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MICROENCAPSULATION OF PROBIOTICS IN FUNCTIONAL DAIRY PRODUCTS DEVELOPMENT

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ABSTRACT

Consumer trends with respect to food choice are changing due to the increasing awareness of the link between diet and health. The health benefits of probiotics have resulted in their increased incorporation in functional dairy foods, leading to creation of a new generation of health foods. Dairy products have been considered as a good carrier for probiotics since fermented foods and dairy products have particularly a positive image. But viability of probiotics in fermented dairy products as well as gastrointestinal conditions is very minimal level, which has encouraged researchers to innovate different methods of probiotics viability improvement. Microencapsulation of the probiotic cells is one of the newest and highly efficient methods, which is now under the special attention and is being developed by various researchers. However, there are still many challenges to overcome with respect to the microencapsulation process and the conditions prevailing in fermented milk products and human gut. In this presentation/paper, the importance of encapsulation of probiotics, methods of encapsulation and wall materials employed in the microencapsulation of probiotics will be reviewed.

I. INTRODUCTION

The word “probiotics” literally means “for life,” and refers to living microorganisms that, when consumed in sufficient numbers, exert health benefits beyond basic nutrition. The first recorded probiotic was fermented milk for human consumption. After that, probiotics became popular with animal nutrition. The role of fermented milk in human diet was known even in Vedic times. But, the scientific interest in this area boosted after the publication of the book entitled "The Prolongation of Life" by Ellie Metchinkoff in 1908, a noble laureate, who attributed the good health and longevity of Bulgarian peasants to sour milk and yoghurt. More recently in a 1981 census, it was noted that Azerbaijan has one of the highest rates of longevity in the world and approximately 48.3 people per 100,000 inhabitants were aged 100 years and above. Again, it was pointed out that yoghurt is a common food in this community. Indeed, the word ‘yoghurt’ is of Azerbaijani origin. Among indigenous people of Africa, yoghurt is highly valued and often used to celebrate special occasions.

The human gastrointestinal tract is home to diverse and vast communities of microorganisms representing over 400 cultivable species. The colonisation of the gastrointestinal tract begins immediately after birth. The mode of

delivery, use of antibiotics, and the level of hygiene are known to exert a significant influence on the number and species of microorganisms that colonise the gut. A change to the adult flora occurs after weaning and by the second year of life the intestinal flora becomes similar to that of an adult and remains relatively stable throughout life. The density and diversity of microbes increases progressively from stomach ($10^{2–3}$ colony forming units (cfu)/g lumenal contents) to colon ($10^{11–12}$ cfu/g lumenal contents). In a healthy adult, the gastrointestinal tract contains 10 times as many bacteria (10^{14} bacteria) as eukaryotic cells in the entire body; the combined genome of the intestinal flora is estimated to be 50–100 times the size of the human genome. This ecosystem gets disrupted when exposed to toxics in the form of polluted water and food as well as injudicious use of antibiotics. This causes destruction of beneficial bacteria leaving resistant ones, pathogenic. Of late it has been realized by health care professionals and prompted them to seek alternative therapeutic options. One such alternative is the use of beneficial bacteria, the probiotics, which stimulate health – promoting indigenous flora and reverting back the change.

According to FAO/WHO [1], Probiotics are "live microorganisms which when ingested or locally applied in sufficient numbers, confer one or more specified demonstrated health benefits for the host."

The most desirable properties of a probiotic culture according to FAO/WHO guidelines (2002) include:

- Human origin.
- The ability to survive harsh environment in the digestive tract i.e. resistance to bile salts, hydrochloric acid, and pancreatic juice.
- Adherence to epithelial cells and intestinal mucosa.
- Colonization potential in the human intestinal tract or the respective target organ.
- Production of antimicrobial substances to kill or suppress pathogens.
- Safety with regard to human use (GRAS status)
- Stability during storage under normal conditions.

The standard for any food sold with health claims from the addition of probiotics is that it must contain per gram at least 10^6 to 10^7 cfu of viable probiotic bacteria FAO/WHO(2001).While there is no universal rule in this regard a count of 10^7 cfu/ml has been found to be acceptable in the case of yoghurt by the vast majority of dairy companies, starter culture producing companies and national regulatory organizations, as well as the International Dairy Federation (IDF) [2].

The bacteria should be metabolically stable, remain active in the product and survive passage through the upper digestive tract in large numbers to bring about the required beneficial effects in the host. Therefore it is of utmost importance to ensure a high survival rate of these microorganisms during the shelf life of the food product to maintain consumer confidence in probiotic products. Several reports have shown that survival of probiotic bacteria is often low in yoghurt.

The viability of probiotics in functional dairy products is greatly affected by their exposure to bacteriostatic/bactericidal factors such as acidity, pH, dissolved oxygen content, hydrogen peroxide, storage temperature, concentration of organic acids etc., A high viable population of probiotic bacteria in food products

at the point of consumption however, does not guarantee the same survival after arrival of the cells in the intestine. The very low pH of the stomach along with the presence of bile salts in the small intestine are the main reasons for the dramatic decline in viability of delivered cells. Their viability can vastly be improved by incorporation of prebiotics , microencapsulation and addition of cryoprotectants.

Microencapsulation of probiotic bacteria can be used to enhance their viability during processing and also for their targeted delivery in gastrointestinal tract. Microencapsulation is a technology of packaging solids, liquids or gaseous materials in miniature sealed capsules that can release their contents at controlled rates under the influences of specific conditions. Microencapsulation improves the probiotic viability and sensory properties due to its protective effects against detrimental environmental period in yoghurt. Electron microscopy could prove to be an useful tool to measure accurately the size of microcapsules and to assess their overall structure. As of now, there are only a few publications regarding electron microscopy techniques used to gain a micro structural description of bacteria loaded microcapsules [3]. Most studies found that large capsule size of 100 µm can negatively influence the texture and mouth feel of a food product [4].

Improving the viability of probiotic bacteria in different food products, as well as under gastrointestinal tract conditions after ingestion has been by far the most significant concern in the field of probiotics during recent years. Their tolerance to pH therefore becomes a critical factor in influencing their probiotic functionality. The low pH of the stomach is an active barrier against entry of most bacteria into the host intestinal system [5, 6]

1.1 Importance of microencapsulation of probiotics

Survival of probiotic organisms such as *L. acidophilus* and *Bifidobacterium* spp has been found to be low in the presence of acid and bile salts[7]. It is essential that probiotic organisms survive the transit in the intestine where they are exposed to acid and bile salts to multiply in the colon and about one million probiotic organisms per gram of product need to be delivered to the colon for health benefits to be obtained by humans.

Microencapsulation is the process of encasing an active component in a shell and is defined as a technology of packaging solids, liquids or gaseous materials in miniature, sealed capsules that can release their contents at controlled rates under the influences of specific conditions [8]. From microbiological point of view, microencapsulation can be defined as the process of entrapment/enclosure of cells of microorganisms by means of coating them with proper hydrocolloid(s) in order to isolate the cells from the surrounding environment, in a way that results in appropriate cell release in the intestinal medium [9]. Factors that release the bacterial cells from the microencapsulated beads include pH changes, mechanical tensions, heat, enzymatic activities, osmotic pressure, slow diffusion of the moisture through the capsule layers, presence of some chemical components and storage time[10].

Microencapsulation protects probiotic organisms during freezing and freeze drying[11, 12]. Micropropagation of probiotic cells has been shown to preserve them from detrimental environmental factors such as high acidity and low pH [13], bile salts [14], cold shock induced by the processing conditions such as deep freezing and freeze drying[12], molecular oxygen in case of obligatory anaerobic microorganisms, heat shock caused by spray drying, bacteriophages [15] and chemical antimicrobial agents [9]. However, other advantages such as

increase in stability of sensory properties and its improvement[16] and immobilization of the cells for their homogenous distribution throughout the product [17] can also be achieved.

Main components used for microencapsulation of probiotics are alginate and its combinations with certain prebiotics (FOS, Hi-maize etc.,), starch, xanthan-gelan mixture, carrageenan and its mixtures, gelatin, cellulose acetate phthalate, chitosan and whey proteins [18]. The survival and multiplication of probiotics in the host strongly affect their probiotic benefits. Studies have shown low viability of probiotics in dairy products including yoghurt and fermented milk [19, 20,21,22]

Protection of the probiotics has been proposed for various dairy fermentations, with microencapsulation in hydro colloidal beads for improving their viability in both the food products and the intestinal tract [23,24,25]

II. ENCAPSULATION METHODS

Encapsulation is a process whereby cells are retained within a wall material to reduce cell injury. Encapsulation in hydrocolloid beads has been investigated as a means to protect and improve viability of probiotic microorganisms in food products and in the intestinal tract [26]. Other benefits of encapsulation includes: protection of probiotics from bacteriophage increased survival during freeze-drying and freezing and greater stability during storage [27]. Hou *et al.*[28] demonstrated that encapsulation of *Lactobacillus delbrueckii* spp. *bulgaricus* increased their bile tolerance, and viability elevated approximately by four log units, using artificial sesame oil emulsions.

Capela *et al.* [29] found improved viability of probiotic organisms encapsulated in 3% v/w sodium alginate in freeze-dried yogurt after 6 months of storage at 4° C (~8 log cfu/g) and 21°C (~6 log cfu/g). Spray drying, freeze drying, fluidized bed drying are the techniques which are used for converting probiotic cultures into a concentrated powdered form.

However, such techniques do not protect the bacteria from the product environment or during their passage through the GIT because they release the bacteria completely into the product. The viability of *Bifidobacterium bifidum* BB-12 and *L. acidophilus* LA-5 encapsulated in Na-alginate by either an extrusion or an emulsion technique in white-brined cheese was monitored and both encapsulation techniques were found to be effective in keeping the numbers of probiotic bacteria higher than the level of the therapeutic minimum ($>10^7$ cfu/g). While the counts of non-encapsulated probiotic bacteria decreased approximately by 3 logs, the decrease was more limited in the cheeses containing microencapsulated cells (approximately1 log).[30]

Depending on the method used to form the beads, the encapsulation techniques applied for probiotics in fermented milk products or biomass production can be classified as either the: i) extrusion or droplet method, and ii) emulsion or two phase systems . Both techniques have been found to increase the survival of probiotic bacteria by up to 80-95% [26,31]. The survivability of encapsulated *Bifidobacterium adolescentis* ATCC 15704 and the changes of organic acids in Kariesh cheese during 2 weeks of cold storage were determined. The study demonstrated that microencapsulation using rennet gelation of milk proteins increased the survival of *Bifidobacterium adolescentis* ATCC 15704 in simulated gastric conditionsThe microencapsulation significantly increased the survival of *Bifidobacterium adolescentis* ATCC 15704 in Kariesh cheese during cold storage.[32]

2.1 Encapsulation by extrusion

In extrusion encapsulation, microorganisms are added into a hydrocolloid solution (alginate) and the suspension is extruded through a syringe needle to form droplets, which free-fall into a hardening solution or setting bath (Figure. 1)

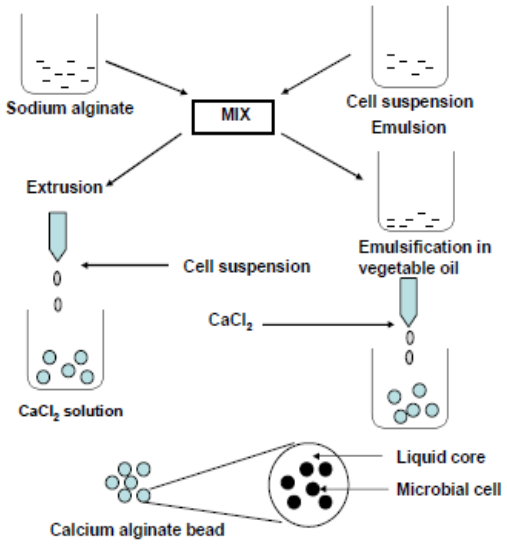

Figure. 1 Flow diagram of encapsulation - extrusion and emulsion techniques (reproduced from Krasaekoopt *et al*., 2003)

The size and shape of the beads depends on the diameter of the needle and the distance of free-fall (King, 1995). Alginate is commonly used supporting material, the concentration varies from a low concentration of 0.6% up to 1–2%. The size and sphericity of the bead also depends on the viscosity of the sodium alginate solution, the distance between the syringe and the calcium chloride collecting solution and the extruder orifice diameter. As the concentration of sodium alginate increases, the size of the beads decreases. The composition of the alginate also influences bead size; small beads result from ‘‘low guluronic’’ alginates [17].

Extrusion is the oldest and most common approach to making capsules with hydrocolloids [33]. It simply involves preparing a hydrocolloid solution, adding microorganisms to it and extruding the cell suspension through a syringe needle in the form of droplets to free fall into a hardening solution (Calcium chloride solution) or setting bath. The method is popular due to its ease, simplicity, low cost, and gentle formulation conditions ensuring high retention of cell viability.

Ozer *et al.* [34] reported that the bead size ranged from 0.5-1.0 mm diameter when 0.6 mm syringe are used for dripping in extrusion method The bead size of extrusion methods of encapsulation by using insulin syringe and starch, low methoxyl pectin and sodium alginate as wall materials are in the rage of 0.89-1.08mm [35] . Li *et al.*[36] reported in their study the mean value of the bead size as 4.0±0.3mm when alginate and gelatin as the wall materials were used.

2.2 Encapsulation by emulsion

Emulsion technique involves, a small volume of the cell polymer suspension (discontinuous phase) added to a large volume of a vegetable oil (continuous phase) such as soybean oil and homogenized to form a water in oil emulsion. Once the water in oil emulsion is formed the water soluble polymer must then be insolubilised (cross linked) to form tiny gel particles within the oil phase. The smaller the internal phase particle size of the emulsion, the smaller the final micro particles and the beads are harvested later by filtration. The size of the beads is controlled by the speed of agitation, and can vary between 25µm to 2 mm (Figure. 2) This technique has been used successfully to encapsulate lactic acid bacteria for batch and continuous fermentation. [24]

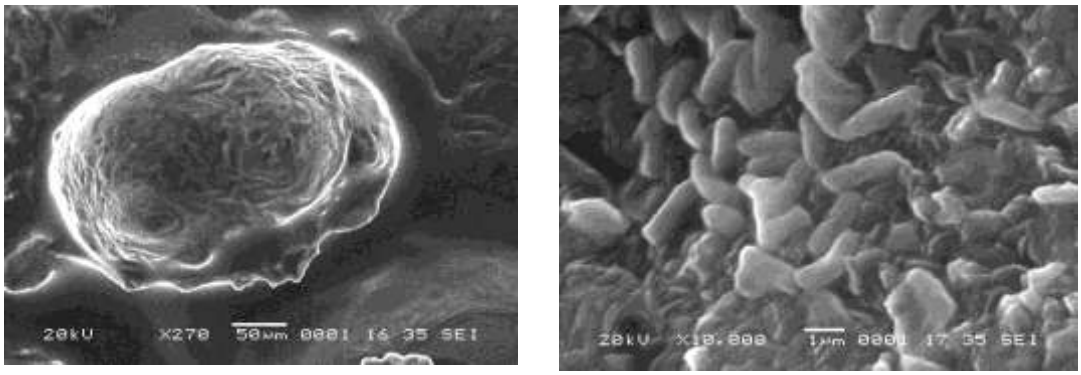

Figure. 2 Microencapsulated bead – emulsion and cross section of bead contains probiotic organisms (Jayalalitha et al., 2011)

2.3 Encapsulation by spray drying

Nowadays new technologies are developed to decrease the size of the microcapsules thus extending its application in industry. Among the latest trends in microencapsulation, new systems are studied by using mixed polymers matrices in order to obtain combined physical-chemical properties, that allowing microencapsulation process being more efficient for the protection and controlled release of active ingredient. Microencapsulation by spray drying is a well-established process that can produce large amounts of material. Nevertheless, this economical and effective technology for protecting materials is rarely considered for cell immobilization because of the high mortality resulting from simultaneous dehydration and thermal inactivation of microorganisms. In response to these limitations, a low cost microencapsulation method, which can be easily scaled up, to improve the stability of probiotic lactic cultures has been proposed [37, 38]

The technique consists of coating milk fat droplets containing powder particles of freeze-dried bacteria with whey protein polymers, using emulsification and spray drying in a continuous two-step process. Successful production of the resulting multiphase low diameter microcapsules requires rigorous control of the size distribution of the different elements constituting the capsules. In particular, the material dispersed in the hydrophobic phase must be larger than the bacterial cells and smaller than the fat globules. In order to decrease the powder particle size, in later studies, Picot and Lacroix [39] micronized the powder particles, produced by spray drying and emulsification methods, using a spiral jet mill as a grinding system.

Lactobacillus brevis was microencapsulated using ultrasonic nozzle atomization at 130°C in sodium alginate and maltodextrin shows higher viability in digestive conditions compared to non-encapsulated bacterial cells (Figure. 3) [40]

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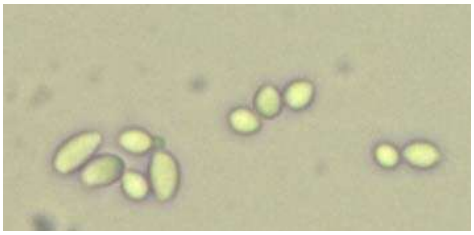

Figure. 3 Microscopic view of spray dried encapsulated cells

(adapted from Both et al., 2012)

Encapsulation by spray-drying, freeze-drying or fluidized bed drying have shown their limitations because the cells encapsulated by these techniques are completely released into the product. Hence, the probiotic bacteria are not protected towards the food environment and in the presence of gastric fluid or bile [7]. This method is commonly used in food industry involves atomization of an aqueous or oily suspension of probiotics and carrier material into a drying gas, resulting in rapid evaporation of water . The spray-drying process is controlled by these temperatures, but also by the product feed and the gas flow [41]. Despite the advantages of spray-drying technique, the high temperatures needed to facilitate water evaporation reduce the probiotics viability and their activity in the final product. The minimum air inlet temperature reported in the literature for probiotic encapsulation is 100 °C while the maximum is 170 °C. The air outlet temperature varies between 45°C and 105°C. At these temperatures, it is unlikely that the cells retain all their probiotic activity.

III.WALL MATERIALS FOR ENCAPSULATION OF PROBIOTICS

3.1 Alginate

Alginate is a linear heteropolysaccharide extracted from different types of algae, with two structural units consisting of D-manuronic and L-guluronic acids. Calcium alginate has been widely used for the encapsulation of lactic and probiotic bacteria, mainly in the concentration range of 0.5 – 4 per cent [42].

Alginate capsules easily form gel matrices around bacterial cells, they are non toxic to the humans (safe or biocompatible), need only cheaper, process conditions for their performance, can be easily prepared and performed (simplicity and ease of handling) and properly resolve in the intestine and release entrapped cells. Alginate gel matrix appropriately surrounds the bacterial cells with a diameter of 1-3µm and the pore sizes formed at the surfaces of alginate beads do not exceed 7nm [43]

Alginate is the most commonly used polymer for immobilizing viable cells and it has been used in various food applications. Maltodextrin functions as an osmotically inactive bulking compound, which causes spacing of the bacterial cells and strengthening of the glassy matrix [44]

3.2 Gelatin

Gelatin, a protein derived from denatured collagen, contains high levels of hydroxyproline, proline and glycine. It is useful as a thermally reversible gelling agent for encapsulation and can be used as encapsulation material

because of its excellent membrane forming ability, biocompatibility and non toxicity. The applicability of gelatin as a hydrogel matrix is limited because of its low network rigidity. However, its physical properties can be improved through the addition of crosslinking agents. Because of its amphoteric nature it is also an excellent candidate for cooperation with anionic polysaccharides such as alginate.

3.3 K Carageenan

Carrageenan is a natural polysaccharide that is extracted from marine macro algae and is commonly used as a food additive. Elevated temperatures (60-80º C) are needed to dissolve the polymer at concentrations ranging from 2 to 5% [40]. Gelation of k-carrageenan is generally dependent on a change in temperature.

Audet *et al.* [31] reported the inhibitory effect of KCl on some bacteria such as *Streptococcus thermophilus* and *L. bulgaricus*. Later they used a combination of k-carrageenan and locust bean gum to encapsulate LAB to enhance their stability during biomass production in dairy products[45,46]. The gel bead strength can be enhanced using another polymer, such as locust bean gum. A ratio of carrageenan to locust bean gum of 2:1, through specific interaction of the galactomannan chains of locust bean gum with carrageenan, has been found to give the synergistic effects and to form the strong gel beads.

3.4 Cellulose Acetate Phthalate (CAP)

This cellulose derivative polymer is insoluble in acid media at pH 5 and lower but is soluble at pH higher than 6. In addition, CAP is physiologically inert when administered in vivo, and is, therefore, widely used as an enteric coating material for the release of core substances for intestinal targeted delivery systems. Rao *et al.*[26] reported the encapsulation of *B. pseudolongum* in CAP using an emulsion technique. Microencapsulated bacteria survived in larger numbers (10^{9} cfu/ml) in an acidic environment than non-encapsulated organisms, which did not retain any viability when exposed to a simulated gastric environment for one hr.

3.5 Chitosan

The polysaccharide chitin, is gaining importance in the food and pharmaceutical field because of its unique polymeric cationic character, good biocompatibility, non-toxicity and biodegradability. Chitosan can be isolated from crustacean shells, insect cuticles and the membranes of fungi. The properties of chitosan vary with its source. The terms chitin and chitosan refer not to specific compounds but to two types of copolymers, containing the two monomer residues anhydro-N-acetyl-D-glucosamine and anhydro-D-glucosamine, respectively. Chitin is a polymer of b-(1-4)-2-acetamido-2- deoxy-D-glucopyranose and is one of the most abundant organic materials on earth and second to cellulose and murein, which is the main structural polymer of the bacterial cell wall. In order to achieve sufficient stability, chitosan gel beads and microspheres can be ionically cross-linked with polyphosphates [47] and sodium alginate[48].

3.6 Starch

Starch is a dietary component that has an important role in colonic physiology and functions and a potential protective role against colorectal cancer[49]. Resistant starch is the starch that is not digested by pancreatic amylases in the small intestine and reaches the colon, where it can be fermented by human and animal gut microflora. The fermentation of carbohydrates by anaerobic bacteria produces short chain fatty acids and lowers the pH in the lumen [50]. Resistant starch can be used to ensure the viability of probiotic populations from the food to the large intestine. Resistant starch also offers an ideal surface for adherence of the probiotics to the starch granule during processing, storage and transit through the upper gastrointestinal tract, providing robustness and resilience to environmental stresses. Bacterial adhesion to starch may also provide advantages in new probiotic technologies to enhance delivery of viable and metabolically active probiotics to the intestinal tract [51]

3.7 Gellan

Gellan gum is a natural anionic microbial polysaccharide obtained from *Pseudomonas elodea*. The primary structure is based on a tetra saccharidic repeating unit consisting of two β-D-glucose, one β-D-glucuronic acid and one α-L-rhamnose residues. The two acyl groups present in the same glucose residue in the native gellan are removed in the commercial preparations. Gellan gum is capable of gelation upon heating and cooling in the presence of a large variety of ions, among which the divalent calcium and magnesium exhibit the greatest efficiency{52,53] Gellan gum can be used as a structuring and gelling agent in a wide range of applications to mimic the texture of existing gelling agents or to create new textures [54]

IV. CONCLUSION

Amongst the various approaches, microencapsulation has emerged as the best alternative so as to overcome the problem of poor survivability of probiotic cultures in the food matrix as well as in the gastrointestinal environment. The process of microencapsulation has started from a simple entrapment to advanced and precise micro capsule formation. Now a days the advancement of microencapsulation have been enormous. However, there are still many challenges in this area, such as developing microencapsulation equipment, clarifying microencapsulation procedures, choosing non-toxic materials for probiotics encapsulation, developing capsules or beads from polymers adapted to the pH of fermented dairy products as well as human digestive tract, determining mechanisms of probiotics release from capsules or beads, carrying out in vitro and in vivo studies and assessing microencapsulation costs. The challenge of equipment refers to beads or capsules sizes, which are crucial and should be carefully controlled. Small capsules or beads under controlled conditions will not affect the texture of food products. In emulsion method of encapsulation, emulsifier or surfactant will be added in oil for emulsification. The residual oil will give detrimental effect to the dairy products in their texture and sensory characters. Modern research should focus towards development of aqueous gelation without oil or emulsifier. Another target of research in developing functional food with probiotic is organoleptic characters like texture, flavour etc. While incorporating extruded beads in to ice cream, yoghurt or cheese, the texture and flavour may vary due to larger size of the beads and associative action of wall materials and dairy ingredients. As there are no commercially available probiotic products that are stable at high temperatures, research may be focused on

naturally heat tolerant probiotics by in vitro techniques or by genetic modification, use of fats or lipids as a coating material for capsules due to their high melting point property. As the viability is an important component of the functionality of probiotic bacteria in foods, the delivery of viable micro encapsulated probiotic bacteria will become important in the near future. In dairy food industry micro-encapsulation will increasingly play a role to protect the viability and enhance the survival of probiotic bacteria in fermented dairy products. New food regulations may specify labelling including the strain and the number of viable probiotic bacteria at the end of shelf life of a food or supplement claimed to be probiotic. Evidence of this delivering must firstly be provided by the results of *in vitro* studies, through simulation of simple and reproducible gastrointestinal tract models. At this level, the lack of standard protocol in the conduct of these tests remains a concern. New research is needed in this direction.

One of the major interests in the future concerns will be the use of probiotic/prebiotic combinations. Nanoencapsulation may assume importance in the near future to develop designer probiotic bacterial preparations which could be delivered to specific parts of the gastro-intestinal tract where they interact with specific receptors. Nevertheless, in general, a lot more good hopes are visualized for the microencapsulation of probiotics in formulation of functional dairy foods in the near future.Last and final conclusion is development of encapsulation method with minimal cost and time is required to produce functional dairy products with microencapsulated probiotics. Probiotic encapsulation technology has the potential to protect microorgansisms and to deliver them into the gut provided problems identified are solved by researchers.

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