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Review article

Application of Emulsion-Based Encapsulation Methods in Food Technology: A Review

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Abstract

Encapsulation is a method of entrapping and protecting sensitive active compounds into the structure of an encapsulation carrier (coating material). Capsules of different dimensions are obtained as encapsulation products. Each capsule consists of two main materials: 1) active compound and 2) carrier (coating material). The main aim of encapsulation is to protect active compounds from degradation influenced by different external factors. Encapsulation is a technique that can increase the stability and improve the usability of many active and biologically valuable natural ingredients. In recent times it has been increasingly used in food technology.

Emulsification is one of the most important encapsulation methods suitable for use in food technology, which can be used alone or in combination with other encapsulation methods. The emulsion formation can be used to encapsulate hydrosoluble and liposoluble liquid substances. The size of droplets within such emulsions ranges from 0.1-5000 μm. The main advantages of this encapsulation method are the protection of the encapsulated substance from high temperatures and oxidation during heat treatment and drying, the possibility of encapsulating liposoluble and hydrosoluble substances, and the controlled release of the active substance. Emulsion-based techniques are widespread encapsulation methods suitable for the food industry. A wide range of active substances can be encapsulated, such as probiotic bacteria, proteins, amino acids, essential oils, flavonoids, vitamin E, lutein, beta carotene, fish oil, omega 3 fatty acids, aspartame and other sweeteners, xylitol and menthol in chewing gum (prolonged cooling effect), curcumin, catechin, vitamin C, vitamin B12 (for the enrichment of dairy products), vitamin B1 and herbal extracts. Obtained capsules can be applied to produce functional milk and dairy products, salad sauces and dressings, fruit juices, dried soup mixtures, functional meat products, the oil industry, and confectionery.

Keywords: Encapsulation, Emulsions, Food technology, Stability, Enrichment.

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INTRODUCTION

Encapsulation is a technique by which a material or mixture of materials is packed into the structure of another material. The beginnings of encapsulation use in industry date back to around 1950. (Madene et al, 2006). Firstly, it was commonly used in the pharmaceutical industry and in recent times in the food industry. For the needs of food products, the most common encapsulated active compounds are vitamins, minerals, dyes, enzymes, probiotics, and flavors. The importance of encapsulation lies in the fact that it is a technique that can improve the stability, usability, and availability of many biologically valuable natural ingredients. The goal of encapsulation is the formation of stable capsules from natural or synthetic polymer materials within which the active substance is encapsulated (Pegg & Shahidi, 1999).

Encapsulation Facts and the Types of Capsules

Each capsule consists of two basic compounds: 1) coating material (carrier, sheath, wall material, shell, or encapsulation matrix) and 2) active substance (encapsulant, inner phase, or capsule core). The active substance is the material that is encapsulated inside the capsule. The coating is the material that coats and protects active compounds in its structure. The role of the carrier is to create a boundary layer between the active substance and the environment. The choice of carrier depends on the size and shape of the capsule, the cost of encapsulation, the encapsulation method, and the mechanism of release of the active substance. Edible and harmless biopolymer substances, which do not react with the active substance, are used as carriers. The most common reasons for applying encapsulation are:

1. Protection of the active substance from external influences, i.e. from degradation under the influence of external factors (heat, oxidation, moisture, air, light, chemical ingredients with which it can react).
2. Enrichment of food products with biologically valuable active ingredients (vitamins, minerals, omega fatty acids), which are often sensitive and easily decomposed.
3. Modification of the physical characteristics and structure of the original material, which makes it possible to achieve easier handling, improvement of the texture of the product into which they are dosed, and better rheological properties. E.g. liquid substances can be inserted into solid materials by encapsulation. It is possible to prevent the formation of lumps, reduce hygroscopicity, improve flowability, and modify the consistency of the medium to which the capsules are added.

4. Controlled release of the active substance (gradual, slow, or at the exact desired moment). The release of the active substance in the product is slower if the substance is encapsulated. E.g. by encapsulating the sweetener, a gradual release of the sweet taste is achieved.
5. Uniform distribution of the active substance when added to different food products.
6. Encapsulation can dilute the concentration of the active substance, which is significant when it is necessary to uniformly mix very low concentrations of the active substance in a larger amount of product.
7. Masking of unwanted taste, smell and aroma (e.g. encapsulated fish oil, bitter substances, etc.).
8. Prevention of unwanted reactions of the active component with the ingredients inside the product.

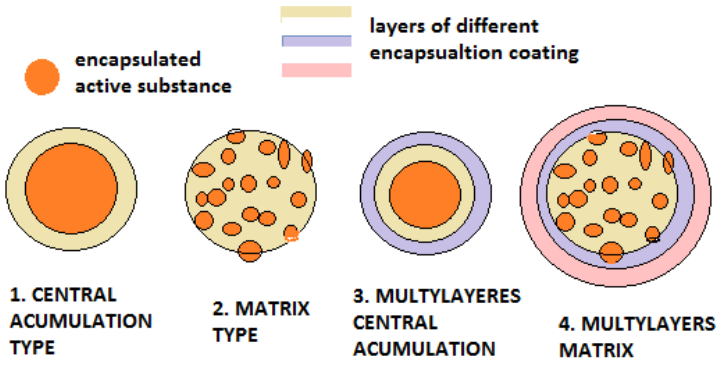

Figure 1. Types of capsules

*Source: the author's private archive

The basic types of capsules are illustrated in Figure 1. Considering the structure and the way the carrier is arranged around the active substance, there are three basic types of capsules (Zuidam & Shimoni, 2010):

1. Aggregate, matrix, or microsphere type – the aggregate structure of the capsule. These are capsules that have several separate cores of the active substance or, more often, a larger number of central particles (molecules or groups of molecules of the active substance that make up the particle) embedded within a continuously distributed matrix or carrier. This is the most common type of capsule and is obtained by applying the largest number of encapsulation techniques. Encapsulant molecules can also be found on the surface layers of the capsule. Therefore, this is the least efficient way of encapsulation.

2. Type of central accumulation. It is the simplest and most obvious form of capsule structure. The core with the encapsulated content is spherical, surrounded by a shell or membrane of uniform thickness like a hen's egg and shell. The thickness of the coating and the proportion of the active substance can be different (Pegg & Shahidi, 1999).
3. Type of multilayer casing. The sheath is wrapped around the core in the form of two or more concentric layers of different materials. Such capsules are designed to allow controlled release of the active substance (Pegg & Shahidi, 1999).

Capsules are not always spherical or other regular shape. The active substance can be evenly or unevenly distributed inside the capsule (Bakry et al, 2015). Also, there are cases where already formed smaller capsules are encapsulated inside larger capsules (capsules within a capsule). The reason for this kind of encapsulation is to achieve the controlled release of the active substance. Therefore, these three basic types of capsules can differ in shape, structure, and size. In addition, depending on the encapsulation carrier and the method of formation, capsules can have a different internal structure (Rodriguez et al, 2016).

A matrix-type capsule (with multiple separate encapsulated regions) is often referred to as a microsphere. Liposome capsules are capsules of the central accumulation type where the encapsulation carrier completely covers the encapsulated substance. In the case of liposomal capsules, the carrier (coating) is formed from a double layer of phospholipid molecules that cover the active substance in the form of a film. The phospholipid bilayer in the liposomal capsule is formed by connecting the non-polar ends of phospholipid molecules, forming a spherical particle (liposome), while the polar groups remain oriented towards the surface and inside the particle. A cyclodextrin capsule is a chemical complex in which the active substance is encapsulated in the empty inner part of the ring of the cyclodextrin molecule. In this way, the active substance forms a chemical complex with the cyclodextrin molecule, which is why this method of encapsulation is called molecular inclusion. Capsules in emulsion represent particles of an active substance coated on all sides with particles of a surface-active substance (emulsifier or stabilizer). In this way, a hydrophobic active substance is stabilized in a hydrophilic environment or vice versa (Rodriguez et al, 2016).

Micelles are complex particles formed by aggregation or self-association of the same or similar molecules (most often proteins such as casein). Aggregation and self-association of molecules in the micellar structure are achieved by hydrophobic and/or electrostatic interactions. The main feature of the micelle is the hydrophilic character on the surface and the hydrophobic interior. Under the influence of physical factors, micelles can dissociate into their constituent parts and reassociate again, i.e. associate. Specifically due to this, the encapsulation of smaller molecules within the micellar structure is possible. Casein micelles are the most common coating material in the formation of micellar-type capsules. Different active compounds with particle size smaller than micelle could be encapsulated inside the

micellar structure of the casein or other proteins. The structure of the casein micelle allows the encapsulation of small molecules of active compounds and the formation of matrix-type capsules. A casein micelle is a spherical particle with a common size of 100-200 nm and consists of smaller spherical particles (submicelles) bound together in a micellar structure. Submicelles are bound together in the micellar structure through pretty strong colloidal calcium phosphate bridges. Beta carotene can be encapsulated inside the micellar casein capsules. Such kind of encapsulation can be improved by high hydrostatic pressure-induced dissociation and reassociation of casein micelle. Dissociation of micelle helps incorporation/encapsulation of beta carotene inside the micellar structure, while reassociation of casein micelle with encapsulated beta carotene inside occurs when pressure decreases (Tahmaz, 2014).

Encapsulation methods can be divided into two main groups: mechanical and chemical. In food technology, the most common encapsulation methods are spray drying, emulsion-based methods, centrifugal separation, coacervation, liposomal capsules, and fluidization (fluid bed coating).

Emulsions – Definitions, Classifications, and Preparation Methods

Emulsification is one of the most common methods in encapsulation, whether it is applied alone or as part of other encapsulation methods. An emulsion is a stable mixture of two liquids between which high surface tension occurs and which therefore cannot mix. A large surface tension occurs as a result of the difference in polarity. Polar and non-polar liquids do not mix. Typical examples of polar liquids are water, salt, and electrolyte solutions, polar organic liquids (alcohols, acids, etc.). The emulsion is obtained by vigorous mixing of polar and non-polar liquid. Vigorous mixing breaks up the layers and breaks up their droplets. In this way, the total contact surface between the particles of polar and non-polar liquid increases and their merging into a mixture occurs. The emulsion created by vigorous mixing is unstable because the particles of polar and non-polar liquids do not show affinity towards each other. Although they are mixed, there is still a high surface tension between them, which is why they repel each other and delamination occurs very quickly. On the other hand, mutual attractive forces between polar and non-polar liquid molecules are activated. As a result of these attractive forces, polar particles are regrouped on one side, non-polar on the other and the emulsions are separated into two phases. In other words, vigorous mixing without the presence of a surface-active substance will result in the formation of an unstable emulsion. Such emulsion is not thermodynamically stable but is only kinetically stable for a short time (Tahmaz, 2019).

Each stable emulsion consists of three basic parts: a polar (hydrophilic, lipophobic) liquid, a non-polar (hydrophobic, lipophilic) liquid, and a surfactant. To obtain a thermodynamically stable emulsion, it is necessary to reduce the surface tension between polar and non-polar liquids so that they remain mixed. Emulsifiers are surface-active substances, whose molecular structure is such that they have polar (charged) and non-polar (non-charged) parts in the same molecule. Polar moieties are mostly functional groups with oxygen such as the OH^- group or some other groups with free electrons. Nonpolar groups

are nonpolar ends (radicals) of organic molecules (e.g. fatty acid radicals). Typical examples of emulsifiers are lecithin, phospholipids, fatty acid monoesters, milk proteins, other proteins, etc. When an emulsifier is added to the unstable emulsion, the polar end of the emulsifier connects to the polar liquid, while the nonpolar end connects to the nonpolar liquid (Tahmaz, 2019).

In the food industry, the emulsification process is often used to obtain stable food products. A large number of food products are typical emulsions. Typical examples of natural food emulsions are milk, butter, and egg yolks. In milk, the milk fat is emulsified in the aqueous phase with lecithin and milk proteins as emulsifiers. Typical food emulsions obtained by artificial emulsification (in the production process) are mayonnaise, margarine, cream, sour cream, cake mixes, baby food, etc.

Emulsions can be divided into different types (Figure 2) depending on the amount and nature of each phase:

1. Single emulsions:
    a. Oil-in-water emulsions - known as o/w emulsions (oil/water)
    b. Water-in-oil emulsions - known as w/o emulsions (water/oil)
2. Double emulsions:
    a. Oil in water and all in oil - o/w/o (oil/water/oil)
    b. Water in oil and all in water - w/o/w (water/oil/water).
3. Multiple emulsions (e.g. o/w/o/w or w/o/w/o).

Oil-in-water (o/w) emulsions have more intense properties of the water polar phase. They can be mixed with water or other polar liquid, and show higher values of electrical conductivity (due to higher water content and higher polarity). Components that are soluble in water can be mixed in them. Emulsifiers soluble in water are added to this type of emulsion to stabilize them. A typical example of such emulsions is milk and cream. In water-in-oil (w/o) emulsions, the dominant phase is oil (non-polar phase). These emulsions are soluble in oils and have low electrical conductivity (due to the low content of polar components). Typical examples of w/o emulsions are butter and margarine.

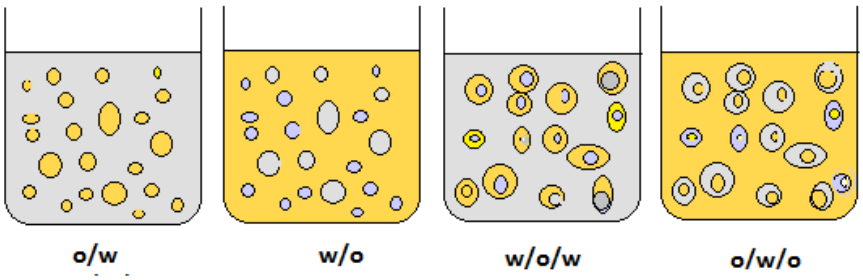

Figure 2. Illustration of emulsion types (o/w-oil in water, w/o-water in oil, w/o/w-water in oil in water, o/w/o-oil in water in oil)

*Source: the author's private archive

Some products show properties of both types of emulsions (w/o and o/w) at the same time. The characteristic of these emulsions is that they can be mixed with both oil and water phases, which means that liposoluble and hydrosoluble active components can be added to them. They are considered stable emulsions because they show good affinity towards polar and non-polar substances. Examples of such emulsions are mayonnaise, cake and pancake mixes, and many other food products. Although it contains a greater amount of oil phase, mayonnaise is defined as oil in water emulsion. These are mixed emulsions, which are very stable because they contain a relatively high proportion of emulsifiers and/or an equal proportion of polar and non-polar phases. This is exactly why they have the properties of o/w and w/o emulsions.

Multiple emulsions (Figure 2) can be double (w/o/w and o/w/o) or even triple (w/o/w/o and o/w/o/w). These emulsions are obtained by the process of double or multiple emulsifying. They can be considered as an emulsion dissolved in an emulsion. The preparation of double or triple emulsions includes several steps. The first step is the preparation of stable primary w/o or o/w type emulsion. The primary emulsion is dosed into another liquid, which cannot be mixed into a stable mixture without emulsifying. But, in the presence of an emulsifier or other surface-active substance, mixing occurs and a multiple emulsion is formed in which the droplets of the original emulsion are dispersed and stabilized. For example, in the case of a w/o/w emulsion, a stable water-in-oil emulsion is first formed. Water in oil emulsion mostly shows properties of the oil phase and it cannot be mixed with the water phase in the stable mixture without emulsifying. Because of that primary water in oil emulsion is added to the polar water phase with vigorous mixing in the presence of an emulsifier. Vigorous mixing help to primary emulsion to be mixed and dispersed in the water phase, while the presence of an emulsifier ménages to stabilize the double emulsion. In an o/w/o emulsion preparation, an oil-in-water type emulsion is first formed and added to the non-polar fat phase with which it is mixed and stabilized in the presence of a suitable emulsifier. These procedures of additional emulsification can be repeated several times according to the same principle so that quadruple emulsions and other more complex emulsions can be obtained.

The key factor for emulsion stability is homogenization, i.e. the better dispersion of particles of the dispersing phase within the dispersing agent (continuous phase). Therefore, the particles of the dispersed phase must be as small as possible. Thus, a larger specific surface area of the continuous phase particles and a larger contact surface between the dispersed phase and the dispersing agent are achieved. The smaller particles in emulsion, cause more stable emulsions. Emulsion homogenization can be done with different devices:

1. High-speed vertical mixers – the mixing speed is not high and they usually form a weak emulsions whose particle sizes are around 10 μm,

2. Homogenizers – devices that operate on the principle of passing liquid material through small holes under high pressure,
3. Ultrasonic homogenizers – for homogenization, that use ultrasonic energy, pulverize dispersed phase particles to very small dimensions (nanoparticles), produce very stable emulsions (nanoemulsions),
4. Colloid mills – which have fast rotation, where they grind particles to colloidal dimensions,
5. Membrane homogenizers where the particles of the dispersed phase are passed through the microporous membrane and
6. Microfluidizers.

With the membrane process, it is possible to obtain fine and stable emulsions. This is why the encapsulation process itself is very successful because very small and stable capsules are formed in such emulsions. Emulsifying using a membrane homogenizer has proven to be a very successful method for encapsulation. It is a process that involves passing the pre-emulsion through a microporous membrane. Depending on the size and character of the pores (hydrophilic or hydrophobic pores), it is possible to encapsulate hydrophobic and hydrophilic active substances. Hydrophilic active substance is encapsulated in w/o, and hydrophobic in o/w emulsion.

Encapsulation in Emulsions

After spray drying, emulsions and emulsifying processes are the second most common encapsulation technique used in industry. The commercial possibilities of using double emulsions are reflected in the production of low-calorie foods (sauces, mayonnaise, whipped cream, etc.) and for the encapsulation of nutritionally valuable ingredients, flavors, and additives (Muschiolik & Dickinson 2017). Some examples of encapsulation using the double emulsification technique are given in Table 1. Emulsifying can be used as the primary encapsulation method or it can be combined with other encapsulation methods like spray drying, freeze drying, and high hydrostatic pressure to obtain more stable particles.

Emulsification can be used to encapsulate water-soluble and lipid-soluble liquid substances. The size of the droplets inside such emulsions ranges from 0.1-5000 μm. The stability of the encapsulated substance can be improved by forming a multilayer shell (Prichapan & Klinkesorn, 2014). Encapsulation proceeds by first dissolving the encapsulation carrier in an organic solvent (non-polar organic phase), and the active substance is added to that solution. An aqueous solution containing an emulsifier is added to the organic phase. By mixing these solutions, an emulsion is formed. The organic solvent is removed by evaporation, while the carrier polymer molecules form a shell around the active substance. The

obtained emulsion can be spry or freeze-dried (Silva et al, 2014). Encapsulation in different emulsion types and images of obtained capsules are illustrated in Figure 3.

Encapsulation by emulsification can be carried out in single or double emulsions. During emulsion base encapsulation, two basic processes are crucial: 1) the emulsification of the active substance in the carrier solution and 2) the precipitation of the obtained capsules (Figure 3). Encapsulation efficiency can be increased with the addition of cross-linking agents. The formation of cross-links can be assisted physically (precipitation of proteins by heating) or chemically (precipitation after the addition of cations - usually Ca^{2+} ions or by lowering the pH value). The encapsulation carrier is dissolved in an aqueous medium containing a cross-linking agent ($CaCl_2$). The resulting solution is mixed with oil or another hydrophobic medium in which the active substance is dissolved. Smaller particles of the active substance remain encapsulated within the cross-linked structure of the encapsulation carrier (Manjanna et al, 2010).

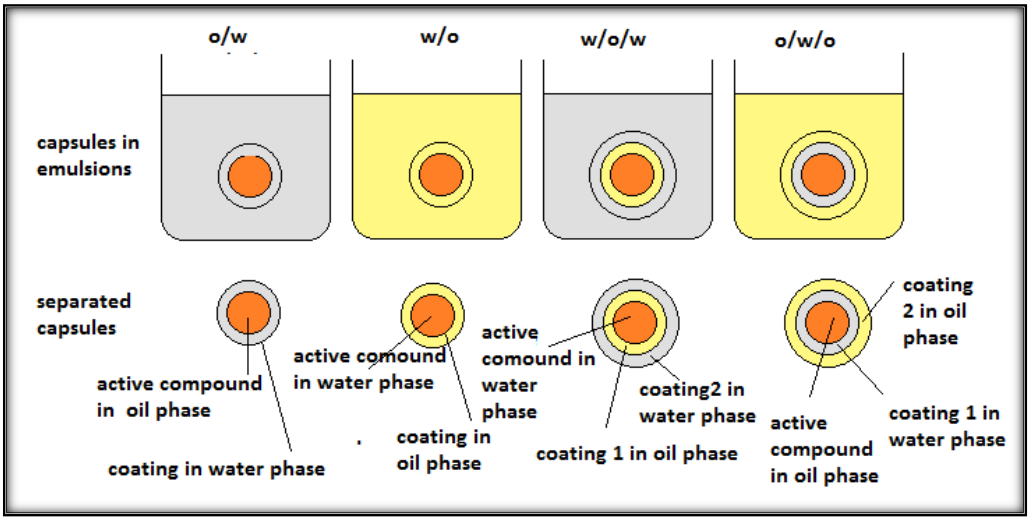

Figure 3. Illustration of capsules formed in emulsions

*Source: the author's private archive

A coaxial microfluidizer can be used as a device, which can form very small microdroplets in emulsions for encapsulation. Using this device, microcapsules of the central accumulation type are obtained in the single, double, and triple emulsion. The advantage of using a coaxial encapsulator is its possibility to form capsules of uniform and regular spherical shape and size with a thin shell with uniform thickness (Li et al, 2018). This method is very similar to encapsulation by co-extrusion because the active substance is coated when leaving the microfluidizer in the same way as in co-extrusion.

Capsules of different types can be formed by encapsulation in an emulsion, which depends on the emulsification method, the type of emulsifier, stabilizer, and biopolymer, the aggregate state of fat droplets, and the water phase. In an ordinary single emulsion, the active substance is stabilized only by a layer of emulsifier molecules. In the case of double emulsions, a single emulsion is first formed, which

is then dissolved in an aqueous or fat phase different from the dispersing agent of the primary emulsion. If the aqueous phase contains a biopolymer that causes gelation (gelatin, pectin, starch, alginate), then an emulsion in the gel state is obtained. In this type of emulsion, a gelled water phase surrounds the fat droplet containing the active ingredient, which is covered in emulsifier molecules. Gelation further stabilizes the emulsion. Encapsulation in an emulsion can be achieved by coating the active component with solid colloidal stabilizer particles (Mao & Miao, 2015).

Pickering emulsion is emulsion stabilized by fine solid particles, which absorb irreversibly on emulsion droplets. It is very applicable for encapsulation in functional food products (Cheon et al, 2023).

Encapsulation in single emulsions

If the active substance is lipophilic (e.g. beta carotene), it can be encapsulated in o/w emulsions. Encapsulation occurs when a hydrophilic shell of surfactant and carrier is formed around the beta-carotene particle. The hydrophilic coating enables the solubility of beta-carotene in the aqueous environment (Ax, 2003; Ribeiro et al, 2010). If the active substance is hydrophilic, then the encapsulation is carried out in a w/o emulsion, and the surface of such a capsule is lipophilic (Manjanna et al, 2010). A schematic representation of the formation of capsules in a single emulsion is shown in Figure 3.

Peng et al (2023) investigated the influence of emulsion particle size on the encapsulation efficiency of encapsulated sweet orange essential oil into maltodextrin and modified starch capsules. The emulsion was prepared by mixing of dispersed phase containing orange oil with a continuous phase containing coating material dissolved in water. After completed homogenization emulsion was divided into three phases with large, small, and nano-sized capsules. The results showed that encapsulation efficiency increased when capsule size decreased.

Fitri et al (2022) reported very high values of encapsulation efficiency (96.6-99.88%) of beta carotene encapsulated in nano cellulose by preparing of o/w single emulsion. The zeta potential of obtained particles ranged between -8.69 and -19.73 mV.

Tessaro, Marteli-Tas, and Sobral (2022) encapsulated polyphenol-rich *Eugenia uniflora* leaf extract in soybean oil using a single w/o emulsion. The obtained particles had sizes between 250 and 4250 μm and improved the stability of antioxidative compounds. The extract firstly was dissolved in aqueous phase. Water in oil emulsion was prepared dropwise addition of aqueous extract into soybean oil containing lipophilic emulsifier under continuous high-speed ultrasound homogenization (15000 rpm for 5 minutes). Emulsion droplet size ranged between 0.25 and 4250 μm depending on the emulsifier and extract concentration. Emulsions with 20% extract concentration had the smallest particles and the highest physical stability.

Encapsulation in double emulsions

Encapsulation using the double emulsification technique is more often used because smaller capsules can be obtained in comparison to single emulsions. The formation of capsules in double emulsions is illustrated in Figure 3. W/o/w emulsions are more often used for encapsulation in food technology in comparison to o/w/o emulsions (Table 1).

Table 1. Application of double emulsion encapsulated active compounds in different foods

Emulsion type	Active compound	Application in foods
w/o/w and o/w/o	Flavors encapsulated in gelatinizing polysaccharides	Addition in preparation of low-calorie salad dressings, margarine, and similar spreads
w/o/w	Vitamins encapsulated in 40% sucrose solution	Enrichment of confectionery products
w/o/w	Polyunsaturated seed oils	In different products that commonly contain milk fat, replacement of milk fat with encapsulated oils
w/o/w	w/o emulsion droplets	Replacement of oil or fat in low-calorie products (e.g. meat dough for the production of sausages, and pate), drops have a lower oil content than oil alone, but give the perception of taste, aroma and texture as oil alone, improved sensory properties of low-calorie products
w/o/w	Na-ascorbate	Enrichment of milk before UHT sterilization. Improved stability of ascorbate under thermal treatment
w/o/w	$CaCl_2$	Fortification of soy milk with Ca without changes in the consistency of soy milk. Encapsulated Ca does not change the consistency of milk, unlike non-encapsulated, which can lead to curdling.
w/o/w	Natural colors	Application in the confectionery industry and ice cream production – coloring of different products. Natural colors (beta carotene, anthocyanins) are mostly antioxidative compounds, very sensitive to oxidation and high temperatures. Because of that these colors are more stable in encapsulated form
o/w/o	Fish oil	Fortification of margarine and other spreads
w/o/w	Polyunsaturated oils	Replacement of pig fat in frankfurter sausages.
w/o/w	Sunflower oil	Fortification of milk
w/o/w	Vitamin B12	Application in technology of functional dairy products
w/o/w	Aspartame and other sweeteners	Application in the production of chewing gums. Encapsulated sweeteners are released more slowly than non-encapsulated, and because of that sweet taste is released gradually.
w/o/w	Xylitol and menthol	In the production of chewing gum - encapsulation achieves a prolonged cooling effect and keeps the menthol aroma longer.
w/o/w	$MgCl_2$	As a coagulant in the production of soy milk cheese - encapsulation masks the bitter taste
w/o/w	Curcumin and catehine	Fortification and coloring of different drinks, juices and beverages
w/o/w	Grape seeds extract and saffron extract	Enrichment of different food products
w/o/w	NaCl	Application in the production of low salt products. Increased perception of salty taste in products with reduced NaCl content, destabilization of the double emulsion with encapsulated NaCl occurs in the mouth, and NaCl is gradually released.

*Source: Muschiolik & Dickinson (2017)

In the case of lipophilic active substance, encapsulation can be achieved in an o/w/o emulsion, and in that case, the encapsulation carrier is dissolved together with the emulsifier in the aqueous phase. The two phases are vigorously mixed to form a primary emulsion, which is allowed to stand for a while before a new amount of the aqueous phase will be added. By adding an aqueous phase, a double (secondary) emulsion of the o/w/o type is formed in which biopolymer particles with an encapsulated substance are deposited. The re-dosed aqueous phase destabilizes the primary emulsion (o/w), causing the capsules to separate. During this process, interactions occur between molecules of the encapsulation carrier, which leads to the formation of cross-links. This process contributes to the encapsulation, and the finer particles of the active substance stabilized in the oil remain encapsulated between the cross-linked carrier molecules (Manjanna et al, 2010). Double o/w/o emulsions are mainly used for encapsulating different oils. Especially it is interesting to encapsulate fish oil in double o/w/o emulsion with purpose to mask its smell. Encapsulating fish oil masks its unpleasant smell and aroma so that it can be added to different food products. In this emulsion type, fish oil is dispersed in water phase, while the secondary oil phase differs from the primary one (o1/w/o2). The most common emulsifier in the primary emulsion (o1/w) is gluten, while in the secondary emulsion, it is Na-caseinate. In this type of emulsion, gluten, and Na-caseinate are not only surfactants but also encapsulation carriers. The encapsulation of fish oil in double o1/w/o2 emulsion takes place in the following order: In the first step, fish oil is dispersed in an aqueous phase containing Na-caseinate, forming a primary emulsion. The primary emulsion is mixed with a second oil phase consisting of equal parts of olive oil and polyglycerol-polyricinoleate (surfactant) to form a secondary emulsion. The double emulsion is combined with an aqueous phase containing dissolved lactose monohydrate and Na-caseinate before drying. (Muschiolik & Dickinson, 2017).

Double w/o/w emulsions are used for encapsulation of hydrophilic active substances such as anthocyanins, phytosterols, betalains, natural colors, probiotics, B group vitamins, vitamin C, mineral salts (NaCl, $CaCl_2$, and $MgCl_2$) and sweeteners. Lecithin is the most often used as an emulsifier, while gelatin, Na-caseinate, and whey protein isolates are used as coating materials). In this type of emulsion, the secondary emulsion water phase can be the same as in the primary (w/o/w), or it can be different (w1/o/w2). The primary emulsion usually contains lecithin, which stabilizes the hydrophobic active substance, while the second aqueous phase w2 represents a solution of a surface-active encapsulation carrier. Encapsulation occurs only after the addition of the second aqueous phase (w2) with another biopolymer, whereby biopolymer 2 simultaneously stabilizes the new secondary emulsion and forms a shell around the droplets of the primary emulsion containing the particles of the active substance. The first water phase often contains NaCl, $CaCl_2$, or glucose. These components help form the coating on the surface of the capsule. NaCl and glucose increase the osmotic pressure, which leads to the precipitation of biopolymers, while Ca^{2+} ions cause aggregation by forming cross-links between carrier molecules and thus help the precipitation of capsules (Muschiolik & Dickinson, 2017).

The encapsulation of fish oil in a two-layer (secondary) emulsion is carried out to prevent oxidation of omega-3 fatty acids and to mask the unpleasant smell of fish oil. The primary emulsion is formed by homogenizing fish oil and the aqueous phase with lecithin at pH 3. First, a negatively charged layer (lecithin) is formed on the surface, and the secondary emulsion can be obtained by adding a cationic stabilizer (chitosan). The anionic layer of lecithin binds chitosan cations and a positively charged particle coated with a lecithin-chitosan layer is formed (McClements et al, 2007).

Dang, Haji, and Kha (2024) encapsulated acerola juice (rich in vitamin C) with maltodextrin and gum arabica as coating materials by preparing double w/o/w emulsion. Firstly, the primary w/o emulsion was prepared by mixing acerola juice with oil in proportion 40:60 and with the addition of lipophilic emulsifier PGPR. The mixture of juice, oil, and emulsifier was vigorously stirred by a mixer speed of 700 rpm. When primary w/o emulsion was prepared and stabilized, the secondary water plus emulsion was finished maltodextrin and gum Arabica dissolved in the water phase were added. The water phase with dissolved wall material and emulsifier (Tween 20) was added slowly drop by drop into the primary emulsion with continuous high-speed mixing (900 rpm). Encapsulation yield of vitamin C ranged between 20.83 and 28.36%.

Encapsulation in multi-layered (multiple) emulsions

The formation of a multilayer shell around the active substance in the emulsion significantly increases the stability of the encapsulated active substance. Multi-layered emulsions are known as m-o/w (multi-layered o/w emulsions) or m-w/o (multi-layered o/w emulsions). Capsules with a multi-layer shell produced most often in oil-in-water (o/w) emulsions are known as m-o/w emulsions. In those emulsions, the active substance is usually hydrophobic (but can also be hydrophilic), dissolved in oil, and encapsulated in a hydrophobic phase. These are central accumulation-type capsules in which the droplet of the hydrophobic phase (oil) is encapsulated by being coated with a three-layer shell. Three-layer coating is achieved by triple homogenization by forming a primary, secondary, and finally tertiary emulsion. In the primary emulsion, the particles of the hydrophobic phase are coated only with emulsifier particles (water-soluble ionized emulsifier with a polar end). The secondary emulsion is created by homogenizing the primary emulsion after adding a surface-active biopolymer solution (hydrocolloid 1). In the secondary emulsion, a second shell of hydrocolloid molecule 1 is formed around the emulsified particles of the fat phase. The third shell will be formed in the tertiary emulsion, i.e. by the third homogenization after the addition of the second biopolymer (hydrocolloid 2) to the secondary emulsion. With this procedure, it is possible to control the amount of encapsulated active component (it depends on the size of the droplets of the fatty phase in the primary emulsion), the thickness of the shell, and the size of the capsule. In the secondary and tertiary homogenization, shell porosity, shell thickness, sign, and strength of capsule charge are controlled (McClements, Decker, and Weiss, 2007).

The stacking of layers around the droplet of the hydrophobic phase with the active substance takes place according to the principle of electrostatic attraction. In the beginning, there are only fat droplets dissolved in the aqueous phase and emulsifier particles that coat around the particles of the fat phase. The non-polar ends of the emulsifier molecule bind to the fat droplet, and the polar ends are directed towards the aqueous phase. In this way, a barrier to the water phase is created. The fat droplet becomes encapsulated in the aqueous phase because there is a polar outer layer of emulsifier molecules on its surface. When biopolymer particles oppositely charged to the emulsifier molecules are added to the primary emulsion, they will be attracted to the coated fat particles due to electrostatic attraction of opposite charges. The third layer is formed after the particles of biopolymer 2 are added to the secondary emulsion, the molecules of which are charged opposite to the molecules of the first biopolymer, and the process is repeated in the same way. According to the principle of electrostatic attraction, the number of layers can continue to increase.

An excess of biopolymer molecules remains in the emulsion, which failed to bind to the surface of the capsule. To finish the coating and obtain the finished surface capsule layer, it is necessary to bind the excess particles to the capsule. The finish surface layer should be the thickest. This can be achieved by adjusting the pH value, commonly by adding Ca^{2+} ions. By changing the pH value, there is a change like the capsule charge (positive or negative), and the capsule can adsorb a new amount of biopolymer of the opposite charge. Also, there can be a change in the charge of the dissolved biopolymer, and then its particles can again bind to the oppositely charged capsule. At the end of the coating, the excess electrolyte is removed from the surface of the capsules (McClements, Decker and Weiss, 2007), and after that, they can be spray-dried.

The main advantages of encapsulation through a multilayer emulsion are reflected in the improved physical and chemical stability of the active substance and the possibility of targeted release through the gradual destruction of the structure of the multilayer shell. Natural biopolymers such as proteins, polysaccharides, and phospholipids are used as coating layers. The basic prerequisite for encapsulation is that their molecules possess polar parts, i.e. charged functional groups. Some examples of such encapsulation carriers are casein micelles, lecithin, and other phospholipids, polysaccharides (OH^-), chitosan (cations), and the like. It is considered an ideal method for the encapsulation of hydrophobic liposoluble active substances (e.g. different oils or some other liposoluble components) (Tahmaz, 2014).

With the described procedure, it is possible to encapsulate hydrophilic substances, although the hydrophilic substance cannot be encapsulated as an integral part of the fat droplet, but as an integral part of one of the coating layers (McClements, Decker and Weiss, 2007). The disadvantage of using this method is that it requires great precision to obtain the desired thickness of the coating, but also to avoid the aggregation of the capsules.

Limonene could be encapsulated by forming a three-layer triple emulsion to prevent it from straightening during spray drying. The primary emulsion is formed by homogenizing the aqueous phase and oil with dissolved limonene. A hydrophilic emulsifier (proteins from lupine seeds - *Lupinus album*) is dissolved in the aqueous phase, the molecules of which are negatively charged. Because of that, a thin negatively charged layer of lupine protein isolate is created around the droplet of oil with dissolved limonene. The next layer from a secondary emulsion is formed when positively charged biopolymers (chitosan) are added to the primary emulsion. Chitosan binds to the free negatively charged ends of the lupine protein, causing the coated oil droplet in the secondary emulsion to become positively charged. The third layer is formed when negatively charged polysaccharides (xanthan gum, pectin, and Na-alginate) are added to the secondary emulsion. Thus, in the end, the coated droplet becomes negatively charged (Burgos-Diaz et al, 2018).

Ribeiro et al (2022) used self-aggregated and cross-linked chitosan nanoparticles and maltodextrin for the encapsulation of roasted coffee oil rich in phenolic compounds. The emulsion was prepared by mixing roasted coffee oil with water suspension of cross-linked and self-aggregated chitosan nanoparticles. To obtain stable capsules maltodextrin was added to the prepared emulsion of coffee oil in the chitosan water phase. The mixture was homogenized by high speed of ultrasonic mixer (12000 rpm for 5 minutes). Obtained emulsions were dried by freeze-drying and spray-drying. The particle size of dried microcapsules ranged between 10.73 and 276.21 μm. Freeze-dried emulsions had significantly larger particles and higher oil retention in comparison to spray-dried. Oil retention ranged from 76.29 – 96.46% in freeze-dried and 19.10 – 25.47 in spray-dried capsules. Total phenolic content and antioxidative activity also were higher in freeze-dried than in spray-dried emulsions.

Application in Food Technology

There are many examples of the application of active compounds encapsulated by emulsion-based methods in the enrichment of different food products. Emulsion-based encapsulation techniques are very applicable to the food industry. Obtained capsules can be used in original liquid emulsion form or could be additionally preserved by spray drying or freeze drying. Encapsulation of active substances in an emulsion is a very suitable encapsulation method for the food industry, especially for products that by their very nature are emulsions. Such emulsion-based products are e.g. mayonnaise, sauces, sausages, meat pates, cheese-based and vegetable-based spreads, dairy products, yogurt, cream, milk, chocolate, etc. Many of these products can be enriched with active substances encapsulated in the original liquid emulsion without drying. For other non-emulsion food products, it is recommended to be enriched with previously dried emulsion-based encapsulated substances.

Bernice et al, (2024) used the o/w Pickering emulsion method to produce nano-fibrillated cellulose nano-capsules with beta carotene, which were used as fat replacements in the formulation of low-fat margarine-like spreads. The obtained capsules had particle sizes between 64.67

and 94.73 nm, zeta potential from -32 and -38 mV, and very high encapsulation efficiency of 86.87-89.90%. Pretty high negative values of zeta potential indicate to good particle stability against aggregation. All values (particle size, zeta potential, and encapsulation efficiency) increased with increasing concentration of coating material (nitrocellulose). The obtained margarine-like spread had increased viscosity with an increased concentration of coating material.

Low-fat spreads and other low-fat products are insufficient in liposoluble vitamins and provitamins content, and because of that it is desirable to be enriched with encapsulated liposoluble vitamins. Encapsulation of beta carotene improved its solubility in water and hydrophilic solutions (Tahmaz, 2014). Some examples of possible applications of emulsion-based encapsulated active compounds in food technology are given in Tables 2-4.

Table 2. Application of emulsion-based encapsulated active compounds in the confectionery products

Food product	**Active compound**	**Coating material & emulsion method**	**Achieved effects**
Jelly and gummies	Chlorophyll from alpha-alpha	Agar and gelatin	Coloring jelly and gummy candies with green pigment chlorophyll, have greater stability of chlorophyll when heated during production (Raei, Yasini and Daneshi, 2017)
Chewing gums	Aspartame	w/o/w emulsion	Gradual/prolonged release of sweet taste
Chocolate	Antioxidant-rich moringa leaf extract	Soybean and coconut oil & w/o single emulsion	Enrichment of chocolate with antioxidants, improved the stability of antioxidants from moringa leaves (Kaltsa et al, 2021)

Table 3. Application of emulsion-based encapsulated compounds in dairy products

Food product	Active compound	Coating material & emulsion method	Achieved effects
Functional ice cream	*L. casei and L. lactis*	Alginate and modified starch	Improved stability of probiotics and acceptable sensory properties (Homayouni et al, 2008)
Cheddar cheese	Bixin	Multi-layered coating: kappa carrageenan, casein, and wax & Multiple o/w/o/w	Improved stability of bixin color (Ravanfar, Celli, and Abbaspourad, 2018)
Melted cheese in slices	Fish oil	Milk proteins	Masked undesirable odor and taste of fish oil, acceptable sensory properties, improved oxidative stability of fish oil (Tolve et al, 2016)
White brine cheese	*L. acidophilus. B. bifidum*	Carrageenan, Na alginate	Improved stability of probiotics (Tolve et al, 2016)
Milk	*L. breve*	Gelatin and starch	Improved stability of probiotics in the digestive tract (Tolve et al, 2016).
Milk	Fe	Fatty acids, phospholipids	Masked undesirable odor and taste, acceptable sensory properties, improved oxidative stability (Tolve et al, 2016)
Yogurt	*L. acidophilus, B. lactis*	Alginate and modified starch	Improved stability of probiotics (Tolve et al., 2016)
Yogurt-like a functional beverage	Vitamin E	Yogurt based emulsion	Improved stability of probiotics in the digestive tract (Tolve et al, 2016)
Tofu	$MgCl_2$	Alginate	Improved hardness and masked bitter taste (Tolve et al, 2016)

Kultsa et al (2021) encapsulated antioxidants rich moringa leaf extract into a single w/o emulsion containing soybean and coconut oil, and emulsifiers/surfactants Span 80 and Tween 80. In the first step, oils were mixed with surfactants at using pretty low speed (600 rpm) until obtain stable homogeny mixture. Then, the extract started to be slowly added drop-by-drop under higher speed stirring (1000 rpm). Obtained emulsion with encapsulated extract was added to chocolate to enrich its antioxidative properties. Emulsion particle size ranged between 15.1 and 82.60 nm and increased by increasing the content of oil in the emulsion.

Table 4. Application of emulsion-based encapsulated active compounds in the ready-to-eat food and margarine

Food product	Active compound	Coating material & emulsion method	Achieved effects
Mayonnaise	*L. acidophylus*	Ca-alginate and resistant starch	Improved probiotic stability after 7 weeks of cold storage (4°C), mostly unchanged sensory properties, significantly lower smoothness in comparison to control (Tolve et al, 2016)
Mayonnaise	*L. acidophilus, L. casei*	Ca-alginate and resistant starch	Improved stability of vitamin E during pasteurization at 65°C, good sensory properties, increased antioxidant activity, good survival of lactic acid bacteria (Raikos et al, 2021)
Mayonnaise	Walnut oil	Pectin and maltodextrin	Improved hardness with a masked bitter taste (Tolve et al, 2016)
Mayonnaise	*L. casei, B. bifidum*	Resistant starch and Ca-alginate	Better survival of probiotics, and improved sensory properties (Mohammadi et al, 2013)
Yogurt sauce	*L. paracasei*	Resistant starch and Na-alginate	Better probiotic stability, lower acidity, and improved sensory properties (Bigdelian & Razavi, 2014)
Soy sauce	*Z. rouxii, T. halophylus*	Alginate & w/o/w	Improved shelf life, lower peroxide number, and lighter color (Akhtar & Masoodi, 2022)
Instant soup mixture	Fish oil	Skimmed milk powder & Emulsification and spray drying	Enhanced survival of probiotics (Fahimdanesh et al, 2012)
Beef burger	*Thymus vulgaris* essential oil	Chitosan & o/w emulsion and ionic gelation	Increased survival of probiotics without negative effects on color, consistency, taste, and acidity (Porjavid et al, 2022)
Beef burger	Safflower oil enriched with acai extract	Amorphophallus konjac and Na-alginate & Hydrogel emulsion technique	Enhanced soy sauce aroma development during fermentation (Dewanthi, 2018)
Meatballs	Cod liver oil	Na alginate and lupin protein isolate & o/w emulsion	Improved oxidative stability of omega-3 fatty acids, acceptable sensory properties, masked fish oil taste (Fakir & Waghmare, 2018)
Fish burger	Lemon essential oil	Chitosan and modified starch	Improved antimicrobial stability and sensory properties, inhibited discoloration (Ghadari et al, 2016)
Margarine like reduced fat spreads	Beta carotene	Palm-originated nano fibrillated cellulose & Pickering o/w emulsion	Extended shelf life of beef burger (Hanula et al, 2022)
Margarine	Anthocyanins from roselle (*Hibiscus sabdariffa*) and red cabbage	Maltodextrin & Microwave melting of maltodextrin and encapsulation in o/w emulsion	A high encapsulation efficiency of 95.62%, improved heat stability of meatballs during heat treatment at 70-100C. acceptable sensory properties (Elsebaie et al, 2022)

Fish burgers produced with the addition of nanocapsules of lemon essential oil encapsulated in chitosan/starch mixture (o/w emulsion) had extended shelf life and better quality during 18 days of cold storage. Nanocapsules with essential oil improved antioxidative properties and inhibited lipid oxidation

of fish burgers during storage. Fish burgers with encapsulated lemon oil had better overall acceptability in comparison to control.

Encapsulated probiotic bacteria *L. acidophilus* added to mayonnaise sauce survived longer in comparison to free bacteria. The *L. acidophilus* cells are very sensitive under to different factors during processing, and because of that encapsulation could serve as a promising method to protect probiotics and to improve the probiotic activity of the yogurt sauce. Besides the improved probiotic activity, yogurt sauce with encapsulated probiotics had better sensory properties (Mohammadi et al, 2013). Encapsulated bacteria *L. acidophilus* and *L. casei* reduce the overall acidity of the mayonnaise-based sauce without significant changes in consistency and rheological properties. After 90 days of refrigerator storage, encapsulated probiotic cells in the sauce can remain preserved in an amount corresponding to the probiotic therapeutic minimum (Bigdelian & Razavi 2014).

Raikos et al (2021) used the emulsion method to formulate a functional yogurt-based drink with encapsulated vitamin E. Vitamin E was dissolved in the corn oil, which represents the oil phase. Yogurt base beverage was formulated by mixing of water, goat milk powder, and freeze-dried lactic bacteria yogurt culture. Obtained yogurt-based mixture represented water phase in emulsion. Both mixtures were mixed, by adding of oil phase slowly into the water phase and stirred using high-speed mixer. Obtained emulsion was fermented at 43ºC. Encapsulated vitamin E had better stability in comparison control. Yogurts with encapsulated vitamin E had better survival of lactic bacteria from culture during pasteurization and storage.

Conclusion

Emulsions are used in almost every encapsulation technique, which is based on the dispersing of active substance in the solution of coating material. Encapsulation methods based on emulsions can be used alone or be combined with other encapsulation techniques like spray drying, freeze drying, microwave heating, and high hydrostatic pressure. The most relevant characteristics of capsules obtained by emulsion-based methods are:

- Capsule types: a) central accumulation-type (encapsulated in double or multi-layer coating emulsions); and b) matrix-type (encapsulation in single or double emulsions).
- Encapsulation carriers: biopolymers, emulsifiers, and other surfactants, e.g. proteins (gelatin, casein, whey proteins), chitosan, lecithin, etc.
- Advantages: good protection of the encapsulated substance from high temperatures and oxidation during heat treatment and drying, the possibility of encapsulating liposoluble and hydrosoluble substances, and controlled release of the active substance.

- Disadvantages: poor encapsulation efficiency for hydrophilic active substances in the case of single-layer emulsion.
- Application possibilities: A widespread encapsulation method in industrial application, a wide range of active substances can be encapsulated, such as probiotic bacteria, proteins, amino acids, essential oils, fish oil, flavonoids, vitamin E, lutein, beta carotene, fish oil, omega 3 fatty acids, aspartame and other sweeteners, xylitol and menthol in chewing gum (prolonged cooling effect), $MgCl_2$ used in the production of tofu to mask its bitter taste, curcumin, catechin, vitamin C, vitamin B12 (for enrichment dairy products), vitamin B1, stevia, saffron, grape seed extracts, dyes, vitamins and minerals. The highest potential is in emulsion-based or emulsion-containing food products such as dairy products, sauces and dressings, mayonnaise, minced meat ready-to-eat products, margarine, and low-fat spreads.

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