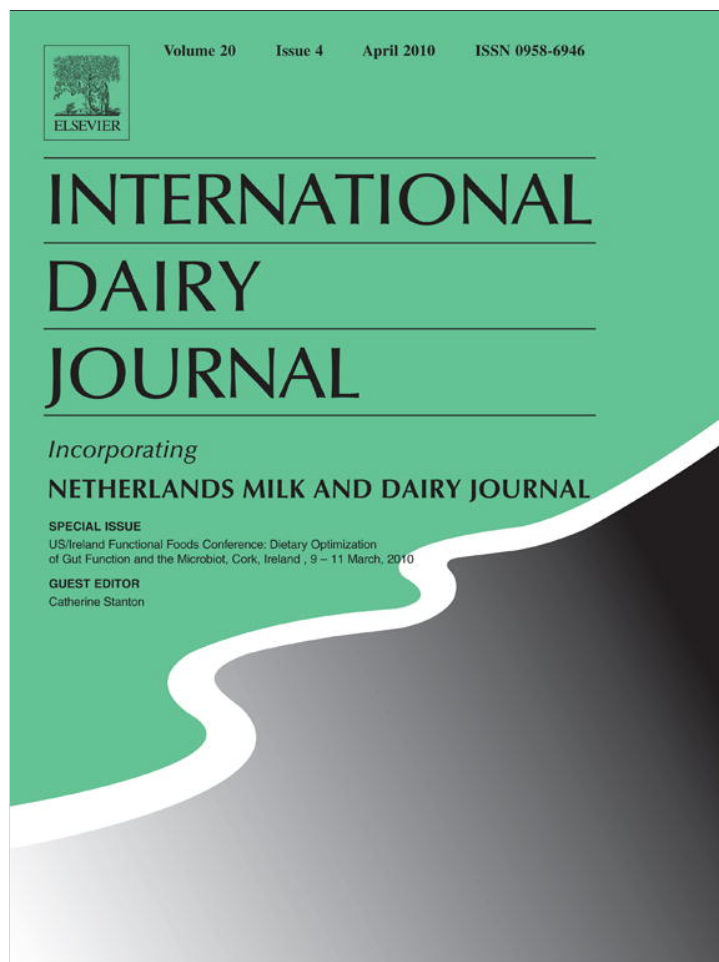


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## Review

## Encapsulation for preservation of functionality and targeted delivery of bioactive food components

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## A B S T R A C T

There has been a tremendous increase in the number of food products containing bioactive components with a health promoting or disease preventing effect. Bioactive food components can be divided into bioactive molecules and bioactive living cells (probiotics). Both bioactive molecules and bioactive living cells may benefit from encapsulation since many report low survival of bioactivity due to adverse effects of (i) processing and storage in the products that serve as vehicles and due to (ii) deleterious circumstances during transport through the gastrointestinal tract. For probiotics, it may even be mandatory to apply protection by encapsulation as the survival of probiotics in traditional products such as in dairy foods and powdered formulas is low. Encapsulation promotes not only viability but more importantly also protects the functionality, and may facilitate targeted release in specific parts of the gut. Different encapsulation approaches qualify for protection of bioactive food components. The most commonly applied technologies are emulsification, coacervation, spray drying, spray cooling, freeze drying, fluid bed coating and extrusion technologies, but also more expensive techniques such as liposome encapsulation, and cyclodextrin encapsulation are used. When targeted release is desired in combination with adequate protection in the product, it is essential to realize which processes in the human gut can be applied to facilitate targeted release. The majority of systems that have been used in the past were either sensitive to mechanical stress, pH, or transport time variations in the gut. More recent systems take advantages of the different enzyme concentrations associated with variations in the composition of the microbiota in different parts of the gut. The latter system should receive more attention in the food industry as it allows for precise release of bioactive food components. The principle of targeted release by enzymatic activity of the microbiota is compatible with many carbohydrates that are generally regarded as safe (GRAS).

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## 1. Introduction

A characteristic of bioactive food components is that they are subject to rapid inactivation or degradation. Many bioactive food components would therefore benefit from an encapsulation procedure that slows down the degradation processes and/or prevents degradation until the product is delivered at the sites where adsorption is desired. These bioactive components include lipids, vitamins, peptides, fatty acids, antioxidants, minerals but also living cells such as probiotics (Burey, Bhandari, Howes, & Gidley, 2008; Champagne & Fustier, 2007; McClements, Decker, & Park, 2009a; McClements, Decker, Park, & Weiss, 2009b).

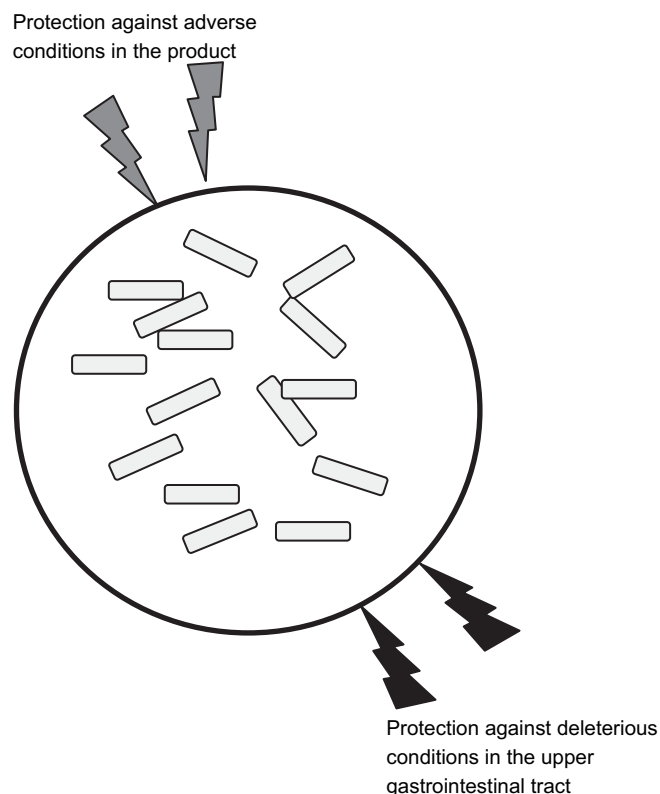
Many encapsulation procedures have been proposed but none of them can be considered as a universally applicable procedure for bioactive food components. This is caused by the fact that individual bioactive food components have their own characteristic molecular structure (Augustin & Hemar, 2009). They demonstrate extreme differences in molecular weight, polarity, solubility, etc. which implies that different encapsulation approaches have to be applied in order to meet the specific physicochemical and molecular requirements for a specific bioactive component (Augustin & Hemar, 2009; Kailasapathy, 2002). However compatibility with the bioactives is not the only requirement an encapsulation procedure has to meet. It also should have specific characteristics to withstand influences from the environment (Augustin & Hemar, 2009).

An important requirement is that the encapsulation system has to protect the bioactive component from chemical degradation (e.g., oxidation or hydrolysis) to keep the bioactive component fully functional. Many will interpret this first requirement as a suggestion that we mainly have to overcome this chemical degradation in the gastrointestinal tract. This, however, is only one of the challenges. A major obstacle in the efficacious delivery of bioactive food components is not only the hazardous events that occur during passage through the gastrointestinal tract but also the deleterious circumstances during storage in the product that serves as vehicle for the bioactive components. Many food components may interfere with the bioactivity of the added bioactive food component. It is therefore mandatory that the encapsulation procedure protects the bioactive component during the whole period of processing, storage, and transport (Gibbs, Kermasha, Alli, & Mulligan, 1999).

Another requirement is that the encapsulation system allows an efficient package load (McClements, et al., 2009a,b). How 'efficient' this package load should be depends of course on the type of molecule that is desired as bioactive component and the specific product that serves as vehicle. Administration of large structures such as probiotics will require a higher efficiency of package than molecular structures such as vitamins. When choosing an encapsulation system with a high package efficiency, it is always essential to choose a system that can be easily incorporated into the food

without interfering with the texture and taste of the food. And, last but certainly not least, it might be necessary to design the encapsulation system as such that the bioactive component is released in a specific site of the gastrointestinal tract (Fig. 1).

In the present review we will discuss the different families of bioactive food components that may benefit from encapsulation. Subsequently we will discuss the different encapsulation approaches that are available for encapsulation of bioactive food components. Subsequently, we will discuss the considerations and requirements a system has to meet in order to combine preservation of functionality and targeted delivery in specific parts of the gut. We will end the overview with discussing commonly applied polymers that are currently proposed as matrix molecule for targeted delivery.



**Fig. 1.** Capsules have to protect bioactive food components against (i) adverse environmental conditions during processing and storage in the product that serves as vehicles for the bioactive components and (ii) against the deleterious conditions during passage through the gastrointestinal tract.

### 1.1. Bioactive molecules

The most commonly applied bioactive food molecules that are already encapsulated in industrial applications are lipids, proteins, and carbohydrates (Augustin & Hemar, 2009; Burey et al., 2008; Champagne & Fustier, 2007; Gibbs et al., 1999). These ingredients are usually encapsulated to resist the high acidity and enzyme activity of the stomach and duodenum but also because of their low water-solubility that interferes with application in many food products. In the following section, we will present the families of bioactive food molecules that will benefit from encapsulation.

Lipids are a broad family of food components that are soluble in organic solvents. This includes fatty acids, phospholipids, carotenoids, and oil-soluble vitamins (Hamalainen, Nieminen, Vuorela, Heinonen, & Moilanen, 2007; Leppala et al., 2000; McClements, et al., 2009a; Morello, Vuorela, Romero, Motilva, & Heinonen, 2005; Stringham & Hammond, 2005). Lipids have benefited from encapsulation approaches already (McClements, et al., 2009a,b). They cannot be easily solved in food products because of their extreme low solubility in water and poly-unsaturated fatty acids, which are highly susceptible to oxidation, and are now widely applied in powdered products thanks to encapsulation processes that form an effective barrier for oxygen. Therefore many different approaches of encapsulation have been proposed for encapsulation of lipids in order to be able to apply them in a large variety of food products (McClements, et al., 2009a,b).

Bioactive proteins are the second family of bioactive molecules that might require encapsulation. Many food derived peptides act as growth factor, anti-hypertensive agent, antioxidant or immune regulatory factor (Hartmann & Meisel, 2007; Kussmann & Affolter, 2006; McClements, et al., 2009a,b; Meisel et al., 2001). Some of these proteins have to reach the site of uptake in the small bowel in an intact conformation in order to exert a beneficial health effect. Proteins have to be considered as the most sensitive bioactive molecules for biodegradation in the gastrointestinal tract. This however should not always be interpreted as a suggestion that all food proteins have to be encapsulated. Most peptides even require hydrolysis in the stomach and small intestine in order to release specific bioactive peptides or amino acids (McClements, et al., 2009a,b; Schlimme & Meisel, 1995; Ward & German, 2004). Thus whether encapsulation for proteins has to be considered depends on the type of protein, its envisioned health effect and the product that serves as vehicle for the bioactive protein.

The third and last family of bioactive molecules that have been proposed to benefit from encapsulation are carbohydrates. With carbohydrates we refer to bioactive carbohydrates that are found in dietary fibers (Meisel et al., 2001; Redgwell & Fischer, 2005). These fibers are very heterogeneous and vary in the type and distribution of polysaccharides as well as in the quantity of monosaccharides. They can be classified according to their molecular or physico-chemical properties, their biological origin, or according to their physiological effects (McClements, et al., 2009a,b; Redgwell & Fischer, 2005). The fibers or its components that would benefit most from encapsulation are the soluble non-digestible polysaccharides. These fibers have been included for cholesterol reduction, reduction of glycemic fluctuations, prevention of constipation, prebiotic effects, and even for the prevention of cancer (McClements, et al., 2009a,b; Redgwell & Fischer, 2005). The main challenge in this area is not to target the fibers to specific parts of the gut but to increase the amount of fibers in food in order to achieve the aforementioned health benefits. According to Redgwell and Fisher (2005) 25–30 dietary fibers are required which rarely if not ever is reached in the diet of the general population in the developed countries. The major encapsulation effort in this area is therefore improving the food load of fibers by packing enough

fibers in capsules without interfering with the product quality such as changes in texture, mouthfeel, or flavor.

### 1.2. Living bioactive food components

Probiotic bacteria are defined as live microorganisms which, when administered in adequate amounts, confer a beneficial physiological effect in the host. Probiotics are at present the driving force in the design of functional foods, especially in dairy products, and maintaining their functional effects for supporting human health are therefore discussed in detail in the present overview.

The application of encapsulation approaches with living probiotics is limited. The size of probiotics (1–5 µm) excludes many of the nanotechnological approaches of encapsulation. Usually we refer to microencapsulation when discussing encapsulation of probiotics. We, like others (Kailasapathy, 2002; McClements, et al., 2009a,b), feel that microencapsulation of many types of probiotics may be mandatory for achieving the promised health benefits. The reasons for that are as follows.

#### 1.2.1. The survival rate of probiotics in traditional dairy products is low

Although many health benefits of probiotics in dairy products have been described, it is far from easy to allow long term survival of probiotics in traditional, including fermented, dairy products (Shah, 2000). The survival of probiotics is influenced by many factors such as variations in pH including the post-acidification in fermented products (Kailasapathy, 2002; Shah, 2000). Also oxygen toxicity during processing and packaging (Kailasapathy, 2002) has been shown to be detrimental for the survival of the probiotics. A final critical issue that limits survival of probiotics in many dairy products is incompatibility with the bacteria involved in the starting culture and lack of a nitrogen source due to the absence of functional proteases in the product (Dave & Shah, 1998; Kailasapathy, 2002; Shah, 2000).

#### 1.2.2. Probiotics bacteria show low survival rates after drying

Due to the complexity of the foregoing issues a number of industries have abandoned the application of probiotics in hydrated products such as dairy products and have moved towards the production of dry products containing probiotics (Kailasapathy, 2002). The number of food supplements belonging to this category is growing fast (Kailasapathy, 2002). However, also here issues associated with the processing interfere with survival. In those rare studies in which the viability count of probiotics was determined it was found that only low numbers could be recovered and in some products that had to contain *Lactobacillus acidophilus* the researchers were not able to recover any viable cell (Kailasapathy, 2002).

#### 1.2.3. Encapsulation promotes not only viability but more importantly functionality

Even if we would be able to enhance the survival rate of probiotics this would not immediately imply that we will increase functional survival. During recent years it has become more and more clear that probiotic effects are determined by the presence of specific bioactive molecules or effector molecules in the cell envelope of probiotic bacteria (van Baarlen et al., 2009; Kleerebezem & Vaughan, 2009; Konstantinov et al., 2008). These effector molecules are (glyco)proteins and have to be preserved in order to achieve functional effects (Konstantinov et al., 2008). The survival of these effector molecules in the product and during passage in the gastrointestinal tract is even more important than the survival of numbers of probiotics. The effector molecules that are presently identified (Konstantinov et al., 2008) are susceptible for the acidic

circumstances and digestive enzymes in the stomach at beginning of the small bowel. Preserving and protecting these effector molecules will be a major challenge in the near future (Ledeboer et al., 2006).

#### 1.2.4. Targeted release is mandatory for probiotics

An overlooked issue in the administration of probiotics is that for full functionality and health effects probiotics have to be delivered in specific areas in the gut. Although the gut is highly organized it is also a very heterogenic organ (Bauer, Muller, & Hamm, 2009; Defonseka & Kaunitz, 2009; Erickson & Hubbard, 2009; Raz, 2009). Its immunological system for example is unique in every single part of the gut (Bauer et al., 2009; Erickson & Hubbard, 2009). Specific immune-cells mature in so-called gut associated lymphoid structures (GALT) that again are different in the varying parts of the gut. Specific cells in the lymphoid structures but also in the lamina propria are in continuous contact with the lumen of the gut to sense immunologically active components (sometimes bioactive food components). A part of the probiotic health effects are facilitated by direct contact between probiotics and the effector/receptor molecules on the surface of the immune cells which we refer to as pattern recognition receptors (Bauer et al., 2009). If probiotics are added to food to stimulate immunoregulation it is mandatory to facilitate the release of the probiotics in the area where the immunological signaling occurs. In most cases this occurs in the ileum where the Peyer patches are located or more distal where other GALTs can be found (Hartmann & Meisel, 2007). This is the site where the immunological exchange between the lumen and immune cells is most intense and also the area where the vast majority of activated and regulatory T-cells are generated. It is the site where 75% of the human T-cell population is present, illustrating the huge possibilities and potential therapeutic consequences when we are successful in designing immunomodulating foods (Hartmann & Meisel, 2007).

#### 1.3. Encapsulation versus immobilization

The foregoing illustrates that encapsulation of bioactive food components may be beneficial and even mandatory for efficacious delivery of microbes. Encapsulation is in most cases nothing more than a technology in which a physical barrier is applied to protect the bioactive components against any adverse environmental condition. It was originally introduced in the area of biotechnology to make production-processes more efficient as the matrix around the cells allows for rapid and efficient separation of the producer cells and the metabolites. Now it becomes more and more recognized that encapsulation can do much more as it has been shown to stabilize cells, as it has a positive influence on the viability of microbes and since it allows simple handling and storage of many probiotics on an industrial scale (Kailasapathy, 2002). Also, some microbes such as lactobacilli and bifidobacteria seem to profit from the encapsulation matrix during dehydration and lyophilization (Kim, Baek, & Yoon, 1996).

Encapsulation of microbes is a natural bacterial response to a stressful environment. It is a bacterial invention that has been developed very early in evolution to protect the cells against toxic components in the microenvironment. Almost all bacteria produce molecules in which they themselves are entrapped and which subsequently serve as a protective membrane by reducing the diffusion of environmental factors that have adverse effects on the viability of the cells (Kailasapathy, 2002). For example, many lactic acid bacteria (Kailasapathy, 2002) produce exo-polysaccharides for protective purposes only. Not surprisingly, researchers have attempted to enhance this exo-polysaccharide production to induce full encapsulation of lactic acid bacteria. Unfortunately,

however, the exo-polysaccharide production is insufficient to warrant this approach as a true encapsulation approach.

Before discussing the concept and considerations for encapsulation in more detail we have to give the concept of encapsulation some further consideration. During recent years encapsulation and immobilization are mentioned in an interchangeable fashion. This however is not completely correct as, they were originally introduced as two separate concepts for different types of technologies. Encapsulation refers to a technology in which the bioactive components such as probiotics are completely enveloped and covered by a matrix without any protrusion of the bioactive components. Immobilization was originally a different technology in which the bioactive component was covered but not necessarily enveloped by the matrix. In immobilized bioactive materials some materials may be exposed at the surface which is not allowed in encapsulation procedures. Although this distinction is rarely being made in the food literature (Kailasapathy, 2002; McClements, et al., 2009a,b) it is mandatory to make this separation when we have to make a choice for a specific encapsulation system for a specific product. An encapsulated product without any chance on leakage of cells may be applied in both dairy products and dry products such as food supplements or dry foods. Immobilized cells hold the risk of leakage of the product which may result for example in undesired growth of probiotics in dairy products which consequently may interfere with the shelf life and the efficacy of the product. Also, immobilization will not always be sufficient to protect cells for hazardous substances in the direct microenvironment. Therefore when discussing encapsulation technologies in the next sections we will always mention whether it is a true encapsulation technology or an immobilization technology.

#### 1.4. Families of encapsulation procedures

The nomenclature for encapsulation procedures varies per field and the list of encapsulation procedures is growing by the month. In order to give an adequate overview we have categorized the technologies in a number of families under which the vast majority of techniques can be categorized. We will shortly explain the principles of the technique and the applicability in the food industry for protection of bioactive food components.

##### 1.4.1. Emulsification

As mentioned before there is not a single universal methodology to encapsulate bioactive molecules because of the enormous difference in chemical structure of the molecules and because of the high variety of products that are being applied. This also applies to families of encapsulation approaches. The type of matrix molecules will vary per bioactive food component. McClements, et al. (2009a,b) recently reviewed and discussed all the interactions that have to be taken into account when designing an encapsulation system by emulsification for bioactive food molecules. Emulsification is defined as a process of dispersing one liquid in a second immiscible liquid. By including the core material in the first liquid we can encapsulate the bioactive component.

In most cases researchers and companies choose to encapsulate the bioactive molecules in food grade (Generally Regarded As Safe (GRAS)) derived molecules by applying electrostatic interactions, hydrophobic interactions, or hydrogen bonding between the bioactive molecule and an encapsulating molecule. In most cases the encapsulating agent is a molecule already present in the food (Augustin & Hemar, 2009). Also addition of a surfactant that induces encapsulation by forming micelles, vesicles, bilayers, and reverse micelles around the bioactive molecules is commonly proposed as a solution (Augustin & Hemar, 2009; McClements, et al., 2009a,b). Usually it protects the bioactive molecules in the



products and facilitates their release in the duodenum as soon as lipase is being released. Another approach of encapsulation is the application of biopolymers such as a variety of proteins and polysaccharides that can envelop the sensitive bioactive molecules by forming a random coil, sheet, or rod like structures around the molecules (Fig. 2). The type and digestibility of the applied biopolymer determines its exact release in the gut (Champagne & Fustier, 2007; Kosaraju, 2005; Madziva, Kailasapathy, & Phillips, 2005; McClements, et al., 2009a,b). Obviously the choice of the biopolymer is determined by the composition of the product. In some cases, to enhance packing efficacy, bulk emulsification approaches are applied. This usually is enveloping the bioactive molecules in fat-droplets, or water-oil-water emulsions (Augustin & Hemar, 2009; McClements, et al., 2009a,b). This bulk emulsification is usually not considered to be a true encapsulation system but more a technique to provide fine controlled release of molecules. A huge amount of food components may be applied as building blocks for emulsions. Depending on the type of molecules for forming the emulsions we can produce simple and very complex emulsions. The possibilities are numerous and have been extensively reviewed (Augustin & Hemar, 2009). Just to illustrate the principle of the emulsion technique we shortly discuss the application of monoglycerides. A characteristic of monoglycerides is that they self-ensemble in water in a wide variety of structures (Fig. 3). By minimal and non-laborious manipulation we can control the formation of micelles, hexagonal, cubic or even lamellar shapes of glycerides that envelope one or more bioactive molecules. It is a simple technology that is already extensively applied for controlled release of aromas and flavors (Augustin & Hemar, 2009).

1.4.2. Coacervation

A modified emulsification technology is coacervation. The principle is relatively simple. When a solution of bioactive components is mixed with a matrix molecule of an opposite charge, a complex is formed. The size of the capsule and its characteristics



Random coil

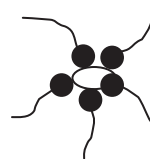


Rod-like conformation



Globular conformation

Fig. 2. By emulsification techniques we can force polymers such as proteins and polysaccharides in the food to undergo transitions in their molecular conformation. This can be applied to encapsulate or protect sensitive bioactive food components.



Simple reverse micelle by addition of monoglycerides



Micelle

Fig. 3. By adding surfactant or a specified amount of monoglycerides reverse micelles or complete micelles can be formed.

can be varied by changing the pH, the ion concentration, the ratio of matrix molecule and the bioactive component, and the type of matrix. The technique is mainly driven by electrostatic interaction but also hydrophobic interactions are involved (Augustin & Hemar, 2009; Girard, Sanchez, Laneville, Turgeon, & Gauthier, 2004; Laneville, Turgeon, Sanchez, & Paquin, 2006). Also this is an immobilization rather than an encapsulation technology and is therefore mostly proposed and applied for bioactive food molecules rather than for bioactive living cells. The technique is applied for flavors, oils but also for some water-soluble bioactive molecules.

1.4.3. Spray drying

One of the most commonly used industrial technologies for encapsulation is spray drying. It is being applied for both bioactive food molecules and living probiotics. It is a fast and relatively cheap procedure that, when adequately performed, is highly reproducible. The principle of spray drying is dissolving the core in a dispersion of a chosen matrix material. The dispersion is subsequently atomized in heated air. This promotes fast removal of the solvent (water). The powdered particles are then separated from the drying air at the outlet at a lower temperature (Fig. 4). The relative ease and also the low cost are the main reasons for the broad application of spray drying in industrial settings. The technology, however, has also some major disadvantages. The first is its small field of application. It is an immobilization technology rather

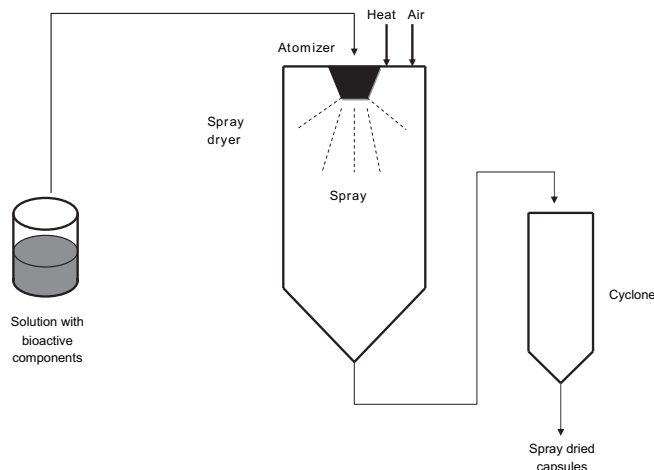


Fig. 4. Schematic presentation of the spray drying procedure.

than an encapsulation technology which implies that some bioactive components may be exposed. This is especially problematic when considering the technique for encapsulation of probiotics, where the bacteria may leak into the product when some hydration occurs. Another limitation is the high temperature that is required during the immobilization process that is not compatible with the survival of all types of probiotics. For example, it has been shown that bifidobacteria were very sensitive for high inlet temperatures (O'Riordan, Andrews, Buckle, & Conway, 2001). Temperatures above 60° C interfered with survival of the bacteria and also influenced the spray drying process as sticky products were reported with this type of bacteria in the cyclone (Kailasapathy, 2002). That this effect of temperature on survival of probiotics is not specific for bifidobacteria but might also occur unnoticed with many more probiotics was shown by Gardiner et al. (2000). It is therefore advisable to investigate the sensitivity of a probiotic for increased temperatures before proposing spray drying as the technology of preference for a specific bacterium. The foregoing should not be interpreted as a suggestion that the technology is inadequate for preserving bioactivity of food components. It has many advantages over other technologies for other bioactive food components such as vitamins, minerals, flavors, unsaturated oils, and enzymes. It is a relative gentle methodology in terms of application of solvents and matrix molecules. Only water-based dispersions are applied in spray drying. Therefore the matrix should have a high solubility in water. In most instances hydrophilic carbohydrate molecules are applied. These carbohydrates undergo a transition to a so-called glass (i.e., an amorphous solid) when the dispersion is rapidly evaporated. Usually the product is very stable and allows for a significant increase in shelf life (Augustin & Hemar, 2009). The fact that the matrix is hydrophilic does not suggest that the procedure is only applicable for hydrophilic molecules. Both hydrophilic and hydrophobic bioactive molecules can be used as the core materials. However, hydrophobic molecules are usually first dissolved in an oil phase after which an oil-in-water emulsion is formed prior to drying.

#### 1.4.4. Spray cooling

A technology in which we apply an opposite principle of spray drying is spray cooling. Also here we make a dispersion of a matrix and the bioactive product but instead of evaporating we cool the dispersion in order to allow immobilization. Usually fats with a high melting point are applied in this technology. Also this is an immobilization rather than an encapsulation technique. Up to now it is only applied for dry products to conserve enzymes, flavors, minerals, and proteins.

#### 1.4.5. Freeze drying

Freeze drying in combination with matrix molecules has been proposed as an alternative for spray drying of heat sensitive bacteria (Augustin & Hemar, 2009). However, drying in general should not be considered to be a very efficacious methodology for preservation of bioactivity of living cells. It has been shown that the recovery rates from administered bacteria from human stools were much lower with dried bacteria than with non-treated bacteria when administered via milk and cheese (Saxelin, Korpela, & Mayra-Makinen, 2003).

#### 1.4.6. Fluid bed coating

A modified spray dry methodology that enlarges the field of application is the fluid bed coating methodology (Fig. 5). In this technology the bioactive food components are suspended in air and the matrix molecules are sprayed onto the bioactive components. This forms a capsule (Champagne & Fustier, 2007). The choice for matrix molecules is broader than for traditional spray drying. It

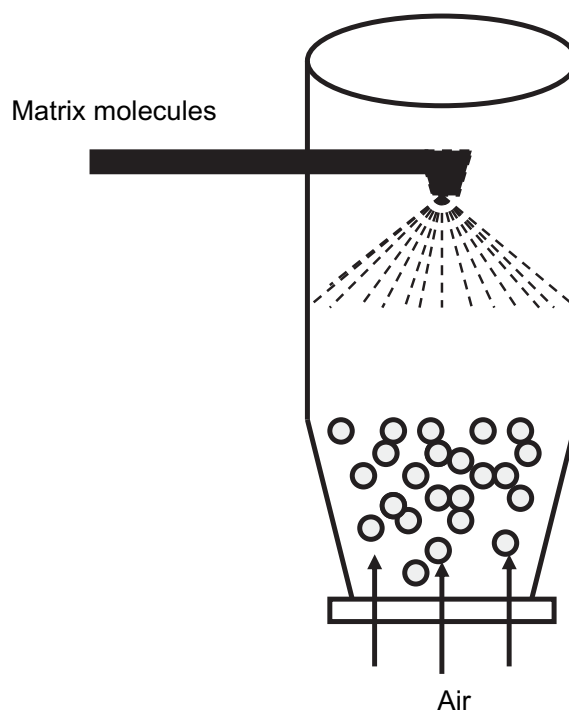
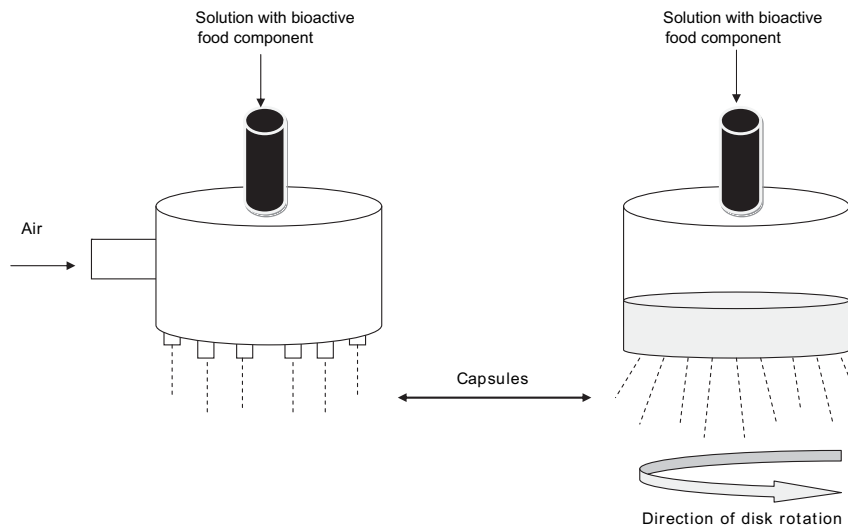


Fig. 5. Schematic presentation of the fluid bed technology. The matrix molecules are sprayed onto a preexisting encapsulated bioactive food product or onto a solid bioactive food particle.

may be fats, proteins, carbohydrates but also emulsifiers. It may even be applied to give spray dried products or a sensitive core of for example oils as a second coating. Also it is useful for applying an additional layer of molecules for targeted release in the gut. In principle the core is always solid.

#### 1.4.7. Extrusion technologies

A less hazardous but also more laborious technology of encapsulation is the category of extrusion technologies. Extrusion is nothing more than producing a small droplet of an encapsulation material by forcing the solution through nozzles or small openings in droplet-generating devices. The smaller the inner diameter of the nozzle or openings, the smaller the capsules. Industry-oriented groups quite often have the incorrect assumption that this procedure does not allow for large scale production and is only suitable for laboratory scale processes. However, enormous advances have been made in the up-scaling of encapsulation processes using extrusion technology. Large scale droplet production can be achieved by multiple-nozzle systems, spinning disc atomizer, or by jet-cutter techniques (De Vos, De Haan, & Van Schilfgaarde, 1997; Kailasapathy, 2002) (Fig. 6). An advantage of the extrusion technology is that it is in most cases a true encapsulation procedure instead of an immobilization technology. By choosing an adequate amount of cells per milliliter of encapsulation material and by selecting materials with low or absent swelling kinetics (De Vos, De Haan, Pater, & Van Schilfgaarde, 1996a; De Vos, De Haan, Wolters, & Van Schilfgaarde, 1996b) during the encapsulation procedure we can reduce protrusion. Extrusion technologies have many advantages for encapsulation of microbes. It is relatively gentle, does not involve deleterious solvents, and can be done under both aerobic and anaerobic conditions. This latter is especially advantages when anaerobic microorganisms are being applied in food products. The modifications to do this are very simple. The extrusion device has to be placed in a sterile cabinet where oxygen is substituted for nitrogen.



**Fig. 6.** Two commonly applied extrusion technologies. Left a multiple needle droplet-generator that usually is air driven. Droplet size can be adjusted by applying small bore needles or by increasing the air-flow through the chamber containing the needle. Right a spinning disk device. By increasing the speed of disk rotation smaller droplet can be produced.

Extrusion technologies are also applied for flavors, enzymes, and proteins.

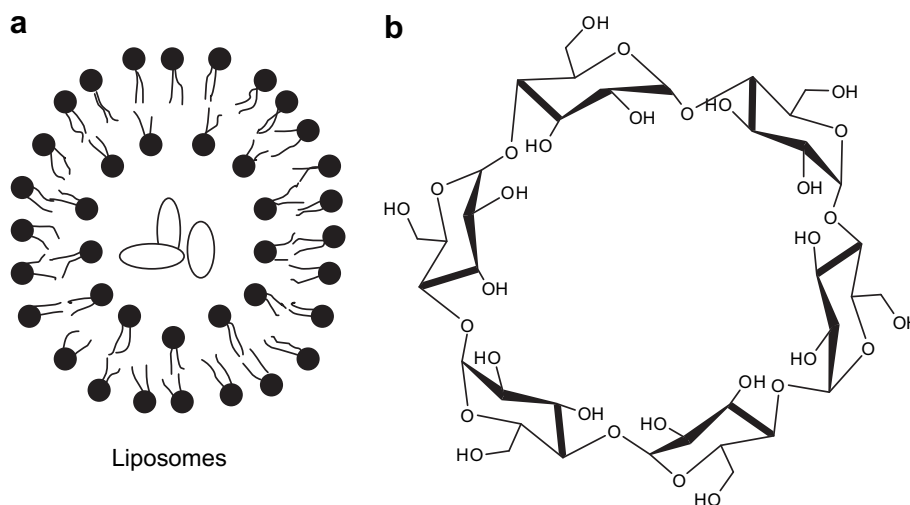
1.4.8. Other encapsulation technologies

For the sake of completeness we also discuss some technologies that due to their high price are rarely applied but may serve as a solution of specific encapsulation issues. One of them is the application of liposomes. Liposomes are spherical bilayers which enclose bioactive molecules. The liposomes are formed by dispersion of polar lipids (mostly phospholipids) in an aqueous solution (Fig. 7a). Another elegant encapsulation method is encapsulation in cyclodextrins. Cyclodextrins can envelop molecular structures by forming molecular inclusion complexes (Fig. 7b). Cyclodextrins have a hydrophobic interior and a hydrophilic exterior. The hydrophobic interior of the capsule can be varied in size by varying the number of glucose units of the cyclodextrin molecules (Bilensky & Hincal, 2009; Harada, Takashima, & Yamaguchi, 2009). It is

applied to increase the solubility of hydrophobic molecules and to protect molecules from inactivation or degradation.

1.5. Principles of targeted release in the gut

When proposing targeted release of bioactive food components in the gut, we have to discuss the processes that facilitate the dissolution of capsules in specific parts of the gastrointestinal tract. The first is the strong peristaltic waves in the colon. In the upper part of the gastrointestinal tract the pressure is relatively low due to the presence of large amount of fluids in the stomach and the small intestine. By increasing the mechanical resistance of capsules as such that they can withstand the pressure in the stomach and small intestine but will break more distal when the pressure in the lumen is increasing we can facilitate the release of bioactive molecules in the lower part of the gastrointestinal tract. The other mechanism is to use the pH variations and the time of transport in the gastrointestinal tract. By applying pH sensitive polymers that stay intact in



**Fig. 7.** Liposomes (a) are phospholipid bilayers enclosing the bioactive food components. Although expensive it has been applied for preserving aromas in the food industry. Cyclodextrins are cyclic oligosaccharides that are broken down by especially the *Bacteroides* in the colon. It facilitates the survival of components in the stomach and small intestine.



the stomach but will be susceptible for digestive enzymes we can facilitate the release in specific parts of either the small intestine or large intestine. The third, and most promising method according to the present authors, is to take advantage of the precise, local activity of enzyme-systems produced by the microbiota. The bacterial populations in the gut and the associated enzyme activities are specific for different parts of the gastrointestinal tract and allow for precise delivery of bioactive food components. The microbiota produce a wide range of enzymes such as  $\beta$ -glucuronidase,  $\beta$ -xylosidase,  $\alpha$ -arabinosidase,  $\beta$ -galactosidase, nitroreductase, azoreductase, deaminase, urea hydroxylase, etc. (Kinget, Kalala, Vervoort, & van den, 1998; Kosaraju, 2005; Shantha, Ravichandran, & Rao, 1995). The variations in redox potential induced by the microbiota of the colon can be applied as a specific tool for solving capsules and delivery of bioactive food components. For drug targeting, this principle has been shown to be very successful. By applying azoreductase sensitive hydrogels, drugs can be delivered very specifically in the colon by allowing degradation of the capsules by azoreductase produced by the colonic microbiota (Saffran et al., 1986; Shantha et al., 1995) (Fig. 8). Many different molecules can be synthesized that allow for degradation but up to now in most cases in the food industry GRAS type molecules are applied. The choice for specific matrix molecules is dependent on the knowledge available about the degradability of the matrix by enzymes produced by the anaerobic bacteria in the colon. Most industries and researchers concentrate on biodegradable natural polymers, which are resistant to degradation in the upper part of the gastrointestinal tract. Most of these molecules are considered to be prebiotics as well since they serve as energy supply for the commensal bacterial populations.

### 1.6. Polymers applied for targeted release in the gut

The vast majority of knowledge about targeted release of molecules in the gut has been obtained from research concerning delivery of drugs rather than from food research (Kosaraju, 2005). Below a number of generally applied polysaccharides coming from pharmaceutical or

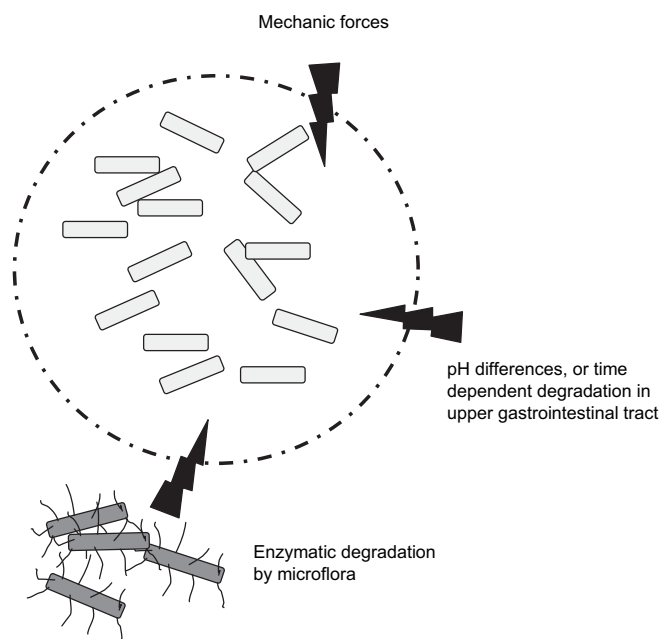


Fig. 8. The three mechanistic forces that are being applied to allow targeted release of bioactive food components in the gut.

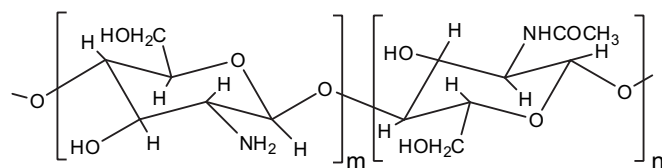


Fig. 9. Structure of chitosan.

biomedical research will be discussed that can serve as matrix molecules to allow for specific delivery of bioactive food molecules by using enzyme activity in the microbiota. We have concentrated mainly on GRAS carbohydrates which are compatible with many food components and therefore potentially combine adequate protection for deleterious events in the product and targeted delivery.

Some of the carbohydrates are already being applied in the food industry. Most of the polysaccharide based targeted delivery systems protect the bioactive food components from the deleterious conditions in the stomach and upper part of the small bowel. The degradation of the polysaccharide matrix molecules mainly depends on the hydrolysis of the glycosidic linkages between the molecules and subsequent release of the bioactive components. Many bacteria from the microbiota contribute to the degradation but the main organisms responsible for this biodegradation are *Bacteroides* and *Bifidobacterium* (Kosaraju, 2005). The most commonly used polysaccharide systems will be discussed in the next section. In many cases, these polysaccharides are modified for optimal compatibility with the food that serves as vehicle or for more precise release.

#### 1.6.1. Chitosan

Chitosan (Fig. 9) is being applied for the targeted release of both bioactive molecules and living cells. It is of special interest for the targeted release of probiotics because of its high compatibility with living cells. Chitosan readily dissolves at low pH. It is often applied in combination with another polymer (often alginate) that withstands the low pH in the stomach. Upon arrival in the small bowel it is degraded by the rich colonic microbiota.

Chitosan is a polycationic molecule derived from naturally occurring chitin by alkaline deacetylation. It is a co-polymer of glucosamine and N-acetylated glucosamine, it is readily degraded into simple metabolising sugars.

#### 1.6.2. Alginates

One of the most commonly applied polysaccharides is alginate. Alginates are linear polymers with 1-4 linked- $\beta$ -D mannuronic acid (M) and  $\alpha$ -L-guluronic acid (G) residues arranged as blocks of either type of unit or as a random distribution of each type (Fig. 10). They can be obtained in different G/M ratios which provides different degrees of mechanical stability. Alginates are compatible with almost all encapsulation procedures but most researchers combine alginates with spray drying and extrusion (Kailasapathy, 2002). Alginates can form gels and can be used to form complexes with

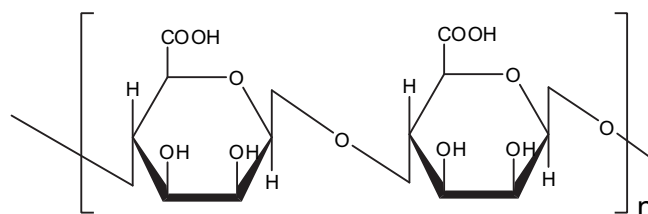


Fig. 10. Structure of alginate.

chitosan to allow for time controlled release of both bioactive molecules and living cells (Kosaraju, 2005). Depending on the chemical characteristics of the capsule, alginate-based capsules are generally applied to facilitate the release of bioactive components in the ileum or colon (Iyer, Phillips, & Kailasapathy, 2005).

### 1.6.3. Pectins

Pectins are plant derived molecules which are mostly linear polymers of mainly  $\alpha$ -(1-4)-linked D-galacturonic acid residues and 1,2-linked L-rhamnose residues (Fig. 11). These polysaccharides are advantageous for targeted delivery because they remain intact in the stomach and the small intestine. The application of pectin as unmodified molecule is limited due to its high solubility in water. In most cases it is combined with a cation or another polymer such as chitosan to form a slowly degrading complex. Its degradation largely depends on enzymes derived from the host microbiota but its speed of degradation can be modified by chemical modifications. It therefore allows for specific delivery in the different parts of the gut.

### 1.6.4. Dextran

Dextrans are bacterial derived sugars that are rapidly degraded by dextranases which are abundantly present in *Bacteroides*. Dextrans are polysaccharides with a linear polymer backbone, with mainly 1,6- $\alpha$ -D-glucopyranosidic linkages (Kosaraju, 2005) (Fig. 12). A beneficial feature of dextrane is that the degradation in the gut can be regulated by varying the structure of the dextrane in the capsules. Dextrans can be obtained as crystalline, hydrogels, and in different molecular weights which all have an influence on their degradation speed and therefore release in the gut. Also combinations of dextrans with other polymers have been shown to be an effective approach to modulate the kinetics of release.

### 1.6.5. Starch

Because of its low price, relative ease of handling and broad application starch is under study as matrix of capsules for targeted delivery of a broad panel of bioactive components. Amylose is the linear constituent of starch, consisting of D-glucopyranose residues linked by  $\alpha$ -(1-4) bond (Fig. 13). The linkages are resistant to pancreatic  $\alpha$ -amylase, but are degraded by colonic bacterial enzymes (Macfarlane & Englyst, 1986). The mechanisms by which degradation of starch can be delayed has been studied in large detail (Cummings, Macfarlane, & Englyst, 2001; Macfarlane & Englyst,

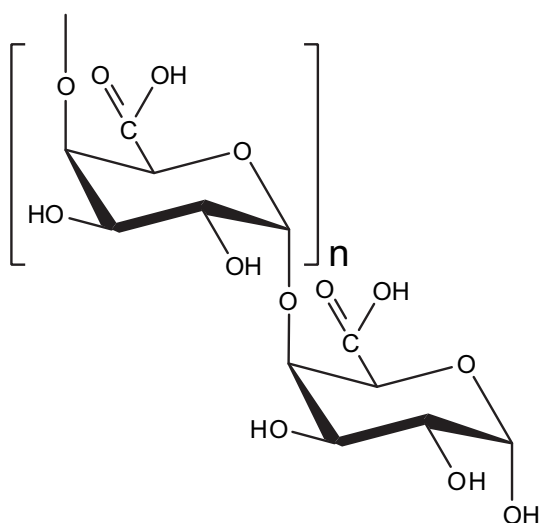


Fig. 11. Structure of pectin.

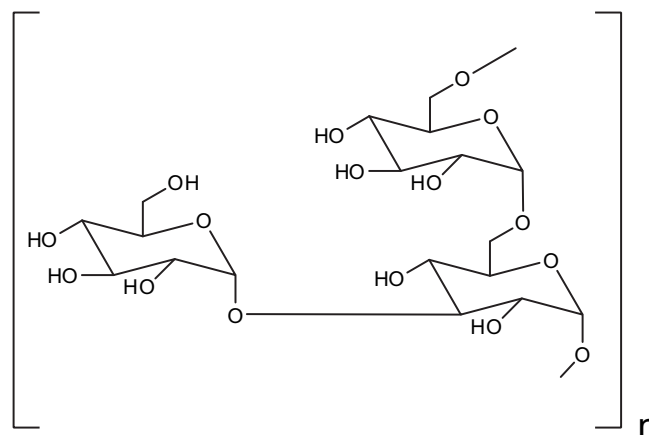


Fig. 12. Structure of dextran.

1986). Starch can be made more resistant by modifying it by etherification, esterification, or acidification. It has been acetylated, hydroxypropylated, octenyl succinylated, or carboxymethylated (Kosaraju, 2005). It is already broadly applied for targeted delivery of probiotics in the colon (Kosaraju, 2005).

### 1.6.6. Inulin

Inulin is a polysaccharide found in many vegetables. It is composed of  $\beta$ -2-1 linked D-fructose molecules (Fig. 14). A feature of inulin is that it is difficult to hydrolyze which qualifies inulin as a matrix molecule for capsules that have to reach the colon and to survive the upper part of the gastrointestinal tract. The microorganisms responsible for degradation of inulin are bifidobacteria, which are abundantly present in the human gut (Gibson, Probert, Loo, Rastall, & Roberfroid, 2004). Inulin should receive more attention for colon specific delivery of bioactive food components as it is cheap, has many health benefits by itself and can be applied in combination with almost all encapsulation techniques.

## 1.7. Concluding remarks

Many bioactive food components will benefit from encapsulation. For some components such as for the driving force behind the design of functional foods, i.e. living probiotics, it may even be mandatory. During recent years it has been demonstrated that probiotic effects are accomplished by interactions between specific host-receptors and functional ligands on the bacteria (Konstantinov et al., 2008). For efficacious health benefits it is mandatory to preserve these functional ligands. We expect that preservation of functionality of probiotics will become a pertinent issue in the near future and even obligatory when products have to pass the very strict regulations of the European Food Safety Authority (EFSA) for health claims associated with probiotics.

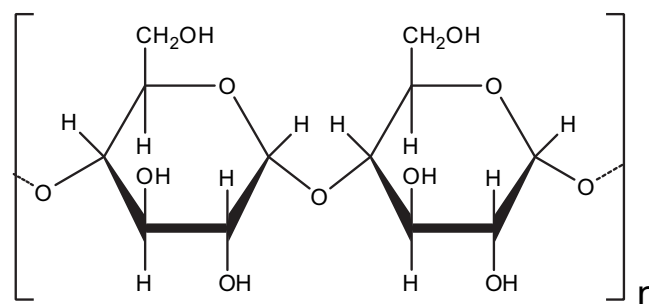


Fig. 13. Structure of starch (amylose).

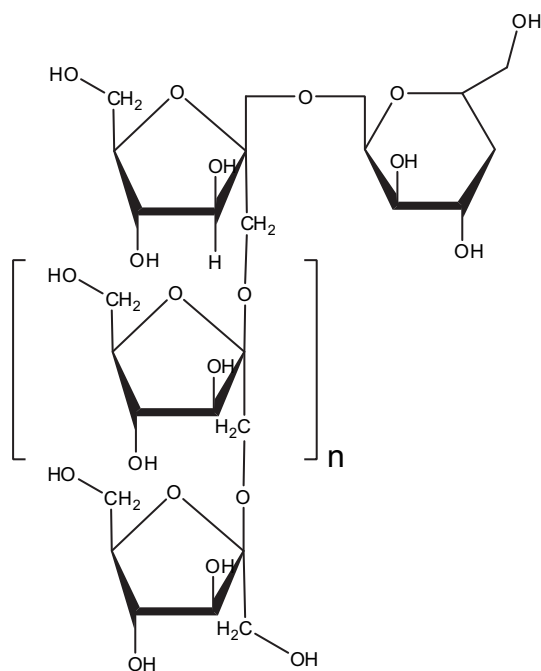


Fig. 14. Structure of inulin.

In the present review we have mainly discussed the matrix molecules that are presently considered to be essential for preserving functionality and allow targeted delivery. There are however many other matrix components that are commonly applied such as gum Arabic, milk proteins, soy proteins but also wax like substances. These molecules have been intensively discussed in previous manuscripts (Augustin & Hemar, 2009; Burey et al., 2008; Champagne & Fustier, 2007; Gibbs et al., 1999; Kosaraju, 2005; Vega & Roos, 2006) and were therefore not discussed in the present paper.

It is impossible to do a recommendation for a specific encapsulation technique or matrix molecule without having detailed insight in the molecular structure of the bioactive molecule, the product that serves as vehicle, and the site in the gut where the product has to be delivered. However, the advances that have been made during recent years in the field of encapsulation are such that it is safe to suggest that there is always a procedure that meets the requirements for application in a specific product. In this review we have given a background of the 'way of thinking' when encapsulation is considered for preserving functionality and targeted delivery of bioactive food components.

A rather novel approach in the targeted delivery is to use the differences in bioactivity and enzyme concentration in the different parts of the gut. This approach is more successful than previous designs that were based on pH or time-dependent release mechanisms (Champagne & Fustier, 2007). In pharmaceutical sciences this approach has been shown to be very successful for delivery of drugs in specific parts of the gut (Kosaraju, 2005). Also for delivery of bioactive food components this approach holds many advantages as it is not only effective but also safe, cheap, and versatile. It is safe since it involves mostly application of carbohydrates that have the GRAS status and are already widely applied in the food industry. It is cheap as most carbohydrates are produced from relative cheap side products obtained from agriculture. It is versatile as most carbohydrates can be applied as dry product or as hydrogel. Moreover, it can be used in all discussed families of encapsulation technologies.

When considering encapsulation of bioactive molecules, we have a wide choice of encapsulation technologies that can be applied as most molecules are resistant to the heat associated with technologies such as spray drying. For living cells this is totally different. Many bacteria require gentle encapsulation technologies that do not interfere with their metabolism and presentation of their functional ligands. More 'gentle' techniques such as extrusion techniques in combination with matrix molecules that preserve the functionality or may even promote the functionality are advisable for probiotics. Much more attention should be paid to encapsulation and preservation of functionality of bacteria when considering probiotics as a bioactive food component with serious health benefits.

Many claimed that health benefits such as stimulation of the immune system, lower cholesterol, improving lactose tolerance (Kailasapathy, 2002) but also prevention of diseases such as cancer (Davis & Milner, 2009; Gibson et al., 2004), inflammatory bowel disease (Hormannspurger & Haller, 2009; Ramakrishna, 2009; Sparrow, Irving, & Hanauer, 2009), ulcerative colitis (Sanges et al., 2009; Sartor, 2009), Crohn's disease (Butterworth, Thomas, & Akobeng, 2008; Sokol et al., 2008; Vavricka & Rogler, 2009; Wallace, 2009) require the delivery of bioactive food components in the colon. Although this distal delivery has been recognized for many years, the number of products that contain bioactive components that are distally released is limited (Kosaraju, 2005). Also the encapsulation approaches that have been applied so far are not very successful in achieving that goal. Most encapsulation approaches are based on pH and time-dependent release principles. In many cases these systems already release their content in the upper part of the gastrointestinal tract due to unpredictable pH variations and variations in the intestinal transit time. These problems can be overcome by applying matrix molecules that withstand the environmental changes in the upper part of the gastrointestinal tract but are rapidly degraded by the enzymes produced by the colonic microbiota. Although this principle is not yet fully employed in the food industry it will without doubt be beneficial for new designs of functional food.

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