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Comparison of fatty acid composition and positional distribution of microalgae triacylglycerols for human milk fat substitutes

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ABSTRACT

Microalgae are renewable and valuable natural sources of triacylglycerol (TAG) for nutritional application or specific purposes. The aim of this study was to evaluate the TAGs produced by twelve microalgae species for production of human milk fat substitutes (HMFs). The investigated species were *Chlorella vulgaris* (CV-15 and CV-395), *Chlorella zofingiensis*, *Chlorella pyrenoidosa*, *Scenedesmus* sp., *Chlorococcum* sp., *Nitzschia laevis*, *Phaeodactylum tricornutum*, *Isochrysis* sp., *Isochrysis galbana*, *Nannochloropsis oceanica* and *Nannochloropsis salina*. The total fatty acids (TFAs) contents of these species varied from 15.9 to 31.1%. TAG was the main lipid class (65.1–91.0%). Based on the evaluation model in view of the fatty acid composition and positional distribution of TAGs by GC–MS and ^{13}C NMR, among the tested species, *Isochrysis*-derived TAGs mimicking human milk TAGs (HMTs) gave the highest G values (deducting score) that were close to that of lard. The G value (69.2) of *Isochrysis* sp. TAG was within the range of G value for local infant formulas; whereas, the G value (61.2) of *I. galbana* TAG was a bit lower than the lower limit of local infant formulas. Moreover, the melting and crystallization properties of TAGs from *Isochrysis* sp. and *I. galbana* were similar to those of HMTs. These results showed that *Isochrysis* TAGs could be promising candidates for HMFs feedstock.

1. Introduction

Extensive review papers and experimental works have shown that microalgae are promising and sustainable sources of natural edible oils for nutritional application or specific purposes in foods [1–5]. Microalgae mainly synthesize lipids as triacylglycerol (TAG) form. It is well known that different microalgae strains can produce TAG with distinct fatty acid composition, due to their individual lipid metabolism [1,6–9]. For example, TAG obtained from *Chlorella zofingiensis* had 25.1% palmitic acid (C16:0, PA), 23.1% oleic acid ($C_{18:1}$, OA), 21.6% linoleic acid ($C_{18:2n-6}$, LA) and 8.9% α-linolenic acid ($C_{18:3n-3}$, ALA) [10]. *Nannochloropsis oculata* under illumination of 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and nitrogen-starvation conditions produced TAG containing 5.1% myristic acid ($C_{14:0}$, MA), 62.2% PA, 19.0% palmitoleic acid ($C_{16:1}$, POA), 0.4% LA and 0.9% eicosapentaenoic acid ($C_{22:5n-3}$, EPA) [7]. Most of the available works analyzed the fatty acid composition of TAGs from investigated oleaginous microalgae for biodiesel production [6,7,10–13]. To date, the existing microalgae biotechnologies for biodiesel have not been viable because of the high investment [1,3,6,14]. Thus, it is important to develop high-value microalgal bioproducts as alternative routes to enhance the economic viability of microalgae oils.

Fat in human milk or infant milk powder is an essential structural ingredient, which accounts for 98% of TAG [15–19]. Human milk TAGs (HMTs) mainly consists of 17.0–24.4% PA, 28.5–42.4% OA and 20.8–33.5% polyunsaturated fatty acids (PUFAs); at the *sn*-2 position, saturated fatty acids (SFAs) comprise 57.7–70.9% and PUFAs comprise 13.2–22.7% [15–21]. Such structure exhibits the maximum health-beneficial properties and nutritional values for infants or babies. Sometimes, the infant formulas are needed to feed babies for medical, religious and unpredictable reasons [15,16,18,22]. Therefore, many studies have recently received much attention in the human milk fat substitutes (HMFs) [15,18,22,23]. In the field of edible oils, lard is considered to be a good natural source of HMFs, due to their structural TAGs resembling HMTs [15,16,24]. Numerous investigations have focused on the modification of lard to yield high quality of HMFs for infant formulas [18,24,25]. However, the fatty acid composition and its position distribution in TAGs from lard are largely influenced by the given oil sources in animal feeds [26], resulting in the inconsistent quality of HMFs. Moreover, many unexpected substances (e.g. antibiotics, hormones) have been detected in lard [27,28]. As a result, many infant-formula producers and consumers have started to doubt the safety of lard [27–29]. Thus, it is necessary to exploit potential

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green alternative(s) to HMFs of infant formulas.

Recently, studies have focused on the production of HMFs using microalgae oils from non-transgenic microalgae [23,30,31]. Wang et al. [23] selected microalgae oil from *Schizochytrium* sp. to produce the HMFs consisting of polyunsaturated fatty acids (PUFAs) at the *sn*-1(3) positions and PA at the *sn*-2 position. Additionally, a review paper [32] showed that microalgae oils obtained from *Arthrospira*, *Porphyridium* and *Crypthecodinium* strains were promising sources to develop the HMFs for full-term or preterm infants. Compared with animals, microalgae yield much higher TAG productivity [1,14,32]. These evidences indicate that natural microalgae oils can be the potential feedstock for HMFs products. However, until now, only a few studies focused on microalgae TAGs for HMFs had been carried out [23,30,31], restricting the microalgae oils in the application of infant formula. Thus, to promote and maintain the sustainable development of infant formulas, it is crucial to develop the potential new sources of microalgae TAGs for HMFs.

Nowadays, among the investigated microalgae, *Chlorella*, *Crypthecodinium cohnii*, *Diacronema lutheri*, *Haematococcus*, *Isochrysis galbana*, *Nannochloropsis* sp., *Odontella aurita*, *Phaeodactylum tricornutum*, *Porphyridium cruentum*, *Schizochytrium* sp., *Spirulina* and *Tetraselmis chuii* have been accepted or authorized by the US Food and Drug Administration and/or European Novel Food Regulation for food and nutraceuticals [14,33,34]. Moreover, the applications for some potential microalgae species are ongoing to deliver unique bioactive compounds for food [1,14,33]. Thus, the purpose of this study was to evaluate the microalgae TAGs obtained from twelve microalgae species for HMFs products. The selected microalgae species were *Chlorella vulgaris* (CV-15 and CV-395), *C. zofingiensis*, *C. pyrenoidosa*, *Scenedesmus* sp., *Chlorococcum* sp., *Nitzschia laevis*, *Phaeodactylum tricornutum*, *Isochrysis* sp., *I. galbana*, *Nannochloropsis oceanica* and *N. salina*. The total fatty acids (TFAs) contents of microalgae biomasses were determined. Then, microalgae TAGs were separated and purified. C-13 nuclear magnetic resonance (^{13}C NMR) and gas chromatography–mass spectrometry (GC–MS) techniques were adopted to analyze the fatty acid composition and its positional distribution in the purified TAGs. The microalgae TAGs were evaluated by a “deducting score” principle in view of fatty acid composition and positional distribution. Differential scanning calorimetry (DSC) was employed to determine the melting and crystallization properties of the potential microalgae TAG(s) for HMFs. Our work will open a new perspective in microalgal bioproducts to exploit the HMFs of infant formula.

2. Materials and methods

2.1. Microalgae and growth media

Chlorella vulgaris (Carolina 15-2075) (named as CV-15) was purchased from Carolina Biological Supply Co. (Burlington, USA). *Chlorella zofingiensis* (ATCC 30412) (named as CZ-30412) was obtained from the American Type Culture Collection (ATCC, Rockville, USA). *Chlorella vulgaris* (UTEX 395) (named as CV-395), *Nitzschia laevis* (UTEX 2047) (named as NL-2047), *Phaeodactylum tricornutum* (UTEX 646) (named as PT-646), *Isochrysis galbana* (UTEX 2307) (named as IG-2307) were purchased from the University of Texas Culture Collection of Algae (UTEX, Austin, USA). *Chlorella pyrenoidosa* (FACHB-9) (named as CP-9) was obtained from Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). *Isochrysis* sp. (named as ISO-FJ) was kindly donated by Prof. Dr. Qiujin Zhang, College of Life Science, Fujian Normal University. *Nannochloropsis oceanica* IMET1 (named as NO-IMET1) was from the Institute of Marine and Environmental Technology, the University of Maryland (IMET, USA). *Nannochloropsis salina* CCMP 537 (named as NS-537) was obtained from the National Center for Marine Algae and Microbiota (NCMA, USA). *Scenedesmus* sp. (named as SS) and *Chlorococcum* sp. (named as CS) were from our laboratory.

BG11 medium (pH, 7.2) [9] was composed of (g/L): $C_6H_{12}O_6$ (5.0), $NaNO_3$ (1.5), K_2HPO_4 (4.0×10^{-2}), $MgSO_4{\cdot}7H_2O$ (7.5×10^{-2}), $CaCl_2{\cdot}H_2O$ (3.60×10^{-2}), Na_2CO_3 (2.0×10^{-2}), $C_6H_8O_7$ (citric acid, 6.0×10^{-3}), EDTA (1.0×10^{-3}), and 1 mL of trace elements solution (solution (g/L): H_3BO_3 (2.86), $MnCl_2{\cdot}4H_2O$ (1.8), $ZnSO_4{\cdot}7H_2O$ (0.2), $NaMoSO_4{\cdot}2H_2O$ (0.4), $CuSO_4{\cdot}5H_2O$ (7.9×10^{-2}) and $Co(NO_3)_2{\cdot}6H_2O$ (4.9×10^{-2})). The modified LDM medium (pH, 8.0) [35] consisted of (g/L): sea salt (10.0), $C_6H_{12}O_6$ (5.0), $NaNO_3$ (1.0), $Na_2SiO_3{\cdot}9H_2O$ (3.0×10^{-2}), Biotin (2.5×10^{-7}), Vitamin B_{12} (1.5×10^{-7}), 100 mL of Bristol's solution ((solution (g/L): $CaCl_2$ (1.0), $MgSO_4{\cdot}7H_2O$ (3.0), K_2HPO_4 (3.0) and KH_2PO_4 (7.0)) and 6 mL of PIV metal solution (solution (g/L): Na_2EDTA, (0.8), $FeCl_3{\cdot}6H_2O$ (9.7×10^{-2}), $MnCl_2{\cdot}4H_2O$ (4.1×10^{-2}), $ZnCl_2$ (5.0×10^{-3}), $CoCl_2{\cdot}6H_2O$ (4.2×10^{-2}) and Na_2MoO_4 (4.0×10^{-3})). The modified f/2 medium (pH, 7.8) [36,37] was composed of (g/L): sea salt (25.0), $C_3H_8O_3$ (5.0), $NaNO_3$ (1.5×10^{-1}), $NaH_2PO_4{\cdot}H_2O$ (6.0×10^{-3}), Na_2EDTA (4.4×10^{-3}), $FeCl_3{\cdot}6H_2O$ (3.2×10^{-3}), $CuSO_4{\cdot}5H_2O$ (1.0×10^{-5}), $ZnSO_4{\cdot}7H_2O$ (2.5×10^{-5}), $CoCl_2{\cdot}6H_2O$ (1.2×10^{-5}), $MnCl_2{\cdot}4H_2O$ (1.8×10^{-4}), $Na_2MoO_4{\cdot}2H_2O$ (7.0×10^{-6}), Vitamin B_1 (1.0×10^{-4}), Vitamin B_{12} (1.0×10^{-6}), Biotin, (1.0×10^{-6}).

2.2. Microalgae cultivation

Previous studies [10,35,38,39] had stated that some microalgae genera such as *Chlorella*, *Scenedesmus*, *Chlorococcum* and *Nitzschia* could effectively utilize glucose (1–30 g/L) to form biomass under mixotrophic conditions. *Phaeodactylum*, *Isochrysis* and *Nannochloropsis* species preferentially consumed glycerol (4.6×10^{-2}-9.2 g/L) to form biomass [37,40,41]. Therefore, 5 g/L of glucose (CV-15, CZ-30412, CV-395, CP-9, SS, CS and NL-2047) and 5 g/L of glycerol (PT-646, ISO-FJ, IG-2307, NO-IMET1 and NS-537) were chosen in this study. CV-15, CZ-30412, CV-395, CP-9, SS and CS were cultivated in the BG11 medium. NL-2047 culture was grown in the modified LDM medium. The modified f/2 medium with 30 mg/L $Na_2SiO_3{\cdot}9H_2O$ was used to cultivate PT-646. ISO-FJ, IG-2307, NO-IMET1 and NS-537 were cultivated in the modified f/2 medium. The microalgal cells were inoculated at 0.1 g/L in 500 mL Erlenmeyer flasks containing 200 mL medium. The experiments were carried out under continuous illumination of 30 μmol photon m^{-2} s^{-1} at 25 °C with 150 rpm orbital shaking speed for 12 d.

2.3. TFAs measurement and TAGs purification

2.3.1. TFAs content of microalgal biomass

After 12 d of cultivation, microalgal cells were harvested and lyophilized for TFAs analysis. About 10 mg of lyophilized biomass was weighted for fatty acid methyl esters (FAMEs). FAMEs were prepared by the previous study [42]. FAMEs were analyzed by a gas chromatography–mass spectrometry, using a GC–MS-QP 2010 SE (Shimadzu, Japan) equipped with a Stabliwas-DA capillary column (30 m × 0.25 mm × 0.25 μm, Shimadzu, Japan) [10]. The initial oven temperature was set at 150 °C and subsequently heated to 180 °C at a rate of 10 °C/min then raised to 220 °C at the speed of 2 °C/min, and finally held for 10 min. The injector temperature was 250 °C. The injection volume was 1 μL. Heptadecanoic acid ($C_{17:0}$; purity, > 98%) was purchased from Sigma-Aldrich (Shanghai, China) and used as the internal standard (1 mg/mL) for the quantification of fatty acids by comparing their peak areas. The TFAs content of microalgae biomass was expressed by the following equation:

$$\text{TFAs content of microalgal biomass} = \frac{m_{TFAs}}{m_{BI}} \times 100\% \quad (1)$$

where, m_{TFAs} was the total fatty acids (TFAs) by GC–MS, mg; m_{BI} was the weight of the treated microalgae biomass, mg.

2.3.2. Microalgae TAG purification

About 1 g of dried microalgal biomass was used to extract microalgal lipid with 100 mL chloroform: methanol: distilled water (2:1:1, v/v/v) for three times according to the method of Sun et al. [9]. After centrifugation at 5000 rpm for 5 min, crude lipid sample was collected, evaporated and stored at −20 °C for TAG purification.

The microalgae TAGs were purified by a silica gel column (diameter, 12 mm, length, 150 mm) based on the method of Bondioli et al. [43]. Two solvents were selected to collected microalgae TAGs: 1) The non-polar compounds like fatty acids hydrocarbons and ester with monoalcohols were obtained by 100 mL *n*-hexane: diethyl ether (98:2, v/v); 2) The microalgae TAGs were collected by eluting 100 mL *n*-hexane: diethyl ether (90:10, v/v). Microalgae TAGs were evaporated to remove organic solvents and stored at −20 °C. The TAG sample was methylated and quantified by GC–MS. The following equation was used to quantify the microalga TAG of microalgal TFAs.

$$\text{The percentage of TAG} = \frac{M_{TAG}}{M_{TFAs}} \times 100\% \quad (2)$$

where, M_{TAG} was the total fatty acid weight, mg.

2.4. Fatty acid positional distribution in microalgae TAGs by GC–MS

TAG sample was hydrolyzed by the pancreatic lipase II on the basis of the modified method of the previous studies [30,31]. TAG (20.0 mg), pancreatic lipase (20.0 mg), 0.1% sodium cholate solution (0.5 mL), 20% $CaCl_2$ solution (0.2 mL) and Tris–HCl buffer (2 mL, pH 7.0) were placed into a centrifuge tube of 10 mL. The hydrolysis was performed at 40 °C for 2 min with 200 rpm in a water bath shaker (MQS-30S, China). Then, enzymatic hydrolysates containing lipid were extracted with diethyl ether (2 mL) for three times. The organic solvent was removed by nitrogen. Lipid products were spotted onto the preparative thin-layer chromatography plate (TLC, silica gel GF UV-254, thickness 250 μm, 20 cm × 20 cm) and developed in chloroform:acetone (1:1, v/v) for 40 min. 2-Monoacylglycerols in TLC plate was scraped, methylated and analyzed by the GC–MS instrument as described in the Section 2.3.1.

2.5. ^{13}C nuclear magnetic resonance (^{13}C NMR) analysis of microalgae TAG

A Bruker Advance 500 MHZ spectrometer (Bruker Co. Ltd., Switzerland) was applied to record the spectra of microalga TAG sample (50.0 mg) placed in a 5 mm NMR tube containing 500 μL deuterated chloroform ($CDCl_3$) [44]. The acquisition parameters were: spectral width 238 ppm, acquisition time 0.37 s, relaxation delay 10 s, delay between scans 2 s, pulse width 45°, required sample temperature 300 K, dummy scans 4 and scan times 1024. The MestReNova 10 software (Santiago de Compostela, Spain) was used to process the ^{13}C NMR spectra.

2.6. Differential scanning calorimetry analysis

The melting and crystallization profiles of TAG sample (around, 5 mg) was performed with a differential scanning calorimeter (DSC-Q2000, TA Instruments, New Castle, DE, USA) [31,45]. The procedures were: 1) the crystallization program was set from 70 to −60 °C at 10 °C/min; 2) the melting program was set from −60 to 70 °C at 10 °C/min. The thermographs were plotted by MicroCal Origin 8.5 Software (Microcal Software Inc., Northampton, USA).

2.7. Statistical analysis

All experiments were conducted in triplicate. Microsoft Excel 2010 and Origin 8.5 were used to process the experimental data presented as means value ($n = 3$) with the standard deviation. The significant differences ($p < 0.05$) between the means were examined by one-way ANOVA and Duncan's multiple range tests.

2.8. Evaluation of microalgae TAG for HMFs

Different microalgae TAG had different fatty acid profiles and positional distribution. The “deducting score” (G) established by Wang et al. [16] was used to evaluate the similarity degree of microalgae TAG compared with the lard and HMTs. The equations were expressed as follows:

$$G = G_1 + G_2 \quad (3)$$

$$G_1 = 50 - 50\sum_{i=1}^{n}\left(\frac{|B_i - A_i|}{A_i}\frac{D_i}{\sum_i^n D_i}\right) \quad (4)$$

$$G_2 = 50 - 50\sum_{i=1}^{n}\left(\frac{|B_{i(sn-2)} - A_{i(sn-2)}|}{A_i(sn-2)}\frac{D_{i(sn-2)}}{\sum_i^n D_{i(sn-2)}}\right) \quad (5)$$

where, the maximum score of G was 100 and 50 each for G_1 (fatty acid of TAG) and G_2 (fatty acid at the *sn*-2 position). B_i was the value of individual fatty acid content (wt%) in the total fatty acid (Table 2 and Table S1); $B_{i(sn-2)}$ was the relative content of individual fatty acid at the *sn*-2 position (Table 3 and Table S2). D_i and $D_{i(sn-2)}$ were the mean values of total fatty acid content and *sn*-2 position fatty acid relative content of TAG (Table S3), respectively. A_i and $A_{i(sn-2)}$ were the lower or the upper limit of 95% reference range of total fatty acid of HMTs and *sn*-2 fatty acids, respectively; moreover, the values of lower or upper limits were obtained from HMTs. The following conditions were used to estimate the results: (1) when the value of B was higher than the upper limit of the corresponding fatty acid content, A was selected as the upper limit; (2) the lower limit was chosen as A when B was lower than the lower limit of the range; (3) the value of B was within the range, $|B_i - A_i|/A_i$ and $|B_{i(sn-2)} - A_{i(sn-2)}|/A_{i(sn-2)}$ were kept at zero.

3. Results and discussion

3.1. The TFAs content of microalgae biomass

The measured TFAs contents of microalgal biomasses were shown in Table 1. It could be seen that the tested microalgae species had TFAs contents in the range from 15.9 to 31.1% (Table 1). Among all microalgae, CS gave the highest TFAs content (31.1%), which was lower than the value (56.0%) reported by Harwati's work [46]. This could be due to the differences in the microalgae species and cultivation conditions. Previous studies had shown that the environmental factors such as temperature, nitrogen concentration, etc. affected the fatty acid synthesis of microalgal cells and resulted in the difference in TFAs content. Four species (CV-15, CZ-30412, CV-395 and CP-9) from the

Table 1
TFAs contents and TAG percentages of twelve microalgae species.

Species	TFAs (wt%)	TAGs percentage[1] (wt%)
CV-15	22.8 ± 1.1^{d}	65.1 ± 2.9^{d}
CZ-30412	28.0 ± 1.1^{b}	76.2 ± 2.6^{bc}
CV-395	26.4 ± 2.3^{bc}	78.9 ± 4.0^{bc}
CP-9	25.1 ± 0.6^{c}	80.3 ± 4.4^{bc}
SS	21.7 ± 1.6^{de}	83.3 ± 5.8^{ab}
CS	31.1 ± 1.4^{a}	77.7 ± 2.3^{bc}
NL-2047	16.6 ± 0.4^{f}	75.1 ± 2.4^{c}
PT-646	22.7 ± 2.5^{de}	84.4 ± 5.5^{ab}
ISO-FJ	22.9 ± 1.0^{d}	91.0 ± 4.4^{a}
IG-2307	19.2 ± 1.3^{e}	86.1 ± 3.1^{ab}
NO-IMET1	15.9 ± 1.1^{f}	81.2 ± 4.2^{bc}
NS-537	19.4 ± 1.2^{e}	78.1 ± 3.8^{bc}

$^{a, b, c, d, e, f}$: The mean values in the same column were significantly different among the microalgae species in the same culture conditions ($p < 0.05$).
[1] The TAGs percentage of microalgal TFAs was estimated by the Eq. (2).

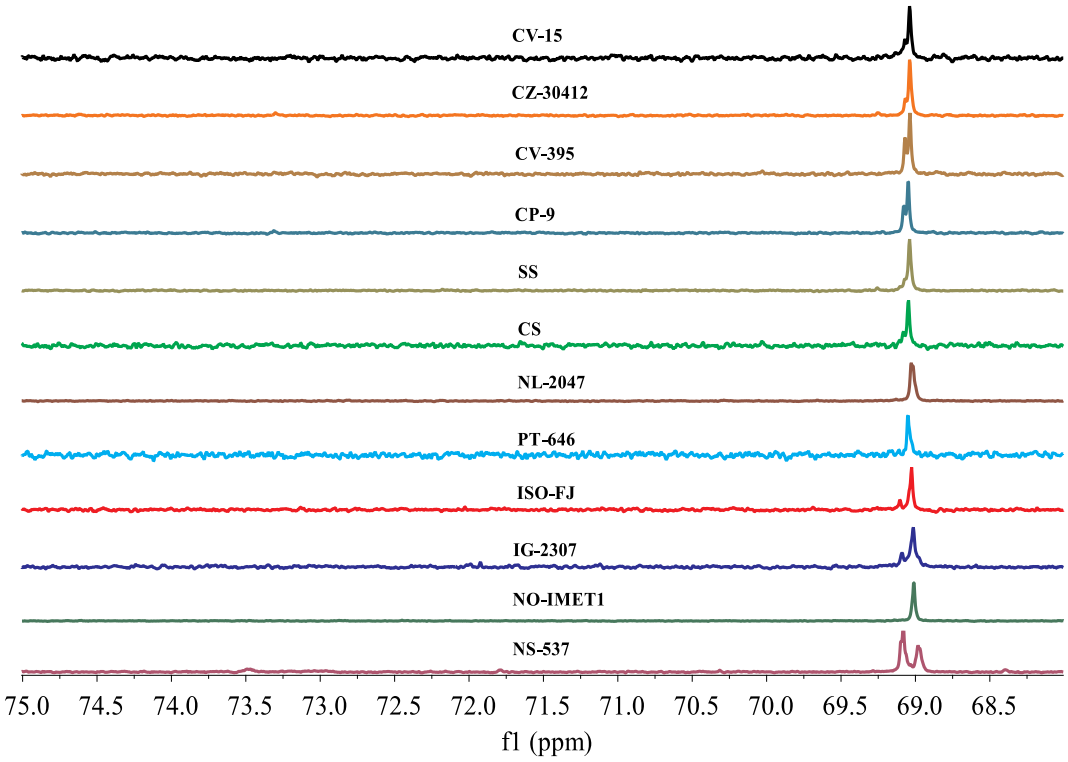

Fig. 1. The glycerol backbone carbon region 68–75 ppm of the purified microalgae TAGs produced by twelve species by ^{13}C NMR analysis.

Table 2
The compositions of four fatty acid species, SFAs, MFAs and PUFAs of twelve microalgae TAGs and HMTs.

Species	$C_{16:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3n-3}$	ΣSFAs[1]	ΣMFAs[2]	ΣPUFAs[3]
CV-15	21.0 ± 1.3^{d}	16.5 ± 0.5^{f}	41.1 ± 2.6^{a}	10.5 ± 0.4^{b}	23.3 ± 0.4^{de}	17.3 ± 0.5^{e}	54.7 ± 2.8^{a}
CZ-30412	20.2 ± 1.4^{d}	33.2 ± 2.5^{c}	23.7 ± 1.1^{c}	9.8 ± 0.5^{b}	24.8 ± 1.8^{de}	34.4 ± 2.1^{d}	38.7 ± 1.6^{b}
CV-395	14.6 ± 1.2^{f}	51.3 ± 2.3^{a}	16.2 ± 1.2^{d}	6.5 ± 0.4^{c}	18.4 ± 0.8^{g}	53.4 ± 2.6^{a}	27.7 ± 1.5^{c}
CP-9	23.4 ± 2.1^{cd}	12.2 ± 1.1^{gh}	29.4 ± 1.3^{b}	12.6 ± 1.1^{a}	27.8 ± 1.5^{d}	14.1 ± 0.9^{f}	47.0 ± 3.3^{a}
SS	27.1 ± 1.7^{b}	28.7 ± 1.4^{d}	21.7 ± 1.9^{c}	9.2 ± 0.9^{b}	31.6 ± 1.8^{c}	31.8 ± 2.2^{d}	36.3 ± 1.8^{b}
CS	22.2 ± 1.5^{e}	38.2 ± 2.6^{b}	9.6 ± 0.4^{e}	13.6 ± 0.9^{a}	26.6 ± 2.0^{d}	45.8 ± 2.3^{b}	27.0 ± 0.9^{ef}
NL-2047	17.4 ± 1.1^{e}	2.4 ± 0.1^{i}	2.2 ± 0.1^{i}	0.4 ± 0.1^{g}	24.1 ± 1.5^{de}	50.9 ± 2.6^{a}	22.6 ± 1.8^{d}
PT-646	15.5 ± 0.4^{f}	20.1 ± 2.2^{e}	0.6 ± 0.4^{k}	0.5 ± 0.1^{f}	34.0 ± 1.8^{c}	42.9 ± 2.6^{b}	18.6 ± 1.4^{ef}
ISO-FJ	24.7 ± 1.5^{bc}	39.4 ± 2.2^{b}	7.5 ± 0.3^{g}	3.1 ± 0.1^{d}	37.5 ± 1.5^{b}	42.0 ± 1.5^{b}	20.1 ± 0.9^{f}
IG-2307	18.5 ± 1.0^{e}	33.0 ± 2.2^{c}	2.0 ± 0.5^{j}	2.7 ± 0.3^{e}	33.9 ± 2.1^{c}	45.2 ± 2.4^{b}	19.7 ± 1.1^{e}
NO-IMET1	42.2 ± 1.5^{a}	16.4 ± 0.7^{h}	3.4 ± 0.3^{h}	0.5 ± 0.0^{f}	51.0 ± 2.1^{a}	38.9 ± 1.8^{c}	6.1 ± 0.2^{h}
NS-537	25.9 ± 1.4^{bc}	13.0 ± 0.8^{g}	5.9 ± 0.3^{f}	0.4 ± 0.0^{g}	53.7 ± 2.5^{a}	34.3 ± 1.2^{d}	10.3 ± 0.8^{g}
HMTs [A]	17.0–24.4	25.0–42.4	10.5–20.3	0.5–2.0	30.3–41.2	31.9–45.4	20.8–33.5

a, b, c, d, e, f: The mean values in the same column were significantly different among the microalgae species in the same culture conditions ($p < 0.05$).

[1] SFAs contain $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$;

[2] MFAs contain $C_{14:1}$, $C_{16:1}$ and $C_{18:1}$;

[3] PUFAs contain $C_{16:2}$, $C_{16:3}$, $C_{18:2}$, $C_{18:3}$, $C_{18:4}$, $C_{20:4}$, $C_{20:5}$ and $C_{22:6}$.

[A] Adapted from Wang et al. [16].

genus *Chlorella* were found to have TFAs contents of 22.8–28.0% (Table 1). Similar results were found in literature for five *Chlorella* species (17.0–30.0%) [47]. SS was found to have TFAs content of 21.7%, which was higher than the values reported by Andruleviciute et al. [48] and Li et al. [47], but lower than the result of Griffiths' work [49]. The TFAs content of NL-2047 (16.6%) was comparable [50] and higher than the one given by Wen and Chen [35]. PT-646 had the TFAs content of 22.7%, which agreed with the study of Li et al. [47]. The TFAs contents of ISO-FJ and IG-2307 were 22.9 and 19.2%, respectively. TFAs values of the genus *Isochrysis* in the range of 17.0–27.7% had been found in previous studies [33,49,51,52]. The TFAs contents of NO-IMET1 and NS-537 from genus *Nannochloropsis* were 15.9 and 19.4%, respectively (Table 1); which were comparable [49] and lower than the ones reported by other authors [33,53].

The method of Bondioli et al. [43] was used to purify the microalgae TAGs by the silica gel column. ^{13}C NMR chemical resonances of the purified microalgae TAGs were around 69 ppm (Fig. 1), which agreed with the summarized study recording the resonances of fish oil and olive oil [44]. These results showed that the purified microalgal oils consisted of TAG molecules and the purity of isolated microalgal TAG was 100%.

TAG percentage of TFAs for the selected microalga was calculated by the Eq. (2) and the results were shown in Table 1. A great diversity was found in view of TAG percentages among all microalgae. CV-15 gave the lowest TAG percentage (65.1%) (Table 1). Five microalgae species CZ-30412, CV-395, CS, NL-2047 and NS-537 showed TAG percentages of 75.1–78.9% (Table 1). TAGs constituted up to over 80% of TFAs in CP-9, SS, PT-646, ISO-FJ, IG-2307 and NO-IMET1 (Table 1).

Breuer et al. [13] selected nine microalgae strains to analyze biomass, TFAs and TAG production under different cultivation conditions and found that TAG obtained from *C. vulgaris*, *C. zofingiensis*, *I. galbana*, *S. obliquus*, and *P. tricornutum* accounted for over 80% of TFAs under nitrogen starvation condition. The results of NO-IMET1 and NS-537 agreed with the results attained by Ma et al. [7]. Many review papers and experimental works [1,6,54,55] had proved that microalgal TAG usually accounted for over 60% of total microalgae lipids.

3.2. Fatty acid composition of microalgae TAG

The fatty acid compositions of purified microalgae TAGs were determined and the results were presented in Table 2 and Table S1. The fatty acid composition of mature HMTs [16] was also listed. HMTs had 1.3–5.3% MA, 17.0–24.4% PA, 3.9–7.4% SA and 30.3–41.2% SFAs. Although MA, PA and SA had different chemical structures in the carbon chain numbers, they showed the similar nutritional characteristics for infants [11,15,20,21]. It could be seen that five freshwater species (CV-15, CZ-30412, CV-395, SS and CS) did not synthesize MA esterified at the glycerol backbone of TAG (Table S1). Similar results were found in the previous studies [9,10,49]. MA content (0.8%) of CP-9 TAG was lower than that (1.3–5.3%) of HMTs. Six marine microalgae species produced TAGs containing 6.0–23.5% MA that were significantly higher than the upper value of HMTs (Table S1). TAGs synthesized by all microalgae (except CV-395 and NO-IMET1) showed 17.4–25.9% PA that were within the PA range of HMTs. Compared with the HMTs, NO-IMET1 TAG had a much higher PA content (42.2%), while CV-395 TAG gave a lower content of PA (14.6%). PA contents of the selected microalgae strains agreed with the previous studies [9,13,35,49]. The SA contents of five microalgae species (CZ-30412, SS, CS, ISO-FJ and NS-537) were between 4.2 and 4.6%, within the SA range (3.9–7.4%) of HMTs (Table S1). The SA contents of the remaining microalgae species were lower than 3.9% of HMTs (Table S1). For the SFAs content, only four species SS, PT-646, ISO-FJ and IG-2307 gave 31.6–37.5% SFAs of TAGs, within the recorded SFAs value of HMTs. NO-IMET1 and NS-537 yielded higher SFAs contents (51.0–53.7%) of TAGs, and CV-15, CZ-30412, CV-395, CP-9, CS and NL-2047 presented lower SFAs contents (18.4–27.8%) of TAGs. Some observational trials had shown that high PA and/or SFAs contents of HMFs (particularly, PA and SFAs were mainly distributed at the *sn*-1 and 3 positions) affected the absorption of minerals for infancy to some extent [15,16,20]. Results in Table 2 indicated that microalgae TAGs obtained from NO-IMET1 and NS-537 could not be used to directly develop the desirable HMFs for infants.

It was clear that NL-2047 TAG had the highest POA content (47.5%), which agreed with the reported value of POA for NL-2047 in the study of Wen and Chen [35]. TAGs obtained from PT-646, NO-IMET1 and NS-537 comprised about 22% POA; the POA contents of TAGs from IG-2307 and CS were 12.2% and 4.9%, respectively. These results were consistent with the studies of Ma et al. [53], Liu et al. [56] and Harvati et al. [46]. It should be noted that these values were much greater than the one (1.1–3.5%) of HMTs (Table S1). The POA contents (1.2–3.1%) of TAGs for five microalgae species (CZ-30412, CV-395, CP-9, SS and ISO-FJ) were close to the POA value of HMTs. CV-15 TAG achieved the lowest POA content (0.8%), likely due to the low activity of palmityl desaturase [6]. OA as the main monounsaturated fatty acids (MFAs) of HMTs was metabolized to provide sufficient energy for infants via mitochondrial fatty acid β-oxidation [15,16,18]. CV-395 TAG presented the highest OA content (51.3%), agreeing with the result (48.2%) of Griffiths et al. [49]. Moreover, this value for CV-395 was 8.9% higher than the upper limit (42.4%) of OA content for HMTs (Table 2). The OA contents (28.7–39.4%) of TAGs from five microalgae species CZ-30412, SS, CS, ISO-FJ and IG-2307 were in the OA range of HMTs; whereas, CV-15, CP-9, NL-2047, NO-IMET1 and NS-537 TAGs achieved lower OA contents (Table 2). For the MFAs, two species (CV-395 and NL-2047) showed higher MFAs contents of TAGs than the upper limit (45.4%) of MFA content for HMTs. The MFAs contents of CV-15 and CP-9 TAGs were lower than 31.9% of HMTs. Eight species (except CV-15, CV-395, CP-9 and NL-2047) could produce 31.8–45.8% MFAs of TAGs, within the accepted MFAs range of HMTs.

As essential fatty acids (EFAs), LA and ALA played critical roles in the growth and development of infants [15,57]. In the tested species, CV-15 TAG had the highest content of LA (41.1%), which was 2.0-fold of the upper limit (20.3%) of LA content for HMTs. CZ-30412, CP-9 and SS TAGs also showed higher LA contents (21.7–29.4%). The LA content within the range of 10.5–20.3% was observed in CV-395 TAG (16.2%). The other species gave lower LA contents (< 10.5%) as shown in the Table 2. For ALA, six freshwater microalgae species achieved over 6% ALA of TAGs, which were much higher than the upper limit of ALA content for HMTs. As given in the Table 2, the ALA values for six marine microalgae species could be acceptable, because some commercial infant formulas had 4.8% ALA based on the total fatty acids. However, high LA and/or ALA contents in infant formulas inhibited the synthesis of long chain PUFAs (LC PUFAs) (e.g. arachidonic acid ($C_{20:4n-6}$, AA) and DHA) [57]. In view of LA and ALA, TAG obtained from ISO-FJ could be the good source of EFAs for infants.

Stearidonic acid ($C_{18:4n-3}$, SDA) as a unique fatty acid could be synthesized by CZ-30412, SS, CS, ISO-FJ and IG-2307. Previous studies also gave similar results [10,49,56]. HMTs did not contain SDA (Table 2). However, SDA like ALA and docosahexaenoic acid ($C_{22:6n-3}$, DHA) could perform many health-beneficial functions [58]. Recently, many researchers had attempted to develop new HMFs containing SDA [18,59]. This evidence suggested that oils with SDA could be used to develop HMFs.

Six marine microalgae species could produce LC PUFAs such as AA, EPA and DHA. Among these species, only NL-2047, NO-IMET1 and NS-537 species could synthesize AA. All marine microalgae TAGs had 0.8–17.6% EPA; particularly, NL-2047 (12.6%) and PT-646 (17.6%) TAGs showed high EPA contents as presented in Table S2. Some studies had revealed that high intake of EPA (> 20 mg kg^{-1} d^{-1}) could interfere with the absorption of bioactive fatty acids (e.g. LA, ALA, AA, DHA) in the baby's early life [15,22,57,60]. These results indicated that NL-2047 and PT-646 TAGs could not be directed exploited to be HMFs for infants. Two *Isochrysis* species could produce DHA. DHA was indispensable for brain growth where it modulated neuronal firing and signaling [19,61].

Moreover, Table S1 also presented the myristoleic acid ($C_{14:1}$, MOA), hexadecadienoic acid ($C_{16:2}$, HDA) and hexadecatrienoic acid ($C_{16:3}$, HTA) contents of some microalgae TAGs. Only microalga NL-2047 could synthesize MOA (1.0%), which was consistent with the previous study [35]. TAGs from six freshwater algal species and NL-2047 had 0.8–6.6% HDA. For HTA, six freshwater algal species TAGs had 1.0–9.1% HTA (Table S1). To date, there are no studies concerning the HMFs containing HDA and HTA. Further studies are needed to investigate the safe assessment and biological functions of HDA and HTA for infants.

3.3. Fatty acid positional distribution in microalgal TAG

Many studies [15–17,20,62] had proven that the composition of fatty acid at the *sn*-2 position of HMFs or HMTs was associated with the assimilation and metabolism of the TAG and the growth of the infant. SFAs at the *sn*-2 position could improve the calcium absorption of babies [15,20]. PUFAs at the *sn*-2 position could increase these PUFAs accumulation, modify structural lipid of infant tissues and improve the immunity and development [58,63–65]. Thus, it was necessary to further analyze the fatty acid positional distribution in these microalgae TAGs for the evaluation of HMFs.

Table 3 and Table S2 showed the fatty acid composition at the *sn*-2 position of microalgae TAGs. To the best of our knowledge, in the tested algal strains, only a few studies reported the fatty acid positional distribution of microalgae TAGs obtained from *C. zofingiensis* and

Table 3
The compositions (wt%) of four fatty acid species, SFAs, MFAs and PUFAs at the *sn*-2 position of TAGs and HMTs.

Species	$C_{16:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3n-3}$	ΣSFAs[1]	ΣMFAs[2]	ΣPUFAs[3]
CV-15	3.8 ± 0.2^{h}	12.7 ± 0.5^{f}	64.0 ± 5.4^{a}	8.1 ± 0.3^{b}	6.3 ± 0.6^{i}	14.7 ± 0.9^{e}	75.6 ± 5. 9^{a}
CZ-30412	7.5 ± 0.5^{g}	27.3 ± 2.1^{e}	45.4 ± 3.0^{b}	6.2 ± 0.6^{cd}	8.8 ± 0.4^{h}	28.9 ± 1.3^{d}	62.1 ± 3.0^{c}
CV-395	1.8 ± 0.1^{j}	69.0 ± 4.3^{a}	17.3 ± 1.1^{e}	1.5 ± 0.1^{f}	2.2 ± 0.2^{k}	71.2 ± 3.2^{a}	26.2 ± 1.8^{fg}
CP-9	9.5 ± 1.4^{f}	12.4 ± 1.0^{f}	39.8 ± 2.8^{b}	6.1 ± 0.5^{cd}	14.6 ± 0.5^{f}	14.1 ± 1.9^{ef}	71.5 ± 2.9^{b}
SS	9.6 ± 0.7^{f}	34.7 ± 2.1^{d}	36.8 ± 2.4^{c}	6.8 ± 0.5^{c}	12.4 ± 0.4^{g}	38.6 ± 2.7^{c}	49.6 ± 2.7^{d}
CS	3.1 ± 0.2^{i}	53.1 ± 2.5^{b}	24.5 ± 0.2^{d}	8.6 ± 0.2^{a}	4.1 ± 0.2^{j}	55.0 ± 2.5^{b}	40.5 ± 2.6^{e}
NL-2047	33.6 ± 2.0^{c}	1.1 ± 0.1^{j}	1.6 ± 0.2^{k}	0.4 ± 0.0^{j}	36.2 ± 2.1^{d}	53.2 ± 1.0^{b}	10.2 ± 0.4^{h}
PT-646	18.5 ± 0.8^{e}	43.6 ± 2.6^{c}	0.7 ± 0.1^{i}	1.0 ± 0.1^{g}	18.6 ± 0.5^{e}	70.2 ± 2.9^{a}	7.0 ± 0.3^{i}
ISO-FJ	36.6 ± 2.1^{c}	4.6 ± 0.2^{h}	7.5 ± 0.4^{f}	5.9 ± 0.2^{d}	61.0 ± 2.9^{b}	8.0 ± 0.4^{g}	30.2 ± 2.1^{g}
IG-2307	28.8 ± 0.9^{d}	4.9 ± 0.2^{h}	3.1 ± 0.1^{h}	5.1 ± 0.2^{e}	51.1 ± 3.0^{c}	11.6 ± 0.8^{f}	37.3 ± 1.2^{f}
NO-IMET1	73.3 ± 3.7^{a}	3.7 ± 0.2^{i}	1.9 ± 0.1^{j}	0.3 ± 0.1^{i}	82.5 ± 2.9^{a}	12.7 ± 1.0^{ef}	4.0 ± 0.3^{k}
NS-537	43.2 ± 2.1^{b}	6.1 ± 0.2^{g}	4.6 ± 0.3^{g}	0.7 ± 0.0^{h}	79.8 ± 3.1^{a}	12.2 ± 1.0^{f}	8.0 ± 0.2^{j}
HMTs [A]	41.8–58.8	11.3–21.4	9.7–17.8	0.3–1.8	57.1–70.9	14.9–24.7	13.2–22.7

a, b, c, d, e, f: The mean values in the same column were significantly different among the microalgae species in the same culture conditions ($p < 0.05$).
[1] SFAs contain $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$;
[2] MFAs contain $C_{14:1}$, $C_{16:1}$ and $C_{18:1}$;
[3] PUFAs contain $C_{16:2}$, $C_{16:3}$, $C_{18:2}$, $C_{18:3}$, $C_{18:4}$, $C_{20:4}$, $C_{20:5}$, $C_{22:5}$ and $C_{22:6}$.
[A] Adapted from Wang et al. [16].

Nannochloropsis strains [7,8,54]. These studies had stated that, in the Kennedy pathway, diacylglycerol acyltransferases (DGAT) from different algal species showed different substrate selectivity towards fatty acids to esterify with the hydroxyl groups at the *sn*-1(3) and *sn*-2 positions of glycerol backbone [3,6,8]. In this study, the results of CZ-30412 and NO-IMET1 were consistent with the data of previous studies [8,54].

For MA, six marine microalgae species TAGs had 1.5–35.2% MA at the *sn*-2 position; the MA value of NO-IMET1 was within the MA *sn*-2 range of HMTs (Table S2). For PA at the *sn*-2 position, the values of all tested species (except NO-IMET1 and NS-537) were less than the lower value of PA *sn*-2 content of HMTs; while NO-IMET1 TAG *sn*-2 yielded 73.3% PA that was much higher than the upper limit of PA *sn*-2 content for HMTs (Table 3). The value of NS-537 TAG *sn*-2 PA was within the PA *sn*-2 range of HMTs. For SA, the contents of SA *sn*-2 of TAGs for seven algal species (CV-15, CZ-30412, CS, NL-2047, PT-646, NO-IMET1 and NS-537) were in the range of 0.6–2.7% for HMTs (Table S2). In view of the SFAs *sn*-2, nine species (except ISO-FJ, NO-IMET1 and NS-537) achieved low SFAs *sn*-2 contents (< 57.1%). On the contrary, NO-IMET1 and NS-537 showed high SFAs *sn*-2 contents (> 70.9%). As aforementioned, the low content of SFAs *sn*-2 could reduce the absorption of minerals in the stomach and the small intestine of infants or babies. On the other hand, SFAs were overloaded at the *sn*-2 position (> 80%), leading to the decrease in PUFAs. Thus, based on the SFAs profiles, ISO-FJ TAG could be the potential source for HMFs.

POA contents of TAG *sn*-2 for six freshwater species and ISO-FJ were in the range of 1.2–5.2% (Table S2). OA contents of TAGs *sn*-2 for CV-15 (12.7%) and CP-9 (12.4%) were between 11.3 and 21.4% (Table 3). Surprisingly, the contents of MFAs at the *sn*-2 position of all microalgae TAGs were beyond the range of 14.9–24.7% for HMTs. In fact, MFAs of typical HMFs were mainly distributed at the *sn*-1 and 3 positions of the glycerol backbone, which improved the metabolism of MFAs to generate energy [15,16,23]. For the MFAs *sn*-2, HMFs with low content of MFAs *sn*-2 could improve the absorption of PUFAs when the SFAs *sn*-2 content was in the range of 57.1–70.9% [16,17,21]. In this case, TAG from CV-15, CP-9, two *Isochrysis* species and two *Nannochloropsis* species could be used to develop HMFs.

The LA *sn*-2 contents of all microalgae TAGs (except CV-395) were beyond the LA *sn*-2 range of HMTs (Table 3). The ALA *sn*-2 contents of CV-395, NL-2047, PT-646, NO-IMET1 and NS-537 TAGs were within the ALA *sn*-2 range of 0.3–1.8% for HMTs (Table 3). With respect to LA and ALA at the *sn*-2 position, CV-395 TAG seemed to be the suitable candidate to meet the excepted values of HMTs. NL-2047 TAG was a good source of AA for HMFs (Table S2). HMFs containing SDA and DHA at the *sn*-2 position could be derived from TAGs produced by two *Isochrysis* species (Table S2).

To verify the above mentioned results by GC–MS, a ^{13}C NMR technique was employed to record the microalgae TAGs. As shown in Fig. 2, the resonances of TAGs acyl chains (carbonyl carbons) were in the range from 172.20 to 173.45 ppm. The resonances of SFAs (e.g. MA, PA, SA, etc.) chains at the *sn*-1(3) and *sn*-2 positions of TAGs were 173.39–173.42 ppm and 173.98–173.01 ppm, respectively. Similar results were reported in a previous study [44]. The chemical shifts of Δ9 fatty acyl residues (e.g., MOA, POA, OA, LA and ALA) of TAGs recorded at the peaks of 173.35–173.39 ppm for the *sn*-1(3) positions and the peaks of 172.94–172.98 ppm for the *sn*-2 position (Fig. 2). The fatty acyl residues of Δ6 (SDA), Δ5 (AA and EPA) and Δ4 (DHA) at the *sn*-1(3) positions of TAGs were 173.18–173.21, 173.13–173.15 and 172.67 ppm, respectively; while they were 172.77–172.81, 172.70–172.74 and 172.28–172.29 ppm at the *sn*-2 position, respectively (Fig. 2). These results agreed with the previous studies recording the fish oil and microalgae oil by the ^{13}C NMR analysis [44]. Moreover, the results of ^{13}C NMR agreed with the results in the Table 3 by GC–MS. For example, the mole ratio of *sn*-1(3) SDA to *sn*-2 SDA was calculated to be 1: 2.0 (((6.6 × 3–13.1) ÷ 259): (13.1 ÷ 259) ≈ 1:2.0) (the molecular mass of SDA was 259 (276–17 = 259)) using the data of ISO-FJ TAG (because the overlapping peaks containing different fatty acid species were existed in all microalgae TAGs). The mole ratio of *sn*-1(3) SDA to *sn*-2 SDA by ^{13}C NMR estimation was 1:2.1 (Fig. 2), which was very close to the ratio by GC–MS. Besides, in Fig. 2, TAGs obtained from six freshwater strains experienced very small peaks at the 172.98–173.00 ppm and broad peaks at the 172.94–172.97 ppm, because of the low SFAs content and high unsaturated fatty acids (UFAs) at the *sn*-2 position as given in the Table 3. Thus, the results in Fig. 2 and Table 3 showed that the employed techniques could support each other and determine the fatty acid positional distribution of microalgae TAGs.

3.4. Evaluation of microalgae TAG for HMFs

Based on the data in the Tables 2–3 and Tables S1-S2, it was difficult to determine the suitable TAG(s) for HMFs. Thus, the established model [16] was applied to evaluate microalgae TAGs and lard by the Eqs. (3)–(5) in view of the fatty acid composition and positional distribution. The results were shown in Table 4. It could be seen that PT-646 TAG gave the lowest value of G (29.0); meanwhile, the G values of NL-2047 TAG was 32.4. These results showed that these two oils were the least similar to HMTs and lard, indicating that they could not be further

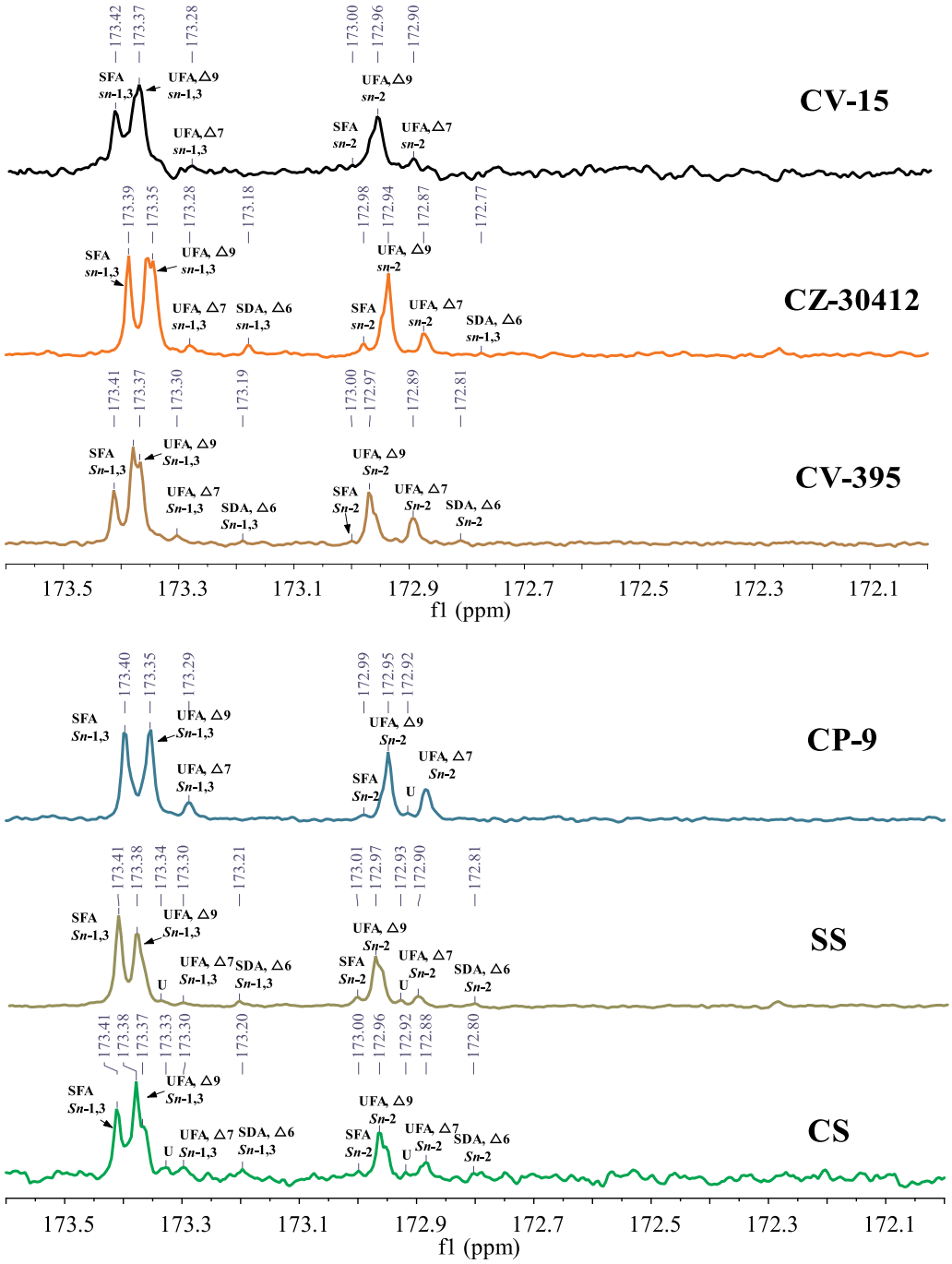

Fig. 2. The glycerol backbone carbon region 173.6–172 ppm of the purified microalgae TAGs by ^{13}C NMR analysis. Note: the unknown fatty acid(s) was/were marked as "U".

developed as acceptable HMFs. Actually, these oils were superior sources for adult consumption [15,32], due to the high EPA content (Table 2).

Among the freshwater microalgae species, CV-15 TAG gave the lowest value of G (32.7). For CP-9 TAG, the values of G1, G2 and G was 34.6, 5.6 and 40.2, respectively. Compared with CV-15 and CP-9, CZ-30412, CV-395, SS and CS TAGs had higher values of G_1 (39.9–44.4) that were close to those of local infant formulas (Table 4). However, the G_2 values of freshwater microalgae TAGs were significantly lower than those of marine microalgae TAGs, lard and local infant formulas (Table 4). This could be ascribed to their low contents of PA and high contents of OA, LA and ALA (Tables 2 and 3). The results showed that these oils could not be used to directly develop as HMFs.

The G values of NO-IMET1 and NS-537 TAGs were 44.8 and 55.0, respectively; which were significantly lower than the value of lard (Table 4). It was found that the G_2 values of NO-IMET1 and NS-537 were close to that of lard. However, they had low G_1 values. To increase the G_1 values of these two oils resembling HMTs, enzymatic method mediated with a *sn*-1,3 specific lipase could be adopted to modify *Nannochloropsis* TAGs to restructure the fatty acids at the *sn*-1 and 3 positions with desired fatty acids [18,19,23]. As carried out in our recent work [31], four commercial immobilized lipases were selected to catalyze TAG of *N. oculata* to decrease the content of PA at the *sn*-1 and 3 positions. In this case, TAGs from CV-15, CZ-30412, CV-395, CP-9, SS and CS could be used to prepare free fatty acids rich in OA, LA and/or ALA for modifying the *Nannochloropsis* TAGs to increase the values of

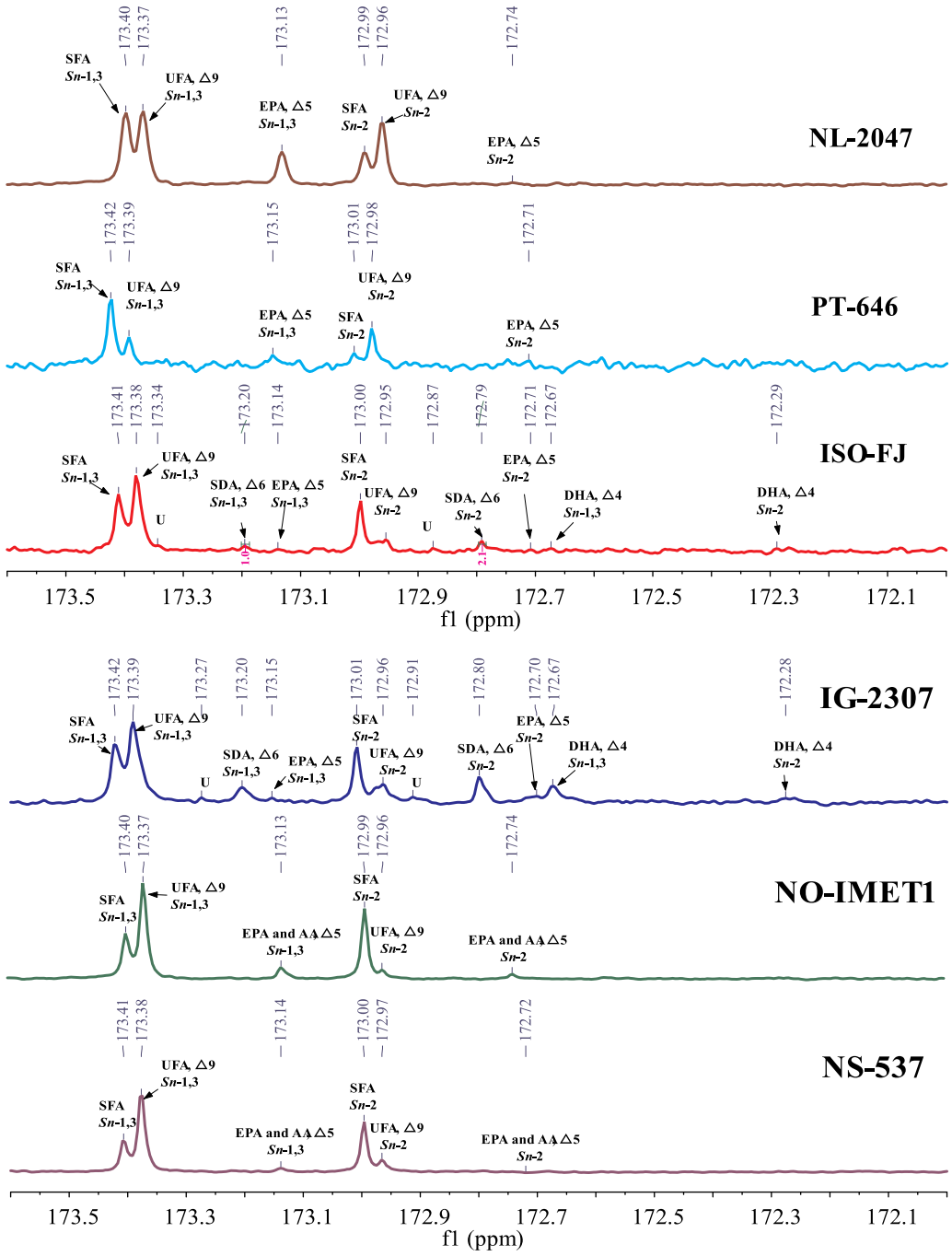

Fig. 2. (*continued*)

G_1 and G by enzymatic acidolysis with the *sn*-1,3 specific lipase. Additionally, HMFs could be produced by enzymatic interesterification of *Nannochloropsis* TAG and freshwater microalga TAG to restructure fatty acids at the *sn*-1(3) and 2 positions for HMFs [15,18]. Of course, the HMFs mediated with enzymatic reaction should be re-evaluated for application of infant formula.

Interestingly, among the tested microalgae TAGs, ISO-FJ TAG showed the highest value of G (69.2), which was higher than the value (61.2) of IG-2307 TAG. However, no significant differences in the G_1 values of ISO-FJ TAG and IG-2307 TAG were observed; meanwhile, the G2 value of ISO-FJ TAG was very close to the one of IG-2307 TAG. Although *Isochrysis* TAGs showed lower G values in comparison to lard, *Isochrysis* TAGs contained some bioactive fatty acids (e.g. SDA and DHA) that did not exist in lard. Moreover, the G value of ISO-FJ TAG was in the range of 67.3–78.2 for local infant formulas; whereas, the G value of IG-2307 TAG was a bit lower than the ones of infant formulas. These results showed that TAGs obtained from two *Isochrysis* species were the most promising source to develop HMFs resembling HMTs. In order to further improve the quality of *Isochrysis* TAGs and promote the application of such superior oil in infant formulas, the following studies are needed to further investigate: (1) The cultivation conditions of *Isochrysis* species are optimized to achieve the balanced fatty acid of TAG resembling HMTs; (2) The safety assessment of *Isochrysis* TAGs for animal mode and infant should be evaluated.

3.5. DSC melting and crystallization records

The characteristics of melting and crystallization profiles of desired

Table 4
Scores of the similarity degree of microalgae TAG, lard and commercial infant formulas according to the Eqs.(3)–(5).

TAGs	G_1	G_2	G
CV-15	27.3 ± 1.8^{c}	5.4 ± 0.7^{e}	32.7 ± 1.7^{f}
CZ-30412	40.8 ± 2.5^{a}	9.7 ± 0.7^{d}	50.5 ± 1.9^{c}
CV-395	39.9 ± 1.7^{a}	9.2 ± 0.4^{d}	49.1 ± 1.4^{c}
CP-9	34.6 ± 2.1^{b}	5.6 ± 0.4^{e}	40.2 ± 1.8^{e}
SS	44.4 ± 2.4^{a}	5.6 ± 0.3^{e}	50.0 ± 2.0^{c}
CS	40.5 ± 2.4^{a}	0.5 ± 0.1^{f}	41.0 ± 1.8^{e}
NL-2047	1.2 ± 0.2^{f}	31.2 ± 1.6^{b}	32.4 ± 1.3^{f}
PT-646	17.6 ± 1.1^{d}	11.4 ± 0.5^{c}	29.0 ± 1.1^{g}
ISO-FJ	38.5 ± 3.1^{ab}	30.7 ± 1.6^{b}	69.2 ± 3.0^{a}
IG-2307	32.5 ± 2.8^{b}	28.7 ± 1.4^{b}	61.2 ± 3.5^{b}
NO-IMET1	6.6 ± 1.0^{e}	38.2 ± 3.1^{a}	44.8 ± 1.8^{d}
NS-537	14.0 ± 1.2^{d}	41.0 ± 3.8^{a}	55.0 ± 4.0^{b}
Lard [A]	37.8	38.1	75.89
Infant formulas [A]	41.1–47.2	20.2–37.1	67.2–78.2
HMTs [A]	50.0	50.0	100.0

a, b, c, d, e, f: The mean values in the same column were significantly different among the microalgae species in the same culture conditions ($p < 0.05$).
[A] Adapted from Wang et al. [16].

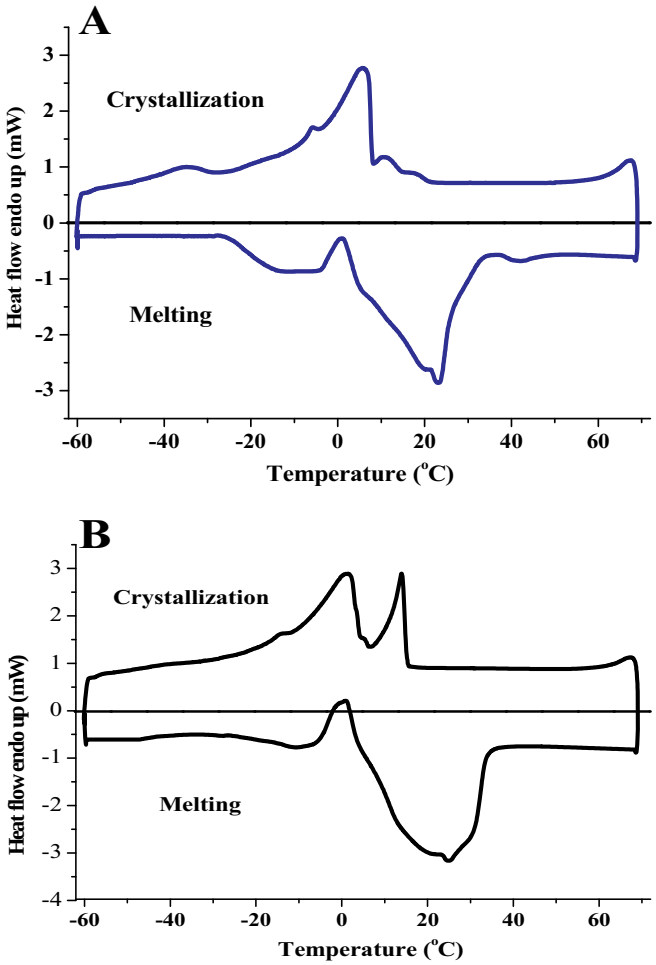

Fig. 3. Melting and crystallization curves of ISO-FJ TAG (A) and ISO-2307 TAG (B). The parameters of DSC were: 1) the crystallization program: the sample (5 mg) was cooled from 70 to −60 °C at 10 °C/min; 2) the melting program: the sample (5 mg) was heated from −60 to 70 °C at 10 °C/min.

oils are useful for the sustainable development of such oils. Fig. 3 showed the melting and crystallization profiles of the *Isochrysis* TAGs in the range from −60 to 70 °C. ISO-FJ TAG experienced the melting profile from −28.5 to 35.8 °C, and the crystallization from – 47.5 to 18.8 °C (Fig. 3A). Similar melting (− 32.0 to 36.0 °C) and crystallization (−31.9 to 15.8 °C) behaviors could be observed in the IG-2307 TAG (Fig. 3B). The phenomenon could be explained by noting that ISO-FJ TAG and IG-2307 TAG had similar fatty acid composition (Table 2). For lard, Cheong et al. [45] showed that lard recorded the melting curve in the range from −18.0 to 42.0 °C and crystallization curve from −36.0 to 23.0 °C. Compared with lard, *Isochrysis* TAGs had lower melting and crystallization temperatures, likely due to the low content of SFAs (Table 2). The melting and crystallization behaviors of *Isochrysis* TAGs were similar to those of HMTs (melting, −10.0 to 37.0 °C; crystallization, −45.0 to 20.0 °C) in the study of Zou et al. [62]. Thus, these details about the physical characteristics of *Isochrysis* TAGs were useful for their plasticity and storage range for developing the sustainable and natural sources of HMFs in the infant formula.

4. Conclusion

Our study represented the first report evaluating the microalgae TAGs of twelve microalgae species for HMFs products. Results showed that, among the tested species, the characteristics of TAGs obtained from *Isochrysis* species (*Isochrysis* sp. and *I. galbana* 2307) could be suitable for HMFs in the application of infant formula with respect to fatty acid composition, fatty acid positional distribution of TAG, and melting and crystallization properties. In all, our work will open a new way to develop high-value microalgae bioproducts in the application of infant formula.

Statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights applicable.

Author contributions

Yongjin He, Tao Wu, Han Sun, Peipei Sun, Bin Liu, Mingfang Luo and Feng Chen conceived the conception and design of this study. Yongjin He, Tao Wu, Han Sun and Peipei Sun preformed the experiments and collected the data. Bin Liu and Mingfang Luo analyzed the GC–MS data. Yongjin He, Tao Wu, Han Sun, Peipei Sun, Bin Liu and Mingfang Luo wrote the manuscript and Feng Chen revised it. All authors read and approved the final manuscript to be submitted to “Algal Research”.

Conflict of interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2018.11.004.

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