Microencapsulation of bacteria: A review of different technologies and their impact on the probiotic effects

María José Martín, Federico Lara-Villoslada, María Adolfina Ruiz, María Encarnación Morales

PII: S1466-8564(14)00160-X
DOI: doi: 10.1016/j.ifset.2014.09.010
Reference: INNFOO 1233

To appear in: *Innovative Food Science and Emerging Technologies*

Received date: 10 April 2014
Accepted date: 30 September 2014

Please cite this article as: Martín, M.J., Lara-Villoslada, F., Ruiz, M.A. & Morales, M.E., Microencapsulation of bacteria: A review of different technologies and their impact on the probiotic effects, *Innovative Food Science and Emerging Technologies* (2014), doi: 10.1016/j.ifset.2014.09.010

MICROENCAPSULATION OF BACTERIA: A REVIEW OF DIFFERENT TECHNOLOGIES AND THEIR IMPACT ON THE PROBIOTIC EFFECTS

María José Martín[1], Federico Lara-Villoslada[2], María Adolfina Ruiz[1] and María Encarnación Morales[1].

[1]Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Granada, 18071 Granada, Spain.

[2] Research and Development Department, Lactalis Puleva, 18004 Granada, Spain.

Address for correspondence:

Mª Encarnación Morales Hernández

Department of Technology and Pharmacy.

Faculty of Pharmacy.

Campus de Cartuja s/n

University of Granada.

Granada, 18071

Spain

Tel +34 958 243905

E-mail maen@ugr.es

Abstract:

Probiotic based products are associated with many health benefits. However, the main problem is the low survival of these microorganisms in food products and in gastrointestinal tract. Providing probiotics with a physical barrier is an efficient approach to protect microorganisms and to deliver them into the gut. In our opinion, microencapsulation, is one of the most efficient methods, and has been under especial consideration and investigation. However, there are still many challenges to overcome with respect to the microencapsulation process. This review focuses mainly on the methodological approach of probiotic encapsulation including materials and results obtained using encapsulated probiotic in food matrices and different pathologies in animal models.

Key word: probiotics, microencapsulation, food, pathologies, protection.

Highlight

The article summarizes the most important and new technologies applied in probiotic encapsulation.

An overview of the materials used in these technologies is given, paying special attention to advantages and disadvantages.

To our knowledge this is the first time that a review described the results obtained using encapsulated probiotic in various pathologies.

1. Introduction

Probiotics are described by the World Health Organization (WHO) as "live organism, which when administered in adequate amounts confer health benefits to the host" (FAO/WHO). In this sense, probiotics have shown in some studies, to be effective in the treatment of several intestinal disorders and to have an impact on the immune system (Kurmann & Rasic, 1991). Considering that these microorganisms are mainly consumed orally, it would be reasonable to believe that its beneficial effects would be mainly apparent in these intestinal pathologies. However, their modulatory effects on systemic immune response may lead to positive effects in systemic disorders such as allergy (Majamaa & Isolauri, 1997) or inflammatory diseases (Malchow, 1997) and they also have demonstrated a beneficial effect in the treatment of vaginitis (Reid, 2000).

The most used probiotics microorganisms are lactobacillus and bifidobacteria strains (Solanki et al., 2013). However, other species, such as *Escherichia coli* and *Bacillus cereus* have also been used to achieve the same objectives, together with some yeast, mainly *Saccharomyces cerevisiae* (Burgain, Gaiani, Linder & Scher, 2011). Some of these species have been incorporated in foods converting them in functional food (Champagne, Gardner & Roy, 2005). These kinds of aliments are defined as modified food or food ingredient that provides a health benefit beyond satisfying traditional nutrient requirements (Sanders, 1998).

To produce these beneficial effects in health, probiotic have to be able to survive and multiply in the host. In this respect, probiotic should be metabolically stable and active in the product, survive passage through the stomach and reach the intestine in large amount (Sanz, 2007). However, several factors have been reported to affect the viability of probiotics, including pH, hydrogen peroxide, oxygen, storage temperature, among others (Shah, Lankaputhra, Britz & Kyle, 1995). Different approaches that increase the resistance of these sensitive microorganisms against adverse conditions have been proposed, including appropriate selection of acid and bile resistant strains, use of oxygen-impermeable containers, two step fermentation, stress adaptation, incorporation of micronutrients such as peptides and aminoacids, and microencapsulation (Sarkar, 2010).

The last option, microencapsulation, is one of the most efficient methods, and has been under especial consideration and investigation. Microencapsulation can be defined as the process in which cells are retained within an encapsulating membrane to reduce cell injury or cell lost, in a way that result in appropriate microorganisms release in the gut (Sultana et al., 2010). Some benefits of microencapsulation of cells include: protection from bacteriphages and detrimental factors increasing survival during freeze drying, freezing and storage and converting them into a powder form easier to use, since it enhance their homogeneous distribution throughout the product (Mortazavian, Razavi, Ehsani & Sohrabvandi, 2007)

Given the importance of microencapsulation, the aim of this article is to review techniques for microencapsulation of probiotics, as well as the components used during encapsulation, and it advantages. In addition, we analyze the effect of encapsulated probiotic in food and in some diseases.

2. Techniques for microencapsulation of probiotics

Currently, there are a lot of encapsulation technologies. Before selecting one of them, industry should have taken into account, the following point (Zuidam & Shimoni, 2010)*:* i) Which conditions affect probiotics viability? (ii) Which processing conditions are used during food production or processing? (iii)What will be the storage conditions of the food product containing the encapsulated prior to consumer use? (iv) Which particle size and density are needed to incorporate it properly in the food product?(v) What are the triggers and mechanisms of release? (vi) What are the cost constraints? We described below the most important technologies used to encapsulated probiotic cells. In this sense, as previously mentioned, it is known that probiotics are affected by different conditions such as moisture content, high temperatures, agitation, etc. In this respect food matrices should be produced in mild conditions, low temperature, controlled agitation, small presence of oxygen and moderate pH. Authors should be test before introducing particles into food matrices the best storage conditions, most of the studies are carried out at 4ºC and room temperature. Particle size should be enough to protect probiotic but not to cause gritty mouthfeel. It has been reported that soft, rounded particles are not perceptually gritty up to about 80 µm (Lawless & Heymann, 2010). The amount of particles that should be incorporated will depend on the dose of probiotic required. The mechanism of release depends on the technology used and the material. In most of the cases, particles release their content because of pH changes (acid or basic), quelating agents and enzymatic action. Finally, the balance between cost and benefit should be taken into account, since some of the technologies described below required specific devices or materials that can increase production cost.

2.1. Extrusion technique

Extrusion technique is the most popular method because of its, simplicity, low cost and gentle formulation conditions that ensure high cell viability (Krasaekoopt, Bhandari & Deeth, 2003). It involves preparing an hydrocolloid solution, adding microorganisms, and extruding the cell suspension through a syringe needle. The droplets are dripped into a hardening solution (Heidebach, Först & Kulozik, 2012)

If the droplet formation occurs in a controlled manner (contrary to spraying) the technique is known as prilling. This is done by pulsation of the jet or vibration of the nozzle. The use of coaxial flow or an electrostatic field is the other common technique to form small droplets. When an electrostatic field is applied, the electrostatic forces disrupt the liquid surface at the needle tip, forming a charged stream of small droplets (Figure 1). The method does not need organic solvents and it is easy to control the size of beads by varying the applied potential. Mass production of beads can either be achieved by multi-nozzle system or using a rotating disc (figure 1). Another process is the centrifugal extrusion which consists on a coextrusion process. It utilizes a nozzle with concentric orifices located on the outer circumference of a rotating cylinder. The core material is pumped through the inner orifice and a liquid shell material through the outer orifice. When the system rotates, the extruded rod breaks up into droplets that form capsules (Kailasapathy, 2002).

2.1.1. Supporting material

Alginate

Alginate is a linear heteropolysaccharide extracted from different types of algae, with two structural units consisting of D-mannuronic (M) and L-guluronic acids(G). Depending on the source, the composition and the sequence in D-mannuronic and L-guluronic acids vary widely. In the same way, the functional properties of alginate as supporting material correlate strongly with the composition and sequence of M –

units and G-units. G-units have a bucked shape while the M-units tends to be as an extended band. In this sense, two G-units aligned side by side, result in the formation of a hole with specific dimension which is able to bind selectively divalent cations.

To form beads, a cell suspension is mixed with a sodium alginate solution and the mixture is dripping into a solution containing a multivalent cations (usually Ca^{+2} in the form of $CaCl_2$). The droplets form gel spheres instantaneously, entrapping the cells in a three dimensional structure. This is because a polymer cross-linking occurs following the exchange of sodium ions from the guluronic acids with divalent cations (Ca^{2+}, Sr^{2+}, or Ba^{2}+). This result in a chain–chain association that constitutes the so-called "egg box model".

The success of this technique is because of the gentle environment that it provides for the entrapped material, and its biocompatibility. The size and spheric shape of the bead depend mainly on the viscosity of the sodium alginate solution and the distance between the syringe and the calcium bath. In this way, high concentration increases the viscosity of the gel but decreases, the size of the beads. The extruder orifice diameter is another important factor, which regulates droplet size. The composition of the alginate also influences bead size, small bead result from low guluronic alginates (Krasaekoopt, Bhandari & Deeth, 2003).

Whey protein

Alternatively to polymeric hydrogles, food proteins can also be used, since their high nutritional value and excellent functional properties (Gunasekaran, Ko, & Xiao, 2007). Whey proteins are a mixture of globular protein isolated from whey, the liquid material created as a result of the production of cheese. These proteins possess the ability to interact with a wide range of active, which offer a wide spectrum of opportunities for protection and reverse binding of active molecules prior to their targeted release in the host. Another potential benefit associated with protein encapsulation matrices involves hydrolysis of food proteins by digestive enzymes. It may generate bioactive peptides that may exert a number of physiological effects *in vivo*. In this sense, Doherty et al. (2011) using whey protein as an encapsulation material. The particles were able to protect probiotic during 3 hours *in vitro* stomach incubation.

Pectin

Pectin is a heteroploysacchride mainly extracted from fruits. It is used as gelling agent in food, in medicines and as a source of dietary fiber. It remains intact in the stomach and the small intestine. Gebara et al. (2013) produced a pectin microparticles coated with whey protein. This microencapsulation system conferred greater protective effect to *L. acidophilus* as compared to the free cells. However, the coating of pectin microparticles with whey protein did not confer additional protection to probiotics when exposed to simulated gastrointestinal conditions. In contrast, Gerez, Font De Valdez, Gigante and Grosso, (2012) found an improvement in the survival of probiotic when they are microencapsulated into pectin particles coated with whey protein after exposure to gastric conditions.

Milk

Pure milk as an encapsulation carrier has been studied too. Shi et al., (2013) developed a milk microparticles coated with carrageenan and locust bean. These milk microspheres showed good

protection for *Lactobacillus bulgaricus.* However, these milk microspheres had irregular shapes and poor mechanical characteristics. To improve it, a mix of alginate with milk was used by these authors (Shi et al., 2013). The studies demonstrated that encapsulation of *L. bulgaricus* in these new microspheres, is an effective way to protect probiotics against extreme simulated gastrointestinal environment.

Human like collagen

Human like collagen (HLC) is produced by recombinant *Escherichia coli* BL21 containing human like collagen cDNA. This collagen is used as an haemostatic material, a scaffolding biomaterial for organs or tissue regeneration and functional foods. Su et al. (2011), prepared microspheres using alginate and HCL by electrostatic droplet generation. The human like collagen incorporated into the solution of alginate forming intermolecular hydrogen bonding or other interactions improving beads stability. The results of these authors showed that the tolerance of probiotic in simulated gastric juice was improved.

2.2. Emulsion technique

In this technique, the discontinuous phase (cell polymer suspension) is added to a large volume of oil (continuous phase). The mixture is homogenized to form a water in oil emulsion. Once the water in oil emulsion is formed, the water soluble polymer is insolubilized (cross-linked) to form the particles within the oil phase (Heidebach et al., 2012).The beads are harvested later by filtration (Figure 1). The size of the beads is controlled by the speed of agitation, and can vary between 25µm and 2 mm.

For food applications, vegetable oils are used as the continuous phase. Some studies have used white light paraffin oil and mineral oil. Emulsifiers are also added to form a better emulsion, because the emulsifiers lower the surface tension, resulting in smaller particles (Krasaekoopt et al., 2003).

2.2.1. Supporting material and technological conditions

There are many supporting materials used with the emulsion technique. We described below the most used ones.

Carrageenan and its mixtures

K-carrageenan is a neutral polysaccharide extracted from marine macroalgae, commonly used as a food additive. Carrageenan requires temperatures comprised between 60 and 90° C for dissolution especially when applied at high concentrations such as 2-5%. Its gelation is induced by temperatures changes. Probiotics are added to the polymer solution at 40-45º C and gelation occurs by cooling to room temperature. After the beads are formed, K^+ ions (in the form of KCl) are used to stabilize the gel and to prevent swelling, or to induce gelation. However, KCl has been reported to have an inhibitory effect on some lactic acid bacteria. As an alternative to KCl, Rb^+, Cs^+ and $NH4^+$ ions have been recommended. These ions, in addition to solve the above mentioned problem, produce stronger gel beads compared with potassium ions (Krasaekoopt et al., 2003).

It has been reported that a proportion of 1:2 for carrageenan and locust gum been gives a strong gel for microencapsulation (Miles, Morris & Carroll, 1984). This mixture has also good efficiency in lactic fermented products (such as yogurt) due to its lower susceptibility to the organic acids. For this reason, it has been widely used for microencapsulation of probiotics in fermented products（Audet, Paquin & Lacroix, 1988; Arnauld, Laroix & Choplin, 1992). However, gel formation of k-carrageenan and locust bean is dependent on calcium ions, which have adverse effects in the viability of *Bifidobacterium* spp. and in the human body because of undesirable effect on the electrolyte equilibrium of liquids in the body (Sun & Griffiths, 2000).

Sodium carboxymethyl cellulose

Sodium carboxymethyl cellulose (NaCMC) is a water soluble-cellulose ether derivative. It consists of linked glucopyranose residues with varying levels of carboxymethyl substitution. The gastric acid resistance and intestinal solubility properties of NaCMC enable its utilization in drugs and probiotics delivery (Kamel, Ali, Jahangir, Shah & El-Gendy, 2008). Chitprasert, Sudsai and Rodklongtan (2012) developed microcapsules using a mix of sodium carboxymetilceulosa and rice bran (RB) as filler. Rice bran is obtained by product of rice milling processes. It is considered to be a good filler. Furthermore, its low cost can help to reduce the production cost of the microcapsules. Microcapsules were prepared using a cell suspension in NaCMC with and without RB emulsified with palm oil and then crosslinking with aluminum ions. The results obtained show that microencapsulation using NaCMC and RB improved the viability of *Lactobacillus reuteri* after heat exposure. For this reasons, these particles could be applied to the development of probiotic products as functional feeds that require heat treatment.

Cellulose acetate phtalate (CAP)

This polymer is used for controlling drug release in the intestine because of its safety nature (Mortazavian et al., 2008). The advantage of CAP is that is insoluble in acid media (pH $\leq$5) but it is soluble when the pH is$\geq$6 as a result of the presence of phthalate groups. In this sense, microencapsulation of bacteria with CAP might offer an effective way of delivering large numbers of viable bacterial cells to the colon (Burgain et al., 2011). Rao, Shiwnavain and Maharaj, (1989) found that preparing an emulsion with starch and oil and adding CAP improved the viability of probiotics in simulated gastric environment. Other authors found similar result using spray drying process (Fávaro- Trindale & Grosso, 2002).

Alginate and its combinations

Calcium alginate has been widely used for the encapsulation of probiotic bacteria, mainly in the concentration range of 0.5-5% (Sheu & Marshall, 1991; Sheu, Marshall & Heymann, 1993; Truelstrup-Hansen, Allan-wojtas, Jin & Paulson, 2002; Kim, Baek & Yoon, 1996; Jankowski, Zielinska & Wysakowska, 1997; Kebary, Hussein & Badawi, 1998; Lee et al., 2000. Shah, & Rarula, 2000; Sultana et al., 2000; Krasaekoopt, Bhandari & Deeth, 2004; Martin, Lara-Villoslada, Ruiz & Morales, 2013).

Alginate microparticles can be obtained by external or internal gelation (Figure 1). In the first case, the microparticles are produced by the formation of a water-in-oil emulsion, usually stabilized by surfactants, such as Tween® 80. The alginate is then gelled by the addition of calcium chloride solution to the

emulsion, as it is explained in section 2.2. Although less common, the microcapsules may also be formed by internal gelation, in which the alginate in solution contains calcium carbonate. A water in oil emulsion is formed and after that an organic acid (acetic acid) is added. As it penetrates into the water phase it reacts with the calcium carbonate releasing calcium ions and carbonic acid. Calcium ions react with the alginate forming the egg-box structure (Cook, Tzortzis, Charalampopoulos & Khutoryanskiy, 2012).

Some drawbacks are attributed to alginate microparticles. For example they are susceptible to acidic environments. They crack and loss their mechanical stability in these environments. Moreover, alginate gel is formed in the presence of calcium ions, thus its integrity is deteriorated when subjected to monovalent ions or chelating agents (phosphates, lactates and citrates). Other disadvantages include difficulties in industrial scale applications. These particles are also very porous, which causes a fast diffusion of moisture and other fluids through the beads. This fact reduces the barrier properties against unfavorable environmental factors (Gouin, 2004). The mentioned defects can be solved by blending alginate with other polymer compounds, coating the alginate with different substances or doing some structural modification of the alginate (Krasaekoopt et al., 2003).

Blending alginate with corn starch has improved the effectiveness of the encapsulation technology using different bacterial cells (Martin et al., 2013; Zou et al., 2011). Starch is a polysaccharide composed by α-D-glucose units linked by glycosidic bonds, produced by all green plants. Resistant starch (RS) is the starch which is not digested by pancreatic enzymes (amylases) in the small intestine. For this reason it can reach the colon where it will be fermented. This specificity provides good enteric delivery characteristic. Moreover, resistant starch is an ideal surface for the adherence of the probiotic cells to the starch granules (Anal & Singh, 2007) and this can enhance probiotic delivery in a viable and a metabolically active state to the intestine (Vivek, 2013). Sultana et al., 2000; Sun et al., 2000; Truelstrup-Hansen et al., 2002; Krasaekoopt et al., 2003) produced particles with high cell viability bleeding the alginate with a resistant starch.

In addition, to improve the survivability of the frozen cells at -20ºC alginate can be blended with glycerol, for its cryogenic effect (Sultana et al., 2000).

Another strategy to improve physical and chemical stability of alginate particles is to form semipermeable layers of chitosan around the capsules. This structure is tolerant against the deteriorative effects of calcium chelating and antigelling agents. Structurally, the beads are also denser and much stronger, thus avoiding breaking and cells release (Krasaekoopt et al., 2003). Low molecular weight is preferred rather than high molecular weight chitosan (Krasaekoopt, Bhandari, & Deeth, 2006), since it diffuses faster into the alginate matrix, resulting in the formation of spheres with higher density and strength.

Another way of coating is using calcium chloride (Chandramouli, Kailasapathy, Peiris & Jones, 2004). This coating causes generation of more stable beads with a higher protective effect on the probiotic cells, and as a result, higher viability.

Polyamino acids can be also used as a coating material. In this sense, poly-L-lysine (PLL) makes strong complexes with alginate matrix and gives it the advantages previously mentioned for chitosan. Generation of multilayer shells of PLL on the alginate capsules has also been investigated. The first layer of PLL on

the particle surface produces positive charge, then the second alginate coat gives negative charge to the beads surface. This technique can be repeated several times. Other alternatives of polycationic polymers, are polyetylenamine and glutaraldehide (Mortazavian et al., 2007).

In addition, modifying alginate itself by fatty acids can be used as an encapsulating material. Amine, et al., 2014) developed palmitolated alginate microparticles using the emulsion technique. Furthermore, Le-Tien, Millette, Mateescu and Lacroix, (2004) elaborated microparticles using extrusion technique (Figure 1). Both kinds of particles were able to improve the stability of probiotic.

Chitosan

Chitosan is a linear polysaccharide with positive charge which is obtained by deacetylation of chitin extracted from crustacean shells. It is water soluble at pH < 6 and like alginate, forms a gel by ionotropic gelation. Chitosan exhibited inhibitory effects on different types of lactic acid bacteria and for this reason is preferred as a coating material as it explained before (Groboillot, Champagne, Darling & Poncelet, 1993).

Gelatin

Gelatin is a protein derived by partial hydrolysis of collagen. It has a special structure and versatile functional properties, and forms a solution of high viscosity in water, which sets to a gel on cooling. Its amphoteric nature gives the ability of having synergistic effects with anionic polysaccharides such as gellan gum. The two mentioned polymers are miscible at pH higher than 6, since they both carry net negative charges and repel one another. However, when the pH is adjusted below gelatin´s isoelectric point, the net charge on the gelatin becomes positive, causing an interaction with the negatively charged gellan gum. Mixture of gelain-toluene diisocyanate makes strong capsules which are tolerant against crackling and breaking, especially at higher concentrations. This can be attributed to the cross-link formation between these polymers. Mentioned mixture has been used for the encapsulation of *Lactobacillus lactis* ssp. Cremoris (Hyndman , Groboillot, Poncelet, Champagne & Neufeld, 1993). Gelatin has also crooslinked with genepin and coated with alginate to prevent the pepsin-induced degradation of the gelatin microspheres in simulated gastric juice (Annan, Borza & Truelstrup Hansen, 2008).

Chickpea protein

Chickpea protein was used as an encapsulating material because of its excellent functional attributes and nutritional importance. Chickpea is also attractive as a result of fewer allergen concerns. This protein is dominated by two salt-soluble globulin-type storage proteins: legumin and vicilinttributes. Wang, Korber, Low and Nickerson (2014) developed a chickpea protein–alginate microcapsules using emulsion technology. The particles offered good protection to *B. adolescentis* within synthetic gastric juice. Beads produced using this design, were <100 μm in size. Thus, there were no perceived adverse effects on the sensory attributes of this ingredient into foods by consumers. The study suggests that chickpea protein-alginate capsule designs could serve as a suitable probiotic carrier intended for food applications. Klemmer, Korber, Low and Nickerson (2011), used a mixture of pea protein and alginate to produce

microcapsules by extrusion. The particles were able to protect *B. adolescentis* within simulated gastric juice and simulated intestinal fluids. However, capsule sizes were too large for foods applications.

2.3. Fluid bed

In this process, cell suspension is sprayed and dried on inert carriers using a Wurster based fluidized bed system. The advantages of this process are total control over the temperature and lower comparable cost. The disadvantages are that this technology is difficult to master and relatively longer duration. Before drying, it is needed that probiotic culture is encapsulated in supporting material such as skimmed milk calcium alginate or fats. Shellac a purified product of the resinous secretion of the insect *Kerria lacca* (Coccoidea), has also been used. The physicochemical properties of shellac are variable depending on the strain of insect, host trees and refining methods (Buch, Penning, Wächtersbach, Maskos & Langguth, 2009). Because of its natural origin, shellac is an acceptable coating material for food supplement products. In general, shellac possesses good resistance to gastric fluid, suggesting its use for enteric coating purposes. However, the low solubility of shellac in the intestinal fluid, especially in the case of enteric coating of hydrophobic substances limits its use as an enteric coating polymer. To improve the enteric coating properties of shellac, Stummer et al., (2010) used sodium alginate, hydroxypropyl methylcellulose and polyvinylpyrrolidone as additional water-soluble polymers, and glycerol and glyceryl triacetate as plasticizers. Fluid bead is easy to scale up. For this reason is one of the mostly encapsulation technologies applied commercially to probiotics. Some companies have develop product using it such as Probiocap® and Duaolac® (Burgain et al., 2011). It can be adapted to give multilayer coatings too. In this respect, Champagne, Raymond and Tompkins (2010) used this method applying a coating with two different fats.

2.4. Rennet-gelled protein encapsulation

Microcapsules can be produced using a food approved enzyme (rennet) and an aqueous milk protein solution. Rennet is a proteolytic enzyme complex, which is capable of cleaving the k-casein molecule, which produces the aggregation of the casein micelles (Heidebach, Först & Kulozik, 2009). Non covalent cross-links are then progressively formed between chains of flocculating micelles to form a final gel above 18º C (Bansal, Fox & McSweeney, 2007). These microcapsules are able to encapsulate probiotics, without significant loss of cells during the encapsulation process. Survival of encapsulated cells can probably be explained by a higher local pH value within the protein matrix of the capsules caused by the protein buffering capacity. It can protect the cells during incubation under simulated gastric conditions at low pH. Furthermore, these proteins alleviate the feasibility to control the capsule size of microcapsules, which is of high importance regarding the sensory impact of the particles in final products. For all that reasons, this technique seems to be a suitable approach for a more effective application of probiotic in food.

2.5. Freeze Drying

Freeze-drying has been used to manufacture probiotic powders for decades but the combination of freeze-drying and encapsulation is relatively new concept. The process is based upon sublimation, occurring in three phases; freezing, primary, and secondary drying. Typically, cells are first frozen and then dried by sublimation under high vacuum (Santivarangkna, Kulozik & Foerst, 2007). As the processing conditions associated with freeze drying are milder than spray drying, higher probiotic survival rates are typically achieved (Wang, Yu, & Chou, 2004). In this technique, the solvent is frozen and removed via sublimation (Solanki et al., 2013). However, freezing causes damage to the cell membrane because of crystal formation and also imparts stress condition by high osmolarity. A variety of protectants have been added to the drying media before freeze drying to protect the viability of probiotics during dehydration, such as skim milk powder, whey protein, glucose, maltodextrine, trehalose among others. Cryoprotectants may also be added to media prior to fermentation to assist in the adaptation of probiotics to the environment (Basholli-Salihu, Mueller, Salar-Behzadi, Unger, & Viernstein, 2014; Capela, Hay & Shah, 2006). The mechanism of cryoprotectants is that they are able to accumulate within the cells, reducing the osmotic difference between the internal and external environments (Kets, Teunissen & de Bont, 1996).

2. 6. Spray drying

Spray drying is the most commonly used microencapsulation method in the food industry, since it is economical and flexible. The energy consumption of spray drying is 6 to10 times lower compared to freeze drying and it produces a good quality product. The process involves the dispersion of the core material, forming an emulsion or dispersion, followed by homogenization of the liquid, and then the atomization of the mixture into the drying chamber (figure 2). This leads to evaporation of the solvent. It is important to underline that in this technique, the product feed, gas flow and temperature should be controlled.

The advantage of the process is that it can be operated on a continuous basis. The disadvantage is that the high temperature used in the process may not be suitable for encapsulating probiotic bacterial cultures. On this point, outlet temperatures greater than 85-90ºC are lethal for probiotics. It is seen that under same inlet temperature conditions a higher inlet feed rate had a lower outlet temperature and an increased survival rate. This indicates that the cell survival is mostly dependent on outlet temperatures. Cellular membrane heat damage is one of the most susceptible target damage during spray drying. These high temperatures during spray drying cause the cellular pores to leak the intracellular substances (Anekella & Orsat, 2013). However, proper adjustment and control of the processing conditions such as the inlet and the outlet temperature can achieve viable encapsulated cultures with a desired particles size distribution (Table 2). Other factors that affect spray dried probiotic viability are the type of strain and their tolerance to stress conditions, the carrier, drying temperature and time of exposure to heat (before spray drying process) and the water activity and storage conditions (after spray drying process).

2.6.1. Two step drying

Normally, probiotics are spray dried at high inlet and outlet temperatures, (Table 2), in order to obtain a dry powder with a moisture content below 4%, required for safe storage. As it is mentioned before, such

drying temperatures are the most probable cause of unsatisfactory survival. Optimization of drying conditions in order to enhance their survival during storage is needed. In this sense, Chávez and Ledeboer (2007) developed a system based on the use of spray drying (Ti= 80º C, to=48º C) and vacuum drying at mild temperatures (45ºC). The result shows that a two-step drying process, is a realistic alternative to freeze drying in order to produce food powders containing viable probiotics. Furthermore, such a two-step process is estimated to be 3 times cheaper than freeze drying.

2.6.3. Spray Freeze drying

Spray freeze drying method combines processing steps that are common to freeze drying and to spray drying. Probiotic cells are in a solution which is atomized into a cold vapour phase of a cryogenic liquid such as liquid nitrogen. This step generates a dispersion of frozen droplets. Frozen droplets are then dried in a freeze dryer (Amin, Thakur, Jain, 2013). This technique presents various advantages, like providing controlled size, larger specific surface area than spray-dried capsules. Moreover capsules can be coated by an additional shell using the fluid bead method to give protection against adverse environmental conditions (Semyonov et al., 2010). However, this process has also some disadvantages including the use of high energy, the long processing time and the cost which is 30–50 times expensive than spray-drying (Zuidam & Shimoni, 2010).

Semyonov et al., (2010) using as a wall matrix maltodextrin, a polysaccharide that contributes reducing the mobility of the cells in the glassy state. Another matrix component was a disaccharide, trehalose, that act as a protective excipient, which is able to improve the cell viability during freezing (cryoprotectant), freeze drying , as well as during the storage of the dried bacteria. Trehalose is known to create hydrogen bonds with proteins and the polar head groups of the lipid membrane of the cells preventing structural damage during dehydration. The authors demonstrated that spray freeze drying is an appropriate process to generate dried microcapsules with *L. paracasei*. These particles are able to retain high viability during the spraying, freezing, and drying stages.

2.6.4. Spray chilling

Spray-chilling is also called spray cooling and spray congealing. This process is similar to spray drying with respect to the production of small droplets. However, spray-chilling is based on the injection of cold air, which enables the solidification of the particle. A molten matrix that contains the bioactive compound is atomized so that it forms drops that quickly solidify when they contact with the cold air (Champagne & Fustier, 2007).

The spray-chilling mainly uses fat matrices as carrier. The microparticles that are produced can present some disadvantages, which include a low encapsulation capacity and the expulsion of core material during storage, as a result of the crystalline structure and polymorphic arrangement characteristic of many lipid materials during the solidification and crystallisation process (Sato & Ueno, 2005). However, spray

chilling is considered to be the cheapest encapsulation technology that has the possibility of industrial scale manufacture (Gouin, 2004) Moreover, this technology could be used to generate smaller beads, which may be desirable in food processing. Pedroso, Thomazini, Barrozo Heinemann and Favaro-Trindade, (2012) using the spray chilling technology to microencapsulate *Bifidobacterium lactis* and *Lactobacillus acidophilus* using as wall materials, interesterified fat with palm and palm kernel. The solid lipid microparticles developed were efficient in protecting the probiotics against the passage through gastric and intestinal fluids, and they could also be stored at low temperatures. In addition, the morphologies and sizes of the microparticles may facilitate the flow of material, while causing no harmful effects towards the food texture.

2.6.5. Ultrasonic vacuum spray dryer

A technique based on spray drying which minimizing the thermal and oxidative stresses during the drying process has been developed. This system uses an ultrasonic nozzle, low temperatures and vacuum atmosphere in the drier chamber. Semyonov, Ramon and Shimoni, (2011) selected as wall material a mix of maltodextrin and trehalose, since as it indicated before, this components can increase the survival by maintaining the probiotic cells membrane integrity during the drying and storage as well as to promote the stabilizing effect of the bacteria's proteins. The results showed that the combination of a protein and a carbohydrate, contributed to retain a high viability after spray drying and to extend survival rates during storage.

2.6. Hybridisation system

The hybridisation system is a dry encapsulation technique. It consists of a high speed rotating rotor with six blades, a stator and a powder recirculation circuit. The powder mixture (host and guest particles) placed in the vessel is subjected to high impaction in air stream generated by the blade rotating at high speed. During the process, the particles form ordered mixture by embedding or filming of the guest particles onto the surface of the host particles. The hybridisation system results in high yields of microcapsules and minimizes heat induced bacterial damage using a cooling system that maintains temperatures below 30 ºC (Takafumi, Honda & Koishi, 1993). Some prebiotic substances have been tested with this technique such as: sorbitol, mannitol, lactulose, xylitol, inulin, fructooligosaccharide and raffinose. The results indicate that double microencapsulation by hybridisation is useful to effectively provide beneficial effects of probiotic for the host (Ann et al., 2007).

2.7. Impinging aerosol technology

Impinging aerosol technology uses two separate aerosols. One with the microbial suspension in alginate solution and the other one with calcium chloride. The mixture of alginate is injected form the top of a cylinder meanwhile the calcium chloride is injected from the base. This technology produces alginate microbeads with an average diameter of less than 40 μm (Sohail, Turner, Coombes, Bostrom & Bhandari, 2011). As no heat or solvent is used, impinging aerosol technology is suitable for encapsulating heat labile and solvent sensitive materials. Moreover, it has a large volume production capacity and

microbeads could be spray or freeze dried. Sohail et al. (2011) demonstrated that microbeads obtained by impiming aerosol technology and extruded macrobeads (approximately 2 mm diameter) offered similar protection to *L. rhamnosus* GG in the acid and bile tolerance study. Moreover, Sohail et al. (2012) investigated the effect of microencapsulation on the survival of *Lactobacillus rhamnosus* GG and *Lactobacillus acidophilus* NCFM and their acidification in orange juice at 25 °C for nine days and at 4 °C over thirty five days of storage. Unencapsulated *L. rhamnosus* GG was found to have excellent survivability in orange juice at both temperatures. However unencapsulated *L. acidophilus* NCFM showed significant reduction in viability. Encapsulation of these two bacteria did not significantly enhance survivability but did reduce acidification at 25° C and 4° C. In conclusion, *L. rhamnosus* GG showed excellent survival in orange juice and microencapsulation has potential in reducing acidification and possible negative sensory effects of probiotics in orange juice and other fruit-based products.

2.10. Electrospinning

The combined use of two techniques namely electrospray and spinning is made use in a highly versatile technique called electrospinning (electrop + spinning). In this technique, a high electric field is applied to a fluid which may be a melt or solution coming out from the tip of a die, which acts as one of the electrodes. This leads to the droplet deformation and finally to the ejection of a charged jet from the tip towards the counter electrode leading to the formation of continuous fibers (Figure 3).

The advantages of electrospinning technique are the production of very thin fibers or capsules to the order of few nanometers with large surface areas. Moreover, the possibility of large scale productions combined with the simplicity of the process makes this technique very attractive for many different applications (Agarwal, Wendorff & Greiner, 2008). In that regards, probiotic encapsulation has been carried through electrospinning using a protein based matrix (whey protein concentrate) and a carbohydrate based matrix (pullulan). Whey protein concentrate microcapsules have proved a greater improvement in cell viability when compared to pullulan structures (López-Rubio, Sanchez, Wilkanowicz, Sanz & Lagaron, 2012).

3. Encapsulated probiotic in food matrices

Although probiotic are normally considered as pharmaceutical products, the current trend is moving toward the health food sector, making true the Hippocrates' statement "let food be your medicine". Most probiotic foods in the current market are refrigerated dairy products. However, the analysis of these products in several different countries has confirmed that probiotic strains exhibit poor survival in food such as fermented dairy products (Shah, 2000). In this respect, probiotic microorganisms present in food should survive in significant number (10^6-10^8 CFU/g), although the number varies from strain to strain. Growth, survival and death of these microorganisms in food is largely governed by properties of the food (water availability, pH, buffering capacity, among others) in addition to the storage conditions (temperature, relative humidity and atmosphere). It has to be pointed that foods matrices should help probiotics to survive through the gastrointestinal tract and regulate the colonization of the gastrointestinal

tract. Therefore, selection of suitable food systems to deliver probiotics is a vital factor that should be considered in developing functional probiotic foods. Microencapsulation can also improve the viability of probiotic in some food matrices. In fact during the past few years, food products containing encapsulated probiotic cells have been introduced on the market (Burgain et al., 2011).

4. Studies of the effects of microencapsulated bacteria on some pathologies

As we mention in section 1, probiotic can produce beneficial effect in some pathologies. However, to get this beneficial effect, they have to reach the gut in adequate amounts. As a result of the harsh condition associated to the gastrointestinal tract, using encapsulated probiotic could be an interesting option. However, only a few *in vivo* studies have been carried out to test the beneficial effect of encapsulated probiotics in various pathologies.

In this respect, probiotic have been used to regulate the glucose concentration. These microorganisms are known to have health effects reducing cholesterol levels (Bhatia, Rana, Sharma, Singla & Randhawa, 2012) and immunomodulation (Kumar, Arora & Bhatia, 2011). In fact, there is a direct correlation between diabetes and immunomodulation. The result obtained by Bhatia, Sharma, Sood and Singla, (2013) using encapsulated Lactobacillus (*Lactobacillu*s (LB10) isolated from healthy buffalo milk and commercial probiotic from LeeBiotic Capsule (LCap) show that encapsulated probiotics have better efficacy as antidiabetic agent than same probiotic in unencapsulated form. Microparticles were prepared using the extrusion technology. In the groups treated with unencapsulated bacteria (LB10) and (LCap) the decrease in glucose level observed was 37.85% and 36.50% respectively whereas in the group receiving encapsulated bacteria LB10 and the encapsulated commercial probiotic, a decrease of 41.84% and 40.97% was observed respectively. Moreover the bacteria reduced the glucose level to normal within 14 days. Glibenclamide reduced the glucose level within 7 days. However this drug created hypoglycemic conditions. This result suggests that encapsulation improves the survival of bacteria under gastrintestinal conditions and produce a significant reduction of total blood glucose level. Hence, for a sustained beneficial health effect of probiotics, encapsulation of bacteria could be an alternative to decrease blood glucose levels.

Another factor which may be responsible for health benefits of probiotics is the induction of the of conversion of linoleic acid (LA) to conjugated linoleic acid (CLA). This fatty acid has shown to have anticholesterolemic action (Schlegel, Ringseis, Windisch, Schwarz & Eder, 2012). Results obtained by Bhatia, Rana, Sharma, Singla and Randhawa (2012), show that encapsulated and unencapsulated *Lactobacillu*s (isolated from healthy buffalo milk) as well as drug (Atorvastatin) reduced the cholesterol level. Microparticles were developed using the extrusion process. The percentage of decrease in cholesterol level in encapsulated an unencapsulated bacteria is almost parallel to that obtained in drug treated mice. The study also indicated that the effect of probiotics is independent from the encapsulation. According to these authors, this result could be because encapsulated bacteria could need a longer period of time to exert the effect since they are release in a slower but maintain way than unencapsulated bacteria.

Furthermore**,** it is known that the micromilieu of solid tumours provides an ideal environment for growth of facultative and strictly anaerobic bacteria (Cheng, et al., 2008). It has been shown that certain species including Lactobacillus and Clostridium can colonize those environments leading to regression of tumour growth (Cheng, et al., 2008; Matsuzaki, 1998; Tuo, et al., 2010; Zabala,et al., 2001; Kim, Oh, Yun, Oh & Kim, 2010). Such observations have given rise to the concept of bacteriolytic therapy where live microorganisms might be used to colonize the tumour and exert a tumorolytic effect. However, these lytic properties of some bacteria could also be detrimental for non-tumor cells. For this reason, it would be advantageous to explore a relatively non pathogenic strain and provide some form of containment that would enable site specific injection and minimise dispersion of the microorganism throughout the host. In testing the feasibility of such an approach, Dwivedi, Nomikou, Nigam, and McHale, (2012) prepared microencapsulated formulations of *Lactobacillus casei* NCDO 161 by external gelation. They demonstrated that these formulations were toxic for tumour cells *in vitro.* Authors also investigated the effects of the microencapsulated formulations on tumour growth *in vivo* following direct intratumoural injection. The study demonstrated significant inhibition of tumour growth and suggested the potential therapeutic benefit of this approach in the treatment of solid tumours.

In addition, Ruan et al., (2007) developed gelatin microparticles to be tested in a hemorrhagic shock model, using *B. longun*, *B. bifidum*, and *B. adolescentis*. Authors demonstrated that rat pretreated with encapsulated and unencapsulated Bifidobacteria, showed a decrease of total aerobes in cecum, magnitude of total aerobes to bacterial translocation levels of plasma endotoxin, and percentage of ileal villous damage when compared with rats treated with phosphate buffered saline. Encapsulated *Bifidobacteria* induced greater decreases than intact *Bifidobacteria* in this model, with the exception of a similar effect on ileal villous damage. Moreover, the incidence of bacterial translocation was decreased in hemorrhagic rats pretreated with *Bifidobacteria* compared with control. However, the magnitude of total anaerobes and *Bifidobacteria* were similar among hemorrhagic shocked rats receiving the different supplements.

5. Conclusion

Microencapsulation has been proved to be one of the most efficient methods for maintaining viability and stability of probiotics, as it protects probiotic during food processing and storage, as well as in gastric conditions. Besides the polysaccharides traditionally used as a matrix in microencapsulation, new materials are being tested and new technologies are developed such as electrospinning. However there is a need to develop new technologies or equipments that produce uniform particles for industrial applications. Further researches also have to be carried out to find appropriate carrier matrices, and bacterial strains. The extra costs incurred by microencapsulation have to be estimated so that they can be minimized. Cost savings can be derived from easier technologies, lower waste of bacterial material and better health impact of the product. Nevertheless, the research is actually focus on expanding the use of encapsulated probiotic in different food matrices.

In addition, only a few *in-vivo* studies have been carried out to test the beneficial effect of encapsulated probiotics. Although these studies show promising results, they have only been carried out in animals. Clinical trials, involving large numbers of patients, will be mandatory to achieve definite evidence of the

preventive and curative role of encapsulated probiotics in medical practice. Information about correct formulations in terms of amount of bacteria and viability and also the capability of these microorganisms to colonize their niche will be required. It is needed to standardize the administration schedule and to achieve homogeneous and comparable results.

6. Acknowledgments

This work has been funded by the University of Granada (FPU-UGR Programme).

7. Bibliography

Agarwal, S., Wendorff, J.H., & Greiner, A. (2008) Use of electrospinning technique for biomedical applications. *Polymer, 49,* 5603–5621.

Al-Otaibi, M.M. (2009). Evaluation of some probiotic fermented milk products from Al-Ahsa markets, Saudi Arabia. *American Journal Of food Technology 4,* 1-8.

Amin, T., Thakur, M., & Jain., S.C. (2013) Microencapsulation the future of probiotic cultures.. Journal of Microbiology Biotechnology and Food Sciences, *3,* 35-43.

Amine, K.M., Champagne, C.P., Salmieri, S., Britten, M., St-Gelais, D., Fustier, P., & Lacroix, M. (2014). Effect of palmitoylated alginate microencapsulation on viability of *Bifidobacterium longum* during freeze-drying, *Food Science & Technology, 56*, 111-117.

Anal, A.K., & Singh, H., 2007. Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *Trends Food Science and Technology*, *18*, 240–251.

Ananta, M. Volkert, & D. Knorr. (2005). Cellular injuries and storage stability of spray-dried Lactobacillus rhamnosus GG. *International Dairy Journal ,15,* 399–409.

Anekella, K., & Orsat, V. (2013). Optimization of microencapsulation of probiotics in raspberry juice by spray drying. *LWT- Food Science and Technology, 50,* 17-24.

Ann, E.Y., Kim, Y., Oh, S., Imm, J.Y., Park D.J., Han, K. S. & Kim, S.H. (2007). Microencapsulation of Lactobacillus acidophilus ATCC 43121 with prebiotic substrates using a hybridisation system. *International Journal of Food Science and Technology , 42,* 411–419.

Annan, N.T., Borza, A.D., & Hansen, T. (2008). Encapsulation in alginate coated gelatin microspheres improves survival of the probiotic *Bifidobacterium adolescentis* 15703T during exposure to simulated gastro-intestinal conditions. *Food Research International, 2,*184-193.

Arnauld, J..P, Laroix, C., Choplin, L. (1992). Effect of agitation rate on cell release rate and metabolism during continues fermentationwith entrapped growing *Lactobacillus casei* subsp. *casei*. *Biotech nology Tecniques*, 6, 265-*270.*

Audet, P., Paquin, C., Lacroix, C. (1988). Immobilized growing lactic acid bacteria with *κ*-carrageenan-locust bean gum gel. *Applied Microbiol ogy and Biotechnol.ogy*, *29*, 11-18.

Bansal, N., Fox, P. F., & McSweeney, P. L. H. (2007). Aggregation of rennet-altered casein micelles at low temperatures. *Journal of Agricultural Food Chemistry, 55,* 3120-3126.

Basholli-Salihu, M., Mueller, M., Salar-Behzadi, S., Unger, F. M., & Viernstein, H. (2014).Effect of lyoprotectants on b-glucosidase activity and viability of Bifidobacterium infantis after freeze-drying and storage in milk and low pH juices. *LWT - Food Science and Technology, 57,* 276-282.

Bhatia, A., Rana, P., Sharma, A., Singla, R., & Randhawa M. K. (2012). Preparation, characterization and hypocholesterolemic effect of sodium alginate encapsulated lab isolate. *Journal of Microbiology and Biotechnology Research, 2,*741-746.

Bhatia, A., Sharma, A., Sood, A., & Singla, R. (2013). Hypoglycemic effect of Encapsulated CLA producing probiotic isolate: An *in vivo* study. *Journal of Microbiology and Biotechnolo*gy *Research, 3,* 157-161.

Buch, K., Penning, M., Wächtersbach, E., Maskos, M., & Langguth, P. (2009). Investigation of various Shellac Grades: Additional analysis for identity. *Drug Development and Industrial Pharmacy, 35,* 694–703.

Burgain, J., Gaiani, C., Linder,M., & Scher, J.(2011). Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *Journal of Food Engineering, 104* , 467–483

Capela, P. Hay, T.K., Shah, N. P. (2006). Effect of cryoprotectants, prebiotics and microencapsulation on survival of probiotic organisms in yoghurt and freeze-dried yoghurt. *Food Research International, 39,* 203–211.

Car, J.P., & Ibrahim, S.A. (2005). Viability of bifidobacteria in commercial yogurts products in North Carolina. *Milchwissenschaft, 60,* 414-416.

Champagne, C. P., & Fustier, P. (2007). Microencapsulation for the improved delivery of bioactive compounds into foods. *Food Biotechnology, 18,* 184-190.

Champagne, C. P., Raymond, Y., & Tompkins, T.A. (2010). The determination of viable counts in probiotic cultures microencapsulated by spray-coating. *Food Microbiology, 27,* 1104-1111.

Champagne, C.P., Gardner, N.J.,& Roy, D. (2005). Challenges in the addition of probiotic cultures to foods. *Critical Reviews in Food Science and Nutrition, 45,* 61–84.

Chandramouli, V., Kailasapathy, K., Peiris, P., Jones, M. (2004). An improved method of microencapsulation and its evaluation to protect *Lactobacillus* spp. in simulated gastric conditions. *Journal of Microbiological Methods,* 56, 27–35.

Chavez, B.E., & Ledeboer, A. M. (2007). Drying of probiotic: Optimization of formulation and process to enhance storage survival. *Drying Technology, 25,* 1193-1201.

Cheng, C.M., Chuang, K.H., Hung, W.C., Shiea, J., Su, Y.C., Kao, C.H., Chen, B.M., Roffler, S.,& Cheng,T.L.(2008). Tumor-targeting prodrug-activation bacteria for cancer therapy. *Cancer Gene Theraphy, 15,* 393–401.

Chitprasert, P., Sudsai, P., & Rodklongtan, A. (2012). Aluminum carboxymethyl cellulose–rice bran microcapsules: Enhancing survival of Lactobacillus reuteri KUB-AC5. *Carbohydrate Polymers, 90,* 78–86.

Cook, M.T, Tzortzis G., Charalampopoulos, D.,& Khutoryanskiy, V.V. (2012). Microencapsulation of probiotics for gastrointestinal delivery. *Journal of controlled release, 162,* 56-67.

Corcoran, B. M., Ross, R.P., Fitzgerald, G. F., &. Stanton, C.(2004). Comparative survival of probiotic lactobacilli spray-dried in the presence of prebiotic substances *Journal of Applied Microbiology, 96,* 1024-1039.

Dianawati, D., Mishra, V., Shah, N. P. (2013). Stability of microencapsulated Lactobacillus acidophilus and Lactococcus lactis ssp. cremoris during storage at room temperature at low a_w. *Food Research International, 50,* 259-265.

Doherty, S. B., Gee, V. L, Ross R. P., Stanton, C., Fitzgerald, G. F., & Brodkorb, A. (2011), Development and characterisation of whey protein micro-beads as potential matrices for probiotic protection. *Food Hydrocolloids 25,* 1604-1617.

Dwivedi, A., Nomikou, N., Nigam, P.S., & McHale, A.P.(2012). The effects of microencapsulated Lactobacillus casei on tumour cell growth: In vitro and in vivo studies. *International Journal of Medical Microbiology, 302*, 293– 299.

FAO/WHO. (2001). Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. Food and Agriculture Organization of the United Nations andWorld Health Organization Expert Consultation Report. Available at: http:// www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf. Last Accessed 19.03.134.

Fávaro-Tindale, C.S., & Grosso, C.R. (2002). Microencapsulation of *L. acidophilus* (La-05) and *B. lactis* (Bb-12) and evaluation of their survival at the pH values of the stomach and in bile. *Journal of microencapsulation, 19,* 485-494.

Fritzen-Freire, C.B., Prudêncio, E.S., Amboni, R.D.M.C., Pinto, S. S., Negrâo-Murakami, A.N., & Murakami, F.S. (2012).Microencapsulation of bifidobacteria by spray drying in the presence of probiotics. *Food Research International, 45*, 306-312.

Gebare, C., Chaves, G.C., Ribeiro, K.S., Chaves, Ribeiro, M.C.E., Grosso, F.N., Gigante, C.R.F., Mirna, L. (2013). Viability of *Lactobacillus acidophilus* La5 in pectin–whey protein microparticles during exposure to simulated gastrointestinal conditions. *Food Research International, 51,* 872–878.

Gerez, C. L., Font De Valdez, G., Gigante, M. L., & Grosso, C. R. F. (2012). Whey protein coating bead improves the survival of the probiotic *Lactobacillus rhammnous* CRL 1505 to low pH. *Letters in Applied Microbiology, 54,* 552–556.

Golowczyc, M.A., Gerez, C.L., Silva, J., Abraham, A.G., De Antoni, G.L., & Teixeira, P. (2010). Survival of spray-dried *Lactobacillus kefir* is affected by different protectants and storage conditions. *Biotechnology Letters 33,* 681-686.

Gouin, S. (2004). Microencapsulation: industrial appraisal of existing technologies and trends. *Trends in Food Science and Technology, 15*, 330–347.

Groboillot, A.F., Champagne, C.P., Darling, G.F., & Poncelet, D. (1993). Membrane formation by interfacial cross-linking of chitosan for microencapsulation of Lactococcus lactis. *Biotechnology and bioengineering, 42*, 1157–1163.

Gunasekaran, S., Ko, S., & Xiao, L. (2007). Use of whey proteins for encapsulation and controlled delivery applications. *Journal of Food Engineering, 83*, 31-40.

Heidebach, T., Först, P., & Kulozik, U. (2009). Microencapsulation of probiotic cells by means of rennet-gelation of milk proteins. *Food Hydrocolloids, 23*, 1670–1677.

Heidebach, T., Först, P., & Kulozik, U. (2012). Microencapsulation of Probiotic Cells for Food Applications *Critical Reviews in Food Science and Nutrition, 52*, 291–311.

Hyndman, C.L., Groboillot, A., Poncelet, D., Champagne, C. & Neufeld, R.J. (1993). Microencapsulation of *Lactococcus lactis* with cross-link gelatin membranes. *Journal of Chemical and Technological Biotechnolology,56*, 259-263.

Hsiao, H-C., Lian, W-C., & Chou, C-C. (2004). Effect of packaging conditions and temperature on viability of microencapsulated bifidobacteria during storage. *Journal of the Science of Food and* Agriculture, *84*, 134-139.

Jankowski, T., Zielinska, M., & Wysakowska, A. (1997). Encapsulation of lactic and bacteria with alginate/starch capsules. *Biotechnology Techniques, 11*, 31-34.

Kailasapathy, K. (2002). Microencapsulation of probiotic bacteria: technology and potential applications. Current Issues in Intestinal Microbiology, 3, 39-48

Kamel, S., Ali, N., Jahangir, K., Shah, S. M., & El-Gendy, A. A. (2008). Pharmaceutical significance of cellulose: A review. *eXPRESS Polymer Letters, 2*, 758–778.

Kebary, K.M.K., Hussein, S.A., & Badawi, R.M. (1998). Improving viability of *Bifidobacterium* and their effect on frozen ice milk. *Journal of Dairy Science, 26*, 319-337.

Kets, E.W., Teunissen, P.J.M., & De Bont, J.A. M. (1996). Effect of Compatible Solutes on Survival of Lactic Acid Bacteria Subjected to Drying. *Applied and Enviromental Microbiology, 62,* 259–261.

Khalil, A.H., & Mansour, E.H. (1998). Alginate encapsulated bifidobacteria survival in mayonnaise. *Journal of Food Sciences. 63,* 702-705.

Kim, I.K., Baek, Y.J., & Yoon, Y.H. (1996). Effects of dehydration media and immobilization in calcium-alginate on the survival of *lactobacillus casei* and *Bifidobacterium bifidum*. *Korean Journal of Dairy Science, 18*, 193-198.

Kim, Y., Oh, S., Yun, H.S., Oh, S., & Kim, S.H .(2010). Cell-bound exopolysaccharide from probiotic bacteria induces autophagic cell death of tumour cells. *Letters in Applied Microbiology, 51,* 123–130.

Klein, G., Pack, A., Bonaparte, C., & Reuter, G.(1998). Taxonomy and physiology of probiotic lactic acid bacteria. *International Journal of Food Microbiology, 41*, 103–125.

Klemmer, K. J, Korber, D. R., Low, N. H, & Nickerson, M. T. (2011). Pea protein-based capsules for probiotic and prebiotic delivery. *International Journal of Food Science and Technology*, *46*, 2248-2256.

Krasaekoopt W., Bhandari B., & Deeth, H. (2004). The influence of coating materials on some properties of alginate beads and survivability of microencapsulated probiotic bacteria. *International Dairy Journal, 14*, 737-743.

Krasaekoopt, W., Bhandari, B. & Deeth, H. (2003). Evaluation of encapsulation techniques of probiotics for yoghurt. *International Dairy Journal, 13*, 3-13.

Krasaekoopt, W., Bhandari, B., & Deeth, H.C. (2006). Survival of probiotics encapsulated in chitosan-coated alginate beads in yoghurt from UHT- and conventionally treated milk during storage. *LWT - Food Science and Technology*, *39*, 177-183.

Kumar R., Arora D., & Bhatia, A.(2011). Therapeutic potential of bioconverted conjugated linoleic acid in drug induced immunosuprressed and infective organism induced plasmodium Berghei. *International Journal of Pharmaceutical Sciencei, 3*, 212-214.

Kurmann, J. A., & Rasic, J.L. (1991).*Therapeutic properties of fermented milks*. London, UK: Elsevier Applied Science Publishers.

Lawless, H. T., & Heymann, H. (2010). Sensory evaluation of food: Principles and practices (2a ed.). Berlin: Springer.

Lee, J.-W., Shin, J.-G., Kim, E.H., Kang, H.E., Yim, I.B., Kim, J.Y., Joo, H.-G., Woo, H.J., Lee, K.I., & Heo, T.R. (2000). Survival of Bifudobacterium longum immobilized in calcium alginate beads in simulated gastric juices and bile salt solution. *Applied and Environmental Microbiology, 66*, 869- 973.

Le-Tien, C., Millette, M., Mateescu, M-A., & Lacroix, M. (2004).Modified alginate and chitosan for lactic acid bacteria immobilization. *Biotechnology and. Applied Biochemistry*, *39*, 347–354.

López-Rubio A.,, Sanchez, E., Wilkanowicz, S. Sanz, Y., & Lagaron, J.M. (2012). Electrospinning as a useful technique for the encapsulation of living bifidobacteria in food hydrocolloids. *Food Hydrocolloids 28*,159-167.

Majamaa, H., & Isolauri, E. Probiotics: a novel approach in the management of food allergy.(1997). *Journal of Allergy and Clinical Immunology, 99*, 179-185.

Malchow, H.A. (1997). Crohn's disease and *Escherichia coli*. A new approach in therapy to maintain remission of colonic Crohn's disease?. *Journal of Clinical Gastroenterology, 25*, 653-658.

Malmo, C., La Storia, A., & Mauriello, G. (2013). Microencapsulation of Lactobacillus reutieri DSM 17938 cell coated in alginate beads with chitosan by spray drying o use as a probiotic cell in a chocolate soufflé. *Food and Bioprocess technology, 6*, 795-805.

Martin, M.J., Lara-Villoslada, F., M.A. Ruiz, M.A.,& Morales, M.E. (2013).Effect of unmodified starch on viability of alginate-encapsulated Lactobacillus fermentum CECT5716. *LWT - Food Science and Technology, 53*, 480-486.

Matsuzaki, T.(1998). Immunomodulation by treatment with Lactobacillus casei strain Shirota. *International Journal of Food Microbiologyl, 41,* 133–140.

Miles, M. J., Morris, V.J., & Carroll, V. (1984). Caron gum k-carrageenan mixed gles: Mechanical properties and X-ray fibre diffraction studies. *Macromelecules, 17,*2443-2447.

Moreno, Y., Collado, M.C, Ferrus, M.A., Cobo, J.M., Hernandez, E., & Hernandez, M . (2006). Viability assessment of lactic acid bacteria in commercial dairy products stored at 4ºC using LIVE/DEAD® BacLight™ staining and conventional plate counts. *International Journal of Food Science and Technology, 41,* 275-280.

Mortazavian, A., Razavi, S. H., Ehsani,M. R., & Sohrabvandi, S. (2007). Principles and methods of microencapsulation of probiotic microorganisms. *Iranian Journal of Biotechnology, 5,* 1- 18.

Mortazavian, A.M., Azizi, A., Ehsani, M.R., Razavi, S.H., Mousavi, S.M., Sohrabvandi, S., & Reinheimer, J.A. (2008). Survival of encapsulated probiotic bacteria in Iranian yogurt drink (Doogh) after the product exposure to simulated gastrointestinal conditions. *Milchwissenschaft 63,* 427–429.

O'Riordan, K., Andrews, D., Buckle, K., & Conway, P. (2001). Evaluation of microencapsulation of a Bifidobacterium strain with starch as an approach to prolonging viability during storage. *Journal of Applied Microbiology,* 91, 1059-1066.

Paéz, R., Lavari, L., Vinderola, G., Audero, G., Cuatrin, A., Zaritzky, N., & Reinheimer, J.(2012). Effect of heat treatment and spray drying on lactabacilli viability and resistance ot simulated gastrointestinal digestión. *Food Research International, 48,* 748-754.

Pedroso D.L, Thomazini, M., Barrozo Heinemann, R. J., & Favaro-Trindade, C.S. (2012).Protection of *Bifidobacterium lactis* and *Lactobacillus acidophilus* by microencapsulation using spray-chilling. *International Dairy Journal, 26,*127-132.

Picinin De Castro-Cislaghi, F.P., Dos Reis E Silva, C., Fritzen-Freire, C.B., Goulart Lorenz, J., Sant´Ánna, E.S. (2012). .*Bifidobacterium* Bb-12 microencapsulated by spray drying with whey: Survival under simulated gastrointestinal conditions, tolerance to NaCl, and viability during storage. *Journal of Food Engineering, 113,* 186-193.

Picot, A., & Lacroix, C. (2003). Production of multiphase water-insoluble microcapsules for cell microencapsulation using an emulsification/spray-drying technology. *Journal of Food Science, 68,* 2693-2700.

Rao, A.V., Shiwnavain, N.,& Maharaj, I. (1989). Survival of microencapsulated Bifidobacterium pseudolongum in simulated gastric and intestinal juices. *Canadian Institute of Food Science and Technology Journal, 22,* 345–349.

Reid G. Probiotic agents to protect the urogenital tract against infection. (2000). *American Journal of Clinical Nutrition, 73,* 437-443

Rokka, S., & Rantamäki, P. (2010). Protecting probiotic bacteria by microencapsulation: challenges for industrial applications. *European Food Research Technology*, *231*, 1-12.

Ruan, X., Shi, H., Xia, G., Xiao, Y., Dong, J., Ming, F., & Wang, S. (2007). Encapsulated *Bifidobacteria* reduced bacterial translocation in rats following hemorrhagic shock and resuscitation. *Nutrition, 23,* 754–761.

Sanders, M.E. (1998).Overview of Functional Foods: Emphasis on Probiotic Bacteria. *International Dairy Journal, 8,* 341-347

Santivarangkna,C., Kulozik,U. and Foerst,P. (2007) Alternative drying processes for the industrial preservation of lactic acid starter cultures. *Biotechnoogy Progress, 23*, 302-315.

Sanz, Y. (2007). Ecologiacal and functional implications of the acid-adaptation ability of Bifidobacterium: A way of selecting improved probiotic strains. *International Dairy Journal, 17,* 1284-1289.

Sarkar, S. (2010). Appoaches for enhancing the viability of probiotics: a review. *Bristish food Jorunal, 112,* 329-349.

Sato, K., & Ueno, S. (2005). *Polymorphism in fats and oils*.. New York, USA: Wiley Interscience.

Schlegel, G, Ringseis, R., Windisch, W., Schwarz, F.J., & Eder, K.(2012).Journal of Dairy Science, *95*, 3905-3918.

Semyonov, D., Ramon, O., Kaplun, Z., Levin-Brener, L., Gurevich, N., & Shimoni, E. (2010). Microencapsulation of Lactobacillus paracasei by spray freeze drying. *Food Research International 43* , 193–202.

Semyonov,D., Ramon,O., & Shimoni, E.(2011). Using ultrasonic vacuum spray dryer to produce highly viable dry probiotics, *LWT - Food Science and Technology, 44,* 1844-1852.

Shah, N. P. (2000). Probiotic bacteria: selective enumeration and survival in dairy foods. Journal of Dairy Science, 83, 894–907.

Shah, N.P.,& Rarula, R.R. (2000). Microencapsulation of probiotic bacteria and their survival in frozen fermented dairy desserts. *Australian Journal of Dairy Technology, 55,* 139-144.

Shah, N.P., Lankaputhra, W.E.V., Britz, M.L., & Kyle, W.S.A. (1995).Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in Commercial Yoghurt During Refrigerated Storage. *International Dairy Journal, Volume 5*, 515-521.

Sheu, T.Y., & Marshall, R.T. (1991). Improving culture viability in frozen dairy desserts by microencapsulation. *Journal of Dairy Scince, 74,* 107-111.

Sheu, T.Y., Marshall, R.T., & Heymann, H. (1993). Improving survival of culture bacteria in frozen dessert by microentrapment. *Journal Dairy Science, 76*, 1902-1907

Shi, L.E., Li, Z-H., Li, D-T., Xu, M., Chen, H-Y, Zhang , Z-L., & Tang, Z-X.(2013).Encapsulation of probiotic *Lactobacillus bulgaricus* in alginate–milk microspheres and evaluation of the survival in simulated gastrointestinal conditions. *Journal of Food Engineering, 117*, 99–104.

Shi, L-E., Li, Z-H., Zhang, Z- L., Zhang, T- T., Yu, W- M., Zhou, M-L., & Tang, Z-X. (2013). Encapsulation of Lactobacillus bulgaricus in carrageenan-locust bean gum coated milk microspheres with double layer structure. *LWT - Food Science and Technology, 54,* 147-151.

Sohail, A., Turner, M.S., Prabawati, E.K., Coombes, A. G.A., & Bhandari, B.(2012). Evaluation of *Lactobacillus rhamnosus* GG and *Lactobacillus acidophilus* NCFM encapsulated using a novel impinging aerosol method in fruit food products. *International Journal of Food Microbiology, 15,* 162–166.

Sohail, A., Turner, M.S., Coombes, A., Bostrom, T. , & Bhandari, B. (2011).Survivability of probiotics encapsulated in alginate gel microbeads using a novel impinging aerosols method. *International Journal of Food Microbiology ,145,* 162–168.

Solanki, H.K., Pawar, D.D., Shah, D.A., Prajapati, V.D., Jani, G.K.,. Mulla, A. M., & Thakar, P. M. (2013). Development of Microencapsulation Delivery System for Long-Term Preservation of Probiotics as Biotherapeutics Agent. *BioMed Research International*, 2013, 1- 21.

Stummer, S., Salar-Behzadi, S. Unger, F.M., Oelzant, S., Penning, M., & Viernstein, H. (2010). Application of shellac for the development of probiotic formulations. *Food Research International, 43* 1312–1320.

Su, R., Zhu, X-L., Fan, D-D., Mi,Y., Yang, C-Y.,& Ji, X. (2011).Encapsulation of probiotic Bifidobacterium longum BIOMA 5920 with alginate–human-like collagen and evaluation of survival in simulated gastrointestinal conditions. *International Journal of Biological Macromolecules 49*, 979– 984.

Sultana, K., Godward, G., Reynolds, N., Arumugaswamy, R., Peiris, P., & Kailasapathy, K. (2000). Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. *International Journal of Food Microbiology,62,* 47–55.

Sun, W.,& Griffiths M.W. (2000). Survival of bifidobacteria in yogurt and simulate gastric juice following immobilization in gellanxanthan beads. *International Journal of Food Microbioliology*, 61, 17-25.

Takafumi, I., Honda, H.. & Koishi, M. (1993). Drug dissolution from indomethacin–starch hybrid powders prepared by the dry impact blending method. *The Journal of Pharmacy and Pharmacology, 45*, 770–774.

Truelstrup-Hansen L., Allan-wojtas PM., Jin Y.L., Paulson, A.T. (2002). Survival of free and calcium-alginate microencapsulated *Bifidobacterium* spp. in simulated gastro-intestinal conditions. *Food Microbiology, 19,* 35-45.

Tuo, Y.F., Zhang, L.W., Yi, H.X., Zhang, Y.C., Han, X., Du, M., Jiao, Y.H., Wang, S.M.(2010). Antiproliferative effect of wild Lactobacillus strains isolated from fermented foods on HT-29 cells. *Journal of Dairy Science, 93,* 2362–2366.

Vivek K. B. (2013). Use of encapsulated probiotics in dairy based foods. *International Journal of Food, Agriculture and Veterinary Sciences, 3,* 188-199.

Wang, J., Korber,D. R., Low, N.H., & Nickerson, M.T.(2014). Entrapment, survival and release of *Bifidobacterium adolescentis* within chickpea protein-based microcapsules. DOI: doi: 10.1016/j.foodres.2013.09.018

Wang, Y.C., Yu, R.C., & Chou, C.C .(2004). Viability of lactic acid bacteria and bifidobacteria in fermented soymilk after drying, subsequent rehydration and storage. *International Journal of Food Microbiology, 93*, 209-217.

Ying, D.Y, Phoon, M.C., Sanguansri, L., Weerakkody, R., Burgar, I., & Augustin, M.A. (2010). Microencapsulated *Lactobacillus rhamnosus* GG powders: Relationship of powder physical properties to probiotic survival during storage. *Journal of Food Science, 75,* 588-595.

Ying, D., Sun, J., Sanguansri, L., Weerakkody, R.,& Augustin, M.A. (2012).Enhanced survival of spray-dried microencapsulated *Lactobacillus rhamnosus* GG in the presence of glucose. *Journal of Food Engineering, 109*, 597-602.

Zabala, A., Martin, M.R., Haza, A.I., Fernandex, L., Rodriguez, J.M., & Morales, P. (2001). Anti-proliferative effect of two lactic acid bacteria strains of human origin on the growth of a myeloma cell line. *Letters in Applied Microbiolog, 32*, 287–292.

Zou, Q., Zhao, J., Liu, X., Tian, F., Zhang, H-p, Zhang, H., & Chen, W. (2011).Microencapsulation of Bifidobacterium bifidum F-35 in reinforced alginate microspheres prepared by emulsification ⁄ internal gelation. *International Journal of Food Science and Technology, 46,* 1672–1678.

Zuidam, N.J., & Shimoni, E. (2010). Encapsulation Technologies for Active Food Ingredients and Food Processing. New York, U. S.A: Springer.

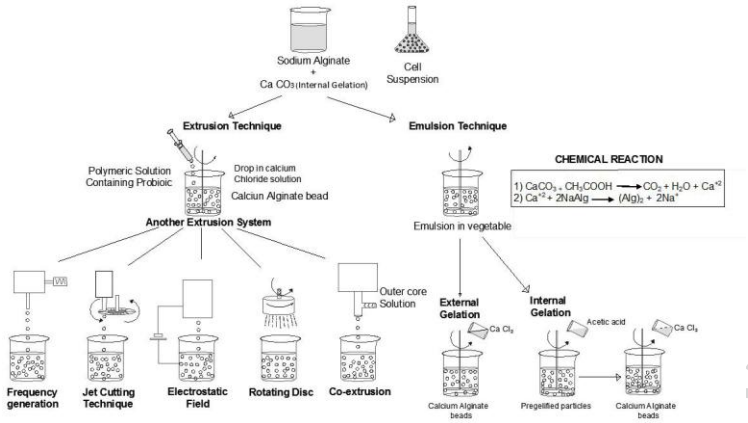

Figure 1

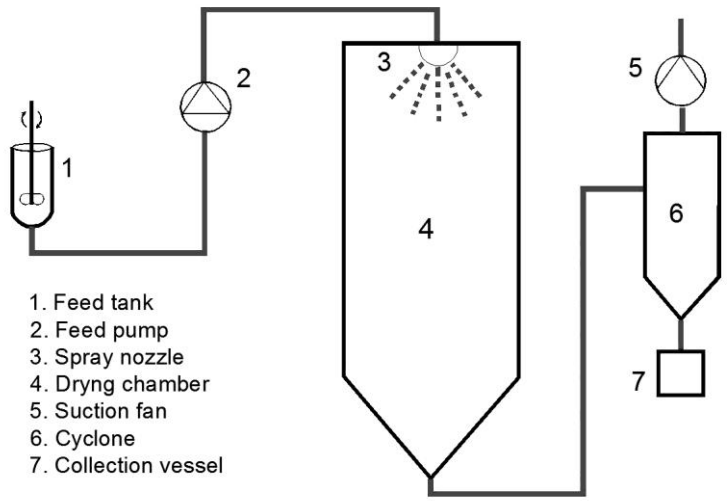

Figure 2

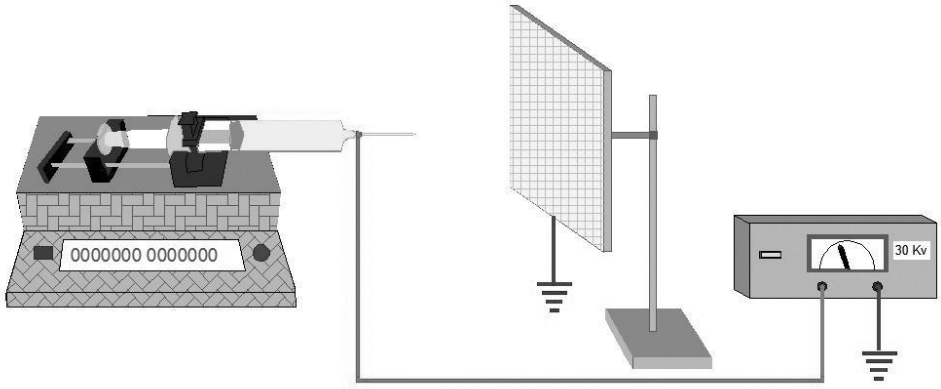

Figure 3

Technique	Particle size	Typical materials	Special treatment	Physical and chemical stability	Technical limitations	[+]Material cost	Food	Authors
Extrusion	1.89mm	Alginate	Used of Low molecular chitosan	Chitosan did not much improve the survival of encapsulated probiotics in the yoghurts	Big Particle size. Difficult to scale up. Particles are not dried.	**	Yogurt	Krasaekoopt et al., (2006)
External gelation	0.5-1mm	Alginate + Hi-maize starch (Resistant starch)	Used of Hi-maize starch and glycerol	Bacteria was not protected from low pH conditions	Not uniform bead size. Perceptible gritty mouthfeel. Particles are not dried.	**	Yogurt	Sultana et al., (2000); Kaisalapathy (2006)
Fluidized bed	15-40 µm	Whey protein	Used of palm oil and cellets®	Encapsulation did not improve probiotic viability in food during storage	Difficult to master Longer duration	*	Infant formula	Weinbreck et al. (2010)
				Sucrose and lactose protect lees than cellobiose and		***		

Freeze- drying	-	Sucrose, lactose, cellobiose, or trehalose	-	trehalose in simulated gastric juice	Freezing damage		Pasteurized low fat milk Grape and red beet juices	Basholli-Salihu, et al. (2014)
Spray-drying	3-75 µm	Whey protein	Used of milk fat	Milk fat did not improve the viability of probiotic	High temperatures (Heat damage of bacteria)	**	Yogurt	Picot & Lacroix (2004)
	11.23 µm	Whey protein		Microparticles did not improve the survival of probiotic in bile salt		*	Dairy dessert (White chocolate flavor vigor Delicatessen®)	Picinin De Castro et al. (2012)
	-	Raspberry juice pulp	Preheating of bacteria	Microorganism were killed during pre-heating in raspberry juice		*	Possible inclusion in non-dairy probiotic food	Anekella & Orsat (2013)
Impinging	10-40 µm	Alginate		Encapsulation was not able to enhance the survival of probiotic in orange juice	Need of a specific equipment Particles are not dried	**	Orange juice and pear and peach snack	Sohail et al., (2012)

[+]Material cost are classified such as: The cheapest one (*) to the most expensive (***)

Table 1. Microparticles produced by different technologies included into food matrices.

Probiotic	Encapsulation matrix	Spray drying Conditions	Reference
***Bifidobacterium* PL1**	Modified waxy maize starch	Ti=100ºC To=45ºC	O'Riordan, Andrews, Buckle & Conway (2001)
***L. acidophilus* La-05** ***B.Lactis* Bb-12**	Celulose Acetate pathalate	Ti=130ºC To= 75ºC	Favaro-Trindade & Grosso (2002)
B. breve R070 ***B.longum R023***	Whey protein isolate (10%w/w) Milk fat	Ti=160ºC To=80ºC	Picot & Lacroix (2003)
Lactobacillus. rhamnosus GG ***L. rhamnosus* E800** **L. salivaris UCC 500**	Skim milk (20% w/v) Polydextrose (20% w/v) Inuline (20% w/v) Skim milk(10%) + Polydextrose (10% w/v) Skim milk (10% w/v) + Inuline (10% w/v) Raftilose® P9 and Synergy1 Raftiline® GR and HP	Ti=140ºC To=85-90ºC	Corcoran, Ross, Fitzgerald & Stanton (2004)
B. longum B6 ***B.infantis* CCRC 14633**	Gelatin (10%) Soluble starch (10%) Skim milk (10%) Arabic Gum (10%)	Ti=100ºC To=50ºC	Hsiao, Lian & Chou (2004)
L. rhamnosus GG	Skim milk (20% w/v) Polydextrose (20% w/v) Inuline (20% w/v) Raftilose® P95 (20 w/v %)	Ti=140ºC To= 70-100ºC	Ananta,. Volkert, & Knorr (2005)
	Skim milk (11% w/v)		

***Lactobacillus kéfir CIDCA* 8321 and 8348**	Skim milk+ Sucrose (2% w/v) Skim milk + Monosodium glutamate (1.25% w/v) Skim milk + Frutooligosaccharides (2% w/v)	Ti=160ºC To= 70ºC	Golowczyc et al. (2010)
L. rhamnosus GG	Whey protein isolate (32.7% w/w) + maltodextrine (65.3% w/w) Whey protein isolate (32.7% w/w) + maltodextrine (32.7% w/w) + Glucose (32.7% w/w) Whey protein isolate (32.7% w/w) +Iinulin (65.3% w/w) Whey protein isolate (32.7% w/w)+ Inulin (32.7% w/w)+ glucose(32.7% w/w)	Ti=160ºC To= 65ºC	Ying et al. (2010)
Bifidobacterium BB-12	Skim milk (20% w/v) Skim milk (10% w/v)+ Inulin (10% w/v) Skim milk (10% w/v)+ Orafti ®Synergy1 (10% w/v) Skim milk (10% w/v)+ Oligofructose	Ti=150ºC To= 55ºC	Fritzen-Freire et al. (2012).
L. casei ***L paracasei*** ***L acidophilus*** ***L. plantarum***	Skim milk (20% w/v)	Ti=170ºC To= 85ºC	Paéz et al. (2012)
Bifidobacterium BB-12	Whey	Ti=150ºC To= 50-60ºC	Picinin De Castro-Cislaghi, Dos Reis E Silva, Fitzeb-Freire, Goulart Lorenz & Sant´Ánna, (2012).
***L. acidophilus* NRRL B-4495** ***L. rhamnosus* NRRL B-442**	Raspberry juice+ Maltodextrine	Ti=100ºC To= 50ºC	Anekella & Orsat (2013).
Lactobacillus acidophilus ***Lactobacillus lactis* ssp**	Vegetable oil (10% w/v) Sodium caseinate (6% w/v)	Ti=99ºC	Dianawati, Mishra & Shah (2013)

	Fructooligasaccharides(2% w/v) D-glucose (3% w/v) Mannitol (3% w/v)	To= 50ºC	
***Lactobacillus reuteri* DSM 17938**	Alginate (1% w/v) Calcium Chloride (1% w/v)	Ti=99ºC To= 50ºC	Malmo, La Storia & Mauriello (2013).

Table 2

Highlight

The article summarizes the most important and new technologies applied in probiotic encapsulation.

An overview of the materials used in these technologies is given, paying special attention to advantages and disadvantages.

To our knowledge this is the first time that a review described the results obtained using encapsulated probiotic in various pathologies.