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ORIGINAL PAPER

A comparative study of lipid composition and powder quality among powdered infant formula with novel functional structured lipids and commercial infant formulas

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Abstract

Pancreatic lipase activity and bile salt concentration are very low in infants. Therefore, infant formula (IF) preparation with structured lipids (SLs) containing essential fatty acids and medium-chain fatty acids (MCFAs) with easy absorption features has attracted great attention. In this work, the lipid composition and physiochemical properties of novel SLs enriched with medium- and long-chain triacylglycerols (MLCTs) and their incorporation into a powdered IF compared to commercial IFs were evaluated. The obtained results showed that the SL contains arachidonic (ARA), caprylic, and capric as major fatty acids (FAs). Further, the evaluation of commercial IFs oil showed that oleic, linoleic, palmitic, and lauric were the most abundant FAs. A novel SL possessed highest levels of MCFAs ($36.33 \pm 1.05\%$) at *sn*-1,3 positions and LCUFAs ($65.91 \pm 1.32\%$) at the *sn*-2 position especially ARA ($42.82 \pm 0.89\%$). A total of 45, 38, and 25 triacylglycerols molecular species were revealed in a novel SL and commercial IFs available in China and the USA, respectively. Thermoprofile revealed that all IFs oil samples melted below the body temperature. The formula produced with microencapsulated SL and the dry-mixing process showed better optical properties and oxidative stability having lower moisture content. The study has potential applications in the development of IF with improved properties.

Keywords Infant formula · Structured lipids · Triacylglycerols · UPLC-Q-TOF-MS · Microencapsulation

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Abbreviations

IF	Infant formula
SLs	Structured lipids
EFAs	Essential fatty acids
ARA	Arachidonic acid
LCUFAs	Long-chain unsaturated fatty acids
MCFAs	Medium-chain fatty acids
MAG	Monoacylglycerol
FA	Fatty acid
LCFAs	Long-chain fatty acids
MLCTs	Medium- and long-chain triacylglycerols
TAGs	Triacylglycerols
DSC	Differential scanning calorimeter
SCFAs	Short-chain fatty acids
FAMEs	Fatty acids methyl ester
UPLC-Q-TOF-MS	Ultra-performance liquid chromatography with quadrupole-time-of-flight mass spectrometry
ECN	Equivalent carbon number

CN	Total carbon number without glycerol
DB	Number of double bonds in the fatty acid
CIFL (1)	Fat extracted from a commercial infant formula available in China
CIFL (2)	Fat extracted from a commercial infant formula available in the USA
IN	Inulin
WPI	Whey protein isolate
FDA	Food and Drug Administration
WHO	World Health Organization
MML	Medium-, medium- and long-chain-type MLCTs
LMM	Long-, medium- and medium-chain type MLCTs
LML	Long-, medium- and long-chain-type MLCTs
MLL	Medium-, long- and long-chain-type MLCTs
LLM	Long-, long- and medium-chain-type MLCTs
MLM	Medium-, long- and medium-chain-type MLCTs

Introduction

Human breast milk is considered as the best food for infants in terms of nutritional value, immunity enhancement, and safety. In addition, it provides about 40—60% of total energy required for the infants through lipids [1]. However, the provision of infants with breast milk is not always possible due to number of reasons, such as time constraints, challenges associated with urbanization, and poor health conditions of the mother or baby, which may influence the early termination of feeding infants breast milk. Therefore, infant formulas (IFs) were developed to imitate the composition of a healthy mother's breast milk [2]. One of the important properties of IFs is the content and type of structured lipids (SLs) that used as a lipid ingredient. These have been reported to supply quick energy and essential fatty acids (EFAs), such as arachidonic acid (ARA), for the construction of an infant's cell membranes and normal function of cells [3]. Most commercial IFs are based on the biological conversion of alpha-linolenic acid and linoleic acid to long-chain unsaturated fatty acids (LCUFAs) containing docosahexaenoic acid and ARA, respectively [4]. Therefore, substitute approaches are used for introducing these useful LCUFAs in IF products [5, 6]. In recent years, the preparation of SLs containing functional LCUFAs in the *sn*-2 position, such as ARA and medium-chain fatty acids (MCFAs), at *sn*-1,3 positions has attracted a lot of attention. LCUFAs' existence at the *sn*-2 position enhances the absorption of monoacylglycerols (2-MAG) [7–9]. ARA is one of the EFAs that exists in human milk but usually not found in IF products. ARA is necessary for the normal growth and function of the brain and retina in infants. Furthermore, ARA constitutes one of the major fatty acids (FAs) in brain, assisting in the protection and development of nervous system functions [3, 10].

Although most conventional IFs are frequently enhanced with lauric acid as a source of MCFAs, the studies on the relationship between the metabolic fate of different kinds of FAs and the chain length have brought misconception about its classification. To this end, some researchers have considered lauric acid as a long-chain fatty acid (LCFA) instead of MCFA [11, 12]. Previous studies recommended the use of capric and caprylic acids as a source of MCFAs in IF products due to their functional and nutritional effects [13, 14]. Medium- and long-chain triacylglycerols (MLCTs) are defined as a type of SLs where the individual triacylglycerol (TAG) molecule consists of both LCFAs and MCFAs attached on the same glycerol molecule [15]. MLCTs are present in food products, such as Resetta™ (Nisshin Oillio Group Ltd., Japan), that have several physiological benefits, like cholesterol maintenance capability and anti-obesity effects [16]. Moreover, MLCTs have the superior ability to provide the body with quick energy and EFAs; hence, they are very beneficial for enhancing human health, especially for infants [17].

Enzymatic interesterification is one of the methods utilized in the production of MLCTs and modification of fats and oils properties for possible IF applications and some other purposes too [18]. Despite the health benefits that MLCTs possess, the incorporation of MLCTs into IFs as a lipid ingredient is a challenge, as these lipid components are prone to oxidation and evolution of unpleasant flavor volatiles affect sensory characteristics [6, 19]. Recently, the late addition of lipid ingredients that have LCUFAs during IF production has been reported to reduce the oxidation of lipids [3]. During the production of powdered IF, SLs containing ARA as LCUFAs can be added after the spray-drying procedure as a microencapsulated powder using a traditional blender [20]. For the microencapsulation of SLs used in IFs, food-grade components appropriate for infants and natural constituents are preferred. Whey proteins are among the milk constituents that improve the nutritional value of IFs. They are widely used as an encapsulating agent in the food industry for emulsifying, building viscosity, and gel formation. Additionally, whey proteins have been used in combination with carbohydrates, such as inulin (IN), to stabilize spray-dried emulsions [21, 22]. IN is a natural source of carbohydrates that is obtained from chicory roots, yacon tubers, wheat, asparagus, Jerusalem artichoke, and onions [23]. IN from chicory roots is available in the market and is used as a refined food ingredient, because of its nutritive

value and other superior properties [24]. IN can also be used as a fat replacer, texture modifier, encapsulation agent, and emulsion stabilizer because of its water-holding capability [25]. IN has many beneficial effects on human health, such as the promotion of gastrointestinal tract function, improvement of minerals absorption, and reduction of appetite modulation [26]. Furthermore, IN is considered as one of the food-grade oligosaccharides for the provision of prebiotics [27]. Previous in vitro and in vivo studies demonstrated that the microencapsulation of oil can protect it from stomach acidic conditions for the delivery and release of the oil into the small intestine. Moreover, it also maintains the bioavailability of the oil [28, 29].

In our previous work [30, 31], a novel SL enriched with ARA and MCFAs was synthesized and encapsulated with different wall materials. The objective of this study was to evaluate the application of a potential novel functional SL as a lipid ingredient in the production of IFs. To identify the relation between SLs enriched with MLCTs and the oil extracted from two commercial IFs samples, the compositions of FAs, *sn*-2 FAs position, and TAG species were determined. In addition, the thermal behaviors of the three oil samples were evaluated using differential scanning calorimetry (DSC). Moreover, UPLC-Q-TOF-MS was utilized as a rapid method to comprehensively quantify TAG species. Powdered IFs with a novel functional SL were prepared by two manufacturing approaches. Later, the quality of powdered IFs was evaluated in terms of oxidative stability (peroxide value), moisture content, water activity, and color. The study has potential applications in the development of IF with improved properties.

Materials and methods

Materials

Purified SLs enriched with MLCTs were produced in our previous studies through the interesterification reaction between ARA-rich single-cell oil with medium-chain triacylglycerols in a solvent-free system using Lipozyme 435 lipase [30, 31]. Two samples of commercial IFs from different companies were used; the first sample CIFL (1) was purchased from China and the second sample CIFL (2) was obtained from the USA. A standard mixture of fatty acids methyl ester (FAMEs), porcine pancreatic lipase (1000–4000 U g^{-1}), and Cumene hydroperoxide standard (80% purity) were obtained from Sigma-Aldrich Chemical Co., Ltd. (Shanghai, China). Silica gel thin-layer chromatography plates (10 × 20 cm; 60 Å mean the diameter of the pore, 2–25 µm mean the size of the particle, 500 µm thickness, with dichlorofluorescein) were bought from Shanghai Shangbang Decoration Materials Co., Ltd. (Shanghai, China). Methanol, chloroform, acetonitrile, isopropanol, and n-hexane used for ultra-performance liquid chromatography (UPLC) analysis were chromatographic pure from Fisher (Fair Lawn, NJ, USA). Whey protein isolate (WPI, BiPro, ~ 92%) was purchased from Davisco Foods International Inc. (Le Sueur, Minnesota, USA). Inulin (IN, degree of polymerization ≥ 23) was obtained from Orafti®HP, BENEO-Orafti. Maltodextrin and lactose were purchased from Xiwang International Trade Co. Ltd., China. Non-fat dry milk was bought from Globe-milk B.V., Holland. Micronutrient premix mainly consisting of vitamins and minerals (Table 1) was obtained from Usp Co., Ltd. (Headquarters Rockville, Maryland, USA). All other organic solvents and chemicals used for extraction and analysis were of analytical and chromatographic grade.

Extraction process of total oil

Total oil was extracted from commercial IF samples according to Floch [32]. Commercial IF samples (16 g) were dissolved in 200 mL mixture of chloroform/methanol (2:1, v/v). The mixture was then shaken for 15 min and centrifuged for 10 min at 4500 rpm. The organic phase that contained the total lipids was collected in separation funnel and then equilibrated by mixing with a one-fourth volume of a saline sodium chloride solution (0.86%, w/w). The lower chloroform layer was separated, filtered, and evaporated at 40 °C under reduced pressure. The resultant total oils were flushed with nitrogen and stored at − 20 °C until further analysis.

The physicochemical properties of a novel SL and infant formula oil

FAs composition analysis

The FAs composition of the purified SLs enriched with MLCTs and the oil extracted from commercial IF samples was analyzed as FAMEs following the procedure reported by Pande et al. [33]. FAMEs were identified and quantified using gas chromatography (Agilent 7820A) equipped with a flame ionization detector and a Trace TR-FAME capillary column (60 m × 0.25 mm × 0.25 µm, Thermo Fisher, USA). The column was initially maintained at 60 °C for 3 min, followed by temperature programming to 175 °C at the rate of 5 °C/min, then held at 175 °C for 15 min, and then raised by 2 °C/min to a final temperature of 220 °C for 10 min. The temperature of the injector and the detector was set at 250 °C. Nitrogen was used as a carrier gas with a flow rate of 1.2 mL/min, a split ratio of 1:100; detector gas 30 mL/min hydrogen, 400 mL/min air and 25 mL/min nitrogen. The running time of each sample was set at 73.5 min. FAMEs were identified by comparing the retention times of sample peaks with the corresponding known standards. The relative

Table 1 Composition of the micronutrient premix

Vitamins	Level[a]	Minerals	Level[a]	Others	Level[a]
Vitamin A (as beta-carotene)	2500 IU	Calcium (as di-calcium phosphate &calcium carbonate)	220 mg	Lycopene	300 µg
Vitamin C (as ascorbic acid)	90 mg	Phosphorous (as anhydrous di-potassium phosphate)	110 mg	Lutein	250 µg
Vitamin D3 (as cholecalciferol)	500 IU	Iodine (as potassium iodide)	150 µg		
Vitamin E (as DL-α-tocopheryl acetate)	50 IU	Magnesium (as magnesium oxide)	50 mg		
Vitamin K (as phytonadione)	30 µg	Zinc (as zinc oxide)	11 mg		
Vitamin B1(as thiamin mono-nitrate)	1.5 mg	Selenium (as sodium selenate)	55 µg		
Vitamin B2 (as riboflavin)	1.7 mg	Copper (as anhydrous copper sulphate)	0.9 mg		
Niacin (as niacinamide)	20 mg	Manganese (as manganese sulfate)	2.3 mg		
Vitamin B6 (as pyridoxine hydrochloride)	3 mg	Chromium (as chromium picolinate)	45 µg		
Pantothenic acid (as calcium dpantothenate)	10 mg	Molybdenum (as sodium molybdate)	45 µg		
Vitamin B12 (as cyanocobalamin)	25 µg	Chloride (as potassium chloride)	72 mg		
Biotin	30 µg	Potassium (as anhydrous di-potassium phosphate)	80 mg		
Folic acid	500 µg	Silicon (as silicon dioxide)	2 mg		
		Nickel (as nickelous sulfate)	5 µg		
		Boron (as sodium borate)	150 µg		
		Vanadium (as sodium metavanadate)	10 µg		

[a] Unit per 1.5 g

contents were expressed as weight percentage (%, w/w) and then calculated. Analyses were performed in triplicate.

Sn-2 FAs composition analysis

Pancreatic lipase catalysis was used to determine FAs composition at the *sn*-2 position of the purified SLs enriched with MLCTs and the oil extracted from commercial IF samples by means of the method described by Sun et al. [34]. Briefly, 1 mL of 1 M Tris–HCl buffer (pH 8.0), 0.25 mL of 0.05% bile salts, 0.1 mL of 2.2% $CaCl_2$, and 20 mg of pancreatic lipase were mixed with each sample (0.1 g). The mixture was placed in a water bath at 40 °C for 3 min with shaking (three times), and 1 mL of 6 M HCl solution and 2 mL of diethyl ether were added and centrifuged for 5 min at 4500 rpm. After that, the upper layer was collected, and diethyl ether was dried by anhydrous sodium sulfate and evaporated under nitrogen to 0.5 mL. The hydrolysis products were then extracted and separated by silica gel thin-layer chromatography plates with a developing solvent system of hexane/diethyl ether/acetic acid (50:50:1, v/v/v). The 2-MAG band was located by viewing under ultraviolet light. Finally, the band corresponding to 2-MAG was scraped off for methylation, and FAs composition was analyzed using gas chromatography as previously described. Analyses for each sample were repeated three times.

Separation and determination of TAG species

TAG species of the purified SLs enriched with MLCTs and the oil extracted from commercial IF samples were identified using an UPLC system (Waters, Milford, Massachusetts, USA) equipped with a BEH C18 analytical column (i.d. 2.1 × 50 mm, 1.9 µm) following the procedure reported by Ali et al. [35]. The temperatures of the column and the sample chamber were set at 45 and 20 °C, respectively. The injection volume of each analysis was 1.0 µL with a concentration of 1 mg/mL. The separation of TAG species from the samples was completed using acetonitrile/isopropanol (1:9, v/v) as a mobile phase (A) and acetonitrile/water (4:6, v/v) as a mobile phase (B). Ammonium acetate (10 mM) was added to the two mobile phases as an electrolyte and the flow rate was set at 300 µL/min. Complete separation of TAG species from the samples was performed with a binary gradient started with 70% of phase (A) and held for 1 min, then raised to 87% for 30 min and held also for 1 min, and then returned to the initial rate of 70% for 1 min and equilibrated for 4 min. Before the beginning of the next analysis, the column was cleared with 5% of the mobile phase (A) for 5 min.

A Q-TOF-MS instrument (Waters, Milford, Massachusetts, USA) with electrospray ionization positive ion mode was applied for TAGs identification and quantification. The optimal conditions of positive ion mode were set as follows: source temperature (400 °C), capillary voltage (3.5 kV), cone voltage (30 V), collision gas (argon), desolvation gas (nitrogen), and flow rate (700 L/h). Full scan spectra were detected for 0.5 s scan duration in the range of m/z 200–1500. The collision energy was set as 5 eV and 20–50 V ramp for low-energy and high-energy scans, respectively. Mass-Lynx software (4.1, Waters) was used to analyze the raw obtained data.

Melting and crystallization profiles

The melting and crystallization properties of the purified SLs enriched with MLCTs and the oil extracted from commercial IF samples were carried on a DSC (Q2000 V4.7A Build 121, TA Instruments, New Castle, DE, USA) equipped with a refrigerated cooling system according to the method reported by Korma et al. [30]. Indium, eicosane, and dodecane standards were used to perform the calibration before the analysis. 5–8 mg of each sample was weighed in 30 µL aluminum pans and sealed hermetically. An empty pan was used as a reference during the measurements. The sample was heated from 25 to 80 °C at a rate of 50 °C/min and kept at 80 °C for 10 min (to destroy any previous crystalline structure), then decreased to − 65 °C at a rate of 10 °C/min and held at − 65 °C for 10 min (for crystallization profiles), and finally heated to 80 °C at a rate of 5 °C/min (for melting profiles). Nitrogen gas was used to purge the system at 20 mL/min during the analysis. Thermal solutions software (TA Instruments) was used to analyze the melting and crystallization profiles.

IFs preparation

Usually, IFs are produced using fat, protein, carbohydrate, and micronutrients premix (minerals and vitamins) to mimic the composition of a healthy mother's breast milk [36, 37]. Purified SLs enriched with MLCT-containing IFs were formulated using two common manufacturing methods: (A) a wet-blending/ spray-drying process and (B) a dry-mixing process. In the wet-mixing/spray-drying process, constituents are mixed together, homogenized, pasteurized, and spray-dried to produce a powdered product as follows: Nonfat dry milk (20 g), WPI (10 g), maltodextrin (30 g), lactose (31 g), and water (800 mL) were mixed at 50–60 °C by stirring mildly and allowed to hydration for 30 min prior addition of SLs enriched with MLCTs (30 g) and micronutrients premix (3.9 g). The mixture was then mixed using a high-speed homogenizer (Ultra-Turrax IKA T18 basic, Wilmington, NC, USA) at 22,000 rpm for 4 min at room temperature, then passed through an ATS AH2100 high-pressure homogenizer (AH- 2010, ATS Engineering Inc., Canada) operated at 40 MPa with 4 processing cycles. After that, the mixture was pasteurized at 65 °C for 30 min, and then spray-dried by a GEA Niro spray dryer (model Mobile MinorTM, Soborg, Denmark), using inlet and outlet temperatures of (180 ± 5 °C) and (80 ± 5 °C), respectively. The airflow rate set at 300 NL min^{-1} and the mixture was fed into the drying chamber at the feed flow rate of 14–15 mL min^{-1} using a peristaltic pump. The dried powder was collected and stored in sealed plastic bags at − 20 °C for further analysis.

In the dry-mixing process, before the mixing step between the ingredients as a dehydrated powdered, SLs enriched with MLCTs were encapsulated by the composition of WPI with IN (1:1), since it was the best combination as a wall material as described in our previous study [31]. The microencapsulated SLs enriched with MLCTs (85 g) were then dry-mixed with the constituents recorded above except for water. The influences of these two methods on product quality were evaluated and compared to two products of commercial IFs.

Characterization of powdered IFs

Determination of peroxide value

The peroxide value of powdered IF samples was determined using a method recommended by Sharif et al. [38] with minor modifications. 0.5 g of each powdered sample was reconstituted in 5 mL purified water and stirred for rehydration. An amount of 1 mL of rehydrated emulsion sample was taken into a 10 mL glass tube. Then, 5 mL of chloroform/methanol (2:1 v/v) was added. The obtained mixture was vortexed three times (10 s each) followed by centrifugation at 2000 rpm for 4 min. The organic solvent phase (0.2 mL) was added to 2.8 mL of chloroform/methanol (2:1 v/v), followed by 15 µL of ammonium thiocyanate solution (3.94 M), and 15 µL of ferrous iron solution. The final mixture was vortexed again, and the absorbance of the samples was measured at 510 nm after kept the samples in dark at room temperature for 20 min. Lipid hydroperoxide concentrations were determined from a Cumene hydroperoxide standard curve [Standard curve eq. $Y = 0.2808\,X + 0.0301$ and coefficient correlation (R^2) = 0.9983]. The analysis was repeated three times.

Determination of moisture content and water activity

The moisture content and water activity were determined by means of the method described by Korma et al. [31]. The moisture content of powdered IF samples was measured using digital moisture balances (Moisture analyzing Lab Equipment, Ohaus). While, the water activity was determined by the Novasina water activity meter (Labswift-aw, Novasina AG, Lachen, Switzerland) at 25 °C. Time of analysis was different from one sample to another and results were recorded after reaching the equilibrium. Samples were placed in the sample dish in a thin layer during the analysis, triplicate for each sample.

Determination of Hunter color values

The color measurements of powdered IF samples were detected using a Colorimeter (Minolta Company Ltd., Osaka, Japan). The findings of L^*, a^*, and b^* were expressed as (lightness), (red to green), and (yellow to blue), respectively. According to Ahmed et al. [39], the values of Chroma (C^*)

and hue angle (h^*) were calculated from a^* and b^* values. The mathematic C^* and h^* are defined as $C^* = [a^{*2} + b^{*2}]^{1/2}$ and $h^* = \tan^{-1}[b^*/a^*]$. Color change (ΔE) was calculated as $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. Prior to the analysis, the instrument was standardized with a white reference tile, triplicate for each sample.

Data analysis

The experimental data were evaluated by one-way analysis of variance (ANOVA) using statistical analysis software (version 6.4, CoStat, Monterey, CA, USA). The findings were stated as mean ± standard deviation. The P values of less than 0.05 were considered statistically significant for the results.

Results and discussion

Composition of FAs

The FA profile of the purified SLs and extracted fat from CIFL (1) and CIFL (2) are presented in Table 2. In the synthesized SLs, the most abundant FAs were ARA (34.46%), caprylic (17.33%), capric (12.69%), and lignoceric (8.16%) acids. The percentages of LCUFAs, LCFAs, and MCFAs were 49.42%, 20.56%, and 30.01%, respectively. The synthesized SLs had high contents of ARA and MCFAs on the same glycerol molecules, and it would provide advantages in the medical field with various therapeutic approaches and also help to maintain the nutritional requirements for the health [18, 40]. In CIFL (1), the most abundant FA was oleic acid (35.16%), followed by linoleic (23.05%), palmitic (14.53%), and lauric (6.09%) acids. The percentages of LCUFAs, LCFAs, MCFAs, and SCFAs were 61.66, 25.31, 8.37, and 4.66%, respectively. The major FA in CIFL (2)

Table 2 FAs composition (%) of structured lipids (SLs) enriched with medium- and long-chain triacylglycerols (MLCTs) compared to fat extracted from commercial infant formulas (CIFLs)

Fatty acids	SLs enriched with MLCTs			CIFL (1) [a]			CIFL (2) [b]		
	Total	*sn*-2	*sn*-1, 3 [c]	Total	*sn*-2	*sn*-1, 3	Total	*sn*-2	*sn*-1, 3
C4:0	ND	ND	ND	4.66 ± 0.38	ND	6.99 ± 0.27	ND	ND	ND
C8:0	17.33 ± 0.07	6.92 ± 1.30	22.53 ± 0.71	1.04 ± 0.18	2.76 ± 0.07	0.18 ± 0.02	1.46 ± 0.22	0.97 ± 0.02	1.71 ± 0.33
C10:0	12.69 ± 0.12	10.47 ± 0.71	13.8 ± 0.38	1.24 ± 0.07	2.73 ± 0.03	0.50 ± 0.08	1.32 ± 0.14	1.62 ± 0.04	1.17 ± 0.21
C12:0	ND	ND	ND	6.09 ± 0.04	16.17 ± 0.01	1.05 ± 0.19	12.79 ± 0.14	35.28 ± 0.18	1.55 ± 0.22
C14:0	0.31 ± 0.00	0.24 ± 0.02	0.34 ± 0.01	4.94 ± 0.03	8.20 ± 0.12	3.31 ± 0.51	5.53 ± 0.02	3.58 ± 0.01	6.51 ± 0.03
C16:0	5.47 ± 0.00	5.55 ± 0.27	5.43 ± 0.13	14.53 ± 0.25	15.78 ± 0.27	13.91 ± 0.27	8.30 ± 0.11	8.68 ± 0.08	8.12 ± 0.16
C18:0	4.57 ± 0.02	5.28 ± 0.27	4.21 ± 0.12	5.65 ± 0.15	5.06 ± 0.08	5.95 ± 0.23	3.86 ± 0.10	10.38 ± 0.31	0.61 ± 0.15
C18:1 *n*-9	4.08 ± 0.01	6.05 ± 0.16	3.09 ± 0.08	35.16 ± 0.38	20.43 ± 0.01	42.53 ± 0.47	42.68 ± 0.29	17.6 ± 0.45	55.22 ± 0.43
C18:2 *n*-6	5.20 ± 0.01	7.84 ± 0.12	3.88 ± 0.05	23.05 ± 0.11	24.58 ± 0.51	22.29 ± 0.12	20.28 ± 0.35	17.23 ± 0.82	21.80 ± 0.53
C18:3 *n*-3	1.81 ± 0.02	2.53 ± 0.03	1.46 ± 0.01	2.85 ± 0.17	3.58 ± 0.06	2.49 ± 0.43	1.97 ± 0.12	1.86 ± 0.04	2.03 ± 0.19
C20:1 *n*-9	ND	ND	ND	0.60 ± 0.17	0.70 ± 0.02	0.55 ± 0.04	1.33 ± 0.08	1.68 ± 0.01	1.16 ± 0.12
C20:3 *n*-6	3.64 ± 0.02	6.52 ± 0.15	2.19 ± 0.10	ND	ND	ND	ND	ND	ND
C20:4 *n*-6	34.46 ± 0.09	42.82 ± 0.89	30.28 ± 0.47	ND	ND	ND	ND	ND	ND
C20:5 *n*-3	0.24 ± 0.00	0.15 ± 0.02	0.28 ± 0.01	ND	ND	ND	ND	ND	ND
C22:0	2.06 ± 0.01	ND	3.09 ± 0.02	0.19 ± 0.06	ND	0.29 ± 0.03	0.47 ± 0.37	1.12 ± 0.04	0.33 ± 0.33
C24:0	8.16 ± 0.03	5.62 ± 0.23	9.43 ± 0.15	ND	ND	ND	ND	ND	ND
∑SCFAs	ND	ND	ND	4.66 ± 0.38	ND	6.99 ± 0.27	ND	ND	ND
∑MCFAs	30.01 ± 0.17	17.39 ± 2.01	36.33 ± 1.05	8.37 ± 0.29	21.66 ± 0.11	1.73 ± 0.29	15.57 ± 0.5	37.87 ± 0.24	4.43 ± 0.76
∑LCFAs	20.56 ± 0.04	16.70 ± 0.72	22.50 ± 0.39	25.31 ± 0.49	29.04 ± 0.47	23.46 ± 1.04	18.16 ± 0.6	23.76 ± 0.44	15.57 ± 0.67
∑LCUFAs	49.42 ± 0.13	65.91 ± 1.32	41.18 ± 0.69	61.66 ± 0.83	49.29 ± 0.6	67.86 ± 1.06	66.26 ± 0.84	38.37 ± 1.32	80.21 ± 1.27

[a]CIFL (1): fat extracted from a commercial infant formula available in China

[b]CIFL (2): fat extracted from a commercial infant formula available in the USA

[c]Fatty acid composition at *sn*-1,3 positions was calculated as (3 × total FA - *sn*-2)/2

ND Not detected; *SCFAs* short chain fatty acids; *MCFAs* medium-chain fatty acids; *LCFAs* long-chain fatty acids; *LCUFAs* long-chain unsaturated fatty acids

FAs data of SLs enriched with MLCTs were cited and reorganized from the results reported by Korma et al. [30]

was oleic acid (42.68%), followed by linoleic (20.28%), lauric (12.79%), and palmitic (8.30%) acids. The percentages of LCUFAs, LCFAs, and MCFAs were making up 66.26, 18.16, and 15.57%, respectively. The high ratios of beneficial LCUFAs are largely necessary for human nutrition. In previous study, it was discovered that LCUFA-rich oils have the ability to avoid several human diseases, such as cardiovascular diseases [41]. The differences in FA percentages of the commercial IFs, CIFL (1) and CIFL (2), were made be due to the variances in sources of plant oils blend used in the composition of the fat product.

Positional distribution of FAs

The FAs distribution in oils acts a crucial role in their availability and functions. Thus, its study has attracted great attention in human nutrition. The absorption, distribution, and metabolism of TAGs are linked to the FAs location. FAs emitted from *sn*-1,3 positions have been known to have different metabolic destinies than those at the *sn*-2 position [42]. The positional distribution of FAs on the glycerol backbone of SLs, CIFL (1), and CIFL (2) samples is displayed in Table 2. The findings of this study showed that ARA (42.82%) was the most abundant FA in SLs that occupied the *sn*-2 position followed by capric (10.47%) and linoleic (7.84%) acids. In CIFL (1), the predominant FA-occupied *sn*-2 position was linoleic acid (24.58%), followed by oleic (20.43%), lauric (16.17%), and palmitic (15.78%) acids. While in CIFL (2), lauric, oleic, linoleic, stearic, and palmitic acids were the major FAs occupied in the *sn*-2 position accounted for 35.28, 17.6, 17.23, 10.38, and 8.68%, respectively. In previous studies, suggested that ARA-enriched oils at the *sn*-2 position show the best intestinal absorption conditions with various benefits in clinical nutrition [42, 43]. Moreover, higher levels of ARA were detected in the brain of newborn rats fed with ARA-enriched oils at the *sn*-2 position rather than randomly distributed [44]. Most commercial IFs are based on the biological conversion of linoleic acid to ARA, but the ratios for these conversions are limited [4]. Additionally, experts in the field of infant nutrition recommend that formulas for infants should provide ARA [45]. Therefore, the synthesized SLs would be a substitute approach to use ARA directly in IF.

At *sn*-1,3 positions of synthesized SLs, ARA, caprylic, capric, and lignoceric acids were the most abundant FAs calculated for 30.28, 22.53, 13.8, and 9.43%, respectively. While at the *sn*-1,3 positions of both commercial IFs, CIFL (1) and CIFL (2), the oleic, linoleic, and palmitic acids were the most abundant FAs accounted for (42.53, 22.29, and 13.91%) and (55.22, 21.8, and 8.12%), respectively. SLs contained higher amounts of MCFAs (caprylic and capric) at the *sn*-1,3 positions and LCUFAs (particularly ARA) at the *sn*-2 position, i.e., making them new and excellent fat substrates for the addition in IFs as a compared with commercial IFs. Additionally, the replacement of LCFAs by MCFAs at the *sn*-1,3 positions might yield SLs with functional characteristics for infants, including rapid energy provision, protection from harmful microorganisms, and improve fat absorption and melting point [46, 47]. Caprylic acid and capric acid were mostly found at the *sn*-1,3 positions in IFs and in mature human milk, except capric acid in IFs prepared using cow milk and goat milk was similarly distributed between *sn*-2 and *sn*-1,3 positions [34]. Numerous studies have used ARA-SCO from *Mortierella alpine* and MCFAs as substrates to produce SLs enriched with MCFAs at *sn*-1,3 positions and ARA at the *sn*-2 position for the potential functional application in IFs [8, 30, 48, 49]. Moreover, FAO/WHO recommended that the formulation of IF should be enhanced with ARA [50]. Hence, the obtained SLs (SLs enriched with MLCTs), containing 42.82% ARA at the *sn*-2 position and 36.33% of MCFAs at the *sn*-1,3 positions, might have potential functional applications in the dairy industry, particularly in IFs.

Profiling of TAGs molecular species composition

The identification of TAGs is one of the characteristics that is used to know the structural and functional features of the final oil products [51]. The parameters of the chromatographic technique were optimized to get the best elution conditions for better separation of TAGs molecular species. The ammoniated TAG ion $(M+NH_4)^+$ was identified as the precursor m/z in electro spray ionization positive mode. Figure 1 shows the UPLC-Q-TOF-MS chromatograms of SL, CIFL (1), and CIFL (2). Table 3 displays the relative percent of TAG ions relative to the total percent of $(TAG+NH_4)^+$ in the three lipid samples. In the SLs enriched with MLCTs, a total of 45 different TAG species were detected. The most abundant TAG species were those of m/z 808.62, m/z 872.74, m/z 676.49, m/z 900.75, m/z 788.72, m/z 968.77, m/z 836.63, m/z 648.48, m/z 740.71, m/z 628.48, and m/z 704.6 with the relative contents 6.44%, 6.39%, 5.68%, 4.36%, 4.01%, 3.44%, 3.36%, 3.33%, 3.27%, 3.16%, and 3.05%, respectively. These findings showed that the types of MLCTs obtained contain a structure (MLM, MML, LMM, LLM, LML, and MLL) with caprylic and capric acids as the MCFAs and ARA as LCUFAs. It might allow appropriate conditions for digestion and absorption. This unique phenomenon makes it more beneficial for human health, particularly for infants [18]. A total of 38 TAG species were identified in CIFL (1). As can be observed, (18:2–18:2–18:2), (18:1–18:2–18:1), (18:1–18:1–16:0), (18:1–18:2–16:0), (18:1–18:3–16:0), (18:0–18:1–18:1), and (12:0–10:0–14:0) were the major TAG molecular species making up 11.88%, 11.70%, 9.43%, 8.62%, 8.23%, 5.02%, and 4.56%, respectively. While twenty-five different TAG species were found

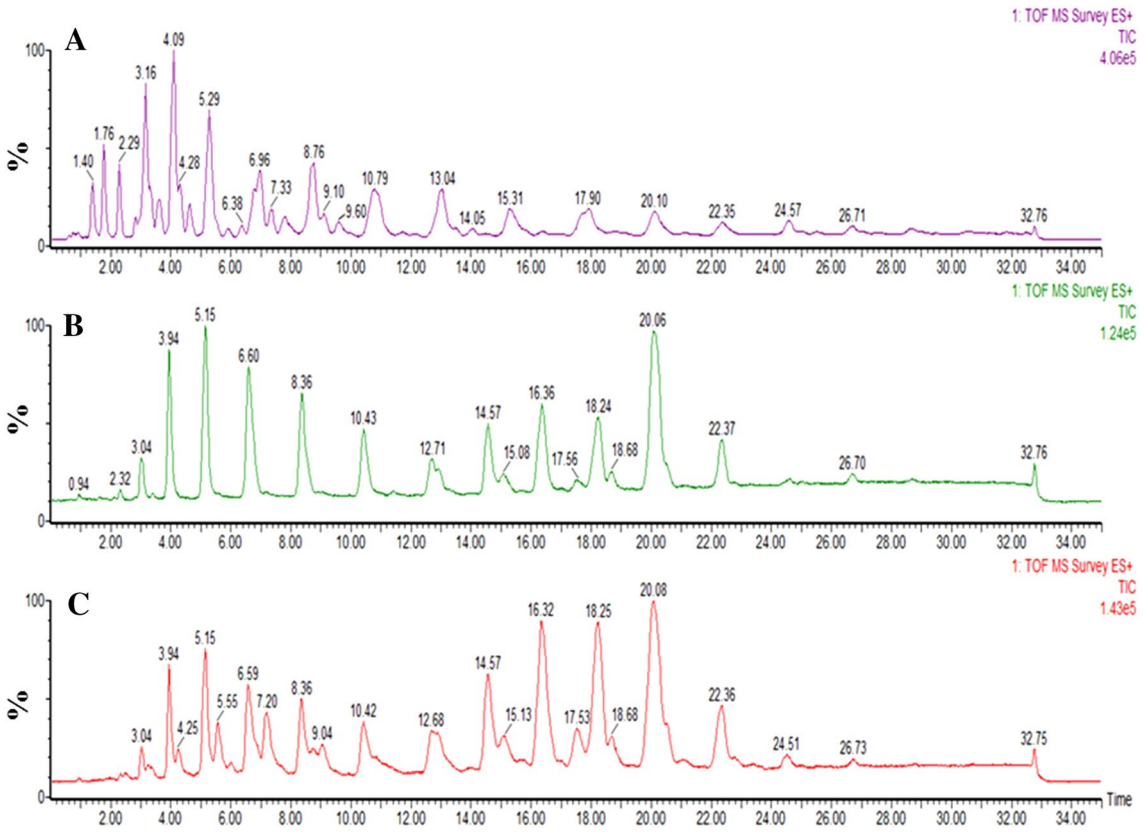

Fig.1 Total ion chromatograms of TAGs in SLs enriched with MLCTs (**a**), fat extracted from a commercial infant formula available in China (**b**), and fat extracted from a commercial infant formula available in the USA (**c**) analyzed by UPLC-Q-TOF–MS in the positive mode

in CIFL (2), and the majority included (18:1–18:2–18:1), (12:0–14:0–10:0), (12:0–12:0–10:0), (18:1–16:0–18:1), (18:0–18:0–18:2), (18:2–18:2–16:0), (12:0–12:0–14:0), (18:1–16:0–18:2), and (12:0–12:0–8:0/12:0–10:0–10:0) accounted for 11.43%, 11.24%, 9.45%, 8.89%, 8.85%, 8.27%, 7.38%, 6.65%, and 6.24%, respectively. Most IFs are usually enriched with oleic, linoleic, and palmitic acids [3, 52, 53]. But, because of the lower level of lipase in newborns, the starter IF was formed with medium-chain triacylglycerols to provide energy directly [54]. Therefore, it was a great relevance to obtain SLs containing EFAs and MCFAs with easy absorption features for infants. Later, Tu et al. [54] reported that TAG species with MCFAs are present in human milk which make attention to the researchers to incorporate MCFAs into the IF due to its health's beneficial effect [18]. There were variances in TAG species (particularly ECN ≤ 40) between the three lipid formulas due to the presence of MCFAs. Similar results were discovered in commercial IFs specially prepared using goat and cow milks [53, 54]. As we know, MCFAs are absorbed more easily than LCFAs by newborns, which can be easily oxidized and produce energy for newborns [18]. So, in general, the medium-chain triacylglycerols are added into formulas for infants with malabsorption and premature infants. Furthermore, some studies revealed that SLs with MCFAs at the *sn*-1,3 positions and EFAs / LCUFAs at the *sn*-2 position could increase absorption of EFAs and LCUFAs in cystic fibrosis syndromes and malabsorption [55, 56].

Melting and crystallization profiles

The melting and crystallization profiles of SLs enriched with MLCTs compared to the fat extracted from CIFL (1) and CIFL (2) are shown, respectively, in Fig. 2a, b. The melting and crystallization points are important to know fats/oils physical conditions inside the human body. The melting and crystallization thermograms of fats/oils are linked to both of FAs and TAGs types. The thermograms of SLs enriched with MLCTs, CIFL (1), and CIFL (2) revealed multiple peaks, representing the complexity of the TAGs distribution. The SLs enriched with MLCTs presented three melting peaks at − 21.24 °C, 11.98 °C, and 29.04 °C and three crystallization peaks at − 40.37 °C, − 0.72 °C, and 24.07 °C. As can be seen, the SLs enriched with MLCTs revealed low melting and crystallization temperatures. This reduction in melting and crystallization temperatures of SLs enriched with MLCTs may have happened due to the high content of medium chains and unsaturated FAs [47]. The vegetable oil

Table 3 TAG molecular species and relative percent (%) in structured lipids (SLs) enriched with medium- and long-chain triacylglycerols (MLCTs) compared to fat extracted from commercial infant formulas (CIFLs) determined by UPLC-Q-TOF–MS

SLs enriched with MLCTs						CIFL (1)[a]						CIFL (2)[b]					
t_R[c] (min)	TAG species	Formula	M+NH_4[d]	ECN[e] (DB)	Content (%)	t_R (min)	TAG species	Formula	M+NH_4	ECN (DB)	Content (%)	t_R (min)	TAG species	Formula	M+NH_4	ECN (DB)	Content (%)
2.252	10:0-10:0-8:0	$C_{31}H_{58}O_6$	544.46	28	1.53	3.891	12:0–8:0–12:0	$C_{35}H_{66}O_6$	600.47	32	2.09	3.891	12:0–12:0–8:0, 12:0–10:0–10:0	$C_{35}H_{66}O_6$	600.47	32	6.24
3.172	8:0–20:4–8:0	$C_{39}H_{66}O_6$	648.48	28	3.33	5.051	12:0–10:0–12:0	$C_{37}H_{70}O_6$	628.48	34	3.50	5.051	12:0–12:0–10:0	$C_{37}H_{70}O_6$	628.48	34	9.45
3.258	8:0–18:2–8:0	$C_{37}H_{66}O_6$	624.48	30	1.97	5.511	16:0–14:0–4:0	$C_{37}H_{70}O_6$	628.48	34	0.49	6.501	12:0–14:0–10:0	$C_{39}H_{74}O_6$	656.58	36	11.24
3.53	8:0–20:3–8:0	$C_{39}H_{68}O_6$	650.48	30	1.59	5.613	18:1–14:0–4:0	$C_{39}H_{72}O_6$	654.58	34	0.62	6.808	18:1–12:0–8:0	$C_{41}H_{76}O_6$	682.59	36	0.54
3.957	10:0–20:4–8:0	$C_{41}H_{70}O_6$	676.49	30	5.68	6.467	12:0–10:0–14:0	$C_{39}H_{74}O_6$	656.58	36	4.56	8.259	12:0–12:0–14:0	$C_{41}H_{78}O_6$	684.59	38	7.38
4.076	8:0–18:1–8:0	$C_{37}H_{68}O_6$	626.48	32	1.40	6.790	18:1–8:0–12:0	$C_{41}H_{76}O_6$	682.59	36	0.20	10.324	14:0–14:0–12:0, 16:0–12:0–12:0	$C_{43}H_{82}O_6$	712.6	40	3.45
4.212	10:0–18:2–8:0	$C_{39}H_{70}O_6$	652.48	32	1.92	8.240	12:0–12:0–14:0	$C_{41}H_{78}O_6$	684.59	38	2.97	10.494	18:1–12:0–12:0	$C_{45}H_{84}O_6$	738.6	40	1.05
4.570	10:0–20:3–8:0	$C_{41}H_{72}O_6$	678.59	32	1.27	8.547	18:1–12:0–10:0	$C_{43}H_{80}O_6$	710.6	38	1.12	11.399	18:3–18:3–18:2	$C_{57}H_{94}O_6$	892.75	38	0.64
5.184	10:0–20:4–10:0	$C_{43}H_{74}O_6$	704.6	32	3.05	9.007	18:0–16:0–4:0	$C_{41}H_{78}O_6$	684.59	38	0.15	12.559	14:0–12:0–16:0, 18:0–12:0–12:0	$C_{45}H_{86}O_6$	740.61	42	1.87
5.201	10:0–16:0–8:0	$C_{37}H_{70}O_6$	628.48	34	3.16	10.304	12:0–12:0–16:0	$C_{43}H_{82}O_6$	712.6	40	1.47	12.764	18:1–14:0–12:0	$C_{47}H_{88}O_6$	766.71	42	0.79

Table 3 (continued)

SLs enriched with MLCTs						CIFL (1)[a]						CIFL (2)[b]					
t_R[c] (min)	TAG species	Formula	M+NH_4[d]	ECN[e] (DB)	Content (%)	t_R (min)	TAG species	Formula	M+NH_4	ECN (DB)	Content (%)	t_R (min)	TAG species	Formula	M+NH_4	ECN (DB)	Content (%)
5.269	10:0–18:1–8:0	$C_{39}H_{72}O_6$	654.58	34	2.4	10.474	12:0–18:1–12:0	$C_{45}H_{84}O_6$	738.6	40	1.00	14.538	18:2–16:0–18:3	$C_{55}H_{96}O_6$	870.74	42	1.56
6.258	18:3–20:4–8:0	$C_{49}H_{80}O_6$	782.62	32	0.90	11.327	18:3–18:3–18:2	$C_{57}H_{94}O_6$	892.75	38	0.42	14.998	16:0–14:0–14:0, 18:0–14:0–12:0	$C_{47}H_{90}O_6$	768.71	44	0.82
6.616	10:0–16:0–10:0	$C_{39}H_{74}O_6$	656.58	36	1.82	12.539	14:0–16:0–10:0	$C_{43}H_{82}O_6$	740.61	40	1.36	15.101	18:1–16:0–12:0	$C_{49}H_{92}O_6$	794.72	44	0.83
6.753	20:4–20:4–8:0	$C_{51}H_{82}O_6$	808.62	32	6.44	12.692	18:1–14:0–12:0	$C_{47}H_{88}O_6$	766.71	42	0.94	16.090	18:2–18:2–16:0	$C_{55}H_{98}O_6$	872.74	44	8.27
6.787	14:0–20:4–8:0	$C_{45}H_{78}O_6$	732.6	34	0.20	12.812	18:3–18:3–18:1	$C_{57}H_{96}O_6$	894.75	40	2.78	17.932	16:0–16:0–18:2	$C_{53}H_{98}O_6$	848.73	46	1.82
7.179	18:2–20:4–8:0	$C_{49}H_{82}O_6$	784.62	34	2.57	14.313	18:2–18:2–18:2	$C_{57}H_{98}O_6$	896.75	42	11.88	17.966	18:1–16:0–18:2	$C_{55}H_{100}O_6$	874.74	46	6.65
7.673	20:3–20:4–8:0	$C_{51}H_{84}O_6$	810.62	34	2.34	14.517	16:0–18:3–18:2	$C_{55}H_{96}O_6$	870.74	42	1.16	18.086	18:1–18:2–18:1	$C_{57}H_{102}O_6$	900.75	46	11.43
8.355	22:4–20:4–8:0	$C_{53}H_{86}O_6$	836.63	34	3.36	14.926	16:0–14:0–14:0	$C_{47}H_{90}O_6$	768.71	44	0.89	19.808	18:1–16:0–18:1	$C_{55}H_{102}O_6$	876.74	48	8.89
8.543	20:4–16:0–8:0	$C_{47}H_{82}O_6$	760.61	36	2.08	15.012	18:1–16:0–12:0	$C_{49}H_{92}O_6$	794.72	44	1.37	19.859	16:0–16:0–18:1	$C_{53}H_{100}O_6$	850.73	48	1.13
8.679	18:1–20:4–8:0	$C_{49}H_{84}O_6$	786.62	36	2.28	15.267	18:1–18:1–12:0	$C_{51}H_{94}O_6$	820.73	44	0.99	20.080	18:0–18:1–16:0	$C_{55}H_{104}O_6$	878.84	50	2.30
8.934	18:2–20:4–10:0	$C_{51}H_{86}O_6$	812.62	36	1.59	15.779	18:2–18:1–14:0	$C_{53}H_{96}O_6$	846.73	44	0.25	22.178	18:0–18:0–18:2	$C_{57}H_{106}O_6$	904.85	50	8.85
9.463	20:3–20:4–10:0	$C_{53}H_{88}O_6$	838.63	36	1.06	16.035	18:1–18:3–16:0	$C_{55}H_{98}O_6$	872.74	44	8.23	22.213	18:1–18:0–16:0	$C_{55}H_{104}O_6$	878.84	50	0.95

Table 3 (continued)

SLs enriched with MLCTs						CIFL (1)[a]						CIFL (2)[b]					
t_R[c] (min)	TAG species	Formula	M+NH_4[d]	ECN[e] (DB)	Content (%)	t_R (min)	TAG species	Formula	M+NH_4	ECN (DB)	Content (%)	t_R (min)	TAG species	Formula	M+NH_4	ECN (DB)	Content (%)
9.479	18:2–18:2–10:0	$C_{49}H_{86}O_6$	788.62	38	2.71	17.313	16:0–16:0–14:0, 16:0–18:0–12:0	$C_{49}H_{94}O_6$	796.72	46	0.90	22.332	18:1–18:1–20:1	$C_{59}H_{108}O_6$	930.86	50	0.92
10.467	20:4–16:0–10:0	$C_{49}H_{86}O_6$	788.72	38	4.01	17.382	18:1–16:0–14:0	$C_{51}H_{96}O_6$	822.73	46	2.19	24.584	18:1–18:1–20:1	$C_{59}H_{108}O_6$	932.86	50	0.88
10.622	18:1–20:4–10:0	$C_{51}H_{88}O_6$	814.72	38	1.25	17.552	18:1–18:1–14:0	$C_{53}H_{98}O_6$	848.73	46	3.47	26.598	18:1–18:1–22:0	$C_{61}H_{114}O_6$	960.86	54	2.04
10.673	22:0–10:0–8:0, 16:0–16:0–8:0	$C_{43}H_{82}O_6$	712.6	40	2.71	17.842	18:1–18:2–16:0	$C_{55}H_{100}O_6$	874.74	46	8.62						
12.702	16:0–16:0–10:0	$C_{45}H_{86}O_6$	740.71	42	3.27	18.012	18:1–18:2–18:1	$C_{57}H_{102}O_6$	900.75	46	11.70						
12.754	18:0–20:4–10:0	$C_{51}H_{90}O_6$	816.73	40	1.57	19.701	16:0–16:0–16:0	$C_{51}H_{98}O_6$	824.73	48	0.86						
12.822	20:4–20:4–20:4	$C_{63}H_{98}O_6$	968.77	36	3.44	19.752	16:0–18:1–16:0	$C_{53}H_{100}O_6$	850.73	48	3.86						
13.316	20:5–20:4–18:1	$C_{61}H_{98}O_6$	944.76	38	1.70	19.82	18:1–18:1–16:0	$C_{55}H_{102}O_6$	876.74	48	9.43						
13.913	20:3–20:3–20:5	$C_{63}H_{100}O_6$	970.77	38	1.24	19.939	18:0–18:1–18:1	$C_{57}H_{106}O_6$	904.85	50	5.02						
15.039	18:0–16:0–10:0, 18:0–18:0–8:0	$C_{47}H_{90}O_6$	768.71	44	1.24	20.467	18:1–20:1–18:2	$C_{59}H_{106}O_6$	928.85	48	0.33						

Table 3 (continued)

SLs enriched with MLCTs						CIFL (1)[a]						CIFL (2)[b]					
t_R[c] (min)	TAG species	Formula	M+NH_4[d]	ECN[e] (DB)	Content (%)	t_R (min)	TAG species	Formula	M+NH_4	ECN (DB)	Content (%)	t_R (min)	TAG species	Formula	M+NH_4	ECN (DB)	Content (%)
15.209	20:4–20:4–18:1	$C_{61}H_{100}O_6$	946.76	40	1.29	22.071	18:0–18:0–14:0	$C_{53}H_{102}O_6$	852.73	50	0.72						
15.243	20:0–20:4–20:4	$C_{63}H_{106}O_6$	844.73	44	1.90	22.122	18:0–16:0–18:1	$C_{55}H_{104}O_6$	878.74	50	2.43						
17.443	20:4-16:0-16:0	$C_{55}H_{98}O_6$	872.74	44	6.39	22.821	18:1–18:1–20:1	$C_{59}H_{108}O_6$	930.86	50	0.25						
18.125	22:0-18:2-10:0	$C_{53}H_{98}O_6$	848.73	46	1.12	24.391	18:0–18:0–16:0	$C_{55}H_{106}O_6$	880.84	52	0.45						
19.797	20:4-16:0-18:0	$C_{57}H_{102}O_6$	900.75	46	4.36	24.442	20:1–18:0–16:0	$C_{57}H_{108}O_6$	906.85	52	0.63						
19.848	10:0-16:0-22:0	$C_{51}H_{98}O_6$	824.73	48	1.21	26.660	18:1–18:1–22:0	$C_{61}H_{114}O_6$	960.86	54	0.72						
19.967	20:3-24:0-10:0	$C_{57}H_{104}O_6$	902.85	48	0.90												
20.018	24:0-18:1-8:0	$C_{53}H_{100}O_6$	850.73	48	0.96												
22.117	10:0-24:0-16:0	$C_{53}H_{102}O_6$	852.83	50	1.47												
24.402	20:4-20:4-24:0	$C_{67}H_{114}O_6$	1032.88	48	2.61												
26.467	24:0-20:4-16:0	$C_{63}H_{114}O_6$	984.87	52	1.03												
28.497	18:0-20:4-24:0	$C_{65}H_{118}O_6$	1012.88	54	0.73												
28.531	24:0-16:0-16:0	$C_{59}H_{114}O_6$	936.86	56	0.85												

Table 3 (continued)

[a]CIFL (1): fat extracted from a commercial infant formula available in China

[b]CIFL (2): fat extracted from a commercial infant formula available in the USA

[c]Retention time (min)

[d]Values are experimental m/z data

[e]Equivalent carbon number (ECN) = CN − 2DB, where CN is carbon number of TAG and DB is total number of double bonds in TAG

UPLC-Q-TOFMS, ultra-performance liquid chromatography with quadrupole-time-of-flight mass spectrometry

blend used in CIFL (1) was formulated from (Corn, rapeseed, coconut, sunflower, soybean, and peanut oils), while in CIFL (2), it was formulated from (sunflower, soybean, and coconut oils) as maintained in the ingredients label. The melting and crystallization temperatures of fat extracted from CIFL (1) were (− 43.04 °C, − 19.16 °C, 12.61 °C) (− 51.43 °C, − 20.84 °C, − 6.25 °C) and for CIFL (2) were (− 37.02 °C, − 15.46 °C, − 1.57 °C, and 20.86 °C) (− 27.01 °C , − 8.50 °C, − 1.67 °C), respectively. The different melting and crystallization temperatures between CIFL (1) and CIFL (2) are related to the source of the plant oils blend. Similar behavior was found by Nagachinta, Akoh [3]. Overall, the melting temperatures of all the samples were below body temperature (37 °C). This confirms that the metabolism of the three samples would be completely melted at the body temperature. Moreover, these findings suggested the use of these SLs enriched with MLCTs as a complementary fat in IFs with a mixture including unsaturated oil (ARA-rich single-cell oil) would be a better substitute for a plant oil mixture.

Characterization of powdered IFs

Generally, powdered IF is produced by one of two kinds of the method, either dry-mixing method or wet-mixing/spray-drying method [36, 37]. Also, some producers use a combination of these methods to spray-drying the base powder (fat and protein constituent) first, then dry-blending process with carbohydrate, mineral, and vitamin constituents. To decide which method is appropriate for SLs enriched with MLCTs application in powdered IF, the powdered IFs were set with SLs enriched with MLCTs as the fat source by these two common processes and then, assessed the peroxide value, water activity, moisture content, and Hunter color values (L^*, a^*, b^*, C^*, h^*, and ΔE^*) of the products. Peroxide value is an index to measure the hydroperoxide amount (primary oxidation products) of oil and fat [57]. The dry-mixing method resulted in products with significantly lower peroxide value, water activity, and moisture content values compared to the wet-mixing/spray-drying method and the commercial IFs are shown in Table 4. The higher peroxide values of commercial IFs may be attributed due to the higher content of LCUFAs in the used oil as mentioned in Table 2. Meanwhile, it was found that the moisture content and water activity of all the powdered IF products were below 3% and 0.30, respectively, which were normally considered ensuring product stability [58].

After the dry-blending process, the lightness (L^*) value of the powder increased, while the redness (a^*) and yellowness (b^*) values decreased in comparison to powder obtained from commercial and wet-mixing/spray-drying method. These findings suggested that the increased lightness of the IF product would be responsible for decreasing

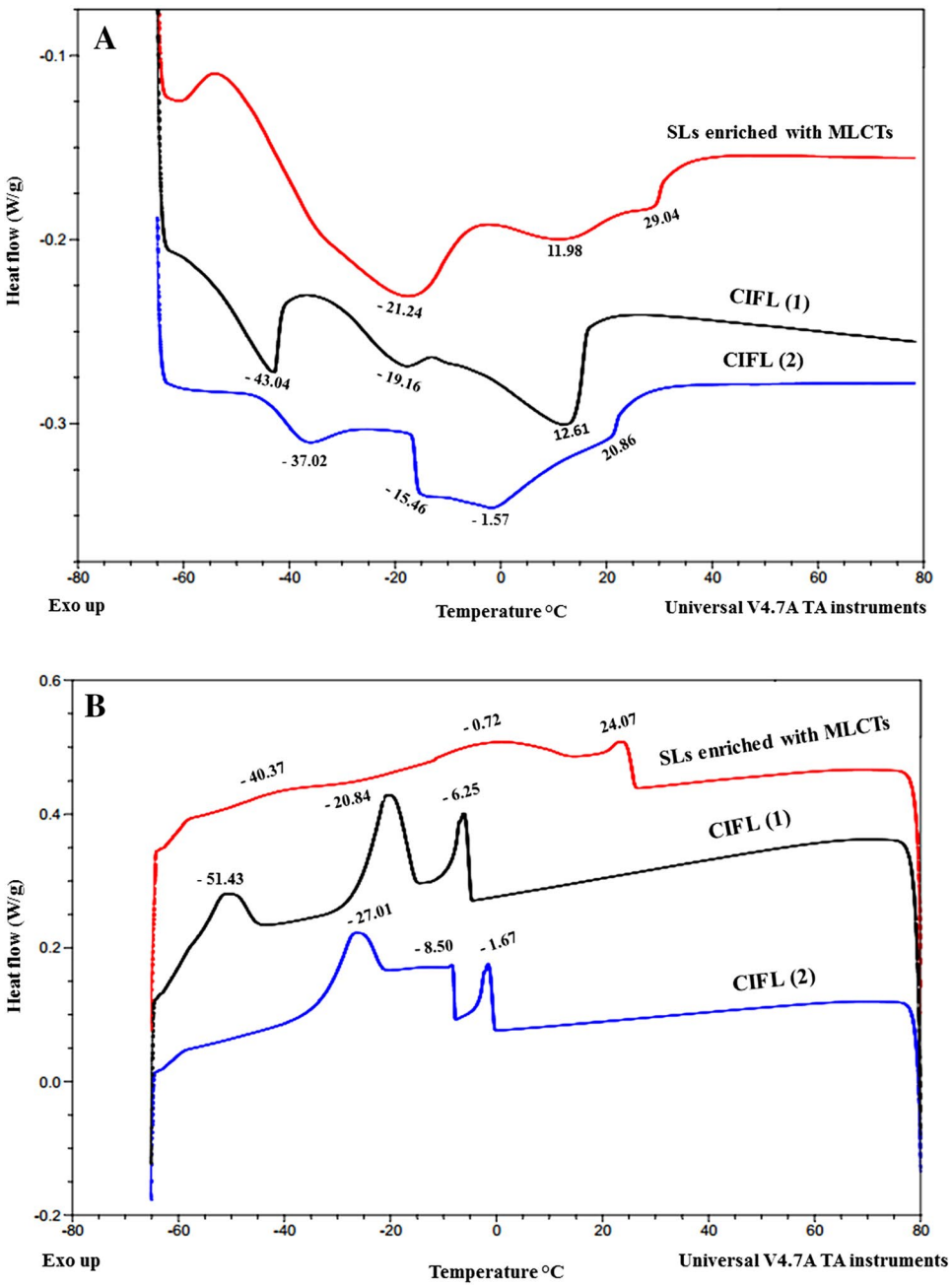

Fig.2 Melting (**a**) and crystallization (**b**) profiles of SLs enriched with MLCTs, CIFL (**1**) fat extracted from a commercial infant formula available in China; and CIFL (**2**) fat extracted from a commercial infant formula available in the USA

the *PV* value. In addition, the microencapsulation process of oils has the ability to improve oxidative stability with a good appearance [59]. Moreover, the Chroma (C^*) values of dry-blending process products were also less, meaning that the color looked light. On the contrary, L^* value decreased, while both a^* and b^* values increased after the wet-mixing/spray-drying process that turns the IF products into dark. The darkness of the IF products may have attributed to increase the peroxide value. A similar finding was reported by Nagachinta, Akoh [3], they stated that the lightness value was negatively associated with PV and TBARS values. Furthermore, the Chroma (C^*) color values of wet-mixing/spray-drying products also were more, meaning that the color looked darker. Overall, the hue (h^*) color values of all the powdered products fall among yellow and green. The total color difference (ΔE^*) was well visible for dry-blending process products, while greatly visible for wet-mixing/spray-drying process products and commercial IFs [60]. These findings are in agreement with Nagachinta, Akoh [3], who reported that the IFs prepared with microencapsulated SL and using dry-mixing method had better color quality and oxidative stability.

Table 4 Characterisation of powdered infant formulas (IFs) prepared using general two processes compared to commercial infant formulas (CIFLs)[a]

Characteristics	Wet-mixing/spray-drying at 180 °C	Dry-blending	CIFL (1)[b]	CIFL (2) [c]
Peroxide value (mM hydro-peroxides)	1.12 ± 0.03[a]	0.42 ± 0.01[c]	0.53 ± 0.12[b]	0.49 ± 0.02[b]
Water activity	0.14 ± 0.00[a]	0.08 ± 0.00[d]	0.11 ± 0.02[b]	0.09 ± 0.01[c]
Moisture content	2.97 ± 0.11[a]	1.83 ± 0.03[c]	2.01 ± 0.01[b]	1.96 ± 0.13[bc]
Hunter color values				
*L**	89.49 ± 0.20[d]	95.20 ± 0.19[a]	93.73 ± 0.13[b]	92.35 ± 0.17[c]
*a**	-0.40 ± 0.10[a]	-0.77 ± 0.07[b]	-2.61 ± 0.09[c]	-0.72 ± 0.01[b]
*b**	14.27 ± 0.09[b]	8.11 ± 0.12[c]	14.01 ± 0.26[b]	14.62 ± 0.05[a]
*C**	14.27 ± 0.09[b]	8.15 ± 0.11[c]	14.24 ± 0.28[b]	14.64 ± 0.05[a]
*h**	88.40 ± 0.40[a]	84.58 ± 0.43[c]	79.45 ± 0.19[d]	87.18 ± 0.05[b]
Δ*E**	11.39 ± 0.15[a]	4.91 ± 0.09[c]	10.60 ± 0.27[b]	11.07 ± 0.05[a]

[a]Means ± SD, with the different superscript letter (within a row) are significantly different at ($p < 0.05$)

[b]CIFL (1): a commercial infant formula available in China

[c]CIFL (2): a commercial infant formula available in the USA

Table 5 Energy contribution in 100 mL of re-suspended potential infant formulas (IFs) prepared using general two processes

Ingredients	Content (g)	Macronutrient contribution (g)			Energy contribution[f] (kcal)
		Lipid	Protein	Carbohydrate	
Non-fat dry milk[a]	2.16	0.01	0.72	1.14	7.53
WPI[b]	1.08	0.00	0.99	0.00	3.96
Lactose	3.35	0.00	0.00	3.35	13.40
Maltodextrin	3.24	0.00	0.00	3.24	12.96
MLCTs-rich SLs	3.24	3.24	0.00	0.00	29.16
Micronutrient premix	0.42	0.00	0.00	0.00	0.00
Total energy contribution[c]	–	–	–	–	67.01
Microencapsulated MLCTs-rich SLs[d]	30	7.50	10.35	11.25	153.90
Total energy contribution[e]	–	–	–	–	191.75

[a]Nonfat dry milk contains 0.60% lipid, 33.50% protein, and 52.60% carbohydrate

[b]WPI, whey protein isolate contains 0.00% lipid, 92% protein, and 0.00% carbohydrate

[c]Total energy contribution of prepared infant formula using wet-mixing/spray-drying method

[d]Microencapsulated MLCTs-rich SLs content MLCTs-rich SLs 25%, WPI 37.5%, and inulin 37.5%

[e]Total energy contribution of prepared infant formula using microencapsulated MLCTs-rich SLs with dry-blending method

[f]Energy density for lipid, protein, and carbohydrate is 9, 4, and 4 kcal/g, respectively [62]

Energy contribution of potential IF

In this work, IF preparation was targeted at a formulation that donates between 60 kcal (250 kJ) and 70 kcal (295 kJ) of energy per 100 mL of re-suspended IF resulting from 5.4 to 8.1% carbohydrates, 3.3 to 6.0% fat, and 1.2 to 3.0% protein. The IF prepared via the wet-mixing/spray-drying method provided the energy contribution by 67.01 kcal/100 mL as shown in Table 5. According to the CODEX STAN, IFs must provide between 60 and 70 kcal of energy per 100 mL [61]. For IF prepared via dry-blending method, microencapsulated SLs enriched with MLCTs contained 25% fat (SLs enriched with MLCTs), 37.5% protein (WPI), and 37.5% carbohydrate (IN). Microencapsulation of SLs enriched with MLCTs improved the final product stability (Table 4); furthermore, the energy donated from carbohydrate and protein ingredients used as encapsulated agents that increased the energy contribution of the final product by 86.4 kcal/100 mL (Table 5).

Conclusion

In this study, SLs enriched with MLCTs contained ARA (42.82 ± 0.89%) at the *sn*-2 position and enriched with MCFAs (36.33 ± 1.05%) at *sn*-1,3 positions, could be used

in preparing a novel commercial formulas to fulfill the nutritional requirements of the infant growth and development. The results indicated that there were differences in the percentages of LCUFAs, LCFAs, MCFAs, and SCFAs between the three IFs oil reported samples. A total of 45 TAG species were detected in a novel SL followed by 38 in CIFL (1), and 25 in CIFL (2). The all IFs oil samples melted below the body temperature. The dry-mixing method was a better method for IF powder production, which provides greater oxidative stability and good color with low moisture content. The physiochemical characteristics of SLs enriched with MLCTs recommended its use as an oil mixture into IF. In the new formulation, the microencapsulation with 25% oil is being studied to develop for microencapsulated SLs enriched with MLCTs on energy contribution with highly functional and nutritional benefits. As we know, the various organizations, such as the European Union, USFDA and WHO, provide the standards to which the infant formulas must adhere. However, in this study, the novel microencapsulated (SL) formula exceeding the recommended standard in terms of energy and macronutrients. Therefore, we believe that the level of nutrients in a newly made formula should be reviewed to meet the references and avoid the possible adverse effect. Nevertheless, the investigation is going on to meet the standard before fair trade practices and to protect the consumers' health.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval This article does not contain any studies with human or animals and complies with ethical requirements.

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