presented in Table 2, and the methyl esters in the distillate were close to the expected values. The free fatty acids recovered after saponification and acidification of short-chain methyl esters had a lower butyrate content than that of the methyl esters (Table 2), which indicated a significant loss of butyric acid occurred during the saponification and acidification.

Fatty acid compositions of SCTG synthesized from commercial and milk fat SCFA are presented in Table 3. SCTG synthesized from both SCFA sources had fatty acid profiles very close to the calculated percentage. SCTG synthesized from milk fat SCFA had a butyrate content lower than that of original milk fat. Using toluene instead of benzene to remove water from the transesterification system and drive the reaction to completion gave satisfactory results. The SCTG synthesized from commercial SCFA had a dark color, while those synthesized from milk fat SCFA were colorless. The color of SCTG can be partially removed by bleaching. The SCTG produced by esterification with glycerol using *p*-toluenesulfonic acid and toluene distillation tasted quite bitter and disagreeable, in agreement with reports by Johnson (11) and Whitehouse (10). Purification of the SCTG by treatment with alumina (25) and deodorization at 80°C for 1.5 h to remove solvent did not remove the disagreeable flavor. This process should remove free fatty acids, partial glycerides, and other polar impurities, and their absence was confirmed by TLC. Interesterifying the SCTG with HOSO resulted in a bland oil with an excellent flavor after deodorization at 100°C for 1.5 h if sufficient (0.5% by weight) sodium methoxide catalyst was used. Whitehouse (10) and Johnson (11), who produced SCTG by a similar process, also noticed a bitter off-flavor in SCTG. Whithouse reported that the off-flavor persisted even after the SCTG was interesterified with HOSO. She removed this flavor by high-temperature deodorization at 240°C for 1 h, but this caused significant loss in SCFA. Probably the greater amount of catalyst used in the present work gave a more complete interesterification of SCTG and HOSO, so less SCTG were left in the mixture.

The SCTG were interesterified with HOSO at ratios that

TABLE 1
Fatty Acid Compositions of Milk Fat Determined With or Without Purification of Decyl Esters Using a Silica Cartridge and Compared to Literature Values[a]

Free fatty acids	With cartridge purification (%)	Without cartridge purification (%)	Milk fat[b] (%)
4:0	3.69 ± 0.15	3.60 ± 0.54	3.4
6:0	2.19 ± 0.07	2.12 ± 0.30	2.1
8:0	1.23 ± 0.03	1.20 ± 0.15	1.2
10:0	2.63 ± 0.05	2.61 ± 0.24	2.6
12:0	3.01 ± 0.04	3.04 ± 0.20	3.0
14:0	10.78 ± 0.46	10.87 ± 0.67	10.6
16:0	30.54 ± 0.51	30.50 ± 0.45	27.7
18:0	14.81 ± 0.77	14.60 ± 0.01	12.8
Pooled 18:1, 18:2, 18:3	30.66 ± 0.35	30.95 ± 0.38	30.5
20:0	0.24 ± 0.04	0.20 ± 0.02	NA[c]
20:1	0.16 ± 0.02	0.21 ± 0.02	NA
22:0	0.06 ± 0.01	0.05 ± 0.01	NA

[a] Means ± SD (n = 5).
[b] From German *et al.* (24).
[c] Not available.

TABLE 2
Fatty Acid Compositions of Milk Fat Methyl Ester Distillates and Short-Chain Fatty Acids (SCFA) Mixture After Saponification and Acidification of Milk Fat Fatty Acid Methyl Esters

Free fatty acids	Milk fat methyl ester distillates (%)[a]	SCFA mixture (%)[a]	Percentage expected (%)[b]
4:0	35.87 ± 0.42	27.45 ± 0.36	38.14
6:0	24.26 ± 0.08	23.82 ± 0.10	22.68
8:0	13.20 ± 0.07	14.33 ± 0.19	12.37
10:0	22.09 ± 0.33	26.30 ± 0.10	26.80
12:0	4.22 ± 0.39	6.81 ± 0.01	0.00
14:0	0.40 ± 0.08	1.30 ± 0.01	0.00

[a] Means ± SD (*n* = 3).
[b] Calculated according to the butter fat fatty acid composition reported in Table 1.

TABLE 3
Fatty Acid Compositions of Short-Chain Triglycerides (SCTG) Synthesized from Synthetic and Natural Milk Fat SCFA

Free fatty acids	Percentage in SCTG synthesized from synthetic source (%)[a]	Percentage expected (%)[b]	Percentage in SCTG from milk fat source (%)[a]	Percentage expected (%)[b]
4:0	36.17 ± 0.14	38.14	25.55 ± 0.18	27.45
6:0	21.98 ± 0.13	22.68	23.29 ± 0.16	23.82
8:0	12.95 ± 0.19	12.37	15.33 ± 0.18	14.33
10:0	28.90 ± 0.43	26.80	27.43 ± 0.45	26.30
12:0	0.00 ± 0.00	0.00	7.07 ± 0.02	6.81
14:0	0.00 ± 0.00	0.00	1.33 ± 0.02	1.30

[a] Means ± SD (*n* = 3).
[b] Calculated according to the butter fat fatty acid composition reported in Table 1. For abbreviation see Table 2.

TABLE 4
Fatty Acid Compositions of Modified High-Oleic Sunflower Oils (HOSO) Before Deodorization

Free fatty acids	HOSO with 100% commercial SCFA (%)[a]	Expected values of SCFA (%)[b]	HOSO with 120% commercial SCFA (%)[a]	Expected values of SCFA (%)[b]	HOSO with 100% milk SCFA (%)[a]	Expected values of SCFA (%)[b]
4:0	3.36 ± 0.03	3.68	3.62 ± 0.02	4.42	2.48 ± 0.06	2.60
6:0	2.16 ± 0.01	2.24	2.34 ± 0.02	2.69	2.26 ± 0.06	2.37
8:0	1.34 ± 0.02	1.32	1.44 ± 0.01	1.58	1.46 ± 0.04	1.56
10:0	2.85 ± 0.04	2.94	3.21 ± 0.23	3.53	2.70 ± 0.02	2.79
12:0	0.00 ± 0.00		0.00 ± 0.00		0.69 ± 0.00	
14:0	0.00 ± 0.00		0.00 ± 0.00		0.17 ± 0.01	
16:0	2.98 ± 0.02		3.53 ± 0.01		3.56 ± 0.07	
18:0	3.76 ± 0.01		3.24 ± 0.09		3.25 ± 0.02	
Pooled 18:1–18:3	81.90 ± 0.05		80.91 ± 0.14		81.55 ± 0.24	
20:0	0.38 ± 0.01		0.43 ± 0.04		0.51 ± 0.06	
20:1	0.23 ± 0.02		0.34 ± 0.02		0.36 ± 0.01	
22:0	1.04 ± 0.03		0.95 ± 0.07		1.01 ± 0.02	

[a] Means ± SD (*n* = 4).
[b] Calculated according to the true SCTG fatty acid compositions (Table 4) and the SCTG/HOSO ratios used in the interesterification. See Tables 2 and 3 for abbreviations.

would give 100 or 120% of the level of the SCFA found in milk fat. The fatty acid compositions of washed and dried modified HOSO are presented in Table 4. The SCFA in modified HOSO were very close to the expected values. The fatty acid compositions of washed, dried, and deodorized modified HOSO are presented in Table 5. No residual toluene was detected by the direct GC method in any of the modified HOSO after deodorization. All deodorized oils had a bland, acceptable flavor as judged by two experienced observers. About 2% oil was lost during deodorization, and this loss might explain the lower SCFA contents in the deodorized oils (Table 5) compared with that of the undeodorized oils (Table 4).

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TABLE 5
Fatty Acid Compositions of Modified HOSO After Deodorization[a]

Free fatty acids	HOSO with 100% synthetic SCFA (%)	HOSO with 120% synthetic SCFA (%)	HOSO with 100% milk SCFA (%)	HOSO[b]
4:0	2.71 ± 0.17	2.81 ± 0.02	2.37 ± 0.01	0.00
6:0	1.78 ± 0.12	2.15 ± 0.02	2.19 ± 0.01	0.00
8:0	1.05 ± 0.05	1.34 ± 0.01	1.48 ± 0.02	0.00
10:0	2.04 ± 0.17	2.67 ± 0.02	2.25 ± 0.02	0.00
12:0	0.00 ± 0.00	0.00 ± 0.00	0.66 ± 0.01	0.00
14:0	0.00 ± 0.00	0.00 ± 0.00	0.17 ± 0.00	0.05
16:0	3.29 ± 0.01	2.98 ± 0.01	3.46 ± 0.01	3.44
18:0	3.63 ± 0.12	3.13 ± 0.12	3.40 ± 0.03	4.01
Pooled 18:1–18:3	83.22 ± 0.60	82.95 ± 0.11	81.84 ± 0.18	90.42
20:0	0.39 ± 0.00	0.33 ± 0.00	0.41 ± 0.00	0.39
20:1	0.30 ± 0.01	0.32 ± 0.00	0.33 ± 0.00	0.31
22:0	1.12 ± 0.01	1.08 ± 0.00	1.15 ± 0.00	1.00
24:0	0.37 ± 0.02	0.33 ± 0.00	0.36 ± 0.00	0.31

[a] Means ± SD (*n* = 4).
[b] Determined as methyl esters. See Tables 2 and 4 for abbreviations.

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