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Significance of probiotic encapsulation and deficiencies within

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Abstract
Health stimulating claims attributed to probiotics are dependent on their viability and numbers in the intestinal tract. Probiotics must survive to reach the small intestine and colonize there for appropriate hindrance and control of several gastrointestinal diseases. Microencapsulation is considered to be a promising approach to improve the survival rates of probiotic microorganisms by providing a physical barrier against harsh conditions mainly against acidity, drying during processing, oxygen toxicity and temperature to protect microorganisms and to deliver them into the gut. Encapsulated probiotics also have been found to augment the sensory properties of probiotics containing products. Specific use of the proper encapsulating material for particular probiotic cells determines the efficacy of the process. Development of carbohydrates or protein based protective matrix compatible to probiotics added more robustness in process. Although, none of the methods used for encapsulation fulfills all the criteria for ideal encapsulated technique. Commonly used encapsulation process are liable to structural defects, high performance cost and not scaled up easily in limited duration. In future, development of more genetically robust strains, recognition of potent applications with minimized large scale microencapsulation techniques can up thrust commercialization.

Keywords: Microencapsulation, Probiotics, Viability, Processing

Introduction
Protection from deterioration is one of the foremost aims while designing for any delivery systems. Microencapsulation is one of such delivery technique which is used for different drug delivery system since a long time and now a days the progression in this field have been significant with nutraceutical and functional food ingredients specially probiotics. Notable magnetism of peoples towards green consumerism and health beneficial foods has swiftly expanded global probiotic market in recent years, which may grow further from 741 million in 2016 to 948 million by 2025 (GPMF, 2017) [1]. Many studies concluded that the oral administration of probiotics lacks the ability to survive harsh gastrointestinal tract (GIT) conditions (Cook *et al*., 2012; Shori, 2017) [2, 3]. Moreover, during processing and storage of probiotic products, a significant count of added probiotic cells was found affected (Anal and Singh, 2007) [4].

In compliance to probiotic definition, there is essential requirement for probiotic product of having specific viable count per gram of the product at the time of consumption to exert health beneficial effect. Microencapsulation approach provides a physical barrier against harsh conditions to protect microorganisms and to deliver them into the gut hence receiving considerable interest for probiotics. Microencapsulation provides segregated structure and innovative system to the core material for probiotics. Different factors where microencapsulation impulse protection and survivability to probiotics are discussed below.

For survival of probiotic during processing and storage
Several detrimental factors such as high temperature (Triphati and Giri, 2014) [5], low pH, heat shocks and cold shocks during spray drying and freezing respectively (Shah and Ravula, 2000) [6], and presence of molecular oxygen (Sunohara *et al*., 1995) [7] were found to influence the survivability of probiotic microorganisms in food products during production, processing and storage. Within this context, microencapsulation technique was observed to improve the stability of probiotic organisms in functional food products (Semyonov *et al*., 2010) [8]. Thermal and osmotic resistance of lactic acid bacteria are species dependent characteristics (Lian *et al*., 2002; Favaro-Trindade and Grosso, 2002; Capela *et al*., 2006) [9, 10, 11].

The type of coating material and its amount directly effects the viability of encapsulated bacteria (Chen and Chen, 2007) [12]. In an encapsulation study by Mosilhey (2003) [13] with different combinations of materials (like acacia gum, gelation and whey protein), non-microencapsulated *Lactobacillus acidophilus* cells showed severe 8 log decrease in viability between 45 and 65°C for 30 min whereas, coated cells was found to increase its resistance to temperature resulting in increase of viability of 10^3 to 10^4 CFU/g at similar condition. Similarly, Altamirano-Fortoul *et al*. (2012) [14] assessed the viability at 65°C, 2 bar of pressure, for 135 min of microencapsulated *L. acidophilus* in a complex matrix of whey protein, cellulose methyl carboxylate, pectin, inulin, and fresh agave syrup via spray-drying for preparation of functional bread baked for 16 min at 180°C. After baking and storage time, microencapsulated *L. acidophilus* cells was reduced by only 1 and 2 log CFU/g respectively.

Bacterial membranes are the main site of damage during spray drying (Ananta *et al*., 2005) [15]. Removal of water may harm cell membranes and linked proteins, as it stabilizes biological molecules. Sugars and certain oligosaccharides are known as good water substitutes to protect dehydrated biomaterials (Leslie *et al*., 1995) [16]. Studies also show that incorporation of gum acacia (gum arabic) in a milk-based medium during storage before spray drying increases viability of *Lactobacillus paracasei* cells (Desmond *et al*., 2002) [17]. Also, work conducted to evaluate the viability of Bifidobacterium BB-12 microencapsulated by spray drying through blending of oligofructose enriched inulin with reconstituted skim milk resulted in better protection of bifidobacteria (Paez *et al*., 2012; Avila-Reyesa *et al*., 2014) [18, 19].

Presence of oxygen and redox potential are another principal factors affecting the viability of probiotics particularly during the storage (Lee and Salminen, 2009) [20]. However, oxygen sensitivity varies among different species of probiotics (Kawasaki *et al*., 2006) [21]. Molecular oxygen affects probiotics by acting self-toxic element, forming peroxides and free radicals production (Korbekandi *et al*., 2011) [22]. Talwalkar and Kailaspathy (2003) [23] estimated the protective role of microencapsulation against oxygen toxicity in *L. acidophilus* and *Bifidobacterium lactis* in both broth medium as well as in prepared yoghurt. Both strains were encapsulated in calcium alginate and grown in oxygen presence. Counts of encapsulated cells in strains were found one log higher than corresponding free cell counts. Presence of oxygen in packed products also effect survival of probiotics (Da Cruz *et al*., 2007) [24]. Most of the dairy probiotic products are stored and sold in the market in plastic packages having high oxygen permeability. The level of dissolved oxygen was found to increase during storage conditions (Dave and Shah, 1997; Jayamanne and Adams, 2004) [25, 26]. Hsiao *et al*. (2004) [27] studied the effect of packaging material and oxygen absorbent at different storage temperature on the viability of microencapsulated Bifidobacteria species. The viability of cells was found improved with the incorporation of deoxidant and desiccant.

Freeze drying is commonly used process to dehydrate probiotics within coating materials or in dairy products (Meng *et al*., 2008) [28]. The amount of water remaining after drying is major factor involved in loss of viability during subsequent storage (Ying *et al*., 2010) [29]. In freeze drying, the drying media have greater protective effect on stability of probiotics than microencapsulation (Ried *et al.,* 2007) [30]. Weinbreck *et al*. (2010) [31] reported that higher water activity of 0.7 after encapsulation of *L. rhamnous* GG with whey protein and palm oil, resulted in more than ten log reduction in viable counts within 2 weeks of storage. Cryoprotectants and prebiotics can be used to protect the viable cells (Sultana *et al*., 2000) [32]. However, in long-term storage, the addition of both has not been found to enhance the viability of microencapsulated cells. Wheat dextrin and polydextrose as carriers are found to protect *Lactobacillus rhamnosus* during freeze drying (Saarela *et al*., 2006) [33]. Microencapsulation in casein-based microcapsules produced by enzymatic gelation with transglutaminase improved the survival of Bifidobacterium Bb12 during storage for up to 90 days at lower temperature while co-encapsulation of resistant starch corns as prebiotic negatively influenced the physical barrier of the protein matrix (Heidebach *et al*., 2010) [34].

For survival of probiotic during gastric transit

After ingestion, through oesophagus probiotic pass quickly to stomach, small intestine and further large intestine. During this journey cells have to transit through harsh acidic conditions of gastric environment followed by bile and various enzymatic actions to enable colonization and proliferation. The journey under these physiological conditions leads to greatest viability loss of bacteria. Microencapsulation offers good protection in the non-cytotoxic, non-antimicrobial and covalently or ionically cross linked polymer networks based matrices (Rokka and Rantamäki, 2010) [35].

Till date studies signifies successful use of alginate, milk proteins, chitosan and plant material based encapsulation under GIT conditions (Nag *et al*., 2011; Burgain *et al*., 2013; Cai *et al*., 2014) [36, 37, 38]. The possible reason behind their effectiveness is the strong level of co-ordination between the compounds extending strong cross linkage which can withstand with harsh environments. Recently, layer-by-layer technique using oppositely charged polyelectrolytes like chitosan and dextran sulfate was used to encapsulate *S. boulardii* yeast cells to protect it against the acidic environment. The cell counts of encapsulated cells were reduced by only 0.5 log cfu/100 mg to whereas control un-encapsulated cells showed about 1.3 log cfu/ 100 mg reductions after 2 hours in simulated gastric juice of pH 2. It was elucidated that the strong electrostatic interaction between the chitosan and dextran sulfate polymer layers led to dense structure for protecting the yeast cells (Thomas *et al*., 2014) [39].

Similar to resistance against processing situations, type of coating and mixtures of suitable biopolymers plays an important role in protection from GIT conditions. Ding and Shah (2009) [40], evaluated the effect of simulated GIT conditions. They tested eight strains of microencapsulated probiotic bacteria in guar and xanthan gums, carob, alginate and carrageenan matrix for their resistance against GIT conditions. The method resulted in better survival of encapsulated cells as compared to control free cells in hydrochloric acid containing MRS. Similarly, for encapsulated cells when exposed to oxgall bile, viability was reduced by 3.36 log CFU ml^{-1} half of the cell concentration lost by free cells. An optimal capsule combination of 3% sodium alginate, 1% pancreatic digested casein and 3% fructooligosaccharides have been reported for probiotic survival in gastric conditions (Chen *et al*., 2006; Ross *et al*., 2008) [41, 42]. Caseinate and fructooligosaccharides along with

dried glucose syrup or resistant starch are also found to provide protection (Crittenden *et al*., 2006) [43]. Microcapsules coated with chitosan and alginate has the ability to bind with bile salts (Murata *et al*., 1999) [44]. An insoluble complex is formed between chitosan and bile salts on the surface leading to restricted diffusion of bile salts into the matrix core thereby protecting the probiotic bacteria (Koo *et al*., 2001) [45]. In an *in vivo* study, survival of *Saccharomyces boulardii* probiotic yeast in alginate microspheres with and without chitosan coating was studied (free cells was fed as control). Here, 13.3% of the uncoated and 9% of coated ingested yeast cells were found viable in rat faeces, whereas only 2% of free cells survived (Graff *et al*., 2008) [46]. The viability of *L. casei* (NCDC-298) was improved with increase in alginate concentration at pH 1.5 for 3 h (Mandal *et al*., 2006) [47]. Similarly, number of alginate layers was found to influence survival of *L. acidophilus* (PTCC1643) and *L. rhamnosus* (PTCC1637) in simulated GI conditions. In this study, encapsulation with a double layer of alginate was found to provide the maximum protection against both in gastric of pH 1.5 for 2 h and intestinal of pH 7.25 for 2 h. Encapsulation of two probiotic isolates (*L. lactis*) was found to increase their survival in simulated gastric/intestinal fluid when compared to free cells. This study used two methods of encapsulation and the formulation was found to support comparable folate production when used for producing folate fortified functional food products (Divya and Nampoothiri, 2015) [48].

For improvement of sensory characters

As explained in previous sections, microencapsulation of probiotics is aimed to increase its shelf life and resistance to harsh gut conditions upon administration. Due to the large size of probiotics, different microencapsulation strategies have been used and have been found successful to varying degrees. A major concern associated with encapsulating any probiotic is whether this modification adversely modulates the functionality of the probiotic and sensory characteristics of the final product. Almost all of the studies involving microencapsulation of probiotics are followed by reassessment of changes in sensory characteristics of the product. The benefits and sensory effects of microencapsulation also depends on the specific type of probiotic strain used (Saxelin *et al*., 2010; Huang *et al*., 2017) [49, 50].

A probiotic fermented product is assessed on various sensory parameters such as taste, texture, smell, appearance and composition. Encapsulated probiotics have been mostly found to augment the sensory properties of probiotics containing products. A study on calcium alginate encapsulated *L. casei* and *B. lactis* showed a 30% increase in probiotic survival with no associated effect on sensory characteristics (Homayouni *et al*., 2008) [51]. Chickpea protein encapsulated *B. adolescentis* microspheres (size <100µm) made by emulsion technology was found to survive better in synthetic gastric juice as compared to free cells and did not produce any perceivable deterioration in sensory score (Wang *et al*., 2014) [52]. Another study on sausage made with alginate encapsulated *P. pentosaceus* and *S. carnosus* was found to have lower probiotic death and no effect on desirable pH, water activity and relative humidity (Muthukumarasamy *et al*., 2006) [53]. *L. acidophilus* cultures encapsulated in alginate-chloride polymer when used in tomato juice were found to have higher cell viability and more pleasant taste (King *et al*., 2007; Tsen *et al*., 2008) [54, 55]. One study has shown that the encapsulation had no detrimental effect on appearance, flavor, taste and acidity of the yogurts over a 7 week storage period. But it also enhanced the survival of the probiotic and improved the texture of the yogurt (Kailasapathy *et al*., 2006) [56].

However, there are some considerations involved with formulation of encapsulated probiotics. Sometimes, the encapsulation polymer may interfere with multiplication of probiotics in fermented product and thus result in different sensory characteristics than the original product. A study reported pH decrease by free probiotics in orange juice got modulated by encapsulation which also led to change in taste of the product (Sohail *et al*., 2012) [57]. Also, the encapsulated probiotic microspheres have size limitations too due to large size of the bacteria. The microspheres of size 1-5µm are preferred which provide protection to the encapsulated probiotic and less interference in sensory perceptions. The larger (0.2-3mm) microspheres are undesirable since they start giving rough and grainy texture to the product leading to poor sensory scores (Engelen *et al*., 2005) [58]. It has been seen that encapsulation with natural components such as gelatin, whey protein, milk and proteins have been found to improve encapsulation and corresponding viability and function of probiotics in the fermented product. Thus, a careful mix of encapsulation material, size of microspheres/beads and the probiotic strain must be made to achieve high probiotic survival and no perceivable change in sensory characteristics (Nazzaro *et al*., 2012) [59].

Limitations

The technology of probiotic encapsulation has the prospective to shield the bacteria and to transport them into the gut and different encapsulation materials has been effectively applied for the micro-encapsulation of the probiotics. Nonetheless, there are still a number of challenges which needs to be overcome with respect to the different microencapsulation process. One of the disadvantage is that microencapsulation of certain probiotics did not significantly upsurge their viability when the probiotic cells come across the simulated gastric juice. Moreover, different technique offers its own advantage and disadvantages. Some of technique like emulsion technique offer advantage that it can be scaled up easily and the produced diameter is substantially smaller (25 µm-2 mm). Yet, it has disadvantage that it requires a higher performance as compared with the other methods like extrusion method. The extrusion method also have a disadvantage such that it is difficult to use this technique for production at larger scale owing to slow formation of the microbeads. Furthermore, extrusion method have a very poor payload of normally 8% and have a higher susceptibility of carbohydrate concerning damage and structural defect. Methods which involve the drying process causes more or less injuries to the formed microbeads and has reducing viability of the probiotics cells. In the freeze drying procedure, injuries to the probiotics cells related to the heat are minimal compared with other procedures. But, this technique is pretty expensive and hard to be achieved on the industrial scale. Additionally, an extra coating is given to the capsule intended for protection against the harsh environmental conditions. The techniques such as spray drying may not be suitable principally for the probiotic bacteria owing to constraint of high temperature drying. The other drying methods, fluidized bed drying also hold disadvantage that it take relatively longer duration for

preparation of this encapsulated probiotics (up to 2 hours). Therefore, none of the methods used for encapsulation provide fulfill all the criteria for ideal encapsulated technique.

Conclusion

Microencapsulation has been proved to be one of the most potential technique for conserving viability and stability of probiotics. These days incorporation of prebiotics, starch, gelatin and milk proteins have been employed as matrix material for better functionality. These components not only protects probiotic during food processing, harsh gastric conditions and storage, also have their own health beneficial effects. Further researches have to be carried out to find suitable carrier matrices and bacterial strains through well-planned clinical trials involving large diverse population. It is needed to calibrate process cost, formulation, waste of bacteria, stability, and shelf life during process to achieve homogeneous and comparable results in each intended application.

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