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Encapsulation of food waste compounds in soy phosphatidylcholine liposomes: Effect of freeze-drying, storage stability and functional aptitude

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ABSTRACT

Liposomes made from soy phosphatidylcholine entrapping food waste compounds (collagen hydrolysate, L-HC; pomegranate peel extract, L-PG; and shrimp lipid extract, L-SL) were freeze-dried and stored for seven months. The freeze-drying process increased the particle size and decreased water solubility. The freeze-dried L-HC and L-PG preparations presented large multivesicular vesicles with spherical and unilamellar morphology. Large multilamellar vesicles were observed in L-SL, coinciding with greater structural changes in the membrane bilayer and increased thermal stability, as observed by ATR-FTIR and DSC. Dynamic oscillatory rheology revealed a slight hardening in the dried liposomes, induced by storage time. A sharp rigidifying effect in the temperature range from 40 to 90 °C was observed in L-SL. The loading with antioxidant compounds prevented freeze-drying-induced lipid oxidation. The storage stability of freeze-dried liposomes and their technological aptitude as a food ingredient varied depending on the chemical nature of the entrapped compounds.

1. Introduction

Nowadays there is growing interest in the use of liposomes in the food industry in a variety of applications (Mozafari et al., 2008). Liposomes carrying bioactive compounds could be incorporated in functional foods for enrichment with healthy components or diseases prevention (Singh, 2016). Soy phosphatidylcholine-based liposomes have been used for encapsulating omega-3, omega-6, and vitamins E and C to fortify chocolate milk (Marsanasco et al., 2016) or orange juice (Marsanasco et al., 2015). The use of natural soybean lecithin for liposomal encapsulation in food applications provides nutritional value owing to its high essential polyunsaturated fatty acid profile with a beneficial role in lipid metabolism (Ramdath et al., 2017), and it does not raise any food legislation concerns (Laye et al., 2008). However, the predominantly highly unsaturated nature of soy lecithin could make this material very susceptible to lipid oxidation (Wang and Wang, 2008).

Liposomes are amphipathic spherical colloidal vesicles composed of one or more phospholipid bilayers around an aqueous core. This feature enables them to entrap and protect both hydrophilic and lipophilic bioactive materials, and to act as target delivery carriers in the organism (Mozafari et al., 2008). There is extensive recently published information on phosphatidylcholine liposomes loaded with a great variety of natural compounds with antioxidant properties, such as gelatin or collagen hydrolysates (Ramezanzade et al., 2017; Mosquera et al., 2014), polyphenolic compounds (Popova and Hincha, 2016; Lopes de Azambuja et al., 2015), or strong lipophilic compounds such as carotenoids (Du et al., 2015), tocopherol (Neunert et al., 2015) or essential polyunsaturated fatty acids (Semenova et al., 2016). However, differences in liposome composition and production procedure make it difficult to compare the physico-chemical properties of the resulting loaded vesicles.

Liposomes are normally presented in the form of aqueous liposomal suspensions, which could lose stability causing vesicle fusion or aggregation, leakage of the entrapped compounds and sedimentation (Sharma and Sharma, 1997). Lyophilisation is an alternative process for increasing the shelf life of liposomes, maintaining their stability by preserving them in a dry state (Stark et al., 2010). Drying liposomes is also a technological way of including vesicles in restructured fish products without negatively affecting their water content (Marín et al., 2018). However, freeze-drying may damage lipid bilayers by ice crystal formation during

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freezing, vesicle fusion/aggregation following dehydration and changes in phase transition during rehydration (Chen et al., 2010). Cryoprotectants, such as carbohydrates or polyalcohols, have been proposed to prevent freeze-drying-induced vesicle damage (Stark et al., 2010).

The aim of this work was to study the effect of freeze-drying and long-term storage on physico-chemical, structural, rheological and oxidative properties of glycerol-added liposomes entrapping various heat-sensitive food waste compounds with high added value, which could be used as functional food ingredients.

2. Materials and methods

2.1. Extraction of food waste compounds

Alcalase acid-soluble collagen hydrolysate (HC) was obtained from frozen squid (*Dosidicus gigas)* tunics following the procedure described in a previous work (Marín et al., 2018). The HC was spray-dried and stored at −20 °C until use. The HC hydrolysate was mostly composed of <1.3 kDa peptide fractions (89%), with major constituent amino acid residues of glycine, glutamic acid, alanine and aspartic acid (56%), and total hydrophobic amino acids accounting for 28% (Marín et al., 2018).

Pomegranate (*Punica granatum*) peel and albedo were dried in an oven at 50 °C overnight and grounded to obtain a fine powder, which was suspended in ethanol/water (70/30) at 1:20 (w/v) ratio and stirred at 40 °C for 4h. The mixture was left to stand at 21 °C overnight and centrifuged at 12000g (at 4 °C for 15 min). The supernatant was filtered through Whatman No.1 paper. The obtained extract (PG) was rotary-evaporated at 40 °C and stored at −20 °C until use. The PG extract was mainly composed of ellagitannins (β-punicalagin, ellagic acid, and α-punicalagin), being rutin and epigallocatechin also identified. The total phenolic content in PG was 166 mg gallic acid eq./g dry extract (Marín et al., 2018).

The ethyl acetate-soluble lipid extract from shrimp (*L. vannamei*) waste (SL) was prepared and characterized in a previous work (Gómez-Estaca et al., 2017). The SL extract was composed of ≈80% fatty acids, ≈13% α-tocopherol, 6.5% cholesterol and 0.7% astaxanthin (free and esterified). The PUFA proportion was 44%. The SL was rotary-evaporated at 60 °C and stored at −20 °C until use.

2.2. Preparation of liposomes

Partially purified phosphatidylcholine (PC) was obtained by dissolving soybean lecithin in ethyl acetate (1:5, w/v) and subsequently performing five washes with acetone (Taladrid et al., 2017). The PC powder was stored at −20 °C until use.

Liposomes L-HC, L-PG and L-SL were produced according to Marín et al. (2018). Each extract (0.8 g) was dispersed in 80 mL of 0.2 M phosphate buffer (pH 7). Then PC (20 g) was added and the dispersions were kept in a water bath at 80 °C for 1 h. Phosphate buffer (68 mL) and glycerol (12 mL) were added, and the mixtures were kept at 80 °C for 1 h. The volume of the suspension was completed with 240 mL of phosphate buffer. The samples were vortexed at 60 °C and sonicated in an ultrasonic cell disrupter (Model Q700, Qsonica sonicators, Newton, CT, USA). The hydrodynamic particle stability of the newly prepared liposomal dispersions was measured during 2 weeks at 4 °C. The freeze-drying process was performed by placing 50 mL of newly prepared liposomal dispersion in plastic cups of 100 mL with perforated caps, which were frozen at −80 °C for 24h. Lyophilisation took place in a VirTis Freeze Drying Equipment (VirTis mod.6K TEL-85, coupled to TRIVAC-E2 pump) operating at a vacuum level of 0.13 mbar, with the collector starting at a temperature of −45 °C up to −80 °C after 48h. All dried liposomes presented a pasty-like consistency rather than a fine powder appearance. The freeze-dried liposomes (liposomal pastes) were stored in darkness at −20 °C for seven months, in order to check their long-term storage stability.

2.3. Size, polydispersity and zeta potential

Particle size (z-average in intensity), polydispersity index (PDI) and zeta potential of fresh and rehydrated liposomes were measured using a Zetasizer Nano ZS (Malvern Instruments Ltd, Worcestershire, UK) in triplicate at 25 °C (Alemán et al., 2016). Freeze-dried liposomes were previously rehydrated by suspending in distilled water (77 mg/mL) at 20 °C for 30 min under magnetic stirring.

2.4. Entrapment efficiency

The entrapment efficiency (EE) was determined following the procedure of Marín et al. (2018), and calculated by the equation:

% EE = encapsulated extract / total extract * 100.

The encapsulated extract was calculated by difference between the total extract and the non-encapsulated extract. The non-encapsulated extract was quantified by: protein content with a LECO FP-2000 nitrogen/protein analyser for HC; phenolic content by the Folin–Ciocalteu method for PG; and astaxanthin content by spectrophotometric absorbance measurement at 470 nm for SL.

2.5. Moisture content and water solubility

Moisture was determined according to method 950.46 (A.O.A.C., 2005). Water solubility was determined after dilution in distilled water (1% w/v) at 20 °C for 150 min under magnetic stirring and centrifugation at 5000*g* at 4 °C for 5 min. The supernatant was dried at 105 °C and the water solubility, expressed as a percentage, was calculated by weight difference with respect to the initial sample weight.

2.6. Cryo-transmission electron microscopy (cryo-TEM)

Cryo-TEM images of freeze-dried liposomes were obtained at −180 °C, using a JEOL JEM-1230 transmission electron microscope operating at 100 kV with a nominal magnification of 30K, as described previously (Taladrid et al., 2017).

2.7. Colour

The colour parameters, L* (lightness), a* (redness) and b* (yellowness) of freeze-dried liposomes, were measured using a Konica Minolta CM-3500d colorimeter (Konica Minolta, Madrid, Spain), with D65 illuminant and D10 standard observer. Hue angle and chroma values were calculated from L* a* b* values. Results were the average of 10 replicates.

2.8. Differential scanning calorimetry (DSC)

DSC analysis was performed using a model TA-Q1000 differential scanning calorimeter (DSC) (TA Instruments, New Castle, DE, USA) (Taladrid et al., 2017). All samples (≈10–12 mg) were scanned at a heating rate of 10 °C/min from −35 °C to 90 °C, under dry nitrogen purge (50 mL/min). Endothermic peak temperatures (T_{peak},°C) and enthalpies of conformational changes (ΔH, J/g) were determined at least in triplicate.