Review

Packaging system and probiotic dairy foods

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Abstract

The consumption of foods containing probiotic cultures has greatly increased over the past years as a result of their benefits to human health. Along with other factors, the choice of the packaging material plays an important role in maintaining viable counts of these microorganisms at sufficiently high levels to assure their therapeutic activity throughout shelf-life. The aim of this article is to provide an overview of the main issues related to the importance of the packaging system and/or materials on the stability of probiotic dairy foods.

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1. Functional foods

Since ancient times, food has been considered essential and indispensable to human life. Food provides all the elements necessary for man to develop not only his physical, but also all intellectual activities. Numerous studies clearly show that quality of life is intimately associated with the type of daily diet, as well as with the lifestyle of each individual (Moura, 2005).

Foods offering some kind of health benefit first appeared on the market in the 1960’s. In those days, the media began to highlight and reinforce the importance of eating right and that food products and processed foods should retain the natural attributes of their ingredients. The general
public began to become aware that there was a link between a series of medical conditions (such as constipation, cardiovascular diseases, obesity and hypertension) and the intake of excessive amount of certain ingredients and that, in addition, the health consequences of bad eating habits and a poor diet were aggravated by stress inherent to modern life (Bello, 1995).

Within this context, a new category of foods called functional foods was launched on the market in the mid 1980's. The age-old eating practices from the Far East, particularly Japan and China, have decisively contributed to the concept of functional foods, i.e. processed foods containing ingredients that aid specific body functions in addition to being nutritious (Buttriss, 2000; Hasler, 1998).

Functional foods are those that provide health benefits beyond basic nutrition and promote health through mechanisms not addressed by conventional nutrition models. It should be noted, furthermore, that the above-mentioned health-promoting effects refer to improving overall health and not to curing diseases (Sanders, 1998; cited by Saad, 2006). Functional foods are also known under other names, such as nutraceuticals, therapeutic foods, medicinal foods, and others (Finley, 1996; cited by Berner & O'Donnell, 1998). Such foods may contain one or even a combination of components that impart desirable physiological effects on the human body. An important class of functional foods encompasses probiotics, prebiotics and synbiotics, a great many of which are dairy products.

In Brazil, the sales of functional foods amount to US$500 million per year, representing about 1% of the total sales of the Brazilian food processing industry, according to the Brazilian Association of Food Industries. This membership organization that represents the interests of the Brazilian food industry further states that functional dairy products, nutraceuticals and soy-based products fill an substantial and promising market niche. ABIA forecasts a substantial growth of 4.5–5% for this segment of the market in 2005 as compared to the production figures of 2004 (Alimento Seguro, 2005).

In order to exert health benefits beyond inherent basic nutrition, it is necessary that the activity of the probiotic culture be maintained at sufficiently high levels throughout the shelf-life of the product. This requires optimization of all phases of the manufacturing process, including the selection of adequate materials to be incorporated into the packaging of the finished food product. With this in view, the objective of this review article is to provide an updated overview of important aspects involved in the selection of packaging materials that protect and assure the therapeutic activity of probiotic foods.

2. Probiotics

2.1. Definition

There are several definitions for the term “probiotic”. Fuller (1989) defines probiotics as biopreparations containing living cells or metabolites that stabilize the autochthonous microbiota that colonize and make up the microflora of the animal and human gastrointestinal tract and exert a stimulating effect on both digestive processes and the immune system of the host. According to Margoles and Garcia (2003), the term “probiotic” refers to cultures of live microorganisms that, when administered to humans or animals (by way of dehydrated cells or fermented foods), benefit the host by improving properties of indigenous microflora. However, the most widely accepted definition of the term is that probiotics are live microorganisms that, when administered in appropriate amounts, confer a series of health benefits (Food And Agriculture Organization of the United Nations, World Health Organization, 2001 cited by Saad, 2006).

A microorganism is considered probiotic if is a normal inhabitant of the gastrointestinal tract, survives the passage through the stomach and maintains its viability and metabolic activity in the intestine (Hyun & Shin, 1998).

2.2. Effects on health

The main beneficial effects of probiotic cultures on the host’s health are: control of intestinal infections, as well as stimulation of intestinal motility with consequent relief of constipation. In addition to these effects, probiotics also promote better absorption of nutrients and help improve the utilization of lactose by the body, thereby providing relief from lactose intolerance symptoms. Other health benefits frequently cited in literature include: reduction of cholesterol levels, anti-carcinogenic effects, stimulation of antibody production and phagocytic activity against pathogens in the intestine and other tissues of the host. Furthermore, probiotics also play an important role in competitive exclusion and the production of antimicrobial compounds (Saarela, Mogensen, Fonden, Matto, & Matila-Sandholm, 2000; Itsaranuwat, Al-Haddad, & Robinson, 2003).

Although probiotic cultures may be incorporated into virtually any kind of food, there is a clear preference for dairy products, since historically the manufacturing processes of these products has been optimized to assure and improve the survival of microorganisms involved in fermentation processes so that only minor changes have to be introduced into already existing manufacturing technologies and/or processes (Heller, 2001).

2.3. Probiotic microorganisms

The microbial species most commonly used in the production of probiotic cultures intended for inclusion in dairy products are all originally isolated from the human intestine, since these species are more appropriate to the physiological needs of the host and are able to colonize more easily and efficiently than bacterial strains from other origins. These species include: Bifidobacterium adolescentis, B. bifidum, B. breve, B. infantis, B. longum, Lactobacillus...
acidophilus, *L. casei* subsp. rhamnosus and *Enterococcus faecium*. Bifidobacteria are generally characterized as gram-positive, non-motile and catalase-negative rods of variable shapes – although some species, such as *Bifidum indicum* and *Bifidum asteroides* are catalase-positive – with optimum growth temperature between 25 °C and 28 °C and optimum growth at pH range 6.5–7.0. *Lactobacilli* are generally characterized as gram-positive, non-spore-forming, mostly non-motile, catalase-negative, rod-shaped bacteria that vary from long to slender forms to short coc-cobacilli, occasionally forming short chains, with optimum growth temperature between 30 °C and 40 °C, and growth pH between 5.5 and 6.2. Both microorganisms have complex nutritional needs, such as the assimilation of free amino acids and peptides, vitamins and nucleotides and certain minerals, in addition to requiring a low oxygen tension environment for optimum growth (Gomes & Malcata, 1999).

### 2.4. Technological aspects

Several factors must be considered when selecting a probiotic strain for food applications. Among other requirements, they should not affect the sensory characteristics of the food in any perceptible way; remain stable throughout processing and storage of the product; and be resistant to the gastrointestinal environment (Mattila-Sandholm et al., 2002).

To ensure the therapeutic activity and effectiveness that provide benefits for the human body, several international organizations issued standards establishing minimum viable counts for probiotic microorganisms to assure the biological effect in the intestinal environment. Most of these organizations introduced standards requiring minimum levels of $10^7$ CFU/mL for *L. acidophilus* and $10^6$ of Bifdobacteria in fermented milk products at the time of sale (Talwalkar & Kallasapathy, 2003). In Japan, the Fermented Milk and Lactic Beverages Association has specified that there be at least $10^7$ CFU/mL of viable Bifdobacteria cells in fermented milk drinks. To achieve the desired effects in probiotic yogurts, it is recommended that the minimum counts of viable probiotic bacteria be at least $10^8$ CFU/mL (Lourens-Hattingh & Viljoen, 2001).

Such high numbers have been suggested to compensate for the possible loss in the numbers of probiotic organisms during passage through the stomach and the intestine. It is therefore recommended that the minimum counts of probiotic bacteria be $10^6$ CFU/g of the product at the expiry date (Kurman & Rasic, 1991).

### 3. Packaging and the food industry

The packaging plays a fundamental role in maintaining the quality and shelf-life of foods. The package is an integrated part of the preservation system and functions as a barrier between the food and the external atmosphere. The package should be designed and developed not only to hold the food product, but also to protect it and add value to it, since its design may directly affect the purchase decision of the consumer (Roberston, 1993).

Azeredo, Faria, and Brito (2004) consider that the protection provided by the packaging is the factor of greatest importance, since it is directly related with the safety of the consumer. These authors emphasize that the package must be appropriate for each food product to minimize undesirable changes that may affect the product during its shelf-life.

Soares et al. (2005) highlight the fact that the design and other features of food packaging have gained enormous importance over the last decades, not only, because of the numerous available packaging material options that offer alternatives for cost reduction while still providing adequate protection to the food product, but mainly, because the packages functions as the first point of contact between the consumer and the product.

Grorski-Berry (1999) report that the content of the package is the basis for consumers to buy again. In other words, if the package draws the attention of the consumer in a first moment, the product will be purchased. If the product is good and lives up to expectations, consumers will be satisfied and buy again.

The Brazilian packaging market is highly profitable, particularly the segment of packagings for non-durable goods, such as foods and beverages. In 2003, total sales of the sector – which directly employs 140,000 people – came to R$ 237 billion. Plastic (35.1% of the total market, R$ 8,307 billion) is the leading packaging material, followed by paperboard (28.2%, R$ 6681 billion), metal (20.9%, R$ 4945 billion) and glass (6.7 %, R$ 1584 billion). Although the packaging sector is highly susceptible to fluctuations in the economic activity of the country, solid growth is expected for the coming years (Pellegrino, 2004).

At present, the so-called “active” and “intelligent” packagings are becoming increasingly important. In addition to the functions described above, active packagings execute several additional functions, such as: (a) absorption of compounds that induce spoilage; (b) release of compounds that extend the shelf-life of the product; and (c) monitoring of the shelf-life and best-before date (Azeredo, Faria, & Azeredo, 2000).

### 4. Packaging and probiotic dairy foods

The incorporation and viability in foods throughout storage of probiotic bacteria which result in health benefits for the host is a constant challenge for the food industry and requires the understanding of all intrinsic and extrinsic factors associated with processing, including the selection of type of packaging material. Mattila-Sandholm et al. (2002) reported that the packaging materials and the storage conditions are important factors for the quality of products containing probiotic microorganisms.

In view of the ecology of the strains normally used in probiotic products – anaerobic and microaerophilic ones
– the level of oxygen within the package during storage of the product should be as low as possible in order to avoid toxicity and death of the microorganism and the consequent loss of functionality of the product. Exposure to dissolved oxygen during processing and storage is highly detrimental to *B. bifidum* and *L. acidophilus*. Contrary to aerobic microorganisms, which completely reduce oxygen to water, the absorption system of this substance is minimal or even completely non-existing. The absence of an electron transport chain results in the incomplete reduction of oxygen to hydrogen peroxide. In addition, these probiotic bacteria do not produce catalase, an enzyme essential to the breakdown of hydrogen peroxide, a characteristic that consequently leads to the accumulation of derived toxic metabolites, such as superoxide anion (O$_2^–$), the hydroxide radical (OH$^–$), hydrogen peroxide (H$_2$O$_2$) in the cell, causing its death. This suggests that probiotic strains may be affected by H$_2$O$_2$ produced by other cultures present in the reaction medium. This has motivated several studies aimed at developing alternatives that minimize these negative effects, among which the most promising are those evaluating the addition of antioxidants, such as ascorbic acid and the elimination of peroxide producing strains (Champagne & Gardner, 2005).

Another important characteristic of the growth and stability of the probiotic species in fermented dairy products is the correlation between the synthesis of enzymes such as NAD-oxidase and NADH-peroxidase. High levels of these enzymes have been reported in aerotolerant species, which is highly significant in view of the fact that these enzymes are responsible for removing oxygen from the intercellular medium (Roy, 2005).

In general, being strictly anaerobic, *Bifidobacterium* sp. are more sensitive to oxygen than *L. acidophilus*, however this sensitivity is exclusively dependent on the specific strain used (Talwalkar & Kallasapathy, 2003). This poses a serious problem, since almost all probiotic dairy products available on the market – mainly fermented milks and yogurts – are practically all contained in plastic packages, which, by nature, are materials with high oxygen permeability.

Studies evaluating the use of packagings for probiotic foods are generally restricted to “active” packages (oxygen absorbers) and plastic films with high oxygen barrier properties. In Brazil, to our knowledge, no studies have been conducted so far relative to this subject. In addition, there have also been no studies on the economic viability of the use of packaging techniques and their exact impact on the final price of the product, which would most probably lead to a reduction of sales volumes.

Dave and Shah (1997) study the behavior of *L. acidophilus* in yogurts filled into glass and high-density polyethylene containers during 35 days. The level of dissolved oxygen in the glass packages remained low, whereas the oxygen levels in the plastic packages significantly increased.

Janson et al. (2002) investigated the survival of bifidobacteria in fermented milk filled into packages with varying degrees of crystallinity and polarity. To evaluate the influence of the first parameter, high-density polyethylene, low density polyethylene and aluminum coated polyethylene were used, whereas the second parameter was studied based on the results produced by packages made of aliphatic polyketone and polyethylene of similar crystallinity. The bacterial counts decreased with storage time and no significant difference was observed independently from the material used, with counts falling below 10$^6$ CFU/g after 10 days storage. These results are surprising in that the increase in crystallinity of a polymeric material directly influences its barrier properties, thereby reducing permeability. However, contrary to the expected, the bacterial counts did not vary proportionally to the degree of crystallinity of the packaging material.

Miller, Nguyen, Rooney, and Kailasapthy (2002) studied the influence of two types of packaging material – (1) high oxygen barrier poly styrene of 300–350 µm thickness and (2) a high gas barrier material with a multilayer structure (HIPS/tie/EVOH/tie/PE, trade name NUPAK) added to high impact poly styrene – on the level of dissolved oxygen in probiotic yogurt throughout shelf-life. Significant differences in the value of this parameter were found between the two materials in the course of the storage period investigated; the oxygen levels in the in poly styrene containers varied from 20 to 40 ppm, whereas the oxygen levels in the second package type decreased, reaching levels below 10 ppm, after 42 days refrigerated storage. The results indicate the efficacy of the materials tested at low storage temperatures in retarding and slowing down the metabolism of the starter culture, which produces acid compounds and consumes all the oxygen that permeates through the packaging material, both of which are key factors directly related to the viability of the probiotic culture.

Miller, Nguyen, Rooney, and Kailasapthy (2003) used an oxygen absorber (ZERO) and high gas barrier material with a multilayer structure (HIPS/tie/EVOH/tie/PE, trade name NUPAK) incorporated into high impact poly styrene to control the level of oxygen in probiotic yogurts. The oxygen content declined to significantly low values with the use of NUPAK, since the amount of oxygen consumed by the lactic culture was not replaced due to the presence of the oxygen absorber, thereby creating an anaerobic environment favoring the growth of the probiotic culture. When only polystyrene was used, values close to 40 ppm oxygen were found. However, the best conditions for creating a favorable anaerobic environment for the growth of viable probiotic cultures was obtained when yogurt was processed in a container made of an oxygen barrier material integrated with an oxygen-scavenging agent.

Jayamanne and Adams (2004) investigated the effect of incorporating *B. longum* NCTC11818 in *mekeki*, a popular fermented product made from buffalo milk and widely consumed throughout South-East Asia. The product was fermented in three different package types (clay pots, plastic cups and glass bottles) and stored at two different temperatures – 29 °C and 4 °C. It was found that the Bifidobacte-
ria survived best in the glass bottles, followed by the plastic packages and the clay pots when stored at 29 °C. The buffalo curd packed in clay pots at 29 °C contained 10^{6} CFU/mL after 4 days, while the curds in the glass and plastic packages exhibited values of 10^{5} CFU/mL for up to 8 days. However, when stored in clay cups and kept at 4 °C, viable counts were observed for up to 6 days. The authors attribute this to the permeability of the packages which allowed diffusion of oxygen into the containers. This, along with the high temperature at which the product was stored, caused the death of the microorganism. Since meekiri is a product that is originally filled into clay pots kept at tropical ambient temperature, the results obtained suggest that the use of packaging materials with low oxygen permeability properties is required to obtain a product that produces beneficial effects on the health of the consumer and a shelf life beyond 4 days.

Talwalkar, Miller, Kailasapathy, and Nugyen (2004) investigated the effect of packaging materials on the survival of probiotic bacteria in yogurt by monitoring the oxygen concentration during storage. Three materials were evaluated: high impact polystyrene (HIPS), a gas-absorbing material (NUPAK, as mentioned previously) and NUPAK along with an oxygen scavenging film (ZERO). The yogurts filled in polystyrene containers exhibited a 30–38% increase of the oxygen concentration after 42 days storage; the oxygen levels in the NUPAK packages declined to values lower than 4.29 ppm, whereas the oxygen concentration in the NUPAK – ZERO containers showed a dramatic drop to 1.4 ppm, reaching values as low as 0.44 ppm in the course of the shelf-life of the product. It was concluded that the level of dissolved oxygen depended on the packaging material used. In addition, the results clearly illustrate the importance and potential of using oxygen absorbers for packaging probiotic foods.

Wang, Yu, and Chou (2004) monitored viable cell counts of S. thermophilus and B. bifidum in fermented soy milk filled into (1) glass packages, (2) polyethylene packages containing an oxygen absorber and a desiccant, and (3) a laminated bag (nylon/aluminum/polypropylene) and stored at 4 °C and 25 °C. Independently of the packaging material used, the total microbial population was found to decrease with storage time. The product stored at 25 °C exhibited higher values compared to the product kept at 4 °C, with the differences being directly proportional to the temperature difference. The laminated material exhibited the best performance, with a reduction of only 0.55 log CFU/g and 29.5% survival rate after 4 months at 4 °C. Next in performance came the glass packagings and the laminated bag. In general, the higher the permeability of the packaging material, the lower the number of viable bacterial cells.

Hisiao, Lian, and Chou (2004) studied the effect of the packaging material and the storage temperature on the viability of microencapsulated bifidobacteria. The authors evaluated samples filled in: (1) glass bottles; (2) polyester bottles with desiccant and oxygen absorber and (3) polyethylene bottles without desiccant and oxygen absorber. The samples in the different packages were stored at 4 and 25 °C. The materials used for microencapsulation were milk powder, soluble starch, gelatin and gum arabic. The inclusion of an oxygen absorber and desiccant improved the viable cell counts, particularly at 25 °C. However, the best results were obtained with the product in glass bottles stored at 4 °C, with a reduction of only 0.15–0.20 log CFU/g after 42 days storage.

Kudelka (2005) analyzed the effect of pasteurization and package type on the acidity of probiotic yogurts made from goat’s and cow’s milk during 21 days refrigerated storage. The yogurt samples were submitted to two different pasteurization processes – 95 °C/5 min and 90 °C/10 min and subsequently filled in plastic packages of polypropylene, polystyrene and polyethylene, as well as in glass containers. Throughout the storage period studied, the yogurt with lowest acidity values was that contained in polystyrene packages as compared to the other package types evaluated, which all exhibited similar values for this parameter.

The use of glass packages favors the survival of probiotic cultures due to its extremely low oxygen permeability. On the other hand, the high cost of glass along with the hazards inherent to its handling make it an inappropriate choice for packaging dairy products. For that reason, the dairy industry prefers to market its products, including probiotic fermented milks and yogurts, in plastic packagings.

Within this context, alternatives should be studied, including changes in processing and manufacturing technologies with the inclusion of an oxygen removal step before filling the product into retail containers. Additional alternatives include the addition of oxygen absorbing compounds, such as ascorbic acid. Active packages with incorporated oxygen barrier materials or films with selective permeability properties also have potential applications in the packaging of probiotic food products. However, additional studies are needed to select and develop the most appropriate packaging material and optimize the manufacturing processes in order to make them both technologically and economically viable.

5. Perspectives

The consumption of probiotic foods has greatly increased over the past years as a result of their benefits for human health. For that reason, it is necessary to optimize several technological and economical aspects of manufacturing process of these products. This includes the development of packaging materials that adequately protect and preserve the therapeutic activity of probiotic foods. The use of appropriate packaging materials and systems is of utmost importance to safeguard the improvements introduced in the manufacturing process as a whole and ensure that the product lives up to the expectations of the people that consume these products. Within this context, research efforts aimed at guaranteeing the
preservation of the full therapeutic potential of the probiotic properties throughout the shelf-life of the products should be encouraged.

References


