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Protein Based Encapsulation of Antioxidants: Methods, Functionality of Components, and Applications

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Abstract

This review focused on the encapsulation of antioxidants as bioactive compound in proteins. Both proteins and antioxidants are well-known for their nutritional, supplementary, and structuring effects in foods. Antioxidants are susceptible to environmental conditions during processing or storage, and thus need to be protected. Besides being good candidates for protection of antioxidants, proteins also have important techno-functional properties, such as texturizing or surfactant properties. An overview of different encapsulation methods of antioxidants was given. Type of encapsulation, such as complex formation or core-and-shell model, depends on the production method. Production methods mainly include a mixture of antioxidant and protein solutions, following a gelation or hardening step for stabilization. Alternatively, depending on the nature of the compounds, hydrophobic or electrostatic interactions were also used for the assembly. Encapsulation of antioxidants can be used for the protection purposes as well as for controlled release systems in food or medical products.

Keywords: Bioactive compound, Complex formation, Microcapsule, Nanoparticle, Phenolic compound.

Protein Temelli Antioksidan Enkapsülasyonu: Metotlar, Bileşenlerin Fonksiyonları ve Uygulamalar

Özet

Bu derleme biyoaktif madde olarak antioksidanların proteinler içinde enkapsülasyonlarına odaklanmıştır. Proteinler ve antioksidanlar gıdalarda besleyici, takviye edici ve yapısal özellik geliştirmeleri ile bilinir. Antioksidanlar proses ve depolama sırasında çevresel faktörlerden çok etkilenir, bu yüzden korunmalıdır. Proteinler, antioksidanları korumaya iyi bir aday olmasının yanı sıra, tekstür geliştirme ve yüzey aktif özellikler gibi tekno-fonksiyonel özelliklere de sahiptir. Antioksidanların farklı enkapsülasyon yöntemleri genel olarak anlatılmıştır. Enkapsülasyon türleri, örneğin kompleks oluşumu ya da çekirdek-kabuk modeli, üretim metoduna bağlıdır. Üretim metotları temelde antioksidan ve protein çözeltilerinin karışımını, sonrasında da jelleşme ve stabilizasyon için katılaşma basamaklarını içerir. Ayrıca, kompleks oluşumu için bileşenlerin yapısına bağlı olarak, hidrofobik veya elektrostatik etkileşimler de kullanılabilir. Antioksidanların enkapsülasyonu, koruma amaçlı kullanılabileceği gibi gıda veya medikal ürünlerinde kontrollü salınım sistemlerinde de kullanılabilir.

Anahtar Kelimeler: *Biyoaktif bileşen, Fenolik bileşen, Kompleks oluşumu, Mikrokapsül, Nanoparçacık*.

1. Introduction

Encapsulation is mainly used for protection and efficient use of bioactive compounds, such as antioxidants or essential oils. Bioactive compounds that need some type of coating can be prone to degradation in the case of exposure to light or air, particularly oxygen or in the case of interaction with other components in the system. For instance, antioxidants as an active compound have less stability in final products and during processing. As another example volatile compounds, such as aroma, are prone to degradation and evaporate quickly, therefore encapsulation as a protection of aroma compound can decrease the evaporation rate (Nedovic et al., 2011). Alternatively, encapsulation can be used to mask odor or off-flavor of some bioactive components (Jansen-Alves et al., 2018). More than one bioactive materials can be used in the same encapsulation system, which could allow use of combined effects or synergistic effect of different bioactive materials (Nedovic et al., 2011).

Encapsulation process can be done via different routes, including physical methods such as spray drying and supercritical fluid applications; physicochemical methods such as emulsification; electrostatic interaction methods such as ionic gelation, acidic precipitation, complex coacervation and layer-by-layer deposition; hydrophobic interaction methods such as micelle and liposome formation; and chemical or enzymatic cross-linking method (Munin & Edwards-Levy, 2011; Nedovic et al., 2011). All of which have their own advantages depending on the food matrix used. Most of the time, micro- or nanoparticles are produced not to disrupt the sensorial properties of food materials. Therefore, the term “microencapsulation” or “nanoencapsulation” is also often used (Jansen-Alves et al., 2018; Silva et al., 2018).

Phenolic compounds are naturally produced by plants as secondary metabolites (Gutierrez-Grijalva et al., 2016). In their chemical composition, aromatic rings and hydroxyl groups are present. In plants, the main function of phenolic compounds is related to the defense mechanism, which is indeed similar to the function of phenolic compounds in human body. Consumption of foods being rich in phenolic compounds was known to have an anticarcinogenic and antimutagenic effects, and also to protect against cardiovascular diseases (Baltacıoğlu & Artık, 2013). Radical scavenging effect of phenolic compounds, which is also known as antioxidant properties, increases the importance of such compounds (Gutierrez-Grijalva et al., 2016). There is still an interest on antioxidant properties of phenolic compounds, their use in food science and food industry, and their bioavailability.

Phenolic compounds and antioxidants, as bioactive materials, were widely studied in encapsulation studies (Jansen-Alves et al., 2018; Zhang et al., 2016). In these studies, the main reason is to protect the phenolic compounds, which are known with their good functional properties to human health, against environmental conditions. These conditions could be the exposure of bioactive compounds to light, heat or oxygen (Lau et al., 2017). In literature, there have been different studies on encapsulated antioxidant, for example curcumin (Yao et al., 2018), β-carotene (Zhang et al., 2016) and tannic acid (Lau et al., 2017). They coated or formed a matrix for antioxidants and obtained higher protection using encapsulation compared to the bare form of them.

As a coating material, also called carrier material, proteins, polysaccharides or the mixtures of them can be used (Nedovic et al., 2011). These systems can give several functional properties to the system. Physical and chemical stabilities of micro-particles or complexes, controlled release from the shell material depending on the environmental conditions, and masking of off-flavor and odors can be counted for such functional properties. Proteins, by their nature, can show many different characteristics as a wall material, such as flexibility, strength, good coverage and/or complex formation with antioxidants (Jansen-Alves et al., 2018). Besides these, proteins have high nutritional value and good emulsifying properties, which increases the possible use as an encapsulation material (Wang et al., 2016; Foegeding et al., 2017). Therefore, in this review the focus is on the protein based carrier materials in encapsulation of antioxidants as bioactive material. Encapsulation methods of antioxidants in a protein containing matrix or shell, functional properties of components separately were also addressed. Finally, possible applications were discussed.

2. Protein based encapsulation methods of antioxidants

There are many techniques for encapsulation of food compounds, particularly phenolic compounds and antioxidants. In encapsulation studies, components are often in liquid form. Therefore, most of the time techniques are based on drying, such as spray drying, spray-bed-drying, fluid-bed coating, spray-chilling, spray-cooling, and melting injection (Gibbs et al., 1999; Zuidam et al., 2009). For example, spray drying has been a commonly used method for encapsulation and it generally produces a few ten µm-sized particles (Ferreira et al., 2007). Spray drying is an old, economic, and flexible method for encapsulation in food systems. Spray-chilling and spray-cooling methods were often used for a coating containing lipid, because of the melting point difference; however protein based coating is also convenient for these methods (Kiyomi Okuro et al., 2013). Fluid-bed coating has been applied to powder particles (bioactive material) by spraying atomized coating material with a controlled airflow and temperature. Vacuum and freeze-drying techniques can also be used for encapsulation (Ballesteros et al., 2017); however, the major disadvantage of these methods is the freeze-fractured or high porosity particle formation, which leads to poor protection of bioactive material. Emulsification, which is similar to injection, is another flexible method in encapsulation studies (Donsi et al., 2011; Nedovic et al., 2011). Depending on the emulsion type, either simple or double emulsion, bioactive material can be present in either oil phase or aqueous phase.

Proteins either formed a shell around the active material or formed complexes with the bioactive material. Although it is difficult to separate the two types of encapsulation completely, one of them often dominates the other.

Proteins, due to their gelation properties, are widely used as a shell for the encapsulation of bioactive compounds (Wang et al., 2016; Yao et al., 2018). One way to create a protein shell is to use two oppositely charged materials by inducing electrostatic attraction between these materials. In a study, zein and curcumin as bioactive materials were encapsulated within alginate and gelatin mixture to increase the bioaccesibility of zein and curcumin (Yao et al., 2018). Alginate and gelatin formed a complex, which was negatively charged. The formed complex was able to bind to the zein particles including curcumin, which were positively charged. This electrostatic deposition used in this study was the one layer deposition. Layer by layer deposition can also be done using materials with different sign of charges. Layer by layer deposition is a commonly used method in in-vitro studies, especially for the simulation of gastro and intestinal tract separately (Lau et al., 2017).

Another method to create a protein shell is to induce the gelation of protein around the bioactive material. Protein gelation can be done via denaturing the protein with heating or enzymatic cross-linking. Du and co-workers (2019) used heating and enzymatic hydrolysis for the gelation and complex formation of α-lactalbumin to encapsulate β-carotene and reported that as long as the formed capsules were in the micelle form, protection of α-lactalbumin was similar. They also showed that encapsulation process increase the stability of β-carotene against heating and UV light.

Protein-based encapsulation also has some limitations, particularly in the case of using some techniques. For instance, the low water solubility of proteins limits the use of spray-drying (Desai & Jin Park, 2005). Besides solubility, heat sensitivity of proteins could also limit the use of several techniques, as with the increasing temperatures proteins could aggregate or gel. Therefore, the technique and nature of protein was reported to be important on the encapsulation characteristics (Ozkan et al., 2019).

3. Functional properties of protein encapsulated antioxidants

3.1. Due to the presence of protein

Proteins in encapsulation systems can serve both physicochemical and nutritional properties. As a very well-known fact, proteins increase the satiety effect, decrease hunger and blood glucose level (Diepvens et al., 2008; Pal & Ellis, 2010). In addition, proteins have many techno-functional properties due to their emulsifying and gelling capacities. Due to the amphiphilic nature of proteins, they have very good surface active properties in emulsions including oil and water (Hoffmann & Reger, 2014). Therefore, proteins in microparticulated form can be used as stabilizer at the interfaces, which is also known as Pickering stabilization. In short, Pickering mechanisms tells surface active properties can be increased with colloidal particles and thereby increasing the stability of emulsions and foams compared to the low molecular weight counterparts (Sagis & Scholten, 2014).

As micro- or nano-sized particles including protein behave as colloidal particles (Zhuang et al., 2018), they can also be used as structuring elements (Jansen-Alves et al., 2018). These particles (i.e. microcapsules in encapsulation systems) are able to modify the physical properties of foods such as texture and viscosity.

As an encapsulation material (carrier material) for the bioactive components, proteins from plant origin, such as pea, rice, soybean and ovalbumin (Jansen-Alves et al., 2018) or animal origin, such as sodium caseinate or whey proteins (Wang et al., 2016; Ebert et al., 2017) can be used.

Studies showed that both types of protein could be convenient for encapsulation systems. More importantly, hydrophilic and hydrophobic characteristics of bioactive and carrier materials can be counted as they affect the solubility, and thereby the compatibility of both materials with each other (Yao et al., 2018). Such chemical properties of proteins became important especially in controlled release systems. Yao and co-workers (2018) prepared a controlled release system that works in simulated gastrointestinal tract fluids using encapsulated curcumin in zein nanoparticles. In their study, an acidic solution was used to simulate the gastro system, whereas a neutral or basic solution was used to simulate the intestinal system together with the corresponding enzymes; and then the behavior of capsules were investigated, most commonly measuring the concentration of released bioactive compound. They have found that increased antioxidant capacity, decreased curcumin degradation, and increased curcumin solubility in gastro intestinal tract.

3.2. Due to the presence of antioxidants

Antioxidants, as the name implies, contributes to prevent from or slow down the adverse effects of oxidative reactive species, such as heavy metals or metalloids. Categories of antioxidants may vary on a very large scale, from carotenoids, enzymes and hormones to vitamins, minerals and phenolic compounds (Flora, 2009). These categories indicate the difference in chemical structure and thus the action mechanism of each are also different.

A commonly known antioxidant, vitamin C (ascorbic acid) is a powerful antioxidant, which works well in aqueous media. Ascorbic acid carries electron pairs between hydroxyl and carbonyl groups. Depending on the pH of the aqueous media, ascorbic acid may be present in different reduced forms, which have different chelating property. Reactive oxygen species undergo sequential reactions with ascorbic acid, forming firstly monodehydroascorbate and then dehydroascorbate. Meanwhile, water and unreactive oxygen species are formed from reactive oxygen species (Flora, 2009).

In addition, an antioxidant prevents or slows down the chain reaction by chelating the metal ions. Toxic and carcinogenic properties of metal ions or heavy metals have known for many years (Flora, 2009). Reducing the effects of metal exposure is as important as the prevention. Therefore, the importance of antioxidants is highly important and their bioavailability should be kept efficiently.

Phenolic compounds from green tea was shown to increase the satiety effect in human (Josic et al., 2010) and in vitro studies polyphenols were reported to increase the inhibition of α-amylase (Oboh et al., 2014). Partial inhibition of digestive enzymes decreased the catabolic reactions and nutrient absorption during digestion, which was possibly increase the satiety effect (Foegeding et al., 2017).

In a recent study, the proteins containing leucine, valine, cystein, methionine, and aromatic amino acids (phenylalanine, tryptophane, and tyrosine) were reported to show strong antioxidant properties (Nwachukwu & Aluko, 2019). Such protein-derived antioxidants could work individually as well as could possess synergistic effects together with other bioactive materials. The gap in literature was reported as the development of increased antioxidant bioaccessibility of protein hydrolysates to be used in food systems (Nwachukwu & Aluko, 2019).

4. Potential applications of antioxidant encapsulation with proteins in food systems

In literature, many studies showed the potential applications for antioxidant encapsulation systems in foods (Aziz & Almasi, 2018; Silva et al., 2018; Du et al., 2019). All these studies showed the possible applications of encapsulated, thereby protected, antioxidants in food systems. For instance, β-carotene was encapsulated with α-lactalbumin and researchers showed that the system could be used for the controlled delivery of hydrophobic compounds (Du et al., 2019). Another study aimed the encapsulation of propolis extract in proteins from different sources using spray drying and analyzing the in vitro digestion (Jansen-Alves et al., 2018). They compared the encapsulation efficiency among different proteins, namely rice, pea, soybean and ovoalbumin and found that rice and pea proteins were the best candidates to encapsulate propolis extract, as the highest encapsulation efficiency and antioxidant activity were obtained in the presence of rice and pea proteins.

Phenolic compounds from pomegranate were encapsulated using soybean protein isolate and a good encapsulation efficiency together with low degradation rate constants were reported. Encapsulated polyphenols were added to yogurt matrix and the stability behavior of phenolic compounds was found to be similar with and without encapsulation (Robert et al, 2010). The reason of this similarity could be that the yogurt protected the biomaterial by some interactions within its complex matrix.

In a different study, curcumin and catechin were encapsulated in a water-in-oil-in-water double emulsion using gelatin as in the inner phase (Aditya et al., 2015). The co-encapsulation of two different phenolic compounds was found to increase the stability against oxidation and the bioaccessibility of these compounds compared to the forms of without encapsulation. Similarly, in another study anthocyanins were encapsulated in water-in-oil-in-water emulsion system, and the increased stability of anthocyanins in the inner phase of the double emulsion was reported (Frank et al., 2012).

Alternatively, nanoencapsulation of thyme extract with the use of whey protein based films was found to increase the mechanical stiffness and decrease water vapor permeability; therefore, the films could be a good alternative for food packaging and could also be used in medical fields (Aziz & Almasi, 2018). However, in this study encapsulation process decreased the antimicrobial activity of antioxidant against *S.aureus* and *E.coli*, due to slow release of antioxidants. Therefore, encapsulation of antioxidants to be used in the active packaging systems can still be an open area for investigation.

The subject of dairy beverages enriched with encapsulated antioxidants is also an important subject, as the interaction of proteins with antioxidants could decrease the bioavailability of antioxidants (Silva et al., 2018). Besides these interactions, antioxidants or on broader scale phenolic compounds often have bitter taste; which may alter the taste of food product. As a result, the potential applications of encapsulated antioxidants in dairy beverages are abundant. For instance, epigallocatechin-3-gallate was encapsulated using beta-lactoglobulin, nanoparticles were formed through heat-denaturation of protein, and the protection of epigallocatechin showed that the system could be used in clear beverage enrichment (Shpigelman et al., 2012). As the particles were small enough, the aggregation was not expected and therefore clear beverages could be obtained.

5. Conclusions

In this review, the importance of antioxidant encapsulation with the use of different proteins was emphasized. Susceptibility of antioxidants to heat, light, and oxygen can be decreased by encapsulation with proteins. This protection for bioactive compounds gives stability not only during storage but also for processing. Basic methods for the encapsulation process and their principles were stated. Drying of the mixture of bioactive and carrier compounds and injection of bioactive compound into a hardening solution were commonly used methods in encapsulation studies. Accordingly, complex formation between protein and antioxidants and core-and-shell model were the main mechanisms. Both protein and antioxidants could supply functional characteristics such as nutritional, textural or structural. Encapsulated antioxidants were shown to be used in controlled release systems and in vitro studies, which implied that such systems were useful for the design of functional beverages and medical drinks.

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