Extension of Shelf Life of Whole and Peeled Shrimp with Organic Acid Salts and Bifidobacteria

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ABSTRACT

Microbiological and sensory characteristics of treated whole and peeled shrimp from the east coast of Saudi Arabia were evaluated. Shrimp samples were treated with organic acid salts with or without Bifidobacterium breve culture and stored in ice. Peeling alone extended the microbiological shelf life by 4 days. Treatment of whole shrimp with sodium acetate alone or potassium sorbate with bifidobacteria prolonged the microbiological shelf life by 3 days and increased the microbial generation time from 12.8 h (control) to 30.1 h or 31.4 h, respectively. The microbiological and sensory shelf life of peeled shrimp treated with sodium acetate was more than 17 days. Sodium acetate extended the microbial lag phase and lengthened the generation time (38.7 h compared to 15.8 h for the control). Micrococci and coryneforms were the predominant microorganisms in whole shrimp during storage. Treatment with sodium acetate maintained better sensory characteristics for peeled shrimp than potassium sorbate combined with bifidobacteria.

Shrimp is becoming an important commodity in the Saudi Arabian diet as can be seen from the increased number of shops selling shrimp along with other seafood products in cities (2). With the exception of the shrimp processed by one company that has the facilities to peel, clean, and process such a valuable product, most of the shrimp is sold whole and refrigerated with no further processing. After arrival at the two major wholesale centers (one in Jeddah on the west coast and the other in Alqatief on the east coast of Saudi Arabia), shrimp is immediately sold, transported at 0°C to the final sales outlets, and then cleaned upon consumer request.

Shrimp is a highly perishable product and its shelf life under refrigerated storage conditions is limited by both enzymatic and microbiological spoilage. Psychrotrophic bacteria are the major groups of microorganisms responsible for spoilage of refrigerated seafoods (1, 30). Due to the perishability of such a product, reliable methods of preservation are sought to extend shelf life and to avoid health hazards. Such methods include cold storage in ice (19, 23, 28), modified ice storage (12, 14), low-dose gamma radiation (10, 29), cook-chill processes (27), and treatment with organic acids and their salts (5, 30). Wide concentration ranges of organic acid salts such as sodium acetate (0.5 to 10.0%, wt/wt), sodium lactate (0.25 to 4.0%), potassium sorbate (0.1 to 10.0%), and sodium citrate (8.0 to 10.0%) have been used, alone or in combination, to extend the shelf life of fresh meat and seafoods (11, 17, 18, 25, 30). Biopreservatives such as lactic acid bacteria and/or their metabolites can suppress the growth of the aerobic bacteria associated with spoilage of refrigerated foods (9, 15, 21). Bifidobacteria are currently used in many dairy products (13, 24). The importance of these bacteria lies in their therapeutic effects of maintaining a normal intestinal balance of microflora, improving lactose tolerance of milk and milk products, reducing serum cholesterol levels, synthesizing B-complex vitamins, and exercising antitumorigenic activity (13). Bifidobacteria produce acetic acid, lactic acid and/or other antimicrobial substances that inhibit many pathogenic microorganisms. Different bifidobacteria strains (combined with sodium acetate) showed similar antimicrobial effects on the aerobic spoilage bacteria of refrigerated catfish fillets (16). Desjardins et al. (8) reported that Bifidobacterium breve was among the highest acid (acetic and lactic) producers grown in reconstituted skim milk.

No studies have reported the use of bifidobacteria (alone or in combination with organic acid salts) as bio-preservatives to improve the shelf life of ice-stored shrimp. This study, therefore, was mainly initiated to evaluate the possibility of extending the shelf life of fresh whole and peeled shrimp treated with combinations of B. breve and food-grade organic acid salts.

MATERIALS AND METHODS

Shrimp samples. Shrimp (Penaeus spp.) samples were brought fresh in crushed ice from Alqatief wholesale market. Upon arrival at the laboratory (within 4 h of purchase), samples were randomly divided in sterile plastic bags and frozen at −40°C until use (within 1 week). The rest of the shrimp samples were peeled and deveined before freezing at −40°C in sterile plastic bags. The average weight of individual shrimp was 13.0 g.

Microorganism and growth conditions. B. breve NCFB 2258 was originally obtained from the National Collection of Food Bacteria (Shinfield, UK). Stock culture was maintained in skim milk medium (SMM) containing (g/liter): skim milk solids, 100; yeast extract (Difco Laboratories, Detroit, Mich.), 5; and glucose (Fisher Scientific, Springfield, N.J.), 5. The culture was
grown anaerobically (anaerobic jar system, BBL, Cockeysville, Md.) at 37°C for 24 h and then refrigerated (4°C) until use. For shrimp treatment, culture was propagated for three consecutive days in SMM before use. Bifidobacteria (48 h after last transfer) in SMM (1.3 x 10^9 CFU/ml) was thoroughly mixed with the solutions of organic acid salts to give a final concentration of 5% (vol/vol). Generation times (GT) during log phase of the aerobic bacteria were calculated as described by Mossel et al. (20).

**Psychrotrophic microbial count.** Each shrimp sample was weighed (ca. 50 g), and sterile peptone water (0.1%) was added and then homogenized using a Braun MX32 blender (Braun, Frankfurt, Germany) for 30 s. From this mixture, serial dilutions using 0.1% peptone water were made. From the appropriate dilution, 0.1 ml was spread plated on plate count agar (PCA, Difco), and the inverted plates were incubated at 7°C for 10 days (6).

**Identification of microorganisms.** At each analysis day, one to three well-isolated representatives of each colony type (color, size, shape) were picked from countable psychrotroph plates (of whole shrimp samples) and subcultured into tryptic soy broth (Difco) at 22°C for 24 h. Cultures were then streaked for isolation onto tryptic soy agar (Difco) and incubated for 24 h at 22°C. Isolated colonies were microscopically rechecked for their purity. Pure cultures were identified to the genus level using the morphological and biochemical tests described by Cowan and Steel (7), and the amount of each colony type was expressed as a percentage of total colonies appearing on countable plates.

**Organic acid salts.** Four solutions of organic acid salts were prepared: sodium acetate, SA (10%, w/w); potassium sorbate, PS (1.5%, w/w); sodium lactate, SL (5%, vol/vol), dilution of 60% sodium lactate solution; and trisodium citrate, TSC (1.5%, w/w). All organic salts were of analytical grade (BDH, Poole, UK) and solutions were sterilized (121°C for 15 min) before use. Each of these solutions was used alone and with bifidobacteria culture (5%, vol/vol).

**Treatment of shrimp.** Shrimp were randomly divided into two lots (two replicates), and each lot was assigned eight treatments for whole shrimp or two treatments for peeled shrimp. For each treatment, 30 whole or peeled shrimp were thawed at room temperature and immersed for 2 min in 500 ml of the treatment solution with gentle swirling using a sterile glass rod to assure complete contact of shrimp with the treatment solution. Shrimp were then removed with sterile tongs and allowed to drain for 2 min on a presterilized metal net. Three treatments were applied as controls: untreated samples (C1), samples immersed in sterile water (C2), and samples immersed in a bifidobacteria (BIF) cell suspension (5%, vol/vol). After draining of the excess solution, samples were placed into sterile stomacher bags (Seward Medical, London) and stored in crushed ice kept at 4°C throughout the storage period. Melted ice was drained daily and was replaced with new ice when needed. At specified time intervals samples were withdrawn and assessed for presence of psychrotrophs and sensory changes.

**Sensory evaluation.** Samples were given three-digit codes and assessed by a seven-member untrained panel (employees of the Food Science and Nutrition Department, College of Agriculture, King Saud University) for color, texture, odor, and general appearance on a seven-point hedonic scale, on which a score of 7 represented attributes most liked; 3 represented attributes at an unacceptable margin; and 1 represented attributes most disliked. On each analysis day, treated samples were compared with a fresh control (from frozen stock). An average of three shrimp per treatment were used per evaluation per day.

**Measurement of pH of whole shrimp.** Shrimp flesh (10.0 g) was homogenized with 20 ml of distilled water for 1 min and pH was measured using a Sargent-Welch pH meter (Model 8000, Skokie, Ill.).

**Statistical analysis.** Data were analyzed using analysis of variance, and means were discriminated using Duncan’s multiple range test (22). All microbial data were transformed to logarithms before analysis.

**RESULTS AND DISCUSSION**

**Psychrotrophic microbial count of whole shrimp.** Populations of psychrotrophic bacteria (log CFU/g) in controls and in treated samples of whole shrimp are presented in Figure 1. In all treatments psychrotrophic counts increased over the 9 days of storage. The added bifidobacteria did not grow on the ice-stored shrimp since their growth requires anaerobic conditions and 37°C as optimum growth temperature. Their expected action against spoilage microorganism can be attributed to compounds (acetic acid, lactic acid, and possibly other antimicrobial substances) produced during culturing (anaerobically at 37°C). The initial psychrotrophic bacterial count was approximately 10^5 CFU/g. The onset of shrimp spoilage is considered to be 10^7 to 10^8 bacteria per g (4); therefore, shrimp used in this study were of acceptable quality. At zero time, C1 had higher (P < 0.05) counts than the other treatments. This was probably due to the washing effect on the surface microorganisms. No statistically significant differences (5% level) were noticed between the controls and other treatments at day 3 of storage. Significant differences between treatments appeared on day 6, where shrimp treated with sodium acetate, sodium acetate with bifidobacteria, and potassium sorbate with bifidobacteria had lower (P < 0.05) psychrotrophic populations compared with the other treatments. In general, the combination of bifidobacteria with the organic acid salts did not significantly lower bacterial counts during the first 6 days of storage. However, the microbial counts were significantly lower on day 9 for treatment with BIF plus PS. Therefore, it is believed that an additive interaction occurred when B. breve culture was combined with PS under experimental conditions. Similar results obtained in our laboratory indicated the same interaction when B. breve was combined with PS and applied to fresh camel meat (unpublished data). Treatments with sodium lactate and trisodium citrate with or without bifidobacteria did not show antimicrobial effects (Fig. 1). Kim et al. (16) reported that the combination of 2.5% B. infantis with SA extended the lag phase and increased the microbial generation time. Results in Figure 1 indicate that the inhibitory effects of SA and PS plus BIF were mainly due to the increase in the microbial generation time (12.8, 30.1, and 31.4 h for C1, SA, and PS plus BIF, respectively).

**Psychrotrophic microbial count of peeled shrimp.** Since shrimp heads and shells harbor large numbers of the spoilage microorganisms (Table 1), it was believed that such treatments would be of greater effect on peeled...
FIGURE 1. Populations of psychrotrophic bacteria (log CFU/g) in controls and treated whole shrimp samples stored in ice. Within each storage time, bars labeled with different letters are significantly different ($P < 0.05$).

shrimp. Treatments with SA and PS plus BIF were the most effective ones used on whole shrimp (Fig. 1), so they were examined on peeled shrimp. Figure 2 illustrates the changes in the psychrotrophic counts (log CFU/g) during storage of peeled shrimp treated with SA and PS plus BIF. Sodium acetate was significantly more effective in suppressing bacterial growth than PS plus BIF. Sodium acetate increased both the lag phase and the microbial generation time (38.7 h compared to 15.8 h for C1). These increases may be attributed to the direct contact of treatment solution with spoilage bacteria on shrimp flesh. Data indicated that untreated controls reached the spoilage onset ($10^7$ CFU/g) after 6 and 10 days of ice storage for whole and peeled shrimp, respectively. This indicated that peeling extended the shelf life of stored shrimp by 4 days. Zhuang et al. (30) reported that neither 2% sodium acetate nor 2% sodium lactate had a significant effect on growth of microorganism naturally present on shrimp. However, on catfish fillets, sodium acetate was effective in controlling the growth of natural microflora.

Previous citations (11, 17, 18, 25, 30) as well as the results obtained in this study suggest that the effectiveness of any surface treatment depends on factors such as treatment technique (tumbling, dipping, spraying), contact time with treatment solutions (2 to 30 min), type of samples (meat, fish fillets, whole and peeled shrimp), and type and concentration of solutions used.

Table 1 illustrates the distribution of microorganisms among shrimp parts. Although the shrimp head is about 36.7% of the shrimp weight, it contained approximately 80% of the total bacteria. On the other hand, the flesh contained only 1% of the total bacteria. Therefore, removing the head and shell eliminated almost 99% of the microbial load of shrimp. The shelf life of the whole shrimp was extended by 3 days after treatment with either SA or PS plus BIF. In contrast, the shelf life of peeled shrimp treated with PS plus BIF was 14 days and was >17 days for samples treated with SA compared to a shelf life of 10 days for untreated control. The expected microbiological shelf life of SA-treated shrimp was calculated using the generation time equation and found to be 26.8 days.

The shelf life of stored shrimp by 4 days. Zhuang et al. (30) reported that neither 2% sodium acetate nor 2% sodium lactate had a significant effect on growth of microorganism naturally present on shrimp. However, on catfish fillets, sodium acetate was effective in controlling the growth of natural microflora.

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Machava (19) reported 5 days as the shelf life of whole shrimp stored on ice, while Kim et al. (16) reported a 3-day shelf-life extension for fish fillets treated with SA plus BIF (0.5%, wt/wt, and 2.5%, vol/wt, respectively). Harrison and Heinsz (12) extended shrimp shelf life by 3.1 days using 0.2% PS containing ice. Gamma irradiation (2 kGy) extended the storage life of peeled shrimp stored at 3°C to 22 days (10).

Types of psychrotrophs on shrimp samples. The following genera were present in the order of predominance: Micrococcus, Corynebacterium, Aerococcus, Streptococcus, and Pseudomonas. Such findings are in agreement with those reported by Ashie et al. (3) and Vanderzant et al. (26). In most treatments (Table 2) micrococci slightly decreased by the ninth day of storage while the number of members of the genus Corynebacterium increased. Unlike the increase in Corynebacterium counts after 9 days of storage in most treatments, shrimp treated with PS and PS plus BIF showed little increase (from 14.4 to 15.9% and from 9.7 to 11.2%, respectively). This result indicated a possible inhibitory effect of sorbate on coryneforms. Members of the genus Aerococcus almost disappeared at day 9, and presence of members of the genus Pseudomonas was of no significance in this investigation. The absence of gram-negative bacteria in frozen shrimp samples was probably due to the killing effect of freezing the shrimp.

Sensory evaluation. No significant differences (P < 0.05) were found up to day 6 of storage between the mean scores of the treatments and the controls for color, texture, odor, and general appearance of whole shrimp samples (data not shown). This was probably due to the effect of shells masking the sensory quality of the shrimp during storage. In general, acceptability decreased as the time of storage increased up to day 6, at which point sensory panellists considered the samples to have reached spoilage.

No significant differences in the general appearance of the peeled shrimp (Table 3) were found between treatments and controls during the first 10 days of storage. After this point, significant differences were evident, and the highest scores favored SA treatment. Within each treatment, acceptability decreased with increasing storage time with the exception of the SA treatment, whereas the scores were not

<table>
<thead>
<tr>
<th>Genus</th>
<th>Time (day)</th>
<th>Treatment</th>
<th>C1</th>
<th>C2</th>
<th>BIF</th>
<th>SA</th>
<th>PS</th>
<th>SL</th>
<th>TSC</th>
<th>SA+BIF</th>
<th>PS+BIF</th>
<th>SL+BIF</th>
<th>TSC+BIF</th>
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</thead>
<tbody>
<tr>
<td>Micrococcus</td>
<td>0</td>
<td>C1</td>
<td>78.9</td>
<td>78.8</td>
<td>78.9</td>
<td>82.3</td>
<td>75.4</td>
<td>73.3</td>
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<td>83.2</td>
<td>79.2</td>
<td>73.6</td>
<td>79.6</td>
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<tr>
<td></td>
<td>9</td>
<td>C2</td>
<td>73.6</td>
<td>67.3</td>
<td>62.8</td>
<td>64.3</td>
<td>75.8</td>
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<td>80.0</td>
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<td>72.5</td>
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<td>I and II</td>
<td>BIF</td>
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<td></td>
<td></td>
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<td>4.5</td>
<td>4.8</td>
<td>4.8</td>
<td>3.0</td>
<td>4.7</td>
<td>4.8</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>SA</td>
<td>5.0</td>
<td>5.0</td>
<td>9.3</td>
<td>5.2</td>
<td>3.6</td>
<td>11.2</td>
<td>8.7</td>
<td>2.0</td>
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<tr>
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<td>I and II</td>
<td>PS</td>
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<td></td>
<td></td>
<td>6.9</td>
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<td>9.8</td>
<td>2.0</td>
<td>6.3</td>
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<td>SL</td>
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<td></td>
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<td>9</td>
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<td>0</td>
<td>0</td>
<td>3.0</td>
<td>0.3</td>
<td>0.8</td>
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<tr>
<td>Pseudomonas</td>
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<td>TSC</td>
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<td></td>
<td></td>
<td>28.0</td>
<td>15.9</td>
<td>18.4</td>
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<td>17.8</td>
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<td></td>
<td>9</td>
<td>SA+BIF</td>
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<td></td>
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<td>PS+BIF</td>
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</table>

* C1 = untreated control; C2 = shrimp dipped in water; BIF = bifidobacteria, SA = sodium acetate; PS = potassium sorbate; SL = sodium lactate; TSC = trisodium citrate.
significantly different during the last 12 days of storage. Again, SA treatment was the best in maintaining acceptable odor, especially during the last 7 days of storage (Table 4). Samples treated with SA and to a lesser degree those treated with PS plus BIF were acceptable until day 17 of storage indicating that sensory shelf life was extended by at least 3 days. Sensory shelf life determined by the panelists was found to be >17 days. This value agreed well with the microbiological shelf life mentioned earlier for peeled shrimp samples treated with SA.

The changes in color and texture of peeled shrimp did not differ significantly from controls during the course of storage (data not shown). Acceptability in both cases decreased as the storage time increased with better stability shown by shrimp treated with SA and to a lesser degree shrimp treated with PS plus BIF.

**Changes in pH of whole shrimp.** No significant differences were noticed between pH levels of controls and treatments at each analysis day. This was probably because shells did not allow for good penetration of solutions of organic acid salts to shrimp flesh. The pH ranges (for the controls and the treatments) were 6.9 to 7.25 at zero time and 7.45 to 7.60 after 9 days of storage.

In conclusion, it appeared that *B. breve*-produced metabolites showed an additive effect when combined with PS in suppressing spoilage organisms on iced whole shrimp. Sodium acetate exhibited significant potential for extending the shelf life of whole and peeled shrimp to 9 and >17 days, respectively. However, further study on sensory quality of cooked, treated samples is needed. Peeling of shrimp was effective in removing most (about 99%) of the bacterial load on shrimp, and as a result the microbiological shelf life of ice stored shrimp was extended by 4 days.

### REFERENCES


### TABLE 3. Mean sensory evaluation scores for the effect of sodium acetate (SA) and potassium sorbate with bifidobacteria (PS+BIF) on the general appearance of peeled shrimp during storage in ice

<table>
<thead>
<tr>
<th>Storage (day)</th>
<th>C1</th>
<th>C2</th>
<th>BIF</th>
<th>SA</th>
<th>PS+BIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.6^A_\pm_0.31</td>
<td>5.7^A_\pm_0.26</td>
<td>6.1^A_\pm_0.23</td>
<td>5.3^A_\pm_0.26</td>
<td>5.8^A_\pm_0.25</td>
</tr>
<tr>
<td>3</td>
<td>4.8^B_\pm_0.25</td>
<td>5.0^AB_\pm_0.30</td>
<td>5.3^B_\pm_0.15</td>
<td>5.0^A_\pm_0.30</td>
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<tr>
<td>6</td>
<td>5.3^AB_\pm_0.15</td>
<td>4.7^BC_\pm_0.21</td>
<td>4.5^B_\pm_0.34</td>
<td>4.5^AB_\pm_0.31</td>
<td>5.3^A_\pm_0.21</td>
</tr>
<tr>
<td>10</td>
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<td>4.1^AC_\pm_0.31</td>
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</tr>
<tr>
<td>14</td>
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<td>3.0^BD_\pm_0.30</td>
<td>3.1^B_\pm_0.31</td>
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<td>17</td>
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<td>2.6^C_\pm_0.37</td>
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<td>3.6^AB_\pm_0.22</td>
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</table>

a Means within a row followed by superscripts containing the same letter are not significantly different at the 5% level.

### TABLE 4. Mean sensory evaluation scores for the effect of sodium acetate (SA) and potassium sorbate with bifidobacteria (PS+BIF) on the odor of peeled shrimp during storage in ice

<table>
<thead>
<tr>
<th>Storage (day)</th>
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<th>C2</th>
<th>BIF</th>
<th>SA</th>
<th>PS+BIF</th>
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<tr>
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<td>4.7^A_\pm_0.21</td>
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<td>4.8^A_\pm_0.25</td>
<td>5.0^A_\pm_0.21</td>
<td>4.4^B_\pm_0.27</td>
<td>4.7^A_\pm_0.27</td>
<td>4.8^A_\pm_0.25</td>
</tr>
<tr>
<td>10</td>
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<td>4.7^A_\pm_0.34</td>
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<td>2.7^B_\pm_0.37</td>
<td>2.6^B_\pm_0.45</td>
<td>4.4^AB_\pm_0.30</td>
<td>3.5^AB_\pm_0.34</td>
</tr>
<tr>
<td>17</td>
<td>2.3^B_\pm_0.37</td>
<td>2.0^B_\pm_0.37</td>
<td>2.2^B_\pm_0.44</td>
<td>3.8^B_\pm_0.29</td>
<td>3.6^A_\pm_0.22</td>
</tr>
</tbody>
</table>

a For sensory scores, 7 = most liked, 3 = unacceptable margin, and 1 = most disliked.

b Means within a row followed by superscripts containing the same letter are not significantly different at the 5% level.

c C1 = untreated control; C2 = immersed-in-water control; BIF = bifidobacteria; SA = sodium acetate; PS = potassium sorbate.


