

## Fruit juices as probiotic carriers.

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#### Editorial

Consumers are increasingly concerned about diet and expect to eat foods that are tasty, attractive, safe and nutritious. They also seek to improve well-being and health, which can be achieved through the ingestion of food added with functional components, such as probiotic cultures or prebiotic components.

Probiotics are living microorganisms that confer beneficial effects on the individual when administered in adequate amounts [1]. This means that the probiotic culture must be alive and present in large quantities in the product, usually in numbers greater than  $10^6$ - $10^7$  cells per milliliter or gram [2]. However, as studies on the population requirement of probiotic cultures to exert health beneficial effects to the consumer are available, it is verified that the amount required varies depending on the strain and the expected beneficial effect.

Probiotic strains of *Lactobacillus*, *Bifidobacterium* and *Saccharomyces* have an extensive safety history for consumption by the healthy population, being considered GRAS (Generally Recognized as Safe). There are few reports in the literature of adverse reactions resulting from ingestion of these microorganisms, and consequently, they are the most studied genera of probiotic cultures. Strains of *Enterococcus*, *Bacillus* and *Escherichia* should be intensively evaluated for safety before use, as they may be opportunistic pathogens and/or capable of transferring genes that confer resistance to antibiotics.

Many studies have reported that probiotic cultures would exert a beneficial effect on the host health, including: reduction in gastrointestinal infections, antimicrobial activity, improvement in lactose metabolism, reduction in serum cholesterol, stimulation of the immune system, antimutagenic, anticarcinogenic and antidiarrheal properties, improvement in symptoms of irritable bowel syndrome, suppression of *Helicobacter pylori* infections, and reduction of obesity and atopic dermatitis [3-7]. It is important to note, however, that the beneficial health effects provided by probiotic cultures are specific to each strain, i.e., no strain will be able to provide all the reported benefits, and even strains of the same species will not be effective against specific health claims.

Fermented dairy products, like yogurts and whey beverages, comprise most of the foods containing probiotic cultures, because they are suitable for the incorporation of probiotics, have a positive image to consumers, do not require changes in the technology involved and in the manufacturing process to include the probiotic culture, and protect the probiotic through the gastrointestinal transit. In addition, the fermentative process acts in the maintenance and optimization of microbial viability; and consumers are familiar with the fact that these products

contain living microorganisms. The refrigerated storage also helps to stabilize the probiotic cultures.

The development of non-dairy probiotic products allows the consumption of these beneficial microorganisms by people intolerant to lactose, allergic to milk proteins, hypercholesterolemia, strict vegetarian or resident in places where dairy products are not accessible.

Fruit juices may be a non-dairy matrix option for the incorporation of probiotics because: (1) they are rich in nutrients (vitamins, antioxidants and polyphenols), (2) they have taste profiles considered pleasant by people of all ages, (3) are consumed regularly, which is essential for the beneficial effects associated with probiotics, (4) are considered healthy products by the general population, mainly due to the presence in the market of fruit juices fortified with vitamins or calcium, (5) do not contain starter cultures that compete for nutrients with probiotics, (6) are generally supplemented with antioxidant ingredients, such as ascorbic acid, which promote anaerobic conditions in the medium, (7) contain significant amounts of sugars, which can be metabolized by the beneficial cultures, (8) do not have allergens, such as lactose and casein, which preclude the consumption of dairy products by some segments of the population, (9) are naturally cholesterol-free, and (10) their transit through the gastrointestinal tract is relatively rapid, which reduces the time of exposure of probiotics to hostile environments, such as the acidity of the stomach.

However, fruit juices have presented limitations to the addition of probiotic cultures, such as: the high acidity, the presence of oxygen and the insufficient amounts of peptides and free amino acids required for probiotics. Probiotic cultures are neutrophils, with pH optimum between 5 and 9, and inhibition of growth at pHs lower than 4.5. When the cells are present in low pH environments, there is an increase in energy required for the maintenance of intracellular pH, and as a result, ATP is lacking for other crucial functions, causing cell death [8]. Furthermore, they are usually anaerobic or microaerophilic and therefore the electron carrier chain and / or the catalase enzyme are absent, and the presence of oxygen may cause formation and accumulation of toxic metabolites in the cell and results in cell death from oxidative damage.

In addition, the presence of preservatives, flavorings and colorings may decrease the viability of probiotic cultures, the probiotic cultures can change the sensory characteristics of the juices, including the development of unpleasant aromas (fragrant, dairy) and flavors (bitter, acid), and probiotic cultures are added as ingredients, not having the ability to multiply in the food, which requires microorganisms with good stability. The metabolism of probiotic cultures may result in the production of components that contribute negatively to the

aroma and flavor of the products. Consequently, probiotic products may be less acceptable when compared to the conventional products, due to the differentiated taste, being characterized as "acid" or "astringent".

The probiotic culture is added to fruit juices after the heat treatment, as it is heat sensitive. The usual form is to add the probiotic culture in its activated form, after successively propagation of the lyophilized culture in Mann, Rogosa and Sharp (MRS) broth [9-14]. Costa et al. [15] proposed the utilization of orange juice in substitution for MRS broth and proved that orange juice was suitable substrate for *Lactobacillus paracasei* propagation. According to the authors, MRS broth is a culture medium with higher cost (US\$ 191.00/500 g) than the orange juice (US\$ 0.15/500 g), increasing the cost of production by US\$ 2.615/L of juice, which precludes the production of probiotic juices by small and medium companies. Future studies should consider the addition of the probiotic cultures in other manners, including the direct inclusion of the lyophilized culture into the juices.

The first challenge in the formulation of probiotic juices is not to alter their physicochemical and sensory characteristics. For this, it is necessary to minimize the metabolization of the juice components by the probiotic microorganisms and to evaluate different probiotic cultures in order to verify their ability to alter the flavor profile of the products. The influence of probiotic addition on the physicochemical and sensory characteristics of fruit juices has not been extensively studied, but the addition can originate products with higher acidity and alteration in the color and turbidity. Probiotic microorganisms can metabolize simple sugars present in the juice and, consequently, small quantities of organic acids are produced. Acidification of juices could also be related to the release of enzymes from bacteria and the hydrolysis of sugars from juices. Changes in color and turbidity are caused by the addition of probiotic, and are visualized in a more effective manner in clarified fruit juices (apple or pineapple) than in cloudy ones (orange). The probiotic culture can stay suspended in the solution, in the form of particles, which are too small to sediment, but enough to refract the light. However, in a general manner, probiotic juices with acceptance similar to the conventional products were obtained using different types of fruits, such as apple [12], orange [15], peach [16], among others.

The second challenge in the development of probiotic fruit juices is to maintain the microorganism viability and functionality during the shelf life of the product and, mainly, in the human organism. The viability of the probiotic cultures in fruit juices is mainly dependent on the type of fruit used in the formulation, the pH and the oxygen content dissolved in the medium, and the probiotic strain used.

In conclusion, the development of probiotic fruit juices is possible, allowing the consumption of these beneficial microorganisms by people who do not like dairy products or with intolerance or allergy to milk components. Furthermore, the inclusion of probiotic fruit juices in the market increases the number of available probiotic products to the general

population. There are two main challenges during manufacture, that is, the maintenance of the probiotic viability during the shelf life of the products and to the gastrointestinal tract and the maintenance of the physicochemical and sensory characteristics of the conventional products.

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