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Chapter 10

Liposomes as Matrices to Hold Bioactive Compounds for Drinkable Foods: Their Ability to Improve Health and Future Prospects

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Additional information is available at the end of the chapter

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Abstract

The aim of this chapter is to describe the use of bioactive compounds with beneficial effects on human health beyond their basic nutritional value. Bioactive compounds like vitamin E, vitamin C, and fatty acids (omega-3 and omega-6) have an important nutritional contribution and are related to the prevention of certain diseases with global impact such as cancer. However, the addition of vitamins in a food product is not easy: E is destroyed by UV-light, and C is dramatically reduced during heat processes. The use of liposomes as matrices to hold bioactive compounds appears to be a promising solution. Liposomes were made of natural soybean lecithin, which has a great nutritional importance, and more so combined with stearic acid or calcium stearate (CaS). Thus, this stabilize liposomes and contribute to the stability of bioactive compounds and to preserve their activity. The stability of bioactive compounds/liposomes incorporated into aqueous food must be demonstrated in properties such as oxidative tendency, morphology, size, and membrane packaging after heat treatment processes. But to make a product applicable at the commercial level, its texture and mouthfeel arising from the ingestion of drinkable foods are all-important to consumer's choice and sensory acceptability must not undergo any modification.

Keywords: bioactive compounds, liposomes, nutrition, healthy, food

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1. Introduction: nutritional properties of soybean lecithin

Phosphatidylcholines can be divided into two types, which differ in their origin: soy phosphatidylcholine (SPC) and egg phosphatidylcholine, both naturally occurring and containing certain polyunsaturated fatty acids (PUFAs) such as linolenic acid (omega-3) and linoleic acid (omega-6), represented by 18:2 and 18:1, respectively [1, 2].

Both linolenic and linoleic acids are essential fatty acids, since they cannot be synthesized by the body and therefore must be obtained by the diet [1–4].

It has been reported that essential fatty acids are highly beneficial in the prevention of diseases such as cardiovascular diseases [2, 5–7], schizophrenia [8], and cancer [9]. In addition, these fatty acids have vasodilator, antihypertensive, anti-inflammatory, and anti-atherothrombotic properties [10].

PUFAs have great human nutritional importance. This is related to the existence of two families of PUFAs: the n-6 family and the n-3 family [4]. n-6 PUFAs are derived from linoleic acid, have two double bonds, and are characterized by having their first double bond at carbon number 6 [1], whereas n-3 PUFAs are derived from linolenic acid, have three double bonds, and are characterized by having their first double bond at carbon number 3. Linoleic acid is metabolized to arachidonic acid, whereas linolenic acid generates eicosapentaenoic acid and docosahexaenoic acid. All of them use the same metabolic pathways and compete for the same elongase and desaturase enzymes [1, 4].

In addition to being a source of energy, the n-6 and n-3 PUFA families are incorporated into cell membranes, where they are precursors of eicosanoids (prostaglandins, prostacyclins, thromboxanes, and leukotrienes), which are involved in numerous physiological processes such as blood clotting or inflammatory and immunological responses [1].

Among vegetable oils, flaxseed oil is considered to be the richest source of linolenic acid (57% of total fatty acids). Rapeseed, soybeans, wheat germ, and walnuts contain between 7 and 13% of the said fatty acid. Some authors consider vegetables (e.g., spinach, lettuce) as a good source of linolenic acid, although their fat content is quite low. Meat, particularly that of ruminants, and dairy products also provide this fatty acid. However, modern farming techniques have led to a decrease in the n-3 fatty acid content in meat (especially in lamb and beef) due to the almost generalized use of n-6-rich grain concentrates to feed cattle [1, 2, 11].

Soybean lecithin is considered a bioavailable source of choline, which was officially recognized as an essential nutrient by the Institute of Medicine in 1998 [12]. This nutrient is needed for the synthesis of neurotransmitters (acetylcholine), cell-membrane signaling (phospholipids), lipid transport (lipoproteins), and methyl-group metabolism (homocysteine reduction) [13, 14]. It plays important roles in brain and memory development in the fetus, and some researchers indicate that choline and methionine intake may be important in reducing the risk of neural tube defects. Studies have also shown that choline supplementation during critical periods of neonatal development can have long-term beneficial effects on memory. Besides, intake of choline has been associated with lower homocysteine levels. This effect is

important because increased levels of homocysteine have been associated with greater risk for several chronic diseases and conditions, including cardiovascular disease, cancer, and cognitive decline and bone fractures [12].

In 2001, the FDA made a statement regarding the Dietary Reference Intakes for thiamine, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline (Food and Nutrition Board, Institute of Medicine (IOM), NAS, 1998, page 390), which stated that choline functions as a precursor for acetylcholine, phospholipids, and the methyl donor betaine [15]. Choline is found in a wide variety of foods like chicken, liver, soy flour, salmon, sockeye, egg, uncooked quinoa, wheat germ, milk, cauliflower, and peas. Among the most concentrated sources of dietary choline are liver, eggs, and wheat germ. In foods, choline is found in free and esterified form (such as phosphocholine, glycerophosphocholine, phosphatidylcholine, and sphingomyelin) [12, 14].

Phosphatidylcholines are obtained by separating the egg yolk, which is generally separated from the whole egg, and then if not used immediately, it is dried or frozen. Soybean lecithin is obtained during the degumming step of oil refining [16], which consists in treating the oil with water at a temperature of 70°C or vapor, so that the phospholipids are hydrated and become insoluble in the fatty phase. Subsequently, the oil is transferred from the mixing tank to a centrifuge, in which the phospholipids, which are hydrated in the excess water, are separated from the degummed oil. The lecithin obtained has commercial value and is especially used, due to its emulgence, in various food industries (chocolate, fine bakery, etc.) [17].

These "raw" lecithins are complex mixtures, which contain significant quantities of triacylglycerols [16]. Also, they may be a mixture of lipids composed largely of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol, combined with other substances such as triglycerides and fatty acids and carbohydrates [18]. The refined degrees of lecithin may contain these components in varying proportions and in combinations depending on the type of fractionation used.

Egg and soybean lecithins may be purified and/or modified to improve their properties [16]. For example, in the case of soybean lecithin, a purification process is required to obtain the highest percentage of phosphatidylcholine to be called soy phosphatidylcholine.

It should be considered that the cost of phospholipids isolated from natural sources is always lower than that of those obtained by synthetic or semisynthetic methods. For natural phospholipids, the more pure they are, the higher the price is [19, 20]. Egg lecithin may be further purified by extraction with ethanol. Solvents may be used to separate lecithin from these triacylglycerols. Soybean lecithin may be precipitated (de-oiled) by acetone and may be enriched in phosphatidylcholine by extraction with ethanol [16, 21].

Lecithin quality is defined by the essays suggested by the "American Oil Chemistry Society":

- Insoluble in acetone: estimates the content of phospholipids.
- Acidity index: measures the free fatty acid content.
- Peroxide index: measures the degree of oxidation.

- Viscosity.
- Gardner color scale.
- Insolubles in hexane: measures the content of solid impurities.

2. Improvement of soybean lecithin by addition of calcium stearate (CaS) or stearic acid

When designing supporting additive matrices for the food industry, it is very important to study membrane stability [2, 22]. The structure of the additives must remain without significant changes over time so activity of the encapsulated component can be assured.

Several authors have reported that cholesterol is a very useful membrane stabilizer, especially when oxidative stability is needed [23, 24]. The effects of cholesterol on the membrane have been very well documented. It is known, for example, that cholesterol modifies the lipid order in membranes: when the concentration of cholesterol in membranes is below 10%, the lipid order in the liquid phase increases, and the lipid order in the gel phase decreases [25]. Cholesterol can also establish hydrogen bonds, thus increasing mechanic resistance [23], and can decrease membrane permeability [26, 27].

All the above mentioned indicate that cholesterol is an interesting candidate to maintain membrane stability. However, cholesterol cannot be used in food industry, because it is very well documented that it is related to atherosclerosis and has a tendency to produce heart diseases [28–31].

Thus, to avoid the use of cholesterol, several other components have been studied as membrane stabilizers. One particular candidate is stearic acid [23]. Hsieh and coworkers studied stearic acid as a membrane stabilizer, by comparing its effect with the one induced by cholesterol [23].

SA is a fatty acid with an 18-carbon-long chain. Because of its hydrophobic character, it is located in between acyl chains of the phospholipids in the bilayer.

The authors reported that liposomes prepared with egg yolk phosphatidylcholine and stearic acid in a 1:0.25 molar ratio present the same encapsulation efficiency and oxidative stability as liposomes prepared with egg yolk phosphatidylcholine and cholesterol in the same molar ratio.

If stearic acid is replaced by calcium stearate, membrane packing requirements would be fulfilled, and also it will contribute with calcium to food-containing additive matrices, which is an additional benefit.

Liposome formulations based on soybean lecithin or soy phosphatidylcholine with stearic acid or calcium stearate have also been studied as food additives, to determine their efficiency to enrich aqueous food with antioxidant vitamins. The molar ratio reported by Hsieh and coworkers was so efficient for the mixture of SPC with stearic acid that this was considered as the ratio to be used. Results from our laboratory have shown that formulations

of SPC with stearic acid or innovative addition of calcium stearate, in the same molar ratio, resulted in an improvement regarding oxidative stability and protection of thermolabile vitamins [32]. On the other hand, these formulations did not induce any unpleasant flavor when added to milk or orange juice, so they can be applied in food commercial products [32–35].

3. Processing issues in adding vitamins E and C protected with lecithin liposomes

This topic is of particular importance in relation to the nutritional value and quality aspects of processed foods as well as in relation to nutrition labeling. A number of general reviews have already been published because it is sought to determine the most suitable processing and time-temperature conditions, to achieve the desired objective, i.e., maximum retention of a specific vitamin or best retention of color or flavor consistent with microbiological stability and safety [36].

In the case of packaged liquid foods, different heat treatment processes can be applied. These include pasteurization, ultra-pasteurization, and ultrahigh temperature. Pasteurization is a heat treatment whose objective is to destroy non-sporulated pathogenic microorganisms and significantly reduce banal microbiota to offer the consumer a safe product with an acceptable shelf life to be consumed in a short term [37]. Ultra-pasteurization is a heat treatment in which the food is subjected for at least 2 s to a minimum temperature of 138°C by a thermal process of continuous flow and immediately cooled below 5°C and packed in a non-aseptic form in sterile and hermetically sealed packaging. Ultrahigh temperature is a process in which the food is subjected for 2–4 s to a temperature between 130 and 150°C, by a continuous flow thermal process, immediately cooled to less than 32°C, and packed under aseptic conditions in sterile packaging and hermetically sealed. This type of food has a shelf life of 5–6 months at room temperature and in closed packaging [18, 38]. The problem with these treatments is that they can generate losses of nutrients, especially of vitamins.

Processing with heat treatment like cooking conditions causes variable losses of vitamins. The losses of these nutrients are related with the cooking method and type of food and reckon on particular experimental arrangements during the culinary process, e.g., temperature, the presence of oxygen, light, moisture, pH, and, of course, duration of heat treatment. Vitamin C, retinol, folate, and thiamine are the most labile vitamins during culinary processes [39].

Concentrated juices are in high demand because they provide a significant amount of nutrients; however, during their elaboration and pasteurization process, they lose flavor, aroma, and nutritional contribution, mainly of vitamins. For this reason, the juices that are marketed in supermarkets present a considerable variation in their vitamin content, mainly of vitamin C (ascorbic acid) [40].

Vitamin C is one of the vitamins most sensitive to heat treatment. With regard to processed foods, there is a loss of vitamin activity that is related to the intensity of the heat treatment. Other factors that influence the loss of this vitamin activity by cooking are the pH, oxygen, surface of exposure to water, conditions of the heat treatment, and the presence of metals such as copper [37, 39, 41].

The losses of this vitamin vary between 40 and 60% in in-bottle sterilized milk, between 20 and 40% after ultrahigh temperature treatment [42, 43], and between 15 and 25% after a pasteurization treatment [44, 45]. Other researchers [46] showed that vitamin C is reduced by different types of pasteurization around 10% for long-term and low-temperature (LTLT) process and 50% for process which included 90°C during 30 min. The authors showed that vitamin C in baobab drink decreased with increasing temperature. These results coincide with that found in orange juice, where a loss of vitamin C of 10 ± 2.5% was observed after slow pasteurization or LTLT process (60 min at 65°C) and of 13.7 ± 1.9% after pasteurization for 45 min at 75°C. With a more extreme heat treatment for 30 min at 90°C, the vitamin loss increased, resulting in a final value of 23.3 ± 3.8% [47].

More current studies have demonstrated a high thermosensitivity of vitamin C against thermal processes that are related to the type of process and food in which this vitamin is found. For example, losses of this vitamin between 29 and 61.45% have been demonstrated in vegetable bleaching processes with temperatures between 94 and 98°C and cooking times between 90 s and 3 min. This shows that the presence of water further favors vitamin loss by leaching [39].

The loss of vitamin C is also related to the type of heat treatment [39, 41]. Other authors [48] demonstrated that the concentration of vitamin C was drastically reduced by various methods of steam cooking, conventional cooking, and high-pressure cooking.

With respect to vitamin E, which is a liposoluble vitamin, it is thermostable but readily oxidizes in the air [49], especially in the presence of ferric ion and other metals. Therefore, the use of some chemical substances such as hydrogen peroxide should be avoided, as it may lead to oxidation and, therefore, loss of vitamin activity [37]. In addition, vitamin E is destroyed by exposure to UV light and is lost, to a large extent, during the refining of oils [17].

During the processing and storage of food, meat and meat products, milk and derivatives, and cereals show few changes in the content of vitamin E. However, during the storage of vegetable foods, vitamin E has a weak antioxidant character, and in the presence of animal fats, it is much more active, especially if there are synergistic substances like vitamin C [37].

Considering the abovementioned, the application of liposomes is a promising solution to avoid losses of vitamins and promote their shelf life and protection [49, 50]. The use of liposomes to encapsulate and protect these vitamins and other bioactive compounds has a number of positive aspects [50]. For example, liposoluble vitamins such as vitamin E mix perfectly with the hydrophobic area of phosphatidylcholine. In addition, the absorption and bioavailability of this vitamin increase when it is encapsulated in liposomes. In particular, vitamin C encapsulated in liposomes retains 50% of its activity after 50 days in refrigerated storage, whereas non-encapsulated vitamin loses its activity after 19 days. Also, liposomes present an important protective effect over thermolabile vitamin C, shown by an antioxidant action after pasteurization [32, 34, 35, 49].

In the case of liposoluble vitamins, the importance of these food systems is that they can be added in aqueous foods [50, 51], such as orange juice, maintaining the stability and preserving the activity of vitamins [34].

4. Addition of improved liposomes containing bioactive compounds to food products: a case study

In order for a food to be considered functional, it must demonstrate (i) that it has a beneficial effect on one or more specific functions of the organism, beyond the usual nutritional effects; (ii) that it improves the state of health and well-being; and (iii) that it reduces the risk of an illness. This means that these foods must necessarily contain some of the so-called functional ingredients or bioactive compounds. It has been shown that, when implemented in aqueous foods, bioactive compounds generate a functional food which can promote health, physical ability, and mental state to benefit consumers of different ages [2, 52–54].

Bioactive compounds, including vitamins, antioxidants, minerals, dietary fibber, essential fatty acids, flavonoids, isothiocyanates, phenolic acids, plant stanols and sterols, polyols, prebiotics and probiotics, phytoestrogens, and soy protein, are the main components of functional foods [52, 53, 55]. Some nutrients have an important nutritional contribution and have been shown to be related to the prevention of certain diseases of great global impact such as cancer. This is the case of essential fatty acids as omega-3 and omega-6 and certain vitamins like vitamins E and C.

Vitamin E or α-tocopherol is the main liposoluble antioxidant in the body. It protects lipids against oxidative damage [56].

Also, it has a desirable effect when blood cholesterol decreases the incidence on atherosclerosis, and the cardiocirculatory system has a positive effect. An additional antioxidant vitamin is ascorbic acid or vitamin C. One of the biological roles of ascorbic acid is to participate in oxidation-reduction processes, blood coagulation, tissue regeneration, and building steroid hormones, inducing free radical inactivation. This vitamin also takes part in the inhibition of nitrosamine formation and participates in the collagen synthesis [17].

The importance of antioxidant vitamins is that several clinical studies have described beneficial effects in a variety of tumors, such as prostate, gastric, and lung tumors. This fact is based on experimental studies that highlight the role of free radicals as key factors associated with the development of cancer, and it is precisely the effectiveness of dietary antioxidants such as vitamin E or vitamin C that play an important role in the prevention of the development and progression of this disease [57–59].

However, most bioactive compounds such as fatty acids, carotenoids, tocopherols, flavonoids, polyphenols, phytosterols, and liposoluble vitamins have hydrophobic nature [52], which makes difficult their application in aqueous foods. Besides, it is not easy to maintain the stability of vitamins. In particular, in the case of functional foods with added vitamins, a number of factors must be taken into account to maintain their stability: their structure (whether they are hydrosoluble or liposoluble); their relation with diverse conditions as pH, the presence of oxygen and metals; the way in which vitamins are added to the food in question; and the heat treatment and storage conditions of the final product [34]. Vitamin E is liposoluble and destroyed by UV light [17], while Vitamin C is dramatically reduced by heat treatment processes [46, 49]. Thus, liposomes, which are microscopic spherical vesicles, composed of polar

lipids that enclose liquid compartments within their structure and enable the encapsulation of both hydrophilic and lipophilic materials [20, 22, 27, 49, 58, 60], may be a promising solution for incorporating bioactive compounds [61] into foods regardless of their affinity for water and for generating a protection over them.

Liposomes are classified into small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs), and large multilamellar vesicles (MLVs), according to their size and lamellarity, the latter of which relates to the method of preparation [62, 63]. The process of forming MLVs consists in mixing the lipids in ethanol, which is then removed by evaporation. Subsequently, the dry lipid film is hydrated, maintaining the temperature above the phase transition temperature of the lipid mixture [2, 22, 27, 60]. So, these liposomes form spontaneously when the dry lipid film is hydrated with water or buffer [27, 62]. Typically, their size distribution ranges from 0.1 μm to a maximum value which may be up to 500 μm in diameter, and they contain hundreds of concentric lamellae [22, 27, 62]. **Figure 1** shows a MLV of soy phosphatidylcholine and calcium stearate with vitamins E and C.

Figure 2 shows the concentric lamellae from MLVs, where liposoluble compounds, such as vitamin E, are located within the lamellae and hydrosoluble ones, such as vitamin C, prefer the aqueous interface. The concentric lamellae are formed by phospholipids such as phosphatidylcholine, which has a phosphate with a choline head and a carbon chain or fatty acids as omega-3 and omega-6, formed by carbon chains with carboxylic acid heads.

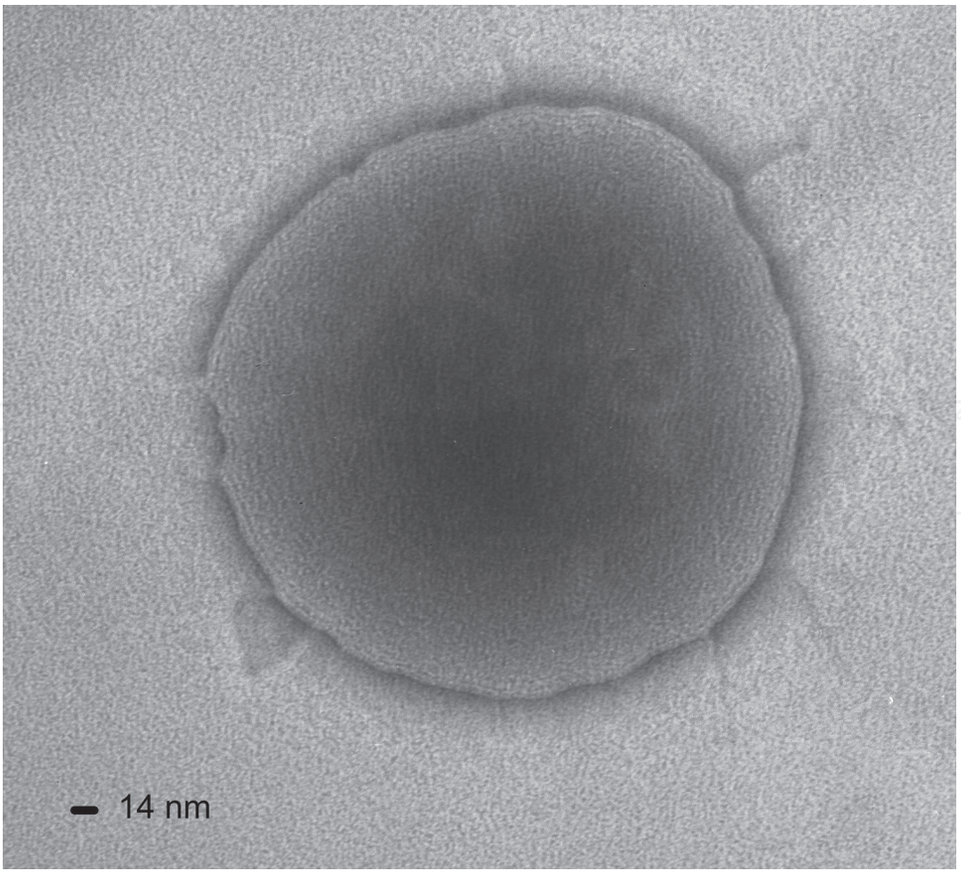


Figure 1. Transmission electron microscopy of soy phosphatidylcholine and calcium stearate liposomes (50 mM, molar ratio of 1:0.25) and vitamin C (90 mM) and vitamin E (5 mM).

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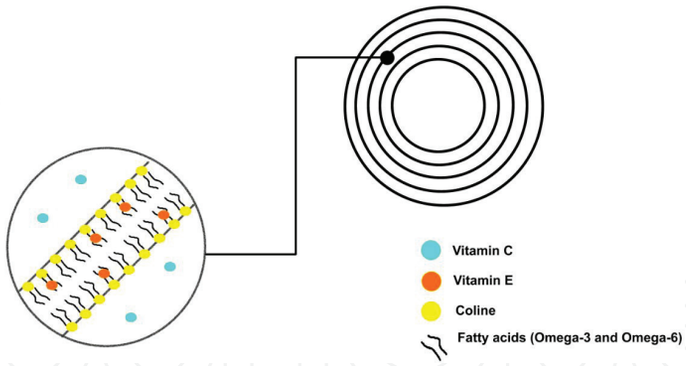


Figure 2. Concentric lamellae from MLVs and the location of vitamins E and C.

Liposomes have been employed as potential carriers to deliver food components and have many applications in food industry including protecting sensitive ingredients, increasing the bioavailability of nutrients and confining undesirable flavors. These types of matrices have been applied as food additives and have the ability to encapsulate vitamins, antioxidants, proteins, peptides, antimicrobials, essential oils, flavors, enzymes, minerals, and fatty acids [58, 60].

Liposomes have been used in the food industry for improving the flavor of ripened cheese using accelerated methods, for promoting antioxidant activity with the synergistic delivery of ascorbic acid and tocopherols in foods of functional food ingredients, and the stabilization of minerals (such as iron) in milk [58]. In respect to the industry of cheese, liposomal entrapment of enzymes offers advantages for cheese applications such as being prepared from ingredients naturally present in this product, because these vesicles can protect casein from early hydrolysis during the production of cheese [49].

Another example of the application is the encapsulation of calcium lactate encapsulation in lecithin liposomes to fortify soymilk with levels of calcium equivalent to those found in cow's milk [51]. Also, liposomes have been applied to encapsulation of lactase because they release lactase in the stomach and, therefore, remove the sweet taste of hydrolyzed milk [2].

Food grade phosphatidylcholine can be applied in food without the need for any clinical study. This aspect is particularly related to the regulation and regulation of food in each country. For example, in Argentina, soy lecithin is approved by local food regulations such as the Argentine Food Code and Resolutions of the Common Marked Group, being endorsed by control agencies such as ANMAT [18, 64].

The main objective of implementing a functional food is to generate a product with a high nutritional value that benefits the health of the population. People generally strive to consume a wide variety of foods and assure the ingestion of compounds such as antioxidants, vitamins, carotenoids, fiber, flavonoids, specific fatty acids, minerals, prebiotics and probiotics, phytoestrogens, soy protein, and vitamins, among others.

In the food industry, several matrices are being applied to encapsulate or associate bioactive compounds. These include liposomes, nanoemulsions, microemulsions, solid lipid nanoparticles, and polymeric nanoparticles [65, 66]. All the matrices that may be applied

in the food industry should have a series of properties like stability, applicability, and sensory evaluation of bioactive compounds in the product [2], which must be considered when incorporating bioactive compounds, especially vitamins, to generate new functional foods. No one would be willing to invest in the development and production on a larger scale of a food that is not acceptable for potential consumers.

In the food industry, considering that phospholipids can be oxidized and that this can limit their shelf life, membrane stability and structure are important factors when designing liposomes [2, 22]. Also, it is very important that liposomes remain stable after pasteurization because the higher the stability, the higher the protection of vitamins [32] and bioactive compounds.

Our research group focuses on the structural study, oxidative stability, and application in food of different liposomal formulations with bioactive compounds (omega-3, omega-6, vitamins E and C) to develop a functional food in commercial pasteurized orange juice. In our studies, the design and strategy of the implementation of these liposomes are based on the use of soy phosphatidylcholine a natural lipid that contains linolenic acid (omega-3) and linoleic acid (omega-6). These essential fatty acids are being added as part of soybean lecithin in the proportion needed for 200 mL of orange juice (38.28 mg for linoleic acid and 3.46 mg for linolenic acid). Soybean lecithin is a commercial product available, described in the Argentine Food Code and approved by ANMAT and INAL (Argentinean Food Quality Organisms) [32–35].

Besides, the design of liposomes has been made for encapsulating bioactive compounds as vitamins E and C. For 200 mL of orange juice, 2 mL of liposome suspension (50 mM) with vitamins was added, which implies that the orange juice was fortified with 4.3 mg of vitamin E (5 mM), equivalent to 43% of the recommended daily intake and 31.70 mg of vitamin C, equivalent to 70.44% of the recommended daily intake according the Argentine Food Code [33, 35].

Liposomes to protect hydrophilic or lipophilic vitamins must not only possess a long circulation time but also maintain the encapsulated vitamins for longer times; this means that they should have low leakage rate. Also, part of our research involved stearic acid (SA) or calcium stearate (CaS) that have been added to stabilize membrane liposomes to contribute to maintaining the stability of bioactive compounds and preserving their activity [32–35].

Food products must also undergo thermal treatment so the structural and oxidative stability of liposomes must be taken into consideration in all of the food-process conditions. Stable liposomes should have conserved size, shape, and surface properties. Size is usually analyzed by light scattering, whereas shape and structure are usually studied by optical and transmission electron microscopy, respectively. Also, to assure that membrane surface is maintained during food manufacturing and processing, a fluorescent probe like merocyanine 540 can be used to monitor surface changes. To complement this, surface charge and oxidative stability can be analyzed by the zeta potential and ORAC method, respectively. Liposomes used in our work as vehicles showed significant stability in all of the parameters mentioned above and conserve an important protective effect over thermolabile vitamin C [32, 34, 35].

Results of our lab regarding the oxidative level in matrices holding liposomes-bioactive compounds obtained showed a high stability in this parameter. The liposomal formulations were resuspended in acetic acid 3% w/v, indicated as food model systems by Argentinean regulations as the Argentine Food Code. The three liposomal formulations without vitamins

(**Table 1**) had the same oxidative stability by the ORAC method without significant differences regarding SPC (Dunnett Test, statistics not shown in **Table 1**) probably because of the low peroxidation of SPC [34].

When the vitamin C was incorporated in the three liposomal systems, it showed a significant higher value than the controls, related to the antioxidant activity after the pasteurization process [34]. In previous results, the percentage of encapsulation efficiency of vitamin C in these liposomes was determined and was c.a. 86% [32]. So, it is possible to infer that with the encapsulation efficiency data and the antioxidant activity these liposomes will protect efficiently most of the vitamin C and hence maintained its antioxidant activity after pasteurization against damage induced by the LTLT process [34, 35]. Noteworthy, liposomes will also exert the vitamin C protection.

Besides, to confirm their capability as commercial functional food, rheological behavior and sensory evaluation of liposomes/bioactive compounds should be performed. In our case, liposomes with bioactive compounds (omega-3, omega-6, vitamins E and C) were added to implement a functional orange juice with all of the above considerations. The sensory evaluation of liposomes in orange juice was performed by the overall acceptability and triangular tests with 40 and 78 potential consumers, respectively.

The three liposomal formulations, soy phosphatidylcholine (SPC), soy phosphatidylcholine and stearic acid (SPC:SA), and soy phosphatidylcholine and calcium stearate (SPC:CaS), studied remained stable even after pasteurization, as demonstrated by morphology, size, membrane packing, and high oxidative stability. Besides, all systems showed protection of the thermolabile vitamin C, which maintained its antioxidant activity after pasteurization. SPC and SPC:SA systems had a rheological behavior similar to a Newtonian fluid, whereas SPC:CaS had a pseudoplastic one; both stages considered excellent for larger-scale production. The incorporation of all liposomal formulation did not change the acceptability of orange juice. From all the aspects covered, it can be concluded that these liposomes with bioactive compounds, especially vitamin C, can be added to orange juice for commercial application with added commercial and nutritional value.

Liposomal formulation	Without vitamins	With vitamin C
SPC	100.30 ± 13.05	180.80 ± 22.95***
SPC:SA	95.75 ± 2.75	186.80 ± 26.55***
SPC:CaS	98.50 ± 4.04	206.80 ± 4.50***

Data correspond to ORAC assay in liposomal formulations (50 mM) in acetic acid 3% w/v after pasteurization. Data correspond to soy phosphatidylcholine (SPC), soy phosphatidylcholine and stearic acid (SPC:SA) 1:0.25 molar ratio, and soy phosphatidylcholine and calcium stearate (SPC:CaS) in 1:0.25 molar ratio without vitamins and with 90 mM of vitamin C. Each column represents the mean ± SD of four independent assays. Statistical comparison was made:

Between each system with vitamin/s with respect to the same system without vitamins (control) through the Dunnett test. Significant differences with respect to the control are shown as ***$p < 0.001$.

With respect to SPC in systems without vitamins with the Dunnett test, no significant differences were observed.

Table 1. Peroxidation assay of ORAC in matrix liposome-bioactive compounds.

5. Importance of bioactive compounds impact in consumers of different ages and economical levels

Nowadays, foods are not intended to only satisfy hunger and provide the necessary nutrients to humans but also to prevent nutrition-related diseases and improve the physical and mental well-being of consumers [67].

Diet quality issues in aging populations are of great concern. Functional foods should be considered as foods with health benefits beyond what is interpreted as nutrients and the challenge of bioavailability [54, 68]. It is very important of combining science with consumer desires when considering how to formulate foods that older consumers will actually purchase and eat [68].

Years of research have demonstrated that diet quality has a huge effect on physical and cognitive condition, bone and eye health, vascular function, and the immune system effectiveness. This can be challenging to achieve for reasons very well known like that aging is often accompanied by a loss of appetite and changes in taste and smell, all of which can lead to more limited food choices and lower intake of healthful foods. In other words, aging also often affects food choice and intake since it is accompanied by general oral health decline and a reduced ability to swallow. On top of this, many older adults experience mobility constraints, which make it difficult to shop for food, lift heavy jars, or even open containers. Also, low income is prevalent in aging populations, making it difficult for many older adults to access high-quality foods that in general tend to be more expensive [68].

Macronutrients, namely, omega-3 fatty acids and fiber, are a must in maintaining health during aging. Dietary fiber is known to be important for maintaining intestinal health and protecting against heart disease and other metabolic conditions. With lipids, epidemiological studies have found that higher intakes of omega-3 fatty acids provide greater protection against many conditions, including cardiovascular events (e.g., arrhythmias, cardiac death, and recurrent myocardial infarction), diabetes, and cognitive decline. The problem is that omega-3 fatty acids are very limited in regular diets of older adults, with the main sources like fatty fish, flax seeds, and walnuts. The health effects associated with this group of fatty acids are an important area of current investigation. With respect to the micronutrients, almost every dietary survey conducted over the past few decades has shown that older adults have inadequate intakes of some essential micronutrients. Moreover, subsets of older adults are often at greater risk of certain micronutrient deficiencies. For example, non-Hispanic black and low-income older adults typically experience micronutrient intake levels lower than the other groups of older adults. According to 2005–2006 data, 92% of adults over the age of 51 years are below the Estimated Average Requirement (EAR) for vitamin E; 67% are below the EAR for magnesium; 46% are below the EAR for vitamin C; 33% are below the EAR for zinc; and 32% are below the EAR for vitamin B6. Only 14.6% are above the al for calcium (1200 mg) [68].

Because of the difficulties in obtaining sufficient levels of vitamin E through the diet, many people are taking vitamin E supplements. The concentrations of certain tocopherols are actually lower in people taking supplements. Also, the larger problem is that negative consequences can occur when supplements are erroneously used as a substitute for food [68].

Bioactive compounds could be used to create functional foods for older adults that improve or maintain taste and smell, digestion, brain health, the immune system, bone and joint health, cardiovascular health, gut flora (i.e., probiotic foods), and eye health [68, 69]. The other important issue arising is if the health-food developers do not relate their products to what is important for consumers; then consumers will not use those products. In order to relate products to those factors that are important to consumers, companies must comprehensively understand aging consumers' needs and accept that understanding into food solutions that consumers want, need, and can afford is not even cost-profitable.

Product development—that is, translating aging consumers' needs into products on the shelf—is a very complex, time-consuming process. It involves everything from "culinary creation" (i.e., making a food that tastes good) to ensuring microbiological stability and regulatory compliance. For a product development, there are four essential "elements":

- Form is a key element of the decision-making. For aging boomer consumers, ease of use and legibility of preparation instructions are additional considerations, like developing new types of easy-to-open packages.
- Function is another key consideration, with the primary goal being to ensure that a product is safe regardless of consumer needs. For older adults, this means that the health benefit is validated with the targeted age group and that the products actually deliver those benefits specifically to older adults.
- Appeal (i.e., taste, texture, and appearance). If a product does not taste or look good, people will not eat it, regardless of its contents. Product development involves extensive sensory work to ensure that the intended benefits are delivered. For aging boomer consumers, additional considerations include vibrancy, potency, and consistency.
- Affordability (i.e., raw materials, manufacturability, distribution). This is a huge concern, especially in today's economic climate and especially for aging boomer consumers. Health food developers should optimize raw material usage, working with suppliers to ensure a cost-effective supply chain and minimizing manufacturing and distribution costs. Also, unit size is important. As people age, they tend to cook only for themselves [68].

The United States is one of the countries that have a clear goal of incorporating functional foods to prevent disease, so it is easy to find cereal bars intended for middle-aged women, supplemented with calcium to prevent osteoporosis or with soy protein to reduce the risk of breast cancer and folic acid to improve heart health. In Europe, "value added" signs are used, and in Germany confections are added with coenzyme Q10 and vitamin E. In Italy, supermarket gondolas offer omega-3 yogurts and vitamins, and in France, there is added sugar added with fructooligosaccharides to promote the development of beneficial intestinal flora [70].

Another author [71] reports that are in accordance with the opinion that consumers in general are hardly willing to compromise on the taste of functional foods for health. For that reason, it is important to evaluate the sensorial aspect in the functional foods. The overall conclusion indicated that consumer demand is undoubtedly in the functional foods market, but the industry must respond with good tasting in the products [72].

In relation to the importance of functional foods in the infant population, international studies from the World Health Organization have informed that 5.9 million children under the age of 5 years died in 2015. The problem is that children under 5 years of age who die annually in the world are from preventable diseases. Pneumonia, diarrhea, and malaria are the main causes of death if considered the period from the end of the neonatal stage through the first 5 years of life. Children are the most vulnerable because of malnutrition, which contributes for about 45% of all child deaths [73].

This problem affects then the socioeconomic opportunities that children will have in adulthood, thus increasing the healthcare maintenance. So, it is useless to convey that prevention is a must in this case. Besides, it is well documented that malnutrition causes a lot of problems in children like delayed growth in height, delayed development, weakening of defenses to infections, and, in the most severe cases, death. The problem in itself effect is far more serious in the first years of life due to the greater need for calories and nutrients and because it is a stage of rapid growth of the body [73, 74].

The publication Maternal and Child Health, developed in collaboration by UNICEF and the Argentine Society of Pediatrics, offers a general statistics about this problem in our country. In these studies, a percentage of people obtained is with unsatisfied basic needs which are from different urban regions which is 36.6% for groups of 0–2 years old and 34.1% for groups of 0–17 years old [75].

In Argentina, the National Nutrition and Food Program was created in compliance with the obligation of the state to ensure citizenship the right to a minimum of food intake and cover the requirements of nutritional benefits of children up to 14 years old, pregnant women, and disabled and elderly (70 years onward, in poverty).

In this way, in Argentina, enrichment of wheat flour, established by Law No. 25.630, enacted in July 2002, where this flour destined for consumption in the national market should be clearly highlighted with the rest of the nutrients and in which concentrations are each: ion (30 mg/kg), folic acid (2.2 mg/kg), thiamine (6.3 mg/kg), riboflavin (1.3 mg/kg), and niacin (13 mg/kg).

The problem is that there are other nutrients of importance for the normal development and functioning of children and that it is necessary that the intake of the nutrients be carried out in Argentina as well as in the worldwide level. Let us not forget that adequate food intake during the first 2 years of life is fundamental. Given the rapid growth of children, which conditions high nutritional requirements, coupled with a limited intake capacity in volume, this stage presents in itself a high nutritional vulnerability [76].

6. Evaluation of the functional and sensory properties of improved liposomes with vitamins in food products

The sensorial analysis allows knowing the organoleptic properties of the food because it is realized through the senses. Sensory evaluation is innate in man since from the moment that

a product is tested, a judgment is made about it, whether it likes or dislikes it, and describes and recognizes its characteristics of taste, smell, texture, etc. [77, 78].

When a food market requires so, a certain product must meet requirements for nutrition, hygiene, safety, quality, and sensory aspects, to be accepted by the consumer. It is from all such properties that sensory analysis of foods is an effective tool for quality control. In such a way, sensory evaluation always gives the same global sensory characteristics and acceptability of a food [77, 78].

There are different sensory methods of evaluations. In general, they can be descriptive, discriminatory, and acceptable and preferable. Discriminatory tests should be used when it is necessary to determine whether two samples are significantly different. It is possible that two samples have chemically different formulations, but the sensory perception of the people is unable to perceive the difference. The development of products is based on this possibility, since in reformulating the ingredients of the food are sought that the consumer does not detect any difference [78, 79].

These tests are widely used in the industry, in quality control procedures, in the study of impact by changes in formulation or process, as well as in the ability of consumers to discriminate between two similar products [79].

The affective tests are those in which the evaluator expresses his/her subjective reaction to the product, indicating if he/she likes or dislikes it, accepts or rejects it, or prefers it or not to another. The main purpose of affective methods is to evaluate the response (reaction, preference, or acceptance) of actual or potential consumers of a product. It is necessary, first, to determine whether one wishes to evaluate simply preference or degree of satisfaction (taste or disgust) or whether one also wants to know what is the acceptance of the product among consumers [77]. The choice of the test to be performed will depend on the objectives of the test. The measure of sensory acceptability is a logical and necessary step before launching a product to the market. No one would be willing to invest in a new product that will be sensory unpleasant [80].

These self-swelling mixtures, obtained in large quantities, can be added to the final commercial product online. Based on this property of liposomes, those containing bioactive compounds (PUFAs, vitamins E and C) were added to commercial orange juice (1:100 ratio) of an Argentinean trademark (Citric® of El Carmen S.A.) was selected for a sensory evaluation. These liposomes with bioactive compounds were prepared the day before the sensory evaluation was programmed, then pasteurized, and finally added to the commercial orange juice (1:100 ratio). Samples obtained were kept refrigerated at 4°C until the sensory evaluation was performed. Specific care was taken so that commercial orange juice always kept the physicochemical, microbiological, and sensory characteristics. If any variation in the flavor exists, it would be from the addition of liposomes. For both sensory tests, samples were given to each evaluator in disposable regular cups of 200 mL; and each cup contained 30 mL of product. Each evaluator was provided with mineralized water and unsalted crackers as flavor neutralizers [34, 81].

Two different tests were used to study the addition of liposomes with bioactive compounds in the commercial orange juice. The first test was the triangle test, which was performed to

compare the differences between commercial orange juice with and without liposomes. To analyze similarities between samples, 78 evaluators were selected [34, 81].

Consumers of commercial orange juice (men and women over 18 years old) were selected and instructed in the test [58]. In each test, two samples were the same, and the third one was different (product with and without liposomes). The orange juice was previously brought up to room temperature, and the randomness of the samples was ensured during the whole procedure. Each evaluator tested nine samples to accomplish the requirements of the triangular test for the three formulations. Each evaluator was requested to drink water to neutralize flavors between samples from the same triangle, as requested in a sensory evaluation procedure. Before changing from one sensory triangle to the next, they were also asked to eat a cracker and drink water to avoid sensory fatigue. To end, each evaluator completed a card with their personal inputs [34, 81].

The other test applied was the affective test, and 40 consumers of the commercial orange juice were selected, men and women over 18 years old [77]. The sensory acceptability of the evaluator is faced with unknown samples to judge. The orange juice sample was kept at room temperature, and the randomness was ensured while the test lasted. Hedonic rating scales associated with score used were as follows: (1) I really dislike it; (3) I dislike it; (5) I neither dislike nor like it; (7) I like it; and (9) I really like it. The evaluators are faced with these possibilities or intermediate ones [81]. As stated before in between samples, evaluators were induced to drink water to abstain sensory fatigue [34].

In the triangular test, the outcome results for the orange juice, containing the liposomes with bioactive compounds, considering favorable/total were 43/78 for SPC, 35/78 for SPC:SA, and 38/78 for SPC:CaS. By favorable answers of the evaluator, they found the difference between the commercial product with and without liposomes/bioactive compounds. Applying the statistical table for the triangular test, and considering a significant level of 0.10 for 78 evaluators, the minimum number of correct answers for samples that showed significant differences is 32 [59]. From the above, it is concluded that there are significant differences between commercial juice with or without liposomes and bioactive compounds [34].

With respect to the affective test, although the significant differences were obtained in the triangular test, the addition of liposomes with bioactive compounds did not change the acceptability of the product. These results are reflected in **Table 2**, where the three added formulations showed no significant differences with respect to commercial juice. The results obtained showed that all three liposomal formulations are potentially applicable in the product [34].

Test knowing the samples					
COJ with SPC	**COJ**	**COJ with SPC:SA**	**COJ**	**COJ with SPC:CaS**	**COJ**
5.93 ± 1.61	5.88 ± 1.81	7.03 ± 1.51	7.08 ± 1.44	7.00 ± 1.76	6.90 ± 1.49

Data correspond to soy phosphatidylcholine (SPC), soy phosphatidylcholine and stearic acid (SPC:SA) in 1:0.25 molar ratio, and soy phosphatidylcholine and calcium stearate (SPC:CaS) in 1:0.25 molar ratio. Qualifications of 40 panelists for commercial orange juice (COJ) with or without liposomes with 5 mM of vitamin E and 90 mM of vitamin C, for each type of formulation. Statistics were performed using the test for paired samples between each commercial orange juice sample with and without liposomes. No significant differences were obtained.

Table 2. Total assay acceptability of liposomal formulations knowing the samples in commercial orange juice (COJ).

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7. Future trends

It should be a community efforts trend. By community it is meant an equal commitment effort from two different levels, governmental and science food developers.

For the development of a new food product, it is important to involve the food industry in devising solutions to certain problems, such as the nutrition and health at different stages of life. In this aspect, the application of functional foods has a promising future considering that it promotes and benefits the health beyond the nutritional contribution.

There is a general consensus with Singh [53] concept that several challenges, including discovering of beneficial compounds, establishing optimal intake levels, and developing adequate food delivering matrix and product formulations, need to be addressed.

The implementation of matrices such as liposomes for the transport of bioactive compounds can facilitate the ingestion of these in diverse foods, especially the aqueous ones, being able to be offered to the sectors of the population of risk such as children and elderly people. These types of matrices must have some stability either by adding bioactive compounds or by being present in the food. There is another aspect of great importance that should not be left aside, and that is the taste of functional foods for health. This issue is intrinsically highly speculative, risky, and deemed to yield a niche market strategy. This conclusion entails a challenging future for food product designers, food technologists, and sensory scientists dealing still with one of the fastest growing segments of the food market.

The juices being so well accepted by young children and adults can result in useful tools to be fortified with iron, calcium, vitamin C, vitamin E, and other antioxidants. Its natural content or the result of its fortification in ascorbic acid facilitates the absorption of iron from vegetables and legumes and on health improvement as a final future goal.

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