

Enrichment of Food Product with Vitamin E Using Different Encapsulation Methods

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ABSTRACT

Vitamin E is a generic term for eight different molecules of tocopherols and tocotrienols including α , β , γ and δ tocopherol and the corresponding tocotrienols. It is a fat soluble vitamin, and its deficiency is associated with risks of cardiovascular, degenerative and several other diseases. This vitamin acts as a lipid-soluble antioxidant with key roles in protecting cell membrane against damaging free radicals. Encapsulation is one of the best methods for delivery of this vitamin as a nutritional supplement or drug delivery system with a good efficiency. There are two main methods for encapsulation: Biopolymer and lipid base methods. Starch, cellulose, pectin, guar gum, chitosan, alginate, dextran, are among biopolymers used in biopolymer and lipid based methods. There are different paths such as emulsions and nanoemulsion, solid-lipid nanoparticles, nanoliposomes, nanococheates, archaeosomes and micro and nanoparticles. Among these, nanoemulsion has the highest encapsulation efficiency of about 99/65. In this review, the methods for encapsulation of this vitamin will be discussed.

Keywords: Vitamin E, Enrichment, Food, Encapsulation

INTRODUCTION

Vitamin E is one the 13 essential vitamins, which is not synthesized by animals. Vitamin E or tocopherol is a generic term for eight different molecules of tocopherols and tocotrienols of which four tocopherols and two tocotrienols have been shown to exhibit vitamin E activity in animals including humans [1]. Tocopherols are solely synthesized by photosynthetic organisms such as plants, algae and cyanobacteria [2]. Vitamin E is found in oils, nuts, germs, seeds and a variety of other plant products [3]. In human diet the main source of vitamin E is plant derived products, notably seed oils. Albeit this vitamin is readily available, dietary studies have shown that human populations for various reasons do not consume enough vitamin E resulting in its deficiency from mild to severe levels [1-3].

Tocopherols or vitamin E are considered as one of the most powerful antioxidants, and these properties are due to their ability to interact with certain molecules including polyunsaturated acyl groups, scavenging lipid peroxyl

radicals and reactive oxygen species (ROS). Therefore, tocopherols can quench ROS and protect fatty acids from peroxidation, consequently preserving cell membrane from subversion [2].

The basic structure of Tocopherols is simple with a polar chromanol ring and a hydrophobic polyprenyl side chain made through shikimate and 1-deoxy-D-xylulose-5-phosphate (DOXP) pathways. The saturated chain is fixed with 16 carbon atoms. Three of these carbon atoms are asymmetric with eight different stereoisomers. The number of methyl groups in chromanol ring is different and this variety is determinant for the type of α , β , γ and δ tocopherols and tocotrienols. α -Tocopherol as the most common isomer in nature has the highest biological activity, while β and γ isomers display less vitamin activity, and δ -tocopherol is nearly inactive [2,4].

The frequency of these sub-forms varies in different products, for example in almond oil this value is 335-337 mg kg⁻¹ for α , 2-50 for γ and 0.1-22 for β tocopherols [5]. Four isomers of tocopherols are called (T) and tocotrienols (T3) in brief. Tocopherol and tocotrienol are also called tocopherols. The tocopherols descending in the order of antioxidant capacity include α , β , γ and δ tocopherols. Between T

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and T3, the descending order of antioxidant activity is: α -T, β -T, α -T3, γ -T, β -T3 and δ -T with γ -T3 and δ -T3 having no function. However, previous researchers showed that α -T3 is more effective than α -T [6]. While, Nukala *et al.* reported that the antioxidant potential of tocotrienols is 1600 times more than that of α -T [7], but for bioavailability of α -T, they can be more effective than other forms.

Also the biological activity of vitamin E is different from its antioxidant activity, and between these isomers are a preference for α -tocopherol. The reason for this preference is associated with a protein called hepatic α -tocopherol transfer protein (α -TTP) with selective degradation and excretion of other vitamin E forms and reserve α -tocopherol, selectively. In fact, Hepatic α -TTP causes α -tocopherol incorporate into circulating lipoproteins easily and consequently distribute the vitamin to non-hepatic tissues. Therefore, α -TTP can be considered as a regulator of vitamin E status in humans [8]. Due to the activity of this regulator, alpha and gamma tocopherols are found in the serum and the red blood cells, with the alpha isoform having the highest concentration and β and δ in much lower concentrations. This high concentration of alpha form can be due to its faster production than other isoforms and α -TTP, and thus the higher binding affinity between α -TTP and alpha isomer thereupon other vitamins secret into the bile and excrete in the feces, while alpha-tocopherol is excreted through urine. α -Tocopherol in human body accumulates in non-hepatic tissues, specially at sites with the greatest free radical production, such as membrane of some organelles including mitochondria and the endoplasmic reticulum in heart and lung cells with a protective role [9].

Bioavailability of vitamin E like other fat-soluble vitamins depend on pancreatic function, biliary secretion, micellar formation and permeability of intestinal membrane. In the stomach, this vitamin is cleaved by the enzyme esterase and partly processed by the gastric lipase [10]. In the gut, tocopherol and tocotrienols are all absorbed by intestinal cells by two routs: passive diffusion and receptor mediated transport. It is then delivered to the lymph and then transported to liver by chylomicron. Here, the efficiency of absorbance for tocotrienols is apparently higher than alpha tocopherol, but α -TTP in liver helps α T to win. Following linkage, α T incorporates into

VLDL/LDL/HDL and disperses to peripheral tissues such as brain, lungs, adipose tissue, bones, skin and muscle with lymphatic system. If not delivered, all forms are metabolized by phase I and II enzymes and excreted as glucuronide or sulfate [10,11].

Despite its antioxidant activity, vitamin E has an essential role in reproductive and neurological processes by regulating cell proliferation and gene expression. Previous studies have demonstrated that this vitamin is necessary for fertility and prevents hemolysis in rats and muscle dystrophy in poultry [11,12]. Also, several studies have reported that vitamin E plays an important role in the reduction of cholesterol levels and acts as an anticancer agent. Its effects on cancer include anti-proliferative, anti-survival, pro-apoptotic, anti-angiogenic and anti-inflammatory activities [13]. α T is very critical for the proper function of brain, and its deficiency is associated with many neurological signs such as ataxia, peripheral neuropathy, myopathy and retinopathy which can lead to neurodegenerative disease and cognitive decline. It has been reported in patients with Alzheimer's disease and mild cognitive disorder that α T levels in plasma and cerebrospinal fluid are reduced [14]. Also, several studies have shown that vitamin E could act as a useful agent in Parkinson disease treatment [15-16] and the cardiovascular system from atherogenic by preserving the oxidation of polyunsaturated fatty acid (PUFA) low-density lipoproteins in arterial walls, In addition, it is effective for prevention and treatment of coronary artery disease [17,18]. Further more, it has been demonstrated that this vitamin acts as a regulator of gene expression and signal transduction and can modulate cell function through specific membrane domains. Therefore, several genetic and signaling disorders can be improved in the presence of vitamin E. In certain disorders with inflammation such as Alzheimer, some forms of vitamin E can expose anti-inflammatory properties and decrease pro-inflammatory cytokines such as IL-1, IL-6 and TNF α [19].

In the deepest layers of human skin, vitamin E is normally distributed in the highest levels. It has been shown that α tocopherol is the main antioxidant in the epidermis, and its deficiency can cause environmental oxidative damage. Indeed, α -tocopherol by inhibiting protein kinase C

activity, prevents the age-dependent increase in collagenase expression and hence postpones the aging process [20]. Enrichment of diet with vitamin E could improve egg laying performance in anti-season breeding goose and so enhance plasma reproductive hormones and the expression of reproductive hormone receptor genes and SWF, LWF and SYF in ovary [21].

Daily requirement of vitamin E was estimated 15 mg, and in 0-13 year children 4-11 mg [8,22]. Due to the vital roles of vitamin E, its importance in food enrichment is necessary for preventing nutrient deficiencies. Thus, in the present study, we review for the methods used for food enrichment with vitamin E. The main obstacle here is the storage and conservation of this vitamin in food products, as it is very unstable in response to light, humidity, oxygen and high temperature conditions [23,24]. The most important methods used to resolve this issue are discussed.

ENCAPSULATION

Encapsulation is a method for encapsulation of bioactive and sensitive compounds such as polyphenols, micronutrients, enzymes, antioxidants and nutraceuticals against severe environmental conditions. Encapsulations may also help in controlling material release at target sites, protection against UV light, incompatibilities with other substances, improve solubility in aqueous systems and hide unsavory taste or odor [25]. In other words, this technique is a process by which sensitive or bioactive materials in particles are packed into thin films of a coating material to build a barrier between the component in the particle and the environment. Stability accretion, transforming liquids into dry, free flowing powders, enhance handling properties, limit loss of volatile materials, and control release of active material are targets of encapsulation [26,27]. So, encapsulation is a good delivery system for vitamin E, and it can include different paths based on biopolymer and lipid such as emulsions and nanoemulsions, solid-lipid nanoparticles, nanoliposomes, nanocochleates, archaeosomes and micro and nanoparticle. Among these, two methods presently used include spray-drying and supercritical fluid extraction for elimination of solvent and creation of capsules [28,29,30].

Encapsulation with Biopolymer-based Particles

Biopolymers are polymers produced by living organisms; in other words, they are polymeric biomolecules and can be used in vitamin E encapsulation. Carbohydrates and proteins due to their enormous molecular structure and ability to entrap bioactive components, and being safe and inexpensive, are suitable for delivery systems. Starch, cellulose, pectin, guar gum, chitosan, alginate, dextran, cyclodextrin are examples of this object [31]. Biopolymer nanoparticles have certain advantages; such as reducing toxicity, enhancing release of drug, improve bioavailability and provide better formulation opportunities. Also, it is important to control particle size, charge, morphology of surface and release rate of loaded molecules [32,33]. Below some of these methods are discussed.

Encapsulation of Vitamin E in Polycaprolactone with SFEE Method

This is a supercritical fluid extraction of emulsions (SFEE) method, in which polycaprolactone (PCL) acts as the encapsulating polymer. PCL is a polymer with high hydrophobicity, high *in vitro* stability and low cost. SFEE is a novel technology of encapsulation that uses supercritical CO₂ to extract the organic phase of an emulsion or solvent rapidly to obtain a suspension of particles in water. This method can produce nanocapsules of liquid lipophilic compounds and expose particles with 90% encapsulation efficiency, narrow particle size distribution and high storage stability [30].

Encapsulation Using Maltodextrin/Sodium Caseinate/Selenomethionine

In this method, vitamin E is incorporated with selenomethionine, a bioactive form of selenium and an essential trace mineral in human diet. Its solubility in water has made it bioavailable in human body. Therefore, its incorporation with vitamin E, increases its bioavailability. Vitamin E and selenomethionine incorporate within a wall consisting of maltodextrin and sodium caseinate at a ratio of 3 maltodextrin:2 vitamin E:1 sodium caseinate. This method successfully obtained a better function and improved vitamin E delivery and bioavailability at different pH values [34]. Use of sodium caseinate and maltodextrin as wall material in the capsules has gained a great success in

vitamin E encapsulation including with high encapsulation efficiency of about 52-70%, a wide retention time of tocopherol and the percent of vitamin E release in simulated gastric fluid and simulated intestinal fluid was being 87% and 42% [35,36].

Encapsulation Using Gliadin

Gliadins, extracted from wheat gluten, is a suitable case for vitamin E encapsulation which generates nanoparticles. The gliadin nanoparticles were prepared by a desolving method. In fact, vitamin loads in gliadin nanoparticles. The resulting nanoparticle had the proper size of < 900 nm with optimum encapsulation rate and an efficiency of more than 77% [37].

Encapsulation with Spray Drying Method Using Octenyl Succinic Anhydride (OSA)

In this technique, initially, vitamin E is made into colloidal suspensions to obtain sufficient chemical, physical stability and high bioaccessibility. an Octenyl Succinic Anhydride (OSA) modified starches that are one of the biopolymers being used as emulsifiers and wall material. This substance is chosen due to its low viscosity, good emulsifying and film forming properties, high oil loading capacity, low molecular weight and oxygen barrier properties. To obtain operational nanocapsules, spray drying is the most popular method due to the availability of the equipment and low cost [38]. This technique is being used for conversion of liquid into dry powder by a nebulizer to disperse the liquid into droplets. This solvent then evaporates in a hot drying medium rapidly to form a dry powder product. It was shown that OSA is able to fabricate nanocapsules with a good vitamin E retention after 60 days of storage at 4-35 °C, and a good suspension in water [38,39]

Encapsulation of Vitamin E with β -Lactoglobulin (BLG) and Hen Egg White Protein (HEW)

In this type of encapsulation, protein hydrogel due to its high nutritional value and safety issued as a carrier particle. There are various preparation techniques to induce gelation of proteins such as acid, heat and ion-induced gelation. For example, in heat-induced gelation, heat makes the globular proteins unfold and exhibit functional groups within the

protein rendering it capable for interaction with active biomaterial. After cooling, for aggregation of protein by electrostatic interaction, salt is added. BLG and HEW can be used here. BLG by its amphiphilic nature can bind to small lipid molecules like vitamin E. HEW, composed of various proteins, has excellent properties including foaming, emulsification, heat setting, and binding adhesion. This method led to an acceptable encapsulation efficiency of 32% in HEW and 20% in BLG, while the release of vitamin E in simulated gastric condition was about 100%. [40,41]

Lipid-based Methods for Encapsulation

These methods are applied system for transmission of bioactive agents particularly antioxidants and can improve their solubility and bioavailability and also protect them from unwelcomed interaction. Several approaches based on this method have been developed that can be useful in food industry such as nanoliposome, colloidosome, nanococheate, nanoemulsion, archaeosome, solid lipid nanoparticles, nanofiber, nanocomposite, *etc.* [31,42]. There are some advantage to using the lipid-based encapsulation which include the ability to entrap material with different solubilities, the possibility of being produced using natural ingredients on an industrial scale, and target ability [43]. Some of these methods will be discussed below.

Nanoliposomes

Liposome is a self-assembled vesicle with a phospholipid bilayer structure in which a fat cover encapsulates a core which could be of aqueous nature or other materials. Liposomes are described as bilayer spherical and continuous structures composed mainly of lipid and phospholipid molecules and presumably proteins or polymers. They are used as carrier system in both nano and micro scales in pharmaceutical, cosmetic and food industries. Research has shown that liposomes are present in breast milk. There are other types of liposomes that include multilamellar (MLV), multivesicular (MUV) and unilamellar vesicles (ULV) [43,44]. Images of different liposomes are shown in Fig. 1 [43].

Liposomes possess both lipid and aqueous phases and therefore can be usable for hydrophilic and hydrophobic materials. Liposomes have been given a great deal of

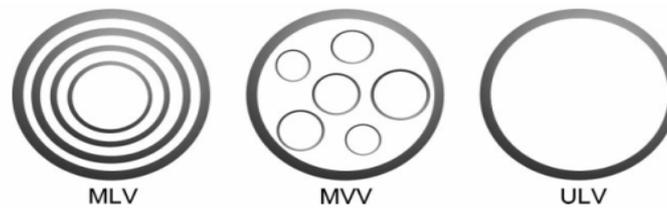


Fig. 1. Three types of liposomes: MLV, MVV, ULV [43].

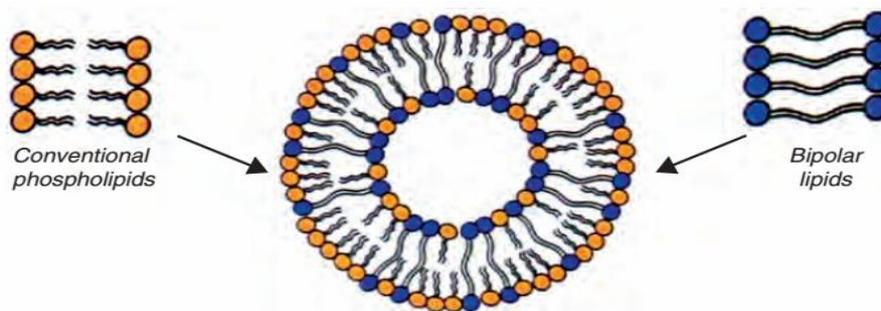


Fig. 2. Structure of an archaeosome [50].

attention since they increase insolubility of drug or nutrient and slow down their release. The size of liposomes is very important and can affect material loading, distribution, targeting, trapping by target organ, therapeutic effect and rate of its clearance. In nanoliposomes, the bioavailability of core is higher than conventional liposomes [44,45]. Indeed, nanoliposomes are nanoscale liposomes with high efficiency due to their small size and high stability. In addition, studies have demonstrated that nanoparticles have a high tendency to be arrested by the reticuloendothelial system [46]. There are various methods in preparation of nanoliposomes such as film hydration, ethanol injection, reverse phase evaporation and other innovative ways of thermal method. In formulation of nanoliposomes, polar lipids such as squalin and lecithin are used. In the case of vitamin E, soybean lecithin can promote stability, absorbance and low rate of release of vitamin E. The encapsulation efficiency of these nanoliposomes by thin film and thermal s in two different studies were 76 and 78%, respectively (Table 1) [47,48].

Nanoemulsions

Emulsions with 100-500 nm size are often prepared with high-pressure valve homogenizers and are thus called

nanoemulsions. These droplets can place bioactive material and functional food within their interfacial region and prevent them from enzymatic reactions and oxidation. Nanoemulsions have several preferences to conventional emulsions including their smaller size, higher stability and a larger surface area which cause superior interaction in conjugation with some biological components such as enzymes in the gastrointestinal tract. Thus, by this technique lipophilic bioactive components can be delivered in food products more conveniently [31,49]. For preparation of vitamin E nanoemulsion, various oils such as mustard oil or palm oil are applied. In addition the functional type of vitamin E, vitamin E acetate, is used. In a successful study with mustard oil and surfactant, the encapsulation efficiency of nanoemulsion was 99/65% (Table 1) [50].

Other Applied Methods: Archaeosomes and Nanocochleate

There are new innovative ways for encapsulation of hydrophilic and hydrophobic materials which can be used for vitamin E encapsulation. Some of these methods are archaeosomes and nanocochleate. Although these methods have not been used in research, however, they present

Table 1. Comparing of Different Methods of Vitamin E Encapsulation

	Encapsulation with	Method	EE	Ref.
Biopolymer-based	Polycaprolactone	SFEE	90%	[30]
	Maltodextrin/Sodium Caseinate	Spray drying	52-70%	[34]
	Gliadin	Desolvation	77%	[35]
	Beta-lactoglobulin (BLG)	Spray drying	20%	[39]
	Hen egg white protein (HEW)	Spray drying	32%	[39]
Lipid-based methods	Nanoliposome with gammaoryzanol and lauric acid	Thermal method	More than 78%	[45]
	Nanoliposome with Lecithin, cholesterol, octadecylamine	Thin film	76%	[46]
	Nanoemulsions with mustard oil	Wash-out method	99/65%	[48]

suitable candidates for vitamin E delivery.

Archaeosomes are a type of liposome extracted from the archaea or synthetic archaeal lipids, and are more stable than normal liposomes due to its ether lipids. Also archaeosomes have higher stability in disparate conditions of the body. Archaeosomes are made of conventional phospholipids and bipolar lipids encompassing an aqueous core [51,52]. The image of an archaeosome is shown in Fig. 2 [52].

Cochleate and nanocochleate are new technologies used as delivery systems, which are more stable than liposomes. They have a cigar-shaped multilayered structure formed of negatively charged phospholipid bilayers, similar to phosphatidylserine rolled up in a spiral fashion through interactions with multivalent counter ions such as Ca^{2+} as a linking agent between the bilayers. Nanocochleate can deliver hydrophobic, amphiphilic, negatively or positively charged molecules [43,53,54,55].

In addition to the aforementioned methods, all of which can be used to deliver vitamin E and other lipophilic materials, there are other methods that can be useful for this application. Colloidosomes, nanofibers, nanocomposites are methods used in the production of vitamin E capsules. In all

of these approaches, there exists a space into which vitamin or other materials can be incorporated [52,53,54].

In several recent studies on enrichment of food with vitamin E, organic tocopherol in natural products has been used. This approach seems to be more economic than the synthetic vitamin. In this approach, tocopherol, extracted by various methods described above, is added to food successfully. Natural products such as certain mushroom types including *Ganoderma lucidum* and *Pleurotus ostreatus*, *Spirulina platensis* microalga, *Ascophyllum nodosum* and many others are rich in tocopherols and their use as a source for enrichment purposes has been successful. [56,57,58].

CONCLUSIONS

Vitamin E is an essential nutrient required in human diet. Research is increasingly demonstrating vitamin E encapsulation as an appropriate method for food enrichment with pharmaceutical use, with an acceptable efficiency. Different methods have been used for this purpose, however, with different efficiencies, among which,

nanoemulsion has shown the highest encapsulation efficiency. The following table shows a comparison of these methods.

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