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The Functionality of Probiotic Bacteria Microencapsulation by Spray Drying: A Literature Review

Fungsionalitas Mikroenkapsulasi Bakteri Probiotik dengan Pengeringan Semprot: Kajian Literatur

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Abstract

Probiotic-based products are associated with many health benefits. The viability of probiotics is necessary to provide health benefits, but it is lost during processing, storage, and gastrointestinal tract. The viability of probiotics can be maintained by applying the spray drying encapsulation technique. This review article discusses probiotic bacteria, encapsulant ingredients, the principle of spray drying in microencapsulation, and the functional properties of spray drying probiotic microcapsules. This article uses a non-research method with a literature review of various sources such as research journals and related books. Based on existing studies, the viability of probiotic spray drying results is influenced by the bacterial culture used, the type and concentration of the encapsulated material, and the spray drying conditions (feed temperature, inlet temperature, and outlet temperature). Probiotic microcapsules spray drying has excellent potential in functional food formulations, and its commercial applications will benefit both the industry and consumers.
Keywords: functionality, microencapsulation, probiotics, spray drying, viability

Abstrak

Produk berbasis probiotik sering dikaitkan dengan banyak manfaat kesehatan. Viabilitas probiotik diperlukan untuk memberi manfaat kesehatan, tetapi hal tersebut hilang selama pemrosesan, penyimpanan, dan melewati saluran pencernaan. Viabilitas probiotik dapat dipertahankan dengan penerapan teknik enkapsulasi pengeringan semprot. Review artikel ini membahas mengenai bakteri probiotik, bahan enkapsulasi, prinsip pengeringan semprot dalam mikroenkapsulasi, dan sifat fungsional mikrokapsul probiotik pengeringan semprot. Penulisan artikel ini menggunakan metode non-research *dengan* literature review *berbagai sumber, seperti jurnal penelitian dan buku yang terkait. Hasil review artikel memberikan informasi bahwa viabilitas probiotik hasil pengeringan semprot dipengaruhi oleh spesies dan strain bakteri yang digunakan, jenis dan konsentrasi bahan enkapsulasi, serta kondisi pengeringan semprot (suhu umpan, suhu masuk, dan suhu keluar). Mikrokapsul probiotik pengeringan semprot memiliki potensi besar dalam formulasi pangan fungsional sehingga aplikasi komersialnya akan menguntungkan industri dan konsumen.*
Kata kunci: *fungsionalitas, mikroenkapsulasi, pengeringan semprot, probiotik, viabilitas*

INTRODUCTION

Probiotics, commonly known as good bacteria, can benefit human health if consumed sufficiently. Probiotics often used in milk processing come from the lactic acid bacteria (LAB) group. The most common probiotics used in food products are Lactobacillus and Bifidobacterium (Eratte et al., 2015). These good bacteria are generally found in dairy products and processed products, such as cheese, buttermilk, ice cream, and yogurt (Fritzen-Freire et al., 2012).

The probiotics addition to food can provide a wide range of benefits in disease prevention efforts, such as stimulating the immune system, stimulating calcium absorption, and inhibiting the growth of foodborne pathogenic microbes. Probiotics can also play a role in lowering cholesterol, balancing the number of microflora in the intestine, increasing the synthesis of vitamins (nico-

tinic acid, folic acid, and vitamin B), and improving cardiovascular disease (Panghal et al., 2019). Probiotics improve health by attaching and colonizing the intestines, strengthening the epithelial barrier, producing metabolites in the form of acids, peroxides, or bacteriocins, inhibiting the adhesion of pathogens, competing with pathogenic bacteria to increase the mucosa, and stimulating the immune system (Huang et al., 2017; Setiarto et al., 2018).

Probiotics must have high viability (number of living cells) and have survival properties in the intestine to benefit human body health. Probiotics are very sensitive to acidic pH, oxygen toxicity (aerobic conditions), time, and storage temperature (Prasanna et al., 2014). The number of probiotic bacteria cells that must be contained in a probiotic food product is at least 106-108 CFU/g of the product (FAO/WHO, 2002). The technological challenge for producing foods with probiotic claims is maintaining the cell viability of microorganisms added to foods under processing, storage, distribution, and consumption conditions.

One way to maintain the viability of probiotics is microencapsulation. Microencapsulation of probiotics is a method of wrapping probiotic cells with a coating material that can provide protection during processing, storage, and human gastrointestinal tract (Sultana et al., 2000; Dolly et al., 2011). Several probiotic microencapsulation techniques such as extrusion, emulsion, freeze-drying, and spray drying have also been proposed to increase the viability of probiotics during food processing, storage, and passage through the human gastrointestinal tract (Anal & Singh, 2007; Amin et al., 2013). Microencapsulation of probiotics by the spray drying method has been widely studied and is commonly used in the food industry because of its lower energy costs, high productivity, fast processing, homogeneous yield, and stability during storage (Sarkar, 2020). This review article discusses the characteristics of probiotics, encapsulation materials, the principle of spray drying in microencapsulation, and the functional properties of probiotic microcapsules using the spray drying method.

METHODS

The methods used in the literature review include searching for research topics, collecting journals for an online database, determining the topics to be reviewed (based on the year of publication, language, sample intervention, learning outcomes, research methods), selecting research methods (covering research design, methods of sampling, data collection techniques, data analysis, results, conclusions), and the last stage is the synthesis of topic search results including current knowledge topics, determination of research topics as needed, explanation of research findings, description of research quality (Fink, 2014).

The topic of microencapsulation of probiotic bacteria by spray drying was selected based on a predetermined method. This determination is because the knowledge gained from this topic can be used for further research development and is an excellent opportunity to implement functional food products. There are 67 libraries identified through a search on the database according to the topic, then sorting and elimination based on duplication so that the libraries considered appropriate according to the predetermined method are 51.

The next step consists of data analysis, interpretation, and confirmation based on the sorted literature. Information related to the functionality of microencapsulated probiotic bacteria with the spray drying method is discussed in more detail in prebiotic characteristics, encapsulation materials, spray drying principles in microencapsulation, and the functional properties of spray drying probiotic microcapsules.

DISCUSSION

Probiotics Characteristics

Probiotics are microorganisms that can provide health benefits to the host when consumed in minimum amounts of 106 – 108 CFU/g of the product (FAO/WHO, 2002). Probiotics can be from the lactic acid bacteria (LAB) and yeast groups. The probiotics from the LAB group that is most widely used include *Lactobacillus sp.*, *Bifidobacterium sp.*, *Lactococcus sp.*, and *Streptococcus sp*, while probiotics from the yeast group that often used in microencapsulation include *Saccharomyces cerevisiae* and *Saccharomyces boulardii* (Arslan, Erbas, Tontul, & Topuz, 2015).

All probiotics can withstand different heating, processing, or storage conditions. *Streptococcus* is usually more resistant than *Lactobacillus* to spray drying. For example, *S. thermophilus* CCRC14085 survives better than *L. acidophilus* CCRC 14079 in spray-drying fermented soy milk (Wang et al., 2004). *Lactobacillus* species such as *L. acidophilus, L. casei, L. fermentum, L. paracasei, L. rhamnosus*,

and L. salivarius are often used in the microencapsulation method by spray drying (Huang et al., 2017). *L. plantarum* is a relatively strong stress tolerance species compared to other genera of *Lactobacillus* (Ferrando et al., 2015). Some probiotics have better resistance to the drying process. *Bifidobacterium longum* ATCC15708 and CCRC 14634 survived better spray drying than *B. infantis* CCRC14661 when using gelatin as encapsulation material. *B. longum* B6 survived better than *B. infantis* CCRC 14633 when using skim milk as encapsulation during drying (Lian, 2002).

The resistance characteristics of a bacterial strain should be an essential criterion when selecting probiotic bacteria. It was aimed to increase the final probiotic viability of the spray drying microcapsules. Thermal stress, osmotic, oxidative, and drying conditions are usually considered the main mechanisms causing bacterial inactivation during and after spray drying, as they affect the intracellular components that are lost from the cell to the surrounding environment (Assadpour & Jafari, 2019). Different bacterial species (or even strains) may exhibit variable tolerance to such stresses.

Encapsulation Material

The encapsulation material is commonly referred to as a coating, shell, carrier, or matrix. A good encapsulated material can play a protective role with the ability to release the probiotic core material at a controlled rate (Panghal et al., 2019). The nature of the encapsulated material, the diameter of the capsule, and the thickness of the coating also affect the viability of the cells. A thinner layer can cause the protective effect lost from encapsulation (Hamaguchi et al., 2018). The capsule walls are getting thicker, and the diameter of the particles formed is getting more significant if the concentration of encapsulating material is higher (Panghal et al., 2019). The choice of encapsulation material is based on physicochemical properties, solubility, viscosity in solution, suitability to the core material, and the desired microcapsule size (Chávez & Ledeboer, 2007). The encapsulation material must be soluble in water, have good film-forming, emulsifying properties, and dry quickly. These materials are usually carbohydrates, lipids, proteins, or other synthetic materials.

Carbohydrates (starch, pectin, maltodextrin, gum Arabic, and alginate) are usually used as encapsulation materials. These materials have poor interfacial properties and must be chemically modified to increase their surface activity. In contrast, proteins have amphiphilic characteristics with the physicochemical and functional properties required to enclose a hydrophobic core material. Protein compounds (sodium caseinate, soy protein isolate, and whey protein concentrate and isolates) are generally considered good encapsulators. Fructooligosaccharides (FOS), maltodextrin, gum Arabic, inulin, polydextrose, and skim milk powder are sources of prebiotics. If the material is mixed with probiotics, it can be considered a very effective synbiotic used as an encapsulation material (Panghal *et al*., 2019).

Many studies have also revealed that carbohydrates (trehalose, maltose, lactose, maltodextrin, inulin, and FOS) help stabilize membranes and proteins by lowering the glass transition temperature during the drying process. Carbohydrate-rich formulations with low molecular weight and high glass transition temperatures will obtain the highest level of protection during drying (Perdana, Fox, Siwei, Boom, & Schutyser, 2014).

Principle of Spray Drying in Microencapsulation

Microencapsulation is considered to be one of the effective methods in protecting the viability of probiotics. This statement is proven because microencapsulation can protect probiotics from adverse environmental factors, such as high and low pH environments, bile salts, oxygen, and other processing conditions (Gbassi & Vandamme, 2012; Sousa et al., 2012).

The basic principles in spray drying include atomization of cells in a polymer solution into the drying chamber via an atomizer, drying liquid droplets in the drying chamber leading to evaporation and formation of microcapsules, and separation of powders through cyclones (Martín et al., 2015). According to Shahidi et al. (2009), the spray drying step in microencapsulation involves four stages: the preparation of a solution or emulsion, homogenization of the solution, atomization of the feed into the drying chamber, and dehydration of atomized particles.

The mixture to be atomized is made by dissolving the core material (hydrophobic probiotics) into an immiscible coating solution. The solution is homogenized with or without the addition of a surfactant or emulsifier. It depends on the emulsifying properties of the encapsulating materials, as some of them have different interfacial activities. The emulsion formed must be stable for a specific time before spray drying (Liu et al., 2001). The feed atomization stage is then carried out continuously with four phases: feed atomization, spray

and air mixing, solvent evaporation, and product separation. The core material retention during microencapsulation by spray drying is affected by the emulsion composition, emulsion properties, and drying conditions. Operating conditions to obtain good viability should pay attention to suitable encapsulation materials and optimal spray drying conditions.

Table 1 shows the encapsulation efficiency (EE) values of various encapsulation materials and different drying conditions. The viability value indicates the EE value. The EE value will be higher if the viability value is high. Viability is usually calculated by comparing the probiotic colony-forming units (CFU) per gram of dry matter from the resulting microcapsules with the cell solution before drying (Heidebach et al., 2012). It indicates that viability is a composite parameter that describes the viability of living cells and successful binding during the encapsulation process. Most of the viability is below 100% due to damage to probiotic cells. Adverse conditions, such as heating, shear stress, or concentrated dissolved materials in the encapsulation process cause damage to the probiotic cells. The difference in viability values are strongly influenced by the species and strain of bacteria used, the type and concentration of the encapsulated material, and the spray drying conditions. The main factors in spray drying that must be optimized are feed temperature, inlet temperature and outlet temperature (Liu et al., 2001).

Viscosity and droplet size should be reduced as the feed temperature increases. Higher temperatures during spray drying result in intracellular membrane damage (Anekella & Orsat, 2013). The inlet temperature is directly proportional to the drying rate of the microcapsules and the final moisture content. If the inlet temperature is low, then the low evaporation rate leads to the formation of microcapsules with high membrane

Table 1. Probiotics microencapsulation with different encapsulation materials and spray drying temperatures

Probiotic Bacteria	Encapsulation Material	Spray Drying Temperature	Viability (%)	Reference
Bifidobacterium BB-12	Liquid whey + inulin (10:1 w/v) Liquid whey + polydextrose (10:1 w/v)	Ti[1] = 150 °C To[2] = 50±3 °C	~95 ~93	Pinto et al. (2012)
Lactobacillus reuteri DSM 20016	Fermented whey (20% w/v)	Ti = 89 ± 1 and 100 + 1 °C	~80	Jantzen et al. (2013)
L. plantarum MTCC 5422	FOS[3] + WPI[4], FOS + DWPI[5] (ratio 1:1 and 1:1.5)	Ti = 110 °C To = 55 ± 3 °C	~93	Rajam & Anandharamakrishnan (2014)
Lactobacillus plantarum ATCC 8014	Maltodekstrin (15% w/w), maltodextrin : chia seed mucilage: chia seed soluble protein (7.5:0.6:7.5% w/w)	Ti = 110 °C To = 75 – 80 °C	83 98	Bustamante et al. (2017)
Lactobacillus plantarum A17	WPI (pH 7.0; 10% w/w) WPI (pH 4.0; 10% w/w) DWPI (pH 7.0; 78 °C , 20 minutes, 10% w/w)	Ti = 110 °C, To = 68 – 70 °C	69 39,3 25	Khem et al. (2016)
Propionibacteria freudenreichii ITG P20	Sweet whey (30% w/w) + casei peptone (0.5% w/w)	Ti = 180 °C To = 73 °C	70	Huang et al. (2016)
Lactoacillus rhamnosus GG	Whey protein + micellar casein + chymosin (90:10 v/v solution, 12.5% w/w)	Ti = 170 °C, To = 55, 70, and 85 °C	~50	Guerin et al. (2017)
Lactobacillus acidophilus NCDC 016	Maltodextrin (20% w/v) + gum Arabic (0; 2.5; 5; 7.5 and 10% w/v)	Ti = 150 °C, To = 55 ± 2 °C	80,61	Arepally & Goswami (2019)
Lactobacillus plantarum NCIM 2083	FOS and whey protein (2:1), FOS and maltodextrin (2:1), FOS, whey protein, and maltodextrin (2:0.5:0.5)	Ti = 110 ± 5 °C, To = 62 ± 5 °C	~89	Yoha et al. (2020)

[1]Ti : Inlet Temperature, [2]To : Outlet temperature, [3]FOS : Fructooligosaccharides, [4]WPI : Whey Protein Isolate, [5]DWPI : Denaturated Whey Protein Isolate

density, high moisture content, poor fluidity, and ease of agglomeration. The high inlet temperature causes excessive evaporation and produces cracks in the membrane leading to premature release and degradation of the encapsulated material. Perdana et al. (2013) reported that the loss of viability of *L. plantarum* WCFS1 during drying occurred due to dehydration inactivation, thermal inactivation, or a combination of both types of inactivation. The outlet temperature cannot be controlled directly because it depends on the inlet temperature. The ideal outlet temperature for probiotic microencapsulation is between 50 – 80 °C (Huang et al., 2017). Using an outlet temperature of 60 - 80 °C in the probiotic encapsulation process with whey protein isolate, in general, will reduce the percentage of viability because the protein is denatured and cannot protect bacterial cells optimally. The viability of the bacteria produced will be lower if the outlet temperature is high.

The result of spray drying is fine microcapsules powder (10 – 400 μm). The results are influenced by the initial feed material and operating conditions (Gharsallaoui et al., 2007). The spray drying method is better than the extrusion method because it can produce more stable microcapsules with a small and homogeneous size and time efficiency. This method only takes 30 minutes, while freeze-drying takes 72 hours to obtain microencapsulations (Wisniewski, 2015). Table 1 summarizes the various encapsulated materials under the experimental spray drying temperature conditions optimized for microencapsulation of probiotics.

Emulsion stability before spray drying can be improved by using amphiphilic macromolecules and stabilizers, such as proteins and carbohydrates (Sapei et al., 2012). Proteins are good encapsulating agents and produce stable emulsions when combined with carbohydrates. Whey protein is often an efficient encapsulating agent for probiotic spray drying. Lutz et al. (2009) stated that the enzymatically charged complex whey protein isolate (WPI) could increase the stability of multiple emulsions and reduce the interfacial tension of oil and water. WPI can help stabilize cell membranes as a hydrophilic emulsifier that will bind to hydrophobic probiotic bacteria. Whey protein is suitable for encapsulation because it can produce high encapsulation characteristics and stability during storage. Whey protein wall material (resistant starch) can also play a role in reducing the solubility and release rate (Pérez-Masiá et al., 2015).

The use of carbohydrates with shorter chains (such as maltodextrin and inulin) acts as filling and matrix-forming agents. The addition of maltodextrin, which has high solubility characteristics with relatively low viscosity, helps protect the core material, minimizing oxygen exposure, creating a protective layer on the surface of the adsorbent particles, increasing the glass transition temperature of the material, and being heat resistant (Valenzuela & Aguilera, 2015).

Inulin is widely applied to food products because it is soluble in hot water (temperature 50-100 °C) and has a relatively low viscosity, so it is widely used as a gelling agent, fat substitute, and emulsion stabilizer (Shoaib et al., 2016). Inulin is prebiotic and can act as a dietary fiber with chemical bonds that enzymes cannot hydrolyze in the human digestive tract. These materials can be hydrolyzed and fermented by *Bifidobacterium* and *Lactobacillus* (Leyva-porras et al., 2014) to be used as encapsulation materials to encourage the proliferation of probiotic bacteria in the human digestive tract (Fernandes *et al*., 2017). Incorporating prebiotics in the encapsulated material may lead to a "synbiotic" effect. One example is the higher viability of *L. plantarum* after spray drying when combining galactooligosaccharides (GOS) in maltodextrin or fructooligosaccharides (FOS) in whey protein isolate (WPI) than without the addition of GOS or FOS (Rajam & Anandharamakrishnan, 2015b). Mucilage and soluble protein from chia seeds and flax seeds were also found to protect *L. acidophilus*, *L. plantarum*, and *B. infantis* during spray drying (Bustamante et al., 2017).

Functional Properties of Probiotic Microcapsules Spray Drying

Probiotics encapsulated by spray drying can maintain the functional properties of probiotics. These properties include being able to extend the shelf life of probiotics during storage, maintain the quality of probiotics, provide convenience for application in food products with their ability to withstand the processing process, be resistant to human digestive tract fluids and have immunomodulating abilities (Chen et al., 2015; Huang et al., 2017).

Spray drying microcapsules of *L. plantarum* MTCC 5422 using fructooligosaccharides (FOS) and whey protein isolate (WPI) as encapsulation materials were able to maintain their viability at levels >107 CFU/g after 60 days of storage at 4

°C. These microcapsules can also develop functional foods (Rajam & Anandharamakrishnan, 2015a). Jantzen et al. (2013) noted a decrease in the number of *Lactobacillus reuteri* after drying (2 log cycles) and after four weeks of storage (1 log cycle), but the survival rate of encapsulated bacteria was 32% higher compared to non-encapsulated when passing through the digestive tract at pH 3.0. The research results by Ivanovska et al. (2012) showed that spray-drying microcapsules of *Lactobacillus casei* using sodium alginate, chitosan, and FOS maintained viability above the therapeutic minimum during 24 hours incubation under simulated gastrointestinal tract conditions (8.31 ± 0.14 log CFU/g). Sultana et al. (2000) conducted a study on *L. acidophilus* which was encapsulated with micro-encapsulated calcium-alginate-starch then added to yogurt and stored for eight weeks at 4 °C. The results showed that the viability during the 8-week storage period in the encapsulated culture was better than that of the free cells.

The resistance of microcapsules during the food processing process was proven by Malmo et al. (2013) research, namely, spray drying microcapsules of *L. reuteri* DSM 17938 encapsulated with alginate and chitosan were significantly better than free bacteria. The percentage of probiotic viability was 10% after roasting in a chocolate souffle for 10 minutes at 180 °C. Bacteria encapsulated by spray drying generally showed higher heat resistance when compared to free bacteria without encapsulation. This statement is supported by Arslan-Tontul *et al*. (2019) research, which showed that the viability of spray-dried *L. acidophilus* microcapsules encapsulated with gum Arabic was better than the viability of free bacteria. The encapsulated bacteria were injected into the center of the cake mixture and baked at 200 °C for 20 minutes. The bacterial viability of the encapsulated bacteria was 2.5 log CFU/g, with the percentage of viability before and after baking was 42.4%, the viability of free bacteria was < 1.0 log CFU/g.

Probiotics or LAB cells (used as starters) are also frequently exposed to an acidic environment in food due to decreased pH during fermentation. Spray-dried probiotic microcapsules often survive better in acidic environments throughout their shelf life. The results of the study of Dimitrellou et al. (2016) showed that *L. casei* ATCC393 had increased viability in fermented milk during refrigerated storage compared to the viability of free bacteria.

Immunomodulatory ability is one of the essential functions of probiotics and has been extensively investigated. Páez et al. (2013) conducted an in vivo study with bacteria *L. acidophilus* A9, *L. paracasei* A13, and *L. casei* Nad encapsulated by spray drying using 20% (w/v) skimmed milk. Probiotic microcapsules were given to mice for 5 and 10 days. The significantly higher number of Immunoglobulin A (IgA) producing cells in the small intestine was induced by spray drying culture than the new culture. Immunomodulation by probiotics is mainly due to molecular interactions between the bacterial cell surface and host cells. The barrier effect of the encapsulated material can modify the interaction between the microbe and the host. The advantages of spray-drying probiotic microcapsules may be related to the barrier effect of the encapsulating material that can protect the bacterial surface structure from the digestive process, including pH-induced conformational changes, enzyme catalysis, and digestive tract shear strength (Huang et al., 2017).

CONCLUSIONS

Microencapsulation of probiotic bacteria by spray drying is an efficient alternative to maintain cell viability and stability during storage, processing, and passing through the digestive tract under various encapsulation materials and drying conditions. The encapsulation of probiotic bacteria by spray drying can maintain 25 - 98% cell viability.

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