ABSTRACT

Branched chain fatty acids (BCFA) are primarily saturated fatty acid with one or more methyl branch on the carbon chain, which are recognized as mono-, di- or multi-methyl BCFA. In monomethyl BCFA, iso- and anteiso-BCFA are predominant. BCFA is known to be high in dairy products and beef. BCFA is one of the major components of bacterial membranes, particularly prominent in \textit{Bacillus}. Some of the food that was fermented with \textit{Bacillus} was analyzed in this study. The BCFA concentration were 0.70±0.08\%, 0.38±0.06\% and 1.71±0.17\% of total fat content in kimchi, miso and natto, respectively. With the consideration of fat content, we conclude that natto could be a BCFA source for Asian countries where dairy consumption is low.
BIOGRAPHICAL SKETCH

Yupeng Yang got her bachelor degree in Food Science from the Ohio State University, in 2013. After graduation from OSU, she worked as an R&D technician for one and a half year at COFCO Nutrition and Health Research Institute in Beijing, China. In 2013, she came to Cornell University and joined Dr. Tom Brenna’s laboratory working on fatty acid analysis of fermented food.
ACKNOWLEDGMENTS

During this project, I received many help from many people. My advisor Dr. Tom Brenna guided me through my project and always gave me great suggestions. My mentor Mr. Donghao Wang taught me how to do the experiment and analyze the data. Mr. Peter Lawrence helped me identify compounds by using electron ionization mass spectrometry. I really appreciate all their efforts helping me through my project.
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CHAPTER 1
INTRODUCTION

Introduction and Classification of Branched Chain Fatty Acids

Branched chain fatty acids (BCFA) are primarily saturated fatty acid with one or more methyl branch on the carbon chain. BCFA are classified as mono-, di- or multi-methyl BCFA. In monomethyl BCFA, iso- and anteiso-BCFA are predominant (Ran-Ressler et al. 2014). iso-BCFA have a methyl branch on the penultimate carbon. anteiso-BCFA have a methyl branch on the antepenultimate carbon (Figure 1).

![Figure 1](image)

**Figure 1** Structure of iso- and antieso-branched chain fatty acids (BCFA). n-(normal) fatty acid has straight hydrocarbon chain without branching. iso-15:0 is 13-methyl tetradecanoic acid. anteiso-15:0 is 12-methyl tetradecanoic acid. (Ran-Ressler et al. 2011)

Branched Chain Fatty Acid in Bacteria

In 1960, occurrence of 13-methyl tetradecanoic acid (iso-15:0) and 15-methyl hexadecanoic acid (iso-17:0), in *Bacillus subtilis* (natto) was first reported (Saito 1960). Many species of genus *Bacillus* were found to contain BCFA as major acid components of lipids. *De novo* synthesis of BCFA is initiated with branched, short-chain acyl-coenzyme A (CoA). Malonyl-CoA serves as
chain extender (Kaneda 1977). BCFA are believed be related to membrane fluidity of bacteria due to the branched chain structure (Kaneda 1991).

**Branched Chain Fatty Acid Presence and Consumption**

Branched chain fatty acids primarily present in dairy and ruminant food products. Fluid cow milk is considered one of the common sources of BCFA, as 2% of total fatty acids are branched (Ran-Ressler et al. 2011). The average BCFA intake is estimated to be 500 mg/d was obtained from dairy and beef food products. BCFA in fermented food such as sauerkraut and miso is appreciable, but they are not major contribution to BCFA since their overall fat content is low (Ran-Ressler et al. 2014). BCFA are known to be a major component in vernix caseosa, the waxy white substance coating the skin of newborns. BCFA also accumulate in the gastrointestinal tract (Ran-Ressler et al. 2008).

**Health Benefit of Branched Chain Fatty Acid**

BCFA is important for microbiota and gut health. Studies have shown that BCFA could reduced the incidence of necrotising enterocolitis (Ran-Ressler et al. 2011). Others have shown that BCFA induce apoptosis in human breast cancer cells, and inhibit tumor growth in cultured cells and in a mouse model (Yang et al. 2000; Wongtangthinatharn 2004). Recent study provides evidence that BCFA may have significant anti-inflammatory effects in the human intestinal epithelium (Yan 2016).
CHAPTER 2
ANALYSIS OF BRANCHED CHAIN FATTY ACID IN KIMCHI, MISO AND NATTO

Materials
Kimchi was homemade with ingredients of napa cabbage, red pepper, carrot and salt. Miso (Rhee Bros., Inc.) and natto (Mizkan Co., Ltd.) were purchased from local supermarkets in Ithaca, NY. Miso and natto are products of Japan.

Methods
Samples (50-250mg) of each food were extracted and methylated according to a modified one-step hydrolysis procedure, with an aqueous phase reagent containing methanol, 2,2-dimethoxypropane and sulphuric acid (85:11:4, by volume) and an organic phase containing heptane and toluene (63:37, by volume). After mixing with the reagents, the samples were heated in a shaking incubator for 2 hours with tricosanoic acid (23:0) added as the internal standard. 2 ml of saturated sodium chloride was added to terminate the reaction. Then the samples were centrifuged for 10 min at 3500 rpm and the organic layer was transferred and evaporated under nitrogen. (Garces et al. 1993; Zhou et al., 2008)

Fatty acid methyl esters (FAME) were identified and analyzed quantitatively, using a Hewlett Packard 5890 Gas Chromatograph (GC), run in splitless mode at 250 °C, and with the flame ionization detector (FID) at 270 °C. A BPX-70 column (25 m × 0.22 mm × 0.25 μm, SGE, Austin, TX) was used with H₂ as the carrier gas. The oven temperature program was initially 80 °C for 1 minute, increased by 30 °C per minute until 170 °C and held for 2 min, then increased by 10 °C per minute until a final temperature of 240 °C and held for 1 min. A mixture of FAME
standard was used to calculate response factors. The results were analyzed in percentage weight.

FAME were identified by electron ionization mass spectrometry (EIMS), using a Varian Star 3400 GC coupled to a Varian Saturn 2000 ion trap MS, based on GC retention times and mass spectra. The GC–MS column was a BPX-70 (60 m × 0.32 mm × 0.25 µm, SGE, Austin, TX). (Ran-Ressler et al. 2011).

**Results**

The BCFA concentration of kimchi was quantified by internal standard. The BCFA concentrations of miso and natto were calculated based on the fat content on their packages and the percentage of BCFA. Table 1 presents the fat content, BCFA concentration and BCFA content per serving of kimchi, miso and natto, compared with fluid milk reported in previous study (Ran-Ressler et al. 2011). According to the results, kimchi has appreciable amount of BCFA, but it is not a primary source of BCFA due to the low fat content. Although miso has much higher fat content, the serving size is small since it is usually used as a sauce. Natto has similar amount of BCFA to fluid milk, and could be a potential source.

<table>
<thead>
<tr>
<th>Fermented Food</th>
<th>Fat (%)</th>
<th>BCFA (mg fatty acid/ g sample)</th>
<th>BCFA (mg fatty acid/ serving)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kimchi</td>
<td>0.5</td>
<td>0.032</td>
<td>(90 g) 2.88</td>
</tr>
<tr>
<td>Miso</td>
<td>6.25</td>
<td>0.24</td>
<td>(16 g) 3.8</td>
</tr>
<tr>
<td>Natto</td>
<td>7.78</td>
<td>1.3</td>
<td>(90 g) 117</td>
</tr>
<tr>
<td>Milk</td>
<td>3.25</td>
<td>0.65</td>
<td>(244 g) 158</td>
</tr>
</tbody>
</table>
The BCFA concentrations were 0.70±0.08%, 0.38±0.06% and 1.71±0.17% of total fat content in kimchi, miso and natto, respectively. Table 2 and Figure 2 show individual branched chain fatty acids distribution. Based on our results, natto had a relatively high amount of BCFA among the samples. Total BCFA concentration in natto is comparable to that in milk, with less anteiso-17:0 BCFA (Figure 3).

**Table 2**  Total and individual branched chain fatty acid concentrations in kimchi, miso and natto

<table>
<thead>
<tr>
<th>BCFA (wt%)</th>
<th>Kimchi</th>
<th>Miso</th>
<th>Natto</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total BCFA</td>
<td>0.70</td>
<td>0.38</td>
<td>1.71</td>
</tr>
<tr>
<td>iso-14:0</td>
<td>0.06</td>
<td>&lt;0.003</td>
<td>0.23</td>
</tr>
<tr>
<td>iso-15:0</td>
<td>0.05</td>
<td>0.20</td>
<td>0.26</td>
</tr>
<tr>
<td>anteiso-15:0</td>
<td>0.07</td>
<td>&lt;0.003</td>
<td>0.56</td>
</tr>
<tr>
<td>iso-16:0</td>
<td>0.13</td>
<td>0.04</td>
<td>0.33</td>
</tr>
<tr>
<td>iso-17:0</td>
<td>0.09</td>
<td>&lt;0.003</td>
<td>0.19</td>
</tr>
<tr>
<td>anteiso-17:0</td>
<td>0.23</td>
<td>0.10</td>
<td>0.14</td>
</tr>
<tr>
<td>iso-18:0</td>
<td>0.06</td>
<td>0.04</td>
<td>&lt;0.003</td>
</tr>
</tbody>
</table>
Figure 2 Distribution of individual branched chain fatty acids in kimchi, miso and natto. Values are means, with their standard deviations represented by vertical bars.

Figure 3 Distribution of individual branched chain fatty acids in natto vs. fluid milk. Fluid milk data were obtained from previous publication (Ran-Ressler et al. 2011). Values are means, with their standard deviations represented by vertical bars.
**Conclusion**

As shown above, natto contains a considerable amount of branched chain fatty acids per serving size. Natto is widely consumed in Japan as a very traditional dish fermented with soybean. We conclude that natto may contribute to BCFA consumption in Asian countries where dairy consumption is low. However, the BCFA distribution is different than fluid milk. Previous studies show structure-specific differences of BCFA in biological properties as an intermediate in development of *Caenorhabditis elegans* (Kniazeva et al. 2004), and different accumulation in human fetal intestinal cells (Ran-Ressler et al. 2008). More study is required for further understanding of BCFA.
REFERENCES


