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Review Paper/Derleme Makale

Materials and methods used in microencapsulation of probiotic microorganisms

Probiyotik mikroorganizmaların mikroenkapsülasyonunda kullanılan materyal ve yöntemler

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Abstract

Objective: Probiotic microorganisms which constitute an important part of functional foods are living creatures that have been proven to benefit human health. However, most of the time they lose their vitality entirely or partly before reaching the human gastrointestinal system due to the various degenerative processes that they are exposed to during food production stages. Those who have been able to maintain their vitality are exposed to destructive bioprocesses in the digestive system.

Conclusion: It is possible to provide the probiotic microorganisms to reach the target point by maintaining their vitality at an optimum level utilizing the microencapsulation method which we could consider as a technological packaging process. In this study, information is given about microencapsulation methods applied to probiotic microorganisms and the coating materials used.

Keywords: probiotics; microencapsulation; coating material; microencapsulation methods

Öz

Amaç: Fonksiyonel gıdaların önemli bir kısmını oluşturan probiyotik mikroorganizmalar insan sağlığına faydalı olduğu kanıtlanmış canlılardır. Ancak çoğu zaman gıda üretim aşamalarında maruz kaldıkları çeşitli dejeneratif işlemler nedeniyle insan gastrointestinal sistemine ulaşamadan canlılıklarını tamamen veya kısmen kaybederler. Canlılığını koruyabilenler sindirim sisteminde yıkıcı biyoproseslere maruz kalırlar.

Sonuç: Teknolojik bir paketleme işlemi olarak değerlendirebileceğimiz mikroenkapsülasyon yöntemi ile probiyotik mikroorganizmaların canlılıklarını optimum düzeyde koruyarak hedef noktaya ulaşmasını sağlamak mümkündür. Bu çalışmada probiyotik mikroorganizmalara uygulanan mikroenkapsülasyon yöntemleri ve kullanılan kaplama malzemeleri hakkında bilgi verilmektedir.

Anahtar kelimeler: probiyotikler; mikroenkapsülasyon; kaplama materyali; mikroenkapsülasyon yöntemleri

1. Introduction

Today, it is expected that foods not only satisfy hunger or meet nutritional needs but also prevent nutritional diseases and improve health. In this respect, functional foods play an important role (Cencic and Chingwaru, 2010).

The usage of functional foods dates back to the 1980s in Japan. In 1991, the concept of FOSHU (Japanese Foods for Specified Health Use) emerged, which is used to name foods that have a positive effect on human health, depending on the components they contain or the removal of allergens from the food (Kumagai, 2014).

In the Turkish Food Codex (Law No. 5179 on the Amendment of the Law Decree on the Production, Consumption and Inspection of Foods), functional foods are defined as "nutritions which are protective, corrective and/or reducing the risk of disease, depending on one or more effective ingredients, in addition to their nutritive effects and whose these effects have been scientifically and clinically proven" (Anonymous, 2004).

Probiotic microorganisms constitute an important class of functional foods. These microorganisms, which are mostly found in dairy products can be found in many food products containing probiotic properties from vegetable and fruit-based products to cereal and legume-based products, from meat and meat products to chocolate (Erem, 2019).

Probiotics are described by the World Health Organization as “live microorganisms having a positive effect on the health of the person when taken in adequate amounts,” (FAO/WHO, 2001). Beneficial intestinal bacteria, which are generally taken into the body together with fermented products and raw fruits and vegetables, perform numerous important functions in a symbiotic interaction with their hosts (Markowiak and Ślizewska, 2017). Usage of probiotics has a positive effect on the advance of targeted microorganisms in the host gastrointestinal tract, discards fungi or harmful bacteria, and enhances the normally occurring defense actions of the host's immune system (Pech-Canul et al., 2020).

Probiotics have some potential benefits such as strengthening the immune system, inhibiting pathogenic organisms, facilitating the metabolism of fats and proteins, providing the synthesis of vitamins, reducing cholesterol and blood pressure, increasing mineral absorption, preventing stress, preventing harmful bacterial growth under colitis, managing urogenital health, playing a role in anti-microbial activities, detoxification and protection from toxins (Amin et al., 2013; Geniş and Tuncer, 2019; Erginkaya et al., 2019). Trying to regulate the intestinal microbiota by the consumption of products containing prebiotics, probiotics and their combined uses (symbiotics) are among the most accepted methods today (Altındiş and Yılmaz, 2017).

In addition to being used while curing many illnesses such as cancer, diarrhea, asthma, celiac, lactose intolerance, allergies, diarrhea, it has been reported that it is effective in reducing the inflammatory intestinal risk, treating *Helicobacter pylori* infections, and preventing antibiotic-associated diarrhea (Arvanitoyannis and Van Houwelingen-Koukaliaroglou 2005; Cencic and Chingwaru, 2010; Shiby and Mishra, 2013; Markowiak and Śliżewska, 2017; Sánchez et al., 2017; Erem, 2019). It is known that the intake of Lactobacillus reduces tumor development by reducing fecal enzymes such as nitroreductase, β-glucuronidase, and azoreductase which can convert pro-carcinogens to carcinogens in the digestive tract in humans (Amin et al., 2013).

Studies put forward that herbal probiotic products provide benefits such as strengthening the immune system, increasing immunoglobulin production, cleansing the colon and liver, and increasing calcium absorption in vegan and vegetarian individuals (Akpınar and Seven, 2019).

The amount of global probiotic trade in 2018 was 45.6 billion dollars. It was projected that this value will rise to 65 billion dollars in 2024 (Anonymous, 2018).

Most of the probiotics used in commercial products today a members of the Lactobacillus or Bifidobacterium genera, which are found in many foods and the human intestine and sensitive to harsh conditions. *Streptococcus citrovorus, Streptococcus lactis, Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus rueteri, Saccharomyces boulardii, Bifidobacterium bifidium* general are important probiotic organisms. It has been reported that Pediococcus, Bacillus, and some yeast strains are also proper probiotic candidates (Çelikel et al., 2018).

It is also stated in Annex 2 of the Turkish Food Codex Regulation on Nutrition and Health Declarations that food must include at least $1.0x10^6$ cfu/g live probiotic microorganisms to be considered probiotic (Anonymous, 2017). However, factors cause a diminution in the number and activity of probiotic microorganisms in food production stages. These factors are pH, oxygen,

water activity, chemicals such as hydrogen peroxide; various parameters used in production such as the presence of salt and sugar, colorants, and artificial flavoring; microbiological parameters (probiotic strain type, amount and inoculation rate; and processing parameters such as incubation temperature, heat application, packaging material, product cooling rate, production scale, and storage method) (Erem, 2019).

In studies evaluating the viability of some commercial probiotics during their passage through the gastrointestinal tract, it was shown that all products used commercially tested suffered a 10^6-fold diminution in colony-forming units (cfu) within 5 minutes (Dodoo et al., 2017). Therefore, it is a big problem that many commercial probiotic products do not provide the expected effect because beneficial bacteria are not able to keep their viability during food processing, storage, and transit through the upper gastrointestinal tract; in addition, even if they reach the targeted point, it is a possibility that they can not identify themselves as part of the intestine microbiome and can only pass into the stool (Yao et al., 2020).

More effective strategies are required to increase the stability of probiotics in foods and to preserve them while they pass through the human intestine. The number of live probiotic microorganisms in the product can be increased by methods such as choosing strains that are resistant to stress and digestive conditions, performing multi-stage fermentation, reducing the oxygen permeability of the product package, adding micronutrients that can be used by probiotics and microencapsulation applications (Çelikel et al., 2018).

Yao et al. (2020) examined the viability of probiotic microorganisms in the intestinal tract using in vitro and in vivo techniques and stated that encapsulation practices are necessary to maintain their viability in storage conditions and the colon. These technologies not only protect probiotics from harsh environmental conditions but also increase the mucoadhesive properties of probiotics. Encapsulation is a method based on the principle of coating with protective material or bonding to a carrier material in nano (≤0.2 µm), micro (0.2-5000 µm), and macro (≥5000 µm) sizes. Basically; it is carried out by adding the bioactive component into the solid or liquid matrix, providing the liquid matrix dispersion, and providing stabilization by a chemical (polymerization), physical (solidification, evaporation, etc.) or physicochemical (gelation) process (Sarao and Arora, 2017).

2. Microencapsulation applications

Microencapsulation can be defined as "the retention of a compound in a compound or emulsion for its immobilization, protection, controlled release, structuring, and functionalization". A microcapsule is the structure of a semi-permeable, spherical, thin, and strong membrane with a diameter ranging from a few microns to 1 mm surrounding a solid or liquid. In addition to its protective feature, it facilitates the transport of cells and allows controlled release (Amin et al., 2013; Yao et al., 2020).

The main purposes of applying microencapsulation are to prevent the encapsulated bioactive material from being damaged by the environment or to prevent its damage to the environment (pH, temperature, enzyme, oxygen, etc.); preserve bioactivity, mask flavor and aroma components; be able to turn liquids into solids and to ensure the release of the encapsulated material at the target point (Ünal and Erginkaya 2010; Aloğlu and Öner, 2010; Yao et al., 2020). It is a method that is used to limit the relationship of bioactive substances that are not desired to be found in the environment (enzymes, etc.) or that are desired to be protected from environmental conditions (probiotics, vitamins, polyunsaturated fatty acids, etc.) with the environment. Microencapsulation increases the bioavailability of bioactive substances and extends the shelf life of products (Chen and Subirade, 2007).

Although microencapsulation is used in many areas, the most common use is in the pharmaceutical industry at a rate of 68%. This is followed by food at 13%, cosmetics at 8%, textile at 5%, biomedical at 3%, agriculture at 2%, and electronics industry at 1% (Uran et al., 2017; Geniş and Tuncer, 2019).

The active substance encapsulated in microencapsulation is described as the core material and can be dissolved, liquid, or solid dispersed. The protective matrix structure is called capsule, shell, wall, or coating (Sarao and Arora, 2017). While the thickness of the protective coating in microcapsules is between 0.5-150 µm, the capsule diameter varies between 0.01 and 1.000 µm, probiotics can survive in multilayer coated microcapsules and a layer thickness of 20 µm is sufficient to maintain this (Li et al., 2017; Geniş and Tuncer, 2019).

Microcapsules can be defined as single-core (mononuclear), multi-core (poly/multinuclear), matrix (matrix), multi-wall, and irregular

according to their morphological structures (Geniş and Tuncer, 2019).

The capability of microcapsules to enhance the survival of probiotics is related to their size. In food products, when the capsules are too large, they may leave a bad taste in the mouth and remain in the stomach. On the contrary, if they are very tiny, it cannot be possible to encapsulate or degrade too quickly due to the large surface area. Cook et al. (2014) stated that capsules should have a diameter of less than about 200 μm for passing effectively throughout the gastrointestinal tract. Li et al. (2017) stated that their diameter should be approximately 500 μm.

2.1. Microencapsulation materials

It is very important to select the correct coating material to carry out a successful microencapsulation process. The coating material to be used in microencapsulation; should not show toxic properties, should be GRAS (generally accepted as safe), should be easily processed, should not react with other substances in the environment, should not react with the active material during and after application, should increase the stability of the active material and protect it from environmental effects, should be biodegradable, should exhibit good rheological properties at high concentrations where sensorial properties are important, should be in a suitable structure to enable the release of the active material at the desired time, and should be cost-effective (Ünal and Erginkaya, 2010; Palamutoğlu et al., 2013, Yavaş et al.,2019; Pech-Canul et al.,2020). It should also have properties such as resistance to high temperature and pressure, low humidity and oxygen permeability, low hygroscopicity (ability to take up water), low solubility in water, low pH, or resistance to the digestive system environment (Amin et al., 2013). As with all other materials to be used in food processes, they must meet the safety criteria of EFSA (European Food Safety Authority) in Europe and the FDA (Food and Drug Administration) in the United States (Palamutoğlu et al., 2013).

Proteins and polysaccharides, which are among the coating materials that can be used in foods on the market, form moisture-permeable films (hygroscopic), especially at high relative humidity values. They generally exhibit good barrier properties against lipids and gases. Lipid-based coatings offer perfect water barrier properties, and latent gas flow, and are comparatively heat resistant (they are compounds with a high melting point). Nevertheless, its mechanical properties are generally weak (Amin et al., 2013).

Generally, biopolymers with good thermal stability, low toxicity, high biocompatibility, and low cost are used in the coating of probiotics (Yao et al., 2020).

While today, alginates, starch, celluloses (carboxymethylcellulose, methylcellulose, nitrocellulose, ethylcellulose, cellulose acetate-phthalate, acetylcellulose, cellulose acetate butyrate-phthalate), carrageenan, gellan gum, xanthan gum, and chitosan among the polysaccharide-based coating materials are being used in the microencapsulation of probiotic cultures; compounds such as whey proteins, gelatin, casein, gluten, chickpea proteins, and cellulose acetate phthalate among the protein-based coating materials and lipids (fatty acids and fatty alcohols, glycerides, waxes and phospholipids) are also suitable materials to be used for encapsulation. However, studies on lipid coating materials are very limited (Wandrey et al., 2010; Aloğlu and Öner, 2010; Martín et al., 2015; Chen et al., 2017; Yao et al., 2020). Table 1 represents some studies on microencapsulation of probiotic microorganisms.

2.1.1. Carbohydrate-based coating materials

The use of carbohydrate-derived coating materials is most common in the spray-drying method. The fact that they are easily accessible and the cost is low increases the attractiveness of these materials (Geniş and Tuncer, 2019).

2.1.1.1. Alginates

Alginate, which is among the most frequently used biopolymer building blocks to form capsules, is a heteropolysaccharide that consists of D-mannuronic acid and L-guluronic acid and can be extracted from the cell wall of brown algae (*Laminaria spp.*) (Altun and Özcan, 2013; Martín et al., 2015; Liu et al., 2016; Sarao and Arora, 2017; Yao et al., 2020). Calcium alginate is a preferred polymer for coating probiotic organisms due to its advantages such as forming thin gels which are effortlessly found in nature, economical, non-toxic, and highly stable, forming a light matrix structure, and appearing simply in alkaline buffer solution (Gökbulut and Öztürk, 2018). On the other hand, forming a porous layer, being sensitive to acids (in stomach conditions), its protective properties being reduced by being affected by the external environment, and being not suitable for large-scale applications are its disadvantages (Gökbulut and Öztürk, 2018; Marcial-Coba et al., 2018). It has been stated that increasing the effectiveness by reinforcing alginate with other coating materials is

a better way to preserve the viability and activity of probiotics (Ünal and Erginkaya, 2010).

Natural biopolymers, for example, p-carrageenan and calcium alginate are suitable materials for gel retention for probiotic applications (Amin et al., 2013). It provides a more stable coating when used with starch, pectin, carrageenan, chitosan, or some synthetic polymers (Martin et al., 2015).

In some studies conducted on the encapsulation of probiotic cells, factors such as alginate and calcium chloride ($CaCl_2$) concentrations, hardening period of the capsules, and cell concentrations were examined. As a result, it was shown that the microorganisms coated with calcium-alginate were better preserved than the unencapsulated ones. In vitro, studies have shown that the viability of encapsulated microorganisms increases with the upsurge in the size of the capsule (Dave et al., 2004).

Chen et al. (2006) measured the resistance of probiotics coated with alginate to gastrointestinal conditions after a one-week storage period and found that microcapsule application can raise the viability of probiotic bacteria under simulated gastric fluid test (SGFT) and 3% sodium alginate combination mixed with 3% fructooligosaccharide and 1% peptide is the best application.

Muthukumarasamy and Holley (2006) microencapsulated *Lactobacillus reuteri* with alginate employing extrusion or emulsion technology, added it to fermented dry sausage dough, and examined its microbiological and sensory properties. While a logarithmic decrease of 2.6 units was observed in dry fermented sausages including *L. reuteri* added without encapsulation, a logarithmic decrease of 0.5 units occurred in dry fermented sausages including encapsulated *L. reuteri*. As a consequence of the consumer taste panel study, no substantial difference was observed with the control sample in terms of sensory properties.

2.1.1.2. **Carrageenan**

Carrageenan which is a hydrophilic, neutral polysaccharide obtained from red seaweed by hot alkaline extraction process is a natural polymer used as an additive in the food sector. Carrageenan is frequently used in food and cosmetic formulations because of contributes positively to the stability of fragrances and aromas. It has six types and κ-carregenan is the most commonly used in encapsulation applications. The fact that carrageenan is cheap and has biopolymer properties causes it to be selected as a coating material in encapsulation applications (Martín et al., 2015). Carrageenan is mostly used as a coating material in emulsion and extrusion methods (Krasaekoopt et al., 2004).

In addition, carrageenan's positive effect on the viability of the microorganism and its low sensitivity to organic acids in fermented products such as yogurt play an active role in the preference of carrageenan in the microencapsulation of probiotic cells (Martín et al., 2015; Sarao and Arora, 2017).

Afzaal et al. (2019) examined the ability of the probiotic *Lactobacillus acidophilus* ATCC4356 strain to survive in ice cream and artificial digestive system conditions by encapsulating it with sodium alginate and carrageenan polysaccharides. Researchers reported that encapsulation application substantially increased the survival level of probiotic cells in ice cream and artificial digestive system conditions compared to free cells.

Carrageenan has three (κ-, ι- and λ) types. The gel properties of κ-carrageenan are enhanced by blending it with different coating materials, for example with calcium alginate, vegetable oils, and also numerous gums (eg, gellan, xanthan, and carob gum) (Pech-Canul et al., 2020).

It is possible to find various studies on the employment of microencapsulated probiotics in fermented or frozen dairy products. In these studies, it was stated that alginate and k-carrageenan were mostly employed as coating materials (Krasaekoopt et al., 2004; Chen and Subirade 2007; Ünal and Erginkaya, 2010; Altun and Özcan, 2013; Yao et al., 2020).

2.1.1.3. **Xantan, gellan and gum arabic**

Anionic polysaccharides like xanthan, gellan, and gum arabic find application in probiotic microencapsulation. Xanthan and gellan gums, which are bacterial extracellular polysaccharides, are respectively produced by *Pseudomonas elodea* and *Xanthomonas campestris*. Also, gum arabic is obtained from acacia family member trees, also known as gumacacia (Arslan-Tontul and Erbas, 2017; Pech-Canul et al.,2020).

Acacia gum (gum arabic) is mostly used for coating flavoring materials with its low viscosity, high solubility, and ability to form emulsions (Merve et al., 2014). The utilization of xanthan gum as a coating agent has been shown to effectively microencapsulate probiotics, protecting against high temperatures and simulated gastrointestinal conditions. (Pech-Canul et al., 2020).

In many studies, xanthan-gellan gum mixture was used in the formation of encapsulated probiotic

cells, and unlike alginate, the mixture showed high resistance to acid conditions and Ca++ ions. (Chen and Subirade, 2007). These polymers can be used alone or with mixtures such as gellan-xanthan, and alginate-starch, and it is aimed to keep the polymer intact in high acid or basic environments (Arslan et al., 2017).

Lactobacillus plantarum LAB12 microencapsulated utilizing xanthan and alginate as coating materials has proven to improve the viability of the probiotic microorganism and provide better protection against elevated temperatures and low pH, compared to its unencapsulated form.

In the microencapsulation of *L.casei*, the survival rate of probiotic microorganisms increased in the environment where gastric fluid and bile salts were simulated by using gellan gum, xanthan gum, and sodium caseinate gellan gum mixtures (Pech-Canul et al., 2020).

2.1.1.4. **Chitosan**

Comprised of glucosamine units, chitosan is the only cationic polysaccharide derived from natural sources. It is not preferred to be used alone in the microencapsulation of probiotic microorganisms. Since chitosan is positively charged while many other polysaccharides are negatively charged, this coating material is among of the most frequently used polysaccharides for this aim (Yao et al., 2020). It is known that coating with chitosan which is an effective antimicrobial agent affects the viability of probiotics (Speranza et al., 2018).

Numerous studies have reported that the incorporation of chitosan into various materials, including but not limited to starch, alginate, whey protein isolate, and xanthan gum, offers protection benefits to a wide range of probiotic microorganisms under simulated in vitro gastrointestinal conditions (Altun and Özcan, 2013; Pech-Canul et al., 2020).

It was defined that the number of live bacteria in milk and yogurt was higher than the uncoated ones during the storage period in which *Lactobacillus* encapsulated in alginate was coated with chitosan (Krasaekoopt et al., 2004).

The status of the whey natural probiotic yeast *Kluyveromyces marxianus* VM004, which was coated with water-soluble chitosan and concentrated whey protein by spray drying method was monitored in storage and simulated gastrointestinal conditions; it was observed that it showed 95% viability in gastrointestinal conditions (Braber et al., 2020).

Several studies have shown that core-shell microgels consisting of a calcium alginate core and a chitosan coating improve the viability of encapsulated probiotics incubated under simulated gastrointestinal conditions (Mirtic et al., 2018).

Various studies have demonstrated the use of core-shell microgels that comprise a calcium alginate core and chitosan coating to improve the viability of encapsulated probiotics incubated under gastrointestinal environments that are simulated (Yeung et al., 2016).

Several studies have highlighted the potential of alginate-chitosan systems for colon targeting due to their ability to be degraded by the colon's intestinal microflora and subsequently release probiotics (Hejazi and Amiji, 2003). Nonetheless, a recent in vitro study stated that there is no enhancement in probiotic viability when alginate microgels were coated with chitosan compared to using solely alginate microgels (Yeung et al., 2016).

2.1.1.5. **Starch and its derivatives**

Resistant starch is a good retainer for probiotic organisms to reach the large intestine (Altun and Özcan, 2013).

The fact that starch contains nonionic, tasteless, odorless, colorless, non-toxic properties, has semi-permeable properties against oxygen, carbon dioxide, moisture, and lipid components, is cheap and easy to find makes it a suitable coating material for probiotics (Açu et al., 2014). The most frequently utilized coating materials in food applications are; starch and its derivatives (syrups, amylose, polydextrose, amylopectin, maltodextrins, and cellulose and their derivatives). The point to be paid to here is the ability of the encapsulated bacteria to decompose starch being taken into account (Wandrey et al., 2010).

It has been shown that alginate-starch microgels increase *Lactobacillus casei* viability under simulated gastrointestinal environments (Pankasemsuk et al., 2016).

In a study, cultures of *B. bifidum* and *L. acidophilus* were coated with a blend of calcium alginate-corn starch; their vitality levels in yogurt and artificial gastrointestinal system were examined, and it was determined that the utilization of corn starch enhanced the vitality, but the increase was not significant with the effect of acidity and bile salts and the death rate of the coated bacteria was lower during the storage period (Açu et al., 2014).

L. rhamnosus KPb7 and *L. acidophilus* KPb4b strains have been coated by electrostatic vibration/dropping method, using

alginate+manukol and alginate+starch, and their various specialties were studied. It has been stated that the most suitable values for storage conditions are alginate+starch coating at 5ºC; alginate+starch microcapsules have the best protection in the toxicity trial and these capsules are the best combination for maintaining vitality (İşleyen, 2010).

2.1.1.6. **Cellulose and its derivatives**

Cellulose acetate-phthalate (CAP), acetylcellulose, carboxymethylcellulose, methylcellulose, hydroxypropyl methylcellulose, hydroxypropyl cellulose, hydroxyethyl cellulose, phthalate, cellulose acetate-butylate, ethylcellulose, microcrystalline cellulose, nitrocellulose are cellulose derivatives which are used as coating material (Geniş and Tuncer, 2019; Pech-Canul et al., 2020).

CAP is insoluble in acidity below pH 5, but soluble in pH 6. Thanks to these features, they enable a large number of living probiotic cells, which are resistant to the acidic conditions of the digestive system, to be transported to the colon (Sarao and Arora, 2017). CAP is successfully applied in the emulsion method.

In a study, CAP and beeswax layered coating were applied on *Bifidobacterium pseudorandom*, and its viability in the stomach environment was monitored. It was observed that the vitality was significantly preserved with the coating application (Geniş and Tuncer, 2019).

Semi-synthetic anionic polysaccharides, namely carboxymethyl cellulose (CMC) and carboxymethyl chitin (CMCH), are derived from cellulose and chitin, respectively. A study conducted by employing these materials for microencapsulation of *L. acidophilus* put forth that these coating materials improved probiotic viability during simulated gastrointestinal transit. In a different examination, *L.plantarum* was coated with CMC and κ-carrageenan and this application was also found to be successful. *Bifidobacterium* coated with CMCH and sodium alginate was examined under simulated in vitro gastrointestinal conditions and it was found that coating application increased the survival rate of bacteria (Pech-Canul et al., 2020).

2.2. Protein-based coating materials

What enables proteins to be a good coating material are their large molecular weights, containing water-soluble and insoluble groups together, interacting within themselves and with a wide variety of substances, and having technological properties such as flexibility of their molecular chains and solubility. Gelatin, whey protein isolate, concentrated whey protein, egg white, casein, and caseinates as animal origin proteins, and pea, chickpea, corn, wheat, and soybean proteins as vegetative proteins are among the proteins used as coating materials (Pech-Canul et al., 2020).

Albumin, whey proteins, and soy proteins come to the forefront with good emulsifying and gelling specialties and are among the ideal materials for microencapsulation by the coacervation method. Coating *Lactobacillus acidophilus* by electrospraying and fluid bed drying method using albumin and stearic acid; encapsulation of *L.plantarum* by extrusion method using soy protein isolate and alginate; coating *Lactobacillus acidophilus* by spray drying method employing maltodextrin and soy extract are some of these studies (Açu et al., 2014).

Soy protein isolates are a high-quality protein source for vegetarians and people with milk allergies. It contains features suitable for coating technology such as emulsification and gelation (Açu et al., 2014; González-Ferrero et al., 2018).

Chickpea proteins are also preferred in microencapsulation applications. The main reasons for this are that it has high nutritional value, does not show allergic properties, has anti-oxidation and GRAS properties, and has emulsifying properties. It has been reported that *Bifidobacterium adolescentis* is protected against artificial gastric fluid in capsules formed with chickpea protein-alginate (Geniş and Tuncer, 2019).

2.2.1. Gelatin

Gelatin, which is used in many areas in the food industry, has capabilities such as foaming and maintaining its foam for a long time, easy emulsion and suspension formation, and film formation (Açu et al., 2014).

For probiotic microencapsulation, gelatin needs to be used in combination with other materials to obtain certain features like desired molecular weight, stickiness, viscosity, and gel strength. In addition, since it is of animal origin, it is not suitable for consumers with vegetarian or kosher tendencies (Açu et al., 2014).

Gelatin is a good coating material in various microencapsulation methods like complex coacervation, extrusion, spray drying, spray cooling, and lyophilization (Açu et al., 2014).

It has been shown that encapsulation of probiotics in alginate-gelatin microgels created by electrostatic complexing increases the viability of

L. salivarus Li01 following elevated temperature applications, longstanding storage, and gastrointestinal transit (Yao et al. 2020).

2.2.2. Whey proteins

Whey proteins, which have good gelling properties, are effectively used in the coating of probiotic organisms. They are preferred as coating materials due to their high nutritional value, easy dissolution, low viscosity in solution, and good emulsification because of create strong gel (Martín et al., 2015). These features enable them to be used in the encapsulation of probiotics and to increase their viability potential even under digestive system conditions (Geniş and Tuncer, 2019).

The ex vivo survival of *Lactobacillus rhamnosus* GG which is coated and uncoated with whey proteins in the digestive tract of pigs was examined; it has been shown that the acid resistance of the coated microorganisms is increased, the adsorption capacity is high and the controlled release of microorganisms occurs within 30 minutes of intestinal incubation, and the usage of whey proteins for this purpose is appropriate (Doherty et al., 2012).

2.2.3. Milk proteins

Milk proteins displaying good gelling properties can be used for the microencapsulation of probiotic microorganisms. Sodium caseinate is widely recognized as the prevalent type of casein utilized as a coating agent and one of its important features is being resistant to heat denaturation (Altun and Özcan 2013; Geniş and Tuncer, 2019). Sweet whey has proved to be an effective alternative in the microencapsulation process through spray drying of *Bifidobacterium lactis* (Açu et al., 2014).

3. Microencapsulation methods

Today, interest in probiotic microorganisms, whose positive effects on health have been proven, is increasing. Unfortunately, these creatures not in a sufficiently stable structure cause inhibition in production, storage, and digestive system and prevent them from providing the expected benefits. Hence, various microencapsulation techniques with different coating materials have been advanced to prepare highly stable probiotics for the food industry. Several research studies have demonstrated that microencapsulation techniques, such as microgels or other different kinds of microcapsules, can be utilized for the development of probiotics (Qin et al., 2014; González-Ferrero et al., 2018; Marcial-Coba et al., 2018; Mirtič et al., 2018; Lee et al., 2019). These particles have sizes in the range of 1 to 1,000 μm. These systems can be designed in various methods to enhance viability in probiotic cells. Firstly; they can be engineered to create a physical barrier that shields probiotics from harmful elements in their surroundings like digestive enzymes, gastric acids, or bile salts. Secondly; it can be designed so that probiotics can be encapsulated with certain micronutrients like proteins, lipids, dietary fibers, carbohydrates, or minerals that help them survive (Haghshenas et al., 2015; Pankasemsuk et al., 2016; Li et al., 2016; González-Ferrero et al., 2018; Yao et al., 2020). Third; they can be designed using additives such as antacids for controlling local pH to provide a favorable environment for probiotics (Li et al., 2016).

The principle in the encapsulation of bioactive materials is to prevent leakage from the inside or the environment. Encapsulation techniques include; extrusion, emulsion, spray cooling, spray freezing (freeze drying), spray drying, liposome entrapment, fluidized bed, coacervation, inclusion complex, rennet gelled protein encapsulation, hybridization system, colliding aerosol technology, electrospinning, electrostatic deposition, ultrasonic vacuum spray drying, two-stage drying, removal of water by centrifugation, co*crystallization, complexing and rotational suspension methods (Ünal and Erginkaya 2010; Qin et al., 2014; Açu et al., 2014; Martín et al., 2015; Geniş and Tuncer, 2019; Yao et al., 2020).

Emulsion, extrusion, phase separation, and spray drying are the most frequently employed techniques for coating probiotic organisms. These methods can also be applied in combination (Cook et al., 2012).

While probiotics are encapsulated in the gas phase by spray drying technique, which is a physicomechanical method, they are encapsulated in liquid in extrusion and emulsion techniques. In all of these methods, probiotics show viability of over 90% (Palamutoğlu et al., 2013).

By using different microencapsulation methods, microcapsules with a wide variety of particle sizes are produced. Microcapsules with particle size of 3-612 μm are achieved by using spray drying; 79-83 μm by spray cooling; 1000-1400 μm by spray freeze drying; 70-80 μm by lyophilization; 0.3-600 μm by electrospray; 56-133 μm with fluid bed drying; 15-3500 μm by extrusion and 110-1250 μm by its improved version: vibrating nozzle technology, 55-2250 μm by emulsification and 10-3000 μm by coacervation method (Pech-Canul et al., 2020).

Table 1. Some studies on microencapsulation of probiotic microorganisms

Probiotic type	Coating material used	Coating method used	Effect on vitality	Reference
Lactobacillus spp.	alginate was coated with chitosan	-	It was determined that the number of live bacteria in milk and yogurt was higher than in uncoated milk and yogurt during the storage period.	Krasaekoopt et al., 2004
Lactobacillus reuteri	alginate	extrusion or emulsion technology	While a logarithmic decrease of 2.6 units was observed in dry fermented sausages, a logarithmic decrease of 0.5 units occurred in dry fermented sausages containing *L. reuteri* added without encapsulation.	Muthukumarasamy and Holley, 2006
Lactobacillus plantarum LAB12	xanthan and alginate	-	It has been proven to increase the viability of probiotic microorganisms and provide better protection against high temperatures and low pH.	Pech-Canul et al., 2020
Lactobacillus plantarum	soy protein isolate and alginate	extrusion	increased probiotic viability	Açu, 2014
Lactobacillus plantarum	CMC and κ-carrageenan	-	increased probiotic viability	Pech-Canul et al., 2020
Lactobacillus casei	gellan gum, xanthan gum, and sodium caseinate gellan gum mixtures	-	It was observed that the survival rate of probiotic microorganisms increased in the environment where gastric fluid and bile salts were simulated.	Pech-Canul et al., 2020
Lactobacillus casei	alginate-starch microgels	-	increases vitality	Pankasemsuk et al., 2016
Lactobacillus acidophilus ATCC4356	sodium alginate and carrageenan	-	increases the survival level of probiotic cells	Afzaal et al., 2019
Lactobacillus acidophilus	albumin and stearic acid	electrosprayin g and fluid bed drying method	increased probiotic viability	Açu et al., 2014
Bifidobacterium bifidum and *Lactobacillus acidophilus*	calcium alginate-corn starch	-	It was determined that the utilization of corn starch enhanced the vitality, but the increase was not significant with the effect of acidity and bile salts and the death rate of the coated bacteria was lower during the storage period.	Açu et al., 2014
Lactobacillus acidophilus	maltodextrin and soy extract	spray drying	increased probiotic viability	Açu et al., 2014
L. acidophilus	carboxymethyl cellulose (CMC) and carboxymethyl chitin (CMCH)	-	probiotic increases vitality	Pech-Canul et al., 2020
L. rhamnosus KPb7 and *L. acidophilus* KPb4b	alginate+manukol and alginate+starch and their various specialities	electrostatic vibration/dropping method	alginate+starch coating at 5°C; has been observed as the best protection	İşleyen, 2010
Lactobacillus rhamnosus GG	whey proteins	-	It has been shown that the acid resistance of the coated microorganisms increases, the adsorption capacity is high, and the controlled release of microorganisms occurs within 30 minutes after intestinal incubation.	Doherty et al., 2012
L. salivarus Li01	alginate-gelatin	electrostatic complexing	High-temperature treatments have been shown to increase the viability of *L. salivarus* LiO1 after long-term storage and gastrointestinal transit.	Yao et al., 2020
Bifidobacterium pseudolangum	CAP and beeswax	-	viability was observed to be significantly preserved	Geniş and Tuncer, 2019
Bifidobacterium	CMCH and sodium alginate	-	found to increase the survival rate of bacteria	Pech-Canul et al., 2020

Bifidobacterium lactis	sweet whey	spray drying	preserves its vitality	Açu et al., 2014
Bifidobacterium adolescentis	chickpea protein-alginate	-	It was reported that it was protected against artificial gastric fluid.	Geniş and Tuncer, 2019
Kluyveromyces marxianus VM004	water-soluble chitosan and concentrate	spray drying	It has been observed that 95% viability is observed in gastrointestinal cases.	Braber et al., 2020

3.1. Emulsion

In the emulsion technique, the suspension containing the microorganisms forming the discontinuous phase is added into vegetable oil (sunflower oil, corn oil, canola oil, or soybean oil) forming the continuous phase, and is continuously mixed to ensure homogenization. Thus, a water-in-oil emulsion is formed. At this stage, it is desirable that the polymers do not dissolve in the oil so that small gel particles can form in the oil phase, and that the internal phase creates a small particle size. In this way, smaller microparticles can be formed. The size of these formed bead-like structures can vary between 25 μm and 2 mm depending on the shaking speed (Heidebach et al., 2012; Martín et al. 2015). It is successfully implemented in the coating of lactic acid bacteria. Emulsifying agents (for example, Tween 80 and lauryl sulfate) reduce the interfacial tension of the two immiscible phases, produce higher homogenization, and may be utilized to prepare smaller capsules. The emulsification method is a low-cost method, unlike extrusion, it is easily scalable (Krasaekoopt et al., 2004; Pech-Canul et al., 2020).

To increase the quality of the emulsion, emulsifiers which will reduce the surface tension and allow the formation of smaller particles can be added to the mixture. Alginate and its combinations, carrageenan, chitosan, sodium carboxymethyl cellulose, gelatin, and chickpea protein are the preferred support materials in this method (Martín et al., 2015).

3.2. Extrusion

Because of being simplicity, ease of application, and low cost, the most commonly used and oldest method is extrusion. However, the most significant drawback of this technique is the slow solidification of the coating of the microcapsules, which makes high-capacity production difficult. In this technique, hydrocolloids are used as coating material. It is applied as microorganisms are added to a prepared solution and hardened by forming small beams of bubbles in the solidifying solution (mostly $CaCl_2$ solution) with the help of a syringe cap. Needle head and free fall distance have an effect on the shape and size of the bead-like structures created. In this technique, generally, alginate is employed as a supporting material (Krasaekoopt et al., 2004; Ünal and Erginkaya 2010; Uran et al., 2017). Extrusion is mostly known as a low-temperature encapsulation method. It is generally used for coating flavoring agents. Generally, sucrose, maltodextrin, glucose syrup, glycerin, and glucose are used as coating material (Anonymous, 2020).

A developing version of the extrusion process is the vibratory nozzle method. It is a mechanical technique and the droplet size varies according to the jet diameter, the velocity of the extruded liquid, viscosity, surface tension, and frequency of vibration. In recent years, it has also started to be utilized in the microencapsulation of probiotics (Pech-Canul et al., 2020).

It has been reported that there is no sensory difference in microcapsules in which emulsion and extraction techniques are applied and even no substantial difference in the viability rates of probiotics. The process step that should be taken into account in the application of these techniques is homogenization due to its effect on the viability of bacteria and capsule size (Ünal and Erginkaya 2010).

3.3. Spray drying

Most methods are based on drying, as the encapsulated ingredients are usually in liquid form. In this method, generally, carbohydrate-based materials are used as coating material or carriers (Anonymous, 2020).

In this method, capsule formation is provided by atomization of the microbial cell suspension in a polymeric solution into hot dry air and quick evaporation of the water in the environment. The microencapsulated dry, powdery product is divided from the carrier air in a cyclone. For product optimization in spray drying, conditions like air flow, inlet and outlet air temperatures, feed temperature, and product feed rate should be adjusted accordingly (Uran et al., 2017).

Since probiotic bacteria are sensitive to temperature, the temperature of the air inlet and outlet of the system should be adjusted very carefully in spray drying applications. While high temperatures may cause inhibition of probiotics, when the inlet air temperature is low, problems may occur in the evaporation of water (Uran et al., 2017). It is possible to obtain probiotics with the desired particle size and preserved viability when combined with other methods (such as double-stage drying, spray freezing, freeze drying, ultrasonic vacuum spray drying) by providing optimum temperature (Martín et al., 2015).

Adjusting the feed temperature well also has an effect on the viscosity. Although it seems like an advantage to be a continuous and simple system, when mass production is planned in spray drying systems, there are also difficulties such as being mechanized in a large area, requiring equipment, and high installation and operating costs (Martín et al., 2015).

There are studies on *Bifidobacterium lactis* treated successfully with the sweet whey in the spray drying method, on the microencapsulation of *L. acidophilus* by spray drying employing maltodextrin and trehalose, and on the successful application of alginate and octenyl succinate starch (E1450) by spray drying method (Pech-Canul et al.,2020).

3.4. Spray cooling

In this method, which was developed to prevent microbial organisms from being damaged by the effect of high temperature applied in the spray drying process, a system similar to the spray drying line is used, but instead of heat application, cold carrier air or a cold chamber is used (Uran et al., 2017).

Spray drying and spray cooling methods for microencapsulation of probiotics are alike in many aspects; both methods contain dispersing the core material through atomization into a chamber that allows the coating to solidify. Then, the created microcapsules are divided from the moist air by a filter or a cyclone so that they are collected in powder form. The basic divergence between these methods is the temperature of the chamber at the stage of solidification of the coating. While hot air causes quick evaporation of the solvent in which the coating material is dissolved during spray drying, in spray cooling solidification is carried out by atomizing the hot melt mixture of coating materials in a cooled environment below the melting point (Pech-Canul et al., 2020).

Spray cooling is also called spray concretion. A molten matrix containing the bioactive compound is atomized in order to form droplets that solidify rapidly in contact with cold air. Spray cooling is accepted as the cheapest encapsulation technology with industrial-scale production capability. This technology can be employed to produce smaller beads, which is desired in the food industry (Martín et al. 2015). These are the technologies that are used to produce lipid-coated active compounds (Anonymous, 2020).

3.5. Spray freezing

It is also called freeze drying, lyophilization or cryodixication. This method is a drying process in which the solvent medium is frozen and later sublimated in a low-pressure environment (direct transition from solid phase to gas phase). In this application, the viability of probiotic cultures is affected by high osmotic pressure and formed crystals. For this reason, materials such as whey protein, milk powder, maltodextrin, and glucose, which exhibit cryoprotectant properties (protecting cells at the freezing point), are added to the medium. The coating material and probiotic cells are sprayed into the chamber (into a cryogenic such as liquid nitrogen) where the spray droplets are quickly frozen. Later on, the frozen droplets are lyophilized to remove the solvent and produce dried particles (Martín et al., 2015; Pech-Canul et al., 2020).

Compared to the spray drying method, this technique offers advantages such as capsule formation with more controlled particle size and specific surface area, prevention of aroma losses, and superior reconstitution qualities of dried products, while having disadvantages such as being 30-50 times more expensive than spray drying, high energy consumption, long processing time. In addition, the capsules can be covered with an additional shell against adverse environmental conditions (Koç et al., 2010; Martin et al. 2015).

3.6. Coacervation

Coacervation occurs as a consequence of the separation of the liquid phase of the coating material from the polymeric solution and the coating phase wrapping the core material in a homogeneous layer. Coacervation technology is not a preferred method in the food industry because of is a very complex process and has a high cost (Anonymous, 2020).

Microencapsulation by coacervation is essentially comprised of three stages carried out under continuous agitation. The first stage contains the creation of three immiscible chemical phases

(mounting fluid, core medium, and coating material). The second stage involves the dispersion of the core material in the polymer coating solution, and the final stage involves the hardening of the coating material to form the coating. Egg whites (albumin), whey proteins, and soy proteins are ideal coating materials for microencapsulation through the coacervation process (Pech-Canul et al., 2020).

3.7. Electrospinning method

The method employed for microencapsulation known as electrospraying is founded on the principle of electrohydrodynamics, so it can also be called as electrohydrodynamic atomization. This method applies electrical forces for the atomization of liquids.

The process typically involves the flowing of a liquid including the core material from the tip of a mold that acts as an electrode and applying a high-voltage electric field to the resulting droplets. Solidification takes place in different ways such as chemical hardening or solvent evaporation. This technology can be incorporated with other microencapsulation techniques to enhance its effectiveness as well. The electrospray extrusion technique has been successfully used for probiotic microencapsulation until today. Using this method, microcapsules can be formed by carbohydrate or protein-based matrices (Zhang, 2020).

The electrospinning technique has advantages such as producing very thin fibers or capsules of a few nanometers with large surface areas, being a one-step, easy-to-apply and inexpensive method. In addition, large-scale productions being able to be performed due to the simplicity of the technique enable this method to be used in many areas (Martín et al., 2015; Pech-Canul et al., 2020).

3.8. Layer by Layer method (LbL)

The Layer-by-Layer (LbL) method relies on the chemical electrostatic attraction of materials with opposite charges. To form microcapsules using LbL, layers are formed by self-assembly via electrostatic adsorption of materials with opposite charges onto the surface of the core material. This approach is very effective as an application for creating multilayer capsules.

Moreover, the number of coating layers can be increased through multiple repetitions of the process. The core material is then subjected to fluidized bed drying, where it is fluidized in the gas phase and combined with the coating material as particles or thin droplets. Because of electrostatic forces, the coating material accumulates on the core material's surface, creating a layer (Pech-Canul et al., 2020).

3.9. Fluidized bed

This method, which was developed in the 1950s, was used for the coating of tablet drugs, and today it is used for coating many solid and functional materials, including probiotic microorganisms.

The fluidized bed provides the drying process with the principle of contact of the product with the hot air. Solid particles applied with pressurized gas act by displaying liquid characteristics. Pressurized gas getting out of the distributor moves along the bed and exerts a force on the particles against gravity. If a drying process is desired, temperature application can be made. The coating of probiotic cells with this method relies on the principle of spraying on inert carriers. The dissolved coating material within a suitable solution is sprayed on the probiotics moving towards the walls in the hot and cold air flow in the coating room and it is ensured to surround the particles suspended in the air. While low cost and temperature control are the advantages of this system, its need for expertise and long process time are its disadvantages. In this technique, starch and cellulose derivatives, gums, and proteins are mostly used as coating material (Açu et al., 2014; Martín et al. 2015).

The highest rate of vitality and bioactivity is expected for bioavailability from probiotics. For this purpose, the use of powder forms of bacterial cultures provides both more resistance to environmental factors and easier use. Amin, et al. (2013) stated that among the many coating techniques, the coating of dried microorganism powders with the fluidized air bed technique for encapsulation is the most promising technology so far.

3.10. Double microencapsulation

To form microcapsules it is beneficial sometimes to use a single and sometimes a multilayer biopolymer. In double microencapsulation, the protective properties of bacterial cells can be increased by further modification of co-encapsulation. For example, double microencapsulation is performed by re-coating and developing co-encapsulated microcapsules containing probiotic+alginate+prebiotic with other coating materials such as chitosan, alginate, or carrageenan. While prebiotics provide a source of carbohydrates, alginate protects the probiotics in the microcapsule, and additional polymers ensure that the surface of the probiotics is completely covered (Palamutoğlu et al., 2013).

Chitosan is the most widely utilized material for this aim due to having a positive charge. It was shown that the capsule which consists of a calcium alginate and chitosan coating enhanced the viability of encapsulated probiotics incubated under a simulated gastrointestinal environment. It has been suggested that alginate chitosan systems are disrupted by the intestinal microflora in the colon, thus probiotics released here have good potential for colon targeting (Mirtic et al. 2018). Nonetheless, a new in vitro study demonstrated that coating alginate-preserved capsules also with chitosan did not enhance probiotic viability compared to employing alginate-coated capsules alone (Yeung et al., 2016). Since it is an effective antimicrobial agent, it is known that direct chitosan coating applications affect the viability of bacteria (Speranza et al., 2018).

Li et al. (2016) showed that the probiotics are retained in the stomach and then released in the small intestine by fortifying bacteria coated with cellulose by calcium alginate. It has been reported that this method significantly increased the survival rates of Bifidobacterium.

More recently, utilizing whey protein and alginate to increase the viability of *L. acidophilus*, a type of microparticle with a diameter ranging from 107 to 222 μm, with a tri-level layer and with an encapsulation efficiency of more than 80% has been developed (de Araújo Etchepare et al., 2020).

Complex coacervation has been stated to be advantageous for high cell capture efficiency, improved functional performance, and availability for scaling (De Prisco and Mauriello, 2016). Furthermore, complex coacervates can be designed to enhance the complete release of encapsulated probiotics from biopolymer microgels (Bosnea et al., 2017).

Recently conducted studies have shown that combinations of various biopolymers are highly suitable for probiotic administration. Yao et al. (2020) reported that combined applications of whey protein isolate/gum arabic; whey protein isolate/carrageenan; whey protein isolate/gum arabic/alginate; gelatin/gum arabic; gelatin/alginate and starch/alginate gave very good results.

Besides, alginate-starch microgels have been proven to increase the viability of probiotics (*Lactobacillus casei*) under a simulated gastrointestinal environment (Pankasemsuk et al., 2016).

Pech-Canul et al. (2020) reported that a coating of egg albumin and stearic acid is used for protecting *Lactobacillus acidophilus* via electrospraying and fluid bed drying; in a similar way, a mixture of alginate and soy protein isolate has been utilized in the form of coating material for microencapsulation of *L. plantarum* by extrusion.

Within the scope of recent studies, probiotic microbes (*Bacillus coagulans*) were coated with chitosan/alginate bilayers employing the LbL method. In in vivo and in vitro experiments conducted, it was observed that the viability of these bacteria increased substantially in the upper gastrointestinal tract. In addition, LbL-coated microbes showed greater adhesion to the mucosal surface than free cells and this has been explained by the strong adhesive specialities of chitosan and alginate which were employed for creating the coatings. The bilayer (chitosan/alginate) coating affected viability in simulated stomach and small intestine environment (Anselmo et al., 2016).

4. Some problems with the design of microencapsulated probiotics

Various factors must be taken into account while designing microcapsules to maintain the viability of probiotics in food products. Attention should be paid to aspects such as low and controlled particle size, dry microcapsules being prepared in higher stability, easier handling, storage of cultures, and limited effect on the sensory specialties of the final product, particularly on the structure. Considering the number of damaging factors faced in the processing and storage process, the improvement of multiphase microcapsules employing coating materials having multiple barrier features seems to be the most promising method to ensure the efficiency of the process. Although promising at a laboratory scale, technologies developed for producing gel capsules pose serious challenges for large-scale production (Amin et al., 2013).

Since the size of particles is so small to contain bacteria, most of the colloidal delivery systems that have been created to encapsulate small molecules (like vitamins, colors, nutraceuticals or flavors) have proven inappropriate for probiotics. Microbial cells typically range in size approximately from 1 to 10 μm, whereas many colloidal systems like nanoemulsions, microemulsions and biopolymer nanoparticles are smaller than approximately 1 μm. Also, the concentration of live probiotics found in products manufactured commercially should ordinarily be higher than approximately 6-7 log10 cfu/g for enhancing health benefits, meaning that the loading capacity of any colloidal system must be high. Probiotics can be encapsulated in the formulation of supplements like tablets that are large enough to contain a large number of

microorganisms. However, the probiotics embedded in these formulations may not reach the human colon properly because they are too large to pass directly through the pyloric sphincter. Therefore, they can break down and release probiotics in the stomach where they are prone to spoilage due to harsh conditions. However, if probiotics are loaded into very large colloidal particles, they can adversely affect the sensory and textural properties of foods. Moreover, many of the colloidal systems developed to encapsulate probiotics do not provide adequate protection in the gut. For example, biopolymer capsules are highly porous and allow the passage of gastric acids and enzymes into their bodies, which can disrupt the structure of probiotics. In addition, many colloidal application systems developed in research laboratories are not suitable for commercial application due to their high cost, elaborate processing requirements, or use of ingredients that are unsuitable for application in the food industry. Any probiotic delivery system should be designed to remain free in the colon. In addition, it must be able to attach to the inner surface of the colon and colonize, otherwise, it will not be bioavailable as it will be excreted with feces. For these reasons, an effective encapsulation process is needed (Yao et al., 2020).

5. Conclusion

Consumption of probiotic foods, which stand out with the functional properties they have, has an important place among today's nutrition trends. In particular, the undeniable effects of colon microbiota on human well-being and health increase the appeal of probiotics. In order to provide the necessary benefit, foodstuffs must include $1.0x10^6$ cfu/g live probiotic cells at the minimum. However, they are inhibited by being catabolised in production, storage, and gastrointestinal system, and they cannot provide the expected benefit sufficiently.

In recent studies aiming to improve the viability and bioactivity of probiotics, the microencapsulation technique, which is used in many areas industrially but has limited application in the food field, draws attention. With these techniques applied in recent years, it is aimed to provide maximum benefit to the consumer by ensuring that probiotic cultures are resistant to adverse conditions for their viability and released at the desired time.

Due to the negativities experienced in the encapsulation of living organisms with the current method and coating materials, it is not yet possible to see the products containing microencapsulated probiotics on the shelves at the desired rate. The methods applied will be developed together with the developing technology and more various coating materials will be evaluated. Another problem is that the studies conducted out remain at the laboratory scale and there are problems in industrial applications. Studies should be conducted on techniques that will allow large-scale and continuous production.

The fact that there are many studies to be done on the microencapsulation of probiotic microorganisms makes this subject an area of research opportunity. In addition, in vivo and in vitro trials should be added to technical studies conducted and deeper information should be obtained about the survival advantages and bioavailability of probiotics.

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