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**Shaikh Uzma A**  
 PG Student, Department of  
 Food Microbiology and Safety,  
 College of Food Technology,  
 Vasantnaik Marathwada  
 Krishi Vidyapeeth, Parbhani,  
 Maharashtra, India

**Deshpande HW**  
 HEAD, Department of Food  
 Microbiology and Safety, College  
 of Food Technology, Vasantnaik  
 Marathwada Krishi  
 Vidyapeeth, Parbhani,  
 Maharashtra, India

**Kulkarni DB**  
 Research associate, Dr.  
 Babasaheb Ambedkar  
 Marathwada University,  
 Aurangabad, Maharashtra,  
 India

**Correspondence**  
**Shaikh Uzma A**  
 PG Student, Department of  
 Food Microbiology and Safety,  
 College of Food Technology,  
 Vasantnaik Marathwada  
 Krishi Vidyapeeth, Parbhani,  
 Maharashtra, India

## A review on probiotic beverages prepared using vegetables

**Shaikh Uzma A, Deshpande HW and Kulkarni DB**

### Abstract

Non-dairy probiotic products have a big worldwide importance due to the ongoing trend of vegetarianism and to a high prevalence of lactose intolerance in many populations around the world. The major probiotic foods available in market are milk based products. Alternatively, 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 vegetable juices and perspective technologies to improve the viability of probiotics and health benefits of probiotic beverages.

**Keywords:** probiotics, lactobacillus, vegetable juice, benefits

### Introduction

Vegetables and fruits have been showed as appropriate for probiotic products as they do not contain any dairy allergens that might prevent usage by part of the population. Also they have several functional food components such as minerals, vitamins, dietary fibers, and antioxidants. In recent years, studies on non-dairy probiotic beverages such as tomato, cabbage, blackcurrant, orange, beet root and carrot juices have been performed in conjunction with different probiotic strains and obtained appealing results (Naga *et al.*, 2014) [15].

The promotion of healthy vegetable products has coincided with a surging consumer interested in the healthy functionality of food. There is an increasing awareness among the general public of the advantages of diets rich in vegetables to ensure an adequate intake of most vitamins and micronutrients, dietary fibers, and phytochemicals that promote health. Consumer's interest in whole foods with enhanced nutritional qualities is at an all-time high, and more consumers are choosing foods on the basis of their healthy benefits (Joao *et al.*, 2012) [7].

Fermented foods are food substrates that are invaded or overgrown by edible microorganisms whose enzymes, particularly amylases, proteases and lipases, hydrolase polysaccharides, proteins and lipids to non-toxic products with flavor's, aromas and textures pleasant and attractive to the human consumer (Steinkraus, 1997) [26].

The lactic acid fermentation of vegetable products, applied as a preservation method for the production of finished and half-finished products, is considered as an important technology and it is further investigated because of the growing amount of raw materials processed in this way in the food industry. The main reasons for this interest are the nutritional, physiological and hygienic aspects of the process and their corresponding implementation and production costs (Karovicova *et al.*, 1999) [8]. While there are 21 different commercial vegetable fermentations in Europe along with a large number of fermented vegetable juices and blends, the most economically relevant of them are the fermentations of olives, cucumbers (pickles) and cabbage (sauerkraut, Korean kimchi) (Caplice and Fitzgerald, 1999) [2].

Fermented foods and beverages have heterogeneity of traditions and cultural preferences found in the different geographical areas, where they are produced. Fermentation has enable dourance stors in temperate and cooler regions to survive during the winter season and those in the tropics to survive drought periods. Fermentation is a slow decomposition process of organic substances induced by microorganisms or enzymes that essentially convert carbohydrates to alcohols or organic acids (FAO, 1998) [4].

In many instances, production methods of different traditional fermented foods were unknown and passed down to subsequent generations as family traditions. Drying and salting are common fermentation practices in the oldest methods of food preservation.

Fermentation processes are believed to have been developed in order to preserve fruits and vegetables for times of scarcity by preserving the food by organic acid and alcohols, impart desirable flavour, texture to foods, reduce toxicity, and decrease cooking time (Rolle, 2002) [22]. 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) [24]. 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 (Shah and Prajapati, 2013) [24].

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) [1]. 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) [3]. 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) [6].

### Health benefits of probiotic beverage

*Lactobacillus plantarum* reduced incidence of diarrhoea in daycare centers when administered to only half of the children Especially effective in reducing inflammation in inflammatory bowel; e.g., *enterocolitis* in rats, small bowel bacterial overgrowth in children, pouchitis reduced pain and constipation of irritable bowel syndrome reduced bloating, flatulence, and pain in irritable bowel syndrome in controlled trial. Positive effect on immunity in HIV+ children (Vanderhoof J. 2000) [28].

In humans, *L. plantarum* 299v can increase the concentration of carboxylic acids in feces and decrease abdominal bloating in patients with irritable bowel disease. It can also decrease fibrinogen concentrations in blood. Should probiotics be administrated through foods, the probiotic organism must remain vigorous in the food until consumption and the food must remain palatable, i.e, the food carrier and the organism must suit each other. *L. plantarum* 299v not only affects the bacterial flora of the intestinal mucosa but may also regulate the host's immunologic defense (Molin G. 2001) [12].

Strain of *Lactobacillus acidophilus* significant decrease of diarrhoea in patients receiving pelvic irradiation decreased polyps, adenomas and colon cancer in experimental animals Prevented urogenital infection with subsequent exposure to three ropathogens *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, Lowered serum cholesterol levels. Beneficial effect of lactic acid bacteria consumption include: improving intestinal tract health; enhancing the immune system, synthesizing and enhancing the bioavailability of nutrients; reducing symptoms of lactose intolerance, decreasing the prevalence of allergy in susceptible individuals; and reducing risk of certain cancers (Parvez *et al.*, 2005) [19].

High intakes of carotenoid- rich fruits and vegetables are associated with a reduced risk of various cancers including colon cancer. A human intervention study with carrot and tomato juice should show whether a diet rich in carotenoids, especially high in b-carotene and lycopene, can modify luminal processes relevant to colon carcinogenesis. In a randomised cross-over trial, twenty-two healthy young men on a low-carotenoid diet consumed 330 ml tomato or carrot juice per day for 2 weeks. 2-week interventions with carotenoid- rich juices led only to minor changes in investigated luminal biomarkers relevant to colon carcinogenesis (Kerstin *et al.*, 2008) [9].

### 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) [29]. 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 (Patel A.R. 2017) [20].

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) [16].

**Table 1:** Some probiotics juices and related beverages are compiled in

juice Base	Probiotic Strains	Outcome Of Experiments	Reference
Carrot juice	B. lactis, Bb-12, B. bifidum B7.1 and B3.2	Show excellent production of lactic acid (15-17 mg/ml) in juice and during the fermentation, 15-45% of carotenoids (a- carotene and B-carotene) were degraded depending on strain used.	Kun <i>et al.</i> , 2008
Carrot juice	<i>Lb. rhamnosus</i> and <i>Lb. bulgaricus</i> with inulin or fructooligosachharides	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. delbrukii</i> D7	<i>Lb. delbrueckii</i> , and <i>Lb. plantarum</i> grew well on cabbage juice, had cell count of 4.1x10 <sup>7</sup> and 4.5x10 <sup>5</sup> per ml after storage at 4°C /4weeks, 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 Lactobacillus spp. except for <i>Lb. acidophilus</i> in the fermented beet juice remained at 10 <sup>6</sup> -10 <sup>8</sup> 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 Lactobacillus spp. in the fermented tomato juice ranged from 10 <sup>6</sup> -10 <sup>8</sup> CFU/ml after 4 weeks at 4°C of storage.	Yoon <i>et al.</i> , 2004
Tomato, orange and grape juice	<i>Lb. plantarum</i> and <i>Lb. acidophilus</i>	Both culture were found to be survive in juice at high acidity and low pH.	Nagpal <i>et al.</i> , 2012
Carrot, celery and apple juice	<i>Lb. Acidophilus</i>	Showed as to be good matrix for growth of <i>Lb. acidophilus</i>	Nicolescu and Buruleanu, 2010

### 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<sup>6</sup>-10<sup>7</sup> 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) [29]. 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) [21].

Several factors could limit probiotic viability and survival in juices. As suggested by Tripathi and Giri (2014) [27], 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) [27].

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) [25].

Different researchers proposed various successful strategies to improve the survival of probiotics in juices; some interesting solutions discussed below:

### 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*.

### 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<sub>2</sub>O<sub>2</sub> or superoxide ion. Commonly, it is noticed that bifidobacteria are more sensitive than LAB (Nag and Das, 2013) [13].

Several authors suggested the modification of product atmosphere by raising the content of CO<sub>2</sub> in the headspace (Corbo *et al.*, 2014) [3]. 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. 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 (Gaudreau *et al.*, 2013) [12].

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) [10].

In standardization of tomato and carrot juices using *Lactobacillus plantarum*, *Lb. fermentum*, *Lb. casei*, *Lysinibacillus sphaericus* and *Saccharomyces boulardii* to enhance the stability, the probiotic cultures were micro-encapsulated using alginate coated chitosan beads. Tomato and carrot juice samples were pasteurized for 20 min at 63 °C. *Lb. fermentum*, *Lb. plantarum*, *Lb. casei* and *Lysinibacillus sphaericus*, *Saccharomyces boulardii* were inoculated and incubated at 37 °C for a period of 72 h. probiotic strains the viable cell count were increased from 6.5 to 8.9 log CFU/mL in *Lysinibacillus sphaericus* and 5.2 to 7.6 log CFU/mL in *Saccharomyces boulardii*, during 24 to 42 hr and later it decreased slowly (Naga *et al.*, 2016) [14].

### Conclusion

Vegetable juices and related beverages represent a suitable carrier for the delivery of probiotics. Since, vegetables are naturally rich in essential macro- and micro-elements; incorporation of probiotics into vegetable 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. So, the probiotic vegetable juices are very beneficial for health for maintaining good health it play an important role in immune system improvement.

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