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Physical and Processing Properties of Milk, Butter, and Cheddar Cheese from Cows Fed Supplemental Fish Meal

C. A. Avramis,* H. Wang,* B. W. McBride,† T. C. Wright,† and A. R. Hill*
*Food Science Department
†Department of Animal and Poultry Science
University of Guelph, Guelph, Ontario, Canada, N1G 2W1

ABSTRACT

Physical, chemical, sensory and processing properties of milk produced by feeding a rumen-undegradable fish meal protein supplement to Holstein cows were investigated. The supplement contained (as fed basis) 25% soft-white wheat, 60% herring meal, and 15% feather meal. The total fat level in the milk decreased to 2.43%. For both pasteurized and ultra-high temperature processed drinking milk, no difference was found between fish meal (FM) milk and control milk in terms of color, flavor and flavor stability; in particular, no oxidized flavor was observed. Cheddar cheese made from FM milk ripened faster after 3 mo of ripening and developed a more desirable texture and stronger Cheddar flavor. The yield efficiencies for FM and control cheese, 94.4 (±2.44 SE) and 96.4 (±2.26 SE), respectively, were not different. Relative to controls, average fat globule size was smaller in FM milk and churning time of FM cream was longer. FM butter had softer texture and better cold spreadability, and butter oils from FM enriched milk had lower dropping points compared to control butter oil (average 32.89 versus 34.06°C). These differences in physical properties of butter fat were greater than expected considering that iodine values were not different. This study demonstrates the feasibility of producing high quality products from milk naturally supplemented with FM, but the results also show that dietary changes affect processing properties.
(**Key words:** milk, butter, Cheddar cheese, fish meal, docosahexaenoic acid)

Abbreviation key: C = control, **CLA** = conjugated linoleic acid, **DLS** = dynamic light scattering, **DHA** = docosahexaenoic acid, **DP** = dropping point, **DSC** = differential scanning calorimetry, **EPA** = eicosapentaenoic acid, **FA** = fatty acid, **FM** = fish meal, **FO** = fish oil, **PUFA** = polyunsaturated fatty acid, **SEM** = scanning electronic microscopy, **SFC** = solid fat content.

INTRODUCTION

Animal studies and epidemiological investigations indicate potential nutritional benefits of n-3 polyunsaturated fatty acids (**PUFA**) and conjugated linoleic acid (**CLA**) in human diets (Simopoulos, 1991; Gibson et al., 1996; McGuire et al., 1999; Pariza et al., 2000). Milk fat composition can be modified by direct addition of fatty acid (**FA**) supplements to milk or milk products, by genetic manipulation, or by feeding special diets to dairy cows. Direct supplementation has the advantages of simplicity and that theoretically, any level of desired FA could be added to milk fat. The major disadvantages are that fishy flavor associated with FA supplements such as fish oil (**FO**) must be reduced to an acceptable level and antioxidants are frequently required to stabilize long-chain unsaturated FA. Further, supplemental FA must be blended with milk fat and then homogenized into skimmed milk, a process that is undesirable for cheese making because homogenization produces cheese with excessive moisture content and reduced firmness (Fox et al., 2000).

Modest differences in fatty acid composition have been observed between breeds of cows and substantial differences exist between species (Palmquist et al., 1993; Jahreis et al., 1997; Kelly et al., 1998). Genetic changes can be accomplished by conventional breeding methods or by transgenic alteration (Gibson, 1991). However, change is slow relative to the large changes required to meet current consumer needs, so it appears that short-term modifications to milk fat composition can be effected only by changing the FA composition of dairy cow diets.

Dietary supplements that contain n-3 PUFA or n-3 PUFA precursors include fish meal (**FM**), FO, algae, and other plant sources. Menhaden oil (Hagemeister et al., 1991), linseed oil (Hagemeister et al., 1988), canola oil (Jenkins et al., 1992; Ashes et al., 1997; Khorasani et al. 1991) and fresh grass (Hebeisen et

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Corresponding author: A. R. Hill; e-mail: arhill@uoguelph.ca.

al., 1993) are all reported to increase long chain (n-3) FA concentrations in milk. For this project, elevated levels of Omega-3 fat were obtained using FM. The composition of FM varies, but it generally contains over 30% of total FA as long-chain polyunsaturated fatty acids, primarily eicosapentaenoic acid [**EPA**, 20:5 (n-3)] and docosahexaenoic acid [**DHA**, 22:6 (n-3)] (Bimbo and Crowther, 1992).

Diets supplemented with FM increased DHA concentration in milk fat and also decreased milk fat content and total milk fat production (Sutton, 1989; Spain and Polan, 1995; Wright et al., 1998 and 2003). Franklin et al. (1999) observed increased concentrations of CLA, DHA and *trans*-vaccenic acids in the milk of cows fed marine algae. DHA levels as high as 0.76g/100g of fat were reported with no effect on milk flavor. Baer et al. (2001) reported higher concentrations of CLA, *trans*-vaccenic acid, and unsaturated FA in milk and butter from cows fed menhaden oil, with no differences in flavor characteristics. Thus far, no other work on physical and processing properties of milk produced with FM or FO has been reported. Results reported in this paper are based on the work of Wright et al. (1998 and 1999) who quantified the apparent transfer rates of DHA from diet to milk by feeding incremental levels of a FM containing supplement. The present study was undertaken to describe the chemical and physical properties relevant to the processing and quality of dairy products made from the modified milk of cows fed FM supplement at 4.5% of DMI (See Wright et al. 2003).

MATERIALS AND METHODS

Experimental Design and Diets

Six multiparous (mean 2.8, SD = 1.2 lactations) Holstein cows were fed a corn-based diet (similar to the basal diet of Wright et al. 2003) and top-dressed with a rumen-undegradable protein supplement containing FM. The top-dressed supplement ingredients were 60% herring meal, 15% feather meal, and 25% wheat. The supplement was pelleted and offered twice daily at feeding. Chemical analysis of the supplement was similar to the corresponding supplement fed in a separate experiment by Wright et al. (2003). The rumen-undegradable supplement was included at 4.5% of DMI. Milk from the six cows was collected 2 or 3 times weekly from March to July 1998. Milk was collected from the bulk tank on the same days, to serve as control milk (**C**). The rest of the herd providing the bulk tank milk was fed the standard lactating cow diet that did not contain FM, as shown in Table 1. The University of Guelph Animal Care Committee, following the guidelines of the Canadian Council for Animal Care, approved the use of the dairy cows.

Table 1. Composition of the diet fed to control cows.

Ingredient	% As-fed
Corn silage	36.8
Haylage	33.2
Dry hay	2.0
High moisture corn	20.8
Soyplus	3.0
Gluten-soybean meal	2.4
Dairy premix	0.66
Limestone	0.49
Sodium bicarbonate	0.34
Salt	0.22

Physical and Chemical Properties of Control and Enriched Milk

FM and C milk were tested for composition each week. Fat, protein and lactose concentration were measured by infrared analysis (Foss System 4000, Hillerod, Denmark), and somatic cell count was determined by a photometric particle counting instrument (Fossomatic System, 440 Hillerod, Denmark).

Proteins were separated and quantified by Sodium Dodecyl SDS-PAGE (Hunt and Dalgleish, 1994) using a 20% polyacrylamide gel at 15°C (Pharmacia Biotech) in a PhastGel system (Pharmacia LKB-PhastSystem, Quebec, Canada). Densitometry was performed on individual protein bands with a Sharp JX-330 scanner and ImageMaster 2.0 software (Pharmacia Biotech, Uppsala, Sweden).

Fat globule sizes were measured by integrated light scattering using a Mastersizer X (Malvern Instruments Ltd., Malvern, UK). Measurements were conducted at room temperature and sample dilutions were approximately 1:1000 for optimum concentration. Instrument software (Malvern Instruments Ltd., Malvern, UK) produced size distribution curves that showed the volume percent of particles in each size interval.

Casein micelle size distribution was determined by dynamic light scattering (**DLS**). Approximately 3 ml of calcium buffer at pH 7.0 (5 m*M* $CaCl_2$ + 50 m*M* $NaCl_2$ + 20 m*M* imidazole) was filtered through a 0.22 μm filter (Milipore Ltd., Mississauga, Ontario, Canada) and transferred into a cuvette. Defatted milk samples were mixed into the buffer solution and tested for average particle size by dynamic light scattering using photon correlation spectroscopy according to Dalgleish and Hallett (1995). Scattering angle was set at 90°, bin time was 20 μs, and the sample temperature was controlled at 25°C. DLS mean sizes for milk sam-

Table 2. Chemical composition of milk from cows fed the control (C) or supplemental fish meal (FM) diet.[1]

	C		FM	
Milk Variable	Mean	SE	Mean	SE
Fat (g/100ml)	4.14[a]	0.33	2.43[b]	0.35
Protein (g/100ml)	3.29	0.13	3.32	0.20
Lactose (g/100ml)	4.62	0.13	4.45	0.30
Somatic cell count (10^3/ml)	211.3	40.09	233.6	40.5

[a,b]Means with different superscript letters within the same row are significantly different ($P < 0.05$).

[1]All values are least square means of n = 15 determinations.

Table 3. Protein distribution of milk from cows fed a control (C) or supplemental fish meal (FM) diet estimated by SDS-PAGE (%).[1]

	C		FM	
Protein	Mean	SE	Mean	SE
α_{s1}-casein	0.71[a]	0.11	0.52[b]	0.15
α_{s2}-casein	0.37	0.04	0.24	0.13
β-casein	0.52[a]	0.10	0.37[b]	0.05
κ-casein	0.15	0.02	0.16	0.12
α-lactalbumin	0.21	0.06	0.22	0.04
β-lactoglobulin	0.41	0.01	0.40	0.11
Blood serum albumin	0.09	0.07	0.10	0.08
Immunoglobulin	0.10	0.03	0.11	0.10

[a,b]Means with different superscripts letters within the same row are significantly different ($P < 0.05$).

[1]All values are least square means of n = 3.

ples used refractive indices and viscosities from Strawbridge and Hallet (1995).

Sensory Evaluation

FM and C milk were pasteurized at 80°C for 2 minutes through a UHT system (NO-BAC UNITHERM IV, Guelph Food Technology Center). The cream was separated with a Westfalia Type LWA-205 cream separator and the cream and skimmed milk were blended to obtain milk standardized to 2.0% fat. The processed milks were bottled and stored in the dark at 4°C. Expert graders evaluated milk samples on d 1, 4, 7, 12, 15 and 21 after processing and butter samples on d 1, 3, 5 and 7 after manufacturing. Each of two graders independently assigned a numeric grade between 1, lowest quality, and 5, highest quality, to each randomly coded milk or butter sample and described any off-flavors. In addition, triangle tests were conducted on milk 1 d after pasteurization and on butter within 1 wk after manufacture. Panels of a minimum of 20 consumers, mostly students and staff of the Department of Food Science, received three randomly coded samples, two of which were the same and a third that was different. Panelists were asked to select the odd sample from each set and comment on the presence of off-flavors. Triangle difference tests with a minimum of 20 untrained panelists were also conducted on cheese samples after 6 mo of ripening.

Cheese Making Properties

Based on the fat and protein composition results, C skim milk was standardized to the same fat concentration as that of FM milk. Milk was pasteurized at 63°C for 30 minutes and cooled. Starter culture, *Streptococcus lactis* and *Streptococcus cremoris* (Superstart concentrated cultures, Madison, WI) was added to the milk at 31°C at the level of 0.16% (v/w) and agitated. Agitation continued until the titratable acidity increased by 0.01%. Single strength rennet (CHR HANSEN, FPC Lot# 20067-21630, Mississauga, ON, Canada) was mixed into the milk at the level of 0.00019% (v/w). Cheese making followed the procedure for rindless Cheddar cheese as described by Kosikowski and Mistry (1997) with the exception of the following adjustments to account for the low fat target composition; whey pH at the draining stage was 6.4 instead of 6.2 and curd pH at milling was 5.5 instead of 5.4 to 5.3. Target cheese moisture content was 40% (v/v). Cheese was ripened at 8°C.

Cheeses were sampled for composition analysis between 7 and 14 d after manufacture and analyzed in duplicate for fat, protein and moisture contents using Babcock, Kjeldahl protein (Büchi Model 121) and oven moisture (3 g, 105°C to constant weight) tests, respectively. Samples for pH and free amino group measure-

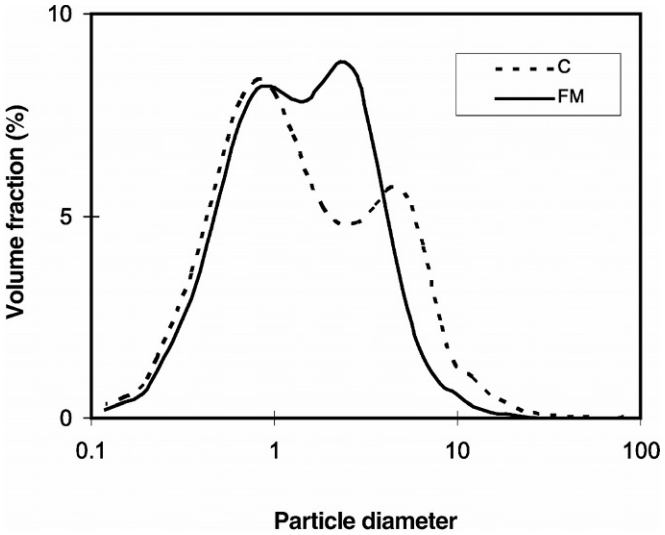

Figure 1. Fat globule size distribution of fish meal and control milks measured by integrated light scattering using a Mastersizer X (Malvern Instruments Ltd., Malvern, UK).

Table 4. Cheddar cheese yield from cows fed a control (C) or supplemental fish meal (FM) diet (%).[1]

	C		FM	
Yield	Mean	SE	Mean	SE
Actual yield	9.22[a]	0.31	8.75[b]	0.39
Expected yield[2]	8.89[a]	0.52	8.26[b]	0.27
Yield efficiency[3]	96.4[a]	2.44	94.4[b]	2.26

[a,b]Means with different superscript letters within the same row are significantly different ($P < 0.05$).

[1]All values are means of n = 2 determinations.

[2]Expected yield is calculated according to van Slyke and Price (1949) assuming a casein number of 77%.

[3]Yield efficiency was calculated as (actual yield/expected yield) × 100.

ments were taken between 7 and 14 d after manufacture and then at 1, 2, 3 and 4 mo of ripening at 5°C. The reaction of 2, 4, 6-trinitrobenzene sulphonic acid (TNBS) with free amines was used to measure free amino groups (Kuchroo et al., 1983).

For observation by SEM, cheese samples were freeze-fractured, sublimated for 45 minutes at –80°C, and sputter coated with gold to a thickness of ca. 300 Å and transferred into a SEM (Model: Hitachi S-570, Hitachi, Ltd., Tokyo, Japan). Temperatures of the microscope and sample chamber were kept at –145°C ± 15°C with liquid nitrogen. Accelerating voltage was 10 kV; t was 0 degree; objective aperture diameter was 50 μm. SEM micrographs were generated by a 100 second image sweep on the photo-CRT and recorded by a Mamiya 6×7 roll film holder assembly.

Physical Properties of Butter

Cream was separated from pasteurized (63°C, 30 min) milk using a Westfalia Type LWA-205 cream separator and its fat content was standardized to 35% w/w. The cream was stored overnight at 4°C and then churned in an 8°C room using a 1 L capacity laboratory churn. Agitation was stopped 10 seconds after emulsion inversion. The butter was transferred to a plastic container with drainage holes in the walls and worked manually to standardize the moisture to 16 to 17% before salting at the rate of 2.5%.

Table 5. Composition of Cheddar cheese from cows fed a control (C) or supplemental fish meal (FM) diet.[1]

	C		FM	
Composition (%)	Mean	SE	Mean	SE
Fat	27.93	0.15	28.28	0.11
Protein	29.98	0.21	29.38	0.32
Moisture	38.50	0.51	39.28	0.47

[1]All values are means of n = 2 determinations.

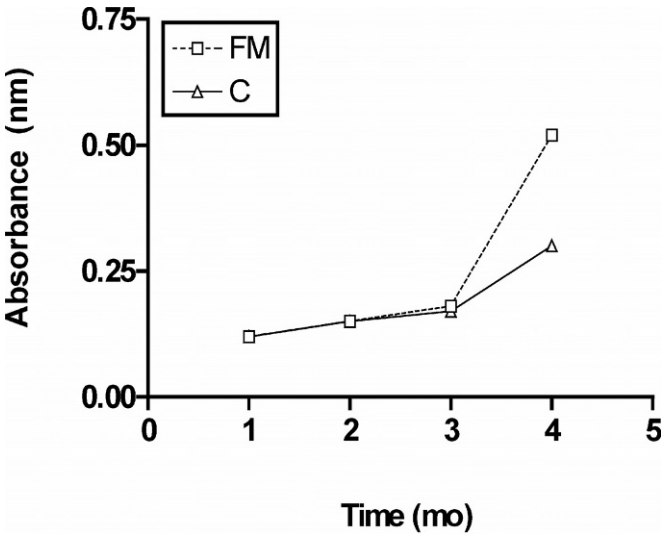

Figure 2. Relative rate of protein breakdown in fish meal and control Cheddar cheese as indicated by the reaction of free amino groups with 2, 4, 6, -trinitrobenzene sulphonic acid (Absorbance). Symbols represent the mean of three replicates.

Solid fat content (**SFC**) was determined according to AOCS official Method Cd 16-81 (AOCS, 1993) by pulsed Nuclear Magnetic Resonance (**pNMR**) using a Bruker PC20 Series NMR Analyzer (Minispec, Milton, Canada). Samples were: tempered at 60°C for 15 min to eliminate any crystal history; cooled and held at 26.7°C for 30 minutes, solidified at 0°C for 15 minutes, tempered again at 26.7°C for another 30 minutes, and, finally conditioned at 5°C for 30 minutes before the first NMR analysis. The sample was then warmed stepwise at 5°C intervals up to 45°C. NMR measurements were taken after 30 minutes of tempering at each temperature.

Dropping point (**DP**) was determined with a Mettler dropping point apparatus (FP83, Mettler, Zurich, Switzerland) according to Wright et al. (2000). Melting properties of butter oil were determined by differential scanning calorimetry (**DSC**) using a DuPont 1090 DSC (Wilmington, DE). Aluminum DSC pans were inserted with 5 mg of liquefied sample, hermetically sealed, heated for 10 min at 80°C to erase crystal history, and stored at 5°C for 48 hours prior to analysis. Samples were melted from 5°C to 60°C at a constant heating rate of 5°C/min. Iodine values were determined by AOCS official method Cd 1-25 (AOCS, 1993).

Statistical Analysis

Two-tailed t-tests were used through out this project to determine significant differences ($P = 0.05$) between control and treatment means using statistical software SAS™ (1991).

RESULTS AND DISCUSSION

Physical and Chemical Properties of Control and Enriched Milk

The fat concentration of FM milk was significantly lower than that of C milk (2.43 versus 4.14 g/100 ml; see Table 2). This agrees with earlier reports that unprotected FO and FM in diets of dairy cows results in milk fat depression (Pennington and Davis, 1975; Sutton, 1989; Chouinard et al., 1999, Baer et al., 2001). There were no differences in protein and lactose contents and somatic cell counts (Table 2). However, other diets supplemented with FO decreased milk protein content (Chilliard and Doreau, 1997; Chilliard et al., 2001).

Protein distribution results for α_{s1}-casein, α_{s2}-casein, β-casein, κ-casein, α-lactalbumin, β-lactoglobulin, blood serum albumin and immunoglobulins are shown in Table 3. There were no differences between FM and C milk, except that α_{s1}-casein and β-casein concentrations were lower in FM milk compared to C milk.

The average fat globule diameter was 1.837 μm and 2.307 μm for FM and C milk, respectively. Bovine milk typically exhibits a bimodal distribution for fat globule size. The distribution for the milk from FM fed cows had a smaller and slightly shifted second peak relative to C milk (Figure 1). The net effect on fat globule size in FM milk is a more uniform distribution with a smaller average globule diameter

With respect to casein micelle size, it remains unclear if the micelles exist in a continuous gradation of sizes or in a series of discrete sizes (Farrell et al., 1990). In the present experiment, the average diameter of casein micelles in FM was 170.8 nm, which is significantly lower, the 187.5 nm value of C milk. Altered size distribution together with the observed altered protein distribution of the casein micelles in FM milk may affect cheese making properties such as coagulation time, curd firmness, and cheese yield.

Sensory Properties

Expert graders detected no differences between FM and C milk regarding quality and flavor; no oxidized flavors were observed in either the FM or C milk prior to d 15 after pasteurization. Consumer triangle tests also indicated no flavor differences between the FM milk and C milk; 8 of 23 panelists correctly identified the odd milk sample. Changes in feed did not affect the quality and flavor of FM milk. In particular, there was no evidence to suggest that FM milk was more susceptible to the development of oxidized flavors. In both descriptive and triangle tests, flavor differences between FM and C butter samples were not significant. With respect to cheese, after 6 mo of ripening panelists indicated both a softer and smoother texture, and stronger Cheddar cheese flavor in the FM cheese compared to the C cheese.

Cheese Making Properties

Significant differences in casein distribution (Table 3) suggest that the treatment milk may also have different cheese making properties. Observations made during Cheddar cheese manufacture did not indicate differences with respect to coagulation time or rate of acidification. However, the rate of firming in treatment milk was slower and the gel in the treated milk, when ready to cut, as indicated by syneresis of clear whey, appeared less firm than the control. Notwithstanding significant differences in casein micelle size and composition, yield efficiency for FM and C cheese was not significantly different (Table 4). There were no differences in fat, moisture and protein contents of the cheeses as summarized in Table 5. Moisture in both cheeses was high resulting from a higher pH at the draining stage. The changes in free amino acids in C cheese during ripening are shown in Figure 2. In the first 3 mo of ripening, there was no significant difference between FM and C cheese in the level of free amino acids. However, after 3 mo of ripening, the level of free amino acids in FM cheese increased more quickly than C cheese. This indicates that the FM cheese ripened much faster than the C cheese. This confirms the sensory observations that the FM cheese had stronger Cheddar flavor, was softer, and had a more desirable texture than C cheese.

Changes in cheese microstructure during ripening have been extensively used to study body and texture development (Ustenol et al., 1995; and Bryant et al., 1995). Micrographs of different ripening stages for C and FM cheese microstructure are shown in Figure 3. Because the fat content was slightly reduced, a denser protein structure that is typically associated with low fat cheese (Ustenol et al., 1995) might be expected. This was true for both FM and C cheese after one month of ripening, but at 6 mo the structure of the FM cheese appears relatively more open. This difference seems to agree with the TNBS and sensory results.

Butter Making Properties

The churning time of FM cream was twice as long as control cream, probably due to both softer fat and smaller and more uniformly sized fat globules in the FM cream. (Figure 1). Note that fat content of both FM and C was standardized to 35% before churning.

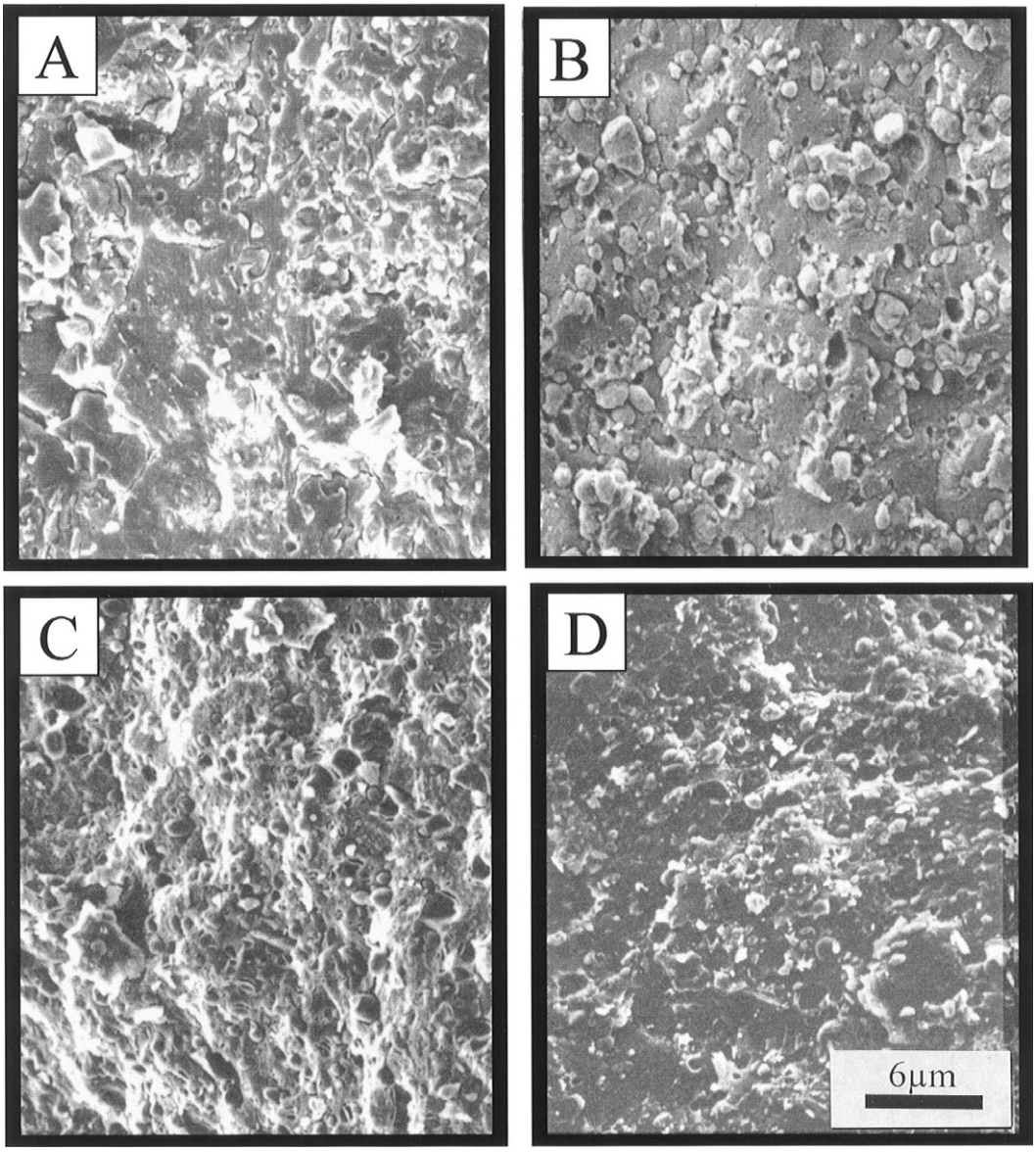

Figure 3. Scanning electron micrographs of freeze fractured control cheese (A and B) and fish meal cheese (C and D) after 1 and 6 mo, respectively.

The dropping point (n = 3) of FM butter oil (32.89°C ± 0.55) was lower ($P < 0.05$) than the dropping point of C butter oil (34.06°C ± 0.29). The melting curves of C and FM butter oil (Figure 4) show that at temperatures below 25°C, FM had a higher proportion of melted fat than C. When the temperature increased above 30°C and became closer to the dropping points of FM and C butter oils, the differences in their volume percentages of melted fat decreased. At 33°C, the dropping point of FM oil, the volume percentage of melted fat in FM oil accounted for 95% (Figure 5). At its dropping point temperature of 34°C, the volume percentage of melted fat in C butter oil was 97%.

Taken together, these physical properties indicate that FM fat is softer and contains less solid fat at temperatures less than 25°C and probably has better cold spreadability than C butter. These differences are certainly due to differences in fatty acid composition, but iodine values (n = 3) for FM (37.87 ± 2.80) and C (35.34 ± 2.60) butter fat were not significantly different, so the level of unsaturation is probably not the principal cause of softer fat. A little more insight on this can be obtained from the DSC thermograms in Figure 6.

The thermograms of FM butter oil have three peaks, while the curves of C butter oil have only two. The

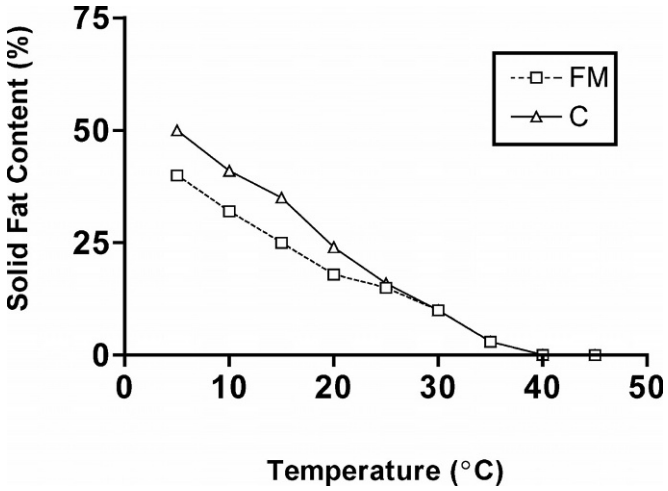

Figure 4. Solid fat content in fish meal and control butter oil measured by pulsed Nuclear Magnetic Resonance (AOCS official Method Cd 16–81). Symbols represent the mean of three replicates.

second and third peaks in the DSC curves of FM butter oil had similar shapes as those in the DSC curve of C butter oil and had similar peak melting temperatures. However, the first peak of C butter oil was steeper which indicates that uniform melting is occurring. The melting temperature of the C peak corresponds to the second peak of FM butter oil that has a broader shape. This indicates that C butter oil had more medium-melting triglycerides than FM butter oil and melted over a wider temperature range. The extra peak in the FM butter oil occurred below 10°C suggesting the presence of low-melting triglycerides. A detailed study of the triglyceride profiles of FM and C butter oils is required to explain the differences in melting properties.

CONCLUSIONS

FM milk had substantially different chemical, physical and processing properties relative to C milk. It appears that these differences can be readily accommodated with minor changes to processing procedures. Reduced fat globule size, increased churning time and improved cold spreadability of FM milk, cream and butter fat, respectively, are of particular scientific and technological interest.

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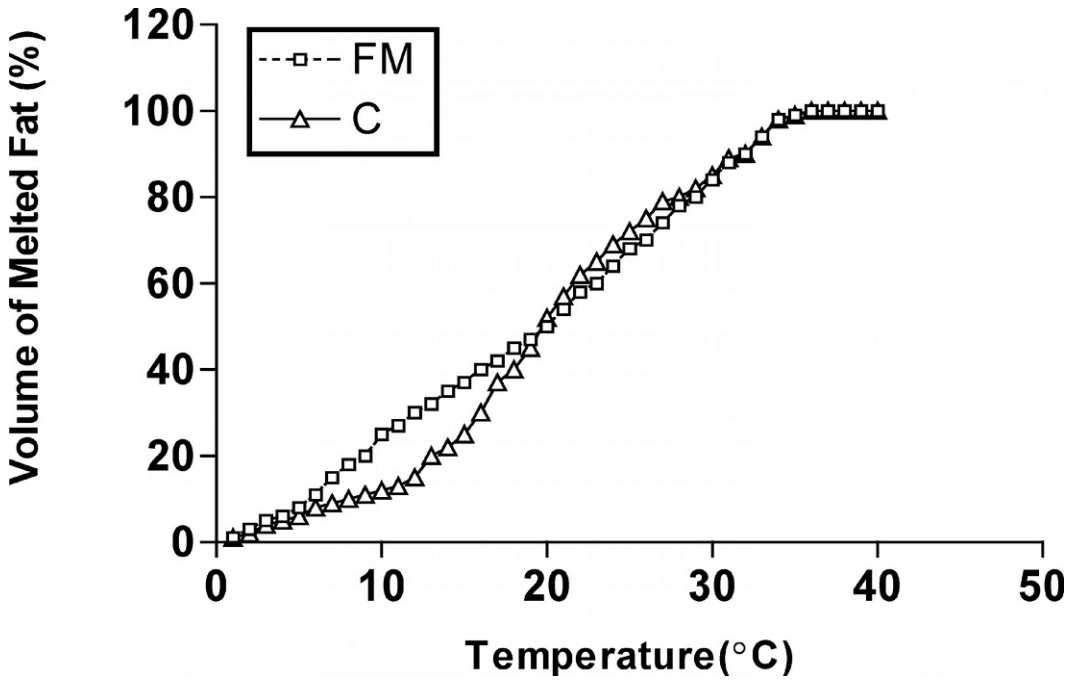

Figure 5. Volume fraction of melted fat content in fish meal and control butter oil measured by pulsed Nuclear Magnetic Resonance (AOCS official Method Cd 16–81). Symbols represent the mean of three replicates.

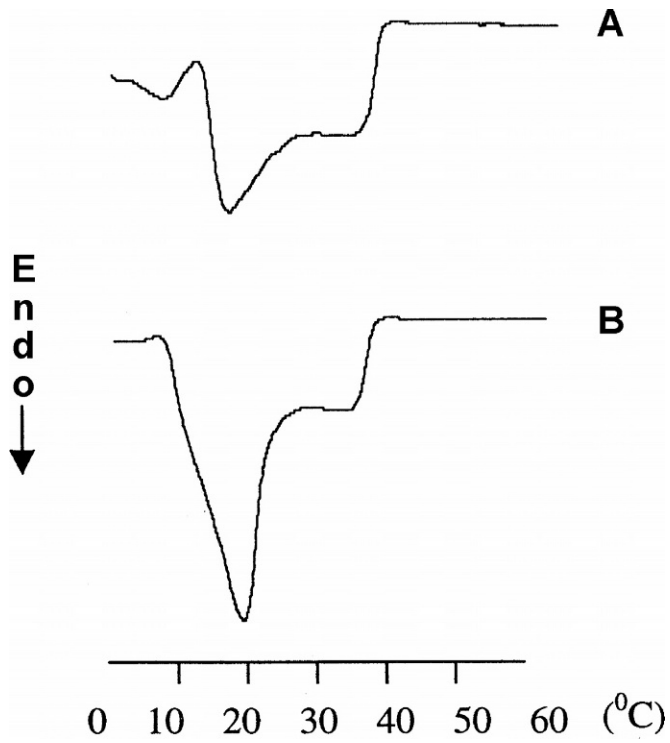

Figure 6. DSC melting curves for fish meal (A) and control butter (B) oil. Samples were crystallized for 48 h and melted at a heating rate of 5°C per minute.

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