Journal of Food Engineering 244 (2019) 47–54

Contents lists available at ScienceDirect

Journal of Food Engineering

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

Changes in structural integrity of sodium caseinate films by the addition of nanoliposomes encapsulating an active shrimp peptide fraction☆

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ARTICLE INFO

Keywords:
Shrimp peptide fraction
Nanoliposomes
Caseinate film
Film structure
Sensory properties
Mucoadhesion

ABSTRACT

A shrimp peptide fraction (ST1) with biological activity (ABTS radical scavenging capacity, angiotensin-converting enzyme and dipeptidyl-peptidase-IV inhibitory activities), was encapsulated in partially purified soy phosphatidylcholine nanoliposomes (L-ST1) using glycerol to preserve the bilayer during the film-forming dehydration step. The z-average, zeta potential and encapsulation efficiency of L-ST1 were, respectively, 99.98 nm, −53.87 mV and 52.37%. Transmisssion Electron Microscopy images showed that the liposomes incorporated in sodium caseinate films caused film matrix disruption, but vesicle structure was well preserved and uniformly distributed along the film matrix. The films with liposomes became more water soluble, adhesive and mucoadhesive, with no changes in thickness or transparency. The film showed a very favourable taste perception, regardless the presence or type of liposomes, while the buccal dissolution was faster with the films carrying the liposomes. The film could be used in different food designs without distorting the sensory acceptance of the final product.

1. Introduction

In the food sector there is currently a growing demand for products of natural origin, with bioactive capacity, low toxicity and preferably environmentally friendly. To prepare this functional food made to meet specific health requirements, a wide variety of biologically active molecules can be used as ingredients. Great part of these molecules could derive from industrial food by-products or waste. Valorisation of this material would result in an economic and environmental benefit, and at the same time, value-added products or even co-products would be obtained (López-Caballero et al., 2016).

Obtaining protein hydrolysates from industrial fish waste has received considerable attention in the last decade (López-Caballero et al., 2013). Such hydrolysates are an important source of bioactive peptides with diverse properties such as antioxidant, antihypertensive or hypoglycaemic activity (López-Caballero et al., 2013, Ketnawa et al., 2016, Ji et al., 2017). Bioactive peptides could be used as potential ingredients in functional food. However, during storage and/or food processing, peptides can be subjected to proteolytic degradation, show instability under extreme conditions (pH, temperature, oxygen …) and/or interact with other components (divalent cations, lipids, proteins …) (Aasen et al., 2003; Chollet et al., 2008; Mozafari et al., 2008). All these factors will greatly limit their potential health benefits, and make necessary the search for protective mechanisms to maintain the peptides in active forms until consumption or even to directly deliver them to the physiological targets. Liposomes have the ability to trap water-soluble, lipid or amphiphilic materials, therefore the encapsulation in liposomes has been reported to be a good way to protect and transport bioactive peptides (Mozafari et al., 2008; Malheiros et al., 2010). The high polyunsaturated fatty acid composition and residual tocopherol content in natural soy lecithin or partially purified phosphatidylcholine provides extra nutritional value for food-grade liposome production (Taladrid et al., 2017). Furthermore, the use of a film matrix as vehicle of peptide-loaded liposomes could represent a versatile strategy to introduce them into a functional food, without the need of restructuring or greatly affecting the food presentation. At the same time, the undesirable effects derived from strong flavour or excessive interactions of bioactive peptides with other constituents present in the film or in the food matrix could be greatly avoided. It should be noted that the film by itself could also be considered as a model system of food with low water content. However, free peptides or liposome-encapsulated peptides could induce physical changes in the film structure that should be taken

☆ ICTAN-CSIC has implemented and maintains a Quality Management System which fulfils the requirements of ISO standard 9001:2015.

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https://doi.org/10.1016/j.jfoodeng.2018.09.024
Received 5 June 2018; Received in revised form 14 September 2018; Accepted 15 September 2018
Available online 17 September 2018
0260-8774/

into account. To this respect, Giménez et al. (2009) observed that the incorporation of peptide hydrolysates induced strong plasticization in gelatin films. The addition of liposomes was also found to change the molecular structure in chitosan films, leading to the decrease in density and tensile strength, with concomitant increase in water solubility (Cui et al., 2017). Casein films have been reported to be an excellent matrix to incorporate nisin-loaded liposomes and halloysite (nanoclay), without altering the film mechanical resistance and thermal degradation properties (Boelter and Brandelli, 2016).

The use of mucoadhesive edible films carrying bioactive peptides-loaded liposomes (either as a quick-dissolving edible strip or as a food wrapping) could be an interesting way for buccal delivery of these bioactive molecules. The buccal and sublingual mucosae are highly permeable, and it would allow the nanocapsules loading bioactive compounds to pass directly to the systemic circulation and further to penetrate into tissues through fine capillaries (Patel et al., 2011). Recent research indicates that these mucoadhesive systems must provide fast release in the oral cavity (Silva et al., 2015; Mašek et al., 2017). Regardless the mode of presentation, the response in mouth of such mucoadhesive films requires being easy-dissolving, avoiding the impression to ingest an artificial plastic.

The objective of the present work was the preparation of a functional sodium caseinate film with mucoadhesive and good sensory properties by introducing nanoliposomes containing an active peptide fraction from discarded shrimp. The peptide fraction was characterised in terms of molecular weight, aminoacid composition and *in vitro* biological activity (antioxidant, antihypertensive and hypoglycaemic capacity).

2. Materials & methods

2.1. Preparation of the peptide fraction

Shrimp (*Penaeus notialis*) frozen during 6 years at −20 °C (non-commercial value) were unfrozen and washed in cold water. Endogenous enzymes were firstly inactivated by heating shrimp (previously vacuum packed) in a water bath at 80 °C for 20 min. Boiled shrimp were then grounded in an Osterix blender (Osterizer, Par Sunbeam, Mod. 867 50 E, USA) and the resulted homogenate was used as raw material. The sample was then mixed with 0.07 M phosphate buffer at pH 8 (1:5, w/v) and the slurry was heated to 38 °C. The enzymatic hydrolysis was started by adding 1% (w/w) of Pancreas trypsin (7 Units/mg, Novozymes, Bagsvaerd, Denmark). During hydrolysis, temperature was controlled, and the pH was kept constant by addition of 1 N NaOH solution using a pH-stat (TIM 856, Radiometer analytical, Villeurbanne, France). After the required digestion time (90 min), the reaction was stopped by heating the sample at 90 °C for 20 min. The hydrolysate was sieved to remove the solid material and the liquid fraction was clarified by centrifuging at 12000 g (Sorvall RT60008 centrifuge, DuPont Co., Delaware, USA) at room temperature for 20 min.

The degree of hydrolysis (DH), determined using the pH-stat method (Adler-Nissen, 1986), was 17.77%.

The hydrolysate was filtered through a 1 kDa MW cut-off (MWCO) membrane of 1 kDa (Amicon Ultra-15 centrifugal filter, Merck Millipore, Billerica, MA, USA). Permeate was freeze-dried and stored at – 80 °C. The peptide fraction obtained was designated as ST1.

2.2. Characterisation of the peptide fraction

The molecular weight distribution of ST1 was determined by SEC-HPLC (SPE-MA10AVP, Shimadzu, Kyoto, Japan) as described by Martínez-Álvarez et al. (2012). Aprotinin (6511 Da), Vitamin B12 (1345 Da), Hippuryl histidyl leucine (429 Da) and glycine (75 Da) were used as molecular weight standards.

The aminoacid composition of the peptide fraction was determined following the procedure described by Alemán et al. (2011).

The Angiotensin converting enzyme (ACE) inhibitory activity and the ABTS radical (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)) scavenging capacity of ST1 were determined according to Alemán et al. (2011). The IC50 value was defined as the concentration of protein (μg protein/mL) required to reduce the hippuric acid peak by 50% (indicating 50% inhibition of ACE activity). The results of the ABTS radical scavenging capacity were expressed as mg Vitamin C Equivalent Antioxidant Capacity (VCEAC)/g protein, based on a standard curve of vitamin C. The amount of protein was calculated from the amino acid analysis. Determinations were carried out in triplicate.

The Dipeptidyl peptidase-IV inhibitory activity (DPP-IV) inhibiting activity of the ST1 was measured according to Tulipano et al. (2011) with slight modifications. The assay was performed in a 96-well plate. Briefly, 10 μL of human DPP-IV (previously diluted in 100 mM Tris HCl buffer, pH 8) were incubated in the absence or in the presence of different concentrations of sample (final volume 300 μL per well) at 37 °C for 15 min. The sample was previously diluted in 100 mM Tris HCl buffer at pH 8. The reaction started after addition of 100 μL of assay buffer containing the chromogenic substrate (H-Gly-Pro-AMC·HBr) at final concentration of 25 μM. The change in fluorescence at 355 nm/460 nm was monitored at 1-min intervals for 15 min in an Appliskan Multimode Microplate Reader (Thermo Fisher Scientific, MA, USA). Recorded data were plotted versus time. The inhibitory activity was calculated from the initial (maximal) slopes obtained in the absence or in the presence of the test sample at different concentrations. Logarithmic regression was used to calculate the IC_{50} value, or concentration of hydrolysate needed to inhibit 50% of DPP-IV activity.

2.3. Purification of crude soybean lecithin

Samples of crude soybean lecithin were provided by Manuel Riesgo, S.A. (Madrid, Spain). Partially purified phosphatidylcholine (PC) was obtained by performing two washes with acetone, following the procedure described by Taladrid et al. (2017).

2.4. Liposome production

PC was milled with a mortar in a porcelain pestle and filtered through a sieve. Five (5) g of PC were then mixed with 0.5 g of ST1. The mixture was dissolved in 20 mL of phosphate buffer (10 mM) at pH 7 and kept in a bath at 80 °C for 1 h. The solution obtained was diluted in 15 mL of phosphate buffer (v/v) containing 5 mL of glycerol; the mixture was kept in a bath at 80 °C for 1 h and then vortexed at 60 °C to produce multilamellar lipid vesicles (MLVs), after to complete the solution with 60 mL of phosphate buffer. Sonication of the preparation was carried out in an ultrasonic cell disrupter (Model Q700, Qsonica sonicators, Newton, CT, USA) with a power rating of 700 W (90% amplitude), where the MLV were exposed to five cycles of sonication for 1 min, followed by 1 min stopped to allow sample cooling. The peptide-loaded liposome suspension (L-ST1) was then filtered through 0.22 μm membrane (Millex-GP Syringe Filter Unit, Millipore, USA). For comparison purposes, empty liposomes (L-E) were prepared in the same way, dissolving PC directly into the phosphate buffer without the peptide fraction.

2.5. Characterisation of liposomes

The mean particle size (z-average in intensity), size distribution (polydispersity index) and ζ-potential of liposome dispersions were measured using a Nanosizer (Nano-ZS, Nanoseries, Malvern Instruments Ltd, Worcestershire, UK). Measurements were performed by triplicate immediately after diluting the formulations in 10 mM phosphate buffer, to avoid particle aggregation.