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Beneficial Microbes in Fermented and Functional Foods

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Probiotics and Prebiotics in Fruits and Vegetables: Technological and Sensory Aspects

Fernanda Galgano, Nicola Condelli, Marisa Carmela Caruso, Maria Antonietta Colangelo, and Fabio Favati

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10.1 Intestinal Microbiota and Probiotic Microorganisms

It has been known for a long time that diet can have a positive or negative effect upon human health. In industrialized countries, a diet based on too much fat is the cause of diseases, such as hypertension and obesity. On the contrary, in developing countries, a poor diet causes serious nutritional deficiencies and death. Therefore, in recent years, there has been an increasing interest in food nutrition, with a view to safeguarding health. In fact, consumers are more aware about the link between food and health; this explains the reason for an increasing demand for foods that promote good health, such as functional foods (Peres et al., 2012).

The term *functional food* was used for the first time in Japan in the mid-1980s (Chonan, 2011). In addition to making nutrients, functional foods have a positive influence on the health of the host by helping to reduce the risk of contracting certain diseases (Cencic and Chingwaru, 2010; Stringheta et al., 2007). The European Commission Concerted Action on Functional Food Science (FUFOSE) has written a document that states that a food can be considered functional if it has been satisfactorily demonstrated that, in addition to its nutritional value, it performs one or more functions beneficial to the body, thus producing a state of well-being and reducing the risk of certain types of diseases (Evangelisti and Restani, 2011). The group of functional foods includes foods with probiotic microorganisms.

Although each area of our body is colonized by microorganisms, most of them lie in our gut. *Gut microbiota* is the terminology used to describe the huge amount of microorganisms that colonize the entire digestive tract (Evangelisti and Restani, 2011). Thus, the original microbiota creates an important barrier between the external environment and the individual, avoiding a wide variety of disorders (Kosin and Rakshit, 2006; Vertuani and Manfredini, 2001).

However, it is possible to ingest some selected bacteria, such as probiotics, which may beneficially affect the human gastrointestinal tract. The first person to realize the positive effect of selected microorganisms on human health was Eli Metchnikoff. He suggested that “the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes” (Morelli, 2000).

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host, influencing positively the gut microbiota (Granato et al., 2010; Sloan, 2004; Ziemer and Gibson, 1998). The term *probiotic* (meaning *for life* in Greek) includes a large range of microorganisms, mainly bacteria, but also yeasts, although their effect on human health is strain specific (Burgain et al., 2011). The main species of probiotic bacteria added to food are *Lactobacillus* and *Bifidobacterium* (Champagne et al., 2011; Saulnier et al., 2009).

In order to be tested for human probiotic use, a microorganism must have the following specific characteristics:

- It must be of human origin.
- It must be nonpathogenic.
- It must be resistant to degradation by gastric and pancreatic juices.
- It must be able to adhere to the intestinal epithelium.
- It must be able to colonize the gastrointestinal tract.
- It must be a producer of antimicrobial substances.
- It must be a modulator of the immune response (Morelli, 2000).

In addition, higher levels of viable microorganisms (1×10^7 CFU/g) are recommended in probiotic foods for better efficacy in human organism (Ranadheera et al., 2010).

It is well known that probiotics are able to modify the intestinal microbiota by reducing the pH, producing substances having antibacterial action, and stimulating the immune system (Agrawal, 2007; Gerritsen et al., 2011; Zubillaga et al., 2001).

Microorganisms commonly used in probiotic foods are *Lactobacillus* species, such as *L. acidophilus*, *L. casei*, *L. reuteri*, *L. rhamnosus*, *L. johnsonii*, and *L. plantarum*, and *Bifidobacterium* species, such as *B. longum*, *B. breve*, and *B. lactis*. Some scientific studies suggest that probiotics are able to treat Crohn's disease and ulcerative colitis (Kruis et al., 2004; Marteau et al., 2006; Prantera et al., 2002; Van Gossum et al., 2007). They also play a protective role against enteric infections caused by *Salmonella*, *Listeria monocytogenes*, and *Clostridium difficile* (Koninkx and Malago, 2008). Selected strains of lactobacilli, *L. casei* GG and *L. reuteri*, as well as *Saccharomyces boulardii*, are effective for reducing the duration of diarrhea associated with gastroenteritis (Bhadoria and Mahapatra, 2011).

L. rhamnosus GG (LGG), *L. plantarum*, and *S. boulardii* strains are most commonly used for infections attributed to *C. difficile* (Marangoni, 2004). However, the intake of *L. johnsonii* is effective for the inhibition and eradication of *Helicobacter pylori* (Sanchez et al., 2009), while *Bifidobacterium animalis* strain seems to have an effect in speeding intestinal transit and combat constipation (Sanchez et al., 2009).

The concept of *bioprotica* derives from analyzing the characteristics that a probiotic must have and its field of action. One of the characteristics of a probiotic is that it must be of human origin. The fact that microorganisms with probiotic characteristics are present not only in the intestine but also in other areas of the body gave rise to the idea of using specific probiotic strains for the treatment of different pathologies. The probiotic strains of the skin, for example, could be used to antagonize the growth and proliferation of pathogenic fungi for the skin. Nonpathogenic strains of human derivation, resistant to the acid pH of the gastric environment, could be used to combat the proliferation of *H. pylori*. According to the selection area of the strain, the probiotics can be used for the treatment of specific disorders. The term *bioprotica* was coined on the basis of these considerations.

Literally, the term *bioprotic* refers to the use of *living bacteria* (bio) for the treatment of a specific sickness (pro stands for *in favor of*) with a well-identified and precise pharmaceutical or nutraceutical (tic) treatment (Di Pierro et al., 2013). Bioprotica means trying to select strains to be used in a specific way; for example, a strain of the oral cavity of *Streptococcus salivarius* K12, which highly produces bacteriocins and also able to colonize the area, has been isolated and tested for clinical evaluation in the prevention of recurrent pharyngitis and/or tonsillitis caused by *Streptococcus pyogenes* in adults (Di Pierro et al., 2013, Tagg, 2004). Moreover, the results of numerous studies on the metabolic profile and toxicity tests have shown the *S. salivarius* K12 to be safe for human use (Burton et al., 2006).

10.2 Prebiotics

> Prebiotic is a non-digestible substance of food origin which, when administered in adequate amounts, is beneficial to the consumer due to the selective promotion of growth and/or activity of one or more bacteria already present in the gastrointestinal tract or taken together with the prebiotic. (Hill et al., 2014)

Foods with prebiotics contain at least one nondigestible ingredient by humans, able to selectively promote the growth and activity of beneficial microbial species that colonize the intestine (Charalampopoulos and Rastall, 2011; Gibson et al., 2004; Roberfroid, 2007).

Prebiotics must have some particular characteristics:

- They must not be hydrolyzed nor absorbed in the upper digestive tract.
- They must represent a selective substrate for one or more beneficial bacterial species in the colon stimulating their growth or activity.
- They must be able to modify the intestinal microflora of the colon promoting a healthy composition.

Prebiotics are used only by beneficial microorganisms that colonize the gut, without promoting the growth and multiplication of pathogenic microorganisms, such as *Escherichia coli* and clostridia.

All the food components that, irrespectively of their chemical nature, are able to reach the colon without any degradation, are not hydrolyzed in the upper digestive tract, stimulate the growth of microbial species present in the gut, and promote a healthy composition of the intestinal microflora can be considered to be prebiotics. Most prebiotics are nondigestible carbohydrates (nondigestible oligosaccharides or fibers), such as fructooligosaccharides (FOS) with bifidogenic action, since these stimulate the growth of bifidobacteria (Evangelisti and Restani, 2011; Ziemer and Gibson, 1998).

Small amounts of FOS are contained in onions, garlic, artichokes, wheat, rye, asparagus, and bananas. However, the food industry produces drinks, cookies, crackers, fermented milks, yogurts, and even herbal teas with added prebiotic fibers. From a technological point of view, the addition of prebiotics does not require special production technologies, as these ingredients are not altered by air and heat (Evangelisti and Restani, 2011). Even products for babies, such as weaning food and milk, often contain added prebiotic components. Infant formula enriched with prebiotics is more similar to breast milk, naturally rich in prebiotics. The prebiotic component of breast milk is of great importance, considering that it is the third most abundant solid component and has a major role in protecting the baby from bacterial infection. Prebiotics act by preventing the adhesion of pathogenic bacteria and preventing urinary tract infections in the child. Breastfeeding is always recommended for both the child's health and for the well-being of the mother. However, for cases in which breastfeeding is not possible, it is necessary to provide replacement formulations containing prebiotics to make them more similar to breast milk (Vandenplas, 2002).

A controversial question regards the effect of the addition of prebiotics on the sensory characteristics of the food. The incorporation of prebiotics in the food matrix has immediate effects on the flavor and texture of the food; therefore, these ingredients could replace the fat component that usually gives softness and creaminess to food (Ranadheera et al., 2010).

Hardi and Slanac (2000) have reported the effect of prebiotics on the stability of the pH and the post-acidification of the fermented milks, showing that in a fermented milk the pH decreases more quickly when inulin is added. On the other hand, Zhu (2004) has reported that the pH and acidity of yogurt do not change after the addition of 4% of FOS. Inconsistent results make further studies and research necessary.

There is synergy between probiotics and prebiotics, as in the colon probiotics use prebiotic compounds as a source of energy. As a consequence, the activity of pathogenic microorganisms in the gut is further reduced (Homayouni et al., 2008).

By a combination of probiotics and prebiotics, it is possible to formulate symbiotic foods. Symbiotic foods contain a probiotic and a prebiotic component. Several studies (Gmeiner et al., 2000; Gomes and Malcata, 1999; Roberfroid et al., 1998; Schaafsma et al., 1998) suggested that the consumption of symbiotic foods affects the human health more than the consumption of a food containing only probiotics or prebiotics alone. The presence of probiotics and prebiotics in a single food improves probiotic viability during storage of the product and during the passage through the gastrointestinal tract. In addition, the symbiotic food allows an efficient implantation of probiotics in the colon due to the stimulating effect of the latter on the growth and activity of the microorganisms (Roberfroid et al., 1998). Unfortunately, the production of symbiotic foods results in increased costs for both the industry and the consumer. The use of probiotic bacteria able to synthesize prebiotics might overcome this limitation. Some authors reported that some bifidobacteria strains are capable to synthesize galactooligosaccharides (Dumortier et al., 1990; Van den Brook et al., 1999).

10.3 Fruits and Vegetables as a Source of Probiotic Microorganisms

The incorporation of probiotic strains in food matrices principally concerns dairy products, such as fermented milks and cheeses (Garcia-Fontan et al., 2006; Hagen and Narvhus, 1999; Laroia and Martin, 1991; Lourens-Hattingh and Viljeon, 2001; Ong et al., 2006; Tharmaraj and Shah, 2004). However, other foods have been studied as potential probiotic carriers, such as fermented sausages, coconut desserts, ice cream, and chocolate mousse (Casale Aragon-Alegro et al., 2007; Homayouni et al., 2008; Lavasani et al., 2011; Madureira et al., 2011).

Although fermented milk and yogurt with probiotic products are the most popular on the market, it is necessary to propose viable alternatives to products that do not contain milk, both to meet the needs of those people suffering from lactose intolerance or hypercholesterolemia and to meet the growing demand for vegetarian products (Peres et al., 2012; Ranadheera et al., 2010). For this reason, there is an increasing demand for vegetarian probiotic products (Ranadheera et al., 2010). Foods based on fruit and vegetables, such as fruit and vegetable juices, represent a new potential carrier and source of probiotic microorganisms (Nicolesco and Buruleanu, 2010; Nualkaekul and Charalampopoulos, 2011; Pereira et al., 2011; Peres et al., 2012; Sheela and Suganya, 2012). Raw and fermented vegetables also represent an excellent vehicle for probiotics due to their natural structure that allows the easy availability of useful nutrients for microbial growth (Espírito-Santo et al., 2011; Peres et al., 2012; Soccol et al., 2010). The tissues of plants contain intracellular spaces, pores, and capillaries. Although the cell walls of plants are very resistant, the microorganisms are able to penetrate through the tissue injury (Brackett and Splittstoesser, 2001). The microorganisms enter the pores, cracks, and lesions of the surface of the fruits. Some operations such as peeling and cutting performed on minimally processed products can favor the availability of nutrients, such as sugars, vitamins, and minerals needed for probiotic growth (de Oliveira et al., 2011; Rößle et al., 2010; Soccol et al., 2010). Lactic acid bacteria isolated from the same plant can be used as probiotics (Alzamora et al., 2005; Betoret et al., 2003; Renadheera et al., 2010). Moreover, most fruits and vegetables contain prebiotic ingredients that promote the growth of beneficial microorganisms.

Some products, processed from fermented vegetables, are already widely consumed in some parts of the world. An example is kinema, obtained by the fermentation of soybeans using *Bacillus subtilis* and consumed by the inhabitants of Nepal (Mugula et al., 2001). The positive growth of lactic acid bacteria in these types of foods shows that it is possible to use grain as a substrate for the growth of probiotics. By obtaining all the benefits from the fermentation process (improving the nutritional and organoleptic characteristics of the food and reducing antinutritional factors), fruits and vegetables represent health-promoting foods thanks to the combination of probiotics and prebiotics naturally present in their structure (Prado et al., 2008). Cereals, for example, may be an excellent substrate for the growth of probiotics, and they can also be used as prebiotics for the presence of nondigestible carbohydrates (Charalampopoulos et al., 2002).

Strains of *Lactobacillus* have been used in this regard for the production of a beverage made of single and mixed fermented cereals. The microorganisms survived producing a large amount of lactic acid.

However, this large production of lactic acid and other organic acids shows that the enrichment of food with probiotics can have a negative effect on its sensory characteristics. Therefore, in order to improve both the survival of the microorganisms and also the sensory characteristics of the food, it is necessary to conduct further studies (Rathore et al., 2012).

A yogurt-like beverage made of a mixture of cereals (rice, barley, emmer, and oat) and soy flours and concentrated red grape must was produced adding two probiotic selected strains of *L. plantarum* (6E and M6). Beverages processed with the mixture of rice and barley or emmer flours were preferred in terms of textural, nutritional, and sensory properties (Coda et al., 2012). Ten panelists evaluated the product and found that the drink had a very intense fruity smell, a dark color, and a very sour taste, with a grainy texture (Coda et al., 2012).

Rößle et al. (2010) reported a study carried out on fresh-cut apple slice wedges enriched with a probiotic microorganism (LGG) and prepared by dipping the fruit in an edible buffer solution containing 10^{10} CFU/mL of LGG. The results showed that dipping apple wedges in a probiotic solution gives the product good physicochemical and sensory characteristics of an acceptable quality and with an adequate number of LGG adsorbed into the surface for a probiotic effect.

One aspect that must not be neglected in a food with probiotics is the survival of microorganisms. In fact, food with probiotics must contain high doses of viable microorganisms throughout the shelf life of the product.

Not all probiotic strains added to fruits and vegetables give good results in terms of survival. The selection of strains of plant origin can help to overcome this and other technological challenges (Karasu et al., 2010). In fact, numerous factors influence microbial growth, such as salt, acidity, and pH. Some strains of LAB, isolated from fermented vegetables and fruits, can be used as probiotics, because they are able to resist high levels of acidity and NaCl during the storage period (Alzamora et al., 2005; Betoret et al., 2003; Fleming and McFeeters, 1981; Renadheera et al., 2010). Microorganisms isolated from fermented vegetables usually belong to the genera *Lactobacillus*, *Leuconostoc*, and *Pediococcus*. Some of these have a resistance to acidic juices of the stomach and to bile and are comparable with the probiotic strains isolated from humans and animals (Chiu et al., 2008; Fleming and McFeeters, 1981; Higashikawa et al., 2010). Potentially, probiotic microorganisms are isolated from many fermented vegetables. Often, these microorganisms produce important compounds to ensure the microbiological safety of food. Some microorganisms isolated from sauerkraut produce bacteriocins (nisin produced by *Lactococcus lactis*) (Harris et al., 1992). Other microorganisms isolated from cucumbers (Atrih et al., 1993) produce bacteria plantaricin C19, which is active against *Listeria grayi*, and those isolated from olives (Delgrado et al., 2005; Mourad and Nour-Eddine, 2006) are producers of bacteriocins, which are active against *Weissella mesenteroides*. Moreover, *Lactobacillus* strains that produce bacteriocins active against *E. coli*, *Staphylococcus aureus*, and *Bacillus cereus* have been isolated from carrots (Joshi et al., 2006). In vivo tests on mice have shown that strains of *L. plantarum* and *Pediococcus pentosaceus* isolated from pickled cabbage have an inhibiting effect upon *Salmonella*. *L. plantarum* strains and *L. acidophilus* have also proved to be good at adhering to the intestinal walls and showing resistance to gastric juices and bile salts (Chiu et al., 2008; Wang et al., 2010).

These results led to the conclusion that the strains isolated from spontaneous plant fermentations can be used as probiotics, having similar characteristics to those of the strains isolated from dairy products. Furthermore, the presence of proteins, minerals, fiber, and other nutrients promotes the survival of microorganisms, introducing a new concept of symbiotic food (Kalui et al., 2010).

Also soybean can be used as a carrier of probiotic microorganisms. Soybean-based foods (oil, milk, and soy cheese) are rich in protein and can be used by vegetarians instead of meat, avoiding high cholesterol levels in blood, preventing osteoporosis, and reducing the risk of contracting certain cancer diseases. Moreover, these products have an unpleasant taste, are rich in antinutritional factors such as phytates and enzyme inhibitors, and contain raffinose and stachyose in high concentrations that can cause flatulence. The fermentation process can enhance the flavor, nutritional characteristics, and digestibility as well as prolong the shelf life of the product (Rivera-Espinoza et al., 2010). In particular, pentanal and n-hexanal are responsible for the undesirable beany flavor of soymilk; a strain of *B. breve* and some strains of *L. acidophilus* are able to metabolize these compounds in fermented soya products (Champagne and Gardner, 2005).

Probiotic microorganisms (*L. acidophilus MJLA 1*, *L. rhamnosus 100-C*, *L. paracasei* ssp. *paracasei 01*, *B. lactis BBDB2*, *B. lactis BB-12*) have been also added into a nonfermented frozen vegetarian soy dessert. The results highlighted that frozen soy dessert is a suitable probiotic food, with an excellent viability of probiotic strains and acceptable sensory characteristics (Heenan et al., 2004).

A further study has also shown enhancement of the isoflavones in soymilk supplemented with lactose by using probiotic bacteria of the *Lactobacillus* and *Bifidobacterium* genus during extended fermentation (Ding and Shah, 2010).

10.3.1 Probiotic Fermented Beverages

Fermented milk and drinks processed from fermented milk are among the most widely used vehicle for probiotics, but also drinks based on fruit and vegetables have been tested as plausible vehicles for probiotics, representing a new technological challenge. Fruit juices are potentially good vehicles of probiotics. They are generally used by a large segment of the population, are appreciated and consumed by individuals of all ages, and are often perceived as healthy (sometimes with added vitamins and minerals) as well as refreshing beverages (Luckow and Delahunty, 2004; Suomalainen et al., 2006). The viability of probiotic microorganisms in food matrices depends on several factors, such as storage temperature, oxygen levels, pH, and the presence of competitor microorganisms, which must all be carefully evaluated before they are added to foods (Gupta and Abu-Ghannaman, 2012). Moreover, it is important to consider food matrix; *L. acidophilus* is reported to grow much better on soymilk than on cow's milk (Champagne and Gardner, 2005).

Also in fruit and vegetable juices, the tolerance to acidity is particularly important. These juices are already naturally acidic; the fermentation process increases the acidity. Yoon et al. (2006) have reported a study regarding the ability of *L. plantarum*, *L. delbrueckii*, and *L. casei* to survive in cabbage juice. It was found that three strains of *L. plantarum* and *L. delbrueckii* are able to grow and maintain good levels of vitality during several weeks of storage, resisting to the low pH, high acidity, and to refrigerated storage at 4°C, with exception of *L. casei*, which loses its vitality after 2 weeks of storage.

In orange, pineapple, and cranberry juices, LGG, *L. casei*, *B. lactis*, *Lactobacillus salivarius* ssp. *salivarius*, and *L. casei* and *L. paracasei* have shown high tolerance to pH, after being kept for 12 weeks at levels of 10^6 CFU/mL. All these strains are often used in the dairy industry for their technological strength. In this case, although all microorganisms have given good results, *L. paracasei* NFBC 43338 is the best in terms of tolerance to acidity, resistance to pasteurization treatments, resistance to pressure, and resistance to refrigerated storage (Sheehan et al., 2007). Also vegetable juices have been tested to assess their suitability to be used as a carrier of probiotics. The carrot is naturally rich in functional components (vitamins and minerals). Carrot juice seems to be suitable for the growth of *B. lactis* and *Bifidobacterium bifidum* without the addition of other nutrients, remaining viable at around 10^8 CFU/mL up to 24 h. Carrot juice is rich in sucrose, glucose, and fructose, possible sources of carbon for bifidobacteria. However, in this case, only glucose and sucrose have been used by microorganisms. The content of fructose remained almost unchanged, indicating that for bifidobacteria this sugar does not constitute a source of nourishment. The slight change in carotenoid content is, perhaps, due to the metabolic activity of microorganisms or to changes in pH, caused by the production of lactic acid, and temperature occurring during the fermentation process. In any case, the degradation of carotenoids was not excessive and the nutritional value of the product has not been affected (Kun et al., 2008).

In cantaloupe melon juice, *L. casei* B-442 survived in high concentrations, due to the fact that this juice contains high levels of fermentable sugars, like sucrose and fructose. In fact, the *L. casei* strain can grow and develop without the addition of other sugars. Moreover, the microorganisms of the genus *Lactobacillus* need amino acids and peptides derived from nucleic acids, vitamins, and fermentable carbohydrates; therefore, even if the cantaloupe melon juice contains little protein, it is rich in amino acids, such as aspartic acid, glutamic acid, arginine, and alanine that favor the growth and survival of probiotics (Fonteles et al., 2012).

Tomato and tomato-based products can be considered as a healthy beverage, being a source of lycopene, vitamins, and antioxidant. It has been reported that the probiotic strains of *L. casei*, *L. acidophilus*, *L. plantarum*, and *L. delbrueckii* are resistant to low pH during storage at 4°C, surviving at levels of 10^6–10^8 CFU/mL in tomato juice. In tomato juice enriched with FOS, low pH did not influence the lycopene content, nor are its chemical properties modified. The addition of FOS also had a positive influence on the sensory characteristics of the product. In fact, in the juice enriched with FOS, the redness increased significantly; furthermore, after an analysis of sensory characteristics, it was observed that the flavor had improved while the off-flavors decreased with the addition of FOS (Koh et al., 2010). Similar results were also obtained for other juices. In pomegranate juice, *L. plantarum* and *L. delbrueckii* survived at their maximum levels (2.9–3.9 × 10^8 CFU/mL) for 2 weeks of storage at 4°C with the number of microorganisms decreasing after 4 weeks; *L. paracasei* and *L. acidophilus* lost their viability after the second week in the same conditions (Mousavi et al., 2011).

A fermented beverage made from whole-grain oat was produced using *L. plantarum* B28. The product, obtained after fermentation for 8 h and stored at 4°C–6°C, was analyzed in order to determine the beta-glucan content, naturally present in oat. Moreover, the microbial count has been performed to determine the viability of the probiotic microorganism in the beverage, and finally, the effect of adding different sweeteners on microbial growth was studied. During fermentation, the beta-glucan content remained unchanged, indicating that the microbial culture did not ferment beta-glucan. The viable cell counts at the end of the storage were 10^6–10^7 CFU/mL.

In order to sweeten the product, making it suitable for consumption by diabetics, several sweeteners, such as sucrose, aspartame, sodium cyclamate, and saccharin, have been added to the probiotic drink. It was found that the sweeteners did not affect the fermentation kinetics nor the viability of the probiotics. The shelf life of this beverage was estimated at 21 days of chilled storage (Angelov et al., 2006).

L. acidophilus was successfully added also to beetroot juice; in this case, the microorganism not only maintained good vitality, but the juice obtained was also better in terms of the pigment, vitamin, and mineral content, compared with the control without probiotic (Rakin et al., 2007).

In Africa and Asia, many fermented cereal-based foods have been produced by using lactic acid bacteria. It has been observed that the fermentation process improves the flavor and aroma of the product (Kalui et al., 2010).

In cashew apple juice, at the end of the cold storage period (42 days), the viable count of probiotic *L. casei* NRRL B-442 was higher than 8.00 log CFU/mL, without important loss of organism viability throughout the storage period. Moreover, the juice was stable without any modification of the characteristic yellow color. In addition, the activity of the enzyme characteristic of the fruit, and responsible for browning, was well controlled without the addition of chemical compounds, such as sodium metabisulfite (Pereira et al., 2011).

However, in some cases, the addition of probiotic microorganisms can negatively affect the sensory characteristics of the food. Luckow et al. (2006) have reported the negative effect that the addition of probiotic microorganisms (*L. paracasei* spp. *paracasei* NFBC 43338, LGG, *L. casei* DN-114001) had on orange juice. The consumers have defined the juice with dairy, metallic, medicinal odor and with bitter, acid, cooked flavor. However, exposure to and familiarity with probiotic drinks, in addition to specific information regarding the health benefits of probiotic ingredients, helps improve consumer acceptance and liking for the sensory characteristics of probiotic juices.

Another possible obstacle to the use of probiotics in fruit juices is an excessively low pH. Fruit and vegetable juices are already acids; in addition, the fermentation process increases the acidity. A useful method for raising the pH of the juice is with the addition of milk ingredients (Sheehan et al., 2007).

Furthermore, it is necessary to implement strategies that help to preserve the viability of probiotic microorganisms, such as the addition of prebiotic components. Prebiotic ingredients extracted from fermented cashew apple juice positively influence the growth of *L. mesenteroides* and *L. johnsonii*. The growth of these two microorganisms is better in apple juice with oligosaccharides than that observed in juice containing only glucose and fructose as carbon sources (Vergara et al., 2010).

Therefore, several studies have shown that the use of probiotics in foods processed from fruits or vegetables is possible. However, the use of protective barriers and microencapsulation for preserving these microorganisms is recommended.

Tables 10.1 and 10.2 summarize the main results regarding the technological and sensory effects of probiotics and prebiotics in fruit- and vegetable-based foods.

TABLE 10.1

Technological and Sensory Aspects That Emerged from the Addition of Probiotics and Prebiotics in Fruit-Based Foods

Microorganisms	Food Products	Technological and Sensory Aspects	References
L. paracasei ssp. *paracasei* NFBC 43338, LGG, *L. casei* DN-114 001	Orange juice	The addition of probiotics in the juice had negative effects. The consumers have defined the juice with dairy, metallic, medicinal odor and with bitter, acid, cooked flavor.	Luckow et al. (2006)
L. johnsonii B-2178	Cashew apple juice	The growth of the probiotic microorganism was threefold higher in apple juice with oligosaccharides than that observed in the juice containing only glucose and fructose as carbon sources.	Vergara et al. (2010)
LGG, *L. acidophilus* NCFM (free cells and encapsulated)	Orange juice and fruit snack	The samples with encapsulated organisms have shown a pH higher than samples free from microorganisms. This aspect could be useful to reduce acidification and possible negative sensory effects in orange juice and fruit snack.	Sohail et al. (2012)
L. casei DN 114001, LGG, *L. paracasei* NFBC 43338, and *B. lactis* Bb-12	Orange, pineapple, and cranberry juices	Although all microorganisms gave good results, *L. paracasei* NFBC 43338 was the best in terms of tolerance to acidity, resistance to pasteurization treatments, resistance to pressure, and refrigerated storage.	Sheehan et al. (2007)
LGG	Fresh-cut apple slices dipped in an edible solution containing a probiotic	Dipping apple wedges in probiotic solution imparts to the product good physicochemical and sensory characteristics, with acceptable quality and with adequate numbers of LGG adsorbed onto the surface for a probiotic effect.	Rößle et al. (2010)
L. casei NRRL B-442	Cantaloupe juice	*L casei* B-442 survived in high concentrations. The reason for this is the high content of fermentable sugar that contains the juice of cantaloupe like sucrose and fructose. An enhancement of the isoflavones in soymilk supplemented with lactose during extended fermentation has been observed.	Fonteles et al. (2012)
L. casei NRRL B-442	Cashew juice	During the 42-day storage, any loss of organism viability was not observed and the juice was stable without any modification of the characteristic yellow color.	Pereira et al. (2011)
L. casei, *L. paracasei*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii*	Pomegranate juice	*L. plantarum* and *L. delbrueckii* were capable to survive for 2 weeks of storage at 4°C at their maximum levels (2.9–3.9 × 10^8 CFU/mL), decreasing the number of microorganisms after 4 weeks; *L. paracasei* and *L. acidophilus* failed their viability after the second week in the same conditions.	Mousavi et al. (2011)

TABLE 10.2

Technological and Sensory Aspects That Emerged from the Addition of Probiotics and Prebiotics in Vegetable-Based Foods

Microorganisms	Food Products	Technological and Sensory Aspects	References
L. plantarum 6E and M6	Yogurt-like beverage made of a mixture of cereals (rice, barley, emmer, and oat) and soy flours and concentrated red grape must	Beverages processed with the mixture of rice and barley or emmer flours were preferred in terms of textural, nutritional, and sensory properties.	Coda et al. (2012)
L. plantarum B28	Fermented beverage based on oat	*L. plantarum* gave good results in terms of survival of microorganisms and sensory characteristics during all the period of storage.	Angelov et al. (2006)
Bifidobacterium strains: *B. lactis*, *B. bifidum*	Pure pasteurized carrot juice	The microorganisms remained viable at around 10^8 CFU/mL up to 24 h. The addition of probiotic leads to a production of lactic acid but without excessive degradation of carotenoids, not affecting the nutritional value of the product.	Kun et al. (2008)
L. casei A4, *L. delbrueckii* D7, and *L. plantarum* C3	Cabbage juice	*L. plantarum* and *L. delbrueckii* were able to grow and maintain good levels of vitality during several weeks of storage, resisting to the low pH, high acidity, and refrigerated storage at 4°C. *L. casei*, however, has proved weaker and therefore has lost its vitality after just 2 weeks of storage.	Yoon et al. (2006)
L. acidophilus MJLA 1, *L. rhamnosus* 100-C, *L. paracasei* ssp. *paracasei* 01, *B. lactis* BBDB2, *B. lactis* BB-12	Nonfermented frozen soy dessert	Frozen soy dessert is a suitable probiotic food, with an excellent viability of probiotic strains and acceptable sensory characteristics.	Heenan et al. (2004)
L. plantarum NCIMB 8826 and *L. acidophilus* NCIMB 8821	Malt beverage	The considerable production of lactic acid and other organic acids has highlighted the possibility of a negative sensory effects in product enriched with probiotics.	Rathore et al. (2012)
L. casei, *L. acidophilus*, *L. plantarum*, *L. delbrueckii*	Tomato juice	In tomato juice enriched with FOS, low pH did not influence the lycopene content, nor are its chemical properties modified. The addition of FOS also had a positive influence on the sensory characteristics of the product. In fact, in the juice enriched with FOS, the redness increased significantly; furthermore, it was observed that the flavor had improved while the off-flavors decreased with the addition of FOS.	Koh et al. (2010)
Lactobacillus and *Bifidobacterium* strains	Soymilk	An enhancement of the isoflavones in soymilk supplemented with lactose during extended fermentation has been observed.	Ding and Shah (2010)
L. acidophilus	Beetroot juice	The microorganism maintained a good vitality. The product with probiotic was found better in terms of pigments, vitamins, and minerals content with respect to the control.	Rakin et al. (2007)
LAB	Fermented cereal-based food	The fermentation process improves the flavor and aroma of the product.	Kalui et al. (2010)

10.4 Microincapsulation

Stability, survival, and resistance to gastric juices and bile salts are essential for probiotic microorganisms, since these qualities can influence their beneficial properties. The therapeutic value of probiotic bacteria generally depends on the viability of these microorganisms. However, the industrial processes used for food preparation, such as freezing, storage temperatures, values of acidity, and oxygen content of food matrices, are not always optimal to ensure the functionality of probiotics (Heidebach et al., 2012).

In order to enhance probiotic survival, microencapsulation successfully creates physical protection during food storage and favors the passage of probiotic through the digestive tract. Microencapsulation is defined as "the technology of packaging of solid, liquid or gaseous materials miniaturized in capsules that can release their contents in a controlled manner and only under certain conditions" (Burgain et al., 2011). Two types of capsules are available:

1. The reservoir type, in which a shell covers the encapsulated substance
2. The matrix type, in which the active agent is dispersed within and on the surface of a support material

A third type of capsule derives from the combination of these two types, in which the active material is coated but also is dispersed within and on the surface of a matrix (Burgain et al., 2011).

The capsule containing the microorganisms is composed of a substance that forms a thin, yet resistant, semipermeable membrane, to protect the content during the passage into the stomach.

Different materials can be used for various microencapsulation techniques: polysaccharides derived from seaweed (carrageenan and alginate), plants (starch and gum arabic), substances of bacterial origin (gellan and xanthan gum), and animal protein (casein). The microencapsulation methods are described in the following.

Extrusion method. This is a method that allows to perform the encapsulation causing minimal damage to the microbial cells. The first step consists of adding probiotics to a hydrocolloid solution, and then the solution is dripped through a syringe needle or nozzle. The dimensions of the nozzle affect the diameter of the microcapsules. Encapsulation materials that are usually used for this technique are alginate, k-carrageenan and k-carrageenan plus locust bean gum, xanthan plus gellan, alginate plus corn starch, and whey proteins (Huq et al., 2013).

Emulsion method. Unlike the extrusion technique, in this case, the diameter of the beads can be controlled and is lower (25 μm–2 mm). This method requires the use of a vegetable oil (soy oil, sun flower oil, corn oil, or millet or light paraffin oil) for emulsion formation. The oil is mixed with a small amount of cell/polymer slurry and is agitated to form an emulsion. The polymer becomes insoluble in solution with the addition of calcium chloride and forms gel particles in the oil phase. The use of emulsifiers that guarantee the formation of beads with a smaller diameter is also possible (Huq et al., 2013).

Drying method. The drying of the encapsulated mixture can be performed by various methods: freeze-drying, spray drying, and fluidized bed drying. Generally, the rate of survival of microorganisms with these techniques is in the range of 70%–85%. However, it is complicated to maintain long product stability. The technique of freeze-drying due to minimal cellular injury but it's necessary to use a cryoprotective agent to protect cells from cold damage. Spray drying allows to treat large volumes of solutions; the technique is relatively cheap, but the loss of viability of the cells is considerable due to the phenomena of heating and dehydration. Best results were obtained by changing the spray-drying technique. The method consists of milk fat droplets containing powder coating particles of freeze-dried cells with polymers of whey proteins, in a condition where an emulsifier is used (Huq et al., 2013). Picot and Lacroix (2004) have reported that a valid method, on the industrial scale with respect to cell viability and economics, is dispersing cells of *Bifidobacterium* spp. in a suspension of whey protein containing milk fat droplets and then proceeding with the spray drying.

Irrespective of the technique used for encapsulation (extrusion, emulsion, or spray drying), microencapsulation is one of the most effective techniques for the defense of probiotic viability.

The success and the validity of the microencapsulation technique is documented by a series of studies showing that there is genuine preservation of the vitality of microorganisms compared to the products in which the probiotics are free (Kebary et al., 1998; Rokka and Rantamaki, 2010; Saxelin et al., 2010; Shah and Ravula, 2000). Microentrapment in gel beads has shown to be very effective to maintain the probiotic viability of frozen foods (Champagne and Gardner, 2005).

Even microencapsulation technology applied to fruits or vegetables has produced good results. Microencapsulation, with alginates and chitosan, protects microorganisms from inhibitors, such as acids and flavonoids (Koo et al., 2001), allowing high levels of vitality and good efficiency during fermentation of the encapsulated bacteria. K-carrageenan and Ca alginate were used successfully for the immobilization of *L. acidophilus* in banana puree (Tsen et al., 2003) and in tomato juice (King et al., 2007; Tsen et al., 2008). In particular, in the study reported by King et al. (2007), sodium alginate and calcium chloride were used to form the immobilizing matrix for the entrapment of cells of *L. acidophilus*. The results showed that the viability of encapsulated microorganisms is higher in tomato juice compared with free cells. Moreover, the juice containing the encapsulated cells was also more pleasant than the juice containing the free cells. This is probably due to the higher viable cell number of immobilized cells and because unfavorable deterioration reaction were inhibited during storage. Excellent results in terms of survival and sensory characteristics of the product formulated with microencapsulated probiotic *L. acidophilus* have been also reported by Tsen et al. (2008) for tomato juice.

The size of the capsules (generally ranging from 1 to 5 μm diameter) is particularly significant. The diameter of the capsules should be wide enough to ensure the protection of the bacterial content but, at the same time, sufficiently small not to create negative changes of the sensory characteristics of the food. Indeed, sensory studies have shown that high concentrations of spherical particles in the food product give sensations of roughness and graininess, which are disfavored by the consumer (Engelen et al., 2005; Imai et al., 1995).

The encapsulated organisms are often added to yogurt and cheese, with a consequent evident increase in the viability of the microorganisms during the storage period. Nevertheless, the protective effect is only obtained with capsules of 0.2–3 mm. With capsules of this size, the negative impact on the sensory characteristics of the product is guaranteed.

A new technique for the encapsulation uses a separate impinging aerosol of sodium alginate solution and calcium chloride cross-linking solution to produce water-insoluble cross-linked alginate microbeads with an average diameter less than 40 μm (Sohail et al., 2012). This technique does not require the use of heat or solvents; therefore, it can also be used for heat- or solvent-sensitive materials. This technique has been used for encapsulation of LGG and *L. acidophilus* NCFM in orange juice, with a pH of 3.80, and in pear- and peach-based fruit snacks. From this study, the following important aspects have emerged:

- Microencapsulation protects the viability of LGG; however, also, free cells have shown a propensity for survival. Conversely, this technique of encapsulation did not favor *L. acidophilus* NCFM survival. Therefore, it is clear that the ability of microorganisms to survive in food matrix largely depends on the characteristics of the strain; the microorganism *L. rhamnosus* is suitable for growth in acidic environments even without protective techniques of microencapsulation, while *L. acidophilus* is decidedly more sensitive.
- Another aspect that emerged regards the effect of microencapsulation on the variation of pH in the food matrix. In a comparison of the pH values of orange juice and fruit-based products produced with free and encapsulated probiotic microorganisms (LGG, *L. acidophilus* NCFM) throughout a 9-day storage period, it was found that the encapsulated microorganisms did not induce as much lowering of pH as free microorganisms present in the food matrix (Sohail et al., 2012). Therefore, microencapsulation in alginate beads could be effective in reducing acidification and could improve the sensory properties of fruit-based products.

Even if microencapsulation may be a good technique for protecting the viability of microorganisms, there are still many obstacles to overcome to improve encapsulation techniques:

- Protection from stress acids must be ensured.
- The capsules should be digested in the intestine so that the content can be released and our health can benefit.
- The capsules must have optimal dimensions in order to serve as valid barriers, but at the same time they should not disturb the palate of the consumer.
- The technical choices for the encapsulation must not reduce the number of live cells or cause sublethal damage in humans.

At the moment, no encapsulation technique can fulfill all these requirements (Heidebach et al., 2012).

10.5 Challenges for Probiotic Foods Formulation

Although research has made great strides, there are many challenges that must be addressed when deciding to make a food that contains probiotics. For example, a consumer who buys a food with probiotics, rightly, expects that food maintains its characteristics unchanged throughout the shelf life; this includes that the microorganisms must remain viable during this period. Different aspects have to be considered: the characteristics of the food matrix, the effects of storage conditions of foods on the microorganisms, and the choice of microbial strains to be used in different foodstuffs. The choice of the probiotic strain is a crucial step. The pH value seems to be a critical factor in the stability of probiotic strains during storage; *L. acidophilus* cultures are generally more resistant to acid environments than bifidobacteria. Moreover, it is also necessary to take into account the fact that a microorganism capable of withstanding the acid pH of a food may not be able to resist the acid pH of the stomach. In fact, the two aspects are not always correlated. For example, some microorganisms, such as *L. lactis*, *S. thermophilus*, and *L. mesenteroides*, grow well in foods with acidic pH, but are not able to grow in the gastrointestinal tract, thus losing their function of probiotics. Also, the time of exposure to low pH is an important aspect. A strain able to resist to the acid pH of the stomach may not be able to resist the acid pH of the product during the storage period. At the moment, it is not well known if there is a link between the ability to survive short-time exposures to high-acid environment and the ability to survive long term in fermented products. In this regard, Chavárri et al. (2010) reported a study on microencapsulation of probiotic microorganisms in simulated gastrointestinal conditions. In this study, chitosan was used as a coating material to improve encapsulation of *Lactobacillus gasseri* and *B. bifidum* in calcium alginate beads. The encapsulation in this type of microspheres significantly increased the viability of the microorganisms. In particular, it was found that the microorganisms encapsulated in chitosan-coated alginate beads are able to withstand the conditions of the gastrointestinal tract, maintaining throughout the exposure time (2 h) a viability of 95%. The present study showed that chitosan is a great ingredient that increases the stability of alginate microcapsules in adverse conditions. The presence of chitosan reduces the porosity of the capsules of alginate and decreases the leakage of the encapsulated probiotic, and accordingly probiotics are more stable during the passage through the gastrointestinal tract.

Another aspect to be considered is the effect that other microorganisms present in the medium may have on probiotics. Probiotics, for example, are particularly sensitive to the presence of oxygen. Probably, the toxic effect of oxygen is due to the accumulation of intracellular hydrogen peroxide favored by the presence of oxygen. Some microorganisms are capable of producing hydrogen peroxide and drop it in the middle, so the presence in the microorganism with this attitude may adversely affect the survival of the probiotic. In this case, it could be useful to replace the microorganism that produces hydrogen peroxide with one that does not. In fermented milks, the elimination of *L. delbrueckii* ssp. *bulgaricus* has had positive effects on the survival of *L. acidophilus*. Another possibility would be to add to the food antioxidants such as ascorbic acid. From these considerations, it is clear that other studies to assess what is the best combination of microorganisms to be used for the production of probiotic foods are still needed (Champagne and Gardner, 2005). Also, the presence of starter cultures in foods used for technological purposes (acidification, flavor, texture) can represent an obstacle for the growth of probiotic strains; the common strategies to reduce competing lactic cultures and to improve the growth or survival of probiotic cultures are the omission of a portion of the starter strains and changes in the respective inoculation rates; in fermented soya milk inoculated with lactic acid starter and bifidobacteria probiotics, this problem has been overcome by the addition of cysteine in the product (Champagne and Gardner, 2005).

Also, food processes that include a heating step above 65°C are highly detrimental for probiotic viability; therefore, it is necessary to add the cultures after thermal treatment. Moreover, microencapsulation of the dried probiotic cultures with lipids could represent a way of protecting the cells against heat treatment (Champagne and Gardner, 2005).

Another important aspect to consider is the dose of microorganisms to be added to the food in order to guarantee the colonization of the gastrointestinal tract. The minimum recommended level of probiotic in foods is 10^6 CFU/g of food product or 10^7 CFU/g at point of delivery or be eaten in adequate amounts to yield a daily intake of 10^8 CFU/g (Chávarri et al., 2010). What still needs to be considered is the effect of the composition of the food on the viability of probiotics. It is difficult to determine the exact number of microorganisms; at the moment, the best approach is still to be based on scientific studies conducted on specific foods (Champagne and Gardner, 2005).

10.6 Conclusions

The market of functional foods is growing; this growth is fueled by an increased level of consumer attention to diet. The consumption of fruits and vegetables is generally associated with the idea of well-being, as these foodstuffs are rich in minerals and vitamins, antioxidants, and fiber; moreover, the addition of probiotic microorganisms enhances the quality of these foodstuffs. In some cases, fruits and vegetables also represent a natural source of prebiotics, which have a protective function toward probiotic microorganisms, preserving their vitality during shelf life of the product. Therefore, fruits and vegetables create a new concept of *symbiotic food*. Several studies have shown that foods made with fruits and vegetables can be successfully used as carriers of probiotics, maintaining good levels of viability of probiotic microorganisms and good sensory characteristics. Microencapsulation of microorganisms can contribute to the maintenance of the functionality of food. However, as shown in this chapter, not all organisms show the same behavior under certain conditions. Therefore, in addition to using protective techniques such as microencapsulation, it is necessary to have a thorough understanding of the food and the physiological characteristics of the probiotic strain. There are still many challenges ahead, and in any case, the choice of probiotic strain to be used in food is essential.

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