Microencapsulation in food science and biotechnology
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Microencapsulation can represent an excellent example of microtechnologies applied to food science and biotechnology. Microencapsulation can be successfully applied to entrap natural compounds, like essential oils or vegetal extracts containing polyphenols with well known antimicrobial properties to be used in food packaging. Microencapsulation preserves lactic acid bacteria, both starters and probiotics, in food and during the passage through the gastrointestinal tract, and may contribute to the development of new functional foods.

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Microencapsulation: principles and use with natural ingredients
Microencapsulation (ME) is the envelopment of small solid particles, liquid droplets or gases in a coating (1–1000 µm). In general, one can differentiate between mononuclear capsules, which have one core enveloped by a shell, and aggregates, which have many cores embedded in a matrix, usually polymers (Figure 1). The development of a successful encapsulation system for a target application is based on the need of a good knowledge about the stability of the chosen component, biomolecules or cells, to be encapsulated (core), the properties of the materials used for encapsulation (encapsulant matrix) and the suitability of the delivery system (microcapsule) for its ultimate application [1]. The most available technologies for microencapsulation use a liquid (complex coacervation, interfacial and in situ polymerization or solvent evaporation from emulsions) or a gas as suspending medium (spray-drying or spray-cooling, fluidized-bed coating or co-extrusion) [2]. ME has an evident impact on the food industry. In food science and biotechnology, it involves the incorporation of natural ingredients, polyphenols, volatile additives, enzymes, bacteria (i.e. lactic acid bacteria acting as starters or probiotics) in small capsules, giving them the chance to be stable, protected and preserved against nutritional and health loss and to eventually act as antimicrobial agents. The matrices in contact with food are generally natural components, but mainly they must be Generally Recognised As Safe (GRAS) for human health.

Microencapsulation of bioactive components is performed through use of different materials, for example water and oil. A usual water-oil-water emulsion is formed with small water droplets, dispersed in large oil droplets, themselves dispersed in an outer aqueous phase. The functional component can be encapsulated within the inner phase, the oil phase or the outer water phase after drying; thus, a single delivery system can contain multiple functional components.

Micro-emulsions, with droplets sizes less than 500 nm diameter, are produced by micro-fluidization or micelle formation techniques. ME can be successfully applied to entrap natural compounds, like essential oils (EOs) or vegetal extracts containing polyphenols with well known antimicrobial properties. This aspect represents an important starting point for industries, which can try out new natural and safe materials or systems of packaging capable to prolong the shelf life of foods, such as highly perishable fresh foods (vegetables, fruits, meat, etc.), without lessening their characteristics in terms of quality and hygiene. ME can be considered as a real resource for food packaging also to mask unpleasant flavors and odors, or to supply barriers between the sensitive bioactive materials and the environment (represented by food or oxygen). Many of the EOs have antimicrobial properties against several foodborne pathogens and can be potentially used in different food matrices, including meat products [3,4]. However, limits to their use are linked to the aroma that can be unpleasant for consumers, or having poor water solubility and volatility.

Encapsulation makes it possible to increase the effectiveness of EOs and to decrease their sensory impact on foods. Microencapsulation of EOs is generally achieved in two steps. Firstly, an emulsion of the volatile compound is made in an aqueous dispersion of a wall material which also functions as the emulsifier. Then, the microencapsulated emulsion must be dried under controlled conditions so as to diminish the loss of the encapsulated
material by volatilization [5]. Encapsulation of rosemary EO has proven to have a more effective antimicrobial activity than rosemary EO alone against *L. monocytogenes* in pork liver sausage [6]. Eugenol and carvacrol encapsulated in micelles of non-ionic surfactant are highly effective against *L. monocytogenes* and *E. coli* O157: H7, thanks to the greater amount of antimicrobial compound exposed to the surface of the bacteria when presented in this form [7,8]. Microencapsulated EOs also have been successfully assessed against *E. coli* O157: H7 inoculated in refrigerated, chopped beef packed under nitrogen [9].

Polyphenols play a well known essential role in food quality and safety, as well as for human health. In food science, their stabilization could be improved using encapsulation and spray drying, which could protect them against oxygen, water or any condition that could affect their stability [10]. Different encapsulating agents are used for spray drying: polysaccharides (starch, maltodextrins, corn syrups and gum arabic), lipids (stearic acid, mono and diglycerides), and proteins (gelatin, casein, milk serum, soy and wheat) [11]. Maltodextrins, the most common used materials for ME, are obtained by an acidic hydrolysis of several starches (corn, potato or others). In general, maltodextrins have high solubility in water, low viscosity, bland flavor and colorless solutions [11]. An interesting possible encapsulation agent is inulin, which owing to its nutritive and prebiotic properties [12,13] would also render ME as helpful in the ideation and development of functional foods.

The limited range of suitable encapsulant materials allowed for food use is still the main challenge in material selection, especially when more complex properties are required by food manufacturers and consumers. New generations of encapsulating systems are continuously emerging for food applications. Polyvinyl alcohol (PVA) might be one of the most promising materials. PVA, a highly polar, nontoxic, water-soluble synthetic polymer prepared by the hydrolysis of polyvinyl acetate, is used in polymer blends with natural polymeric materials. Furthermore it is utilized for a variety of biomedical applications [14], because of its inherent non-toxicity, non-carcinogenicity, good biocompatibility and desirable physical properties, in addition to excellent film forming properties. PVA has been recently used to entrap a hydro-alcoholic extract obtained from rosemary that is used as an antimicrobial agent against different foodborne pathogens like *E. coli* [14,15] (Figure 2). The excellent results suggest its suitability of use in food packaging, to prolong the shelf life of highly perishable fresh foods, thereby enhancing their quality and safety (Nazzaro et al., unpublished data).

**Microencapsulation of bacteria**

In food biotechnology, ME can be also used to entrap or enclose microorganisms by segregating them from the external environment with a coating of hydrocolloids, such that the cells are released in the appropriate gut compartment at the right time. The technology protects lactic acid bacteria (LAB) in food and during the passage through the gastrointestinal tract [16,17,18,19,20]; other advantages include prevention of interfacial inactivation, stimulation of production and excretion of secondary metabolites, and continuous utilization. Additionally, ME may enhance microbial survival and operating efficiency during fermentation [21]. Generally, LAB present two sets of problems: their size, which immediately excludes nanotechnologies, and the fact that they must be kept alive [22]. Viability of encapsulated cells is
affected by the physico-chemical properties of the capsules: type and concentration of the coating material, particle size, initial cell numbers and bacterial strains are some of the parameters to be considered [23].

The main purpose of probiotic encapsulation is to protect cells against an unfavourable environment, and to allow their release in a viable and metabolically active state in the intestine [24]. Microparticles should be water-insoluble to maintain their structural integrity in the food matrix and in the upper part of the GI tract; above all, particle properties should allow progressive liberation of the cells during the intestinal phase [24,25]. For ME of microorganisms, the most used polymers (all natural, inexpensive, biocompatible and GRAS) are chitosan (obtained from arthropods), alginate (a polymer extracted from seaweed), carrageenan, whey proteins, pectin, poly-L-lysine, and starch. Different types of starch and modified starches have been tested as entrapping agents of probiotics [26]; unfortunately, in some cases, the low pH and the presence of proteases, two of the conditions commonly experienced by probiotic organisms during their passage through the stomach, diminish their adhesion to starch [27]. Resistant starch is not degraded by the pancreatic amylase and arrives at the intestine in an indigestible form. This provides a good release of bacterial cells in the large intestine and offers them prebiotic functionality [28**]. The materials are used alone (monolayer) or in combination: in this last case, coating the microcapsules with an additional film can avoid their exposure to oxygen during storage and can enhance their stability at low pH. For example, one of the most common double (or triple) layer strategy is represented by an inner layer of alginate, containing the entrapped microorganisms, then covered by a monolayer of chitosan that might be eventually contained in a further outer layer of alginate, chitosan or other polymer [29–31]. Chitosan-coated alginate beads give better protection in simulated gastric conditions than other outer coating films [27,32].

Different techniques are used in microencapsulation of probiotics: coacervation, emulsion, extrusion, spray-drying, and gel-particle technologies (including spray-chilling). Coacervation is a fluid–fluid phase separation of an aqueous polymeric solution, where a change in pH enables the formation of the shell by the polymer complex. Microcapsules are then dried by spray-drying or freeze-drying [33,34]. When using the emulsion technique [35], the encapsulating material is added to the bacterial cells; then, the mixture is suspended in an oil bath containing Tween 80 (acting as emulsifier). The emulsion is then broken by adding CaCl₂ and microcapsules are collected by centrifugation. For extrusion, probiotics are added to a hydrocolloid solution, then the solution is dripped through a syringe needle or nozzle [27], which influences the size of microcapsules.

Spray drying involves atomization of a suspension of probiotics and carrier material into a drying gas, giving rise to a rapid evaporation of water. Spray-drying precondition the cells so that they can be better stress-adapted to subsequent environmentally adverse conditions, such as high temperatures, acidic environment, or presence of bile salts. However, despite these advantages, the high temperatures needed to facilitate water evaporation also lower the viability of the probiotics and reduce their activity in the final product.

Spray chilling involves the dispersion of the core material into a warm coating material and the subsequent spraying through a heated nozzle into a controlled environment, where the encapsulant solidifies to form the microcapsule particles [27]. The process is performed in equipment similar to that used in spray drying except that the process air is not heated.

Sometimes, microcapsules have a certain number of particles located at their surface. This type of microencapsulation is known as matrix encapsulation, and often provides more protection during spray drying and storage of probiotics, as well as during their passage through the stomach [27].

The size of the microcapsules is an important parameter that affects the sensory properties of foods. The size of alginate beads ranges from 20 μm to 4 mm. However, a bead size of 150 μm can be obtained with the new extrusion technologies, thereby allowing the achievement of more uniformly shaped microcapsules than those reached through the emulsion technique. Spray drying produces capsule sizes ranging between 5 and 80 μm. Bead size also affects the adhesion property of probiotics, and is optimal with granules of starch that are 50 μm in diameter [27,28**,29,36–37].

Co-encapsulation of microorganisms and other important components like prebiotics protects probiotics in food systems and in the gastrointestinal tract much better, owing to symbiosis [38–40]. To date, dairy-based products have emerged as the main carriers for the delivery of probiotics to humans [18**]. However, increasing demand by consumers has opened a trend in using nondairy-based products as potential carriers of probiotics. Most of the foods containing probiotic microorganisms are found in the refrigerated section of supermarkets; this being owing to the fact that the bacteria are sensitive and can be destroyed by heat.

Research is ongoing to develop new envelopes, especially formed by double or triple layers of alginate-chitosan, that allow a better resistance of bacteria to heat (e.g. to pasteurization). Ding and Shah [25*] demonstrated, in fact, that the heat tolerance of microencapsulated probiotic bacteria incubated at 65 °C for up to 1 h survived at
Lactobacillus acidophilus microencapsulated in alginate + inulin + xanthan and grown in berry + grape juice (F. Nazzaro, figure not published).

30 min with good protection. Use of probiotics is continuously expanding in the food industry. From that point of view, ME can achieve a wide variety of functionalities according to the development of the technology, and nowadays encapsulated probiotic cells can be incorporated into many types of food products.

Different foods containing encapsulated probiotic cells are present on the market. Belgium group Barry Callebaut produces chocolate containing encapsulated probiotic cells that do not negatively affect the taste, texture or mouth feel of the final functional product, and which, at doses of 13.5 g per day, might be sufficient to positively affect the gut microbiome. In some cases, inulin or other prebiotics have been added to probiotics in the manufacturing of the bar called ‘Attune’ (www.attunefoods.com), into yogurt-covered raisins, nutrient bars, chocolate bars, or tablets (www.balchem.com). The ice cream industry is viewing the probiotic market with much interest. Unilever, Hansen, and company Dos Pinos, have developed a probiotic ice cream having multiple health benefits (www.chr-hansen.com). Many products containing encapsulated probiotic cells are available in a tablet/capsule form or in a powder form [22], ensuring the probiotic cells to be preserved against the acidic juices of the stomach and able to reach the intestine, and with a shelf life over 24 months if stored at refrigerated temperature (www.cerbios.ch).

Today, new foods such as cereal-based products, soy-based products, fruits, vegetables and meat products are considered as potential carriers of probiotics. However, growth in non-dairy products such as sausage and fruit juices is diminished by the presence of inhibitory substances such as nisin, organic acids and curing salts. Appropriate selection of cultures to be microencapsulated can improve their viability without affecting the sensory property of the final products [41–44,45**], opening new frontiers in the use of ME in food industry. Fratianni et al. [46*] used the microencapsulated L. acidophilus to ferment a fruit juice (berry + grape). The strain not only was capable to grow in fruit juice and ferment it, creating a new functional product but, after fermentation, microcapsules exhibited a noticeable microbial viability and an amount of anthocyanins similar to that present in a litre of red wine (Figure 3). One of the increasing interests in the future will concern mainly the use of new probiotic/prebiotic combinations. Special attention is needed to design future carrier matrices and technology, to keep pace with the increasing consumer interest in health issues, food safety and environmental consciousness.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


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