Encapsulation of Lipid-Soluble Bioactives

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Lipid-soluble bioactives, such as vitamins A, E, D and K, carotenoids, polyunsaturated fatty acids (PUFA) and essential oils, are important nutrients in foods. However, their addition in food formulations, is often limited by limited solubility and high tendency for oxidation. Among the different encapsulation technologies, nanoemulsions are one of the most promising for protecting lipid-soluble bioactives.

Keywords: nanoemulsions ; vitamins ; lipid-soluble bioactives ; emulsions ; food ; antioxidants

1. Introduction

Lipid-soluble bioactives are essential nutrients that play significant role in human diet [1]. This group includes lipophilic vitamins (e.g., vitamin A, D, E and K), carotenoids, polyunsaturated fatty acids (PUFA) and essential oils. However, their direct use in food and beverage products is limited by their low water solubility and high sensitivity towards oxidation. For these reasons, these bioactives need to be encapsulated. A number of innovative technologies are fit for this purpose. Examples include coacervation, spray-drying, freeze drying, spray cooling and fluidized bed coating [2]. Alternatively, emulsification technology is also commonly used.

Emulsions can be also categorized depending on the diameter size of the droplets. Droplet size greatly influences the optical rheology, physical and chemical properties of the emulsions. Conventional or macroemulsions have a droplet size range between 100 nm and 100 µm and are thermodynamically unstable and opaque. Nanoemulsion can be defined by a smaller droplet size with a mean diameter between 20 to 100 nm and they are still categorized as thermodynamically unstable systems. Microemulsions unlike the other two are thermodynamically stable systems with a particle size between 5 nm and 50 nm [3]. Regardless to the type and size of the emulsions, the technology used for their preparation includes high or low energy methods depending on the type of equipment and power needed to produce emulsions.

Although a number of studies have investigated the possibility to improve the oxidative stability of encapsulated bioactives against environmental stress factors and increase their biological and nutritional properties [4], their high surface to volume ratio and the high oxygen diffusion in the aqueous phase may increase the lipid oxidation process [5].

To overcome such drawback, several technologies have been proposed in recent years. Among others, nanoemulsion technology seems one of the most promising, not only for delivering lipid-soluble bioactives in foods, but also for protecting them from oxidation processes [6][7]. A growing number of studies suggest that smaller particle size of the droplets containing lipid-soluble bioactive compounds increase their uptake in biological systems [8][9]. For these reasons, nowadays, many food products, such as soft drinks, butter, ice-cream, milk, dressings, sauces, and creams are produced with application of nanoemulsion technologies. Nanoemulsions with desired composition, stability and functional properties can be prepared using commercial emulsifiers, oils and water using simple operations, such as mixing and homogenization.

2. Encapsulation of Lipid-Soluble Bioactive Compounds by Nanoemulsions

2.1. Vitamin A

The encapsulation of vitamin A in nanoemulsions is challenging because of its low water solubility and high sensitivity to oxidation. Only a few studies have been reported. Hwang and colleagues prepared a phospholipid-based microemulsion system to overcome the solubility problem of all—trans retinoic acid. Their microemulsion was prepared by mixing soybean oil and phospholipids with a high-pressure homogenizer. Eight cycles at 150 MPa were needed. However, the resulting stability was still limited. In 1 h, 9% of all-trans-retinoic acid was degraded. After 7 h, around 59% of all trans retinoic acid was retained [10].

2.2. Vitamin E

Vitamin E defines a group of liposoluble vitamins widely used as antioxidants in food, pharmaceutical, and cosmetic formulations [11]. Various structural forms of vitamin E can be classified as tocopherols derivatives (α, β, γ, and δ) or as tocotrienols (α, β, γ, and δ). The α-tocopherol is considered the strongest antioxidant form among tocopherols. Although vitamin E is one of the most important oil-soluble antioxidants, hydrophobic properties limit its direct dispersion in beverages and food products containing high amounts of water. In addition, vitamin E easily degrades when exposed to oxygen, heat and light. Thus, its encapsulation in nanoemulsions facilitates its incorporation in food products [12].

2.3. Vitamin K

Vitamin K includes a family of lipophilic compounds with a same chemical structure containing 2-methyl-1,4-napthoquinone. Vitamin K1 (2-methyl-3-phytyl-1,4-naphthoquinone; VK1) is a molecule, which exists in a numerous number of green species of vegetables and was shown to prevent skin diseases.

Currently the number of studies on encapsulation of vitamin K by nanoemulsion technology is still limited. A study performed by Campani and colleagues aimed to solve some problems related to semisolid vitamin K1 for incorporation into aqueous lipid free formulation. In this study the nanoemulsions were prepared by using spontaneous emulsification method using Tween 80 as a surfactant and ethanol as organic solvent. The oil phase consisted of α-tocopherol and vitamin K. The organic phase was slowly mixed by a syringe pump at the flow rate 50 mL/min into an aqueous phase, while stirring at 700 rpm in the beginning and at 1400 rpm for the last 5 min. The prepared nanoemulsion was found to be a good option for commercial development of tropical vitamin K1 delivery in both liquid and aqueous formulations [13].

2.4. Vitamin D

Vitamin D is a precursor of a hormone and has two forms: the first, ergocalciferol (D2) is present in fish and plants, while the second cholecalciferol (D3), is synthesized in skin when exposed to the sun.

Studies published on nanoemulsion for the encapsulation of vitamin D are all quite recent. They have the objective to obtain a fortified system to be used as supplement for all health interventions, which are designed for micronutrient delivery. One of the first studies was published in 2015 by Guttoff et al. They produced vitamin D nanoemulsions by the method of spontaneous emulsification. The organic phase was prepared with vitamin D, medium chain fatty acids (MCT), Tween 20, 40, 60, 80 and 85 as surfactant. Aqueous phase at pH 3 consisted of 0.8% citric acid and 0.08% sodium benzoate. In the next step, the organic phase was titrated into the aqueous phase at a fixed speed using a magnetic stir bar. The results also showed that with the spontaneous emulsification method was able to obtain stable system (particle size lower than 200 nm) with droplet growth lower than 10% in diameter after 1 month of storage [14].

2.5. Carotenoids

Carotenoids are another important group of liposoluble antioxidants, which act as filters for blue light in a human eye. Carotenoids are also natural precursors of vitamin A and its metabolites and play an important role in the immune system and formation of cells and tissues [15]. Carotenoids are contained in many vegetables and fruits (e.g., tomatoes, peppers and carrots) and are responsible for their orange, yellow and red color. Carotenoids can be categorized into two main groups: carotenes—α-carotene, β-carotene, γ-carotene, and lycopene; and, xanthophylls—lutein, zeaxanthin, α-cryptoxanthin, and β-cryptoxanthin [16]. Most carotenoids can be found in vegetables and fruits, but they can be in some microbial and edible animal products. Carotenoids have been proved to have a wide a range of positive biological activities [17]. The antioxidant property of carotenoids is due to its binding ability with a singlet oxygen by conjugated double bonds systems [18]. Despite of many health benefits potential of carotenoids, their chemical instability and low water solubility limits their application in many functional beverage and food products [19]. Thus, carotenoids encapsulation by nanoemulsions has been reported to overcome the solubility problems and increase their bioavailability.

Several studies describe encapsulation of carotenoids using nanoemulsions. In the study of Ha and colleagues, nanoemulsions of lycopene were prepared in order to preserve the antioxidant activity and enhance tomato extract bioaccessibility [20]. The nanoemulsion was already enriched with lycopene (contained 6% of lycopene) by the method of emulsification evaporation. For this, tomato extract enriched with lycopene was dissolved in ethyl acetate while stirring for 3 h at 500 rpm. The organic solution and aqueous solution containing 0.5% (w/w) Tween 20 in distilled water were mixed with each other at constant stirring. The mixture was then homogenized at 5000 rpm for 5 min by a shear homogenizer, and accordingly by the high-pressure homogenizer for 1, 2, and 3 cycles at various pressures of 60, 80, 100, and 140

MPa. Under homogenization pressure in between 60 and 140 MPa (3 cycles), the mean droplet diameter of the resulting nanoemulsion was between 96 and 282 nm. The results showed that the lycopene encapsulated in nanoemulsions with droplet size less than 100 nm had the highest in vitro bioaccessibility.

2.6. β-Carotene

Studies related to the encapsulation of β-carotene in aqueous based formulations are limited due to their low water solubility. In 2012, Qian and colleagues studied the effect of the antioxidants on chemical degradation of β-carotene encapsulated in nanoemulsions. The compound was incorporated into an O/W nanoemulsions stabilized by a globular protein (β-lactoglobulin) or Tween 20 as a non-ionic surfactant. A strong chelating agent, such as ethylenediaminetetraacetic acid (EDTA), water-soluble antioxidants such as ascorbic acid or an oil-soluble antioxidant (vitamin E acetate)were added to this formulation along with coenzyme Q10. Nanoemulsions were then kept at neutral pH and both their physical and chemical stability were investigated at 55°C. The degradation of β-carotene was monitored by a nondestructive method which was color reflectance measurements. The results showed that oil in water nanoemulsions were prone to color fading in about 3 days of storage time. The degradation was related to the chemical degradation of the carotenoid. The addition of water-soluble antioxidants effectively retarded the degradation. In detail, EDTA had a high influence on inhibition of the color loss. This effect was due to its ability to strongly chelate and inactivate the metals such as iron transition which normally can promote the oxidation of carotenoid. Ascorbic acid could slow the color fading, but it was found to be less effective than EDTA. In between oil soluble antioxidants, coenzyme Q10 could bring a higher protection against color loss in comparison to vitamin E. One reason for this can be its ability to reproduce other antioxidants which presented in the system [21].

2.7. Polyunsaturated Fatty Acids (PUFA)

Polyunsaturated fatty acids (PUFA) are responsible for reducing risk of chronic diseases, such as cardiovascular disease, inflammation, immune response disorders, mental disorders, and poor infant development can be mentioned [22]. The most important PUFA in human diet are linoleic, alpha-linolenic acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Thus, there is an increased interest in encapsulation of PUFA for food fortification [23].

2.8. Essential Oils and Flavor Compounds

Essential oils are botanical products, which principally derive from whole or specific parts of plants which includes flowers, roots, barks, leaves, seeds, peel, fruits and wood. Apart from their discovered aromatic and coloring properties, essential oils found to have antimicrobial and antioxidant activity [24]. The hydrophobic, volatile and reactive nature of essential oils reduces the possibility of their incorporation directly into food matrices. Encapsulation in nanoemulsions can help to overcome the challenge of essential oils incorporation into food formulations. Common techniques for production of nanoemulsions with essential oils include two methods [25].

References

1. Bengü Öztürk; Nanoemulsions for food fortification with lipophilic vitamins: Production challenges, stability, and bioavail ability. *European Journal of Lipid Science and Technology* **2017**, *119*, 1500539, 10.1002/ejlt.201500539.
2. Paul De Vos; Marijke M. Faas; Milica Spasojevic; Jan Sikkema; Encapsulation for preservation of functionality and targ eted delivery of bioactive food components. *International Dairy Journal* **2010**, *20*, 292-302, 10.1016/j.idairyj.2009.11.008.
3. David Julian McClements; Emulsion Design to Improve the Delivery of Functional Lipophilic Components. *Annual Review of Food Science and Technology* **2010**, *1*, 241-269, 10.1146/annurev.food.080708.100722.
4. David Julian McClements; E.A. Decker; Jochen Weiss; Emulsion-Based Delivery Systems for Lipophilic Bioactive Com ponents. *Journal of Food Science* **2007**, *72*, R109-R124, 10.1111/j.1750-3841.2007.00507.x.
5. D. J. McClements; E. A. Decker; Lipid Oxidation in Oil-in-Water Emulsions: Impact of Molecular Environment on Chemi cal Reactions in Heterogeneous Food Systems. *Journal of Food Science* **2000**, *65*, 1270-1282, 10.1111/j.1365-2621.2000.tb10596.x.
6. Chakraborty, S.; Shukla, D.; Mishra, B.; Singh, S. Lipid—An emerging platform for oral delivery of drugs with poor bioav ailability. Eur. J. Pharm. Biopharm. 2009, 73, 1–15.
7. McClements, D.J.; Li, Y. Structured emulsion-based delivery systems: Controlling the digestion and release of lipophilic food components. Adv. Colloid Interface Sci. 2010, 159, 213–228.

8. Sjöström, B.; Bergenståhl, B.; Kronberg, B. A Method for the Preparation of Submicron Particles of Sparingly Water-Soluble Drugs by Precipitation in Oil-in-Water Emulsions. II: Influence of the Emulsifier, the Solvent, and the Drug Substance. J. Pharm. Sci. 1993, 82, 584–589.
9. Sjöström, B.; Kronberg, B.; Carlfors, J. A Method for the Preparation of Submicron Particles of Sparingly Water-Soluble Drugs by Precipitation in Oil-in-Water Emulsions. I: Influence of Emulsification and Surfactant Concentration. J. Pharm. Sci. 1993, 82, 579–583.
10. Seung Rim Hwang; Soo-Jeong Lim; Jeong-Sook Park; Chong-Kook Kim; Phospholipid-based microemulsion formulation of all-trans-retinoic acid for parenteral administration. *International Journal of Pharmaceutics* **2004**, *276*, 175-183, 10.1016/j.ijpharm.2004.02.025.
11. Y.C. Chiu; W.L. Yang; Preparation of vitamin E microemulsion possessing high resistance to oxidation in air. *Colloids and Surfaces* **1992**, *63*, 311-322, 10.1016/0166-6622(92)80253-x.
12. Shanshan Lv; Yanhua Zhang; Haiyan Tan; Ruojie Zhang; David Julian McClements; Vitamin E Encapsulation within Oil-in-Water Emulsions: Impact of Emulsifier Type on Physicochemical Stability and Bioaccessibility. *Journal of Agricultural and Food Chemistry* **2019**, *67*, 1521-1529, 10.1021/acs.jafc.8b06347.
13. Virginia Campani; Marco Biondi; Laura Mayol; Francesco Cilurzo; Michele Pitaro; Giuseppe De Rosa; Development of nanoemulsions for topical delivery of vitamin K1. *International Journal of Pharmaceutics* **2016**, *511*, 170-177, 10.1016/j.ijpharm.2016.07.004.
14. Marrisa Guttoff; Amir Hossein Saberi; David Julian McClements; Formation of vitamin D nanoemulsion-based delivery systems by spontaneous emulsification: Factors affecting particle size and stability. *Food Chemistry* **2015**, *171*, 117-122, 10.1016/j.foodchem.2014.08.087.
15. von Lintig, J.; Babino, D. Vitamin A and Other Carotenoids. In Principles of Nutrigenetics and Nutrigenomics; Caterina, R.D.E., Martinez, J.A., Kohlmeier, M., Eds.; Academic Press: Cambridge, MA, USA, 2020; pp. 237–244
16. Seung Jun Choi; David Julian McClements; Nanoemulsions as delivery systems for lipophilic nutraceuticals: strategies for improving their formulation, stability, functionality and bioavailability. *Food Science and Biotechnology* **2020**, *29*, 149-168, 10.1007/s10068-019-00731-4.
17. Tilman Grune; Georg Lietz; Andreu Palou; A. Catharine Ross; Wilhelm Stahl; Guangweng Tang; David Thurnham; Shi-An Yin; Hans K. Biesalski; β-Carotene Is an Important Vitamin A Source for Humans. *The Journal of Nutrition* **2010**, *140*, 2268S-2285S, 10.3945/jn.109.119024.
18. Bernardo Dias Ribeiro; Daniel Weingart Barreto; Maria Alice Zarur Coelho; Technological Aspects of β-Carotene Production. *Food and Bioprocess Technology* **2011**, *4*, 693-701, 10.1007/s11947-011-0545-3.
19. Cheng Qian; Eric Andrew Decker; Hang Xiao; David Julian McClements; Physical and chemical stability of β-carotene-enriched nanoemulsions: Influence of pH, ionic strength, temperature, and emulsifier type. *Food Chemistry* **2012**, *132*, 1221-1229, 10.1016/j.foodchem.2011.11.091.
20. Thi Van Anh Ha; SaeHoon Kim; Yeri Choi; H.S. Kwak; Sung Je Lee; Jingyuan Wen; Indrawati Oey; Sanghoon Ko; Antioxidant activity and bioaccessibility of size-different nanoemulsions for lycopene-enriched tomato extract. *Food Chemistry* **2015**, *178*, 115-121, 10.1016/j.foodchem.2015.01.048.
21. Cheng Qian; Eric Andrew Decker; Hang Xiao; David Julian McClements; Inhibition of β-carotene degradation in oil-in-water nanoemulsions: Influence of oil-soluble and water-soluble antioxidants. *Food Chemistry* **2012**, *135*, 1036-1043, 10.1016/j.foodchem.2012.05.085.
22. Nabila Belhaj; Elmira Arab-Tehrany; M. Linder; Oxidative kinetics of salmon oil in bulk and in nanoemulsion stabilized by marine lecithin. *Process Biochemistry* **2010**, *45*, 187-195, 10.1016/j.procbio.2009.09.005.
23. H. Tapiero; G Nguyen Ba; P Couvreur; K.D Tew; Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies. *Biomedicine & Pharmacotherapy* **2002**, *56*, 215-222, 10.1016/s0753-3322(02)00193-2.
24. Giovanna Ferrentino; Ksenia Morozova; Christine Horn; Matteo Scampicchio; Extraction of Essential Oils from Medicinal Plants and their Utilization as Food Antioxidants. *Current Pharmaceutical Design* **2020**, *26*, 519-541, 10.2174/1381612826666200121092018.
25. Qingqing Liu; He Huang; Honghong Chen; Junfan Lin; Qin Wang; Food-Grade Nanoemulsions: Preparation, Stability and Application in Encapsulation of Bioactive Compounds. *Molecules* **2019**, *24*, 4242, 10.3390/molecules24234242.