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Functional properties of cream and butter oil from milk of Holstein cows abomasally infused with increasing amounts of high-oleic sunflower fatty acids

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Abstract

This research paper addresses the hypothesis that there is an optimal amount of intestinally available oleic acid (provided *via* abomasal infusion) to produce higher-oleic acid milk fat with satisfactory functional characteristics of cream and butter oil. A control and four increasing doses of free fatty acids from high oleic sunflower oil (HOSFA) were infused into the abomasum of four lactating dairy cows in a crossover experimental design with 7-d periods. Treatments were: (1) control (no HOSFA infused), (2) HOSFA (250 g/d), (3) HOSFA (500 g/d), (4) HOSFA (750 g/d), and (5) HOSFA (1000 g/d). All treatments included meat solubles and Tween 80 as emulsifiers. Viscosity, overrun and whipping time as well as foam firmness and stability were evaluated in whipping creams (33% fat). Solid fat content (from 0 to 40°C), melting point and firmness were determined in butter oil. Whipping time of cream increased linearly and viscosity decreased linearly as infusion of HOSFA increased. Overrun displayed a quadratic response, decreasing when 500 g/d or more was infused. Foam firmness and stability were not affected significantly by HOSFA. For butter oil, melting point, firmness, and solid fat content decreased as HOSFA infusion increased. Changes in 21 TG fractions were statistically correlated to functional properties, with 6–10 fractions showing the highest correlations consistently. Decisions on the optimal amount of HOSFA were dependent on the dairy product to which milk fat is applied. For products handled at commercial refrigeration temperatures, such as whipping cream and butter oil, the 250 g/d level was the limit to maintain satisfactory functional qualities. Palmitic acid needed to be present in at least 20% in milk fat to keep the functional properties for the products.

In moving toward a more nutritionally desirable milk fat composition, replacement of a portion of the saturated fatty acids (SFA) with monounsaturated fatty acids (MUFA) should be a desirable change. Oleic acid is the most abundant MUFA in milk and in the human diet. Although olive oil probably is the most recognized source of oleic acid, the contribution of animal fats to total intake is of much more importance. An increase in the MUFA content and decreased SFA of milk fat might lead to an improved consumer perception of milk fat products.

The fatty acid (FA) and triglyceride (TG) composition of milk can be altered by manipulating the diet of lactating dairy cows (Banks *et al.*, 1989b; Lin *et al.*, 1996; DePeters *et al.*, 2001; Pacheco-Pappenheim *et al.*, 2022). Oleic acid content of milk fat increases linearly in response to increased intestinal supply of oleic acid to cows (Drackley *et al.*, 2007; LaCount *et al.*, 1994). Butters prepared from milk of cows fed high oleic sunflower seeds or regular sunflower seeds were softer and more unsaturated than control butter, yet exhibited acceptable flavor, manufacturing and storage characteristics (Middaugh *et al.*, 1988). Feeding calcium-protected high oleic sunflower oil to cows resulted in milk with substantially greater oleic acid content but no change in milk flavor (Lin *et al.*, 1996). The amide of oleic acid (oleamide) provides intestinally available oleic acid to cows, which resulted in increased oleic acid in milk fat (Jenkins, 1999, 2000). Thus, the potential exists on a practical level to produce milk with substantially increased contents of oleic acid. It is important that the responses of functional characteristics of such milks be described adequately if they are to be useful in production of human food products.

In addition to the FA composition of milk fat, the TG composition affects solid fat content and functional properties of milk fat (Banks *et al.*, 1989b; Gresti *et al.*, 1993; Pacheco-Pappenheim *et al.*, 2022). Effects of increasing oleic acid on milk TG profile might impact properties of dairy products (Banks *et al.*, 1989b), and may be the key for determining

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the type and amount of FA to be included in an altered milk fat to improve the nutritional quality of milk without risking the processing characteristics of a specific dairy product.

The purpose of this study was to evaluate the effect of increasing amounts of oleic acid in milk fat on the functional properties of that fat. To avoid effects of ruminal biohydrogenation of oleic acid, milk fat enriched with oleic acid was obtained by abomasally infusing lactating dairy cows with high oleic sunflower FA (HOSFA). The experiment was conducted as a dose-response trial, so that an optimum content of oleic acid might be identified at which functional properties are maintained for processing purposes and consumer acceptance of milk fat dairy products, such as whipping cream and butter oil. Our hypothesis was that an amount of intestinally available oleic acid exists that will increase oleic content in milk fat but not compromise physical qualities of butter oil and whipping cream.

Materials and methods

Experimental design and treatments

The University of Illinois Institutional Animal Care and Use Committee approved all procedures using animals. Cannulation, housing and management of cows as well as preparation of treatments were as described by Drackley *et al.* (2007). Briefly, experimental infusions were prepared weekly. The ingredients (described below) were mixed and heated to 72°C in steam-jacketed stainless steel vats and then homogenized, cooled and stored at 4°C until use. The treatments consisted of continuous abomasal infusions of: (a) control; 240 g/d of meat solubles (Milk Specialties Co., Dundee, IL) plus 11.24 g/d of Tween 80 (Sigma Chemical Co., St. Louis, MO) in 10 l of tap water; or (b) HOSFA (91.4% C_{18:1cis}, 2.4% C_{18:2cis-cis}, 2.4% C_{16:0}, and 1.8% C_{18:0} by weight; Henkel Corporation, Emery Division, Cincinnati, OH) at increasing amounts of 0, 250, 500, 750, and 1000 g/d, each homogenized with 240 g/d of meat solubles and 11.24 g/d of Tween 80. The meat solubles and Tween 80 served to emulsify the FA for aqueous infusion. Each amount of HOSFA was infused for 7 d in succession. Four cows received treatments according to a crossover statistical design; two cows were randomly selected to receive the set of increasing HOSFA infusions, while the other two served as controls (0 g/d) and were infused only with carriers. After 2 wk of washout at the end of the sequential set of treatments, the control cows were changed to HOSFA and HOSFA cows were changed to controls, and the sequence of increasing amounts of HOSFA was repeated.

Compositional and functional analysis: cream

Milk was heated to 32°C and cream was separated in an open centrifugal cream separator. Cream was standardized with its own skim milk to a fat content of 33% and pasteurized (63°C for 30 min) in 18.9 L stainless-steel buckets set in a small water-jacketed pasteurizer. The cream was cooled and held for 24 h at approximately 4°C before being whipped.

Compositional and functional analysis: butter oil

Whipping of cream was continued until butter granules formed and churning occurred. After eliminating most of the buttermilk, the remaining water was removed by warming the samples in a commercial microwave oven (30 s at high power), and separating

the water and protein phases by centrifugation. Samples of liquid butter oil were poured into Petri dishes, tempered for 1 h, and then stored in a refrigerator at 4°C for 2 wk until testing.

Physical evaluation of cream

The overrun (O) is defined as

$$O = ((V2 - V1)/V1) \times 100$$

where V1 is the initial volume of the cream and V2 is the maximum volume obtained upon whipping. The time taken to reach maximum overrun is called whipping time, and the serum leakage is a measurement of whipped cream stability. The firmness of the cream can be estimated by using constant speed penetrometry, which involves measurements of the force required to push a cylindrical punch moving at constant speed through the sample.

Experimentally, these parameters were measured using a domestic Kitchen-Aid mixer (model K5-A; Hobart MFG Co., Troy, OH). Because foam firmness is affected by temperature, the bowl was cooled to 4°C, a pre-weighed volume of cream (ca. 350 ml) at the same temperature was added, and the beaters (speed 8) and a stop-clock were set in motion. Once the whipping point was reached, stop-clock and mixer were stopped. The weight for the original volume was measured, and a sample (ca. 50 ml) of whipped cream was transferred to a fast filtration funnel. The funnels were left for 1 h at room temperature (ca. 20°C), and the seepage was collected. The volume of this serum was measured with a 10-ml graduated cylinder and represents stability of the foam. The firmness of the whipped cream was measured with a texture analyzer (model TA-XT2; Texture Technologies Corp., Scarsdale, NY) fitted with a 5-cm diameter stainless-steel cylinder and a 5.5-cm diameter stainless-steel cup. Whipped cream was placed in the cylindrical container and the cream surface was leveled with a spatula. Readings of the force and force by time were taken at 10 s from the initial count.

Viscosity was measured in creams at 7°C by using a Brookfield DVI-RVT roto-viscometer (Brookfield Engineering Lab, Inc., Stoughton, MA) with a UL low-viscosity device. Readings were made at 10, 20, 50, and 100 RPM. Because in some samples churning occurred at speeds over 50 RPM, viscosity at this speed was considered for comparison among treatments.

Physical evaluation of butter oil

Heating thermograms were obtained using differential scanning calorimetry (Modulated DSC model 2920; TA-Instruments Inc., New Castle, DE) calibrated with an indium standard. Samples (9 ± 2 mg) were encapsulated in hermetically sealed alodined aluminum pans (TA-Instruments, Inc., New Castle, DE). The sample cell was purged with He (25 ml/min) and cooled with N₂ (150 ml/min) during the analysis. Samples were held at 60°C for 3 min to melt any crystal present, cooled to -60°C at 10°C/min, held for 3 min, and then heated to 60°C at 10°C/min to obtain a melting profile (Kaylegian and Lindsay, 1994). The melting point was taken as the temperature at the top of the peak for the high melting fraction. Solid fat content was calculated by dividing the partial area under the melting curve by the total area from -40 to 40°C multiplied by 100. Solid fat content from 0 to 40°C was calculated at 5°C intervals.

Butter hardness was determined by constant speed penetration using the same instrument as for whipped cream. In this case, the probe was a TA-55 cylindrical punch probe. The samples were removed from the refrigerator (4°C) and taken to the instrument for reading. The force required for the stainless-steel cylinder to break the butter oil surface was determined at a speed of 10 mm/min. Three penetration tests were done in each of the Petri dish samples. Hardness results were expressed in g_f and the force by time ($g_f \times s$).

Statistical analysis

Data were analyzed using the SAS general linear models procedure (v. 9.2, SAS Institute, Inc., Cary, NC). Analysis of variance for a crossover design was performed, with a model where: $Y = \text{sequence} + \text{cow} + \text{sequence} + \text{period} + \text{treatment} + \text{treatment by period by cow} + \text{sequence} + \text{week} + \text{treatment by week}$. We tested the interaction of infusate treatment with linear, quadratic, and cubic effects of amount. By design, week within periods was confounded with infusate amount. Least-squares means were calculated and are reported. Significance was declared at $P < 0.05$. Pearson correlation analysis was conducted to find relationships among functional properties and milk fat composition.

During wk 5 and the 1000 g/d HOSFA infusion, two cows receiving this treatment stopped eating and the infusion was suspended. Therefore, results for the 1000 g/d infusion represent only two cows.

Results and discussion

Effect of infusions on composition of milk fat

The FA profile of the butter oil was reported in Drackley *et al.* (2007) and the TG profile of butter oil was presented in Ortiz Gonzalez *et al.* (2022). Briefly, milk fat became progressively enriched with oleic acid to a maximum of 57.4% of total FA as infusion increased, whereas concentrations of FA from C4 to C16:0 decreased linearly. Palmitic acid decreased to a low of 15.2% of total FA at the 1000 g/d infusion (Drackley *et al.*, 2007). A total of 41 TG fractions were collected and identified. The most significant changes in milk fat TG as HOSFA infusion increased were a decrease in TG with SFA and increases in dioleoyl TG and triolein (Ortiz Gonzalez *et al.*, 2022).

Effect of infusions on functional properties of cream

An important question to be answered is the amount of oleic acid that can be present in milk fat to maintain good functional and processing characteristics of cream while decreasing the hypercholesterolemic SFA in milk fat. The dairy industry has tended to associate poor whipping characteristics with periods in which milk fat has a lower melting point due to the presence of elevated contents of oleic acid. However, some experiments showed that composition of FA is not the only factor influencing whipping ability (Banks *et al.*, 1989a).

Results for the whipping cream functional properties as impacted by the increasing HOSFA infusions are in Table 1. Whipping time increased linearly as HOSFA infusion increased from 0 to 1000 g/d. A good whipping cream should reach its maximum overrun within 90 s (Aggarwal, 1975). Therefore, creams resulting from 500 g/d of HOSFA represented the limit for a good whipping cream, although other characteristics must be

Table 1. Functional properties of cream and butter oil made from milk of cows abomasally infused with increasing amounts of high-oleic sunflower fatty acids (HOSFA)^a

Functional characteristic	HOSFA infused (g/d)					Treatment by amount, $P =$			
	0	250	500	750	1000	SEM	Linear	Quadratic	Cubic
Cream									
Whipping time (s)	98.4 (87.4)	78.8 (69.0)	91.5 (81.1)	129.0 (93.1)	150.0 (91.8)	5.1	0.03	0.21	0.91
Overrun (%)	132.0 (126.2)	135.5 (132.8)	110.7 (123.8)	81.2 (119.7)	63.8 (132.7)	3.7	<0.001	0.02	0.65
Foam firmness (g at 5 s)	79.0 (81.8)	79.3 (84.1)	60.4 (70.0)	59.3 (66.1)	64.3 (74.0)	1.9	0.54	0.85	0.90
Foam firmness area (g _s at 5 s)	289.4 (296.6)	293.1 (310.8)	215.9 (246.6)	199.0 (238.4)	215.4 (267.9)	8.0	0.26	0.99	0.98
Foam stability (mL)	2.87 (2.71)	3.22 (2.55)	4.47 (2.71)	4.38 (2.34)	4.49 (2.66)	0.26	0.19	0.57	0.72
Viscosity (cps)	37.5 (33.1)	32.3 (34.2)	29.0 (34.65)	19.7 (30.5)	16.8 (36.2)	1.3	0.001	0.71	0.63
Butter oil									
Melting point (°C)	30.9 (29.9)	28.6 (29.4)	24.5 (29.6)	20.9 (29.2)	8.3 (29.7)	0.9	<0.001	<0.001	0.04
Firmness (g)	1558 (1031)	1049 (1781)	370 (1846)	110 (1881)	-242 (1433)	129	<0.001	0.01	0.89
Solid fat content, 5°C (%)	67.2 (62.7)	59.6 (65.4)	47.2 (67.6)	38.1 (69.0)	31.0 (64.9)	2.0	<0.001	0.14	0.22
Solid fat content, 20°C (%)	29.3 (23.9)	20.5 (27.4)	9.9 (32.8)	4.6 (28.1)	0.2 (26.5)	1.8	<0.001	0.03	0.91

^aValues within parentheses are least-squares means for cows infused with control infusate for each infusion level.

considered to reach a complete conclusion. Whipping time was negatively correlated (Table 2) with overrun ($r = -0.51$, $P = 0.001$), which is logical considering that the increase in lower-melting point FA delays the whipping speed and the capacity of the foam emulsion to retain air because of the antifoaming characteristic of the greater content of liquid fat. This liquid fat increased as HOSFA infused, but also increased because of the increased time of whipping, which increased the temperature in the whipping cream. As expected from previous correlations, maximum peak ($r = -0.39$, $P = 0.02$) and area ($r = 0.36$, $P = 0.03$) of firmness were also negatively correlated to the whipping time, demonstrating that the longer the time of whipping, the lower the values for texture parameters of whipped cream. Foam stability was positively correlated to whipping time ($r = 0.46$, $P = 0.004$), as more unstable foams were formed with the increase in whipping time. Viscosity of the cream at 7°C was also correlated negatively with the whipping time ($r = -0.37$, $P = 0.035$). Viscosity decreased as HOSFA infusion increased because of the decreased solid fat content in milk fat. Whipping time, as expected, was directly correlated (Table 3) with the increase in oleic acid ($r = 0.54$, $P < 0.001$). Triglyceride fractions (Ortiz Gonzalez *et al.*, 2022) including 14 (butyryl-palmitin-palmitin or caproin-myristin-palmitin; $r = -0.68$, $P < 0.0001$) and 17 (butyryl-palmitin-stearin; $r = -0.66$, $P < 0.0001$), were negatively correlated with whipping time (Table 4), since the decrease in these fractions corresponds to the increase in the speed of whipping. On the other hand, fractions 12 (butyryl-olein-olein; $r = 0.35$, $P = 0.04$), 26 (laurin-olein-olein; $r = 0.54$, $P = 0.007$) and 36 (palmitin-olein-olein; $r = 0.60$, $P < 0.001$) were directly correlated with the increase in whipping time. Surprisingly, F35 (triolein) was not significantly correlated ($P = 0.12$) with whipping time, which is probably due to the low degree to which triolein increased as HOSFA infusion increased.

Overrun decreased linearly as HOSFA infusion increased (Table 1). This variable was directly correlated with the maximum peak ($r = 0.61$, $P < 0.001$) and area of firmness ($r = 0.68$, $P < 0.001$) of whipped cream (Table 2). As mentioned before, the increase in solid fat caused an increase of viscosity, which also was directly correlated ($r = 0.73$, $P < 0.001$) with overrun. Overrun was correlated positively with short-chain FA, longer SFA, and C18:1*trans* and correlated negatively ($r = -0.80$, $P < 0.001$) with oleic acid (Table 3). Triglyceride fractions related to SFA were positively correlated with overrun, with the highest positive correlations for fractions 10 (butyryl-myristin-palmitin; $r = 0.71$, $P < 0.001$), 17 (butyryl-palmitin-stearin; $r = 0.81$, $P < 0.001$), and 18 (caproin-palmitin-palmitin; $r = 0.77$, $P < 0.001$). The most important fractions associated with increasing oleic acid that had a negative correlation to overrun were fractions 26 (laurin-olein-olein; $r = -0.73$, $P < 0.001$), 35 (palmitin-olein-olein; $r = -0.94$, $P < 0.001$) and 36 (triolein; $r = -0.83$, $P < 0.001$). According to these correlations, triolein seems to have an important effect on overrun, but not on whipping time, which means that this fraction influences the amount of air retained in the foam more than the time for the emulsion to form.

Maximum peak and the area under the curve for foam firmness did not respond linearly to the increase in infused HOSFA (Table 1). The lack of clear differences in texture among the different creams may be because a stiffer foam with higher overrun compensates for softer foams with lower overruns. Peak of foam firmness was correlated with the area under the curve ($r = 0.98$, $P < 0.001$) of firmness, since as this peak decreases the area is also decreased. As this peak of firmness was also directly

associated with the amount of solid fat, a correlation with other parameters affected by the presence of lower melting fat was expected. In agreement, we found peak of firmness correlated negatively with stability ($r = -0.33$, $P = 0.05$) and positively to viscosity of cream at 7°C ($r = 0.38$, $P = 0.03$). Peak firmness and firmness area were correlated positively with C16:0, C18:0, and C18:1*trans* and correlated negatively with oleic acid (Table 3). Once more TG fractions 17 ($r = 0.49$, $P = 0.002$) and 18 ($r = 0.55$, $P = 0.001$) among others related to SFA resulted in the highest positive correlations to the peak of firmness, while fractions 12 ($r = -0.52$, $P = 0.037$), 23 (caproin-olein-olein; $r = -0.55$, $P = 0.002$) and 26 ($r = -0.66$, $P = 0.0004$) were negatively correlated with this variable (Table 4). Correlation analysis also showed a positive correlation to fraction 31 (olein-olein-linolein; $r = 0.57$, $P = 0.0002$), which is not logical because of the low melting point of the FA included in this fraction that should be more strongly correlated negatively. This correlation may be biased by unidentified factors. Area under the curve of firmness was also correlated with the other functional parameters for whipping cream (Table 2). Since the correlation with the maximum peak of firmness was 0.98, the same TG fractions affecting the maximum peak also affected the area under the curve of firmness.

Foam stability was not significantly affected by increasing HOSFA (Table 1). Foam stability was related negatively to short-chain FA and SFA and positively with oleic acid (Table 3). Again the fractions 12 ($r = 0.45$, $P = 0.006$), 15 (caproin-olein-olein; $r = 0.46$, $P = 0.004$) and 26 ($r = 0.52$, $P = 0.008$) were mainly associated with an increase in the liquid recovered, negatively affecting the stability of whipped cream. Fractions associated with short- and medium-chain SFA improved the stability of cream. The TG fractions 2 ($r = -0.46$, $P = 0.005$; butyryl-caprylin-palmitin or butyryl-caprylin-olein), 6 ($r = -0.48$, $P = 0.003$; butyryl-laurin-olein) and 10 ($r = -0.52$, $P = 0.001$; butyryl-myristin-palmitin), which in previously discussed parameters also exhibited important correlations, resulted in the highest negative correlations with stability of the whipped product.

For the majority of the functional parameters related to whipping cream, doses of infused HOSFA over 250 g/d resulted in a detectable change as compared to control creams. At the 250 g/d level, functional properties of whipping cream were even better than for control, which is in agreement with the findings of Shamsi *et al.* (2002), who found more stability in palm oil-based than in regular whipping creams. Since most of the functional parameters for whipping cream were correlated, the TG fractions strongly affecting whipping parameters in a positive or negative way are reduced to six (fractions 11, 14, 16, 17, 29, and 36; Ortiz Gonzalez *et al.*, 2022) out of as many as twenty statistically significant fractions.

Effect of infusions on functional properties of butter oil

The isolation of milk fat from the other components of the milk, which occurs in the conversion from butter to butter oil, allowed us to work with a practically pure component. We were able to analyze the results from the melting point determinations with the understanding that the other functional properties should respond in a similar way as the melting point behavior. This was confirmed by functional parameters of butter oil being highly correlated with each other (Table 2).

The melting point of milk fat varies from 32 to 36°C. Within this range, milk fat becomes visually clear and free of crystals. The comparative results for the melting properties evaluated in butter

Table 2. Correlations among cream and butter oil functional properties^a

Functional property	Cream overrun	Cream foam firmness peak	Cream foam firmness area	Cream foam stability	Cream viscosity at 7°C	Butter melting point	Butter solid fat content, 5°C	Butter solid fat content, 20°C	Butter oil firmness
Cream whipping time	−0.52 0.001	−0.39 0.02	−0.36 0.03	0.46 0.004	−0.37 0.03	−0.47 0.005	−0.53 <0.001	−0.49 0.002	0.71 <0.001
Cream overrun		0.61 <0.001	0.68 <0.001	−0.16 0.34	0.73 <0.001	0.68 0.008	0.78 <0.001	0.70 <0.001	0.68 <0.001
Cream foam firmness peak			0.98 <0.001	−0.33 0.05	0.38 0.03	0.41 0.01	0.50 0.002	0.44 0.007	0.37 0.03
Cream foam firmness area				−0.29 0.09	0.43 0.01	0.42 0.009	0.53 0.001	0.46 0.005	0.42 0.02
Cream foam stability					−0.34 0.06	−0.30 0.08	−0.50 0.002	−0.45 0.006	−0.38 0.02
Cream viscosity at 7°C						0.75 <0.001	0.71 <0.001	0.63 <0.001	0.01 0.99
Butter melting point							0.87 <0.001	0.93 <0.001	0.88 <0.001
Butter solid fat content, 5°C								0.80 <0.001	0.88 <0.001
Butter solid fat content, 20°C									0.92 <0.001

^aTop number is Pearson correlation coefficient, bottom is *P*-value.

Table 3. Correlations among functional properties of cream and butter oil with fatty acid composition of milk^a

Functional property	C4 – C10	C12	C14	C16:0	C18:0	C18:1 <i>cis</i>	C18:1 <i>trans</i>	C18:2	C18:3
Cream whipping time	–0.47 0.004	–0.47 0.004	–0.59 <0.001	–0.49 0.002	–0.40 0.01	0.54 <0.001	–0.24 0.15	–0.09 0.61	–0.52 0.002
Cream overrun	0.73 <0.001	0.64 <0.001	0.74 <0.001	0.68 <0.001	0.78 <0.001	–0.80 <0.001	0.65 <0.001	0.28 0.11	0.43 0.01
Cream foam firmness peak	0.23 0.17	0.22 0.19	0.32 0.06	0.44 0.007	0.58 <0.001	–0.47 0.004	0.48 0.003	0.14 0.43	0.26 0.15
Cream foam firmness area	0.30 0.07	0.27 0.12	0.34 0.04	0.47 0.004	0.65 <0.001	–0.51 0.002	0.48 0.003	0.12 0.48	0.25 0.16
Cream stability	–0.36 0.03	–0.41 0.01	–0.48 0.003	–0.52 0.001	–0.21 0.21	0.49 0.002	–0.26 0.12	–0.22 0.20	–0.71 <0.001
Cream viscosity at 7°C	0.60 <0.001	0.60 <0.001	0.71 <0.001	0.66 <0.001	0.64 <0.001	–0.74 <0.001	0.58 <0.001	0.40 0.02	0.56 0.001
Butter melting point	0.80 <0.001	0.80 <0.001	0.86 <0.001	0.83 <0.001	–0.67 <0.001	–0.90 <0.001	0.58 <0.001	0.26 0.13	0.58 <0.001
Butter oil firmness	0.68 <0.001	0.70 <0.001	0.79 <0.001	0.91 <0.001	0.69 <0.001	–0.87 <0.001	0.48 0.003	0.09 0.60	0.47 0.006
Butter solid fat content, 5°C	0.75 <0.001	0.82 <0.001	0.88 <0.001	0.96 <0.001	0.75 <0.001	–0.97 <0.001	0.61 <0.001	0.28 0.11	0.68 <0.001
Butter solid fat content, 20°C	0.71 <0.001	0.81 <0.001	0.85 <0.001	0.94 <0.001	0.73 <0.001	–0.93 <0.001	0.61 <0.001	0.25 0.15	0.61 <0.001

^aTop number is Pearson correlation coefficient, bottom is *P*-value.

oil are in Table 1. Melting point as determined by differential scanning calorimetry decreased linearly (and cubically) as HOSFA infusion increased from 0 to 1000 g/d. Linear decreases in short- and medium-chain SFA were directly correlated with the decrease in melting point (Table 3). Furthermore, the linear increase in C18:1*cis* FA to a maximum of 294% of control at the 1000 g/d HOSFA infusion (Drackley *et al.*, 2007) decreased melting point as expected because of the low melting point of oleic acid and the decrease in SFA, mainly palmitic (reduced by 50%). Our results were in agreement with those of Kaylegian and Lindsay (1994) and also with our previous study (Ortiz-Gonzalez *et al.*, 2007) in which we infused different long-chain FA mixtures and found that increases in short-chain FA and long-chain unsaturated FA with concurrent decreases in long-chain SFA resulted in milk fats with lowered melting points.

Because melting point was significantly correlated with most of the FA, a large number of significant correlations with TG fractions resulted (Table 4). Taking into account those correlations in which *P* < 0.001, we found the following fractions directly correlated with melting point: 2, 7, 10, 14, 16, 17, 18, 21, 25, 29, and 38. These fractions were the most abundant fractions associated with SFA (Ortiz Gonzalez *et al.*, 2022). Similarly, fractions 12, 26, 35, 36, and 39, which were identified as diolein fractions (Ortiz Gonzalez *et al.*, 2022), promoted a decrease in the melting point as they were increased by more HOSFA infused. In a previous study that attempted to determine the TG fractions that exert the main influence on butter texture, Bornaz *et al.* (1992) contended that there were mainly four TG fractions responsible for changes in butter texture. The first fraction was palmitin-olein-olein; the second was myristin-olein-olein; the third includes caprin-laurin-olein, caprylin-myristin-olein and caproin-palmitin-linolein; and the fourth fraction constituted butyrin-palmitin-olein, caproin-myristin-olein and caproin-palmitin-linolein. These data indicated that most of the TG fractions responsible for texture in

butter are related to oleic acid, which is the most abundant when green grasses are present in the diet of cows. The first mentioned fraction is the most abundant and, thus, has the largest influence on spreadability of butter. Results obtained in our study were similar to those reported by Bornaz *et al.* (1992).

Firmness of butter oil also decreased linearly in response to the increase of HOSFA infused (Table 1). Changes in texture were marked as a result of the increase of infused HOSFA. High melting TG were mainly reduced, so low and mid-melting fractions became abundant. This can be concluded from the linear reduction in solid fat content as determined from differential scanning calorimetry at 5 and 20°C. Butter oils from cows infused with 500 g/d or more turned into spreadable oils as compared to those from control or 250 g/d infusions.

In conclusion, the effect of the increasing amount of HOSFA on functional properties of dairy products where fat plays an important functional role has to be analyzed according to the type of dairy product under study, since functional requirements may be different for each product. For whipping cream, we conclude that 250 g/d level of HOSFA infusion represents the limit for keeping adequate functional properties of this product. The amount of liquid fat present strongly affects whipping properties such as whipping time, overrun, viscosity, and stability. Results from functional properties of butter oil indicated that the proper level of HOSFA infusion for a spreadable product under refrigeration conditions, and a stable product at ambient (20°C) temperature conditions, is close to 250 g/d. At this level, the firmness of butter oil was decreased 33% with respect to control, while at 500 g/d the firmness decreased by 80% and the butter oil was practically liquid under ambient conditions due to a very low solid fat content. Under the conditions of this experiment, palmitic acid needs to be present at a minimum of 20% to maintain milk fat functionality. In both products and their functional properties investigated, we found nearly the same TG involved: around

Table 4. Partial list of correlations between milk fat triglyceride fractions and functional properties of cream and butter oil^a

Triglyceride fraction ^b	Cream whipping time	Cream overrun	Cream foam firmness peak	Cream foam firmness area	Cream foam stability	Cream viscosity at 7°C	Butter melting point	Butter oil firmness	Butter solid fat content, 5°C	Butter solid fat content, 20°C
2	-0.48 0.003	0.49 0.002	0.16 0.34	0.18 0.2	-0.46 0.005	0.54 0.001	0.63 <0.001	0.57 <0.001	0.71 0.001	0.70 <0.001
3	-0.34 0.04	0.32 0.06	-0.03 0.84	-0.01 0.94	-0.27 0.11	0.32 0.08	0.47 0.004	0.23 0.17	0.40 0.02	0.37 0.03
4	-0.44 0.008	0.41 0.01	0.16 0.36	0.15 0.38	-0.45 0.007	0.45 0.01	0.60 <0.001	0.54 0.001	0.68 <0.001	0.65 <0.001
6	-0.46 0.005	0.39 0.02	0.17 0.32	0.19 0.27	-0.48 0.003	0.41 0.02	0.51 0.001	0.47 0.004	0.58 <0.001	0.57 <0.001
7	-0.47 0.004	0.55 <0.001	0.23 0.18	0.26 0.12	-0.44 0.006	0.58 <0.001	0.72 <0.001	0.65 <0.001	0.77 <0.001	0.74 <0.001
8	-0.38 0.06	0.41 0.04	0.04 0.83	0.03 0.89	-0.09 0.67	0.47 0.02	0.53 0.005	0.24 0.24	0.38 0.05	0.39 0.05
9	-0.39 0.02	0.48 0.003	0.25 0.14	0.28 0.10	0.37 0.02	0.40 0.02	0.53 0.001	0.41 0.01	0.54 <0.001	0.48 0.003
10	-0.50 0.002	0.71 <0.001	0.39 0.02	0.43 0.009	-0.52 0.001	0.67 <0.001	0.81 <0.001	0.81 <0.001	0.88 <0.001	0.84 <0.001
11	-0.57 0.001	0.57 0.001	0.27 0.14	0.26 0.16	-0.22 0.23	0.64 <0.001	0.63 <0.001	0.44 0.01	0.53 0.003	0.54 0.002
12	0.35 0.04	-0.68 <0.001	-0.52 0.001	-0.57 <0.001	0.45 0.006	-0.62 <0.001	0.82 <0.001	-0.77 <0.001	-0.88 <0.001	-0.84 <0.001
14	-0.68 <0.001	0.64 <0.001	0.44 0.007	0.445 0.006	-0.42 0.01	0.37 0.04	0.68 <0.001	0.82 <0.001	0.81 <0.001	0.77 <0.001
15	0.10 0.54	-0.32 0.06	-0.35 0.04	-0.40 0.02	0.46 0.004	-0.35 0.05	-0.35 0.04	-0.55 <0.001	-0.59 <0.001	-0.57 <0.001
16	-0.58 <0.001	0.58 <0.001	0.38 0.02	0.36 0.03	-0.39 0.02	0.61 <0.001	0.76 <0.001	0.58 <0.001	0.73 <0.001	0.70 <0.001
17	-0.66 <0.001	0.81 <0.001	0.49 0.002	0.53 0.001	-0.32 0.06	0.66 <0.001	0.78 <0.001	0.82 <0.001	0.86 <0.001	0.81 <0.001
18	-0.47 0.004	0.77 <0.001	0.55 <0.001	0.59 <0.001	-0.22 0.20	0.58 <0.001	0.68 <0.001	0.60 <0.001	0.69 <0.001	0.65 <0.001
19	-0.09 0.84	0.48 0.23	-0.48 0.23	-0.52 0.19	0.70 0.05	0.24 0.57	0.67 0.07	-0.24 0.57	0.04 0.93	-0.13 0.76
21	-0.50 0.002	0.62 <0.001	0.28 0.10	0.30 0.08	-0.43 0.009	0.57 <0.001	0.77 <0.001	0.79 <0.001	0.86 <0.001	0.84 <0.001
22	-0.17 0.35	0.47 0.005	0.13 0.47	0.20 0.26	-0.12 0.52	0.36 0.06	0.56 <0.001	0.65 <0.001	0.55 0.001	0.54 0.001
23	0.36 0.06	-0.51 0.005	-0.55 0.002	-0.56 0.002	0.26 0.18	-0.44 0.02	-0.44 0.02	-0.39 0.04	-0.54 0.003	-0.58 0.001

(Continued)

Table 4. (Continued.)

Triglyceride fraction ^b	Cream whipping time	Cream overrun	Cream foam firmness peak	Cream foam firmness area	Cream foam stability	Cream viscosity at 7°C	Butter melting point	Butter oil firmness	Butter solid fat content, 5°C	Butter solid fat content, 20°C
24	0.24 0.15	0.31 0.07	0.10 0.56	0.11 0.51	-0.25 0.14	0.26 0.16	0.47 0.004	0.27 0.11	0.45 0.007	0.42 0.01
25	-0.46 0.005	0.58 <0.001	0.24 0.15	0.27 0.11	-0.43 0.009	0.58 0.001	0.73 <0.001	0.86 <0.001	0.88 <0.001	0.87 <0.001
26	0.54 0.007	-0.73 <0.001	-0.66 <0.001	-0.68 <0.001	0.52 0.008	-0.72 <0.001	0.71 <0.001	-0.76 <0.001	-0.86 <0.001	-0.84 <0.001
27	-0.17 0.31	0.36 0.03	0.22 0.20	0.24 0.16	-0.35 0.04	0.40 0.02	0.50 0.002	0.31 0.07	0.50 0.002	0.46 0.005
29	-0.53 0.001	0.54 0.001	0.37 0.03	0.37 0.03	-0.58 <0.001	0.62 <0.001	0.63 <0.001	0.84 <0.001	0.85 <0.001	0.88 <0.001
31	-0.02 0.92	0.19 0.26	0.57 <0.001	0.55 <0.001	-0.19 0.26	-0.02 0.92	0.12 0.50	0.04 0.80	0.15 0.37	0.20 0.25
33	-0.18 0.30	0.37 0.02	0.29 0.09	0.31 0.06	-0.17 0.31	0.46 0.008	0.44 0.007	0.23 0.18	0.34 0.04	0.35 0.04
35	0.39 0.12	-0.94 <0.001	-0.54 0.02	-0.63 <0.001	0.13 0.61	-0.78 <0.001	-0.97 <0.001	-0.73 0.001	-0.86 <0.001	-0.78 <0.001
36	0.60 <0.001	-0.83 <0.001	-0.38 0.02	-0.43 0.009	0.33 0.05	-0.71 <0.001	-0.91 <0.001	-0.71 <0.001	-0.85 <0.001	-0.80 <0.001
38	-0.14 0.52	0.58 0.005	0.33 0.14	0.32 0.14	-0.15 0.50	0.51 0.02	0.88 <0.001	0.70 <0.001	0.81 <0.001	0.75 <0.001
39	0.28 0.24	-0.65 0.003	-0.22 0.36	-0.28 0.24	0.08 0.76	-0.44 0.07	-0.81 <0.001	-0.43 0.07	-0.54 0.02	-0.47 0.04
40	-0.10 0.62	-0.11 0.58	0.46 0.01	0.43 0.02	-0.11 0.57	-0.18 0.37	-0.29 0.12	-0.28 0.14	-0.17 0.38	-0.22 0.26

^aTop number is Pearson correlation coefficient and bottom number is *P*-value.

^bFrom Ortiz Gonzalez et al. (2023).

21 fractions were statistically correlated, but 6 to 10 fractions consistently had the highest correlations.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0022029924000037>

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