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Review

Challenges for the Production of Probiotic Fruit Juices

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Abstract: Fruit juices represent a promising carrier for probiotic bacteria; however, there are some drawbacks and limits that could preclude their production at the industrial level, namely the survival of probiotics throughout storage, and the possible impact of bacteria on the sensory traits and overall acceptance. This review addresses the inoculation of probiotics in juices; with a special focus on the possibilities and challenges for future; *i.e.*, why probiotics in juices and which kind of microorganisms; some drawbacks and how to improve the viability of probiotics; and some ideas on the sensory impact.

Keywords: juices; probiotics; survival; improvement; overall acceptance

1. Introduction

Today foods have many aims: satisfying hunger and providing the necessary nutrients for humans, promoting a state of physical and mental well-being, improving health, preventing and/or reducing nutrition-related diseases. Moreover, in recent years, consumers' awareness towards the relationship between food and health has led to an explosion of interest in "healthy foods"; this phenomenon could be partly attributed to the increasing cost of healthcare, the steady increase in life expectancy, and the desire of older people for an improved quality of their later years [1].

Nowadays, healthy foods mean "functional foods", and we generally label a food as functional if it exerts beneficial effects or more specific body functions, in addition to the traditional nutritional effects. Well-known examples of functional foods are those containing or prepared with bioactive compounds, such as dietary fiber, oligosaccharides, and active "friendly" bacteria that promote the equilibrium of intestinal bacterial strains. In addition to the well-established functional ingredients, such as vitamins, minerals, and micronutrients, probiotics belong to an emerging generation of active ingredients, which includes prebiotics, phytonutrients, and lipids [2].

The label "functional food" was introduced in 1980 in Japan, which was the first country that stated a specific regulatory approval process for functional foods, known as Foods for Specified Health Use (FOSHU) [3]. On the other hand, in Europe, the interest in functional foods started in the 1990s, when the European Commission created a commission called Functional Food Science in Europe (FuFoSE) to explore the concept of functional foods through a science-based approach [4].

Several critical factors have been recognized as the key factors leading to the diffusion of functional foods: health deterioration, due to busy lifestyles, low consumption of convenience foods and insufficient exercise, increased incidence of self-medication, increased awareness of the link between diet and health, and a crowded and competitive food market [4].

Thereafter, functional foods represent one of the most interesting areas of research and innovation in the food field, as suggested by the increasing number of scientific papers dealing with this topic since 2007. The market of functional foods is characterized by an increasing trend, and some researchers reported that probiotic foods represent *ca.* 60%–70% of functional foods [5].

2. Why and Which Kind of Probiotics in Juices

The word probiotic comes from the Greek word "προ-βίος" that means "for life"; thus, probiotics are live microorganisms (mainly bacteria but also yeasts) that confer a beneficial effect on the host if administered in proper amounts [6]. Dairy fermented products have been traditionally considered as the best carriers for probiotics; but, nowadays, up to 70% of the world population is affected by lactose-intolerance. Furthermore, the use of milk-based products may be also limited by allergies, cholesterol diseases, dyslipidemia, and vegetarianism; therefore, several raw materials have been extensively investigated to determine if they are suitable substrates to produce novel non-dairy functional foods [6].

Recently, beverages based on fruits, vegetables, cereals, and soybeans have been proposed as new products containing probiotic strains; particularly, fruit juices have been reported as a novel and appropriate medium for probiotic for their content of essential nutrients. Moreover, they are usually referred as healthy foods, designed for young and old people [7]. Many authors reported on the effects of juices on health; for example, Sutton [8] demonstrated that aqueous extracts of kiwifruit and avocado had very low cytotoxicity and high anti-inflammatory activity in a Crohn's gene-specific assay. Non-aqueous extracts of kiwifruit, blueberry and avocado had similarly high anti-inflammatory activity, with slightly higher cytotoxicity than the aqueous extracts.

Fenech *et al.* [9] carried out the effect of the intake of nine micronutrients (vitamin E, calcium, folate, retinol, nicotin acid, β-carotene, riboflavin, pantothenic acid and biotin) on genome damage and repair; these compounds can be easily found in juices. Therefore, juice fortification with probiotic microorganisms is a challenge and a frontier goal, as juices could combine nutritional effects with the added value of a healthy benefit from a probiotic.

Furthermore, fruit juices have shown negative effects on some pathogenic microorganisms, while improving the growth of probiotics because berries, such as blueberry, blackberry and raspberry, possess antimicrobial effects towards many pathogens [10].

The most commonly probiotic bacterial genera are *Lactobacillus* and *Bifidobacterium*, while yeasts mainly belong to *Saccharomyces cerevisiae* var. *boulardii*; different strains from *Lactobacillus acidophilus*, *Lb. casei*, *Lb. crispatus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. fermentum*, *Lb. gasseri*, *Lb. johnsonii*, *Lb. paracasei*, *Lb. plantarum*, *Lb. reuteri*, *Lb. rhamnosus*, *Lb. helveticus*, *Lb. lactis*, *B. bifidum*, *B. breve*, *B. infantis*, *B. longum*, *B. lactis*, *B. adolescentis*, *B. essensis*, *B. laterosporus*, and other species like *Escherichia coli* Nissle, *Saccharomyces cerevisiae* var. *boulardii*, *Streptococcus thermophilus*, *Enterococcus faecium*, *Propionibacterium* spp., *Pediococcus* spp. and *Leuconostoc* spp. could be considered as main targets used as probiotics both in dairy and non-dairy functional foods [11].

Maintaining the viability (the recent trend is to have one billion viable cells per portion—*i.e.*, 100 g of product) and the activity of probiotics in foods to the end of shelf-life are two important criteria to be fulfilled in juices, where low pH represents a drawback.

Several strains of *Lb. plantarum*, *Lb. acidophilus* and *Lb. casei* can grow in fruit matrices due to their tolerance to acidic environments [12]; Table 1 lists the suitability of some species in different kinds of juices.

Table 1. Probiotics in juices *.

Juice	Probiotic
Strawberry	Negative effects on probiotic except for *Lb. casei*
Pineapple	Viability loss by *Lb. plantarum* Prolonged survival of *Lb. casei*, *Lb. rhamnosus*, *Lb. paracasei* and *Lb. reuteri*
Kiwi	Viability loss by *Lb. plantarum*
Peach	No effect on probiotics
Orange	Prolonged survival of *Lb. casei*, *Lb. rhamnosus*, *Lb. paracasei*
Cranberry	Reduced survival of *Lb. casei*, *Lb. rhamnosus*, *Lb. paracasei*
Pomegranate	*Lb. plantarum* and *Lb. delbrueckii* were more resistant than *Lb. paracasei* and *Lb. acidophilus*
Tomato	Suitable medium for *Lb. acidophilus*, *Lb. casei*, *Lb. plantarum* and *Lb. brevis*
Carrot	Suitable medium for *B. lactis*, *B. bifidum*, *Lb. rhamnosus* and *Lb. delbrueckii* subsp. *bulgaricus*

* Sources: [12–15].

3. Drawbacks for Probiotic Survival in Juices

The health benefit of probiotics mainly relies upon their concentration in foods, as well as on their ability to survive to adverse conditions of the gastrointestinal tract. Hence, even if the probiotic viability is to be strain-dependent [4,5,13], they should be at least 10^7 CFU/mL in the product at the end of the shelf life, which approximately corresponds to 10^9 CFU per portion [16].

Although juices contain some essential nutrients (minerals, vitamins, dietary fibers, antioxidants), there are some strong factors that could limit probiotic survival in juices; Tripathi and Giri [5] grouped them as follows:

(1) food parameters: pH, titratable acidity, molecular oxygen, water activity, presence of salt, sugar and chemicals, like hydrogen peroxide, bacteriocins, artificial flavoring and coloring agents;

(2) processing parameters: heat treatment, incubation temperature, cooling rate, packaging materials and storage methods, oxygen levels, volume;
(3) microbiological parameters: strains of probiotics, rate and proportion of inoculation.

pH is one of the most important factors affecting the survival of probiotics. Juices contain a high level of organic acids and the low pH increases the concentration of undissociated form, thus, in juices, we could presume the existence of a combined action of acidic conditions and the intrinsic antimicrobial effect of acids. Lactobacilli are generally resistant and survive in juices with pH ranging from 3.7 to 4.3; on the other hand, bifidobacteria are less acid tolerant, and a pH of about 4.6 is detrimental for their survival [5].

However, pH cannot explain the trends experienced by some probiotics in different kinds of juices. Nualkaekul *et al.* [17] investigated the factors that affected the survival of *B. longum* in model solutions and in fruit juices (orange, grapefruit, blackcurrant, pineapple, pomegranate and strawberry). They reported that after six week of storage at 4 °C, bifidobacteria in orange, grapefruit, blackcurrant, and pineapple juices decreased by less than 0.8 log CFU/mL, with the highest cell count found in orange and pineapple juice. Moreover, they found some controversial data on the effects of pH, as the decrease in grapefruit was only 0.5 log CFU/mL, despite the low pH (3.21) and the high concentration of citric acid (15.3 g/L). On the other hand, the probiotic was below the detection limit after one week in pomegranate and four weeks in strawberry juice. These results suggest that the survival was the result of the synergistic and antagonistic action of some parameters, and that phenolic compounds could play a significant role. Generally, pH exerts a detrimental effect, but protein and dietary fiber could protect cells from acidic stress; the role of citric and malic acids is controversial, as they seemed to protect probiotics, whereas phenols could cause a strong viability loss.

Although the pH is a drawback for probiotic survival in juices, Ranadheera *et al.* [10] assumed that the incorporation of lactic acid bacteria into fruit juices with low pH may enhance the resistance of bacteria to subsequent stressful acidic conditions, such as those found in gastrointestinal tract.

4. Improving Probiotic Survival in Juices

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.

4.1. Fortification with Prebiotics

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. Rakin *et al.* [18] enriched beetroot and carrot juices with brewer's yeast autolysate before lactic acid fermentation with *Lb. acidophilus*; the addition of autolysate enhanced the growth of *Lb. acidophilus* during the fermentation, reduced fermentation time, enriched the juices with amino-acids, vitamins, minerals and antioxidants and exerted a positive effect on the survival of probiotics.

Other researchers fortified juices with glucans, e.g., Saarela *et al.* [19] reported that oat flour with 20% of β-glucan could protect *Lb. rhamnosus* during refrigerated storage in apple juice.

4.2. Adaptation and Induction of Resistance

Gobetti *et al.* [20] pinpointed that the exposure of probiotics to a sub-lethal stress could induce a kind of resistance and an adaptive stress response. Perricone *et al.* [3] successfully tested this kind of approach; they evaluated the viability of *Lb. reuteri* DSM 20016 in pineapple, orange, green apple, and red fruit juices and found that the probiotic experienced a strong viability loss in red-fruit juice, due probably to a combined effect of low pH and phenols. Thus, they used two different strategies: strain cultivation in a lab medium containing different amounts of red 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 *Lb. reuteri* by 5 (phenol stress) or 11 days (pH stress).

Saarela *et al.* [21] improved the survival of *B. breve* in a blended juice (orange-grape and passion fruit) generating an acid tolerance variant of the microorganism by UV mutagenesis, combined with cultivation at sub-lethal pHs.

4.3. Storage under Refrigeration and Use of Antioxidants

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; for example, Sohail *et al.* [22] improved the viability of *Lb. rhamnosus* and *Lb. acidophilus* using a novel microencapsulation method, thus, they reduced the acidification and assured the survival of the probiotics at 25 °C for at least nine days in orange juice. Moreover, the level of oxygen within the package during foods storage should be as low as possible to prevent toxicity and death of the probiotics, although the extent of sensitivity is strongly variable. Generally, bifidobacteria are more sensitive than lattobacilli [23].

Oxygen induces an oxidative damage by the formation of reactive oxygen species (ROS), such as H_2O_2 or superoxide ion. Many researchers proposed the modification of product atmosphere, increasing the content of CO_2 in the headspace [4]. In addition, antioxidant compounds could be a good tool to limit negative effects of oxygen exposure. Some researchers evaluated the effects of different concentrations of (+)-catechin, green tea epigallocatechin gallate, and green tea extracts (GTE) on the growth and survival of some probiotic strains with different oxygen sensitivities; thus, they found that the growth of *Lb. helveticus* was strongly enhanced [23]. Moreover, Gaudreau *et al.* [24] improved the stability of *Lb. casei* CRL 431 during 20 week storage period at 25 °C by fortification of vitamin-E in the stabilization food matrix.

4.4. Microencapsulation

Finally, microencapsulation technologies have been designed and successfully applied using various matrices to protect the bacterial cells from the damage caused by the external environment.

Several studies reported that microencapsulation might provide a more favorable anaerobic environment for sensitive probiotic bacteria, as well as a physical barrier from the harsh acidic conditions of the fruit juice [25].

Tsen *et al.* [26] reported that *Lb. acidophilus* immobilized in Ca-alginate can carry out a fermentation of banana puree, resulting in a novel probiotic banana product.

Gaanappriya *et al.* [27] evaluated the viability of encapsulated *Lb. plantarum* in sapodilla, grapes, orange and watermelon juices, and maintained the probiotic at 7 log CFU/mL or more for one month.

Ding and Shah [25] highlighted that fruit juices containing microencapsulated probiotic bacteria were more stable than those containing free probiotic organisms. In particular, the encapsulated probiotics (*Lb. rhamnosus*, *Lb. salivarius*, *Lb. plantarum*, *Lb. acidophilus*, *Lb. paracasei*, *B. longum*, *B. lactis* type Bi-04 and Bi-07) were protected from the acidic environment of the orange juice and did not undergo a strong viability loss, as, after six weeks, they showed a residual cell count of 5 log CFU/mL.

Finally, King *et al.* [15] found that *Lb. acidophilus* immobilized in Ca-alginate showed a higher survival rate than free cells in tomato juice during cold storage at 4 °C; moreover, the sensory evaluation revealed that the overall acceptance of immobilized cell fermentation was higher than free cells during storage.

5. Sensory Traits

A challenge for probiotic fortification of juices is product acceptance by consumers [28]. The effects of probiotics on the sensory traits of juices rely upon the kind of microorganism and juice, storage temperature, addition of other compounds. Some researchers showed that probiotics did not affect the overall acceptance of juices, e.g., Perricone *et al.* [3] for pineapple juice containing *Lb. reuteri*, Rodrigues *et al.* [29] for a fresh apple beverage fermented by *Lb. casei*, and Ellendersen *et al.* [28] for apple juice.

A possible solution for the juices where probiotic could negatively affect the overall acceptance is masking, *i.e.*, the addition of pleasant aroma and volatile compounds, able to “mask” the presence of probiotics. Luckow *et al.* [7] reported that the addition of tropical fruit juices, mainly pineapple, but also mango or passion fruit (10% *v*/*v*), might positively contribute to the aroma and flavor of the final product and might avoid the identification of probiotic off-flavors by consumers.

Finally, Ranadheera *et al.* [10] underlined that some fruit juices could naturally mask the “medicinal” taste of probiotics.

6. Conclusions

Juices can represent a suitable carrier for probiotics, as they can combine the appearance of healthy and fresh foods, designed for a wide range of consumers, and the healthy benefits from probiotics.

There are some challenges to overcome, *i.e.*, the survival of probiotics, and the effects on the sensory traits; however, there are some possible solutions that show that there is a promising way. Some probiotics juices are already on the market (Table 2), but many other products can be launched on the market. Which is the winning strategy? Perhaps, the combination of probiotics with some new methods (encapsulation, fortification with other ingredients, using non conventional juices or non conventional probiotics) able to catch up with consumers.

Table 2. Examples of probiotic juices easily found on sale.

Label	Producer	Traits
GoodBelly	Next Foods (USA)	Produced from mango, blueberry acai, pomegranate, blackberry, tropical green, cranberry, watermelon, tropical orange, and coconut water juices. Inoculated with *Lb. plantarum* 299v (50 billion cells per portion). Without sugar added.
Proviva	Skane Dairy (Sweden)	Strawberry or blackcurrant juice, fortified with 5% ota meal. Inoculated with *Lb. plantarum* 299v (50 million cells per portion)
Biola	Valio Gefilus Ltd. (Finland)	Produced from seven varieties of juices. Inoculated with *Lb. rhamnosus* GG and fortified with vitamins C and D

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Author Contributions

M. Perricone, A. Bevilacqua, C. Altieri, M. Sinigaglia, and M.R. Corbo equally contributed to the paper.

Conflicts of Interest

The authors declare no conflict of interest.

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