Formulation and characterization of liposomes containing fatty acids omega-3 for food aplications.

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Introduction

- ✓Omega-3 polyunsaturated fatty acids have shown benefits for health.
- ✓Biological activities of these fatty acids can be low down by food processing and environmental conditions (i.e. light, temperature).
- ✓Liposomes constitute a very promising technological alternative for omega-3 delivery.
- ✓Liposomes are self-closed vesicles with one or several concentric phospholipid bilayers with an aqueous phase inside and between bilayers.
- ✓Food applications are limited because of colloidal instability and irreproducibility of preparations.

Objectives

The objectives of the present work were to formulate liposomes containing omega-3 fatty acids, to characterize them in size and morphology and to evaluate the effect of physical treatments (ultraturrax and sonication) in size distribution. In addition, the morphology of liposomes coated with whey or caseins from different milks (cow, sheep and goat) was characterized.

Abstract

The objective of the present work was to formulate liposomes containing eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), and characterize them in encapsulation efficiency, size and morphology. The effect of physical treatments (ultraturrax and sonication) on size distribution was also studied. Besides, whey and caseins fractions of different types of milk (cow, sheep and goat) were utilized to coat liposomes. The lipid film hidration was the method employed to prepare liposomes utilizing phosphatidilcholine, cholesterol and esters of EPA and DHA. The omega-3 fatty acids were determined by gas chromatography. The size distribution of liposomes was determined by dynamic light scattering: vesicles had an average size of 5 microns, which decreased to 60-70 nm after applying the physical treatments. Transmission electron microscopy (TEM) images of liposomes showed conventional liposomes (uncoated) had a smooth and even surface; while the coated liposomes had irregular surfaces. All images showed high structural integrity. The efficiency of incorporation of fatty acids was 30% for EPA and 20% for DHA. These studies are preliminary and currently there are undergoing trials to improve the efficiency of encapsulation and their application in dairy foods.

Material and Methods

Obtention of milk protein fractions to coat liposomes

✓ Protein milk fractions used to coat liposomes were isolated from goat, sheep and coat milk. For that, milks were acidified at 4.6 and centrifuged: two fractions were obtained (one of soluble whey proteins and one of insoluble caseins).

Preparation of multilamellar liposomes by "film hydration method"

✓ Preparation of liposomes without coating: Phosphatidylcholine (161 mg), cholesterol (9.7 mg) and ethyl esters of omega 3 fatty acids (EPA and DHA) (100 mg) were dissolved in chloroform (6 ml). Solvent was eliminated by rotary evaporation (50ºC/6 h) in order to obtain a lipid film which was then hidrated with PBS buffer (pH 7, 30 ml). Liposome dispersion was reserved at 4ºC.

✓Preparation of coated liposomes: liposome suspensions were incubated with PBS solutions of the protein milk fractions obtained. Seven types of liposomes were obtained: uncoated (L1), coated with goat whey proteins (L2), goat caseins (L3), sheep whey proteins(L4), sheep caseins (L5), cow whey proteins (L6) and cow caseins (L7).

Physical treatments

Sonication (180, 60, 120 min) and homogenization in Ultraturrax (4, 8, 12, 15 and 20 min) was applied in uncoated liposome dispersions to reduce its size and obtain oligolamellar liposomes.

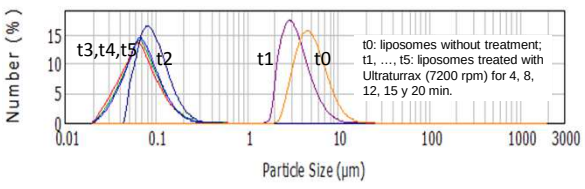
Analysis

- ✓ ***Size Distribution:*** by Dynamic Light Scattering (DLS).
- ✓ ***Morphological characterization:*** by negative staining transmisison electronic microscopy (TEM).
- ✓ **Encapsulation efficiency:** liposome dispersions were filtere by 100 KDa membranes and centrifuged. Omega-3 fatty acids were analyzed in supernatant by GC .
- ✓ ***Gas chromatography–mass spectrometry (GC-MS):*** EPA and DHA were extracted by ethyl ether and analyzed by gas chromatography coupled to mass spectrometry (GC / MS) (scan from 45-450 Da). Quantification was performed with methyl stearate as an internal standard.

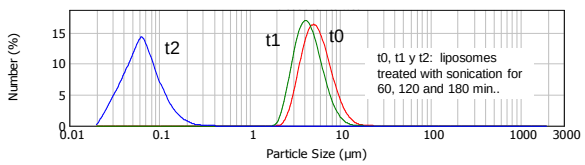
Results and Discussion

Effect of physical treatments on size distribution

✓ Size distribution of no-coated liposomes diminished with increasing time of homogenization in Ultraturrax. Mean values obtained were 5.4; 3.3; 0.09; 0.07 y 0.07 µm for 4, 8, 12, 15 y 20 min of treatment, respectively.



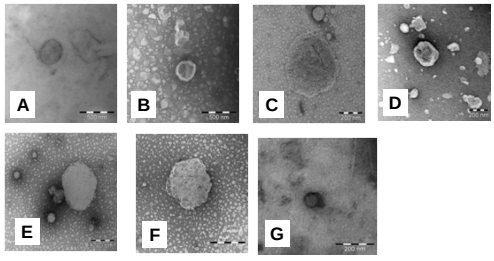
✓ A decrease in the size distribution of no coated liposomes by increasing sonication time was also observed. The mean size values were 5.3, 4.3 and 0.06 µm after 60, 120 and 180 min of treatment, respectively.



Morphological characterization

✓ Size variability was observed in the different liposome suspensions.

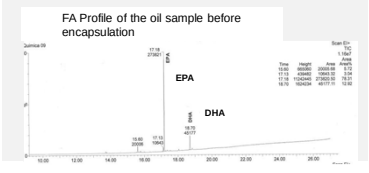
✓ Liposomes without coating presented smooth and even surface; while the coated liposomes had irregular surfaces. All images showed high structural integrity.

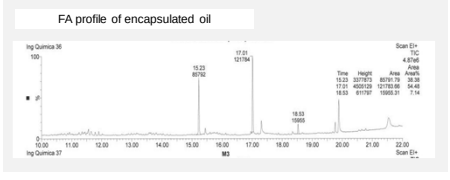


Photomicrographs of 7 types of liposomes: uncoated (A), coated with goat whey proteins (B), goat caseins (C), sheep whey proteins (D), sheep caseins (E), cow whey proteins (F), and cow caseins (G).

Encapsulation efficiency

✓ 30% of EPA and 20% of DHA were incorporated in liposomes.





Conclusions

- In this work we developed and characterized stable liposome carriers of EPA and DHA.
- It could be possible to control size distribution of liposomes by Ultraturrax homogenization and sonication.

The present work constitutes a preliminary study of liposome structures containing omega-3 FA with a view of potential applications in food formulations.

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