Enzyme microencapsulation technique in food industry: A Review

N. Abdollahi¹, A. Shahab Lavasani^{2,*}

¹ Department of Nutrition and Food Industry, Islamic Azad University, North Tehran Branch, Tehran. ² Innovative Technologies in Functional Food Production Research Center, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran

Received: 20 June 2021; Accepted: 23 August 2021

ABSTRACT: Some of the important microbial enzymes used in the food industry are lipases, amylases, proteases, cheeses, pectinases, invertases, cellulases and glucose oxidases. The use of enzymes as a strategy to produce food and increase efficiency and improve production processes can play an important role in industry. Microencapsulation (encapsulation) is the process by which a thin, continuous coating is formed around various compounds. As the wall of the capsule (dunk) contains the encapsulated material, Enzymes due to their wide application in the food industry and the possibility of change if mixed with food, today various techniques are used to preserve enzymes and release them in a specific part of the production process. Microencapsulation is a major technique for enzyme stabilization in the food industry. The present study is an overview of the microencapsulation process of enzymes in the food industry and deals with different methods of encapsulation of enzymes.

Keywords: Enzyme; Food industry; Microencapsulation; Microencapsulation.

INTRODUCTION

Thin capsules

A microcapsule is a term used for particles with a size of 0.2 to 500 micrometers. While those larger than 500m are classified as macro and those smaller than 0.2m are classified as nanocapsules [1]. The product of the microencapsulation process is generally called a capsule. Such capsules are micrometer in size and have a spherical or irregular shape. Each microcapsule consists of two main parts: the core and the wall. The nucleus (inner part) contains active components, while the shell or wall (outer part) permanently or temporarily protects the nucleus. The microcapsule shape is shown in Fig. 1 [2].

The core material in microcapsules may be solid, liq-

(*) Corresponding Author - e-mail: (*) shahabam20@yahoo.com

uid or gaseous. Core materials are often used in the form of a solution, dispersion or emulsion. The compatibility of the core material with the wall is an important factor to increase the efficiency of the microcoating. The amount of nuclear material also plays an important role in controlled distribution, penetration or release. Due to the variety of microcoating applications, a wide range of core materials can be microcoated, such as enzymes, drugs, pigments, monomers, catalysts, cofactors, plasticizers, and flavor-forming compounds [3]. The abundance of natural and synthetic polymers and the variety of their structural properties make it possible to select the appropriate wall with the desired properties. Walls are usually made in three forms: permeable, semi-permeable or impermeable. Permeable walls are used for

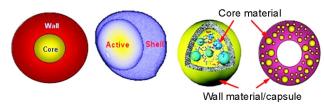


Fig. 1. Microcapsule structure

release applications. While semi-permeable capsules are usually impermeable to core material, they are less permeable to low molecular weight liquids; therefore, these capsules are often used to absorb certain compounds from one environment and release them into another environment. Unlike the previous two; the impermeable wall completely encloses the core material and protects it from the outside environment. Usually, in order to release the contents of the core material, the wall must be disintegrated by pressure, melted, dried, dissolved in a solvent, or decomposed by light. The rate of release of the core material from the permeable wall is mainly controlled by the wall thickness and the size of the pore [4].

The shape of tiny capsules

The shape of the microcapsules depends mainly on the material of the nucleus and the process of wall formation. Microcapsules may have regular or irregular shapes and can therefore be divided into single-core, multi-core, and lattice types (Fig. 2). Single-core (core-wall) microcapsules contain a wall around the core; while in multinucleated capsules, there are many nuclei inside the wall. They are walls or may be in addition to these three shapes, microcapsules can form a single nucleus with several clusters of microcapsules [5].

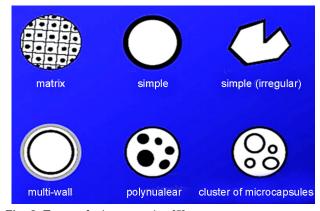


Fig. 2. Types of microcapsules [5]

Table 1. Types of food components that are finely coated.

Type of ingredient :
Flavoring agents such as oil, spices, seasonings and sweeteners
Acids, alkalies, buffers
Lipids
Redox agents(bleaching,maturing)
Enzymes or microorganisms
Artificial sweeteners
Leavening agents
Antioxidants
Preservative
Colorants
Cross linking and setting agents
Agents with undesirable flavors and odors
Essential oils ,amino acids ,vitamins and mineral

Benefits of microfinance

The main reasons for using microcoating are:

- Protection of unstable and sensitive materials against environmental factors

- Better process capability to improve solubility, dispersibility and buoyancy

Release (controlled or temporal release)

- Safe and convenient use of toxic substances
- Hide the smell or taste
- Increase the shelf life by preventing decomposition reactions (oxidation, dehydration)
- Stabilization of enzymes and microorganisms
- Controlled and targeted distribution of drugs
- Changing the surface properties of materials
- Reduction of volatility and flammability of liquids Separation of reactive materials

Types of finely coated food components

The types of food ingredients that can shred are shown in Table 1

Most applications of micropropagation in food are to preserve enzymes and other compounds during the processing process. Capsules are usually soluble in water and therefore dissolve when added to water. From this group of capsules can be mentioned types containing enzymatic compounds. Microencapsulation can also trap components such as enzymes so that their activity is maintained over time. Usually, the use of unprotected enzymes in food exposes them to ions, protons, radicals, inhibitors, etc., which causes instability and inactivation, but the microencapsulation of enzymes protects them against these factors and thus reduces their activity. It prevents them. Other types of food ingredients that can be fine-grained include enzymes, vitamins and minerals, sodium bicarbonate sweeteners, citrus essential oils, and flavorings [6]

Compounds used in the microencapsulation system of enzymes

In the process of microencapsulation of enzymatic compounds by means of a wall that separates them from the outside environment; are protected. The size of the microcapsule can be less than a millimeter to less than 1 micrometer. The simplest microcapsules may contain only one nucleus covered by uniformly or non-uniformly thick walls. The wall may also be simple or a mixture of several different materials. In addition, the wall can be composed of one or more layers [7]. Microcapsules are usually added to food systems as additives and must therefore be compatible with that system; therefore, these points should be considered when producing microcapsules, especially in choosing the type of wall. In general, different materials can be used as walls. These substances include proteins, carbohydrates, lipids, gums and cellulose. Each of these substances has disadvantages and advantages. To select the wall material, factors such as the type of brain material (nucleus), the type of microencapsulation process, economic issues, the type of product consumption and also the conditions provided in the regulatory laws should be considered [8]. On the other hand, it should be noted that in the process of microencapsulation of enzymatic compounds, the wall material should not react with the core material and be easy to work with [9]. The most important properties and characteristics that a wall material should have are the following:

- At high concentrations, show low viscosity and have good rheological properties, - Separation of solvent from it at any stage of the operation is required to be **Table 2.** Characteristics of wall materials used for microen-
capsulation of enzymatic compounds [5]

Wall material	interest	
Maltodextrin(DE<20)	film forming	
Corn syrup solid (DE>20)	film forming ,reducibility	
Modified starch	very good emulsifier	
Gum Arabic	emulsifier, film forming	
Modified cellulose	film forming	
Gelatin	emulsifier, film forming	
Cyclodextrin	encapsulation, emulsifier	
Lecithin	emulsifier	
Whey protein	good emulsifier	
Hydrogenated fat	barrier to oxygen and water	

done easily,

- Provide maximum protection of the inner core material against external factors such as light, temperature and humidity,

- Produce stable emulsion, - show appropriate behavior when redissolved in order to release enzymatic compounds in the desired place and time,

- Does not react with the coated material or the core during the process and during the storage period,

Have the ability to dissolve in solvents used in the food industry such as water and ethanol [5].

- It is cost effective. Adequate knowledge of the physicochemical interactions that occur between the enzymatic compounds used and the major constituents of foods such as lipids, polysaccharides, and proteins is essential to controlling the quality and taste of food [10]. The properties of the materials that are often used as a wall in the microencapsulation system for enzymatic compounds are shown in Table 2.

Because there is almost no coating material that has all the necessary properties and conditions, a mixture of several coating materials as well as other compounds such as antioxidants, chelating agents and surfactants are usually used. In addition, by changing the wall

 Table 3. Coating materials used in food microfiber [5].

Carbohydrates	starch, maltodextrins, corn syrup, dextran, sucrose, cyclodextrins		
Celluloses	carboxyl methylcellulose, methylcellulose, ethyl cellulose, acetyl cellulose,		
	nitrocellulose, cellulose acetate phthalate, cellulose acetate butyrate phthalate		
<u>Gums</u>	gum acacia, agar, sodium alginate, carrageenan		
Lipids	wax, paraffin, beeswax, tristearic acid, monoglycerides, oils, fats, hardened oil		
Protein	gluten, casein, gelatin, albumin, hemoglobin, peptides		

thickness and structure, the desired protection goals and physical properties can be achieved to a large extent [11]. Some types of coatings are listed in Table 3. Given the importance of the role of the wall in the microencapsulation of enzymes, this section provides a further explanation of some of the main compounds used in the wall.

Carbohydrates

In the microencapsulation technique of enzymes, different types of carbohydrates are often used as a wall material or carrier. Carbohydrates such as starches, maltodextrins, corn syrup, and acacia gum are widely used in the microencapsulation of enzymatic compounds due to their variety and variety, cheap price, and many applications in the food industry. In addition, these compounds have properties such as low viscosity at high concentrations and optimal solubility, which makes them suitable for use in optimally enzymatic microencapsulation systems. Starch and its derivatives such as maltodextrin, betacylcodextrin and modified starches are the best compounds used as a wall. Therefore, the use of these compounds and their reactions with enzymatic compounds has been highly regarded by researchers. The following is an introduction to the most important polysaccharides used in the microencapsulation process of enzymes [12].

Alginate

This polysaccharide is found in the cell wall and intercellular space of brown algae and provides the plant with flexibility and structural strength. Structurally, alginates are composed of units of manuronic acid D (M) and L-gluconic acid (G), which are joined together by glycoside bonds (Fig. 3). There is no specific order in terms of number and position of these units in the polysaccharide chain, and therefore the sequence of these units creates homo and heteropolymer sections in the alginate structure. The ratio of manuronic acid to gluconic acid and the structure of the polymer determine the physicochemical properties of alginate [5].

In the food industry, alginates are used to form gels (such as puddings), absorb water (such as soups), create texture and consistency (such as ice cream), and form films (such as coatings). Alginates are imperme-

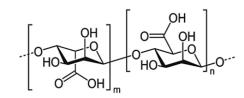
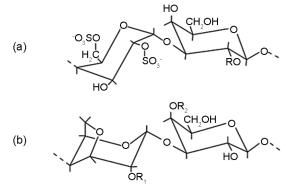


Fig. 3. General structure of alginates [5].

able to oils and fats, but they are permeable to moisture. Alginate coatings are a good protector against oxygen; they can delay the oxidation of lipids in food and prevent loss of taste and texture change. Alginates were first used for coating in the United States in the 1920s. Alginates can also be used to make liquid capsules. In addition, alginates are used in microencapsulation of microbial cells. For example, microencapsulation of probiotic bacteria with an improved alginate-starch mixture increases survival in gastric and yogurt conditions. It has also been reported that microfibers of bifidobacteria with alginate increase the likelihood that they will survive in mayonnaise. In one study, the survival of bifidobacteria trapped in alginate cells in frozen ice milk was investigated. Their results showed that stabilization of bifidobacteria in calcium alginate globules increases their viability in gastric extract and enhances bile salt secretion [13]. Alginate microencapsulation is easily reversible because the structure of calcium alginate gel depends on ionic interactions; therefore, the coating may be easily disintegrated by chelating agents such as phosphate. Of course, the amplitude of these interactions depends on the type and concentration of cations and the pH of the environment. The results of research indicate that cells coated with calcium alginate are able to survive after freeze-drying and their activity is maintained after a long storage period [14].

Carrageenan

Carrageenan is a natural polysaccharide found as filler in the pores of the cellulose structure of red seaweed. This polysaccharide has a high molecular weight and a linear structure consisting of repeats of disaccharides [1, 4, 15], B and (units B) galactose (Fig. 4). B-units are often in the form of anider sugars due to the ether bond between carbons 3 and 6. Carrageenans are found in nature in four forms: kappa (k), Utah (i), lambda [15] and beta (b). The structural difference



Repeatig disaccharide structures of (a) λ -carrageenan (R=H or SO₃⁻) and (b) t-carrageenan (R₁=R₂=SO₃⁻), κ -carrageenan (R₁=H; R2=SO₃⁻), and β -carrageenan (R₁=R₂=H).

Fig. 4. General structure of Carrageenans

of these compounds is in the substitutions on units A and B; it affects their gel strength, texture formation, solubility, especially in brine, and melting point. All carrageenans are soluble in hot water and have a very low viscosity. With the exception of lambda, this is highly sulfated and does not have the ability to form gels, other forms of carrageenan, all of which contain the anider form of unit B, have the ability to form gels. Also, except for lambda, only the salts of Utah sodium and kappa carrageenan; it is soluble in cold water. Potassium and calcium are essential for the formation of gels by carrageenans. Carrageenan jellies are stable at room temperature and melt with increasing temperature to about 5 to 10 degrees above the gel formation temperature (40-70 °C) [9].

Considering that carrageenans belong to a hydrocolloid family whose components have different properties; therefore, a wide range of applications are envisaged for them. The most important uses of these carbohydrates are in the dairy industry, cakes and cookies, chocolate drinks and the like. In addition, carrageenan films are used in food coatings to prevent microbial activity, moisture loss, and oxidation reactions. Because of their ability to react with proteins; Low concentrations (usually 0.1 to 0.3%) of these carbohydrates can be used to stabilize protein-containing emulsions, as well as carrageenans used to microbial bacterial cells [13].

Starch

Natural starch and its derivatives (modified starches, maltodextrins and betacylcodextrins) are widely used

in the food industry to protect enzymatic compounds. These carbohydrates are widely used as a wall material for microencapsulation of enzymatic compounds. Recent research on these compounds has led to the production of new starch compounds that can be used for microencapsulation and storage of enzymes [15]. Natural starches are hydrophilic molecules that lack surface activity, but modified starches have emulsifying properties due to the presence of hydrophobic groups in their structure, and after being placed on the joint surface of oil-water droplets, stabilize the emulsion by creating a spatial repulsion. Therefore, these emulsions are not very sensitive to changes in pH, ionic strength and us. On the other hand, due to the low surface activity of the modified starches, large amounts of them should be used to stabilize the emulsion to ensure the complete coverage of the droplet surfaces [5]. Starch granules, especially starches that have been dried in a spray dryer; they have surface pores with a diameter of 1-3 micrometers. Recent studies suggest that the presence of these pores in starch granules allows them to bind to proteins and trap them internally; Therefore, modifying the structure of starch granules by various methods, including the use of amylase enzyme, which can increase the porosity, improves the retention and trapping percentage of the material. Binding of enzymes to starch is done in two ways: by the amylase helix and by hydrophobic bonds, which are considered as trapping and trapping points; they bind to starch or through polar interactions Hydrogen bonds of hydroxyl groups of starch and enzymes of these compounds are stabilized. Today, it has been shown that amylose has the ability to trap and trap a wide range of different substances, including enzyme compounds [13].

Maltodextrin

Maltodextrins are produced by partial acidic or enzymatic hydrolysis of corn flour. The degree of hydrolysis of starch and its breakdown is measured against maltodextrins by a unit called the dextrose equivalent (DE). Different are identified. Maltodextrins are considered as a wall due to their ability to form networks in various microencapsulation methods [5]. High efficiency of maltodextrins, low viscosity of their solution even at high concentrations, their availability in different molecular weights and their low price are other important factors in the use of these compounds in microencapsulation. The main disadvantage of these materials is that they do not have emulsifying properties and therefore less storage capacity. However, studies have shown that the survival rate of twelve different compounds depends on the DE of maltodextrins. Maltodextrins with DE showed about 10 best retention properties and with increasing DE the amount of retention of compounds decreased. In addition, enzyme retention increased by DE during storage. Various studies have shown that maltodextrin with high DE protects orange peel oil from oxidation. In this study, the effect of DE on the performance of wall systems was well explained. At the same time, the use of DE number cannot be used as a sufficient predictive factor in the production of different products with different applications. It has recently been shown that molecular weight is an important factor in predicting the main properties of maltodextrin, but its use is limited to certain cases. In one study, the persistence of enzymatic compounds was investigated using different ratios of maltodextrins at three temperatures of 60, 70 and 80 °C. Maltodextrin solution with a DE of 5 (10% w/w) showed that the persistence rate depends on the degree of hydrophobicity of the enzymes, which improves with increasing temperature [15].

Gum

Arabic gum is the most important gum that is used as a wall in the microencapsulation system of enzymatic compounds. This gum is a natural exudate of the Acacia Senegal tree. In terms of structure, gum arabic consists of arabino-galactane chains that are attached to a single polypeptide strand as side substitutions (Fig. 5 and Table 4). The protein part is hydrophobic and the polysaccharide is hydrophilic, so the whole molecule is emulsified. It is believed that the fixation of oil emulsions in water by gum arabic is done by creating a spatial repulsion between the droplets; however, electrostatic interactions are not ineffective in this regard. It has also been found that the tendency of this gum to settle at the oil-water interface is relatively low compared to most biopolymers, and therefore should be used at high concentrations to create a stable emulsion. Accordingly, its use as an emulsifier is often limited to emulsions in which the droplet density is low (such as beverages, especially since compared to most other polysaccharides, gum arabic is very resistant to acidic conditions. However, desirable solubility, low viscosity, the emulsifying properties and high holding capacity of volatile compounds by this gum have caused it to be considered in many micro-coating processes. In addition, this material is very suitable for micro-coating of fat droplets, as it plays both the active role of surface and wall in At the same time, its use in the food industry is slightly limited due to the high cost of this polysaccharide, which has led to further studies to find a suitable alternative [13].

Various studies have been performed on the effectiveness of gum arabic or its mixture with other colloids in the microenvironment of food components and enzymes. In this regard, we can mention the micro-coating of proteases with a mixture of gum arabic and maltodextrin with a spray dryer. In a similar study, a mixture of ethyl propionate, ethyl butyrate, orange oil, aldehydes and benzaldehyde was finely encapsulated in the walls of gum arabic and maltodextrin. The results of both studies showed that the shelf life of the

Table 4. Chemical	composition of	f gum arabic	[5]
-------------------	----------------	--------------	-----

Compound	% in gum
Galactose	36.0
Arabinose	30.0
Rhamnose	12.6
Glucuronic acid	19.2
Protein	2.2

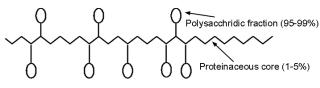


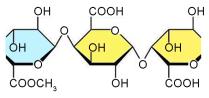
Fig. 5. Chemical structure of gum arabic [5]

materials in the capsules increased with increasing the percentage of gum arabic. Dried powders mixed with gum arabic and maltodextrin with a spray dryer are usually about 10-200 nm in size and can retain about 80% of the enzymes. However, factors such as spray dryer inlet temperature, emulsion concentration and viscosity, and the ratio of maltodextrin to gum arabic are influential in producing these results [15]. In one study, different ratios of gum arabic and maltodextrin were used to finely coat 1-2-acetyl-1-pyrrole with a spray dryer. These researchers showed that the 70: 30 ratio of gum arabic - maltodextrin has the best quality among the manufactured capsules [5].

Pectin

Pectin is a major component of plant cell wall compounds that is responsible for a wide range of different biological activities in plants. Pectin compounds are effective in controlling cell growth, counteracting the invasion of microorganisms and maintaining the physical and sensory properties of fresh fruits. The structure of pectin is shown in Fig. 6. Pectins are polymers of D-galacturonic acid that are bonded together (1-1). The difference between pectin compounds is in the content of methyl esters or their degree of esterification [13].

Pectin forms gels under suitable pH conditions and solids (percent sugar) and is therefore considered as a gel and thickener in the food industry. Pectin is not usually used alone in microencapsulation but is often used in combination with other polymers, especially proteins. The results of various studies indicate that electrostatic interactions between protein and pectin





strengthen the wall and prevent droplets from sticking together. However, the ratio of protein to pectin, pH and ionic strength of the environment affect the amount and type of these interactions and sometimes cause the emulsion to disintegrate. Pectin may be used in conjunction with other polysaccharides, including alginates, neutral polysaccharides such as starch, galactomannans, and dextran to encapsulate enzymes. It should be noted that in mixing polysaccharides with each other, attention should be paid to their thermodynamic compatibility; otherwise, if there is no thermodynamic compatibility between them, they will quickly separate from each other and cause the system under study to break and become two-phase [15].

Proteins

Although carbohydrates, especially polysaccharides, are often used as wall compounds in enzymatic microfilaments, dietary proteins such as sodium caseinate, whey protein, and soy protein isolates are also used for this purpose. Having different functional groups, amphiphilic properties, self-aggregation and self-structuring ability and interaction with various types of compounds, high molecular weight and flexibility and protein molecular chain flexibility have led to desirable functional properties such as solubility, emulsifying viscosity and layer formation. And therefore have been considered in the fine-tuning process. Creating a stable emulsion is crucial to the success of microencapsulation of enzymatic compounds, and proteins do it well. Usually during the formation of the emulsion, the protein molecules are rapidly absorbed by the surface of the oil-water droplets, and thus, by forming a stable layer, the oil droplets in the emulsion are protected against each other and become larger, thus resulting in physical stability of the emulsion in the formation stage are maintained [15].

Gelatin

Gelatin is one of the proteins commonly used to coat food components. Non-toxic gelatin is inexpensive and commercially available immediately. It is a high molecular weight polypeptide protein obtained by the hydrolysis of acidic (gelatin A) or alkaline (gelatin B collagen). Gelatin acts on the surface and is used as an emulsifier in the stabilization of oil-in-water emulsions, but gelatin itself is not used for this purpose because it forms large droplets; the degree of hydrophobicity is altered or mixed with anionic surfactants. In general, gelatin has the ideal chemical and physicochemical properties for the microcoating process; it is also used in film and food coatings [5].

Cheese proteins

Whey proteins have special functional properties. These properties have led to them being mentioned as a suitable compound for the capsule wall. The use of whey proteins in micronutrients has been reported by many researchers. In global markets, whey proteins are available as whey protein isolate powders or whey protein concentrate (50-WPC and WPC70) [13].

Research has shown that whey protein isolates are a good protector for lipases and amylase finely coated against oxidation and can form a suitable wall for the coating of volatile compounds by spray drying. However, researchers in another study concluded that WPC microencapsulation properties for amylase protection are lower than lipases. Also, various researches have been done on the impermeability and insolubility of protein walls in water, especially whey proteins, in order to enable the controlled release of microcoated compounds [15]. In addition to whey proteins alone; Mixtures of these proteins and carbohydrates have also been used as the capsule wall in the process of coating volatile compounds in such systems. And the next layers play a role. It is noteworthy that lactoglobulin is the most important compound in whey protein that has good emulsifying and foaming properties and is widely used in pure form in food industry [5].

Enzymatic microencapsulation methods

There are many techniques and methods for enzymat-

ic microencapsulation. However, three main goals are pursued in all these processes: the formation of a suitable wall around the material, preventing the penetration and penetration of the coated material into the surface of the capsule during storage, preventing contact and reaction of environmental factors with the core. In general, enzymatic microencapsulation techniques are divided into two main physical and chemical groups and physicochemical, physical and mechanical subgroups [15]. Fig. 7 shows the diagram of the microencapsulation process of enzymatic compounds. Table 5 also shows some of the important methods used in microcoating.

Physicochemical processes

Conservation

The method of cohesion or fuzzy separation was first introduced by Bangburg and Cleus. In this method, microcoating and trapping of the desired compounds is done by transferring polymers and macromolecules from the solution phase and their aggregation in another phase. As a result of this transfer and due to the interaction between the polymer molecules, a layer is formed around the microcoating material (nucleus) which subsequently solidifies and thus the coating and trapping is completed [13].

This process is not widely used in the food industry; because it is a complex and costly method, small quantities of food polymers are also commercially available for this purpose. The distinguishing feature of conservation is its high efficiency and the creation of microcapsules with larger sizes than other methods. This method has already been used to fine-tune oxidoreductases, glycosidases and lipases. Fuzzy separation is possible in both aqueous and non-aqueous forms. In the first case, the coating material is hydrophobic and

Table 5. Some important methods of enzyme microencapsulation [5]

Chemical processes:	Physical processes	Physio mechanical:
<u>Chemieur processes.</u>	<u>Physio_chemical:</u>	
. Suspension, dispersion and		. Spray drying
emulsion polymerization	. Coacervation	. Multiple nozzle spraying
. Polycondensation	. Layer by layer	. Fluid bed coating
	(L.B.L) assembly	. Centrifugal techniques
	.Sol gel encapsulation	. Vacuum encapsulation
	Supercritical $\mathrm{CO}_{\!_2}$ assisted microencapsulation	. Electrostatic encapsulation

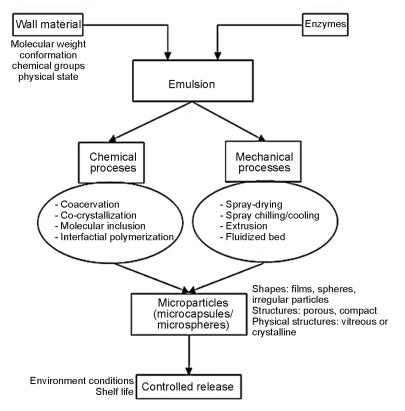


Fig. 7. Diagram of the microencapsulation process of enzymes [5]

therefore the wall materials are water-soluble such as gelatin, acacia gum or gum arabic; in non-aqueous phase separation, however, the nucleus contains water-soluble compounds surrounded by a hydrophobic wall such as ethylcellulose copolymers or styrene maleic acid.

There are generally two methods for conservation: simple conservation and composite conservation [5]. The mechanism of microcapsule formation is the same for both processes but the way of fuzzy separation in them is different from each other. In simple cohesion, a polymer solubilizing agent such as salt or ethanol is added for fuzzy separation; whereas in composite cohesion, fuzzy separation occurs through the formation of a polyelectrolytic complex by mixing two polymers with opposite electric charges.

Composite cohesion

As mentioned, composite co-curing is performed by mixing a solution of two polymers with opposite electric charges. The scheme of this process is shown in Fig. 8 [13].

Composite cohesion takes place in three stages

A) Preparation of dispersion or emulsion

B) Microencapsulation of the desired enzyme

C) Fixed particle stabilization.

In this method, the desired coating material is first dispersed in the first polymer solution. They are also used to facilitate the dispersion and increase the micro coating efficiency of hydrophobic surfactants. The second polymer solution, which has an electric charge opposite to the first polymer, is then added to the prepared dispersion. When a complex of two polymers is formed by electrostatic interactions, precipitation and particle separation take place by adding salt or changing the pH of the temperature, or by suddenly diluting the medium. The wall thickness and structure can be controlled by changing the concentration and type of the second polymer, creating intermolecular lateral connections and transverse bridges, and changing environmental conditions [5]. The process of composite cohesion and the structure of walls composed of a mixture of proteins and polysaccharides are influenced by various factors, such as ambient pH, ionic strength, protein and polysaccharide charge density, degree of flexibility of proteinaceous chains, and proteinaceous chains. He referred to polysaccharides, process temperature and time [15]. Composite cohesion can be used to make microcapsules containing enzymes, various oils, flavorings, dyes, and flavorings. The important point in using this technique is that the environmental conditions should be adjusted in such a way as to prevent the agglomeration of the capsules after formation [5].

Microencapsulation in alginate globules

Alginate globules are widely used for in vitro microencapsulation of various components and compounds. Microcoating with this method is simple and easy to apply and can be done in sterile conditions. Alginate globules can be used to fine-tune almost any material, including hydrophobic and hydrophilic compounds, dilute and concentrated liquids, solids, and heat-sensitive components. However, the application of this process in the food industry faces two main problems: (1) Its implementation on an industrial scale, unlike laboratory conditions, is very complex and difficult and requires a lot of money. (2) Alginate globules are highly porous and easily allow water and other liquids to diffuse in or out. Although this property is highly desirable for living enzymes and cells that are stabilized in alginate globules and must have access to the environment, they are not suitable for sensitive components that must be protected by microencapsulation and separation from reactive compounds [13].

Microcoating using supercritical fluids

Supercritical fluids are highly compressed gases that exhibit both the properties of liquids and the properties of gases above a critical temperature and pressure. The use of these fluids in industrial processes has received much attention in recent years. The most common supercritical fluids used are carbon dioxide, alkanes (Ca-C2) and nitrous oxide. The distinctive feature of these fluids is that with a slight change in temperature with pressure near the critical point; Their density, viscosity, diffusion coefficient, solubility and dielectric constant vary greatly. Therefore, by adjusting the temperature and pressure, the solubility of the supercritical fluid for the material can be maintained at an optimal level. Supercritical fluids have so far been used to fine-tune various substances, including

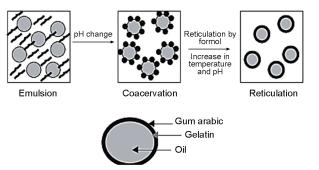


Fig. 8. Basis of conservation process in microencapsulation of enzymes [5]

enzymes, drugs, vitamins, flavors, and dyes. The most common supercritical fluid ever used to coat food components is CO₂; Because its critical temperature is low, it is non-toxic and non-flammable, it has a high degree of purity, it is easily accessible and it is cheap. It should be noted that sometimes the use of other supercritical fluids such as ethane and propane is preferable to carbon dioxide, but in that it is difficult to prepare food types of these fluids and are also flammable; Their application in the food industry is associated with problems. In general, microcoating using supercritical fluids involves exposing the mixture of wall material and the microcoating compound to the supercritical fluid and spraying it through a nozzle into the collection chamber [15]. This process is often done in three ways:

Rapid expansion of supercritical fluid (RESS)

Gaseous anti-solvent (GAS)

Particle separation from solution or gas saturation dispersion (PGSS)

Rapid expansion of the supercritical solution

In this process, the supercritical fluid containing the microcoating compound and the wall material is released through a capillary nozzle or with very small orifices at atmospheric pressure. Due to the rapid drop in pressure, the solubility of the wall material is lost and thus, by depositing around the active components (core), it forms the desired wall. To be. Therefore, this process has little application in the food industry; Because the number of polymers that can be dissolved in supercritical fluid is very limited, in addition to its low efficiency. It should be noted that sometimes solvent or other compounds are used to increase the solubility of the wall material. A schematic of the microcritical process using supercritical carbon dioxide is shown in

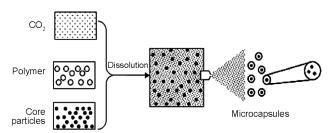


Fig. 9. Microcoating by rapid expansion method of supercritical fluid [5]



Gas anti-solvent process

This process is also called supercritical fluid solvent (SAS). In this method, supercritical fluid is injected under pressure into a solution containing the wall material and the components to be finely coated; As a result, the volume of the solution increases rapidly and reaches a supersaturated state. Under these conditions, the soluble material (core and wall material) precipitates and the wall material surrounds the microcoated components. It should be noted that in this method, both the core and the wall material should be soluble in the solvent used, but should not be dissolved in the mixture of solvent and supercritical fluid. On the other hand, solvent and supercritical fluid must be miscible with each other. This process is unsuitable for microcoating of water-soluble components, as water has low solubility in supercritical fluids [15].

Particle separation from solution or gas saturation dispersion (PGSS)

In this process, the core and wall material are pressurized with supercritical fluid. Under these conditions, the supercritical fluid penetrates into the wall material and causes it to swell. The mixture is then heated above the glass transition temperature of the wall material to melt. After this step, the mixture is sprayed through a nozzle into a chamber to deposit the wall material on the microcoating material. To do this, the core and wall material do not necessarily have to be soluble in supercritical fluid [13].

Liposomal trapping

Liposomes are mainly used in the pharmaceutical industry (for example, to deliver vaccines, hormones, enzymes, and vitamins to body tissues, or to diagnose cancer cells with MRI) and health and beauty, but have also recently been used in the food industry. Structurally, liposomes consist of one or more phospholipid layers (Fig. 10) that range in size from 25 nm to several microns. The most common phospholipids used to make liposomes are lecithin or phosphatidylcholine, which is derived from soy or egg yolk. Research shows that the most suitable liposomes for micronutrient micronutrients are large single-lamellar vesicles (LUVs) that are easy to produce, stable to environmental conditions, and have high micropolar efficiency. To release the microscopic components of liposomes, their walls can be designed to disintegrate at a certain temperature and release their contents. For example, a pair of phospholipid membranes at their transition temperature, about 50 °C, degrades rapidly and expels all the coated components [15].

There are two major challenges to the use of liposomes in the food industry: (1) their industrial production at an acceptable cost, and (2) how micronutrients are supplied. The first case has been solved by introducing the process of microfluidization and its industrialization. Using this method is efficient, costeffective and solvent-free, and has a high micro-coating efficiency. In addition, research is underway to replace phospholipids with lower cost compounds such as a mixture of high and low HLB glyceride emulsifiers such as Menu hydrophobic emulsifiers and diglycerides or lactate ester, acetate and 3H monoethyl citrate (H) Hydrophilic emulsifiers (HLBs between 8 and 15) such as sucrose esters or stearoyl lactylates increase the cost-effectiveness of this process. Are accompanied. At present, the only way to dry liposomes is freeze-drying, which is not economically justified, while not all liposomes can be used, and their solution is difficult to reconstruct. On the other hand, it is not possible to use antimicrobial compounds in the storage of liposomes, because they cause their structure to disintegrate. Storing and transporting liposomal solutions at low temperatures also increases costs and does not justify cheap products. Regardless of these problems, liposomes have a major advantage over other microcoating methods, which is the protection of water-soluble microcoated components in environments with high water activity; However, microcapsules prepared by other methods, despite having a relatively high sta-

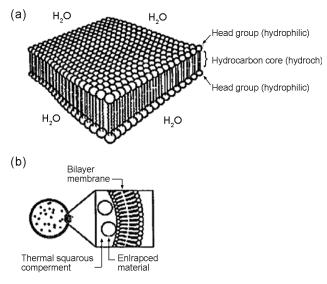


Fig. 8. Scheme of placental membrane membrane (A) and liposomal microcapsule (B) [5]

bility in dry conditions, lose their structure and release their contents as soon as they are exposed to moisture. Another unique feature of other liposomes is that they can be used for targeted delivery of fine-grained components. Studies have reported that the microencapsulation of flavor-producing enzymes with liposomes helps to retain them in the cheese clot, while the lack of microencapsulation causes the loss of most of them (up to 96%) during the process. In addition to flavorproducing enzymes, meat-crushing enzymes (bromelain), nizin, and vitamin C have also been finely coated with liposomes [15].

Chemical methods

Interfacial and extracellular polymerization

In both of these methods, the capsule walls are formed by the polymerization of monomers that are added to the medium. In surface coating polymerization (IFP), monomers such as isocyanates and acid chlorides are added to the microcoating material and then dispersed in droplets in the aqueous phase. If amino compounds are added to this mixture, polymerization occurs rapidly at the droplet joint and a wall is formed. Chlorides are used to make the wall of polyurea, isocyanates, and the wall of polyamide or nylon. Polyurethane wall is also formed if isocyanates react with compounds with hydroxyl groups [13]. Unlike interfacial polymerization, in extracellular polymerization microsurgery, no monomer is added to the core material, but after the formation of dispersion, all monomers and reactants are added to the continuous phase. At the beginning of the reaction, a light molecular weight prepolymer is formed, which grows over time and precipitates on the core material. Used in non-food industries [15].

Physicomechanical processes Extrusion

The extrusion process was first introduced in 1957 by Sweiser et al., And evolved almost a decade later with modifications by Schultz. This method can be used to fine-tune volatile and enzymatic fragrance-producing components such as citrus essential oils in glassy carbohydrate coatings. The main advantage of this process is that it protects and prolongs the oxidationsensitive compounds, because the release of atmospheric gases into the glass carbohydrate coatings is very slow and slow, so that these walls can be said to be impermeable to these gases. It is estimated that the shelf life of finely coated citrus essential oil with this method is 5 years, while for varieties prepared by dry method (spraying is estimated to take 1 year. One of the disadvantages of this method is the low capacity of finely coated or loaded 8. at best) which causes a large amount of it to be used in the formulation to create the desired aroma in the product, regardless of its economic considerations, which upsets the balance of the components in the formula and also increases the caloric content of the food. However, there are reports that efforts have been made in recent years to address this problem, for example Motka and Nelson (1988) showed that if modified starches have hydrophobic lateral substrates If used, the fine-grained capacity can be increased up to 40%, which is more than twice the loading capacity by spray-drying, while the microcoated compounds have a higher shelf life and higher oxidation resistance. Losses, high operating costs of this process are also compensated [15]. Another drawback of extrusion microparticles is the large particle size (500-1000 microns) that are easily felt in the mouth; And therefore make use of them in food products. In addition, the variety of compounds that can be used as a wall in this method is limited, although the results of researchers suggest that in addition to carbohydrates, a mixture of other compounds can be used. It should be noted that the extrusion coating pro-

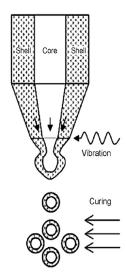


Fig. 11. Extrusion process design by coaxial capillary tube method [5].

cess can be done in three simple ways: coaxial capillary tube (Fig. 11), centrifugal and centrifugal by recycling the coating material [13].

Spray drying

Spray drying is one of the most common and common methods of micro-coating that has been used since the late 1950s at the cost of a wide range of materials including enzymes, essential oils, extra special and medicinal components, aromatic compounds and special oils. The process includes the availability of equipment and its low operating costs, the possibility of using a wide range of walls, the optimal preservation of volatile compounds, the appropriate quality of the final product and the ease of its implementation on an ongoing basis. Modified starch, maltodextrin, various gums such as acacia and gum arabic, proteins (whey protein, soy protein and sodium caseinate) as well as mixtures thereof are among the compounds often used as a carrier or wall in this process (W). In general, the compound used as a wall should have high solubility in water, low viscosity at high concentrations, good

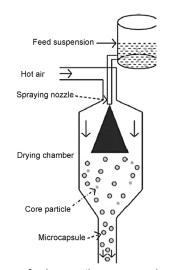


Fig. 12. Scheme of microcoating process by spray drying method [5].

emulsifying power and film formation, and finally its drying properties should be desirable. The basis of spraying by spray drying method is to prepare an oil emulsion in the water of the core material and spray it in hot air of the drying chamber (Fig. 12). As the solvent evaporates, the core droplets become entangled in a solid network of wall material, forming fine capsules. Because the drying time in this process is short, the core temperature does not rise much and can therefore be used to microcoat heat-sensitive components. However, volatiles that have a low boiling point may be low and may be lost during the process. Table 6 lists the advantages and disadvantages of this method [15].

The efficiency of microencapsulation and the retention rate of enzymatic compounds by spray drying method depends on the physical and chemical properties of the wall and core material, process temperature, emulsifying power and film-forming ability of the wall material, final humidity of the microcapsules and humidity of the air leaving the dryer. Microencapsulation by spray drying is the retention of the core ma-

Table 6. Advantages and disadvantages of using spray dryer [5]

Advantages:	Disadvantages :
Low operating cost	Produce no uniform microcapsules
High quality of capsules good yield	Limitation in the choice of wall material
Rapid solubility of the capsules	(low viscosity at relatively high concentrations)
Small size	Produce very fine powder which needs further processing
High stability capsules	Not good for heat sensitive material

terial on the surface of the microcapsules during the process or its spread to this area during storage, which gradually oxidizes and changes the taste and smell of the product. Another disadvantage of this method is the small particle size of the powder produced (10-100 microns), which prevents its rapid solubility in water. To solve this problem, the powder is agglomerated in bed dryers and converted into particles with a size of 0.3 to 1 mm. Over the years, many efforts have been made to increase the efficiency of microencapsulation in this way and to prevent the loss of microencapsulated components from microcapsules [16].

CONCLUSIONS

One of the qualitative features of desirability in the food industry is the use of enzymes that improve food as well as its shelf life. Enzymes are composed of a complex group of protein compounds and are lost during the process and storage of food products due to their unique physicochemical properties. Encapsulation is the process of preventing evaporation, oxidation, migration, and adverse reactions of these compounds to food components by covering the aneurysms with suitable wall materials. Many materials have been proposed for the wall structure of microcapsules, including carbohydrates and proteins, as well as many techniques such as coagulation, spray drying, etc. for enzymatic microencapsulation. The results of the present study show that in many enzymes, the use of carbohydrates (such as gums) to coat the enzymes is superior to other methods and also different methods of enzymatic microencapsulation of spray drying method due to low cost. And the workforce to perform operations continuously and produce capsules with high stability is the most common method of enzymatic microencapsulation in the food industry. The use of microencapsulated food compounds for controlled release purposes is a promising alternative to solving the main food problem facing the food industry. Despite the wide range of encapsulated products that have been successfully marketed in the pharmaceutical and cosmetic industries, microencapsulation has a relatively smaller market in the food industry. Learn how to solve this problem widely in the food industry.

REFERENCES

- Hu, H. (2020). Recent advances of polymeric phase change composites for flexible electronics and thermal energy storage system. Compos. Part B- Eng., 195, 108094.
- [2] Zuluaga, M. et al. (2018) 'Astaxanthin-antioxidant impact on excessive Reactive Oxygen Species generation induced by ischemia and reperfusion injury. Chem.-Biol. Interact, 279, 145–158.
- Zia, K.M. et al. (2018). Recent trends on gellan gum blends with natural and synthetic polymers: A review. Int. J. Biol. Macromol., 109, 1068– 1087.
- [4] Adil, M.M. and Schaffer, D.V. (2017). Expansion of human pluripotent stem cells. Curr. Opin. Chem. Eng., 15, 24–35.
- [5] Wypych, G. (ed.) (2019). Substitution of solvent by safer products, in Handbook of Solvents (Third Edition). Chem. Tec Publishing, 1455–1634.
- [6] Wang, J. et al. (2020). Deodorizing for fiber and fabric: Adsorption, catalysis, source control and masking. Adv. Colloid Interface Sc., 283, 102243.
- [7] Paulo, F. and Santos, L. (2017). Design of experiments for microencapsulation applications: A review. Materials Science and Engineering: C, 77, 1327–1340.
- [8] Santos, P.D. de F. et al. (2021). Microencapsulation of carotenoid-rich materials: A review. Food Res. Int., 147, 110571.
- [9] Martín, M.J. et al. (2015). Microencapsulation of bacteria: A review of different technologies and their impact on the probiotic effects. Innov. Food Sci. Emerg. Technol., 27, 15–25.
- [10] Ojha, K.S. et al. (2015). Technological advances for enhancing quality and safety of fermented meat products. Trends Food Sci. Technol., 44(1), 105–116.
- [11] Shinwari, K.J. and Rao, P.S. (2018). Stability of bioactive compounds in fruit jam and jelly during processing and storage: A review. Trends Food Sci. Technol., 75, 181–193.
- [12] Orive, G. et al. (2015). Cell encapsulation: technical and clinical advances. Trends Pharm. Sci., 36(8), 537–546.
- [13] Nooshkam, M. and Varidi, M. (2020). Maillard

conjugate-based delivery systems for the encapsulation, protection, and controlled release of nutraceuticals and food bioactive ingredients: A review. Food Hydrocolloids, 100, 105389.

- [14] Zanetti, M. et al. (2018). Use of encapsulated natural compounds as antimicrobial additives in food packaging: A brief review. Trends Food Sci. Technol., 81, 51–60.
- [15] Devi, N. et al. (2017). Encapsulation of active

ingredients in polysaccharide- protein complex coacervates. Adv. Colloid Interface Sci., 239, 136-145.

[16] Wijerathna, T., Gunathunga, S. and Gunathilaka, N. (2020). Recent developments and future directions in the paratransgenesis based control of Leishmania transmission. Biol. Control, 145, 104260.

AUTHOR (S) BIOSKETCHES

Neda Abdollahi, Ph.D., Food Science and Technology, Department of Nutrition and Food Industry, Islamic Azad University, North Tehran Branch, Tehran.

Alireza Shahab Lavasani, Associate Professor, Innovative Technologies in Functional Food Production Research Center, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran. *Email: shahabam20@* yahoo.com