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Enzymatic Interesterification of Tripalmitin with Vegetable Oil Blends for Formulation of Caprine Milk Infant Formula Analogs

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ABSTRACT

The structure of triacylglycerols in vegetable oil blends was enzymatically modified, and the blends were incorporated into skim caprine milk to produce goat milk-based infant formula analogs, homologous to human milk. A modified lipid containing palmitic, oleic, and linoleic acids, resembling the composition of human milk fat, was synthesized by enzymatic interesterification reactions between tripalmitin and a vegetable oil blend containing a 2.5:1.1:0.8 ratio of coconut, safflower, and soybean oils. A commercial *sn*-1,3-specific lipase obtained from *Rhyzomucor miehei*, Lipozyme RM IM, was used as the biocatalyst. The effects of substrate molar ratio and reaction time on the incorporation of palmitic, oleic, and linoleic acids at the *sn*-2 position of the triacylglycerols were investigated. The fatty acid composition and *sn*-2 position of the experimental formulas were analyzed using gas chromatography. Results showed that the highest incorporation of palmitic acid was obtained at 12 h of incubation at 55°C with a substrate molar ratio of 1:0.4 of tripalmitin to vegetable oil blend. However, the modified milk interesterified for 12 h at a 1:1 molar ratio had a greater resemblance to human milk compared with the other formulas. The level of oleic acid incorporation at the *sn*-2 position increased with the molar ratio of tripalmitin to vegetable oil blend. It was concluded that, unlike the original goat milk and other formulas, the formulated caprine milk with a molar ratio of 1:1 and a 12-h incubation was similar to the fatty acid composition of human milk.
Key words: enzymatic interesterification, tripalmitin, vegetable oil blend, caprine milk

INTRODUCTION

Human milk is considered nature's best infant food from nutritional, immunological, and food safety points of view (Megraud et al., 1990). However, time constraints and urbanization may cause the early termination of breast-feeding. Furthermore, some infants are not breast-fed because of a short supply of human milk, insufficient nutrition or a health condition of the nursing mother, the death of the mother during or after childbirth, and the necessity of some mothers having to work.

Therefore, the need exists to provide an alternative means of feeding for those infants who cannot be breast-fed. As the demand for an alternative to breast milk continues, a substitute for human milk should, as closely as possible, meet the nutritional requirements of the rapidly growing infant (Forsyth, 1998). The ultimate goal when designing an infant formula is to achieve the same outcomes as seen in breast-fed infants (British Department of Health, 1996).

Human milk consists of 4.4% of total lipids, and human milk fat (**HMF**) consists mostly of long-chain fatty acids such as palmitic, oleic, linoleic, and stearic acids. Unlike the palmitic acid in vegetable oils and ruminant milk fats, the palmitic acid in HMF constitutes the highest proportion (53 to 70%) of saturated fatty acids at the *sn*-2 position of the triacylglycerol (**TAG**) backbone, and unsaturated fatty acids are at the *sn*-1 and *sn*-3 positions (Jensen, 1989; Innis et al., 1995; Xu, 2000). The location of palmitic acid at the *sn*-2 position of TAG in HMF increases the absorption of palmitic and stearic acids in the lumen of infants and decreases the loss of calcium in their feces (Quinlan et al., 1995; Kennedy et al., 1999). This is due to the preservation of the *sn*-2-positional palmitic acid during digestion, absorption, and biosynthesis of TAG in the intestinal wall.

Milk fat can be modified for infant feeding by redesigning its physical, chemical, and nutritional properties. Modified lipids resembling the TAG of human milk can be produced by interesterification, using *sn*-1,3-specific lipase as the biocatalyst (Sahin et al., 2005a,b), and use of this lipase gives high selectivity and mimics the natural pathways of metabolic processes. Because a major portion of the energy required by infants is provided by lipids, the modified fats and oils in infant formulas should have both the correct fatty acid compo-

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sition and the same positional acyl distribution as in HMF.

Caprine milk has been recommended as a potential alternative to human milk for infant feeding (Birkbeck, 1978; Kirke, 1979; Taitz and Armitage, 1984) because it is less allergenic and more digestible compared with bovine milk (Park, 1991, 1994; Park and Haenlein, 2006). A significant amount of caprine milk produced in the world is consumed by infants and patients who suffer from an allergy to cow's milk (Park, 1994; Park and Haenlein, 2006). Infants' diets in Australia, Italy, South Wales, and North Africa have been reported to consist chiefly of caprine milk (Brande, 1972).

However, the positions of fatty acids (especially at *sn*-2) in the TAG of goat milk fat are different from those of HMF. It is beneficial to modify the TAG of goat milk to become homologous to HMF so that the absorption of milk fats in caprine milk infant formulas will be similar to that of human breast milk. Therefore, the objective of this study was to produce caprine milk infant formula analogs containing a fatty acid profile and TAG composition similar to that of human milk fat for infant feeding.

MATERIALS AND METHODS

Preparation of Materials and Experimental Procedures

Preparation of Chemicals. Tripalmitin (glycerol tripalmitate, minimum purity of 85%) and porcine pancreatic lipase (type II, crude) were purchased from Sigma Chemical Co. (St. Louis, MO). An immobilized 1,3-specific lipase, Lipozyme RM IM, was purchased from Novo Nordisk A/S (Bagsvaerd, Denmark). Organic solvents and thin-layer chromatography (**TLC**) plates were purchased from JT Baker Chemical Co. (Phillipsburg, NJ) and Fisher Scientific (Fair Lawn, NJ), respectively. All solvents and reagents used for sample analyses were of chromatographic or analytical grade.

Preparation of Goat Milk and Vegetable Oil Blends. Fresh goat milk was obtained from the bulk tank milk of midlactation Saanen, Nubian, and Alpine dairy goat breeds at the Georgia Small Ruminant Research and Extension Center, Fort Valley State University, Fort Valley, Georgia. The average daily milk yield was 1.1 L/head and the average somatic cell count of the milk was 550,000/mL. The goats were fed bermudagrass hay ad libitum, and 0.453 kg of concentrate was fed twice daily. The concentrate contained 5.0% of crude fat and 2.72% of digestible energy, which ensured 17% of fiber in the total diet.

On the basis of our preliminary studies (Maduko et al., 2005, 2006) and the results of Packard (1982), a vegetable oil blend was prepared by mixing coconut oil, safflower oil, and soybean oil at a ratio of 2.5:1.1:0.8, to achieve a fatty acid profile comparable to that of HMF. Coconut, safflower, and soybean oils were purchased from local retail outlets in Athens, Georgia. Coconut oil was melted to a liquid form at 40°C before mixing. The mixture was stirred vigorously to ensure uniform distribution, then stored at 40 ± 2°C to prevent recrystallization of the fat particles.

Interesterification of TAG. Two similarly formulated lipid groups were prepared, with each group containing 2 separate mixtures of tripalmitin and vegetable oil blends at different substrate molar ratios of 1:0.4 and 1:1, respectively. The mixtures were placed in screw-capped test tubes containing 3 mL of *n*-hexane and Lipozyme RM IM enzyme (10 wt% of total reactants). The sample mixtures were incubated separately for 12 and 24 h at 55°C in an orbital shaking water bath at 200 rpm, giving rise to 4 (2 × 2) samples. All formulated lipids were prepared in 4 replicates and were chemically analyzed in duplicate. The enzyme and products were passed through a sodium sulfate column to stop the reaction and remove the enzyme from the reaction products, as described in Sahin et al. (2005b).

The reaction products were applied to TLC plates (20 × 20 cm) coated with silica gel G, and were developed in a TLC tank using petroleum ether-ethyl ether-acetic acid (80:20:0.5 vol/vol) as the developing solvent, as described in Sahin et al. (2005b). The bands were sprayed with 0.2% 2,7-dichlorofluorescein in methanol and visualized under UV light. The TAG band produced was scraped into a screw-capped test tube and the TAG were extracted from the band by vortexing the scraped band twice with 3 mL of hexane and centrifuging at 800 × *g* for 5 min. The solvent was evaporated in a 25-mL evaporator flask using a rotary evaporator at 60°C. The 4 fat samples (from 2 molar ratios and 2 reaction times) produced from each replicate were then stored separately at –85°C. This interesterification of TAG was repeated 4 times.

Preparation of Infant Formula Analogs. Fresh caprine milk was pasteurized at 72°C for 30 min, and the fat was separated from the milk using a cream separator (Armfield FT 15; Armfield, London, UK). The resultant caprine skim milk was subdivided into 4 equal portions, in 4 replicates, and each portion was separately combined with 1 of the 4 prepared lipid group samples at a rate of 0.4 g of fat/10 g of milk to produce 4 different infant formula analogs: **F1** (1:0.4 molar ratio of tripalmitin-vegetable oil blend, esterified for 12 h), **F2** (1:1 molar ratio of tripalmitin-vegetable oil blend, esterified for 12 h), **F3** (1:0.4 molar ratio of tripalmitin-vegetable oil blend, esterified for 24 h), and **F4** (1:1 molar ratio of tripalmitin-vegetable oil blend, esterified for 24 h). These products were then freeze-dried at a

temperature of –40°C and a pressure of 133×10^{-4} kPa, using a freeze-drier (VirTis Freeze Mobile 25; VirTis, Gardiner, NY).

Chemical Analysis

Fat Extraction. Lipids were extracted from the freeze-dried infant formula analogs using the procedure of Folch et al. (1957). One gram of the freeze-dried sample was homogenized with 12 mL of prechilled chloroform-methanol (2:1 vol/vol) containing 0.005% butylated hydroxytoluene. The sample tubes were centrifuged at $800 \times g$ for 5 min to separate insoluble particles, and the solvent layer was passed through a sodium sulfate column. The solvent was evaporated in 50-mL evaporation flasks using a rotary evaporator at 60°C, and the fat extracts were stored in a freezer (–85°C).

Preparation of Fatty Acid Methyl Esters. The fat extracted was reconstituted with 2 mL of chloroform-methanol and flushed with nitrogen using a nitrogen evaporator (N-EVAP model 111; Organomation, Berlin, MA) as described in Park and Washington (1993). A 200-μL subsample was pipetted from each reconstituent and transferred to a screw-capped reaction tube. A 20-μL quantity of heptadecanoic acid was added to each tube to act as an internal standard. Excess solvent was evaporated from each tube, after which 3 mL of 6% HCl in methanol was added, and the mixture was methylated by incubating at 75°C for 2 h in a preheated oven. Thereafter, the mixture was extracted twice with 2 mL of hexane and 1 mL of 0.1 *M* KCl and centrifuged at $400 \times g$ for 3 min, and the upper (hexane) layer was separated and combined for each sample, then passed through a sodium sulfate column as described in Sahin et al. (2005b). The excess solvent was evaporated under nitrogen until about 1 mL of aliquot was obtained.

Quantification of Fatty Acids by Gas Chromatography. The fatty acid composition of the extracts was quantified by an Agilent 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a flame-ionization detector. Helium was the carrier gas and the gas flow rate was 1.7 mL/min. The oven temperature was initially held at 80°C for 3 min, and was then programmed to 215°C for 10 min at a rate of 10°C/min and held isothermally for 20 min, as described in Sahin et al. (2005b). The column used was a fused-silica Heliflex capillary column (Alltech-AT-225, 30 mm × 0.25 mm × 0.25 μm film thickness; Alltech, Deerfield, IL). The different amounts of fatty acid methyl esters (**FAME**; mol%) were analyzed and integrated by an integrator (model G2070AA; Agilent Technologies) with reference to 17:0 as an internal standard.

sn-2 Positional Analysis of TAG by Pancreatic Lipase. Fatty acids at the *sn*-2 position were analyzed according to the method described in Sahin et al. (2005b). Quantities of 20 mg of pancreatic lipase, 1 mL of Tris buffer (pH 8.0), 0.25 mL of bile salts (0.05%), and 0.1 mL of calcium chloride (2.2%) were added to a test tube (25 × 200 mm) containing 0.1 g of fat sample extracted from each of the infant formula analogs as described above. The sample reaction mixture was incubated at 40°C in a water bath for 3 min. One milliliter of 6 *M* HCl and 1 mL of diethyl ether were then added and the tube was centrifuged. The diethyl ether layer was evaporated under a stream of nitrogen (N-EVAP model 111; Organomation) to a volume of approximately 200 μL.

The 200-μL aliquot was spotted onto a silica gel G TLC plate and developed in a TLC tank by using hexane-diethyl ether-acetic acid (50:50:1 vol/vol) as the developing solvent. The 2-monoacylglycerol (**2-MAG**) band produced was sprayed with 0.2% 2,7-dichlorofluorescein in methanol and identified under UV light. The 2-monoolein standard (Sigma) was used to confirm the separation by TLC of 2-MAG in the reaction products. The 2-MAG band was then scraped off into a screw-capped test tube, extracted twice with 1 mL of hexane, and the FAME were derivatized as mentioned; the FAME were then analyzed by gas chromatography to evaluate the enzymatic interesterification of fatty acids at the *sn*-2 position of the TAG.

Statistical Analysis

All data collected from the 4 experimental infant formula analogs in 4 replicates of enzymatic interesterification were analyzed by ANOVA for mean differences between treatments. The effects of substrate molar ratio, reaction times, and their interactions were analyzed using the GLM procedure of SAS (SAS Institute, 1996).

RESULTS AND DISCUSSION

Fatty Acid Composition of Infant Formula Analogs

The fatty acid profiles and *sn*-2 fatty acid distribution of the 3 vegetable oils are shown in Table 1. Our data revealed that coconut oil was an important source of medium- and long-chain saturated fatty acids, whereas safflower and soybean oils were integral sources of polyunsaturated fatty acids (**PUFA**), in agreement with the results of Packard (1982). With regard to the *sn*-2 position of fatty acids in the vegetable oils, coconut oil had 81% as 12:0, safflower oil had 65% as 18:1, and soybean oil had approximately 69% as 18:2 fatty acids (Table 1).

The fatty acid profiles of the formulated goat milk analogs (F2, F3, and F4) appeared to be similar to that of human milk ($\alpha = 0.05$) by consisting mainly of medi-

Table 1. Fatty acid and *sn*-2 positional fatty acid profiles of vegetable oils (mol%/100 g)

	Coconut oil		Safflower oil		Soybean oil	
Fatty acid	Fatty acid profile	*sn*-2 profile	Fatty acid profile	*sn*-2 profile	Fatty acid profile	*sn*-2 profile
8:0	15.62 ± 0.09	0.64 ± 0.05	—	—	—	—
10:0	10.27 ± 0.03	2.73 ± 0.11	—	—	2.63 ± 0.32	0.19 ± 0.02
12:0	50.57 ± 0.12	81.01 ± 0.47	—	—	0.17 ± 0.04	0.15 ± 0.01
14:0	12.92 ± 0.08	9.78 ± 0.25	—	—	0.44 ± 0.04	2.0 ± 0.17
16:0	3.27 ± 0.04	0.87 ± 0.08	9.22 ± 0.15	1.19 ± 0.14	16.34 ± 0.84	1.18 ± 0.10
18:0	3.44 ± 0.01	0.13 ± 0.03	1.96 ± 0.05	0.66 ± 0.01	3.87 ± 0.41	0.10 ± 0.01
18:1	1.29 ± 0.08	3.47 ± 0.06	74.01 ± 0.20	65.03 ± 5.30	12.02 ± 0.98	22.84 ± 7.35
18:2	0.34 ± 0.01	1.40 ± 0.02	14.82 ± 0.11	33.50 ± 4.85	49.05 ± 1.03	68.68 ± 6.87
18:3	—	—	Trace	Trace	6.23 ± 0.15	4.74 ± 0.55
20:1	—	—	—	—	0.37 ± 0.03	0.12 ± 0.01

um- and long-chain fatty acids, with unsaturated fatty acids ranging from oleic acid to trace amounts of arachidonic acid (Table 2). There were differences ($P < 0.05$ or 0.01) in the fatty acid contents (mol%) of the goat milk infant formula analogs, with palmitic acid being highest in the F1 and F3 formulas and oleic acid being the highest in F2 and F4. Oleic acid in human milk accounts for 38% of the total fatty acids, whereas other higher fatty acids include palmitic acid (23%), linoleic acid (9%), and myristic acid (8%; Table 2). These 4 major fatty acids in human milk account for approximately 78% of the total fatty acids, with oleic and palmitic acids contributing more than 75% of the total. In the infant formula analogs, F2 had the closest similarity to HMF in this aspect, followed by F4, F3, and F1, in that order. The oleic, palmitic, linoleic, and myristic fatty acids of these 4 formula analogs accounted for about 73.3, 75.8, 78.1, and 86.6% of their total fatty acids, respectively.

Incorporation of Palmitic Acid

The fatty acids in F1 and F3 consisted predominantly of palmitic acid, with F1 having the highest palmitic acid content (Table 2). The high contents of palmitic acid in these samples, in comparison with the other 2 groups (F2 and F4), can be attributed to the high ratio of tripalmitin reacting with the vegetable oil blends, which subsequently resulted in a higher level of incorporation of palmitic acid into the TAG of the samples. Although F2 and F4 had lower levels of palmitic acid incorporation, their mole percentages of palmitic acid (25.9 and 28.2%, respectively) were closer to that of

Table 2. Fatty acid profiles (mol%) of infant formula analogs and human milk[1]

Fatty acid	GM	F1	F2	F3	F4	HM
4:0	2.24 ± 0.04	—	—	—	—	—
6:0	2.43 ± 0.05	—	—	—	—	—
8:0	2.71 ± 0.06^{ab}	1.1 ± 0.81^{a}	2.49 ± 0.41^{ab}	1.54 ± 1.46^{a}	1.75 ± 0.67^{a}	0
10:0	9.89 ± 0.20^{b}	2.14 ± 0.58^{a}	3.65 ± 0.13^{a}	3.26 ± 1.23^{a}	3.34 ± 0.26^{a}	2^{a}
12:0	4.89 ± 0.11^{ab}	3.8 ± 2.72^{a}	7.1 ± 0.53^{b}	8.9 ± 2.34^{b}	10.9 ± 0.99^{b}	7^{b}
14:0	9.69 ± 0.02^{a}	5.13 ± 0.61^{a}	7.04 ± 0.15^{a}	6.89 ± 3.22^{a}	6.8 ± 0.10^{a}	8^{a}
16:0	30.1 ± 0.19^{ab}	43.2 ± 6.31^{b}	25.9 ± 1.02^{a}	32.7 ± 6.36^{b}	28.2 ± 1.65^{ab}	23^{a}
16:1	1.59 ± 0.05^{a}	—	—	—	—	3^{a}
18:0	8.00 ± 0.51^{b}	6.41 ± 0.15^{a}	6.03 ± 0.05^{a}	6.4 ± 0.07^{a}	6.04 ± 0.10^{a}	7^{ab}
18:1	18.2 ± 0.42^{a}	28.69 ± 1.57^{ab}	31.69 ± 0.27^{b}	30.14 ± 1.66^{b}	31.84 ± 0.42^{b}	38^{b}
18:2	3.33 ± 0.09^{a}	8.28 ± 0.73^{b}	9.72 ± 0.20^{b}	8.84 ± 0.94^{b}	9.67 ± 0.19^{b}	9^{b}
18:3	0.37 ± 0.04^{a}	1.32 ± 0.08^{b}	1.44 ± 0.02^{b}	1.36 ± 0.13^{b}	1.57 ± 0.04^{b}	1^{b}
20:1	—	Trace	Trace	0.15 ± 0.03^{a}	Trace	1^{b}
20:4	—	Trace	Trace	Trace	Trace	0.8

[a,b]Means with different superscripts in the same row are different ($P < 0.05$ or $P < 0.01$).

[1]GM = Goat milk; F1 = infant formula analog produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio) and esterified for 12 h; F2 = infant formula analog produced with tripalmitin and vegetable oil blend (1:1 molar ratio) and esterified for 12 h; F3 = infant formula analog produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio) and esterified for 24 h; F4 = infant formula analog produced with tripalmitin and vegetable oil blend (1:1 molar ratio) and esterified for 24 h; HM = human milk (source: USDA, 1976; Packard, 1982).

human milk (23%), in comparison with the levels in F1 (43.2%) and F3 (32.7%).

Incorporation of Oleic and Stearic Acids

Oleic acid accounted for approximately 28.7, 31.7, 30.1, and 31.8% of total fatty acids in F1, F2, F3, and F4, respectively, whereas stearic acid accounted for 6.4, 6.03, 6.4, and 6.04% of the total fatty acids in the corresponding formula groups. These outcomes were similar to the oleic and stearic acid contents of human milk fat, which account for 38 and 7% of the total fatty acids, respectively. The higher oleic acid incorporation of F2 and F4 can be explained by the higher content of vegetable oil in F2 and F4 in comparison with the other 2 analogs. Reaction time also appeared to have an effect on the oleic acid content of the formulated samples (Table 2). Increasing the reaction time from 12 to 24 h also resulted in a subsequent increase in the oleic acid contents of F3 and F4 compared with those of F1 and F2, in agreement with other previous reports (Fomuso and Akoh, 1997; Lee and Akoh, 1998; Sahin et al., 2005a).

Incorporation of Linoleic Acid

The respective linoleic acid contents of the infant formula analogs F1, F2, F3, and F4 were 8.28, 9.72, 8.85, and 9.67% (Table 2). Linoleic acid is the main essential PUFA in human milk (Agostoni, 2003). The linoleic acid content in human milk is approximately 9% of total fatty acids, and it also makes up approximately 83% of the PUFA in HMF (Figure 1). Although there was no significant difference ($P > 0.05$) in the linoleic acid content between human milk and the formula analogs, F2 had the highest mean linoleic acid content, whereas F1 appeared to have the lowest (Figure 1 and Table 2). On the other hand, linoleic acid contributed about 85% of the PUFA in these infant formula analogs. In addition, there were increases in the linoleic acid levels of the formula analogs as the substrate molar ratios and reaction times increased (Figure 1). The F2 analog had the greatest incorporation of linoleic acid after the esterification reaction (Table 2), which was due to its high substrate molar ratio as well as its higher reaction time in comparison with F4 and the other 2 formula groups.

Polyunsaturated-to-Saturated Fatty Acid Ratio

The polyunsaturated-to-saturated fatty acid ratios (**P/S**) of the formulated analogs were comparable to that of human milk (Figure 2). The saturated fatty acid levels of the formulas are of considerable importance

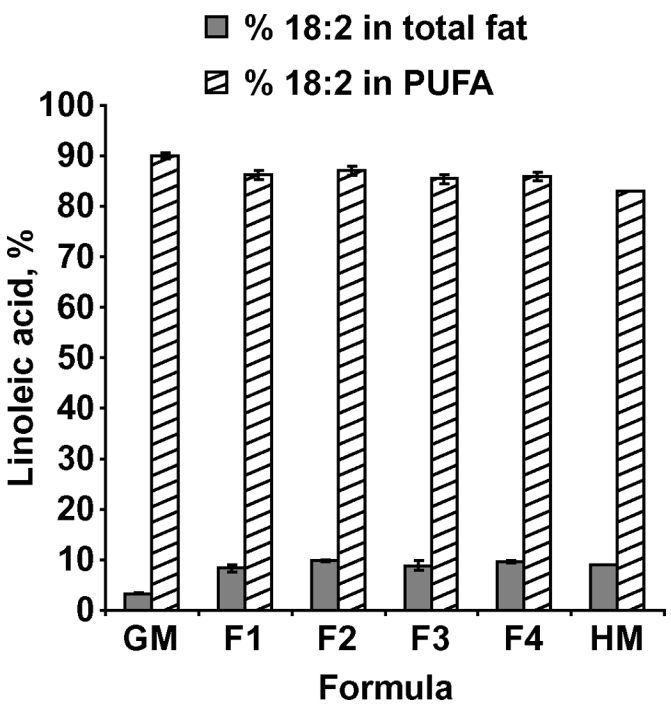

Figure 1. Percentage of linoleic acid content in polyunsaturated fatty acids (PUFA) and total fat of human milk and infant formulas. GM = Goat milk; F1 = infant formula produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio, 12 h); F2 = infant formula produced with tripalmitin and vegetable oil blend (1:1 molar ratio, 12 h); F3 = infant formula produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio, 24 h); F4 = infant formula produced with tripalmitin and vegetable oil blend (1:1 molar ratio, 24 h); HM = human milk. Formulas were interesterified at 55°C; the enzyme amount was 10 weight percent of total substrates. Incubation was at 200 rpm in *n*-hexane. Analogs FI, F2, F3, and F4 were not different from HM, whereas GM was different from HM ($P < 0.05$).

because of certain health concerns from the influence of saturated fatty acids on low-density lipoproteins in human metabolism (Shepherd et al., 1980; Spady and Dietschy, 1985). Koletzko and Bremer (1989) indicated that most manufacturers of infant formulas aim for a P/S ratio close to 0.2 to 0.5. The P/S ratio in HMF is about 0.23 (Jensen, 1989), which is not significantly ($P > 0.05$) different from those of the infant formulas in this study.

Monounsaturated Fatty Acid vs. PUFA Content

The monounsaturated fatty acid (**MUFA**) and PUFA contents and their ratios in the infant formula analogs and human milk are shown in Figure 3. Human milk fat contains about 39% MUFA and 10.8% PUFA, with both accounting for approximately 50% of total milk lipids. These fat moieties in the F2 analog were 43.3% of total lipids, which appeared to have a better resemblance to those of human milk compared with other

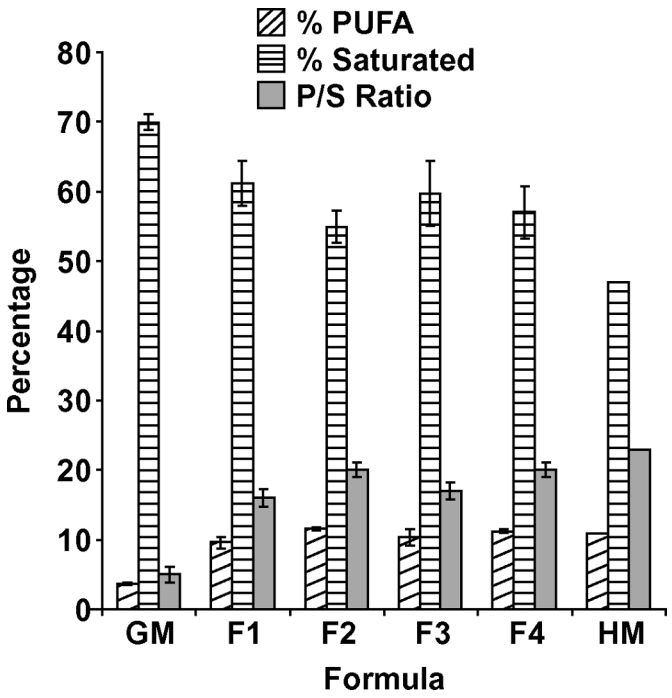

Figure 2. Polyunsaturated-to-saturated (P/S) fatty acid ratio of human milk and infant formulas. GM = Goat milk; F1 = infant formula produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio, 12 h); F2 = infant formula produced with tripalmitin and vegetable oil blend (1:1 molar ratio, 12 h); F3 = infant formula produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio, 24 h); F4 = infant formula produced with tripalmitin and vegetable oil blend (1:1 molar ratio, 24 h); HM = human milk. Formulas were interesterified at 55°C; the enzyme amount was 10 weight percent of total substrates. Incubation was at 200 rpm in *n*-hexane. Analogs FI, F2, F3, and F4 were not different from HM, whereas GM was different from HM ($P < 0.05$).

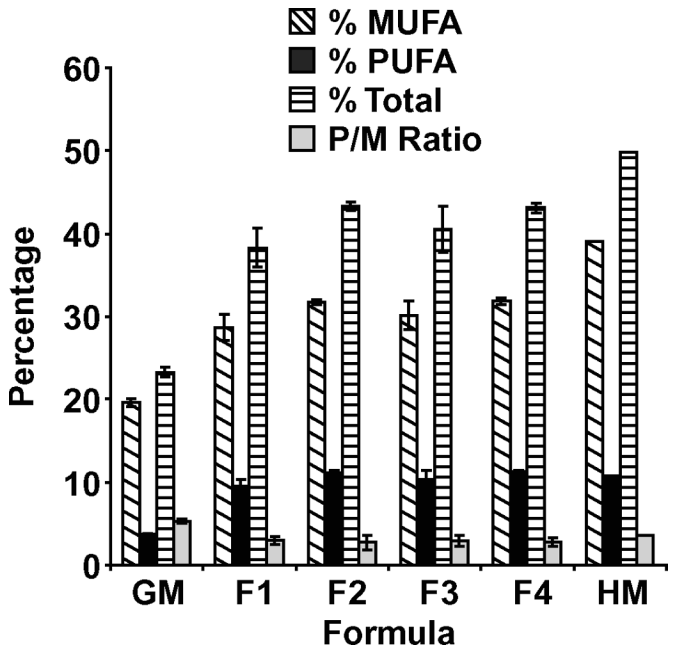

Figure 3. Percentage totals and ratios of monounsaturated and polyunsaturated fatty acid content (P/M) of human milk and infant formulas. GM = Goat milk; F1 = infant formula produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio, 12 h); F2 = infant formula produced with tripalmitin and vegetable oil blend (1:1 molar ratio, 12 h); F3 = infant formula produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio, 24 h); F4 = infant formula produced with tripalmitin and vegetable oil blend (1:1 molar ratio, 24 h); HM = human milk. Formulas were interesterified at 55°C; the enzyme amount was 10 weight percent of total substrates. Incubation was at 200 rpm in *n*-hexane. Analogs FI, F2, F3, and F4 were not different from HM, whereas GM was different from HM ($P < 0.05$).

formulated fats. The F1, F3, and F4 analogs had slightly lower values of total MUFA and PUFA contents, with F1 having the lowest value. These values may have resulted from the high rate of tripalmitin inclusion in the F1 analog sample, in comparison with the other analog samples formulated.

Oleic-to-Palmitic Acid Ratio

The oleic-to-palmitic acid ratio (**OPR**) of the formulated infant formulas, compared with that of human milk, is depicted in Figure 4. In human milk, oleic acid has the highest content, followed by palmitic acid, resulting in a 1.65 ratio of these 2 fatty acids. The OPR of F2 and F4 infant formulas were more similar to that of human milk, whereas the OPR values of the F1 and F3 groups were relatively low. The lower OPR in the F1 and F3 groups were actually attributable to their levels of palmitic acid incorporation at the substrate molar ratio at which they were reacted. According to Xu (2000), modified lipids with OPR values of approximately 1.2 are desirable for infant food formulations.

sn-2 Positional Analysis with Pancreatic Lipase

The *sn*-2 positional fatty acid profiles of the infant formula analogs are shown in Table 3. The 4 formulated analogs contained *sn*-2-positional fatty acid profiles in descending order of palmitic acid, oleic acid, linoleic acid, lauric acid, myristic acid, and stearic acid. These fatty acid profiles of the formulated milks were quite similar to human milk. These formula analogs revealed a high percentage of palmitic acid at the *sn*-2 position, with F4 containing the highest (66.4%) and F2 the lowest (63.7%). The high incorporation of palmitic acid at the *sn*-2 position of F3 and F4 may have been due to their longer reaction times in comparison with those of F1 and F2 (Table 3). The higher palmitic acid content of F1 compared with F2 was also attributable to the high substrate molar ratio of F1, which enabled a better retention of palmitic acid at the *sn*-2 position of the TAG backbone. However, there were no significant dif-

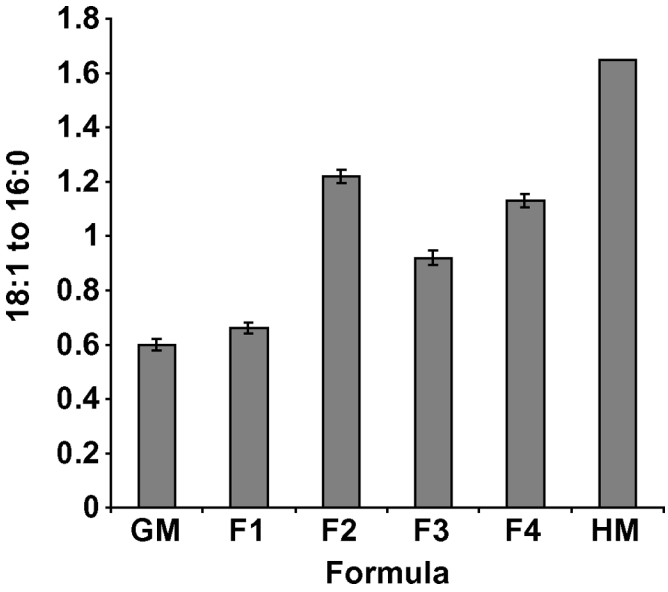

Figure 4. Oleic acid-to-palmitic acid ratio (OPR) of human milk and infant formulas. GM = goat milk; F1 = infant formula produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio, 12 h); F2 = infant formula produced with tripalmitin and vegetable oil blend (1:1 molar ratio, 12 h); F3 = infant formula produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio, 24 h); F4 = infant formula produced with tripalmitin and vegetable oil blend (1:1 molar ratio, 24 h); HM = human milk. Formulas were interesterified at 55°C; the enzyme amount was 10 weight percent of total substrates. Incubation was at 200 rpm in *n*-hexane. Analogs F2 and F4 were not significantly different from HM, whereas GM, F1, and F3 were significantly different from HM ($P < 0.05$).

ferences in the palmitic acid contents among the 4 formula groups.

The oleic acid distribution at the *sn*-2 position of the F2 and F4 samples (Table 3) appeared to better simulate that of human milk than those of F3 and F1. The higher substrate molar ratio of the F2 and F4 samples might have resulted in subsequent increases in oleic acid incorporation. These results are in agreement with Sahin et al. (2005a), indicating that substrate molar ratio, temperature, and time have positive effects on oleic acid incorporation with Lipozyme RM IM. The incorporation level of linoleic acid at the *sn*-2 position in the infant formula analogs ranged from 8.37 to 8.93%. These values were higher than the *sn*-2 linoleic acid content of human milk (7.3%).

CONCLUSIONS

The enzyme Lipozyme RM IM was used in interesterification because of its *sn*-1,3-specificity, which would result in incorporation of the unsaturated fatty acids oleic and linoleic acids at these specific *sn*-1,3 positions of the TAG backbone. Quinlan et al. (1995) showed that unsaturated fatty acids at these positions would guarantee the maximum fat and calcium absorption in infants.

We concluded that the formulated caprine milk infant formula analogs were similar to human milk in terms of their fatty acid contents and acyl distribution on the glycerol backbone. The F2 caprine milk infant formula, produced with fat interesterified at 55°C for 12 h with a 1:10 molar ratio of tripalmitin-vegetable oil blend (2.5 coconut, 1.1 safflower, and 0.8 soybean oil), had a better resemblance to human milk than did the other formulas under the conditions used for formulating the caprine milk analogs in this study.

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Table 3. *sn*-2 Positional distribution of fatty acids (mol%) in goat milk, infant formula analogs, and human milk[1]

FA	GM	F1	F2	F3	F4	HM*
8:0	—	0.55 ± 0.05^{a}	0.53 ± 0.13^{a}	0.57 ± 0.05^{a}	0.67 ± 0.14^{ab}	—
10:0	11.18 ± 2.7^{b}	0.66 ± 0.01^{a}	0.63 ± 0.04^{a}	0.61 ± 0.04^{a}	0.55 ± 0.05^{a}	0.2^{a}
12:0	7.54 ± 0.62^{b}	2.53 ± 0.09^{a}	2.58 ± 0.33^{a}	2.66 ± 0.43^{a}	2.47 ± 0.33^{a}	2.1^{a}
14:0	14.8 ± 4.97^{b}	7.06 ± 0.09^{a}	7.51 ± 0.46^{a}	7.25 ± 0.52^{a}	7.28 ± 0.30^{a}	7.3^{a}
16:0	40.3 ± 5.57^{a}	65.6 ± 1.7^{bc}	63.7 ± 2.4^{bc}	66.0 ± 0.7^{bc}	66.4 ± 0.36^{bc}	58.2^{b}
16:1	—	—	—	—	—	4.7
18:0	26.14 ± 3.52^{b}	2.55 ± 0.05^{a}	2.20 ± 0.23^{a}	2.70 ± 0.50^{a}	2.35 ± 0.43^{a}	3.3^{a}
18:1	—	12.19 ± 1.4^{a}	12.42 ± 0.7^{a}	12.28 ± 0.4^{a}	12.87 ± 0.18^{a}	12.7^{a}
18:2	—	8.66 ± 0.80^{ab}	8.37 ± 0.79^{ab}	8.45 ± 0.23^{ab}	8.93 ± 0.43^{ab}	7.3^{a}
18:3	—	0.33 ± 0.27^{a}	0.46 ± 0.05^{ab}	0.51 ± 0.08^{b}	0.51 ± 0.03^{b}	0.6^{b}
20:1	—	Trace	Trace	Trace	Trace	0.7
20:4	—	Trace	Trace	Trace	Trace	0.9

$^{a–c}$Means with different superscripts in the same row are different ($P < 0.05$ or $P < 0.01$).

[1]GM = goat milk; F1 = infant formula analog produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio, 12 h); F2 = infant formula analog produced with tripalmitin and vegetable oil blend (1:1 molar ratio, 12 h); F3 = infant formula analog produced with tripalmitin and vegetable oil blend (1:0.4 molar ratio, 24 h); F4 = infant formula analog produced with tripalmitin and vegetable oil blend (1:1 molar ratio, 24 h); HM = human milk (source: Jensen, 1989).

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