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REVIEW

Encapsulation of Bioactive Ingredients by Extrusion with Vibrating Technology: Advantages and Challenges

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Abstract

Bioactive ingredients are present in functional foods and provide benefits to consumer health. However, for these compounds to be effective in the human organism, their bioactive features must be preserved during food processing and uptake by the organism. An effective technology to maintain bioactivity is encapsulation, which uses a coating material to protect bioactive ingredients. Not all encapsulation techniques are suitable for the effective protection of bioactive ingredients due to stages in the technique that might damage the bioactivity of the encapsulated ingredient. However, extrusion with vibrating technology has proven to be a technique with simple stages, thus enabling the formation of resistant microcapsules and maintaining the bioactivity of the encapsulated material. The aim of this revision is to show the state of the art on protection of bioactive ingredients using the encapsulation process by extrusion with vibrating technology. Therefore, the characteristics of vibrating technology in encapsulation by extrusion shall be addressed as well as the wall materials used, highlighting the features of the microcapsules obtained. For that, recent studies that have used the method in question specifically to protect bioactive ingredients will be discussed. Vibrating technology associated with extrusion, combined with a previous determination of parameters of suitable equipment and wall materials, allows for obtaining homogenous-sized and shaped microcapsules which provide effective protection to the bioactive compound.

Keywords Microencapsulation · Probiotics · Bioactive compounds · Microspheres · Microcapsules

Introduction

Functional foods are considered to be conventional foods that are consumed in everyday life as part of the human diet and are health beneficial as well as nutritious, thus contributing to improve well-being and life quality, and possibly reducing risks of diseases (El Sohaimy 2012; Ghosh et al. 2014). This definition considers that the use of these dietary items must be part of a varied diet in order to provide effective results (Crowe 2013). According to Del Castillo et al. (2018), functional foods are regulated by the European Food Safety Authority (EFSA), which defines them as foods that are beneficial to one or more functions in the organism (aside from their nutritional effects), thus providing benefits to health and well-being and/or reducing the risk of diseases, when ingested in amounts that can usually be consumed in the diet. Additionally, they can be either natural food items or foods to which a component has been added or removed through technological or biotechnological processes. The authors (Del Castillo et al. 2018) state that the Food and Drug Administration (FDA) does not have a specific definition for these food items, and therefore, the definition of Nutraceutics includes functional foods, i.e., foods that provide a specific benefit to health based on its ingredients.

Bioactive ingredients that provide benefits to consumer health are either naturally present in these foods or might be incorporated in food processing. These ingredients have attracted consumer attention due to the toxic and carcinogenic effects of their chemical additives (Jacobsen et al. 2018). Bioactive ingredients, such as polyphenols, isoflavones,

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carotenoids, minerals, vitamins, phytosterols, fatty acids, prebiotics, and probiotics, have pharmaceutical and nutritional properties and provide potential benefits to human health. However, the great challenge is to maintain the bioactive properties of these ingredients, preserving bioaccessibility and bioavailability, after industrial processing conditions (temperature, shaking, osmotic stress, water activity, and antibacterial substances), transport, storage, and during their exposure to the gastrointestinal tract environment (Champagne and Fustier 2007; Moura et al. 2018).

Given the beneficial potential of foods containing bioactive compounds and the emphasis on developing a correct dietary behavior, functional foods formulated with bioactive ingredients have been developed in matrices with the purpose of improving the stability, bioavailability, and bioactivity of these compounds. Thus, the food industry along with studies that have been developed seek to mitigate the degradation of these compounds to improve their effectiveness and prevent nutritional and functional losses (Moudache et al. 2017; Crowe 2013; Tavares et al. 2014; Wen et al. 2017).

In order to propose new forms of use, higher bioavailability, and preservation of the beneficial features of bioactive substances, encapsulation technology becomes a useful tool of great interest in scientific studies. The food industry has been demanding the incorporation of functional compounds to several products. These compounds are generally susceptible to environmental and gastrointestinal conditions and encapsulation arises as an alternative for the effective protection of these substances (El-Abbassi et al. 2016; Siegrist et al. 2007). The primary objective of encapsulation is to protect, isolate, and control the release of bioactive ingredients during food processing and their metabolization in the human organism (Ezhilarasi et al. 2013; Vos et al. 2010). In encapsulation, the bioactive compound to be encapsulated comprises the core that is retained inside the membrane formed by the encapsulant material (Fávaro-Trindade et al. 2008), which must be chemically compatible and non-reactive with the component to be encapsulated. It must also provide the desired coating properties, such as porosity, resistance, flexibility, impermeability, and stability (Maresca et al. 2016). Encapsulation, via several techniques, produces particles with different sizes and features (Iyer and Kailasapathy 2005). The most frequently used encapsulation techniques might be divided in classes, e.g., spraying, which includes sample drying; coacervation through the interaction of superficial charges; liposomes (lipid vehicles for bioactive compounds); emulsion, which requires the gelation of the system; molecular inclusion (using cyclodextrin molecules); and extrusion, which results in particles with different features depending on the type of equipment used (Trifković et al. 2016).

Extrusion techniques might be identified according to the equipment used for dripping, as follows: electrostatic extrusion, coaxial airflow extrusion, jet extrusion or with vibrating nozzle, and extrusion with rotational automizer discs. Equipment influences the size of the drop obtained and, in general, the particles produced via extrusion technologies lie within the size range from 0.2 to 0.5 mm. Among these techniques, encapsulation by extrusion with vibrating technology has proven to be effective and reproducible for the protection and stability of bioactive ingredients (Trifković et al. 2016; Semba and Trusek-Holownia 2017). It is a simple and low-cost process as it consists of mixing the bioactive ingredient with the encapsulant material, which results in the formation of droplets as the material passes through an injector nozzle to which is applied a defined vibrational frequency; these droplets are immediately solidified in capsules through a physical or chemical process (Heidebach et al. 2012).

Based on the abovementioned, the aim of the present revision is to show the state of the art on protection of bioactive ingredients via encapsulation by extrusion with vibrating technology, and for that, recent studies that have used this technique have been listed. Encapsulated and wall materials tested will be shown as well as the different pieces of equipment and their corresponding parameters, forms of storage, assessments carried out, and primary results.

Encapsulation

Over time, encapsulation has received a significant interest from the food, pharmaceutical, nutraceutical, and cosmetic industries due to its wide application in the development of functional products, either food or ingredients (Vinceković et al. 2017). In encapsulation, bioactive ingredients are completely entrapped and protected by a physical wall (Vos et al. 2010). This method might also be applied to change the physical features of the original material, in order to allow for easy handling, help sort out the ingredients of a mixture, which in turn might react with each other, and finally, provide the suitable concentration and uniform dispersion of an active agent (Nedovic et al. 2011). In the food area, this technique helps reduce the interactions between the core and environmental factors, delaying changes that might result in loss of aroma, color, or nutritional value (Stephen et al. 2006; Trojanowska et al. 2017). Among the encapsulation methods, spray drying is the most frequently employed for food products, and among the materials that stand out are starch wall, gum Arabic, maltodextrin, and cheese serum proteins (Bakry et al. 2016; Maresca et al. 2016).

Encapsulation Methods

There are several encapsulation methods, each generating particles of different sizes, comprised of a round, thin, and resistant membrane that entraps a solid or liquid core (Anal and Singh 2007). The most frequently employed processes in the encapsulation of bioactive substances are extrusion, spray

drying, lyophilization, coacervation, and co-crystallization. The particles generated in each one of these processes have different features, such as biocompatibility, reduced bioactivity, protection degree, and production cost. Wall materials generally include carbohydrates and their by-products (pectin, alginate, amylose, and chitosan) and proteins and their by-products, as well as synthetic polymers (Chawda et al. 2017). In order to select the encapsulation method to be used, some requirements indicated by Whelehan and Marison (2011) must be considered, such as the size of the particle and where it will be applied, the features of the bioactive ingredient to be encapsulated, the release mechanism, and the properties of the wall material. Since some techniques use chemical products and extreme process parameters, this might affect viability and the beneficial and sensorial features of bioactive ingredients (Fávaro-Trindade et al. 2008). Among the existing encapsulation methods are the following:

- Emulsion: this technique is based on the principle of forming an emulsion between a continuous phase (generally, some plant oil) and a discontinuous phase (solution with a bioactive ingredient and a hydrocolloid), as well as the addition of an emulsifier or tensoactive agent. The advantage of this method is its large-scale application and the formation of capsules with small diameter. However, the disadvantage is the formation of different sized and shaped microcapsules, and its high cost due to the use of plant oil, surfactant, and emulsifier (Solanki et al. 2013; El-Salam and El-Shibiny 2012).
- Lyophilization: The principle of this process is to freeze the material, which is subsequently subjected to vacuum and gradual increase in temperature, thus allowing water to be eliminated through sublimation. The advantages of this technique are short processing time and easy handling. However, bioactive ingredients might be damaged due to the formation of crystals and to the stress caused by high osmolarity. High cost is another limiting factor for this process (Heidebach et al. 2010).
- Spray drying: It consists of the atomization of a suspension of the material to be encapsulated, which is submerged into a polymer, and is subsequently subject to hot air for rapid evaporation of water and drying, thus generating encapsulants in the form of dry powder. One advantage is the cost-benefit ratio and the ability for high-scale production. On the other hand, a disadvantage is the application of high temperatures, which might damage the bioactivity of the ingredients (Rathore et al. 2013; Maresca et al. 2016).
- Extrusion: This process consists of mixing the bioactive substance with the encapsulant material, which goes through the extrusion nozzle of the equipment, resulting in droplets that are immediately transformed into capsules in a solidification bath (Whelehan and Marison 2011; Heidebach et al. 2012). The advantages of this technique are the absence of solvents and extreme temperatures. However, one disadvantage is the difficulty in broadening the process to the industrial level, as the speed of the microsphere production process is low (Heinzen et al. 2004; Burgain et al. 2011).

A successful encapsulation process depends on the wall materials selected and the encapsulation method, which result in different values of encapsulation efficiency, distribution of particle size, system feeding rate, and system rheology. These factors also influence the stability of the encapsulated material obtained and in the release profile of the bioactive compound (Chawda et al. 2017).

Encapsulation by Extrusion with Vibrating Technology

Not all techniques are suitable for the encapsulation of bioactive ingredients intended for the development of functional food since some techniques use organic solvents or employ encapsulating agents that affect sensorial features, which hinders the subsequent application in food matrices. However, encapsulation by extrusion with vibrating technology has proven to be effective for this purpose (Islam Shishir et al. 2018; Wang et al. 2017).

In the encapsulation process by extrusion, the encapsulant mixture is extruded through the equipment nozzle, forming a laminar jet, which freely breaks due to natural and irregular disturbances/vibrations, leading to the formation of different sized and shaped microcapsules, which is not desirable (Haas 1992). Therefore, in order to correct this issue during the formation of capsules, the vibration technology has been combined with the extrusion method, as shown in Fig. 1. This extrusion technique is based on the principle of laminar jet break-up by applying a vibratonal frequency, as shown in Fig. 2, thus generating homogeneous-sized and shaped particles, less porous than those obtained by the spray-drying process (De Vos et al. 2010; Whelehan and Marison 2011).

Aside from the use of vibrating technology, other ways of controlling the size of microcapsules in extrusion are the viscosity of the encapsulant mixture, concentration of the wall material and of the solidification solution, the size of the extrusion nozzle, and the distance between the dripping system and the gelling solution (Brun-Graeppi et al. 2011; Heidebach et al. 2012). The nozzle consists of a stainless steel cone with a hole through which the extruded polymer passes, with a diameter that ranges from 50 to 1000 μm, which allows for the production of 100 to 2000-μm microcapsules (Nemethova et al. 2014). The higher the viscosity of the encapsulant mixture, the lower the effect of the vibration on the

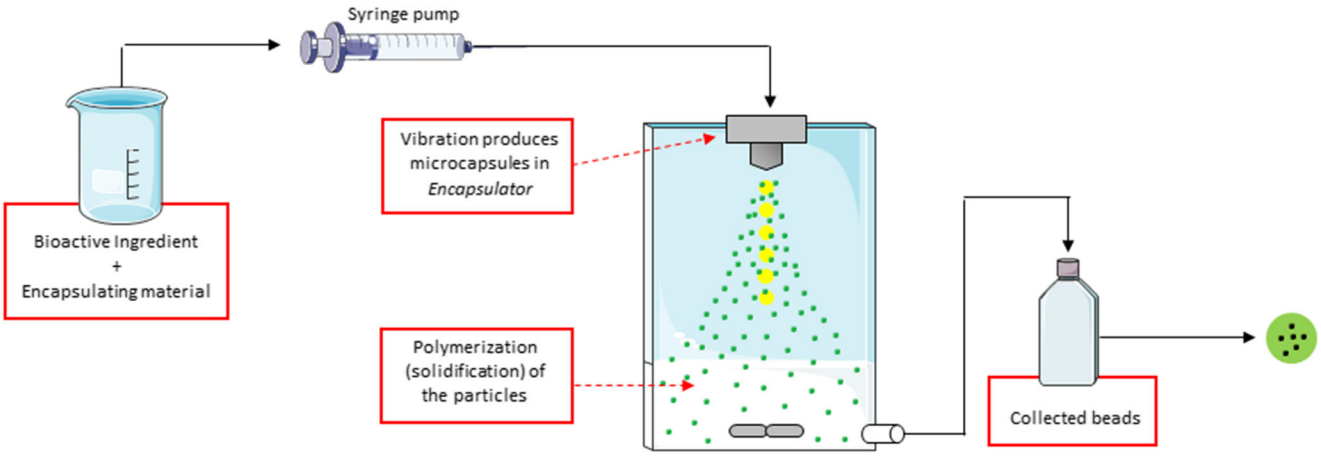

Fig. 1 Stages of the encapsulation process of bioactive ingredients by extrusion with vibrating technology

production of microcapsules, and the higher the probability of clogging the nozzles, which in turn determine the size of the particles (Razga et al. 2014). However, low viscosity might cause limitations in the production of completely spherical particles. To contextualize the parameter viscosity, the following example is used: a 120-μm nozzle for which the viscosity indicated by the equipment manufacturer ranges from 25 to 75 cP, which corresponds to a solution of 1% sodium alginate at 25 °C, approximately (Nemethova et al. 2014; Whelehan 2014).

Pieces of Equipment and their Parameters

Equipment for encapsulation with vibrating technology (coined Encapsulators) of the following brands is available in the market: Nisco Engineering AG, Inotech Biotechnologies AG,

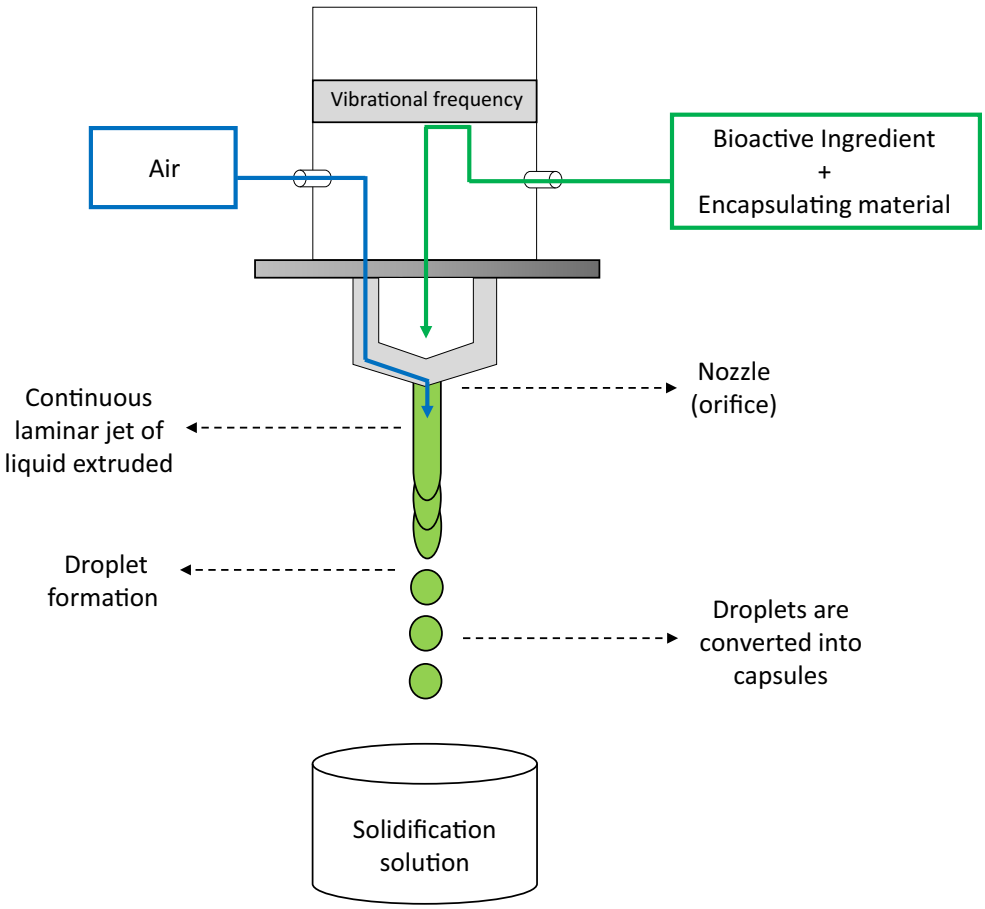

Fig. 2 Diagram of use of vibrational frequency in the extrusion encapsulation process intended for the controlled sorting of laminar jet and formation of round, same-sized capsules

Brace Technologies, and Buchi. All of them are comprised of the following main elements:

- Nozzle feeding pump, where the material to be encapsulated is inserted together with the wall material;
- Nozzle or nozzles that produce the laminar jet;
- Vibrating device coupled to a control system, which through the parameters frequency and breadth, enables the controlled sorting of the laminar jet;
- Stroboscopic light, which allows for coupling and viewing the formation of microcapsules, for adjustments in frequency;
- Gelling/solidification bath, which has a stirring system that allows for the polymerization of droplets and formation of microcapsules;
- Collector device to retrieve the microcapsules produced.

Overall, pieces of equipment are comprised of a capsule producing-unit and a control unit. All pieces that are in contact with the polymer mixture are autoclavable, i.e., they allow for a sterile process. Encapsulants available in the market are designed for research studies and for the development of small productions for commercial purposes, as their maximum production is 40 mL/min. They are therefore indicated for the study and development of encapsulation conditions to be employed subsequently at a large scale, with the help of equipment with a larger number of nozzles (Semba and Trusek-Holownia 2017).

Studies Conducted on the Encapsulation of Bioactive Ingredients by Extrusion with Vibrating Technology

Studies that have used the extrusion process with vibrating technology to encapsulate bioactive ingredients are discussed below. Table 1 highlights some data related to processing stages, main assessments, and results in each scientific study.

Wall Materials

Several food-grade materials are associated with the encapsulation of bioactive compounds. These coating materials work in structurally different manners, thus varying their ability for protection (Fathi et al. 2014; Jain et al. 2016). The efficacy of any coating material depends on its ability to form capsules and on the resistance of these structures, as this material must preserve the core from processing, storage, and gastrointestinal conditions (De Souza Simões et al. 2017). The main criteria to select a wall material for encapsulation are the bioactive features of the core, the application of encapsulants, and cost (Jain et al. 2016).

Sodium Alginate

Among the options of wall materials are the hydrophilic polymers that have functional groups with non-ionic charge, capable of forming hydrogen bonds with the mucosa surface (Dhawan et al. 2004); this is an excellent mucoadhesive property, important in the in situ release of bioactive ingredients throughout the gastrointestinal tract (Gombotz and Wee 2012; Chen et al. 2013). Among these coating materials, sodium alginate stands out, as it is non-toxic, biocompatible, and absorbs water quickly, and it is used in different concentrations (Chávarri et al. 2012).

Chew and Nyam (2016) observed that increased concentration of this biopolymer caused a higher degree of reticulation and formation of a more stringent structure. On the other hand, the concentration of 2% (m/m) caused clogging in the extrusion nozzle; therefore, the concentration of 1.5% (m/m) was used. This study shows that it is necessary to establish the threshold for the concentration of sodium alginate solution that provides a stringent coating structure, consequently providing higher protection to the encapsulated agent while having no impact on the extrusion process and not causing clogs and loss.

Under acidic conditions, as in the gastrointestinal tract, there is shrinkage of the sodium alginate gel structure due to the stretching of the carboxyl groups, which decreases its protection efficacy as wall material (Gouin 2004; Mortazavian et al. 2008). However, this disadvantage might be compensated by mixing it with other polymers, or by entrapping the capsules with another compound, or yet by the structural change in alginate using different additives (Krasaekoopt et al. 2003; Burgain et al. 2011).

Chitosan

Another frequently used biopolymer is chitosan, obtained primarily from crustacean exoskeletons, which are biodegradable and biocompatible (Goy et al. 2009). When mixed to a positively charged polymer, it forms hydrogels due to the addition of anions, e.g., pentasodium tripolyphosphate, and interacts with negatively charged polymers such as alginate (Chávarri et al. 2012). However, the application of chitosan as wall material for the encapsulation of probiotics must be assessed with caution, considering its antibacterial activity (Peniche et al. 2003), which is the reason why it is usually applied as a second microcapsule coating and not directly onto the encapsulated mixture (Chávarri et al. 2012).

Milk Proteins

Milk proteins are also an alternative wall material as they are biocompatible, biodegradable, capable of forming gels, and capable also of bonding with ions and having interactions with

Table 1 Description of studies that used the extrusion process with vibration technology to encapsulate bioactive ingredients

Reference	Encapsulated material	Wall material	Storage	Parameters	Assessments	Main results
Bioactive ingredients						
Moura et al. (2018)[a]	Anthocyanins of *Hibiscus sabdariffa* L. cups	2.0% pectin (in a previously conducted emulsion method)	$CaCl_2$ solution (pH 3, at 5, 15, and 25 °C, with no light, for 50 days)	Nozzle: 300 μm Breadth: 3 Frequency: 100–2200 Hz Electric power: 400–2000 V Pressure: 200 mbar Flow: 11.5 mL/min	Morphological characterization of microcapsules; content of bioactive ingredient; encapsulation efficiency; stability of bioactive ingredients at storage; analysis of encapsulation parameters in the microcapsules.	Microcapsules with 600-μm diameter. Better encapsulation conditions: 300 μm nozzle, 2000 V tension, and 1150 Hz frequency. Encapsulation efficiency was 80.0 and 95.6% for polyphenols and 84.3 and 93.9% for anthocyanins. Encapsulation provided the highest stability to the material.
Chew and Nyam (2016)[a]	Kenaf oil seed (*Hibiscus cannabinus* L.)	Sodium alginate (1.5% m/m) and alginate-pectin (1.5% m/m)	Subject to different drying processes (storage conditions have not been mentioned)	Nozzle: 150–300 μm Breadth: 3 Frequency: 500, 1000, 1500, and 2000 Hz Electric power: 1500 V Pressure: 600 mbar Flow: -	Morphological characterization of microcapsules; encapsulation efficiency; analysis of encapsulation parameters in the microcapsules.	Size of microcapsules 513 to 900 μm (before drying) and 300 to 513 μm (after drying). The best effectiveness (74.25%) was obtained for capsules with alginate-pectin (flow of 7.2 mL/min and frequency of 500 Hz).
Maresca et al. (2016)[b]	Nysine	Sodium alginate (16 g/L)	Residual $CaCl_2$ solution (0.5 mol/L, pH 6.0)	Nozzle: 80 μm Breadth: - Frequency: 2000 Hz Electric power: 950 V Pressure: - Flow: 2.91 mL/min	Morphological characterization of microcapsules; content and activity of bioactive ingredient; encapsulation efficiency; stability of bioactive ingredient at storage;	The alginate coating protected nysin from protease activity. The antimicrobial activity of the microcapsules was effective in different temperature and pH conditions, and was highly preserved at 4 °C and pH 4.5 and 6.0. Encapsulation efficiency from 71 to 76% was observed.
Waterhouse et al. (2014)[c]	Canola oil	Sodium alginate (1.0 and 2.0% m/m); sodium alginate strengthened with quercetin (6.18 mg of di-hydrated quercetin/100 mL water); quercetin in the oil core at	Collected in a 50-μm *nylon* mesh, rinsed with water at 30 °C and then, lyophilized. Stored	Nozzle: 200 μm Breadth: - Frequency: 1750 Hz	Characterization of microcapsules; microcapsule resistance to different pH levels; stability of bioactive ingredient at storage.	Microcapsule diameter, from 343 to 370 μm. In pH 2.0 and 6.5, the microcapsules remained intact, there was a 10–15% decrease in their size in pH 3.0 and 6.5.

Table 1 (continued)

Reference	Encapsulated material	Wall material	Storage	Parameters	Assessments	Main results
		200 ppm encapsulated with sodium alginate	at 20 and 38 °C, for 30 and 60 days.	Electric power: 1500 V Pressure: - Flow: -		The oil was protected, regardless of antioxidant addition. The incorporation of quercetin into the oil was less effective in preventing deterioration compared with encapsulation with alginate and quercetin.
Sun-Waterhouse et al. (2012)[c]	Avocado oil (*Persea americana Mill.*)	Sodium alginate (2.0% m/m) and Hydroxypropylmethylcellulose (0.4% m/m)	Collected in a 50 μm *nylon* mesh, rinsed with water at 30 °C and then, freeze-dried. Stored at 37 °C, for 90 days.	Nozzle: - Breadth: - Frequency: 1706 Hz Electric power: - Pressure: - Flow: -	Characterization of microcapsules; content of bioactive ingredient; stability of bioactive ingredient at storage.	Alginate microcapsules were more uniform and had a spherical, smooth, and regular surface, while alginate--hydroxypropylmethylcellulose provided a rough and wrinkled surface. After lyophilization, both microcapsules became wrinkled and shrank, and there was break of 5 to 10% of the microcapsules analyzed. The use of coextrusion improved the oxidative stability of avocado oil, suppressing the hydrolytic rancidity.
Zhang and Rochefort (2010)[c]	Lactase and glucose oxidase enzymes	Poly (ethyleneimine) 0.2 to 1.6%	Solution of 1% ciclohexane, after filtering and rinsing with ultra-pure water (storage conditions were not mentioned)	Nozzle: - Breadth: - Frequency: high Electric power: - Pressure: - Flow: 3.8 mL/min	Morphological characterization of microcapsules; encapsulation efficiency.	Microcapsules with approximately 200 μm and encapsulation efficiency of 83%.
Microorganisms as bioactive ingredients						
Eckert et al. (2018)[b]	*L. plantarum* ATCC8014, *L. paracasei* ML33, *L. pentosus* ML82	Cheese serum, serum permeated, sodium alginate (1.50%), and pectin (1.25%)	Rinsed with ultra-pure water and resuspended in 100 mL of phosphate buffer (10 mM, pH 7.0), stored at 4 °C.	Nozzle: 80 μm Breadth: - Frequency: 1740 Hz Electric power: 950 mV Pressure: - Flow: 5 mL/min	Morphological characterization of microcapsules; viability of bioactive ingredient; stability of bioactive ingredient at storage; microcapsule resistance to different pH levels; potential acidification of microcapsules.	Reduced viability of microorganisms after the encapsulation process between 0.07 and 0.74 log UFC/mL. Cell viability of encapsulated microorganisms higher than 6 UFC/mL, after 3 months of storage. The approximate size of microcapsules was 160 μm, and all wall materials testes were observed to be uniform and reproducible.
Olivares et al. (2017)[a]	*L. casei* (DSM20011),	Sodium alginate (2%)	Drained through a mesh and dried with	Nozzle: 450 μm	Morphological characterization of microcapsules; viability of	The three microcapsules had similar sizes (600–800 μm).

Table 1 (continued)

Reference	Encapsulated material	Wall material	Storage	Parameters	Assessments	Main results
	L. reuteri (DSM20016), and *L. delbrueckii* subsp. *bulgaricus* (DSM20081) freeze-dried		paper (storage conditions were not mentioned)	Breadth: - Frequency: 500–3000 Hz Electric power: 250 V Pressure: - Flow: 10–30 mL/-min	bioactive ingredient; evaluation of encapsulation parameters; microcapsule resistance to different pH levels.	Encapsulated *L. casei* had survival of 75% (pH 2.0/30 min) and 77.7% (pH 2.5/90 min). Encapsulated *L. reuteri* had survival of 84.5% (pH 2.5/60 min) and in free form, it survived for only 30 min. In pH 4.0, there was, on average, a cell survival higher than 96%. The conditions of 1000 Hz and 20 mL/min resulted in the best capsules regarding size, viability, round shape, and smooth surface.
Yeung et al. (2016)[b]	Bifidobacteria	Sodium alginate (1%) and Chitosan (0.4%)	Saline physiological solution (4 °C)	Nozzle: 120 μm Breadth: 3 Frequency: 800 Hz Electric power: 800 V Pressure: 250–-300 mbar Flow: -	Morphological characterization of microcapsules; stability of bioactive ingredient at storage; microcapsule resistance to different pH levels.	*Particle size varied from* 135 to 292 μm. *Alginate-based microcapsules* survived 7 to 8 log for 3 weeks, and there was reduction of only 1.4 log cycles in simulated gastrointestinal tract. The performance observed for alginate-chitosan microcapsules was not better compared to alginate alone.
De Prisco et al. (2015)[b]	*L. reuteri*	Sodium alginate (20 g/L) and Chitosan-alginate (7 g/L, dissolved in acetic acid 0.14 mol/L, pH 3.2)	Skimmed milk (− 18 °C); afterwards, lyophilized and at 4 and 20 °C	Nozzle: 80 μm Breadth: - Frequency: 1740 Hz Electric power: 950 mV Pressure: - Flow: 2.91 mL/m-in	Morphological characterization of microcapsules; stability of bioactive ingredient at storage; content of bioactive ingredient; encapsulation efficiency; microcapsule resistance to different pH levels and osmotic stress.	The bacterial load of microcapsules had an encapsulation efficiency of 100% immediately after lyophilization. Microcapsules with 110-μm diameter, with no differences in size due to addition of chitosan, only in shape. Alginate coating provided higher viability to the microorganism compared to chitosan-alginate. After storage (4 °C/28 days), microcapsules had reduction of 1 log cycle.
Shi et al. (2013a)[c]	*L. bulgaricus*	Sodium alginate (low viscosity—not specified) and pure milk (3.1% protein, > 3.5% fat, > 8.0% total solids)—with different ratios between them.	Distilled water, filtered and placed in sterile tubes, at 4 °C for 1 month.	Nozzle: 200 and 450 μm Breadth: - Frequency: - Electric power: - Pressure: -	Morphological characterization of microcapsules; stability of bioactive ingredient at storage; viability of bioactive ingredient; microcapsule resistance to different pH levels and osmotic stress.	Results showed that the mean diameters of milk-alginate microspheres with nozzle of 0.45 and 0.20 mm were 830 and 381 μm, respectively. High encapsulation yield: 10 log UFC/mL of free cells, and 9.95 to 9.98 log UFC/mL of encapsulated cells.

Table 1 (continued)

Reference	Encapsulated material	Wall material	Storage	Parameters	Assessments	Main results
				Flow: -		Reduction of 9.98 to 9.24 and 8.48 log UFC/mL after exposure of microcapsules to a 1% bile saline solution for 1 and 2 h, respectively, while free cells did not resist after 1 h of contact.
Shi et al. (2013b)[c]	*L. bulgaricus*	Pure milk (3.1% protein); carrageenan (0.03%); locust bean gum (0.25%, pH 7.0)	Distilled water, filtered and placed in sterile tubes, at 4 °C for 1 month.	Nozzle: 200 and 450 μm Breadth: - Frequency: - Electric power: - Pressure: - Flow: -	Morphological characterization of microcapsules; stability of bioactive ingredient at storage; viability of bioactive ingredient; microcapsule resistance to different pH levels.	530 μm, round shaped and irregular microcapsules. Free microorganisms did not survive after 1 min in pH 2.5; on the other hand, the microcapsule preserved it after 2 h in pH 2.0. Reduction of 1.5 log cycles in the viability of microcapsules after 2 h in a solution of 2% bile salts while free cells did not resist after 1 h in 1% bile solution.
Graff et al. (2008)[c]	*Saccharomyces boulardii*	Acrylic acid (4 g) and sodium alginate (1.8 g) dissolved in 70 mL of buffer phosphate solution pH 8.0; and chitosan (0.4% dissolved in acetic acid)	Sterilized water, dried in furnace for 4 days at 25 °C (storage conditions were not mentioned)	Nozzle: 300 μm Breadth: 3 Frequency: 765 Hz Electric power: 1000 V Pressure: - Flow: 11 mL/min	Morphological characterization of microcapsules; microcapsule resistance to different pH levels; viability of in vitro and in vivo bioactive ingredient.	Chitosan coating increased diameter, changed the shape, and decreased microcapsule yield. After administering the microcapsules in rats, the microorganisms were evident in 50% of fecal samples (after 6 h), and its viability was not detected after 5 days.
Iyer and Kailasapathy (2005)[c]	*L. acidophilus*	Sodium alginate (1.8% m/v) and prebiotic (poly-L-lysine, chitosan, and alginate) (1.0% m/v)	–	Nozzle: 300 μm Breadth: - Frequency: - Electric power: - Pressure: - Flow: -	Morphological characterization of microcapsules; microcapsule resistance to different pH levels; application of encapsulant in yogurt elaboration.	Chitosan coating increased microcapsule diameter (500 μm). Resistance of coencapsulated bacteria with prebiotic increased 0.6 log cycles in pH 2.0/3 h compared to free-form bacteria and bacteria encapsulated with sodium alginate and poly-L-lysine.

Data with the symbol "-" mean that the information has not been shown in the paper

Equipment used: [a] Encapsulator B-390 (Buchi, Switzerland); [b] Encapsulator B-395 (Buchi, Switzerland); [c] Encapsulator (Inotech Biosystems Intl. Inc., Switzerland)

other polymers to form complexes (Livney 2010; El-Salam and El-Shibiny 2012). Shi et al. (2013a) observed that the milk-based encapsulant formulations were effective in protecting the microorganisms as the encapsulated material had higher survival in pH levels of 2.0 and 2.5 and in concentrations of bile salts of 1.0 and 2.0%, even after 1 month of storage compared to free-form microorganisms. Eckert et al. (2018) also emphasized that the cheese serum used as wall material is a natural habitat for encapsulated probiotic milk bacteria, which turns the microparticles into an environment with suitable physicochemical and biological properties for these microorganisms.

Secondary Coating/Coencapsulation

Among the alternatives used to make the capsules more resistant and provide higher protection to the core is the secondary coating used by Shi et al. (2013b). The authors observed that microorganism viability after the encapsulation process remained nearly unchanged, and that despite the irregular spherical shape of the capsules, the coating was capable of preserving the microorganism from the acidic conditions of the gastric tract. This protection derives from the milk protein used as wall material, which provides low porosity to the microsphere surface, even though it is irregular. As for the double layer, the authors observed that it caused a decrease in porosity and provided a thicker structure, preventing bile salts from entering the microcapsule and reducing stress on the bacteria. It also improved stability at storage, having preserved the viability of the encapsulated microorganism, whereas viability in free-form microorganisms was observed to be reduced by 3 log cycles after the same period.

On the other hand, Yeung et al. (2016) subjected part of the alginate-based microcapsules to coencapsulation with chitosan and observed diameters of 135 to 216 μm in the calcium alginate coating and of 191 to 292 μm in the second chitosan coating. In the same study, they also observed that free-form microorganisms and coencapsulated microorganisms remained stable in saliva fluids (reduction of 1 log cycle after 30 min). In the gastric phase, however, encapsulation provided higher protection, with a decrease of 1.4 log cycles, whereas the free-form microbial cells decreased by 2.7 log cycles after exposure to pH 2.5/5 min.

Graff et al. (2008) observed that the secondary chitosan-based coating increased the diameter of microcapsules, and it changed their shape and caused decreased yield, similar to Yeung et al. (2016). Therefore, based on these studies, it is evident that the secondary coating might provide higher protection to the encapsulated material. However, it must be previously analyzed in order to avoid equipment clogging and decrease in the final microcapsule yield. Moreover, it is necessary to set the acceptable diameter of microcapsules considering their subsequent application.

Combination of Two or More Wall Materials

Another alternative for optimizing protection of microcapsules is using the combination of two or more wall materials, such as chitosan-alginate studied by De Prisco et al. (2015). These authors observed that the viability of alginate-based microcapsules was less affected compared to the chitosan-alginate-based microcapsule. On the other hand, there was no difference in mean diameter resulting from the addition of chitosan. Therefore, coencapsulation might affect morphology, increasing microcapsule diameter, but it provides higher protection to the bioactive ingredient compared to the use of two or more materials combined. The latter technique must be assessed with caution to optimize protection and not cause negative effects to the core.

Encapsulation Parameters

To preserve encapsulated bioactive ingredients, aside from establishing the best wall material, it is also important to determine the operational conditions of the equipment. For that purpose, there are determining and adjustable parameters for encapsulants, namely feeding liquid supply (1.1–40.0 mL/min), vibrational frequency (40–3000 Hz), electric power (250–2500 V), breadth (1–9), and pressure (maximum 700 mbar) (Whelehan 2014; Nemethova et al. 2014; Semba and Trusek-Holownia 2017).

Chew and Nyam (2016) assessed the effects of vibrational frequency with the same flow rate and concluded that microcapsules produced at 500 Hz were more spherically shaped compared to higher frequencies, which provided irregular shapes, double microcapsules, or microcapsules with a tail. On the other hand, Moura et al. (2018) determined that a higher frequency (of 1150 Hz) provides stability to the capsules. There are some differences between both studies, which might explain this disparity between the frequencies used. Moura et al. (2018) performed the emulsion method in pectin solution prior to the encapsulation by extrusion, while Chew and Nyam (2016) mixed the encapsulated and wall material, and then, submitted them to extrusion. Another difference is in the pressure used to inject the material into the encapsulator, a pressure of 200 mbar (for 1150 Hz) (Moura et al. 2018) and 600 mbar (for 500 Hz) (Chew and Nyam 2016). Therefore, the parameters pressure and vibrational frequency are correlated and must be previously tested in order to obtain microcapsules with suitable features.

In the studies that employed the vibrating technology, flow, vibrational frequency, electric power, and breadth are determining for the formation of a laminar jet, thus avoiding the formation of a spray or jet that increases the impact of microcapsules in the gelling solution, consequently deforming them (Maresca et al. 2016; Nemethova et al. 2014). A flow rate at low velocity might cause adherence of the material to the

extruding nozzle, whereas a higher velocity might pulverize the jet and cause an unregulated release. To avoid droplet coalescence during jet break-up and input into the gelling bath, an electrical charge is induced onto their surface using a electrostatic tension system, which applies an electric potential between the nozzle and the electrode, causing the droplets to be deviated from their vertical position, and to spread out throughout the falling process (Fig. 2). Consequently, the droplets are distributed in a more homogeneous manner in the solidification solution. This allows for the formation of monodisperse capsules with size with a maximum standard deviation of 5% (Whelehan 2014). In the chamber, prior to extrusion through the nozzle, the vibrational frequency is applied to a pre-defined breadth of the jet, causing the drops to repel each other when passing through the electrode, resulting in a more uniform distribution of the microcapsules, as they tend to agglomerate. Therefore, both parameters are responsible for breaking the jet in uniform and same-sized drops (Heinzen et al. 2004; Maresca et al. 2016). It is important to emphasize that aside from equipment parameters, the operator is required to be knowledgeable on which are the suitable jet features to be attained, since this parameter can be controlled via observation through the stroboscopic lamp coupled to the encapsulator.

Types of Capsules

The capsules obtained through encapsulation must be spherical and have a fine and resistant membrane (Anal and Singh 2007). There is still no consensus regarding the definition of capsule size; however, Whelehan and Marison (2011) defined the following classification: nanocapsules (< 1 μm), microcapsules (1–1000 μm), and macrocapsules (> 1000 μm). The dimension of capsules with bioactive ingredients influences the features of functional foods elaborated with that encapsulated ingredient. Large capsules might negatively affect the texture of food. On the other hand, if they are too small, they might not provide enough protection to the bioactive ingredients (Shi et al. 2013a, b). Furthermore, De Prisco et al. (2015), studying encapsulation of microorganisms, highlighted that smaller microcapsules with lower concentration of microbial cells ensure a more homogeneous behavior of the bacterial population both regarding resistance to stressful conditions and to metabolic activity.

Some authors relate capsule dimension with the sensorial features of food. For instance, Kailasapathy (2006) observed that 300-μm microspheres containing probiotic bacteria result in an expressive increase in yogurt softness, compared to free-form microbial cells. On the other hand, Truelstup-Hansen et al. (2002) suggested that microcapsules with size smaller than 100 μm could prevent negative sensorial impact on food matrices.

Morphology and Size of Microcapsules

Most of the studies in Table 1 defined microcapsule morphology and size, as these are essential parameters to ensure protection to the encapsulated material while enabling the incorporation of food matrices. Irregular morphology decreases the microcapsule ability for protection, hence the importance of the assessment performed by Sun-Waterhouse et al. (2012). The authors emphasized that microcapsules coated only with alginate were more uniform and had a spherical, smooth, and regular surface, while alginate-hydroxypropylmethylcellulose provided a rough and wrinkled surface.

Yeung et al. (2016) used two pieces of equipment to view the microcapsules; using the scanning electron microscope, the result was a wrinkled surface while the images in the optical microscope showed smooth surfaces. Scanning electron microscope uses electron beams that hit the sample and are reflected, thus allowing for a higher image enhancement and resolution of the microcapsule. However, the equipment applies high temperatures and vacuum to the capsules, which might affect their morphological features and size. On the other hand, the optical microscope uses visible light, causing less impact on the structure of microcapsules, but its enhancement capacity is smaller, thus hampering the definition of features of the layer formed. Therefore, there are variations in the interpretation of parameters such as microcapsule morphology and size according to the method of analysis chosen, and it is necessary to weigh up which one suits each study better.

Microcapsule Yield, Efficacy, and Sampling

Some authors have assessed encapsulation yield, which might be related to how the bioactive ingredient has been encapsulated. For instance, Olivares et al. (2017) obtained a low result in the process, approximately 50%, probably because the authors used microorganisms in the form of lyophilized powder; depending on the size of powder granules, they might have clogged the extrusion nozzle, and consequently, caused low yield.

Another factor is the retention of microcapsules in the encapsulator flask and even loss of microcapsules during the collection stage, since the more stages/transfers there are in the process the higher the loss of material, and consequently, the lower the yield. In this regard, Zhang and Rochefort (2010) compared two encapsulation methods, and observed that emulsion had higher yield, of 94%, compared to the extrusion process with vibrating technology, with 83%. The authors observed that in this process, losses inevitably occurred due to the system of releasing aqueous solution of $CaCl_2$. Therefore, the collection method must be analyzed with caution, since microcapsules yielded with size smaller than the sieve mesh might be lost in this stage. Additionally, moisture

removal with filter paper might carry or break the microcapsules generated.

In order to collect microcapsules, Maresca et al. (2016) carefully discarded the $CaCl_2$ solution until there was a 30-mL volume left. Chew and Nyam (2016) also used a different collection method for sorting microcapsules: these were collected by using a sieve, rinsing, and removing moisture with filter paper. On the other hand, Shi et al. (2013a) entrapped the microcapsules in tubes containing only moisture of the rinsing water. The results obtained emphasized that the methods employed did not interfere with the viability of the encapsulated material, as the encapsulated microorganisms showed higher survival compared to free-form microbial cells, in pH of 2.0 and 2.5 and in 1.0 and 2.0% biliar saline solutions, even after 1 month of storage.

Differential Definitions

Aside from definitions commonly carried out in studies on encapsulation of bioactive ingredients by extrusion with vibrating technology, which are described in Table 1, some authors have performed other assessments to determine the most specific features regarding the microcapsules obtained.

For instance, Yeung et al. (2016) analyzed electrophoretic light dispersal with the purpose of determining the electrical properties of microcapsules. This analysis emphasized the negative impact on alginate-based capsules and potential positive impact on capsules with the chitosan layer, indicating that cation molecules of chitosan form a secondary layer around the anion capsules of alginate. Similarly, De Prisco et al. (2015) analyzed the resistance to osmotic stress, inoculating the microcapsules in apricot jam (high concentration of sugar), and observed a small reduction in viability of the encapsulated microorganisms. The authors also analyzed the production of reuterin, an antimicrobial substance produced by the encapsulated bacteria, and observed that none of the coatings interfered with the production of this compound. Graff et al. (2008) conducted in vivo experiments to assess the viability of encapsulated leaven in the organism and concluded that alginate-based microencapsulation limits its degradation inside the gastrointestinal tract while microsphere coatings with chitosan did not offer additional benefit. Iyer and Kailasapathy (2005), on the other hand, produced symbiotic yogurt with free-form, encapsulated, and coencapsulated microbial cells, observing decreases in microorganism viability by 4, 2, and 1 log cycles, respectively. Based on the practical assessment of the encapsulated material in a leavened product, they concluded that coencapsulation has higher potential for application in functional food matrices than encapsulation with only alginate.

Conclusions

Currently, the food and pharmaceutical industries are facing challenges to maintain the bioactive properties of ingredients, and based on this revision, we can conclude that:

I. The extrusion technique with vibrating technology, under optimal conditions, allows for obtaining homogeneous microcapsules with controllable sizes, and with satisfactory efficiency and reproducibility;
II. It is essential to previously establish parameters for the encapsulation equipment, as it directly affects microcapsule characteristics, and consequently, the effective protection of the bioactive ingredient;
III. This process provides protection to bioactive ingredients, having emphasized the use of different pieces of equipment and parameters, as well as different wall materials;
IV. This technique is suitable for the encapsulation of bioactive ingredients due to its advantages over other methodologies, namely non-use of either high/low temperatures or organic solvents.

However, some deficiencies and future perspectives have also been identified, and developing further knowledge and research is required to tackle the following topics:

I. Encapsulation of two or more bioactive ingredients that might be combined to provide a synergistic effect;
II. The use of different wall materials that enable higher yield, low cost, and protection of the bioactive ingredients;
III. Improvement of advanced coencapsulation techniques and/or use of two or more wall materials, without compromising core protection and microcapsule morphology;
IV. Establishing a suitable methodology to determine size and morphology of microcapsules, without altering their structure, and consequently, obtaining results as close to the real as possible;
V. In-depth evaluation of the interaction between bioactive ingredient and wall material, determining reactions that occur between these components and how microcapsules are protected when submitted to adverse conditions;
VI. In vivo evaluation of the stability of encapsulated material under gastrointestinal conditions, since studies have conducted their analyses using in vitro techniques;
VII. Broadening the scale of the encapsulated production process, as the studies cited have been developed for laboratory-scale equipment;
VIII. In order to promote advancements in the development of functional foods with encapsulated bioactive ingredients, a better understanding of parameters, such as

extrusion nozzle diameter, feeding rate, and vibration frequency, which involve the extrusion process with vibrating technology at an industrial level must be targeted.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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