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CHALLENGES AND STRATEGIES IN FRUIT JUICE PROBIOTICATION: A REVIEW

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Abstract

Probiotic cultures are successfully applied in various types of food matrices. Numerous food products including dairy, meats, beverages, cereals, vegetables and fruit are utilized as delivery vehicles for probiotics. Fruits are exhibited as appropriate for probiotic products as they don't have any dairy allergens that may prevent usage by part of the population. Also, they contain numerous functional food components like as minerals, vitamins, dietary fibers, and antioxidants. Even though the fruit juices contain various vital nutrients (minerals, vitamins, dietary fibers, antioxidants), there are some strong factors that could limit probiotic survival in juices like food parameters (titratable acidity, molecular oxygen, water activity, presence of salt, sugar, pH and chemicals, like hydrogen peroxide, bacteriocins, artificial flavoring and coloring agents), processing parameters (heat treatment, incubation temperature, cooling rate, packaging materials and storage methods, oxygen levels, volume) and microbiological parameters (strains of probiotics, rate and proportion of inoculation). Different authors proposed successful strategies to improve the survival of probiotics in juices. An easy way to improve probiotic stability in fruit juice could be the fortification of juice with some prebiotics.Induction of Resistance could be achieved through two different strategies: strain cultivation in a lab medium containing different amounts of fruit juices (up to 50%) or added with vanillic acid (phenol stress) or acidified to pH 5.0 (acid stress). These approaches resulted in a prolongation of the viability of lactic cultures by 5 (phenol stress) or 11 days (pH stress).

Introduction

Foods are not only designed to fulfil hunger and to supply necessary nutrients for humans butalso, to prevent or reduce the advancement of nutrition-related diseases and to boost physical and mental well-being (Shori, 2014). With respect to this, functional foods play animportant role. The term functionalfood was first utilised in Japan, where the concept of food designed to be medically beneficial to thecustomer evolved during the 1980s (Siro *et al*., 2008). Functional food can be defined as food or dietary componentsthat may provide a health benefit beyond the basic function of providing nutrients (Cencic and Chingwaru, 2010). Probiotic foods are one amongest the fastest growing field of functional food production. Probiotics are live microorganisms that when consumed in sufficient amounts exert their health benefits. Probiotic cultures are successfullyapplied in various types of food matrices. Numerous food products including dairy, meats, beverages, cereals, vegetables and fruit are utilized as delivery vehicles for probiotics. Fruits are exhibited as appropriate for probiotic products as they don't have any dairy allergens that may prevent usage by part of the population. Also, they contain numerous functional food components like as minerals, vitamins, dietary fibers and antioxidants. Juice is defined as “the extractable fluid contents of tissues or cells.” Each juice has particular, chemical, nutritional and sensorial characteristics, depending upon the kind or nature of fruit or vegetable used. In recent years, studies which were carried out on non-dairy probiotic beverages such as tomato, cabbage, blackcurrant, orange, beet root and carrot juices are performed in combination with different probiotic strains showed the appealing results (Naga *et al.,* 2014). Consumer's interest in whole foods with enhanced nutritional qualities is at an all-time high, and more consumers are opting foods on based on their health benefits (Joao *et al*., 2012) Fermented foods are food substrates that are invaded or overgrown by edible microorganisms whose enzymes, mainly amylases, proteases and lipases, hydrolase polysaccharides, proteins and lipids to non-toxic products with flavor's, aromas and textures pleasant and attractive to the consumers (Steinkraus, 1997). Number of health benefits is linked to the consumption of probiotic products like as treatment of diarrhea, alleviation of symptoms of lactose intolerance, reduction of blood cholesterol, treatment of irritable bowel syndrome, and inflammatory bowel disease, anti-carcinogenic properties, synthesis of vitamins, and enhancing immunity (Kerry *et al.,* 2018). The market of functional foods is identified by an

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increasing trend; some researchers reported that probiotic foods represent ca. 60-70% of functional foods (Kidist *et al.,* 2019). Generally, the concentrations of 10^6 and 10^7-10^8cfu mL^{-1} (cfu g^{-1}), respectively, are accepted because of the minimum and satisfactory levels (Karimi *et al.,* 2011).

Challenges for Probiotic Survival in Fruit Juice

Even though the fruit juices contain various vital nutrients (minerals, vitamins, dietary fibers, antioxidants), there are some strong factors that could limit probiotic survival in juices like food parameters (titratable acidity, molecular oxygen, water activity, presence of salt, sugar, pH and chemicals, like hydrogen peroxide, bacteriocins, artificial flavoring and coloring agents), processing parameters (heat treatment, incubation temperature, cooling rate, packaging materials and storage methods, oxygen levels, volume) and microbiological parameters (strains of probiotics, rate and proportion of inoculation) (Perricone *et al.,* 2015).

Mostly, pH exerts a negative effect, but protein and dietary fiber could protect cells from acidic stress; the role of citric and malic acids is divisive, as they appeared to protect probiotics, whereas phenols could cause a much viability loss. Even if the pH is a problem for probiotic survival in juices, Ranadheera *et al.* (2014) assumed that the addition of lactic acid bacteria into fruit juices with low pH may improve the resistance of bacteria to consequent stressful acidic conditions, as those found in GIT.

Strategies for Improving Survival of Probiotic Strains

Different authors proposed successful strategies to improve the survival of probiotics in juices; in this section there is a focus on some case-studies dealing with interesting solutions. An easy way to improve probiotic stability in fruit juice could be the fortification of juice with some prebiotics (dietary fiber, cellulose) or with some ingredients able to exert a protective effect. Adaptation and Induction of Resistance could be achieved through two different strategies: strain cultivation in a lab medium containing different amounts of fruit juices (up to 50%) or added with vanillic acid (phenol stress) or acidified to pH 5.0 (acid stress). These approaches resulted in a prolongation of the viability of lactic cultures by 5 (phenol stress) or 11 days (pH stress). The viability of probiotic bacteria in juices is negatively related to storage temperature, as refrigeration could assure a longer survival, whereas a thermal abuse could show a detrimental effect. Some authors proposed different strategies to fight against the effects of a thermal abuse. Micro encapsulation technologies have been designed and successfully applied using various matrices to protect the bacterial cells from the damage caused by the external environment. Few more inventions in the way of improving viability of probiotic cell count in fruit juices given bellow.

Sensory Characteristics and Consumer Acceptance of Probiotic Fruit Juice

Many researchers showcased that overall acceptance remained unaffected due to the probiotication of fruit juice like Rodrigues *et al.* (2009) for a fresh apple beverage fermented by *Lb. casei*, Perricone *et al.,* (2014) for pineapple juice containing *Lb. reuteri and* Ellendersen *et al.,* (2012) for apple juice fermented with *Lb. casei.* Luckow and Delahunty (2004) observed that the sensory characteristics of probiotic black currant juice were perfumery and dairy in odour and sour and savoury in flavour. Wunwisa and Kamolnate (2010) found that the addition of probiotic beads enhanced the sensory properties of the product by increasing the swallowing difficulty and remaining particles of the products. The orange and grape juices containing probiotic beads (82.3 and 84.3%, respectively) was accepted by the maximum consumers. Pineapple with Consortium *(Pediococcus pentosaceus LaG1, Pediococcus pentosaceus LBF2* and *Lactobacillus rhamnosus GG* was preferred over the single culture pineapple juice (Tayo and Akpeji, 2016). Luckow *et al.* (2006) explicit that the incorporation of tropical fruit juices, principally pineapple, however cojointly mango or passion fruit (10% *v*/*v*), may completely contribute to the aroma and flavor of the end product and may avoid the identification of probiotic off-flavors by consumers. Ranadheera *et al.* (2014) underlined that some fruit juices might naturally mask the “medicinaltaste of probiotics.

Safety Issues and Assessment of Probiotic

Probiotics are more often considered as food supplements, and not pharmaceuticals, which suggests the avoidance of extremely thorough testing which are obligatory for all pharmaceuticals. If the probiotic manufacturer makes any specific heath claim, probiotics will be categorized as food supplements, meaning that the focus on safety c be underestimated. Furthermore, if any health claimsfound on the packaging of probiotics, regulatory bodies will examine mainly the validity of such claims and not the safety of the product (De Simone, 2019).

Most probiotic bacteria are marketed in foodstuffs or feed supplements hence probiotic bacteria safety is very important. Through a long research of safe use in food as starter cultures these microbes are confirmed as safe. Bacteria like *Lactobacillus, Leuconostoc* and *Pediococcus* species have been used in food processing throughout the history. LAB areclassified as generally recognized as safe (GRAS), and there were no reports of any harmful effects from the consumption of these bacteria through many processed products (fermented dairy, fermented vegetables etc.) (Naidu *et al.,* 1999).

In the EU, European Food Safety Authority (EFSA) oversees reviewing health claims of probiotics, which are usually stated on the label while the Food Products Directive and Regulation (2000/13/EU, 178/2002/EC) controls both probiotics and food supplements. The EFSA also issues the Qualified Presumption of Safety (QPS) for various bacterial strains. The word "presumption" is the only criteria linked with the actual true safety of probiotics, meaning that safety valuation is not the primary concern (Binnendijk and Rijkers, 2013).

Many of the clinical case studies reported that the increased usage of probiotic products of lactobacilli did not causeany increase in incidence or frequency of bacteremia in Finland and few reported the adverse effects of it. Naturally, bifidobacteria are the key bacteria in the intestinal tract of breast-fed infants and are believed to contribute to the good health of infants. As the reports of a harmful effect of these microbes on the host are very sporadic so till now, the safety of the bifidobacteria has not been questioned.

Use of probiotic bacteria in ill persons is restricted to the strains and indications with proven effectiveness. A multidisciplinary approach is needed to judge the toxicological, immunological, gastroenterological, pathological, infectivity, the inherent properties of the microbes, virulence factors comprising metabolic activity, and microbiological effects of probiotic strains. Various methods have been established to evaluate the safety of LAB through the use of in vitro studies, animal studies, and human clinical studies.

Safety considerations of probiotic bacteria comprise of antibiotic resistance profiles, infectivity in immune-compromised animal models, toxin production: probiotic bacteria must be tested for toxin production. The EU Scientific Committee on Animal Nutrition has recommendedoneof the possible schemes for testing toxin production *i.e.*, hemolytic activity, metabolic activities (D-lactate, bile salt de-conjugation), genetic and pathological side effects, epidemiological surveillance of adverse incidents in consumers (post market).

Potential probiotic health risk can be viewed in two ways (Sanders *et al.,* 2016). The first way encompasses the adverse effects of probiotic per se, while the second way is safety concerns, due toundefined quality standards and manufacturing procedures. However, the only standardization ofaccurate probiotic safety assessment is a retrospective epidemiologic study, accompanied by thoroughpharmacological and toxicological post-marketing vigilance of the product, in order to evaluatefurther probiotic safety (Kothari *et al.,* 2019).

Labelling Requirements for Probiotic Product

Appropriate labelling and health claims are a pre-requisite for the consumer to make aninformed choice. Genus, species and strain, the serving size that delivers the effective dose of probiotic bacteria related to thehealth claim, minimum viable numbers of each probiotic strain at the end of shelf life, an precise description of the functional effect, as far as is permissible by law with therequired scientific evidence, appropriate storage conditions including the temperature at which the product should bestored, corporate contact details for consumer information, safety in the conditions of recommended use and label information must not mislead the consumers to understand that consumption ofthe food, ingredient or nutrient of such food, can treat, relieve, cure or prevent adisease should be mentioned on the label of probiotic products.

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Table 1: Examples of Fruit and vegetable Probiotic Product

Product	**Probiotic Strain**	**References**
Apple	*Lactobacillus casei,* *Lactobacillus plantarum ATCC14917*	de Souza *et al.,* 2012, Li *et al.,* 2019
Apricot Juice	*Bifidobacterium lactis Bb-12, Bifidobacterium longum Bb-46, Lactobacillus casei 01 and Lactobacillus acidophilus La-5*	Bujn *et al.,* 2018
Banana puree	*Lactobacillus acidophilus*	Tsen *et al.,* 2009
Beet Juice	*Lactobacillus plantarum, Lactobacillus casei, Lactobacillus delbrueckii*	Yoon *et al.,* 2005
Beverage with jucara fruit	*Bifidobacteriumanimalis subsp. lactis BB-12 and Lactobacillus acidophilus LA-5*	de Oliveira Ribeiro *et al.,* 2019
Blended orange, carrot, apple and Chinese jujube juice	*Lactobacillus plantarum CICC20265, Bifidobacterium breve CICC6184 and Streptococcus thermophilus CICC6220*	Xu *et al.,* 2019
Cabbage Juice	*Lactobacillus plantarum C3, Lactobacillus delbrueckii D7*	Yoon *et al.,* 2006
Carrot and orange juice	*Lactobacillus plantarum*	Valero-Cases *et al.,* 2017
Cashew apple juice	*Lactobacillus plantarum, Lactobacillus casei*	Kaprasob *et al.,* 2018, Pereira *et al.,* 2011
Cantaloupe juice	*Lactobacillus casei NRRL B-442*	Fonteles *et al.,* 2012
Cherry juice	*Lactobacillus plantarum, Lactobacillus casei, Lactobacillus paracasei and Lactobacillus rhamnosus*	Ricci *et al.,* 2019
Cornelian cherry juice	*Lactobacillus plantarum ATCC 14917*	Mantzourani *et al.,* 2018
Fruit smoothies	*Lactobacillus acidophilus LA-5, Bifidobacterium animalis spp. lactis BB-12*	Rodgers, 2008
Honeydew melon juice	*Lactobacillus casei NCIMB 4114*	Saw *et al.,* 2011
Litchi juice	*Lactobacillus casei*	Zhen *et al.,* 2014
Mango and guava juice	*Lactobacillus casei, Streptococcus thermophillus, Lactobacillus bulgaricus*	Maldonado *et al.,* 2018
Moringa leaves and beetroot beverage	*Lactobacillus plantarum, Enterococcus hirae*	Vanajakshi *et al.,* 2015
Orange, grapefruit, black currant, pineapple, pomegranate, cranberry and lemon juice	*Lactobacillus plantarum*	Nualkaekul *et al.,* 2012
Orange, pineapple and cranberry juice	*Lactobacillus casei DN 114001, Lactobacillus rhamnosus GG, Lactobacillus paracasei NFBC 43338, Bifidobacterium lactis BB-12*	Sheehan *et al.,* 2007
Passion fruit juice	*Bifidobacterium animalis subsp. lactis BB-12*	Dias *et al.,* 2018
Pear Juice	*Lactobacillus acidophilus*	Ankoleka *et al.,* 2012
Pineapple Juice	*Lactobacillus plantarum 299V, Lactobacillus acidophilus La5, Bifidobacterium lactis Bb-12*	Nguyen *et al.,* 2019
Plum Juice	*Lactobacillus kefiranofaciens, Candida kefir, Saccharomyces boluradii*	Sheela and Suganya, 2012
Pomegranate juice	*Lactobacillus plantarum ATCC 14917, Lactobacillus plantarum, Lactobacillus delbrueckii, Lactobacillus acidophilus, Lactobacillus paracasei*	Mantzourani *et al.,* 2019 Mousavi *et al.,* 2011
Pumpkin juice	*Lactobacillus reuteri*	Semjonovs *et al.,* 2013
Sohiong juice	*Lactobacillus plantarum MCC 2974*	Vivek *et al.,* 2019
Star fruit juice	*Lactobacillus helveticus L10, Lactobacillus paracasei L26, and Lactobacillus rhamnosus HN001*	Lu *et al.,* 2018
Table olives	*Lactobacillus GG, Lactobacillus paracasei, Lactobacillus plantarum*	Lavermicocca *et al.,* 2005, Hurtado *et al.,* 2012

Table 2: Examples of Cereal Probiotic Products

Cereal Probiotic	**Product Probiotic Strains**	**Reference**
Barley malt fermented beverage	*Weissellacibaria*	Zannini *et al.,* 2013
Beverage from rice, barley, oats, wheat, soy flour and red grape juice	*Lactobacillus plantarum* 6E and M6	Coda *et al.,* 2012
Breadfruit Flour beverage	*Lactobacillus plantarum DPC 206, Lactobacillus acidophilus* "de Winkel", *Lactobacillus caseiShirota*	Gao *et al.,* 2019
Cashew juice	*Lactobacillus casei* NRRL B 442	Pereira *et al.,* 2011
Fermented beverage from maize and rice	*Lactobacillus plantarum, Torulasporadelbrueckii, Lactobacillus acidophilus*	Freire *et al.,* 2017
Fermented oat flour	*Streptococcus thermophilus* TH-4, *Lactobacillus acidophilus* LA-5	Duru *et al.,* 2019
Fermented oat flour beverage	*Lactobacillus plantarum*	Gupta and Bajaj, 2017
Legume sprouts	*Lactobacilllus plantarum* 299V	Swieca *et al.,* 2018
maize-based substrate	*Lactobacillus paracasei* LBC-81, *Saccharomyces cerevisiae* CCMA 0731, *Saccharomyces cerevisiae* CCMA 0732 and *Pichiakluyveri* CCMA 0615	Menezes *et al.,* 2018
Malt beverage	*Lactobacillus plantarum* NCIMB 8826, *Lactobacillus acidophilus* NCIMB 8821	Rathore *et al.,* 2012
Millet-Based Probiotic Fermented Food	*Lactobacillus rhamnosus* GR-1 and *Streptococcus thermophilus* C106	Di Stefano *et al.,* 2017
Oat based symbiotic drink	*Rhizopus oryzae, L. acidophilus*	Gao *et al*., 2012
Oat-based probiotic drink	*Lactobacillus plantarum* B28; *Lactobacillus reuteri* ATCC 55730	Angelov*et al.,*2006
Peanut milk	*Bifidobacterium pseudocatenulatum* G4	Kabeir *et al.,* 2009
Soymilk	*Lactobacillus acidophilus*	Donkor *et al.,* 2007
Soymilk with apple juice	*Lactobacillus acidophilus*	Icier *et al.,* 2015
Wheat based probiotic Beverage	*Lactobacillus acidophilus* NCDC-14, *Lactobacillus acidophilus* NCDC-16	Sharma *et al.,* 2014
Wheat/rice cereal infant products	*Bifidobacteriumanimalis subsp. lactis* BB-12®	Lebos-Pavunc *et al.,* 2019

Table 3: Strategies for Improving Survival of Probiotic Strains

Examples of Probiotic	Probiotic Strain	Strategy	Reference
Beetroot and carrot Juice	*Lb. acidophilus*	*Fortification with* brewer's yeast autolysate	Rakin *et al., 2007*
Apple Juice	*Lb. rhamnosus*	Fortification with Glucan	Saarela *et al., 2006*
apple cider, orange, and grape juice	*Lb. rhamnosus*	Fortification with long and short chain Inulin	Jessica and Sharareh, 2018
pineapple, orange, green apple	*Lb. reuteri*	*Adaptation and Induction of Resistance (Acid and Phenol Stress)*	Perricone *et al., 2014*
blended juice (orange - grape and passion fruit)	*B. breve*	*Adaptation and Induction of Resistance (Acid Stress)*	Saarela *et al., 2011*
Freeze Dried Strains	*Lb. helveticus*	*Addition of Tea Extract*	Nag and Das, 2013
	Lb. helveticus	*Addition of Vit E*	Gaudreau *et al.,*2013
Model Juice	*L. rhamnosus* HN001, HOWARU *B. Lactis* HN001 and *L. Paracasei* LPC 37	Addition of grape seed extract, green tea extract and vitamin C	Shah *et al.,* 2010
Banana puree	*Lb. acidophilus*	Microencapsulation with Ca-alginate	Tsen *et al*
Sapodilla, grapes, orange and watermelon juices	*Lb. plantarum in,*	Microencapsulation with Na - Alginate	Gaanappriya *et al., 2013*
orange and apple juices	*Lactobacillus rhamnosus, Bifidobacteriumlongum, L. salivarius, L. plantarum, L. acidophilus, L. paracasei, B. lactis type Bi -04 and B. lactis type Bi-07*	Microencapsulation with Na - Alginate	Ding and Shah, 2008.
tomato juice	*Lb. acidophilus*	Microencapsulation with Ca - alginate	King *et al., 2007*
Orange and Apple Juice	*Lb. casei*	Microencapsulation with Chitosan	Wunwisa and Kamolnate , 2010
mulberry, maoberry, longan, and melon juices	*L. casei* 01b, *L.acidophilus*LA5 and *B. lactis Bb-12*	Alginate Encapsulation with cashew flower, pennywort, and yanang	Chaikham, 2015