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Original article

Fatty acid and triglyceride molecular species of milk fat fractionated by short-path molecular distillation

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Summary Fractionation is important for the application of milk fat (MF). In this study, the contents of fatty acid (FA) and triglyceride (TAG) of MF and its fractions distilled by short-path molecular distillation (SPMD) were detected. The results showed that a total of 19 FAs and 109 TAG molecular species were detected. Moreover, the short-chain saturated FA, medium-chain saturated FA, low-molecular-weight TAG, medium-molecular-weight TAG, S_3 (TAG molecular species with three saturated FAs) and L_2S (TAG molecular species with two long-chain FAs and one short-chain FA) were easily accumulated in the distillate, and the percentage of these components all increased first and then decreased during the whole distilling process. Compared with the distillate, polyunsaturated FA (PUFA) and high-molecular-weight TAG (HMW-TAG, with the carbon numbers: 41–54 and molecular weight: 704–888) were enriched in the residue, and the increasing ratio of PUFA and HMW-TAG was 393.75% and 8.58% respectively. Further analysis showed that the 16:0/4:0/16:0, 16:0/4:0/14:0, 16:0/12:0/4:0, etc. were the discrepant TAG molecular species during SPMD. Therefore, these results demonstrated that different fractions of MF could be obtained by adjusting the fractionation temperature, and it also would provide more important theoretical guidance for regulating MF fractionation, enriching the nutritional information of MF fractions.

Keywords Fatty acid, Molecular species, Short-path molecular distillation, Triglyceride.

Introduction

Undoubtedly, milk fat (MF) is an important element of milk, which accounts for 3%–5% of total milk. The composition of MF mainly consisted of triglyceride (TAG), with 98% of total MF, and the remaining components include glycerol diester, glycerol ester, free fatty acid (FFA), phospholipid, a small amount of sterol and fat-soluble vitamins (Tzompa-Sosa *et al.*, 2018). About 200 kinds of TAG molecular species have been identified in MF based on the abundant species of FA, with more than 400. Moreover, the unsaturation, length of carbon chains and structure and position of double bonds of FA all make TAG exhibit differences in homogeneous polycrystals, melting points, etc. Some physiological active substances, which are beneficial to human health, such as conjugated linoleic acid and sphingomyelin, are contained in the MF, and these components besides enhancing immunity of the human body, also regulate cardiovascular and gastrointestinal diseases (Agyare & Liang, 2021; Wei *et al.*, 2022).

The melting point of MF ranges from −40 to 50 °C, resulting in the stability of MF practical applications, such as the instability of plasticity and hardness during the process of crisp pastries (Lopez *et al.*, 2006). Therefore, physical modification technology is used to obtain MF components with different melting points to develop their application value. Dry fractionation is widely used because of its simple operation characteristics, and the Tirtiaux and Desmet methods are the most common approaches (Boudreau & Arul, 1993; Mohan *et al.*, 2021). Studies have reported that following dry fractionation, the short-chain saturated FA (SC-SFA) and unsaturated FA (UFA) have accumulated in the liquid fraction (Wang *et al.*, 2019; Si *et al.*, 2023). In recent decades, some new technologies,

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such as supercritical fluid extraction, ultra-high-pressure, short-path molecular distillation (SPMD) are studied in the production of MF to obtain more fractions with different properties (Mohan *et al.,* 2021). Compared with other separation methods, SPMD is an effective liquid–liquid separation technology, which is dependent mainly on the different mean free paths of the various components in distilling substrate. The SPMD has a lot of advantages, such as low pressure, short heating time and high separation efficiency, so it is especially suitable for oil, heat-sensitive substances and bioactive substances (Boudreau & Arul, 1993; Mahrous & Farag, 2021). Campos *et al.* (2003) reported that the distillate yield increased from 0.30% to 42.70% (wt/wt) when the distillation temperature increased from 125 to 250 °C, and the short- and medium-chain FA and low-molecular-weight TAG were accumulated in the distillate, while the percentage of long-chain saturated and unsaturated fatty acid and high-molecular-weight TAG in the residue was higher (Campos *et al.,* 2003). Furthermore, another study verified that the sensory properties of distillation products were similar to those of unsettled MF (Berti *et al.,* 2018).

The TAG molecular species play an important role in the processing and nutrient characteristics of MF, and more and more studies had focused on the TAG stereochemical structure of MF in recent years. Wang *et al.* (2022) reported that there are 116 individual TAG molecular species detected in the MF (Wang *et al.,* 2022), and another study reported that there were more than 100 TAG molecular species in mature bovine milk (Liu *et al.,* 2017). In addition, the TAG was influenced by the season and region, which might be caused by the composition of raw material milk, grow lush grass, normal nutrition feed, the age and lactation period of cows, etc. (Mohan *et al.,* 2021). For the distilling fractions of MF, Arul *et al.* (1988) and Campos *et al.* (2003) have reported that there were 16 and 19 TAG molecular species, with total carbon numbers, such as C24, C36, C44 and C52, existed in the MF and its distilling products respectively (Arul *et al.,* 1988; Campos *et al.,* 2003). However, these results are limited for the current nutritional requirements of people, so it is necessary to obtain more TAG molecular species and information about the stereochemical structure and FA composition of TAG and to further explore the content variation of TAG molecular species during the distilling process. In this study, the anhydrous MF (AMF) was separated by SPMD at 165, 180, 190, 200, 210, 225 and 240 °C, and then the distillate and residue were detected for FA and stereochemical structure of TAG. These results will provide more theoretical reference for MF fractionation, and further explore nutritional information for the application of distilling fraction of MF.

Materials and method

Materials

Anhydrous MF (AMF; fat content ≥99.99%) was purchased from New Zealand Milk Brands Limited; and 37 fatty acid methyl ester (37 FAMEs) was obtained from Shanghai Anpu Experimental Technology Co., Ltd. HCL, methanol, hexane, acetonitrile, 98% formic acid and ammonium formate all were bought from Fisher Scientific, Inc. (Pittsburgh, PA, USA). The internal standard 1,3(d5)-diheptadecanoyl-2-heptadecenoyl-glycerol was purchased from Avanti (Birmingham, AL, USA).

Short-path molecular distillation

The AMF was cut into small pieces with 1 cm^3 and then heated at 60 °C until melted. After that, the AMF sample was transferred to the SPMD machine (KDL-5, UIC, Germany). For the specific setting condition, the inlet temperature (oil bath), inlet speed, vacuum degree and film scraping speed were set as 60 °C, 2.0 mL min^{-1}, 1×10^{-3} mbar and 150 rpm respectively. The distillation temperatures were 150, 165, 180, 190, 200, 210, 225 and 240 °C. Furthermore, the equipment should be thoroughly cleaned with absolute ethanol before every distillation temperature change, and the ethanol contaminated on the inner wall of the instrument evaporated naturally to avoid contaminating the fraction. Finally, the AMF, light-phase (distillate) and heavy-phase (residue) fractions were collected and stored at −80 °C for FA and TAG analyses.

Fatty acid analysis

The FA analysis was referred to in previous studies (Zhu *et al.,* 2022; Si *et al.,* 2023). Briefly, the samples (30 mg), MeOH (2 mL), solution (HCL/MeOH, 1/3, 2 mL) and n-hexane (1 mL) were added into the glass tube. The mixture was vortexed for 2 min and heated by water bath (100°C) for 1 h, and then the tube was stood at room temperature and 2 mL pure water was added subsequently. In the next step, the mixture was shaken thoroughly for 1 min and centrifuged for 5 min (1500 ***g***). Finally, the liquid supernatant was obtained and analysed using gas chromatograph flame ionisation detector (GC-FID, Agilent 8890B, USA) with the DB-23 column (60 m × 0.25 mm × 0.25 µm; Sigma-Aldrich). The setting condition of GC-FID was as follows: the temperature of the injection port and FID were set as 250 °C. For the temperature programming, the initial temperature was 50 °C and remained for 1 min, and it then increased to 175 °C with a speed rate of 20 °C min^{-1}. After that, the temperature of the column container continually increased to 230 °C at the rate of 1.3 °C min^{-1} and kept for 5 min.

Triglyceride molecular species analysis

The detection method for TAGs was described in our previous study (Zhao *et al.,* 2018; Wang *et al.,* 2022). The samples (5 mg) were dissolved in 1 mL solution mixed with chloroform and MeOH (2:1, V/V), and then it was diluted 50-folds with the mixed solution (acetonitrile: water (7:3, V/V)) containing 10 mM ammonium formate and 0.1% formic acid. The mobile phases A and B of ultra-high-performance liquid chromatography (UPLC, I-class ACQUITY, Waters Corporation, Milford, USA) were composed of isopropanol:acetonitrile (9:1, V/V) and acetonitrile:water (7:3, V/V), respectively, and they all contained 10 mM ammonium formate and 0.1% formic acid. The BEH C18 column (1.7 μm, 2.1 mmID × 100 mm, Waters Corporation, Milford, USA) was used, and the column temperature was set as 60 °C. For the elution programme, the flow rate was 0.3 mL min^{-1}, and the percentage of solvent B increased from 70% to 85% at 28 min, then it was changed to 70% at 28.1 min and kept for 1.9 min. Finally, sample solution (5 μL) was injected and analysed using mass spectrum (MS, API 4500 Q-Trap, AB SCIEX, Framingham, MA, USA).

The parameters of MS were as follows: the electrospray voltage, curtain gas, ion source gas 1 and ion source gas 2 were 5500 V, 25, 45 and 50 respectively. For the detection mode, the multiple-reaction monitoring information-dependent acquisition enhancer ion (MRM-IDA-EPI) was selected, which included 80 of de-clustering voltage, 10 of inlet voltage, 13 of outlet voltage, 30 of collision energy and 15 of collision energy dispersion. The retention time of each TAG molecular species on the column and the corresponding fragment ions were detected, and 17:0/17:1/17:0 (d5) was used as the internal standard for the quantitative analysis of specific TAG.

Statistical analysis

Each data was expressed by mean values ± SD, and all samples were detected three times. One-way ANOVA and Duncan multiple-range test were used to analyse the significant differences among groups ($P < 0.05$) using IBM SPSS Statistics 22 (SPSS, Chicago, IL, USA). The principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) performed by SIMCA-P software (14.1, Demo Umetrics, Umea, Sweden) were used to further find the different TAG molecular species during SPMD.

Results and discussion

The yield of distillate

Distillation temperature played a key role in the process of SPMD (Arul *et al.,* 1988). The yields of light-phase fractionated products at different distillation temperatures are shown in Fig. 1. When the distillation temperature was 165 °C, the lower-molecular-weight component of MF was distilled and the yield was low, which was only 1.19% of AMF. The yield of distillate increased sharply from 180 °C with the increase in distillation temperature, and the yield of distillate increased from 2.39% at 180 °C to 37.42% at 240 °C, which was similar to the results reported in a previous study (Campos *et al.,* 2003). Furthermore, to know the correlation between the yield of distillate and distilling temperature, a regression analysis was conducted and it was calculated by the formula: $Y = 0.9052e^{0.5401x}$ (Y: the yield of distillate and x: the distilling temperature), $R^2 = 0.9793$.

Fatty acid of distillation products

A total of 18 FA species were detected in MF and its distilling products at different distillation temperatures (Tables 1 and 2). Among these FA species, palmitic acid (C16:0), myristic acid (C14:0), stearic acid (C18:0) and oleic acid (C18:1n9c) were the major FAs, accounting for 27.00%, 14.32%, 9.68% and 17.47% of total FA in AMF respectively. The percentage of FA in distillate and residue was affected significantly by the SPMD temperature when compared with the AMF. For the distillate, the percentage of short- and medium-chain saturated FA (SC-SFA and MC-SFA) all increased sharply from 4.02% and 18.96% to 6.30% and 37.69% of total FA at 165 °C, respectively, and then they all declined continuously to 4.84% and 21.84% of total FA

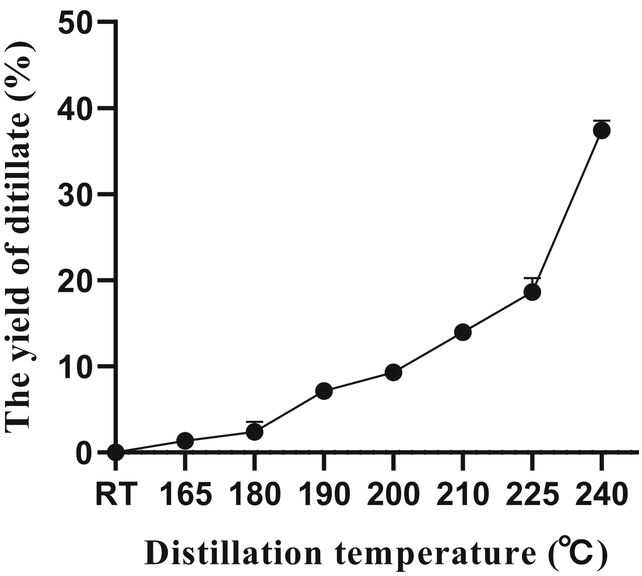

Figure 1 The yield of distillate at different distillation temperatures. RT, room temperature.

Table 1 Fatty acids in the distillate of each temperature

Fatty acids	Distillation temperature (°C)							
	Room temperature	165	180	190	200	210	225	240
C4:0	4.02 ± 0.02a	6.30 ± 0.08b	5.98 ± 0.07c	5.54 ± 0.06d	5.31 ± 0.19ef	5.00 ± 0.19f	4.81 ± 0.21f	4.84 ± 0.06f
C6:0	4.35 ± 0.09a	7.99 ± 0.00b	7.63 ± 0.02c	6.76 ± 0.01d	6.42 ± 0.13ef	6.34 ± 0.13f	6.20 ± 0.35f	5.57 ± 0.10g
C8:0	2.53 ± 0.03a	6.24 ± 0.03b	5.73 ± 0.02c	3.96 ± 0.00d	3.66 ± 0.04e	3.45 ± 0.04ef	3.24 ± 0.10f	3.08 ± 0.06g
C10:0	5.19 ± 0.04a	11.07 ± 0.07b	10.36 ± 0.04c	7.50 ± 0.02d	6.96 ± 0.05e	6.55 ± 0.04f	5.94 ± 0.06 g	5.68 ± 0.08g
C11:0	0.09 ± 0.00a	0.19 ± 0.00b	0.18 ± 0.00b	0.14 ± 0.00c	0.13 ± 0.00c	0.13 ± 0.00c	0.11 ± 0.00 ac	0.10 ± 0.00ac
C12:0	6.80 ± 0.02a	12.21 ± 0.07b	11.90 ± 0.05b	9.89 ± 0.01c	9.42 ± 0.03d	8.94 ± 0.03e	7.55 ± 0.29f	6.90 ± 0.18a
C13:0	0.11 ± 0.00a	0.19 ± 0.00b	0.19 ± 0.00b	0.17 ± 0.00b	0.16 ± 0.00b	0.16 ± 0.00b	0.13 ± 0.01a	0.11 ± 0.00a
C14:0	14.32 ± 0.01a	17.52 ± 0.04b	18.01 ± 0.08c	17.52 ± 0.10b	17.22 ± 0.03d	17.19 ± 0.04d	15.2 ± 0.63e	14.64 ± 0.22a
C14:1	0.81 ± 0.00	0.79 ± 0.04	0.79 ± 0.01	0.80 ± 0.01	0.86 ± 0.00	0.82 ± 0.00	0.81 ± 0.02	0.81 ± 0.02
C15:0	1.02 ± 0.00a	1.22 ± 0.00b	1.28 ± 0.01bc	1.33 ± 0.01bc	1.34 ± 0.00c	1.36 ± 0.00c	1.25 ± 0.04b	1.20 ± 0.00b
C16:0	27.00 ± 0.05a	20.61 ± 0.06b	21.92 ± 0.08c	25.25 ± 0.24d	25.67 ± 0.08d	26.51 ± 0.11e	26.49 ± 0.23e	26.25 ± 0.10e
C16:1	1.07 ± 0.00a	0.69 ± 0.13b	0.72 ± 0.13b	0.94 ± 0.17ab	1.12 ± 0.00a	1.09 ± 0.00a	1.01 ± 0.22ab	0.72 ± 0.01b
C17:0	0.01 ± 0.00a	0.23 ± 0.00b	0.25 ± 0.01b	0.29 ± 0.01bc	0.30 ± 0.00bc	0.29 ± 0.00bc	0.37 ± 0.02c	0.39 ± 0.00c
C18:0	9.68 ± 0.04a	4.16 ± 0.04b	4.62 ± 0.06c	5.69 ± 0.05d	5.98 ± 0.03de	6.34 ± 0.04e	7.65 ± 0.43f	8.21 ± 0.10g
C18:1n9t	2.74 ± 0.01a	1.03 ± 0.03b	1.07 ± 0.03b	1.34 ± 0.04c	1.65 ± 0.01d	1.61 ± 0.01d	1.84 ± 0.10de	2.02 ± 0.01e
C18:1n9c	17.47 ± 0.07a	6.62 ± 0.14b	6.40 ± 0.05b	8.23 ± 0.17c	8.58 ± 0.04 cd	8.64 ± 0.05d	10.91 ± 0.81e	12.32 ± 0.14f
C18:3n3	0.06 ± 0.00a	0.21 ± 0.00b	0.16 ± 0.01ab	0.32 ± 0.01c	0.21 ± 0.00b	0.32 ± 0.00c	0.42 ± 0.00d	0.42 ± 0.01d
C20:1	0.04 ± 0.00a	0.21 ± 0.02b	0.20 ± 0.03b	0.28 ± 0.02bc	0.32 ± 0.00c	0.33 ± 0.00c	0.38 ± 0.01cd	0.41 ± 0.00d
C18:2n6c	2.70 ± 0.01a	2.53 ± 0.19b	2.60 ± 0.50a	4.04 ± 0.30c	4.70 ± 0.02d	4.92 ± 0.03d	5.70 ± 0.51e	6.32 ± 0.12f
SC-SFA	4.02 ± 0.02a	6.30 ± 0.08b	5.98 ± 0.07c	5.54 ± 0.06d	5.31 ± 0.19de	5.00 ± 0.19e	4.81 ± 0.21e	4.84 ± 0.06e
MC-SFA	18.96 ± 0.19a	37.69 ± 0.23b	35.81 ± 0.14b	28.26 ± 0.03c	26.59 ± 0.25 cd	25.42 ± 0.24d	23.04 ± 0.80de	21.33 ± 0.11e
LC-SFA	52.06 ± 0.04a	43.81 ± 0.15b	46.14 ± 0.16c	50.13 ± 0.47d	50.56 ± 0.06d	51.74 ± 0.12ad	51.00 ± 0.50ad	50.74 ± 0.03ad
SFA	75.04 ± 0.14a	87.80 ± 0.10b	87.93 ± 0.35b	83.93 ± 0.45c	82.45 ± 0.15c	82.16 ± 0.17c	78.85 ± 1.27d	76.91 ± 0.07ad
MUFA	22.13 ± 0.08a	9.33 ± 0.04b	9.18 ± 0.22b	11.59 ± 0.06c	12.53 ± 0.05c	12.50 ± 0.07c	14.95 ± 0.72d	16.28 ± 0.14e
PUFA	2.76 ± 0.01a	2.75 ± 0.19a	2.76 ± 0.49a	4.36 ± 0.32b	4.91 ± 0.02bc	5.24 ± 0.03c	6.11 ± 0.51d	6.74 ± 0.12e
UFA	24.89 ± 0.09a	12.08 ± 0.14b	11.94 ± 0.27b	15.95 ± 0.37c	17.44 ± 0.08d	17.74 ± 0.09d	21.06 ± 1.23e	23.02 ± 0.02ae

[a-g]Different letters within the same row are significantly different ($P < 0.05$).

SFA (saturated fatty acid) = ∑(C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0); SC-SFA (short-chain saturated fatty acid) = ∑(C4:0); MC-SFA (medium-chain saturated fatty acid) = ∑(C6:0, C8:0, C10:0, C11:0, C12:0); LC-SFA (long-chain saturated fatty acids) = ∑(C13:0, C14:0, C15:0, C16:0, C17:0, C18:0); MUFA (monounsaturated fatty acid) = ∑(C14:1, C16:1, C18:1n9t, C18:1n9c, C20:1); PUFA (polyunsaturated fatty acid) = ∑(C18:2n6c, C18:3n6); and UFA = ∑(MUFA and PUFA).

at 240 °C. This phenomenon might be caused by short-chain and medium-chain FAs, with small-molecular-weight compared with long-chain FA, first gathered in the distillate with a sudden rise in temperature. After that, according to the principle of SPMD, the long-chain FA with large molecular weight accumulated easily at higher distillation temperature, leading to decrease in the content of short-chain and medium-chain FAs. Furthermore, the proportion of SC-SFA and MC-SFA decreased slightly in residue. A similar result was also reported by previous studies (Arul *et al.*, 1988; Campos *et al.*, 2003). The long-chain saturated FA (LC-SFA) was the main component in total FA, including C14:0, pentadecanoic acid (C15:0), C16:0, heptadecanoic acid (C17:0) and C18:0. The percentage of LC-SFA showed opposite variation with SC- and MC-SFA, which decreased sharply at 165 °C and then increased from 43.81% to 50.74% at 240 °C. However, the percentage of LC-SFA in residue declined during the whole process. The C18:0 was verified it be beneficial for humans, like prevention of cardiovascular disease, and many studies reported that there were no bad effects of C18:0 on the health of humans when compared with other SFAs (Gómez-Cortés *et al.*, 2018; Van Rooijen & Mensink, 2020).

The monounsaturated FA and polyunsaturated FA (MUFA and PUFA) were mainly composed of C18:1n9c, trans-oleic acid (C18:1n9t) and linoleic acid (C18:2n6c) in this study. The proportion of UFA and MUFA in distillate was similar to LC-SFA, which decreased dramatically at 165 °C and then increased with the growth of distilling temperature. By contrast, the percentage of PUFA increased from 2.76% at room temperature to 6.74% and 13.89% of total FA in distillate and residue at 240 °C, with a growth rate of 144.20% and 403.26% respectively. These results were higher than that of previous studies, which was caused by the difference between original samples and

Table 2 Fatty acids in the residue of each temperature

Fatty acids	Distillation temperature (°C)							
	Room temperature	165	180	190	200	210	225	240
C4:0	4.02 ± 0.02a	3.50 ± 0.04b	3.49 ± 0.09b	3.23 ± 0.07b	3.22 ± 0.03b	2.84 ± 0.06c	2.73 ± 0.04c	2.74 ± 0.08c
C6:0	4.35 ± 0.09a	3.69 ± 0.08b	3.69 ± 0.08b	3.43 ± 0.02b	3.46 ± 0.03b	3.45 ± 0.03b	2.87 ± 0.15c	2.54 ± 0.01c
C8:0	2.53 ± 0.03a	2.10 ± 0.03b	2.09 ± 0.05b	1.97 ± 0.01b	1.99 ± 0.01b	2.00 ± 0.01b	1.77 ± 0.09bc	1.62 ± 0.01bc
C10:0	5.19 ± 0.04a	4.37 ± 0.03b	4.33 ± 0.10b	4.13 ± 0.03b	4.16 ± 0.02b	4.22 ± 0.02b	3.91 ± 0.14bc	3.60 ± 0.08c
C11:0	0.09 ± 0.00	0.07 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00
C12:0	6.80 ± 0.02a	5.72 ± 0.02b	5.70 ± 0.10b	5.42 ± 0.02b	5.40 ± 0.02b	5.46 ± 0.02b	5.27 ± 0.04bc	5.02 ± 0.15c
C13:0	0.11 ± 0.00	0.09 ± 0.00	0.10 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.00
C14:0	14.32 ± 0.01a	11.97 ± 0.01b	11.98 ± 0.10b	11.48 ± 0.13b	11.49 ± 0.00b	11.42 ± 0.08b	11.5 ± 0.19b	11.41 ± 0.18b
C14:1n5	0.81 ± 0.00	0.80 ± 0.00	0.82 ± 0.01	0.81 ± 0.00	0.79 ± 0.00	0.83 ± 0.00	0.86 ± 0.01	0.82 ± 0.03
C15:0	1.02 ± 0.00	1.08 ± 0.00	1.07 ± 0.00	1.06 ± 0.00	1.05 ± 0.00	1.04 ± 0.00	0.92 ± 0.08	0.83 ± 0.00
C16:0	27.00 ± 0.05a	23.90 ± 0.05b	24.40 ± 0.01b	24.06 ± 0.09b	23.88 ± 0.01b	23.97 ± 0.05b	23.24 ± 0.12b	23.13 ± 0.04b
C16:1n7	1.07 ± 0.00ab	0.78 ± 0.00a	1.36 ± 0.01b	1.37 ± 0.00b	1.37 ± 0.01b	1.36 ± 0.01b	1.38 ± 0.01b	1.42 ± 0.02b
C17:0	0.01 ± 0.00a	0.41 ± 0.00b	0.39 ± 0.00b	0.25 ± 0.08c	0.35 ± 0.07b	0.41 ± 0.01b	0.42 ± 0.00b	0.42 ± 0.00b
C18:0	9.68 ± 0.04a	10.11 ± 0.04b	10.00 ± 0.08b	10.30 ± 0.11b	10.35 ± 0.15b	10.27 ± 0.03b	10.88 ± 0.24c	11.17 ± 0.09c
C18:1n9t	2.74 ± 0.01	2.95 ± 0.01	2.69 ± 0.02	2.06 ± 0.37	2.72 ± 0.16	2.81 ± 0.10	2.87 ± 0.11	2.78 ± 0.03
C18:1n9c	17.47 ± 0.07a	15.69 ± 0.06b	15.40 ± 0.11b	15.98 ± 0.11b	16.01 ± 0.24b	16.03 ± 0.06b	16.85 ± 0.48ab	17.84 ± 0.29a
C18:3n3	0.06 ± 0.00a	0.57 ± 0.00b	0.60 ± 0.00b	0.63 ± 0.0b2	0.63 ± 0.00b	0.65 ± 0.00b	0.68 ± 0.04b	0.72 ± 0.01b
C20:1	0.04 ± 0.00a	0.54 ± 0.00b	0.52 ± 0.00b	0.54 ± 0.04b	0.55 ± 0.00b	0.52 ± 0.02b	0.57 ± 0.02b	0.61 ± 0.01b
C18:2n6c	2.70 ± 0.01a	11.65 ± 0.04b	11.29 ± 0.34b	13.13 ± 0.24b	12.41 ± 0.20b	12.57 ± 0.02b	13.13 ± 0.11b	13.17 ± 0.20b
SC-SFA	4.02 ± 0.02a	3.50 ± 0.04ab	3.49 ± 0.09ab	3.23 ± 0.07ab	3.22 ± 0.03ab	2.84 ± 0.06b	2.73 ± 0.04b	2.74 ± 0.08b
MC-SFA	18.96 ± 0.19a	15.95 ± 0.16ab	15.89 ± 0.33ab	15.02 ± 0.07ab	15.09 ± 0.08ab	15.20 ± 0.09ab	13.89 ± 0.43b	12.84 ± 0.23b
LC-SFA	52.06 ± 0.04	47.51 ± 0.05b	47.87 ± 0.03b	47.17 ± 0.18b	47.15 ± 0.19b	47.14 ± 0.08b	46.98 ± 0.20b	46.99 ± 0.04b
SFA	75.04 ± 0.14a	66.96 ± 0.15b	67.25 ± 0.39b	65.41 ± 0.05b	65.46 ± 0.08b	65.18 ± 0.08b	63.60 ± 0.58b	62.58 ± 0.14b
MUFA	22.13 ± 0.08	20.76 ± 0.07	20.79 ± 0.09	20.76 ± 0.21	21.44 ± 0.08	21.55 ± 0.06	22.53 ± 0.39	23.47 ± 0.32
PUFA	2.76 ± 0.01a	12.22 ± 0.04b	11.89 ± 0.34b	13.76 ± 0.27b	13.04 ± 0.2b	13.21 ± 0.02b	13.81 ± 0.15b	13.89 ± 0.21b
UFA	24.89 ± 0.09a	32.99 ± 0.11b	32.68 ± 0.44b	34.52 ± 0.05b	34.48 ± 0.12b	34.76 ± 0.04b	36.34 ± 0.54bc	37.36 ± 0.11c

[a–g]Different letters within the same row are significantly different ($P < 0.05$).

SFA (saturated fatty acid) = ∑(C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0); SC-SFA (short-chain saturated fatty acid) = ∑(C4:0); MC-SFA (medium-chain saturated fatty acid) = ∑(C6:0, C8:0, C10:0, C11:0, C12:0, C14:0); LC-SFA (long-chain saturated fatty acids) = ∑(C13:0, C14:0, C15:0, C16:0, C17:0, C18:0); MUFA (monounsaturated fatty acid) = ∑(C14:1, C16:1, C18:1n9t, C18:1n9c, C20:1); PUFA (polyunsaturated fatty acid) = ∑(C18:2n6c, C18:3n6); and UFA = ∑(MUFA and PUFA).

distilling temperature. For the individual FA species, they showed a similar variation with the FA groups, such as C18:2n6c, accumulated in the distilling fractions, with the increasing ratio of 134.07% and 387.78% in the distillate and residue respectively. The C18:2 n6c was not synthesised in the human body, it must be supplemented by food or formed by specific precursor substances to maintain normal physiological function, which was called human essential fatty acid. The combination of cholesterol and linoleic acid plays a important role during the transport process, resulting in metabolic disorders were happened when it was scarce in the human body (Limongi *et al.*, 2018; Minieri *et al.*, 2020; Denis *et al.*, 2022).

Triglyceride composition of distilling products

Based on the UPLC-MS technology, a total of 109 individual TAG molecular species were detected in the current research (Tables S1 and S1). These TAG molecular species could be classified into three groups (Tables 3 and 4), including low-molecular-weight TAG (LMW-TAG, with the carbon numbers: 24–34 and molecular weight: 498–610), medium-molecular-weight TAG (MMW-TAG, with the carbon numbers: 35–40 and molecular weight: 624–694) and high-molecular-weight TAG (HMW-TAG, with the carbon numbers: 41–54 and molecular weight: 704–888) (Smiddy *et al.*, 2012). Moreover, there were 38 TAG molecular species with a relative distribution of more than 1%, and 16:0/4:0/16:0 was the most abundant molecular species, accounting for 4.19% of total TAG, followed by 16:0/6:0/16:0 (4.17% of total TAG), 18:1/16:0/18:1 (3.98% of total TAG), 16:0/16:0/18:1 (3.33% of total TAG), 18:1/14:0/18:1 (3.23% of total TAG), *etc.*, which were in accordance with previous studies (Liu *et al.*, 2020; Wang *et al.*, 2022).

It was evident that the percentage of TAG molecular species in MF was influenced significantly by SPMD (Tables 3 and 4). The percentage of LMW-

Table 3 Different groups of triglycerides in the distillate of each temperature

TAG groups	Distillation temperature (°C)							
	Room temperature	165	180	190	200	210	225	240
LMW	9.25 ± 0.11a	66.27 ± 0.09b	67.40 ± 0.20a	46.09 ± 0.40c	42.78 ± 0.25c	38.45 ± 0.07d	29.34 ± 0.44e	17.76 ± 0.19f
MMW	30.88 ± 0.20a	31.89 ± 0.09a	30.85 ± 0.16a	49.24 ± 0.32b	51.56 ± 0.18b	54.81 ± 0.04c	59.34 ± 0.06d	53.1 ± 0.05bc
HMW	59.87 ± 0.31a	1.84 ± 0.00b	1.74 ± 0.04b	4.67 ± 0.08c	5.66 ± 0.07d	6.74 ± 0.03e	11.32 ± 0.38f	29.15 ± 0.24g
S_3	48.45 ± 0.31a	73.48 ± 0.03b	77.07 ± 0.28b	69.41 ± 0.23c	68.69 ± 0.20cd	68.31 ± 0.24cd	65.47 ± 0.03d	60.2 ± 0.07e
U_2S	19.59 ± 0.17a	2.07 ± 0.03b	1.68 ± 0.05c	4.04 ± 0.14d	4.47 ± 0.04de	4.92 ± 0.11e	6.80 ± 0.01f	11.05 ± 0.02g
S_2U	29.68 ± 0.19a	24.44 ± 0.07b	21.24 ± 0.23c	26.53 ± 0.09d	26.84 ± 0.17d	26.76 ± 0.13d	27.7 ± 0.02d	28.61 ± 0.05ad
U_3	2.28 ± 0.06a	0.01 ± 0.00b	0.01 ± 0.00b	0.01 ± 0.00b	0.01 ± 0.00b	0.01 ± 0.00b	0.03 ± 0.00c	0.14 ± 0.00d
M_3	0.76 ± 0.00a	8.27 ± 0.05b	8.14 ± 0.00b	4.96 ± 0.01c	4.53 ± 0.05d	3.94 ± 0.02e	2.74 ± 0.04f	1.47 ± 0.02g
LM_2	2.07 ± 0.03a	11.52 ± 0.09b	11.56 ± 0.26b	8.01 ± 0.08c	7.98 ± 0.07c	7.08 ± 0.06d	5.91 ± 0.05e	3.71 ± 0.00f
LMS	3.63 ± 0.04a	29.26 ± 0.02b	29.06 ± 0.33b	19.74 ± 0.35c	17.98 ± 0.04d	15.93 ± 0.02e	11.93 ± 0.09f	7.00 ± 0.02g
L_2M	33.56 ± 0.19a	10.33 ± 0.05b	9.42 ± 0.10c	20.04 ± 0.33d	22.3 ± 0.24de	24.65 ± 0.05e	30.78 ± 0.29f	40.38 ± 0.28g
L_3	40.13 ± 0.50a	0.97 ± 0.01b	0.86 ± 0.02b	1.99 ± 0.00c	2.32 ± 0.05d	2.63 ± 0.01e	4.03 ± 0.16f	11.13 ± 0.26g
M_2S	0.06 ± 0.00a	1.68 ± 0.00b	1.51 ± 0.02b	0.46 ± 0.00c	0.41 ± 0.01 cd	0.30 ± 0.00d	0.22 ± 0.00e	0.11 ± 0.00f
L_2S	19.79 ± 0.24a	37.97 ± 0.08b	39.44 ± 0.17b	44.80 ± 0.11c	44.48 ± 0.45c	45.47 ± 0.02c	44.39 ± 0.35c	36.2 ± 0.05b

[a–g]Different letters within the same row are significantly different ($P < 0.05$).

LMW (low-molecular-weight triglyceride) = ∑(carbon numbers from 26 to 34); MMW (medium-molecular-weight triglyceride) = ∑(carbon numbers from 35 to 40); HMW (high-molecular-weight triglyceride) = ∑(carbon numbers from 41 to 54); S_3: triglyceride with three saturated fatty acids, U_3: triglyceride with three unsaturated fatty acids, S_2U: (SSU and SUS) triglyceride with one unsaturated fatty acid and two saturated fatty acid, including SSU and SUS; SU_2: triglyceride with one saturated fatty acid and two unsaturated fatty acids, including SUU and USU; SM_2: triglyceride with one short-chain fatty acid and two medium-chain fatty acids, including MMS, SMM and MSM; LMS: triglyceride with one short-chain fatty acid, one medium-chain fatty acid and one long-chain fatty acid; M_2L: triglyceride with one long-chain fatty acid and two medium-chain fatty acids, including MML, LMM and MLM; L_2M: triglyceride with two long-chain fatty acids and one medium-chain fatty acid, including LLM, MLL and LML; L_2S: triglyceride with two long-chain fatty acids and one short-chain fatty acid, including LLS, SLL and LSL; M_3: triglyceride with three medium-chain fatty acids; and L_3: triglyceride with three long-chain fatty acids.

TAG in the distillate increased significantly from 9.25% to 66.27% of total TAG at 165 °C when compared with the AMF, and then it decreased with the growth of distilling temperature and reached 17.72% at 240 °C, which was still about twice than that of AMF. A similar phenomenon was also reported in previous study (Campos *et al.*, 2003). However, the variation of HMW-TAG in the distillate was opposite to LMW-TAG. The percentage of HMW-TAG decreased from 59.87% to 1.84% of total TAG at 165 °C, indicating that almost only the LMW-TAG and MMW-TAG remained in distillate at this distilling temperature. After that, the percentage of HMW-TAG gradually increased, and it reached finally 29.15% of total TAG at 240 °C. This phenomenon was likely caused by that the LMW-TAG was first gathered in the distillate with a sudden rise of distilling temperature, and the percentage of HMW-TAG in the distillate was rare; as the principle of SPMD mentioned above, the LMW-TAG was excluded gradually and HMW-TAG was accumulated in the distillate. For the MMW-TAG, its percentage in distillate showed an increasing trend during the whole distillation process. In addition, the LMW-TAG and MMW-TAG in residue were impacted slightly in comparison with that of distillate, and the percentage of LMW-TAG and MMW-TAG all decreased from 9.25% and 30.88% of total TAG to 7.81% and 27.18% of total TAG respectively. The increasing trend was observed in the proportion of HMW-TAG, which increased from 59.87% to 65.01% of total TAG at 240 °C. These results were in accordance with the phenomena found in previous studies (Arul *et al.*, 1988; Campos *et al.*, 2003).

For the individual TAG molecular species, the 16:0/4:0/16:0 and 16:0/6:0/16:0 were the major molecular species, taking up more than 8% of the total TAG. The percentage of 16:0/4:0/16:0 showed a similar variation with MMW-TAG in the distillate, which had the highest value (11.14%) at 190 °C. However, the percentage of 16:0/6:0/16:0 declined in distillate at the early stage of the distillation (165 and 180 °C), and then its content increased. This result showed that there was a difference among different TAGs molecular species while they belonged to the same type of TAGs, this phenomenon was induced by the FA composition of TAG which was different. In the remaining TAG molecular species, the percentage of 18:1/16:0/18:1, 16:0/16:0/18:1 and 18:1/14:0/18:1 was more than 3% of total TAG, and the variation in them all was

Table 4 Different groups of triglycerides in the residue of each temperature

TAG groups	Distillation temperature (°C) Room temperature	165	180	190	200	210	225	240
LMW	9.25 ± 0.11a	9.38 ± 0.40a	8.97 ± 0.50b	6.72 ± 0.04c	6.59 ± 0.06c	6.09 ± 0.29d	6.11 ± 0.28d	7.81 ± 0.12e
MMW	30.88 ± 0.20a	34.57 ± 1.03b	34.55 ± 0.63b	30.45 ± 0.25a	31.80 ± 0.21a	31.15 ± 1.05a	30.16 ± 1.10a	27.18 ± 0.25c
HMW	59.87 ± 0.31ab	56.05 ± 1.43a	56.48 ± 1.13a	62.84 ± 0.29b	61.61 ± 0.26ab	62.76 ± 1.33b	63.73 ± 1.38b	65.01 ± 0.38c
S_3	48.45 ± 0.31a	53.34 ± 0.35b	54.55 ± 0.47b	49.12 ± 0.16a	50.13 ± 0.40ab	48.87 ± 0.04a	46.59 ± 0.00a	44.95 ± 0.24c
U_2S	19.59 ± 0.17a	17.27 ± 0.43b	16.67 ± 0.40b	19.67 ± 0.12a	19.36 ± 0.06a	20.12 ± 0.04a	21.85 ± 0.15a	22.83 ± 0.02c
S_2U	29.68 ± 0.19a	27.57 ± 0.01b	27.2 ± 0.03b	29.06 ± 0.06a	28.39 ± 0.3ab	28.74 ± 0.06ab	28.96 ± 0.25ab	29.15 ± 0.26a
U_3	2.28 ± 0.06a	1.82 ± 0.06ab	1.59 ± 0.10b	2.14 ± 0.10a	2.11 ± 0.04a	2.27 ± 0.02a	2.60 ± 0.10c	3.07 ± 0.04d
M_3	0.76 ± 0.00a	0.65 ± 0.01b	0.60 ± 0.06b	0.43 ± 0.01c	0.43 ± 0.01c	0.37 ± 0.02d	0.42 ± 0.00c	0.56 ± 0.03bc
LM_2	2.07 ± 0.03a	1.95 ± 0.12ab	1.86 ± 0.03b	1.54 ± 0.03c	1.52 ± 0.02c	1.41 ± 0.04d	1.41 ± 0.07d	1.52 ± 0.03c
LMS	3.63 ± 0.04a	3.34 ± 0.09b	3.19 ± 0.16c	2.45 ± 0.01d	2.26 ± 0.02e	2.09 ± 0.10e	2.20 ± 0.07e	2.84 ± 0.00f
L_2M	33.56 ± 0.19a	34.23 ± 0.13ab	34.58 ± 0.24ab	33.96 ± 0.10ab	35.08 ± 0.14ab	35.66 ± 0.00b	34.85 ± 0.68ab	30.42 ± 0.01c
L_3	40.13 ± 0.50a	36.73 ± 1.02b	36.75 ± 0.99b	42.34 ± 0.33c	40.77 ± 0.27ac	41.16 ± 1.15ac	42.18 ± 1.57c	45.60 ± 0.28d
M_2S	0.06 ± 0.00a	0.03 ± 0.00b	0.03 ± 0.00b	0.03 ± 0.00b	0.03 ± 0.00b	0.03 ± 0.00b	0.03 ± 0.00b	0.04 ± 0.00ab
L_2S	19.79 ± 0.24a	23.07 ± 0.93b	23.00 ± 0.98b	19.25 ± 0.18a	19.92 ± 0.09a	19.29 ± 0.99a	18.9 ± 0.75a	19.01 ± 0.24a

[a–g]Different letters within the same row are significantly different ($P < 0.05$).
LMW (low-molecular-weight triglyceride) = ∑(carbon numbers from 26 to 34); MMW (medium-molecular-weight triglyceride) = ∑(carbon numbers from 35 to 40); HMW (high-molecular-weight triglyceride) = ∑(carbon numbers from 41 to 54); S_3: triglyceride with three saturated fatty acids, U_3: triglyceride with three unsaturated fatty acids, S_2U: (SSU and SUS) triglyceride with one unsaturated fatty acid and two saturated fatty acid, including SSU and SUS; SU_2: triglyceride with one saturated fatty acid and two unsaturated fatty acids, including SUU and USU; SM_2: triglyceride with one short-chain fatty acid and two medium-chain fatty acids, including MMS, SMM and MSM; LMS: triglyceride with one short-chain fatty acid, one medium-chain fatty acid and one long-chain fatty acid; M_2L: triglyceride with one long-chain fatty acid and two medium-chain fatty acids, including MML, LMM and MLM; L_2M: triglyceride with two long-chain fatty acids and one medium-chain fatty acid, including LLM, MLL and LML; L_2S: triglyceride with two long-chain fatty acids and one short-chain fatty acid, including LLS, SLL and LSL; M_3: triglyceride with three medium-chain fatty acids; and L_3: triglyceride with three long-chain fatty acids.

similar to HWM-TAG. Specifically, as the distilling temperature increased, the percentage of 18:1/16:0/18:1, 16:0/16:0/18:1 and 18:1/14:0/18:1 decreased in the distillate, with a declining rate of 88.44%, 83.18% and 79.32% respectively. On the contrary, they demonstrated an increasing trend in the residue, with the growth ratio of 39.20%, 24.92% and 30.56% respectively. It was reported that C18:1/C16:0/C18:1 (OPO) and C18:1/C16:0/C18:2 (OPL) were the major components in human milk, with 20%–40% of the total TAG, and they played an important role for the growth of infants (Zhao *et al.*, 2018; Zhu *et al.*, 2021; Lan *et al.*, 2022). However, the percentage of OPO and OPL was low in infant formula based on cow milk; many manufacturers want their concentration to be close to that of human milk and so take some measures, such as adding soybean oil and palm oil (Zou *et al.*, 2016; Hageman *et al.*, 2019; Hokkanen *et al.*, 2022). However, the TAG molecular species was different in vegetable oils and human milk; it is necessary to find other ingredients to substitute the vegetable oils, such as dairy milk fat and goat milk fat. In this study, the percentages of OPO and OPL have been observed to show an increasing trend in the residue, which increased from 3.98% and 1.09% to 5.56% and 1.25% of total TAG at 240°C, respectively, with the increasing ratio of 39.70% and 14.68%. These results indicated that these distilling fractions would be the infant ingredient in the infant formula powder.

Characteristic of TAG

The application of MF is mainly based on its physical properties at present, MF is usually divided into three groups with high melting point (with the melting point > 38 °C), medium melting point (with the melting point 28 - 38 °C), and low melting point (with the melting point < 28 °C) according to its application characteristics. The high melting point fraction is often used to produce crisp cream, which is used to remain solid and prevent becoming brittle of the product. The medium-melting-point fraction is generally used to prepare whipped cream to make the product taste better, and the low-melting-point fraction is usually applied for egg tart cream and flavours and fragrances. However, there are few researches on the nutritive characteristics of MF and the molecular structure of TAG after fractions (Shimamura *et al.*, 2013; Mohan *et al.*, 2021). The saturation and carbon chain length of FA are important for TAG, they can further influence the physical property of MF, such as melting point and crystal texture, and they also provide more

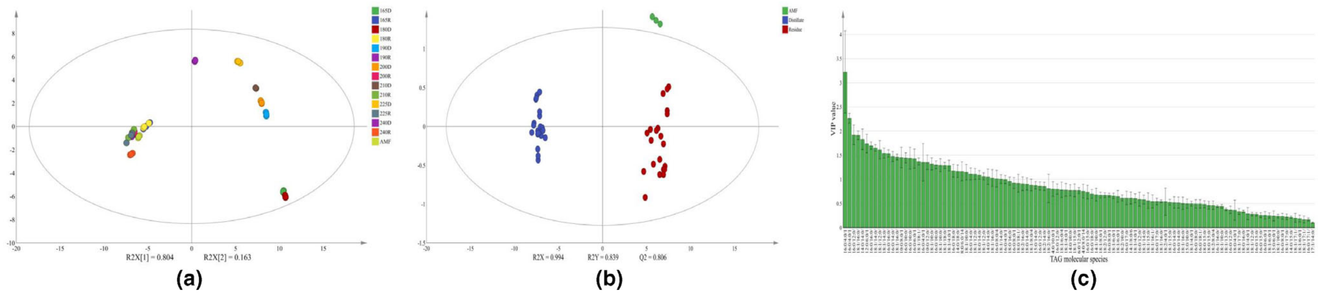

Figure 2 Score plot of PCA (a), score plot of PLS-DA(b) and VIP value plot of PLS-DA (c) based on triglyceride molecular species of milk fat and its distillation fractions (distillate and residue). R2X and R2Y are the cumulative modelled variation in the X and Y matrixes, respectively, and Q2 is the cumulative predicted variation in the Y matrix. OPLS-DA, orthogonal partial least-squares-discriminate analysis; PCA, principal component analysis; VIP, variable importance in projection.

nutritional information (Martini *et al.,* 2012; Tzompa-Sosa *et al.,* 2016; Zhao *et al.,* 2018; Wang *et al.,* 2019). Based on the saturation of FA, the TAG of MF was classified into four groups: S_3 (SSS) with three SFAs; U_3 (UUU) with three UFAs; S_2U (SSU and SUS) with one UFA and two SFAs; and SU_2 (SUU and USU) consisted of one SFA and two UFAs (Yuan *et al.,* 2019; Yu *et al.,* 2022). It was evident that the TAG with different saturation of FA in the residue was not changed significantly compared with those of AMF and distillate (Tables 3 and 4). In the distillate, the percentage of S_3 increased rapidly at the initial distillation temperatures (165 and 180 °C), which increased from 48.44% to 77.07% of total TAG, while the proportion of U_3, S_2U and SU_2 all decreased at the same time. After that, the declining trend was observed in S_3 with the increase in distilling temperature. By contrast, the U_3 and SU_2 all showed an increasing trend in the residue, with an increasing ratio of 34.65% and 16.54% at 240 °C respectively. As regards the reason for these phenomena, it was likely caused by the fact that there were two or three UFAs in the SU_2 and U_3 and UFAs were generally medium- and long-chain FA, so the molecular weight of these two types of TAG was larger. The HMW-TAG was mostly accumulated in residue, so the percentage of SU_2 and U_3 in the distillate was reduced after SPMD. Moreover, it was reported that the percentage of U_3 in colostrum human milk was higher, and UFAs, with a lower melting point in comparison with the human body temperature, were easily absorbed by infants (Zhao *et al.,* 2018). Therefore, the increase in U_3 and SU_2 in the residue could be used in the infant formula to provide more nutrients for the newborns.

The TAG molecular species could be divided into seven groups according to the length of FA, including SM_2 (one short-chain FA and two medium-chain FAs), LMS (one short-chain FA, one medium-chain FA and one long-chain FA), M_2L (one long-chain FA and two medium-chain FAs), L_2M (two long-chain FAs and one medium-chain FA), L_2S (two long-chain FAs and one short-chain FA), M_3 (MMM, three medium-chain FAs) and L_3 (LLL, three long-chain FAs) (Table 3) (Zhu *et al.,* 2021). After SPMD, it was evident that the percentage of SM_2, LMS, M_2L, L_2S and M_3 significantly increased in distillate at 165 °C, and then it decreased. However, the percentage of L_2M and L_3 decreased sharply at 165 °C, which was changed from 33.56% and 40.13% to 10.33% and 0.98%, respectively, and they all showed an increasing trend at the remained distillation temperature, the distribution of L_2M was the highest at 240 °C. Compared with the distillate, the percentage of SM_2, LMS, M_2L, L_2M and M_3 all decreased during the whole distilling process, and L_3 increased from 40.13% at room temperature to 45.60% at 240 °C.

To know the precise influence made by SPMD on the TAG molecular species, PCA and OPLS-DA were used to look for the differential TAG molecular species during SPMD. We could clearly see that there was an apparent difference among groups (plot a of Fig. 2), which indicated that TAG molecular species were impacted significantly by SPMD. Moreover, the OPLS-DA result (plot b of Fig. 2) demonstrated that TAG molecular species belonged to distillate, residue and AMF, respectively, had obvious difference, and the variable importance in projection (VIP) (plot c of Fig. 2) of 16:0/4:0/16:0, 16:0/4:0/14:0, 16:0/12:0/4:0, 18:1/16:0/18:1, 14:0/14:0/4:0, 12:0/6:0/14:0, 16:0/16:0/18:1, 18:1/14:0/18:1, 18:1/12:0/4:0, 18:1/14:0/16:0, 16:0/16:0/16:0, 16:0/14:0/16:0, etc. all were than 1 ($P < 0.05$), indicating that these TAG molecular species were affected obviously by SPMD and they could be used in the quality control in the separation of MF.

Conclusion

The MF was separated into different fractions (distillates and residues) through SPMD and the yield of distillate increased from 1.36% at 165 °C to 37.42% at 240 °C. The distillate had more SC-SFA and MC-

SFA, but LC-SFA and PUFA were more accumulated in the residue. Moreover, the SC-SFA and MC-SFA all showed a sharp increasing trend at the initial temperature (165 °C) and then the proportion of these components decreased with the increase in distillation temperature. For the TAG of AMF and its distillation products, a total of 109 TAG molecular species were detected, including 22 kinds of LMW-TAGs, 35 kinds of MMW-TAGs and 52 kinds of LMW-TAGs. Among these TAG molecular species, LMW-TAG, MMW-TAG and S_3 were easily accumulated in the distillate, and their content all showed similar variation with SC-SFA when the distillation temperature increased. Compared with the distillate, HMW-TAG, such as OPO and OPL, were enriched in the residue. The dimension reduction analysis (PCA and OPLS-DA) demonstrated that the 16:0/4:0/16:0, 16:0/4:0/14:0, 16:0/12:0/4:0, 18:1/16:0/18:1, 14:0/14:0/4:0, 12:0/6:0/14:0, 16:0/16:0/18:1, 18:1/14:0/18:1, etc. were the discrepant TAG molecular species during SPMD, which enriched more accurate data for the quality control of SPMD. In a word, these results provide more basic theoretical data for the use of SPMD in MF fractionation, and it also provided more nutritional information on MF distilling fractions for its further utilisation in the infant formula.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

Huiquan Zhu: Methodology; Formal analysis (equal); writing – original draft. **Xiaodan Wang:** Formal analysis (equal). **Wenyuan Zhang:** Formal analysis (equal). **Yumeng Zhang:** Formal analysis (equal). **Xixi Gao:** Data curation (equal); software (equal). **Yunna Wang:** Software (equal). **Peng Gao:** Formal analysis (equal). **Xiaoyang Pang:** Conceptualization (equal); resources (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal). **Shuwen Zhang:** Conceptualization (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal). **Jiaping Lv:** Conceptualization (equal); resources (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal).

Ethical statement

Ethics approval was not required for this research.

Peer review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ijfs.16476.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

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This article discussed the variation in chemical components of anhydrous milk fat after short-path distillation at 245°C and 265°C. They found that short-chain (C4–C8) fatty acids gradually decreased from liquid to solid fractions and the trend was reversed for long-chain (C14–C18) fatty acids, both saturated and unsaturated. We feel that this article was important for our research because this article was the first paper to use the short path in the fractionation of milk fat.

Berti, J., Grosso, N.R., Fernandez, H., Pramparo, M.C. & Gayol, M.F. (2018). Sensory quality of milk fat with low cholesterol content fractioned by molecular distillation. *Journal of the Science of Food and Agriculture*, **98**, 3478–3484.

This article discussed the amount of cholesterol from anhydrous milk fat by molecular distillation and analysed the sensory properties of the obtained product. They found that the best results were obtained in a three-stage arrangement, in which ~60% of cholesterol was removed with a 30.48% distillate yield. The sensory properties of this distillation cut were also most similar to those of the untreated milk fat. We feel that this article was important for our research because this article showed that the sensory properties of distilling products were hardly affected by molecular distillation.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Different triglyceride molecular species in distillate of each temperature.

Table S2. Different triglyceride molecular species in residue of each temperature.