**A novel functional wrapping design by complexation of ϵ -polylysine with
liposomes entrapping bioactive peptides**

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Abstract

An innovative edible wrapping with potential use for designing functional foods with antimicrobial capacity was developed by complexation of ϵ -polylysine with peptide-loaded liposomes. Unmarketable long-term frozen cooked shrimp (*L. vannamei*) muscle was used as a source of both bioactive peptides and complex liposomal suspension carrier, producing a sustainable value-added protein wrapping material with desirable sensory properties. A <10 kDa peptide fraction (SH) with antioxidant and angiotensin-converting enzyme inhibitory capacity was encapsulated in partially purified phosphatidylcholine (PC) liposomes (LSH) with an entrapment efficiency of 85%. The average size and zeta-potential of LSH were 164 ± 2 nm and -37.0 ± 1.7 mV, respectively. The LSH surface changed from electronegative to electropositive upon adsorption of ϵ -polylysine (PL) with an optimal concentration of 0.5%. The average diameter and zeta-potential of the resulting complex ϵ -polylysine-adsorbed liposomes containing the peptide hydrolysate (PL-LSH) were 216 ± 5 nm and $+51.1 \pm 1.1$ mV, respectively. The ϵ -PL proved to be effective as liposome stabilizing and antimicrobial agent. The PL-LSH suspension was incorporated in the formulation of the protein wrapping to provide it with both bioactive and antimicrobial properties. The wrapping showed low water solubility ($\approx 30\%$) and low mechanical resistance (tensile strength = 0.23 ± 0.06 MPa; elongation at break = 0.91 ± 0.19 %) properties that allowed it to be very versatile for varied food design, and was effective against *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus* and *Yersinia enterocolitica*.

Keywords: Liposome complexation, bioactive peptides, edible film, ϵ -polylysine, antimicrobial, shrimp.

1. Introduction

There is emerging consumer demand for safe, health-promoting foods with potential additional functions beyond basic nutrition. Moreover, foods are also required to possess an innovative layout and design. A great variety of biologically active molecules can be included as ingredients in processed functional food products to meet requirements for specific health claims. In this context, liposomes have been extensively developed as stable carriers of bioactive ingredients for two main reasons: i) to overcome efficacy losses associated with interactions with other food components or exposure to extreme pH or temperature conditions, and ii) to improve controlled delivery of the bioactive ingredients to the target cells (Taylor, Davidson, Bruce, & Weiss, 2005; Mozafari, Johnson, Hatziantoniou & Demetzos, 2008). Liposomes, which are colloidal structures, composed of one or more phospholipid bilayers encapsulating a volume of aqueous medium, can be used in a wide range of applications owing to their unique physical and chemical properties and their ability to incorporate lipophilic, amphiphilic and/or hydrophilic compounds (Sharma & Sharma, 1997). Liposomes have been shown to encapsulate proteins, enzymes, vitamins, antioxidants, minerals and flavours, and are of great interest for a variety of food applications (Rao, Chawan, & Veeramachaneni, 1995; Taylor et al., 2005; Mozafari et al., 2008; McClements, 2015). Recently, a peptide fraction from sea bream scales with high antioxidant and angiotensin I-converting enzyme (ACE)-inhibitory activity was efficiently encapsulated into stable phosphatidylcholine nanoliposomes (Mosquera et al., 2014). In a later work, the incorporation of nanovesicles loaded with ACE-inhibitory peptides from squid tunics was shown to confer the bioactive potential to fish gelatin gels, without detriment to the rheological and thermal properties (Mosquera, Giménez, Montero & Gómez-Guillén, 2015). The entrapment of antimicrobial peptides in liposomes has also been offered as a solution to overcome a possible decrease in activity related to their direct application in food (Malheiros, Daroit & Brandelli, 2010). Furthermore, nisin-loaded liposomes have

been incorporated into biopolymer films to confer the property of antimicrobial nano-active packaging material, although this may cause some loss of efficiency (Imran et al., 2012).

ϵ -Polylysine (PL), a naturally occurring non-toxic biomaterial produced by *Streptomyces albulus*, has well recognized potent antimicrobial activity on the basis of its strong polycationic property (Shih, Shen & Van, 2006; Yoshida & Nagasawa, 2003). The safety of PL as an antimicrobial preservative in food has been confirmed by in vivo studies in rats, and in fact it is approved for food use in Japan (Hiraki et al., 2003). It has been also granted GRAS (Generally Recognized as Safe) status by the USFDA for a broad range of food applications (FDA, 2004). Moreover, ϵ -PL does not provide any peculiar smell to the treated food at concentrations tested up to 1.5% w/v (Li et al., 2014).

PL has been successfully added to silver carp surimi films (Weng et al., 2014) and starch films (Zhang et al., 2015), providing them with effective antimicrobial activity. Moreover, PL has also been reported as a liposome stabilizer by adsorption at the outer membrane surface (Volodkin, Ball, Schaaf, Voegel & Mohwald, 2007a; Malcher et al., 2008). PL-coated vesicles are considered a new kind of carrier that could be used for drug delivery and controlled release (Volodkin, Mohwald, Voegel & Ball, 2007b). Surface-induced modification of liposomes by complexation with PL has been shown to avoid vesicle fusion when included in polyelectrolyte films (Michel, Vautier, Voegel, Schaaf & Ball, 2004), however, no information is available regarding their potential use for functional food applications.

Enzymatic hydrolysates from shrimp processing by-products are an interesting source of bioactive peptides with antioxidant and ACE-inhibitory capacities (Guerard, Sumaya-Martinez, Laroque, Chabeaud & Dufossé, 2007; Cheung & Li-Chan, 2010). Bioactive peptides have been also found in shrimp cooking effluents (Pérez-Santín, Calvo,

López-Caballero, Montero, Gómez-Guillén, 2013; Amado, González, Murado & Vázquez, 2015). The present work proposes the valorisation of very long-term frozen cooked shrimp, an unmarketable material frequently accumulated by the crustacean industries, as a source of both bioactive peptides and protein material for developing the wrapping carrier. The use of muscle protein from cooked shrimp for the production of edible films has not previously been reported. Due to the desirable flavour, this could be an excellent material to be used for specific gastronomic applications and also for the design of functional foods in haute cuisine.

The general aim of the present work was to develop an antimicrobial edible wrapping material with antioxidant and ACE-inhibitory potential by including a complex of ϵ -polylysine-adsorbed liposomes entrapping bioactive peptides. The various tasks were, therefore: i) to prepare an enzymatic hydrolysate with antioxidant and ACE-inhibitory capacity from cooked shrimp muscle ii) to encapsulate the active hydrolysate (bioactive core) in phosphatidylcholine liposomes (first shell), iii) to adsorb ϵ -polylysine (second shell) onto the liposomal surface membrane to enhance vesicle stability and provide the antimicrobial property, and iv) to include the complex liposomal system in the formulation of the cooked shrimp wrapping, as an example of potential functional food application. The wrapping antimicrobial capacity against several foodborne pathogens, namely *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus* and *Yersinia enterocolitica* was evaluated.

2. Materials and Methods

2.1. Materials and chemicals

Unmarketable very long-term cooked shrimp (*L. vannamei*) (more than 2 years) were kindly provided by Angulas Aguinaga Burgos, S.A. (Burgos, Spain). Commercial ϵ -polylysine (Epsiliseen-H) was from Siveele (Breda, The Netherlands). The ABTS

radical [2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)], L-ascorbic acid, norleucine, Angiotensin I-converting enzyme (ACE; 1 U), hippuryl-L-histidyl-L-leucine (HHL), hippuric acid (HA) and Alcalase 2.4 L were purchased from Sigma-Aldrich (St. Louis, MO, USA). Crude soybean lecithin was provided by Manuel Riesgo, S.A. (Madrid, Spain). All other reagents were obtained from Panreac (Madrid, Spain).

2.2. Preparation of the enzymatic hydrolysate

The peeled muscle from cooked shrimps (20% w/v) was homogenized with distilled water at 10000 rpm for 6 min in a Thermomix (Vorwerk España M.S.L., S.C., Madrid, Spain) and subjected to hydrolysis by Alcalase 2.4 L, with an enzyme/substrate ratio of 1:20 (w/w), in optimal conditions for enzymatic activity (pH 8, 50 °C) for 3 h. The pH of the reaction was adjusted initially by addition of 1 N NaOH solution to the reaction medium and kept constant using the same solution in a pH-stat (TIM 856, Radiometer Analytical, Villeurbanne Cedex, France). Afterwards, the enzyme was inactivated by heating at 90 °C for 10 min. After centrifugation at 7000 g for 15 min at 4 °C (Heaerus Labofuge 400, DJB Labcare, Bucks, UK), the supernatant was fractionated with 10 kDa molecular weight cut-off (MWCO) membrane (Macrosep® Centrifugal Devices 10 K, Pall Corporation, Madrid, Spain). The shrimp hydrolysate permeate fraction was freeze-dried and stored at -20 °C (SH). The degree of hydrolysis was calculated according to Adler-Nissen (1977) and found to be 17.6%.

2.3. Properties of the hydrolysate

2.3.1. Amino acid composition

SH was dissolved at a concentration of 5 mg/mL using Milli-Q water. An amount of 20 µL of sample was dried and hydrolysed in vacuum-sealed glass tubes at 110 °C for 24 h in the presence of continuously boiling 6 N HCl containing 0.1% (v/v) phenol with norleucine as internal standard. After hydrolysis, samples were again vacuum-dried, dissolved in a sodium loading buffer pH 2.2 (Ref. 80-2037-67, Biochrom Ltd.,

Cambridge, UK), and injected onto a Biochrom 30 amino acid analyser (Biochrom Ltd.). A mixture of amino acids was used as the standard. The amino acid separation method is based on ion exchange chromatography with post column derivatization with ninhydrin. Amino acid composition was expressed as number of residues per 1000 residues. The percentage of hydrophobic amino acids was calculated on the basis of the relative amounts of glycine, proline, alanine, valine, methionine, isoleucine, leucine and phenylalanine. The amount of taurine was expressed as mg/g of dry sample.

2.3.2. Antioxidant activity

The ferric ion reducing ability (FRAP) and ABTS radical scavenging capacity assays were used to measure the antioxidant activity of SH as described previously (Alemán, Giménez, Pérez-Santin, Gómez-Guillén & Montero, 2011a). Results for the FRAP assay were expressed as $\mu\text{mol Fe}^{2+}$ equivalents/g of sample based on a standard curve of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. Results for the ABTS assay were expressed as mg Vitamin C Equivalent Antioxidant Capacity (VCEAC)/g of sample based on a standard curve of vitamin C. All determinations were carried out at least in triplicate.

2.3.3. ACE-inhibition activity

Reversed-phase high performance liquid chromatography (RP-HPLC) was used to determine the ACE-inhibition capacity of SH by monitoring the elution at 228 nm of hippuric acid (HA) and hippuryl-L-histidyl-L-leucine (HHL). The method was performed as described previously (Alemán et al., 2011a). Determinations were carried out at least in triplicate. The IC_{50} value was defined as the concentration of hydrolysate (mg/mL) required to reduce the HA peak by 50% (indicating 50% inhibition of ACE activity).

2.4. Liposome preparation

Partially purified phosphatidylcholine powder (PC) was obtained from soybean lecithin following the procedure described by Mosquera et al. (2014). The PC was milled with a mortar in a porcelain pestle and filtered through a 500-mesh sieve. PC (5% w/v) was mixed with SH solution (0.5% w/v) and gently stirred for 1 minute. Glycerol (3% w/v) was then added and the mixture was again stirred for 1 minute. The solution obtained was kept in a water bath at 80 °C for 2 hours and then vortexed above the phase transition temperature (60 °C) to produce multilamellar lipid vesicles (MLVs). Sonication of the preparation (in order to reduce the size and homogenize liposomes) was carried out in an ultrasonic cell disrupter (Model Q700, Qsonica sonicators, Newton, CT, USA) where the MLVs were exposed to ten cycles of sonication (10% amplitude) for 10 seconds, followed by 30 seconds stopped to allow sample cooling. The peptide-loaded liposome suspension (L-SH) was adjusted to pH 4 with 0.1M citric acid solution. For comparison purposes, empty liposomes (LE) were prepared in the same way by dissolving PC in distilled water without the peptide fraction.

2.4.1. Liposome complexation with ϵ -polylysine

Surface modification of negatively charged liposomes by positively charged ϵ -polylysine (PL) was largely mediated by electrostatic interactions. Surface modification of liposomes was accomplished by dropping 5 mL of liposomal suspension (pH 4) into 5 mL of PL solutions at increasing concentrations (0.2–2%) under magnetic stirring at around 250 rpm (Multimatic 5S, J.P Selecta, S.A. Barcelona, Spain). Liposome complexation with PL was monitored by measuring changes in the zeta-potential values of the suspension from electronegative to electropositive. From the resulting electric charge profile, the suspension containing SH-loaded liposomes + 0.5% (final concentration) of PL (PL-LSH) was selected for checking of antimicrobial properties and for inclusion in the edible wrapping.

2.5. Entrapment efficiency

The entrapment efficiency (EE) of the liposome-encapsulated peptide fraction was determined by measuring the protein content with a LECO FP-2000 nitrogen/protein analyser (LECO Corp., St. Joseph, MI, USA), using a nitrogen-to-protein conversion factor of 6.25. Encapsulated SH was separated from non-encapsulated SH by centrifugal ultrafiltration at 5000xg for 60min with Macrosep® Centrifugal Devices 10 K (Pall Corporation, Madrid, Spain). EE was calculated using the following equation:

$$EE (\%) = [\text{Encapsulated SH} / (\text{Encapsulated SH} + \text{Non-encapsulated SH})] * 100.$$

2.6. Characterization of nanovesicles

A Zetasizer Nano ZS (Malvern Instruments Ltd, Worcestershire, UK) was used to determine the zeta-potential of empty liposomes (LE), liposomes entrapping the shrimp hydrolysate (LSH) and ϵ -polylysine-coated LSH (PL-LSH), using Laser Doppler Velocimetry. The Huckel approximation was used to determine the zeta potential through the electrophoretic mobility, considering the values of the viscosity and the dielectric constant of the solvent (water) at room conditions. Particle size measurements were performed by Dynamic Light Scattering using the same equipment (Zetasizer Nano ZS, Malvern Instruments Ltd., UK). Average diameter was provided as z-average mean for the liposomal hydrodynamic diameter (nm). Measurements were carried out at 25°C after 10-fold dilution of the liposomal suspensions in water at pH4, using 0.1M citric acid solution for pH adjustment.

2.7. Edible wrapping preparation

The peeled muscle from the unmarketable cooked shrimps (60% w/v) was homogenized at 10000 rpm for 6 min in a Thermomix (Vorwerk España M.S.L., S.C., Madrid, Spain) with distilled water and NaCl (2% w/w). Glycerol (20% w/w) was used as plasticizer. The resulting filmogenic solution was mixed 3:1 (v/v) with PL solution (W-PL: wrapping with free PL), PL-LSH solution (W-PL-LSH: wrapping with PL-surface-modified liposomes loaded with SH) and distilled water (W-C: control wrapping). The

final concentration of PL in each of the filmogenic solutions (S-PL and S-PL-LSH) was 0.5% w/v. Amounts of 50 mL of the filmogenic solution were poured on polymethyl methacrylate plates (12 × 12 cm), and dehydrated in an oven with air renewal and circulation (Binder FD240, Tuttlingen, Germany) at 45 °C for 15 h. Prior to characterization, the wrappings were conditioned at 58% relative humidity and 22 °C for 2 days.

2.8. Wrapping characterization

2.8.1. Physicochemical properties

The thickness was determined using a micrometer (MDC-25M, Mitutoyo, Kanagawa, Japan). Six measurements taken randomly on each wrapping were averaged.

The moisture content of each conditioned wrapping was determined by drying samples (0.5 g) at 105 °C for 24 h (A.O.A.C, 1995). Determinations were done at least in triplicate. Water content was expressed as a percentage of total weight.

For water solubility determination, wrapping pieces measuring 2 × 2 cm were placed in aluminium capsules (7 cm diameter) with 15 mL of distilled water for 15 hours at 22 °C. The contents were filtered through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, UK). The remaining undissolved material was desiccated at 105 °C for 24 h. Water solubility, expressed as a percentage, was calculated using the expression $[(W_o - W_f)/W_o] \times 100$, where W_o is the initial weight of the sample expressed as dry matter and W_f is the weight of the undissolved desiccated residue. All tests were carried out at least in triplicate.

For transparency measurement, a rectangular piece was cut from each wrapping and placed directly in the spectrophotometer test cell. An empty test cell was used as the reference. The measurement of light absorption was performed at 600 nm using a spectrophotometer (Model CPS -240, Shimadzu, Kyoto, Japan). The opacity index of

was calculated by the equation $O = \text{Abs}_{600}/x$, where Abs_{600} is the value of absorbance at 600 nm and x is the thickness (mm).

A Konica Minolta CM-3500d colorimeter (Konica Minolta, Madrid, Spain) was used for determining the colour parameters L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness), using D65 illuminant (Daylight) and D10 standard observer. Results are expressed as the mean value of at least 15 measurements.

Water vapour permeability (WVP) was determined gravimetrically in triplicate at 21 ± 1 °C using a desiccator with distilled water (100% RH). Water vapour permeability was calculated using the formula: $w \cdot x \cdot t^{-1} \cdot A^{-1} \cdot \Delta P^{-1}$, where w is the gained weight every hour for at least 7 h (g), x is the wrapping thickness (mm), t is the time (h), A is the exposed area (cm^2) and ΔP is the partial vapour pressure difference between the atmosphere and silica gel (2642 Pa at 22 °C). Results were expressed in $\text{g mm h}^{-1} \text{ cm}^{-2} \text{ Pa}^{-1}$.

Mechanical properties (Tensile and Puncture tests) were measured using a TA.XT2 plus texture analyser (Texture Technologies Corp., Scarsdale, NY, USA) equipped with a 5 kg static load cell. At least three rectangular-shaped samples were cut (100 mm × 20 mm). The grip separation was set at 60 mm and the cross-head speed at 100 mm/min. The tensile strength (TS, MPa) and elongation at break (EAB, %), were determined from the stress vs strain curves at the breaking point.

The puncture test was performed by fixing the wrapping in a 35 mm diameter cell and puncturing to breaking point with a round-ended stainless steel plunger (5 mm) at a cross-head speed of 60 mm/min. Puncture force (PF, N) and puncture deformation (PD, %) were determined. Measurements were carried out in sextuplicate at room temperature. Samples were kept at 58% RH until the test was performed.

2.8.2. Antimicrobial properties

The antimicrobial properties of both wrappings and filmogenic solutions were determined by the disk diffusion method in agar (Giménez, Gómez-Guillén, López-Caballero, Gómez-Estaca & Montero, 2012). The wrappings were cut into discs of 10 mm in diameter, and sterile filter paper discs (5 mm diameter) were impregnated with 40 µL of the filmogenic solutions. Paper discs with 40 µL of distilled water were used for control purposes. Plates of Brain Heart Infusion Agar (Oxoid, Basingstoke, UK) were inoculated with 100 µL of different microorganisms (10^5 - 10^6 cfu/mL). Both the cut wrappings and the soaked paper discs were laid on the surface of agar previously inoculated with the microorganisms. The strains selected for their importance in health as foodborne pathogens were obtained from the Spanish Type Culture Collection (CECT): *Escherichia coli* CECT 515 (EC), *Listeria monocytogenes* CECT 4032 (LM), *Staphylococcus aureus* CECT 240 (SA) and *Yersinia enterocolitica* CECT 4315 (YE). After incubation for 24–48 h at 37 °C, the appearance of a clear inhibition zone was considered as a positive antimicrobial activity.

2.9. Statistical analysis

Statistical tests were performed using the SPSS® computer program (SPSS Statistical Software, Inc., Chicago, IL, USA). Tukey's test was used to determine the level of significance at $p \leq 0.05$

3. Results and Discussion

3.1. Shrimp protein hydrolysate

The <10 kDa molecular weight Alcalase hydrolysate obtained from cooked shrimp (SH) was used as the potential bioactive ingredient to be included in the formulation of the functional edible food wrapping material. The amino acid composition of SH is presented in Table 1. The most abundant amino acid residues were, in descending

order, glutamic acid + glutamine (Glx), Gly, aspartic acid + asparagine (Asx) and Ala. The percentage contribution of hydrophobic residues to the SH amino acid profile was 44%, the most abundant being Gly (12.5%), Ala (10.5%), Leu (6.8%), Pro (5.6%), Phe (4.3%), Met (2.9%), Val (2.8%) and Ile (1.9%). The absence of Hyp residues indicates that the amount of collagenous material in the shrimp muscle hydrolysate was negligible, but a trace amount of Hyl was detected. Although the amino acid composition may vary depending on species, age and type of feed, relatively high amounts of Gly, Pro, Arg, Glx, Asx and Ala have been also reported in different crustacean species and shrimp process by-products hydrolysates (Mente et al., 2002; Cheung, Cheung, & Li-Chan, 2012). In particular, Glu, Gly, Pro, Asp and Arg, in descending order, were found to be the most abundant amino acids in an industrial cooking effluent from *L. vannamei* (Pérez-Santín et al., 2013), whereas Pro, Arg and Phe accounted for 84% of the total free amino acids in cooked commercial shrimp waste (Mandeville, Yaylayan & Simpson, 1992).

With regard to the bioactive potential of the protein hydrolysate, SH exhibited noticeable antioxidant activity, with values of 122.16 ± 4.95 mg VitC Eq/g for ABTS radical scavenging capacity, and 16.65 ± 0.45 μ mol Fe Eq/g for ferric ion reducing capacity (FRAP). The FRAP value of SH was 2–3 fold higher than the value reported for fish gelatin hydrolysates prepared under similar conditions in a previous study (Alemán, Giménez, Montero & Gómez-Guillén, 2011b). Interestingly, the ABTS value in the present work was 20 times higher than the value of the gelatin hydrolysates just mentioned. The high radical scavenging capacity of SH might be attributable to the contribution of residual carotenoids, which was presumably high in view of the orange-coloured appearance of the shrimp hydrolysate. Carotenoids – more specifically, in crustaceans, astaxanthin – are well recognized as potent antioxidants (Liñán-Cabello, Paniagua-Michel & Hopkins, 2002). Furthermore, there was a noticeable presence in SH of taurine (2.0 mg/g), which has also been reported as a biologically active

biomolecule in the regulation of oxidative stress (Jong, Azuma & Schaffer, 2012). High levels of taurine (or 2-aminoethanesulfonic acid) are normally present in muscle tissue of crustaceans and molluscs (Carr, Netherton, Gleeson & Derby, 1996), and they have also been reported in oyster cooker effluents (Kim et al., 2000).

As far as its potential as an antihypertensive agent is concerned, SH showed a reasonably high ACE-inhibition capacity, a concentration of 0.118 ± 0.004 mg/mL being necessary to inhibit 50% of the ACE enzyme activity (IC_{50}). Other hydrolysates were found to inhibit 50% of the enzyme activity using higher concentrations, as reported for raw shrimp hydrolysate (0.97 mg/mL) (He, Chen, Sun, Zhang & Zhou, 2006) or squid gelatin hydrolysate (0.34 mg/mL) (Alemán et al., 2011a). The reasonably high antioxidant and ACE-inhibitory activities found in SH suggest that shrimp cooking treatment and long-term frozen storage do not represent an impediment to proper hydrolysis of the muscle protein. This peptide fraction was used as bioactive core to confer the potential functional nature to the liposome complex system.

3.2. Encapsulation of SH in liposomes

Empty liposomes (LE) and liposomes entrapping the shrimp hydrolysate (LSH), with an average diameter of 168 ± 3 nm and 164 ± 2 nm, respectively, were prepared from partially purified phosphatidylcholine (PC). The monomodal liposomal size distributions of LE and LSH are shown in Figs. 1a and 1b. The polydispersity index (PDI) decreased from 0.4 ± 0.01 in LE to 0.3 ± 0.01 in LSH. The narrower distribution of particle size in LSH was mainly at the expense of shortening of the smaller vesicle fraction, strongly suggesting some liposomal agglomeration, probably favoured by a certain degree of peptide binding to liposomes (Moeller, Holst, Nielsen, Pedersen & Østergaard, 2010). The SH was encapsulated with an entrapment efficiency of 85%, which was higher than the value reported in previous work on PC liposomes encapsulating other

peptides, such as calcein (71%) (Were, Bruce, Davidson & Weiss, 2003) or a peptide fraction from sea bream scales (75%) (Mosquera et al., 2014). It is worth noting that the average PC vesicle diameter in the present work is greater than in the above-mentioned studies, where particle sizes of 85 and 90 nm, respectively, were reported.

The surface charge of the LSH suspension at pH 4 was -37.0 ± 1.7 mV, slightly less electronegative than the charge observed for the liposomes without peptides (-47.4 ± 1.1 mV), but within the limits ($> +30$ mV or < -30 mV) that define a dispersion as physically stable (Müller, Jacobs & Kayser, 2001). The acidic pH in the liposome suspension was used to allow optimal conditions for complexation with the ϵ -polylysine in the next step. According to the amino acid composition of SH, the percentage of total hydrophilic amino acid residues was slightly higher than the hydrophobic percentage (56% vs 44%), strongly suggesting that the greater part of the SH amino acid residues was included in the inner core of the liposome, but also that a significant amount was immobilized in the vesicle lipid bilayer. With regard to the non-entrapped peptide fraction, most of the amino acid residues would be protonated to a certain extent at acidic pH, and therefore they could have been responsible for the slight reduction in the electronegative zeta-potential of the liposomal suspension. Both types of liposomes (LE and LSH) showed high stability, which could be partly attributable to the glycerol included in their formulation (Harrigan, Madden & Cullis, 1990). Glycerol was mainly used to prevent later possible vesicle fusion, and accompanying peptide leakage, during the dehydration of the filmogenic solution required to produce the edible wrapping. Furthermore, in addition to the antimicrobial effect specifically sought by the ϵ -polylysine (PL) in the edible wrapping, as discussed below, PL was also used in the present work as a liposome surface-modifier forming a complex system, in order to ensure optimal liposome stabilization for enhanced peptide protection inside the nanostructured wrapping matrix.

3.3. Liposome complexation with ϵ -polylysine

The stable, high electronegative charge that LSH exhibited at pH 4 was a crucial factor allowing subsequent adsorption with ϵ -polylysine (PL). The positively charged side chains of PL are an important prerequisite for the initial association with the negatively charged components of the vesicle phospholipid membrane (Finogenova, Filinsky, Ermakov & Yu, 2008). More specifically, interactions of the positively charged lysine residues with the outer membrane phosphate groups have been reported to cause perturbation and eventual permeation of the phospholipid bilayers (Sasaki, Karasawa, Hironaka, Tahara, Tozuka, & Takeuchi, 2013). Increasing PL concentrations were added to the LSH suspension, and changes in the liposomal surface charge were determined (Fig. 2). The zeta-potential values showed that the liposomal surface changed from electronegative to electropositive, strongly suggesting that, from a certain concentration, an effective adsorption of LSH by ϵ -polylysine molecules took place (Volodkin et al., 2007a,b; Sasaki et al., 2013). A plateau was reached from 0.5% upwards, indicating that liposomes were totally coated with PL at this concentration, preventing further adsorption of excess PL by saturation of the bilayer membrane binding sites (Volodkin et al., 2007a,b; Finogenova et al., 2008). Residual PL amino groups, which do not interact with the liposomal surface, would presumably be available to exert a direct antimicrobial effect.

The average diameter and zeta-potential of the resulting ϵ -polylysine-adsorbed (at 0.5% PL concentration) liposomes entrapping the peptide hydrolysate (PL-LSH) were 216 ± 5 nm and $+51.1 \pm 1.1$ mV, respectively. The particle size distribution of PL-LSH, with a PDI value of 0.4, is depicted in Figure 1c. The bimodal distribution may be due to deagglomeration of the small liposome fraction. The adsorption of PL on LSH implies stabilization at short distance, and then the LSH remains separated by the steric effect of the adsorbed PL. However, the shift of the average peak size towards higher values, compared with the non-coated LSH liposomes, would indicate that some vesicles might have been aggregated (Efimova, Sybachin & Yaroslavov, 2011; Sasaki et al., 2013).

Complexation between liposomes and PL might lead to the formation of stable PL-covered single liposomes but also to stable small liposome aggregates (Volodkin et al. 2007a; Malcher et al., 2008). Vesicle aggregation may occur owing to non-homogeneous overcompensation of the liposomal surface charge by the adsorbed PL. From the particle size distribution of PL-LSH shown in Fig 1c, single and aggregated PL-coated vesicles appeared to coexist. The strong electropositive surface charge of the PL-coated liposomal suspension indicates that the complex vesicles produced were of high stability. The surface-induced modification of liposomes by complexation with PL has been shown largely to avoid vesicle deformation and possible fusion when included in polyelectrolyte films (Michel et al., 2004). Nevertheless, liposomes located on the film surface could lose their integrity upon drying, owing to water evaporation (Malcher et al., 2008).

3.4. Properties of the functional wrapping

The complex PL-LSH suspension was included in the formulation of an edible wrapping with the aim of producing a functional covering material that would provide a food product with potential bioactive properties and improved preservation ability. In this context, thermally aggregated muscle protein of cooked shrimp was used as the biopolymer delivery system, considering that it would be suitable for wrapping shrimp tails or restructured muscle on the basis of its desirable flavouring and textural properties. Moreover, the design of an innovative and original food was also taken into account. The physico-chemical properties of wrappings containing the complex liposomal system (W-PL-LSH) were compared with a wrapping without liposome addition (W-C) and another added with free PL (W-PL) (Table 2). Wrappings with relatively high thickness (around 0.35 mm) were produced in order to provide the capacity to support a high load of bioactive compound. All the wrappings were flexible and easy to handle, did not break upon folding and resembled the appearance of a pasta sheet. The W-PL-LSH sheet would be intended to take part of the functional

food, imitating the appearance of spring rolls, cannelloni or Won ton. The addition of PL-complex liposomes or free PL did not induce any significant change ($p>0.05$) in thickness, although the moisture of the respective wrappings was somewhat ($p\leq 0.05$) reduced (Table 2). In general, addition of free PL or PL-complex liposomes did not dramatically change the overall appearance. All the tested wrappings presented rather low light transmission (Table 2), which was attributed to the heat-induced aggregated nature of the muscle protein (cooked shrimp) used as the biopolymer matrix. However, a slight decrease ($p\leq 0.05$) in opacity was observed when PL was incorporated in the complex form (W-PL-LSH) rather than the free form (W-PL), indicating that liposomes favoured this slightly lower opacity. With regard to colour parameters, lightness increased significantly ($p\leq 0.05$) with added liposomes (W-PL-LSH), which also showed higher yellowness. The water solubility of the single wrappings (W-C) was around 30%, which is in the range of values reported for films produced from various raw seafood muscle proteins (Blanco-Pascual, Fernández-Martín, F & Montero, 2013; Gómez-Estaca, Montero & Gomez-Guillen, 2014). Relatively low water solubility is a desirable property, since it prevents the wrapping from disintegrating upon contact with a moist food surface, making it more versatile for incorporation in or as a covering for various kinds of food. This allows designing products in which the wrap take part of the food, not being used just as a cover film. The water vapour permeability (WVP) of W-C was considerably higher than with other types of proteins, such as raw shrimp muscle protein (Gómez-Estaca et al., 2014) or squid muscle (Blanco-Pascual et al., 2013), possibly owing to the thermally-aggregated nature of the protein, which hampers protein interactions upon drying. The addition of PL or PL-LSH did not induce significant ($p\leq 0.05$) changes in either water solubility or WVP.

With regard to mechanical properties, the wrappings obtained from cooked shrimp muscle showed values of elongation at break and tensile strength much lower than those reported for fish protein films (Sobral, Dos Santos & García, 2005), attributed to

the difficulty for the thermally-aggregated protein to cross-link within the film matrix. Moreover, the addition of PL or PL-LSH reduced ($p \leq 0.05$) the elongation at break, as well as both puncture force and deformation. A decrease in mechanical resistance resulting from the addition of lecithin nanoliposomes encapsulating antimicrobial essential oils was reported in starch–sodium caseinate films (Jiménez, Sánchez-González, Desobry, Chiralt & Tehrany, 2014) and in gelatin films (Wu et al., 2015), attributed to a space steric effect of liposomes hindering hydrogen bonding cross-linking of the gelatin molecules (Wu et al., 2015). Moreover, discontinuities in the polymer matrix introduced by lecithin nanovesicles and changes in polymer chain interactions were also found to cause weakening of HPMC film mechanical response (Imran et al., 2012). In the present work, the lipid PC bilayers, in the form of single or aggregated vesicles, were largely covered by PL, so the strong polycationic property of PL seemed to have more influence on the mechanical properties than the liposome-nanostructured matrix at the amount added. The addition of ϵ -polylysine to silver carp surimi films has been shown to present a concentration-dependent behaviour in the mechanical properties, an increase from 0.15% to 0.20% (% in the film-forming solution), causing a significant decrease in both tensile strength and elongation at break (Weng et al., 2014). In the present work, the PL concentration is considerably higher than 0.2%, but differences between W-C and the two PL-containing wrappings are fairly low, partly owing to the low mechanical resistance of the single control film, but also to the low presence of available free reactive –OH groups in the thermally-aggregated protein, thus hindering intermolecular hydrogen-bonding interactions with positively charged amino groups of lysine residues. From a sensory point of view, the low mechanical properties would be an advantage, since they would presumably favour greater chewiness, a desirable feature, in the resulting wrapped food product.

Figure 3 shows the antimicrobial capacity of W-PL and W-PL-LSH wrappings, and the corresponding filmogenic solutions (FFS), against several Gram-negative and Gram-

positive foodborne pathogens, i.e., *E. coli*, *L. monocytogenes*, *S. aureus* and *Y. enterocolitica*. As could be expected, the FFS and wrappings without PL (W-C) showed no inhibition zone, whereas strong activity was found for the microorganisms tested with the presence of PL. It is worth noting the great similarity in microbial growth inhibition between FFS and wrappings, in both cases indicating the diffusion of PL. Moreover, differences in water availability, solid vs fluid state of nanovesicles and free chain mobility did not significantly influence the antimicrobial effectiveness. In addition, no differences were observed regarding the effect of PL in the free form (W-PL) or in the complex liposomal form (W-PL-LSH), which means that the bioactive hydrolysate could be effectively protected within the liposome without losing the antimicrobial capacity provided by the PL in the wrapping. In this connection, silver carp surimi films with added ϵ -polylysine have been found to exhibit a wide range of antimicrobial activity, especially against Gram-positive bacteria (Weng et al., 2014). Films based on HPMC activated with nisin-loaded nanoliposomes, at 1 mg/mL of final nisin concentration in the film forming solution, also demonstrated strong activity against *L. monocytogenes* (Imran et al., 2012). The same authors reported better control of the pathogen when both free and encapsulated nisin were present in the film rather than when nisin was 100% encapsulated. In contrast, no antilisterial activity was observed in starch–sodium caseinate films incorporating orange essential oil and limonene-loaded nanoliposomes, which was partly attributed to the low antilisterial activity of the compounds used (final concentration of 5 mg/g in the film forming dispersion), but also to the encapsulation effect, which hindered release of the antimicrobial from the matrix (Jiménez et al., 2014). In another work with fish gelatin films, the incorporation of cinnamon essential oil encapsulated in nanoliposomes (50 mg/mL of essential oil in the film forming solution) was reported to produce a decrease in the release rate of the antimicrobial compound (Wu et al., 2015). The type of active compound used is undoubtedly a key factor governing the antimicrobial efficacy of the film. The potent antibacterial activity of ϵ -polylysine has been related to its strong polycationic property,

which allows electrostatic adsorption and perturbation of the microbial cell surface, causing physiological damage (Yoshida & Nagasawa, 2003). In addition, the availability of the antimicrobial compound inside the film matrix is another aspect to be considered, since possible interactions between the active compound and the biopolymer may impair the antimicrobial efficiency, as reported in gelatin films with added clove essential oil (Giménez, Gómez-Guillén, López-Caballero, Gómez-Estaca & Montero, 2012) or in various food systems with added antimicrobial peptides (Malheiros et al., 2010). In the present wrappings with added complex PL-liposome system, part of the PL was immobilized by adsorption to the liposomal surface, but most of the positively charged amino groups of lysine residues remained available to exert their antimicrobial effect, while the encapsulated bioactive hydrolysate was presumably protected from interactions with other food matrix components.

4. Conclusions

An innovative edible wrapping material with antimicrobial activity was produced by introducing a complex system based on ϵ -polylysine-adsorbed liposomes entrapping a bioactive peptide fraction. The ϵ -PL in the present study proved to be effective as liposome stabilizer and antimicrobial agent. In view of the relatively low water solubility and mechanical resistance, the resulting functional wrapping, mostly derived from a sustainable natural source, was suitable for the versatile design of functional foods, without the need of product restructuring or reformulation.

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References

535 A.O.A.C. (1995). Official methods of analysis. Method number 934.01. Maryland, USA:
 536 Association of Official Analytical Chemistry.

537 Adler-Nissen J (1977). Enzymatic hydrolysis of food proteins. *Process Biochemistry*, 8,
 538 18–32.

539 Alemán A, Giménez B, Pérez-Santin E, Gómez-Guillén MC & Montero P (2011a).
 540 Contribution of Leu and Hyp residues to antioxidant and ACE-inhibitory activities of
 541 peptide sequences isolated from squid gelatin hydrolysate. *Food Chemistry*, 125 (2),
 542 334-341.

543 Alemán A, Giménez B, Montero P & Gómez-Guillén MC (2011b). Antioxidant activity of
 544 several marine skin gelatins. *LWT - Food Science and Technology*, 44 (2), 407-413.

545 Amado IR, González M, Murado MA & Vázquez JA (2015). Shrimp (*Penaeus*
 546 *vannamei*) cooking wastewater as a source of astaxanthin and bioactive peptides.
 547 *Journal of Chemical Technology and Biotechnology*, in press.

548 Blanco-Pascual N, Fernández-Martín F, & Montero MP (2013). Effect of different
 549 protein extracts from *Dosidicus gigas* muscle co-products on edible films development.
 550 *Food Hydrocolloids*, 33(1), 118—131.

551 Carr WES, Netherton JC, Gleeson RA, Derby CD (1996). Stimulants of feeding
 552 behavior in fish: Analyses of tissues of diverse marine organisms. *Biological Bulletin*,
 553 190 (2), 149-160.

554 Cheung IWY, Li-Chan ECY (2010). Angiotensin-I-converting enzyme inhibitory activity
 555 and bitterness of enzymatically-produced hydrolysates of shrimp (*Pandalopsis dispar*)
 556 processing byproducts investigated by Taguchi design. *Food Chemistry*, 122 (4), 1003-
 557 1012.

558 Cheung, L.K.Y., Cheung, I.W.Y., Li-Chan, E.C.Y. (2012). Effects of production factors
 559 and egg-bearing period on the antioxidant activity of enzymatic hydrolysates from

560 shrimp (*Pandalopsis dispar*) processing byproducts. Journal of Agricultural and Food
 561 Chemistry, 60 (27), 6823-6831.

562 Efimova AA, Sybachin AV & Yaroslavov AA (2011). Effect of anionic-lipid-molecule
 563 geometry on the structure and properties of liposome-polycation complexes. Polymer
 564 Science - Series C, 53 (1), 89-96.

565 FDA (2004). Food and Drug Administration. Agency Response Letter GRAS Notice No.
 566 GRN 000135.

567 Finogenova OA, Filinsky DV & Ermakov, YA (2008). Electrostatic effects upon
 568 adsorption and desorption of polylysines on the surface of lipid membranes of different
 569 composition. Biochemistry (Moscow) Supplement Series A: Membrane and Cell
 570 Biology, 2 (2), 181-188.

571 Giménez B, Gómez-Guillén MC, López-Caballero ME, Gómez-Estaca J & Montero P
 572 (2012). Role of sepiolite in the release of active compounds from gelatin-egg white
 573 films. Food Hydrocolloids, 27 (2), 475-486.

574 Gomez-Estaca J, Montero P & Gomez-Guillen MC (2014). Shrimp (*Litopenaeus*
 575 *vannamei*) muscle proteins as source to develop edible films. Food Hydrocolloids, 41,
 576 86-94.

577 Guerard F, Sumaya-Martinez MT, Laroque D, Chabeaud A & Dufossé L. (2007).
 578 Optimization of free radical scavenging activity by response surface methodology in the
 579 hydrolysis of shrimp processing discards. Process Biochemistry, 42 (11), 1486-1491.

580 He HL, Chen XL, Sun CY, Zhang YZ & Zhou BC (2006). Analysis of novel angiotensin-
 581 I-converting enzyme inhibitory peptides from protease-hydrolyzed marine shrimp
 582 *Acetes chinensis*. Journal of Peptide Science, 12 (11), 726-733.

583 Harrigan PR, Madden TD & Cullis PR (1990). Protection of liposomes during
 584 dehydration or freezing. Chemistry and Physics of Lipids, 52, 139-149.

585 Hiraki J, Ichikawa T, Ninomiya S, Seki H, Uohama K, Seki H, Kimura S, Yanagimoto Y
 586 & Barnett Jr JW (2003). Use of ADME studies to confirm the safety of ϵ -polylysine as a
 587 preservative in food. *Regulatory Toxicology and Pharmacology*, 37 (2), 328-340.

588 Imran M, Revol-Junelles AM, René N, Jamshidian M, Akhtar MJ, Arab-Tehrany E,
 589 Jacquot M & Desobry S (2012). Microstructure and physico-chemical evaluation of
 590 nano-emulsion-based antimicrobial peptides embedded in bioactive packaging films.
 591 *Food Hydrocolloids*, 29 (2), 407-419.

592 Jiménez A, Sánchez-González L, Desobry S, Chiralt A & Tehrany EA (2014). Influence
 593 of nanoliposomes incorporation on properties of film forming dispersions and films
 594 based on corn starch and sodium caseinate. *Food Hydrocolloids*, 35, 159-169.

595 Jong CJ, Azuma J & Schaffer S (2012). Mechanism underlying the antioxidant activity
 596 of taurine: prevention of mitochondrial oxidant production. *Amino Acids*, 42(6), 2223-
 597 2232.

598 Kim DS, Baek HH, Ahn CB, Byun DS, Jung KJ, Lee HG, Cadwallader KR & Kim HR
 599 (2000). Development and characterization of a flavoring agent from oyster cooker
 600 effluent. *Journal of Agricultural and Food Chemistry*, 48 (10), 4839-4843.

601 Li, Y.-Q., Feng, J.-L., Han, Q., Dai, Z.-Y., Liu, W., Mo, H.-Z. (2014). Effects of ϵ -
 602 Polylysine on Physicochemical Characteristics of Chilled Pork. *Food and Bioprocess*
 603 *Technology*, 7 (9), 2507-2515.

604 Liñán-Cabello MA, Paniagua-Michel J, & Hopkins PM (2002). Bioactive roles of
 605 carotenoids and retinoids in crustaceans. *Aquaculture Nutrition*, 8 (4), 299–309.

606 Malcher M, Volodkin D, Heurtault B, André P, Schaaf P, Möhwald H, Voegel JC,
 607 Sokolowski A, Ball V, Boulmedais F & Frisch B (2008). Embedded silver ions-
 608 containing liposomes in polyelectrolyte multilayers: Cargos films for antibacterial
 609 agents. *Langmuir*, 24 (18), 10209-10215.

610 Malheiros P, Daroit DJ & Brandelli A (2010). Food applications of liposome-
 611 encapsulated antimicrobial peptides. Trends in Food Science and Technology, 21 (6),
 612 284-292.

613 Mandeville, S., Yaylayan, V., Simpson, B.K. (1992). Proximate analysis, isolation and
 614 identification of amino acids and sugars from raw and cooked commercial shrimp
 615 waste. Food Biotechnology, 6 (1), 51-64.

616 McClements, D.J. (2015). Encapsulation, protection, and release of hydrophilic active
 617 components: Potential and limitations of colloidal delivery systems. Advances in Colloid
 618 and Interface Science, 219, 27-53.

619 Mente, E., Coutteau, P., Houlihan, D., Davidson, I., Sorgeloos, P. (2002). Protein
 620 turnover, amino acid profile and amino acid flux in juvenile shrimp *Litopenaeus*
 621 *vannamei*: Effects of dietary protein source. Journal of Experimental Biology, 205 (20),
 622 3107-3122.

623 Michel M, Vautier D, Voegel JC, Schaaf P & Ball V (2004). Layer by layer self-
 624 assembled polyelectrolyte multilayers with embedded phospholipid vesicles. Langmuir,
 625 20 (12), 4835-4839.

626 Moeller EH, Holst B, Nielsen LH, Pedersen PS & Østergaard J (2010). Stability,
 627 liposome interaction, and in vivo pharmacology of ghrelin in liposomal suspensions.
 628 International Journal of Pharmaceutics, 390 (1), 13-18.

629 Mosquera M, Giménez B, Mallmann da Silva I, Ferreira Boelter J, Montero P, Gómez-
 630 Guillén MC & Brandelli A (2014). Nanoencapsulation of an active peptidic fraction from
 631 sea bream scales collagen. Food Chemistry, 156, 144-150.

632 Mosquera, M., Giménez, B., Montero, P., Gómez-Guillén, M.C. (2015). Incorporation of
 633 liposomes containing squid tunic ACE-inhibitory peptides into fish gelatin. Journal of
 634 the Science of Food and Agriculture. DOI: 10.1002/jsfa.7145.

635 Mozafari MR, Johnson C, Hatziantoniou S & Demetzos C (2008). Nanoliposomes and
 636 their applications in food nanotechnology. *Journal of Liposome Research*, 18 (4), 309-
 637 327.

638 Müller RH, Jacobs C, & Kayser O (2001). Nanosuspensions as particulate drug
 639 formulations in therapy: Rationale for development and what we can expect for the
 640 future. *Advanced Drug Delivery Reviews*, 47, 3-19.

641 Pérez-Santín E, Calvo MM, López-Caballero ME, Montero P & Gómez-Guillén MC
 642 (2013). Compositional properties and bioactive potential of waste material from shrimp
 643 cooking juice. *LWT - Food Science and Technology*, 54 (1), 87-94.

644 Rao DR, Chawan CB & Veeramachaneni R (1995). Liposomal encapsulation of beta-
 645 galactosidase - comparison of two methods of encapsulation and in vitro lactose
 646 digestibility. *Journal of Food Biochemistry*, 18, 239-251.

647 Sasaki H, Karasawa K, Hironaka K, Tahara K, Tozuka Y & Takeuchi H (2013). Retinal
 648 drug delivery using eyedrop preparations of poly-l-lysine-modified liposomes. *European*
 649 *Journal of Pharmaceutics and Biopharmaceutics*, 83 (3), 364-369.

650 Sharma A & Sharma US (1997). Liposomes in drug delivery: Progress and limitations.
 651 *International Journal of Pharmaceutics*, 154 (2), 123-140.

652 Shih, I.-L., Shen, M.-H., Van, Y.-T. (2006). Microbial synthesis of poly(ϵ -lysine) and its
 653 various applications. *Bioresource Technology*, 97 (9), 1148-1159.

654 Sobral PJDA, Dos Santos JS & García FT (2005). Effect of protein and plasticizer
 655 concentrations in film forming solutions on physical properties of edible films based on
 656 muscle proteins of a Thai Tilapia. *Journal of Food Engineering*, 70 (1), 93-100.

657 Taylor TM, Davidson PM, Bruce BD & Weiss J (2005). Liposomal nanocapsules in food
 658 science and agriculture. *Critical Reviews in Food Science and Nutrition*, 45 (7-8), 587-
 659 605.

660 Volodkin D, Ball V, Schaaf P, Voegel JC & Mohwald H (2007a). Complexation of
 661 phosphocholine liposomes with polylysine. Stabilization by surface coverage versus
 662 aggregation. *Biochimica et Biophysica Acta - Biomembranes*, 1768 (2), 280-290.

663 Volodkin D, Mohwald H, Voegel JC & Ball V (2007b). Coating of negatively charged
 664 liposomes by polylysine: Drug release study. *Journal of Controlled Release*, 117 (1),
 665 111-120.

666 Weng WY, Tao Z, Liu GM, Su WJ, Osako K, Tanaka M & Cao MJ (2014). Mechanical,
 667 barrier, optical properties and antimicrobial activity of edible films prepared from silver
 668 carp surimi incorporated with ϵ -polylysine. *Packaging Technology and Science*, 27 (1),
 669 37-47.

670 Were LM, Bruce BD, Davidson PM & Weiss J (2003). Size, Stability, and Entrapment
 671 Efficiency of Phospholipid Nanocapsules Containing Polypeptide Antimicrobials.
 672 *Journal of Agricultural and Food Chemistry*, 51, 8073-8079.

673 Wu J, Liu H, Ge S, Wang S, Qin Z, Chen L, Zheng Q, Liu Q & Zhang Q (2015). The
 674 preparation, characterization, antimicrobial stability and in vitro release evaluation of
 675 fish gelatin films incorporated with cinnamon essential oil nanoliposomes. *Food*
 676 *Hydrocolloids* 43, 427-435

677 Yoshida T & Nagasawa T (2003). Epsilon-Poly-L-lysine: microbial production,
 678 biodegradation and application potential. *Applied Microbiology and Biotechnology*,
 679 62(1), 21-26.

680 Zhang, L., Li, R., Dong, F., Tian, A., Li, Z., Dai, Y. (2015). Physical, mechanical and
 681 antimicrobial properties of starch films incorporated with ϵ -poly-L-lysine. *Food*
 682 *Chemistry*, 166, 107-114.

683 Legend to figures

684 Figure 1. Liposomal size distributions of a) Empty liposomes (LE); b) Liposomes
685 entrapping the shrimp hydrolysate (LSH) and c) ϵ -polylysine-adsorbed liposomes
686 entrapping the shrimp hydrolysate (PL-LSH).

687

688 Figure 2. Changes in the liposome surface charge with increasing concentration of ϵ -
689 polylysine

690

691 Figure 3. Antimicrobial capacity of wrappings (W-) and corresponding filmogenic
692 solutions (S-) against *Yersinia enterocolitica* (YE), *Escherichia coli* (EC), *Listeria*
693 *monocytogenes* (LM) and *Staphylococcus aureus* (SA).

694 1: W-C (control wrapping), 2: W-PL (with free ϵ -polylysine), 3: W-PL-LSH (with ϵ -
695 polylysine -surface-modified liposomes loaded with shrimp hydrolysate); 4: S-C (control
696 filmogenic solution), 5: S-PL (with free ϵ -polylysine), 6: S-PL-LSH (with ϵ -polylysine -
697 surface-modified liposomes loaded with shrimp hydrolysate), 7: Deionized water

Figure 1

Figure 2

Figure 3

Table 1. Amino acid composition of shrimp hydrolysate (SH)

Amino acid residue	Abbreviation	residues (‰)	g /100g hydrolysate
Aspartic acid + asparagine	Asx	109	4,74
Threonine	Thr	34	1,30
Serine	Ser	56	1,85
Glutamic acid + glutamine	Glx	150	7,33
Glycine	Gly	125	2,69
Alanine	Ala	105	2,82
Cysteine	Cys	4	0,17
Valine	Val	28	1,05
Methionine	Met	29	1,42
Isoleucine	Ile	19	0,83
Leucine	Leu	68	2,90
Tyrosine	Tyr	27	1,68
Phenylalanine	Phe	43	2,38
Hydroxylysine	Hyl	6	0,32
Histidine	His	15	0,78
Lysine	Lys	62	3,01
Arginine	Arg	62	3,63
Hydroxyproline	Hyp	0	0,00
Proline	Pro	56	3,06

Table 2. Physicochemical properties of wrappings **W-C** (control), **W-PL** (with free PL) and **W-PL-LSH** (with PL-surface-modified liposomes loaded with SH).

	W-C	W-PL	W-PL-LSH
Thickness (mm)	0.34 ± 0.06 ^a	0.35 ± 0.05 ^a	0.37 ± 0.04 ^a
Moisture (%)	33.80 ± 2.55 ^c	25.76 ± 0.67 ^b	26.29 ± 0.63 ^a
Opacity	3.94 ± 0.24 ^{ab}	4.33 ± 0.32 ^b	3.69 ± 0.10 ^a
L*	45.37 ± 5.04 ^b	48.79 ± 4.36 ^b	54.04 ± 1.12 ^a
a*	1.72 ± 0.32 ^a	0.98 ± 0.99 ^b	1.81 ± 0.33 ^a
b*	5.73 ± 0.97 ^b	6.88 ± 1.99 ^b	8.54 ± 1.00 ^a
Water solubility (%)	29.76 ± 5.55 ^a	31.07 ± 0.25 ^a	33.38 ± 0.92 ^a
WVP (x10 ⁻⁷ g·mm·h ⁻¹ ·cm ⁻² ·Pa ⁻¹)	4.72 ± 0.56 ^a	4.43 ± 0.83 ^a	3.74 ± 0.96 ^a
Tensile strength (MPa)	0.36 ± 0.12 ^a	0.11 ± 0.03 ^a	0.23 ± 0.06 ^a
Elongation at break (%)	1.48 ± 0.31 ^b	0.98 ± 0.20 ^a	0.91 ± 0.19 ^a
Puncture force (N)	2.54 ± 0.64 ^b	0.66 ± 0.20 ^a	0.93 ± 0.10 ^a
Deformation (%)	8.63 ± 1.76 ^b	5.68 ± 1.22 ^a	5.63 ± 1.05 ^a

Different letters a,b,c indicate significant differences (p≤0.05) among samples.