Mini Review

Probiotic fruit and vegetable juices- recent advances and future perspective

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Abstract

The major probiotic foods available in market are milk based products. Alternatively, fruit and vegetable juices represent promising carrier for probiotic bacteria; however, probiotic bacterial stability is difficult to maintain during cold storage that could preclude their industrial production. Current review discusses the various factors affecting the survival of probiotics throughout storage period in diverse fruit and vegetable juices, the possible impact of probiotics on the sensory attributes as well as on the overall acceptance of the products, and perspective technologies to improve the viability of probiotics.

Introduction

Foods have many roles such as satisfying hunger, providing necessary nutrients, improving health, promoting a state of physical and mental well-being as well as preventing or reducing nutrition-related diseases. Moreover, consumers’ awareness towards the association between food and health has flare-up interest in “healthy foods” in recent years (Shah and Prajapati 2013). In addition to the traditional nutritional effects, “functional foods” exert beneficial health effects on body. Well-recognized examples of functional foods are those containing bioactive compounds like dietary fibers, oligosaccharides, vitamins, minerals and active “friendly” bacteria, called probiotics that promote the equilibrium of intestinal microflora (Jankovic et al., 2010; Shah and Prajapati 2013).

The functional foods market is growing globally and represents one of the most fascinating areas of investigation and innovation in the food sector, as suggested by the increasing number of scientific literatures. According to one survey probiotic market will rise up to worth $46.55 Billion by 2020, incorporating probiotics in different kind of food products (dietary supplements, functional foods, specialty nutrients, animal feed); in medicinal relevance (regular, therapeuetic, preventive health care); or by any other convenient mode of application (Anon, 2016). Certain critical factors have been identified as the key reasons for enhanced trend towards the uptake of functional foods which includes health deterioration due to busy lifestyles increased awareness of the connection between diet and health, low consumption of handiness foods and insufficient exercise, increased prevalence of self-medication, and a crowded competitive food market (Corbo et al., 2014). Further, this could be partly attributed to the growing healthcare cost, the steady increase in life expectancy, and the aspiration for an improved quality life in later years (Granato et al., 2010).

Probiotics in fruit and vegetable juices

As defined by FAO/WHO (2001), probiotics are live microorganisms (mainly bacteria and few yeast strains) that confer a beneficial heath effect on the host if administered in appropriate amounts. Fermented milk products have been conventionally considered as the most excellent carriers for probiotics; however, the use of milk-based products may be also limited by lactose-intolerance, allergies, dyslipidemia and vegetarianism. Hence, in recent time several raw materials have been extensively explored to determine if they are appropriate substrates to produce novel non-dairy functional foods (Vasudha and Mishra, 2013). Beverages based on fruits, cereals, vegetables and soybeans have been proposed as new products containing probiotic strains; essentially, fruit and vegetable juices have been reported as a novel suitable carrier medium for probiotic.
Naturally, fruits and vegetables are rich in carbohydrates, dietary fibers, vitamins, minerals, polyphenols and phytochemicals; referred as healthy foods (Sutton, 2007). Numerous researchers reported on the beneficial health effects of juices; for example, aqueous extracts of kiwifruit and avocado had very less cytotoxicity plus high anti-inflammatory activity in a Crohn’s gene-specific assay (Sutton, 2007). Similarly non-aqueous extracts of kiwifruit, avocado and blueberry had high anti-inflammatory activity, with slightly higher cytotoxicity than the aqueous extracts. Fenech and co-workers (2005) demonstrated the positive effect of the intake of nine micronutrients that can be easily found in fruits viz. calcium, retinol, vitamin E, folate, nicotin acid, riboflavin, pantethenic acid, β-carotene and biotin on genome damage and repair. Therefore, juice fortification with probiotics and/or prebiotics is a challenge and a frontier goal, as juices could combine nutritional effects in addition provides specific health benefit through added probiotic strain. Furthermore, fruit juices have shown negative effects on some pathogenic microorganisms, conversely improves the growth of beneficial bacteria. The berries, such as blueberry, blackberry and raspberry, possess antimicrobial effects towards many foodborne pathogens (Ranadheera et al., 2014).

While looking for different food matrices, many researchers have been investigated the suitability of various fruit and vegetable juices, such as tomato, mango, orange, apple, grape, peach, pomegranate, Watermelon, carrot, beet root and cabbage juices as raw material for the production of probiotic juices or related beverages. The most commonly employed probiotics includes different strains from Lactobacillus acidophilus, Lb. helveticus, Lb. casei, Lb. paracasei, Lb. johnsonii, Lb. plantarum, Lb. gasseri, Lb. reuteri, Lb. delbrueckii subsp. bulgaricus, Lb. crispatus, Lb. fermentum, Lb. rhamnosus, B. bifidum, B. longum, B. adolescentis, B. infantis, B. breve, B. lactis, B. laterosporus, and other species like Escherichia coli, Nissle, Streptococcus thermophilus, Weissella spp., Propionibacterium spp., Pediococcus spp., Enterococcus faecium, Leuconostoc spp. and Saccharomyces cerevisiae var. boulardii (Nagpal et al., 2012; Patel et al., 2013). Some probiotics juices and related beverages available in market are compiled in Table 1.

### Major factors affecting probiotic survival in juices

The health benefit of probiotics mainly relies upon their concentration in foods plus on their ability to endure the unfavorable conditions of the gastrointestinal tract. Maintaining the viability (at least 10^6-10^7 cells/ml) and activity of probiotics in food products at the end of shelf-life are two important criteria to be fulfilled in fruit juices, too. The low pH of fruit juices is a shortcoming in favoring the total viable counts and activities of probiotics (Vasudha and Mishra, 2013). However, probiotic viability is strain-dependent, i.e. some strains of Lb. plantarum, Lb. acidophilus and Lb. casei can grow in fruit matrices due to their tolerance to acidic environments (Peres et al., 2012). The brief summary of experiments conducted so far by various researchers is comprised in Table 2, which also suggests suitability of some probiotic strains in diverse kinds of vegetable and fruit juices.

Several factors could limit probiotic viability and survival in juices. As suggested by Tripathi and Giri (2014), the major influencing parameters can be categorized as, (1) intrinsic food parameters, such as titratable acidity, pH, molecular oxygen, water activity, presence of salt, sugar, artificial flavoring and coloring agents, and chemical or microbial preservatives like hydrogen peroxide and bacteriocins; (2) processing parameters- extent of heat treatment, incubation temperature, cooling rate, volume, packaging materials and storage techniques; (3) microbiological factors which mainly includes kind of probiotic strains, compatibility of different strains, inoculums proportion and rate.

Among all these, pH is one of the chief significant factors affecting the probiotic viability. Fruit juices naturally have a low pH and high level of organic acids, which increases the concentration of undissociated form. It is presume that combined action of acidic environment and the intrinsic antimicrobial activity of accumulated organic acids affect probiotic bacteria. Among various probiotics, lactobacilli generally found to resist and survive in fruit juices with pH ranging from 4.3 to 3.7, while bifidobacteria are less acid tolerant; even about pH 4.6 is unfavorable for their survival (Tripathi and Giri, 2014). On the other hand, this trend differs with kind of probiotic strain. For instance, strains of lactobacillus and bifidobacteria revealed wide differences regarding acid resistance into orange, pineapple and cranberry juice, the strains screened survived for longer in orange and pineapple juice than cranberry (Sheehan et al., 2007).

Lactobacillus casei, Lb. rhamnosus, Lb. paracasei display a great robustness surviving at levels above 7.0 log cfu/ml and 6.0 log cfu/ml in orange and pineapple juice, respectively for at least 3 months. However, after thermal pasteurization at 76°C for 30 s and 90°C for 1 min, an additional 5 min high-pressure treatment (400 MPa) observed that these strains were not able to withstand the
treatments required to achieve >6.0 log cfu/ml in juice (Sheehan et al., 2007).

Nualkaekul and Charalampopoulos (2011) investigated the factors that affected B. longum survival in model solutions and in fruit juices (orange, pineapple, grapefruit, blackcurrant, strawberry and pomegranate). The orange, pineapple, grapefruit and blackcurrant juices reduced (less than 0.8 log CFU/mL) viability of bifidobacteria, with the highest cell count found in orange and pineapple juice while storage at 4°C after six week. Further, the decrease in grapefruit was only 0.5 log CFU/mL, despite of the low pH (3.21) and the high concentration of citric acid (15.3 g/L) suggesting some controversial effects of pH. The probiotic was below the detection limit after one week in pomegranate and four weeks in strawberry juice. These results are suggestive of the synergistic as well as antagonistic action of some parameters on the survival of bacteria. Fruits are naturally rich in phenolic compounds, which strongly found to affect the viability of probiotic bacteria. Some food components like proteins and dietary fiber could protect cells from acidic stress at low pH. According to several researchers the incorporation of LAB into fruit juices with low pH may boost the resistance of bacteria to subsequent stressful acidic conditions, such as those observed in gastrointestinal tract (Ranadheera et al., 2014).

A major challenge during fortification of probiotics in fruit juices or beverages is the product acceptance by consumers (Ellendersen et al., 2012). The kind of microorganism and juices type, storage conditions, and addition of other compounds may influence on the sensory traits of finished product. The addition of pleasant aroma and volatile ingredients may able to “mask” the presence of probiotics. Fermented juices with sugar had more acceptable taste and flavor than the sugar free juice; further, when sucrose was added at the beginning of fermentation, flavors seemed to be reduced and the taste was more acceptable (Sivudu et al., 2014). Luckow et al. (2006) mentioned that the addition of tropical fruit juices, mainly pineapple, but also mango or passion fruit (10% v/v), might optimistically contribute to the aroma and flavor of the final product and might avoid the identification of probiotic off-flavors by consumers. According to

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<td>Skane Dairy, Sweden</td>
<td>Orange, strawberry or blackcurrant juice contains Lb. plantarum 299v (50 million cells/portion) and fortified with 5% oat flour and a fruit juice with Lb. reuteri MMS3</td>
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Table 2. Experiments conducted by various researchers to study the suitability of various probiotics in different kinds of vegetable and fruit juices

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<tr>
<th>Fruit base</th>
<th>Probiotic strain(s)</th>
<th>Outcome of the experiments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amla (Emblica officinalis)</td>
<td>Lb. reuteri, Lb.</td>
<td>Fermented juices was healthy enough to stabilize the oxidized form of the metal ion; results were suggestive of development of fermented probiotic juice enriched with bioactive compounds</td>
<td>Peerapan et al., 2016</td>
</tr>
<tr>
<td>Sweet Lime and Lb. acidophilus</td>
<td>Lb. debrueckii, Lb.</td>
<td>The viable cell counts in the fermented control of Sweet Lime and Lb. acidophilus juice with whey (50% <em>Lb. acidophilus</em> grown at 4°C and 7°C)</td>
<td>Katoono and Katoono, 2015</td>
</tr>
<tr>
<td>Peach juice</td>
<td>Lb. debrueckii, Lb.</td>
<td>After 4 weeks, Lb. debrueckii were 1.72 × 10^9 CFU/mL, with Lb. acidophilus juice at 3°C</td>
<td>Pekkin et al., 2014</td>
</tr>
<tr>
<td>Tomato and Lb. casei</td>
<td></td>
<td>After four weeks of storage at 4°C, L. fermentum grown at lower temperature (30°C) and L. casei grown at higher temperature (37°C) survived better</td>
<td>Srudu et al., 2014</td>
</tr>
<tr>
<td>Mango, sapota, Lb. casei, grape</td>
<td>Probiotic strain could survive and capable of rapidly utilizing the nutrients of different fruit juices without adding additional nutrients</td>
<td>Kumar et al., 2013</td>
<td></td>
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<tr>
<td>and cantaloupe</td>
<td>Lb. plantarum and Lb.</td>
<td>Both cultures were found to be able to survive in fermented juices with high acidity and low pH</td>
<td>Nagpal et al., 2012</td>
</tr>
<tr>
<td>Pomegranate juice</td>
<td>Lb. plantarum, Lb.</td>
<td>Lb. plantarum and Lb. debrueckii showed higher viability during the storage; viable cells remained at their maximum level within 2 weeks, but decreased dramatically after 4 weeks</td>
<td>Mousavi et al., 2011</td>
</tr>
<tr>
<td>Carrots, celery, and apples Lb.</td>
<td>Showed to be a good matrix for the growth of L. acidophilus</td>
<td>Nicolacu and Buneleusa, 2010</td>
<td></td>
</tr>
<tr>
<td>Noni juice</td>
<td>Lb. casei, Lb.</td>
<td>All tested strains grew well on noni juice (about 107 cfu/mL), noni juice fermented with <em>B. longum</em> had a high antioxidant capacity</td>
<td>Wang et al., 2009</td>
</tr>
<tr>
<td>Carrot juice</td>
<td>Lb. acidophilus</td>
<td>Showed excellent production of lactic acid (15–17 mg/mL) in juice and during the fermentation, 15–45% of carotenoids (α-carotene and β-carotene) were degraded depending on the strain used</td>
<td>Kun et al., 2008</td>
</tr>
<tr>
<td>Carrot juice</td>
<td>Lb. rhamnosus and Lb.</td>
<td>The viable cell counts of the two lactobacilli in the fermented juice after 4 weeks of storage at 4°C, demonstrated good survival of the two strains at low pH</td>
<td>Nazzaro et al., 2008</td>
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Ranadheera et al. (2014), some fruit juices could naturally mask the “medicinal” taste of probiotics. On the other hand, several researchers confirmed that probiotics did not affect the overall acceptance of juices (Rodrigues et al., 2009; Ellendersen et al., 2012; Perricone et al., 2014).

**Strategies to improve probiotic survival in juices**

Different researchers proposed various successful strategies to improve the survival of probiotics in juices; some case-studies dealing with interesting solutions discussed in this section.

**Fortification with prebiotics**

The most attractive and straightforward way to improve probiotic stability in fruit juice could be the fortification with some prebiotics such as dietary fiber, cellulose or with some ingredients able to exert a protective effect within the fruit juice. In connection to this, Rakin and co-workers (2007) enriched beetroot juice and carrot juice with brewer’s yeast autolysate before fermentation with Lb. acidophilus. It was noticed that autolysate enhanced the growth of Lb. acidophilus during the fermentation, decreased fermentation time, enriched the juices with minerals, vitamins, amino-acids, and antioxidants as well as positively influenced probiotics survival. Another group of researchers fortified juices with glucans and demonstrated that in apple juice, oat flour with 20% of β-glucan could protect Lb. rhamnosus during refrigerated storage (Saarela et al., 2006).

**Storage under refrigeration, use of antioxidants and microencapsulation**

The level of oxygen within the package foods during storage should be as low as possible in order to avoid oxidative damage to the probiotics, however the extent of sensitivity is strongly strain variable. Oxygen induces an oxidative damage by the creation of reactive oxygen species (ROS) like H$_2$O$_2$ or superoxide ion. Commonly, it is noticed that bifidobacteria are more sensitive than LAB (Nag and Das, 2013; Tamminen et al., 2013).

Several authors suggested the modification of product atmosphere by raising the content of CO$_2$ in the headspace (Corbo et al., 2014). Additionally, antioxidant compounds could help to limit the harmful effects of oxygen. In this connection, a group of researchers evaluated the effects of different amounts of (+)-catechin, green tea epigallocatechin gallate, and green tea extracts on the growth and survival of B. longum ATCC 15708, B. longum subsp. infantis ATCC 15697 and Lb. helveticus R0052, having different oxygen sensitivities (Gaudreau et al., 2013). They found that the growth of Lb. helveticus was strongly enhanced. Moreover, fortification of vitamin-E improved the stability of Lb. casei CRL 431 in the food matrix during 20 week storage period at 25°C.

LAB are highly sensitive to fluctuation in storage temperature. The viability of probiotic strains in fruit juices is also found to influenced, as refrigeration could promise a longer survival, whereas a thermal abuse could demonstrate a harmful effect. Different authors proposed numerous strategies to resolve such issues. Microencapsulation technologies have also been successfully applied using various matrices to protect the probiotic bacterial cells from the damage caused by the external environmental factors. For instance, a novel microencapsulation method reduced the acidification and improved the viability of probiotic strains Lb. rhamnosus and Lb. acidophilus at 25°C for at least 9 days in orange juice (Sohail et al., 2012). In a recent investigation, Lb. acidophilus immobilized in Ca-alginate carried out normal banana puree fermentation and resulted in a novel probiotic
fruit product (Tsen et al., 2004). In tomato juice, Lb. acidophilus immobilized in Ca-alginate showed a higher survival rate than free cells during cold storage at 4°C. Further, the overall acceptance of immobilized cell fermentation was higher than free cells as noticed by the sensory evaluation during storage (King et al., 2007). Recently, Chaikham (2015) investigated the effect of alginate encapsulation with Thai herbal extracts including cashew flower, pennywort and ylang ylang on the viability of probiotic L. casei 01b, Lb. acidophilus LA5 and B. lactis Bb-12 bacteria suspended in mulberry, maoberry, longan and melon juices. It was noticed that the survival rate of L. casei 01 cells entrapped with 0.05% (w/v) cashew flower extract were notably higher than those encapsulated with pennywort and ylang ylang extracts, after 30 days storage.

On the other hand, Gaanappriya et al. (2013) evaluated the viability of encapsulated Lb. plantarum in sapodilla, grapes, orange and watermelon juices which successfully maintained the viable probiotic count at 7 log CFU/mL or more for one month. Ding and Shah (2008) emphasized that microencapsulated probiotic bacteria were more stable in compare to free probiotic cells in fruit juices. In principle, the encapsulated probiotics (Lb. rhamnosus, Lb. acidophilus, Lb. paracasei, Lb. plantarum, Lb. salivarius, B. longum, B. lactis type Bi-04 and Bi-07) were protected from the acidic environment of the orange juice, did not allowed a strong viability loss and showed a residual cell count of 5 log CFU/mL even after 6 weeks. Some studies reported that microencapsulation might provide a more favorable anaerobic environment for susceptible probiotic strains, as well as a physical barrier from the harsh acidic conditions of the fruit juice (Ding and Shah, 2008).

**Adaptation and induction of resistance**

According to several authors, the exposure of probiotic strains to a sub-lethal stress could induce a sort of resistance and an adaptive stress response (Gobetti et al., 2010; Perricone et al., 2014). In context to that, Perricone et al. (2014) successfully evaluated the viability of Lb. reuteri DSM 20016 in orange, pineapple, green apple, and red fruit juices and observed strong loss of probiotics viability in red-fruit juice, perhaps due to a combined effect of low pH and phenols. Consequently, authors used two different strategies: growth of strain in a lab medium containing different amounts of red fruit juices (up to 50%) or else added with vanillic acid (phenol stress) or acidified to pH 5.0 (acid stress). These approaches resulted in a prolongation of the Lb. reuteri viability by 5 (phenol stress) or 11 days (pH stress).

Alternatively, authors reported an improvement in the survival of B. breve in a blended juice (orange-grape and passion fruit) generating an acid tolerance variant of the bacterium by UV mutagenesis, combined with cultivation at sub-lethal pH values (Saarela et al., 2011).

**Conclusion**

Fruit juices and related beverages represent a suitable carrier for the delivery of probiotics. Since, fruits are naturally rich in essential macro- and micro-elements; incorporation of probiotics into fruit juices makes them healthier. There are several challenges to overcome, such as the survival of probiotics and their effects on the sensory attributes. Preliminary outcomes of the various strategies (encapsulation, fortification with prebiotics, etc.) used to overcome the issues are very promising and fascinating.

**References**


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