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Green Tea and Rosemary Extracts in Free and Encapsulated Liposome Forms: Natural Antioxidants in Sports Science

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ABSTRACT

Natural Antioxidants are compounds with many advantages, but their applications have some limitations, including bioavailability and instability. Liposomes are appropriate carriers that can be used for antioxidants to protect them from unfavorable conditions. Therefore, they are used in various cosmetic, pharmaceutical and food industries. One of the most critical applications of liposome antioxidants in medicine is cancer prevention, which happens by reducing oxidative stress. Encapsulated liposome antioxidants increase the therapeutic effects because the higher drug concentrations reach the cancer tissue. Also, due to the harmful effects of synthetic antioxidants, the use of natural antioxidants has increased. Polyphenols have strong antioxidant properties. This study critically reviews the green tea and rosemary extracts as natural antioxidants and their encapsulation. Green tea and rosemary extracts indicated excellent antioxidant properties in food oils. Also, the encapsulated green tea and rosemary extracts in liposomes had more substantial antioxidant features than their free forms. Green tea extracts in both free and encapsulated liposomes had strong antioxidant properties in canola oil, while liposomes containing green tea extracts acted better than free forms of extracts and synthetic antioxidants and caused the production of the first and second products to be delayed. Some of the natural antioxidants have more potent antioxidant properties than synthetic antioxidants, such as BHT, which causes the oxidation ratio in food oils to be delayed. The controlled release and the better performance of natural antioxidants cause the encapsulation of polyphenols.

Keywords: Antioxidant, Encapsulation, Liposome, Liposomal sports supplements, Natural antioxidants in athletics, Oil, Oxidation, Performance nutrition.

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1. Introduction

Nowadays, the extensive advantages of natural antioxidants have caused their applications in various fields such as food, pharmaceutical, and cosmetics to increase. Also, novel maintenance methods such as antioxidants have been widespread in food science. One of the most effective methods to decrease oxidation reactions includes antioxidants in oils and fats. To preserve desired quality and increase the shelf-life of oils and fats, synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole are added to foods, including more harmful than beneficial effects. Due to increasing consumer awareness and worries about consuming chemical additives, the consumption of natural additives has significantly increased. Adverse effects of synthetic antioxidants, such as mutation, poisoning, and carcinogenicity, have replaced synthetic with natural antioxidants, including polyphenols, vitamins, and plant extracts with protective effects against chronic diseases, cardiovascular diseases (CVD), and diabetes (1). Most herbal extracts are biologically unstable and sensitive to light, heat, and oxygen. Therefore, encapsulation is used to increase their solubility, bioavailability, efficiency, and controlled release (2). Encapsulation is trapping active compounds inside other materials or walls, producing particles at picometer, nanometer, micrometer, or millimeter sizes. Encapsulation technology is used in food science as an appropriate carrier for susceptible food compounds such as flavorings,

vitamins, and antioxidants to protect them against environmental factors, including light, heat, and free radicals. The choice of encapsulation method should be proportionate to the molecular and physicochemical properties of bioactive substances. The most important purposes of using encapsulation technology can be summarized as follows (3):

- 1- Protection of encapsulated compounds by decreasing the interaction of bioactive compounds with the environment.
 - 2- Prevention or delaying evaporation of volatile compounds by decreasing transfer rates.
 - 3- Modification in physical properties of encapsulated compounds.
 - 4- Controlled releasing encapsulated compounds.
 - 5- Covering unfavorable tastes of encapsulated compounds.
 - 6- Possible separating internal compounds of mixtures that can react with each other.
 - 7- Uniform suspending encapsulated compounds.
- One of the important problem in the applying of natural antioxidants in pharmaceutical

In literature, polyphenol encapsulation has carried out using spray drying (4), liposome (5, 6), and freeze-drying methods to overcome limitations of using plants extracts, including phenolic compounds. The conventional and new methods to prepare liposomes containing polyphenols (7) were listed in Table 1 (8-14) and Table 2 (15-22), respectively.

Table 1. Conventional methods for the production of liposomes

Conventional method	Advantage	Disadvantage	Application	Reference
Bangham method	Simple operation	-Time-consuming of the solvent removal stage -The liposome production with micron size -Low entrapment efficiency, especially for water-soluble compounds -Small scale production -Heterogeneous in size and shape	To encapsulate liposomal and nanoliposomal polyunsaturated fatty acids	(7, 9-11)
Detergent depletion method	A mild and homogeneous process to produce of the liposomes	-The low concentration of final production - Low encapsulation efficiency for hydrophobic compounds -Detergent remaining in final production	To prepare proteo-liposome formulations	(9-12)
Ethanol injection	-Simple operation -Control to particle size	-The poor solubility some of lipids in ethanol - heterogeneous liposomes	To encapsulate of hydrophobic and hydrophilic drugs	(8-12)
Reverse phase evaporation	-Simple process -The high encapsulation method	-Contact the encapsulated compound with organic solvent	To prepare Amphotericin B liposomes	(9)
Microfluidic channel	-Control size and size distribution of the liposomes -A mild and homogeneous process to produce of the liposomes	- Using of organic solvent -Improper for bulk production,	Drug encapsulation	(13)
Detergent dialysis	-The production of homogenous liposomes	-The applying of organic solvent	–	(14)

Table 2. Novel methods for the production of liposomes

Method	Advantage	Disadvantage	Application	Reference
Heating method	- Simple operation - Not use of hazardous any chemical - Without the need to the pre-hydration of the applied compounds	Applying high temperatures	To encapsulate food-grade antimicrobial Nisin	(17, 18, 22)
Spray drying	-The usable method in industry -Fast single step method	- Need to expensive equipment -Requires high expertise	Microencapsulation of green tea extracts in mango drink	(19)
Freeze drying	-The homogenous formation of lipid particles in water-soluble carrier	-Time consuming - Need to the expensive equipment	–	(16)
Membrane Contactor	-Simple operation - Large scale production - The size control of the solid-lipid nanoparticle	-Use of high temperature - High cost	To produce liposome in large scale	(21)
Super Critical Reverse Phase Evaporation (SCRPE)	-One-step new method -The high encapsulation efficiency for both water-soluble and oil-soluble compounds -The narrow particle size distributions	-Expensive Method	Pharmaceutical powder systems	(15, 23)

2. Lipid Oxidation

Lipid oxidation is an important concern of the food industry. Formed compounds by oxidation create adverse effects on tissues, appearance, and nutrients of foods (23). Moreover, oxidation of biomolecules may cause chronic diseases such as CVD, cancers, and diabetes (24). Hydroperoxides are the first products of lipid oxidation. These compounds are unstable and decomposed to alkoxy and peroxy radicals, which are active and can initiate autoxidation. Autoxidation includes oxidative degradation of unsaturated fatty acids (USFA) through catalytic processes and chain reactions of free radicals (25). The oxidation rate of lipids is affected by internal and external factors such as fatty acid (FA) composition, FA quantity and activity, pH, the ionic composition of the water phase, temperature, oxygen concentration, the surface area in contact with oxygen, and water activity. The lipids oxidation in foods can occur by free radical (autoxidation), photooxidation, and lipoxygenase (26).

3. Antioxidants

A protection method against oxidation includes the use of compounds with antioxidant properties. Lipid oxidation by removing hydrogen atoms and addition of oxygen is progressed, producing peroxy radicals. Antioxidants are significant factors that can neutralize free radicals and reactive oxygen species (ROS). Furthermore, antioxidants can sequester metal ions, which are needed to produce active oxygens. Although the human body has defensive

mechanisms against oxidation, the addition of these compounds to human diets improves these mechanisms. Free radicals in high quantities can attach to other chemical compounds, destroying chemical structures and producing free radicals. By transferring electrons to free radicals, antioxidants stabilize these components and disrupt oxidation chain reactions (27). Antioxidants can be categorized as synthetic and natural antioxidants based on their origins. Synthetic antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole, and tert-butylhydroquinone are widely used worldwide to delay or inhibit oxidation. In recent years, increased consumer awareness of the harmful effects of synthetic antioxidants has decreased consumption of these chemicals despite their compelling performances, low prices, and high stabilities in foods (28). The side effects of synthetic antioxidants have been investigated in various studies, and their use has been questioned due to their possible toxicity and carcinogenicity(29). Therefore, strong synthetic antioxidants such as TBHQ are not used. Moreover, BHA synthetic antioxidants have been removed from the generally recognized as safe (GRAS) list (30). Thus, consumption of natural antioxidants with plant origins instead of synthetic antioxidants has increased. In this regard, extensive studies have been carried out to find natural antioxidants. Natural antioxidants include lower volatility and higher acceptability rates compared to those synthetic antioxidants. The former biochemicals act as preservatives and decrease synthetic additives (31). The most important natural antioxidants,

which various methods have encapsulated, have been shown in Table 3.

Table 3. Physicochemical properties of the natural antioxidant liposomes

Antioxidant	Preparation Method	Particle size (nm)	Z-potential (mV)	Entrapment Efficiency (%)	Reference
Green tea polyphenol	- High-pressure homogenization	140	-20	60	(18, 19, 32, 33)
	- Thin film ultrasonic dispersion method	160.4	-67.2	60.09	
	-Mozafari method	419	-57	51.34	
	-Spray drying	—	—	71.41 -88.04	
Green tea epigallocatechin gallate and catechin	-Reverse-phase evaporation method	180	-42.4 to -	85.79	(34, 35)
		133	46.1	70	
	- High pressure homogenisation	—	—	—	
Curcumin	-Dynamic high-pressure microfluidization	68.1	-3.16	57.1	(36, 37)
		135.5	—	65	
	- High-pressure homogenizer	—	—	—	
Rosemary	Freeze Drying	154.9	18.4	58.71	(38)
Origanum dictamnus	Thin film and ultrasonic method	275	-10.5	—	(39)
Grape seed polyphenols	High-pressure homogenization	66.7	+65	99.5	(6)

One of the most important natural antioxidants is phenolic compounds. Phenolic compounds are bioactive materials widely detected in plants and act as free radicals and metals inhibitors. These compounds prevent lipid oxidation. Therefore, phenolic compounds are promising compounds and strong protectors against lipid peroxidation. Effects of natural antioxidants derived from several plants, such as rosemary (40) and green tea (41), have been studied. Nevertheless, their activities are naturally limited due to their poor bioavailability and instability against unfavorable conditions. Various methods have been described to improve their antioxidant properties. Of these methods, liposome carrier systems are a novel promising method to solve the problems of using phenolic compounds.

4. Green Tea as Natural Antioxidant

Antioxidants can be grouped into first and second groups. The first group causes lipidic free radical stability and disruption of oxidation chain reactions. The second group decreases lipid oxidation through inactivating single oxygens, chelate metal ions, absorption of ultraviolet rays, and recovery of the antioxidants within the first group. Polyphenols of green tea include the highest antioxidant power. This property is majorly linked to chemical structures of aromatic rings and hydroxyl groups that are combined with free radicals to neutralize them. Extensive researches have shown that green tea polyphenols act effectively in the entrapment of active oxygens such as superoxide anions, peroxy radicals, and single oxygens by donating electrons

(42). Phenolic compounds can chelate copper and iron ions via hydroxyl and carboxyl groups (43). Moreover, these compounds can show their antioxidant properties by inhibiting peroxidase enzymatic activities (44). The antioxidant function of green tea catechins is majorly linked to the substrate types (45). Catechins show the highest antioxidant activity in oils while acting as peroxidants in oils/water emulsions (46). Zhong and Shahidi reported that the antioxidant activity of epigallocatechin gallate increased through lipophilicity enhancement and esterification of epigallocatechin gallate with selected FAs, including stearic acid eicosapentaenoic acid and docosahexaenoic acid. Produced lipophilic derivations included better antioxidant properties compared to those the primary epigallocatechin gallate did (47). Jahanfar et al. assessed the effects of green tea extracts entrapped in liposomes at 200, 600, and 1000 mg/L concentrations on the oxidative stability of canola oil. Peroxide, anisidine, TOTOX values, and thiobarbituric acid were assessed. Results showed that increases in the green tea extract concentrations to 600 mg/L decreased the oxidation rates of canola oil. In contrast, green tea extracts imported into the liposome at 1000 mg/L concentrations included peroxidant activities (48). Wanasundara and Shahidi used 500 and 1000 mg/L concentrations of green tea extracts in menhaden oil. They reported that green tea extracts at 1000 mg/L concentrations included prooxidant properties due to the further presence of chlorophyll (49). Gramza et al. assessed the antioxidant properties of green tea's ethanolic and water extracts on sunflower and lard oil oxidations. Oxidation of oils at 100 °C was investigated using the

Rancimat test. Results demonstrated that the most antioxidant activity as induction time (hour) was linked to ethanol green tea extract at 1000 mg/L concentrations, while the highest induction time was linked to lard oil for ethanolic extracts at 500 and 1000 mg/L concentrations. The antioxidant property of green tea extract in the Rancimat test was associated with epicatechin gallate, epicatechin, and catechin. Various studies revealed synergistic and antagonistic effects of the compounds, called the mixture effect (50). Korczak et al. stated that the antioxidant effects of plant extracts depended on polyphenolic compounds and their potential interactions. Better understandings of these interactions result in improved extraction processes, improving the antioxidant properties of the compounds (51). Chen et al. investigated antioxidant activities of white, green, oolong, black, yellow, and dark-green teas on canola oil at 100 °C via oxygen consumption and linoleic linolenic acid changes of the oil. Green, white and yellow ethanol tea extracts were more potent than butylated hydroxytoluene to prevent canola oil oxidation due to the presence of natural polyphenols in the extracts (52). Wanasundara and Shahidi studied the antioxidant function of green tea catechins on fish oil. They reported that catechins had more oxidative stabilities than α -tocopherol, BHA, and BHT. The catechin antioxidant activity increased in epicatechin gallate, epigallocatechin gallate, epigallocatechin, and epicatechin, respectively (53).

5. Rosemary as Natural Antioxidant

The phenolic diterpenes of rosemary extract possess the antioxidant activity which can scavenge hydroxyl radicals, singlet oxygen, and lipid peroxy radicals. Therefore, these components will be able to prevent of lipid oxidation (54). The most important phenolic diterpenes of rosemary extract are carnosic acid, carnosol, and rosmarinol and carnosic acid (55). Chen et al. assessed the antioxidant effects of rosemary ethanol extracts (REE) on the storage of sunflower oil at 60 °C and compared with synthetic antioxidants. Results showed that the peroxide ($75.7\text{ meq/kg} \pm 0.47$), thiobarbituric acid ($0.161\text{ }\mu\text{g/mL} \pm 0.002$), FA ($0.45\text{ mg/g} \pm 0.04$), and anisidine (12.4 ± 0.02) values were lower than those of oil samples containing synthetic antioxidants such as BHA,

BHT and TBHQ at 200 mg/L concentrations after three weeks of storage at 60 °C. They reported that rosemary extract included more potent antioxidant properties compared to others (56). Turan studied the effects of rosemary methanol, ethanol, and acetone extracts on canola oil oxidative stability and pure triacylglycerols. The REE included the highest antioxidant activity at 250 $\mu\text{g/mL}$ concentrations, followed by the methanol and acetone extracts (57). The antioxidant properties of rosemary extracts depend on diterpene compounds such as carnosol, carnosic acid, and rosmarinic acid (58). Moreno et al. reported total quantities of phenolic compounds such as rosmarinic acid, carnosol, and carnosic acid in rosemary ethanol and acetone extracts as 52.2 and 36.5%, respectively (59). Yang et al. used rosemary extract at 400 mg/kg concentrations as a natural antioxidant and a combination of BHA and BHT at 200 mg/L concentrations as synthetic antioxidants and studied their effects on soybean rice bran and cottonseed oil oxidative stabilities. Rosemary extract was more effective than synthetic antioxidants in decreasing peroxide values. Furthermore, they reported that all oil samples with rosemary extract included higher induction times in the Rancimat test. Increases in induction times indicated greater oxidative stabilities of the oil samples (40). Abramovic and Abram investigated the antioxidant properties of rosemary extracts at 0.2% concentrations on oxidative stability of the *Camelina sativa* oil. They assessed the peroxide values every three months for 11 months. The peroxide value of the samples was lower than that of the controls. Moreover, the antioxidant function of the rosemary extracts in oil was studied using the Rancimat test. Results determined that the induction time of the oil samples with rosemary extracts was 60% higher than that of other samples (60).

6. Liposome as Encapsulation Vesicle

One of the encapsulation methods includes the entrapment of materials inside liposomes. Liposomes can be used as appropriate carriers due to their protection and controlled release of bioactive compounds and nutraceuticals (41, 61, 62). The constituent compounds of liposomes have shown in Figure 1.

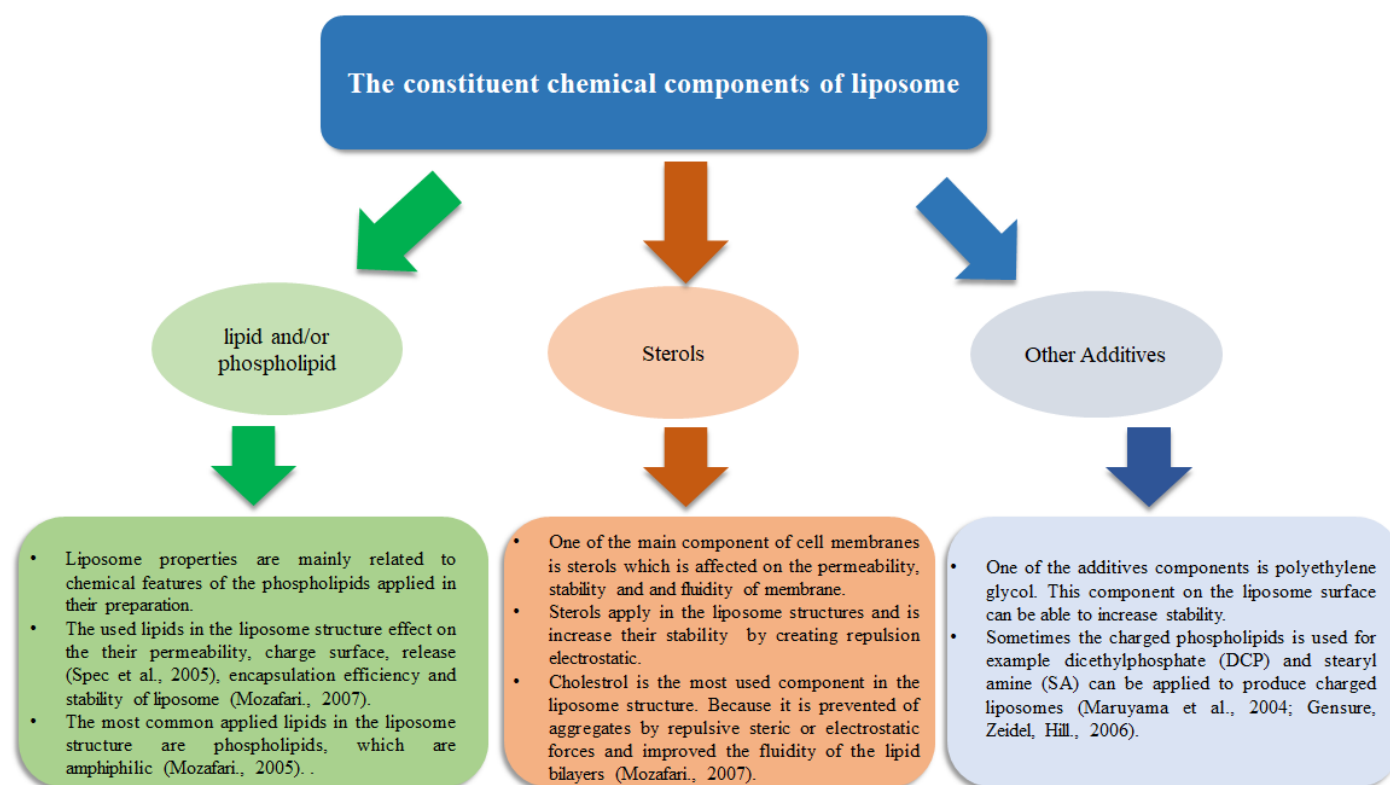


Figure 1. The main constituent compounds of liposome structure

One of the most important components of liposome are phospholipids. When phospholipid molecules are placed in an aqueous environment, the cause of the amphiphilic property are formed a linear structure. In this case, hydrophobic sections are moved away from the water molecules and placed side by side while are in contact with water molecules by hydrophilic head groups. If enough energy (such as heat, homogenization, sonication and etc) is provided, phospholipid molecules are rearranged and formed a spherical structure which are called liposome (Figure 2). Liposomes include liquid spherical structures

with aqueous cores surrounded by single (unilamellar) or several (multilamellar) lipid bilayers. To prepare liposomes containing green tea extract by Mozafari method, in the first, green tea extract was mixed with distilled water. Then phosphatidylcholine (2.5-4.5 %) and glycerol (3% v/v) were added to the green tea extract solution. The mixture was agitated on a hot plate stirrer at temperatures of (50° - 70°C) for different time intervals (30-60 min) at a mixing speed of 1,000 rpm. Finally, the mixture was placed at room temperature for one hour in order to increase the stability of the liposomes (18).

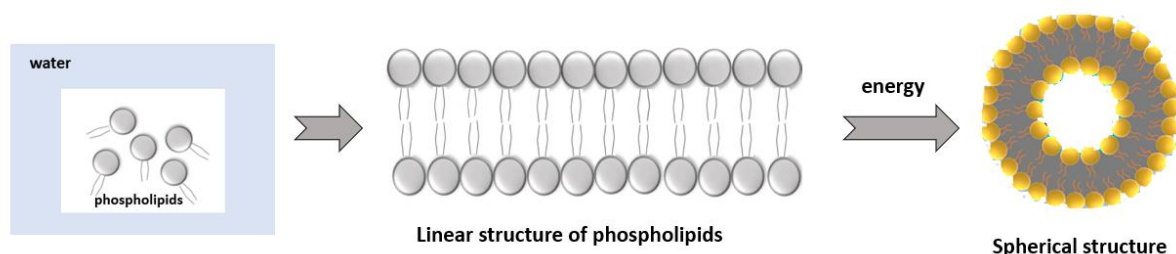


Figure 2. A simple schematic diagram of liposome formation

As can be seen in Figure 3, hydrophilic components are placed in the center of liposome, while hydrophobic compounds are entered to lipidic bilayers. One of the significant advantages of liposome structure includes its ability to imprison hydrophilic and hydrophobic bioactive compounds simultaneously. Another advantage of liposomes is their natural constituents. Phospholipids are amphiphilic molecules, which possess a hydrophilic polar head and two non-polar hydrophobic chains. Phospholipids include a strong tendency to form membranes in suspended aqueous solutions. While polar heads react with the aqueous

environment, non-polar aliphatic chains tend to react with each other (63). Various methods have been described as preparing liposomes, categorized as mechanical and non-mechanical methods. Important mechanical liposome preparation methods in food science are microfluidization, high-pressure homogenization, ultrasonication, and extrusion (64). The most critical non-mechanical methods include reverse-phase evaporation (63), detergent dialysis (65), freeze-drying, freeze-thawing (63), and heating methods (66).

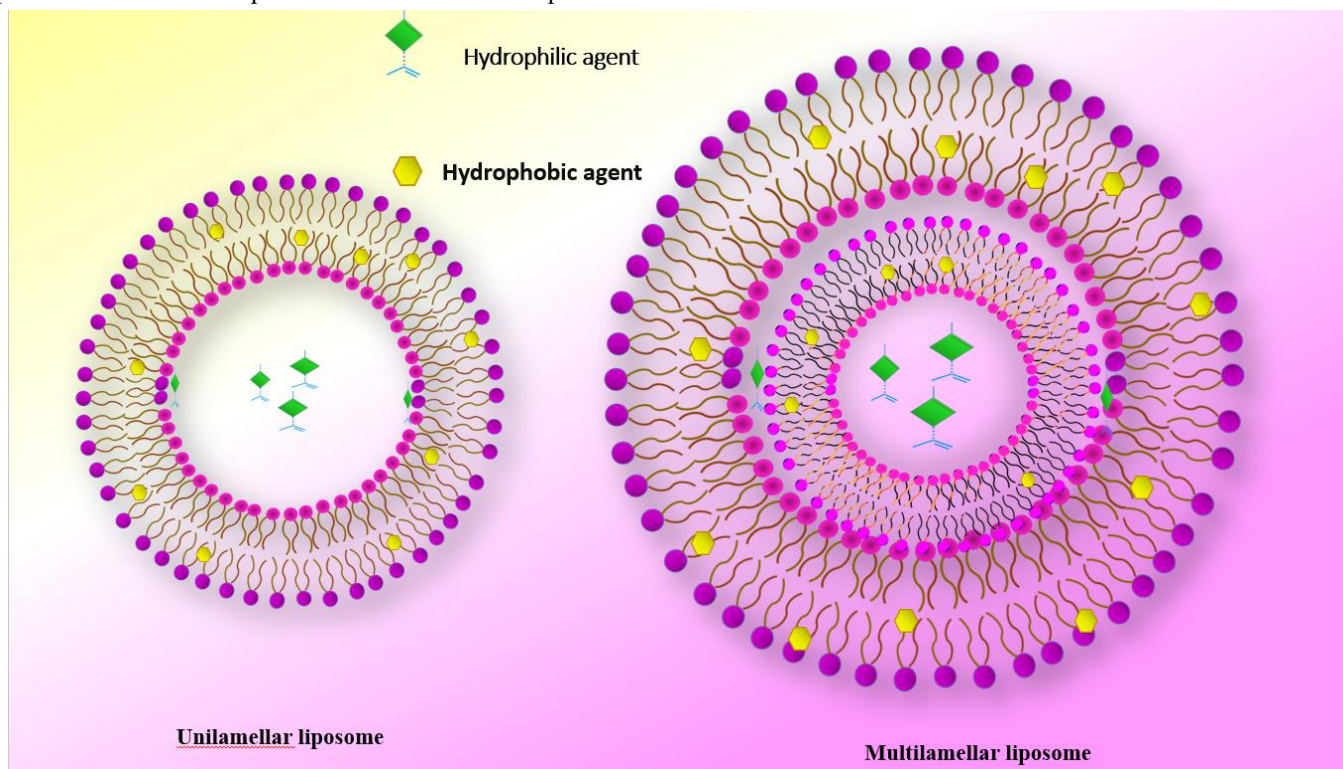


Figure 3. Schematic drawing of lipophilic or hydrophilic antioxidant entrapment models in unilamellar and multilamellar liposomes

7. Liposome Use in Food Industries

In recent years, liposomes have widely been researched as carrier systems in medicine (67-69). Liposome technology has attracted attention from food scientists for the encapsulation of nutrients, including vitamins (70), enzymes such as proteases (71-74) and lipases (75) and antimicrobial agents (76-78). In addition, liposomes can be used to improve novel tastes and colors and change the textures of food compositions. Liposomes can increase the absorption and availability of food compounds and healthy complements. Liposomes can participate in structures of

novel packaging materials for food products, improving their inhibitory and antimicrobial properties (64).

8. Antioxidant Entry Mechanisms of Liposomes

Since phospholipids of liposomes include amphiphilic properties, they can entrap hydrophilic and hydrophobic bioactive agents. Therefore, hydrophobic antioxidants are encapsulated in aqueous parts, while hydrophobic antioxidants are entrapped in lipid bilayers (79). Two standard methods are available to encapsulate hydrophilic antioxidants, including passive and active methods (80). In the passive loading method, lipids are dissolved in aqueous solutions as dried thin films, including target antioxidants

(81). The loading efficiency in this method is low, while the water phase and added cholesterol during liposome formation affect the encapsulation efficiency. In contrast, the encapsulation efficiency of the active method to imprison hydrophilic antioxidants is high. The pH gradient of the membrane in the active loading method transfers the antioxidant molecules (82). In contrast, hydrophobic antioxidants enter the lipid bilayers when liposomes are formed (80). The critical reason for this is linked to their chemical structures, affecting the membrane properties (83). Moreover, extensive studies have revealed that the position and orientation of hydrophobic antioxidants inside bilayer lipids positively affect delayed oxidation processes of the membranes (84).

9. Antioxidant Release Mechanism of Liposomes

Low physical stability is one of the significant important reasons that involves controlled releases of antioxidants from liposomes. Two major factors, which cause physical instability, include (1) changes in particle sizes that can occur because of aggregation and fusion of the vesicles, and (2) ruptures in liposome membranes and hence leakage of the entrapped antioxidants. Decreases in liposome surface loads cause particle instability. With low surface charges of the particles, the particles tend to aggregate or fuse, compared to liposomes with further surface loads (85). Two major factors, which affect liposome stability, include particle size and structural composition of the liposomes. Further stabilities of the liposomes cause slow releases of the entrapped antioxidants in liposomes, increasing antioxidant effects on oxidation processes. Nowadays, nanotechnology techniques are used as appropriate delivery systems for the encapsulation, protection, and controlled releases of bioactive components and nutraceuticals (63). In 2015, Chen et al. encapsulated curcumin in nanoliposomes. Curcumin is a natural multiple-function compound. However, the compound used in food and pharmaceutical industries has been limited due to its physicochemical instability and low availability. Results of the study showed that encapsulation of curcumin in nanoliposomes increased their solubility and stability. Encapsulation efficiency, particle size, polydispersity index, and Z-potential of curcumin in liposomes included 57.1%, 68.1 nm, 0.246%, and -3.16 mV, respectively. Compared to the free form, encapsulated curcumin included good stability against alkaline pH, metal ions, and storage at 4 °C (36). Gortzi et al. studied the antioxidant activity of *Myrtus communis* extracts before and

after encapsulation in liposomes. Assessment of the extract antioxidant activity on sunflower oil stability was carried out using the Rancimat test. Then, malonaldehyde content was assessed and compared with commercial antioxidants such as BHT and α -tocopherol. Results demonstrated that the extract at 160 mg/L concentrations included more potent antioxidant activity than the commercial antioxidants. Moreover, encapsulated extracts showed higher antioxidant properties compared to those the free extracts did. The most common methods to produce liposomes are thin-film hydration and ethanol injection, including disadvantages such as organic solvents, heterogeneous particle sizes, and inappropriateness for industrial-scale productions. In contrast, the Mozafari method can produce liposomes and nanoliposomes in one step with no organic solvents using simple equipment (39). Zou et al. stated green tea polyphenols as susceptible antioxidant compounds affected by oxygen level, alkaline pH, and concentration. Liposomes entrapped green tea extracts increased the stability of the phenolic compounds. Although green tea polyphenols were primarily susceptible to destruction at high and low pH, results showed that polyphenols were protected in liposomes due to two factors: oxygen level and controlled concentration of polyphenols. Since lipid bilayers surround polyphenols in liposomes, controlled releases of polyphenols in liposomes prevent their destruction (86). Rashidinejad et al. encapsulated catechin and epigallocatechin of green tea in liposomes of soy lecithin at 0, 0.125, 0.25, and 05% (v/w) concentrations and then inoculated these encapsulations at 0 and 0.25% (v/w) concentrations into cheeses. The average diameter of empty liposomes was 133 nm, which increased significantly with the encapsulation of catechin and epigallocatechin. The composed liposome Z-potential showed no significant differences between various treatments in ranges of 42.4–46.1 mV that caused liposome system stability. Nanoliposomes containing antioxidants in hard and low-fat cheeses acted as an appropriate carrier to transport antioxidants. They reported that the encapsulation efficiency of the antioxidants at 0.5% concentrations was lower than that at 0.125 and 0.25% concentrations (35). Gulsern and Corredig prepared liposomes with green tea polyphenols using milk phospholipids and homogenization methods. They assessed the physical properties and stability of the green tea polyphenol liposomes. Encapsulation efficiency increased (58%) by increasing the concentration of green tea polyphenols to more than 4 mg/mL. However, increasing this concentration to more than 6mg/mL resulted in

instability in liposomes. The size and Z-potential of the composed liposomes were 140 nm and -20 mV, respectively. Results revealed that quantities of the encapsulated polyphenols depended on the initial concentration of green tea polyphenols. Polyphenols at low concentrations were entirely encapsulated in liposomes (32). Lu et al. prepared nanoliposomes using the thin-layer ultrasonic suspension method of green tea polyphenols to increase their accessibility and study their physicochemical properties. The optimal concentration achieved for the ratio of polyphenols to lecithin was 0.125:1 and for the ratio of lecithin to cholesterol was 4:1, pH was 6.62, and ultrasonic time was 3.5 min. Theoretical and practical encapsulation efficiencies included 60.36 and 60.09, respectively. The mean size of liposomes containing polyphenols was reported as 160.4 nm and Z-potential as -67.2 mV. The epigallocatechin gallate level was higher in liposome systems with lecithin than in liposome systems with other components such as epicatechin, epigallocatechin, epicatechin gallate, and catechin. All catechin compounds in oil emulsions in the water included antioxidant properties (34). Antioxidant activity of the green tea catechins in liposomes (compared to emulsions) included a greater tendency of polar catechins to polar surfaces of the lecithin lipid bilayers with better protections (87).

10. Encapsulation of Rosemary Extract in Liposomes

Rashidaie Abandansarie and Ariaii assessed the antioxidant properties of rosemary extract at 800 and 1600 mg/L concentrations in free and encapsulated forms in liposomes oxidative stability of beef meats during cold storage. They reported that rosemary extract in the two forms decreased peroxide and thiobarbituric acid contents, followed by increased production stability. Peroxide and thiobarbituric acid included lower values when concentrations of the rosemary extract increased (38).

11. Mozafari Method for the Production of Liposomal Antioxidants

Jahanfar et al. encapsulated green tea extract in liposomes through the heating method and optimized the process conditions. Based on the results, the optimal levels for the variables were verified as follows: lecithin proportion of 4.5%, green tea extract concentration of 0.7%, and mixing temperature and time of 50°C and 30 min, respectively. Physicochemical properties of the optimal liposomes included encapsulation of 51.34%, the particle size of

liposome-loaded green tea extract of 419 nm, and Z-potential of -59.7 mV. Findings demonstrated that the antioxidant properties of the green tea extracts increased after encapsulation. The antioxidant activities of the green tea extracts in free and loaded liposomes were 86.4 and 93.37%, respectively (18). Rasti et al. investigated liposomes' physical and oxidative stabilities and nanoliposome with polyunsaturated fatty acid (PUFA), including docosahexaenoic acid eicosapentaenoic acid prepared using thin-film hydration and Mozafari method. The most outstanding physicochemical stability was observed in prepared liposomes by the Mozafari method, thin-film hydration, and free PUFA, respectively. Comparisons between liposomes and nanoliposomes showed that the surface load, physical stability, and oxidative stability of the encapsulated PUFA increased with decreasing particle sizes (11). Jahadi and Khosravi Darani. optimized the effects of operational variables to accelerate the production of the Persian white cheeses. Results showed that the presence of 0.3% nanoliposome containing flavourzymes increases the intensity of proteolysis index and depth of proteolysis by 1.45 and 2.22%, respectively. The best encapsulation efficiency (26.5%) was achieved using lecithin of 4.5%, flavourzyme to lecithin ratio of 5%, pH of 6, the temperature of 45° C, and time of 30 min (88). Colas et al. produced nanoliposomes containing nisin Z using the Mozafari method. They used various compounds such as phosphatidylcholine, dipalmitoyl phosphatidyl-choline, stearyl amine, diacetyl phosphate, and cholesterol with various molar to prepare liposomes ratios. Results demonstrated that cationic vesicles included lower encapsulation efficiency than the anionic vesicles owe to electrostatic repulsion between cationic vesicles and positively charged nisin. While cholesterol increased from 10 to 20%, the molar ratio decreased encapsulation efficiency. Encapsulation efficiencies of various formulations included 12–54%, and the particle sizes included 190–284 nm (17).

12. Antioxidants in Sports Nutrition and Recovery

Intense physical activity increases oxidative stress due to the overproduction of free radicals, leading to muscle fatigue, inflammation, and delayed recovery. Natural antioxidants (e.g., green tea and rosemary polyphenols) could help mitigate exercise-induced oxidative damage. On the other hands encapsulated antioxidants may improve bioavailability, ensuring sustained release during prolonged

exercise, potentially enhancing endurance and reducing muscle damage. Liposomal antioxidants may show enhanced bioavailability, while athletes require rapid and efficient nutrient absorption. Liposomal encapsulation could improve the delivery of antioxidants post-exercise, aiding faster recovery. Besides, liposomes could be engineered to deliver antioxidants directly to muscle tissues, reducing inflammation and accelerating repair

13. Natural vs. Synthetic Antioxidants in Sports Supplements

Many athletes rely on synthetic antioxidants (e.g., BHT, BHA) in supplements. The shift toward natural antioxidants (green tea, rosemary) aligns with the growing demand for clean-label, non-toxic sports nutrition products. Synergistic Effects caused by combining green tea catechins and rosemary diterpenes could offer superior antioxidant protection compared to single compounds, benefiting high-performance athletes.

14. Applications in Sports Drinks and Functional Foods

Liposomal encapsulation could protect antioxidants from degradation in sports drinks, gels, and protein bars, ensuring prolonged shelf life and efficacy. Also, encapsulated antioxidants could be designed to release gradually during endurance events, maintaining oxidative balance.

Future Research Directions in Sports Science

Investigating whether liposomal antioxidants enhance post-exercise muscle recovery and protein synthesis seems a new direction. Also, dosing strategies for athletes needs more investigation to optimizing concentrations for different sports (endurance vs. strength training. Interaction with other sports supplements needs investigation to illustrate how liposomal antioxidants interact with creatine, beta-alanine, or caffeine for synergistic effects .

15. Conclusion

Green tea and rosemary have many polyphenol compounds. The most crucial function of polyphenol compounds is antioxidant properties, which can be interrupted by chain oxidation reactions or chelating metals. Also, polyphenols able to prolong the shelf life of foods. However, the sensitivity of these compounds against light and oxygen limits their application as natural antioxidants. The encapsulation methods will be able to overcome this problem. Some of these methods have disadvantages, such

as the various applying solvents, expensive equipment, low encapsulation efficiency, and large particle sizes, which leads to instability of the capsules. Liposomes are made up of membrane-like lipids in aqueous solvents. The most crucial advantage of liposomes is that because of their constituent compounds. They can encapsulate both types of hydrophobic and hydrophilic bioactive agents. Liposomes can withstand various environmental conditions and chemical changes. However, they can enhance the efficiency of bioactive agents by increasing ingredient solubility. The extensive research showed that liposome technology for the encapsulation of phenolic compounds from plant extracts might improve their antioxidant properties. This can be controlled release and the better distribution of the encapsulated green tea extract in oil. It is noteworthy to conclude that the liposomal encapsulation of natural antioxidants results in better efficiencies of these antioxidants than synthetic antioxidants such as BHT. These extracts have shown abilities to delay or decrease oxidation rates in food oils. Further research is needed to achieve further progress and extensive use of liposomes containing the herbal extracts as a natural antioxidant and applying in food oils to delaying the oxidation process.

It is better to do more research on the following in the future: Another unsolved novel subject is a study on the interaction between ingredients of liposomes and natural antioxidants during processing, storage and its effect on stability and releases the antioxidant compounds of the liposome.

Besides all aspects on food and pharmaceutical applications, the intersection with sport science opens new avenues for research. Liposomal green tea and rosemary extracts could revolutionize sports nutrition by improving antioxidant delivery, reducing exercise-induced oxidative stress, and enhancing recovery—making them a promising area for future studies in athletic performance and recovery .

Authors' Contributions

All authors equally contributed to this study.

Declaration

In order to correct and improve the academic writing of our paper, we have used the language model ChatGPT.

Transparency Statement

Data are available for research purposes upon reasonable request to the corresponding author.

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Declaration of Interest

The authors report no conflict of interest.

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Ethical Considerations

Not applicable.

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