Microencapsulation of Bifidobacterium longum can change the characteristics of the orange juice
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ABSTRACT
Bifid bacterium longum bb536 was prepared from Iranian developed collection of bacteria and fungi. The samples were cultured at 70 °C and on the De-Man-Rogosa-Sharpe agar (MRSA) medium enriched with 0.5 grams per liter of L-cysteine (HCL and MRSA + cys), and then are incubated at 37 °C. This bacteria was microencapsulated and compared to non-encapsulated form under different population (ml cfu. 6E8, 6E7 and 6E6 and). The results showed that the encapsulated probiotics increased the viability of the bacteria in probiotic orange juice in comparison with their free form. The final numbers of probiotic bacteria in both free and encapsulated ones after twenty-five days of storage at 4 °C of orange juice were more than the minimum amount recommended for therapeutic effects on human health. During the storage phases, the results of colorimetric and Sensory evaluation of probiotic juice showed that encapsulate probiotic Bifidobacterium has a significant difference with not-capsulated one, where the capsulated one was better. Not-encapsulated probiotic significantly sowed a reduce pH in compare to the form of encapsulates bacteria. Bifidobacterium encapsulated form not only affecting physicochemical and sensory properties, did not improve the taste and increase shelf life of probiotic properties in compared with the free form during storage at 4 °C for 25 days. Therefore, the use of alginate capsules / isolated whey can be a proper carrier for Bifidobacterium longum.

Introduction
There is much biomedical interest in citrus fruits, as their consumption has been linked to a lower risk of colorectal (Jaganathan et al., 2014) esophageal (Chen et al., 2002), gastric (Palli et al., 2001), and cancers (McCullough et al., 2001) and stroke (Cassidy et al., 2012). Orange juice contains flavones and narirutin, which display a wide spectrum of pharmacological effects, including anti-tumor (Aranganathan and Nalini, 2013), antioxidant (Cai et al., 2004), and hypcholesterolemic and hypoglycemic activities (Akiyama et al., 2009). On the other hand, Probiotics, primarily represented in humans by bacteria from the genera Bifidobacterium (Sarao, 2015) offer health benefits to a host, including promotion of digestion and mineral absorption, maintenance of an intestinal microbial balance, improvements in immune system response and resistance to pathogens, reduction of the risk of cardiovascular disease, cancer, obesity, and type-2 diabetes, regulation of lipid and serum cholesterol levels, and treatment of inflammation, autoimmune responses, and allergies (Hooper et al., 2001). A decline in the number of naturally occurring probiotics in the gut could be caused by antibacterial drugs, stress, gastrointestinal disorders, and aging, which can further lead to chronic gastrointestinal diseases with symptoms of abdominal cramps, diarrhea, fever, and vomiting (O’Mahony et al., 2005). Supplementation with probiotics via oral administration in medicine and food products has to be concerned with promotion of delivery of viable probiotics to a target organ and proper protection during transit through the gastrointestinal tract.

Encapsulation has been reported to provide probiotic living cells with a physical barrier against the adverse environmental conditions encountered in foods and within a host (Vidhyalakshmi et al., 2009). Use of encapsulated ingredients has increased over the past few years, and includes use of polysaccharides originating from seaweed (carrageenan and alginate), plants (starch and derivatives, gum Arabic), bacteria (gellan and xanthan), animal proteins (milk caseins, whey, protein, and gelatin), and as polycations (chitosan) as encapsulation agents (Rokka and Rantamäki, 2010). Whey proteins are valuable by-products from the cheese industry. They are used widely in a variety of foods primarily for their superior gelling and emulsification properties. The physicochemical properties of the whey proteins suggest that they are suitable for food and nonfood applications (Gunasekaran et al., 2006).

Because of the importance of the orange juice and its high consumption, this research was conducted to expanding the feasibility study of Non-dairy probiotic to enhance the health of people who are sensitive to dairy products by using encapsulated probiotic orange juice.

Materials and methods
Preparation and testing of microencapsulation
Bifidobacterium longum bb536 was prepared from Iranian developed collection of bacteria and fungi. The samples were cultured at 70 °C and on the De-Man-Rogosa-Sharpe agar (MRSA) medium enriched with 0.5 grams per liter of L-cysteine (HCL and MRSA + cys), and then are incubated at 37 °C.
Whey isolate powder of 8% w/v of water was stirring at room temperature for one hour and then soaked up for two hours to be fully hydrated. The resulting solution having pH = 8 was heated to 80 °C for 30 minutes to fully denature its complex protein. Whey isolate powder solution is then cooled and kept at room temperature for 2 hours (Chen and Subirade, 2006). Bifidobacterium with growing population ratio of probiotics 6E8, 6E7 and 6E6 (cfu.ml) were drop to each sample and then sodium alginate powder was added at the rate of 2% w/v was stirred overnight and the resulting solution was added to the microspheres by internal emulsification / gelation (Poncelet, 2001).

To evaluate the size and size distribution, microspheres freshly prepared in distilled water containing 80 Tween 1% w / w filtered through 2.0 mm filter solved (Ding and Shah, 2008). Finally the number of bacteria trapped in the capsules were counted by the method of (Sultana et al., 2000). Orange juice and its Testing during storage

The orange juices were got from SunStar Company of Iran and they held during the experiment without any maintenance. Fruit juices at a 4 °C temperature were maintained for one night before insemination. The juice's samples containing bifidobacterium encapsulated by the three bacterial population ml cfu. 6E8, 6E7 and 6E6 and bifidobacterium free samples population with three before mentioned bacterium ratio as control were taken as the experiment treatment with three replications. Then the tests were done for 25 days and on days zero and the fifth, tenth and fifteenth, twentieth and twenty five (Ding and Shah, 2008). Assess the viability of bacteria

According to Tamminen et al. (2013) with little change, survival ability of bacteria to grow in the fruit juices (treated and control), free and encapsulated on the environment RCM-agar, Oxoid anaerobic in The temperature 37 °C was measured. Measured traits

Brix and pH of the sample

pH and brix during storage at zero, and the fifth, tenth and fifteenth, twentieth and twenty five days after starting the experiment were measured.

Color evaluation

Calorimeter (D25-DP9000) to measure colorimetric with the reference plate in black and white were test (Umesha et al., 2014).

Sensory evaluation

The sensory properties such as color, appearance, taste, texture and overall acceptability by 25 trained panelists were evaluated at the Research Institute of Agricultural Engineering Branch. Evaluation was performed based on five-point hedonic scale. In this study (5=excellent, 4=good, 3=neither good nor bad 2=bad, 1=very bad) was evaluated (Krasaekoopt and Kitsawad, 2010).

Statistical analysis of data

The data were analyzed using Completely Randomized Design and then the means were compared by LSD test at 5% of probability. SPSS 22 were used for data analyses.

**Results and Discussion**

**Particle size**

As it is clear in Table 1, Percentage increase in the size of the bacterial population showed a significant difference (p<0.05) related to size of bacteria capsule. Percentage of the population with the highest size of population (Cfu / ml 108) showed highest size of capsule while minimum size of the sample population (Cfu / ml 106) had the lowest size of capsule. Increasing the population of probiotics affects the microencapsulated of capsules, so that if the initial kernel used for encapsulation are larger, the size of the capsules will increased. This results was in line with the results of Ying et al. (2013). Ying et al. (2013) also showed higher levels of Lactobacillus rhamnosus accumulation in starch capsules / whey isolates showed a significant increase in the size of microcapsules. They also showed the effects of other factors such as formartion environment pH, electrostatic equilibrium of carrier compounds and zeta potential capsules, and the temperature of microencapsulation formation, on the size of microencapsulation.

<table>
<thead>
<tr>
<th>Treatment C</th>
<th>Treatment B</th>
<th>Treatment A</th>
</tr>
</thead>
<tbody>
<tr>
<td>515±0.27</td>
<td>415±0.28</td>
<td>325±0.14</td>
</tr>
</tbody>
</table>

Table 1. Mean comparison for size of whey capsules in different treatment

**Capsule morphology**

Figure 1 shows morphological characteristics of microencapsulation for treatments. It could be observed in this figure that morphological properties of capsules are spherical nanoparticles and soft and without any agglomeration. The sizes of capsules are different. These results confirmed the formation of spherical nanoparticles, and biopolymers having relatively uniform size. Nano particles were separated, spherical form, and uniform. On the other hand, use of probiotic cells in appearance and morphological characteristics of microencapsulation were not effective. The results of morphological characteristics are coincided with Nualkaekul et al. (2012).

![Microscopically images of Alginate microspheres electron isolated from whey containing bifidobacterium](image)

**Number of bacteria trapped in the capsules**

Figure 2 shows the final number of bacteria trapped in the capsules. Based on this result, the concentration of bacteria trapped within the capsule formulation used in microencapsulation is equal to their initial rate. As the results showed, carrier alginate / whey isolate has the ability to trap or the accumulation of 100 percent. One of the reasons for such situation can be ability of alginate to create an oval networks and trap the compounds in its structure (Gunasekaran et al., 2006). The results of this study is in line with the results of the Zandi et al. (2014).
Figure 2. Number of bacteria trapped in the capsules.

Table 2. Twenty-five days after the results of Bifidobacterium longum.

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>Treatment A</th>
<th>Treatment B</th>
<th>Treatment A</th>
<th>Treatment B</th>
<th>Treatment A</th>
<th>Treatment B</th>
<th>Treatment A</th>
<th>Treatment B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5 × 10^9 a</td>
<td>5 × 10^9 a</td>
<td>5 × 10^9 a</td>
<td>5 × 10^9 a</td>
<td>5 × 10^9 a</td>
<td>5 × 10^9 a</td>
<td>5 × 10^9 a</td>
<td>5 × 10^9 a</td>
</tr>
<tr>
<td>5</td>
<td>4 × 10^9 ab</td>
<td>5 × 10^9 ab</td>
<td>5 × 10^9 ab</td>
<td>5 × 10^9 ab</td>
<td>5 × 10^9 ab</td>
<td>5 × 10^9 ab</td>
<td>5 × 10^9 ab</td>
<td>5 × 10^9 ab</td>
</tr>
<tr>
<td>10</td>
<td>4 × 10^9 c</td>
<td>5 × 10^9 b</td>
<td>5 × 10^9 b</td>
<td>5 × 10^9 b</td>
<td>5 × 10^9 b</td>
<td>5 × 10^9 b</td>
<td>5 × 10^9 b</td>
<td>5 × 10^9 b</td>
</tr>
<tr>
<td>15</td>
<td>4 × 10^9 cd</td>
<td>4 × 10^9 cf</td>
<td>4 × 10^9 cd</td>
<td>5 × 10^9 b</td>
<td>5 × 10^9 b</td>
<td>5 × 10^9 b</td>
<td>5 × 10^9 b</td>
<td>5 × 10^9 b</td>
</tr>
<tr>
<td>20</td>
<td>3 × 10^9 tf</td>
<td>4 × 10^9 td</td>
<td>4 × 10^9 td</td>
<td>5 × 10^9 b</td>
<td>5 × 10^9 b</td>
<td>5 × 10^9 b</td>
<td>5 × 10^9 b</td>
<td>5 × 10^9 b</td>
</tr>
</tbody>
</table>

Table 3. The brix samples after twenty-five days at 4 °C.

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>Treatment A</th>
<th>Treatment B</th>
<th>Treatment A</th>
<th>Treatment B</th>
<th>Treatment A</th>
<th>Treatment B</th>
<th>Treatment A</th>
<th>Treatment B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>63± 0.03 a</td>
<td>63± 0.03 a</td>
<td>63± 0.03 a</td>
<td>63± 0.03 a</td>
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</tr>
<tr>
<td>5</td>
<td>60± 0.09 b</td>
<td>60± 0.09 b</td>
<td>60± 0.09 b</td>
<td>60± 0.09 b</td>
<td>60± 0.09 b</td>
<td>60± 0.09 b</td>
<td>60± 0.09 b</td>
<td>60± 0.09 b</td>
</tr>
<tr>
<td>10</td>
<td>58± 0.19 c</td>
<td>58± 0.19 c</td>
<td>58± 0.19 c</td>
<td>58± 0.19 c</td>
<td>58± 0.19 c</td>
<td>58± 0.19 c</td>
<td>58± 0.19 c</td>
<td>58± 0.19 c</td>
</tr>
<tr>
<td>15</td>
<td>56± 0.21 d</td>
<td>56± 0.21 d</td>
<td>56± 0.21 d</td>
<td>56± 0.21 d</td>
<td>56± 0.21 d</td>
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</tr>
<tr>
<td>20</td>
<td>44± 0.23 e</td>
<td>44± 0.23 e</td>
<td>44± 0.23 e</td>
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<td>44± 0.23 e</td>
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<tr>
<td>25</td>
<td>40± 0.32 f</td>
<td>43± 0.32 g</td>
<td>52± 0.32 h</td>
<td>52± 0.32 i</td>
<td>52± 0.32 j</td>
<td>52± 0.32 k</td>
<td>52± 0.32 l</td>
<td>52± 0.32 m</td>
</tr>
</tbody>
</table>

Table 4. The pH of samples during twenty-five days at 4 °C.

Viability of bacteria

According to Table 2 in twenty-five days of probiotic orange juice in some treatments, the number of bacteria increased, while some of treatments have decreased over time. The highest and lowest number of live bacteria were belonging to the treatment C and control A, respectively. Encapsulated cells need more time to a cycle of log reduction in the number of cells with the viability potential. Storage temperature of juice is effective the on survival of probiotic bacteria. On the other hand, Probiotics showed 30% more survival in the alginate coating / whey were isolated than probiotics without any cover. The results of the study of (Zandi et al., 2014) showed a significant difference related to survival of microencapsulation of cells for all races between coated and not-coated with whey. Prie and pH of samples.

Sensory evaluation of probiotic juice
Brix and pH changes are presented in Tables 3 and 4. Encapsulating with Bifidobacterium longum showed a significant trend during the time of measurement. The brix and pH were decreased during the time. Decrease in the pH value during storage indicating that cells in the formulation Bifodobacterium in the formulation of orange juice, metabolized substrates sugar and produce acid compounds which leads to a decrease in pH in probiotic orange juice. With increasing population, decreasing pH of the probiotic showed higher rate losses. pH changes in treated samples were not significant. One of the reasons for this matter could be the existence of isolated proteins from whey and alginate. These isolated could be used by the probiotics in the orange juice instead of the sugar compound of juice leads to lower decrease of pH.

Colorimetric of samples

The results of colorimetric showed that the color of the control samples A, B, C had significant difference in brightness index (L*) and color difference samples (ΔE) was observed (0.05> p). Control C had higher decrease than control sample of A and B related to brightness index. The rate of this decline was more severe after tenth day in compare to before days. ΔE trend was similar to the trend of the control samples. The samples L* and Δa showed no significant difference in treatment. In addition, other parameters such as b* and a* color measurement in samples of treated and control groups did not show significant differences.

The results of sensory evaluation showed that the samples with encapsulation of probiotics form significantly have better taste than free form encapsulation samples. There was no significant differences between the three types of treated sample (A, B and C) related to their taste. But among the control samples (A, B and C) Control C did not show a good taste while the best flavors was obtained in control sample A, in other words there is revers relationship between population of probiotics and orange juice taste. Sensory or organoleptic characteristics of the probiotic products, are the first condition for product to be accepted by consumers. One of the reasons for lower acceptance of probiotic orange juice by the customers is compounds produced by probiotics. Grattapanche et al. (2008) showed that high doses of probiotic bacteria in cheese due to the unfavorable compounds produced have negative effects on their sensory characteristics. Probiotic products are usually acidic, bitter and sharp, which they may affect the sensory properties (Grattapanche et al., 2008).

The overall results showed that the encapsulated probiotics increased the viability of the bacteria in probiotic orange juice in comparison with their free form. The final numbers of probiotic bacteria in both free and encapsulated ones after twenty-five days of storage at 4 °C of orange juice were more than the minimum amount recommended for therapeutic effects on human health. During the storage phases, the results of colorimetric and Sensory evaluation of probiotic juice showed that encapsulate probiotic Bifidobacterium has a significant difference with not-encapsulated one, where the encapsulated one was better. Not-encapsulated probiotic significantly sowed a reduce pH in compare to the form of encapsulates bacteria. Bifidobacterium encapsulated form not only affecting physicochemical and sensory properties, did not improve the taste and increase shelf life of probiotic properties in compared with the free form during storage at 4 °C for 25 days. Therefore, the use of alginate capsules / isolated whey can be a proper carrier for Bifidobacterium longum.

References

women. Cancer Epidemiology Biomarkers & Prevention 10, 1201-1205.


Cassidy, A., Rimm, E. B., O'Reilly, É. J., Logroscino, G., Kay, C., Chiue, S. E., and Rexrode, K. M.


