International Journal of Fermented Foods: v.2 n.1 p.47-61. June, 2013

Research Paper

Microencapsulation of probiotic cultures for preparation of yoghurt

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Received: 08th February 2013 Accepted: 27th April 2013

ABSTRACT

Probiotics are live microorganisms which transit the gastrointestinal tract and in doing so benefit the health of the consumer. Therapeutic benefits have led to an increase in the incorporation of probiotic bacteria such as lactobacilli and bifidobacteria in dairy products, especially yoghurts. Microencapsulation enhanced the survival of probiotic cultures compared to free cells in yogurts stored for a longer periods. It also protects the bacteria from harsh conditions (low pH, antibiotics, bacteriocins, bile salt concentration etc). Microencapsulation of various bacterial cultures including probiotics has been a common practice for extending their storage life and converting them into a powder form for ease of their use.

Keywords: Probiotics, Yoghurt, Microencapsulation, Spray drying, Polymers

INTRODUCTION

The efficiency of added probiotic bacteria in food and beverages depends on dose level and their viability throughout production and storage, and their ability to survive in gut environment. Hence, viability of probiotic bacteria is of paramount importance in the marketability of probiotic-based food products. Several reports have shown that survival and viability of probiotic bacteria is often low in yoghurt (Kailasapathy and Rybka, 1997; Lourens-Hattingh and Viljoen, 2001; Shah, 2000) and results in less than 10^8–10^9 cells daily recommended intake.

According to FAO /WHO (1977) – "Yoghurt is a coagulated milk product obtained by lactic acid fermentation through the action of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, from milk and milk products (pasteurized milk or concentrated milk) with or without optional additions (milk powder, skimmed milk powder, whey powder etc.). The micro-organisms in the final product must be viable and abundant". The basic ingredients of yoghurt are milk and micro-flora, *which* grow symbiotically. In some countries, apart from these two bacteria, the

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culture also contains lactose fermenting yeasts, *Leuconostoc* strains, *Lactococcus lactis* subsp. *lactis biovar diacetylactis* and *Lactobacillus acidophilus*. The method of maintaining purity of the yoghurt culture is very important and it is better to maintain the mother cultures individually rather than in mixed form. The optimum pH and temperature for growth of *S. thermophilus* is 6.8 and 38°C. These strains usually reach acidities of 0.80 to 0.95 per cent. In case of *L. bulgaricus,* the optimum temperature for growth is 42°C and the acidity obtained is 1.25 to 1.50 per cent. The extreme sensitivity of both these microorganisms to antibiotics especially, penicillin, calls for selection of milk intended for yoghurt manufacture to be free from residual penicillin. More over, extra care has to be exercised in maintaining the purity of the yoghurt cultures since they can be easily overgrown by contaminating organisms.

Types of Yoghurt

At present the retail markets of Europe, North America, Australia and elsewhere are dominated by two types of yoghurt one variant has a firm gel like structure together with a clean, mildly acidic and slightly aromatic flavour-"natural set yoghurt", while the other has the consistency of double cream and the background flavour is modified by the addition of fruit/ flavours and sugar-"stirred yoghurt". More recently yoghurt with a high level of milk solids (~20% total solids with ~10% fat) has appeared on the commercial market, and this so called "greek style" yoghurt has proved popular.

Classification of yoghurt

a) Based on the manufacturing method: i) Set yoghurt ii) Stirred yoghurt iii) Drinking yoghurt

b) Based on the fat content: i) Normal yoghurt (3.0% fat) ii) Low fat yoghurt (1.5% fat)

ii i) Non fatty yoghurt (<0.1% fat).

c) Based on style of yoghurt: i) Inverted sundae style (fruits are settled at the button) and ii) Swiss style (fruits are mixed throughout the container)

Traditional method of yoghurt making

Traditionally yoghurt is made from cow milk which is evaporated to 1/3 to 1/4 water content so that it attains the required concentration. Alternatively, 4-5% non-fat dried milk (NFDM) can be added to the whole milk. It is heated to 82-93°C for 30 minutes. Then milk is cooled to 42-43°C and inoculated with 2-3% starter culture followed by incubation at 42-43°C for 3 hours or until a titratable acidity of 0.75% lactic acid or coagulation occurs. The product is chilled to 5°C. Further acidity of 0.9% lactic acid may develop while the product is being chilled. The product can be stored satisfactorily for 1-2 weeks at 5°C.

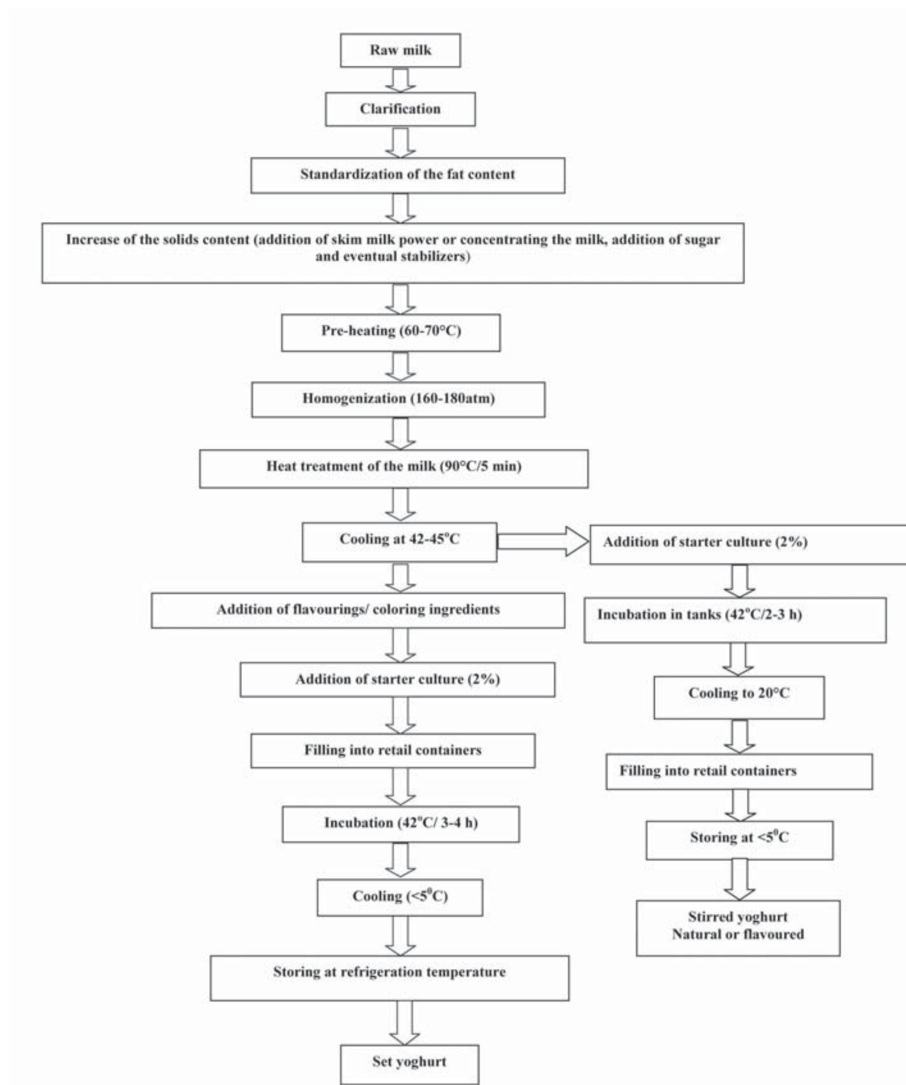

Fig 1. Method of manufacture of Set and Stirred Yoghurt

Microencapsulation of probiotic organisms

Probiotics are live bacteria that are administered in order to provide a health benefit to the host. Bacterial strains selected as probiotics are predominantly from the genera *Bifidobacterium* and *Lactobacillus*, which are indigenous to the human gastrointestinal tract. The environmental sensitivities of many potential probiotic strains currently limit their practical use in non-refrigerated foods and pharmaceutical-type supplements. Hence, technologies that can protect the viability of probiotics during manufacture, storage and gastrointestinal transit are highly

desired. Entrapment of cells in a gel matrix of alginates is the most popular system of encapsulation. Encapsulation is the process of forming a continuous coating around an inner matrix that is wholly contained within the capsule wall as a core of encapsulated material, encapsulation occurs naturally when bacterial cells grow and produce exo-polysaccharides (EPS). The microbial cells are entrapped within their own secretions that act as a protective structure or a capsule, reducing the permeability of material through the capsule and therefore less exposed to adverse environmental factors. Many lactic acid bacteria synthesize EPS, but they produce insufficient EPS to be able to encapsulate themselves fully (Shah, 2002). Microencapsulation helps to separate a core material from its environment until it is released. It protects the unstable core from its environment, thereby improving its stability, extends the cores' shelf life and provides a sustained and controlled release. The structure formed by the micro-encapsulation agent around the core substance is known as the wall. The properties of the wall system are designed to protect the core and to release it at controlled rates under specific conditions while allowing small molecules to pass in and out of the membrane (Franjione and Vasishtha, 1995; Gibbs *et al*., 1999). The capsules may range from submicron to several millimeters in size and can be of different shapes (Shahidi and Han, 1993; Franjione and Vasishtha, 1995). Compared to immobilization/ entrapment techniques, micro-encapsulation has many advantages. The microcapsule is composed of a semi-permeable, spherical, thin and strong membranous wall. Therefore the bacterial cells are retained within the microcapsules (Jankowski *et al*., 1997). Moreover, compared to an entrapment matrix, there is no solid or gelled core in the microcapsule and its small diameter helps to reduce mass transfer limitations. The nutrients and metabolites can diffuse through the semi permeable membrane easily. The membrane serves as a barrier to cell release and minimizes contamination. The encapsulated core material is released by several mechanisms such as mechanical rupture of the cell wall, dissolution of the wall, melting of the wall and diffusion through the wall (Franjione and Vasishtha, 1995). Microencapsulation separates the microbes from their environment, thereby maximizing the substrate's survivability. Microencapsulation is the best available technology to preserve the potency of probiotic products to be delivered into the gastrointestinal tract. The other benefits of encapsulation of bacterial cells include protection of cells from bacteriophages (Steenson *et al.*, 1987; Tanaka *et al.*, 1989), increased survival during freeze-drying and freezing (Sung, 1997; Sha and Ravula, 2000), greater stability during the storage (Koo *et al.*, 2001) and increased survival after exposure to gastric solution (Lee *et al*., 2001).

Methods used for encapsulating of bacteria

Spray Drying

Spray drying is the most commonly used microencapsulation technique in food industry, as this is an economical and flexible process, which produces good quality product (Dziezak, 1988). Process involves the dispersion of the core material into a

polymer solution, forming an emulsion or dispersion, followed by homogenization of the liquid, then atomization of the mixture into the drying chamber (Jackson and Lee, 1991). After evaporation of water microcapsules are formed. Advantage of the process is that it can be operated on a continuous basis. Disadvantage is that the high temperature causes damage to probiotic bacteria. However, by adjusting the processing conditions such as inlet and outlet temperatures, viability of bacteria can be increased. At an inlet temperature of 100°C and low outlet temperature of 45°C, *Bifidobacterium* cells were encapsulated satisfactorily to produce micro spheres with gelatinised modified starch as encapsulating material (O'Riordan *et al*., 2001). Gardiner *et al*. (2000) reported lose viability of probiotic cells occurs at higher inlet temperatures.

Extrusion, Emulsion and Phase Separation

The capsules or beads are formed in a two-step procedure involving dispersion and hardening. The dispersion can be performed either by extrusion or by emulsification (Groboillot *et al*., 1994). In extrusion method, bacterial cells and polymer suspension is extruded through a needle or a nozzle, generating spherical droplets that fall into a hardening solution. Micro-encapsulation by extrusion involves projecting an emulsion core and coating material through a nozzle at high pressure. Extrusion of polymer solutions through nozzles to produce capsules is mainly reported on a laboratory scale, where simple devices such as syringes are applied. If the droplet formation occurs in a controlled manner (contrary to spraying) the technique is known as prilling (Heinzen, 2002). This is preferably 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 droplets. Mass production of beads can either be achieved by multi-nozzle systems, rotating disc atomizers or by the jet cutting technique. Centrifugal systems using either a multi-nozzle system or a rotating disc have also been developed for the mass production of microcapsules (Heinzen, 2002). The centrifugal extrusion process is a liquid co-extrusion process utilizing a nozzle consisting of concentric orifices located on the outer circumference of a rotating cylinder (Schlameus, 1995). A liquid or core material is pumped through the inner orifice and a liquid shell material through the outer orifice forming a co-extruded rod of core material surrounded by shell material. As the system rotates, the extruded rod breaks up into droplets that form capsules.

The emulsion technique involves the dispersion of an aqueous phase containing the bacterial cells and polymer suspension into an organic phase, such as oil, resulting in water-in-oil emulsion. The dispersed aqueous droplets are hardened by cooling or by addition of a gelling agent or a crosslinking agent in the case of polyacrylamide gels. Following gelation, the beads are partitioned into water and washed to remove oil. The emulsion technique results in smaller diameter beads, and is better suited to scale up applications. Residual oil in the capsules, however, may not be suitable for development of low-fat food product applications.

For encapsulation of viable bacterial cells, the materials used should be of food grade, gentle and non-toxic. Most commonly used bio-gum for micro-encapsulation of bacterial cells is alginate. The advantages of using alginate as an encapsulating agent includes: non-toxicity, forms gentle matrices with calcium chloride to trap sensitive materials such as probiotic bacteria, the viability of bacteria is not affected during the encapsulated shelf life and the reversibility of immobilization as gels can be solubilized by sequestering calcium ions thus releasing the entrapped cells (Sheu and Marshall, 1993; Shah and Ravula, 2000). Several gelling agents including pectate, kappa-carrageenan-locust bean gum, gellan gum and agar were tested for gel strength. The results showed that alginates formed a firmer gel with good mechanical stability and demonstrated easy release of the encapsulated bacteria when suspended in an alkaline buffer (Nicetic *et al*., 1999). When used in lactic acid fermentation, chelating agents such as lactate may cause weakening of calcium-alginate gel and may allow cell leakage (Smidsrod and Skjack, 1990). Since alginates are a heterogenous group of polymers, with a wide range of functional properties, their success as immobilization matrices will rely on an appropriate choice of materials and methodology for each application. Alginates with a high content of guluronic acid blocks (G blocks) are preferable for capsule formation because of their high mechanical stability, high porosity and tolerance to salts and chelating agents (Nicetic *et al*., 1999).

Additional treatment of microcapsules

To reduce mass transfer effects, poly-L-lysine (PLL) membrane coating of alginate beads followed by liquefaction of the alginate core is reported (Lim, 1983), however leakage of cultures from the matrix was still observed with PLL-alginate beads (Champagne *et al*., 1992). Synthetic polymers such as nylon or cross-linked polyethyleneimine membranes are unsuitable for live cell immobilisation due to toxicity of the reagents and harsh conditions of encapsulation (Larisch *et al*., 1994). Rao *et al*. (1989) reported a microencapsulating procedure for *Bifidobacterium pseudolongum* which involved mixing the bacterial cells with starch and suspending the powder in light paraffin oil. Solution of cellulose acetate phthalate is added to the suspension and stirred to obtain the microspheres. The microspheres are then coated with beeswax. For better stability of capsules and improvement of probiotic survival and reducing the loss of probiotics, different treatments are given below:

i) Cross linking with cationic polymers

Alginate beads have been stabilized by cross-linking with cationic polymers such as polyethyleneimine and glutaraldehyde (Marx, 1989). Membrane formations around the beads and the spraying the beads with glutaraldehyde have been proposed as stabilizing techniques which minimizes the cell release (Kolot, 1988). Groboillot *et al*. (1993) reported that the membrane formed with 4% chitosan cross-linked with hexamethylene-di-isocyanate or glutaraldehyde resulted in stronger

microencapsules. The reaction of the bifunctional reagent with chitosan resulted in bridge formation linking the chitosan molecules and the length of the bridge depends on the cross linking of agent. Hyndman *et al*. (1993) obtained a similar result when toluene –di-isocyanate is used to cross-link the gelatin.

ii) Coating with other polymers

Additional coatings to the microcapsules with polymers including chitosan, poly-l-lysine, cell free gel matrix, etc. reduce the cell loss and improve the viability of entrapped cells. The chitosan coated alginate beads are produced by dropping the alginate solution into a mixture of calcium chloride and chitosan solution (Overagaard, 1991). McKnight *et al*. (1988) coated the alginate beads with chitosan film. Suspending of alginate beads in low molecular weight chitosan solution formed a membrane, which reduce cell release by 40 % (Zhou *et al*., 1998). Chitosan a positively charged polyamine forms semi permeable membranes around the negatively charged polymers such as alginates, which is not dissolved in the presence of Ca^{+2}-chelators or antigelling agents and thus protect the gel stability (Smidsrod and Skjak-Braek, 1990) and acts as barrier to release of entrapped cells. Champagne *et al*., 1992 found that coating of alginate beads with a single layer of poly-L-lysine did not significantly reduce the release of cells but a composite coating with poly-L-lysine and alginate decreased cell release.

iii) Incorporation of additives

Additives are incorporated into the hydrocolloids to improve the encapsulation efficiency with incorporating of cryoprotectants such as glycerol, adonitol etc. Survival of encapsulated cells after freeze drying and rehydration is improved due to the additional protection during the processing (Kushal, 2000). The survival of *Bifidobacteria* increased significantly to 88.5 % (Kebary, 1998) and *L. delbrueckii* ssp. *bulgaricus* to 90% (Sheu *et al*., 1993) because these reduce the formation of ice-crystal by binding with free water. The beads with glycerol exhibited a 43% decrease in size due to the higher concentration of alginate per unit volume in offer the ability to encapsulate *L. acidophilus* without loss of viability and fermentation ability (Jankowski *et al.*, 1997). Addition of glycerol and mannitol during preparing of alginate beads increases the survival of bifidobacteria from 58.8 to 88.5%.

Microencapsulation of lactic acid bacteria and their application

Sue and Marshall (1993) reported an emulsion method to encapsulate lactobacilli in calcium alginate gels, in which the cells were mixed with sodium alginate solution (one part of the culture concentrate with 4 parts of 3 % sodium alginate). One part of this mixture was added dropwise to 5 parts of vegetable oil containing Tween 80 (0.2%), and stirred. Calcium chloride solution (0.05M) was then added to this turbid emulsion down the sides of a beaker until the waterin-oil emulsion is broken. The formed calcium-alginate beads are collected by gentle centrifugation and washed with sterile water. Similar procedures are used to encapsulate *B. bifidum, B.infantis*

and *B. longum* for incorporation in the production of Crescenza cheese (Gobbetti *et al.*, 1997), encapsulating *B. bifidum* and *B. infantis* into mayonnaise and frozen milk (Khalil and Mansour, 1998; Kebary *et al.*, 1998) and microencapsulating L. acidophilus LAMJLA1 and Bifidobacterium spp. BBBDBB2 for incorporation into frozen dairy desserts (Shah and Ravula, 2000). A two-polymer micro-encapsulation reported by Khalida *et al*. (2000) used a mixture of 2% alginate solution and 2% HiMaize resistant starch and 1% probiotic culture cells (*L. acidophilus* and *Bifidobacterium* spp). The encapsulation of Lactobacillus casei subsp. rhamnosus (ATCC 10863) was reported using alginate capsules that possess an interphasic membrane and a liquid core (Yoo *et al*., 1996). Compared to gels, micro capsules consist of a liquid core surrounded by a semi permeable membrane that retains the cells, reduces mass transfer limitations and minimize phage contamination (Hsu and Chu, 1992). Jankowski *et al*. (1997) developed biocompatible capsules consisting of a liquid core with calcium alginate membranes for encapsulating lactic acid bacteria. They concluded that the alginate-starch liquid core capsules offer the possibility to encapsulate lactic acid bacteria without loss of viability. Prevost and Divies (1992) reported a double needle system to entrap lactococci in cream fermentation. In this procedure, a concentric flow of sterile sodium-alginate solution through 0.5 mm tubing was used to mix alginate cells off the nozzle into a well-mixed 0.2M calcium chloride solution. The resulting capsules contained a two-layered calcium alginate gel. Chitosan, a water-soluble polymer (pH <6) has been used to microencapsulate *Lactococcus lactis* (Groboillot *et al*., 1993). In this study, a cross-linked chitosan membrane was formed by emulsification/ interfacial polymerisation using biocompatible reagents with oil soluble cross-linking agents at low concentrations to minimise cell contact. The anti-bacterial property of chitosan, however, limits its use as coating material in encapsulation (Sudarshan *et al*., 1992). *Lactococcus lactis* cells were encapsulated within gelatin membrane cross-linked with toluene-2, 4 diisocyanate at an oil/water interface. Reagent toxicity was avoided by use of vegetable or silicone oil as a dispersant, and by minimising cell exposure to the water insoluble cross-linking agent during membrane formation (Hyndman *et al*., 1993). Larisch *et al*. (1994) however, observed toxic effects with solvents and reagents when *Lactococcus lactis* subspp. *cremoris* was encapsulated within poly-L-Lysine membranes formed on alginate micro spheres. Another bio gum that has been used as an encapsulant was gellan gum. Camelin *et al*. (1993) observed that the gellan gum produced by dissolving in a citrate solution was a better entrapment matrix for temperature-sensitive cells such as mesophilic lactic acid bacteria. In this study, *B. longum* (ATCC 15707) cells were successfully immobilized using a sterilised 2.5% gellan gum solution supplemented with 0.2% citrate or metaphosphate to prepare gel beads with immobilised living cells. In some other studies, equal volumes of bacterial suspension and kappa-carrageenan were mixed and the mixture was added drop wise to a gently stirred 0.1M potassium chloride solution, the beads were collected by gentle centrifugation (Buyukgungor, 1992). A similar procedure for micro entrapment of

B. bifidum and *B. infantis* was reported using kappa-carrageenan as the gelling polymer. In this procedure, one part of bacterial cells was mixed with 3% kappa-carrageenan and the mixture is then added drop-wise using a syringe into 10 parts of 3M potassium chloride solution to form the gelled beads (Kebary *et al*., 1998). Adhikari *et al*. (2000) reported an emulsion method involving kappa-carrageenan for encapsulating *B. longum* ATCC 14708 for incorporation into set yoghurt. The method used was similar to that reported earlier (Kebary *et al*., 1998), however, in this method the bacterial cell/kappa-carrageenan mix was added to vegetable oil to which Tween 80 was added as an emulsifier, and stirred. Potassium chloride solution (0.3M) was then added down the sides of a beaker to break the emulsion. The oil phase as removed from the top with an aspirator and the micro capsules were harvested from the potassium chloride solution by gentle centrifugation.

Bifidobacterium infantis strain is microencapsulated within a novel, film-forming protein-carbohydrate-lipid emulsion. Nondigestible prebiotic carbohydrates, which specifically stimulate the growth and/or activity of beneficial populations of bacteria within the gut formed part of the encapsulant formulation to produce a synbiotic combination. The capacity of the encapsulant to protect the viability of the bacteria is evaluated during nonrefrigerated storage and during simulated gastrointestinal transit. *Bifidobacterium infantis,* the freeze-dried bacteria are microencapsulated within a film-forming protein-carbohydrate-oil emulsion. Briefly, oil-in-water emulsions are prepared, containing canola vegetable oil, caseinate and prebiotic fructo-oligosaccharides (FOS) (Raftilose P95; ORAFTI, Belgium) plus either dried glucose syrup (DGS) (Maltostar 30; Manildra, Australia) or microfluidized resistant starch (RS) (Hylon VII; National Starches). The emulsions are heated to 98°C for 30 min to promote maillard reaction products, which improve film-forming and oxygen-scavenging properties. The mixture is then cooled to 10°C before the addition of the probiotic bacteria at 8% (wt/wt) prior to spray drying. Both encapsulated and nonencapsulated *B. infantis* cells are spray dried using a Drytec (Tonbridge, United Kingdom) laboratory-scale spray dryer with an inlet temperature of 160°C and an outlet temperature of 65°C. The final formulation (wt/wt) of the dried powders is follows: 8% probiotic, 32% oil, 20% caseinate, 20% FOS, and either 20% DGS or 20% RS.

A variation of the method proposed by Sun and Griffiths (2000) is used. Gellan (0.1 g), (Sigma) and xanthan (0.2 g) (Sigma) are added to 20 ml distilled water. The solution is mixed using a magnetic stirrer (Ikamag) and heated to 80°C for 1 h. The gel mix is then autoclaved at 121°C for 15 min. *B. lactis* is grown in a Bactron 1.5 anaerobic chamber at 37°C overnight in 250 ml TYG broth to an OD600 0.9–1.1. All subsequent procedures are done under aerobic conditions. Cells are harvested by centrifugation at 8000·g for 10 min in a Beckman J21 centrifuge at 4°C. The pellet of cells is washed three times by resuspension in 20 ml aliquots of sterile distilled water, followed by centrifugation. Finally, the cells are suspended in sterile distilled water, to give a volume of 2.5 ml. One ml of this concentrate is mixed into

20 ml sterile gellan: xanthan gum at 55°C. Microcapsules are generated by a bead entrapment method, using a superposed airflow together with mono-axial extrusion of the gum/bacteria mixture (Huebner and Buccholz, 1999; Park and Chang, 2000). The gum/cell mix is manually extruded through a 27.5 G bevelled needle fitted on to a sterile 20 ml syringe (Promex). Gel and sterile air flow rates are 10 ml / min and 250 ml/ s respectively. Micro-droplets formed by this method are hardened into spheres by free fall into a sterile 0.1 M $CaCl_2$ solution (100 ml). After 1h, beads are separated from the solution by aseptic filtration through sterile Whatman filter paper No 1. Microcapsules remaining on the filter paper are washed thoroughly with sterile 0.1 M $CaCl_2$.

Viability of microencapsulated probiotics in yoghurt

Microencapsulation of bacterial cells is currently gaining attention to increase viability of probiotic bacteria in acidic products such as yoghurt. Microencapsulation segregates the cells from adverse environment, thus potentially reducing cell injury. There is a need for encapsulation or enteric coatings for probiotic bacteria to survive human gastric juice in the stomach, where the pH can be as low as 2. It has been reported that microencapsulation using calcium-induced alginate–starch polymers (Godward and Kailasapathy, 2003), in potassium induced kappa-carrageenan polymers (Adhikari *et al*., 2000) and in whey protein polymers (Picot and Lacroix, 2004) have increased the survival and viability of probiotic bacteria in yoghurt during storage. The encapsulant materials used in these studies such as alginate, starch, carrageenan and whey protein are commonly used food stabilizers in the manufacture of stirred yoghurts to prevent syneresis. For example, alginate is a natural polysaccharide extracted from brown seaweeds and it enhances viscosity and binds water hence reduces syneresis in stirred yoghurts. Divalent cations such as calcium bind preferentially to the alginate polymer and hence increase viscosity or form gels depending on the concentration (Kailasapathy, 1996). Modified starches have improved thickening and gelling properties and bind water and thicken when added to yoghurt hence prevent syneresis and improve textural properties (Rapaille and Vanhemelrijck, 1994). Microbial exopolysaccharides (EPS) are also employed as additives to a wide variety of food products, where they serve as thickening, stabilizing, emulsifying or gelling agents. EPS produced by lactic acid bacteria, which carry the GRAS status, are used *in situ* to improve body and texture of fermented products (Faber, 2000). It has been reported that most lactic acid bacteria produce a small amount of EPS (De Vuyst and Degeest, 1999). By incorporating polymer encapsulated bacteria it may be possible to not only increase viability but also improve viscosity/gel properties of yoghurt. However, it has been reported that encapsulated cultures with EPS produced from *in situ* ropy strains could produce void spaces around bacteria and when they reach sufficient numbers and dimensions, could affect the integrity of the yoghurt matrix (Hassan,Frank *et al*., 1996). Probiotic effects are influenced very strongly by the ability of the organism to survive in the product and also survive and multiply within the host. To achieve

the benefits, probiotics should be metabolically stable and active both in the product and host. However, the survival of *Bifidobacteria* in yogurt is quite low because the pH of yoghurt ranges from 4.2 to 4.6. Lankaputhra *et al*. (2006) reported the survival of only three out of nine bifidobacterial strains in the pH range of 3.7 to 4.3. The presence of yoghurt cultures adversely affect the growth of bifidobacteria. Because the minimum suggested level of viable bifidobacterial cells at the time of consumption is approximately 10^7 cfu/g of product, and the suggested daily intake is approximately 10^9 viable cells (around 100 g of product per day), this poses a problem for the yogurt industry. Strawberry yogurt containing probiotic beads has evaluated by the trained panelists (Krasaekoopt and Tandhanskul, 2008). Nine attributes (2 odors, 1 appearance, 1 texture, and 5 flavors) were established.

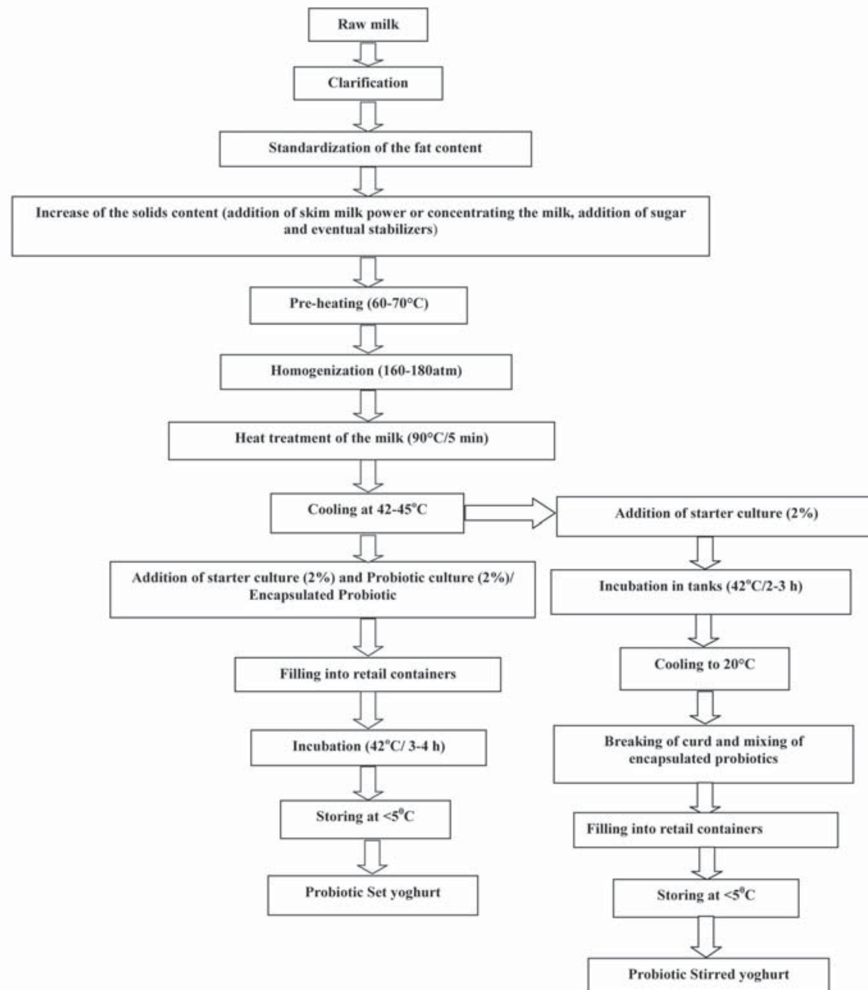

Fig 2: Flow-Diagram of Microencapsulated Probiotic Yoghurt

Addition of probiotic beads in strawberry yogurt significantly ($p<0.05$) influenced the viscosity of the product, with score of 7.6 compared to without beads (5.3). While color, flavor, taste and oiliness showed similar trends (Krasaekoopt and Tandhanskul, 2008). Microencapsulation enhanced the survival of probiotic cultures compared to free cells in yogurts stored over 7 weeks (Kailasapathy, 2003).

CONCLUSION

The human gastrointestinal tract, a kinetic micro ecosystem facilitates normal physiological functions in an optimal balance of micro flora. A favourable dynamic balance through predominance of beneficial microflora is of substantial improvement of the host health that can be accomplished by an efficient supplementation of the diet with the probiotics, prebiotics or combination of these (Synbiotics). For this probiotic foods should be regularly consumed in sufficient quantities to deliver the relevant dose of live bacteria to GUT, keeping in mind the losses in cells viability typically encountered during processing of food and GIT transit. Probiotics have to survive in unfavorable condition during food processing and storage and in the GIT. Yoghurt having inherent nutritional and functional attributes and is widely accepted to all age groups. The studies have demonstrated that cultures can be significantly protected by microencapsulation through yoghurt which includes milk proteins, food hydrocolloids and complex carbohydrates in many cases; the resultant products not only have better probiotic viability but can also be regarded as synbiotics given the presence of probiotics and prebiotics. There may be the chance of settling down of encapsulated probiotics in case of Set yoghurt when kept as such so to prevent this defect, Stirred yoghurt is suitable for encapsulated probiotics as because stirring causes homogenous mixing of encapsulated beads throughout the product.

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