Growth and viability of probiotic Weissella kimchi R-3 in fruit and vegetable beverages

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Probiotics, due to their multitude of health benefits, have become the focus of intense research in recent years. Fruits and vegetable juices have emerged as potential probiotic vehicles for human consumption in view of issues associated with dairy products as traditional probiotic carriers viz. lactose intolerance, casein allergies and high fat content. Fruits and vegetable juices as healthy foods may serve as promising non-dairy probiotic carriers. In this study, the fruit/vegetable juices (orange, pomegranate, carrot juice) and soymilk were fermented with a probiotic bacterium Weissella kimchii R-3, previously characterized in our laboratory, and studied for several functional attributes. Viability of probiotic organism was examined during fermentation, as well as during storage at refrigerating conditions (4°C) and room temperature (25°C). Changes in the biochemical properties of beverages viz. acidity, pH, sugar content, were also analyzed. The results have shown that W. kimchii R-3 had good growth and viability in the carrot juice (3.15-9.87 log cfu/mL) and soymilk (3.0-11.97 log cfu/mL) during fermentation, and storage for four weeks compared to pomegranate and orange juices. Furthermore, acid production, pH reduction and sugar utilization have indicated good metabolic activity of the probiotic organism in the fermented beverages. It may be concluded from the results that fruit juices/soymilk may serve as good vehicles for probiotic delivery and may gain as much acceptance as dairy products.

Keywords: Carrot juice, Fermentation, Orange juice, LAB Pomegranate juice, Probiotic, Soymilk, Weissella kimchii R-3

The gut microbiota composition and number is vital for overall good health and averting pathologies1. Ingestion of probiotics in viable numbers helps maintaining a good gut health2. Probiotics are live microorganisms which upon ingestion in adequate quantities exert wide range of health benefits on the host by stimulating growth of other microorganisms, modulating the mucosal and systemic immunity, and improving the nutritional and microbial balance in the intestinal tract3,4. Most commonly used probiotics include lactic acid bacteria, LAB (Lactobacillus spp. and others), Bifidobacterium spp. and yeast Saccharomyces boulardii apart from certain other bacterial and yeast species5. Recently, probiotics, due to their multifaceted health benefits such as prevention and treatment of diarrheal diseases, systemic infections and various allergies, management of inflammatory bowel disease, hypercholesterolemia, immunomodulation, anticancer effects and alleviation of lactose intolerance, among others, have gained researchers attention, particularly of medical and nutritional scientists6.

Considering that probiotics intake is practiced mostly through various food types, growth as well as survival of probiotics in such food products is extremely important. Health benefits of foods with added ‘live microbes’ (probiotics) are being increasingly promoted by health professionals, precisely among children and other populations6. Dairy products have most extensively been used as carriers of probiotics4,5,7. However, a trend of non-dairy probiotic carriers is growing due to some concerns with dairy products, such as lactose intolerance, high cholesterol content, presence of potential allergens like casein, requirement of cold storage during shelf life and growing popularity of vegetarianism8,9. Apart from this, higher frequency of allergies to milk proteins has also been reported among infants, than adults8, and has encouraged development of non-dairy products as potential vehicles for probiotics. Several foods such as coconut milk, fruit drinks, nutrition bars, soy products and cereal based products, etc. have been examined as potential probiotic carriers10.

Fruit juices are considered healthy drinks and may serve as ideal carriers for probiotics. Fruit juices enjoy an established market sector, consumed regularly and are microbiologically safe due to their low pH and storage under refrigeration11. Soymilk is now an

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accepted healthy substitute worldover for high level of phenolic content, and anticarcinogenic, anti-inflammatory, antioxidant and antimutagenic attributes. Furthermore, soy foods have a high dietary fiber and low cholesterol content with balanced composition of protein and are lactose free. Different studies have been carried out to explore the suitability of fruit/vegetable juices, such as apple, beet, guava, litchi, mango, melon, orange, passion fruit, pineapple, pomegranate and tomato as carriers for production of probiotic products. Soy milk has also been evaluated previously for its potential as a probiotic carrier. Although the minimum recommended level of viable number of probiotic organisms in foods may vary, food industry has adopted the recommended level of 10^6 colony forming units (cfu) per mL or per gram at the time of consumption. However, per day consumption of probiotic cells could be from fewer cells to up to 10 billion cells.

Several genera and species of LAB from diverse sources have been reported to possess admirable probiotic attributes. Weissella spp. represents the LAB that are Gram-positive, catalase-negative, non-endospore forming cells with coccoid or rod-shaped morphology. Weissella spp. have been isolated from different sources and characterized for their probiotic attributes.

Weissella kimchii R-3, an isolate from poultry gut was previously characterized for its functional probiotic characteristics in our laboratory. W. kimchii R-3 possessed several desired probiotic attributes like high hydrophobicity, autoaggregation, coaggregation ability and antimicrobial activity against bacterial pathogens, and phytase-producing ability. Here, we studied the growth and viability of W. kimchii R-3 in various fruit/vegetable juices and soymilk, and assessed their potential as prospective non-dairy vehicles for probiotics. The juices and soymilk were subjected to fermentation with W. kimchii R-3. The fermented beverages were examined for growth/viability of probiotic organism and changes in various biochemical parameters during the period of fermentation and after storage at different temperatures.

**Materials and Methods**

**Chemicals, media components and probiotic microorganism**

Various media, reagents, and chemicals employed in the experiments for current investigation were procured from HiMedia Laboratories, Merck and Co., Ranbaxy Fine Chemicals and Sigma-Aldrich. All the chemicals and media components were of analytic grade.

The lactic acid bacterium Weissella kimchii R-3 used here was an isolate from poultry gut that has been previously characterized in our laboratory as mentioned already.

**Fruit/vegetable juices and soymilk**

Commercially available ‘ready to drink’ packaged orange juice (OJ) and pomegranate juice (PJ) were bought from the local supermarket. Both OJ and PJ had no added sugar and had a long shelf life of approximately 12 months from the date of manufacturing. Ready to drink soymilk (SM) having added calcium but no preservatives was also purchased from the local supermarket. The fruit juices and soymilk were refrigerated (4 ± 1°C) immediately for further use.

Carrot juice was prepared in the laboratory itself. Carrots purchased from local vegetable store were washed thoroughly, peeled and chopped into pieces of approximately 1 cm thickness. Chopped carrots were processed in the laboratory juice extractor (Bajaj, India), and the juice obtained was sieved through a cheesecloth. The filtered juice was centrifuged (Eppendorf centrifuge 5804R) at 5000 g for 20 min at 4°C to obtain a clear pulp-free juice. The clear carrot juice was transferred to sterile containers and refrigerated.

**Fermentation of fruit juices/soymilk with Weissella kimchii R-3**

Various fruit juices viz., orange, carrot, pomegranate juice, and soymilk were suitably heat treated (90°C for 1 min) in water bath, cooled and stored at 4°C. Prior to probiotic bacterial inoculation, the juices and soymilk were allowed to equilibrate to room temperature by incubating for 1 h at 25°C. W. kimchii R-3 was activated by cultivating it in MRS broth for 24 h at 37°C. The activated culture was inoculated at 1% (v/v) into fresh MRS broth and allowed to grow for 18 h at 37°C. The exponential phase biomass was harvested by centrifugation (5000 g for 10 min), and the cell pellet was washed twice with sterile phosphate buffered saline (pH 7.4), and finally the cells were inoculated in different juices and soymilk at a final concentration of ~10^9 cfu mL^-1. Inoculated juices and soymilk were subjected to fermentation at 37°C under anaerobic conditions for 72 h. Samples were drawn after every 24 h for analysis of viable cell count, and for assessment of biochemical parameters.

**Viable cell count assay**

The fermented/stored samples of juices/soymilk were suitably diluted with sterile saline and pour plated on MRS agar, and plates were incubated
anaerobically at 37°C for 48-72 h. The colonies appeared on MRS-agar were counted, and viable cell count was expressed as log cfu mL⁻¹.

**Biochemical analysis of the fermented beverages**

Biochemical changes that occurred in the fruit juices and soymilk (OJ, PJ, CJ and SM) during fermentation and storage were examined with respect to several parameters viz. pH, acidity and sugar concentration.

**Analysis of the fermented beverages for pH**

The pH changes in the fermented beverage samples were monitored using pH meter. pH meter was properly calibrated with standard buffers. The unfermented fruit juices and soy milk were used as controls.

**Determination of total acidity**

For the titratable acidity assay of fermented/stored beverages, a 10 mL of the sample was titrated against sodium hydroxide (0.1 N NaOH) using phenolphthalein (2-3 drops) indicator (pH 8.3). The amount of NaOH used (titre) was recorded. The results were interpreted according to the following formula

\[
\text{Percentage acid, g/100 ml} = \frac{T \times N \times A \times 100}{V}
\]

Where T shows mL of NaOH, N indicates normality of NaOH, A refers to acid factor, and V corresponds to volume of sample.

**Determination of total sugar**

Total sugar content of fermented/stored beverages (juices/soymilk) was quantified by phenol sulfuric acid method. In a hot acidic medium glucose is dehydrated to hydroxymethyl furfural and forms a coloured product with phenol that has absorption maximum (λₘₐₓ) at 490 nm. The absorbance of yellow-orange colour obtained was measured at 490 nm. The amount of total sugars present in the sample solution was determined using the standard curve of glucose.

**Analysis of fermented juices/soymilk after storage**

After 72 h of fermentation of fruit juices and soymilk at 37°C, the fermented samples were stored at refrigeration temperature (4°C) and room temperature (25°C). The samples were analyzed for viable cell count of probiotic bacterium, and biochemical properties of fermented beverages like total sugar content, lactic acid production and changes in pH, at regular intervals. The fermented juices/soymilk stored at 4°C were analyzed weekly up to 5 weeks, and those stored at 25°C were examined up to 12 days at 3 days interval.

All the analytical experiments were conducted in triplicates, and data presents the mean ±SD of three different experiments.

**Results and Discussion**

**Fermentation of beverages with Weissella kimchii R-3**

Fermentation of different fruit juices viz. soymilk (SM), pomegranate juice (PJ), orange juice (OJ) and carrot juice (CJ) with *W. kimchii* R-3 was performed and the survival of the organism during fermentation for 72 h at 37°C was determined. A decline in the *W. kimchii* R-3 population in PJ and OJ was observed during the first 24 h of fermentation (Fig. 1A). This could be due to the stress induced by differences between the MRS broth (pre-culture) and the pomegranate and orange juices. Pre-culture had a pH of about 6.5, however, the initial pH of PJ and OJ were much lower (about 2.3 and 3.21, respectively). Loss of viability of probiotic organism could be attributed to decrease in the pH of the medium and accumulation of organic acid as a result of growth and fermentation. Low pH of medium can lead to decreased growth rate and extended lag phase. However, in contrast to these observations, an increased cell number (log cfu/mL) of

![Fig. 1](image-url)
A good growth medium for probiotic microorganisms. Juice, as potential probiotic carriers. Studies have examined tomato juice, cabbage juice, apple juice and orange vegetable juices have been examined like carrot juice, because of the promising health benefits of the probiotics, a number of fruit and vegetables have been tested for probiotic delivery. Thus, fruit/vegetable juices may be good delivery vehicles. Because of the promising health benefits of the probiotic drinks, a number of fruit and vegetable juices have been examined for probiotic delivery vehicles, like carrot juice, tomato juice, cabbage juice, apple juice and orange juice.

**Biochemical analysis of beverages during fermentation**

Physicochemical properties of fermented juices such as pH, and titratable acidity, may affect the viability of probiotics in fortified products. During the initial fermentation at 37°C, pH of juices decreased from 5.96 to 5.05 for SM, 2.87 to 2.68 for PJ, 3.47 to 3.30 for OJ, and from 4.66 to 4.47 for CJ. Carrot juice (CJ) and soymilk (SM) showed a considerable reduction in pH during the first 24 h, compared to the orange (OJ) and pomegranate juices (PJ) (Fig. 1B). As CJ and SM are less acidic than OJ and PJ, therefore, both SM and CJ supported excellent growth and metabolic activity of *W. kimchii* R-3.

All LAB have the inherent tendency to produce acids majorly lactic acid and other acids. The titratable acidity (TA) represents the measure of the acid content. Generally, higher is the growth and metabolic activity of LAB more is the TA, and correspondingly more reduction in pH of the medium. SM and CJ showed substantial increase in TA during first 24 h of fermentation (Table 1). However, during next 48 and 72 h of fermentation, though, TA increased but to a lesser extent. On the other hand, OJ and PJ exhibited only marginal variations in TA. OJ and PJ being highly acidic, TA of these decreased from initial 5.05 to 4.61 for OJ and from 5.05 to 4.47 for PJ.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>Time (h)</th>
<th>SM</th>
<th>PJ</th>
<th>OJ</th>
<th>CJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>24</td>
<td>0.252±0.5</td>
<td>0.579±0.1</td>
<td>0.685±0.2</td>
<td>0.374±0.4</td>
</tr>
<tr>
<td>38</td>
<td>24</td>
<td>0.306±0.2</td>
<td>0.603±0.1</td>
<td>0.739±0.3</td>
<td>0.347±0.2</td>
</tr>
<tr>
<td>37</td>
<td>72</td>
<td>0.324±0.5</td>
<td>0.649±0.5</td>
<td>0.707±1.4</td>
<td>0.414±0.05</td>
</tr>
</tbody>
</table>

*Control. [SM: Soymilk; PJ: Pomegranate juice; OJ: Orange juice; CJ: Carrot juice]
acidic in nature supported less growth of *W. Kimchii* R-3 that is why TA also did not show any variation.

Total sugar content of fermented beverages was examined during fermentation. Sugar content in all the fermented beverages decreased to a variable level due to its utilization by *W. Kimchii* R-3 for growth and metabolic activity. During first 24 h of fermentation, maximum sugar reduction was observed in PJ (42.0%), and was followed by SM (36.7%), and OJ (22.7%). However, in CJ sugar reduction was meager during the first 24 h of fermentation (9%), but decreased substantially (50%) during the next 48-72 h (Table 2). Sugar reduction in the probiotic fermented beverages is directly related to the growth and metabolic activity of the probiotic organism *W. kimchii* R-3. Furthermore, sugar utilization by probiotic organism *W. kimchii* R-3 may also be determined by the nutritional quality of the medium (fruit/vegetable juices) i.e. presence of various growth supporting factors, such as vitamins, minerals and other nutrients, that may support adequate growth and metabolic activity. It appears that all the beverages currently used for probiotic fermentation served as good media for growth of the probiotic organism *W. kimchii* R-3. Similar to the present study, fermentation of drumstick leaves and beetroot with *Lactobacillus plantarum* and *Enterococcus hirae* for 48 h at 37°C, showed a reduction in total sugar content from 8.71 to 3.33 g/L.31.

### Analysis of the probiotic fermented beverages during storage

Probiotic strains intended for commercial application must retain high viability and functional activity throughout the shelf life in the designated delivery product. The fruit/vegetable juices and soymilk fermented by probiotic *W. Kimchii* R-3 for 72 h at 37°C, were stored for a prolonged time period at refrigeration (4°C) and room temperature (25°C) and then analyzed for cell viability and other biochemical parameters. The changes observed for cell viability during storage of fermented beverages at 25 and 4°C are presented in Fig. 2 A and B. The *W. kimchii* R-3 showed a decrease of 1.37 and 0.82 log cfu mL⁻¹ in soymilk (SM) and carrot juice (CJ), respectively, during the first week of cold storage (4°C). Although, the viable cell counts of *W. kimchii* R-3 gradually decreased in the fermented SM and CJ during cold storage, but considerable viability was retained even after 5 weeks. Similar studies by Corona et al.14 on fermentation of carrot juice at 25°C for 48 h recorded a viable cell count of 5.7 log cfu mL⁻¹ after storage at room temperature. In another study, soymilk was fermented with *L. acidophilus*, and analyzed for survival during storage at 4°C for 21 days. It was observed that *L. acidophilus* exhibited good viability (8.73- 9.11 log cfu/g) after 21 days of storage at 4°C. No significant decrease in the number of *L. acidophilus* was observed during storage32.

In PJ and OJ, the cell concentration decreased gradually during storage and reached zero after 2 weeks of cold storage. Pomegranate (PJ) and orange juices (OJ) showed a log decrease of 2.52 and 2.37 (log cfu mL⁻¹), respectively, during the first week and lost viability after 2 weeks of cold storage. Loss of viability could be attributed to stressful conditions.

### Table 2 — Changes in the total sugar (glucose) of fruit/vegetable juices and soymilk during fermentation with *W. Kimchii* R-3

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>Time (h)</th>
<th>SM (28.3 ±0.00)*</th>
<th>PJ (69 ±0.00)*</th>
<th>OJ (61.5 ±0.00)*</th>
<th>CJ (44 ±0.00)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>18.0±0.01</td>
<td>40±0.01</td>
<td>47.5±0.01</td>
<td>40.0±0.01</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>17.0±0.01</td>
<td>35.5±0.01</td>
<td>47.0±0.02</td>
<td>22.0±0.1</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>15.0±0.1</td>
<td>30.1±0.02</td>
<td>45.0±0.01</td>
<td>22.5±0.01</td>
<td></td>
</tr>
</tbody>
</table>

*Control. [SM: Soymilk; PJ: Pomegranate juice; OJ: Orange juice; CJ: Carrot juice]
like extremely low pH (pH 2.9 and 3.51) of the juices, and of course relatively low temperature (4°C). Exposure to multiple stresses simultaneously leads to more drastic effects on the viability of the process organism. In contrast to present study, Randazzo et al. reported that the pomegranate juice fermented by commercial starter microbial preparation (LAB and yeast, Lactobacillus fermentum, L. kefiri, Lactococcus lactis, Leuconostoc mesenteroides and Saccharomyces cerevisiae) supported adequate microbial viability (cfu/mL of 7.8) even after prolonged storage. Barbosa et al. studied the survival of different Lactobacillus spp., Pediococcus acidilactici HA-6111-2 and Lactobacillus plantarum 299v during storage in spray dried orange juice (4°C). They observed that the probiotic organisms proved to be stable with no significant difference in viability even after storage for 180 days.

The probiotic organism W. kimchii R-3 exhibited better survival in fermented CJ and SM when stored at 25°C for 12 days as compared to PM and OJ. Viability of cells decreased after 6 and 3 days of storage at 25°C in PJ and OJ, respectively (Fig. 2A). The loss of viability might be due to adequate cellular growth and metabolic activity at 25°C, and hence adequate acid production (pH reduction) and even exhaustion of nutrients. The fermented probiotic beverages stored at refrigerated temperatures had more bacterial counts due to poor metabolic and cellular activity. Thus, refrigeration temperature had a substantial impact on viability of the probiotic organism. Therefore, storage temperature may help enhancing probiotic viability in fruit juices.

Biochemical analysis of the fermented beverages during storage

Acid production property of LAB constitute one of the main technological probiotic characteristics. Thus, pH-reduction and lactic acid production was monitored in the probiotic fermented beverages during storage at different temperatures (Fig. 3 A&B). In case of PJ and CJ, there was a slight decrease in the pH from 2.68 to 2.54 and 3.3 to 3, respectively at the end of cold storage (5 weeks). The pH decrease in case of SM was more pronounced i.e. from 5.05 to 4.6, where as the CJ showed a mild slight increase in pH (4.47 to 4.8) after completion of cold storage. At room temperature also a similar trend was observed for pH changes in various fermented beverages. Yacon fermented with lactic acid bacteria showed a decrease in the pH from 6.00 to 3.50 after 52 days of storage. The pH values of fermented soymilk with added apple juice decreased during storage from 5.17 to 3.86 after 21 days of storage.

The titratable acidity (TA) of the fermented soymilk and fruit/vegetable juices was increased during storage at different temperatures (Table 3). During storage at room temperature SM, OJ and CJ showed a considerable increase in the TA from 0.324 to 0.432, 0.707 to 0.966 and 0.414 to 0.685, respectively. At refrigerated temperature, SM and OJ also showed a considerable increase in the TA from 0.324±0.5 to 0.649±0.5 and 0.707±0.7 to 0.966±0.3, respectively.

**Table 3** — Changes in titratable acidity (%) of W. kimchii R-3 fermented fruit/vegetable juices and soymilk during storage with at different temperatures

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>SM</th>
<th>PJ</th>
<th>OJ</th>
<th>CJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0</td>
<td>0.324±0.5</td>
<td>0.649±0.5</td>
<td>0.707±1.4</td>
<td>0.414±0.05</td>
</tr>
<tr>
<td>D3</td>
<td>0.315±1.1</td>
<td>0.419±0.3</td>
<td>0.705±1.1</td>
<td>0.405±0.1</td>
</tr>
<tr>
<td>D6</td>
<td>0.378±0.1</td>
<td>0.513±0.2</td>
<td>0.798±0.5</td>
<td>0.490±0.5</td>
</tr>
<tr>
<td>D9</td>
<td>0.396±0.1</td>
<td>0.477±0.5</td>
<td>0.944±0.3</td>
<td>0.540±0.4</td>
</tr>
<tr>
<td>D12</td>
<td>0.432±0.5</td>
<td>0.678±1.2</td>
<td>0.966±0.3</td>
<td>0.685±0.2</td>
</tr>
<tr>
<td>W0</td>
<td>0.324±0.5</td>
<td>0.649±0.5</td>
<td>0.707±1.4</td>
<td>0.414±0.05</td>
</tr>
<tr>
<td>W1</td>
<td>0.333±0.1</td>
<td>0.536±0.2</td>
<td>0.802±0.05</td>
<td>0.450±0.1</td>
</tr>
<tr>
<td>W2</td>
<td>0.378±0.2</td>
<td>0.581±0.05</td>
<td>0.923±0.4</td>
<td>0.626±0.05</td>
</tr>
<tr>
<td>W3</td>
<td>0.513±0.1</td>
<td>0.792±0.3</td>
<td>0.959±0.1</td>
<td>0.649±0.2</td>
</tr>
<tr>
<td>W4</td>
<td>0.513±0.3</td>
<td>0.806±0.1</td>
<td>0.594±0.2</td>
<td>0.293±0.5</td>
</tr>
<tr>
<td>W5</td>
<td>0.450±0.5</td>
<td>0.720±0.4</td>
<td>0.540±1.4</td>
<td>0.270±0.7</td>
</tr>
</tbody>
</table>

[SM: Soymilk; PJ: Pomegranate juice; OJ: Orange juice; CJ: Carrot juice; D: days; W: weeks]
respectively, whereas in case of PJ, TA increased only slightly. In cold storage, TA had increased in SM and PJ (from 0.324 to 0.450 and 0.649 to 0.720, respectively) while in case of OJ and CJ, TA had reduced i.e. from 0.707 to 0.540 and from 0.414 to 0.270, respectively after 5 weeks of storage. Icier et al.\textsuperscript{32} observed an increase in the TA from 0.760 to 1.332 in fermented soymilk beverage with added apple juice after storage at 4°C for 21 days. Apple juice fermented with \textit{Lactobacillus plantarum} PCS 26 strain showed an increase in lactic acid content from 2 to 8g/L after 17 days of storage at low temperature (4–7°C)\textsuperscript{25}. Post-acidification i.e. decrease in pH and accumulation of organic acids during refrigerated storage has been identified as one of the most detrimental factors for the viability/stability of probiotics during shelf-life\textsuperscript{35}. Several factors such as pH, storage temperature, oxygen level and water activity create a great challenge for incorporating probiotics in fruit juices\textsuperscript{36}. The physicochemical properties of many probiotic products have been reported to change during their shelf life. A low but significant reduction in pH was also observed in soy yoghurt during refrigerated storage due to fermentation of carbohydrates by probiotic\textsuperscript{37}.

### Total sugars in the fermented juices and soymilk during storage at different temperatures

The ability to metabolize sugars and fermentation pattern are important for the selection of appropriate probiotic strain intended for incorporation into a particular food product\textsuperscript{37}. Therefore, the utilization of sugars by \textit{W. kimchii} R-3 in fermented soymilk and fruit juices was evaluated and results are shown in Table 4. At room temperature, all the four fermented products, SM, PJ, OJ and CJ showed reduction in total sugar content. However, in case of OJ and CJ the sugar level had considerably decreased as compared to SM and PJ. At cold storage also similar trends were recorded wherein the sugar concentration had decreased in all the beverages (SM, PJ, OJ and CJ). But unlike the room temperature storage, OJ and CJ showed a higher reduction in sugar level than SM and PJ. Moreover, there was a higher reduction in the sugar concentration of SM and PJ during 12 days of storage at 25°C compared to reduction in the sugar concentration under refrigerated temperature (4°C for 5 weeks). This can be due to better utilization of sugar by \textit{W. kimchii} R-3 at 25°C compared to the low storage temperature (4°C). Contrary to this, in OJ and CJ the sugar concentration had effectively reduced even after 5 weeks of cold storage. Thus, the biochemical properties of the probiotic fermented fruit/vegetable juices and soymilk got changed during storage.

Fermented probiotic mango juice showed a reduction in total sugar content from 397.75 mM to 221.79 mM after 30 days of storage at 4°C\textsuperscript{30}. Nagpal et al.\textsuperscript{38} found \textit{L. plantarum} and \textit{L. acidophilus} were not only able to survive but utilize fruit juices for their cell synthesis as evident by decrease in fruit sugar and pH, and increase in acidity. \textit{Lactobacillus plantarum} survived in orange, blackcurrant and pineapple juices (pH about 3.8), but failed to survive in grapefruit and pomegranate juices during storage at 4°C. Besides pH and citric acid, other factors such as proteins and dietary fiber also influence the survival of cells during storage. Lower cell survival in pomegranate juice was due to presence of antimicrobial compounds such as phenolic compounds\textsuperscript{39}.

### Conclusion

The results of current study suggest that fruit and vegetable juices and soymilk adequately support the growth and survival of probiotic bacterium \textit{W. kimchii} R-3, and may potentially be employed as proficient probiotic vehicles. The soymilk and carrot juice were found to be better media for viability of probiotic organism \textit{Weissella kimchii} R-3 during storage as compared to orange and pomegranate juice. Further work on organoleptic characteristics of probiotic fermented beverages is under way.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>Time</th>
<th>SM</th>
<th>PJ</th>
<th>OJ</th>
<th>CJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0</td>
<td>15.0±0.1</td>
<td>30.1±0.02</td>
<td>45.0±0.01</td>
<td>22.5±0.01</td>
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</tr>
<tr>
<td>D3</td>
<td>12.0±0.03</td>
<td>29.5±0.1</td>
<td>43.0±0.03</td>
<td>18.5±0.00</td>
<td></td>
</tr>
<tr>
<td>W0</td>
<td>11.0±0.03</td>
<td>21.0±0.01</td>
<td>13±0.2</td>
<td>3.0±0.1</td>
<td></td>
</tr>
<tr>
<td>W1</td>
<td>13.7±0.01</td>
<td>22.0±0.03</td>
<td>42.8±0.03</td>
<td>15.0±0.1</td>
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</tr>
<tr>
<td>W2</td>
<td>13.3±0.02</td>
<td>24.0±0.00</td>
<td>37±0.1</td>
<td>11.3±0.01</td>
<td></td>
</tr>
<tr>
<td>W3</td>
<td>12.0±0.03</td>
<td>22.0±0.1</td>
<td>29±0.03</td>
<td>11.9±0.01</td>
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</tr>
<tr>
<td>W4</td>
<td>10.0±0.01</td>
<td>20.0±0.1</td>
<td>26.0±0.1</td>
<td>7.0±0.00</td>
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</tr>
<tr>
<td>W5</td>
<td>11.0±0.03</td>
<td>21.0±0.01</td>
<td>13±0.2</td>
<td>3.0±0.1</td>
<td></td>
</tr>
</tbody>
</table>

[SM: Soymilk; PJ: Pomegranate juice; OJ: Orange juice; CJ: Carrot juice; D:days; W:weeks]
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Conflict of interest
The authors declare that they have no conflict of interest.

References
BHAT et al.: NON-DAIRY BEVERAGES AS PROBIOTIC VEHICLES


