Liposomes: Composition, preparation techniques, and diverse applications in food science

A. Semra Aksoy[1*]

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Abstract

In this review, the fundamental characteristics of liposomal constituents required for the development and application of liposomes, as well as the techniques employed for liposome preparation, are explained, along with examples of their use in foods. Liposomes are composed of two-layered membranes formed spontaneously by molecules called phospholipids in an aqueous environment. Molecules of phospholipids have a structure that includes polar (water-associated) head groups and nonpolar hydrocarbon tails. Liposomes can carry both hydrophilic (water-soluble) and hydrophobic (water-repellent) compounds because they have both aqueous and lipid phases. Liposomes can be categorized into various groups based on their sizes and the number of layers. Two main types are referred to as multilamellar vesicles (MLVs) and unilamellar vesicles (ULVs). Various methods are used for the preparing of liposomes, detergent removal technique, including the thin-film method, extrusion method, injection method, heating method, supercritical fluid method, microfluidization, and ultrasonication. The application of liposomal coating is extensively utilized in cosmetics and pharmaceuticals for delivering bioactive substances, medications, and vaccines. Over the last twenty years, food liposomes have become a focal point in food science research, with the anticipation of discovering broad applications in the food industry. Liposomes are used in the food industry for the transport and controlled release of bioactive compounds. Liposomes are particularly intriguing due to their resemblance to cell membranes in terms of composition and structure, which makes them valuable for improving the bioavailability

[1] *Bezm-İ Âlem Vakıf Üniversitesi, Sağlık Bilimleri Fakültesi, Beslenme ve Diyetetik Bölüm, asemraksoy@gmail.com ORCID: 0000-0002-4708-3195*

of encapsulated functional compounds. Diversifying the materials and methods used in liposome preparation and developing high-stability, low-cost liposomes, as in methods such heating method and sonication are important for expanding potential application areas.

Keywords: *Liposome; Bioactive compounds; Stability; Phospholipid; Edible Coating*

Lipozomlar: Bileşimi, hazırlama teknikleri ve gıda biliminde çeşitli uygulamaları

Öz

Bu derlemede, lipozomların geliştirilmesi ve uygulanması için gerekli olan lipozomal bileşenlerin temel özellikleri ile lipozomların hazırlanmasında kullanılan teknikler, gıdalardaki kullanım örnekleriyle birlikte açıklanmaktadır. Lipozomlar, sulu bir ortamda fosfolipid adı verilen moleküller tarafından kendiliğinden oluşan iki katmanlı zarlarla oluşur. Fosfolipid molekülleri, polar (su ile ilişkili) baş gruplarını ve apolar hidrokarbon kuyruklarını içeren bir yapıya sahiptir. Lipozomlar, hem sulu hem de lipid fazlarına sahip oldukları için hem hidrofilik (suya çözünür) hem de hidrofobik (suya dirençli) bileşikleri taşıyabilirler. Lipozomlar, boyutlarına ve katman sayısına göre çeşitli gruplara ayrılabilir. İki ana tip çok katmanlı veziküller (MLV'ler) ve tek katmanlı veziküller (ULV'ler) olarak adlandırılır. Lipozom hazırlamak için ince film yöntemi, ekstrüzyon yöntemi, enjeksiyon yöntemi, ısıtma yöntemi, süperkritik akışkan yöntemi, mikroakışkanlaştırma ve ultrasonikasyon gibi çeşitli yöntemler kullanılır. Lipozomal kaplamaların uygulanması, biyoaktif maddelerin, ilaçların ve aşıların iletilmesi için kozmetik ve ilaçlarda yaygın olarak kullanılmaktadır. Son yirmi yılda, gıda lipozomları gıda bilimi araştırmalarında odak noktası haline gelmiş ve gıda endüstrisinde geniş uygulama alanlarının keşfedilmesi beklenmektedir. Lipozomlar, biyoaktif bileşiklerin taşınması ve kontrollü salımı için gıda endüstrisinde kullanılmaktadır. Lipozomlar, bileşim ve yapı bakımından hücre zarlarına benzediğinden, enkapsüle edilmiş fonksiyonel bileşiklerin biyoyararlanımını artırmada değerli kılmaktadır. Lipozom hazırlamada kullanılan malzemeleri ve yöntemleri çeşitlendirmek, yüksek stabiliteye sahip, düşük maliyetli lipozomlar geliştirmek ve potansiyel uygulama alanlarını genişletmek önemlidir.

Anahtar Kelimeler: *Lipozom, Biyoaktif bileşikler, Stabilite, Fosfolipid, Yenilebilir kaplama*

Introduction

Liposomes are encapsulated sacs characterized by a spontaneously formed phospholipid bilayer structure that distinguishes them from the surrounding watery medium (Deshpande et al., 2016). The discovery of the liposome structure dates back to the early 1960s. During his research into the role of biological membranes, particularly phospholipids, in blood coagulation using electron microscopy, Dr. Alec D. Bangham became fascinated by the ways in which egg lecithin structures interacted with water, as he described it himself (Weissig, 2017).

The term 'liposome' originates from the Greek words 'lipos,' which signifies fat, and 'soma,' which signifies body or structure (Sharma and Agrawal, 2021). Liposomes, having both aqueous and lipid phases, can encapsulate hydrophobic, hydrophilic, or amphiphilic molecules. The encapsulation of hydrophilic and lipophilic molecules occurs within the aqueous core and between the lipid bilayers, respectively, while amphiphilic molecules can be retained in the aqueous/lipid phase depending on their affinity for the liposomal formulation (Laouini et al., 2012).

Liposomes, formed by the self-assembly of lecithin molecules into a bilayered spherical structure, possess the ability to encapsulate functional components soluble in both water and oil within their interiors (Taylor et al., 2005; Laye et al., 2008). Encapsulation of functional components within liposomes has been demonstrated to enhance the stability of the encapsulated substance, reduce its interaction with the environment, thereby allowing it to maintain its activity for longer periods, especially in conditions prone to degradation (Chun et al. 2013).

This review article offers a general overview of liposome structures and liposomal coating materials. Liposome preparation methods, along with their advantages and disadvantages, have been discussed. Promising developments of liposome technology in the field of food, which has found extensive application in pharmaceutical and cosmetic domains, are also exemplified.

Liposome structures

Liposomes are spherical entities constructed from amphiphilic components, typically phospholipids, capable of autonomously organizing into bilayers when in water-based environments. These bilayers consist of two molecular layers oriented so that their hydrophobic regions face each other

(McClements, 2015). The particle size spans a broad spectrum, ranging from 10 nm to several micrometers (Akhavan et al., 2018).

Phospholipid molecules have a hydrophilic polar head group that is attracted to aqueous phases, both internally and externally, and a hydrophobic nonpolar tail group composed of a lengthy hydrocarbon chain that is water-repellent (Liu et al., 2015). The polar head consists of choline ($C_5H_{14}NO^+$), phosphate (PO_4^{3-}), and glycerol ($C_3H_8O_3$), while the nonpolar tail is composed of a hydrocarbon chain (fatty acid) (Daraee et al, 2016). When in contact with water, the hydrophilic head groups of phospholipid molecules orient themselves toward the aqueous phase, establishing a 'water-facing' configuration, whereas the hydrophobic tails of phospholipids generate a 'water-avoiding' surface (Sharma and Agrawal, 2021).

Liposome structures can consist of both small and large single bilayer structures, as well as multilayered structures, and even multilamellar spherical structures containing multiple inner layers. Liposomes can also be classified into two primary groups depending on their size and the quantity of bilayers they possess (Subramani and Ganapathyswamy, 2020; Sharma and Sharma, 1997) : 1. Multilamellar vesicles (MLVs): Distinguished by the existence of numerous lipid bilayers. 2. Unilamellar vesicles (ULVs): Additionally, it can be subdivided into three categories: A. Giant unilamellar vesicles (GUVs), a category of ULV with a solitary lipid bilayer and a size of 1 μm. B. Small unilamellar vesicles (SUVs), a type of ULV with vesicle sizes ranging from 20 to 200 nm. C. Multivesicular vesicles (MVVs), belonging to the ULV category and comprising numerous vesicles that are less than 1 μm in size.

Liposome coating material

The encapsulation capacity, stability, and release of bioactive compounds are strongly linked to liposomes to the components used in liposome preparation (Abdur Rauf, 2023). Various classes of phospholipids are employed in the preparation of liposomes (Abdur Rauf, 2023; Storm and Woodle, 1998). These encompass purified natural phospholipids like phosphatidylethanolamine (PE), phosphatidylcholine (PC) and sphingomyelin; partially or fully hydrogenated natural phospholipids, which offer partially saturated acyl chains; and synthetic phospholipids, including dipalmitoyl phosphatidylgycerocholine (DPPC), distearoyl phosphatidylgycerocholine (DSPC) and dimyristoyl phosphatidylglycerol (DMPC) (Hoogevest and Wendel, 2014).

Synthetic lipids are typically produced through the deacylation or reacylation of natural lipids. Cholesterol is the primary sterol utilized in liposomes, and it easily integrates into lipid bilayers, improving their stability and reducing their permeability. Liposome formation is not a result of covalent bonding between lipid molecules; instead, it emerges from the interactions between hydrophilic and hydrophobic regions of these molecules within an aqueous environment. These interactions encompass: (i) the affinity between water and hydrophilic head groups, (ii) repulsion between water and hydrophobic acyl tails, and (iii) the attraction between the tails. In terms of function, liposomes can be categorized as traditional liposomes, anionic liposomes, cationic liposomes, targeted liposomes, elastic liposomes and stealth liposomes. Liposomes that lack any specific feature or, in other words, are neutral and without surface modifications, are referred to as traditional liposomes or simply liposomes. These represent the most prevalent liposome varieties.

As an alternative in liposome coating, polymers such as polyethylene glycol (PEG), biopolymers like chitosan, and certain antimicrobial peptides are used.

Liposomes are employed to enable the controlled release of bioactive compounds in foods, and regardless of their solubility characteristics, bioactive substances can be encapsulated within liposomes; hydrophobic substances can be encapsulated within the lipid bilayer, whereas hydrophilic substances can be housed within the core. Simultaneous loading of hydrophilic and lipophilic compounds into the same liposome is achievable. After encapsulation, liposomes should retain their payloads until reaching the target, both *in vivo* and *in vitro*, and release them as intended (Abdur Rauf, 2023; Briuglia et al., 201; Kulkarni, 2005).

Methods of liposome preparation

The typical and essential steps in liposome preparation involve four key stages: (1) Drying of Lipids Dissolved in Organic Solvent: To achieve a uniform distribution of lipids in the mixture, a solvent or a combination of solvents is used to dissolve the phospholipid. If the bioactive compounds (BACs) exhibit lipophilic characteristics, a solvent can be introduced and subsequently evaporated. Conversely, if the BACs possess hydrophilic attributes, they can be integrated into the desiccated lipids while hydrating with the aqueous phase (Nkanga et al., 2019; Alavi et al., 2017) . (2) Exposure of Lipids to the Aqueous Environment: Hydrophilic BACs are

introduced into the aqueous phase for encapsulation, where they bind with the hydrophilic constituents of phospholipids during the lipid phase hydration. Hydrophobic BACs may be retained within the phospholipid bilayer (Alavi et al., 2017). (3) Refining of the Formed Liposome: Effective entrapment of the wanted substance is accomplished by eliminating undesired substances, such as non-entrapped BACs and residual solvents. Frequently employed purification techniques encompass dialysis, column chromatography, centrifugation, ultrafiltration and ion-exchange chromatography (Alavi et al., 2017; Dimov et al., 2017). (4) Analysis of the Final Product: The critical parameters for liposome characterization involve encapsulation efficiency (EE), zeta potential, size dispersion, visual observation and polydispersity index (PDI) (Panahi et al., 2017).

EE represents the proportion of encapsulated BAC relative to the overall BAC content within the liposomes. Discrepancies in the quantities of total and unbound BAC can be employed to calculate EE. An exceptionally high encapsulation efficiency indicates the effectiveness of the liposome production technique in use (Shao et al., 2017; Amin et al., 2017; Dasharath and Niteshkumar 2020). The findings regarding zeta potential represents the effective surface charge that influences aggregation and stability on the liposome. Decreasing particle size leads to an enhancement in the surface-to-volume ratio, thereby improving bioavailability. Dynamic light scattering can gauge the size of liposomes, while electrophoretic light scattering can determine their zeta potential (Shao et al., 2017; Amin et al., 2017). PDI quantifies the breadth or dispersion of sizes. Alterations in liposome dimensions following BAC loading can be assessed through this metric by gauging the consistency of size distribution (Amin et al., 2017; Dasharath and Niteshkumar 2020).

Traditional techniques

Thin film rehydration method-The thin film rehydration method is a frequently used approach for crafting liposomes that encapsulate BACs. In this procedure, phosphatidylcholine (PC), cholesterol, and BACs are dissolved in a predetermined organic solvent. Subsequently, these components are arranged either on a surface or within a container to form a thin film. Following this, hydration is carried out with an appropriate buffer solution, such as 4-(2-hydroxyethyl), for example. For the formation of liposomes, 1-piperazineethanesulfonic acid (HEPES), phosphate buffer, or simply a water-based medium can be utilized. The most significant

advantage of this method is the ability to achieve high encapsulation rates with a simple approach. However, this technique comes with significant difficulties, including the potential for residual solvents due to the substantial use of organic solvents, the labor-intensive nature of the procedure, and sometimes the need for sterilization steps. In this method, organic solvents can be completely removed during lyophilization. It can be used to produce SUVs with high EE and high stability. (Maja and Mateja, 2020; Wagner and Voruer-Uhl, 2011).

Injection method-The injection of ethanol or ether is a crucial approach in the creation of liposomes. This technique involves injecting lipids that are soluble in the organic phase into the aqueous medium to produce liposomes (Wagner et al., 2006; Velez et al., 2017). One advantage of this approach efficiently eliminates harmful solvents - such as chloroform - during liposome development. Additionally, this method is easy to scale up. Ethanol, generally recognized as safe (GRAS), is commonly used in the ethanol injection method, providing a more favorable environment for liposome formation (Wagner et al., 2006). This method shows low capacity efficiency (Riccardi et al., 2024). The main disadvantage of this approach is the existence of lingering organic solvent remnants. Additional drawbacks include the risk of breast plugging in ether systems and the need for sterilization (Maja and Mateja, 2020). In the injection procedure, the desired substances (such as BAC and phosphatidylcholine) are dissolved in ethanol and introduced into water to create liposomes. This technique has been effectively used, as an example, to produce liposomes containing conjugated linoleic acid (CLA) isomers and soy phosphatidylcholine (SPC) (Velez et al., 2017).

Extrusion method-In the extrusion method, liposomes are formed through the controlled extrusion of lipid suspensions. The extrusion method involves passing lipids suspensions means of a small-scale extrusion apparatus to create small unilamellar vesicles from multilamellar vehicles. This process facilitates the extrusion of vesicles through small-pore-sized polycarbonate filters using high pressure. Factors such as the pressure of the filters used, the number of cycles, and the pore size affect the characteristics of the extruded liposome, including its average diameter and polydispersity. This technique entails the fragmentation of larger vesicles into smaller pieces as they traverse the pores under the applied pressure (Ong et al., 2016).

The primary advantage of the extrusion method is its ability to produce liposomes with a narrow size distribution and precise control over particle size. It is particularly suitable for applications where size homogeneity is important, such as drug delivery systems (Daraee et al, 2016). This approach provides a straightforward, rapid, consistent, and relatively uniform size distribution (Costa and Santos, 2017). However, the primary limitations of this method manifest as pore clogging, product loss, and restrictions in large-scale production (Costa and Santos, 2017; Maja and Mateja, 2020).

In a study, the extrusion method was used with a polycarbonate membrane featuring a pore size of 200 nm to produce liposomes loaded with tea catechins in egg phosphatidylcholine. The observed encapsulation efficiency (EE) values were 98.1% for EGCG, 57% for (+)-catechin, and 64.7% for (–)-epicatechin (Fang et al., 2006).

Detergent removal method-The detergent removal technique is alternatively referred to as the micelle-vesicle transition procedure (Ollivon et al., 2000). This method allows for the formation of enlarged micelles and achieving a crucial balance between detergent and lipid, facilitating the production of membrane bilayers and liposomes. This technique, used to control particle size, provides a homogeneous product through a simple process. Nonetheless, this approach faces significant drawbacks such as being a time-intensive process, leaving unwanted e substances left in the end product, and potential interactions between the detergent and the enclosed substance (Schubert, 2003). Additionally, the detergent removal process through dilution has been associated with decreased EE for hydrophobic compounds (Meure et al., 2008).

Heating method-Heating processes are used in various areas, from the preparation of liposomes to their stability, drug loading, and controlled release. This technique involves the hydration of phospholipids, and with the addition of a hydrating agent (such as propylene glycol, glycerol, or sorbitol), they are heated above the phase transition temperature. Liposomes are formed directly via the hydration process without the need for dissolving phospholipids in organic solvents. Glycerol, because of its water-solubility, safety, physiological compatibility, and isotonic characteristics, can serve as a hydrating agent. At the same time, it can enhance the stability of lipid vesicles, preventing clotting and sedimentation (Mozafari, 2005).

Within traditional methods, the heating method for preparing liposomes is preferred due to its simplicity and speed, as well as containing fewer organic solvent residues (Khorasani et al., 2018). Liposomes produced using the heating method exhibit minimal deterioration of bioactive lipids due to the higher temperatures, which range from 60 to 120°C. Additionally, a benefit of the heating approach is that the resultant liposomes necessitate less supplementary sterilization, leading to decreased time and procedural intricacies (Maherani et al., 2011).

In a study, nanoliposomes loaded with algal extract were produced using the heating method. The phenolic compounds derived from the algal extract were encapsulated within soy lecithin nanoliposomes, achieving an EE of 50.2% (Savaghebi et al. 2020).

New methods

Supercritical fluid method-Supercritical fluid extraction technology is a process method that offers a green approach using supercritical fluids such as supercritical carbon dioxide ($ScCO_2$). It has been demonstrated that $ScCO_2$, as a non-toxic substance, can replace organic solvents, making it a promising application in the pharmaceutical sector, such as micro and nanocapsule formation of drugs (Trucillo et al., 2019; Santo et al., 2014). Furthermore, GRAS solvents like ethanol can also be utilized to alter the system and enhance efficiency of the procedure (Tsai and W.-C., 2016). The primary appeal of supercritical fluid methods is the reduced solvent usage compared to traditional evaporation methods. However, these techniques come with some disadvantages, notably the high cost and pressure requirements, as well as the use of complex instrumentation(Nkanga et al., 2019).

Ultrasonication sonication-Sonication, a commonly employed method, is frequently utilized in the formulation of liposomes due to its simplicity. This technique involves employing acoustic energy to transform large multilamellar vesicles (MLVs) and clusters into smaller unilamellar liposomes. In this procedure, the duration of application and the pressure wave's intensity are critical factors that govern the size of the resulting vesicles (Cho et al., 2013).

Ultrasonication is considered a sustainable technology employed in emulsion preparation, with the goal of enhanced control over the physical characteristics of emulsions. In this method, size reduction is achieved

using ultrasonic waves, and the growth, formation, and collapse of microbubbles and voids occur through a phenomenon called cavitation. These small bubbles grow to their maximum size and then rapidly collapse, discharging substantial amounts of energy (Wrenn et al., 2012).

These phenomena lead to the formation of high-pressure and high-velocity liquid jets. This process occurs through a liquid medium and results in intense interactions among particles (Cheaburu-Yilmaz et al.,2019; Kumar, 2019). Probe sonication and bath sonication represent two fundamental techniques within the realm of sonication. Both techniques yield liposomes exhibiting comparable traits. Nonetheless, bath sonication offers an advantage in terms of better regulation of process parameters (Akbarzadeh et al., 2013; Pattni et al., 2015).

Products produced by bath sonication or probe sonication are kept under inert conditions (e.g., under nitrogen or argon) at elevated temperatures for 1 hour to obtain a stable ultimate outcome (Panahi et al., 2017). This is a highly efficient, fast, and simple technique. However, this method has some disadvantages, such as limited inner volume, the risk of lipid and compound degradation, and the possibility of contamination of the liposome preparation by the metal probe during probe sonication (Akbarzadeh et al., 2013; Panahi et al., 2017; Cho et al., 2013).

Ultrasonication sonication is used in the production of liposomes for encapsulating therapeutic agents such as anticancer drugs, antibiotics, and anti-inflammatory medications. It is also applied in the production of supplements like Omega-3 fatty acids (EPA and DHA) and vitamins (vitamin C, vitamin E), as well as for carrying neuroprotective agents (e.g., coenzyme Q10) within liposomes.

Microfluidization-A microfluidizer is a high-pressure processing apparatus that transforms elevated liquid pressures into powerful shear forces. This method is recognized as a non-thermal process method that employs a divided pressure flow through a small orifice to guide the flow within the microfluidizer chamber (Devrim et al., 2016).

Utilizing a stationary interaction chamber with a consistent design, liposomes are effectively manufactured by converting elevated pressure into strong shear and impact forces, alongside significant energy dispersion and hydrodynamic cavitation (Davidson et al., 216; Alizadeh et al., 2015).

The production of liposomes involves placing a suspension of liposomal constituents in an aqueous phase inside a dedicated chamber within the microfluidizer. This method allows for the continuous or repeatable production of large quantities of liposomes without the use of detergents, sonication, or toxic chemicals (Yu et al., 2015; Tabatabaei Mirakabad et al., 2016). Microfluidic technology enhances homogeneity by providing uniform-sized desired small ULVs (ultra-low volume). It also offers advantages such as repeatability and reducing post-production processing steps. This method has a minor drawback of requiring the use of elevated pressures throughout the procedure (Al-Amin et al., 2020).

Final product analysis techniques

After the production of liposomes, especially when using new methods, characterization is essential to ensure product quality. There are several characterization techniques, including radiotracers, fluorescence quenching, electron spin resonance spectroscopy, electron microscopy, and nuclear magnetic resonance, each with unique advantages and disadvantages. The key factors in liposome characterization are size distribution, visual appearance, encapsulation efficiency, and Zeta potential (Panahi et al., 2017).

Size Determination Techniques-The size and size distribution of nanoliposomes are crucial for their stability. Electron microscopy is commonly used to determine morphology and stability, while light scattering methods are advantageous for analyzing large numbers of vesicles in an aqueous phase. Both techniques, alongside other cost-effective laboratory methods, should be used together to achieve comprehensive characterization (Panahi et al., 2017).

Visualization Techniques-Various imaging methods are used to visualize nanoliposomes. Phase-contrast optical microscopy is suitable for detecting particles over 290 nm, while polarizing microscopy reveals liposome lamellarity. Scanning electron microscopy, freeze-fracture, and negative staining are standard techniques for structural characterization. Advanced methods like atomic force microscopy (AFM) and scanning tunneling microscopy (STM) provide high-resolution images of biological and non-biological samples (Panahi et al., 2017).

Zeta Potential-Zeta potential measures the total charge on lipid vesicles, indicating the degree of attraction or repulsion between particles. Assessing

zeta potential helps predict the stability and in vivo behavior of liposomes. This measurement is also valuable for monitoring surface modifications, which can affect circulation time and stability (Panahi et al., 2017).

Encapsulation Efficiency-Encapsulation efficiency is often measured using hydrophilic markers like fluorescent dyes or radioactive sugars, with methods including fluorescence spectroscopy, spectrophotometry, and enzyme-based techniques. These methods are also useful for tracking storage stability and retention under biological conditions. The type of liposome and lipid composition influence the retention and leakage of encapsulated material (Panahi et al., 2017).

Food Applications

In order to improve quality, food safety, nutritional content, and longevity by utilizing various constituents that act as antioxidants, antimicrobials, food supplements, and flavor enhancers are frequently added to foods. These are known as functional food ingredients, including minerals, vitamins, polyphenols, bioactive proteins, peptides and carotenoids (Ajeeshkumar et al., 2021). These substances can also enhance the potential of foods to improve health by exhibiting pharmaceutical characteristics such as properties that combat inflammation and inhibit tumors (He et al., 2019).

On the other hand, these substances are sensitive to environmental factors like light and heat, and they typically exhibit limited bioavailability. This situation makes it challenging to utilize them effectively in foods. One a possible resolution for these issues is liposomal encapsulation. Functional food ingredients are protected from environmental factors by liposomal encapsulation (Ajeeshkumar et al., 2021). Liposomes, given their similarity in composition and structure to cell membranes, elevate the bioavailability of the encapsulated functional components (He et al., 2019).

Today, numerous instances of liposome applications in milk and dairy products. The predominant objective in these uses is typically to augment the effectiveness of antimicrobial peptides. Numerous antimicrobial peptides, like those generated by Lactobacillus sakei subsp., exhibit limited stability and can readily interact with constituents of food, resulting in a decline in their efficacy. The use of liposomes in antimicrobial peptides can yield beneficial results in preserving their stability and extending shelf life (Liu et al., 2020).

The application of liposomes in cheese was initially done to accelerate maturation and prevent taste defects caused by the addition of protease. The economy of cheddar cheese maturation using liposome-coated enzymes is widely practiced and is 100 times more efficient than other methods (Law and King, 1985). Nisin, primarily employed as a food preservative in milk and dairy items, is an antimicrobial agent derived from lactic acid bacteria and based on peptides. However, when it comes into contact with food components, it can enzymatically degrade and become ineffective (Pan et al., 2020). *Clostridium tyrobutyricum* is an agent that causes cheese spoilage. Upon the application of liposomal nisin to a cheese mixture contaminated with 3.5 log CFU g^{-1} of *Clostridium tyrobutyricum*, the spoilage-causing bacteria were entirely suppressed within 2 weeks at 4°C, and no subsequent growth occurred for up to 4 weeks (Hassan et al., 2021).

A study reported that the addition of cholesterol to liposomal bilayers in a double-layered structure could affect the stability and distribution of nisin in food matrices compared to liposomal membranes without cholesterol, without disrupting the milk fermentation process. Studies have demonstrated that encapsulating two or more antimicrobial agents within liposomes produces a synergistic impact in dairy items (Laridi et al., 2003).

Lysozyme is a naturally occurring antibiotic known for its potent antimicrobial properties, particularly against Gram-positive bacteria. Specifically, the concurrent use of lysozyme and nisin results in synergistic antimicrobial effects. Utilizing liposomal delivery of the lysozyme-nisin complex in whole milk contaminated with *L. monocytogenes* demonstrated prolonged release and increased antimicrobial effectiveness compared to non-liposomal administration (Lopes et al., 2019).

They encapsulated doum fruit extract powder for application in yogurt, and adding it to yogurt increased the stability of phenolic compounds. However, they observed that as the load of doum fruit extract powder increased, certain properties such as stickiness and gumminess were adversely affected. Ultimately, it has been concluded that the addition of liposomes containing 5% doum fruit extract powder can provide higher antioxidant activity to yogurt without significantly altering its fractional characteristics (El-Said et al., 2018).

To extend the shelf life of rice flour, curry leaf oil, a natural preservative,

was used. It was observed that when curry leaf oil was encapsulated in liposomes and added to rice flour, it exhibited antibacterial activity against the food pathogen *B. cereus* (Cui et al., 2017a).

An aqueous extract derived from garlic is a well-known natural antimicrobial substance that exhibits effectiveness against various foodborne pathogens. Using liposomes to apply garlic extract has shown a consistent release of the extract and a continuous inhibition of *Escherichia coli* in simulated food environments. Consequently, it has led to a more substantial decrease in *E. coli*., especially during long-term storage (30 days) at 25°C, compared to non-liposomal applications (Nazari et al., 2019). The applied of liposomal garlic extract on bread displayed antifungal properties, ultimately extending its shelf life (Pinilla et al., 2019).

Clove oil, when encapsulated in liposomes and applied to the surface of vegetables, has been used as a biopreservative to inhibit the formation of *Escherichia coli* (Cui et al., 2020). It has been observed that liposome-coated chitosan added to beef did not cause any changes in sensory characteristics and exhibited high antibacterial activity against *E. coli* O157:H7 (Cui et al., 2017a; Cui et al., 2017b).

Vitamins, commonly used as food additives for antioxidants and dietary supplements, are extremely sensitive to light and heat. Liposome technology has been shown to enhance the bioavailability and storage performance of vitamins. Liposomes shield encapsulated vitamin A from light-induced degradation. When vitamin A is enclosed within liposomes and subjected to fluorescent light at 25°C for 2 days, more than 80% of the vitamin A remains intact, whereas non-liposomal vitamin A is diminished to below 20% (Lee, et al., 2021). Today, liposomal forms of vitamins C and D are preferred as dietary supplements, offering up to 80% bioavailability. Studies have demonstrated that liposomes efficiently safeguard encapsulated vitamins C and E from deterioration when subjected to pasteurization at 65°C for 30 minutes. The antioxidant properties of liposomal vitamins C and E were retained for a duration of 7 days when stored at 4°C. Furthermore, the addition of liposomal C and E vitamins to chocolate milk hardly altered its organoleptic characteristics (Marsanasco and Alonso 2022). Waters enriched with D vitamin-loaded liposomes are another example. Studies have shown that lipid peroxidation in fruit juice during storage significantly decreased after the addition of C vitamin-loaded liposomes to the fruit

juice. Liposomal vitamin C has been shown to increase skin permeability and enhance collagen synthesis by fibroblasts (Maione-Silva et al., 2019).

Carotenoids and polyphenols, which are found in abundance in spices and vegetables, have been linked to potential health advantages, including the potential to lower the risks of various diseases like cardiovascular ailments, cancer, and neurodegenerative conditions. Nonetheless, their susceptibility to environmental influences like oxygen, heat, acids, light, and their limited bioavailability pose challenges for their utilization in the fields of food and pharmaceuticals (Boonlao et al., 2022). The use of liposomal coating has shielded carotenoids enclosed within it from lipid oxidation and ensured their continuous release (Rostamabadi et al., 2019). Following a 30-day storage period at 4°C, findings indicated that 85.2% of the encapsulated beta-carotene remained intact, and there was an enhancement in beta-carotene bioavailability during gastrointestinal digestion (Ji et al., 2023). After encapsulation of phenolic compounds obtained from grape seed oil and pomegranate seed oil residues through extraction, they were added to salad dressing. It was determined that they exhibited natural antioxidant properties and had a positive impact on the product's shelf life (Aksoy et al., 2021). When carrot carotenoids and phenolic compounds are coated with liposomes with high encapsulation efficiency, they are effectively preserved without thermal degradation (Esposto et al., 2022).

The study explored the encapsulation of fish protein hydrolysate (FPH), fish oil (FO), and shrimp lipid extract (SHE), and subsequently applied various coatings to the prepared nanoliposomes. Whey protein concentrate (WPC) and chitosan (CS) were applied singly/doubly and in a composite mannerDuring storage, the physicochemical properties and oxidative stability of liposomes were investigated, and the results have shown that uncoated liposomes are unstable for a short period and that the presence of coating has a positive impact on the physical stability of liposomes. The encapsulation of marine bioactive compounds in liposomal carriers and their encapsulation with WPC and CS in single/double layers have been demonstrated to be a potential approach for enhancing the antioxidant properties and application in food products (Amiri et al., 2023). The impact of coating liposomes with chitosan and hydrolysates from pea protein isolate was assessed, with flaxseed oil liposomes serving as a representative model. The findings indicated that liposomes covered with chitosan and hydrolysates from pea protein isolate exhibited enhanced

resistance to oxidation throughout storage and a more gradual release of flaxseed oil during simulated digestion (Song et al., 2023).

Conclusion

The application of liposome coatings has found widely employed in delivering bioactive substances, medications, and vaccines within the realms of cosmetics and pharmaceuticals. Food liposomes have been a focus of research in the field of food science over the last twenty years, with the anticipation that they will have extensive use in various food products. Liposomes are of particular interest for enhancing the bioavailability of enclosed functional components because they share similarities in composition and structure with cell membranes. Nonetheless, their utilization in food products has been restricted due to a variety of obstacles. Among these challenges, the cost of key raw materials like phospholipids used in liposomes, as well as the storage stability of food liposomes greatly influenced by the physicochemical properties of phospholipids, stands out. The development of highly stable and low-cost liposomes for use in the food industry may be possible through systematic research. Additionally, the development of faster methods for liposome preparation is seen as a necessity.

Overall, a combination of traditional and innovative techniques can be utilized to prepare liposomes that contain bioactive compounds (BACs) for use in functional foods. In particular, advanced methods like ultrafiltration, supercritical fluid extraction, and microfluidization offer advantages over conventional techniques when it comes to generating high-quality liposomal products and streamlining the scaling process.

In conclusion, liposome technology holds significant potential in food applications, particularly in terms of improving bioavailability, antioxidant activity, and antimicrobial properties. Future research could explore the preparation of liposomes with different components and methods. Additionally, efforts to develop high-stability and cost-effective liposomes could expand their potential application areas.

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