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New technologies for improving probiotic survival rates in yogurt

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Abstract

In this review we aim to present the current trends in the development of techniques for encapsulating probiotic microorganisms to maintain cell viability in dairy products with special reference to yogurt. The use of probiotic microorganisms in dairy products is difficult because there are several factors responsible for the loss of cell viability during production (fermentation), processing (low pH, oxygen, fermentation / incubation temperature, cooling rate, food matrix composition, nutrient availability). and storage (packaging materials, storage methods, temperature). Gastrointestinal tract conditions (low pH of the gastric juice and the presence of bile salts in the small intestine) can also affect the viability of probiotics. All this has necessitated the development of appropriate technologies to maintain cell viability. Of all the existing technologies in this study, the following encapsulation techniques were analysed: extrusion, emulsion, and spray drying. All these methods have both advantages and disadvantages in encapsulating probiotics.

Keywords: probiotics, yogurt, encapsulation, viability, survival conditions.

1.Introduction

In recent years, probiotics - marketed in the form of food, food supplements with live bacteria or pharmaceuticals - have increasingly attracted the interest of both researchers and consumers. The general public interest in discovering a healthy lifestyle, together with the concern of physicians to discover non-invasive therapies, have shifted attention to the potentially beneficial role [1, 2, 19]. The term "probiotic" was originally used as an antonym for the word "antibiotic". The etymology of the word is in Greek: προ = for and βιοτοσ = life [34]. In 1953, Kollath, first used the term to describe the healing of malnourished patients with various organic and inorganic supplements. Then, in 1954 Vergin claims that the imbalance produced in the body by antibiotic treatment can be restored by a diet rich in probiotics; a suggestion later cited as the first reference to probiotics as they are defined today [38]. Similarly, in 1955, Kolb recognized the undesirable effects of post-antibiotics and proposed prophylactic probiotic therapy.

Later, in 1965, *Lilly and Stillwell* [30] defined probiotics as compounds metabolised by a microorganism that support the growth of other microorganisms. Similarly, experts in 1971 and in 1973, describe probiotics as compounds that either stimulate microbial growth or enhance the host's immune response without inhibiting culture growth in vitro. Another definition given by Parker in 1974 is similar to the recent descriptions of probiotics - organisms or substances, which contribute to maintaining the intestinal microbial balance. This definition has been challenged by many authors because multiple substances could be included in this category, including antibiotics [15]. The late 1980s and early 1990s saw a wave of new and different definitions of probiotics.

A frequently cited definition is that of Fuller in 1992, who defined probiotics as supplements to feed living animal organisms, which have beneficial effects on the animal host by improving the balance of the intestinal microbial flora. This definition seemed to address and is more applicable to animals than humans. Other authors have followed this line, offering their own versions.

Following the FAO / WHO recommendation on the evaluation of probiotics as food additives, in 2002, the suggested definition described probiotics as live microorganisms, which, administered in appropriate

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doses, are beneficial to the host's health. So, a multitude of microorganisms appear to have probiotic properties [7, 20].

Based on its advantageous benefits, probiotics have been increasingly included in yogurts and fermented dairy products for the last two decades. Therapeutic benefits have led to an increase in the incorporation of probiotic bacteria such as lactobacilli and bifidobacteria into dairy products, especially yogurts [32]. Now days, yogurt industry is the largest food sector with the probiotics highest number and diversity used for. The addition of probiotics into dairy products, focuses two directions: (1) milk fermentation with using pure probiotics or mixed traditional strains and probiotics; (2) incorporation of probiotic strains directly into yogurt matrix, improving the product quality. By 2010, worldwide sales of probiotic yogurt had risen to $ 500 from $ 294 million in 2007 [23].

The effectiveness of adding probiotic bacteria depends on the dose and their viability, which must be maintained throughout storage, and they must survive in the gut [24]. Therefore, the viability of probiotic bacteria is of paramount importance in the marketing of probiotic-based foods. The beneficial effect of probiotic microorganisms occurs when they reach the large intestine in sufficient numbers and viable, after having withstood the environmental conditions such as: free acids, hydrogen ions, molecular oxygen or various antibacterial components [21]. The minimum number of probiotic cells (cfu / g) that must exist in the product at the time of consumption for a therapeutic effect has been quantified as the "minimum of bio-value" (MBV) [36]. The International Dairy Federation (IDF) recommends that this index be higher than 10^7 cfu / g, until the last moment of validity of the probiotic food product. In other countries, such as Argentina, Paraguay and Brazil, the standard is at least 10^6 cfu / g but refers only to bifidobacteria, and in Japan the total number of probiotic cells must exceed 10^7 cfu / g. The literature also records 10^6 cfu / g (with strict reference to probiotic microorganisms) as the minimum value for which a product can be called probiotic, and if it refers only to bifidobacteria as probiotic microorganisms the minimum recorded and accepted value is 10^7 cfu / g. In addition to the MBV index, the DI (daily intake) index has also been defined, which refers to the total daily number of probiotic bacteria introduced consciously and voluntarily into the body, an index that is also crucial for the effectiveness of probiotic treatment. The minimum value of DI was set at 10^9 viable cells per day [28, 44] The specific culture medium used in counting probiotic bacteria is also considered to be a particularly important factor in determining the viability of these cells, especially due to the different degree of selectivity of the various media used.

The loss of bacterial viability in dairy fermented products, as well as the action of acids and bile salts in the gastrointestinal tract, has led to numerous studies and research to find new and effective methods to improve the viability of probiotic bacteria [4]. The inclusion of probiotics in dairy products and in particular in yoghurt, in encapsulated form by various techniques (emulsification-gelation, extrusion or spray drying and, more recently, the use of fluidized bed.), raises many more challenges to maintain their viability [25]. All these technical encapsulation processes are based on the use of polymers as efficient as possible and adapted to direct human consumption. Consumption safety, gelling properties as well as resistance to the actions of gastric juice and bile salts are the main criteria for choosing encapsulating biopolymers. Currently, a number of chemical compounds such as alginate (sodium or calcium) are used as encapsulating biopolymers. Substances such as starch, xanthan, carrageenan, gelatin, chitosan, carob flour, whey protein and even calcium chloride are also tested. Sodium alginate is one of the most widely used polymers as an encapsulating material because it forms an extremely matrix accessible, biocompatible and non-toxic for ingredient protection especially probiotic microorganisms and cells sensitive to various factors to which food is exposed during processing and storage. Although sodium alginate is suitable for encapsulation, the gel is porous and sensitive to extreme pH values, thus affecting both release and protection of compounds [35]. There are several ways to overcome this obstacle and improve stability of microorganisms, for example, addition of a nutrient substrate in the composition of the capsule [12].

In this study we will first discuss the history and evolution of yogurt over time, then we will review the main probiotic cultures used in the manufacture of various varieties of probiotic yogurt and their benefits for consumer health. Furthermore, we intent to refer to the factors that affect their viability. In the last part of this review, we will focus on the main encapsulation techniques.

2. A brief history of yogurt

Yogurt is a popular dairy product usually produced by fermentation of lactose by *Lactobacillus delbrueckii ssp. Bulgaricus* and *Streptococcus thermophilus ssp. Salivarius.* It is a major source of macro- and micronutrients that contribute to the daily energy intake and as such, is an important part of the human diet [13]. Yogurt was for millennia the only product for preserving milk, other than cheese. Over time, yogurt has come to be known under various names such as: matsoni (Georgia, Russia, respectively Japan), dahi (India), zabadi (Egypt), mast (Iran), leben raib (Saudi Arabia), laban (Lebanon, Iraq), roba (Sudan), iogurte (Brazil), katyk (Armenia), cuajada (Spain), coalhada (Portugal), dovga (Azerbaijan) [17]. The Indian Ayurvedic archives, mentions the mixture of yogurt and honey called "food of the gods" [9]. The Greeks also included in their diet, a dairy product known as "Oxygala", which is considered a form of Greek yogurt. Galen mentioned that "Oxygala" is consumed with honey, just as Greek yogurt is consumed today. Today it is belived that the word yogurt drift from the Turkish “yog˘urmak, which means to thicken or coagulate. In fact, the Turks were the first to mention in their writings the therapeutic effects of yogurt in combating diarrhea, cramps and skin burns [26]. Yogurt has remained a commonly used dairy product in daily food of the people of India, Central Asia, the Middle East region, Central and Southeast Europe until the 1900s, when a Russian biologist Ilya Ilyich Mechnikov theorized that heavy yogurt consumption is responsible for longevity long life of the Bulgarians farmers. Considering that lactobacilli are important to maintain people's health, Mechnikov made a constant effort to promote yogurt as a food product in Europe. The task of industrializing yogurt production felt to a Spanish entrepreneur named Isaac Carasso. In 1919 he opened a commercial yogurt factory in Barcelona, and his son Daniel later founded the Danone business in France. Fruit jam yogurt was patented in 1933 by the dairy company Radlicka Mlekarna in Prague. Yogurt was first made in the United States in the first decade of the twentieth century, the production being boosted by the writing “Prolonging Life; Optimistic Studies” by Elie Metchnikoff. By the end of the century, yogurt had become a common food product in the United States, and the Colombo Yogurt company was sold in 1993 to General Mills, which discontinued the brand in 2010 [6, 7].

Last but not least, a new technology was developed in Switzerland only after 1950 which led to the marketing of fruit-flavored and sweetened yogurt.

In the 2000's a variety of yogurts based on plant milk appeared, such as soy milk, rice milk, hazelnut milk, almond milk or coconut milk. The products are intended for vegans, but also for people who prefer plant milk or those with dairy intolerance. Annual per capita consumption is high and in Bulgaria in particular it is 31.5 kg / year. The FAO / WHO Codex Alimentarius Commission defines yogurt as “a dairy product coagulated” obtained by fermenting lactose from milk to lactic acid by the action of *Lb. Delbrueckii subsp. bulgaricus* and *St. thermophilus* (pasteurized or concentrated milk) with or without added additives (milk powder, skim milk powder, etc.). According to the FAO, the yogurt must contain a large number of active and living microorganisms that play an important role in promoting it as a healthy food and also an abundant and viable microflora of endogenous origin at the time of consumption. Thus, in the food legislation of many countries, yogurt contains a minimum number of viable lactobacilli (LAB) between 10^6 and 10^8 cfu/ml. Before 1950, the acceptability of yogurt by communities in other parts of the world (West Europe and North America) was limited to very small minorities and some ethnic groups of Balkanic or Middle Eastern origin. The reason for this lack of popularity was attributed to many factors:

- Preference for other fermented dairy products, e.g cheese;
- Limited diversity of yogurt and related products available in the markets;
- Lack of consumer knowledge about the beneficial properties of yogurt or fermented milk obtained by inoculation with probiotic cultures.

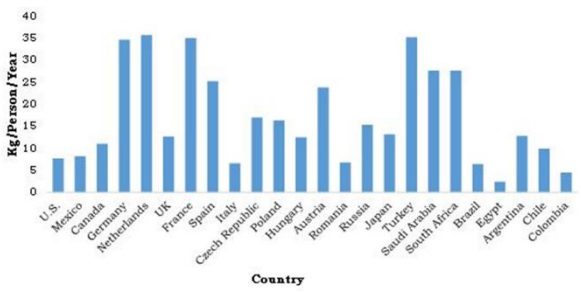

Figure 1. Consumption of yogurt per capita in different countries in 2020 (source: Yogurt Market Trends, Share, Industry Growth and Global Statistics (alliedmarketresearch.com))

Since then, the popularity yogurt has spread to other parts of the world and consumption has increased significantly (Figure 1).

2.1. Probiotic cultures used for the manufacturing of functional yogurt

Probiotics are represented by a wide range of microorganisms in the field of bacteria, prokaryotes genus *Lactobacillus* (about 116 species in 2008) and genus *Bifidobacterium* (about 30 species), but also in the field of fungi (certain yeasts). The species *Lactobacillus acidophilus, L. casei, L. paracasei* and *Bifidobacterium,* are used predominantly in yogurt [22]. Manufacturers of therapeutic fermented dairy products commonly use five species of Bifidobacterium (*B. adolescentis, B. bifidum, B. breve, B. infantis* and *B. Longum* [5]. The characteristics of probiotic strains vary and each strain must be studied individually. Some probiotic strains are proteolytic enough to grow excellently in milk, but others need growth stimulants. Those who do not ferment lactose need monosaccharides. Sometimes the texture or taste of a dairy product fermented with a probiotic does not meet consumer approval or is technologically impractical. For this reason, it is common to use probiotic bacteria together with standard initial cultures in yogurt [43]. Most Bifidobacterium species cannot ferment milk on their own because they require low redox potential and peptides caused by the breakdown of casein. Moreover, when co-cultured with lactobacilli, they are inhibited as the pH decreases [27]. Several factors such as strain characteristics, food matrix, temperature, pH, and accompanying microbes affect the viability of probiotics [18]. A symbiotic product that contains probiotic bacteria and prebiotic in a single food can improve the survival of bifidobacteria during product storage and during passage into the intestinal tract and can also reduce competition with microorganisms in the intestinal flora [29]. The theoretical basis for the selection of probiotic microorganisms includes: safety, functional (survival, adherence, colonization, production of antimicrobial substances, stimulation of the immune system, antigenotoxic activity and prevention of pathogens) and technological aspects (growth in milk, sensory properties, stability , phage resistance, process viability). Safety features include the following specifications such as: they are isolated from the healthy human GI tract. are non-pathogenic, have no history of association with diseases, does not de-conjugate bile salts and possess no antibiotic-resistant genes. To become functional, probiotics must meet certain conditions, such as: tolerance to acid and human gastric juice. bile salts, adhesion to epithelial surfaces and persistence in the human GI tract, immunostimulation, but not proinflammatory effects [41]. The technological aspects for the probiotic selection, include: good sensory characteristics. resistance to bacteriophages. viability during processing and stability in the product during storage. The problem of processing lactic acid bacteria in their commercial form encounters various obstacles related to the release at the target site of a fixed amount of microorganisms, in viable and active form, to fulfill their predicted therapeutic effect. Viability is a basic requirement, but there are studies that have demonstrated the exercise of functional properties, such as immunomodulation, by non-viable microorganisms [42]. A unanimously accepted concentration of microorganisms has not been reached to be effective, with the levels studied ranging from 10^6 to over 10^8 cfu / mL [32]. These high concentrations are desired precisely to ensure the delivery of a sufficient quantity to the target site, given the decrease in concentration during processing and storage, as well as the effect of passing through the gastrointestinal tract. The combination of milk with lactic acid bacteria has established a concentration of 10^7 viable bacteria per gram of product to constitute a probiotic yogurt [37]. However, a lot of researches have shown that probiotic strains grow slowly in milk, decrease to very low concentrations in yogurt, or lose their full viability through prolonged cold storage, especially bifidobacteria [50]. The viability of probiotics is a particularly important issue, as they must retain their functional properties during storage, transit through gastric acid, enzymatic degradation and the action of bile salts. Several media were proposed in 1990 for *L. acidophyllus*, L. casei and *Bifidobacterium* spp., but these methods were based only on pure cultures of these organisms and were therefore considered inaccurate. More recently, Tharmaraj and Shah, 2003 [51] recommended certain media for the selective enumeration of S. thermophyllus, L. delbrueckii spp. Bulgaricus, L. acidophyllus, L. casei, L. rhamnosus and Bifidobacterium spp, which can be used even under mixture. of bacteria. Today, a variety of assortments of probiotic yogurts are known globally (Table 1).

Table 1. Examples of probiotic yogurt brands available in different parts of the world

Yogurt brand	Type of probioticstrains	Country of origin	Producing company
Actimel	*L. casei* DN-114 001	France	Danone
Activia yogurt	*B. animalis lactis* DN-173 010/CNCM I-2494	France	Danone
Laura Chenel Probiotic Goat Milk Yogurt	*B. bifidum* Bb12	USA	Laura Chenel
Nestle A+ ActiPlus Dahi	*L. acidophilus* (LA-5)	India	Nestle
Yeo valley organic yogurt	*B. infantis*, *B. lactis*, *L. fermentum*, *L.rhamnosus* *L. acidophilus*, *Leuconostoc pseudomesenteroides*, *La lactis*, and *L. paracasei*	United Kingdom	Yeo Valley Organic
La Yogurt Probiotic Cherry Original Blended Low-Fat Yogurt	*L. acidophilus* (LA-5), *B. Animalis lactis* (BB-12), L. casei	The Netherlands	Goisco
Greek yogurt (Greek Pro+)	GanedenBC$^{30®}$ (*Bacillus coagulans* GBI-30, 6086)	USA	Norman's dairy
Yoba	*L. rhamnosus* GG (*L. rhamnosus* Yoba, 2012)	Uganda	Yoba for Life
YoyiC yogurt	*L.* casei	Indonesia	Yoyic
Meng Niu Guan Yi Milk	*B. bifidum* Bb12	China	Mengniu
Vaalia yogurt	*L. rhamnosus* GG	Australia	Parmalat Professional

3.Encapsulation

3.1.General considerations

Encapsulation has been used in the food industry for more than seven decades. In a narrow sense, encapsulation involves either covering a particle with a role in the diet such as acidifiers, yeasts, artificial sweeteners, minerals, vitamins, antioxidants, essential oils, flavours and others. or covering whole ingredients such as raisins, nuts or products. of confectionery. The first is microencapsulation, and the second is macro coating. Encapsulation is generally defined as the action of a perfect coating of a substance in another substance that forms the outer shell, the capsules themselves. Microencapsulation refers to the performance of encapsulation operation at micro level (capsules with dimensions smaller than 1 mm and reaching up to 2-4μm). More recently, controlled release nanoparticle systems are beginning to make their presence felt in the food industry as well. The nanoparticles could be used to make drinks that do not disturb, even if they have added various ingredients. In nanoencapsulation the particle size is around 30 nm. Therefore, encapsulation is a protection by covering a substance with a value higher than the capsule shell, but which, compared to this shell, is labile in nature, easily destructible under certain environmental conditions. Regarding the probiotic survival rate, microencapsulation can be understood as the technique of capturing / embedding the microorganism' cells by covering them with one or more layers of certain hydrocolloids, to delimit and protect the cell from the action of the external environment, in a way that its cell can be released into the intestinal environment [3, 47]. The microencapsulation operation is an efficient method of increasing the viability of microorganisms and it has a special practical importance. The probiotics microencapsulation takes place in three stages or technological phases: the first stage involves the incorporation of the bioactive component (probiotics in the environment growth) in a liquid or solid matrix. If the matrix is liquid, incorporation involves dispersion or dissolution in matrix, while in the case of solid core incorporation involves agglomeration or adsorption. In the second phase, the liquid matrix is dispersed. In the final stage there is the stabilization that can be chemical (polymerization), physico-chemical (gelling) or physical (evaporation, solidification) [40]. Bioencapsulation benefits of the fundamental principles of encapsulation and involves effective coating of a living form in a membrane which is inert, non-toxic for the cell, and stable to the internal conditions of the biochemical reactions for agitation. The choice of an appropriate bioencapsulation technique depends on the final destination of the product and the processing conditions involved in obtaining the final product. Pfutze S. (2003) [39] considers that encapsulation technologies can be classified into two categories:

- capsule matrix formation: an active and protective ingredient forms homogeneous granules. The active product is evenly distributed in the granule being surrounded in abundance by the protective material, forming the active matrix.
- capsule shell formation: the active material is granulated and covered with a protective layer. The active and protective material is well separated.

The main goal is to build a barrier between the component particles and the environment. This barrier is a protection against oxygen, water, light; avoid contact with other particles or ingredients; or

control their release over time. Protection of bioactive compounds during processing and storage, as well as controlled release in the gastrointestinal tract is a priority in exploiting the beneficial potential of many bioactive compounds.

It is generally believed that there are a number of both intrinsic and extrinsic factors for which probiotics should be pre-encapsulated when added to the manufacture of yogurt. The encapsulation of probiotic strains used in the manufacture of yogurt is applied to prolong their viability due to unfavourable factors in the yogurt matrix such as: lactic acid and acetic acid, low pH, the presence of hydrogen peroxide, and the high oxygen content [14]. Also, encapsulation of probiotics has become a very attractive technology, being adequate for the protection of probiotics in the acidic environment of the stomach [8, 45], and ensuring targeted release in the colon. To improve the viability and resistance of probiotics over time and at different temperatures, but also to ensure the minimum dose required after their passage through the upper and lower gastrointestinal tract, probiotic encapsulation is required [10].

3.2. Materials used in microencapsulation technology of probiotics

The substances with a polymeric structure used to encapsulate probiotics require certain conditions:

- Adequate rheological properties, which means it flows easily, even at high concentrations;
- Have the ability to disperse or emulsify the active ingredient;
- Do not react with it during the process or during storage;
- To keep the encapsulated material active throughout the encapsulation process and then during the storage period;
- May be free of solvents or other auxiliary materials used during the encapsulation process;
- Provide maximum protection for the encapsulated probiotic;
- To be soluble in solvents accepted by the food industry (water, ethanol);
- Do not react chemically with the encapsulated material
- Observe the solubility standards of the capsules and release the active microorganism in a controlled manner;
- Low cost.

As in practice it is impossible to find only one material that meets all these conditions, it frequently resorts to mixtures of materials, or additives which alter their properties. Among the most common encapsulation materials are:

- Carbohydrates (starch, maltodextrin, dextran, modified starch, sucrose, cyclodextrins);
- Cellulose (Carboxymethylcellulose, methyl cellulose, ethyl cellulose, nitrocellulose, acetyl cellulose phthalate);
- Other polysaccharides (agar, sodium alginate, k- carrageenan, gellan gum);
- Lipids and Wax (paraffin, beeswax, tristearic acid, oils, fats, hardened oils);
- Proteins (Gluten, milk protein, gelatin, albumin, peptides)

The dimensions of the microcapsules can vary between a submicron and a few millimetres, and the ideal shape is spherical. Their shape depends largely on the structure of the encapsulation material and its method of production. The active portion of a capsule is called the core, internal phase, or filling. The thickness of the encapsulation material and the number of layers are variable.

Alginate is the most common biomaterial used to encapsulate probiotics. Natural polysaccharide extracted from different types of algae (especially brown algae), composed of αL-guluronic acid (G) and β-D-mannuronic acid (M), respectively, is the most studied material. The M/G ratio determines the technological functionality of the alginate. Gel resistance is given by the high proportion of G groups. Alginate microcapsules can be obtained by extrusion and emulsion - alginate gel is susceptible to precipitation in the presence of excess Ca^{2+} or chelating agents. The alginate solution can be mixed with probiotic-containing liquid media (MRS) and then dropped into $CaCl_2$ solution to solidification. The maximum cell load in microcapsules is limited to 25% by volume [48].

Chitosan is a positively charged polysaccharide and is formed by deacetylation of chitin. It is preferred to be used as a wrapper and not as a capsule. It is often used in conjunction with alginate. The chitosan capsule provides the best protection in the bile salt solution due to the ion exchange at the absorption of bile salt. Chitosan appears to have inhibitory effects on lactic acid bacteria as well as in other bacteria, viruses, fungi. The lack of solubility in water represents a disadvantage in preventing the complete

release of the encapsulated probiotic in intestine, which has a higher pH (5.4), so its applicability is practically limited [11].

Carrageenan can be obtained from red seaweed. They are linear anionic sulfated polysaccharides composed of D-galactopyranose residues linked by alternating α - (1/3) and β - (1/4). The carrageenan is presented in 3 variant types: k, l, λ after the enzymatic modification of the substrates (fraction - μ, fraction - δ, fraction - σ) which differ in their disaccharide structure.

K-Carrageenan together with immobilized lactic acid bacteria can be emulsified into a stable vegetable oil in a thermostatic reactor.

Gellan gum is a linear anionic heteropolysaccharide having a repetitive tetrasaccharide unit consisting of rhamnose, D-glucose and D-glucuronic acid in a ratio of 1:2:1 Has the potential to replace partial or total gelling agents [48].

Xanthan gum is a polysaccharide synthesized by aerobic fermentation by *Xanthomonas campestris*. Consists of 1,4 β-linked D-glucose residues with a side trisaccharide chain attached to successive d-glucosyl residues 0-3. The side chains are tied in position α D-manopyranose, in position β D-mannopyranose (4-1) and in position β-β-D-glucuronic acid (2-1) which causes anionic properties of this hydrocolloid. It is used in combination with gelatin for encapsulation of probiotics) [12].

Cellulose acetate phthalate is a highly hydrophilic polymer in the composition of plants and bacteria being safe to use; is used to control the release of functional compounds in the gut. The advantage of this compound is its insolubility at acidic pH (pH <5), but soluble at pH> 6, which gives it excellent protective properties for microorganisms under conditions of gastric acid environment. The disadvantage of this compound is that it cannot form gel particles by ionotropic gelation. Only capsules by emulsification and interfacial polymerization were developed. Cellulose acetate phthalate is widely used as a microcapsule coating [16].

Starch is a hydrocolloid biopolymer produced from plants in the form of granules of different hydrophilic sizes. Sources of starch are represented by: potatoes, corn, rice, wheat. Resistant starch is the one that resists the action of pancreatic amylases in the small intestine and which reaches the level of the colon where it can be fermented. This specificity allows a better release capacity in the large intestine. Starch also has prebiotic functions for encapsulated bacteria. Starch-based microcapsules were obtained by researchers mainly by emulsification cross-linking methods [3].

Gelatin is a protein obtained by hydrolysing collagen from bones and skin. Gelatin makes a heat-sensitive gel and was used to encapsulate probiotics, alone or in combination other compounds. Thanks to its amphoteric nature, it is an excellent candidate for cooperation with anionic polysaccharides (alginate or gellan gum). It does not form particles, however, can be considered as microencapsulation material as coating material. Gelatin and K-carrageenan are polymers very often used for the coating of alginate microcapsules and chitosan since it does not have satisfactory encapsulation properties [3].

Table 2. Encapsulated probiotics used in yogurt

Probiotic	**Encapsulated technic**	**Materials**
B. infantis	Extrusion	Gellan/xanthan gum
B.longum	Emulsification	K-Carrageenan
L. acidophifus	Emulsification	Alginate—starch
B. adolescentis	Emulsification	Alginate
B. fongum	Emulsification	K-Carrageenan
L.acidophilus	Emulsification	Alginate/starch
B. infantis		
B. breve	Emulsification	Milk fat and whey protein
B. longum	Spray-drying	
L. acidophilus	Extrusion	Ca-alginate
L. acidophilus	Extrusion	Raftilose, raftiline and starch
B. infantis		
L. acidophilus	Emulsification	Ca-alginate
L. casei		
L. rhamnosus		
B. infantis		

Milk proteins They are natural vehicles for probiotics, thanks to their structural and physicochemical properties. They have excellent gelatinizing properties and this specificity has recently been studied by Heidelbach and Livney [31] for encapsulation of probiotics. Their physicochemical properties (low viscosity, indefinite aroma, ability to form gel) promote them as being ideal encapsulation matrices.

Probiotic strains and microcapsule types used for in yoghurt are summarised in Table 2.

3.3. Methods of microencapsulation of probiotic bacteria

3.3.1. Extrusion technique

Extrusion, also called the drip method is a physical method of encapsulating probiotics in hydrocolloids (alginate and carrageenan). The extrusion method is the oldest and most commonly used technique for producing hydrocolloid capsules. The probiotic medium is mixed with the alginate solution, then the suspension is dripped in a solution of $CaCl_2$ for solidification. The mixing solution can be projected through a tip at high pressures. Extrusion of polymeric solutions through the tips to produce capsules at the level of laboratory is performed with the help of syringes, by pulsation of the jet or vibration of the tip or by use of coaxial flux or an electrostatic field. In general, it is a cheap and easy to use method at the laboratory level, being also the method that affects to the least the viability of bacterial cells. The defining terms for this method are said to be biocompatibility and flexibility [33, 49]. Unfortunately, this technique cannot be applied in industrial production, due to the weight with which the particles are formed and the low working speed [12].

3.3.2. Emulsification and ionic gelification

The principle of the method is to form a mixture between the continuous oily phase (oil from maize vegetable, soybean, sunflower, light paraffin) and batch phase (dispersed) consisting of a mixture of the encapsulating material and the probiotic medium adding in small volume. The emulsification technique has been successfully applied for the microencapsulation of probiotic lactic acid bacteria. Unlike the extrusion technique, this technique can be easily transposed to the industrial level, and the particle size is considerably smaller (25.1.m-2 mm). However, the method requires high costs to make good quality capsules, compared to the extrusion method due primarily to the use of an oil to form the emulsion. In this technique, a small amount of polymer-cell mixture is added to a large volume of oil (soybean, sunflower, corn or any other vegetable oil may be used). The resulting solution should then be homogenized by appropriate stirring until a water emulsion is formed in oil. For a better homogenization, emulsifiers such as Tween 80 can be added, in concentrations of 0.2% (recommendation made by Sheu and Marshal, 1993 [46]. Once the emulsion is formed, the soluble polymer is insolubilized by the addition of calcium chloride. The smallest possible size of the water particles in the emulsion will lead to the small size of the capsules obtained [10].

3.3.3. Spray drying

Atomization is an economical, flexible method that offers a variety of possibilities regarding the materials that make up the encapsulation matrix. It can adapt to ordinary equipment in the food industry and produces good quality particles. Spray drying consists of several steps, the first of which is the preparation of the dispersion or emulsion. For this, the material that constitutes the encapsulation matrix must be hydrated. Together with the encapsulating material it forms a dispersion or an emulsion, if a surfactant is added. The mixture is fed with a pump into the atomizing chamber. If necessary, the dispersion is homogenized in order to have as uniform drops as possible. Next is the atomization step which is done by means of a nozzle that rotates at high speed. In the atomization chamber the drops finely dispersed, they meet with hot air, either concurrently or countercurrently, taking place a dehydration process. The water evaporates from the surface of the droplets and the encapsulation material hardens. The aroma or encapsulated substance remains trapped in the core of the dehydrating capsule. The rapid evaporation of water from the surface causes the temperature inside the capsule to be below 100 degrees. The particles are kept at temperatures of 100-180 degrees for a few minutes, which is why some volatile compounds can be lost. Granules with 100 μm diameter are obtained which are separated in a cyclone. Drops occur and the obtained capsules are in the form of a dry powder. High temperatures reduce viability and reduce their activity in the final product. Both gellan gum and starch are preferred because they tend to form spherical microcapsules during drying.

Spray freeze drying process is similar to spray drying because in this case the encapsulated probiotic is dispersed in the encapsulating material and then sprayed in a controlled environment. In this case, the water does not evaporate, because the coating material is a grease. Because of this, there are other differences between these procedures. Spray drying uses hot air to evaporate water from the initial dispersion, while in the last two processes cold air or very cold air is used to solidify coating material. The microcapsules obtained by this process are insoluble in water. Therefore, the mentioned processes are approved for the encapsulation of probiotics. Probiotics are in a solution that is vaporized at low temperatures (vapor phase of a cryogenic liquid such as liquid nitrogen). It's generating practically a dispersion of frozen drops. There are problems with the loss of substances that are soluble in fat, and over time can be lost some volatile substances [14].

4.Conclusions

The use of microencapsulated probiotic cultures facilitates the growth of beneficial microorganisms, decreases the flora of potentially harmful microorganisms and improves the body's immune system. Before a probiotic can benefit human health, it must meet several criteria: optimum technological qualities, so that they can be manufactured and incorporated into food without losing viability and usefulness or generating unpleasant odours or textures, but must survive the passage through the upper segment of the gastrointestinal tract and reach life at its level of action; and it must be able to function in the intestinal environment.

The size of the microcapsules is important when using them in the manufacture of yogurts, by adding them directly to the finished product or to the milk to be fermented, because they must not be detected by the taste buds.

For the encapsulation of living probiotic bacteria, emulsification-gelification, extrusion or spray drying and, more recently, the use of a fluidized bed are used as basic methods.

The application of microencapsulation techniques involves the use of polymers as efficient adapted to direct human consumption. Alginate (sodium or calcium) is currently the most commonly used for encapsulating living organisms.

The results published in numerous scientific articles show that the extrusion and emulsification encapsulation techniques are not reliable for industrial applications regarding the probiotics, and the product resulting from the application of these techniques cannot be used for the intended purpose (microcapsules obtained are uneven, very hygroscopic and very difficult to dry). However, the spray drying technique generated clearly superior results. Depending on the encapsulating biopolymer or the mixture of biopolymers, depending on their concentration and the nature of the gel generated, this technique has led to obtaining homogeneous products from a structural and compositional point of view, but also to easily reproducible results.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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