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Improving the viability of probiotics by encapsulation methods for development of functional dairy products

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Abstract

The popularity of functional foods among scientists and common people has been increasing day by day. Awareness and modernization make the consumer think better regarding food and nutrition. Now a day's individual knows very well about the relation between food consumption and disease prevalence. Humans have a diversity of microbes in the gut that together form the gut microflora. Probiotics are the health-promoting live microbial cells improve host health through gut and brain connection and fighting against harmful bacteria. *Bifidobacterium* and *Lactobacillus* are the two bacterial genera which are considered to be probiotic. These good bacteria are facing challenges of viability. There are so many factors such as sensitivity to heat, pH, acidity, osmotic effect, mechanical shear, chemical components, freezing and storage time as well which affects the viability of probiotics in the dairy food matrix as well as in the gut. Multiple efforts have been done in the past and ongoing in present for these beneficial microbial population stability until their destination in the gut. One of a useful technique known as microencapsulation makes the probiotic effective in the diversified conditions and maintain these microbe's community to the optimum level for achieving targeted benefits. Dairy products are found to be an ideal vehicle for probiotic incorporation. It has been seen that the encapsulated microbial cells show higher viability than the free cells in different processing and storage conditions as well as against bile salts in the gut. They make the food functional when incorporated, without affecting the product sensory characteristics.

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Introduction

Food provides energy for growth and development. Presently, consumers have serious concern due to their understanding regarding diet consumption and health in diet selection. The demand for functional foods has been increased (Tripathi and Giri, 2014). Food is said to be a functional food, which exerts additional positive health effects along with nourishing the host (Siro *et al.*, 2008; Prosapio *et al.*, 2016). Different functional ingredients such as probiotics and prebiotics are used in functional foods. Functional foods are lactose-free and provide a lot of health benefits (Chuayana *et al.*, 2003; Suvarna and Boby, 2005).

Functional foods exert positive effects on mood and behaviour, regulate the physical activity, antioxidant and improve immune functions (Lin, 2003). However, probiotic functional food products facing issues for the survival of the probiotic at different stages of production (Boylston *et al.*, 2004). Different functional foods such as probiotics dairy products are available. In fact, the phrase "You are what you eat" compel the people for consumption of functional foods (Verbeke, 2005). Fermented milk products are being used for their nutritional as well as the therapeutic role (Roberfroid, 1999).To incorporate dairy products with probiotics (good bacteria) is such a big task for the researcher as well as for industry (Karthikyan *et al.*, 2014).

Probiotics are the living microorganism which provides numerous health benefits to the consumers when ingested to an adequate extent. *Bifidobacterium* and *Lactobacillus* are the two bacterial genera (Solanki *et al.*, 2013) which are commonly used in the preparation of ready to eat (RTE) functional foods (Gawkowski and chikindas, 2013). Both genera are GRAS (Siro *et al.*, 2008). Probiotics boost up the immunity when ingested to an adequate amount and protect against various diseases (Collado *et al.*, 2009). Probiotics prevent the intestine from pathogenic bacteria and prevention against intestinal infection (Tuomola *et al.,* 2006). In fact, Probiotic strains are specific in their function and exert positive health effects to the host (Oelschlaeger, 2010). The probiotic food products must be safe and have a significant viable number at the time of utilization. According to recommendations, viable probiotics bacteria should be present in the product at a minimum level from 10^6 to 10^7CFU/ml or g for their best performance. Functional foods have the economic scope and huge market gains. Market share of probiotic functional foods contribute about 60% to 70% (Holzapfel, 2006; Kołozyn-Krajewskaa and Dolatowski, 2012; Stanton *et al.,* 2001). Several probiotic dairy products have been developed such as dairy beverages, dairy desserts, flavored milk, powder milk, buttermilk, fermented milk, sour cream, baby foods, ice cream, cheese and yoghurt. (Mohammadi and Mortazavian, 2011). Fermented milk-based products are good tools for the incorporation of probiotics but viability losses occur at different stages of production, freezing and storage. Viability losses are more in freezing than storage process. (Mohammadi *et al.*, 2011). The use of functional ingredients (probiotics) in the products have been increasing day by day (Karthikeyan *et al.*, 2013).

There are so many factors such as sensitivity to heat, pH, acidity, osmotic effect, mechanical shear, chemical components, freezing and storage time (Krasaekoopt *et al.*, 2003; Martin *et al.*, 2015) which affects the viability of probiotics in the dairy products. These factors motivate researchers to develop some innovative methods for their survival in the product as well in G.I system (luckow and Delahunty, 2004). Encapsulation may enhance the survivability of probiotics in the product and the G.I tract as well (Mohammadi *et al.*, 2011). Microencapsulation (ME) is a technique to augment the viability of probiotics (Heidebach *et al.*, 2012) and considered very effective (Martín *et al.*, 2015). Microencapsulation is wrapping the probiotics to save them from external environment (Sultana *et al.*, 2000). The most appropriate and usually used wrapping material is Alginate (Krasaekoopt *et al.*, 2004). Encapsulation reduces injury to bacteria and protects probiotics against bacteriophages (Burgain *et al.,* 2011). The aim of this current review is to highlight the probiotic overview, factors affecting their survivability in various conditions, materials and methods used for encapsulation and some probiotic foods, especially fermented dairy products.

Probiotics

Probiotics are well known for their various health possession activities (Kerry *et al.*, 2018). Beneficial microbes have been used in the fermentation of different products for a long time ago (Cross *et al.*, 2001). By the definition of probiotic, promote the health of the host when dispensing to a sufficient extent (Bagchi, 2014). At industrial scale, probiotics illustrated as "live microbes being the part of product exhibiting health advantages" (Clancy, 2003). Prerequisites, for the microorganisms to be appraised as "probiotics" it should retain viability in the product as well as in the stomach and gastrointestinal tract (G.I), present in sufficient number for its activity at the delivery site (Hyun and Shin, 1998),

Benefits of probiotics

Probiotics have an application to be eaten as a pharmaceutical product or be administered in nutraceuticals and functional foods (Hoch and Saad, 2009; Radulović *et al.*, 2017). These beneficial microorganisms improve immune functions (Song *et al.*, 2013), prevent the intestine from pathogenic bacteria (Jandhyala *et al.*, 2015; Tsai *et al.*, 2019) as well as prevent against intestinal infection (Tuomola *et al.,* 2006). Probiotics exceed food nutritional value by increasing their bioavailability and enhance lactose digestion (Marco *et al.*, 2010), reduce blood pressure as well as lower serum lipids proportion. By the gut-brain connection they have importance in the disorder of mood, mitigate anxiety (Huang *et al.*, 2016; Messaoudi *et al.*, 2011) and depression (Huang *et al.*, 2016).

Probiotics also have a therapeutic role such as reduce the chances of cancer by binding the carcinogens (Rasic, 2003). They also ameliorate lactose intolerance, treat diarrhoea (Reid, 2015; Appel-da-Silva *et al.*, 2017) and prevent urinogenital diseases (Lourens-Hattingh and Viljoen, 2001; Mattila-Sandholm *et al.*, 2002). Probiotics (a mixture of *L. rhamnosus, B. breve* and *P. freudenreichii*) used to minimize inflammatory bowel diseases (Bakirtzi *et al.,* 2016). They were found to be better in comparison of placebo in treating the irritable bowel syndrome (Kajander *et al.*, 2005). Probiotics (*B. breve, L. casei* and *L. plantarum*) have been used to treat the patient from infection after surgery and significantly reduce postoperative bacterial infections (Rayes *et al.*, 2005; Kanazawa *et al.,* 2005).

Probiotics have their important role to treat and prevent some allergic diseases (Begum *et al.*, 2017) such as specific strains of *L. rhamnosus, B. longum* and *L. Reuteri* remarkably diminish the rhinorrhea and eczema after treating the host with such probiotics strains to about 6 months (Kalliomaki *et al., 2001;* Rosenfeidt *et al.*, 2004).

Well known probiotics

There are several species and genera of microorganisms evaluated as prospective "probiotics" (Shah and Ravula, 2004). Several species of lactic acid bacteria (LAB) and non-lactic acid and some yeasts remain viable in the intestine and exhibit adequate health activities (Holzepfel *et al.*, 2006). Chiefly used bacterial genera are *Bifidobacterium* and *lactobacillus* (both are gram-positive and anaerobic). Both genera are designated as GRAS (generally recognized as safe) and have their habitat in the intestine of humans (Boumis *et al.*, 2018).

Others bacteria used as probiotics are belonging to the genera *Streptococcus, lactococcus, Propionibacterium, Bacillus, Enterococcus* and *Pediococcus*. Some yeasts such as *saccharomyces boulardii, saccharomyces cerevisiae* and fungi (Aspergillus oryzae) deliberating health-promoting effects to host are evaluated as probiotics. (Rivera-Espinoza and Gallardo-Navarro, 2010; Vinderola and Reinheimer, 2003: Fijan, 2014; Tsai *et al.*, 2019).

Probioticsselection

In fact, *Lactobacillus* and *Bifidobacterium* are commercially used bacterial genera. Probiotics strains selection is such a worthwhile consideration to uproot some major health-related benefits. Several determinants should be considered to choose bacterial strains as probiotics. Adequate amount and appropriate strains selection for the development of probiotics food products is some prerequisites, which should remain viable during processing and storage as well as in stomach and gastrointestinal tract (Ventura and Perozzi, 2011).

Specific bacterial strains have certain effects and have the ability to bear harsh processing operations, survive at low pH in the stomach and G.I tract as well as in food matrices. *Lactobacillus* (most probably occurs in fermented products) strains have more resistibility against such conditions than *Bifidobacteria*. Due to such ability they have more uses regarding technological aspects in the food industry (Tripathi and Giri, 2014).

Probiotic Doses

Different probiotic strains are commercially available in the market in the form of a variety of fermented products (*S. thermophilus* as culture in fermented milk and yoghurt) and in capsule (*L. rhamnosus, B. longum, B. bifidum*) as well (Fuller, 1992). Probiotics confer health benefits only in case when present in ample quantity and retain their viability. So, it's necessary to declare their suitable count at different stages for their effectiveness. Probiotics minimum viable count in the product should be as low as106 CFU/g or mL (Boylston *et al*., 2004), probiotics must be consumed daily at the rate of 108 CFU/day (Lopez-Robio *et al*., 2006) and their minimum count should be 107 CFU/mL at the point of action (Doleyres and Lacroix, 2005; Tripathi and Giri, 2014).

Factors affecting probiotics viability and survival

Bacteria to be a probiotic, it's a necessary to remain viable and survive in the product in active form to its enough population at the time of utilization for its beneficiary effects to host health (Korbekandi *et al.,* 2011). So, their viability was found to be a supreme Importance for action. Several parameters were found, which significantly affects the probiotic viability and survival rate in the food products during different stages of production until consumption.

Some known factors like food factors (amount of sugar and salt, pH, dissolved oxygen, water activity, H_2O_2, a fermentation by-product, acidity), processing factors such as exposure to heat, cooling, incubation time and temperature, use of anti-microbial ingredients, freezing, drying, material used in packaging and storage environment and microbial factors (probiotics strains and amount used) creates challenges to probiotic survival (Tripathi and Giri, 2014). Food matrix is important for probiotic incorporation.

Selection of appropriate food product is a vehicle to carry probiotic, much necessary for their viability. Low stomach pH and bile acid secretions into the G.I tract such an inappropriate environment for probiotic to be functional and maintain viability. They would have to show metabolic activity and resist susceptibility to enzymatic degradation (Ranadheera *et al*., 2012).

To overcome the probiotic viability issues in different harsh conditions of food manufacturing and in G.I tract, there is a serious need of technological advancement to develop such techniques, which enhance probiotic survival and viability in such competitive environment to perform functions (Mattila-Sandholm *et al*., 2011). Microencapsulation is an advanced method to augment the probiotic viability (Philips *et al*., 2006). Its makes probiotic to be viable even in an acidic environment and functional in the food matrix as well.

Microencapsulation

It's an important technique in which the defined substance is just entrapped into a specific material to form a capsule ranges from few millimetres to nanometres, a protective covering membrane save the transferring material from outer severe environmental conditions (Vivek, 2013). Microencapsulation is such an effective approach to make sure the safe transfer of probiotic microorganisms to the target site. It ensures their viability during processing and storages as well as in the G.I tract (Cook *et al*., 2013). The main purpose is to separate the reactive substances from unfavourable surroundings.

The technology of encapsulation enhance the survival of live microbes against unfavourable conditions such as high bile salt concentrations, oxygen, achieve longer shelf life, reduces evaporation rate (Borgogna *et al*., 2010; Madene at el., 2006). It protects the material as well as permits the controlled release in the intestine in biologically active form (Zuidam and Shimoni, 2009) and immobilization of cell results in uniform apportionment all over the product (Krasaekoopt *et al*., 2003).

There are several challenges in the development of probiotic microcapsule, one of them is their size (diameter about 1-5µm) exert difficulties in advancement, which directly inhibit nanotechnologies. Larger capsule size negatively influence the properties of food products. Even different methods of encapsulation applied gives heterogeneous capsule size ranges and shapes (De Vos *et al.*, 2010). Physicochemical characteristics of encapsulated material greatly affect probiotic viability and concentration as well. The most pivotal is the selection of probiotic strains (Krishnan *et al.*, 2005).

This unique technology is accomplished into three steps. The first step is to assimilate the bioactive material in matrix which may be liquid or solid. If the encapsulated material is liquid, incorporation will be dissolution but if the material is solid it would be adsorption. Next and second step involves liquid matrix dispersion. Third and the last step involves stabilization by a gelification, physical (solidification, coalescence) and chemical (polymerization) method (Poncelet and draffier, 2007). After encapsulation of microbial cells the micro-beads are formed having a resemblance to that of oval and spherical shapes. Every single bead retains one to diverse numbers of bacterial cells (Zinedine and Faid, 2007).

Studies revealed the effect of encapsulation of microbial cell in comparison to free cell. Encapsulated strains of *Lactobacillus* and *Bifidobacterium* exhibit better stability to low stomach pH (2.0) and bile salts and reached to about 58.9% mean value in contrast to non-encapsulated cells (Champagne *et al.*, 2015). Acidic pH, lactic acid concentration, high amount of molecular oxygen and hydrogen peroxide makes the environment unfit for probiotic persistence in the dairy products. Microencapsulation enables them to linger in such an unfriendly surrounding (Picot and Lacroix, 2004).

Free cells of bacterial strains of *L. acidophilus LA-5* and *Bifidobacterium lactis BB-12* should not survive in the fermented dairy drinks at pH<4.2 after fortnight period of storage and their viable count were restricted to 102 CFU ml-1. But when both strains were encapsulated their viable populace increased and remain viable in such habitat (Mortazavian *et al.*, 2008).

Encapsulated material

Material being used in encapsulation should be safe, non-toxic and food grade (Ei-Salam and Ei-shibiny, 2012). Polymers (starch, chitosan, gelatine), celluloses (CMC), gums (xanthan gum. Gum arabic), milk proteins (casein and whey) and fats are extensively used materials in microencapsulation (Mokarram *et al.*, 2009; Chavarri *et al.*, 2010; Cook *et al.*, 2013).

Starch

Starch is a polysaccharide made up of the large number of glucose units connected through glucosidic linkage. Amylose and amylopectin comprise starch composition. Resistant starch is a type of starch which is indigestible by pancreatic enzymes (Sajilata *et al.*, 2006). Moreover, resistant starch permit the control liberation to the intestine causes good microbial survival in this type of application (Naulkaekul and Charalampopoulos, 2011). Due to such successful survival of bacteria, it is appreciably used in the food industry (Anal and Singh, 2007).

Chitosan

In the presence of anions and poly anions glucosamine molecules cross-link to form a linear polymer known as chitosan. Chitosan itself is not effective for encapsulation, but it is good coating material. It gives excellent protection in G.I tract when used in combination with alginate (Mortazavian *et al.*, 2008; Chavarri *et al.*, 2010).

Gelatin

It is a non-toxic, commercially available and valuable material used for microencapsulation. Usually obtained from bones having good protective properties (Imeson, 1997). It is observed to be a protein gum forming a thermoreversible gel. Due to its amphoteric nature it can be used in combination with other ionic polysaccharides like gellan gum (Sendra *et al.*, 2008).

Milk proteins

Milk proteins like whey protein have excellent gelling and emulsifying properties (Cayot and Lorient, 1997).

Moreover, physicochemical characteristics of milk protein enables them good carrier matrix for probiotics. Biologically it's a good material for probiotic encapsulation (Livney, 2010).

Gum Arabic

A plant source gum highly soluble in water and have low viscosity. It is a good encapsulation material which maintain the original characters of the core material and keep the probiotic survivability in storage (Reyes *et al.*, 2018). It does not affect the organoleptic properties of the product and resist to processing and acidic conditions (Kravtchenko,1998).

Alginate

A naturally occurring polysaccharide consist of beta-D-mannuronic acid and alpha-L-glucuronic acid (Sohail *et al.*, 2011). For encapsulation of microbial cell alginate is extensively used (Hansen *et al.*, 2002). Calcium and Sodium alginate are most commonly used due to non-toxicity and economical price (Krasaekoopt *et al.*, 2003). Sodium alginate is most suitable material for probiotic encapsulation due to high retention of viability, heat stability, readily form gel and naturally occurring (Lee and Heo, 2000). Using Alginate is disadvantage is that beads produced are sensitive to acidic conditions, but this can be overcome by applying another coating or mixing with other material or applying additives (Krasaekoopt *et al.*, 2003).

Encapsulated methods

Extrusion method

A physical approach to protect probiotic to maintain its integrity. It's a simple and economical process causes no damage to microbial cell (Krasaekoopt *et al.*, 2003). Prepared hydrocolloid suspension (Na or Ca alginate in water) having probiotic strains in it. It is entitled to pass through a narrow opening under pressure. Droplets thus formed are collected in a hardening solution of CaCl2 and permit to stay in solution about an half-hour. The solution is then centrifuged to separate the micro-beads for further application or stored at 39.2 °F. Some challenges to use this method. First, it form beads at very slow rate and the second one is that the bead produced are relatively larger in size which disturbs the product textural attributes (Kailasapathy,2009; De Vos *et al.*, 2010; Jayalalilha *et al.*, 2011).

Spray drying and spray freeze-drying method

The operational procedure for both of these methods is same except solidification of the micro beads. Suspension of probiotic cells and polymer is projected to form mist in controlled chamber by atomizing the mixture through nozzle. Rapidity of the method and acceptable for industrial implementation are pros of the method. In case of spray drying the solvent is vaporized by hot gas in the chamber and micro-capsule is obtained by cyclone separator. Whereas, in spray freeze drying frozen droplets are obtained in a freezing chamber and dried through freeze-drying. Cons of both of these methods are that additional coating is required for protection against surroundings (Semyonov *et al.*, 2010; Kailasapathy, 2009; Zuidam and Shimoni, 2009).

Emulsification Method

This chemical method of emulsification involves two phases, one is continuous (oil) and other is discontinuous (mixture of probiotic and coated material) phase are involved. Mixture of both phases is then homogenized to make water-in-oil emulsions or oil-in-water emulsions. Emulsifier and surfactant are added for emulsion stability. For solidification $CaCl_2$ is added. This method is easy to scale-up but beads produced are relatively larger size that is undesirable (Chen and Chen, 2007; Kailasapathy, 2009; De Vos *et al.*, 2010).

Probiotic foods

Numerous food products have been developed to be claimed as probiotics. Several qualitative parameters should be considered in the development of probiotic food products such as it should be safe, have unique sensory characteristics, economical, beneficial to health and have enough population of such beneficial microbial cells for effectiveness at target point (Rose *et al.*, 2005: Burgain *et al.*, 2011). During the recent decades trend has been increased in the development of probiotic (cheeses, buttermilk, yoghurt and ice cream) dairy products (Radulović *et al.*, 2017).

Following are some commercially available probiotic dairy based food products which are overviewed.

Yoghurt

Probiotic, the live microbes are poorly adopted to yoghurt because of vulnerability to low pH (4.2-4.6). Microencapsulation found to be much better approach for their persistence in such competitive environment (Kailasapathy, 2009). Greek style lactose free probiotic yoghurt were prepared which proved good survivability of encapsulated probiotic strains over one month storage at 4°C (Pinto *et al.,* 2019). Two strains of *Bifidobacterium* (*B.breve 070*, *B.longum R023*) when encapsulated using whey protein by spray drying technique, exhibits remarkable viability at low yoghurt pH (stored at 4°C for 4 weeks) and in-vitro digestion in contrast to free cells (Arnaud Picot and Christophe Lacroix, 2004). Effects of microencapsulation on strains of *Lactobacillus* and *Bifidobacterium ssp*. shows high rate of viability than non-encapsulated strains. The reduction in serviceability of *B. infantis17930* and *L. Rhamnosus GG* was 0.07 log, while that of *L. casei 1520* number lowered by 0.28 log and *B.longum 1941* was decreased by 0.39 log. Alginate based microencapsulation enhance viability of combined selected probiotic by 0.31 log in freeze-dried yoghurt after 28 days storage at 21°C (Capela *et al.*, 2006).

Yoghurt was prepared from goat milk using encapsulated strains besides free strains of *Bifidobactrium* along with prebiotic insulin. Results showed good survival of encapsulated combination of probiotic with prebiotic in goat milk yoghurt (Pradeep Prasanna and charalampopoulos, 2019). Moreover study investigated that symbiotic microcapsule of *S. thermophiles* and *L. bulgaricus*in yoghurt remained viable to standard count than free-state (Wattananapakasem *et al.,* 2018). However, studies shows that the problem exist in the larger size (22 to 50µm) of the capsule cause's grittiness in mouth which is undesirable in the product (Adhikari *et al.*, 2003). The issue can be mitigated by developing the technologies that may produce the minimum capsule size to improve the textural characteristics.

Cheese

Cheese is one of the nutritional and fermented dairy product suitable for the consumption of all age groups. It creates buffer in gut against acidic environment. Additionally such type of food matrix impart suitable protection for survivability in stomach (Ross *et al.*, 2002; Bergamini *et al.*, 2005). Cheese found to be good food matrix for probiotic incorporation because of its high value of pH (5.5) especially the cheddar cheese. Moreover its high fat content lowers the probiotic susceptibility to low pH and degradation by enzymes. Beads of microbial strains of *Bifidobacterium bifidum* were obtained by emulsion method. It was found that there was no significant change in sensory attributes of cheddar cheese after 24 weeks of storage in contrast to control. Even *Bifidobacterium* survive at low ripening temperature (6-7°C) and persist for 6 months (Dinakar and Mistry, 1994). Literature showed that supplementation of microencapsulated probiotic strains of *L. paracasei* maintained their viability in feta cheese to their optimum population (Kia *et al.*, 2018). The viability of *L. plantarum* 564 was checked in both free and encapsulated form by spray dried method in soft goat cheese over a period of 2 month. Results were in favor of encapsulated form that exhibited higher viability (Radulović *et al.*, 2017). Another study confirmed the encapsulated probiotic stability higher than free cell in the cream cheese (Ningtyas *et al.*, 2019). Work was done on encapsulation of *L. acidophilus* showed higher viability in Manaba cheese than free strains at 25 °C (Santacruz and Castro, 2018). Microencapsulated *L.acidophilic* (LA-5) remained viable in UF white cheese (Nejati *et al.,* 2017).

Ice cream

Ice cream is a popular dairy product and consumed worldwide and popular among people of all age especially in children's. To enhance its nutritional value is a serious concern of today research. Probiotic added to ice cream do not affect its sensory profile except slightly show acidic characters causing sour taste (Salem *et al.*, 2005). Study was conducted to evaluate the effect of microencapsulation on microbial viability. Experiments showed that

encapsulated probiotic showed high survival rate more than 30 percent as compared to free probiotic cell in ice cream without effecting its sensory characteristics (Karthikeyan *et al.,* 2014). Bacteria incorporated to ice cream face harsh challenges to variety of technological procedure. To combat such problems they were encapsulated for their survivability in variety of conditions and remain biologically active.

The viability of two bacterial strains *L. casei (NCDC-298)* and *Bifidobacterium animalis ssp. Lactis (BB-12)* was tested in four types of ice cream in encapsulated and non-encapsulated form. The viable count for *Lactobacillus casei was* 5.3 ± 0.2 ×10^9cfu/ ml at first day and then reduced to 4.5 ± 0.2 × 10^6cfu/ml after 180 days of storage. 4.6 ± 0.2 × 10^9cfu/ ml was viable count for *Bifidobacterium animalis ssp. Lactis* at first day and then lessen to 2.1 ± 0.1× 10^7cfu/ ml after 180 days of storage at -23°C. Encapsulation of these strains using calcium alginate enhance their viability up to 30 percent under similar conditions without affecting organoleptic properties of ice-cream (Karthikeyan *et al.*, 2013). It has been evaluated that the probiotic strains of *B. adolescentis and L. casei*improved viability to their standard count by microencapsulation in ice cream without effecting product sensorial attributes after storage up-to 100 days (Zanjani *et al.*, 2018). Study evaluated the probiotic viability and similar results were obtained by Champagne *et al.* (2015) and Afzaal *et al.* (2019).

Conclsusion

Dairy food products are found to have an ideal profile of food matrix for safe delivery of probiotic living cells to the gut and proliferate their growth. High fat content of dairy products provide protection to living microbial cell in the G.I tract up to limit. Their viability issues have been resolved to a certain extent by using the advance technology of microencapsulation, which enables them to remain biologically active till consumption to accomplish their functions and provide control release and ultimately they promote the host health. But bacterial size causes obstruction in probiotic food product development which ultimately diminish sensory attributes of the product. So, its dire need to develop more innovative technologies for production of Nano-size capsule, for incorporation to the product.

Recommendations

The care should be taken in selection of material for coating and interaction among matrix, encapsulation material as well as probiotics should be evaluated for long term stability of probiotics and product safety. Besides, their viability should be assessed in G.I transit as *In-vivo* and *in-vitro* models for probiotic efficacy.

Abbreviation

GRAS (generally recognize as safe), G.I. (gastrointestinal)

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