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THE USE OF WHEY PROTEINS AS COATING MATERIAL IN NANOENCAPSULATION AND MICROENCAPSULATION APPLICATIONS

Uso de proteínas de soro de leite como material de revestimento em aplicações de nanoencapsulação e microencapsulação

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ABSTRACT

Encapsulation technology has applications in various fields, including the food, agriculture, pharmaceutical, and cosmetic industries. In the food field, encapsulation is carried out using bioactive components such as flavor components, micronutrients, fatty acids, vitamins, enzymes, antioxidants, polyphenols, known as the core materials, and carbohydrates, gums, and proteins such as shell (coating) material. Among coating materials, whey proteins are commonly used in microencapsulation and nanoencapsulation applications. Whey proteins used as coating material protect food against external influences and increase the nutritional value of the food and the health functionality of the foods to which they are added. However, there are some limitations due to the low solubility of whey proteins in aqueous media. High hygroscopicity, likely to promote unpleasant taste. To overcome these, researchers are continuing optimization studies for technologies using these hydrolysates and isolates in different food matrices. The use of whey proteins as a capsule material for encapsulating food ingredients with micro/nanoencapsulation techniques is becoming increasingly common. This review is focused on the nanoencapsulation and microencapsulation techniques and the use of whey proteins as coating material for different substances in the food industry is evaluated.

Keywords: encapsulation methods; controlled release; bioactive compounds.

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RESUMO

A tecnologia de encapsulamento tem aplicações em vários campos, incluindo as indústrias alimentícia, agrícola, farmacêutica e cosmética. Na área de alimentos, o encapsulamento é realizado usando componentes bioativos, como componentes de sabor, micronutrientes, ácidos graxos, vitaminas, enzimas, antioxidantes, polifenóis, conhecidos como materiais do núcleo, e os carboidratos, gomas e proteínas, como material de casca (revestimento). Entre os materiais de revestimento, as proteínas do soro são comumente usadas em aplicações de microencapsulação e nanoencapsulação. As proteínas de soro de leite usadas como material de revestimento protegem os alimentos contra influências externas e aumentam o valor nutricional dos alimentos e a funcionalidade saudável dos alimentos aos quais são adicionadas. No entanto, existem algumas limitações devido à baixa solubilidade das proteínas do soro em meio aquoso e alta higroscopicidade, susceptível de promover sabor desagradável. Para superá-los, os pesquisadores realizam estudos de otimização de tecnologias que usam esses hidrolisados e isolados em diferentes matrizes alimentares. O uso de proteínas de soro de leite como material de cápsula, para encapsular ingredientes alimentícios com técnicas de micro/nanoencapsulação está se tornando cada vez mais comum. Esta revisão é focada nas técnicas de nanoencapsulação e microencapsulação e avalia o uso de proteínas de soro de leite como material de revestimento para diferentes substâncias na indústria de alimentos.

Palavras-chave: métodos de encapsulação; liberação controlada; compostos bioativos

INTRODUCTION

The encapsulation technique is a process of coating the core material (food ingredients or biologically active material) in solid or liquid form with a shell material in the polymer matrix. In other words, encapsulation is the retention of an active substance in a carrier substance (wall material). It is a rapidly expanding technology due to its wide application areas in the food, nutrition, pharmaceutical, agriculture, and cosmetic industries (EZHILARASI *et al.*, 2013; FANGMEIER *et al.*, 2019). The encapsulated material is called the core; whereas the encapsulating material is called a coating, shell, membrane, capsule, carrier material, matrix or, outer phase (DEVI *et al.*, 2017; MURTHY *et al.*, 2018). This technique is used for various purposes, such as increasing the stability of the coated components, protecting and facilitating their use, avoiding undesirable reactions, and providing a controlled release of active ingredients. (XU *et al.*, 2017; CARVALHO *et al.*, 2019). This technique also helps to reduce the interaction between environmental and core factors. It can prevent changes that can cause the loss of properties really important to the sensory, such as aroma, colour, or nutritional value (FANGMEIER *et al.*, 2019).

The choice of wall materials in the encapsulation process is an important step in developing capsules because it determines the strength and stability of the obtained capsules. In food applications, milk proteins, natural biopolymers, natural gums, and starches must be compatible with the food and have safe properties used as wall materials (NORKAEW *et al.*, 2019).

Whey protein is widely used in food applications because it contains essential amino acids valuable for nutritional physiology and other functional properties in the human body. Whey proteins are a mixture of α-lactalbumin and β-lactoglobulin globular proteins. In addition to their nutritional properties, they have some other functional properties as coating material. For instance, whey proteins can trap hydrophobic compounds such as vitamin D3. Whey protein concentrates can be used as a wall material for encapsulation due to their surfactant properties and can form a protective coating around core materials due to hydrophobic interactions, as mentioned above (KHAN *et al.*, 2019; LEKSHMI *et al.*, 2019).

Some encapsulation methods enter a solid or liquid core, each producing particles of different sizes. These capsules consist of a round, thin, and resistant membrane. The methods used to encapsulate bioactive substances are spray drying, spray cooling, extrusion, co-crystallization, fluidized bed coating, coacervation, and freeze-drying. The particles produced due to these applications have different properties such as reduced bioactivity, biocompatibility, production cost, and degree of protection (CARVALHO *et al*., 2019; FANGMEIER *et al*., 2019; NIU *et al*., 2020; TOLUN *et al*., 2020).

Using whey proteins in encapsulation applications in the food industry enables to increase the nutritional value and health functionality of the foods to which they are added. Consumers who value healthy eating habits consider whey proteins to have lower allergenicity and higher bioavailability so they can be consumed in their diet. This review focuses on the studies that use whey proteins as coating materials and supply information about the manufacturing processes with different technologies.

Encapsulation Methods

When packing the solid, liquid, or gas phases of food components or microorganisms with protein, the carbohydrate-based coating material can be defined as encapsulation (YU *et al*., 2016). Encapsulation techniques can be divided into two classes such as chemical and physical encapsulation methods (Figure 1).

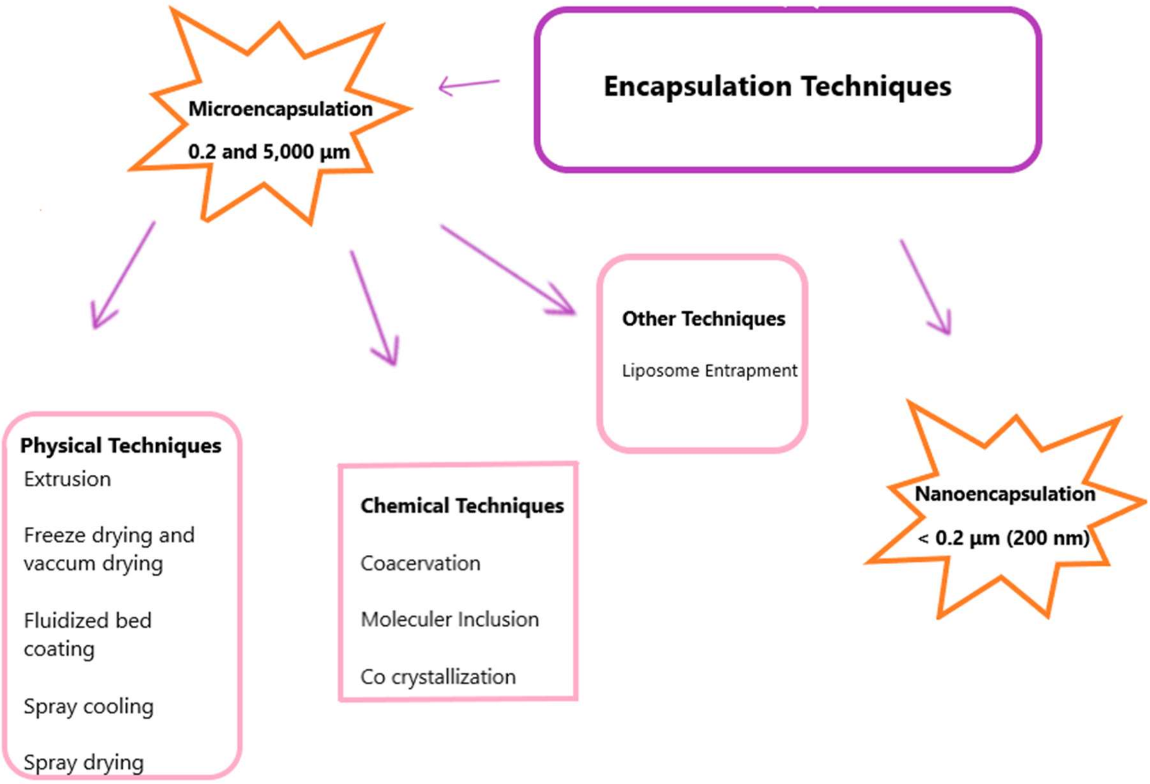

Figure 1. Encapsulation techniques.
Source: made by the authors, 2023.

Coacervation, molecular inclusion, and co-crystallization techniques are chemical encapsulation techniques. Among the physical encapsulation methods, the most widely used techniques are spray cooling, spray drying, fluidized bed coating, extrusion, freeze drying, and vacuum drying (SONAWANE *et al*., 2020). Furthermore, the encapsulation techniques can also be classified into two methods according to the size of the capsule: microencapsulation and nanoencapsulation (EL-KADER; HASHISH, 2020). In encapsulation techniques, if the particle size range is between 0.2 and 5000 µm, it is called microencapsulation; if smaller than 0.2 µm, it is called nanoencapsulation (KING, 1995). The particle sizes of the capsules produced by spray drying, fluid bed coating, spray cooling, extrusion, and inclusion encapsulation technologies are as follows, respectively: (10-400 µm), (5-5000 µm), (20-200 µm), (150-800 µm), (0.001-0.01 µm) (EL-KADER; HASHISH, 2020). In Figure 1, the widely used encapsulation techniques are shown.

When choosing an encapsulation method for detailed application in the food industry, the required particle size must be determined first. And then, the parameters of the encapsulation material, its physical and chemical properties, active ingredient and carrier, cost, and production scale must be considered.

Physical Techniques

<u>Spray Drying</u>

Spray drying is one of the most economical, simple, and widely used techniques in food and medicine for encapsulating various substances such as probiotics, nutraceuticals, flavor compounds, enzymes, peptides, etc. Active ingredients (food flavors, oils, or drugs) and polymer coating material suitable for encapsulation dissolved in a solvent or the active ingredient suspended in the polymer solution. When the suspensions encounter hot air, the atomized droplets' solvent evaporates to obtain dry powder capsules. One of the most important factors limiting the application of spray drying is the selection of suitable shell materials for applying this technique. The important advantages of this method are the high production rate and the cost-benefit ratio. If high temperatures are applied while using this technique, the biological effectiveness of the components may be impaired. Today, spray drying is considered one of the most preferred and low-cost techniques among other methods (KOÇ; SAKIN, 2010; XU *et al.*, 2017; ZANONI *et al.*, 2020).

The spray drying technique is widely used to prevent the chemical and/or microbiological deterioration of the products, ensure their microbiological stability, protect the products' specific properties, and reduce storage and transportation costs. At the same time, spray drying technology is the most widely used encapsulation technique due to its high drying performance. (GÖKMEN *et al.*, 2012).

In the spray drying technique, carbohydrates (cellulose and maltodextrin derivatives), natural gums (alginates, gum Arabic, carrageenan), proteins (milk and soy proteins, gelatins), and oils are used as carriers (CARNEIRO *et al.*, 2013). The particle size of the capsules obtained by spray drying is 10-100 micrometers (FANG; BHANDARI, 2012).

<u>Spray Cooling</u>

Spray cooling consists of an atomization source, particle formation chamber, and collection zone. The most important difference from spray drying is the particle formation area. In the particle formation zone, the particles are formed not by solvent evaporation, but by cooling and hardening the droplets (OXLEY, 2012). The core material is melted or dispersed in a molten carrier with spray cooling. The final preparation is fed through an atomizing nozzle and sprayed into a cooling chamber. Finally, the molten droplets in contact with the cooled air harden. Spray cooling can also be optimized by making changes such as changing the temperature of the supply line and cooling the air used in the operation. After this technique, particles are formed and the core material is evenly distributed (GAVORY *et al.*, 2014; BAMPi *et al.*, 2016). The spray-cooling encapsulation process has been reported for food ingredients, active pharmaceutical ingredients, and sweeteners (GIBBS *et al.*, 1999). The spray cooling technique is well suited for encapsulating active pharmaceutical ingredients as it is a low-cost process; It is easy to scale up and does not need organic solvents (FAVARO-TRINDADE *et al.*, 2021).

Spray cooling technique has advantages such as speed, performance, and relatively low cost. Elimination of solvents is not necessary, as its application does not require the use of organic solvents. It is also considered a reproducible physical process with easy particle size adjustments (ALBERTINI *et al.*, 2004).

Extrusion

This technique is mainly applied for encapsulating living cells without other options. One of the 'gentle' approaches to encapsulation, the extrusion technique, is particularly recommended for probiotics or other microorganisms (BILUŠIĆ *et al.*, 2021). The extrusion method is the oldest and most widely used in hydrocolloid encapsulation processing. With this technique, the bioactive materials are mixed with the encapsulating material to form capsules immediately in a solidification bath of the droplets (FANGMEIER *et al.*, 2019; SANDOVAL-MOSQUEDA *et al.*, 2019). Extrusion technology has assisted in producing extrudate with a small particle size that can be used in food applications. It provides many solutions to the problems encountered during the encapsulation of bioactive compounds. Carbohydrates (Starch, Maltodextrins, Gum Arabic, Alginate, and Cyclodextrins) are used as wall material in extrusion technology. For flavor compounds, and other carbohydrates especially maltodextrins and cyclodextrins; Alginates (Sodium alginate) are widely used during the extrusion of encapsulation of probiotic bacteria (BAMIDELE; EMMAMBUX, 2020). The most important advantage of this technique is the absence of solvent use and the absence of excessive heat. Due to the low microspherical processing speed in this method, there are difficulties in carrying the technique to the industrial level (GÖKMEN *et al.*, 2012).

Fluid Bed Coating

With the fluidized bed coating technique, the solid core material is suspended in a gas stream at a specific temperature. A liquid film is formed on the particle by spraying the liquid into the coating material as fine droplets. Next, the core is gradually wetted and dried to form a solid homogeneous layer that the particles can encapsulate. This method is used to encapsulate various minerals and vitamins used as nutritional supplements in the food industry and to improve the color and flavor of various organic acids in the meat industry (NEDOVIC *et al.*, 2011). One of the most important advantages of fluid bed spray technology is that it provides more uniform coatings using melt or liquid coating materials. The process is controlled by adjusting the spray rate, coating cycle and temperature variables (WANG *et al.*, 2020). Some disadvantages of the method are expensive equipment, long residence time, prone to filter clogging, higher probability of solvent explosion, and poor performance with larger-sized granules as they affect the trajectory (LAWRENCIA *et al.*, 2021).

Freeze Drying

Freeze drying is a method of evaporating moisture from a frozen substrate. This method is preferred over the dehydration process in heat-resistant compounds. Freeze drying has three main steps, namely, freezing, primary drying, and secondary drying. Since the temperature used in this method remains low throughout the whole process, it offers better quality products in terms of taste, texture, and aroma. The most commonly used coating materials are maltodextrin, emulsifying starches, whey proteins, and gum arabic (BORA *et al.*, 2019; VAHIDMOGHADAM *et al.*, 2019; WALIA *et al.*, 2019). This method is more suitable for sensitive bioactive compounds. For example, polyunsaturated fatty acids, probiotics, rich in essential oils, proteins and derived peptides, polyphenolic compounds, vitamins, and anthocyanin components in plant seed oils and plants. Because minus temperature is used to freeze the emulsion. The frozen solution is subjected to very low pressures and the ice crystals formed are sublimated. It is also considered an expensive drying technology due to applying a pump that can provide vacuum conditions (REZVANKHAH *et al.*, 2020).

Chemical Techniques

Coacervation

Encapsulation by coacervation has recently received increasing attention due to its practical applications. In this technique, phase separation occurs first to separate the polyelectrolyte and/or polyelectrolyte mixture from a solution, then a coacervate phase is formed and the core is completely coated. It has been observed that the potency of coacervate is increased by adding enzymatic/chemical crosslinkers such as transglutaminase or glutaraldehyde. Coacervations consisting of a single biopolymer are called simple coacervation, and those composed of two or more biopolymers are called complex coacervation. (DEVI *et al.*, 2017; SAMAKRADHAMRONGTHAI *et al.*, 2019; WALIA *et al.*, 2019). These technical variants include species using one or more shell types respectively and have been successfully used to capture sensitive bioactive. For example, omega-3 lipids, pharmaceuticals, and plant extracts. Complex coacervation appears to be an effective method for encapsulating sensitive food ingredients. This method allows proteins and carbohydrates to form a complex shell surrounding the nucleus (TIMILSENA *et al.*, 2019).

Co-crystallization

The co-crystallization technique uses sucrose syrup as a matrix surrounding the core material. The sucrose syrup is f saturated by concentrating and the temperature is adjusted to a level that prevents crystallization. Then the encapsulated material is dried to the appropriate moisture content. Between sucrose crystals of non-sucrose materials or microcrystalline clusters ranging from 3 to 30 µm are formed during entrainment. (KOÇ; SAKIN, 2010; GÖKMEN *et al.*, 2012).

Studies on the encapsulation of food compounds by co-crystallization, covering samples of honey, peanut butter, orange peel oil, cardamom oleoresin, yerba mate extract, marjoram extract, and chokeberry, are relatively few (CHEZANOGLOU; GOULA, 2021).

Emulsion methods are frequently used to encapsulate lipophilic bioactive components in the food industry. The size of the droplets produced in emulsion-based encapsulation systems depends on the composition of the system and the homogenization method. An average droplet particle size is <100 nm for nanoemulsions and >100 nm for conventional emulsions. It has been reported that nanoemulsions have better stability against particle aggregation and gravity separation than conventional emulsions due to their small droplet size (AHMED, 2012). Thermodynamically labile emulsions use amphiphilic compounds (lipids, some proteins, peptides, and polymers) to stabilize the dispersed phase, which prevents systems from phase separation. Whey oil-in-water emulsion is one of these compounds that can be dried by different drying methods such as spray or freeze drying (KAKRAN; ANTIPINA, 2014). Such dried capsules can be a ready-made formulation for numerous food products (NEDOVIC *et al.*, 2011). The simulation of the emulsification process with whey proteins is illustrated in Figure 2.

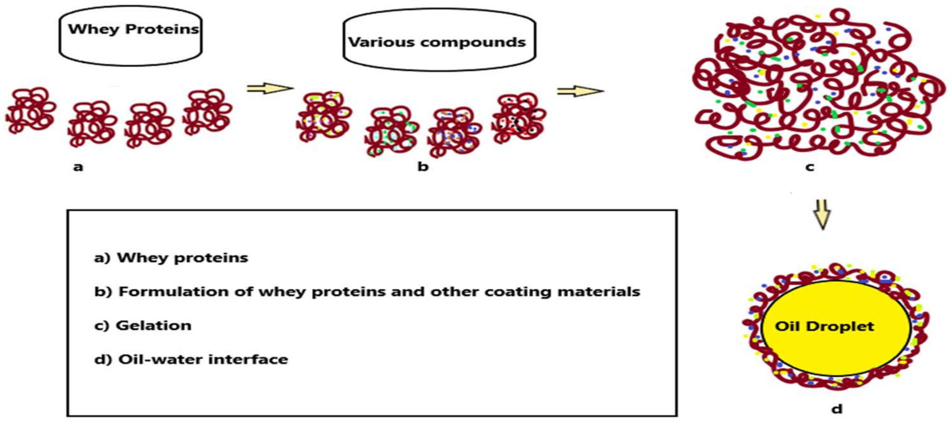

Figure 2. Simulation of emulsification with whey
Source: made by the authors, 2023.

Use of Whey Proteins as Coating Material

Whey is the liquid obtained after removing the casein fraction from milk. It is a blend of proteins with high nutritional, therapeutic, and functional properties. Whey proteins are found in milk at about 0.6-0.7%. Whey proteins are composed of β-lactoglobulin, α-lactalbumin, bovine serum albumin, and immunoglobulins. These proteins are classified into major and minor components. As the main component, whey proteins vary in amount, the concentration of β-lactoglobulin in whey is about 58%, consists of 162 amino acids, and has a molecular weight of 18.4 kDa. α-lactalbumin has a molecular weight of 14.2 kDa, 123 amino acids, and constitutes 12% of whey proteins. Bovine serum albumin is approximately 1.5%, while immunoglobulins are about 1% in whey. Minor components of whey include lactoferrin, protease-peptone components, lactoperoxidase, and milk fat globule membrane proteins (SINGH *et al.*, 2009; KRUNIĆ *et al.*, 2019).

Whey is generally obtained from two sources, which are known as sweet and acidic whey. Sweet whey is produced from rennet cheeses; acidic whey is usually made from acid-precipitated cheeses such as cottage cheese (RISNER *et al.*, 2019). The commonly used whey protein products are whey protein concentrates, whey protein isolates, and native whey proteins produced from milk using microfiltration/diafiltration in combination with ultrafiltration techniques (KULOZIK; KERSTEN, 2002; POONIA, 2017). In addition, it is possible to produce native whey proteins from acid whey, sweet whey, lactose-reduced whey, and demineralized whey by using other fractionation methods such as chromatography and precipitation. Protein concentration in whey concentrate is between 25-89%, whereas protein concentration in whey protein isolate is 90% or higher than 90% (GÜZELER *et al.*, 2017; BOEVE; JOYE, 2020).

In addition, whey proteins are widely used in the food industry due to their functional and nutritional properties such as foaming, emulsification, hydration, and gelling (ALOĞLU; ÖNER, 2010; KEVIJ *et al.*, 2019; KAUR *et al.*, 2020). Unlike caseins, whey proteins undergo thermal denaturation when heated at temperatures above about 65 °C and associate with each other through disulfide bond formation and hydrophobic attraction (FATHI *et al.*, 2018). These functional properties of whey proteins make them suitable for capsule wall use. Bioactive molecules have hydrogel-forming abilities and surface activity properties to form encapsulation systems.

Microencapsulation and Nanoencapsulation Techniques

Coating of different food bioactive components by using micro/nanoencapsulation techniques play a critical role in protecting nutrients against adverse processing and storage conditions such as excessive humidity, high temperatures, certain pH values, light, and high oxygen levels. Moreover, these technologies can produce commercialized additives, ingredients, and supplements with a long shelf life that can be applied to food products, cosmetics, and pharmaceuticals. The encapsulation is used for the targeted/controlled release of bioactive compounds within commercial products or the human body (ASSADPOUR; JAFARI, 2019), another advantage of this technique.

Commercial microcapsules typically have a diameter of 3-800 µm, whereas nanocapsules vary between 10 and 1000 nm particle size. Nanoencapsulation is superior to microencapsulation with potential properties such as increasing bioavailability, improving controlled release, and enabling more precise targeting of bioactive compounds (EZHILARASI *et al.*, 2013; FANGMEIER *et al.*, 2019). The size of capsules containing bioactive ingredients affects the properties of functional foods prepared with encapsulated substances. The large capsules can adversely affect the texture of the food. On the other hand, if they are too small, they may not provide adequate protection for the bioactive components. The products formed after the encapsulation process have a micrometer diameter to the nanometer. It is called nanocapsule and microcapsule (YE *et al.*, 2018; AHANGARAN *et al.*, 2019). It can be stated that nanoencapsulation technology has become more attractive than microencapsulation due to its advantages, such as encapsulation efficiency and increased product yield.

Microencapsulation and Nanoencapsulation studies with whey proteins

Technologically, whey protein-based systems have been used to encapsulate some bioactive ingredients, such as β-carotene, caffeine, epigallocatechin-3-gallate, bilberry extract, palm extract, and encapsulation of probiotics (FATHI *et al.*, 2018). The important studies about microencapsulation and nanoencapsulation, which were carried out with whey proteins are summarised in Table 1.

Tan *et al.*, (2019) originally (WPI-Whey Protein Isolate) hydrolyzed (WPI) and gelled whey was used to encapsulate the pepsin enzyme. The study suggested that pepsin could be encapsulated, and its release controlled by combining WPI and gelled WPI in capsules or sandwich tablets (outer gelled WPI-pepsin layers covering the WPI pepsin layer). In another study, Rosolen *et al.*, (2019) evaluated the production of *Lactococcus lactis* R7 microencapsulated with whey and inulin using the spray drying technique and the ability of microencapsulation to protect against adverse conditions. It was determined that the combination of whey and inulin used as encapsulation material protected bacteria against adverse conditions and showed potential for application as a coating material in foods.

Table 1. Microencapsulation and nanoencapsulation studies with whey proteins

Active compound	**Encapsulation content**	**Encapsulation method**	**Key findings**	**References**
Beetroot extract	Inulin (IN), Maltodextrin (MD), and whey protein isolate (WPI)	Microencapsulation/Spray drying	Moisture, betalain content, and retention values of WPI were 4.24%, 385.47mg/100g (dry basis) and encapsulation efficiency was 95.69%, respectively.	Carmo *et al.*, (2018)
Betalain pigment	Whey protein concentrate (WPC)	Microencapsulation/Spray drying	5-15% WPC, inlet air temperatures (160-180°C), and feed flow rate to produce powder (400-600 mL/h). It has been stated that it can be added to functional foods as both an antioxidant and a red colorant.	Bazaria; Kumar, (2016)
Black rice extract	MD, WPI, gum arabic (GA), and their combinations	Microencapsulation/Spray drying	Maltodextrin (88%) and its combination with WPI exhibited the best anthocyanin retention.	Norkaew *et al.*, (2019)
Chia essential oil	Mesquite gum/gum Arabic-whey protein	Microencapsulation/Spraydrying	The encapsulation efficiency was calculated to be higher than 70% for all microcapsules (2.32 and 3.35 μm).	Rodea-González *et al.*, (2012)
Chia oil and fish oil	Whey protein	Microencapsulation/Spray drying	Microencapsulated chia oil and fish oil using whey protein as wall material was investigated in the 50-65°C. Oil sprayed at 55°C showed maximum encapsulation efficiency and minimum peroxide value	Lavanya *et al.*, (2020)
Curcumin	Whey protein isolate (WPI)	Microencapsulation/Spray-drying	Curcumin with whey protein isolate (WPI) via hydrophobic interactions; Soluble complexes were formed in solution. Approximately 100% curcumin was kept amorphous, and the microparticles were rehydrated regardless of drying temperature.	Liu *et al.*, (2016)
Curcumin	Whey protein	Nanoencapsulation	It has been stated that curcumin nano-encapsulated with whey protein may be a potential application that should be considered for clinical applications.	Jayaprakasha, *et al.*, (2016)
D-limonene	Pectin-whey protein	Nanoencapsulation /Complexation	The best encapsulation efficiency reported is that there is a 4 to 1 ratio between WPC and pectin (4% WPC and 1% pectin at pH = 3).	Ghasemi *et al.*, (2018)

EPA-rich oil	WPC	Nanoencapsulation	This is the first time that EPA-rich oil has been successfully encapsulated in within WPC. The EPA oil is not oxidised during encapsulation at 25°C and in the presence of air.	Escobar-García *et al.*, (2021)
Fish oil	Maltodextrin/modified starch-whey protein	Nanoencapsulation /Spray drying	The results showed that microfluidization is an effective emulsification technique in the nano-range (d43, 210-280 nm).	Jafari *et al.*, (2008).
Flaxseed oil/protein hydrolysate	Gellan gum-WPI	Microencapsulation/ Emulsification	It has been reported that microgels obtained by encapsulating 1.5% whey protein isolate, 0.56% calcium chloride solution, flaxseed oil (15%), and protein hydrolyzate are sufficient to encapsulate bioactive compounds to be released in the small intestine and pass through the stomach without deterioration.	Kuhn *et al.*, (2019)
Gallic acid	Pectin-whey protein	Nanoencapsulation /spray-drying	From the obtained nanoencapsules, it has been revealed that the pectin-WPC complex has the same resistance to precipitation and creaming as Tween 80.	Gharehbeglou *et al.*, (2019)
Garlic extract	Chitosan-whey protein isolate	Microencapsulation/ Coacervation	The phenolic compound retention efficiency for the encapsulated garlic extract powders ranged from 51% to 61%.	Tavares *et al.*, (2019)
Grape skin extract	Whey protein concentrate (WPC)	Microencapsulation/Spray-drying	By using whey protein as a carrier agent in grape skin extract, a powder with high anthocyanin retention was obtained after spray drying, showing the potential application in food products.	Oliveira *et al.*, (2018)
Infant formulae	Maltodextrin and oil WPH	Microencapsulation/ Emulsification	During covalent bonding and heat treatment between proteins/peptides in hydrolyzed whey protein and maltodextrin; A performance-enhanced component was created during the heat treatment of the model baby food emulsion.	Drapala *et al.*, (2016)
Lactobacillus acidophilus and blackberry juice	Gum Arabic (GA), maltodextrin (MD), and whey protein concentrate (WPC)	Microencapsulation/ Spray-drying	After encapsulation, total phenolic compounds (TPC) and total monomeric anthocyanin content (TMAC) were higher at 98.4±1.0% and 99.0±1.0%, respectively, when the GA-MD mixture was used as the encapsulation agent. The viability of LA was higher when WPC was used (93.3±0.9%)	Colín-Cruz *et al.*, (2019)
Lactobacillus casei ssp. paracasei and anthocyanins from black currant extract	Whey protein isolate, inulin, and chitosan	Microencapsulation/ Freeze-drying	Anthocyanins from black currant extract and lactic acid bacteria were co-microencapsulated using a gastrointestinal resistant biocomposite composed of whey protein isolate, inulin, and chitosan, with an encapsulation efficiency of 95.46%±1.30% and 87.38% ± 0.48%, respectively. It was tested by adding it to yoghurt and the results showed satisfactory stability of the biologically active compounds when stored at 4°C for 21 days.	Enache *et al.*, (2020)

Lactobacillus delbrueckii subsp. bulgaricus and Lactobacillus paracasei subsp. Paracasei	Gum Arabic (GA), maltodextrin (MD), and whey protein concentrate (WPC)	Microencapsulation/ Freeze-drying	Ca-Alg-WPI microcapsules were helpful in protecting and deliveringhelpfulpreserve *L. bulgaricus* and *L. paracasei.*	Han *et al*., (2020)
Linseed oil	Gum Arabic/ maltodextrin/ methyl cellulose-whey protein isolate	Microencapsulation/Spray drying	As a result, the highest protection against oxidation and microencapsulation efficiency is greater than 90%.	Gallardo *et al*., (2013).
Orange peel oil	WPC, low methoxyl pectin (LMP), and MD solutions	Nanoencapsulation	Optimum nanocomplex suspensions containing orange peel oil were formulated at three different pH values (3, 6 and 9) and freeze-dried into powder. Analysis of the size and zeta potentials of the nanocomplexes, the encapsulation efficiency of the smallest particles formed at pH = 6. At pH = 3, 6 and 9, the encapsulation efficiency of the powders was 88%, 84% and 70%, respectively.	Ghasemi *et al*., (2017)
Palm fruit feeds	Whey protein isolate (WPI)	Nanoencapsulation	The study aimed to encapsulate the aqueous extract of palm kernel as a rich source of phenolic compounds. It has been claimed that protein-protein and polyphenol-protein interactions are attenuated at higher extract/WPI ratio.	Bagheri *et al*., (2013)
Peptide (in yogurt fermentation)	Chitosan and gelatin (1:1) Whey protein concentrate hydrolysate (WPCH)	Microencapsulation/ Spray-drying	The effects of microencapsulation were investigated in terms of preservation of peptides during gastrointestinal digestion and lactic acid fermentation, and it was stated that chitosan was more effective than gelatin in stabilizing peptides during milk fermentation.	Gómez-Mascaraque *et al*., (2016)
Pumpkin seed oil	Different carbohydrates with WPI	Microencapsulation/ Spray-drying	A microencapsulation efficiency of 94.5% was achieved with a mixture of whey protein and maltodextrin.	Le *et al*., (2017)

Red Pepper Waste (RPW)	WPI	Microencapsulation/Spray-drying	A faster initial release of carotenoids was observed from whey protein matrices than phenolics. Encapsulation of RPW showed a protective effect against pH changes and enzymatic activities during digestion and contributed to increased bioavailability in the gut.	Vulić *et al.*, (2019)
Resveratrol	Gum Arabic-whey protein	Microencapsulation/ Emulsification	The study in which a mixture of gum arabic (GA) and whey protein (WP) was used showed that the stabilized emulsions had >50% encapsulation efficiency for resveratrol.	Shao *et al.*, (2019)
Riboflavin	Whey protein isolate (WPI)	Microencapsulation/Spray-drying	It has been reported that riboflavin-loaded whey protein isolate (WPI) microparticles can be kept dry upon desolvation by spray drying. The sample prepared from 30% v/v ethanol exhibited rapid peptic digestion in less than 30 minutes. Samples from 30% v/v ethanol at 1 and 2 mM Ca^{2+} exhibited excellent gastric resistance and intestinal release.	Ye *et al.*, (2019)
Saffron extract (crocin, safranal, and picrocrocin)	Whey protein concentrate (WPC), pectin and maltodextrin	Nanoencapsulation /Multiple emulsification	A primary saffron water extract in oil (W/O) microemulsion containing 10% (w/w) saffron extract was reemulsified to prepare W/O/W multiple emulsions. Stabilized using 0.25 mass fraction and protein (whey protein concentrate (WPC))/polysaccharide (pectin). With the sequential adsorption of WPC/pectin, good encapsulation efficiency and efficiency for crocin, picrocrocin, and saffron, smooth surfaces in final powders were obtained.	Esfanjani *et al.*, (2015)
Thymol	Almond gum-whey protein isolate (WPI)	Nanoencapsulation/ Emulsification	In the obtained emulsions, thymol's encapsulation efficiency of >50% was calculated.	Sedaghat Doost *et al.*, (2019)
Lactobacillus casei and tuna oil	Whey protein isolate-gum Arabic	Microencapsulation/ Spray-drying + freeze-dried	The oil encapsulation efficiency of freeze-dried WPI-PO-GA microcapsules (76.28%) was significantly lower ($p > 0.05$) than spray-dried WPI-PO-GA microcapsules (93.35%).	Eratte *et al.*, (2015)
Vitamin E	whey protein isolate (WPI)	Microencapsulation/ Spray-drying	Vitamin E microcapsules prepared by spray drying, freeze drying and spray freeze drying techniques showed maximum encapsulation efficiency of 89.6 ± 2.58, 86.1 ± 1.44 and 89.3 ± 2.56, respectively.	Parthasarathi; Anandharamakrishnan, (2016)

Milea *et al.*, (2019) microencapsulated anthocyanin extracted from sweet cherry peels with whey protein isolate by combining chitosan with freeze-drying. The resulting powders improved products such as yoghurt and marshmallows. Anthocyanins were microencapsulated in a whey protein isolate-chitosan matrix in a 78% yield.

According to Tavares *et al.*, (2019), complex coacervates were freeze-dried by complex coacervation method to obtain microencapsulated garlic extract microparticle powders using whey protein isolate (WPI)/chitosan (CH) and gum arabic (GA)/CH combinations as wall materials. Encapsulation with complex coacervation followed by freeze-drying preserved the heat-sensitive phenolic compounds found in the garlic extract. As a result of the study, it has been shown that microparticles have the potential to be used as an ingredient in the preparation of food products such as soups and bakery products.

Ghasemi *et al.*, (2018) investigated the volatile compounds of chemically unstable D-lime in the presence of air, light, humidity, and high temperature. Therefore, it was subjected to nanoencapsulation with a whey-pectin mixture at different pH values (3, 6, and 9). In the study, the production of nanocapsules loaded with D-limonene was 0.5% pectin content; 0.75; 1, and whey protein content was studied with 4; 6; 8 %. The study aims to produce an optimized nanocomplex based on viscosity, color, and stability. The researchers determined that the encapsulation efficiency was about 88%. As a result of the study, the optimum whey concentrate-pectin nano complex containing D-limonene was used in cakes, biscuits, fruit juices, etc. They argued that it could be used in products and that the aroma of this product can be used in this way.

The study of Reddy *et al.*, (2019) aimed to nanoencapsulate coffee bean oil roasted by nanospray drying (NSD). They characterized the structural properties and thermal behavior of the roasted coffee oil capsule obtained from the NSD technique by comparing it with the encapsulation obtained by the conventional spray drying (CSD) process. From the particle size measurement based on dynamic light scattering, they found that the average particle diameter of the nanospray-dried capsules was 304.9 ± 99.4 nm. Conventional spray dryer has been shown to produce heterogeneous particle size distribution and micron-sized capsules, and image analysis shows that nanocapsules are approximately 11 times smaller than microcapsules. NSD showed that the coffee oil nanocapsules have a more uniform particle size distribution than their microencapsulated counterparts.

In a study in which the pressurized gas (EAPG) assisted emulsion electrospraying method was performed for the first time to encapsulate bioactive eicosapentaenoic acid (EPA) rich oil in whey protein concentrate (WPC), submicron droplets of EPA oil are encapsulated in WPC spherical microparticles that are approximately 5 m in size. At the end of the research, oils enriched with 80% PUFA at room temperature with emulsion EAPG technology were provided with thermal stability and oil protection without affecting the bioactivity of EPA oil. It is stated that these capsules can be used as personalized medicines or nutraceuticals (ESCOBAR-GARCÍA *et al.*, 2021).

D-limonene is a volatile compound commonly used in food flavoring chemically unstable to light, air, humidity, and high temperatures. D-limonene was nano-encapsulated with 4, 6, and 8% w/w whey proteins and 0.5, 0.75, and 1% w/w pectin at pH 3, 6, and 9. The study determined that encapsulation efficiency was approximately 88% (GHASEMI *et al.*, 2018).

In another study in which they obtained the aqueous extract of palm kernel by desolvation with ethanol, they aimed to encapsulate the nanoparticles in whey protein as a rich source of phenolic compounds. It has been stated that the obtained extract-charged particles can be added to beverages without impacting the taste and appearance of the beverages (BAGHERI *et al.*, 2013).

Adsare; Annapure, (2021) studied the coconut with a spray-dried technique and mixing whey powders, coconut milk whey, and gum arabic mixture (0. 5, 10, or 15%) with/without curcumin, they obtained different combinations. As a result of the study, they stated that the prey drying process is suitable for the microencapsulation of curcumin, and coconut whey can be used as an alternative encapsulation agent for encapsulating bioactive compounds such as curcumin.

In a study performed by Esfanjani *et al.*, (2015), it was shown that pectin and maltodextrin were able to produce nanoemulsions containing picrocrocin, safranal, and crocin using whey protein concentrate. Fioramonti *et al.*, (2017) found that a multilayer emulsion of flaxseed oil with maltodextrin and whey protein isolate provides >90% encapsulation efficiency, 0.14-0.33 water activity.

In nanoencapsulation experiments performed at three different ratios, 70:30, 50:50, and 35:65, it was observed that curcumin's bioavailability has increased and prevented colon cancer by using whey protein. In this study, it was stated that whey protein, which is used to improve the biological activities of curcumin and provide stability for more extended periods, will have the potential to be considered for clinical applications in future studies (JAYAPRAKASHA *et al.*, 2016). In a study using the spray drying method, whey protein concentrate (WPC) and whey protein concentrate admixture of microencapsulated curcumin (TWPC); WPC and TWPC, without changing the technological properties of curcumin, showed a spherical, irregular particle morphology with agglomeration points (GOMES *et al.*, 2021). In another study on curcumin, Adsare; Annapure (2021) investigated both spray-dried coconut whey powder and curcumin-enriched coconut whey powders for physicochemical properties. As a result, the capsule efficiency for encapsulation in a powder containing 5% whey, coconut milk, and gum arabic 92% with a spray drying efficiency of 66.72% supplied the best conditions in this process.

Due to the increasing awareness of functional food products, it has focused on encapsulating probiotic bacteria with bioactive compounds. Many studies have stated that the co-encapsulation of bioactive compounds and probiotic bacteria in a single product provides synergistic health benefits and improves the adhesion of probiotic bacteria to the intestinal wall. In a study encapsulated in a single whey protein isolate (WPI) – gum Arabian (GA) complex microcapsule with a combination of tuna fish oil (T) and *Lactobacillus casei* (L), Co-microcapsules (WPI-L)-T-GA) and microcapsules containing *L. casei* (WPI-P-GA), powdered using spray and freeze drying, and the oxidative stability of Tuna oil in spray-dried auxiliary capsules compared to freeze-dried ones calculated higher. On the contrary, it showed lower probiotic viability (56.19%) (ERATTE *et al.*, 2015). Co-encapsulation of the probiotic strain *Lactobacillus casei* ssp. with black currant extract resulted in encapsulation efficiency of 87.38% and 95.46%, respectively, when using inulin, whey protein isolate, and chitosan as coating materials (Enache *et al.*, 2020).

CONCLUSIONS

Based on the numerous health benefits of bioactive compounds, the production of functional food products with the application of bioactive compounds has increased significantly in recent years. Therefore, encapsulation applications are crucial for preserving and stabilizing bioactive compounds. Micro-/nanoencapsulation methods are widely used in the food industry.

The delivery of any bioactive compound to various sites in the body is directly affected by its particle size. The encapsulation method is used to protect bioactive compounds from adverse environmental factors such as high temperatures, high oxygen levels, high humidity, exposure to light, and certain pH values, from storage conditions and for controlled release. Nano-encapsulation can potentially increase bioavailability, improve controlled release, and enable more precise targeting of bioactive compounds than microencapsulation.

The coating materials used in the encapsulation of bioactive components work in different ways structurally, thus they change their protection capabilities. The effectiveness of any coating material depends on the strength of these structures and their ability to form encapsulation. The main criteria for choosing a wall material for encapsulation are the application of the encapsulants, the bioactive properties of the core, and its cost.

Whey proteins, one of the most important by-products of dairy technology, have an important place in the food industry with their important functional and nutritional properties. It is particularly suitable for creating encapsulation systems for bioactive molecules due to their surface activity and hydrogel-forming abilities in encapsulation applications.

The use of whey proteins in micro/nanoencapsulation is an important technology due to its biological, physicochemical, and technological properties. Moreover, whey proteins are dietary supplements containing all essential amino acids, have high bioavailability, and have a wide range of commercial use. Using whey proteins as a capsule material for encapsulating food ingredients by micro/nanoencapsulation techniques is becoming increasingly common. This reduces the use of whey as a waste material and enables these proteins to show their functional properties as coating material. Therefore, encapsulation applications are of great importance in this respect. At the same time, more research is needed on how the stability of whey proteins against digestive enzymes and their bioavailability may be affected.

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