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REVIEW ARTICLE

Microencapsulation of bioactive compounds in the food industry

Microencapsulación de compuestos bioactivos en la industria alimentaria

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Abstract The microencapsulation of bioactive compounds is a widely used technology in the food industry to protect and enhance the functionality of bioactive ingredients such as vitamins, antioxidants, probiotics, and essential fatty acids. This process involves encapsulating bioactive particles in a matrix, usually made of natural or synthetic polymers, forming microcapsules that improve the compounds' stability, controlled release, and bioavailability. Among the most commonly used techniques are spray-drying, coacervation, and extrusion, chosen based on the properties of the compound to be encapsulated and the desired applications. During food processing and storage, these technologies protect sensitive compounds from adverse factors such as oxidation, moisture, light, or extreme pH. Microencapsulation allows for the controlled release of bioactive compounds at the right time and place, improving their effectiveness in the body, an essential property in functional foods and nutraceuticals. This review aimed to analyze the microencapsulation techniques used in the food industry to protect and improve the functionality of bioactive compounds such as vitamins, antioxidants, probiotics, and essential fatty acids.

Keywords microencapsulation, bioactive compounds, probiotic microorganisms, stability, controlled release, food industry.

Resumen La microencapsulación de compuestos bioactivos es una tecnología ampliamente utilizada en la industria alimentaria para proteger y mejorar la funcionalidad de ingredientes bioactivos como vitaminas, antioxidantes, probióticos y ácidos grasos esenciales. Este proceso implica encapsular partículas bioactivas en una matriz, generalmente de polímeros naturales o sintéticos, formando microcápsulas que mejoran la estabilidad, liberación controlada y biodisponibilidad de los compuestos. Entre las técnicas más empleadas destacan el spray-drying, coacervación y extrusión, seleccionadas según las propiedades del compuesto a encapsular y las aplicaciones deseadas. Estas tecnologías permiten proteger los compuestos sensibles frente a factores adversos como la oxidación, la humedad, la luz o el pH extremo durante el procesamiento y almacenamiento de alimentos. La microencapsulación facilita la liberación controlada de los compuestos bioactivos en el momento y lugar adecuado, mejorando su efectividad en el organismo, propiedad esencial en los alimentos funcionales y nutracéuticos. El objetivo de esta revisión fue analizar las técnicas de microencapsulación utilizadas en la industria alimentaria para proteger y mejorar la funcionalidad de compuestos bioactivos, como vitaminas, antioxidantes, probióticos y ácidos grasos esenciales.

Palabras clave microencapsulación, compuestos bioactivos, microorganismos probióticos, estabilidad, liberación controlada, industria alimentaria.

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PUBLIS EDITORIAL

Introduction

Microencapsulation is a technology that involves enclosing solid, liquid, or gaseous materials in small capsules, which release their contents in a controlled manner under the influence of specific factors. The microcapsules comprise a thin semipermeable membrane surrounding a core, where the material of interest is located (Naveed et al., 2021). According to Rios-Aguirre and Gil-Garzón (2021), most microcapsules are small spheres with diameters ranging from 0.2 to 5000 µm. The structures of the microcapsules can be spherical or irregular, with the core distributed within a matrix of wall material (Choudhury et al., 2021). The release of the internal material can be triggered by factors such as temperature, pH, enzymatic action, or mechanical stress (Kamaly et al., 2016).

Bioactive food components, such as vitamins, antioxidants, and probiotics, are sensitive to degradation, making microencapsulation a suitable option for protecting them. This technology benefits bioactives like lipids, carbohydrates, proteins, and probiotics (Zabot et al., 2022). The microencapsulation of lipids, for example, allows their inclusion in food products, protecting them from oxidation and improving their solubility (Calderón-Oliver & Ponce-Alquicira, 2022).

The benefits of microencapsulation include improved stability of the core material, protection against oxidative stress, masking of undesirable flavors, and extending the shelf life of food products. It also facilitates the handling and uniform distribution of bioactives in food mixtures (Pattnaik et al., 2021). Despite its success in the pharmaceutical and cosmetic industries, microencapsulation has yet to have as significant an impact in the food industry, mainly due to concerns over costs. However, it can be cost-effective when applied to active ingredients in functional foods (Piñón-Balderrama et al., 2020).

This review aimed to analyze microencapsulation techniques used in the food industry to protect and enhance the functionality of bioactive compounds, such as vitamins, antioxidants, probiotics, and essential fatty acids. The review seeks to evaluate the benefits of this process in terms of stability, controlled release, and bioavailability of encapsulated compounds, as well as the specific applications of technologies such as spray drying, coacervation, and extrusion. Additionally, it aims to discuss how these strategies contribute to innovation in the design of functional foods and nutraceuticals, optimizing their quality and response to the demands of health-conscious consumers.

Microencapsulation methods

The microencapsulation process has been carried out using various techniques, and it is estimated that over 200 methods are documented in the patent literature (Vieira et al., 2020). The classification of these methods varies significantly, and creating a universal categorization system is becoming increasingly complex. In this review, the classification proposed by de Vos et al. (2010) has been adopted, which groups the methods into families. The selection of the appropriate method depends on factors such as budget, costs, properties of the core material and coating, the desired size of the microcapsules, the final application, and the expected release mechanisms (Choudhury et al., 2021).

Emulsion

Emulsion is one of the most commonly reported techniques for obtaining microcapsules in small quantities. This process is carried out in two steps: dispersion and hardening. First, an aqueous phase containing the core and the coating material is dispersed in an organic phase, such as oil, resulting in an oil-in-water emulsion. The dispersed aqueous droplets are hardened by cooling or adding a gelling or cross-linking agent. After the formation of microspheres, they are transferred to an aqueous system to be washed, and the oil is removed from their surface. This technique allows for a reduction in the size of the microspheres compared to extrusion and does not present significant challenges for scaling up. However, the residual oil in the microcapsules limits their use in the development of low-calorie foods (Ayyaril et al., 2023).

Coacervation

Coacervation is a variant of emulsification technology, which involves mixing a solution of the bioactive compound with a molecular matrix of opposite charge to form a complex. The size and characteristics of the microcapsules can be modified by adjusting the pH, ionic concentration, and concentration ratio between the core and the coating material. This technique is primarily based on electrostatic interactions, although hydrophobic interactions also play a role (de Vos et al., 2010).

Complex coacervation occurs when two polymers with opposite charges interact, resulting in a complex whose solubility is so low that it precipitates, forming a film around the core to be coated. This process is carried out by pH changes that alter the charges of one or both polymers or by dilution processes that promote interaction between substances with opposite charges.

Spray drying

Spray drying is one of the most commonly used microencapsulation methods in the food industry due to its low cost and the high quality and quantity of products obtained. The process involves dispersing the core material in a polymer solution, forming an emulsion or dispersion, then homogenized and atomized into a drying chamber. In this chamber, the water evaporates, and the microcapsules are collected (Mohammed et al., 2020). However, this method's main disadvantage lies in high temperatures during drying, which can damage bioactives, especially probiotics. Despite this, some studies suggest that drying parameters can be optimized to achieve the desired results (Arebo et al., 2023). Other researchers point out that spray drying is suitable for heat-sensitive materials due to the short exposure to high temperatures (Drozłowska et al., 2023).

Spray drying only applies to dispersions in aqueous systems, so the coating material must be highly soluble in water. For this reason, carbohydrates are most often used as the outer phase. Although this method favors hydrophilic substances, it can also be applied to lipophilic substances. These substances are dissolved in a lipophilic phase that is added to the aqueous phase to form an oil-in-water emulsion before drying. The microcapsules obtained by this method are highly stable due to their low water activity, which allows for an extended shelf life.

Freeze drying

Fluidized bed coating is a technology that involves suspending the core to be microencapsulated, usually in a solid state, in an airflow directed from the bottom to the top inside a chamber. The coating material is atomized onto the bioactive component using another device. This method offers a wider variety of coating materials than spray drying, as lipidic, protein, polysaccharides, or emulsifying materials can be used (Zhang et al., 2020).

Many microcapsules produced by other methods undergo this process to apply a second layer, which can influence the controlled release of the core, provide additional protection, improve the compatibility of the first layer with the food matrix, mask flavors, or direct the release to the specific desired site (de Vos et al., 2010).

Extrusion

The extrusion method consists of three main steps: first, the core material is dispersed in the coating material; then, this dispersion is divided into tiny droplets using a fine-diameter needle or a suitable device for this purpose; and finally, the resulting droplets pass through a dehydrating liquid or a solution that promotes polymer cross-linking. This procedure is effective for thermolabile materials or those sensitive to harmful solvents and can be performed under anaerobic conditions (Bamidele & Emmambux, 2020).

Due to its complexity, it is generally considered a method suitable only for laboratory scales. However, significant advancements have been made in scaling it up, such as using multiple needle systems, rotating atomization discs, or interrupted flow techniques. This method is primarily applied to probiotics but is also used to microencapsulate flavors, enzymes, and proteins (Kowalska et al., 2022).

Other microencapsulation technologies

Several additional technologies besides those previously mentioned are rarely used due to their high cost despite their high efficiency. However, they can help solve specific problems in the field of microencapsulation. One example is liposome technology, which consists of a spherical lipid bilayer that encapsulates the bioactive compound to be protected. This liposome is formed by dispersing polar lipids, usually phospholipids, in aqueous dispersions (de Vos et al., 2010).

Another unique method is microencapsulation in cyclodextrins, circular polymeric molecules composed of glucose monomers. The exterior of these molecules is hydrophilic, while their interior has hydrophobic characteristics, which can be enhanced by decreasing the number of glucose monomers in the cyclodextrin structure. This method increases the solubility of nonpolar molecules in polar matrices and prevents their inactivation or degradation (Poulson et al., 2022).

Coating materials

The biomaterials used in microencapsulation vary in their chemical composition and natural source. Among the protein coatings applied to probiotics, gelatin, and bovine whey proteins stand out, having been used as encapsulating agents, either combined by cross-linking with polysaccharides (Koh et al., 2022) or individually (Picot & Lacroix, 2004). There is a greater variety of encapsulating agents of polysaccharide origin, with alginate, derived from seaweed, being widely used in probiotic encapsulation; its limitation is being affected by the lactic acid produced by lactic acid bacteria (Mahmoud et al., 2020), cellulose derivatives (Lukova et al., 2023), starch (Lukova et al., 2023), and chitosan, a polysaccharide obtained from chitin found in the exoskeletons of crustaceans, insects, and in the cell walls of filamentous fungi (Meng et al., 2023).

Alginate

Alginate acid is a natural polyuronic acid extracted from seaweed, composed of β-D-mannuronic acid (M) and α-L-guluronic acid (G), linked by bonds between carbon atoms 1 and 4. The ratio of these residues varies depending on the source of alginate acid extraction (Abka-Khajouei et al., 2022). This polymer and its salts are block copolymers, including homopolymers MM and GG, which can combine or with individual residues. The ability of alginate acid or its salts to bind to monovalent cations (such as sodium alginate) and divalent cations (such as Ca^{2+}) favors gel formation, a process dependent on the composition and arrangement of the blocks (Malektaj et al., 2023).

GG blocks have specific sites to bind to divalent cations, and their interaction with other GG blocks promotes the polymer cross-linking responsible for gelation. Thus, when sodium alginate is added to a solution with dissociated calcium salts, immediate interfacial polymerization occurs, resulting in calcium alginate precipitation and gradual gelation as the Ca^{2+} cations diffuse inward (Hurtado et al., 2022).

Factors affecting the preparation of microcapsules have been studied, such as alginate and $CaCl_2$ concentrations, hardening time, and cell concentrations in probiotic encapsulation (Lotfipour et al., 2012). The conventional encapsulation method uses sodium alginate in calcium chloride ($CaCl_2$) to encapsulate *L. acidophilus* and protect this organism from the acidic conditions of gastric juice. Studies have shown that cell cultures immobilized in calcium alginate offer better protection, reflected in increased bacterial survival under various conditions, compared to their unencapsulated state. Additionally, the results indicate that the viability of bacteria encapsulated in simulated gastric fluid increases as capsule size increases (Lotfipour et al., 2012).

Chitosan

Chitosan is a deacetylated derivative of chitin, obtained by treating chitin with a concentrated sodium hydroxide or potassium hydroxide solution at high temperatures (Aranaz et al., 2021). This process leads to the hydrolysis of the N-acetyl bond of chitin, a natural polymer abundant after starch and cellulose. Chitosan comprises composed units of 2-deoxy-2-acetoamido-α-D-glucose (Piekarska et al., 2023). Chitin, a fibrous polymer, provides highly chemical and mechanical resistance materials. This polysaccharide, a homopolymer of N-acetyl-D-glucosamine with β(1-4) bonds, is commonly found as a white to yellowish powder or flakes, non-toxic, biodegradable, and processable into various derivatives (Piekarska et al., 2023).

Chitosan is widely used in the food and pharmaceutical industries due to its film-forming properties, good biocompatibility, biodegradability, and low cost (Jiménez-Gómez & Cecilia, 2020). It is harmless (Jiménez-Gómez & Cecilia, 2020) and a renewable resource. Its application in probiotic encapsulation has been limited due to its antimicrobial properties (Yan et al., 2021). However, it has been successfully used as an additional layer in alginate microcapsules, providing hardness and improving their sensory characteristics. Lactobacilli have been encapsulated with chitosan using the emulsion method, successfully encapsulating starter microorganisms that could be recovered and reused with satisfactory results (Călinoiu et al., 2019).

Probiotics

Probiotics have been defined in various ways, depending on how their mechanisms of action and health benefits are interpreted. The beneficial effects of probiotics are mainly grouped into two categories: antagonistic effects, which inhibit the growth of pathogenic microorganisms, and immunological effects, which strengthen the body's natural defense mechanisms (Latif et al., 2023).

The antipathogenic mechanism of probiotics includes the reduction of intestinal luminal pH through the production of short-chain fatty acids such as acetic, lactic, or propionic acid; restriction of essential nutrients for pathogens; alteration of the redox potential and the production of hydrogen peroxide, bacteriocins, and other inhibitory substances (Plaza-Díaz et al., 2019). Probiotics can induce cell-mediated immune responses, such as activation of the reticuloendothelial system and cytokine release, as well as the pro-inflammatory response through the regulation of tumor necrosis factors and interleukins, in addition to directly activating macrophages (Mazziotta et al., 2023).

In recent years, probiotic-enriched foods have been proposed to treat various intestinal disorders in humans, such as lactose intolerance, Crohn's disease, acute gastroenteritis, food allergies, atopic dermatitis, rheumatoid arthritis, and colon cancer (Kiousi et al., 2019). Among the most notable probiotic microorganisms are the lactic acid bacteria of the genus *Lactobacillus* and the bifidobacteria of the genus *Bifidobacterium*.

Microencapsulation of probiotics

In order for probiotic foods to achieve the desired therapeutic effects, they must remain metabolically stable in the product and pass through the upper gastrointestinal

tract without losing viability or undergoing physiological changes (Mendonça et al., 2023) so that they reach the intestine in sufficient quantities to ensure their survival and multiplication.

There are challenges related to the low viability of probiotic bacteria in fermented products. Various factors have been identified that affect probiotic viability, from the production stage to passage through the gastrointestinal tract. During fermentation, factors such as the composition of the growth medium, toxicity generated by the accumulation of metabolites (organic acids, hydrogen peroxide), dissolved oxygen concentration, and high biomass can affect viability. Before being incorporated into foods, probiotics may undergo mechanical, osmotic, or oxygen stress, and if subjected to spray drying or freezing treatments, they may be exposed to extreme temperatures and pronounced cellular dehydration (Mendonça et al., 2023).

During storage, microorganism viability can be affected by storage temperature, incompatibilities with starter cultures, and intrinsic characteristics of the food matrix, such as pH, moisture content, dissolved oxygen, and concentrations of proteins and sugars. Viability is also affected by the adverse conditions of the upper gastrointestinal tract, extreme pH, enzymatic activity, and bile salts. Proper design of the food matrix can mitigate these effects (Ulrika, 2022).

Commercial probiotic strains are typically selected for their technological properties rather than their probiotic potential, as some intestinal strains face difficulties in producing large quantities of biomass. There is a growing demand for new technologies that optimize scaling, ensure microorganism stability in food, allow the incorporation of new strains, and expand food matrix options while ensuring economic profitability (Terpou et al., 2019).

Strategies to increase probiotic resistance to adverse conditions include sublethal stress during fermentation to induce cross-resistance, the use of oxygen-impermeable packaging, the addition of micronutrients, osmoprotectors such as betaine, and microencapsulation (Agriopoulou et al., 2023). Microencapsulation, which occurs naturally through the excretion of exopolysaccharides during bacterial growth, effectively protects microorganisms against osmotic changes and adverse environmental factors. However, many lactic acid bacteria do not produce exopolysaccharides in sufficient quantities for complete encapsulation (Jurášková et al., 2022).

Microencapsulation in biodegradable polymer matrices offers numerous advantages (Table 1). It simplifies the quantification and handling of microorganisms, allows the incorporation of growth factors, prebiotics, osmoprotectants, and thermoprotectors into the capsule, and the microcapsules can be coated with other polymers to provide desired physical or sensory properties. Additionally, the microcapsules can be designed to release their contents in different areas of the body, protecting probiotics during storage and passage through the gastrointestinal tract to release them in the small intestine, maintaining their viability and probiotic properties (Lakshmi et al., 2023).

Figure 1 shows the percentages of occurrence of different encapsulation materials in the formulation of the microcapsules; the most commonly used material is alginate, and its concentration in the microcapsule is directly related to the survival of the probiotics, especially when exposed to high temperatures. The most used proteins are serum proteins and casein, derived from milk, and gelatin obtained from the partial hydrolysis of animal collagen tissue. Prebiotics (FOS, inulin, IMO, agave nectar) are incorporated into the formu-

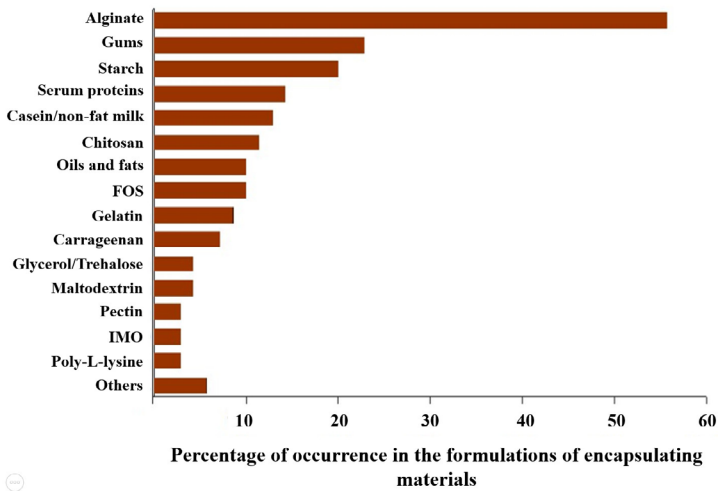

Figure 1. Most commonly used microencapsulating materials.

Table 1. Encapsulated probiotics studied in foods

Microencapsulation materials	Method	Encapsulated microorganism	Microcapsule dimensions	Food matrix	Reference
Carrageenan		*B. longum* B6 and *B. longum* ATCC 15708		Yogurt	Adhikari et al. (2000)
Alginate		*Lactobacillus acidophilus* MJLA1 and *Bifidobacterium* sp. BDBB2	1.77 mm, 0.064 with Tween and SDS	Frozen fermented dairy desserts	Shah & Ravula (2000)
Alginate, starch, glycerol	Emulsion	*Lactobacillus acidophilus* and *Bifidobacterium* spp.	0.5 – 1 mm	Yogurt	Sultana et al. (2000)
Gellan gum and xanthan gum	Extrusion	*Bifidobacterium infantis* ATCC 15697	3 mm	Yogurt	Sun & Griffiths (2000)
		L. acidophilus CSCC2401, *B. infantis* CSCC1912, *L. acidophilus* 910, and *B. lactis* 920		Cheddar cheese	Godward & Kailasapathy (2003)
Dairy fat and/or serum proteins	Emulsion and/or spray drying	*B. breve* R070 and *B. longum* R023	3 – 75 µm	Yogurt	Picot & Lacroix (2004)
Alginate coated with chitosan	Extrusion and coating	*L. acidophilus* 547, *B. bifidum* ATCC 1994, and *L. casei* 01	1.89 mm	Stirred yogurt	Krasaekoopt et al. (2004)
Chitosan, poli-L-lysine, alginate, starch, and/or FOS	Extrusion and coating	*L. acidophilus* CSCC2400 and *L. acidophilus* CSCC2409	~1 mm	Yogurt	Iyer & Kailasapathy (2005)
Alginate and starch		*L. acidophilus* DD 910 and *B. lactis* DD 920		Feta cheese	Kailasapathy & Masondole (2005)
Gellan gum and xanthan gum	Extrusion	*B. lactis* DSM 10140	20 – 2200 µm	Yogurt	McMaster et al. (2005)
Alginate, starch, FOS, and inulin	Emulsion	*L. acidophilus*, *L. casei*, *L. rhamnosus*, and *Bifidobacterium* spp.		Yogurt	Capela et al. (2006)
Alginate and starch	Emulsion	*L. acidophilus* and *B. lactis*		Yogurt	Kailasapathy (2006)
Alginate	Extrusion or emulsion	*L. reuteri*	40 µm (emulsion), 2 – 3 mm (extrusion)	Sausages	Muthukumarasamy & Holley (2006)
Carrageenan		*B. longum*		Stirred yogurt	Adhikari et al. (2006)

Microencapsulation materials	Method	Encapsulated microorganism	Microcapsule dimensions	Food matrix	Reference
Alginate	Extrusion	*L. reuteri* and *B. longum*	2 – 3 mm	Sausages	Muthukumarasamy & Holley (2007)
Serum proteins	Extrusion followed by freeze-drying	*L. rhamnosus* R011	2.8 mm	Biscuits, frozen cranberry juice, and vegetable juice	Ainsley et al. (2007)
Alginate and starch	Emulsion	*L. casei* and *B. lactis*		Ice cream	Homayouni et al. (2008)
Alginate	Emulsion	*B. bifidum* BB-12 and *L. acidophilus* LA-5	340 µm	Iranian yogurt	Mortazavian et al. (2008)
Alginate or carrageenan, corn oil	Extrusion or emulsion	*B. bifidum* BB-12 and *L. acidophilus* LA-5	0.5 – 1.0 mm	Kasar cheese	Özer et al. (2008)
Alginate or carrageenan	Extrusion or emulsion	*B. bifidum* BB-12 and *L. acidophilus* LA-5	0.3 – 0.4 mm	White cheese	Özer et al. (2009)
Alginate, lecithin, and starch	Extrusion followed by freeze-drying	*Lactobacillus* spp., *Bifidobacterium* spp., and *Lactococcus lactis*		Yogurt	Donthidi et al. (2010)
		L. helveticus CNCM I-1722 and *B. longum* CNCM I-3470		Chocolate, milk	Possemiers et al. (2010)
Alginate and pectin	Extrusion	*L. casei*	~1 mm	Yogurt	Sandoval-Castilla et al. (2010)
Serum proteins and palm oil	Spray drying	*L. rhamnosus* GG		Infant formula powder	Weinbreck et al. (2010)

lations to protect the microorganisms during microencapsulation, storage, and gastrointestinal transit, and the results obtained have been satisfactory.

Figure 2 shows different techniques for producing microcapsules at the laboratory scale. There is a marked tendency toward extrusion, emulsification, and spray drying. These techniques are directly related to the size of the microcapsules, which in turn has consequences on the levels of protection, which often increase with the diameter of the microcapsules, and the changes in the textural properties of foods with incorporated microcapsules, which on the contrary, decrease as the size decreases. Another factor influencing the choice

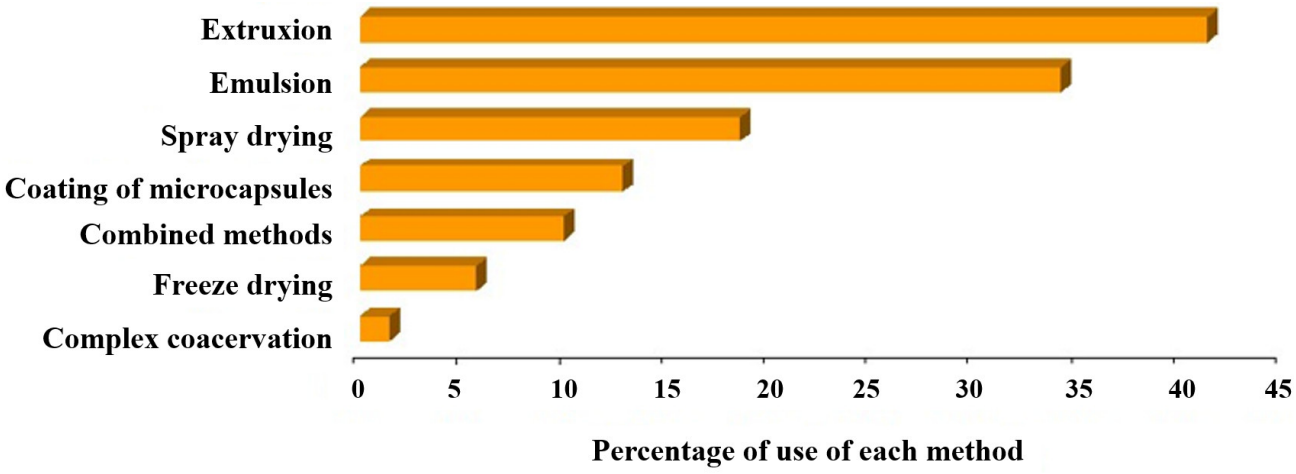

Figure 2. Methods used in the production of microcapsules.

of one technique over another is the thermal resistance of the species, as well as the available microencapsulating material and the possibility of scaling up the product.

Conclusions

Microencapsulation is an alternative to increase the viability of probiotics under simulated gastrointestinal conditions and during the storage of the microcapsules or the food matrix itself. The materials for microencapsulation must be selected according to the characteristics of the food in which they will be used and the applied method. The most commonly used microencapsulation method is extrusion, which produces microcapsules of acceptable size, but there are difficulties in scaling up the process. The most significant limitation of using probiotics in the food industry lies in the variation of the food's textural properties. The most studied probiotic bacterial genera are Lactobacillus and Bifidobacterium. However, studies have also been reported on species from the genera *Lactococcus* and *Pediococcus*, as well as the yeast *Saccharomyces boulardii.*

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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