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High levels of branched chain fatty acids in nātto and other Asian fermented foods

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ABSTRACT

The mean intake (500 mg/day) of branched chain fatty acids (BCFA) in western countries is from dairy products and beef. We hypothesized that Asian countries with low dairy consumption have an alternative source of BCFA and report the characterization of nātto and selected fermented foods for BCFA content. Nātto BCFA averaged 0.6 (range 0.21–1.43) mg BCFA per g natto (wet weight) and was highly variable. Nātto major BCFA are C14-17 *iso-* and *anteiso*-BCFA similar to fluid milk. BCFA concentrations were 1.00 ± 0.64%, 1.63 ± 0.72% and 0.65 ± 0.07%, of total fatty acids in nātto, shrimp paste and fish sauce, respectively. In contrast, saturates, monounsaturates, and major polyunsaturates were more constant (coefficient of variation = 21%, 26% and 4% compared to 64% for nātto BCFA). Detection of fatty acid ethyl esters were confirmed in miso and found in homemade kimchi. Habitual nātto and/or fermented seafood consumption could support BCFA intakes similar to dairy consumption.

1. Introduction

Fermented foods play important roles in the diets and culture of Asian countries. Some varieties are as common as yogurt in the western world. For instance, fermented vegetables known as kimchi is a daily part of Korean diets, and fermented soy miso is a common food in Japan. Douchi is a fermented black soybean product considered an indispensable ingredient in Chinese kitchens across the country. Nātto is a traditional Japanese food made from fermented cooked soybeans, consumed by many Japanese weekly. Limited data on chronic disease prevention is available for some foods. For instance, nātto intake is associated with less bone loss in Japanese postmenopausal women (Ikeda et al., 2006). Non-vegetarian fermented foods are available in most parts of Asia, for example shrimp paste, employing *Acetes* as the fermentation organism, and fish sauce made from fermented anchovy. While dairy consumption in Asian countries is low, these fermented foods contribute protein, fat and vitamins in a traditional cereal based diet.

BCFA are mostly saturated fatty acids with a terminal propan-2-yl (isopropyl) group (*iso*) or butan-2-yl (sec-butyl) group (*anteiso*) (Favre & Powell, 2013). Food BCFA originate primarily from bacterial action. U.S. milkfat contains 2% BCFA derived from ruminal fermentation (Ran-Ressler et al., 2011), though milkfats of some animals such as yak and sheep contain > 3% (Or-Rashid, Odongo, Subedi, Karki, & McBride, 2008) and 2.7%, respectively (Ran-Ressler, Bae, Lawrence, Wang, & Brenna, 2014). Together with beef, BCFA daily intake for an average American is about 500 mg (Ran-Ressler et al., 2014), greater than other bioactive fatty acids such as omega-3 EPA and DHA averaging 100 mg for Americans (Brenna & Lapillonne, 2009; Ervin, Wright, Wang, & Kennedy-Stephenson, 2004). High BCFA intake from dairy and beef products may support gut health. Early evidence indicates that BCFA alter nascent microbiota and reduce the incidence of necrotizing enterocolitis in a neonatal rat study (Ran-Ressler et al., 2011), and reduce pro-inflammatory markers in a human intestinal cell line (Yan et al., 2017).

In Asian countries where dairy consumption is relatively low, intake of potential health-promoting BCFA might be expected to be low. However, a recent study found a comparable amount of BCFA in the breast milk from Chinese mothers and American mothers (Dingess et al., 2017). We speculate that fermentation may be a source of BCFA in Asian foods. The nātto organism *Bacillus subtilis* biosynthesizes BCFA to supply membranes to about 95%,w/w of total fatty acids (Kaneda,

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1977). This observation suggests that nātto may be rich in BCFA. Kimchi is fermented by lactic acid bacteria (Lee, 1997) which are another group of BCFA producers (Kaneda, 1991). Fermented foods may also have other distinct fatty acid properties from the unfermented raw food, specifically a high concentration of fatty acid ethyl esters (FAEE) as are found in miso (Yamabe, Kaneko, Inoue, & Takita, 2004).

Numerous reports are available on the BCFA contents of ruminant milks and meat (Bravo-Lamas, Barron, Farmer, & Aldai, 2018; DePooter, Decloedt, & Schamp, 1981; Dreiucker & Vetter, 2011; Klir et al., 2017; Ran-Ressler et al., 2011; Rawdah, Zamil El-Faer, & Koreish, 1994). Other than our previous work (Ran-Ressler et al., 2014), reports of fatty acid profiles of other foods, including fermented foods, rarely specify BCFA levels, and no studies have systematically considered the content of BCFA in fermented foods. The goal of this study is to determine the BCFA composition of popular fermented Asian foods that are fermented by BCFA producing microorganisms and/or high in fat content. Nātto and kimchi were chosen mainly because they are fermented by BCFA producing microorganisms while miso and douchi are high in fat that could contribute significant amount of BCFA even if the concentration is low. Although it is unclear if *Acetes* species are naturally rich in BCFA, as seen in some true krill such as Antarctic krill (*Euphausia superba*) (Fricke, Gercken, Schreiber, & Oehlenschlager, 1984), shrimp paste undergoes complex bacterial fermentation that could also contribute to BCFA content.

2. Materials and methods

2.1. Sampling

A total of 9 different brands and/or preparations of nātto were purchased from Asian grocery stores for the study purposes. Nos.1–7 nātto brands/preparations were purchased in Austin, TX (Nos.1–3 are three preparations of Shirakiku brand, Japan; No.4 is Hime brand, Japan; No.5 is Azuma Brand, Japan; No.6 is Kazoku brand, Japan and No.7 is imported by JFC from Japan). Nātto No.8 and No.9 were two different preparations (Mizkan Co., Ltd., 2–6, Nakamura-cho, Handa-shi, Aichi-ken, Japan) purchased from local Asian stores in Ithaca, NY. A total of three shrimp paste brands were purchased from Asian supermarkets in Austin (Huong Vi Que Nha, Vietnam; Cincalok, Malaysia and Lee Kum Kee International Holdings Ltd., Hong Kong, China) with the exception of the two Lee Kum Kee shrimp paste, one of which were obtained from Amazon online. three brands of anchovy fish sauce were also purchased from Asian supermarkets in Austin (two products from Saigon International brand, Thailand and another from Phu-Quoc brand, Bangkok, Thailand). Kimchi was homemade by a traditional recipe, including napa cabbage, red pepper, carrot, salt and a small amount of shrimp paste. Miso (Aka miso, Kabuto Inc.) was purchased from a local supermarket in Ithaca, NY (Distributed by Rhee Bros., Inc., 7461 Coca Cola Drive, Hanover, MD, USA) and originated in Japan. Douchi was obtained from a local supermarket in Ithaca, NY (Koon Chun, Inc., Hong Kong, China). Dried shrimps (Acetes species) were purchased from a local Asian grocery in Ithaca, NY (Eastern Oceanic Enterprise, Brooklyn, NY). Aliquots of shrimp paste, dried shrimp, fish sauce, miso, kimchi, douchi and nātto No.8–9 were processed and analyzed in duplicate. All other nātto brands/preparations were analyzed from two packages to explore their variance within brands/preparations. All samples were stored in 4 °C refrigerator and analyzed as soon as practical after purchase.

2.2. Fatty acid analysis

Samples (50–250 mg) of each food were extracted, saponified and methylated according to a modified one-step hydrolysis procedure described previously (Garces & Mancha, 1993; Zhou et al., 2008). Fish sauce in liquid form was extracted by Bligh & Dyer method before one-step procedure (Bligh & Dyer, 1959). Methylated fatty acids were then analyzed with a Hewlett Packard 5890 Gas Chromatograph (GC) equipped with a split/splitless injector run in splitless mode at 250 °C, and with the flame ionization detector (FID) set at 270 °C. A BPX-70 column (25 m × 0.22 mm × 0.25 lm, SGE, Austin, TX) was used with hydrogen as the carrier gas. The oven temperature program was initially 80 °C for one minute, increased by 30 °C per minute to 170 °C and held for 2 min, then increased by 10 °C per minute until a final temperature of 240 °C which was held for 1 min. An equal weight FAME mixture (GLC462; Nu-Chek Prep, Elysian, MN) was used to calculate response factors. BCFA were identified by electron ionization mass spectrometry (EIMS) and EIMS/MS described previously (Ran-Ressler, Lawrence, & Brenna, 2012). Several pure BCFA were also used as reference standards: *iso*-14:0, *anteiso*-15:0; *iso*-16:0, *anteiso*-17:0, *iso*-18:0 and *iso*-20:0 (Larodan Fine Chemicals AB, Malmo, Sweden). Where statistical comparisons were made, Student's two tailed, equal variance *t*-test was conducted in Microsoft Excel with significance level set at $p < 0.05$.

3. Results and discussion

Nātto BCFA concentrations were 1.00 ± 0.64%, w/w of total fatty acids (mean ± SD). Fermented shrimp paste had the highest concentration of BCFA at 1.63 ± 0.72%, similar to the proportion of 2% BCFA in fluid milk in the USA (Ran-Ressler et al., 2011). BCFA were 0.62 ± 0.05, 0.73 ± 0.04, 0.37 ± 0.06%, 0.64 ± 0.08% and 0.08 ± 0.01% in fish sauce (liquid), fish sauce (paste), miso, homemade kimchi and douchi, respectively. *iso*-14:0, *iso*-15:0, *anteiso*-15:0, *iso*-16:0, *iso*-17:0, *anteiso*-17:0 and *iso*-18:0 were all detected in shrimp paste, kimchi and nātto, except *iso*-18:0 was below detection limit in nātto. In addition, shrimp paste also contained *iso*-20:0 and *iso*-24:0. Two types of fish sauce had all the aforementioned C15–C18 BCFA. *Only iso*-15:0, *iso*-16:0, *anteiso*-17:0 and *iso*-18:0 were found in miso. Low concentrations of *iso*-15:0, *anteiso*-15:0, *iso*-16:0, *iso*-17:0 and *anteiso*-17:0 were found in douchi. *iso*-17:0 was the highest at 0.5% in shrimp paste and *anteiso*-15:0 was highest at 0.35% in nātto. However, *iso*-15:0 and *anteiso*-17:0 were highest in miso and kimchi, respectively. Despite *anteiso*-15:0 alone made up a third of BCFA in nātto, total iso-BCFA were slightly higher than *anteiso*-BCFA in nātto and this trend applied to kimchi, miso and douchi as well. In the case of shrimp paste and both types of fish sauce, iso-BCFA were much higher than *anteiso*-BCFA (Table 1).

The nātto products contain about 8% fat according to the package. Using this figure, one gram of nātto would provide 0.63 ± 0.51 mg BCFA, resembling that of the 0.67 ± 0.05 mg BCFA in 1 g of whole milk. Some nātto products have up to 1.43 mg BCFA per gram of sample, which is more than twice the concentration in whole milk. Shrimp paste, fish sauce (paste-like) and miso are also good sources of BCFA at 0.2–0.4 mg per gram of the foods. Liquid fish sauce, kimchi and douchi had < 0.1 mg BCFA per gram of the foods. One half cup serving of nātto is about 90 g, and one serving of whole milk is about one cup (244 g). When serving size is taken into account, a serving of nātto provides 19–129 mg BCFA as compared with 158 mg in cow's milk. Shrimp paste, fish sauce, kimchi, miso and douchi provide small amounts of BCFA (~0.1–6 mg/serving, Table 2). Not surprisingly linoleic acid comprises more than 50% of nātto total fatty acids, followed by oleic acid, palmitic acid, alpha-linolenic acid and stearic acid (Supplementary Table 1). All other fatty acids are below 1%. Saturated fatty acids comprise about a fifth of total fatty acids by weight. The two polyunsaturated fatty acids (PUFA), linoleic acid and alpha-linolenic acid, in nātto represent about 65% of total fatty acids, all consistent with the fatty acid profile of soybeans.

Fig. 1 shows that nātto BCFA varied more than the other lipid classes, specifically normal saturates, monounsaturates and polyunsaturates, and across different groups (brands/preparations) and even remarkably within groups. Nātto BCFA ranged from 0.2 to 3.0% of total fatty acids. Since BCFA originate with *B. subtilis* (95% BCFA

Table 1
BCFA concentrations (wt% of total fatty acids) in nātto, shrimp paste, fish sauce, miso, kimchi and douchi (mean ± SD).

BCFA (wt%)	Nātto	Shrimp paste	Fish sauce liquid	Fish sauce (paste-like)	Miso	Kimchi[a]	Douchi
iso-14:0	0.05 ± 0.10	0.02 ± 0.01	< 0.003[b]	< 0.003	< 0.003	0.06 ± 0.01	< 0.003
iso-15:0	0.15 ± 0.08	0.14 ± 0.05	0.12 ± 0.01	0.14 ± 0.00	0.20 ± 0.00	0.04 ± 0.01	0.01 ± 0.01
ai-15:0	0.35 ± 0.25	0.06 ± 0.01	0.05 ± 0.01	0.04 ± 0.00	< 0.003	0.07 ± 0.00	0.02 ± 0.01
iso-16:0	0.17 ± 0.12	0.11 ± 0.08	0.07 ± 0.00	0.07 ± 0.00	0.04 ± 0.00	0.12 ± 0.01	0.01 ± 0.00
iso-17:0	0.14 ± 0.07	0.50 ± 0.25	0.09 ± 0.02	0.28 ± 0.01	< 0.003	0.08 ± 0.00	0.02 ± 0.00
ai-17:0	0.12 ± 0.11	0.16 ± 0.06	0.11 ± 0.02	0.08 ± 0.00	0.10 ± 0.03	0.21 ± 0.02	0.01 ± 0.00
iso-18:0	< 0.003	0.25 ± 0.10	0.18 ± 0.02	0.11 ± 0.02	0.04 ± 0.02	0.06 ± 0.02	< 0.003
iso-20:0	< 0.003	0.05 ± 0.07	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003
iso-24:0	< 0.003	0.36 ± 0.13	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003
Total *iso*	0.52 ± 0.33	1.42 ± 0.68	0.46 ± 0.02	0.60 ± 0.03	0.28 ± 0.02	0.36 ± 0.05	0.04 ± 0.01
Total *ai*	0.48 ± 0.35	0.22 ± 0.06	0.16 ± 0.03	0.12 ± 0.00	0.10 ± 0.03	0.28 ± 0.02	0.03 ± 0.01
Total BCFA	1.00 ± 0.64	1.63 ± 0.72	0.62 ± 0.05	0.73 ± 0.04	0.37 ± 0.06	0.64 ± 0.08	0.08 ± 0.01
CV, total BCFA	64.3%	44.2%	8.3%	N.A	N.A	N.A	N.A

[a] The addition of shrimp paste into homemade kimchi is mainly for flavor and such small addition made negligible contribution to BCFA concentration in kimchi based on our calculations.
[b] Reflects the lower limit of quantification.

Table 2
BCFA content per serving in nātto, shrimp paste, fish sauce, miso, kimchi and douchi in comparison with milk, expressed as the ranges (low–high) for each sample analyzed here.

Fermented Food	Fat (%)[a]	BCFA (mg fatty acid/g sample)	BCFA (mg fatty acid/serving)
Milk	3.25	0.67 ± 0.05[b]	(244 g) 158
Nātto	4.57–8.38	0.21–1.43	(90 g) 19–129
Shrimp paste	0.52–1.55	0.06–0.39	(16 g) 0.9–6.3
Fish sauce (liquid)	0.13–0.19	0.01	(16 g) 0.13–0.18
Fish sauce (paste-like)	4.11–4.17	0.29–0.31	(16 g) 4.6–5.0
Miso	6.25	0.23–0.24	(16 g) 3.7–3.8
Kimchi	0.50–0.52	0.03	(90 g) 2.8–3.1
Douchi	9.23–9.60	0.06–0.08	(16 g) 1.0–1.3

[a] Percent fat of miso was obtained from product information and nātto, shrimp paste, fish sauce, kimchi and douchi from the addition of an internal standard.
[b] Milk BCFA concentration is calculated from reporting by Ran-Ressler (Ran-Ressler et al., 2011).

(Kaneda, 1991), the data suggest that nātto products varied in the extent of fermentation and can achieve substantially greater BCFA than U.S. dairy milk fat (Ran-Ressler et al., 2011). Within groups, the mean coefficient of variation (CV) and 95% confidence limits around the CV for BCFA was 37.0% (14.5%, 59.5%) while normal saturates, monounsaturates and polyunsaturates had CV of 1.8% (1.0%, 2.5%), 5.0% (2.3%, 7.7%) and 1.5% (0.7%, 2.3%), respectively. Because our analytical error for technical replicates was small, 0.6% for BCFA and < 0.1% for the other fatty acid classes, we conclude that BCFA variability from product to product was significantly greater than the other fatty acid classes. Nātto BCFA also varied more across groups. CV between different brands or preparations was 64.3% for BCFA, 20.8% for normal saturated fatty acids, 26.4% for monounsaturated fatty acids and as low as 4.0% for polyunsaturated fatty acids.

The BCFA profile of nātto shows some similarity with milkfat (Fig. 2a). Most BCFA are lower in nātto except for *iso-15:*0. Notably, *iso-17:*0 and *anteiso-17:*0 are significantly lower in nātto than cow's milk, with *anteiso-17:0* at only a fourth of the concentration in cow's milk. While bacterial contribution to milkfat is the net result of the complex rumen microbiota, nātto is a product characteristic of *B. subtilis* fermentation. The comparison of nātto fatty acid profile with the compositional fatty acid profile of *B. subtilis* shows that they have the identical chain length for BCFA (Kaneda, 1977). When each BCFA is expressed as percent weight of total BCFA, *anteiso*-15:0 is the predominant BCFA, comprising one third of total BCFA for both nātto and its fermenting bacteria. The remaining *iso-14:0, iso-15:0, iso-17:0* and *anteiso-17:0* are in the same proportions while *iso-16:0* is more variable (Fig. 2b). This variability may be due to differences in fermentation substrates, specifically soybean for nātto and medium for *B. subtilis* incubation. Considering the above findings, we conclude that BCFA producing bacteria can significantly modify and increase the BCFA content of a fermented food, as seen with *B. subtilis* contributing its compositional BCFA to nātto.

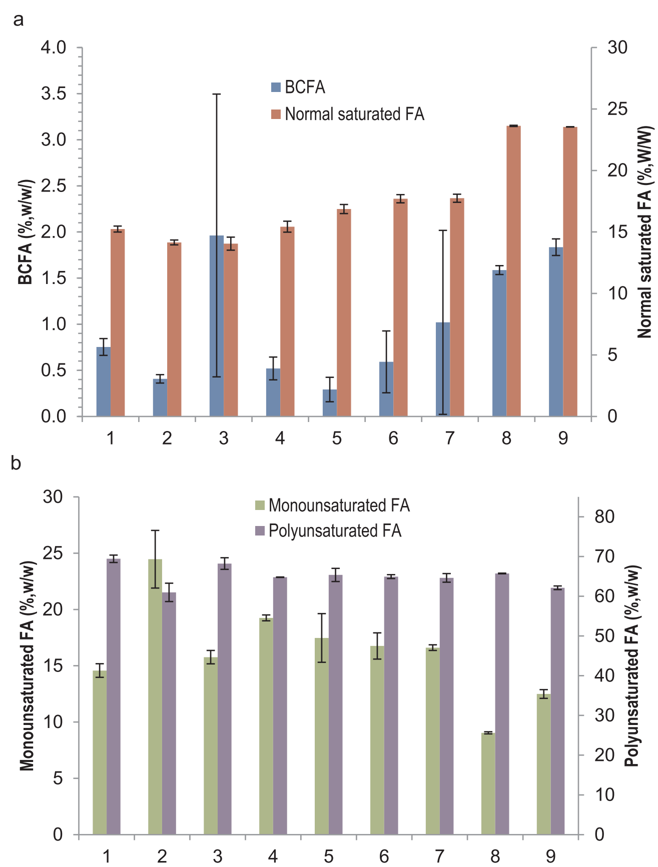

Fig. 1. Classified fatty acids concentration of 9 different nātto brands/preparations presented individually to show variation within groups (standard deviation) and across brands/preparations. a. Total branched chain fatty acids and total normal saturated fatty acids. b. Total monounsaturated fatty acids and total polyunsaturated fatty acids. Two separate packages of nātto brand/preparation 1–7 were analyzed and two portions of 8–9 were analyzed.

Shrimp paste has a relatively high concentration of BCFA at

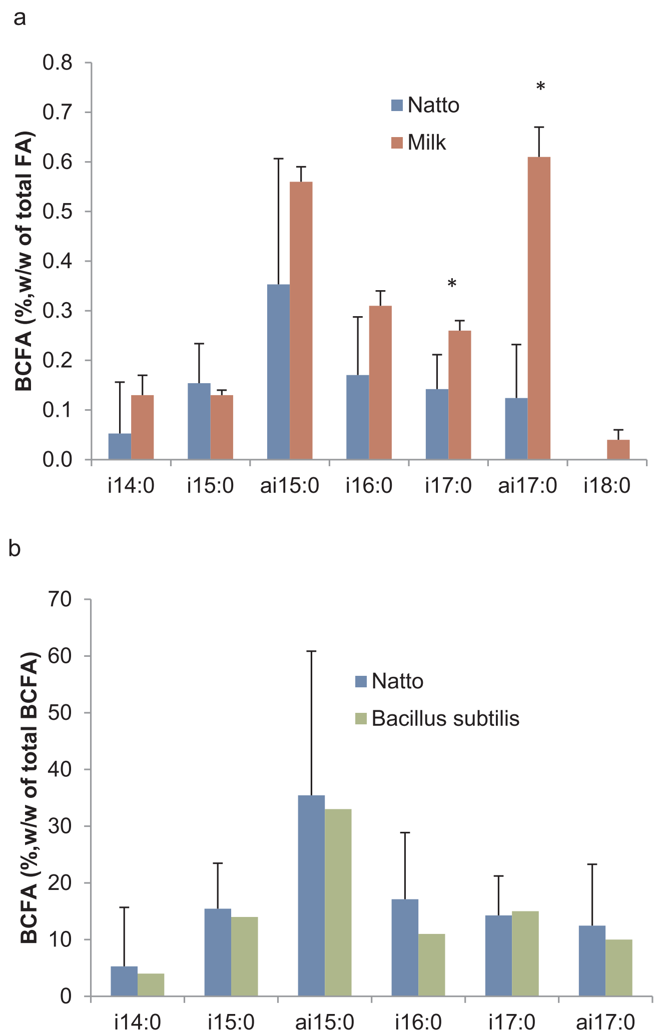

Fig. 2. Comparison of BCFA concentrations in nātto, milk and *B. subtilis.* (a) Nātto mimics milk BCFA distribution (Ran-Ressler et al., 2011) but at a lower concentration for most BCFA. *iso-17:0* and *anteiso-17:0* are significantly lower in nātto. (b) Nātto BCFA profile resembles its fermenting agent, *B. subtilis* (Kaneda, 1977).

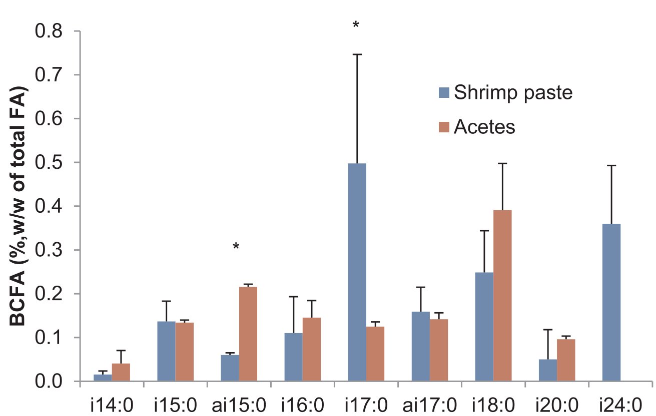

Fig. 3. BCFA comparison of fermented shrimp paste and a commercially available dried shrimp (*Acetes* species). *iso*-17:0 is significantly enriched and *anteiso*-15:0 is significantly lower in shrimp paste than dried *Acetes* shrimp. High levels of *iso*-24:0 are only detected in shrimp paste. Clearly, fermentation alters and increases the total BCFA concentration in shrimp paste.

1.63 ± 0.72%, w/w of total fatty acids. This BCFA abundance could be due to the BCFA naturally present in *Acetes* species and the fermentation process. The fatty acid profile of dried *Acetes* was determined and the BCFA proportion presented with shrimp paste, its fermented product, in Fig. 3. Dried *Acetes* has 1.29 ± 0.10% total BCFA of total fatty acids, which already was a rich source of food BCFA. Compared with dried *Acetes,* shrimp paste is significantly higher in *iso*-17:0 and lower in *anteiso*-15:0. Two very long chain BCFA, *iso*-20:0 and *iso*-24:0 were found in shrimp paste and *iso*-20:0 in dried *Acetes*. The fermentation process of shrimp paste is complex, and many studies describe its fermenting organisms. Surono et al. reported that the most abundant microflora in “Terasi” shrimp paste was in the order of *Bacillus, Pseudomonas, Micrococcus, Kurthia* and *Sporolactobacillus* (Surono & Hosono, 1994). Other lactic acid bacteria such as halophilic *tetragenococcus halophilus* and *T. muriaticus* were also found in “Terasi” shrimp paste (Kobayashi et al., 2003). Species in the genera of *Bacillus, Pseudomonas, Micrococcus* and many lactic acid bacteria are well known to incorporate extremely high amounts of BCFA in their membranes (Kaneda, 1991). Two strains of *Lentibacillus kapialis* were directly isolated from a fermented shrimp paste in Thailand and found to contain 98% cellular BCFA (Pakdeeto et al., 2007). These observations are further evidence that bacterial fermentation altered and further enriched the BCFA content in small shrimp.

Our analyses naturally provide all ancillary data on fatty acids and related compounds on which we comment here. The levels of EPA and DHA in shrimp paste and its original dried small shrimp as well as fish sauce are also remarkable. The total EPA and DHA in shrimp paste, dried small shrimp and fish sauce are 29%, 27% and 25% of total fatty acids respectively, the same level as the average of 27 freshwater fishes in the northeastern US (Wang et al., 2016), and higher than farmed salmon fed either fish oil or palm oil/rapeseed oil (Bell et al., 2002, 2001). Fermented shrimp paste, dried small shrimp and anchovy fish sauce are therefore shelf-stable omega-3 fatty acid sources, important for those who do not have access to live or frozen seafood, or omega-3 supplements. Both shrimp paste and dried small shrimp are very low in omega-6 fatty acids, especially linoleic acid at < 2% and arachidonic acid at 4%. Anchovy fish sauce had linoleic acid and arachidonic acid both at 3%. These omega-6 levels are lower than most farmed fish, and comparable or lower than wild freshwater fish (Wang et al., 2016). Frequent consumption of these foods would contribute to lower relative omega-6/omega-3 status.

Fatty acid ethyl esters (FAEE) are esters of ethanol and free fatty acids, similar to fatty acid methyl esters which contain a methanol moiety. Though they are the most common form for omega-3 concentrates, a lipid class that is normally at very low concentration in living tissue and thus raw foods. In miso, we found 39.5 ± 0.3% total FAEE as a fraction of total fatty acids, in line with Yamabe et al. who reported 35% total FAEE of total lipids in maturated miso (Yamabe et al., 2004). Linoleic acid (18:2n-6) ethyl ester is the highest at 21.18 ± 0.31% of total fatty acids, followed by 16:0, 18:1n-9 and 18:3n-3 ethyl ester, again consistent with the previous report (Yamabe et al., 2004). In addition to those ethyl esters reported previously, we also found 0.72 ± 0.00% and 0.10 ± 0.01% 22:0 and 20:0 ethyl esters in miso. Based on the fat content of this miso product, total FAEE content is estimated at 24.68 ± 0.20 mg/g of sample. PUFAs in miso are 33.53 ± 0.01% of total fatty acids with linoleic acid dominating (Supplementary Table 4). Saturated fatty acids comprise a total of 13.60 ± 0.33% and palmitic acid is the highest at 8.57 ± 0.14%.

Incidental to this BCFA study we detected FAEE as a significant components of kimchi, 6.6 ± 0.10% of total fatty acids, which has not been reported previously to our knowledge. Similar to miso, linoleic acid ethyl ester is at the highest concentration, 3.69 ± 0.03%. For saturated fatty acids, palmitic acid is the highest and comprises 21.62 ± 0.07% of total fatty acids. Linoleic acid and alpha-linolenic acid dominates the polyunsaturated portion. Interestingly, 0.91 ± 0.05% total EPA and DHA were found in homemade kimchi, probably as a result of minor shrimp paste addition and could be a significant amount for those consuming kimchi daily and not obtaining these fatty acids from other sources, e.g. vegetarians. Notably, our kimchi was a properly fermented homemade product, unlike many commercial products available in the west, that are not fermented but instead acidified with vinegar. Such pickled kimchi is not expected to have fatty acids or lipid classes (e.g. no FAEE) substantially different from the cabbage from which it is made. Indeed, the presence of FAEE may be a simple means to test whether a product labeled as kimchi has been fermented.

Douchi has low BCFA content compared with nātto. Theoretically, there is little food chemical difference between their fermentation substrates, black soybean or white soybean. The discrepancy in BCFA content would come from the fermentation process. Douchi is produced in numerous fermentation processes, for instance, one relying on action of molds *Aspergillus oryzae* and *Rhizopus oryzae*, and another relying on *B. subtilis*. Our purchased product is consistent with fermentation by an organism other than *B. subtilis*. A Chinese language publication reports that most of the douchi products are fermented by the aforementioned molds and presented as a dry form (Jiang, 2013). Bacteria fermented douchi is usually produced on a small scale or homemade and we were not able to obtain genuine samples of this form of douchi, though we expect such a product to be similar to our nātto results. To the best of our knowledge, molds do not produce BCFA as many bacterial genera do. This could well explain why “dry” douchi and tempeh (Ran-Ressler et al., 2014) are low in BCFA. Further research can compare the fatty acid composition of nātto and bacterial fermented “wet” douchi since the same bacteria species is involved in their production.

Fermented foods have numerous benefits compared with their non-fermented counterparts, most notably that fermented foods are better preserved. Nutritional benefits have long been known. For example, the protein digestibility of nātto is higher than for raw soybeans which have a trypsin inhibitor. It is also remarkable that fermented shrimp paste still maintains an appreciable level of EPA and DHA. In recent years, the probiotic and prebiotic properties of foods have been more clearly recognized. Nātto is fermented by *Bacillus subtilis* which was found to improve the growth and survival of pigs (Guo, Li, Lu, Piao, & Chen, 2006) and various fish and shrimp (Aly, Abdel-Galil Ahmed, Abdel-Aziz Ghareeb, & Mohamed, 2008; Balcazar & Rojas-Luna, 2007; Kumar, Mukherjee, Prasad, & Pal, 2006). The increasing appreciation of the benefits of fermented foods could lead to a rise in their consumption and the intake of BCFA. In fact, BCFA intake is already high for diets with habitual consumption of nātto, in an amount similar to the 63 mg DHA that an American would consume in a day on average (Papanikolaou, Brooks, Reider, & Fulgoni, 2014).

4. Conclusion

Our data show that branched fats are more widely distributed in human diets than previously appreciated. Nātto is the first fermented food discovered that has comparable concentration of BCFA as milkfat, averaging 0.6 or 0.21–1.43 mg/g. We found that fermentation itself can dramatically increase BCFA compared to the known composition of the parent food. Fermented foods may provide significant BCFA in diets low in milk, beef, and other ruminant products. Our study supports further research into the health effects of these little studied branched fats.

Declaration of interests

None.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.02.018.

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