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ARTICLE

Establishment of an Evaluation Model for Human Milk Fat Substitutes

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Fatty acid composition and distribution of human milk fat (HMF), from mothers over different lactating periods in Guangzhou, China, were analyzed. The universal characteristics were consistent with previously reported results although the fatty acid content was within a different range and dependent on the local population (low saturated fatty acid and high oleic acid for Guangdong mothers' milk fat). Based on the composition of the total and *sn*-2 fatty acids of mature milk fat, an efficient evaluation model was innovatively established by adopting the "deducting score" principle. The model showed good agreement between the scores and the degree of similarity by assessing 15 samples from different sources including four samples of HMF, eight samples of human milk fat substitutes (HMFSs) and infant formulas, and three samples of fats and oils. This study would allow for the devolvement of individual human milk fat substitutes with different and specific fatty acid compositions for local infants.

KEYWORDS: Human milk fat; fatty acids; evaluation model; human milk fat substitute

INTRODUCTION

There is no doubt that from a nutritional and health point of view, human milk fat (HMF) is regarded as the best nutriceutical for newborn babies (*1*). Not only does it provide more than 50% of the dietary energy requirements for the infant (*2*) but it acts as a vehicle for the provision of fat-soluble vitamins and essential fatty acids (FAs) in the diet (*3*). The structure of human milk fat is rather unique. Human milk fat contains a triacylglycerol (TAG > 98%) core surrounded by a trilayer of polar lipids (*4*).The TAG consists of seven fatty acids in amounts greater than 1% in mature milk, and the distribution of fatty acids on the glycerol skeleton is not random, but unique (**Table 1**). In the main composition of *sn*-2 fatty acids, saturated fatty acids (SFAs) (C12:0, C14:0, C16:0, C18:0) comprise about 68.84%, of which C16:0 accounts for about 52.30%, and unsaturated fatty acids (UFAs) (C18:1, *n*−9, C18:2, *n*−6, and C16:1, *n*−7) comprise about 26.80%. In the total fatty acids, most of the C16:0 (> 70%) are at the s*n*−2 position and the UFAs (C18:1, *n*−9 and C18:2, *n*−6) and SFAs (C10:0, C12:0 and C18:0) are mainly found at the *sn*-1,3 positions. Such a structure helps the simultaneous absorption of fatty acids in the gut lumen of the infant and the loss of calcium through the feces (*5*, *6*).

When human milk fat cannot be provided to the babies by some mothers for reasons such as poor health condition, insufficient nutrition, short supply of human milk, working necessity, and fitting requirement (especially for young mothers), an alternative food formula for feeding babies is needed. Therefore, recently the studies regarding the development of human milk fat substitutes (HMFSs) have been more focused. Some research groups have reported the enzymatic production of human milk fat substitutes using different sources (e.g., soybean oil, fish oil, borage oil, and lard) by different reaction systems (e.g., acidolysis, transesterification) with various lipases (*15*−*18*). The obtained HMFSs had different fatty acid compositions and distributions. However, it is very difficult to evaluate the quality of HMFSs or the degree of similarity (**Table 2**). Moreover no reports relating to the recommendation of the evaluation standard for HMFSs have been published so far.

The aim of this study was to establish the evaluation model for HMFSs, which was compared against local infants. Forty samples of human milk fat (20 for colostrum milk and 20 for mature milk) from healthy mothers in Guangzhou of South China were withdrawn, and fatty acid compositions and distributions of these samples were analyzed. Based on the data from the analysis, the evaluation model was established and its degree of accuracy was explored using different fats with various fatty acid compositions and distributions. Thus, this work provided a simple and feasible model for the evaluation of HMFSs quality and will greatly improve developments in the HMFSs production industry.

MATERIALS AND METHODS

Subjects and Sample Collection. Forty samples of human milk were obtained from the First Affiliated Hospital, Sun Yat-sen University and were donated by apparently healthy and well nourished women in

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Guangzhou city, Guangdong province, China. The project was approved by Guangdong Provincial Hospital Ethics and Scientific Committees. The milk samples, each of which represented a single full breast expression, were collected by hand and frozen immediately and stored at −20 °C until required. Samples obtained between the first and fifth day postdelivery were assigned to the colostrum group (n = 20) and samples obtained after the 15th day postdelivery were assigned to the mature milk group (n = 20). The composition and distribution of total fatty acids and *sn*-2 fatty acids of all of the samples were then analyzed.

Milk Lipid Extraction. Before analysis, frozen samples from each mother were thawed at room temperature (25 °C). Lipid from a 10 g sample was extracted into 30 mL of $CHCl_3$/MeOH (2:1, v/v), sonicated using an ultrasonic wave (100 W, 5 min), thoroughly mixed, and centrifuged (4000 rpm, 5 min), and the organic phase was transferred to a 25 mL round-bottom flask containing heptadecanoic acid (20 μL) as an internal standard. Excess solvent was evaporated under nitrogen.

Fatty Acid Composition of Human Milk Fat. After extraction, the milk lipids were converted to their fatty acid methyl esters (FAME) according to ISO 5509:2000(E) (Animal and vegetable fats and oils-Preparation of methyl esters of fatty acids). Sample was introduced into a 50 mL flask, then 4 mL of 0.5 mol/L methanolic sodium hydroxide solution was added and was boiled under reflux for about 10 min, after an addition of 5 mL of methanolic boron trifluoride solution (12−15% as BF_3). The boiling was continued for 3 min. Two milliliters of isooctane was added into the boiling mixture at the top of the condenser. The flask was removed immediately and 20 mL of saturated sodium chloride solution was added. The flask was covered and shaken vigorously for at least 15 s. More saturated sodium chloride solution was added to make the liquid level of the mixture to the neck of the flask. As the two phases (the isooctane phase and the saturated sodium chloride solution phase) were separated, 1 mL of the upper isooctane layer was transferred into a 4 mL vial, and a small amount of anhydrous sodium sulfate was added to remove any traces of water, and then the sample was stored at −20 °C until required for gas chromatography (GC) analysis.

Fatty Acid Composition of Human Milk Substituted at the *sn*-2 Position. Lipids extracted from human milk were converted into 2-monoacylglycerol (2-MAG), substituted at *sn*-2 position, by means of the enzymatic process described by Sahin et al. (*19*). Pancreatic lipase (20 mg), Tris HCl buffer (pH 8.0, 1.0 mL, 1.0 M), bile salts (0.25 mL, 0.05%), and calcium chloride (0.1 mL, 2.2%) were added to a test tube containing a fat sample (150 mg). The reaction mixture was incubated at 40 °C for 5 min, and then HCl (6 M, 1.0 mL) and diethyl ether (1.0 mL) were added, and the tube was centrifuged. The ether layer was separated, concentrated to 200 μL under a stream of nitrogen, and then spotted on a silica gel G thin layer chromatography (TLC) plate and developed in a TLC tank with hexane−diethyl ether−acetic acid (70:30:1 v/v/v). The 2-MAG was visualized with 0.2% 2,7-dichlorofluorescein in methanol under UV light. Standard 2-monoolein (Sigma) was used on the TLC plate to confirm the presence of 2-MAG in the reaction products. The 2-MAG band was then scraped into a screw-capped test tube, and extracted into hexane (2 mL). Excess solvent was removed under nitrogen, and the residue was methylated as described above and analyzed by GC.

Fatty Acid Analysis by GC. The fatty acid methyl esters were analyzed by GC with a DM−FFAP capillary column (30 m × 0.25 mm i.d., 0.20 μm) in a Hewlett-Packard 7890 series gas chromatograph equipped with a flame-ionization detector. The temperatures of injector and of detector were 250 and 300 °C, respectively. The column oven was initially held at 170 °C (2 min), heated from 170 to 200 °C at a rate of 5 °C/min, and then increased to 230 °C at a rate of 10 °C/min. Hydrogen was used as the carrier gas at a head pressure of 0.5 MPa.

Statistical Analysis. All determinations were made in duplicate. Since results for some fatty acids appeared to have a skewed distribution, the data was chosen as median values (IQR) and ranges and processed with the Statistical Program for Social Sciences for Windows 13.0. Analysis of variance followed by two-independent-samples tests where applicable was used for statistical evaluation of significant differences between groups.

Establishment of Evaluation Model for Human Milk Fat Substitute. Human milk triacylglycerol has a specific chemical structure that contributes to its special assimilated and nutritional function. Its chemical structure could be determined by two equally important elements: total fatty acid composition (sector I) and *sn*-2 fatty acid composition (sector II). A "deducting score" principle was used to evaluate the degree of similarity of the HMFSs (**Table 6**), and the model was expressed by following equations:

$$G = G_1 + G_2 \quad (1)$$

$$G_1 = 50 - \sum_{i=1}^{n} E_i \quad (2)$$

$$G_2 = 50 - \sum_{i=1}^{n} E_{i(\text{sn-2})} \quad (3)$$

It was assumed that the maximum score for G is 100 and 50 each for G_1 and G_2; E_i and $E_{i(sn\text{-}2)}$ were the corresponding scores calculated from the following equations:

$$E_i = 50\left(C_i \frac{D_i}{\sum_{i=1}^{n} D_i}\right) \quad (4)$$

$$E_{i(\text{sn-2})} = 50\left(C_{i(\text{sn-2})} \frac{D_{i(\text{sn-2})}}{\sum_{i=1}^{n} D_{i(\text{sn-2})}}\right) \quad (5)$$

Table 1. Fatty Acid Composition and Distribution in Human Mature Milk

FA	FA profile of TAG[a] (%)	FA profile of *sn*-2[b] (%)	relative content of *sn*-2 fatty acids in the total fatty acids[b,c] (%)
C10:0	1.01−2.00	0.36	8.26
C12:0	3.20−7.43	4.81	24.61
C14:0	3.61−8.93	9.66	52.77
C16:0	17.30−23.23	52.30	87.86
C16:1, *n*−7	0.00−3.79	1.88	38.99
C18:0	5.43−9.20	1.71	9.04
C18:1, *n*−9	25.00−36.49	13.97	12.22
C18:2, *n*−6	10.45−20.30	10.95	22.07
SFAs	34.27−47.14	68.84	
UFAs	42.44−54.72	26.80	

[a] Adapted from refs *7*−*14* and processed with the Statistical Program for Social Sciences for Windows 13.0. [b] Adapted from López-López et al. (*7*). [c] Relative content of *sn*-2 fatty acids in the total fatty acids = (100 × *sn*-2 fatty acid)/(3 × total fatty acid).

Table 2. Fatty Acid Composition in TAG and at the *sn*-2 Position for HMFSs

	FA profile of TAG (%)				FA profile of *sn*-2 (%)			
FA	HMFS-1[a]	HMFS-2[b]	HMFS-3[b]	HMFS-4[b]	HMFS-1	HMFS-2	HMFS-3	HMFS-4
C12:0		0.20	12.7	0.80		0.40	14.0	0.40
C14:0	1.30	1.65	4.60	5.60	2.00	4.50	4.70	9.50
C16:0	29.00	31.20	22.30	29.60	71.1	71.90	31.70	9.70
C18:0	8.90	8.60	2.90	3.90	5.60	3.40	1.40	0.70
C18:1, *n*−9	34.70	27.10	38.30	35.90	15.30	10.10	28.30	50.20
C18:2, *n*−6	23.70	23.80	13.60	15.70	4.10	4.80	16.40	23.20

[a] Adapted from Yang et al. (*17*). [b] Adapted from Nielsen et al. (*18*).

Table 3. Fatty Acid Composition of Colostrum and Mature Milk Fat (%)

	colostrum milk (n = 20)		mature milk (n = 20)	
fatty acids	median (IQR)[a]	range (95%)	median (IQR)	range (95%)
C10:0	0.24 a (0.22)	0.10−0.90	0.91 (0.38)	0.52−1.64
C12:0	1.59 a (1.29)	0.97−4.31	3.61 (2.16)	1.23−6.41
C14:0	3.46 (1.61)	2.20−7.45	3.50 (2.32)	1.20−5.29
C15:0	0.11 (0.04)	0.00−0.19	0.08 (0.11)	0.00−0.18
C15:1	0.06 (0.14)	0.00−0.21	0.00 (0.06)	0.00−0.14
C16:0	24.17 a (2.55)	21.30−29.71	20.22 (3.29)	17.02−24.39
C16:1, n−7	1.98 a (0.34)	1.49−2.85	2.44 (1.44)	1.12−3.47
C16:2	0.00 (0.06)	0.00−0.23	0.00 (0.08)	0.00−0.21
C17:0	0.21 (0.08)	0.00−0.33	0.19 (0.07)	0.00−0.28
C17:1	0.13 (0.06)	0.00−0.23	0.11 (0.07)	0.00−0.19
C18:0	5.89 a (0.69)	3.34−9.23	5.29 (1.55)	3.89−7.37
C18:1, n−9	38.10 (2.47)	32.72−41.42	36.96 (3.31)	28.50−42.37
C18:2, n−6	15.93 a (2.34)	10.67−23.61	20.85 (4.60)	16.58−27.29
C18:3, n−6	0.00 a (0.00)	0.00−0.03	0.07 (0.18)	0.00−0.20
C18:3, n−3	0.51 a (0.37)	0.32−1.15	0.83 (0.67)	0.46−2.04
C20:0	0.21 (0.05)	0.00−0.39	0.21 (0.17)	0.12−0.53
C20:1, n−9	0.99 a (0.28)	0.62−1.38	0.53 (0.10)	0.38−0.95
C21:0	0.00 (0.00)	0.00−0.20	b	b
C20:2, n−6	1.11 a (0.40)	0.85−1.79	0.48 (0.18)	0.05−1.89
C20:3, n−6	0.66 a (0.34)	0.29−1.13	0.38 (0.16)	0.18−0.63
C20:4, n−6	0.87 a (0.54)	0.42−1.60	0.54 (0.19)	0.37−1.77
C20:3, n−3	0.00 (0.08)	0.00−0.15	0.00 (0.05)	0.00−0.85
C20:5, n−3	0.07 a (0.11)	0.00−0.36	0.17 (0.19)	0.00−0.87
C22:0	0.00 (0.06)	0.00−0.10	0.00 (0.04)	0.00−0.35
C22:1, n−9	0.24 a (0.12)	0.00−0.38	0.10 (0.18)	0.00−1.80
C22:2, n−6	0.15 (0.36)	0.00−0.89	0.07 (0.13)	0.00−1.14
C23:0	0.13 (0.22)	0.00−0.30	0.10 (0.23)	0.00−1.39
C22:4, n−6	0.54 a (0.50)	0.00−1.12	0.14 (0.12)	0.00−2.74
C22:5, n−3	0.14 (0.22)	0.00−0.50	0.12 (0.17)	0.00−0.51
C22:5, n−6	0.43 a (0.39)	0.00−1.33	0.23 (0.21)	0.00−1.15
C22:6, n−3	0.83 a (0.43)	0.26−1.50	0.44 (0.58)	0.00−3.69
C24:0	0.00 (0.22)	0.00−0.80	0.10 (0.46)	0.00−1.68
C24:1	0.00 (0.24)	0.00−0.67	0.00 (0.19)	0.00−0.41
SFAs	36.47 a (4.84)	28.61−48.55	33.73 (4.75)	30.34−41.20
MUFAs	41.78 (2.38)	35.94−44.88	39.86 (3.16)	31.86−45.44
PUFAs	22.12 a (3.96)	15.47−27.31	24.98 (5.66)	20.84−33.46
PUFAs n−3	1.74 (0.82)	1.16−2.40	1.80 (1.52)	1.11−4.55
PUFAs n−6	20.27 a (3.57)	14.17−25.99	23.35 (5.61)	19.13−30.17
n−6/n−3	11.10 (3.43)	8.37−20.46	12.38 (8.66)	4.20−25.97
LC-PUFAs n−3	1.11 (0.54)	0.26−1.65	1.13 (0.89)	0.47−4.15
LC-PUFAs n−6	3.91 a (1.44)	2.15−7.30	2.11 (0.92)	1.27−7.34
LC-PUFAs	4.96 a (1.70)	2.56−8.66	3.30 (1.39)	1.90−9.53
LA/LnA	28.40 (14.33)	14.02−51.33	26.32 (17.51)	8.84−44.17
AA/DHA	1.07 (0.46)	0.66−2.22	1.18 (0.95)	0.12−3.28
PUFAs/SFAs	0.62 a (0.18)	0.32−0.95	0.74 (0.21)	0.52−1.10
MUFAs/SFAs	1.12 (0.16)	0.74−1.54	1.17 (0.20)	0.83−1.44
LC-PUFAs n−6/LC-PUFAs n−3	3.43 a (1.30)	2.59−8.85	1.67 (1.33)	0.42−4.28

[a] The a in an entry denotes significant differences (P <0.05) between colostrum and mature milk groups. [b] Not detectable.

$D_i/\sum_{i=1}^{n} D_i$ and $D_{i(sn\text{-}2)}/\sum_{i=1}^{n} D_{i(sn\text{-}2)}$ were the weights of fatty acid for sector I and sector II. It was assumed that D_i and $D_{i(sn\text{-}2)}$ were mean values of total fatty acid content and *sn*-2 positional content from the above mature milk TAG, respectively. C_i and $C_{i(sn\text{-}2)}$ were the floating rates for sector I and sector II, respectively, and were calculated by the following equations:

$$C_i = \frac{|B_i - A_i|}{A_i} \quad (6)$$

$$C_{i(\text{sn-2})} = \frac{|B_{i(\text{sn-2})} - A_{i(\text{sn-2})}|}{A_{i(\text{sn-2})}} \quad (7)$$

B_i and $B_{i(sn\text{-}2)}$ were values of different fatty acid content in the total fatty acids and *sn*-2 fatty acids from different samples. A_i and $A_{i(sn\text{-}2)}$ were the lower or the upper limit of 95% reference range of total fatty acids and *sn*-2 fatty acids, and the value of lower or upper limits were obtained from above human mature milk TAG. When B is higher than the upper limit of the corresponding fatty acid content, A was selected as the upper limit of the range; but the lower limit was given as A if B was lower than the lower limit of the range. If the values of B were within the range, floating rate (C) was kept at zero.

The accuracy of established model was evaluated using different fats and oils such as natural HMF, different HMFSs, infant formulas, lard, and vegetable oils (corn oil and soybean oil). The above information relating to the different kinds of fatty acid content of mature milk fat was used as fundamental data to give the lower and upper limits of the range of total fatty acids and *sn*-2 fatty acids. In order to simplify the calculation, important fatty acids and fatty acids with amounts higher than 1% were selected. Eight fatty acids (C10:0, C12:0, C14:0, C16:0, C16:1, n−7, C18:0, C18:1, n−9, and C18:2, n−6) made up the components for sector I, and six fatty acids (C12:0, C14:0, C16:0, C18:0, C18:1, n−9, and C18:2, n−6) made up the components for sector II. The score (G_1 and G_2) of each sector

Table 4. Sn-2 Fatty Acid Composition (%) of Colostrum and Mature Milk Fat

	colostrum milk (n = 20)		mature milk (n = 20)	
fatty acids	median (IQR)[a]	range	median (IQR)	range
C10:0	0.13 a (0.34)	0.00−2.97	0.58 (0.29)	0.00−0.99
C12:0	1.95 a (1.50)	0.24−4.43	4.32 (2.28)	1.42−7.48
C14:0	5.34 (2.72)	2.74−9.60	5.55 (1.65)	2.06−10.12
C15:0	0.25 (0.21)	0.00−1.07	0.17 (0.14)	0.00−1.10
C15:1	b	b	b	b
C16:0	49.25 (4.62)	39.45−56.40	49.06 (5.49)	41.79−58.84
C16:1, n−7	2.11 a (0.83)	1.12−3.08	3.22 (1.18)	1.23−5.23
C16:2	b	b	b	b
C17:0	0.26 (0.16)	0.00−0.76	0.28 (0.14)	0.00−3.84
C17:1	0.00 (0.06)	0.00−0.29	0.06 (0.19)	0.00−0.22
C18:0	2.21 a (0.99)	1.52−3.36	1.60 (0.52)	0.55−2.68
C18:1, n−9	16.49 a (2.30)	13.51−20.32	14.91 (1.97)	11.32−21.35
C18:2, n−6	11.49 (2.52)	8.36−23.73	12.95 (2.83)	9.66−17.76
C18:3, n−6	0.00 (0.00)	0.00−0.26	0.00 (0.16)	0.00−0.31
C18:3, n−3	0.45 (0.40)	0.00−2.02	0.58 (0.27)	0.28−1.78
C20:0	0.17 (0.37)	0.00−0.97	0.24 (0.15)	0.00−0.51
C20:1, n−9	0.62 a (0.45)	0.13−1.90	0.40 (0.10)	0.00−1.56
C21:0	0.42 a (0.38)	0.00−0.97	0.16 (0.28)	0.00−0.64
C20:2, n−6	0.51 a (0.17)	0.00−0.98	0.25 (0.21)	0.00−0.74
C20:3, n−6	0.55 a (0.47)	0.00−1.37	0.26 (0.08)	0.12−0.50
C20:4, n−6	1.16 a (0.81)	0.62−2.97	0.54 (0.23)	0.19−0.81
C20:3, n−3	b	b	b	b
C20:5, n−3	0.00 (0.00)	0.00−0.12	0.00 (0.24)	0.00−0.38
C22:0	0.00 (0.02)	0.00−0.33	b	b
C22:1, n−9	0.13 (0.23)	0.00−0.47	0.00 (0.16)	0.00−0.28
C22:2, n−6	0.39 (0.68)	0.00−1.37	0.32 (0.66)	0.00−2.66
C23:0	0.40 a (0.34)	0.00−1.58	0.00 (0.29)	0.00−0.34
C22:4, n−6	0.40 a (0.52)	0.00−1.72	0.32 (0.25)	0.00−0.58
C22:5, n−3	0.78 (1.38)	0.00−2.47	0.27 (0.38)	0.00−0.67
C22:5, n−6	0.47 a (0.68)	0.00−1.65	0.31 (0.46)	0.00−0.84
C22:6, n−3	0.75 a (1.00)	0.60−3.82	1.06 (0.71)	0.00−2.44
C24:0	b	b	0.36 (0.73)	0.00−1.73
C24:1	b	b	0.00 (0.33)	0.00−1.37
SFAs	61.48 (8.60)	49.73−67.93	62.94 (5.86)	57.07−70.94
MUFAs	19.66 (2.16)	16.66−23.22	19.32 (2.69)	14.86−24.66
PUFAs	18.29 (6.25)	14.46−34.62	17.10 (2.56)	13.22−22.71
PUFAs n−3	2.43 a (1.49)	1.29−4.63	1.91 (1.21)	0.73−3.90
PUFAs n−6	15.79 (5.12)	12.78−29.99	15.11 (2.92)	11.67−21.41
n−6/n−3	6.58 (2.38)	4.18−10.68	8.33 (3.27)	3.46−21.67
LC-PUFAs n−3	2.23 a (1.18)	0.60−3.82	1.19 (0.96)	0.00−3.30
LC-PUFAs n−6	4.73 a (2.76)	1.94−7.43	2.06 (1.26)	1.21−4.38
LC-PUFAs	6.99 a (3.26)	2.54−10.89	3.34 (1.97)	1.38−5.57
LA/LnA	23.13 (19.05)	11.75−68.59	21.73 (9.37)	7.69−34.50
AA/DHA	0.74 a (0.65)	0.45−3.96	0.52 (0.48)	0.11−3.33
PUFAs/SFAs	0.30 (0.15)	0.22−0.70	0.27 (0.08)	0.19−0.38
MUFAs/SFAs	0.32 (0.07)	0.25−0.40	0.31 (0.04)	0.22−0.43
LC-PUFAs n−6/LC-PUFAs n−3	2.18 (0.99)	1.40−3.35	1.63 (1.32)	0.68−7.12

[a] The a in an entry denotes significant differences (P < 0.05) between colostrum and mature milk groups. [b] Not detectable.

was evaluated, so the total score ($G = G_1 + G_2$) was used to evaluate the degree of similarity of different HMFSs.

RESULTS AND DISCUSSION

Total Fatty Acid Composition of the Colostrum and Mature Milk Fat. Total fatty acid composition of both the colostrum (20 samples) and mature milk (20 samples) fat was analyzed, and the results are presented in **Table 3**. A significant increase for C10:0 and C12:0 was observed between the colostrum and the mature milk group, which was consistent with similar findings in Spain (*7*, *20*), Chongqing, and Hong Kong (*21*), while there was a significant reduction in C16:0 and C18:0. The sum of the medium-chain fatty acids (MCFAs, C10:0−C14:0) is greater in the mature group (23.78%) than in the colostrum group (14.51%). C16:0 accounted for just over half of the total SFAs, and the SFAs average in colostrum (36.47%) and in mature (33.73%) milk was lower than that in other reports (*8*, *21*, *22*) but in the range of those reported from Spain (*7*). C18:1 was a major component in the mono-unsaturated fatty acids (MUFAs), of which it accounted for about 91%. C18:2 (accounted for about 73.29% in PUFAs) was a major and important polyunsaturated fatty acids (PUFAs) since it is an essential fatty acid and must be supplied in the diet. C18:2 was significantly increased with the stage of lactation. The fatty acid composition in PUFAs was usually connected with the people's diets during their daily life. From the **Table 3**, more information about the human milk fat of Guangdong mothers can be seen such as SFAs/UFAs, MUFAs/PUFAs, n−6/n−3.

***sn*-2 Positional Fatty Acid Composition of the Colostrum and Mature Milk Fat.** Positional distributions of different fatty acids had a great impact on the metabolism, assimilation, and nutrition

Table 5. Relative Percentage of Each Fatty Acid at *sn*-2 Position in Colostrum and Mature Fat

	colostrum milk (n = 12)		mature milk (n = 12)	
fatty acids	median (IQR)[a]	range	median (IQR)	range
C12:0	23.89 (2.77)	20.65−25.32	21.97 (2.02)	20.10−25.31
C14:0	54.40 (27.60)	27.72−88.71	58.97 (17.00)	47.08−82.32
C16:0	72.08 a (7.16)	62.01−77.27	81.41 (7.41)	71.08−84.40
C18:0	10.30 a (4.40)	8.54−17.65	9.15 (1.37)	5.70−10.71
C18:1, n−9	14.73 (2.50)	13.19−17.74	13.76 (1.65)	13.00−16.80
C18:2, n−6	23.94 (2.91)	20.65−28.18	21.97 (2.02)	20.09−25.31
C18:3, n−3	22.76 (3.13)	20.00−36.37	29.08 (6.59)	16.01−32.75

[a] The a in an entry denotes significant differences (P < 0.05) between colostrum and mature milk groups.

of TAG (*23*, *24*). The *sn*-2 position fatty acid composition of both the colostrum and the mature milk is shown in **Table 4**. The contents of C10:0, C12:0, and C16:1 were significantly increased with the lactation changing from the colostrum stage to the mature stage. However, a significant decrease was found in the long and extra-long chain fatty acids such as C18:0, C18:1, n−9, C20:1, n−9, C21:0, C20:2, n−6, C20:3, n−6, C20:4, n−6, C23:0, and C22:5, n−6. The relative content of different total fatty acids in different position were calculated and are listed in **Table 5**. It could be seen that SFAs C12:0 and C18:0 and UFAs C18:1, n−9, C18:2, n−6, and C18:3, n−3 were mostly substituted at *sn*-1,3 positions, while C16:0 was substituted mainly at the *sn*-2 position (up to 81.41%). Comparing the fatty acid distribution in the colostrum milk fat with that in the mature milk fat showed that

Table 6. The Total Fatty Acid Evaluation Model (Sector I)

	fatty acids	C10:0	C12:0	C14:0	C16:0	C16:1, n−7	C18:0	C18:1, n−9	C18:2, n−6
	range (%)[a]	0.52−1.64	1.23−6.41	1.20−5.29	17.02−24.39	1.12−3.47	3.89−7.37	28.50−42.37	16.58- 27.29
B_i	HMF-1[b]	0.24	1.59	3.46	24.17	1.98	5.89	38.10	15.93
	HMF-2[c]	1.63	6.28	6.00	19.48	1.78	6.25	36.35	16.29
	HMF-3[c]	0.66	3.49	4.75	21.17	1.36	6.29	38.83	16.10
	HMF-4[c]	1.66	6.97	6.94	19.35	1.52	6.2	35.84	15.74
	HMFS-1[b]	0.53	6.99	3.25	29.17	0.00	7.78	34.60	16.16
	HMFS-2[d]	0.00	12.70	4.60	22.30	0.00	2.90	38.30	13.60
	HMFS-3[e]	0.00	0.20	1.65	31.20	0.00	8.60	27.10	23.80
	lard[e]	0.00	0.10	1.80	29.50	0.00	16.20	35.30	9.20
	IF1[c]	0.74	7.74	4.39	23.87	0.52	4.55	40.40	13.88
	IF2[c]	1.14	5.57	5.11	23.86	0.78	6.72	38.09	13.55
	IF3[c]	0.84	5.65	4.48	26.76	0.53	4.82	35.59	16.42
	IF7[c]	0.81	5.19	4.14	27.42	0.51	4.95	36.24	17.02
	IF9[c]	0.89	12.64	5.91	22.98	0.14	3.05	41.52	8.93
	corn oil[f]	0.00	0.00	0.00	10.30	0.00	1.70	30.40	57.1
	soybean oil[f]	0.00	0.00	0.00	11.50	0.00	2.90	21.30	56.6
C_i	HMF-1	0.54	0.00	0.00	0.00	0.00	0.00	0.00	0.04
	HMF-2	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.02
	HMF-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
	HMF-4	0.01	0.09	0.31	0.00	0.00	0.00	0.00	0.05
	HMFS-1	0.00	0.09	0.00	0.20	1.00	0.06	0.00	0.03
	HMFS-2	1.00	0.98	0.00	0.00	1.00	0.25	0.00	0.18
	HMFS-3	1.00	0.84	0.00	0.28	1.00	0.17	0.05	0.00
	lard	1.00	0.92	0.00	0.21	1.00	1.20	0.00	0.45
	IF1	0.00	0.21	0.00	0.00	1.00	0.00	0.00	0.16
	IF2	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.18
	IF3	0.00	0.00	0.00	0.10	1.00	0.00	0.00	0.01
	IF7	0.00	0.00	0.00	0.12	1.00	0.09	0.00	0.00
	IF9	0.00	0.97	0.00	0.00	1.00	0.22	0.00	0.46
	corn oil	1.00	1.00	1.00	0.39	1.00	0.56	0.00	1.09
	soybean oil	1.00	1.00	0.92	0.32	1.00	0.25	0.25	1.07
D_i		0.91	3.61	3.50	20.22	2.44	5.29	36.96	20.85
E_i	HMF-1	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	HMF-2	0.00	0.00	0.24	0.00	0.00	0.00	0.00	0.22
	HMF-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33
	HMF-4	0.00	0.17	0.58	0.00	0.00	0.00	0.00	0.56
	HMFS-1	0.00	0.17	0.00	2.16	1.30	0.17	0.00	0.33
	HMFS-2	0.49	1.89	0.00	0.00	1.30	0.71	0.00	2.00
	HMFS-3	0.49	1.62	0.00	3.02	1.30	0.48	0.99	0.00
	lard	0.49	1.77	0.00	2.26	1.30	3.38	0.00	5.00
	IF1	0.00	0.40	0.00	0.00	1.30	0.00	0.00	1.78
	IF2	0.00	0.00	0.00	0.00	1.30	0.00	0.00	2.00
	IF3	0.00	0.00	0.00	1.08	1.30	0.00	0.00	0.11
	IF7	0.00	0.00	0.00	1.29	1.30	0.25	0.00	0.00
	IF9	0.00	1.87	0.00	0.00	1.30	0.62	0.00	5.11
	corn oil	0.49	1.92	1.87	4.20	1.30	1.58	0.00	12.12
	soybean oil	0.49	1.92	1.72	3.45	1.30	0.71	4.93	11.89

[a] B_i represents the content of different fatty acids for different samples. C_i represnts the floating rate. D_i represents the content of different fatty acids from mature milk fat. E_i represts the deducting score. [b] Values of colostrum human milk and HMFS from our data. [c] Values of colostrum, transitional, and mature human milk and infant formulas adapted from López-López et al. (*7*). [d] Commercially produced structured HMFS, adapted from Nielsen et al. (*18*). [e] Adapted from Nielsen et al.(*18*). [f] Values adapted from Tautorus et al. (*27*).

Table 7. Relative Content of *sn*-2 Fatty Acids in the Total Fatty Acid Evaluation Model (Sector II)

	fatty acids	C12:0	C14:0	C16:0	C18:0	C18:1, *n*−9	C18:2, *n*−6
	range (%)[a]	20.10−25.31	47.08−82.32	>70	5.70−10.71	13.00−16.80	20.09−25.31
$B_{i(sn\text{-}2)}$	HMF-1[b]	23.89	54.40	72.08	10.30	14.73	23.94
	HMF-2[c]	24.61	52.77	87.86	9.04	12.22	22.07
	HMF-3[c]	29.24	55.32	80.30	8.98	14.10	23.84
	HMF-4[c]	25.04	52.88	86.25	9.05	13.03	23.54
	HMFS-1[b]	4.38	27.21	71.33	23.27	13.85	23.05
	HMFS-2[d]	36.75	34.06	47.38	16.09	24.63	14.81
	HMFS-3[e]	66.67	90.91	76.82	13.18	12.42	6.72
	lard[e]	66.67	81.48	83.50	6.38	8.97	11.59
	IF1[c]	31.45	24.73	41.06	9.54	25.88	36.86
	IF2[c]	53.03	41.66	18.92	9.16	32.24	45.58
	IF3[c]	86.62	19.64	7.33	4.68	47.71	39.40
	IF7[c]	30.07	36.84	16.09	9.34	43.22	50.82
	IF9[c]	30.19	31.47	62.37	25.96	21.14	30.39
	corn oil[f]	0.00	0.00	7.44	33.33	30.26	39.99
	soybean oil[f]	0.00	0.00	5.80	0.00	33.33	41.76
$C_{i(sn\text{-}2)}$	HMF-1	0.00	0.00	0.00	0.00	0.00	0.00
	HMF-2	0.00	0.00	0.00	0.00	0.06	0.00
	HMF-3	0.16	0.00	0.00	0.00	0.00	0.00
	HMF-4	0.00	0.00	0.00	0.00	0.00	0.00
	HMFS-1	0.78	0.42	0.00	1.17	0.00	0.00
	HMFS-2	0.45	0.28	0.32	0.50	0.47	0.26
	HMFS-3	1.63	0.10	0.00	0.23	0.04	0.67
	lard	1.63	0.00	0.00	0.00	0.31	0.42
	IF1	0.24	0.47	0.41	0.00	0.54	0.46
	IF2	1.10	0.12	0.73	0.00	0.92	0.80
	IF3	2.42	0.58	0.90	0.18	1.84	0.56
	IF7	0.19	0.22	0.77	0.00	1.57	1.01
	IF9	0.19	0.33	0.11	1.42	0.26	0.20
	corn oil	1.00	1.00	0.89	2.11	0.80	0.58
	soybean oil	1.00	1.00	0.92	1.00	0.98	0.65
$D_{i(sn\text{-}2)}$		21.97	58.97	81.41	9.15	13.76	21.97
$E_{i(sn\text{-}2)}$	HMF-1	0.00	0.00	0.00	0.00	0.00	0.00
	HMF-2	0.00	0.00	0.00	0.00	0.20	0.00
	HMF-3	0.85	0.00	0.00	0.00	0.00	0.00
	HMF-4	0.00	0.00	0.00	0.00	0.00	0.00
	HMFS-1	4.15	5.98	0.00	2.59	0.00	0.00
	HMFS-2	2.40	3.93	6.35	1.11	1.55	1.39
	HMFS-3	8.64	1.42	0.00	0.51	0.13	3.55
	lard	8.64	0.00	0.00	0.00	1.03	2.23
	IF1	1.29	6.69	8.05	0.00	1.79	2.44
	IF2	5.83	1.71	14.34	0.00	3.05	4.24
	IF3	12.83	8.25	17.68	0.40	6.11	2.97
	IF7	1.01	3.13	15.12	0.00	5.21	5.35
	IF9	1.01	4.70	2.16	3.13	0.86	1.06
	corn oil	5.30	14.23	17.48	4.66	2.66	3.07
	soybean oil	5.30	14.23	18.07	2.21	3.25	3.45

[a] $B_{i(sn\text{-}2)}$ represents the content of *sn*-2 positional fatty acids for different samples. $C_{i(sn\text{-}2)}$ represents the floating rate. $D_{i(sn\text{-}2)}$ represents the mean content of *sn*-2 positional fatty acids from mature milk fat. $E_{i(sn\text{-}2)}$ represents the deducting score. [b] Values of colostrum human milk and HMFS from our data. [c] Values of colostrum, transitional, and mature human milk and infant formulas adapted from López-López et al. (*7*). [d] Commercially produced structured HMFS, adapted from Nielsen et al. (*18*). [e] Adapted from Nielsen et al. (*18*). [f] Values adapted from Tautorus et al. (*27*).

the relative content of C16:0 at the *sn*-2 position was significantly increased while that of C18:0 was significantly decreased. The other fatty acids showed no significant difference. The positional specificity showed consistence with the reported results although the contents of fatty acids were within a different range (*7*).

Establishment of Evaluation Model for HMFS. Fatty acid composition of HMF varies greatly since it is influenced by various factors, such as the dietary composition and habits of lactating mothers, stages of lactation, and health status. (*14*, *21*, *25*, *26*). Although universal characteristics [(i) seven main fatty acids in amounts greater than 1%; (ii) C16:0 as a major component of SFAs and principally esterified at the *sn*-2 position of TAG; (iii) C18:1 as a major component of PUFAs and mainly distributed at *sn*-1,3 positions; (iv) C12:0, C18:0, C18:1, and C18:2 mainly found at the *sn*-1,3 positions] from **Tables 3**−**5** existed in the chemical structure of HMF TAG and were consistent with those reported in the literature, it was still very difficult to evaluate the quality of HMFSs in the market.

Actually if the total fatty acids and *sn*-2 positional fatty acids were within the range of those in HMF TAG, all the parameters, such as contents of SFAs and PUFAs and the ratio of SFAs/PUFAs, could meet the required standard. Thus, the "deducting score" principle was the best to be used to evaluate the degree of similarity of HMFSs. If the contents of fatty acids were within the standard range of corresponding fatty acids of HMF, it was considered best to get the maximum score. Contrarily lower score

Table 8. Scores of the Degree of Similarity of Different Samples[a]

samples	G_1	G_2	G
HMF-1	49.74	50.00	99.74
HMF-2	49.54	49.80	99.34
HMF-3	49.67	49.15	98.82
HMF-4	48.69	50.00	98.69
HMFS-1	45.87	37.29	83.16
HMFS-2	43.62	33.27	76.89
HMFS-3	42.11	35.74	77.85
lard	37.79	38.10	73.89
IF1	46.52	29.74	76.26
IF2	46.70	20.83	67.53
IF3	47.51	1.77	49.28
IF7	47.15	20.17	67.32
IF9	41.10	37.08	78.18
corn oil	26.52	3.49	30.01
soybean oil	23.60	2.60	26.20

[a] G_1, score of total fatty acid composition; G_2, score of *sn*-2 fatty acid composition; G, the total score of different samples.

(calculated by eqs **4** and **5**) would be given according to eqs **6** and **7**. The scores for sector I and for sector II (G_1 and G_2) were calculated, respectively, by eqs **2** and **3**, and the total score (G) was the sum of G_1 and G_2.

Efficiency of the model was evaluated by analyzing the fatty acid composition and distribution of different fats [four natural HMFs, three HMFSs, one lard, five infant formulas, and two kinds of vegetable oils (corn and soybean oils)]. Seven fatty acids (C12:0, C14:0, C16:0, C16:1, $n-7$, C18:0, C18:1, $n-9$, and C18:2, $n-6$) were used as factors for sector I because their amounts were greater than 1%. C10:0 was also considered as a factor for sector I since it was a useful fatty acid to provide energy to infants rapidly after being assimilated. In the analysis of sector II, C12:0, C14:0, C16:0, C18:0, C18:1, $n-9$, and C18:2, $n-6$ were selected since C10:0 and C16:1, $n-7$, had less content at the *sn*-2 position. The results are shown in **Tables 6−8**. It can be seen that samples from HMF (HMF-1, HMF-2, HMF-3, and HMF-4) got highest score, which referred to the highest degree of similarity. HMFS-1, HMFS-2, and HMFS-3 scored well for total fatty acid composition but had a lower degree of similarity in *sn*-2 fatty acid composition. For lard, which was a potential substrate for the production of HMFSs by enzymatic catalysis, its chemical structure had some similarity with the HMF. Infant formula such as IF1, IF2, IF3, IF7, and IF9 had a lower degree of similarity, which was mainly related to the *sn*-2 fatty acid composition. Corn oil and soybean oil had the least parallel with HMFSs compared with other samples.

Nowadays more attention is being paid to produce HMFSs for infants who are in different growth stages. But no evaluation system was established to justify the quality of HMFSs products, which was a drawback in the development of HMFSs for infant food and oil industries. An efficient model was established and evaluated in the study, and more advantages could be concluded: (1) the complicated data could be dealt with easily and quickly, and the degree of similarity could be reflected directly by different scores; (2) The established model was generalized since, though only eight fatty acids were selected for assessment, more than this could be accommodated. Based on this study, it was suggested to encourage the quick development of individual HMFSs with different and specific fatty acid composition for local infants.

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