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Encapsulation "The Future of Probiotics"-A Review

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Abstract: In the recent past, there has been an explosion of probiotic health-based products. Many reports indicated that there is poor survival of probiotic bacteria in these products. Further, the survival of these bacteria in the human gastro-intestinal system is questionable. Providing probiotic living cells with a physical barrier against adverse environmental conditions is therefore an approach currently receiving considerable interest. The technology of microencapsulation of probiotic bacterial cells evolved from the immobilized cell culture technology used in the biotechnological industry. The use of encapsulated ingredients has increased markedly over the past few years. This increase has been the result of several factors, but the primary factor has been the increased awareness on the part of the food industry of the real advantages offered by encapsulated ingredients. Clearly, they do not offer a panacea for all the problems, but they do offer properties, which cannot be achieved by other routes and should be in the repertoire of those charged with the development of new and better products. Added convenience and reduced packaging costs may also be used to offset the cost of encapsulating one or more ingredients. Encapsulated forms of ingredients achieve longer shelf life of the product.

Key words: Microcapsules · Encapsulation · Probiotics

INTRODUCTION

There are a variety of techniques available for the production of encapsulated materials. Few are co-extrusion and spray drying to prepare capsules, spray chilling, matrix entrapment, gel formation and fluid bed processing. All of these methods are applicable to food ingredients as well as to other materials. a wide range of materials are available including fats, waxes, glyceride derivatives, sugars, starches and modified starches, dextrin's, vegetable gums, gelatins, zein and other proteins, cellulose derivatives, caseinates, non-fat milk solids and others. With this variety of materials available, it is possible to form capsules, which will release under a variety of conditions [1].

Immobilization and Encapsulation: Entrapment of cells in a gel matrix of alginates is the most popular system of immobilization [2]. The terms immobilization and encapsulation were used interchangeably in most reported literature. While 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, immobilization refers to the trapping of material within or

Throughout a matrix. A small percentage of immobilised material may be exposed at the surface, while this is not the case for encapsulated material [3]. Encapsulation occurs naturally when bacterial cells grow and produce exo-polysaccharides. 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 exo-polysaccharides, but they produce insufficient exo-polysaccharides to be able to encapsulate themselves fully [4]. 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 core's 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 [5]. The capsules may range from submicron to several millimeters in size and can be of different shapes [6, 5]. Compared to immobilization/ entrapment techniques, micro-encapsulation has many

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advantages. The microcapsule is composed of a semi permeable, spherical, thin and strong membranous wall. Therefore the bacterial cells are retained within the microcapsules [7].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 [5].

Techniques to Manufacture Microcapsules

Physical Methods

Pan Coating: The pan coating process, widely used in the pharmaceutical industry, is among the oldest industrial procedures for forming small, coated particles or tablets. The particles are tumbled in a pan or other device while the coating material is applied slowly [8].

Air-Suspension Coating: Air-suspension coating of particles by solutions or melts gives better control and flexibility. The particles are coated while suspended in an upward-moving air stream. They are supported by a perforated plate having different patterns of holes inside and outside a cylindrical insert. Just sufficient air is permitted to rise through the outer annular space to fluidize the settling particles. Most of the rising air (usually heated) flows inside the cylinder, causing the particles to rise rapidly. At the top, as the air stream diverges and slows, they settle back onto the outer bed and move downward to repeat the cycle. The particles pass through the inner cylinder many times in a few minutes [8].

Centrifugal Extrusion: Liquids are encapsulated using a rotating extrusion head containing concentric nozzles. In this process, a jet of core liquid is surrounded by a sheath of wall solution or melt. As the jet moves through the air it breaks, owing to Rayleigh instability, into droplets of core, each coated with the wall solution. While the droplets are in flight, a molten wall may be hardened or a solvent may be evaporated from the wall solution. Since most of the droplets are within ± 10% of the mean diameter, they land in a narrow ring around the spray nozzle. Hence, if needed, the capsules can be hardened after formation by catching them in a ring-shaped hardening bath. This process is excellent for forming particles 400-2,000 μm (16-79 mils) in diameter. Since the drops are formed by the breakup of a liquid jet, the process is only suitable for liquid or slurry. A high production rate can be achieved, i.e., up to 22.5 kg (50 lb) of microcapsules can be produced per nozzle per hour per head. Heads containing 16 nozzles are available [8].

Vibrational Nozzle: Core-Shell encapsulation or Micro granulation (matrix-encapsulation) can be done using a laminar flow through a nozzle and an additional vibration of the nozzle or the liquid. The vibration has to be done in resonance of the Rayleigh instability and leads to very uniform droplets. The liquid can consists of any liquids with limited viscosities (0-10,000 mPa•s have been shown to work), e.g. solutions, emulsions, suspensions, melts etc. The solidification can be done according to the used gelation system with an internal gelation (e.g. sol-gel processing, melt) or an external (additional binder system, e.g. in a slurry). The process works very well for generating droplets between 100-5,000 μm (3.9-200 mils), applications for smaller and larger droplets are known. The units are deployed in industries and research mostly with capacities of 1-10,000 kg per hour (2-22,000 lb/h) at working temperatures of 20-1,500°C (68-2,700°F) (room temperature up to molten silicon). Nozzles heads are available from one up to several hundred thousand are available [8].

Spray-Drying: Spray drying serves as a microencapsulation technique when an active material is dissolved or suspended in a melt or polymer solution and becomes trapped in the dried particle. The main advantages is the ability to handle labile materials because of the short contact time in the dryer, in addition, the operation is economical. In modern spray dryers the viscosity of the solutions to be sprayed can be as high as 300 mPa•s. [8].

Chemical Methods

Interfacial Polymerization: In Interfacial polymerization, the two reactants in a polycondensation meet at an interface and react rapidly. The basis of this method is the classical Schotten-Baumann reaction between an acid chloride and a compound containing an active hydrogen atom, such as an amine or alcohol, polyesters, polyurea, polyurethane. Under the right conditions, thin flexible walls form rapidly at the interface. A solution of the pesticide and a diacid chloride are emulsified in water and an aqueous solution containing an amine and a polyfunctional isocyanate is added. Base is present to

neutralize the acid formed during the reaction. Condensed polymer walls form instantaneously at the interface of the emulsion droplets [8].

In-situ Polymerization: In a few microencapsulation processes, the direct polymerization of a single monomer is carried out on the particle surface. In one process, e.g. Cellulose fibers are encapsulated in polyethylene while immersed in dry toluene. Usual deposition rates are about 0.5ìm/min. Coating thickness ranges 0.2-75 µm (0.0079-2.95 mils). The coating is uniform, even over sharp projections [8].

Matrix Polymerization: In a number of processes, a core material is imbedded in a polymeric matrix during formation of the particles. A simple method of this type is spray-drying, in which the particle is formed by evaporation of the solvent from the matrix material. However, the solidification of the matrix also can be caused by a chemical change [8].

Encapsulation of Probiotic Bacteria

Alginate-starch Encapsulation: A modified method using calcium alginate for the microencapsulation of probiotic bacteria is reported in this study. Incorporation of Hi-Maize starch (a prebiotic) improved encapsulation of viable bacteria as compared to when the bacteria were encapsulated without the starch. Inclusion of glycerol (a cryo-protectant) with alginate mix increased the survival of bacteria when frozen at-20°C. The acidification kinetics of encapsulated bacteria showed that the rate of acid produced was lower than that of free cultures. The encapsulated bacteria, however, did not demonstrate a significant increase in survival when subjected to *in vitro* high acid and bile salt conditions. A preliminary study was carried out in order to monitor the effects of encapsulation on the survival of *Lactobacillus acidophilus* and *Bifidobacterium* spp. in yoghurt over a period of 8 weeks. It showed that the survival of encapsulated cultures of *L. acidophilus* and *Bifidobacterium* spp. showed a decline in viable count of about 0.5 log over a period of 8 weeks while there was a decline of about 1 log in cultures which were incorporated as free cells in yoghurt [9].

Direct Compression Encapsulation: The potential use of compression coating is an alternative method for the encapsulation of probiotic bacteria *Lactobacillus acidophilus* to improve their storage stability. Microbial cell containing powders were first compressed into a pellet, which was then encapsulated with a coating material of a combination of sodium alginate and hydroxypropyl cellulose by further compression. The effect of compression pressure on cell viability was studied. Results showed that compression of the microbial cell containing powders at pressures up to 90 MPa caused little loss of viability of the bacteria. Beyond 90MPa, the cell viability decreased almost linearly with the compression pressure. Further compression to form a coating did not cause significant reduction in the cell viability. The stability of the encapsulated bacteria using the compression pressures up to 60MPa was approximately 10 times higher than free cell containing powders and cell pellets after 30 days storage at 25°C [10].

Rennet-gelled Protein Encapsulation: Gelling of milk proteins using the food-approved enzyme rennet can produce microcapsules capable of encapsulating healthy ingredients like probiotics; rennet could be used to prepare water-insoluble microcapsules based on milk-proteins without significant loss of cells during the encapsulation process. Creating microcapsules from highly concentrated aqueous milk protein solutions enables the formation of microcapsules with a high density gel network, able to offer a favourable micro-milieu for the encapsulated probiotic strains and can therefore be a suitable approach for a more effective application of probiotics in food. 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, protecting the cells during incubation under simulated gastric conditions at low pH. Furthermore the unique functional properties of proteins alleviate the feasibility to control the capsule size of protein-based microcapsules, which is of high importance regarding the sensory impact of microcapsules in final food products [11].

Whey Protein Gel Particles Encapsulation: Encapsulation of probiotics in whey protein gel particles could offer protection during processing and storage, as well as extending the food applications of the bacteria to biscuits, vegetable and frozen cranberry juice. The potential of the *L. rhamnosus* strain encapsulated in a whey protein isolate (WPI). Beads were prepared by extruding the denatured WPI-concentrated bacteria solution and 96 per cent of the probiotic cells were in the whey protein particles.

The protein-based technique can provide an alternative to microencapsulation (ME) with alginate-type gels or spray-coating with fats, the two most widely-used probiotic encapsulation methods. The protein matrix would have different cell release properties than the other ME methods (polymer or fat based). Thus, applications can extend to other foods for protection during processing as well as stability during storage but also in nutraceuticals for protection and cell release in the GI tract [12].

Prebiotic Encapsulates: Adding the prebiotic inulin to yoghurt boosted the growth of probiotic bacteria and, when used in a novel double-microencapsulation, extended the survival rates of the friendly bacteria. The various prebiotic fibres protect the stability and viability of probiotic *Lactobacillus rhamnosus* strains during freeze-drying, storage in freeze-dried form and after formulation into apple juice and chocolate-coated breakfast cereals. The prebiotics studied were: sorbitol, mannitol, lactulose, xylitol, inulin, fructooligosaccharide FOS and raffinose.

L. acidophilus ATCC 43121 is able to effectively utilize fructooligosaccharide FOS, lactulose and raffinose as a source of carbohydrates to promote growth but is unable to process the other tested substrates. Scanning and transmission electron microscopy showed that the double-microencapsulated bacteria exhibited smooth, rounded external surfaces, with a thick external coating composed of the prebiotic substrates. The efficacy of this double-encapsulation was studied over a 36-day storage period, with the highest survival rates observed for the bacteria double-microencapsulation with FOS. Interestingly, after 36 days of storage at 25 degrees Celsius, the bacteria that were double-microencapsulated with FOS maintained a [stable] cell count. These results indicate that double-microencapsulation of L. acidophilus ATCC 43121 by hybridisation is useful to effectively provide beneficial effects of probiotic bacteria for the host [13].

Alginate-coated Gelatin Microsphere Encapsulation: Alginate-coated gelatin microspheres were produced to encapsulate the probiotic *Bifidobacterium adolescentis* 15703T for enhancing survival during exposure to the adverse conditions of the gastro-intestinal tract. Gelatin microspheres were cross-linked with the non-cytotoxic genipin and coated with alginate cross-linked by Ca^{2+} from external or internal sources. The alginate coat prevented pepsin-induced degradation of the gelatin microspheres in simulated gastric juice (pH 2.0, 2 h), resulting in significantly ($P < 0.05$) higher numbers of survivors due to the buffering effect of intact microspheres. After sequential incubation in simulated gastric (1 h) and intestinal juices (pH 7.4,4 h), number of surviving cells were 7.6 and 7.4 log cfu ml^{-1} for alginate coated microspheres by the internal and external Ca^{2+}-source methods, respectively, while 6.7 and 6.4 log cfu ml^{-1} were obtained for cells in uncoated gelatin microspheres and free cells, respectively. This is a novel microencapsulation method, which protects probiotic *Bifidobacterium* during exposure to adverse environmental conditions [14].

An Interpolymer Complex Encapsulation in Supercritical Carbon Dioxide: Traditional encapsulation methods in fortified foods and drug delivery applications present difficulties for 'actives', such as probiotics, sensitive to exposure to water, solvents, heat or oxygen, where 'active' refers to a material, chemical or organism that has some potential benefit when consumed. A novel encapsulation technology, based on interpolymer complex formation in supercritical carbon dioxide, avoids such exposure during the encapsulation process. The method was used to encapsulate indomethacin and *Bifidobacterium longum* in a poly (vinyl pyrrolidone)-poly (vinyl acetate-co-crotonic acid) interpolymer complex. Polymer complexation was confirmed by Fourier Transform infrared and moisture absorption studies. Polymer plasticization and release of encapsulated probiotics were studied with scanning electron microscopy. It was shown that the encapsulation matrix is stable at low pH, but disintegrates at higher pH, triggering release of the encapsulated material. The technology could find application in encapsulation of sensitive actives in the food and pharmaceutical industry [15].

Effect of Various Encapsulating Materials on the Stability of Probiotic Bacteria: Ten probiotic bacteria, including *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *L. salivarius*, *L. plantarum*, *L. acidophilus*, *L. paracasei*, *B. lactis* type Bl-04, *B. lactis* type Bi-07, HOWARU *L. rhamnosus* and HOWARU *B. bifidum*, were encapsulated in various coating materials, namely alginate, guar gum, xanthan gum, locust bean gum and carrageenan gum. The various encapsulated probiotic bacteria were studied for their acid and bile tolerance. Free probiotic organisms were used as a control. The acid tolerance of probiotic organisms was tested at pH 2 over

a 2-h incubation period. Bile tolerance was tested with taurocholic acid over an 8-h incubation period. The permeability of the capsules was also examined using a water-soluble dye, 6-carboxyflourescin (6-CF). The permeability was monitored by measuring the amount of 6-CF released from the capsules during a 2-w storage period. Results indicated that probiotic bacteria encapsulated in alginate, xanthan gum and carrageenan gum survived better ($P < 0.05$) than free probiotic bacteria, under acidic conditions. When free probiotic bacteria were exposed to taurocholic acid, viability was reduced by 6.36 log CFU/mL, whereas only 3.63, 3.27 and 4.12 log CFU/mL was lost in probiotic organisms encapsulated in alginate, xanthan gum and carrageenan gum, respectively. All encapsulating materials tested released small amounts of 6-CF; however, alginate and xanthan gum retained 22.1% and 18.6% more fluorescent dye than guar gum. In general, microcapsules made of alginate, xanthan gum and carrageenan gum greatly improved the survival of probiotic bacteria when exposed to acidic conditions and bile salts [16].

Effect of encapsulation on the survival of probiotic microorganisms under high acid and bile conditions

L. acidophilus and *Bifidobacterium* were isolated from yoghurt samples. The isolates were tested for their acid and bile salt tolerance under *in vitro* conditions. The susceptible isolates viz., La.3 (*L.acidophilus*) and Bi. 1 (*Bifidobacterium*) were encapsulated by using 2% alginate. The encapsulation enhanced the survival of *L.acidophilus* by 15.9% and *Bifidobacterium* sp. by 16.6% [17].

Techniques for the Preservation of Probiotics: A novel encapsulation method for probiotics, which excludes the use of organic solvents. The efficiency/potential of this new method increases stability of sensitive probiotic cultures, specifically *Bifidobacteria*.

Early studies using both culture dependent and culture independent techniques showed reduced numbers of viable cultures in probiotic products, mainly yoghurts, from all around the world. These results were confirmed in this study for similar products sold in South Africa. Most of the product labels did not specify viable numbers of probiotics nor the identity (genus and species names) of the microorganisms incorporated.

Successful encapsulation of *Bifidobacteria* was achieved using this method. Complete encapsulation was indicated by absence of cells on surfaces of the encapsulated particles and production of a product with an acceptable particle size distribution was obtained. The encapsulation process produced no visible morphological changes to the bacterial cells nor did it have a negative effect on cell viability over time. The potential of interpolymer complex formation in scCO2 for the encapsulation of sensitive probiotic cultures was demonstrated for the first time.

Once ingested, probiotic cultures are exposed to unfavorable acidic conditions in the upper gastrointestinal tract. It is desired that these cultures be protected from this in order to increase the viability of the probiotics for efficient colonization. Interpolymer complex encapsulated *B. longum* Bb-46 cells were therefore exposed to simulated gastric fluid (SGF) and subsequently to simulated intestinal fluid (SIF).

It was found that the interpolymer complex protected *Bifidobacterium* from gastric acidity, displaying pH-responsive release properties, with little to no release in SGF and substantial release in SIF. Thus the interpolymer complex demonstrated desirable characteristics retaining the encapsulated bacteria inside when conditions were unfavorable and only releasing them under favorable conditions. Survival was improved by the incorporation of glyceryl monostearate (GMS) in the matrix and by use of gelatine capsules. Protection efficiency of the interpolymer matrix was better when higher loading of GMS was used. Use of polycaprolactone (PCL) as an alternative to poly (vinylpyrrolidone) (PVP) and incorporation of ethylene oxide-propylene oxide triblock copolymer (PEO-PPO-PEO) affected the interpolymer complex negatively, rendering it swellable in the low pH environment exposing the *Bifidobacterium* to gastric acidity. The use of beeswax seemed to have a more protective effect though results were inconclusive.

Probiotic cultures must also remain viable in products during storage. Encapsulated bacteria were either harvested from the reactor after 2 h of equilibration followed by depressurization and then ground to a fine powder or after 2 h of equilibration the liquefied product was sprayed through a capillary tube with a heated nozzle at the end, into the product chamber. Encapsulated bacteria were stored in either sterile plastic bags or glass bottles under different conditions and then viable counts were determined over time. Survival of bacteria was generally better when the products were stored in glass bottles than in plastic bags. Bacteria encapsulated in an interpolymer complex formed between PVP and vinyl acetate-crotonic acid copolymer (VA-CA), (PVP: VA-CA) survived better than non-encapsulated bacteria under all

storage conditions when the product was recovered from the reaction chamber. When the product was recovered from the product chamber, numbers of viable non-encapsulated bacteria were higher than the encapsulated bacteria for all interpolymer complex formulations. This was probably due to some exposure to high shear during spraying into the product chamber. The interpolymer complex between PCL and VA-CA i.e. PCL: VA-CA seemed weaker than the PVP: VA-CA interpolymer complex as viable counts of bacteria released from it were lower than those from the latter complex. Addition of PEO-PPO-PEO to both the PVP: VA-CA and PCL: VA-CA complexes decreased the protection efficiency. However, results indicated that sufficient release of encapsulated bacteria from the interpolymer complexes was obtained when the encapsulated material was incubated in SIF rather than in Ringer's solution. When SIF was used for release of encapsulated bacteria, the shelf life of *B. longum* Bb-46 was doubled. Encapsulation in an interpolymer complex therefore provided protection for encapsulated cells and thus has potential for improving shelf life of probiotic cultures in products. Further studies will investigate the effects of encapsulating probiotics together with prebiotics in the interpolymer complex as well as effects of encapsulating combinations of different probiotic strains together, both on survival in simulated gastrointestinal tract and during storage.

The unique particles produced using the patented encapsulation technique increased the stability of probiotic cultures. This technique may find significant application in industries manufacturing probiotic products, especially food and pharmaceuticals, thereby improving the well being of consumers [18].

Encapsulating Probiotics May Help Survival in Gi Tract: Ten probiotic bacteria were encapsulated using various coating materials. The acid tolerance of probiotic organisms was tested at pH2 over a two-hour incubation period; bile tolerance was tested with taurocholic acid over an eight-hour incubation period. Free probiotic organisms were used as a control. All probiotic organisms tested showed a gradual loss in viability when exposed to acidic conditions, although the encapsulated bacteria survived better than the control group. *Lactobacillus acidophilus* and *L. salivarius* were the most acid-tolerant strains.

Microencapsulation may prove to be an important method of improving the viability of probiotic bacteria in acidic food products to help deliver viable bacteria to the host's gastrointestinal tract. Furthermore, the various encapsulating materials in particular xanthan gum and carrageenan gum appeared to be as effective as alginate in protecting probiotic cells from harsh environmental conditions [19].

CONCLUSION

Use of different encapsulation technologies for protection of health ingredients achieved high ingredient efficiency. It not only depends on developing or choosing the right encapsulation technique but also requires expertise in food processing. The technology of micro-encapsulation has developed from a simple immobilization or entrapment to sophisticated and precise micro capsule formation. The advances in this field have been tremendous with nutraceuticals and food ingredients; however, as to the micro-encapsulation of live probiotic bacterial cells, the technology seems to be not well developed. Probiotic therapy (or microbial intervention) is based on the concept of healthy gut micro flora. The delivery of viable micro encapsulated probiotic bacteria will become important in the near future. Micro-encapsulation will assume importance in delivering viable strains of probiotic bacteria in large numbers to consumers. It will be used as tools to co encapsulate both prebiotic ingredients and probiotic bacteria within the same capsule to enhance growth and multiplication of these bacteria through symbiotic effects when they are released in the gastro-intestinal tract. In the future multiple-delivery may be developed, such as co-encapsulating prebiotics and probiotics as well as nutraceuticals, thus a new area of more complex nutritional matrices will need to be investigated. In the food processing industry, preservation and storage and micro-encapsulation will increasingly play a role to protect the viability and enhance the survival of bacteria against adverse environmental conditions. New food regulations may specify labeling 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. Studies (clinical data) will need to be conducted on the effect of encapsulation on the safety of probiotic bacteria. Fermented and non-fermented dairy, cereals, meat small goods, sous-vide products as well as prepared home meal solutions could become food vehicles using microencapsulation technology to protect probiotic bacteria as a means of delivering large quantities to consumers. In the health food industry, capsules, tablets, suspensions, creams and powders will be increasingly using micro-encapsulation technology for direct

Consumption and for external application of probiotics. They will increasingly be used to treat patients with medical problems. The technology of micro-encapsulation, however, needs to be developed with more precise machinery, capsule and better delivery systems. Nanoencapsulation may assume importance in the near future to develop designer probiotic bacterial preparations that could be delivered to certain parts of the gastro-intestinal tract where they interact with specific receptors. These nano-encapsulated designer probiotic bacterial preparations may act ac de novo vaccines, with the capability of modulating immune responses. Improved techniques need to be developed to track these micro-or nano-encapsulated probiotic bacterial cells for their delivery, persistence, sustained release and immune enhancing effects in the gastro-intestinal system. More in vivo studies should be conducted using human subjects to confirm the efficacy of micro or nano encapsulation in delivering probiotic bacteria and their controlled release in the gastro-intestinal system.

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