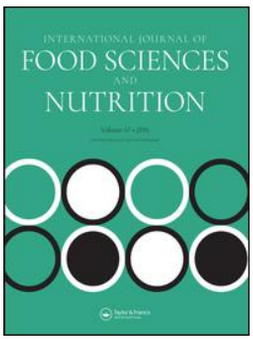

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COMPREHENSIVE REVIEW

Encapsulation of health-promoting ingredients: applications in foodstuffs

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ABSTRACT

Many nutritional experts and food scientists are interested in developing functional foods containing bioactive agents and many of these health-promoting ingredients may benefit from nano/micro-encapsulation technology. Encapsulation has been proven useful to improve the physical and the chemical stability of bioactive agents, as well as their bioavailability and efficacy, enabling their incorporation into a wide range of formulations aimed to functional food production.

There are several reviews concerning nano/micro-encapsulation techniques, but none are focused on the incorporation of the bioactive agents into food matrices. The aim of this paper was to investigate the development of microencapsulated food, taking into account the different bioactive ingredients, the variety of processes, techniques and coating materials that can be used for this purpose.

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Introduction

In the last several decades, the concept of feeding has further evolved. Diet is no longer necessarily linked to the satisfaction of nutritional needs, but it is directed to the prevention of diseases related to nutrition and improvement of human physical and mental well-being. Bioactive food agents responsible for these positive effects include phytosterols, flavonoids, carotenoids, phenolic compounds, bioactive peptide and lipid, antimicrobials, probiotics, oligosaccharide and fibres (Table 1). They may be chemically synthesised or isolated from plants and from animal sources though natural ingredients are generally preferred. These compounds may be naturally present in foods or added, as functional ingredients (Rein et al. 2013). However, the addition of health-promoting ingredients to food products, to improve nutritional value and functionality, presents several challenges associated with their physical/chemical instability and incompatibility with the product matrix. These challenges can be overcome by encapsulating the bioactive compound into a matrix or shell material before it is introduced in the final product. A further challenge is to identify and design the right encapsulation system using the appropriate technique that would make this possible without adversely affecting the bioactive agent activity and bioavailability while maintaining or improving the product sensory attributes such as physical appearance, taste and texture. Many bioactive food agents may benefit from encapsulation (Davidov-Pardo et al. 2012; Umesha et al. 2015; Liu et al. 2015) (Table 1). For example, some bioactive agents can slowly degrade, lose their activity, become hazardous by oxidation reactions and can react with other components present in the food system, which may limit their bioavailability (Dubey et al. 2009). Foods fortified with probiotics present challenges: the probiotics can lose their viability during processing, storage and when passing through the human stomach with a strong acidic gastric fluid. Due to the use of encapsulation, it is possible to increase the survival rate of probiotics in food products and to have a targeted release in the intestine (de Vos et al. 2010; Nazzaro et al. 2012). Fortification with vitamins and minerals is a challenge because of their susceptibility to degradation during processing and storage, these compounds are generally sensitive to temperature, moisture, oxygen, light, pH and their effect is often compromised by the interaction with other ingredients. Fat-soluble bioactive compounds cannot be easily incorporated in food products because of their low solubility in water and highly susceptibility

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Table 1. Examples of bioactive compounds that may benefit from encapsulation before they can be successfully utilized within food stuffs (adapted from Kris-Etherton 2002; Champagne & Fustier 2007; McClements 2015).

Bioactive compounds	Examples	Putative beneficial biological effects	Potential advantage of encapsulation
Phytosterols	Sitostanol, stigmasterol, campesterol	↓ TC and LDL-C, AOx, ↓ cholesterol absorption. Adverse effect: ↓ carotenoid absorption	Prevent/retard chemical degradation Facilitate storage and utilisation Allow incorporation into aqueous medium Increase bioavailability and bioefficacy
Flavonoids	Epicatechin, epigallocatechin, epicatechin-3-gallete, epigallocatechin-3-gallete		Increase solubility in aqueous medium and bioavailability
Phenolic compounds	Hydroxytyrosol Resveratrol	Antioxidant Anti-inflammatory Slowing the aging process ↓ LDL-C oxidation, ↓ platelet aggregation/thrombosis, ↓ eicosanoid synthesis, AOx, carcinogen detoxification, antimutagen, ↓ tumor initiation/ promotion, estrogen/antiestrogen	Increase oral bioavailability (e.g. resveratrol Increase solubility limited stability Reduce metabolization rate & enhance efficacy at target sites
Carotenoids	Lycopene	Antioxidant ↓ LDL-C and LDL-C oxidation, AOx, antimutagen	Avoid or reduce oxidation and improve stability
Bioactive lipids	ω-3 fatty acid Conjugated linoleic acid		Avoid chemical degradation (oxidation) Allow incorporation in aqueous medium Improve ease of utilisation Control delivery in the GIT
Bioactive Peptides and enzyme	Milk peptide Meat peptide Plant peptide	Immunomodulatory, opioid, anxiolytic, antihypertensive, antimicrobial,	Mask bitterness and astringency Retard degradation in the stomach Avoid premature proteolysis
Probiotics	Lactic acid bacteria Yeast		Improved stability during storage in dried form Improved survival during their incorporation into products, storage and exposure in GIT environment Target delivery Protection against bacteriophages and yeast contaminants (e.g. Fermented milks)
Oligosaccharides and fibers	β-Glucan, pectin, psyllium, inulin Arabinoxylans, lactulose	Prebiotics ↓ TC, TG, LDL-C	Avoid adverse ingredient interactions Improve product texture, and stability Control delivery in the GIT

AOxm: antioxidant activity; BP: blood pressure; CVD: cardiovascular disease; HDL-C: high-density lipoprotein cholesterol; HMGR: HMG CoA reductase; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; TG: triglycerides.

to oxidation (de Vos et al. 2010). To circumvent these drawbacks, encapsulation has proved to be an effective technology (Banville et al. 2000; Toniazzo et al. 2014). Phytochemicals are active compounds extracted from plant source and in various ways contribute to the reduction of chronic disease risk. The phytochemicals of interest to the food industry include phytosterol, coenzyme Q10, curcumin, garlic extracts and polyphenols. For instance, phenolic compounds are very useful for human health because of their free radical scavenging property. However, the unsaturated bonds in the molecular structure make them susceptible to oxidants, light and heat (Dias et al. 2015). Nano/micro-encapsulation appears as a response to overcome this problem, reducing the degradation of the polyphenols and protecting its antioxidant activity (Munin & Edwards-Lévy 2011; Aizpurua-Olaizola et al. 2016). Many studies have reported the use of nano- or microencapsulation for the production of functional foods containing fatty acids, such as dairy products, cereal products, beverages and bakery products (Sanguansri & Augustin 2010; Burgain et al. 2011; Shi et al. 2014). Fatty acids, particularly docosahexaenoic acid, eicosapentaenoic acid, α-linolenic and conjugated linoleic acid have attracted significant attention due to their potential health benefits. Direct addition of fatty acids aiming to supplement food products is limited due to their hydrophobic nature, oxidative instability and fishy taste and odour (de Vos et al. 2010; Sanguansri & Augustin 2010). Lycopene has generated great interest because of its possible role in the prevention of chronic diseases, such as atherosclerosis, skin cancer and prostate cancer. Due to the high number of conjugated double bonds, it is considered to be one of the most potent antioxidants among the carotenoids. However, lycopene can be susceptible to oxidants, light and heat.

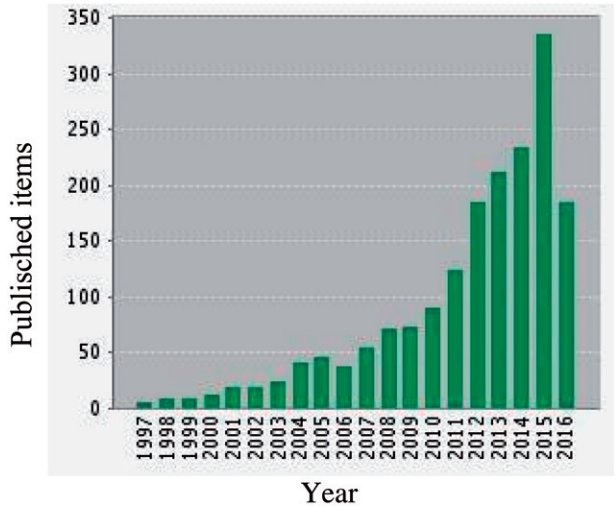

Figure 1. Number of research and review papers published in the last 20 years regarding the applications of encapsulation in food (obtained on web of science, June, 2016; keyword: encapsulation food).

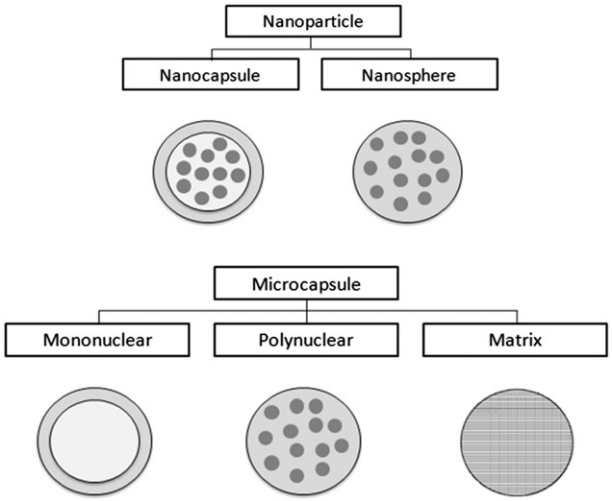

Figure 2. Types of nanoparticles (size 10–1000 nm) and microcapsules (size 3–800 μm).

Encapsulation can be effective to increase lycopene stability while enabling its dispersion in an aqueous medium (Chiu et al. 2007). Encapsulation can also be used to add antimicrobial substances, such as allyl isothiocyanate or rosemary essential oils, in food products. These compounds are unstable, and they can undergo oxidation or volatilization if they are not encapsulated. Their stability can be increased by applying the nano- or microencapsulation technique (Chacon et al. 2006; Teodouro et al. 2014). The technologies are already well known but none of these practices can be considered as universally applicable for bioactive food compounds. The choice of the most suitable technique to use should be carefully considered according to the characteristics of the bioactive molecules such as molecular weight, polarity and solubility (Augustin & Hemar 2009).

In view of the increasing demand of functional food, and taking into account the potential positive effects of encapsulation on stability, bioavailability and bioefficacy of bioactive agents, a great number of scientific papers regarding microencapsulation for food applications have been published (Figure 1). Therefore, the aim of this paper is to present an overview on the studies that brought to the development of microencapsulated foods taking into account the different bioactive ingredients, the variety of encapsulation techniques and coating materials that can be used for this purpose.

Nano/microencapsulation is a rapidly expanding technology through which a bioactive or functional ingredient is packaged within a secondary material in order to form small capsules. The substance that is encapsulated may be called core material, the active ingredient, nucleus or internal phase. The material encapsulating the core is referred to as the coating, membrane, shell or wall material. The core may be composed of one or more ingredients and the wall may be single or double layered (Poshadri & Aparna 2010; Pillai et al. 2012; Gunasekaran 2014). The core can be of a spherical or irregular shape, and its composition can be liquid droplets, solid particles or even gas bubbles (Umer et al. 2011). Based on the capsule size, the name and the technology of the encapsulation are different: nanocapsules or nanospheres are characterized by a small size (10–1000 nm) and the technology is called nanoencapsulation. In particular, nanocapsules are vesicular systems in which the bioactive compound is surrounded by a unique polymer membrane, while nanospheres are matrix systems where the bioactive compound is uniformly dispersed (Figure 2) (Ezhilarasi et al. 2013b). If the particle size ranged from 3 to 800 μm, these are called microspheres and the technology involved is termed microencapsulation (Gunasekaran 2014). According to their morphology, microspheres can be classified such as mononuclear, polynuclear or matrix types.

Mononuclear (core-shell) microcapsules contain the shell around the core, while the polynuclear capsules have many cores enclosed within the shell. In the matrix types, the core material is distributed homogeneously into the shell material. In addition to these three basic morphologies, microcapsules can also be mononuclear with multiple shells, or they may form clusters of microcapsules (Figure 2) (Jyothi et al. 2010; Umer et al. 2011; Jyothi et al. 2012).

Nano/microencapsulation can provide a physical barrier between the core and the environment and can be utilized in many applications in the food industry: to mask odours, colours or tastes, to protect reactive substances from the environment, to separate incompatible components, to provide a controlled release, etc. Therefore, as the core is protected from the environment and from other components in the food, the use of encapsulation can improve the nutritional content of food without altering the taste, aroma or texture of food (Augustin & Hemar 2009; Gallo & Corbo 2010). Nano/microencapsulation can improve the availability of bioactive compounds in foods. Some of the compounds successfully microencapsulated in the last few years include probiotics, minerals, vitamins, lutein, fatty acids, lycopene and antioxidants (Sanguansri & Augustin 2010; McClements 2015). However, the encapsulation of bioactive agents is still performed at low payloads (1–5%) (Đorđević et al. 2014) and it is important to know the various factors involved in the process, in order to optimize, as much as possible, the concentrations of the bioactive molecules encapsulated.

The choice of encapsulant material is very important, depending on the properties of the core and the purpose of microencapsulation. The matrices in contact with food may be natural or synthetic polymers, but in both cases they have to be Generally Recognised as Safe (GRAS) for human health (Nazzaro et al. 2012). The choice of the coating material is dependent on a number of factors, including its compatibility with the target food application and its influence on the sensory properties of the final product. The encapsulant materials must also have a number of specific requirements as the following:

- chemically inert;
- non-toxic;
- sterilized;
- stable from a physical-chemical point of view;
- mechanically resistant;
- must not release impurities or other residue.

Materials can be chosen from a wide variety of substances and may be carbohydrates (starch, modified starch, dextrin, sucrose, cellulose and chitosan), gums (arabic gum, alginate and carrageenan), lipids (wax, paraffin, mono and diglycerides, oils and hydrogenated fats) or proteins (gluten, casein, gelatine and albumin) (Silva et al. 2014). These materials may be used alone or in combination, with or without other additives, such as emulsifiers, plasticizers, channeling agents, surfactants or deforming agents (Sanguansri & Augustin 2010).

Encapsulation processes

Processes used in the food industry for encapsulation are generally adapted from methods initially used in the pharmaceutical industry. However, compared with the pharmaceutical industry, the food industry is more obligated to cut production costs (Sanguansri & Augustin 2010). Several processes can be applied to the microencapsulation of bioactive agents and each of them provides microcapsules with different characteristics in terms of microcapsule size and morphology (Burgain et al. 2011). The selection of the microencapsulation process is governed by the physical and chemical properties of core and coating materials as well as by the intended application of food ingredients (Poshadri & Aparna 2010). Microencapsulation processes can be classified into three categories physical, chemical and physico-chemical processes (Mishra 2015). The physical methods include spray drying, spray-chilling, rotary disk atomization, fluid bed coating, stationary nozzle coextrusion, multiorifice-centrifugal process, submerged nozzle coextrusion, pan coating, air suspension coating and centrifugal extrusion. Chemical processes are those involving mainly polymerization of monomers at the surface of the dispersed core material (interfacial polymerization) or *in situ* formation of the shell with no reactants included in the core material (*in situ* polymerization). Conversely, physico-chemical processes refer to the formation of the microcapsule/microsphere shell from a preformed polymer involving processes such as ionotropic gelation, polyelectrolyte complexation, coacervation, phase separation and solvent removal (Umer et al. 2011).

Nanoencapsulation techniques are more complex than those normally used in the microencapsulation. The techniques which can produce capsules in the nanorange are coacervation, nanoprecipitation, inclusion complexation and supercritical fluid extraction techniques (Gunasekaran, 2014).

The encapsulation techniques, as well as the encapsulant materials of interest have recently been reviewed elsewhere (Gunasekaran 2014; Augustin & Sanguansri 2015; Dias et al. 2015). Most of the commonly used microencapsulation techniques in the food industry are described below.

Spray drying

Spray drying is the most common technology used in the food industry to microencapsulate bioactive food ingredients; in fact, about 90% of microencapsules have been prepared by this technique (Đorđević et al. 2014; Gouin 2004). The process consists of the

transformation of a liquid active material into a dry powder (Gallo & Corbo 2010). Core materials are dispersed in a polymer solution, forming a dispersion or emulsion, and then sprayed into a hot chamber. The solvent evaporation causes solidification of the shell material. This procedure allows the formation of polynuclear or matrix-type capsules. Depending on the initial concentration of the feed solution and operating conditions, the size of the microcapsules produced ranges from 10 to 400 µm (Zuidam & Shimoni 2009; Vandamme et al. 2014). Spray drying is a relatively simple encapsulation process that enables the choice among different coating materials. Other advantages are the rapidity, the low-cost and the ease scale-up (Shi et al. 2014). However, although spray drying is widespread in the food industry, there are several disadvantages associated with this process, such as the high temperature used in the process for solvent evaporation. This is especially problematic when considering the encapsulation of probiotic bacteria or heat-sensitive molecules (Gallo & Corbo 2010). This drawback can be overcome by applying lower operating temperatures and selecting appropriate coating materials able to interact with the bacteria to preserve their survival. For example, Khem et al. (2016) deduced that a high survival of *Lactobacillus plantarum* encapsulated in Whey Protein Isolate (WPI) with spray drying is attributable to the hydrophobic interactions between bacterial cells and the exposed hydrophobic portions of the encapsulation matrix during processing. Another limitation is that only water-based dispersions are applied in spray drying. Therefore, the coating should have a high solubility in water, while both hydrophilic and hydrophobic bioactive molecules can be used as the core materials. However, hydrophobic molecules prior to drying are usually first dissolved in an oil phase (Augustin & Hemar 2009), which is emulsified in an aqueous medium by homogenization and stabilization with surfactants (Di Battista et al. 2015).

Spray cooling

This process, also called spray congealing or spray chilling, consists of making a dispersion or emulsion of bioactive products in the coating material, which is then atomized through a nozzle into a chamber where its contact with injected cold air or liquid nitrogen results in the solidification of particles. This process can be implemented with spray drying equipment. Lipophilic materials, such as fatty acids, alcohols, triacylglycerols and waxes that have a high melting point are usually applied to encapsulate bioactive molecules by this process. Spray cooling produces matrix-type solid lipid microparticles (SLMs), from 20 to 200 µm, which appear to be dense and not empty, different from those obtained with the spray drying. Spray chilling is a good choice for encapsulation of thermosensitive ingredients, such as ω-3 fatty acids, enzymes and probiotics; it also reduces energy use and operation time. However, spray chilling presents some disadvantages, such as low encapsulation efficiency of active materials and the possibility of expulsion of bioactive ingredients during storage (Okuro et al. 2013; Vandamme et al. 2014). Moreover, spray drying and spray chilling often produce microcapsules with some of the core material located at the surface, especially when a solid is encapsulated. This drawback can be overcome by subsequently coating the encapsulate with other methods, such as fluid bed coating.

Fluid bed coating

Fluid bed coating is an encapsulation process where a coating is applied onto solid particles. Solid particles are suspended by an air stream and then covered by a spray of liquid containing coating material. In general, aqueous solutions of hydrocolloids, such as gum, protein and melted fats or waxes have been used as coating formulations. It is a very efficient way to apply a uniform layer of coating material onto solid particles, obtaining reservoir type microspheres. According to the position/location of the spray nozzle, it is possible to distinguish three spray modes for microencapsulation by fluidized bed: top spray, bottom spray or tangential spray. In the fluid bed, drops and particles have sizes which ranging from 0.01 to 0.04 µm and from 100 µm to several millimeters, respectively (Gouin 2004; Guignon et al. 2002). This process can be used to add a secondary coating to improve the protection of the active molecules in the core or for their target release in the gut. Moreover, due to an efficient heat and mass transport in a fluidized bed, it is possible to dry the coated particles at lower temperatures compared with spray drying which results in greater protection of bioactive components (Augustin & Hemar 2009).

Emulsification

Emulsification is defined as "a process of dispersing a liquid in a second immiscible liquid". By including a core material in the first liquid, it is possible to encapsulate bioactive agents (de Vos et al. 2010). There are two combinations of emulsions: water/oil or oil/water emulsion and water/oil/water or oil/water/oil double emulsions. Therefore, it is possible to use emulsions as

a delivery vehicle for either water-soluble or lipophilic active agents in food products (Zuidam & Shimoni 2009; Nedovic et al. 2011). The size of the particles is about 50–500 µm (Gallo & Corbo 2010) but could be far smaller depending on the formulation and processing conditions. The advantage of the emulsification is that it is easy to scale-up and it is possible to choose different coating materials. However, it involves several additional procedures, such as emulsification and separation of oil (Shi et al. 2014). As a substitute to traditional emulsions, the multiple or multilayered emulsions can be used. Multiple oil-in-water-in-oil (O1/W/O2) emulsions can be prepared using a hydrophilic emulsifier to stabilize the O1/W interface and a hydrophobic emulsifier that stabilizes the O2/W interface. In these emulsions, the two oil phases may have equal or different nature and the encapsulated bioactive molecule is in the inner oil phase. Moreover, multilayered emulsions have recently been used for the encapsulation of ω-3 oils (Jiménez-Martín et al. 2015).

Extrusion

The extrusion process produces droplets by forcing a biopolymer liquid containing the dispersed active core through an orifice into a hardening bath. The smaller the diameter of the orifice, the smaller the size of the capsule. A typical process is syringe-extrusion to form alginate beads using a solution containing alginate and the bioactive molecule that is extruded into a calcium chloride solution. It is possible to obtain a scale-up of droplet production by the use of a multiple nozzle system, spinning disc atomizer or by jet-cutter techniques, even if the throughput is still limited in comparison with the emulsification in stirred vessels (Augustin & Hemar 2009; de Vos et al. 2010). The microencapsulation based on extrusion process has widely been used for the encapsulation of volatile and unstable flavours in glassy carbohydrate matrices (Poshadri & Aparna 2010). Moreover, the extrusion technique has many advantages for microencapsulation of microorganisms: it is relatively gentle, does not require the use of solvents and can be conducted in aerobic and anaerobic conditions (Kiran et al. 2015).

Coacervation

Coacervation, often called "phase separation", is a physical process of phase separation and consists of three stages, all carried out under constant stirring. The first stage includes the formation of a solution composed of three immiscible phases: the core (active molecule), the coating material and the solvent. After the addition of a third component, or after a variation of the parameters such as temperature, pH or salt addition, two phases are formed: one which is polymer-rich (coacervate) and another primarily consisting of the solvent. The second step includes the deposition of liquid polymer upon the core material, and in the third stage, the coating layer is solidified thermally or by evaporation (Jyothi et al. 2010). A large number of coating materials have been evaluated for applying coacervation, but the most common coating system is gelatine/gum acacia (Poshadri & Aparna 2010) that has recently been used for the microencapsulation of poppy seed oil (Yang et al. 2015).

Application of encapsulated bioactive compounds in foodstuffs

The incorporation of bioactive agents in foodstuffs is a challenge due to the different characteristics and properties of the molecules. In the last 20 years, researchers have identified many bioactive agents but surprisingly few nutraceuticals have been incorporated as ingredients in functional food (Augustin & Sanguansri 2015). As reported by Dias et al. (2015), the majority of the studies about the nano/microencapsulation of the health-promoting ingredients do not include their incorporation into food matrices. However, this step is very important for the food industry, especially for the assessment of the stability and the acceptability of the final product. In general, the most studied matrices are dairy products, followed by cereals and bakery products. Fruit, meat and cream are other food matrices that were evaluated for incorporation of microencapsulated bioactive compounds.

Dairy products

Milk

Milk has commonly been used as a carrier for microencapsulation of active compounds (Table 2). Indeed, milk is a good candidate for fortification, not only due to its worldwide consumption but also because of its high nutritional value, the buffer effect in the digestion and absorption processes and the positive effects on growth. Liquid milk fortification with vitamins A, C, D and E is performed in several countries. Also, some milk has been fortified with different micronutrients such as calcium, zinc and iron. Unfortunately, vitamins are sensitive to heat, light and humidity, as well as oxidation. Nevertheless, fortification with minerals, in general, less sensitive than vitamins to chemical factors, may give problems of bioavailability, due to their

Table 2. Microencapsulation of bioactive agents in milk.

Product	Bioactive agent	Method	Material	Sensory aspects	Stability of the microencapsulated bioactive compound	References
Milk	Iron	Solvent evaporation	Arabic gum, maltodextrin and modified starch	Data not reported	The iron beads showed significant difference in colour, appearance, odour and taste after 5 days of storage	Gupta et al. (2015a)
Milk	Iron	Liposome, Fatty acids esters, freeze-drying, emulsion	Polyglycerol monostearate, sodium alginate, modified starch	Fortified milk presented sensory characteristics similar to unfortified sample	Data not reported	Gupta et al. (2015b)
Milk	Iron	LiposomeFatty acids esters	Polyglycerol monostearate	No significant difference in terms of astringency, colour, bitterness was found.	Lipid oxidation was less rapid in the milk containing the microencapsulated iron	Abbasi & Azari (2011)
Milk	*B. breve*	Emulsion	Outer layer: gelatine and starchInner layer: hard oil made from coconut oil	Data not reported	Number of microencapsulated bacteria remained quite stable under gastric and small intestine conditions	Jung et al. (2007)
Milk	Korean mistletoe extract	Emulsion	Polyglycerol monostearate and medium chain triacyl-glycerol	After 12 days of refrigerated storage, no difference for bitter-ness, flavour and yellowish colour between control and microencapsulated groups was observed	The TBA test showed that the chemical oxidation in encapsullated extract was significantly lower than that of unencapsulated extract	Kim et al. (2008)
Milk	Vitamin C and iron	Spray dryer	Polyglycerol monostearate	After 5 d of refrigerate storage, no differences for most aspects between control and microencapsulated groups was observed	The TBA test showed the lowest value in the milk sample with added encapsulated iron and unencapsulated L-ascorbic acid up to 5 d of storage	Lee et al. (2004)

interaction with milk proteins and lipids. In addition, they may have adverse effects on sensory properties such as colour, odour and taste. Microencapsulation has enabled the production of more stable fortified milk. For example, this technology has been used to protect iron from oxidation by forming an impermeable membrane as a barrier to oxygen diffusion, to mask the flavour and to increase bioavailability. This was demonstrated by Gupta et al. (2015b) who have also evaluated the sensory characteristics and oxidative stability of fortified milk and proved that milk with non-haeme iron microcapsules has a high *in vitro* bioavailability of iron as compared with unfortified milk as a control. Moreover, from the first to the fifth day, the sensory scores of iron salt fortified milk were significantly lower as compared with milk fortified with microencapsulated iron. The encapsulation process used involved formulating ferrous sulphate arabic gum, maltodextrin and modified starch to favour the interaction between positively charged ferrous ions and the carboxylic group of arabic gum followed by spraying the mixture into chilled alcohol. This allowed the extraction of water by using the non-solvent solution for the shell material and the formation of microparticles loaded with iron. Although the process involved a large quantity of ethanol which may make it less attractive for mass production, results suggested a promising encapsulation system which could be used with different encapsulation processes such as combination of polyelectrolyte complexation and spray drying. In order to have a large-scale production of milk fortified with microencapsulated iron, it is necessary to obtain a high encapsulation efficiency and, as shown by Gupta et al. (2015a), this is also dependent on the type of formulation and process. They found higher encapsulation efficiency by microencapsulating iron using the emulsification process when compared with liposome, fatty acid esters, freeze-drying methods.

Moreover, the sensory properties of the milk obtained with the addition of these microcapsules containing 10 mg L^{-1} were similar to unfortified milk. Nevertheless, the choice of the most suitable microencapsulation process cannot be made only on the basis of encapsulation efficiency but also other parameters such as, the stability of microcapsules in a real food system. In the light of these considerations, Abbasi and Azari (2011) studied the thiobarbituric acid (TBA) absorbance of milk as an indicator of oxidation, demonstrating that in the first hour of storage, TBA absorbance of milk fortified with iron microencapsulated with liposome and with fatty acid esters did not increase with rising iron concentrations, while in the milk fortified with non-encapsulated iron, the TBA absorbance increased significantly. Moreover, after 3 days of storage under fridge conditions, albeit the TBA absorbance of all samples increased, the microencapsulation was able to reduce the rate of lipid oxidation by about 60%. Similar results have been reported by Kwak et al. (2003) utilizing ferric ammonium sulphate as core material. The authors have also studied the *in vitro* digestion of the microcapsules coated with polyglycerol monostearate and observed a good resistance after incubation in simulated gastric fluid (release of 3-5%) and an abundant release of 95.7% in simulated intestinal fluid. These results suggested that microcapsulation would be desirable for iron-fortified milk due to an increase of iron absorption by favouring the uptake and effective release in the intestine. Lee et al. (2004) encapsulated vitamin C by spray drying using polyglycerol monostearate as wall material and obtained encapsulation efficiencies between 80.7 and 94.2%. Milk, fortified with prepared microcapsules, was stored at 4 °C for 12 days and the release rate of vitamin C from the microcapsules was 6.6% and 9.2% after 5 and 12 days of storage, respectively, which indicated that encapsulation of vitamin C in polyglycerol monostearate was a potential fortifier for milk and milk products.

Moreover, microencapsulation can be useful to reduce post fermentation acidification and possible negative sensory effects of probiotic food products (Sohail et al. 2012; Ranadheera et al. 2015). Non-fermented milk is also a good carrier for probiotics because it does not contain starter cultures which may compromise the survival of the probiotics. Microencapsulation of *Bifidumbacterium breve* with double layers was used to increase the survival rate during digestion utilizing a starch and gelatine outer layer to endure gastric and intestinal conditions, and an inner layer composed of a hard oil (Jung et al. 2007). Microencapsulation was also used for the fortification of milk with an extract from the European mistletoe (*Viscum album* L.), widely used in cancer prevention, to overcome the problems regarding its instability in processing, its high viscosity and its undesirable flavour and colour. Kim et al. (2008) by using polyglycerol monostearate and medium-chain triacylglycerol as coating materials have demonstrated that microcapsules of Korean mistletoe extract could be incorporated in milk to improve the sensory characteristics with respect to colour and flavour. Moreover, these results also indicate that microencapsulation would also be desirable for lectin fortification, because of an increase of absorption and an effective release in the intestine.

Cheese

Among the traditional dairy foods, cheese is one of the most widely consumed. Cheese is an interesting food-based vehicle of different bioactive compounds, such as omega 3, enzymes, polyphenols, flavours and vitamins (Pothakamury & Barbosa-Canovas 1996; Bermúdez-Aguirre & Barbosa-Cánovas 2012; Ye et al. 2009; Banville et al. 2000; Seneweera & Kailasapathy 2010; Amighi et al. 2013; Rashidinejad et al. 2014; Da Costa et al. 2015) (Table 3). In particular, due to its acidic pH (4.8–5.6), high-fat content and solid consistency, cheese offers advantages over fermented milk products in terms of delivering a viable probiotic micro-organism in human gut (Anjani et al. 2007; Zuidam & Shimoni 2009). Several types of cheeses have been tested as vehicles for probiotics, such as Iranian white cheese (Zomorodi et al. 2011), Kasar cheese (Özer et al. 2008), Turkish white-brined cheese (Özer et al. 2009), Ricotta cream (Fritzen-Freire et al. 2013), Fiordilatte (Angiolillo et al. 2014) and Cheddar cheese (Amine et al. 2014).

Zomorodi et al. (2011) studied the survival of *Lactobacillus casei*, *L. plantarum* and *Bifidobacterium bifidum* in Iranian white cheese in free and microencapsulated forms. The result indicated that there were a sufficient number of viable probiotic cells both in free and microencapsulated forms in the cheese for the therapeutic minimum (10^7 cfu g^{-1}). However, during ripening, the survival of micro-organisms in microencapsulated form was higher than free cell. The same results were obtained by several authors (Özer et al. 2008; Özer et al. 2009; Mirzaei et al. 2012). Moreover, the addition of probiotic cultures to food did not result in lower acceptance of the food compared with the conventional product, as shown in the development of various Iranian white cheeses (Zomorodi et al. 2011), Kasar Cheese (Özer et al. 2008) and Ricotta cream

Table 3. Microencapsulation of bioactive agents in cheese.

Product	Bioactive Agent	Method	Material	Sensory aspects	Stability of the microencapsulated bioactive compound	References
Cheddar	Flavourzyme	Extrusion and subsequent coating with alginate or po-ly- L-lysine	Alginate, Alginate:starch, Alginate:pectin, K-carrageenan	Data not reported	Uncoated and poly L-lysine coa-ted capsules were stored after freeze-drying and air-drying at 4 C and the frozen capsules at −20 °C to evaluate shelf life. both uncoated and poly L lysine coated, retained most flavour-enzyme than air dried capsules stored at 4 °C	Pothakamury and Barbosa-Canovas (1996)
Cheddar	*B. longum*	Extrusion or emulsion	Native Alginate Palmitoylated Alginate	Data not reported	*B. longum* encapsulated cells in cheddar by emulsion were more stable after 21 days of storage at 4 °C than free cells	Amine et al. (2014)
White-brined cheese	*B.bifidum BB-12 and L. acidophilis*	Extrusion or emulsion	K-carrageenan or Na-alginate	Microencapsulation did not affect the appearance, the co-lour, the texture and overall acceptability of the experimental cheese	After 90 days of storage at 4 °C cells microencapsulated in cheese showed a higher survival than free cells	Özer et al. (2009)
Iranian white cheese	*L. casei, L. plantarum B. bifidum*	Extrusion	Na alginate	No significant difference between the experimental samples and the control was observed in terms of texture and flavour	After 60 days of storage at 8-10° C the survival of encapsulated probiotic bacteria was higher than free cells	Zomorodi et al. (2011)
Cheddar	Vitamin D	Data not reported	Liposome	Data not reported	Vitamin D concentration was significantly higher when en-trapped in liposomes compared with cheese made with cream fortified with vitamin D or with a water soluble vitamin D preparation	Banville et al. (2000)
Iranian white brined cheese	*L. acidophilus LA5*	Extrusion	Na-alginate Hi-maize resistant starch	The addition of free and encapsulated probiotics had no significant effect on sensory properties of probiotic Iranian white brined cheese	After 182 days the reduction of *Lactobacillus acidophilus* in cheese containing microencapsulated cells was significantly lower than cheese containing free cells	Mirzaei et al. (2012)
Kasar Cheese	*L. acidophilus LA-5 and B.bifidum BB-12*	Emulsion or extrusion	κ-carrageenan Sodium alginate	No difference between cheese with encapsulated and free cells in terms of appearance,	After scalding the number of probiotic bacterium showed a slight decline, while the number of	Özer et al. (2008)

(*continued*)

Table 3. Continued

Product	Bioactive Agent	Method	Material	Sensory aspects	Stability of the microencapsulated bioactive compound	References
				texture, aroma, flavour and overall acceptability was observed	unencapsulated probiotic bacteria decreased continuously	
Processed cheese slices	Tuna fish oil	Emulsion	Milk protein complexes	There was no significant difference in the sensory perception of samples containing a low level of fish oil and the control sample containing no fish oil	The peroxide value of samples fortified with fish oil emulsion was lower than that of samples fortified with fish oil	Ye et al. (2009)
Cheddar	Peptidase from *Aspergillus oryzae*	Extrusion and subsequent coating with alginate or poly-L-lysine	Alginate, alginate:starch, algina-te: pectin, K-carrageenan	Data not reported	Data not reported	Seneweera and Kailasapathy (2010)
Swiss cheese	Bioaroma	Spray drying	Maltodextrin, modified starch	Data not reported	Data not reported	Da Costa et al. (2015)
Kariesh Cheese	*B. adolescentis ATCC 15704*	Rennet gelation of milk proteins.	Data not reported	Data not reported	Microencapsulation protected *B. adolescentis* ATCC 15704 in *Kariesh* cheese during cold storage at 9 °C for 2 weeks as compared with free cells	Abd-Elhamid (2012)
Mozzarella	*L. paracasei LBC-1e*	Extrusion	Na-alginate	Data not reported	During the storage at 4 °C for 6 week no statistically significant decrease was observed in LBC-1e	Ortakci et al. (2012)
Ricotta cream	*B. lactisBB-12*	Spray drying	Reconstituted skim milk, insulin, oligofructose enriched with inulin	The results of the sensory analysis showed that the addition of bifidobacteria in the ricotta cream samples, whether in the free or in the microencapsulated form, presented a good acceptability of the product	The viability count for the ricotta cream samples with microencapsulated bifidobacteria was much greater after 60 days of storage than for those with free cells	Fritzen-Freire et al. (2013)

(Fritzen-Freire et al. 2013). The microencapsulation of *Lactobacillus rhamnosus* and fructo-oligosaccharide in an edible sodium alginate coating, which was incorporated in Fiordilatte cheese, also guaranteed high bacterial survival. Furthermore, probiotic and prebiotic substances have a slight antimicrobial effect against *Pseudomonas spp.* and *Enterobacteriaceae,* thus improving the final taste of the product and prolonging its shelf life (Angiolillo et al. 2014). Other researchers evaluated the survival of *Lactobacillus paracasei* LBC-1e during the manufacturing of Mozzarella cheese and simulated gastric digestion. Lactic acid bacteria were added in free and microencapsulated forms in alginate to the cheese and LBC-1e, and total lactic acid bacteria survival was evaluated during the production, storage and simulated gastric and intestinal digestion. Hot stretching during Mozzarella cheese manufacturing caused slight reductions in the numbers of free and encapsulated *L. paracasei*, with values slightly greater in microencapsulated form. During storage, a decrease was observed in total number of viable LAB, but no statistically significant decrease was observed in LBC-1e. The simulated gastric digestion included incubation in 0.1 M HCl. After this treatment, the mixture was converted to simulated intestinal juice by adding pancreatin and bile salts suspended in phosphate buffer.

The alginate microcapsules did not provide any protection against HCl, but unexpectedly increased the survival of LBC-1e in H3PO4 (Ortakci et al. 2012). Abd-Elhamid (2012) reported that microencapsulation of *Bifidobacterium adolescentis* ATCC 15704 with rennet gelation of milk not only increased the survival and vitality in Kariesh Cheese but also the production of acetic and lactic acids during cold storage. Opposite results were obtained from Godward and Kailasapathy (2003) who claimed that microencapsulation of *Lactobacillus acidophilus* and *Bifidobacterium infantis* with alginate and starch by using the emulsification technique caused a higher cell loss during ripening and storage period of cheddar cheese. This result could be explained by the fact that the capsules prevented cell interaction with the external environment and cellular metabolites accumulated inside the capsules may cause the death of the bacteria. Microencapsulation was also used to increase the activity of enzymes involved in cheese ripening. In fact, most of these enzymes are water soluble, and their activity loss (up to 90%) may influence the yield of cheese production, casein degradation and loss of nitrogen in whey (Mohammadi et al. 2014). Moreover, the microencapsulation allowed us to increase the antioxidant activity of cottage cheese fortified with extracts of *Foeniculum vulgare* Mill. (fennel) and *Matricaria recutita* L. (chamomile). In particular, Caleja et al. (2016) used the microencapsulation to preserve the extracts from degradation, indeed the antioxidant effect was limited to 7 days in cheese fortified with non-encapsulated extract while samples functionalized with microencapsulated extracts showed higher antioxidant activity after the 7th day.

The addition of encapsulated enzymes avoided the problems associated with direct enzyme addition and prevented the immediate and extensive proteolysis (Anjani et al. 2007). Many studies have already used the microencapsulation in order to modulate cheese ripening and cheese aroma by the immobilization of flavourzyme, proteinases and lipases (Picon et al. 1997; Kheadr et al. 2000; Kheadr et al. 2002; Kailasapathy and Lam 2005). Cheese ripening involves a series of complex biochemical reactions including proteolysis, lipolysis and glycolysis, which determine changes in smell, taste and texture of different types of cheeses (Mohammadi et al. 2014). Anjani et al. (2007) reported that the immobilization of flavourzyme in alginate matrix, followed by gelling in chitosan containing cationic solution is a good way to prepare encapsulated enzymes for applications in the cheese industry aiming at a controlled release during ripening. Moreover, the addition of capsules before rennetting determined a more even distribution when compared with the addition of capsules before salting. Using the same technique, other researchers microencapsulated flavourzyme from *Aspergillus oryzae* in order to enhance the proteolytic maturity of cheddar cheese, and showed that encapsulated fungal peptidase not only accelerated cheese ripening but also presented the potential to enrich the bioactive peptide profile in cheddar cheese. In fact, after 3 months of ripening, the proteolysis was higher with respect to cheese not microencapsulated and the α and k casein were largely degraded. Moreover, a great number of low molecular weight peptides in experimental cheeses compared with the control cheese were identified (Seneweera & Kailasapathy 2010). Similarly spray-dried microparticles containing Swiss cheese bioaroma produced by *P. freudenreichii* showed greater retention of short chain organic acids flavouring agents when encapsulated with 50% modified starch and 50% maltodextrin spray-dried at 175 °C compared with the formations with modified starch concentrations of 100% or 14.5% and spray drying conditions of 180 °C and 163 °C (da Costa et al. 2015). Moreover, the characteristics of Swiss cheese fortified with microencapsulated of fermented whey permeate produced by *Propionibacterium freudenreichii* was investigated. In this way, it was possible to enhance the shelf life of the short chain organic acids flavouring agents, since they are volatile and chemically unstable in air, light, moisture and high temperatures. The use of cheese as a food for the delivery of phenolic compounds was also investigated. In particular, Rashidinejad et al. (2014) demonstrated that the encapsulation of green tea catechin and epigallocatechin gallate in soy lecithin liposomes is a promising technique to protect and deliver antioxidants to the gut when incorporated into low-fat hard cheese. Microencapsulation has also been used to fortify different types of cheeses with omega-3, to mask undesirable fishy odour or taste and to prevent chemical reactions during food processing. Bermúdez-Aguirre and Barbosa-Cánovas (2013) evaluated the incorporation of omega-3 from vegetable and animal sources in Queso fresco, Cheddar and Mozzarella cheese. They evaluated three stages of cheese making for fortification: after milk pasteurization, during curdling and salting. A better retention was observed with microencapsulated oil, after milk pasteurisation in Queso fresco, during salting in cheddar, and during curdling in Mozzarella cheese. In order to minimize the loss of vitamin D activity in whey during cheese production, Banville et al. (2000) entrapped liposoluble or hydrosoluble vitamin D in milk fat or in liposomes, respectively, then added them to cheese milk, and compared them with a commercial water-soluble vitamin D preparation (Vitex D) added

to cheese milk. The results showed that the stability of vitamin D during cheese making and ripening over a 7-month period was higher by applying the encapsulated vitamin D into liposomes.

Yogurt

Yogurt is considered as the most popular vehicle for probiotics fortification; the incorporation of living cells in yogurt enhances its therapeutic value (Burgain et al. 2011; Mousa et al. 2014) (Table 4). The physical protection offered by microencapsulation can be explained through the limited diffusion of inhibitory substances, such as metabolic products from the starter cultures, lactic acid and bacteriocin into the beads. In addition, the beads protect probiotics from the gastrointestinal conditions and enhance their survival during fermentation (Ziar et al. 2012), without affecting the safety and the tolerability of yogurt (Jones et al. 2012). The incorporation of encapsulated probiotic living cells in yogurt has been widely studied. Pinto et al. (2012) added microcapsules of *Bifidobacterium* BB-12 produced with reconstituted skim milk and/or inulin in samples of frozen yogurt and have demonstrated that the number of cells remained stable in the samples added with microcapsules, while in the frozen yogurt with free cells a reduction of about 34% after 90 days of storage occurred. On one hand, in order to increase the survival of lactic acid bacteria it is possible to encapsulate them in double layer beads, as reported by Mousa et al. (2014), who reported that beads with a two-layer coating of sodium alginate and whey protein significantly increases the *B. bifidum* F-35 count. Moreover, the presence of alginate as polysaccharides has improved the sensory property obtaining a creamier yogurt which was preferred by the panelists. On the other hand, the double-layer beads produced yogurt with a slightly bitter taste due to the addition of calcium chloride as a cross linking agent. These results are in agreement with those obtained by Kailasapathy (2006), who worked on *L. acidophilus* and *Bifidobacterium lactis* microencapsulated in alginate-starch beads and by Krasaekoopt and Tandhanskul (2008), who conducted a study on *L. casei* TIST 390 using alginate as the supporting matrix.

The various health benefits offered by probiotic bacteria depend on their ability to survive the passage through the gastrointestinal tract in sufficient number, generally accepted as 10^7 cfu g^{-1} or ml^{-1}. For this reason, it is important to evaluate how the microencapsulated probiotics survive gastro-intestinal conditions. Ziar et al. (2012) investigated the protective effect of calcium alginate and resistant starch on *L. rhamnosus* LBRE-LSAS and *Bifidobacterium animalis* subsp. *lactis* Bb12 and evaluated the integrity of the beads, by calculating the percentages of cells released upon α-amylase exposure, and probiotic survival when exposed in simulated gastrointestinal conditions. The researchers found that the Ca–alginate–resistant starch beads are resistant to α-amylase and, for both strains, the microencapsulation increases their survival in simulated gastric and intestinal fluids. Moreover, the microencapsulation improved the viability of bacteria and maintained a suitable post-acidifying activity of these beneficial organisms in yogurt after 1 month of storage at 4 °C. The same was shown by Urbanska et al. (2007) and by Ortakci and Sert (2012). On the contrary, Brinques and Ayub (2011) reported no significant difference in cell survival in simulated gastrointestinal conditions between free *L. plantarum* BL011 and the micro-organism immobilized with sodium alginate or pectin, coated with sodium alginate or chitosan. The different results reported by these studies may be attributed both to the different species considered and the different simulated gastric fluid used. For the simulation of gastric juice, Ortakci and Sert (2012) used sodium chloride solutions with adjusted pH, while Brinques and Ayub (2011) used simulated gastric juice with the addition of enzymes. To stimulate the growth and the activity of prebiotic cells in gastrointestinal tract, it is also possible to produce a co-encapsulation of probiotic and prebiotic. Krasaekoopt and Watcharapoka (2014) added to *L. acidophilus* 5 and *L. casei* 01 alginate beads coated with chitosan, galattooligosaccarides, demonstrating that the addition of prebiotics during microencapsulation of probiotic cells increased their resistance to low pH and bile salt in simulated gastrointestinal fluid. This can be due to the presence of carbon and nitrogen sources for the growth of probiotic bacteria. In this case, the selection of complementary prebiotics is very important. For example, Iyer and Kailasapathy (2005) have investigated the effect of co-encapsulation of different prebiotics on the viability of *L. acidophilus* CSCC 2400 and CSCC 2409. The selected prebiotics were inulin, oligofructose and high amylose cornstarch; moreover, the growth of *Lactobacillus* species was evaluated on glucose, fructose, lactose, and also in a carbon-free medium, with this latter used as a control. Compared with inulin and oligofructose, the addition of high amylose cornstarch to capsules containing *Lactobacillus spp.* provided a maximum protection to the encapsulated bacteria after 3 h of incubation at pH 2.0.

β-Carotene is a hydrophobic bioactive compound with important nutritional value but its high tendency to degradation and low bioavailability can reduce its

Table 4. Microencapsulation of bioactive agents in yogurt.

Product	Bioactive Agent	Method	Material	Sensory aspects	Stability of the microencapsulated bioactive compound	References
Frozen Yogurt	*B. lactis* BB-12	Spray drying	Reconstituted skim milk/inulin	Data not reported	During 90 days of storage the counts of bifidobacteria are constant	Pinto et al. (2012)
Yogurt	*B. animalis* subsp. *Lactis* Bb 12 and *L. rhamnosus* LBRE-LSAS	Emulsion	Alginate and resistant starch	Integrity of beads was acceptable under α-amylase and simulated gastrointestinal model	Data not reported	Ziar et al. (2012)
Yogurt	*B.bifidum F-35*	Transglutamminase method and Ca^{2+} cross-linking	One-layer with whey protein and second-layer with alginate	The double-layer beads gave better texture but greater bitter taste. The one layer yogurt had a better overall acceptability	Microencapsulation enhanced the survivability of *B. bifidum* F-35 after 14 days of storage	Mousa et al. (2014)
Yogurt	*L. acidophilus and B. lactis*	Emulsion	Alginate and Hi-Maize starch	After 7 weeks of storage at 4 °C no significant differences in terms of appearance, colour, and flavour were observed. However, significant differences in smoothness of the yogurt were found. Overall, the micro-encapsulation did not modify the sensory characteristics of the product	Approximately 4 and 3 log cycle losses in number of cells of both free *L. acidophilus* and *B. lactis*, respectively were observed. Conversely, the encapsulated bacteria showed only a 2 log decrease in cell number	Kailasapathy and Lam (2005)
Yogurt	*L. acidophilus* ATCC 4356	Extrusion	Alginate	The addition of probiotic cultures in free or alginate-encapsulated form did not significantly affect appearance/colour or flavour/odour of the yogurts. Yogurts that contained en-capsulated ATCC 4356 had the lowest overall li-king score	High survival of microencapsulated probiotic in artificial gastric juice. Moreover, statistically significant reductions of both free and encapsulated ATCC 4356 were observed during 4 weeks refrigerated storage of yogurts	Ortakci et al. (2012)
Yogurt	*L. acidophilus*	Extrusion	Alginateand chitosan	Data not reported	Integrity of microcapsules was preserved after 76 h of mechanical shaking and after 12 h and 24 h in simulated gastric and intestinal fluids. The microcapsules provided the highest bacterial survival after 4 week of storage	Urbanska et al. (2007)
Yogurt	*L. plantarum*	Emulsion	Na-Alginate:pectinNa-Alginate:chitosan	Data not reported	Simulated gastric fluid reduced the viability of free and microencapsulated cell. Under	Brinques and Ayub (2011)

(*continued*)

Table 4. Continued

Product	Bioactive Agent	Method	Material	Sensory aspects	Stability of the microencapsulated bioactive compound	References
					refrigerated conditions, microencapsulated cells showed better survival than free cells	
Yogurt	*L. acidophilus* 5 and *L. casei* 01 and galattooligo-saccarides (GOS)	Extrusion	Alginate chitosan	Data not reported	Addition of GOS during microencapsulation providing the better protection to probiotics, and enhanced the growth of these microorganisms in simulated digestive system	Krasaekoopt and and Tandhanskul (2014)
Yogurt	β-carotene	Multilamellar liposome	Spray dryer and xanthan and guar gum as thickener	There was no significant difference in terms of texture in yogurt with microencapsulated β-carotene	After 95 days of storage, about 90% of the encapsulated com-pounds were preserved	Toniazzo et al. (2014)
Yogurt	*L. acidophilus* CSCC 2400 and CSCC 2409 inulin, oligofructose and high amylose corn starch	Encapsulator	Poly-L-lysine, chitosan, and alginate	Data not reported	Addition of high amylose corn starch to capsules containing *Lactobacillus* spp. provided maximum protection to the en-capsulated bacteria after 3 h of incubation at pH 2.0	Iyer and Kailasapathy (2005)
Yogurt	Fish oil	Complex coacervation	Gelatine acacia gum	Data not reported	Data not reported	Tamjidi et al. (2014)
Yogurt	Fish oil	Spray drying	Barley protein	Data not reported	After 8 weeks at 40 °C fish oil microencapsulated was better protected from oxidation	Wang et al. (2011)
Yogurt	β-carotene	Spray dryingExtrusion	Maltodextrin, chitosan and alginate	Data not reported	Data not reported	Donhowe et al. (2014)
Yogurt	Red beetroot extract	Spray drying	Maltodextrin	The addition of micro- capsules was not perceived in terms of flavour by panelists	Data not reported	Azeredo et al. (2007)
Yogurt	Phenolic extracts of *R. ulmifolius*	Atomization	Alginate	Data not reported	Data not reported	Martins et al. (2014)

health benefits related to dietary intake. Toniazzo et al. (2014) have microencapsulated the β-carotene by the dispersion of multilamellar liposomes stabilized by the addition of xanthan and guar gums as thickeners and tested their incorporation in yogurt. This study revealed that the liposomes are suitable for the protection of β-carotene from degradation for 95 days of storage and about 90% of the encapsulated compound were preserved after this time. The results enabled these researchers to conclude that microencapsulated β-carotene can replace part or all of a commercial strawberry mix made with artificial flavours and colourants used for yogurt production. Furthermore, in addition to protecting the β-carotene from degradation, the microencapsulation is able to increase its bioavailability as shown by Donhowe et al. (2014) who compared *in vitro* the release and bioavailability of three types of β-carotene: a spray-dried powder of β-carotene and maltodextrin, commercially available water-dispersible β-carotene powder, and chitosan-coated β-carotene alginate produced using extrusion techniques and incorporated them in a yogurt matrix. Microencapsulation significantly influences bioavailability, as emerged from *in vitro* digestion. The commercial water dispersible β-carotene had the highest extent of release, the chitosan-alginate microcapsules had the lowest release and lower incorporation into micelles phase during digestion, while the spray drying

β-carotene represented an acceptable compromise between the β-carotene preservation and its bioavailability. Haham et al. (2012) reported the development of functional yogurt fortified with nanoencapsulated vitamin D. This substance, essential for the proper functioning of the human body, is often deficient (Banville et al. 2000). Researchers demonstrated that the encapsulation in re-assembled casein micelles confer better protection to vitamin D against degradation during heat treatment and shelf life, compared to that in polysorbate-80 (Haham et al. 2012). Moreover, the presence of vitamin D nanoencapsulated in casein micelles improved both the viscosity and the gel-rebuilding ability of yogurt as shown by rheological studies (Levinson et al. 2016).

Azeredo et al. (2007) have shown that microencapsulation of red beetroot extract by maltodextrin based on spray drying is useful to decrease the degradation during storage without affecting the sensory characteristics of yogurt. Having the same purpose to achieve antioxidant benefits for yogurt, Martins et al. (2014) microencapsulated the hydroalcoholic extract of *Rubus ulmifolius* Schott in alginate beads, obtaining a better preservation of the antioxidant activity over time. Microencapsulation has also been used to formulate yogurt enriched with microencapsulated fish oil, as reported by Tamjidi et al. (2014), who have microencapsulated fish oil in gelatine-gum acacia by a complex coacervation method, and Wang et al. (2011) who used a spray drying technique and barley protein as wall material. The addition of fish oil microcapsules to yogurt may be useful for improving its health-promoting effect and the rheological properties of the product.

Ice-cream

Ice-cream is one of the most consumed dairy products in the world, and consists of ice crystals, air cells, and fat droplets dispersed in a continuous freeze-concentrated aqueous phase containing polysaccharides, proteins and minerals (Soukoulis et al. 2014) (Table 5). It was reported that ice-cream can serve as a good carrier for delivering probiotic bacteria to consumers (Homayouni et al. 2008). On one hand, probiotics can be incorporated into ice cream either in free or microencapsulated forms. In the first case, probiotics can be supplied by either blending an acidified/fermented milk base (e.g. probiotic yogurt, acidified milk or cream) or by direct inoculation of the ice cream prior to the whipping-freezing step. On the other hand, using microencapsulated probiotic bacteria facilitates the manufacturing process (no need for a cultured milk base preparation). However, it can also effectively reduce the mechanical or osmotic stress that would induce injury to living cells (Soukoulis et al. 2014). Homayouni et al. (2008) monitored the survival for 180 d at −20 °C in symbiotic ice cream with free and encapsulated *Lactobacillus casei* and *Bifidobacterium lactis*. Probiotic cells were microencapsulated in alginate and hi-maize resistant starch by using an emulsion technique. Comparing the survival of microencapsulated cells with free cells it was found that the probiotic survival was raised by 30% during storage at −20 °C for 180 d. Furthermore, from a sensory point of view, the addition of free and encapsulated probiotics had no effect on colour, body texture and taste of ice cream. With the same purpose, Karthikeyan et al.

Table 5. Microencapsulation of bioactive agents in ice-cream.

Product	Bioactiveagent	Method	Material	Sensory aspects	Stability of the microencapsulated bioactive compound	References
Ice-cream	*L. casei* and *B. lactis*	Emulsion	Alginate and resistant starch	In terms of colour, body-texture and taste, there was no significant difference between free and encapsulated pro-biotic	The probiotics survival rate increased by 30% when they were microencapsusulated	Homayouni et al. (2008)
Ice-cream	*L. acidophilus* and *L. casei*	Extrusion	Na-alginate and starch, or Na-alginate and whey protein concentrate	In terms of colour, body-texture and taste, there was no significant difference between free and encapsulated probiotics	The probiotics survival rate increased by 30% when they were microencapsulated	Karthikeyan et al. (2014)
Ice-cream	Pomegranate peel phenolics	Spray drying	Maltodextrin	No significant difference between the mean scores of free and microencapsulated ice-cream was observed	Data not reported	Çam et al. (2014)

(2014) have microencapsulated *L. acidophilus* and *L. casei* with sodium alginate and starch or with whey protein concentrate and starch using an extrusion technique. The microcapsules obtained and free *L. acidophilus* and *L. casei* were added to the ice cream and the survival of probiotics was evaluated throughout the storage period of 180 d at −23 °C. The ice cream containing alginate and whey protein concentrate beads showed more than 30% survival rate over the ice cream with free cells during the same period of storage. Moreover, the sensory analysis of the ice cream showed that the addition of probiotic micro-organisms, in free or encapsulated forms, does not influence the colour, texture and taste of samples. Recently, the feasibility of using pomegranate (*Punica granatum L.*) peel rich in phenolic compounds and PUFAs for producing functional ice cream was investigated (Çam et al. 2014) by microencapsulating phenolic extract of pomegranate peels by using maltodextrin and a laboratory scale spray dryer. Because phenolics are prone to oxidation when exposed to excessive temperatures, the authors studied the effects of different inlet air temperatures on the yield. At low temperatures (130–140 °C), there was an insufficient yield due to ineffective evaporation of water from microcapsules. The better yield was observed when the inlet temperature was 160 °C and the phenolics/maltodextrin ratio was 1/1 or 1/3.

Cereal and bakery products

As reported by Sanguansri and Augustin (2010), cereal and bakery products account for about 20% of the functional food market. After dairy products, this category is the most popular vehicle for the delivery of bioactive compounds due to its market size, convenient format and easy addition to food. The ingredients used for microencapsulation include fatty acids, folic acid, colouring agents, probiotic cells and polyphenols (Verardo et al. 2009; Altamirano-Fortoul et al. 2012; Davidov-Pardo et al. 2012; Liu et al. 2012; Vitaglione et al. 2012) (Table 6).

Bread enriched with a low level of microencapsulated tuna oil is available in some countries (Yep et al. 2002). On one hand, Davidov-Pardo et al. (2008) have shown that it is possible to add fish oil to bread without affecting sensory and technological characteristics, using methyl cellulose as a coating material to produce microcapsules with spray drying. Moreover, from *in vivo* studies conducted on omnivore and vegetarian consumers, it emerged that the oil of microencapsulated tuna used for bread fortification is able to increase Omega-3 long chain-polyunsaturated fatty acid (ω3 LC PUFA) levels in the plasma (Yep et al. 2002).

On the other hand, Gallardo et al. (2013) have microencapsulated linseed oil by spray drying used arabic gum (GA) alone or combined with maltodextrin (MD), methyl cellulose (MC) and whey protein isolate (WPI). Microcapsules made of GA and ternary mixture of GA, MD and MC showed a poor oxygen permeability which minimized lipid oxidation. However, the addition of GA capsules during bread manufacturing produced a decrease of α-linolenic (Muthyala et al. 2004) content probably due to the addition of water, the incubation at 80% RH or the baking at 220 °C, as also reported by Serna-Saldivar et al. (2006), who prepared functional bread fortified with commercial microencapsulated algae, fish oils and flax oil. Opposite results have been reported by Henna Lu and Norziah (2011), who studied the effect of the replacement of shortening with commercial microencapsulated PUFA powders in bread. They have shown that microencapsulated ω-3 PUFA powder is stable in bread, with high recovery of eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA) (80–89%), and low lipid oxidation in bread after baking and storage. This result was confirmed by de Conto et al. (2012) who have reported a good resistance of microencapsulated ω-3 to baking process temperatures. Moreover, bread containing 1.0% of microencapsulated ω-3 PUFA powders was acceptable from a sensory point of view, while in bread with 2.5% of microencapsulated ω-3 PUFA powders the fishy flavour increased and the palatability decreased, especially after storage for a period of time.

The bread was also used as a vehicle for fortification with curcumin, a colourant with several pharmacological activities. Wang et al. (2012) spray dried a mixture containing curcumin, gelatine and porous starch in order to produce microcapsules embedded into the mixture of the bread. The final result showed that not only the microencapsulated curcumin is more resistant to heat, but also reduced mould spore growth. Moreover, from *in vivo* studies, it emerged that the use of microencapsulated bread can increase the curcumin bioavailability by preventing its biotransformation and this phenomenon increases its circulating concentration when compared with the ingredient-free formulation (Vitaglione et al. 2012). Considering the importance of probiotic intake for health benefits, Altamirano-Fortoul (2012) have examined the possibility to include them in a product like bread, which is a staple food in many countries. The authors incorporated *L. acidophilus* in a watery dispersion of whey protein isolate, inulin, pectin, fresh agave sap and carboxymethylcellulose, via spray drying. Subsequently, the microcapsules were

Table 6. Microencapsulation of bioactive agents in cereal and bakery products.

Product	Bioactive Agent	Method	Material	Sensory aspects	Stability of the microencapsulated bioactive compound	References
Bread	Fish oil	Spray dryer	Methyl cellulose, soybean protein isolates, calcium gelatine casein, whey protein concentrate	The microencapsulation with Methyl cellulose and soybean protein isolates did not cause significant modification of bread sensory characteristics	Soybean protein isolates beads showed the lower oxidative rate	Davidov-Pardo et al. (2008)
Bread	Linseed oil	Spray dryer	Arabic gum, maltoxedtrin, methyl cellulose and WPI	Data not reported	Microcapsules made of arabic gum showed a poor oxygen permeability which minimized lipid oxidation	Gallardo et al. (2013)
Bread	Different sources of DHA and/or ω-3 rich oils	Data not reported	Data not reported	The acceptability of the pro-duct was closely related to the period of storage	All breads lost texture throughout 14 days of storage	Serna-Saldivar et al. (2006)
Bread	ω-3	Data not reported	Data not reported	Bread containing 1.0% microencapsulated ω-3 PUFA powder was acceptable, while in bread with a 2.5% microencapsulated ω-3 PUFA, the fishy flavour increased	Greater stability of the microencapsulated bread after 7 days was observed with higher recovery of EPA and DHA and lower lipids oxidation than control bread	Henna Lu and Norziah (2011)
Bread	ω3	Data not reported	Data not reported	The fortified bread had good sensory acceptance even at the maxi-mum dosage of ω-3 microcapsules	The microencapsulated ω -3 presented good resistance to the baking process temperatures	de Conto et al. (2012)
Bread	Curcumin	Spray drying	Gelatine, porous starch	Data not reported	Microencapsulation preserved curcumin even if it was boiled	Wang et al. (2012)
Bread	*L. acidophilus*	Spray drying	WPI, inulin, pectin, fresh agave sap, carboxymethylcellulose and starch	Good acceptability of functional bread	Viable microorganisms remained after the baking process in all the coatings. But was observed a reduction in the microbial counts during the storage period in all the treated breads, independently on the coating treatment	Altamirano-Fortoul et al. (2012)
Bread	L-5MTHF	Spray drying	Modified starch	Data not reported	Coencapsulation with ascorbate significantly improved the stability during storage	Liu et al. (2012)
Bread	L-5MTHF	Spray drying	Skim milk powder	Data not reported	Coencapsulation with ascorbate significantly improved the stability during storage	Tomiuk et al. (2012)
Bread	HCA	Freeze drying	WPI and maltodextrin	WPI microcapsules gave the best	Data not reported	Ezhilarasi et al. (2013a)

(*continued*)

Table 6. Continued

Product	Bioactive Agent	Method	Material	Sensory aspects	Stability of the microencapsulated bioactive compound	References
				colour and sensory attributes to bread		
Bread	HCA	Spray drying	WPI and maltodextrin	The microencapsules incorporated in bread enhanced crust colour, shape, crumb colour, taste, texture and grain score that significantly resulted in higher sensory score.	Data not reported	Ezhilarasi et al. (2014)
Pasta	HCA	Spray drying	WPI	Pasta whit microcapsules showed better mouthfeel, appearance, strand quality and overall quality score	Data not reported	Pillai et al. (2012)
Noodles	L-5MTHF	Spray drying	Modified starch	Data not reported	Coencapsulation with ascorbate significantly improved the stability during storage	Liu et al. (2016)
Pasta	ω3	Data not reported	Corn starch	Data not reported	The type of storage substantially affected the onset of peroxidation in spaghetti	Verardo et al. (2009)
Cookies	Garden cress seed oil	Spray drying	WPC	The sensory score rating of colour, crumb colour and surf-face characteristics of biscuits with microcapsules were com-parable to the control biscuits but were moderately harder in texture due to the presence of WPC and Maillard reaction	Microencapsulation led to a reduced ALA oxidations	Umesha et al. (2015)

resuspended in various starch solutions and the bread was baked for 16 min at 180 °C. After baking and 24 h of storage, there was good survival of LA in the bread crust. The authors concluded that additional layers and materials with a different chemical nature can provide improved thermo-tolerance of microencapsulated probiotics. Moreover, the sensory evaluation of the enriched breads revealed a good acceptability of the product. Recent studies have also proposed the possibility of microencapsulated acid folic and hydroxycitric acid (HCA) to produce several health benefits to consumers. Folic acid is a synthetic form of folate. The fortification of food with folic acid successfully reduced the incidence of neural tube defects. In contrast, the fortification with folic acid may reduce the absorption of Vitamin B12. A potential alternative is to use a reduced form of folate, the L-methyltetrahydrofolate (L-5MTHF) for fortifying foodstuffs, which unlike folic acid should not involve a vitamin B12 deficiency (Tomiuk et al. 2012). In order to fortify wheat for bread production, Liu et al. (2012) microencapsulated L-5MTHF, alone or in combination with sodium ascorbate, in modified starch beads, produced using spray drying. The authors reported that this approach was an effective way to prevent L-5MTHF loss during the production and the storage of bread. Moreover, researchers reported that the presence of sodium ascorbate with L-5-MTHF allowed a further recovery of L-5-

MTHF during the microencapsulation process, showing that the reducing capacity of sodium ascorbate is an important characteristic for stabilizing L-5-MTHF. Also Tomiuk et al. (2012) studied the microencapsulation of L-5-MTHF using skim milk powder with and without sodium ascorbate for bread fortification. The efficacy of wheat fortification with L-5MTHF or equimolar folic acid compared with wheat without folat, was demonstrated by the fact that both forms of microencapsulated folic acid increased blood folate. The assumption of HCA, the major acid present in fruit rinds of *Garcinia cowa,* is related to several health benefits. Various studies reported that HCA regulates the synthesis of fatty acids, lipogenesis, appetite and weight loss. The free HCA, naturally available in fruit, is thermally sensitive and is converted to lactone during drying and evaporation. So the use of microencapsulation aimed to make HCA a shelf stable ingredient, was investigated (Ezhilarasi et al. 2014). Ezhilarasi et al. (2013a) studied the effect of freeze dried microencapsulated *Garcinia* fruit extract on bread quality characteristics and HCA content. *Garcinia cowa* extract was microencapsulated using whey protein isolate (WPI), maltodextrin (MD), and a combination of WPI and MD. Final results showed that incorporated beads significantly enhanced the qualitative characteristics of the product exhibiting higher volume, softer crumb texture, desirable colour and sensory attributes and a greater amount of HCA. In particular, the use of WPI as wall material significantly gave a high volume, soft crumb texture and high desirable sensory attributes to bread. This was attributed to the good encapsulation efficiency and may be due to the denaturation of WPI during bread baking. In a subsequent study, the same authors evaluated the possibility of using the same coatings to immobilize the HCA with a different technology, spray drying. In particular, Ezhilarasi et al. (2013a) reported that different encapsulation techniques have different product yields. Indeed, the encapsulation of *Garcinia* fruit extract with freeze drying yielded high HCA recovery due to the use of low temperatures (−30 °C to 40 °C). Moreover, also in this case, the water extract incorporated in bread had undesirable qualitative characteristics due to the direct effect of *Garcinia* extract on bread while all three encapsulates enhanced qualitative characteristics of bread. The *Garcinia* extract was also microencapsulated in pasta. Some studies have been carried out to develop pasta with enhanced nutritional attributes (Kaur et al. 2004; Prabhasankar et al. 2007). Pillai et al. (2012) investigated the effect of spray drying conditions and whey protein isolates to *Garcinia* fruit extract. The microcapsules obtained were incorporated in pasta and it was found that spray-dried at a 90 °C outlet temperature and 1.5:1 wall-to-core ratio exhibited higher antioxidant activity and better cooking and sensory characteristics when compared with a higher outlet temperature (105 °C) and a wall-to-core ratio of 1:1. Noodles have been used as a vehicle for the addition of folate. L-5-MTHF was microencapsulated by spray drying with modified starch in the presence of sodium ascorbate as a stabilizer. As also previously reported by Tomiuk et al. (2012), microencapsulation of L-5-MTHF with sodium ascorbate was useful for maintaining the stability of L-5-MTHF during noodle making and cooking (Liu et al. 2015). Microencapsulation was also studied to enrich spaghetti with ω-3 PUFA by addition to semolina of an integrator containing EPA and DHA. Two oxidative parameters were evaluated: peroxide value (PV) and oxidized fatty acids. Spaghetti with microencapsulated PUFA had a shelf life comparable with the control pasta. Moreover, ω-3 PUFA were not significantly implicated in the onset of oxidation in spaghetti stored under daylight and accelerated oxidation in a laboratory heater. Indeed, the rate of lipid oxidation was affected by storage conditions more than lipid integration (Kadam & Prabhasankar 2010). Also the cookies have been used for the production of functional foods, because they are popular bakery item and are consumed by whole population (Davidov-Pardo et al. 2012). Umesha et al. (2015) have recently microencapsulated Garden cress seed oil, rich in ALA, in whey protein concentrate (WPC) and incorporated the beads in biscuits, while biscuits fortified with free Garden cress seed oil were used as control. In both samples, the addition of Garden cress seed oil increased the nutritional quality of biscuits, but biscuits containing the bioactive compound in free form showed a higher rate of ALA oxidation. Moreover, the sensory evaluation of microencapsulated biscuits showed that they were acceptable. Similar results have been obtained by Jeyakumari et al. (2016) who fortified cookies with fish oil in order to enhance the dietary intake of omega-3 fatty acids. Davidov-Pardo et al. (2012) demonstrated that it is possible to create functional cookies with an antioxidant activity 10 times higher than regular biscuits. They microencapsulated grape seed extract (GSE), a rich source of polyphenol compounds, with mesquite gum and zein or with maltodextrin and zein by using spray drying. The addition of free GSE resulted in darker cookies, effect masked by microencapsulation. Moreover, biscuits containing GSE beads showed a significant higher antioxidant activity. On the other hand, Fiore et al. (2012) have used the microencapsulation in order to prevent the formation of Maillard reaction products. Some of

these compounds, such as 5-hydroxymethylfurfural (HMF) and acrylamide, are potentially toxic. It has been shown that the addition of NaCl may influence the Maillard reaction through the dehydration of various intermediates (Claus et al. 2008). The microencapsulation of NaCl was performed by using spray drying and three different coatings: melted fatty acid blend, candelilla wax and carnauba wax. Fiore et al. (2012) have shown that increasing NaCl concentration from 0 to 0.65% led to an HMF concentration augment up to 75%, whereas in the presence of encapsulated NaCl the reduction of HMF varied from 18 to 61%. Therefore, they concluded that microencapsulation was a useful approach to prevent the formation of potentially harmful compounds in thermally processed foods.

Animal products

Meat is an important source of protein, vitamins and minerals but also of fat, saturated fatty acids, salt and cholesterol and it has often been fortified by using the microencapsulation technique (Table 7). One of the challenges in the food industry concerns meat products with healthier image replacing, for examples, the quality of lipid fraction (Josquin et al. 2012). The substitution of saturated fatty acids (SFA) with PUFA has already been carried out in dry fermented sausages by Valencia et al. (2006), but they found that the products are more susceptible to lipid oxidation. The encapsulation of fish oil may be a way to achieve this purpose. Josquin et al. (2012) showed that the addition of commercial encapsulated oil to dry sausage lead to a higher firmness rating, lower ω6/ω3 ratio and a lower lipid oxidation compared with control sample. Moreover, a sensory analysis showed that the addition of microencapsulated fish oil masked fishy taste and smell. Also Pelser et al. (2007) replaced pork backfat by flaxseed oil and canola oil, pre-emulsified with soy protein isolate. To this mixture a commercial encapsulated flaxseed oil and encapsulated fish oil were also added. Generally, PUFA/SFA ratio increased in all the samples compared to the control. Moreover, the addition of canola oil and encapsulated flaxseed oil did not reduce the shelf life in terms of lipid oxidation, while the addition of flaxseed oil and encapsulated fish oil showed an increase of lipid oxidation during storage. No significant differences were observed between sausage samples with and without encapsulated fish and flaxseed oils and subjected to sensory and physical analyses.

In order to extend the shelf life of meat, by inhibiting undesirable microorganisms, several bacterial preparations can be added (Lemay et al. 2002). In addition, Pérez-Chabela et al. (2013) have added four thermotolerant lactic acid bacteria (TLAB) (*Aerococcus viridans* UAM21, *Enterococcus faecium* UAM10a, *L. plantarum* UAM17 and *Pediococcus pentosaceus* UAM11) to cooked meat in free and encapsulate form. The microcapsules were obtained by using spray drying and acacia gum as coating material. As expected, the samples inoculated with encapsulated spray dry lactic acid bacteria have significantly higher viable cells than samples inoculated TLAB as free cells. Moreover, inoculation of spray dried LAB caused a concomitant *Enterobacteria* reduction. However, there was a significant difference in terms of colour, resulting in lighter and less red meat batter, but no difference in terms of hardness of samples. On the contrary, Lemay et al. (2002) studied the effect of microencapsulated cultures of *Lactobacillus sakei* (sensitive to cooking temperature) in lyophilized alginate beads in meat system. The researchers showed that the encapsulation of probiotic cells is effective in protecting cells against heat treatment in a liquid medium, but not in a control meat sample. In order to increase the safety characteristics of meat products, Muthukumarasamy and Holley (2006) fortified the dry sausages both with free and microencapsulated *Lactobacillus reuteri* with alginate by using both extrusion and emulsification techniques. *Lactobacillus reuteri* is active against undesirable micro-organism and is also considered as probiotic organism able to produce bacteriocins and other antimicrobials compounds of low molecular weight. Both techniques showed that the survival of *L. reuteri* is greater than free cells. Another problem of dry fermented sausage is that they are traditionally consumed without cooking and some pathogen micro-organisms, such as *Escherichia coli* O157:H7, are able to survive after dry process. Muthukumarasamy and Holley (2007) studied the survival of *L. reuteri* and *Bifidobacterium longum* in dry sausages and showed that microencapsulation of lactic bacteria in alginate beads increases the survival of probiotic bacteria, even if reduces their inhibitory action against *E. coli* O157:H7. Other antimicrobial agents, that can be used against *E. coli* O157:H7 in meat products, are lactoferrin and Ally-Isothiocyanate (AIT) (Muthyala et al. 2004). Al-Nabulsi and Holley (2007) evaluated the effect of lactoferrin in free or microencapsulated form against *E. coli* O157:H7 in dry sausage, obtaining an emulsion of lactoferrin and corn oil. To this emulsion was also added an aqueous phase of WPI and xanthan. This mixture was subsequently lyophilized to produce microcapsules. The results highlighted that, despite LF inhibited the growth of *E. coli* without affecting the growth of starter cultures, microencapsulated

Table 7. Microencapsulation of bioactive agents in animal products.

Product	Bioactive agent	Method	Material	Sensory aspects	Stability of the microencapsulated bioactive compound	References
Fermented sausage	Commercial encapsulated fish oil	Data not reported	Data not reported	The addition of fish oil microencapsulated did not determine the presence of fi-shy taste and smell in final product	The degree of lipid oxidation, in sausage containing fish oil microencapsulated, was reduced	Josquin et al. (2012)
Fermented sausage	Flaxseed oil and canola oil	Emulsion	Soy protein isolate	Sausage containing microencapsulated oil has been appreciated by consumers as well as control	Flaxseed oil and encapsulated fish oil showed increased lipid oxidation	Pelser et al. (2007)
Meat batters	*Aerococcus viridans* UAM21, *Enterococcus faecium* UAM10a, *L. planta-rum* UAM17, and *Pediococcus pentosaceus* UAM11	Spray drying	Acacia gum	Data not reported	The microencapsulation increased survival of bacteria	Pérez-Chabela et al. (2013)
Chicken meat model	*L. sakei*	Lyophilization	Alginate	Data not reported	Microencapsulation didn't protect cells from heat	Lemay et al. (2002)
Fermented sausage	*L. reuteri*	Extrusion and emulsion	Alginate	Data not reported	The survival of microencapsulated *L. reuteri* was greater than free cells	Muthukumarasamy and Holley (2007)
Dry sausage	*L. reuteri and B. longum*	Extrusion techniques	Alginate	Data not reported	Microencapsulation of lactic bacteria increased the survival of pro-biotic bacteria but, on the other side, reduced their inhibitory action against *E. coli* O157:H7	Muthukumarasamy and Holley (2007)
Dry sausage	Lactoferrin	Emulsion	WPI and xantan	Data not reported	Microencapsulated LF showed a lower level of antimicrobial activity	Al-Nabulsi and Holley (2007)
Fermented dry sausages	Ally isothiocyanate (AIT)	Freeze-drying	Arabic gum	Sausage containing 1000 ppm had a strong bitter mustard flavour, brittle texture and slight yellow colour	A greater antimicrobial effect was found in sausages with AIT micro-encapsulated, in proportion to the concentration, compared to control without AIT	Chacon et al. (2006)
Ham	Nisin, oregano essential oil and cinnamon essential oil	Alginate nanocrystal	cellulose	Data not reported	The results showed that nisin microencapsulated was 20 times more available than free nisin	Huq et al. (2014)
Fish burgher	Propolis	Spray drying	Arabic gum and a chemically modified starch	The microencapsulation of propolis enhanced texture, tenderness, juiciness and taste	Data not reported	Spinelli et al. (2015)
Mayonnaise	*B. bifidum* *B. infantis*	Emulsion	Alginate	Mayonnaise containing encapsulated probiotic had higher scores for flavour, texture, colour and overall palatability	Microencapsulation improved probiotics survival	Khalil and and Mansour (1998)

lactoferrin showed a lower level of antimicrobial activity. This unexpected result was attributed to the dilution of lactoferrin made when microcapsules were incorporated in dry-sausage batters. Instead, Chacon et al. (2006) evaluated the survival of *E. coli* O157: H7 in fermented dry sausages when AIT was added to meat. AIT is a potent inhibitor of a large number of pathogenic microorganisms, but its application in food industry is limited due to its volatility. This obstacle could be overcome by applying a microencapsulation technique. For this purpose, Chacon et al. (2006) microencapsulated the AIT with arabic gum by using freeze-drying process. *E. coli* was reduced by 6.5 $\log_{10}$ CFU/g in sausage containing 750 and 1000 ppm of AIT after 21 and 16 d of storage, respectively. The effect of microencapsulated AIT on the total number of viable bacteria was minimum. Despite the microencapsulation, dry sausage containing 1000 ppm of AIT had a strong mustard flavour. Also the addition of lower concentrations of AIT (500 and 750 ppm), significantly affected the sensory attributes, like flavour, appearance, texture and overall impression, probably due to the development of slight yellow colour and more brittle texture in AIT samples. In order to inhibit the growth of *Listeria monocytogenes* in ready-to-eat ham, Huq et al. (2014) microencapsulated nisin in alginate–cellulose nanocrystal microbeads. The results showed that microencapsulated nisin is 20 times more available than free nisin during 28 d of storage at 4 °C. To evaluate the antimicrobial activity in ham, alginate microcapsules containing, nisin, oregano essential oil and cinnamon essential oil have been formulated. The products were also radiated with γ-irradiation at 1.5 kGy to enhance the effect against *L. monocytogenes.* This treatment showed a synergistic anti listerial effect with γ-irradiation on ready-to-eat meat products. In particular, the microencapsulation of essential oil improved the radiosensitivity of *L. monocytogenes* compared with the control (Huq et al. 2015). Microencapsulation techniques have also aimed to minimize the losses of volatile substances in meat products. For example, Jeon et al. (2003) evaluated the ability of different wall materials to retain efficiently flavours isolated from meat processing industry. For this purpose, flavours selected, like benzaldehyde, dimethyl trisulphide, 2-mercaptopropionic acid and benzothiazole, have been microencapsulated in native corn and barley starches. The results showed that all starches could be used as wall material for the microencapsulation of flavour. Moreover, over a 4-week storage period, the tested starches retained flavours better than β-cyclodextrin, which is commonly used for the encapsulation of essential oils and flavour. To enhance the antioxidant properties of fish burgers, Spinelli et al. (2015) have instead microencapsulated propolis with arabic gum and capsul, a chemically modified starch, by using a spray-drying technique. Propolis represents a natural substance very rich in phenolic compounds, but its application in food products is still limited, because of its strong and unpleasant odour that generally compromises the food acceptability. Final results indicated that by using capsule as carrier during the spray drying, it is possible to retain a greater amount of propolis and mask the characteristic smell. Microencapsulation allowed not only an increase in phenolics and antioxidant activity but also a good acceptability of sea bass fish burger by consumers. Microencapsulation process has also been used to give nutritional and health benefit to mayonnaise. The characteristics of mayonnaise, such as elevated concentration of acetic acid and low pH (3.6–4.6), inhibit the growth of micro-organisms, ensuring the safety of the product. However, these mean conditions could be a hurdle to probiotic bacteria growth. For example, bifidobacteria grow slowly in pH <5 and in order to provide benefits, they must be alive in the intestinal tract. Khalil and Mansour (1998) added free and microencapsulated *B. bifidum* and *B. infantis* in mayonnaise. The beads were obtained by feeding a spray dryer with a mixture of probiotic bacteria and alginate. Free bifidobacteria decreased markedly during refrigerated storage and no viable cells were recovered after 2 weeks. Conversely, microencapsulated bacteria survived well up to 12 weeks of storage. Moreover, in samples containing microencapsulated cells, there was a decline of total viable bacterial count due to the ability of bifidobacteria to produce antimicrobial substances. In terms of sensory characteristics, mayonnaise samples containing encapsulated bacteria had higher scores than control samples in terms of flavour, texture, colour and overall palatability.

Vegetables

Microencapsulation was also used to increase the nutritional value of tomato and its derivatives, such as juice, soup, puree, ketchup, sauce and canned that are important sources of vitamins and minerals in diet (Table 7). In particular, tomato juice, already known as a health beverage, could be used to obtain probiotic juice by lactic acid fermentation. The probiotic tomato juice could be used by consumers who are allergic to dairy products and by vegetarians. In order to raise the survival of bacteria, Tsen et al. (2008) immobilized *L. acidophilus* in k-carrageenan and showed that the encapsulated cells could survive to unfavourable low

Table 8. Microencapsulation of bioactive agents in vegetables.

Product	Bioactive agent	Method	Material	Sensory aspects	Stability of the microencapsulated bioactive compound	References
Tomato juice	*L. acidophilus*	Extrusion	k-Carrageenan	Microencapsulated tomato juice showed a better overall palatability	The number of living cells after storage for 10 weeks at 4 °C due to microencapsulation was greater	Tsen et al. (2008)
Fresh cut tomato	Garlic oil	Precipitation method	β-Cyclodextrin	The panelists evaluated as "unacceptable" the smell of tomatoes with free GO, while there was no significant difference between the control and the tomatoes with GO microencapsulated	Garlic oil microencapsulated showed the lowest microbial growth	Ayala-Zavala and González-Aguilar (2010)
Mature green tomato	Isothiocyanate	Complex coacervation	Gelatine and arabic gum	Data not reported	Data not reported	Wu et al. (2015)
Tomato seeds	*B. subtilis*	Extrusion	Alginate	Data not reported	Data not reported	Suarez et al. (2011)

pH. Moreover, a better overall palatability was obtained, comparing the fortified tomato juice with that obtained by adding free cells during 10 weeks of storage at 4 °C. Tomato has also been used as a vehicle for the encapsulation of a known compound for its antimicrobial properties, such as garlic oil (GO) (Golding et al. 2011). An alternative that could solve the issue of high volatility of essential oils, including GO, and facilitate its application as food additive is their encapsulation in β-cyclodextrin. Ayala-Zavala and González-Aguilar (2010) obtained GO:β-CD capsules (12:88 [w/w] ratio) and investigated their effect on microbial growth and sensory quality of fresh-cut tomato. For experimental design, on the tomato slice was applied a filter paper impregnated with GO or GO capsules at different concentrations, 0, 50, 100 and 200 µg/100 g and 0, 0.25, 0.5 and 1 g/100 g, respectively. The results showed that the release of GO volatiles from β-CD capsules is connected with relative humidity (RH) and close to 70% of GO the volatiles were released from capsules when exposed to 100% RH during 5 weeks. The most effective antimicrobial concentration of free oil applied to tomato samples did not correspond with an acceptable sensory quality for panellists. On the contrary, the highest concentration of GO encapsulated showed the lowest microbial growth and the highest sensory quality. Having the same purpose to achieve the healthiness of tomatoes, Wu et al. (2015) microencapsulated isothiocyanate in gelatine and arabic gum by complex coacervation. The results showed that the isothiocyanate microencapsulated could effectively control fungi growth and prolong significantly the tomato preservation. Furthermore, Suarez et al. (2011) evaluated the possibility to microencapsulate the antagonist rhizobacteria belonging to *Bacillus* genus with sodium alginate, in order to increase their inhibitory properties, to stimulate of plant growth and increase crop yield. The beads containing bacterial strains were inoculated into tomato seeds and the microcapsules were applied again after the plants emerged. Results showed an effective biocontrol of growth of phytopathogenic fungi.

Fruits

Fruit juices may represent an alternative functional food for probiotic bacteria incorporation, because of their high content of essential nutrients, like vitamins, antioxidants and polyphenols. Moreover, they do not contain starter cultures that compete with probiotics for nutrients (Table 8). However, fruits are generally considered too acid to enable a good stability of cells during storage (Rodrigues et al. 2012; Sohail et al. 2012). The protection of probiotics by microencapsulation is a method to improve their viability in fruit juice matrix (Galgano et al. 2015). Ding and Shah (2008) conducted a study to determinate the survival of free and microencapsulated in alginate *L. rhamnosus, B.longum, L. salivarius, L. plantarum, L. acidophilus, L. paracasei, B. lactis* type Bi-04 *and B. lactis* type Bi-07 in orange and apple juices. The results showed that there is a rapid decline in viability of all free strains both in orange and apple juices. Conversely, microencapsulated bacteria, after 6 weeks of storage, showed a concentration $>10^5$ CFU mL^{-1}. There were changes in soluble solid concentration during 6 weeks of storage

Table 9. Microencapsulation of bioactive agents in fruits.

Product	Bioactive Agent	Method	Material	Sensory aspects	Stability of the microencapsulated bioactive compound	References
Orange and apple juice	*L. rhamnosus, B. longum, L. salivarius, L. plantarum, L.acidophilus, L. paracasei, B. lactis* type Bi-04 and *B. lactis* type Bi-07	Emulsion	Alginate	Data not reported	After 5 weeks of storage at 4 °C, probiotic bacteria showed better survival in microencapsulated form than free form	Ding and Shah (2008)
Banana	*L. acidophilus*	Extrusion	K-carrageenan	Data not reported	Data not reported	Tsen et al. (2004)
Orange and peach juice	*L.paracasei*	Extrusion	Double coating of chitosan and dextrans-sulphate	Data not reported	After 50 days of storage at 5 °C, the survival of microencapsulated probiotic bacteria in juice was higher than free cells	Rodrigues et al. (2012)
Apple juice	*L. rhamnosus* GG	Spray drying	WPI and physically modified resistant starch	Data not reported	Microencapsulation protected probiotic bacteria at low environmental pH over 5 weeks of storage	Ying et al. (2013)
Orange pear and peach juice	*L. rhamnoosus* and *L. acidophilus*	Aereosol method	Alginate	Data not reported	Their results showed that the microencapsulation did not protect probiotic bacteria	Sohail et al. (2012)
Cranberry juice	*L. rhamnosus* GG	Electrostatic deposition	Pectin, citrus pectin, sodium alginate, k-carrageenan, iota-carrgeeanan, inulin and whey protein	Data not reported	Pectin and whey protein beads were more resistant and provided the best cell protection du-ring juice storage and gastric incubation	Doherty et al. (2012)
Pomegranate and cranberry juice	*L. plantarum* and *B. longum*	Extrusion technique	Alginate, pectin, chitosan, glucomannan and gelatine	Data not reported	The beads with double coating of gelatine and pectin improved considerably the cell survival	Nualkaekul et al. (2013)

of free and microencapsulated probiotics in both orange juice and apple. The final °Brix of fruit juices with encapsulated probiotic bacteria was greater than juices inoculated with free cells. These results indicated that free probiotic bacteria are more readily prone to utilize the sugar compared with cells immobilized inside microcapsules. Moreover, microencapsulated probiotic bacteria produced less malic acid than free cells, suggesting that encapsulation may make a more stable food product. Greater survival of lactic acid bacteria was also highlighted by Tsen et al. (2004), who immobilized *L. acidophilus* by k-carrageenan to enhance the fermentation efficiency of ripe banana. The same results were obtained by Rodrigues et al. (2012). Sohail et al. (2012) evaluated the addition of free or microencapsulated *L. rhamnosus* GG and *L. acidophilus* NCFM in alginate beads added to orange juice. However, the encapsulation of *L. rhamnosus* GG and *L. acidophilus* NCFM did not significantly enhance the survivability, but was only useful to reduce the acidification of orange juice. The survival of probiotics in the juice was also closely related to the coating material. Nualkaekul et al. (2013) reported that a double-layer coating of alginate and pectin beads with chitosan or gelatine was significantly better than single-layer coating for increasing the survival of *L. plantarum* and *L. longum* in pomegranate and cranberry juice. These results indicated that the right selection and combination of encapsulant coating polymers is very important. Different combinations of core and coating polymers gave different results, due to the formation of a dense polyelectrolyte complex between the core and the coating polymers, with consequent increase of buffering effect of the beads. Similarly, the combined pectin-jelly was found to offer the best protection to probiotic bacteria. Fruit juices were also used as a vehicle for other bioactive compounds. For example, the microencapsulated ethanolic extracts of

Bactris guineensis in maltodextrin showed greater thermal stability compared with non-microencapsulated extract, with an advantage for their use in food industry (Osorio et al. 2010) (Table 9).

Chocolate

Chocolate, like some other confectionary products, is often perceived negatively by consumers and is associated with the development of potential health problems, such as obesity. It should be considered that although it contains fat and sugar, chocolate is a good source of polyphenols, which are important for heart and vascular protection due to their antioxidant activity (Thamke et al. 2009; Đorđević et al. 2014). Due also to high acceptability, chocolate may represent an interesting vehicle for incorporation of bioactive compounds (Botelho et al. 2014) (Table 10). It was demonstrated that the encapsulation of cells, in particular two *B. longum* strains, in cocoa butter lipid fraction of cocoa butter increased the plate counts during storage (Lahtinen et al. 2007). Moreover, Possemiers et al. (2010) evaluated the possibility to use chocolate as a carrier to microencapsulate a mixture of *Lactobacillus helveticus* CNCM I-1722 and *B. longum* CNCM I-3470. For this purpose, the researchers evaluated the protection of the cells during the passage through the stomach and the small intestine, when embedded in two prototypes of products, milk and dark chocolate. Both chocolates offered good protection for cell viability, higher in the milk chocolate than in the dark chocolate. This may be due to the antimicrobial effect of the polyphenol content in milk chocolate, which is five-fold higher than in dark chocolate. The researchers also showed that coating the probiotics in chocolate is a good solution to boost the ability of the probiotics to temporally colonize the colon. Satisfactory results were obtained by Also Malmo et al. (2013) who tried to develop a hedonistic probiotic product by adding *L. reuteri* DSM 17938, microencapsulated in alginate-chitosan matrix, in chocolate soufflè. The microencapsulated probiotic cells were more resistant to heat stress and simulated gastrointestinal conditions compared to free cells but nevertheless, the survival rate did not allow them to define chocolate soufflé as a probiotic product.

Other products

In addition to the above-mentioned foods, other products that may be considered less traditional have been obtained by the incorporation of microencapsulated compounds. For example, a soup powder enriched with encapsulated linseed oil was shown to be oxidatively stable, able to provide a source of ω-3 and widely accepted by consumers (Rubilar et al. 2012). Microcapsules containing olive oil prepared by freeze-drying were successfully used in instant salad sauce (Silva et al. 2013) while the citric acid powder was microencapsulated by casein and inulin in chewing gum to obtain a product, which from the sensory point of view, is even much better than commercial ones (Abbasi et al. 2009). Sardar et al. (2013) microencapsulated the cardamom oleoresin with a sucrose wall matrix and obtained small flavouring cubes for tea beverages utilizing a co-crystallization method and recently Chranioti et al. (2016) have microencapsulated steviol glycosides to mask the bitter taste, which was also used as a sweetener with acceptable sensorial characteristics.

Conclusion and outlooks

Microencapsulation is a useful technology to enhance the nutritional and health promoting food properties, through the addition of bioactive compounds. Microencapsulation can offer significant advantages to improve delivery and protection of bioactive

Table 10. Microencapsulation of bioactive agents in chocolate.

Product	Bioactive agent	Method	Material	Sensory aspects	Stability of the microencapsulated bioactive compound	References
Chocolate	*L. helveticus* CNCM I-1722 and *B. longum* CNCM I-3470	Data not reported	Milk chocolate, dark chocolate or liquid milk	Data not reported	The coating of probiotic in chocolate improved their stability in the gastrointestinal environment	Possemiers et al. (2010)
Soufflé	*L. reuteri* DSM 17938	Spray drying	Alginate	Data not reported	Microencapsulated probiotic cells are more resistant to heat stress compared to free cells and simulated gastrointestinal conditions	Malmo et al. (2013)

ingredients in food, which would not be possible to produce otherwise. There are important issues to consider for successful microencapsulation of bioactive micro-organisms into food products, such as the specific product features and the production process, any interactions between bioactive and coating or between bioactive and other ingredients, storage conditions and shelf-life. Finally, the sensory properties have a crucial role in the acceptability of the food product. One of the most important challenges is the choice of the appropriate microencapsulation techniques and encapsulating material. An attractive possibility is to use coatings which have a nutritional or health promoting function, besides a protective function. According to the present review, the active components which have mainly been microencapsulated include lipids, vitamins, colourants, antioxidants, minerals, probiotics and prebiotics. Likewise, dairy products, fruit juices, cereals and bakery products were often fortified with the addition of microcapsules, but there are other foods, such as meat, chocolate and vegetables that may represent an interesting vehicle for the incorporation of bioactive compounds. In order to verify the target release and the effective protection of the ingredients after ingestion, it may be also necessary to carry out *in vivo* studies on bioavailability in order to demonstrate the effect of the incorporation of bioactive compounds in microcapsules for healthy outcomes. However, despite the wide range of encapsulated products developed, few are fortified food products containing the microcapsules in mention.

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