The Probiotic Bacteria Viability under Different Conditions

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Abstract
This review summarized the current knowledge on probiotics and on the effects that different conditions have under this type of bacteria.

The purpose of this review was to evaluate the survival rate/resistance or viability of different probiotic bacteria under several conditions, such as: processing, food composition, storage, freezing, thawing, refrigeration, temperature, oxygen, pH, gastrointestinal environment and package.

Nowadays, the demand on probiotic functional foods is increasing rapidly, as the consumers became more aware about the potential health benefits, due to the fact that probiotics help in maintaining the balance and composition of intestinal flora and protect it from pathogens. A daily ingestion of $10^8$–$10^9$ CFU ml⁻¹ probiotic microorganisms is crucial in order to be able to demonstrate an effect in our organism, considering the dose and the effect of storage/gastrointestinal environments on the probiotic viability.

Microencapsulation of probiotics in different polysaccharides was proven to be an ideal way to preserve and protect the cells from detrimental factors during processing, storage or resistance in the gastrointestinal transit, as many studies demonstrate it.

There is a general interest in the improvement of the physical and mechanical stability of the polymers used in probiotics encapsulation, to ensure high population of probiotics not only in food during storage, but also after gastrointestinal digestion. Also, the carrier plays a very important role and should be carefully examined.

Keywords: conditions, microencapsulation, probiotic bacteria, survival, viability

INTRODUCTION: PROBIOTICS

Probiotics have been extensively studied for their claimed health benefits. According to the currently international FAO/WHO definition, generated by a group of experts, probiotics are: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO, 2001). The Ministry of Health from Italy has defined probiotics as "microorganisms which, once ingested in adequate amounts, have beneficial effects on the organism" essentially resuming the above definition (Italy Ministry of Health, 2001). It should be noticed that the FAO/WHO definition does not state the human origin of the bacterial strain, therefore it is not a criterion for definition of probiotics and, instead, it is underlined the type of effect caused (FAO, 2002). This aspect, the switch from scientific to regulatory, has led to consider the term “probiotic” which in its original definition implies a health benefit, as a real “health claim” in accordance to Regulation (EC) no.1924/2006;

Probiotics, either as a food matrix fermented by a “beneficial” bacterium, either as a “concentrated” bacterial supplementation of diet as it was described from tradition, can provide consumers with live bacteria that are able to pass the gastrointestinal environments and to reproduce themselves in the large intestine. The term probiotic should be used only for products containing living and vital cells. As it is well stipulated in the AFSSA (2005) (Agence Francaise de Sécurité Sanitaire...
des Aliments) that “The quantity of probiotics passing live through the gut depends on the strain, the dose ingested, factors related to the host and the vector food”, different strains of the same species may exert different effects on the host or strains of the same species can exert different and sometimes opposite actions.

In order to have beneficial health effects, the food products enriched with probiotics should contain the required minimum viable microorganisms when consumed. It has been agreed by the responsible ones from the food industry that the minimum recommended level of probiotics should be $10^6$ CFU ml$^{-1}$ at the time of consumption (Boylan, Vinderola, Ghodusi and Reinheimer, 2004; Kailasapathy and Rybka, 1997). A daily ingestion of $10^8–10^9$ CFU ml$^{-1}$ probiotic microorganisms is crucial in order to be able to demonstrate an effect in our organism, considering the dose and the effect of storage on the probiotic viability (Knorr, 1998). It has also been shown that an approximate amount of 100 g of probiotic food per day should be consumed in order to deliver $10^9$ viable cells into the intestine (Karimi, Mortazavian and Cruz, 2011).

The International Scientific Association for Probiotics and Prebiotics (ISAPP, 2014) has settled that “Prebiotics target the microbiota already present within the ecosystem acting as a ‘food’ for the target microbes seen as beneficial.”

Nowadays, the demand on probiotic functional foods is increasing rapidly, as the consumers became more aware about the potential health benefits, due to the fact that probiotics help in maintaining the balance and composition of intestinal flora and protect it from pathogens. The food products used as source of probiotics are the fermented milks flavored with juice, ice creams, cheese, yoghurts, chocolate, chewing gum, oat or soy-enriched milk, infant formula, and fermented meats (Pennachia et al., 2006; Sendra, 2008).

The most commonly used probiotic bacteria belong to the *Bifidobacterium*and *Lactobacilli* species, which are also dominant in the human intestine (Lactobacilli in the small intestine and Bifidobacterium in the large one).

Probiotics use prebiotics as a “food”, which are dietary fibers that occur naturally in foods, but in order to have prebiotic effects the intake should be large, consequently, it is more convenient to fortify food stuffs with a defined amount. The most popular prebiotics are fructans and resistant starches (Sendra et al., 2010).

### EFFECTS OF PROCESSING, STORAGE AND FOOD COMPOSITION ON PROBIOTIC BACTERIA VIABILITY

It is crucial that the foods containing probiotics maintain the number of bacteria (over 7 log CFU/g), till the end of shelf life, as there are several environmental factors than can affect the viability of probiotics, such as acidity, oxygen stress, storage temperature, co-culture competition, osmotic pressure, moisture content, etc; (Stanton et al., 2003).

Scientists use advanced technology to stabilize the viability of probiotics during processing and storage, the most common technique being the encapsulation method (spray-drying, extrusion, emulsion, and phases separation) (Ananta et al., 2004).

As outlined before, there are a lot of factors during production, processing and storage that affect the number of viable or active probiotics cells per gram that can trigger the beneficial effects. (Korbekandi, Mortazavian & Iravani, 2011). They include food parameters (pH, titratable acidity, molecular oxygen, water activity, presence of salt, sugar and chemicals like hydrogen peroxide, bacteriocins, artificial flavoring and coloring agents); processing parameters (heat treatment, incubation temperature, cooling rate of the product, packaging materials and storage methods scale of production); microbiological parameters (strains of probiotics, rate and proportion of inoculation). When probiotic cells are present in low pH environments (<4.5), a lot of energy is required to maintain the intracellular pH, resulting in an insufficient quantity of ATP necessary for other critical functions and thereby causing cell death (Nualkaekul, Salmeron & Charalampopoulos, 2011). In addition, the presence of oxygen can cause formation and accumulation of toxic metabolites in cells, which can lead to cell death by oxidative damage (Boza-Mendez et al., 2012; Talwalkar and Kailasapathy, 2004).

Microencapsulation of probiotics in different polysaccharides, was proven to be an ideal way to preserve and protect the cells from detrimental factors during processing, storage or resistance in the gastrointestinal transit, as many studies demonstrate it. (Anal and Singh, 2007; Burgain et al., 2006; Champagne and Fustier, 2007; Heidebach...
et al., 2010; Mohammadi et al., 2011; Vodnar et al., 2010; Vodnar et al., 2014; Wenrong and Griffiths, 2000).

Concerning food composition, the matrix to be supplemented with probiotics plays an important role. For instance, the process of juice supplementation is more complicated than the process of dairy products supplementation. A possible explanation could be the insufficient quantities of peptides and free amino acids present in juice which are necessary for the metabolism of probiotic cultures (Sheehan et al., 2007). Moreover, probiotic cultures may change juices sensory characteristics (Granato et al., 2010). The required minimal concentration of protein to maintain probiotic viability is 0.3% (Nualkaekul, Salmeron and Charalampopoulos, 2011). Michael et al. (2010) reported that supplementation of yogurt with 0.014% and 0.028% L-cysteine (Cys) maintained higher viability of L. bulgaricus (46 log cfu g⁻¹) than non-Cys-supplemented yogurt during 50 days of storage.

In a previous study was detected that enrichment of yogurt with flavoring agents such as strawberry, vanilla, peach and bananas extracts did not affect the growth of the probiotic bacteria in the optimum concentrations commonly used in dairy industry (Vinderola et al., 2002).

High nutritional value in carbohydrates, salts, minerals, dietary fiber, vitamins, fatty acids, aminoacids and protein concluded that increase the viability of B. bifidum (7.10 log cfu g⁻¹) in supplemented yogurt enriched with 2% of date syrup compared to plain yogurt (6.81 log cfu g⁻¹) during 10 days of storage (El-Nagga and Abd El-Tawab, 2012).

Zarea et al. (2012) reported that cereal ingredients such as soy and lentil flours increased the lactobacilli fraction in the yogurt.

Oliveira et al. (2011) reported that inulin (40mg/g) sustained the growth of B. lactis in fermented skim milk which had a dramatically increase (p<0.05) from 7.5 to 9.1 log cfu ml⁻¹ during 7 days storage at 4 °C. Moreover, the combination of inulin in fermented skim milk with probiotic cocktails of L. rhamnosus, L. bulgaricus and B. lactis improved the quantity of these strains compared to the absence during two weeks’ storage. In addition, during milk fermentation, inulin stimulated biomass growth of both S. thermophilus and L. rhamnosus and increased the percentage of metabolic end-products like lactic acid (26.1%), acetic acid (33.5%) and ethanol (241%). Another study reported that barley, wheat and malt extracts supported well the growth of L. plantarum and its survival in the fermented product during refrigerated storage. In addition, the presence of a high residual sugar in fermented malt extracts resulted in the best survival of L. plantarum compared to barley and wheat extracts over 70 days of storage at 4 °C. (Charalampopolus and Pandiella, 2010).

Chocolate mousse is indicated to be an effective food matrix for the incorporation of L. paracasei subsp. paracasei LBC 82 (Aragon-Alegro et al., 2007). The neutral pH values (pH 6.3–5.7) of probiotic chocolate mousse correspond to an appropriate condition for L. paracasei survival during 28 days of storage at 4 °C.

Considering the types of chocolates, the probiotic cells could remain highest in dark chocolate (50% cocoa), followed by milk (10% cocoa) and white (0% cocoa) chocolates (Kemsawasd et al., 2016). The same author explained that this fact might be primarily due to the property of dark chocolate containing higher levels of cocoa and antioxidant compounds and activities than milk and white chocolates.

Chestnut purees verified to be the suitable media for probiotic growth. (Blaiotta et al., 2012).

**EFFECT OF TEMPERATURE AND OXYGEN ON PROBIOTIC BACTERIA VIABILITY**

Fermentation temperature is crucial for the viability of probiotics, the favorable temperature for most probiotics ranges between 37–43 °C, but over 45 °C will destroy at least a fraction of the population (Boylston et al., 2004; Champagne and Gardner, 2005; Korbekandi et al., 2011; Lee and Salminen, 2009;).

Oxygen is another toxic factor for the survival of probiotics, so scientists have discovered methods to lower oxygen levels, for example the fermentation under vacuum, or intelligent packaging like the multi layer with oxygen scavengers in the material. (Cruz, Faria and Van Dender, 2007). The continuous exposure to oxygen under acidic conditions during storage is the main reason for the reduction in probiotic counts (Sheehan et al., 2007).
EFFECT OF FREEZING, THAWING AND REFRIGERATED CONDITIONS ON PROBIOTIC BACTERIA VIABILITY

Freezing and thawing are affecting the probiotics, as the integrity of the cell membrane is damaged during the freezing process so metabolic life can be stopped, additionally, during thawing process, the microbial cells can attack the probiotics due to osmotic effects. (Akin, Akin and Kirmacı, 2007).

Khalf et al. (2010) showed by plate counting that maple sap was able to sustain survival of a probiotic combination (BB12 and L. rhamnosus GG each inoculated at \( \sim 10^8 \) CFU/ml) throughout cold storage at 4 °C.

As described by Kemsawasd et al. (2016) in his study, after 60 days of storage at 4 °C, the survival rates of probiotics (L. casei and L. acidophilus) in refrigerated chocolate remained rather high at more than 6 log CFU/g.

As it is found in literature, low storage temperatures allow better maintenance of probiotic viability over prolonged storage (Champagne et al., 2005).

The effect of refrigerated storage temperature (at 2, 5, and 8 °C) on the viability of probiotics (L. acidophilus, Bifidobacterium animalis subsp. lactis BB-12) in yogurt has been studied (Mortazavian et al., 2007). Within this study, after 20 days storage at 2 °C resulted in the highest viability of L. acidophilus, whereas for B. lactis, the highest viability was obtained when yogurt was stored at 8 °C. However, although bifidobacteria are less tolerant to low temperatures than lactobacilli, low storage temperatures favor the survival of probiotics as L. bulgaricus growth and post-acidification are restricted (Lourems-Hattingand Viljoen, 2001). Although tolerance to frozen stress is strain-dependent, most lactobacilli survive well in frozen storage. Ice cream, which is subject to freezing and has high pH, seems a good product for the delivery of probiotics.

VIABILITY OF DIFFERENT PROBIOTIC BACTERIA DURING EXPOSURE TO SIMULATED GASTROINTESTINAL CONDITIONS

In order to obtain a resistance/survival of probiotics bacteria under gastrointestinal conditions an encapsulation method is required.

The study of Chavarri et al. (2010) showed that, microencapsulation of Lactobacillus gasseri and Bifidobacterium bifidum with alginate and a chitosan coating, improved survival during exposure to adverse conditions of the gastrointestinal tract.

Laličić-Petronijević et al. (2015) demonstrated that chocolate was an ideal matrix for protecting the probiotic cells (L. casei and L. acidophilus) during shelf storage and for enhancing their property to resist over the gastrointestinal tract environments.

Several researchers reported that the viability of probiotic bacteria under in vitro gastrointestinal conditions shown an increase when they were combined with chocolates (da Silva et al., 2012; Possemiers et al., 2010). Yonejima et al. (2015) reported that, when combined with chocolate, the Lactococcus brevis ssp. coagulans could survive in gastric juice (pH 2.5) better than combined with commercial beverages (i.e. fruit juices and fermented milks). In accordance with this were also Maillard and Landuyt (2008) who reported that chocolates were the best carriers for probiotics Lactobacillus Rosell-52 and Bifidobacterium Rosell-175 while exposed to gastrointestinal conditions in a dynamic human gut model (SHIME reactor).

EFFECT OF DIFFERENT PACKAGE TYPE ON PROBIOTIC BACTERIA VIABILITY

A positive effect on the viability of probiotic cultures have been attributed to food packaging because they are generally anaerobic or micro-aerophilic bacteria (Talwalkar and Kailasapathy, 2004). The glass package due to its low oxygen permeability favors the survival of probiotic cultures. The polyethylene is permeable to gases and allows the diffusion of oxygen into products during storage (Talwalkar and Kailasapathy, 2004). However, food manufacturers prefer the use of plastic packages due to the high cost of glass and the dangers inherent to its use (Cruz, Faria and Van Dender, 2007).

The package for storage plays an important role because oxygen may affect a number of active probiotic cells and thus trigger the reduction of cell survivability over the entire storage period (Chaikham, 2015).

Tatiana Colombo Pimentel et al. (2015) concluded that the glass package was more appropri-
ate than the plastic package in maintaining the viability of the probiotic culture, with no effect of packaging (glass or plastic) on the physicochemical characteristics and acceptability of clarified apple juice with probiotic *Lactobacillus paracasei ssp. paracasei*.

**CONCLUSIONS**

In order to improve the viability of probiotic microorganisms during foods production, microencapsulation is shown to be the key process. There is a general interest in the improvement of the physical and mechanical stability of the polymers used in probiotics encapsulation, to ensure high population of probiotics not only in food during storage, but also after gastrointestinal digestion.

The carrier plays a very important role and should be carefully examined, considering the main constraints for the survival of probiotics: low oxygen tolerance, low acidity tolerance and the convenience of the addition of prebiotics or growth promoting factors in the formulation of probiotic foods.

It is crucial to be aware of probiotic strain's technological properties. They are usually selected based on this aspect. Also, when a mix of probiotic bacteria is chosen always should be kept in mind the antagonistic relationship between them.

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