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MICROENCAPSULATION OF PROBIOTIC: TECHNIQUES & BIOMATERIALS

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ABSTRACT

The oral administration of most of the probiotics results in the lack of ability to survive in a high proportion of the harsh conditions of acidity and bile concentration commonly encountered in the gastrointestinal tract of humans. Providing Probiotic living cells with a physical barrier against adverse environmental conditions is therefore an approach currently receiving considerable interest. Probiotic encapsulation technology has the potential to protect microorganisms and to deliver them into the gut. This review focuses mainly on the methodological approach of Probiotic encapsulation including biomaterials selection.

KEY WORDS: Probiotics, Biomaterials, Microencapsulation

INTRODUCTION

In recent years, increasing evidence indicating numerous health benefits associated with the intake of probiotic bacteria has created a big market of probiotic foods worldwide. The biggest challenge in the development of probiotic products is to maintain the adequate number of viable cells during the shelf life of the product as well as during the gastrointestinal (GI)-tract transit after consumption, so that the claimed health benefits can be delivered to the consumer.[1,2,3,4] Consequently, there has been a growing interest in developing techniques to enhance the survival of probiotic bacteria particularly during the GI-tract transit of the cells.[5,6,7,8]

Probiotic encapsulation technology is an exciting field of biopharmacy that has emerged and developed rapidly in the past decade. Based on this technology, a wide range of microorganisms have been immobilized within semi permeable and biocompatible materials that modulate the delivery of cells. The terms immobilization, entrapment and encapsulation have been used interchangeably in most reported literature.[9]

To make a health claim, the therapeutic minimum level should be at least 10^7cfu/g or ml of the product[10], which can be achieved by using microencapsulation technology. Simultaneously, low level or poor survival of free probiotic bacteria was demonstrated by many studies.[11]

Probiotics

Probiotics are defined as the living microorganisms administered in a sufficient number to survive in the intestinal ecosystem. They must have a positive effect on the host [12]. The term 'probiotic' was first described by Lilly and Stillwell in 1965 as: “substances secreted by one microorganism that stimulate the growth of another”. In 1974 Parker [13] proposed that probiotics are “organisms and substances which contribute to intestinal microbial balance” .In more modern definitions, the concept of an action on the gut microflora, and even that of live microorganisms disappeared. Salminen et al.[14] defined probiotics as the 'food which contains live bacteria beneficial to health whereas Marteau et al.[15] defined them as 'microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being'. Some modern definitions include more precisely a preventive or therapeutic action of probiotics. Charteris et al. for example, defined probiotics as microorganisms which, when ingested, may have a positive effect in the prevention and treatment of a specific pathologic condition'. Finally, since probiotics have been found to be effective in the treatment of some gastrointestinal diseases[15], they can be considered to be therapeutic agents. It is clear that a number of definitions of the term 'probiotic' have been used over the years but the one derived by the Food and Agriculture Organization of the United Nations/World Health Organization and endorsed by the International Scientific Association for Probiotics and Prebiotics[16] best exemplifies the breadth and scope of probiotics as they are known today: 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host'

Table 1: Probiotics and their therapeutic applications

S.No	Probiotics strains	Therapeutic application
1	L. rhamnosus GG [17], S. boulardii[18]	Antibiotic-Associated Diarrhea
3	Bifidobacterium infantis[19] L. rhamnosus GG [20] , B. longum[21,22]	Inflammatory Bowel Syndrome19
4	L. rhamnosus GG [23]	Atopic Dermatitis
5	L. rhamnosus GR-1 and L. fermentum RC-14[24,25]	Genitourinary Disorders in Women
6	Lactobacillus rhamnosus GG[26]	Nonsteroidal anti-inflammatory Drug
7	Lactobacillus fermentum RC-14[27]	Urinary tract infection
8	Lactobacillus plantarum 299v,[28]	Giardia infection
9	B.coagulans[29]	Rheumatoid arthritis
10	Lactobacillus rhamnosus GG, Saccharomyces cerevisiae[30,31]	Crohn's disease
11	Lactobacillus rhamnosus GG, Lactobacillus acidophilus, Lactobacillus paracasei,Lactobacillus acidophilus, and Lactobacillus delbrueckii ssp. bulgaricus strain LB-51[32,33,34,35,36]	Prevention of colon cancer

Microencapsulation

Microencapsulation is described as a process of enclosing micron-sized particles of solids or droplets of liquids or gasses in an inert shell, which in turn isolates and protects them from the external environment. Microencapsulation has been developed approximately 40 years ago and widely used in the food industry due to its capabilities to provide good protection from moisture, heat or other extreme conditions which are undesirable factors for maintaining stability and viability of core materials.[37]

Probiotics (usually 1-4 μm) are too big for nano-technology[38] , so microencapsulation is a useful tool for improving the delivery of the active probiotics.

The best application of probiotic encapsulation technology is the controlled and continuous delivery of cells in the gut. The potential benefit of this therapeutic strategy is to maintain greater cell viability despite the acidity into the stomach. In their viable state, probiotics may exert a health benefice on the host.[17, 39]

Physicochemical properties of coating material affect the viability of encapsulated probiotic cells. Type and concentration of coating material, particle size, initial cell number, and bacterial strains are important during formulation.

Methods for Probiotic Microencapsulation

(a) Extrusion

Extrusion is the oldest & most common technique for converting hydrocolloids into microcapsules.The property of certain biopolymers such as alginate,carrageenan & pectin to form gel in presence of mineral such as calcium and potassium has been successfully applied to entrap probiotics using extrusion method.Bonding of multiple free carboxlic radicals by gelling ions leads to the formation of gels.[38] (Champagne & Fustier 2007).

Extrusion involves projecting an emulsion core and coating material through a nozzle at high pressure.On a laboratory scale, this can be done by a simple devices such as syringes are applied. If the droplet formation occurs in a controlled manner the technique is known as prilling.[40] This is preferably done by pulsation of the jet or vibration of the nozzle.

In extrusion method Hydrocolloid solution was prepared & probiotics are added into the prepared solution to form cell suspension. These cells suspension is passed through the syringe needle to form droplets which are directly dripped into the hardening solution containing cations like calcium. When the droplets come in contact with hardening solution, alginate polymers surround the core to form a three dimensional lattice structure by cross-linking calcium ions.[41]

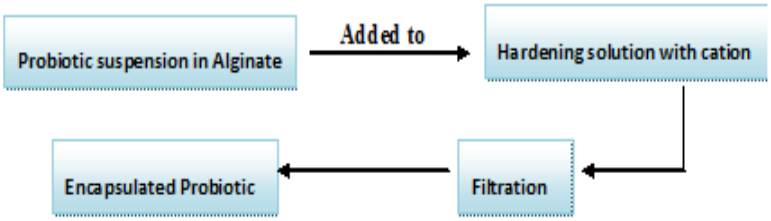

Fig 1: Microencapsulation of Probiotics by Extrusion

(b) Emulsification

A small volume of the cell polymer suspension (i.e., the discontinuous phase) is added to a large volume of vegetable oil (i.e., the continuous phase). The mixture is then homogenized to form a water-in-oil emulsion. Once the water-in-oil emulsion is formed, the water-soluble polymer must be insolubilizing to form tiny gel particles within the oil phase. Microbeads produced by emulsion method are usually recovered by the membrane filtration technique. In contrary with the extrusion technique, it can be easily scaled up and the diameter of produced beads is considerably smaller (25 μm-2 mm). However, this method requires more cost for

performance compared with the extrusion method due to need of using vegetable oil for emulsion formation.[41]

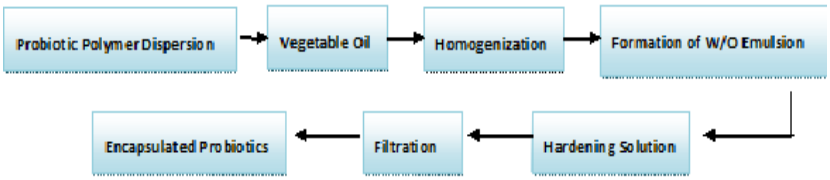

Fig 2: Microencapsulation of Probiotics by Emulsification

(c) Spray Drying

The 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.[42] This leads to evaporation of the solvent (water) and hence the formation of matrix type micro capsules.

The advantage of the process is that it can be operated on a continuous basis. The disadvantage is that the high temperature used in the process may not be suitable for encapsulating probiotic bacterial cultures

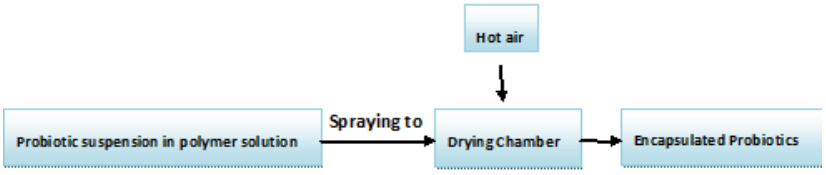

Fig 3: Microencapsulation of Probiotics by Spray drying

(d) Freeze Drying

In this technique, the solvent is frozen and removed via sublimation.[43] The techniques of spray-drying, freeze-drying or fluidized bed drying have shown their limitations because the cells encapsulated by these techniques are completely released into the product. Thereby, the cells are not protected towards the food matrix environment and in the presence of gastric fluid or bile.[44] *Lactobacillus* F19 and *Bifidobacterium Bb12* cells were first encapsulated into enzymatically gelled sodium caseinate, and gel particles were freeze-dried to study the storage stability.[45]

The process is performed by freezing probiotics in the presence of carrier material at low temperatures, followed by sublimation of the water under vacuum. lyophilisation or freeze-drying is a very expensive technology, significantly more than spray-drying.[46] However, most of freeze-drying process only provide stability upon storage and not or limited during consumption. Because of that, this technique is used as a second step of encapsulation process. The freeze-drying is useful to dry probiotics previously encapsulated by other different techniques, as emulsion [45] or entrapment in gel microspheres. In this way it is possible to improve the stability in the gastrointestinal tract and optimize the beneficial effect of probiotic consumption.

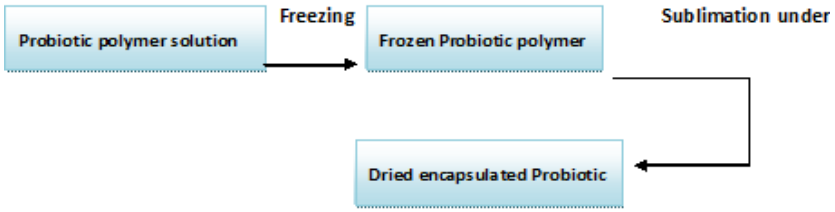

Fig 4: Microencapsulation of Probiotics by Freeze drying

Selection of Biomaterials for Microencapsulation

(a) Pectin

Pectins are non-starch, linear polysaccharides extracted from the plant cell walls.[47] It is major cell wall component in plants, playing a role in the control of cell growth & the defence against the invasion of microorganism. Pectin are composed of α-D-galacturonic acid, which are interrupted by single α –L – rahamnose residue.A difference between pectin is their content in methyl ester.High methoxy pectin forms gels due to hydrophobic interaction and hydrogen bonding between pectin molecules.Low methoxy pectin forms gel in the presence of di and polyvalent cations, which cross link and neutralize the negative charge of pectin molecule.[48]

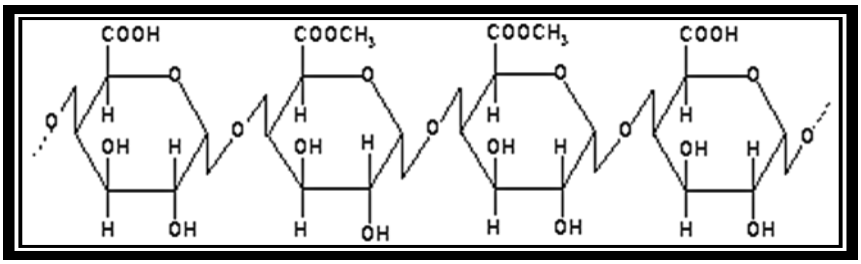

Fig 5: Chemical structure of Pectin

(b) Alginate

Alginate, is an anionic polysaccharide distributed widely in the cell walls of brown algae, where through binding with water it forms a viscous gum. In extracted form it absorbs water quickly; it is capable of absorbing 200-300 times its own weight in water.[49] Chemically it is a linear polymer of heterogeneous structure composed of two monosaccharide units: acid α-L-guluronic (G) and acid β-D-mannuronic (M) linked by β (1–4) glycosidic bonds.[50,51]

It is the most widely used hydrocolloid for microencapsulation of probiotcs due to its gentle environment, low cost, simplicity, biocompatibility with the probiotics and properly resolve in the intestine to release encapsulated cells.[52, 53,54]

Calcium alginate has been widely used for the encapsulation of lactic acid- and probiotic bacteria, mainly in the concentration range of 0.5-4% .[55,56,57,58]

Alginate gels are insoluble in acidic media[1, 59] usually alginate is used in concentration range of 0.5–4%.[56]

Advantages of alginates are

- Easily form gel matrices around bacterial cells.
- They are not poisonous to the body (is safe or biocompatible).
- Alginates are cheap, mild process conditions (such as temperature) are needed for their performance.
- They can be easily prepared and performed (simplicity and ease of handling) and properly resolve in the intestine and release entrapped cells.
- Alginate gel matrix appropriately surrounds the bacterial cells with a diameter of 1-3 µm.

Dis advantages

- Susceptible to acidic environments, crackling and loss of mechanical stability in lactic acid.[60]
- Fast diffusion of moisture and other fluid through the capsule which reduces their barrier properties against unfavorable environmental factor.[54]
- Due to their high expenses, and a weak ability of scaling up as well as the formation of crackled and porous bead surfaces, which leads to fast diffusion of moisture and fluids through capsules which reduces their barrier properties against unfavorable environment factor, it is difficult in industrial scale applications.[54]

These can be overcome by blending of alginate with another polymer like starch, coating other compound on its capsules.[61, 62, 63]

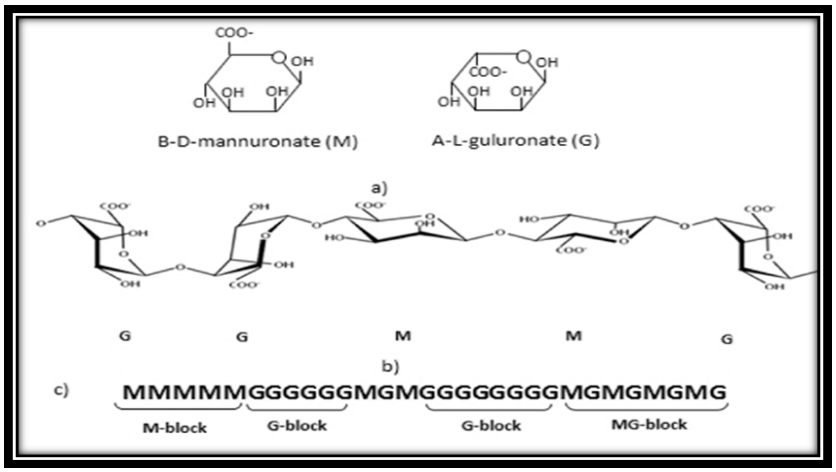

Fig 6: Chemical Structure of Alginate monomer (a) and chain (b) conformations and a schematic alginate chain sequence (c).

(c) Chitosan

Chitosan is a linear polysaccharide with negative charge arising from its amine groups obtained by deacetylation of chitin. Chitosan can be isolated from crustacean shells, insect cuticles and the membranes of fungi. The properties of chitosan vary with its source. In order to achieve sufficient stability, chitosan gel beads and microspheres can be ionically cross-linked with polyphosphates and sodium alginate.[64]

Advantages

- Show excellent biological properties such as biocompatibility, biodegradability, lack of toxicity, and adsorption, as well as relatively high percentage of nitrogen.[65, 66]

Disadvantage

- It is water insoluble at a pH higher than 5.4. This insolubility presents the drawback of preventing the complete release of this biomaterial into the gut which pH is greater than 5.4.[67]
- Antibacterial property of chitosan limits its use as coating material in encapsulation.[68]

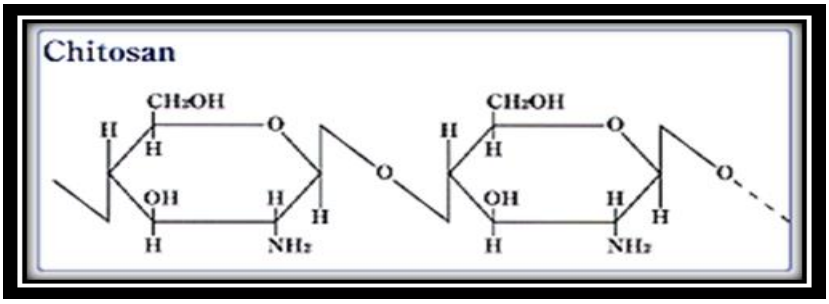

Fig 7: Chemical structure of Chitosan

(d) κ Carageenan

Carageenans are gel-forming and viscosifying polysaccharides, which are obtained by extraction from certain species of red seaweeds. All carrageenans are high-molecular-weight polysaccharides made up of repeating galactose units and 3,6 anhydrogalactose (3,6-AG), both sulfated and nonsulfated. The units are joined by alternating α-1, 3 and β-1,4 glycosidic linkages. Ҡ-carrageen an requires high temperatures (60-90 ºC) for dissolution, especially when applied at high concentrations such as 2-5%. However, this material used for encapsulating probiotics requires a temperature comprised between 40 and 50 ºC at which the cells are added to the polymer solution. Beads by this polymer can be prepared by extrusion as well as emulsion technique.[69, 70] Gelation of C-carrageenan can also be performed using chemical method by reacting with monovalent ions such as potassium (KCl), providing brittle gel, with low ability to withstand stresses. Addition of KCl inhibits the growth of some lactic acid bacteria such as Streptococcus thermophilus and L. delbrueckii ssp. Bulgaricus[71], which can be eliminated by the combination with locust bean gum with the ratio of)-carrageenan to locust bean gum as 1:2.[72]

Advantages: The encapsulation of probiotic cells in j-carrageenan beads keeps the bacteria in a viable state.[73]

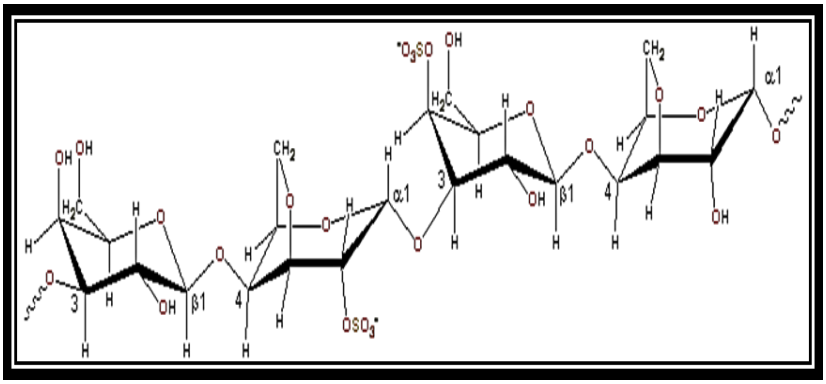

Fig 8: Chemical structure of κ Carageenan

Dis Advantages

- Scale up difficulty
- Susceptibility to environment factors
- Lack of mechanical strength

(e) Xanthan gum

Xanthan gum is a microbial exopolysaccharide consisting of a cellulosic backbone with side chains of two mannose and one glucuronic acid on every second glucose residue.[74,75] Xanthan gum is stable over a wide range of temperatures and pH, which finds many applications in food, pharmaceutical, cosmetic, and oil-drilling industries.[76] Side chains of xanthan gum represent a very high proportion of the molecule (60%). Due to the side chains, the polymer completely hydrates in water.

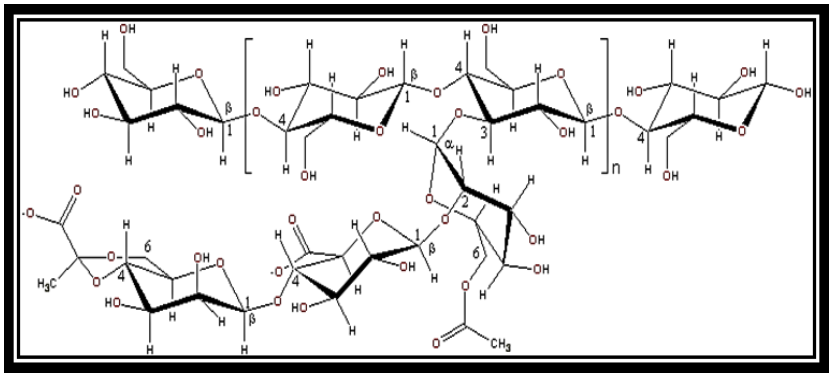

Fig 9: Chemical structure of Xanthum gum

(f) Cellulose acetate phthalate

Cellulose acetate phthalate is most widely used encapsulating material due to its acid resistance. Its structure composes of cellulose polymer that 50% of hydroxyl groups esterified with acetyls and 25% is esterified with one or two carboxyls of a phthalic acid. It is soluble at pH ≥ 6, but insoluble at pH ≤ 5.[77] Because of having a safe nature for purpose human ingestions, it is being widely used for drug capsulation in pharmacy.[78, 41]

There are several studies which shows successful microencapsulation of probiotics using CAP can be achieved by both chemical and physical methods. For example, Rao et al. reported high survivability as high as 109 cfu mL-1 after incubation in simulated gastric juice of B. pseudolongum encapsulated in CAP using emulsion technique. The similar result was obtained from the studies of Favaro-Trindale and Grosso when B. lactis (Bb-12) and L. acidophilus (La-05) were encapsulated in CAP using spray drying method.

Advantage

- Insoluble at pH below 5 & soluble at pH above 6, thus suitable for microencapsulation of probiotic.[79]

Disadvantage

- The disadvantage of CAP is that it cannot form gel beads by ionotropic gelation; only capsules have been developed by emulsification using this biomaterial.

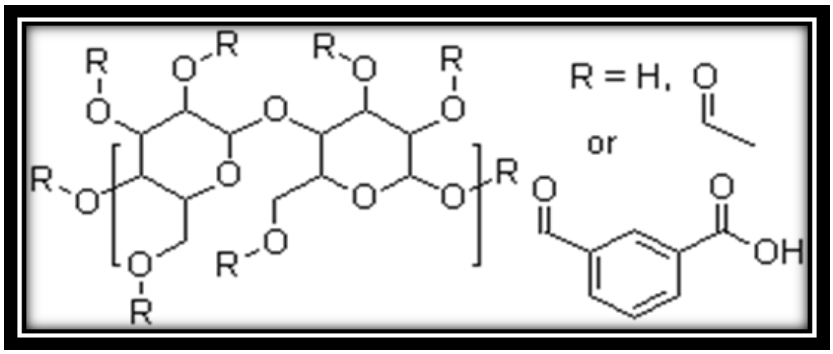

Fig 10: Chemical structure of Cellulose acetate phthalate

(g) Gelatin

Gelatin is a mixture of peptides and proteins produced by partial hydrolysis of collagen extracted from the skin, bones, and connective tissues of animals such as domesticated cattle, chicken, pigs, and fish. Its chemical composition is, in many respects, closely similar to that of its parent collagen.Commercial gelatins can be divided into two groups depending on the treatment to obtain as type A (acid pre-treatment) and type B (basic pre-treatment).[80] Gelatin is water-soluble, providing high viscosity. Gelation of gelatin is caused by conversion of random coli-helix when it is cooled.

Gelatin gum has been used for the microencapsulation of probiotics, alone or in mixture with other gums.[81]

Advantages

- Gelatin has a very special structure and versatile functional properties, and orms a solution of high viscosity in water, which sets to a gel during cooling.[82]
- Its amphoteric nature gives the ability of having synergistic effects with anionic polysaccharides such as gelan gum.

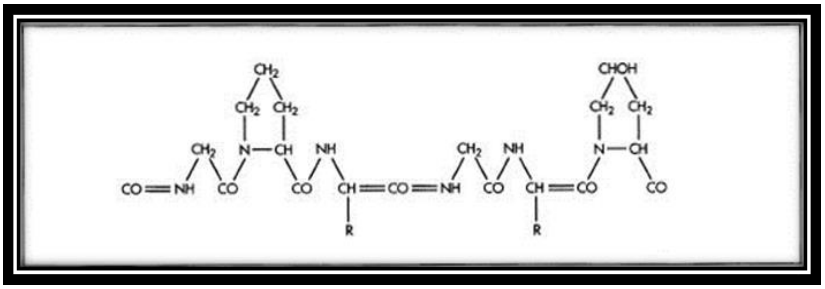

Fig 11: Chemical structure of Gelatin

(h) Whey protein

Whey protein is the collection of globular proteins isolated from whey, a by-product of cheese manufactured from cow's milk. There are four main classes of proteins in whey: β-lactoglobulin, α-lactalbumin, serum albumin and several immunoglubins. Most of these proteins have a globular conformation and are susceptible to denaturation and aggregation induced by heat and high pressure.[83] Whey protein are obtain by ultrafiltration ,during which the low molecular weight compounds such as lactose, minerals, vitamins & non protein nitrogen are removed in the permeate while the proteins become concentrated in the retentate.Whey protein is very popular for film forming characteristics & is used as a protective material in spray drying, resulting in water soluble microcapsule system.[84] They can be easily mixed with negatively charged polysaccharides such as alginate, carrageenan or pectin.[85] Whey protein appears as a potential candidate as coating agent as it is entirely biodegradable and frequently used in many types of food products.

(i) Starch

Starch consists of D-glucose unit joint together with glycosidic bonds. It has been used as a material for coating of alginate capsules. High-amylose corn starch (HACS) can be applied for enhancing functions of capsule or shell/coat formation.[86] Blending alginate with starch is a common practice and it has been shown that encapsulation effectiveness of different bacterial cells especially lactic acid bacteria were improved by applying this method.[87] Lyophilized corn starch (LCS) has been reported to be used as capsule-forming material; however, it decomposes by pancreatic enzymes.[88] Resistant starch (RS) is not degraded by the pancreatic amylase enters the intestine in the indigestible form. This specification apart from giving the microbeads good enteric delivery characteristic (good release of bacterial cells in the large intestine), also gives them prebiotic functionality as they can be used by the probiotic bacteria in the intestine.[89] Consumption of resistant starch reduces the risk of intestinal cancer because of having dietary fiber functionality.[90]

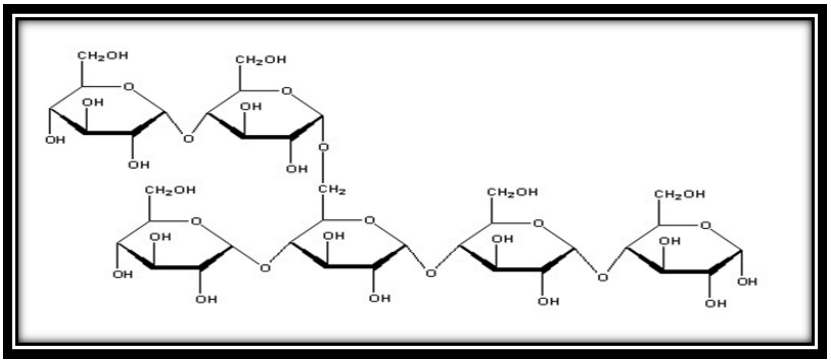

Fig 12: Chemical structure of Starch

Table 2: Different probiotic strain, biomaterial, and microencapsulation techniques

S.No	Probiotic	Encapsulation method	Encapsulating Material	Reference
1	*Lactobacillus acidophilus*	Spray drying	Chitosan	[91]
2	*Lactobacillus Plantrum*	Spray freeze drying	Whey Protein	[92]
3	*Lactobacillus casei*	Spray drying	Chitosan Calcium alginate	[93]
4	*L. rhamnosus*	Gel beads	Alginate	[61]
5	*L. casei*	Emulsification	Carrageenan/locust bean gum	[94]
6	Saccharomycese Boulardii	Emulsification	Alginate was blended with mucilage and inulin	[95]
7	*Bifidobacterium*	Gel beads	Alginate/chitosan	[64]
8	*Bifidobacterium*	Gel beads	Carrageenan/locust bean gum	[85,96
9	*Bifidobacterium longum*	Extrusion	Alginate	[59]
10	*L. acidophilus* (La-05)	Gel beads	Cellulose acetate phthalate	[97]

CONCLUSION

Probiotics, live cells with different beneficiary characteristics, have been extensively studied and explored commercially in many different products in the world. Their benefits to human and animal health have been proven in hundreds of scientific research. Viability of probiotic bacteria in a product at the point of consumption is an important consideration for their efficacy, as they have to survive during the processing and Shelf life of food and supplements, transit through high acidic conditions of the stomach and enzymes and bile salts in the small intestine. Microencapsulation is most widely used technology to retain the potency of probiotic to be delivered orally into the GI system to maintain their potency. The viability of probiotics is a key parameter for developing probiotic food products. New technologies have been developed to enable high cell yield at large scale and ensure probiotic stability for a long period in food.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.

Future research could be concentrated on the aspects such as applying more efficient encapsulation materials or improving the common used ones, The technology of micro-encapsulation, needs to be developed with more precise machinery, capsule and better delivery systems, *in vitro* and *in vivo* studies using human being should be carried out to confirm the efficacy of microencapsulated probiotics, We should also look forward to curtail cost and provide more cost effective techniques for Probiotic encapsulation .

Emerging and promising research work serving mankind for a better health is visualized for the microencapsulation of probiotics in the future.

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