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Identification and quantification of branched-chain fatty acids and odd-chain fatty acids of mammalian milk, dairy products, and vegetable oils using GC/TOF-MS

Xue Yang [a, b], Emad Karrar [a, b], Fang Cong [c], Huijia Lu [a, b], Qingzhe Jin [a, b], Xuebing Xu [c], Thom Huppertz [d], Wei Wei [a, b, *], Xingguo Wang [a, b]

[a] State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, China
[b] Collaborative Innovation Centre of Food Safety and Quality Control in Jiangsu Province, School of Food Science and Technology, Jiangnan University, Wuxi 214122, China
[c] Wilmar (Shanghai) Biotechnology Research & Development Centre, 200137, Shanghai, China
[d] Food Quality and Design Group, Wageningen University & Research, Wageningen, the Netherlands

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ABSTRACT

Branched-chain fatty acids (BCFAs) and odd-chain fatty acids (OCFAs) in mammalian milk, dairy products, and vegetable oils were investigated; 72 fatty acids, including 16 BCFAs and 10 OCFAs, were identified and quantified using GC/TOF-MS. Human milk showed the lowest content of BCFA (0.4% of total FA) and the content in other milk ranged from 2 to 6%, of which *ai*17:0, *i*17:0, *ai*15:0 and *i*15:0 were most abundant. Differences in the content of OCFA in mammalian milk trended similarly to differences in BCFA content. The molecular species of BCFA in cow milk-derived dairy products were identical, but the content was different, with higher content in fermented dairy products. Apparent differences in BCFA and OCFA composition were found in infant formulas, strongly related to their fat sources. Vegetable oils contained no BCFA and a small amount of OCFA. A scientific basis for further investigation of high-BCFA and OCFA dairy products is provided.

1. Introduction

Branched-chain fatty acids (BCFAs) are primarily saturated fatty acids with one or more methyl branches. Fatty acids (FAs) with a methyl branch on the penultimate or antepenultimate carbon, forming a terminal isopropyl or isobutyl group, are *i*BCFA and *ai*BCFA, respectively (Ran-Ressler et al., 2011a; Ran-Ressler, Glahn, Bae, & Brenna, 2013). Monomethyl BCFA (mmBCFA) is the main BCFA form and is usually near the terminal methyl group. The BCFA with methyl branching at the middle position of the carbon chain is a non-terminal BCFA (ntBCFA). BCFAs comprise about 2% of US retail milk's total FA of milk fat (Ran-Ressler et al., 2011b). The concentration of BCFA in cow, yak, sheep and goat milk fat is about 0.5–1.0%, 4.0–5.5%, 2.2% and 1.5% of total FA, respectively (Bainbridge, Cersosimo, Wright, & Kraft, 2016; Nudda, Correddu, Cesarani, Pulina, & Battacone, 2021; Sun, Luo, Wang, Kothapalli, & Brenna, 2019). Some Asian fermented and plant-based foods have high levels of BCFAs; e.g., fermented foods such as nātto, shrimp paste, and fish sauce have 0.6–1.7% BCFAs of total FA (Wang et al., 2019). The fat in plant-based foods such as brussels sprouts and chia seeds have 3.7% and 0.4% BCFAs (Wang, Wang, Chen, Kothapalli, & Brenna, 2020b).

Odd-chain fatty acids (OCFAs) are a group of straight-chain fatty acids having an odd number of carbon atoms (Ran-Ressler, Devapatla, Lawrence, & Brenna, 2008; Xin et al., 2020). Although OCFAs are low in content, they are widely present in higher animals, plants, and micro heterotrophs. Dairy products are considered one of the human diet's primary dietary sources of OCFAs. The most common saturated OCFA is pentadecanoic acid (15:0) and heptadecanoic acid (17:0). Of these, 15:0 accounts for 0.62–1.75% of total FAs in milk, whereas 17:0 accounts for 0.46–2.52% of total FAs in milk (Dąbrowski & Konopka, 2022). A human cannot synthesise 15:0 and 17:0, which are therefore recognised as biomarkers of milk fat intake (Nudda et al., 2021). The concentration of OCFAs in cow, yak, sheep, and goat milk fat in several studies ranged from 1.2% to 4.3% of total FAs (Bainbridge et al., 2016; Nudda et al., 2021; Sun et al., 2019).

BCFAs and OCFAs have recently been of interest due to their potential benefits to human health, especially early life. The BCFAs in vernix (as high as ~30% of total FAs) support the growth and

* Corresponding author.
E-mail address: weiw@jiangnan.edu.cn (W. Wei).

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metabolism of intestinal cells and the intestinal health of infants (Liu et al., 2017; Ran-Ressler et al., 2008). Levels of BCFAs are also related to the anti-inflammatory in cell study (Yan et al., 2017). In a neonatal rat pup model for necrotising enterocolitis (NEC), lipids with 20% of BCFA reduced the incidence of NEC to less than half of the control level while also increasing the abundance of nascent bacteria species reliant on BCFAs for membrane lipids (Ran-Ressler et al., 2011a). A very recent study shows that phospholipids containing BCFAs (*ai*15:0 and *i*15:0) of an intestinal bacterium (*Akkermansia muciniphila*) affect the body's immune response (Bae et al., 2022). There is some evidence that OCFAs may be beneficial to health. A study has shown that 15:0 is an active, exogenous fatty acid with anti-inflammatory, antifibrotic, red blood cell-stabilising, and mitochondrial−reparative properties, resulting in lower cholesterol, triglycerides, and glucose, as well as reduced inflammation, anaemia, and liver fibrosis (Venn-Watson, Lumpkin, & Dennis, 2020). Also, 15:0 suppresses the stemness of MCF-7/SC human breast cancer stem-like cells through JAK2/STAT3 signalling (To, Nguyen, Moon, Ediriweera, & Cho, 2020).

Previous studies indicated the potential nutritional and health properties of BCFAs and OCFAs in developing and maintaining the microbiota, enterocyte health, skin, and possibly other functions. Numerous reports are available on mammalian milk BCFA or OCFA contents (Ran-Ressler et al., 2011b; Sun et al., 2019; Vlaeminck, Fievez, Cabrita, Fonseca, & Dewhurst, 2006; Watkins et al., 2021). Commercially available dairy products, most of which have added vegetable oils, are becoming more diversified, but their BCFA and OCFA contents are unclear.

This study aims to evaluate two kinds of bioactive fatty acids, the BCFAs and OCFAs, in mammalian milk, dairy products, and vegetable oils generally used in infant formula. These two fatty acids content and molecular species were analysed and compared in different samples. These data provide a scientific basis for studying the association of BCFAs and OCFAs in dietary fat sources and human health and developing high-BCFA and -OCFA food.

2. Materials and methods

2.1. Materials

Analytical grade diethyl ether, petroleum ether, ammonia, ethanol, and potassium hydroxide were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). High-performance liquid chromatography-grade hexane and methanol were purchased from J&K Scientific Ltd (Beijing, China). Fatty acid methyl esters (FAME) standard (C_4−C_{24}, 37 fatty acid methyl esters) was purchased from Sigma−Aldrich (St. Louis, MO, USA). BCFA standards of *i*15:0, *ai*15:0, *i*16:0, *i*17:0, *ai*17:0, *i*18:0 and a mixture standard (14:0, 15:0, 16:0, *i*14:0, *ai*15:0, *i*16:0, and *ai*17:0) were purchased from Larodan Fine Chemicals (Malmö, Sweden).

Human milk samples (>3 months of exclusive breastfeeding) were obtained from six volunteer mothers at the Wuxi Maternal and Child Health Hospital (Wuxi, China). The experiments were approved by the Ethics Committee of the Medical Research Board of Jiangnan University (JNU20210318IRB06). All donors received detailed information about the study and were provided written informed consent.

All food samples are shown in Supplementary material Table S1. Food samples were purchased from local supermarkets in Wuxi, China or online-market. Four mammalian kinds of milk, cow milk, goat milk, yak milk, and camel milk (pooled milk samples of 40−50), were purchased from pastures. From the dairy products group, twenty-seven types of commercially available dairy products, including four yoghurts, three blended milk, high-fat, low-fat, regular, non-fat milk, organic milk, processed cheese, buttermilk powder, anhydrous milk fat, cream cheese, condensed milk, ice cream, five kinds of infant formula, yak ghee, two Chinese cheese, and cheese crisp were also analysed. The vegetable oils include ten kinds (coconut oil, rice bran oil, soybean oil, flaxseed oil, sunflower seed oil, corn oil, palm oil, olive oil, peanut oil, and rapeseed oil). All samples were delivered to the lab and analysed within 24 h.

2.2. Lipid extraction

Before lipid extraction, about 0.50−3.00 g of materials were weighed and mixed with 5 mL of distilled water, respectively. Lipids were extracted with the modified Mojonnier method (Barbano, Clark, & Dunham, 1988). Briefly, 5 mL of the above sample was mixed with 1 mL of ammonia, and then the mixture was incubated at 65 °C for 20 min. After cooling to room temperature, 5 mL of ethanol, 10 mL of diethyl ether, and 10 mL of petroleum ether were added with vigorous mixing. Then the upper layer was transferred into a new tube. The second extraction was similar, except the volume of solvents was halved. Upper solutions from the two extractions were combined, dried with nitrogen, and then stored at −80 °C until analysis. Lipids were directly analysed without extraction for yak ghee, anhydrous milk fat, and vegetable oils.

2.3. Preparation of fatty acid methyl esters

Methylation of the extracted lipids was performed according to the method reported by Christopherson and Glass (1969). Briefly, 14 mg of lipid sample extract was dissolved in 2 mL hexane, then 400 μL of 2 mol L^{-1} potassium hydroxide in methanol was added. The mixture was vortexed for 2 min and centrifuged at 8000×*g* for 3 min. The upper ether layer was collected and filtered through a 0.22 μm membrane. The resulting samples were used for GC−MS analysis.

2.4. GC−MS analysis

FAMEs were analysed by Pegasus BT GC (LECO, Saint Joseph, MI, USA) equipped with a fime-of-flight mass spectrometer (TOF-MS). A DB-5 column (30 m × 0.25 mm × 0.25 μm, Agilent, Santa Clara, CA, USA) was used. The injector ran in split mode at 260 °C, the split ratio was 30, and the injection volume was 0.5 μL. High-purity helium (99.999%) was the carrier gas with a 1.0 mL min^{-1} flow rate. The oven temperature program was initially at 170 °C for 0.5 min, increased by 6 °C min^{-1} to 210 °C, increased by 3 °C min^{-1} to 230 °C, and then increased by 15 °C min^{-1} until a final temperature of 280 °C which was held for 5 min. Electron ionisation MS determined the identities of FAME. The electron energy was 70 eV, and the ion source temperature was 230 °C. The transmission line temperature was 300 °C, and the emission current was 1 mA. The full-scan mode collected the mass spectrum data with a scan range from 50 to 450 *m/z*, and the solvent delay was 160 s.

2.5. Identification of the branched-chain fatty acids and odd-chain fatty acids

The identification and quantification of FA were performed according to the reported method (Jie et al., 2018; Yan et al., 2015). LECO Chroma TOF software (v5.40.12.0, Saint Joseph, MI, USA) for Pegasus BT was used to process and analyse GC−MS data. By comparing the mass spectrum with the spectra of reference compounds in the NIST, Replib, mainly, and Wiley spectral libraries, along with the retention time and peak order of recognised substances in the standards, as well as relevant literature, it is possible to determine the chemical structure of fatty acids. Comparing the standards of *i*15:0/*ai*15:0/15:0 and *i*17:0/*ai*17:0/17:0, it is concluded

the elution order of FAMEs with the same molecular formula is *i*BCFA, *ai*BCFA, and straight-chain fatty acids. The respective peak areas were multiplied by response factors and then normalised to obtain the concentration of each FA as a percentage of the total FAs.

2.6. Statistical analysis

Each sample was analysed in triplicate. The results for each sample are expressed as the mean ± standard deviation. Results were evaluated with IBM SPSS 21 (Armonk, New York, USA). The comparison between multiple data groups used the Duncan test method in the one-way analysis of variance (ANOVA) for significance analysis, with $P < 0.05$ declared significant. Graphing was performed using Origin 2021 (OriginLab, Northampton, MA, USA).

3. Results and discussion

3.1. Fatty acid molecular structure identification by EI mass spectrum

Liquid chromatography with secondary mass spectrometric detection (Tanno, Sassa, Sawai, & Kihara, 2021) and two-dimensional gas chromatography with mass spectrometric detection (Chin, Che Man, Tan, & Hashim, 2009) and other methods have been used to determine BCFA concentration. However, GC–MS is still the most common method for determining BCFAs. It is challenging to separate *i*BCFA from the corresponding monoenoic acids when the gas chromatographic column is polar, such as BPX-70 or CP-Sil-88 (Dewhurst, Moorby, Vlaeminck, & Fievez, 2007). The DB-5 column is a weakly polar low-loss column, which we have previously used to study BCFAs in breast milk (Jie et al., 2018) as well as in vernix caseosa and meconium (Li et al., 2021). This column can well separate BCFAs from straight-chain fatty acids. This study analysed BCFAs and OCFAs in mammalian milk, dairy products, and vegetable oils with a DB-5 column and a different temperature program from our previous study.

This study found 72 FAs, including 16 BCFAs and 10 OCFAs, within 23 min. Fig. 1a gives the total ion current diagram of FAME in yak ghee. FAME (C_2–C_3) bond through γ-hydrogen migration and McDonald's rearrangement to generate fragment ions $[CH_3–O–C(OH) = CH_2]^+$ (*m/z* 74), which is the base peak ion of linear saturated FAME. $[M–43]^+$ and $[M–31]^+$ are the characteristic ions of straight-chain saturated fatty acid (*n*-SFA) methyl esters (Zhang, Tan, Zeng, Lu, & Liang, 2012). The *i*BCFA methyl esters produce an ion of $[M–43]^+$ corresponding to the terminal isopropyl moiety in the original *i*BCFA methyl esters. *ai*BCFA methyl esters produce two ions, corresponding to the loss of both sides of the methyl branch, $[M–29]^+$ and $[M–57]^+$ (Ran-Ressler, Lawrence, & Brenna, 2012; Wang, Wang, & Brenna, 2020a). The elution order for all FAMEs of the same molecular formula is *i*BCFA, *ai*BCFA, and straight-chain fatty acid. The mass spectrums of 17:0, *i*17:0, and *ai*17:0 methyl esters are shown in Fig. 1b–d. Polymethyl BCFA elutes before mmBCFA of the same molecular structure (Ran-Ressler et al., 2012). The ions produced by polymethyl BCFA methyl esters reflect bond breakage around the branch points. The two ions on either side of the branch point differ by $[-C_2H_4-]$. In the mass spectrum, the mass-to-charge ratio of the ions varies by 28 (Ratnayake, Olsson, & Ackman, 1989; Zirrolli & Murphy, 1993). The mass spectrum of 4,8,12-trimethyltridecyl acid methyl esters is shown in Fig. 1e.

3.2. BCFA and OCFA in mammalian milk

The fatty acid composition of all samples is shown in Supplementary material Table S1. The *i*BCFA had odd and even carbon-numbered fatty acids from *i*13:0 to *i*19:0, *ai*BCFA only had odd carbon number FA from *ai*13:0 to *ai*17:0, and several ntBCFA were found in all samples. The level of *ai*15:0 was the highest of BCFAs, followed by *ai*17:0, *i*17:0, and *i*15:0. *ai*15:0 and *ai*17:0 accounted for about half of the total BCFAs, and 15:0 nearly accounts for half of OCFAs.

As shown in Table 1, the BCFA and OCFA content of five mammalian milk samples were determined. Camel milk had the highest BCFA tested in this study, with a BCFA concentration of 6.04%. The BCFA concentration of yak milk, goat milk, and cow milk were 3.91%, 2.30%, and 2.27%, respectively. Human milk had the lowest BCFA content of mammalian milk detected in this study at 0.37%, consistent with a previous study (0.28–0.55%) (Jie et al., 2018). The content of ntBCFA in goat milk was 0.15% of the total FA, which was the highest content of ntBCFA in mammalian milk. 4,8-Dimethylnonanoic acid and 4-methyldodecanoic acid were detected in goat milk (Supplementary material Table S1). 4-Methyl BCFAs are characteristic BCFAs in goat milk; at the same time, they are the signature flavour of goat milk (Watkins et al., 2021).

The OCFA concentrations of camel, yak, goat, cow and human milk were 4.34, 3.95, 2.61, 2.53, and 0.81% of total FAs, respectively. The difference in the content of OCFAs in mammalian milk has a similar trend to the difference in BCFA content. According to prior studies, the OCFA concentrations in camel, yak, goat, cow, and human milk fat were 1.5–5.0%, 2.9–5.9%, 1.7–2.3%, 1.2–2.5%, and 0.5–0.9% of total FAs, respectively (Bainbridge et al., 2016; Ereifej, Alu'datt, AlKhalidy, Alli, & Rababah, 2011; Karrar et al., 2022; Nudda et al., 2021; Qi et al., 2018; Sun et al., 2019; Zhang, Wei, Tao, Jin, & Wang, 2021).

In ruminants, BCFAs are mainly from the diet and de novo synthesised by rumen microorganisms, particularly in bacteria and protozoa (Tanno et al., 2021; Taormina, Unger, Schiksnis, Torres-Gonzalez, & Kraft, 2020). *i* even chain, *i* odd chain, *ai* odd chain BCFAs are derived from branched-chain amino acids (BCAAs) valine, leucine and isoleucine, respectively (Tanno et al., 2021; Taormina et al., 2020). Synthesis of mmBCFAs is also maintained in the absence of microbiota in ruminants. mmBCFAs are generated de novo by mitochondrial BCAA catabolism, exported to the cytosol through adipose-specific carnitine acetyltransferase (CrAT) expression, and elongated by FA synthase (Wallace et al., 2018).

OCFAs are mainly derived from the ruminal bacteria cell wall and then partitioned into organs and tissues (Nudda et al., 2021). Typically, OCFAs are biosynthesised from odd-chain precursors or by chain shortening driven by α-oxidation of even-chain fatty acids (Zhang, Liang, Zong, Yang, & Lou, 2020). Bacteria synthesise OCFAs by elongating the carbon chain of propionate or valerate (Or-Rashid, Odongo, & McBride, 2007). And the mammary gland plays a role in their synthesis using propionate (Nudda et al., 2021). The variations in rumen microorganism species, the effectiveness of fatty acid synthesis and absorption, and the uptake and storage of fatty acids in blood circulation by the mammary gland may all contribute to the variations in OCFA and BCFA concentrations in these ruminant milk (Nudda et al., 2021; Taormina et al., 2020).

3.3. BCFA and OCFA in cow milk-derived samples

The BCFA and OCFA contents of cow milk-derived dairy products are shown in Table 2. The molecular species of BCFAs in cow milk-derived dairy products are similar, but the content is different (Supplementary material Table S1). Among all cow milk-derived dairy products, Chinese cheese 1, Chinese cheese 2 and processed cheese show the highest range of BCFA at 2.95, 1.94, and 1.94% of total FA, respectively. The BCFA content in sheep and goat cheese was relatively high among all dairy food detected by Ran-Ressler, Bae, Lawrence, Wang, and Brenna (2014).

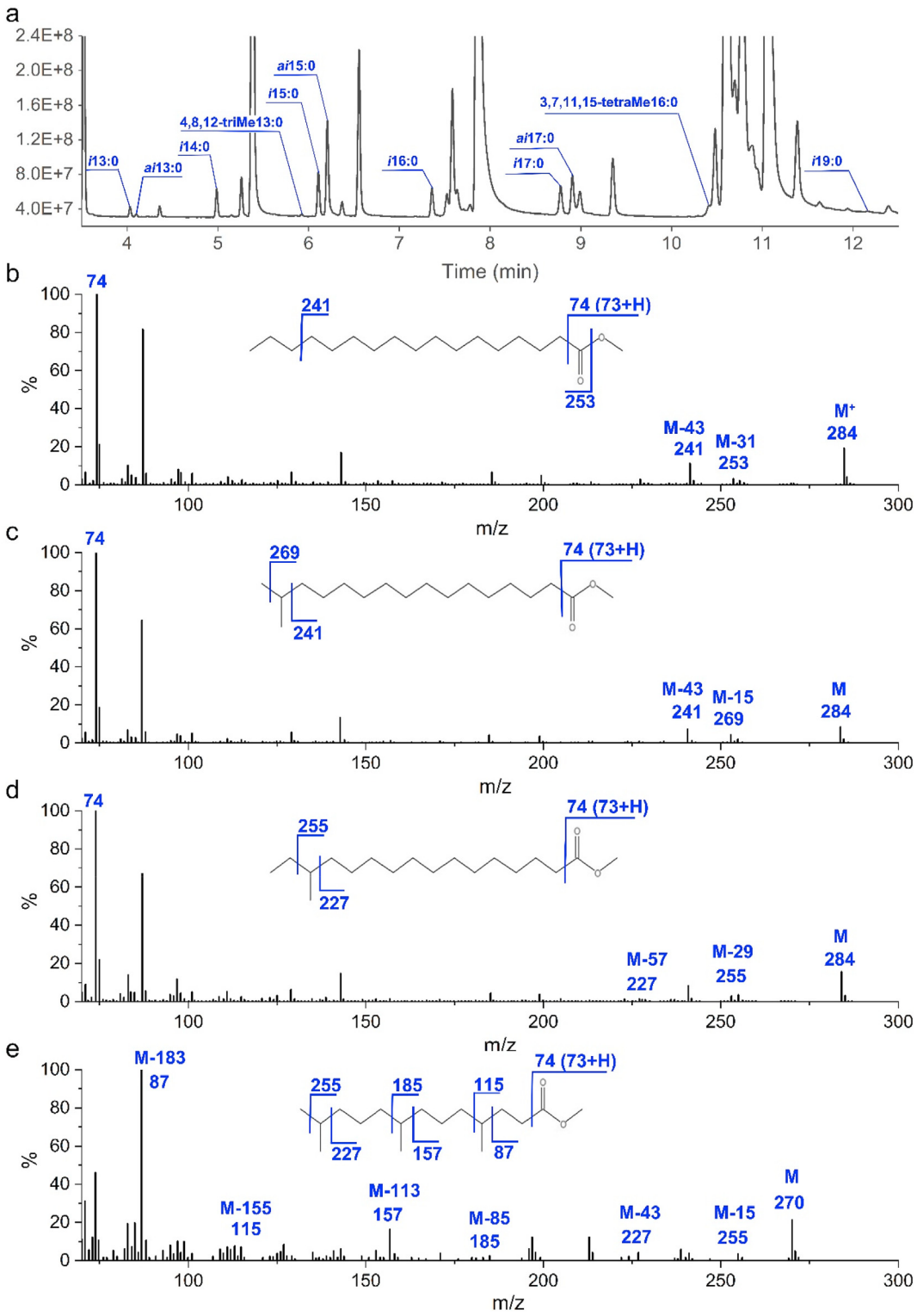

Fig. 1. The representative total ion current diagram of fatty acid methyl ester (a) and the mass spectrum of 17:0 (b), *i*17:0 (c), *ai*17:0 (d), 4,8,12-trimethyltridecyl acid methyl esters (e) in yak ghee.

BCFAs are essential components of the cell membrane of some microorganisms, which can help individuals adapt to the external environment (Kaneda, 1991). The bacteria introduced during the fermentation process might increase the content of BCFAs in dairy products, resulting in higher BCFA content in fermented dairy products. But Nudda et al. (2021) examined raw milk and processed fresh cheese fat and found no difference in individual OCFA and BCFA concentrations. This indicates if processing affects dairy products' OCFA and BCFA concentrations and needs further study. The diet, lactation stage, and breed of dairy cows affect dairy

Table 1
Branched-chain fatty acids and odd-chain fatty acids profile (wt% of total fatty acids) of mammalian milk samples.[a]

Fatty acid	Human milk	Cow milk	Goat milk	Yak milk	Camel milk
*i*BCFA	0.18 ± 0.05	1.01 ± 0.06	1.12 ± 0.02	1.77 ± 0.11	2.72 ± 0.05
i13:0	nd	0.04 ± 0.00	0.02 ± 0.00	0.07 ± 0.00	0.07 ± 0.00
i14:0	0.02 ± 0.01	0.10 ± 0.00	0.10 ± 0.00	0.30 ± 0.02	0.27 ± 0.01
i15:0	0.02 ± 0.01	0.20 ± 0.00	0.19 ± 0.00	0.44 ± 0.03	0.90 ± 0.02
i16:0	0.05 ± 0.01	0.20 ± 0.00	0.30 ± 0.00	0.38 ± 0.02	0.55 ± 0.01
i17:0	0.05 ± 0.02	0.34 ± 0.00	0.42 ± 0.00	0.47 ± 0.03	0.70 ± 0.01
i18:0	nd	0.12 ± 0.06	0.09 ± 0.02	0.12 ± 0.01	0.20 ± 0.01
i19:0	0.04 ± 0.03	0.20 ± 0.00	nd	nd	0.04 ± 0.00
*ai*BCFA	0.19 ± 0.05	1.26 ± 0.02	1.04 ± 0.02	2.12 ± 0.13	3.24 ± 0.06
ai13:0	nd	0.01 ± 0.00	0.01 ± 0.00	0.03 ± 0.01	0.05 ± 0.00
ai15:0	0.04 ± 0.03	0.69 ± 0.01	0.49 ± 0.02	1.37 ± 0.08	1.84 ± 0.04
ai17:0	0.15 ± 0.04	0.56 ± 0.01	0.54 ± 0.00	0.72 ± 0.05	1.36 ± 0.03
ntBCFA	0.01 ± 0.09	0.01 ± 0.00	0.15 ± 0.00	0.01 ± 0.00	0.08 ± 0.00
Total BCFA	0.37 ± 0.09	2.27 ± 0.08	2.30 ± 0.04	3.91 ± 0.24	6.04 ± 0.11
OCFA	0.81 ± 0.11	2.53 ± 0.03	2.61 ± 0.02	3.95 ± 0.24	4.34 ± 0.08

[a] Abbreviations are: BCFA, branched-chain fatty acid; ntBCFA, non-terminal branched-chain fatty acid; OCFA, odd-chain fatty acid; nd, not detected.

Table 2
Branched-chain fatty acids and odd-chain fatty acids profile (wt% of total fatty acids) in cow milk-derived dairy products.[a]

Sample	*i*BCFA	*ai*BCFA	ntBCFA	Total BCFA	OCFA
Yoghurt 1	0.54 ± 0.07	0.67 ± 0.10	nd	1.20 ± 0.18	1.67 ± 0.31
Yoghurt 2	0.51 ± 0.04	0.62 ± 0.07	nd	1.13 ± 0.11	1.77 ± 0.17
Yoghurt 3	0.60 ± 0.03	0.75 ± 0.01	nd	1.35 ± 0.02	2.18 ± 0.01
Yoghurt 4	0.44 ± 0.04	0.47 ± 0.05	nd	0.92 ± 0.09	1.57 ± 0.28
Blended milk 1	0.52 ± 0.04	0.55 ± 0.08	nd	1.08 ± 0.12	1.34 ± 0.20
Blended milk 2	0.67 ± 0.10	0.84 ± 0.15	0.19 ± 0.01	1.70 ± 0.26	1.62 ± 0.28
Blended milk 3	0.45 ± 0.08	0.61 ± 0.15	nd	1.07 ± 0.22	1.71 ± 0.33
High-fat milk	0.60 ± 0.03	0.70 ± 0.04	nd	1.29 ± 0.06	1.63 ± 0.12
Low-fat milk	0.57 ± 0.05	0.67 ± 0.07	nd	1.24 ± 0.12	1.67 ± 0.11
Regular milk	0.53 ± 0.03	0.64 ± 0.01	nd	1.17 ± 0.05	1.67 ± 0.02
Non-fat milk	0.43 ± 0.03	0.36 ± 0.03	nd	0.79 ± 0.06	1.07 ± 0.14
Organic milk	0.52 ± 0.06	0.65 ± 0.09	nd	1.17 ± 0.15	1.58 ± 0.21
Chinese cheese 1	1.45 ± 0.02	1.50 ± 0.03	nd	2.95 ± 0.05	2.10 ± 0.04
Chinese cheese 2	0.92 ± 0.02	1.00 ± 0.03	0.01 ± 0.00	1.94 ± 0.06	2.29 ± 0.26
Processed cheese	0.79 ± 0.11	1.00 ± 0.17	0.16 ± 0.02	1.94 ± 0.30	2.02 ± 0.35
Buttermilk powder	0.88 ± 0.02	0.68 ± 0.03	0.24 ± 0.01	1.80 ± 0.06	1.94 ± 0.05
Anhydrous milk fat	0.78 ± 0.04	0.94 ± 0.05	nd	1.71 ± 0.09	1.80 ± 0.04
Cream cheese	0.47 ± 0.05	0.59 ± 0.04	nd	1.06 ± 0.09	1.63 ± 0.04
Condensed milk	0.32 ± 0.01	0.33 ± 0.01	nd	0.65 ± 0.02	0.82 ± 0.01
Ice cream	0.31 ± 0.00	0.34 ± 0.00	nd	0.64 ± 0.01	0.73 ± 0.01
Cheese crisp	0.22 ± 0.01	0.22 ± 0.02	nd	0.44 ± 0.03	0.69 ± 0.04

[a] Abbreviations are: BCFA, branched-chain fatty acid; ntBCFA, non-terminal branched-chain fatty acid; OCFA, odd-chain fatty acid; nd, not detected.

products' BCFA and OCFA content (Bainbridge et al., 2016; Corazzin et al., 2019). In addition, the distinction between conventional and organic milk has been demonstrated (Schwendel et al., 2015). But the FA composition of organic milk detected in this study had no difference from regular milk (Supplementary material Table S1).

The BCFA concentrations of high-fat, low-fat, regular, and non-fat milk from the same brands were 1.29%, 1.24%, 1.17% and 0.79% of total FAs, respectively. The OCFA concentrations of the four were 1.63%, 1.67%, 1.67%, and 1.07% of total FAs, respectively. The total BCFAs and OCFAs of total FAs in high-fat, low-fat, and regular milk were not significantly different ($P < 0.05$). The total BCFAs and OCFAs of total FAs in non-fat milk were much lower than the other three. Buttermilk powder, as a co-product of butter production, has a similar total BCFA and total OCFA content to anhydrous milk fat. Yak ghee BCFA and OCFA contents were higher, accounting for 2.93% and 2.75% of total FA, respectively (Supplementary material Table S1). The fat sources highly influenced the content of BCFAs and OCFAs. Condensed milk, ice cream, and cheese crisp had the lowest BCFAs and OCFAs, 0.44–0.65% and 0.69–0.82%, respectively. Yoghurt and blended milk had BCFA amounts of 0.92–1.35% and 1.07–1.70% of total FAs, respectively, whereas the OCFA contents were 1.57–2.18% and 1.34–1.71% of total FAs, respectively.

3.4. BCFA and OCFA in infant formulas of different fat sources

This study selected five IFs with different oil compositions (Table 3). Among the IFs, two contain cow milk fat (IF A and IF C); one has goat milk fat (IF B), while the others contain mainly a blend of vegetable oils. All IFs were supplied with long-chain poly-unsaturated fatty acids. Fig. 2 shows the contents of BCFAs and OCFAs of total FAs in five IFs with different fat sources and similar processing. There were apparent differences in the composition of BCFAs in IFs that were highly related to their fat sources. BCFAs were not detected in IF E, which contained vegetable oils, ARA from *Mortierella alpina* and DHA from *Crypthecodinium cohnii*. A small amount (0.04% of total FAs) of BCFAs was detected in IF D, which contained OPO, ARA and DHA in addition to vegetable oils. Recent studies show the potential benefits of BCFAs to infant gut health (Liu et al., 2017; Ran-Ressler et al., 2008). Considering the BCFA level, vegetable oils without mammalian fats are not recommended due to differences from human milk.

IF C with raw cow milk and vegetable oil as the lipid source, IF B with goat milk powder and vegetable oil, and IF A with anhydrous milk fat and vegetable oil, their concentrations of total BCFAs were 0.24, 0.47, and 0.61%. IF A had the highest concentration of each

Table 3
Oil composition of infant formulas.[a]

Formula	Oil composition
IF A	Anhydrous milk fat, vegetable oils (rapeseed oil, sunflower oil, coconut oil), ARA, DHA, phospholipids
IF B	Goat milk powder, vegetable oils (palm oil, sunflower oil, soybean oil, coconut oil), DHA, ARA, soy lecithin
IF C	Raw cow milk, vegetable oils (*sn*-2 palmitate, sunflower oil, linseed oil), ARA, DHA, phospholipids
IF D	Vegetable oils (sunflower oil, coconut oil, corn oil, rapeseed oil, soybean oil), *sn*-2 palmitate, ARA, DHA, phospholipids
IF E	Vegetable oils (sunflower oil, soybean oil, coconut oil), ARA (source of *Mortierella alpina*), DHA (source of *Crypthecodinium cohnii*), phospholipids

[a] Abbreviations are: DHA, docosahexaenoic acid; ARA, arachidonic acid. The oils in infant formulas are ordered by content from high to low.

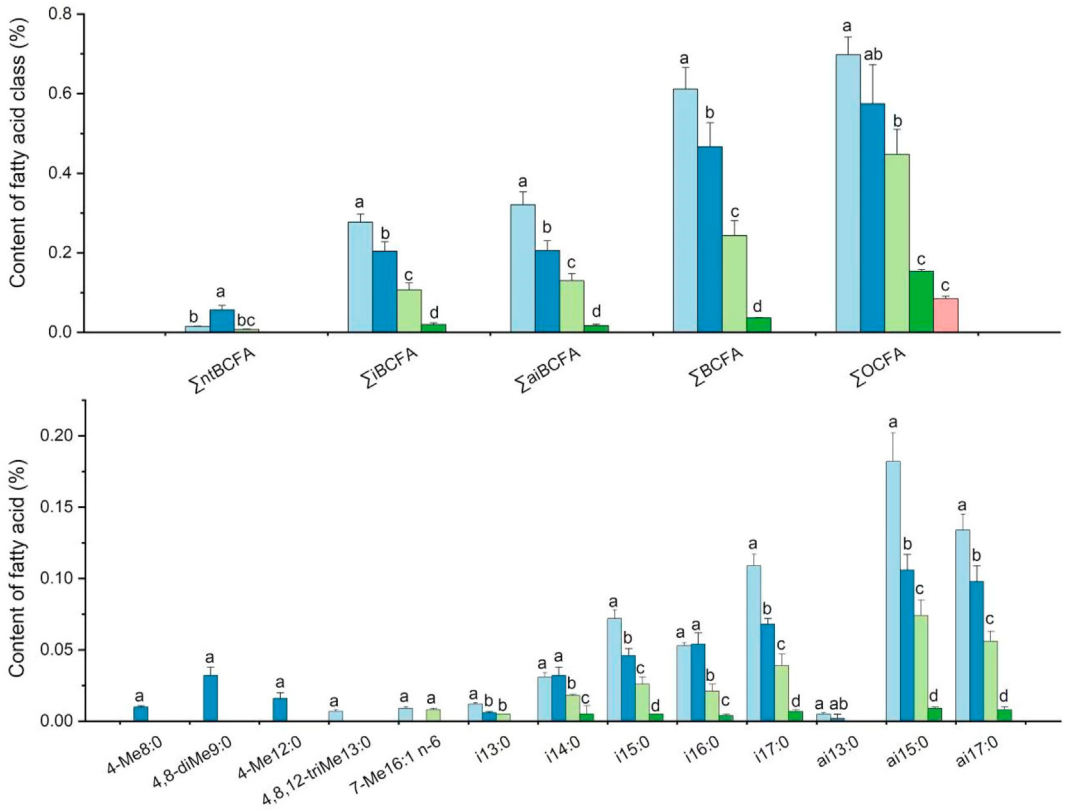

Fig. 2. Contents of BCFA and OCFA (wt% of total fatty acids) in five infant formulas with different fat sources: ■, IF A; ■, IF B; ■, IF C; ■, IF D; ■, IF E. Different letters (a–b) indicate significant differences ($P < 0.05$) among the samples.

BCFA, except for 4-methyl BCFA. IF B had the highest concentration of ntBCFA. Only IF B had 4-methyloctanoic acid, 4,8-dimethylnonanoic acid, and 4-methyldodecanoic acid, which was consistent with goat milk containing 4-methyl BCFA, as shown in Supplementary material Table S1 (Watkins et al., 2021).

The OCFA concentrations of IF A–E were 0.70, 0.58, 0.45, 0.15 and 0.09% of total FA, respectively. The difference in the content of OCFAs in IFs has a similar trend to the difference in BCFA content.

3.5. OCFAs in vegetable oils

Supplementary material Table S1 shows no BCFAs were detected in all 10 types of vegetable oil often added to dairy products. This was consistent with the IF result with vegetable oil as the sole source of lipids discussed above and other research findings (Chin et al., 2009; Chowdhury, Banu, Khan, & Latif, 2007; Giakoumis, 2018). The levels of OCFAs in vegetable oil were low at 0.05–0.22% of total FAs. BCFAs were not detected in vegetable oils in this study, but was caught in chia seeds (Wang et al., 2020b), brussels sprouts (Eibler, Seyfried, Kaffarnik, & Vetter, 2015), and natto (Wang et al., 2019).

3.6. BCFA and OCFA profiles of different samples

Fig. 3 shows the concentration of BCFAs and OCFAs in samples from various fat sources. According to the fat source, the samples were classified into the plant, human, cow, goat, yak, and camel milk. Camel milk fat has the highest levels of BCFAs and OCFAs, followed by yak milk fat, goat milk fat, and cow milk fat. BCFAs in mammalian and cow milk-derived dairy products are almost all iBCFA, and aiBCFA and each one accounted for ~50% of total BCFAs. The samples with vegetable oil as the sole source of fat contained no BCFAs but very low OCFAs. The concentrations of BCFA and OCFA in the samples of the milk category analysed in this study were quite different, which may be due to the addition of varying content vegetable oils, the processing, or the diet, lactation stage, breed of dairy cow and so on. The samples with more milk-derived fat seem to have more BCFAs and OCFAs.

4. Conclusions

BCFAs and OCFAs were evaluated and compared in lipid samples of different sources using GC. This study provides an effective

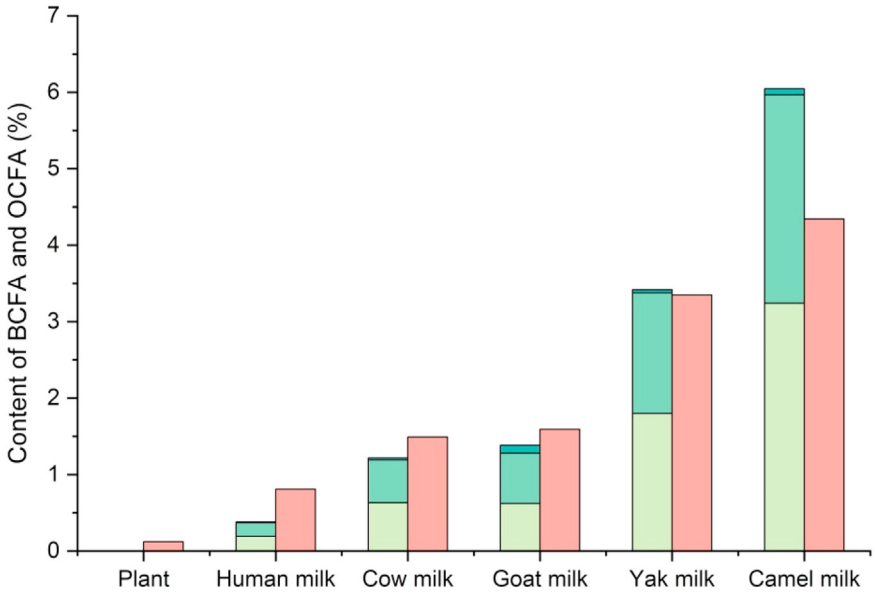

Fig. 3. Contents of BCFA (*i*BCFA, ■; *ai*BCFA, ■; ntBCFA, ■) and OCFA (■) (wt% of total fatty acids) in samples from different fat sources.

GC–MS method for as many as 72 kinds of FAs identified using a facile pre-processing method. The content and molecular species of BCFAs and OCFAs in samples from different fat sources are other. BCFAs are rich in mammalian milk, with the highest range in camel milk (~6%) and the lowest in human milk (~0.4%). The content of BCFAs and OCFAs in mammalian milk fat and dairy products is the same. Processes such as fermentation may affect the BCFAs content in cow milk-derived dairy products. The BCFAs content in infant formula significantly differs between different fat phase sources. Vegetable oils do not contain BCFAs but contain a small amount of OCFAs. The main BCFAs molecular species in dairy are *i*BCFA and *ai*BCFA, accounting for ~50% of each type. This study provides data for the development of high BCFA/OCFA-foods. The association between BCFA content and molecular configuration and human health function needs further investigation.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.idairyj.2023.105587.

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