
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.

Keywords

Functional foods
Juices
Antioxidants
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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, therapeutic, 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 health 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.

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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, pantothenic 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 bifidobacterium 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

Table 1. Commercially available probiotic juices

Product Label	Manufacturer	Major Attributes
Proviva	Skane Dairy, Sweden	Orange, strawberry or blackcurrant juice contains <i>Lb. plantarum</i> 299v (50 million cells/ portion) and fortified with 5% oat flour and
Rela	Biogaia, Sweden	a fruit juice with <i>Lb. reuteri</i> MM53
Bravo Friscus	Skane Dairy, Sweden	Orange and apple juice with billions of <i>Lb. plantarum</i> HEAL9 and <i>Lb. paracasei</i> 8700:2
Biola	Tine BA, Norway	orange–mango and apple–pear flavours containing <i>Lb. rhamnosus</i> GG
Biola	Valio Gefilus Ltd., Finland	Seven varieties of juices, fortified with vitamins C and D and contains <i>Lb. rhamnosus</i> GG and <i>Propionibacterium freudenreichii</i> ssp. <i>shermanii</i> JS
Probiotic Naked Juices	Naked® Juice, USA	Apple juice, mango puree, orange juice, pineapple juice, banana puree fortified with fructooligosaccharides and <i>bifidobacterium</i> sp.
GoodBelly	Next Foods, USA	Mango, pomegranate, blueberry, blackberry, tropical orange, tropical green, cranberry, watermelon, and coconut water juices contains <i>Lb. plantarum</i> 299v (50 billion cells/portion)
KeVita	H-E-B, USA	Sparkling lemon ginger probiotic drink containing <i>Bacillus coagulans</i> GBI-306086, <i>Lb. paracasei</i>, <i>Lb. plantarum</i>, <i>Lb. rhamnosus</i>
Nomva	Phenomenal Foods, California, USA	Banana and strawberry juice with probiotics
Healthy life Probiotic	Golden Circle Healthy Life™, Australia	Apple and mango juice containing 1 billion of <i>Lb. paracasei</i> 8700:2 and <i>Lb. plantarum</i> HEAL 9
Malee Probiotic Juices	Malee Probiotics, Thailand	White grape and orange juice containing <i>Lb. paracasei</i>

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

Table 2. Experiments conducted by various researchers to study the suitability of various probiotics in different kinds of vegetable and fruit juices

Fruit base	Probiotic strain(s)	Outcome of the experiments	Reference
Amla (<i>Phyllanthus emblica</i> fruit)	<i>Lb. paracasei</i> H1101	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	Peerajan <i>et al.</i> , 2016
Sugarcane and Sweet Lime juices	<i>Lb. acidophilus</i>	the viable cell counts in the juices of Sugarcane control, Sugarcane juice with Wheat grass juice and <i>Withnia somnifera</i> (SOF1) and Sugarcane juice with green tea and whey (SOF2) were 4.0×10^8 , 2.0×10^8 and 5.5×10^8 CFU/ ml, respectively after 3 weeks at 4°C storage; culture viability in Sweet Lime juices was lost after 2 nd week of storage	Khatoon and Gupta, 2015
Peach juice	<i>Lb. delbrueckii</i> , <i>Lb. casei</i>	the viable cell counts of <i>Lb. delbrueckii</i> were 1.72×10^7 CFU/mL during storage at 4°C after 4weeks, in fermented peach juice, <i>Lb. casei</i> could not survive in fermented fruit juice after the cold storage	Pakbin <i>et al.</i> , 2014
Watermelon and tomato juice	<i>Lb. fermentum</i> and <i>Lb. casei</i>	After four weeks of storage at 4°C, <i>L. fermentum</i> grown at lower temperature (30°C) and <i>L. casei</i> grown at higher temperature (37°C) survived better	Sivudu <i>et al.</i> , 2014
Mango, sapota, grape and cantaloupe	<i>Lb. casei</i>	Probiotic strain could survive and capable of rapidly utilizing the nutrients of different fruit juices without adding additional nutrients	Kumar <i>et al.</i> , 2013
Tomato, orange and grape juices	<i>Lb. plantarum</i> and <i>Lb. acidophilus</i>	both cultures were found to be able to survive in fermented juices with high acidity and low pH	Nagpal <i>et al.</i> , 2012
Pomegranate juice	<i>Lb. plantarum</i> , <i>Lb. delbrueckii</i> , <i>Lb. paracasei</i> , <i>Lb. acidophilus</i>	<i>Lb. plantarum</i> and <i>Lb. delbrueckii</i> showed higher viability during the storage; viable cells remained at their maximum level within 2 weeks, but decreased dramatically after 4 weeks	Mousavi <i>et al.</i> , 2011
Carrots, celery and apples	<i>Lb. acidophilus</i>	showed to be a good matrix for the growth of <i>L. acidophilus</i>	Nicolescu and Buruleanu, 2010
Noni juice	<i>Lb. casei</i> , <i>Lb. plantarum</i> and <i>B. longum</i>	All tested strains grew well on noni juice (about 10^7 cfu/ml), noni juice fermented with <i>B. longum</i> had a high antioxidant capacity	Wang <i>et al.</i> , 2009
Carrot juice	<i>B. lactis</i> Bb-12, <i>B. bifidum</i> B7.1 and B3.2)	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	Kun <i>et al.</i> , 2008
Carrot juice	<i>Lb. rhamnosus</i> and <i>Lb. bulgaricus</i> with inulin or fructooligosaccharides	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	Nazzaro <i>et al.</i> , 2008

Cabbage juice	<i>Lb. plantarum</i> C3, <i>Lb. casei</i> A4, and <i>Lb. delbrueckii</i> D7	<i>Lb. delbrueckii</i> , and <i>Lb. plantarum</i> grew well on cabbage juice, had cell count of 4.1×10^7 and 4.5×10^5 per ml after storage at 4 °C/4 weeks, but <i>Lb. casei</i> could not survive low pH and lost complete cell viability after 2 weeks	Yoon <i>et al.</i> , 2006
Beet root juice	<i>Lb. acidophilus</i> LA39, <i>Lb. plantarum</i> C3, <i>Lb. casei</i> A4, and <i>Lb. delbrueckii</i> D7	viable cell counts of all <i>Lactobacillus</i> spp. except for <i>Lb. acidophilus</i> in the fermented beet juice remained at 10^6 – 10^8 CFU/ml after 4 weeks of storage at 4°C	Yoon <i>et al.</i> , 2005
Tomato juice	<i>Lb. acidophilus</i> LA39, <i>Lb. plantarum</i> C3, <i>Lb. casei</i> A4, and <i>Lb. delbrueckii</i> D7	viable cell counts of the four <i>Lactobacillus</i> spp. in the fermented tomato juice ranged from 10^6 – 10^8 CFU/ml after 4 weeks of storage at 4°C	Yoon <i>et al.</i> , 2004

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_2O_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 yanang 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 yanang 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.

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