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MICROENCAPSULATION: AN OVERVIEW FOR THE SURVIVAL OF PROBIOTIC BACTERIA

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ABSTRACT

For maintaining good health, one needs a proper balance and composition of intestinal microflora which can be achieved by supplementing probiotics. A noteworthy issue in creating helpful and valuable probiotic food items is bacterial survival, amid capacity and ingestion. Several gastrointestinal diseases can be reduced by colonizing Probiotic supplement as the appropriate barrier in the small intestine. Probiotic is characterized as a suitable microorganism with several medical advantages to the consumer when administrated in a satisfactory amount. The poor survival and steadiness of the probiotic microorganisms as revealed from the earlier reports is an essential question to that impact. Diverse natural components like oxygen toxicity, an intolerant condition of acidity and travel through the gastrointestinal tract offers a variety of extreme conditions to the probiotic microorganisms. Therefore, the current review is more emphasized upon the microencapsulation of the probiotics that enhance their viability against different parameters like oxidation, light, moisture, and temperature. Recent advancements in ensuring microorganism survival rate and their colonization in the gut as gut microflora using microencapsulation enhance probiotic supplements for better health. Hence, the present review also emphasis on the methodological systems used for probiotic alive by the encapsulation process advance technologies used to stabilize their viability during storage including the selection of biomaterial and decision for proper innovation.

Keywords: microencapsulation, probiotic, health benefits, gastrointestinal, polymeric matrix, survival

INTRODUCTION

Probiotics are generally microbial supplements of beneficial microorganisms which surpasses the gastrointestinal tract and provide the medical advantages to the host when managed in the satisfactory amount by enhancing the properties of microflora **(Ammor *et al.*, 2007**). The remarkable choice of the microorganism to be considered as probiotic depends on the fluke that it is a typical inhabitant of the gastrointestinal tract, stay active along the route through the gastrointestinal tract and draw out its suitability and reliability in the digestive system **(Cook *et al.*, 2012)**. Important probiotics give various medical advantages identified with the counteractive action of harmful microscopic organisms by the aggressive prohibition against gastrointestinal pathogens and through the preservation of typical intestinal microflora. Besides, these probiotics built the resistant framework, cover the treatment of lactose bigotry and create vitamin B **(Rasic, 2003)**. The investigations of the prominent countless microorganisms comprise of various strains of *lactobacillus and Bifidobacteria***(Theodorakopoulou*et al.*, 2013)**. Bacterial culture used as a probiotic enhances the development of the favored microbes, removes unwanted microbes and builds up the normal functions of the body. The general soundness of the individual relies upon an individual way of life or dietary patterns. In various food products, probiotic microbes have been enhanced as an approach to expand their good quality and probiotic characteristics **(Sullivian, 2005).** In the present era, these are of great importance in many food industries to develop new products with probiotic characteristics **(Doherty *et al.*,2012)**. Further, the application of microencapsulation techniques upgrades the security of the probiotic product**(Tolun *et al.*, 2016).**

UTILIZATION OF MICROENCAPSULATION FOR THE SURVIVAL OF PROBIOTIC BACTERIA

Microencapsulation of microorganism is one of the most recent and effective techniques to secure microbes against serious ecological elements and coat them with proper biomaterial for suitable release in the intestinal medium **(Mortazavian*et al.*, 2008)**. Microencapsulation help in segregating Probiotic bacteria from the harsh environment of the gastrointestinal tract. Microencapsulation of probiotic bacteria as exemplified in Fig 1,shows the core material based on proteins as a useful nourishment for the probiotic cells which is a promising alternative to polysaccharide hydrogels. These biomaterials frame a boundary to secure the center material against the gastrointestinal condition **(Zuidam and Shimoni, 2007)**.

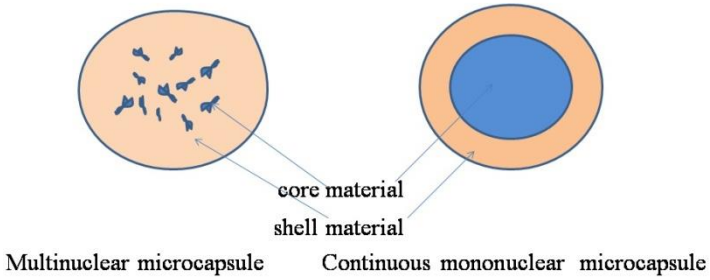

Figure 1 Schematic demonstration of a Microcapsule.

MICROCAPSULE AND A MICROBEAD

Altered polymers of sugars, gums, proteins, and lipids are diverse bioactive components that are utilized to shape microcapsule and can be distinguished as reservoir type, matrix type and coated matrix type as outlined in Figure 2. The shape and smoothness of the sporadic microcapsules enhances their productivity **(Mortazavian *et al.*, 2007)**. Each microbead (likewise called the capsule) comprises of hydrocolloids that are secured around the cell. The gel-like structure of the core called gel-globule. In terms of the size of the particle and type of capsule, microbead comprises different characteristics. The microbead covered with the layer of the chemical compound expands the effectiveness of

microencapsulation. The constituent capture encompassed by the coat called "core" **(Sultana *et al.*, 2000 and Truelstrup-Hansen *et al.*, 2002)**.

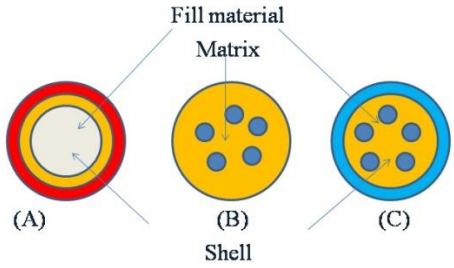

Figure 2 Structure of Microcapsule (A) reservoir type (B) matrix type (C) coated matrix type

ADVANTAGES OF MICROENCAPSULATED PROBIOTICS

The microencapsulation of the probiotics improves the survival and safety of microbes in food. The 40% of probiotic strains survive in dairy products when incorporated in a calcium alginate sphere than free cells **(Sheu and Mrashall, 1993)**. Encapsulation is a quick, adaptable method as it permits delivery of good quality particles under 40 µm with steady activity for the number of industrial applications**(Fang and Bhandari, 2010; Zuidam and Heinrich, 2009)**. Further, the system is utilized to diminish the instability and exhibit the improvement of survival in the gastrointestinal tract. Microencapsulation has been utilized to diminish the conceivable threat of harmful substances such as fumigants, herbicides and pesticides.

BIOMATERIALS UTILIZED FOR THE MICROENCAPSULATION OF PROBIOTICS

Alginate

Alginate and its mix are regularly utilized as an exemplifying material because of its non-harmful nature and being promptly accessible. Alginate is removed and filtered from various sorts of green growth. It is a straight heteropolysaccharide comprising of two basic units D-mannuronic acid and L-guluronic acid. As alginate can retain water and simple to control because of the capacity to ingest and control, it is utilized as a typifying material. Due to its diverse properties such as gelling, balancing out and thickening it is utilized in various applications among the food and pharma industries (**Goh and Chang *et al.*, 2012**). Further, because of its non-harmful nature and minimal effort, alginate is utilized for embodying material for probiotic microorganisms that upgrade the feasibility of microscopic organisms when presented to different ecological conditions **(Burgin *et al.*, 2011)**.

Chitosan Polymer

Chitosan has basically deacetylated polymer of N-acetyl-glucosamine which comprises mainly chitin, a material found in algae and molluscs with effective biocompatibility and biodegradability. In addition, it enhances the antibacterial efficacy of the probiotic microorganisms. The property of chitosan takes care of the survival of microbes enhancing the ability and, furthermore resist in the gastrointestinal tract **(Capela, 2006 and Chavarri *et al.*, 2010).** Hence, it is the most ideal approach to transport the sensible cells of the colon **(Zhou *et al.*, 1988).**

Xanthan gum

It is a heteropolysaccharide that comprises of rehashed structures of the pentasaccharide units framed by two glucose units, two mannose units, and one glucuronic corrosive unit. It is synthesized by the aging of microbe*Xanthomonascampsetris*. Xanthan is considered as essentially gelling gum and has been utilized for the embodiment of probiotic microorganisms and gives high resistance towards acidic conditions in the stomach **(Sultana *et al.*, 2000 and Chen, 2007)**.

Starch Polysaccharide

Starch, a polysaccharide manufactured by every green plant comprising of α-d-glucose units connected by glycosidic bonds. The probiotic cells can be embodied to the starch granules by the grip in the granules. The surface of starch granule and safe starch for the probiotic cells can achieve the condition of gastro intestine and colon when embodied. One of the important properties of safe starch is the better release of the bacterial cells in the intestinal tract **(Haralampu,2000)**. However, altered starch has greater coating properties. Microencapsulation of ascorbic acid using starch granules has been proved in maintaining high amid capacity ascorbic acid**(Gupta *et al.*, 2015)**.

Cellulose Acetate Phthalate (CAP)

Due to its safe nature and physically inert characteristics to the gastrointestinal tract, Cellulose Acetate Phthalate (CAP) is employed for encapsulation of probiotic bacteria. Generally, this compound is insoluble at acidic hydrogen ion concentration via due to its ionizable phthalate groups **(Mortazavian *et al.*, 2007).** The addition of spray-dried *Bifidobacterium animalis* encapsulated in CAP together with inulin considerably increased probiotic viability **t**hroughout storage at 5°C for 30days **(Antunes *et al.*, 2013).**

METHODS FOR PREPARATION OF MICROCAPSULES

Physical Methods

Air Suspension Covering Method

In this technique, the central material is strongly dispersed into supporting air stream as the suspended particles are covered with unstable polymer discharge leaving a thin layer of it on the center. The procedure is repeated until the required parameters are achieved, such as covering thickness is accomplished. The rate of drying is specifically relative to the temperature of an air stream as the air stream dries the particles in the suspension. The covering chamber is arranged as such that the particles move upwards through covering zones and disperse into moving air and revert back to the chamber base making the point of desirable thickness when the process is accomplished **(Jackson *et al.*, 1991)**. Along thickness, the different process factors to be considered such as the concentration of covering material, solubility, melting point, surface zone, density, volatility of central material, the temperature of the air stream and the measure of the fluidizing air stream.

Coacervation Process

In this process, the active central material is spread in such an arrangement of covering material that the core material doesn't break in the dissolvable medium. Coacervation occurs when there is a difference in pH of the medium, which is done either by including sulfuric acid, hydrochloric acid and natural acids. However, later it diminishes the solvency of shell material and continues the shape support from the microcapsule. The shell material structures a consistent covering around the center and shell to solidify. As a result here is the formation of simple and complex shapes of microcapsule coacervate **(Kruif *et al.*, 2004).** The solidifying agents like formaldehyde might also be added to the procedure after which the suspension was dried in the fluidized bed dryer **(Nihant *et al.*, 1995)**.

Pan Coating

One of the most established strategies utilized in the pharmaceutical industries for microencapsulation. In this technique, the particles are tumbled in a pan or a device while the covering material is applied in the spray form. Further, the particles are mixed with the coating material and increased temperature results in the melting of coating material which can be gradually applied to core particles. From the start of encapsulation, core particles were wholly mixed in tumbling vessel rather than being mixed with the core particles**.** The arrangement associated with the particle size > 600µm usually fit for pan coating microcapsules **(Kasturagi *et al.*, 1995)**.

Divergent Expulsion Process

The divergent expulsion process is reasonable for fluid and slurries. In this process, the encapsulation occurs by utilizing divergent expulsion which contains concentric microbeads. The stream of central fluid encompasses by the sheath of microbead arrangements. As the stream travels through the air zones it breaks into microbeads of center each covered with wall arrangement. As the beads are in fluidized liquid, the divider is solidified and may vanish from wall arrangement. Since the beads are inside ±10% mean distance across the center, they settle as a limited ring around the microbead. In this way, a container can be solidified after development by holding them in a ring formed called solidifying microbead. This procedure is capable of varied size particles of 400-2000µm and with diverse coating or polymers materials **(Venkatesan *et al.*, 2009).**

Spray drying and hardening strategy

This technique is reasonable for labile medications due to minimum contact time in the spray dryer and is efficient. In spray drying, the active material is broken and suspended in polymer arrangement which is caught as the dried molecule. Both the strategies of spraying and hardening are comparable in the procedure of dispersion of the center and covering the molecule. However, there is a difference in the rate of hardening of covering. In spray drying, there is a fast dissolution of

dissolvable, as a result of breaking of covering material. However, during spray hardening by hot solidifying a non-dissolvable covering material is obtained. Expulsion of non-dissolvable is by absorption, extraction and vanishing **(Aparna, 2010)**.

Dissolvable vanishing strategy

This technique is broadly utilized for water-soluble and water-insoluble compounds to deliver strong fluid center microbeads. In this technique, the covering material (polymer) is broken up in an unstable dissolvable form which is immiscible with the fluid medium phase. In other words, a central material or microencapsulated form is broken down in the covering polymer arrangement. With unsettling, the center covering materials blend or spread in the fluid medium phase to get the proper microcapsule size. The dissolvable vanishing strategy is accomplished by constant disturbance and by using external heat supply **(Jain, 2002)**.

METHODS FOR MICROENCAPSULATION OF PROBIOTIC MICROSCOPIC ORGANISMS

Probiotic microscopic organisms are formed by various procedures like extrusion, emulsion and spray drying strategies. In these strategies, by utilizing the different systems probiotic microbes are trapped in the gel lattice **(Champagene and Fustier, 2007)**. The conditions for actualizing innovation are intended to keep up cell suitability of the probiotic microorganism. In any case, the solvents occupied with the exemplification innovation ought to be non-lethal **(Gbassi and Vandamme, 2012)**. These procedures are isolated into two segments:

(A) Encapsulation Process
(B) Drying Process

Encapsulation Process

The strategies utilized for the encapsulation procedure are extrusion or bead technique and emulsion or two-stage framework strategy **(King, 1995)** and the carrier material is obtained by several methods such as spray chilling, spray drying, cocyrystallization, lyophilization, coacervation and thermal gelation **(Poshadri and Kuna, 2010).**

Extrusion Technique

Extrusion is the most common physical strategy for delivering hydrocolloid capsules **(King, 1995)**. However, it is a poor and simple process with direct and straightforward tasks, which make the cell harm minimum and causes a relatively high suitability of probiotic microorganism. Some different particulars of this strategy are biocompatibility and adaptability **(Klein and Vorlop, 1985; Martinsen *et al.*, 1989)**. All things considered, the vital disadvantage of this technique is that it can't be utilized for real generation on account of its relaxed improvement of microbeads. In another way, it is difficult to scale up. The arrangement of beads size of breadth 2-5 mm is maximum, delivered in the emulsion technique. Probiotic encapsulated bacteria showed enhanced survival rate by ionic gelation to the microbeads with electrostatic extrusion under simulated gastric conditions in the gastrointestinal tract **(Kim *et al.*, 2016).**

Emulsion Technique

Emulsion technique is viably utilized for the microencapsulation of lactic microbes **(Audet *et al.*, 1988)**. Similar to the extrusion system, it tends to scale up the process and the measurement of shaped globules is particularly little (25µm-2mm). All the same, this includes extra expense for execution contrast among the extrusion procedure along with the utilization of vegetable oil for emulsion arrangement **(Krasaekoopt *et al.*, 2003)**. In this strategy, the expansive amount of vegetable oil (as a ceaseless stage) for example soy, sunflower, corn-millet or light paraffin oil is added to the least volume of a cell **(Gismondo *et al.*, 1999)**.In the emulsion technique, the arrangement turns out to be fine, reliable and blending by actual increase with easy scale-up and high survival of bacteria, focusing on the estimation of microencapsulation. The best decision of Tween 80 at the grouping of 0.2% has been recommended for the arrangement of the capsule **(Sheu and Marshalla, 1993)**. The strategy for the planning of microcapsule by emulsion appears in Fig 3. Microparticles of encapsulated probiotic bacteria produced by the emulsification process using sodium alginate as biomaterial are effective in protection under simulated gastric condition **(Holkem *et al.*, 2017).**

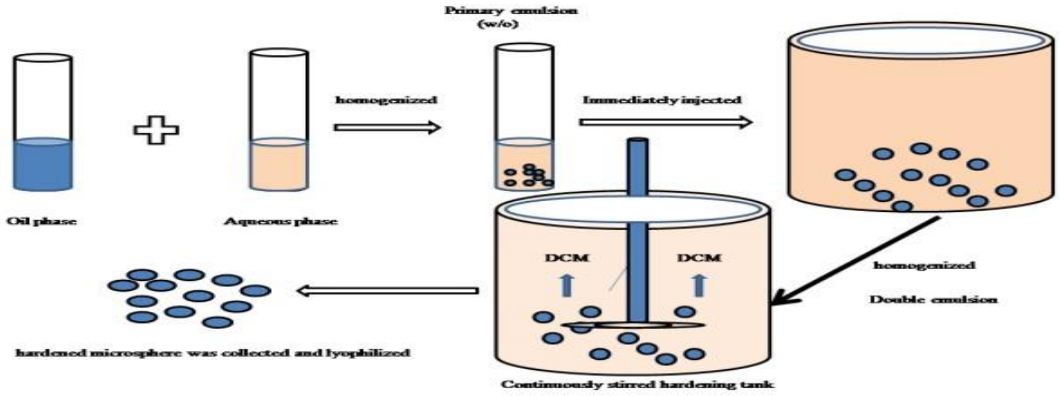

Figure 3 Method for preparation of microcapsule by emulsion technique.

Drying Process

The system of spray drying or fluidized bed drying has been broadly utilized for the drying of embodied microbes. The cells exemplified by these strategies have accomplished discharge into the item. In spite of the fact that the cells are not anchored towards the food environment and remain within the sight of gastric liquid or bile after drying process **(Lankaputhra and Shah, 1995)**. Probiotics in solidifying dried form shape demonstrate similarity with different starter cultures, for example, cheddar cheese; however, results in realistic usability with their cell slurry formation. With specific reference to spray drying, ongoing production makes it viable in ensuring microencapsulated probiotics **(Kitamura *et al.*, 2009)**. This technique is normally utilized in the food industry requires atomization of fluids or efficient suspension of probiotics and transporter material into a drying gas, that outcomes in a quick dissolution of water. Water dissolution is resolved as the contrast between the air delta temperature and air outlet temperature. The spray drying process is managed by these temperatures in addition by the gas stream **(Rokka and Rantamaki, 2010)**. The spray drying strategy requires high temperatures to encourage water dissipation that decreases the probiotic viability and their progress in the food product. As per the earlier results, it was found that the base air delta temperature ought to be 100°C, while the most extreme 170°C for the probiotic exemplification life form. The air outlet temperature changes somewhere in the range of 45°C and 105°C. At these temperatures, the cells hold all their probiotic action. The action of probiotic must be separated from probiotic survival. The drying process doesn't decrease cell survival and doesn't repress the stability of probiotic cells within the gastrointestinal and intestinal mucosa conditions **(Piano *et al.*, 2008)**. The impact of various drying strategies alongside their methods on the molecule measure has been presented in Table 1.

Table 1 Different drying technique along with major steps and particle size used for the encapsulation

Drying Method	Mechanism	Major steps	Particle size(µm)	Cost	Example
Spray drying	Dehydration	Preparation of dispersion, homogenization, atomization of feed dispersion, dehydration of atomized particle.	3-100	Low	Dry flavorings, vitamin, mineral, Colorant, fat oil flavor, aroma compounds, enzyme etc.
Spray freeze Drying	Lyophilization and dehydration	Atomization of dispersion and low temperature drying	400-1400	High	Mainly probiotic bacteria.
Lyophilization	Sublimation drying	Mixing of core in coating solution and freeze-drying of the mixture.	-	High	All heat sensitive materials and aromas, water-soluble essences.
Fluidized bed Coating	Coating of the solution	Preparation of coating solution, fluidization of core particle and coating of core material, dehydration and cooling.	5-5000	Moderate	Any kind of shell material like polysaccharides, proteins, emulsifiers, fats, complex formulations, enteric coating, powder coatings, yeast cell extract etc.

Source: Laohasongkram *et al.*,2011.

SURVIVAL OF PROBIOTIC BACTERIA

Covering of microcapsules with chitosan was found best in protecting probiotic microscopic organisms from the intestinal juice. Different variables were found to influence the viability of probiotic microorganisms in nourishment items amid handling, generation, and capacity as appeared in Fig 4. **Krasaekoopt *et al.* (2004)** reported that the probiotic microscopic organisms covered with alginate chitosan covering upgrade the feasibility and conveyance in the gastrointestinal tract. Various investigations of researchers portrayed that covering with chitosan gives the best security in bile salt. **Murata *et al.* (1999), Koo *et al.* (2001), Krasaekoopt *et al.* (2004), Lee *et al.* (2004), Chavarri *et al.* (2010)** demonstrated that the microencapsulated *Lactobacillus casei* and *Lactobacillus gasseri* surrounded with chitosan covering results in much reasonability as compare to microcapsules without covering with chitosan. **Sultana *et al.*, (2000)** showed with the purpose of coating with alginate *L. acidophilus* and *L. casei* diminished in log cycle as compared with the free cell by different bile salt concentrations. The preventive result of high amylose maize starch on the bile corrosive resistance was computed by **Wang *et al.* (1999)**. It shows that amylomaize advance the suitability of probiotics with the different concentrations of bile and with the addition of starch granules. The gelatinized starch substance is utilized as the coating material for encapsulated probiotics. This swollen and gelatinized starch, along these lines, adds to expanding a consolidated structure **(Slaughter *et al.*, 2001 and Mohammadi *et al.*, 2012)**. The reason for starch gel consolidates with chitosan covering is to enhance and grow the modern purposes. It has been postulated that the major reason for that is the microcapsules inundated in the bile salt and hence, the penetrability of bile salt in the microcapsules gets restricted. The different investigations demonstrated that the approach of prebiotics is better recovered with calcium alginate**(Capela *et al.*, 2006, Homayouni *et al.*, 2008, Nazzaro*et al.*,2009, Zanjani *et al.*, 2012)**. In other research, it was observed that the microencapsulation method had a positive aftereffect of inulin in human medical studies **(Capela *et al.*, 2006 and Nazzaro*et al.*, 2009)**.

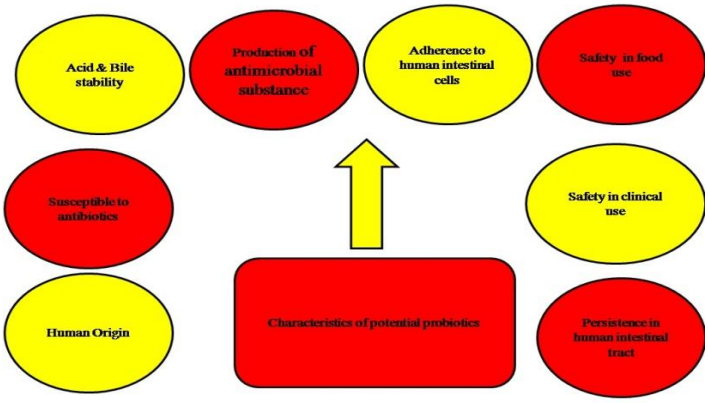

Figure 4 Various characteristics affecting the viability of probiotics in food products.

SURVIVAL OF PROBIOTICS DURING PROCESSING AND STORAGE

Development of foods with appropriate viability of probiotic as a result of many factors through out process and storage affect the stability of probiotic microorganisms **(Korbekandi *et al.*, 2011)**. Due to the high survival rate of probiotics enhance the stability of probiotic microorganism in food products **(Saxelin *et al.*,1999 and Cruz *et al.*, 2010)**. Various endeavors have been made to enhance the feasibility of probiotics in various nourishment items amid their creation until the season of utilization. Numerous factors were found to impact the suitability of probiotic microorganisms in nourishment items amid creation, handling, and capacity. The distinguished variables incorporated in food products along with various parameters such as pH, titratable acidity, oxygen, water activity, salt concentration, sugar and synthetic substances like hydrogen peroxide, bacteriocins, handling parameters (warm treatment, hatching temperature, cooling rate of the item, packing materials and capacity techniques, and size of capsule along with, microbiological parameters (strains of probiotics, rate and extent of inoculation) which helps in the enhancement of probiotic microorganisms.

VARIABLES INFLUENCING SURVIVAL OF PROBIOTICS DURING PROCESSING

Fermentation conditions

For influencing the viability of probiotic microorganisms fermentation temperature is one of the essential variables and other subjective parameters of probiotic food products with appropriate temperature ranges from 37– 43°C **(Boylston *et al.*, 2004; Lee and Salminen, 2009 and Korbekandi *et al.*, 2011)**. Despite the fact that at temperatures of 45 °C the specific species of lactobacillus like *L. acidophilus* can grow, however, the ideal growth happens between 40– 42 °C. Temperatures above 45– 50 °C have a negative impact on the survival rate of probiotic cultures. The arrangement time must be shorter at a higher temperature with the end goal to spare the probiotics **(Lee and Salminen, 2009)**. Management to oxygen during aging assumes a noteworthy job in loss of feasibility of oxygen-sensitive microorganisms **(Gaudreau *et al.*, 2013)**. A few techniques have been utilized to diminish oxygen content during aging. The most essential one is achieving temperature under vacuum **(Cruz *et al.*, 2007)**. The obstruction of probiotic microscopic organisms to warm pressure can be expanded by gentle heat treatment preceding their utilization. Application of non-lethal heat shock enables microbes to tolerate pressure higher in force and it has been discovered that the heat adjustment builds the warm buoyancy of *Lactobacilli* **(Teixeira *et al.*, 1994)**. The study showed that the heat adjustment of live microorganisms preceding heat thrust has a positive impact to enhance the warm buoyancy of *Lactococci* and *Lactobacilli* with the untreated strains **(Desmond *et al.*, 2001)**.

Solidifying and defrosting activities

The earlier studies showed that different strains of probiotic microorganisms can survive in solidified items. The cell films of probiotics get harmed as a result of the solidifying process because of the mechanical burdens of the ice crystal formation in the intracellular and extracellular form of the cells. In these conditions, the solutes condensate and the cells get dried out results in amid solidifying. Thus, the crucial metabolic actions of the cells were decreased **(Akin and Kirmaci, 2007)**. The rate of solidifying influences cell survival, as bigger ice crystals delivered by moderate solidifying cause more prominent harm to the cells and fast solidifying aides in better upkeep of the microorganisms in the item **(Fowler and Toner, 2005; Gill, 2006; Mortazavian *et al.*, 2011)**. Mortality additionally happens through defrosting of solidified items because of the presentation of the microbial cells to osmotic impacts and also to the high concentrations of hindering components such as hydrogen particles, natural acids, oxygen and other harming segments in liquefying media **(Jay *et al.*, 2005)**.

EXPANSION OF CELL PROTECTANTS

In drying medium, the substances are added which facilitate in securing the practicality of probiotic cells. Some of those substances include skim powder, whey protein, glycerol, betaine, adonitol, lactose and polymers, for example, dextran **(Hubalek, 2003)**. Perfect cryoprotectants as an example, glycerol was added to the medium for freeze-drying that helped within the regulation of probiotics to the adverse conditions by decreasing the osmotic characteristics **(Capela *et al.*, 2006). Desmond *et al.*, 2002** utilized gum arabic (10%) in the spray drying medium with an outlet temperature of 100– 105 °C for upgrading probiotic survival of *L.paracasei* NFBC 338. The results showed less improved survival in gum arabic treated cells than the control cells. Skim protein in a reconstituted skim milk medium will stop the damage to the external covering of the cell and hence proved to be an appropriate medium for efficient spray drying of probiotic bacteria**(Ananta *et al.*,2005)**. The reconstituted skimmed milk medium has the ability to form a protective covering on the proteins and use up calcium for survival after drying out **(King and Su, 1993).** In another experiment, the addition of polydextrose and inulin in the spray drying reconstituted skimmed milk medium did not improve the stability (**Corcoran *et al.*, 2005)**. The defensive effect of recipients on the spray drying and capacity was assessed**Salar-Behzadi et al. (2013)**. Gum arabic and gelatin demonstrated the best defensive effect. Cells pre-treated with these biomaterials appeared diminished with upgraded reliability and along with multi-month of capacity time. It is expressed before that starches have defensive impacts for probiotic microscopic organisms intending to stop drying. They assist in raising the temperature and consequently creating a difference with free cells to attain the microencapsulated stage**(Fowler and Toner, 2005)**. The safety of probiotics in smooth protein-starch relies upon the arrangement of the various factors **(Hoobin *et al.*, 2013)**. The incomplete substitution of maltodextrin with glucose (D-or L-) enhanced microbial survival at 33% RH as a result of pointed sub-atomic versatility and lower water take-up. It has likewise been illustrated that trehalose is a good cryoprotectant used as a solidifying agent because of its good parameters with high change temperature, the solid ion-dipole associations and hydrogen bonding among trehalose and therefore the biomolecules allow higher survival of *L. acidophilus* **(Conrad *et al.*, 2000)**. Perfect solutes have additionally demonstrated useful in probiotic viability and safety in acidic conditions. **Corcoran *et al.* (2004)** found that the concentration of 19.4mM glucose brought about up to 6-log improved survival following 90 min enhanced the stability of probiotic bacteria in digestive juice at pH 2.0 as analyzed to the control. **Santivarangkna *et al.* (2006)** detailed that the survival of *L. helveticus* under vacuum drying was the best method by the increase of 1% sorbitol.

APPLICATIONS OF ENCAPSULATION IN THE FOOD INDUSTRY

Microencapsulation method is broadly utilized in different fields, essentially food industries, since; it can upgrade strength, increase dissolvability and enhance the properties of probiotic products, for example, cancer prevention agents and chemicals. The food industries use fundamental components to enhance texture, flavor, surface, and timeframe of the realistic usability of items. The principal objective in the food is to create a high-productivity microcapsule with an ease generation. Despite the fact that a more wide-time of genera and types of different probiotic microorganisms are considered as potential probiotics. The principal microbes from the genera *Lactobacillus* and *Bifidobacterium* are economically utilized in probiotic food items is clearly seen from Table 2 **(Shah and Ravula, 2004)**. Further, the cells of various probiotic life forms were epitomized and conceivably utilized in various sustenances and biotechnological applications (Table 3). In the field of food innovation relatively few investigations detailed for the embarrassment of live microorganism in dairy-based items and protein microcapsules, because of the reason of heat-sensitive and gelation of food based protein. Hence, heat treatment was not found for heat sensitive barrier or center materials like live organisms **(Chen *et al.*, 2006)**. The healthful and practical estimation of proteins of grains (oat, wheat, grain, and corn) is more gainful for production reason and their vital useful properties or different food applications and hence, these proteins were used as a biomaterial for microencapsulation **(Ducel*et al.*, 2004 and Nur, 2010)**.

Table 2 Commonly used species of lactic acid bacteria in probiotic preparation

Probiotic bacteria	Species
Lactobacillus sp.	*L. acidophilus, L. casei,* *L. delbrueckii ssp.,* *L. cellobiosus, L. curvatus,* *L. fermentum, L. lactis,* *L. plantarum, L. reuteri,*
Bifidobacterium sp.	*B. bifidum, B. adolescentis,* *B. animalis, B. infantis, B. thermophilum, B. Longum*
Enterococcus sp.	*Ent. faecalis, Ent. faecium*
Streptococcus sp.	*S. cremoris, S. salivarius,* *S. diacetylactis, S. Intermedius*

Source: Shah and Ravula, 2004.

MICROENCAPSULATION AND RELEASE OF PROBIOTICS

Microencapsulation is a technique characterized for the entrapment of a compound or a substance (active agent) into another substance (wall material) for its grip, safety, controlled discharge and its structural function **(Poncelet, 2006)**. The core or payload of the microcapsule is the encapsulated active substance in microencapsulation where the active agent is known as coating or carrier material. The wide range of substances can be utilized by microcapsules: solids, fluids, drugs, proteins, bacterial cells, undifferentiated cells. Due to a huge scope of free substances, microcapsules can have a combination of goals and applications in health. Regardless of whether for medication conveyance, catalyst recovery and simulation of cells and artificial organ conveyance and as depicted in this review, for the conveyance of live probiotic microorganisms. There is a number of microcapsule conveyance frameworks that have been proposed for the oral intake of live bacterial cells. In 2000, **Sun and Griffiths** explored the utilization of an acidic stable capsule composed of gellan and xanthan gum for the control of *the Bifidobacterium* release. The results showed that capsulated cells survived altogether superior to the free cells after refrigeration in purified yogurt for a maximum time of 5 weeks. The use of calcium alginate as a polymer for microencapsulation is the normal strategy. In case the utilization of alginate is troublesome as these are not safe and due to low pH conditions experienced in the stomach, they show critical shrinkage and a decline in mechanical quality and their passage **(Krasaekoopt *et al.*, 2004)**. Various techniques using polymer cross-connection have been proposed for microencapsulation by utilizing carrageenan, alginate-poly-L-lysine, starch polyanhydrides, polymethacrylates, and enteric covered polymers. Microencapsulation techniques are being created and enhanced to take account for expanded gastrointestinal survival and immunoprotection in the tract. One recently created kind of microcapsule that showed promising outcomes in terms of mechanical solidness and pH obstruction is cross-linked- alginate-chitosan microcapsules. The most ordinarily used plans for microencapsulation are the alginate-poly- L-lysine-alginate (APA) microcapsule for micro-coating **(Prakash and Chang, 1996)**. APA sort of microcapsule has been utilized for various applications including drugs, undifferentiated organisms, and bacterial cell delivery. This technique depends on the polyelectrolyte complexation instrument for the association of the polymers to the alginate and poly-L-lysine (PLL). Alginate is a normal occurring biocompatible polymer extracted from brown-green algae that are progressively utilized in the field of biotechnology for extensive uses. Alginate is an unbranched polysaccharide which contains 1, 4 - connected β-D-mannuronic acid and α-L-guluronic acid chain which are inter-dispersed with areas of the substituting structure of β-L-mannuronic and α-L-guluronic chain **(Haug and Larsen, 1962)**. PLL is a polypeptide of amino acid, L-lysine that is accessible in a variable number of chain lengths, defined by its sub-atomic weight. It is a polycationic polymer that can be utilized for covering the venture of microencapsulation. The expansion of polycationic polymer prompts the development of a product that gives particular penetrability and immunoprotection to the microcapsules. The alginate bead cannot withstand the harsh condition of the gastrointestinal tract without PLL, which furnishes it with an extended mechanical safety and targeted delivery.

Table 3 Different food applications of encapsulated microorganism

Microorganism	Encapsulation material	Food	Reference
B. bifidum, B. Infantis	Calcium alginate	Mayonnaise	Khalil; Mansour 1998 & Kasipathy Kailasapathy, 2002
L. paracasei	Milk fat	Cheddar cheese	Stanton *et al.*, 1998
Enterococcus faecium	Milk fat	Cheddar cheese	Gardiner *et al.*, 1998 & Kasipathy Kailasapathy, 2002
B. bifidum, B. adolescentis and B.breve	Cream	White brined cheese	Ghoddusi and Robinson, 1998; Cook *et al.*, 2014
B. bifidum, B. infantis, and B. Longum	Calcium alginate gels	Crescenza cheese	Gobbeti *et al.*, 1997 & Chavarri *et al.*, 2010
L. lactis	k-Carrageenan and locust bean gum	Fresh cheese	Sodini *et al.*, 1997
L. casei	Liquid core alginate capsule	Fresh cheese	Li *et al.*, 2011
Lactobacilli	Calcium alginate	Frozen ice cream	Sheu and Marshall, 1993
Lactobacillus acidophilus	Calcium alginate	White brined cheese	Ozer *et al.*, 2009

MICROENCAPSULATED PROBIOTICS

In the field of microencapsulated probiotics, the interest rate has been increased in recent years. Microencapsulated probiotics keep their practicality finer to free cells under concern in gastrointestinal conveyances; this has been demonstrated by recent research. Microencapsulated probiotics provide promising results for the usual treatment of various gastrointestinal infections, increases gut microflora and hence is very useful for maintaining the balance of the digestive system.

Microencapsulated Probiotics and Colon Cancer

The microencapsulated *L. acidophilus* was examined for antitumorigenic properties in different intestinal neoplasia mice assigning a germline APC change which, treats various pretumoric intestinal neoplasms **(Urbanska, 2009)**. The mice were injected with APA *L. acidophilus* microencapsulates for a duration of 12 weeks pursued with the identification, grouping and the histopathology of adenomas. No huge difference was observed between the treated and control group of immense intestinal adenomas. In addition, there was a measurable difference between the control and treatment study of the digestive tract, furthermore, resulting in the treatment of gastrointestinal intraepithelial neoplasias. This study on mice results in effective colon growth with the help of microencapsulated probiotic microorganisms.

Microencapsulated Probiotics for Use in Cardiovascular

Microencapsulated probiotic microorganism lowers the cholesterol level in humans. Previous research has shown that specific species of *Lactobacilli* have a bile salt hydrolase (BSH) chemical which can add and results in cholesterol level down impact in vivo in cardiovascular infections **(Anderson and Gilliland, 1999)**. This chemical adds to the deconjugation of bile salts in the digestive tract. The oral conveyance of *Lactobacillus* has, in this way, rose as a potential component for actuating cholesterol bringing down. **Martoni** and **Prakash, 2008** showed that microencapsulated BSH-dynamic microscopic organisms can make due in a reenacted human gastrointestinal demonstrate while keeping up cell practicality and catalyst action, which would not be conceivable with the immediate conveyance of non-microencapsulated bacterial cells. Another microencapsulated probiotic *Lactobacillus* containing feruloyl esterase protein helps in bringing down the hypercholesterolemic activities (**Bathena *et al.*, 2009).** Hypercholesterolemic mice were injected with *Lactobacillus fermentum*, twice every day by oral dose, for a time of 18 weeks and further histological investigations were additionally performed which showed that the microencapsulated probiotic reduces the progress of atherosclerotic injuries in the mice and was subsequently appeared to be viable in controlling the serum cholesterol and triglyceride level. The study evaluated that the microencapsulation of probiotics is very helpful for the improvement of cardiovascular diseases. Microencapsulation can possibly be valuable in other applications. It has been demonstrated that *Lactobacillus acidophilus* affected colon tumorigenesis colonize the probiotic microorganism in the gastrointestinal tract which is helpful in solving the problem of tumorigenesis. Therefore, the viability is vital to the activity of the probiotic organism ingested and survived by 1%in the gastric environment, regulating the impact of orally conveyed bacterial microorganisms. Microencapsulation shows the beneficial increase in viability with protected probiotic microorganisms **(Pool, 1996)**.

CONCLUSION

Encapsulation is one of the most emerging technologies and has the ability to enhance the shelf life of food products further, providing consumers with convenient and healthier foods. Although, on a laboratory scale various technologies exists for encapsulation are efficient but on a large scale, it is very difficult to produce microencapsulated microorganisms of food grade. In the present article, the important strategies utilized in the epitome of probiotic cells are discussed. The survival of cells can be enhanced by the microencapsulation of probiotic microorganisms in calcium alginate-gelatinized starch with the covering of chitosan after re-sanctioned in gastrointestinal condition when appeared differently in relation to free cells. Further, microencapsulation can be used for increasing the survivability of probiotic bacteria in the food matrix. Biomaterials utilized in the encapsulation techniques such as calcium alginate, gellan gum; xanthan and starch provide a smooth surface to the final functional food. Along these lines, the connected methodology in this review may demonstrate value for the conveyance of probiotic microbes to the reproduced individual gastrointestinal tract.

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