Journal of Food Engineering 166 (2015) 55–63

Contents lists available at ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Bioactive compounds as functional food ingredients: characterization in model system and sensory evaluation in chocolate milk

Marina Marsanasco [a], Andrés L. Márquez [b], Jorge R. Wagner [b], Nadia S. Chiaramoni [a], Silvia del V. Alonso [a,*]

[a] Laboratorio de Biomembranas, Grupo de Biología Estructural y Biotecnología (GBEyB), IMBICE-CONICET, Universidad Nacional de Quilmes, Roque Sáenz Peña 352, (B1876BXD) Bernal, Buenos Aires, Argentina
[b] Laboratorio de Investigación en Funcionalidad y Tecnología de Alimentos (LIFTA), Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Sáenz Peña 352, (B1876BXD) Bernal, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 10 September 2014
Received in revised form 20 February 2015
Accepted 10 May 2015
Available online 12 May 2015

Chemical compounds studied in this article:
Stearic acid (Pubchem CID: 5281)
Calcium stearate (Pubchem CID: 1532)
Vitamin E (Pubchem CID: 14985)
Folic acid (Pubchem CID: 6037)
Laurdan (Pubchem CID: 104983)
Merocyanine 540 (Pubchem CID: 5702743)

Keywords:
Liposome
Bioactive compounds
Vitamins
Preservation
Stability
Acceptability

ABSTRACT

The objective was the design and the structural and functional characterization of bioactive compounds (BC) as functional food ingredients for a future industrial application in chocolate milk. Liposomes were made of soy phosphatidylcholine, containing BC (omega-3, omega-6, vitamin E) and encapsulating folic acid (FA). Stearic acid and calcium stearate were added as liposome stabilizers.

The oxidative stability, size and shape were analyzed by thiobarbituric acid method, light scattering, and light microscopy, respectively. Membrane packing was also studied. Rheological behavior of liposomes and encapsulation efficiency of FA were analyzed after pasteurization. Studies were performed in food-model. Liposomes showed significant stability of all parameters and a protective effect over thermolabile FA remaining half of this vitamin encapsulated.

Sensory evaluations were studied in chocolate milk with BC, demonstrating positive effects on acceptability. For all the above, BC formulations are suitable for a future application and/or scaling up in dairy industry.

1. Introduction

Bioactive substances present as natural constituents in food provide health benefits beyond the basic nutritional value of the product (Biesalski et al., 2009). They are extranutritional constituents that typically occur in small quantities in foods and they are being intensively studied to evaluate their effects on health (Kris-Etherton et al., 2002). For example, it is important to consume essential fatty acids because human body cannot produce those (López and Suárez, 2003). However it is not the only quality because they have proven benefits in preventing cardiovascular disease (Lee and Lip, 2003), schizophrenia (Sivrioglu et al., 2007) and cancer (Jenski et al., 1995), among others. Also, they have properties of vasodilators, antihypertensive, anti-inflammatory, and anti-atherothrombotic (Baguma-Nibasheka et al., 1999). Essential fatty acids like linolenic acid (ω-3) and linoleic acid (ω-6) are contained in soy phosphatidylcholine (SPC) which is a natural lipid.

Besides, vitamins have important functions in certain metabolic processes in the human body. Vitamin E (VE) or α-tocopherol is the major fat soluble antioxidant in the body. It protects the lipids against oxidative damage (Atkinson et al., 2008), reduces the formation of hydroperoxides and delays the initial phase of the oxidative process (Ordóñez et al., 1998). Also, it is related to the decrease in blood cholesterol, having a positive effect on the incidence of atherosclerosis and cardio-circulatory system (Primo Yúfera, 1998). Another antioxidant vitamin, which is hydrosoluble, is the folic acid (FA) or vitamin B9. This vitamin acts as a cofactor in carbon transfer reactions (formyl, hydroxymethyl, and methyl) nucleotide biosynthesis (purine bases and pyrimidine), amino acid metabolism (methionine, histidine) and metabolism

* Corresponding author.
E-mail address: salonso@unq.edu.ar (S.d.V. Alonso).

http://dx.doi.org/10.1016/j.jfoodeng.2015.05.007
0260-8774/

neurotransmitters (serine, choline) (Bekaert et al., 2008). Deficiency of FA is related to neural tube defects, heart disease and megaloblastic anemia (López and Suárez, 2003). And an important discovery in recent years is that there are studies linking FA intake with a decreased risk of heart disease and cancer (Bailey et al., 2003; Malinow et al., 1998).

However, most of the bioactive compounds: e.g. fatty acids, carotenoids, tocopherols, flavonoids, polyphenols, phytosterols, oil soluble vitamins have hydrophobic nature (Kris-Etherton et al., 2002). Besides it is not easy to add vitamins to aqueous foods while retaining their activity. For instance, VE is easily oxidized in the air (Ordóñez et al., 1998), while FA is thermolabile and is degraded after thermal treatments like pasteurization and light exposure (Fennema, 2000). The addition of these vitamins to aqueous foods using liposomes appears to be a promising solution. Liposomes are microscopic spherical vesicles composed of polar lipids like phospholipids, which enclose liquid compartments within their structure (consisting of lipid bilayers) and enable the encapsulation of both hydrophilic and lipophilic materials (Keller, 2001). With respect to VE, it was demonstrated that this vitamin mixes perfectly with the phosphatidylcholine of the bilayer (Wang and Quinn, 2000). With respect to hydrosoluble vitamins, liposomes promote the protection and activity of vitamins like vitamin C (Marsanasco et al., 2011). Liposomes could be done with SPC allowing the incorporation of bioactive compounds (essential fatty acids, VE) and FA in food like milk, generating a functional food. In recent years, functional foods are defined as the substances which provide benefits to the health of the consumers. Typically, a food marketed as functional contains added, technologically developed ingredients with a specific health benefit (Niva, 2007).

In the food industry, for a given industrial application, membrane stability and structure are important factors when designing liposomes (Keller, 2001), considering that phospholipids can be oxidized, limiting their lifetime (Grit and Crommelin, 1993). That is why several studies include characterization of liposomes by several methodologies. Besides, for preservation of liposomes is necessary to apply a heat treatment used in food industry like pasteurization. And it is very important that liposomes remain stable after a heating process because the higher the stability, the higher the protection of vitamins (Marsanasco et al., 2011) and bioactive compounds.

For an industrial application and production line of a new food, it is very important to assess its acceptability and ease of production. 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.

The aim of this work was the design and the structural and functional characterization of different liposomal formulations based on SPC with bioactive compounds (omega-3, omega-6, VE) and VC for developing a functional food in pasteurized chocolate milk. Essential fatty acids like linolenic acid (ω-3) and linoleic acid (ω-6) are contained in SPC and in the formulations, the stearic acid (SA) and the calcium stearate (CaS) were incorporated for stabilize the lipid bilayer by increasing rigidity. Besides, addition of CaS incorporated a mineral (calcium) that increases the nutritional value. Oxidative stability was determined with the thiobarbituric acid method. The size and the shape were analyzed by light scattering and light microscopy, respectively. And the packing of membrane was studied with two probes (merocyanine 540 and Laurdan). All studies were performed with or without vitamins and before and after pasteurization in order to have information about the effect of the heat treatment. Pasteurization was applied for preserve the liposomes. Also, encapsulation efficiency of FA was determined after 72 h of dialysis in pasteurized formulations. The experiments mentioned were made in a food model system in order to avoid fluctuations in data due to other components of the food product. Finally, applicability in the product was studied by the rheological behaviors of liposomes and by the sensorial tests. The sensory evaluation in commercial chocolate milk, with and without liposomes, was done by the overall acceptability with hedonic scale and triangular tests with 40 and 78 potential consumers, respectively.

2. Materials and methods

2.1. Raw materials

SPC was purchased from Avanti Polar Lipids (Alabaster, USA). SA and CaS were purchased from Vitalquim (Buenos Aires, Argentina). VE was obtained from Parafarm (Buenos Aires, Argentina) and FA was obtained from Anedra (Buenos Aires, Argentina).

2.2. Liposome preparation

Multilamellar liposomes were prepared by the dehydration–rehydration method (Bangham, 1972). Briefly, 40 μmol of lipids were dissolved in 500 μL ethanol in a round bottom flask, solvent was dried in a rotary evaporator at 37 °C. Dry lipid film composed by SPC, SPC:SA (1:0.25, mol ratio), and SPC:CaS (1:0.25, mol ratio) was rehydrated with 2 mL distilled water to a final 50 mM lipid concentration.

In order to prepare liposomes with VE, a stock solution of this vitamin diluted in ethanol was prepared. Stock concentration was 22.4 mM. Then, 0.445 mL of this stock was mixed with a proper amount of lipids. Solvent was evaporated and lipid film was obtained. When the film was rehydrated in 2 mL of distilled water, a final concentration of 5 mM was reached.

In the case of FA, fresh solutions of this vitamin were prepared at the moment of rehydration. FA was weighted and diluted with distilled water to reach a 0.136 mM concentration.

Samples were prepared with the main goal of fortify food with mentioned vitamins. According to Argentina regulations (Article 1363 of the Argentine Food Code), the percentage of recommended daily intake (RDI) in a portion of fortified food must be between 20% and 50% for fat soluble vitamins and between 20% and 100% for hydro-soluble vitamins. The RDI of VE is 10 mg and for FA is 400 μg. In this work, chocolate milk was used for the sensory evaluation assay. In order to fortify this beverage, 2 mL of liposome suspension (50 mM) with vitamins was added in each serving of chocolate milk (200 mL), which implies that it was fortified with 4.3 mg of VE (5 mM) equivalent to 43% of the RDI and 120 μg of FA equivalent to 30% of the RDI. Thus, 1 L of chocolate milk contained 21.5 mg of VE and 600 μg of FA.

2.3. Preparation of food model systems

Assays were performed in distilled water, classified as food simulant according to local food regulations (Mercosur Resolution No. 30 of 1992). This solution simulates aqueous food having a pH higher than 5, such as chocolate milk.

2.4. Lipid peroxidation stability

Lipid peroxidation was followed by the thiobarbituric acid (TBA) method, as described before (Marsanasco et al., 2011).

2.5. Particle size distribution

Particle size distributions were determined in the range 0.1–1000 μm by laser scattering using a Particle Analyzer (Malvern Mastersizer 2000E, Malvern Instruments Ltd, UK).