Chemical Characterization of Tomato Juice Fermented with Bifidobacteria

Jong-Ho Koh, Younghshik Kim, and Jun-Hyun Oh

Abstract: The objective of this research was to characterize the chemical properties of tomato juice fermented with bifidobacterial species. Tomato juice was prepared from fresh tomatoes and heated at 100 °C prior to fermentation. Bifidobacterium breve, Bifidobacterium longum, and Bifidobacterium infantis were inoculated in tomato juice and kept at 35 to 37 °C for up to 6 h. Fructooligosaccharide (FOS) was added to tomato juice prior to fermentation. The analyses for brix, total titratable acidity (TTA), pH, color, and lycopene content were conducted to characterize tomato juices fermented with bifidobacterial species. Heat treatment of tomato juice did not cause any significant changes in brix, pH, and TTA. Only the redness of tomato juice was significantly increased, as the heating time increased to 30 min. The tomato juices fermented with B. breve and B. longum exhibited significant decreases in pH (3.51 and 3.80, respectively) and significant increases in TTA (13.50 and 12.50, respectively) (P < 0.05). B. infantis did not cause any significant change in the chemical properties of tomato juice. The addition of FOS further improved the fermentation of tomato juice by bifidobacterial species. The lycopene contents of tomato juice were significantly increased from 88 to 113 μg/g by heat treatment at 100 °C (P < 0.05), however, did not exhibit any significant change after fermentation with bifidobacterial species.

Keywords: bifidobacteria, fermentation, juice, tomato

Introduction

Tomato is one of major vegetables widely consumed around the world. The health benefits of tomatoes are attributed to the abundant antioxidant components present in tomatoes (Giovannucci 1999; Willcox and others 2003; Ramandeep and Savage 2005), such as lycopene and provitamin A (Rodriguez-Amaya and Tavares 1992; Mayeux and others 2006), ascorbic acid (Hanson and others 2004), vitamin E (Lee and others 2000), and other flavonoids (Dewanto and others 2002). Tomatoes are consumed mainly as fresh or processed products. In 1999, approximately 17.6 pounds of fresh tomatoes and 72.8 pounds of tomato products were consumed annually per capita in the United States (Willcox and others 2003). Tomatoes are processed for wide ranges of products such as canned and sun-dried tomatoes, ketchups, pastes, purées, salads, sauces, soups, and juice products, supplying significant sources of vitamins and minerals to the consumers (Tsen and others 2008). Tomato products are playing an important role in supplying lycopene to the consumers; more than 80% of dietary lycopene consumed in the United States come from a wide variety of tomato products (Willcox and others 2003).

Among tomato products, tomato juices are well recognized as healthy beverages (Yoon and others 2004). In fact, tomato juices as well as tomato paste and sauces are the main contributors of dietary lycopene, accounting for 25% total daily lycopene intake in Canadian population (Rao and others 1998). There are extensive efforts to enhance the functionality of tomato juice products with the trials of introducing value-added components or health-improving compounds to tomato juice products (Anese and others 1999). One recent effort in improving the functionality of tomato juice products is to utilize lactic acid bacteria (LAB) to ferment tomato juice as probiotics (Yoon and others 2004; Tsen and others 2008; Cagno and others 2009). Although the probiotic products are usually marketed in the forms of fermented dairy products, fruit or vegetable juices may serve as good media for cultivating probiotics (Yoon and others 2004; Wang and others 2009).

Probiotic microorganisms are defined as live microorganisms that confer a health benefit on the host, when administered in adequate amounts (Sindhu and Khetarpaul 2004; Corrêa and others 2008). Microorganisms from several selected bacterial genera such as Lactobacillus are currently utilized as probiotics, however the members of genus Bifidobacterium are also believed to possess probiotic activities (Ouwehand and others 2002). Bifidobacteria are gram-positive procaryotes that naturally inhabit the gastrointestinal tract of humans and other warm-blooded animals (Leahy and others 2005). Bifidobacteria possess health-improving properties by maintaining improved intestinal microflora, reducing serum cholesterol, inhibiting the growth of potential pathogens, stimulating the immune responses, and exhibiting anticarcinogenic activity (Jeon and others 2002). Recently, as the market for functional food grows, probiotic products are gaining a great attention by the consumers.

Nonetheless, there is very limited information on the probiotic potentials of bifidobacteria in vegetable juices such as tomato juice. Regarding the probiotic potentials of bifidobacteria, coconut flan (Corrêa and others 2008) and noni juice (Wang and others 2009) were used for fermentation with the mixture of both LAB and bifidobacteria. However, there is no research on the fermentation of...
of tomato juice with bifidobacteria alone. Therefore, the objective of this research was to characterize the chemical properties of tomato juice product fermented with bifidobacteria species.

Materials and Methods

Materials

Fresh raw tomatoes (Lycopersicon esculentum) were purchased from local markets and farms. Bifidobacteria selected in this research were Bifidobacterium breve, Bifidobacterium longum, and Bifidobacterium infantis isolated from the commercial probiotic products manufactured by Mediogen Co. (Chungbuk, South Korea). The bifidobacterial culture was cultivated and maintained in either MRS broth or MRS agar (Difco Laboratories, Detroit, Mich., U.S.A.). Fructooligosaccharide (FOS) was obtained from Samyang Genex Co. (Incheon, South Korea). The other chemical reagents and solvents used in this research were analytical grades and high-performance liquid chromatography (HPLC) grades.

Preparation and fermentation of tomato juice

The purchased fresh tomatoes were stored in the box at room temperature for further maturation. The tomatoes were washed with tap water to remove soil and other impurities, dried at room temperature prior to use, and treated with steam for 3 to 4 min for easy peeling. The tomato fruit was ground and filtered through a pulper (AG-5500, Angel Juicer Co, Pusan, South Korea) with a sieve (0.8 to 1.1 mm pore size) to separate tomato juice and cake containing peel and seed. The tomato juice was homogenized using a D-500 homogenizer (Hauser, Berlin, Germany) for 10 min. The tomato juice was then heated at 100 °C for selected time periods (5, 30, 60, 90, and 120 min) prior to fermentation. The selected concentrations of FOS (0.25% and 0.50%) were added to tomato juice prior to fermentation. The starter of bifidobacterial species was activated in the MRS media for overnight to possess approximately 10^9 CFU/mL before inoculation to tomato juice. The cultured bifidobacteria (5%, v/v) were finally inoculated into the tomato juice in 250 to 500 mL flasks. The fermentation of tomato juice was performed anaerobically in the dark for up to 6 h in a shaking incubator with a shaking rate of 120 rpm at 35 to 37 °C.

Characterization of fermented tomato juice

The solid contents of tomato juice were determined by the standard AOAC method (AOAC 1997). The recovery rate was expressed as the percentage calculated from the ratio of the weight of tomato juice obtained after each processing step and the weight of the fresh tomatoes. The total titratable acidity (TTA) of tomato juice was determined by the standard AOAC method, and expressed as % lactic acid (AOAC 1997). The pH was directly measured in the tomato juice using a pH meter (Seven Easy S20, Metter Toledo, Greifensee, Switzerland). The total sugar content expressed as brix of tomato juice was measured using a CM-3500d Minolta Chromameter (Minolta Camera Co., Osaka, Japan). The light source was illuminant C, and the white plate (CR-A43; L^* = 96.86, a^* = -0.02, and b^* = 1.99) provided by the manufacturer was used for calibration and background.

Analysis of lycopene

The extraction and analysis of lycopene from tomato was conducted following the general procedures described by Kozukue and Friedman (2003). The homogenized tomato juice (2 g) was mixed with 10 mL 6% pyrogallol in ethanol, and 8 mL 60% KOH was added into the mixture under nitrogen gas. The mixture was saponified at 70 °C and cooled down to room temperature. The concentration of ethanol was adjusted to possess approximately 30% (v/v) using 2% NaCl solution. Lycopene was extracted from tomato juice using a mixed solvent (hexane:acetone:methanol = 50 : 25 : 25, v/v/v) in the presence of BHT as an antioxidant. The hexane layer containing lycopene was separated from the mixture, and recovered several times. The optical density was measured at 470 and 502 nm using a spectrophotometer (UV/VIS Lambda35, Perkin Elmer, Waltham, Mass., U.S.A.). Total lycopene content was calculated from the optical density based on a standard curve obtained from lycopene standard (Sigma, St. Louis, Mo., U.S.A.). Figure 1 represents the spectral profiles of lycopene standard (A) and the standard curves obtained at selected wavelengths (B and C).

Statistical analysis

Each experiment was performed 3 times in duplicates. Data were analyzed using the general linear models procedure in SAS software program (Statistical Analysis System Inst., Inc., Cary, N.C., U.S.A.). Multiple mean comparisons among the samples were carried out by Duncan’s multiple range tests at 5% significance level (P < 0.05).

Results and Discussion

Solid content and recovery rate of tomato juice

Approximately 840 g tomato juice was obtained after steaming 1000 g fresh tomato juice at 100 °C for 5 min, exhibiting an increase of solid contents of tomato juice from 4.5% to 4.7% (w/v) (Table 1). The tomato juice was then further concentrated to possess the solid content approximately 9.3% prior to use for fermentation.

Chemical characterization of tomato juice prior to fermentation

The brix, pH, TTA of tomato juice heated at 100 °C for 30 to 120 min were not significantly different, when compared to the tomato juice heated at 100 °C for 5 min (control) (Table 2). Regarding the color properties, the lightness (L^* value) and yellowness (b^* value) of tomato juice were not significantly changed, however, the redness (a^* value) was significantly changed by the heating time (P < 0.05). As the heating time increased until 60 min, the redness of tomato juice also significantly increased, however, decreased after 60-min heating. The red color of tomatoes is known mainly due to the large lycopene contents in tomatoes (Mayeaux and others 2006). The significant changes of lycopene contents in tomatoes during storage and processing such as drying, paste, or juice processing were reported by several researchers (Dewanto and others 2002; Hackett and others 2004; Seybold and others 2003; Mayeaux and others 2006). Dewanto and others (2002) reported that the lycopene contents in cooked tomatoes after heating for 2, 15, and 30 min at 88 °C were increased to 3.11, 5.35, and 5.32 mg/g, respectively, as compared to 2.01 mg/g in raw tomatoes. Therefore, the significant increase in red color of
Tomato juice fermented by bifidobacteria...

Figure 1—The spectral profile of lycopene standard (A), and the standard curves at 470 nm (B) and 502 nm (C).

Table 1—Solid content and recovery rate of tomato juice during selected process steps.

<table>
<thead>
<tr>
<th>Processing step</th>
<th>Solid content (%)</th>
<th>Recovery rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh tomatoes</td>
<td>4.5</td>
<td>100.0</td>
</tr>
<tr>
<td>Processed tomato juice after steaming at 100 °C for 5 min</td>
<td>4.7</td>
<td>84.0</td>
</tr>
<tr>
<td>Processed tomato juice concentrate after steaming at 100 °C for 5 min</td>
<td>9.3</td>
<td>83.0</td>
</tr>
</tbody>
</table>

Chemical characterization of tomato juice fermented with selected bifidobacterial species

The brix, pH, TTA, and color of tomato juice fermented with selected bifidobacterial species are summarized in Table 3. The pH of tomato juice was significantly decreased by the fermentation with \( B. \) breve and \( B. \) longum \( (P < 0.05) \), indicating that these 2 bifidobacterial species could grow in tomato juice. The growth of both \( B. \) breve and \( B. \) longum resulted in a significant increase in TTA of the tomato juice \( (P < 0.05) \). In addition, the fermentation of tomato juice with \( B. \) breve and \( B. \) longum significantly increased the \( L^* \), \( a^* \), and \( b^* \) values of tomato juice \( (P < 0.05) \). However, since there were no significant changes of pH and TTA in tomato juice inoculated with \( B. \) infantis, it was assumed that \( B. \) infantis did not grow in the tomato juice.

The probiotic properties of LAB in tomato juice were reported by several investigators \( (\text{Yoon and others 2004; Tsen and others 2008}) \). The viable cell counts of LAB in tomato juice reached...
Tomato juice fermented by bifidobacteria...

1 × 10^9 to 9 × 10^9 CFU/mL after 72 h-fermentation, simultaneously reducing the pH of tomato juice to 4.1 or below, and increasing the acidity to 0.65% or higher (Yoon and others 2004). Tsen and others (2008) also reported that the fermentation of tomato juice was significantly improved by using k-carrageenan immobilized with Lactobacillus acidophilus, exhibiting better L. acidophilus growth during the k-carrageenan mobilized fermentation than during the free cell fermentation. Both research groups suggested that the probiotic tomato juice fermented with LABs could serve as a healthy beverage alternative to fermented dairy products, specifically for vegetarians or consumers allergic to dairy products.

There is very limited information on the probiotic properties of bifidobacteria alone in the literature. Most investigators focused on the probiotic effects from the combination of both LAB and bifidobacteria. Corrêa and others (2008) reported the probiotic potentials of coconut flan supplemented with B. lactis in the presence of Lactobacillus panacaei. Recently, Wang and others (2009) reported the combined probiotic effects of both B. longum and Lactobacillus plantarum in fermented noni juice. The results in this research demonstrated the potential probiotic effects of selected bifidobacterial species in tomato juice, suggesting that bifidobacterial species such as B. breve and B. longum could be used for tomato juice fermentation.

Chemical characterization of tomato juice fermented with bifidobacteria with the addition of FOS

Yoon and others (2004) observed a rapid reduction in sugar contents of tomato juice as LAB fermentation progressed, indicating that LABs consumed sugar to promote tomato juice fermentation. In fact, a large number of bifidobacteria isolated from human and animal origins was able to ferment and degrade complex carbohydrates, mainly D-galactosamine, D-glucosamine, amylose, and amylpectin (Crociani and others 1994). FOS contains oligosaccharides that are smaller than complex carbohydrates, and is well known as a substrate for microflora in the large intestine, increasing the overall gastrointestinal tract health (Yun 1996). The main health-improving effects of FOS are believed to attribute to the prebiotic effects of FOS (Yun 1996; Alles and others 1997; Xu and others 2003). Therefore, it was hypothesized that FOS might not only promote the fermentation process with bifidobacteria, but also provide the additional prebiotic effects to fermented tomato juice. The addition of 0.50% FOS into tomato juice resulted in a significant reduction in pH and increase in TTA of tomato juice after 6-h fermentation (P < 0.05), as compared to the addition of 0.25% FOS, indicating that FOS could promote the fermentation with B. breve (Figure 2). The color properties of tomato juice with the addition of FOS are also presented in Table 4. When 0.50% FOS was added, the a' and b' values of fermented tomato juice were significantly increased (P < 0.05). Xu and others (2003) also reported that FOS enhanced the growth of lactic acid bacteria such as Bifidobacterium and Lactobacillus, however, inhibited the growth of E. coli in the small intestine and colon in chicken. FOS also exhibited 83% apparent fermentability in the colon, and significantly increased the fecal weight, when fed to the patients with large bowel disease (Alles and others 1997).

The addition of FOS in tomato juice might also improve the taste of tomato juice fermented with bifidobacteria. Although an extensive sensory evaluation was not conducted in this research, the preliminary sensory test implied that the tastes and off-flavors of the tomato juice were apparently improved by the fermentation with bifidobacterial species and the addition of FOS into tomato juice (data not presented). Therefore, FOS could be recommended to use for the improvement of tomato juice fermentation with bifidobacteria.

Lycopene content of fermented tomato juice

The lycopene contents of fresh tomato and tomato juices are presented in Table 5, exhibiting comparable amounts reported by Rao and others (1998). The lycopene content of tomato juice after heating at 100 °C for 5 min was significantly increased from 88 to 113 μg/g (P < 0.05). Although there are still controversies on the changes in lycopene contents caused by heating, it was reported that the lycopene contents could be increased after heat processing, especially when processed at relatively low temperature such as approximately 80 to 90 °C (Thompson and others 2000). Dewanto and others (2002) also reported that the lycopene contents of raw tomatoes were significantly decreased by heating at 88 °C for selected times. Our results also supported the increase in lycopene contents in processed tomato juice, also evident from the significant increases in redness in color (P < 0.05) (Table 2).

However, the lycopene content of fermented tomato juice was not significantly different from the lycopene contents of unfermented tomato juice, indicating that bifidobacterial fermentation did not cause any adverse effects on lycopene content in tomato juice. Therefore, it was thought that the fermentation of tomato juice with bifidobacteria would not affect the health benefits of lycopene in tomato juice.

| Table 1–The brix, pH, TTA, and color of tomato juice fermented by selected bifidobacterial species. |

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Brix (%)</th>
<th>pH</th>
<th>TTA (%)</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>No fermentation</td>
<td>4.40 ± 0.15a</td>
<td>4.32 ± 0.11a</td>
<td>6.00 ± 0.21a</td>
<td>L' = 29.48 ± 0.20b, a' = 10.58 ± 0.31b, b' = 21.63 ± 0.31b</td>
</tr>
<tr>
<td>B. breve</td>
<td>4.10 ± 0.21a</td>
<td>3.51 ± 0.12a</td>
<td>13.50 ± 0.33a</td>
<td>38.02 ± 0.31a, 15.45 ± 0.27a, 26.29 ± 0.18a</td>
</tr>
<tr>
<td>B. longum</td>
<td>4.00 ± 0.26a</td>
<td>3.80 ± 0.15a</td>
<td>12.50 ± 0.28a</td>
<td>36.72 ± 0.19a, 14.84 ± 0.19a, 25.23 ± 0.11a</td>
</tr>
<tr>
<td>B. infantis</td>
<td>4.20 ± 0.11a</td>
<td>4.30 ± 0.21a</td>
<td>5.90 ± 0.17a</td>
<td>31.36 ± 0.25a, 11.61 ± 0.21b, 22.32 ± 0.29a</td>
</tr>
</tbody>
</table>

Different letters within the column indicate significant differences between the means (P < 0.05).
Figure 2—The changes of pH (A) and TTA (B) in tomato juice during fermentation with _B. breve_ in the presence of selected concentrations of FOS.

### Table 4—The color properties of tomato juice fermented with bifidobacterial species in the presence of FOS.

<table>
<thead>
<tr>
<th>Bifidobacteria</th>
<th>FOS content (%)</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. breve</em></td>
<td>0.25</td>
<td>24.33±0.33</td>
<td>15.36±0.23</td>
<td>14.21±0.23</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>24.95±0.24</td>
<td>19.36±0.29</td>
<td>16.37±0.31</td>
</tr>
<tr>
<td><em>B. longum</em></td>
<td>0.25</td>
<td>24.35±0.21</td>
<td>15.88±0.31</td>
<td>16.69±0.41</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>26.80±0.15</td>
<td>21.98±0.19</td>
<td>20.06±0.21</td>
</tr>
</tbody>
</table>

Different letters within the column indicate significant differences between the means (P < 0.05).

### Table 5—The lycopene content in tomato juice fermented with _B. breve_.

<table>
<thead>
<tr>
<th>Processing step</th>
<th>Lycopene content (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh whole tomatoes</td>
<td>88.0±4.80</td>
</tr>
<tr>
<td>Tomato juice after steaming at 100 °C for 5 min</td>
<td>113.0±4.21</td>
</tr>
<tr>
<td>Tomato juice fermented by <em>B. breve</em></td>
<td>112.1±4.17</td>
</tr>
</tbody>
</table>

Different letters within the column indicate significant differences between the means (P < 0.05).

### Conclusions

This research demonstrates that tomato juices can be fermented with selected bifidobacterial species alone such as _B. breve_ and _B. longum_ as probiotic microorganisms. The addition of sugars such as FOS into tomato juice promoted the fermentation process, and presumably improved the tastes and off-flavors of fermented tomato juice. During fermentation, the lycopene contents in tomato juice were not affected by the growth of bifidobacterial species. Therefore, this research suggests that the tomato juice fermented with bifidobacteria can be developed as a potential probiotic product, and may benefit the consumers searching for an alternative beverage to replace fermented dairy products.

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### References


References