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Oxidative stability and physical properties of mayonnaise fortified with zein electrosprayed capsules loaded with fish oil

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Abstract

This study investigated the oxidative stability and physical properties of low-fat mayonnaise fortified with electrosprayed zein capsules loaded with fish oil. A high encapsulation efficiency (EE) (83 ± 1 %) was achieved in the encapsulates and semi-spherical capsules with rough surface and mean diameter of 2.4 ± 0.7 μm were obtained. The amount of primary oxidation products of the zein capsules did not increase over the storage, even though the initial content was higher compared to neat fish oil. Cryo-SEM images revealed that the capsules remained intact when they were incorporated into a water-based food matrix like mayonnaise. This explained the lower content of hydroperoxides at the end of the storage for the capsule-enriched mayonnaise (Mayo-C) when compared to mayonnaise enriched with neat fish oil (Mayo-F) (2 meq/kg oil vs. 6 meq/kg oil, respectively). Even though, volatile compounds from the secondary oxidation of omega-3, such as 2-ethylfuran and 1-penten-3-one were found in higher amounts in Mayo-F, a higher overall increase in all other secondary oxidation products was observed in Mayo-C. This increase was attributed to the formation of oxidation volatile compounds from the zein material, together with the oxidation of un-encapsulated fish oil droplets. Moreover, Mayo-C presented higher viscosity and droplet size than Mayo-F, which also increased during storage, although no further alterations of the emulsion like creaming were observed. Overall,

these findings indicate the benefits of using zein as hydrophobic shell material for the development of omega-3 loaded-electrosprayed capsules intended to the enrichment of water-based food matrices when compared to the use of hydrophilic wall materials.

Keywords: *Omega-3, Encapsulation, Electrospraying, Lipid oxidation, Zein, Mayonnaise*

1. Introduction

Long chain omega-3 polyunsaturated fatty acids (omega-3 PUFA), which include eicosapentaenoic (EPA, 20:5n-3), docosapentaenoic (DPA, 22:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids, are found in marine lipids (Bimbo, 2013). Health benefits such as the prevention of cardiovascular disease, the reduction of symptoms in rheumatoid arthritis and the prevention of the promotion and progression stages of some types of cancer have been associated to the intake of EPA and DHA (Arab-Tehrany et al., 2012). Moreover, DHA is essential for the development of brain and nervous system in fetus and infants (Arab-Tehrany et al., 2012). Due to the limited synthesis of EPA and DHA by humans from the essential α -linolenic fatty acid (ALA, C18:3n-3), the incorporation of omega-3 through the diet is highly important (Brenna et al., 2009). Nevertheless, Western populations present a consumption of omega-3 PUFA rich products (e.g. fatty fish, krill, algae) below the recommended intake (Calder, 2013). Hence, the food industry has gained an increasing interest in incorporating omega-3 to emulsified food. However, EPA and DHA are highly prone to lipid oxidation and consequently, oxidation of foods containing omega-3 PUFA is a serious concern (Jacobsen, 2015). Besides the loss of nutritional value and health safety issues associated with specific lipid oxidation products (e.g. malonaldehyde, α,β -unsaturated carbonyls), secondary volatile oxidation compounds give rise to off-flavors that negatively affect consumer acceptability (Vieira et al., 2017) (Arab-Tehrany et al., 2012).

To minimize lipid oxidation when enriching food with omega-3 PUFA, the encapsulation of these beneficial lipids has been widely explored. Spray drying is the most common encapsulation technique used in the food industry to encapsulate omega-3 PUFA (Drusch and Berg, 2008). Even though this technique implies short residence time of the encapsulate in the drying chamber, oxidation of omega-3 PUFA still occurs due to temperature increase of the encapsulate (up to 60-80 °C) during the falling-rate period of the spray-drying process (Woo and Bhandari, 2013). On the other hand, freeze-drying, which works at mild conditions (e.g. vacuum, low temperature), has low throughput and it is costly. In addition, the size of the encapsulates obtained by spray-drying and freeze-drying processes are considerable large (10-100 μm), which may negatively affect the organoleptic properties of the final enriched product (Barrow et al., 2013).

Alternative techniques such as electrospraying, which does not require heat to dry and results in encapsulates with reduced size (1-5 μm), is gaining relevance for the encapsulation of thermosensitive bioactives (Jacobsen et al., 2018). This technique consists of charging the surface of a polymer solution using a high voltage electrostatic field to produce ultrathin droplets, which after solvent evaporation at room temperature results in nano- and micro-sized dried capsules (Bhushani & Anandharamakrishnan, 2014; García-Moreno et al., 2018a). Recently, high-throughput electrospraying assisted by pressurized gas (EAPG) has been developed. EAPG is based on the atomization of the polymer solution in a pneumatic injector (e.g. by using compressed air/gas) that nebulizes within a high electric field. During this process, air at room temperature is used to evaporate the solvent in a drying chamber and the encapsulated material is then collected as a free-flowing powder (Lagaron et al., 2017). Thus, EAPG enhances the productivity of the common electrospraying process and makes it feasible for the industrial production of omega-3 loaded-electrosprayed capsules for application in food (Busolo et al., 2018).

Food-grade biopolymers, mainly proteins and polysaccharides have been studied as capsule shell material (García-Moreno et al., 2017; García-Moreno et al., 2018b). Zein prolamine, is a hydrophobic corn protein that presents interesting features such as low moisture absorption, high thermal resistance, oxygen barrier and antioxidant properties (García-Moreno et al., 2018a). This makes zein attractive to be used as shell material for the encapsulation of omega-3 PUFA. For instance, zein capsules loaded with DHA and produced by electrospraying had higher oxidative stability during storage than bulk DHA (Busolo et al., 2018). Moreover, shell materials to produce omega-3 encapsulates should be carefully selected taking into account the food matrix and the processing conditions. These are important aspects determining the stability of the encapsulate in the fortified food product and thereby the protection of the bioactive compound (Jacobsen, 2010).

Recently, we reported the use of glucose syrup as main wall material to encapsulate fish oil by electrospraying (García-Moreno et al., 2018). Nevertheless, the enrichment of oil-in-water emulsion-based food matrices such as mayonnaise with electrosprayed glucose syrup capsules loaded with fish oil (stabilized or not with natural antioxidants) did not enhance its oxidative stability when compared to mayonnaise enriched with neat fish oil (Hermund et al., 2019). This was attributed to the disintegration of glucose syrup capsules in a water-based food matrix like mayonnaise during production, which led to the release of unprotected and already oxidized fish oil favoring propagation of oxidation (Hermund et al., 2019). As an alternative and considering that zein is one of the few hydrophobic biopolymers approved for oral use by the Food and Drug Administration (Patel and Velikov, 2014), the investigation of zein as shell material for omega-3 encapsulates targeted to be incorporated into water-food matrices is of special interest. Zein capsules loaded with DHA-enriched fish oil were efficiently incorporated into milk powder, and the organoleptic characteristics of the reconstituted milk were reported unaltered when measured by sensory analysis (Busolo et al., 2018).

However, the physicochemical properties (e.g. rheology, color, droplet size, content of specific oxidation products) of food enriched with zein electrosprayed capsules loaded with omega-3 PUFA remains to be investigated. There are very few works in the literature dealing with the incorporation of electrosprayed capsules to food matrices, and most of them study the use of electrosprayed encapsulates produced with hydrophilic biopolymers as shell materials (Hermund et al., 2019; Gómez-Mascaraque et al., 2017). Hence, there is need for evaluating the effect of electrosprayed encapsulates on fortified products obtained at real food processing conditions, especially of those capsules having hydrophobic wall materials (e.g. microstructure of the enriched food, viscosity, color, physical stability, and ability of the capsules to protect the bioactive compound encapsulated).

In the context of the above, this study aimed to investigate the oxidative stability and physical properties of low-fat mayonnaise enriched with zein electrosprayed capsules loaded with fish oil. Mayonnaise was selected as model of a water-based food matrix where lipid oxidation is a challenge due to the release of iron bounded to the protein phosvitin from egg yolk, which catalyzes lipid oxidation (Jacobsen et al., 2001). Thus, in this work, we first characterized the fish oil-loaded zein capsules and assayed their oxidative stability during storage. Secondly, we evaluated the physical properties of the fortified mayonnaise such as droplet size distribution, viscosity and color of the, as well as the physical status of the electrosprayed encapsulates once incorporated into the mayonnaise matrix. Finally, the oxidative stability of the omega-3 fortified mayonnaises, either with fish oil-loaded zein electrosprayed capsules or with neat fish oil, was evaluated during storage.

2. Materials and methods

2.1 Materials

Zein from maize, grade Z3625, was purchased from Sigma-Aldrich S.A. (Madrid, Spain). Commercial cod liver oil was donated by Maritex A/S, subsidiary of TINE, SA (Sortland, Norway) and stored at -40 °C until use. The major fatty acid composition of the fish oil was: C16:0, 9.5%; C16:1, 8.7%; C18:1, 16.3%; C20:1, 12.6%; C20:5, 9.2% and C22:6, 11.4%. The tocopherol content of the fish oil was: α -tocopherol, 200 $\pm$ 3 μ g/g oil; β -tocopherol, 5 $\pm$ 1 μ g/g oil; γ -tocopherol, 96 $\pm$ 3 μ g/g oil and δ -tocopherol, 47 $\pm$ 1 μ g/g oil. Peroxide value (PV) of the fish oil was 0.4 $\pm$ 0.1 meq/kg oil. The rapeseed oil used in the production of the mayonnaise was donated by AAK Sweden AB (Karlshamn, Sweden). The major fatty acids of the rapeseed oil were: C16:0, 4.4%; C18:1, 58.3%; C18:2, 18.9%; and C18:3, 9.3%. This oil had a PV of 0.2 $\pm$ 0.0 meq/kg oil and a tocopherol content of 193 $\pm$ 1 μ g α -tocopherol/g oil and 96 $\pm$ 3 μ g γ -tocopherol/g oil. The properties of the oils used were determined as described elsewhere (Hermund et al., 2019). Potassium sorbate was purchased from Merck (Darmstadt, Germany). Stabilizer Grindsted FF was purchased from Danisco (Copenhagen, Denmark). Egg yolk, salt, vinegar and sugar were purchased from the local supermarket. All other chemicals and solvents used were of analytical grade.

2.2 Production of capsules

Zein capsules containing 20% of fish oil, were provided by Bioinicia S.L. (Valencia, Spain) and stored at -40°C when received. They were prepared as described in a previous study (Busolo et al., 2018) by adding fish oil into a zein solution (5 wt% zein, ethanol 85%) under a nitrogen atmosphere. The mix was emulsified using an Ultraturrax T-25 homogenizer (IKA, Satusfen, Germany) for 5 minutes at 17,500 rpm. The zein solution containing fish oil was subjected to electrospraying assisted by pressurized air using the pending patent FluidnatekTM LE500 Capsultek pilot tool (Bionicia S.L., Valencia, Spain). Temperature was maintained at 24°C and relative humidity at 40%. The solution was

pumped at of 1.4 mL/min to a nebulizer that worked with an air pressure of 10 L/min. The nebulized was connected to an electric voltage of 10 kV and the resulting droplets dried in their travel towards the collection unit. The collection of the encapsulated powder was carried out in a grounded cyclonic collector as a free flowing powder. The production batches had a duration of 40 minutes. The powder collected in three different batches was blended together in order to have a homogeneous final sample.

2.3 Physicochemical properties of capsules

2.3.1 Morphology of capsules

Morphology of the zein capsules containing fish oil was analyzed using scanning electron microscopy (SEM) in a Hitachi S-4800 FE-SEM from Hitachi High Technologies Corp. (Tokyo, Japan) with an electron beam acceleration of 5 KV. The samples were coated with a gold/palladium layer prior to SEM analysis. Capsule diameters were determined measuring 120 random capsules by ImageJ (National Institutes of Health).

2.3.2 Encapsulation Efficiency

This assay was performed to quantify the efficiency of the encapsulation process. The conventional method consists in the removal of non-encapsulated oil, which is mainly attached to the shell surface, by soaking the capsules in a solvent (e.g. heptane) where the oil can be solubilized (Moomand and Lim, 2014). In this case, a few modifications were done since the zein capsules were totally degraded when they were completely soaked into the solvent. In brief, approximately 25 mg of capsules were placed on a filter paper and 5 mL of isooctane was softly poured over the capsules. The absorbance of the solvent resulting after washing the capsules was measured at 284 nm. A calibration curve was

prepared in the range of 0.1-0.5 mg/mL using fish oil as standard and isooctane as solvent. Measurements were carried out in triplicate. The encapsulation efficiency was calculated using Eq. 1, where A is the theoretical quantity of fish oil and B is the amount of oil present in the washing solvent.

$$Efficiency (\%) = \left[\frac{(A-B)}{A} \right] * 100 \quad (1)$$

2.3.3 Oxidative stability of capsules

Fish oil-loaded zein electrosprayed capsules (2.2 g) and un-encapsulated fish oil (6.4 g) were stored during 35 days in 8 mL vials at 20°C in the dark. Equal headspace was kept for all vials. Samples were taken at 0, 3, 8, 14, 21 days and 35 days, they were overlaid with N₂ and stored at -40°C until analysis.

2.3.3.1 Determination of oil content and peroxide value (PV)

Approximately 0.5 g of capsules were dissolved in 15 ml ethanol/water 85 wt.%, followed by vortex for 5 min. Afterwards, 15 mL of isooctane was added, then vortex was applied for 2 min and the resulting mixture was centrifuged at 2,800 rpm, 18°C for 10 minutes (Sigma 4-16KS, Osterode, Germany). The upper phase, which contained the isooctane and the oil, was carefully extracted and approximately 70% of the total volume was weighted and dried overnight (approximately 4.5 g) in order to determine the oil content. The oil content was determined according to Bligh and Dyer method with modifications according to the sample volume used (Bligh and Dyer, 1959). Analyses were carried out in duplicate.

The rest of the extract obtained, approximately 2 g, was evaporated under N₂ atmosphere and used for PV analysis. PV of the fish oil extracted from the capsules and PV of the neat fish oil, were determined based on the measurement of the colorimetric ferric-thiocyanate complex at 500 nm,

that results from the oxidation of Fe^{+2} to Fe^{+3} by peroxides compounds present in the oil (Shantha and Decker, 1994). Results were expressed as miliequivalents of peroxides per kg of oil. Analyses were carried out in duplicate.

2.3.3.2 Determination of volatile secondary oxidation products

The volatiles secondary oxidation products of neat fish oil and zein electrosprayed capsules loaded with fish oil were determined by dynamic headspace gas chromatography-mass spectrometry (GC-MS). For the electrosprayed zein capsules and zein protein, the volatiles were determined as described in our previous work for glucose syrup and dextran capsules with some modifications (García-Moreno et al., 2018). Approximately 0.4 g of capsules or zein protein, 30 mg of internal standard (4-methyl-1-pentanol, 30 $\mu\text{g/g}$ water) and 5 mL of ethanol/water 85 wt.% were added to 100 mL purge bottle. Vortex was applied (3 min) to dissolve the capsules into the solvent and afterwards the bottle was connected to a flow of N_2 (250 mL/min) in a water bath at 45° C for 30 min. The released volatiles compounds were collected in a Tenax®GR tube. The tube was dried under N_2 (50 mL/min) for 30 min to remove the excess of ethanol. The volatiles were desorbed by heating at 200°C in an Automatic Thermal Desorber (ATD-400, Perkin Elmer, Norwalk, CN), cryofocused on a cold trap (-30°C), released again (220°C), and led to a gas chromatograph (HP 5890IIA, Hewlett Packard, Palo Alto, CA, USA; Column: DB-1701, 30 m x 0.25 mm x 1.0 μm ; J&W Scientific, CA, USA). The oven program had an initial temperature of 45°C for 5 min, increasing at 1.5°C/min until 70°C, at 2.5°C/min until 90°C, and at 12.0°C/min until 220°C, where the temperature was kept for 4 min. The individual compounds were analyzed by mass-spectrometry (HP 5972 mass-selective electron ionization mode, 70 eV; mass to charge ratio scan between 30 and 250). The individual compounds were identified by

both mass spectrometry (MS) -library searches (Wiley 138 K, John Wiley and Sons, Hewlett-Packard) and by authentic external standard and quantified through calibration curves.

For neat fish oil, approximately, 4 g oil and 30 mg of internal standard (4-methyl-1 pentanol, 30 µg/g water) were weighted out in a 100 mL purge bottle and then it was connected to flow of N₂ (150 mL/min) in a water bath at 60°C for 30 min. The released volatiles compounds were collected in Tenax® GR tubes. The volatiles from neat fish oil were analyzed at the conditions described elsewhere (García-Moreno et al., 2016).

Two different calibration curves were carried out, one for the neat fish oil and another for the zein capsules. The external standards used were 2-ethyl-furan, 1-penten-3-one, pentanal, 1-penten-3-ol, (*E*)-2-pentenal, hexanal, (*E*)-2-hexenal, 3-methyl nutanal, limonene, 2-pentyl-furan, (*E,E*)-2,4-heptadienal and nonanal (Sigma-185 Aldrich, Brøndby, Denmark). The standard solutions were directly injected to the Tenax®GR tube and dried for 5 min under N₂ (50 mL/min). Samples were analyzed in triplicate.

2.4 Production of capsule-enriched mayonnaise

Low-fat mayonnaise (40 wt.% of total oil) was enriched with fish oil (2.5 wt.%). Fish oil was incorporated either as neat oil or fish oil-loaded zein electrosprayed capsules. For the production of capsule-enriched mayonnaise, 120 g in total, water (52.7 wt.%) salt (0.3 wt.%) sugar (1 wt.%), potassium sorbate (0.1 wt.%) and egg yolk (4 wt.%) were poured into a blender (Turbo, Molinex, Lyon, France) and dissolved for 15 s. Vinegar (1 wt.%), lemon juice (0.4 wt.%) and Grindsted FF (0.5 wt.%) were added to rapeseed oil (37.5 wt.%). Grindsted FF was previously dissolved in a small portion of the total amount of rapeseed oil. The resulting oil mixture was slowly poured into the blender to be mixed for 30 s with the other ingredients previously added to form an emulsion. Finally, 15g of

capsules were added to the blender and the mixture was mixed by 60 s. A 30 s period was used to pour down the materials attached at the blender's walls and the mixture was blend for other 60 s. A second mayonnaise containing equal amount of fish oil, but un-encapsulated, was prepared as a control. The same recipe was followed with the difference that neat fish oil (2.5 wt. %) was mixed to rapeseed oil and then blend with the rest of ingredients as it was stated before. Both mayonnaises were stored in 50 mL brown bottles at 25 °C in the dark for 21 days. Samples were taken at 0, 7, 14 and 21 days, subdivided into two brown bottles (6 and 9 g, respectively), overlaid with N₂ and stored at -40°C until PV and volatile analyses were carried out.

2.5 Characterization of capsule-enriched mayonnaise

2.5.1 Microstructure of enriched mayonnaise

The microstructure of the mayonnaise samples was observed by cryo-scanning electron microscopy. Droplets of mayonnaise were frozen in liquid nitrogen on day 0 and stored for later sample preparation and imaging. Frozen mayonnaise samples were removed from the liquid nitrogen, quickly adhered to SEM stubs with a 50:50 mixture of colloidal graphite powder (Agar Scientific, Stansted, United Kingdom) and Tissue-Tek OCT compound (Ted Pella, Redding, USA), and re-frozen in liquid nitrogen. Frozen samples were then loaded onto a Leica EM VCT 100 Cryo Transfer Shuttle and transferred to a Leica EM MED020 freeze fracture and high vacuum coating system (Leica, Wetzlar, Germany). Samples were then fractured, sublimated for 1 minute at -90 °C and sputter coated with 6 nm of carbon/platinum. After coating, the samples were transferred via the VCT 100 Cryo Transfer Shuttle under vacuum and at -140°C to a FEI Quanta 3D FEG FIB/SEM for subsequent SEM imaging (Thermo Fisher Scientific, Waltham, USA). Imaging was performed at high vacuum at -140°C with an accelerating voltage of 2 kV.

2.5.2 Droplet size, viscosity and color

The droplet size distribution of light-mayonnaise samples was measured by laser diffraction (Mastersizer 2000, Malvern Instruments Ltd) at days 0 and 21. A few drops of light-mayonnaise were diluted in recirculating water (3000 rpm), reaching an obscuration of approximately 10%. Mayonnaise samples were not previously diluted in sodium dodecyl sulfate (SDS) buffer since no differences in droplet size were observed when diluting in distilled water when compared to diluting in SDS buffer (results not shown). The refractive indices of sunflower oil (1.469) and water (1.330) were used as particle and dispersant, respectively. Results were given in surface area mean diameter ($D [3.2]$), and volume weighted mean diameter, ($D [4.3]$). Measurements were made in triplicate.

The viscosity of light-mayonnaise samples was measured at 25 °C using a StressTech HR High Resolution Oscillatory rheometer (Reologica Instruments, Lund, Sweden) at days 0 and 21. Serrated bottom base and upper plate were used (P30 Serrated), with a gap of 2 mm. An increasing gradient of stress was applied up to 65 Pa. Measurements were carried out in duplicate.

A Konica Minolta CR-300 Chroma colorimeter was utilized to measure the color of mayonnaise samples at day 0 and after 21 days of storage (Minolta, Tokyo, Japan). The data were recorded by the instrument using the CIEL^{*}*a*^{*}*b*^{*} color system space, where *L*^{*} refers to lightness, *a*^{*} to red/green, and *b*^{*} to yellow/ blue. Yellowness index (YI) was calculated from these values according to the equation: $YI = 142.86 \cdot b^* / L^*$ (Francis and Clydesdale, 1975). All measurements were carried out in duplicate.

2.5.3 Oxidative stability of capsule-enriched mayonnaise

2.5.3.1 Determination of peroxide value and tocopherol content

The determination of PV in the mayonnaise samples was carried out by extracting the lipids according to Bligh and Dyer method using a reduced amount of the chloroform/methanol (1:1, wt.%) solvent (Bligh and Dyer, 1959). Two extractions were made from each sample. Peroxide value was determined on lipid extracts using the colorimetric ferric-thiocyanate method at 500 nm as described by Shantha and Decker (1994). Results were expressed as milliequivalents of peroxides per kg of oil.

The tocopherol content was determined according to AOCS (1998) using an Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA, USA), equipped with a fluorescence detector. About 2 g of the chloroform extract from the Bligh and Dyer extract were evaporated under nitrogen and dissolved in 10 mL n-heptane and from this, 1 mL samples were taken into separate vials before injection of an aliquot (40 μ L) on a Spherisorb S5 W column (250 x 4.6 mm) (Phase Separation Ltd., Deeside, UK). Elution was performed with an isocratic mixture of n-heptane/2-propanol (100:0.4, v/v) at a flow of 1 mL/min. Detection was done using a fluorescence detector with excitation at 290 nm and emission at 330 nm. Measurements were performed in duplicate and quantified by authentic standards. Results were expressed in μ g tocopherol/g mayonnaise.

2.5.3.2 Analysis of secondary volatile oxidation compounds by dynamic head space GC-MS

The measurement of the volatiles secondary oxidation products was performed by dynamic headspace GC-MS. Approximately, 3 g of mayonnaise and 30 mg internal standard (4-methyl-1-pentanol, 30 μ g/g water) were weighed out in a 100 mL purge bottle, to which 5 mL of distilled water and 1 mL antifoam (Synperonic 800 μ L/L water) were added. The bottle was heated to 60°C in a water bath while purging with nitrogen (flow 150 mL/min, 30 min). Volatile secondary oxidation products were trapped on Tenax GR tubes. Volatile acids were removed by 0.1 g KOH during the headspace collection by using an S-tube with KOH. The volatiles were desorbed in the gas chromatograph as

described above. The temperature program was as follows: 3 min at 35 °C, 3 °C/min from 35 to 120 °C, 7 °C/min to 120–160 °C, 15 °C/min 160–200 °C and hold for 4 min at 200 °C. The individual volatiles were analysed by MS, identified by both library and external standards and quantified through calibration curves as indicated above. Results were expressed in ng/g of mayonnaise.

2.6 Statistical analysis

Statgraphics 18 (Statistical Graphics Corp., Rockville, MD, USA) was used for data analysis. Data were expressed as mean $\pm$ standard deviation. Firstly, multiple sample comparison analysis was performed to identify significant differences between samples. Secondly, mean values were compared by using the Tukey's test. Differences between means were considered significant at $P < 0.05$.

3. Results and discussion

3.1 Physicochemical properties of capsules

3.1.1 Morphology and diameter distribution of capsules

The zein electrosprayed capsules loaded with fish oil consisted of particles with a semi-spherical shape (Fig. 1a). It is worth mentioning that fibers interconnecting the capsules were not observed. They presented a rough-like surface as observed in a magnified SEM image (Fig. 1b). Approximately 60% of the capsules had a diameter in the range of 2-3 μm , with nearly 25% in the range of 1-2 μm and 15% above 3 μm (Fig. 1c). The appearance of the particles obtained in this study is in agreement with those reported by Busolo *et al.*, 2018, where zein was used as shell material to encapsulate DHA-rich oil using electrospraying assisted by pressurized air. It should be noted that the particles obtained in this work have larger diameter ($2.4 \pm 0.7 \mu\text{m}$) when compared to the average particle diameter of the study mentioned ($1.4 \pm 0.8 \mu\text{m}$). This difference is related to the ratio of zein: fish oil used. Busolo

et al. (2018) used a zein: fish oil ratio of 2:1, while we decreased the fish oil load of the capsules by employing a ratio zein: fish oil of 4:1. The capsule diameter is mainly determined by the type of encapsulating method, the oil load as well as the type, the concentration of shell material and the electrospray processing conditions. However, similar ranges of capsules average diameter (1-3 μm) were reported for fish oil-loaded capsules with glucose syrup or dextran as main wall materials when obtained by electrospraying assisted by pressurized air (Hermund et al., 2019)(Busolo et al., 2018)(García-Moreno et al., 2018b). Nonetheless, the size of the electrosprayed capsules reported in this work was significantly lower when compared to fish oil-load capsules obtained by alternative encapsulation techniques such as spray-drying (Mahdi et al., 2008)(Drusch et al., 2007).

3.1.2 Encapsulation efficiency (EE) of capsules

The zein electrosprayed capsules loaded with fish oil presented an encapsulation efficiency (EE) of $83 \pm 1\%$. This value indicates that the encapsulation method used in this study was effective to retain most of the fish oil within the zein microstructure. However, it also denotes that a considerable part of the omega-3 rich oil (17%) could be distributed on the surface of the capsules or in a layer close to the surface allowing its extraction when using a hydrophobic solvent (Drusch and Berg, 2008). Likewise, Busolo *et al.*, 2018 obtained a similar EE ($84 \pm 1\%$) when producing zein electrosprayed capsules loaded with DHA-rich oil, even when the capsules obtained by these authors had higher fish oil load capacity than the ones reported in this work. Similar EE values (78-92%) have been reported previously for fish oil-loaded electrosprayed capsules with 20% of loading capacity, but when water-soluble biopolymers such as glucose syrup or dextran were used as shell material (García-Moreno et al., 2018b).

Although high EE values (e.g. EE > 95%) are desired in order to minimize lipid oxidation of oil droplets sitting on the surface of the capsules, non-encapsulated oil may also protect other fractions of the extractable oil from oxidation (Drusch and Berg, 2008). In any case, EE values obtained for electrosprayed capsules produced with hydrophobic shell materials should be interpreted carefully. This is because hydrophobic solvents used to extract the non-encapsulated oil could also favor the disintegration of the capsules by solution of the wall material, allowing the extraction of encapsulated oil droplets (Busolo et al., 2018).

3.1.3 Oxidative stability

Fig. 2 shows the peroxide value (PV) of both neat fish oil and zein electrosprayed capsules loaded with fish oil during storage. The un-encapsulated fish oil presented a very low initial concentration of primary oxidation products ($PV = 0.5 \pm 0.0$ meq/kg oil), which increased during the first 21 days of storage and then leveled off at $PV = 6.2 \pm 0.1$ meq/kg oil. A different behavior was observed for the zein capsules loaded with fish oil, which presented considerably higher initial PV than neat fish oil (14.7 ± 0.2 meq/kg oil). This indicates that fish oil was oxidized during electrospraying encapsulation, mainly due to the higher specific surface area of the resulting encapsulates, together with the exposure of un-encapsulated oil to atmospheric oxygen (García-Moreno et al., 2018b). Thus, further research is still needed to reduce lipid oxidation during electrospraying. For that, better entrapment of oil within the zein matrix either by improvement of homogenization, which allows lower droplet size, or by using a co-axial process, which provides an extra shell to the encapsulate, should be further investigated. Nevertheless, it should be highlighted that the content of primary oxidation products of the capsules did not increase during storage, with no significant differences ($P > 0.05$) between PV for un-encapsulated fish oil and fish oil-loaded capsules at day 35. Although Busolo et al. (2018) reported

an initial PV of ~1.5 meq/per kg oil for zein electrosprayed capsules loaded with DHA-rich oil, the authors found that the encapsulates increased its PV up to 20 meq/kg oil after one week storage at 23 °C in the dark. These differences might be attributed to the higher DHA content of the oil used by Busolo et al. (2018), which led to an exponential increase in lipid oxidation as also denoted by the higher PV for the un-encapsulated oil found by these authors. However, when comparing the results shown in Fig. 2 with those obtained in our previous study (García-Moreno et al., 2018), a higher protective effect of zein to the fish oil was clearly observed compared to glucose syrup or dextran when used as main shell materials in electrospraying. We previously reported PV up to 20 meq/kg oil for fish oil-loaded electrosprayed capsules (oil load of 20%) with glucose syrup or dextran as main wall materials after 21 days storage. These values are considerably higher than those shown in Fig. 2 for zein electrosprayed capsules loaded with fish oil (even after a longer storage of 35 days).

Moreover, we also investigated the formation of secondary volatile oxidation products formed as a consequence of the decomposition of the unstable lipid peroxides. In order to obtain results comparable to our previous studies (García-Moreno, Pelayo, et al., 2018; Hermund et al., 2019), we selected dynamic headspace GC-MS as the analytical method, although in this work the capsules were dissolved in a mixture of water-ethanol due to the hydrophobic character of zein. As it is shown in Fig 1S, an increasing formation of volatile compounds originating from omega-3 oxidation (1-penten-3-ol, 1-penten-3-one, 2,4-heptadienal) was observed in neat fish oil during storage. Similar volatile compounds were not identified in zein electrosprayed capsules loaded with fish oil (Fig 2S). Instead, most of the volatile products detected in the capsules were also found in high concentration in the zein protein powder, such as 3-methyl-butanal (978.7 ± 168.2 ng/g zein), hexanal (171.1 ± 30.4 ng/g zein) and 2-pentylfuran (97.0 ± 29.2 ng/g zein). Hence, it is deduced that volatile oxidation products of interest were not properly collected in the Tenax tubes since they may become saturated with

ethanol used as solvent to dissolve the capsules. This assumption is supported by the lower concentration of internal standard, 4-methyl-1-pentanol (0.8 ± 3.0 ng/g zein) detected in the zein powder when compared to the analysis of fish oil (> 200 ng/g fish oil). Therefore, further optimization of dynamic headspace GC-MS method to analyse volatiles compounds in zein capsules (non-water soluble shell material) is required. Torres-Giner *et al.* (2010) did report volatile oxidation products from fish oil oxidation in zein capsules, such as 2,4-heptadienal and propanal using headspace solid-phase micro-extraction (HS-SPME) (Torres-Giner *et al.*, 2010). HS-SPME method has a high affinity for low molecular weight compounds, such as 3-methyl-butanol. Nevertheless, this method is suggested only for less-complex matrices since volatile compounds of similar molecular weight can be lost (Lu *et al.*, 2013).

3.2 Physicochemical properties of mayonnaises enriched with fish oil

Mayonnaise was selected as an aqueous-based food matrix in order to study the incorporation of the zein electrosprayed capsules loaded with fish oil into a food product. For that purpose, the physicochemical properties of mayonnaise fortified with fish oil-loaded zein electrosprayed capsules or neat fish oil were investigated.

3.2.1 Microstructure of enriched mayonnaises

The study of the microstructure of fortified food products is essential in order to determine how efficient is the incorporation of the encapsulates as well as to elucidate the effect of enrichment on the physicochemical properties of the final product. Fig. 3 shows a freeze fractured surface of both types of mayonnaises (enriched with capsules or with neat fish oil) obtained via cryo-SEM. In the capsule-enriched mayonnaise (Fig. 3a), the presence of spherical particles, which correspond to the zein electrosprayed capsules, were clearly visible. The diameter of these structures coincided with the

size range of the zein capsules reported in section 3.1.1. Furthermore, as expected, these particulates were not present in the mayonnaise fortified with neat fish oil, which was used as a control and where only the dispersed oil droplets were observed (Fig. 3b). Hence, the appearance of these particles in Fig. 3a reveals that the fish oil-loaded electrosprayed capsules were preserved intact during processing of the mayonnaise. Additionally, the presence of solid spheroids and spherical voids within the particles are indicative of the encapsulation of fish oil droplets within the zein matrix. These results show for first time ever that zein electrosprayed capsules did not disintegrate when incorporated into an aqueous-food matrix like mayonnaise, which will affect the protection of the encapsulated bioactive.

3.2.2 Droplet size, viscosity and color

The physical stability of the mayonnaise samples was first investigated by measuring its droplet size after production and after 21 days storage. Table 1 shows that the mayonnaise enriched with zein electrosprayed capsules loaded with fish oil (Mayo-C) presented significantly ($P<0.05$) higher D_{4,3} values after production when compared to the mayonnaise fortified with neat fish oil (Mayo-F) (although no significant differences were observed in the D_{3,2} values). D_{4,3} reveals information about the size of the bulk of the droplets constituting the mayonnaise, whereas D_{3,2} indicates mainly the size of small droplets present in the emulsion-like product (McClements, 2005). Hence, the reduction of droplet size during preparation of the mayonnaise was considerably limited in the presence of zein particles when compared to the presence of only neat oil. Likewise, Hermund et al. (2019) reported higher droplet size for mayonnaise enriched with glucose syrup electrosprayed capsules when compared to mayonnaise enriched with neat fish oil. Nevertheless, zein capsules-enriched mayonnaise had larger droplets than glucose syrup capsules-enriched mayonnaise (Hermund et al.,

2019). This may be attributed to the fact that zein capsules remained intact in the mayonnaise matrix, limiting the reduction of droplet size during homogenization. On the other hand, glucose syrup capsules may have disintegrated when incorporated into the mayonnaise aqueous-based matrix, favoring homogenization. It is also noteworthy that, although no creaming was observed, the droplet size of Mayo-C significantly increased during storage as shown by D_{4,3} values in Table 1. On the contrary, the D_{4,3} of Mayo-F decreased after 21 days storage. This denoted physical destabilization by flocculation/coalescence in Mayo-C, whereas the potential floccules present in mayo-F after production disappeared during storage. Both phenomena have been previously reported in mayonnaise products (Hermund et al., 2019; Sørensen et al., 2010).

Fig. 4 shows the viscosity results for the mayonnaise samples. A pseudoplastic behavior was observed in Mayo-C and Mayo-F. Mayo-C presented a higher apparent viscosity due to the presence of intact capsules, which thickened the aqueous phase of the emulsion. Hermund *et al.* (2019) also observed an increase in viscosity for mayonnaise enriched with capsules due to the thickening effect of the carbohydrate used as shell material when capsules disintegrated. The slight increase of the viscosity after 21 days of storage in Mayo-F, can be explained by the decrease in droplet size during storage, which increases friction between droplets, as a consequence of a reduced surface-to-volume ratio of the dispersed phase (Pal, 1996). On the other hand, Mayo-C presented higher viscosity after storage, even though an increase in the droplet size was observed after storage. This is contrary to the results obtained by Hermund *et al.* (2019), where the viscosity of syrup capsule-enriched mayonnaise decreased after storage (Hermund et al., 2019).

As expected, Mayo-C presented a significantly ($P < 0.05$) higher yellowness index (YI) than Mayo-F both after production and after 21 days storage (Table 1). This is attributed to the impurities such as

xanthophylls and carotenoids contained in the zein protein used (Shukla and Cheryan, 2001). Therefore, further processed zein prolamine protein (e.g. subjected to removal of undesirable color-compounds) (Cook et al., 1996) is required to avoid change in the fortified food product. This is of special importance taking into account consumers' perception of the food product.

3.2.3 Oxidative stability

3.2.3.1 Peroxide value and tocopherol content

Fig. 5a shows the content of primary oxidation products in the mayonnaise samples during storage. The initial PV of capsule-enriched mayonnaise (2.1 ± 0.1 meq/kg oil) was significantly ($P < 0.05$) higher than mayonnaise enriched with neat fish oil (0.5 ± 0.2 meq/kg oil). However, the PV of Mayo-F increased significantly after 14 days storage, reaching a value of 6.3 ± 0.4 meq/kg oil at 21 days. On the other hand, the PV of Mayo-C did not change significantly ($P > 0.05$) compared to the initial PV, with a final value at day 21 of 2.0 ± 0.1 meq/kg oil, which was 2-fold lower when compared to Mayo-F. It is noteworthy that the initial PV of zein capsules-enriched mayonnaise observed in this study (~ 2 meq/kg oil, Fig. 5a) was considerably higher than the one reported in our previous study for glucose-syrup capsules enriched mayonnaise (< 1 meq/kg oil) (Hermund et al., 2019). Nevertheless, the content of primary oxidation products of zein capsules-enriched mayonnaise was considerably lower after 14 days storage when compared to glucose syrup capsules-enriched mayonnaise (8 meq/kg oil at day 21) (Hermund et al., 2019). This may be attributed to a better protection of the encapsulated oil within the zein matrix due to the presence of intact zein electrosprayed capsules in the mayonnaise matrix (Fig. 3a), which was not the case for the glucose syrup capsules where potential disintegration of the capsules with a hydrophilic shell material could have occurred (Hermund et al., 2019).

The tocopherol content of the mayonnaise samples was also evaluated during storage (Fig. 5b). Among the four tocopherols homologues, results for only α - and γ -tocopherols are shown since they were the most abundant in the fish oil used (β -tocopherol content changed from 13.4 to 20.1 and δ -tocopherol content varied from 4.0 to 6.0 ng/g mayonnaise). Interestingly, an opposite behavior for tocopherol content was observed when compared to the PV curves. The initial content of α - and γ -tocopherols was higher in Mayo-F than in Mayo-C, which could be attributed to a loss of α - and γ -tocopherols during electrospraying. However, a decrease in the content of tocopherols was found for Mayo-F after 14 days storage (which was not observed in Mayo-C) (Fig. 5b). This reduction could be a result of the natural antioxidant role of tocopherols in preventing the oxidation of the oil by donation of an hydrogen to scavenge free radicals (Alemán et al., 2015). This correlated well with the PV results (Fig. 5a) since after 14 days storage the Mayo-F suffered a significant increase in PV, which denoted a more pronounced oxidation, which was due to the consumption of natural antioxidants present in the fish oil (e.g. α - and γ - tocopherols).

3.2.3.2 Secondary volatile oxidation products

Fig. 6 shows the formation of secondary oxidation products in fish oil-enriched mayonnaises during storage. An increasing concentration of volatile compounds from oxidation of omega-3 fatty acids such as 2-ethylfuran and 1-penten-3-one, was observed in Mayo-F, whereas they were not detected in Mayo-C (Fig. 6a,b). However, the concentration of these volatiles were considerably lower than those of other volatiles derived from oxidation of omega-3 PUFA such as 1-penten-3-ol (Fig. 6c), (*E,E*)2,4-heptadienal (Fig. 6d) and (*E*)-2-pentenal (results not shown) obtained in Mayo-F, which were also detected in higher concentrations in Mayo-C. Other volatile oxidation products such as hexanal (Fig. 6e) and 3-methyl-butanal (Fig. 6f) were present in higher concentrations in Mayo-C from day 0

when compared to Mayo-F, but they did not increase their concentration during storage. A similar trend was observed for (*E*)-hexenal, heptanal, benzaldehyde, nonanal (data not shown). Therefore, the presence of these volatiles was attributed to the zein powder used, where these volatiles were formed. This reveals the importance of the quality of the ingredients used to produce the encapsulates, and should be borne in mind when selection the shell material. Additionally, other volatile compounds were derived from lipid oxidation occurring in the un-encapsulated fish oil portion of the capsules (17%), which oxidized during production and accelerated lipid oxidation in the mayonnaise matrix.

Concentration of alcohols such as 3-methyl-1-butanol (Fig. 6g), 1-pentanol (Fig. 6h) and 2,4-hexadienal-1-ol (results not shown) increased considerably after 14 days of storage for Mayo-C sample, whereas they were not detected in mayo-F. The considerably high concentration of 3-methyl-1-butanol (approximately 3000 ng/g mayonnaise) could be related to the growth of fungal species observed in this sample. For instance, 3-methyl-1-butanol was one of the main volatile compounds found in spoilage fungal genera such as *Aspergillus*, *Penicillium* and *Fusarium* cultured with grains (corn, wheat, barley) as substrates (Magan and Evans, 2000). This compound is originated from the degradation of leucine (Jeleń and Wąsowicz, 1998), which is in agreement with our results since leucine is the main non-polar amino acid (20% of total amino acids) found in zein protein (Shukla and Cheryan, 2001). The fungal degradation of mayonnaise containing zein capsules needs to be further investigated as well as the optimization of the preservation method.

Overall, the higher content of secondary oxidation products found for Mayo-C during storage compared to Mayo-F was consequence of the volatile compounds present in the zein protein, together with the formation of other volatiles originated from the fungi spoilage and oxidation of non-encapsulated oil. Therefore, further research is still needed (e.g. on the reduction of lipid

oxidation during encapsulation) to prove the advantages of the enrichment of mayonnaise with electro sprayed capsules. Nonetheless, zein capsules-enriched mayonnaise was considerably less oxidized than glucose syrup capsules-enriched mayonnaise (Hermund et al., 2019). For instance, the amount 1-penten-3-ol detected at 21 days was above 1000ng/g mayonnaise for mayonnaise fortified with glucose syrup capsules (Hermund et al., 2019), whereas for mayonnaise enriched with zein capsules it was around 60 ng/g mayonnaise.

4. Conclusion

Encapsulation of fish oil in zein electro sprayed capsules reduced lipid oxidation during storage when compared to fish oil-loaded electro sprayed capsules produced with hydrophilic biopolymers (e.g. glucose syrup or dextran). Cryo-SEM images revealed that zein capsules remained intact after incorporation into a water-based matrix like light-mayonnaise. This implies that the use of non-water soluble compounds as capsule shell, such as zein prolamine, is a key factor to preserve the capsule-structure when incorporated into an oil-water emulsion, which enhanced oxidative stability of the fortified product when compared to the use of other water soluble electro sprayed encapsulates. On the other hand, a noticeable amount of secondary oxidation products was observed in capsule-enriched mayonnaise, mainly due to the presence of volatiles formed in the zein protein powder, volatiles derived from fungi-spoilage of this type of mayonnaise; as well as volatiles derived from lipid oxidation of un-encapsulated oil droplets distributed on the surface of the capsules. Finally, addition of antioxidants and antimicrobial compounds to the mayonnaise should be considered for enhancing the shelf-life of the final omega-3 enriched product.

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Table 1. Droplet size: surface (D[3,2]) and volume (D[4,3]) mean diameters, and yellowness index (YI) of mayonnaise enriched either with zein electrospayed capsules loaded with fish oil (Mayo-C) or with neat fish oil (Mayo-F).

Sample	D[3,2], μm		D[4,3], μm		YI	
	day 0	day 21	day 0	day 21	day 0	day 21
Mayo-C	1.0 $\pm$ 0.4 ^a	0.7 $\pm$ 0.0 ^{a,ns}	39.7 $\pm$ 0.2 ^a	47.6 $\pm$ 1.9 ^{a,*}	37.8 $\pm$ 0.0 ^a	35.6 $\pm$ 1.6 ^{a,ns}
Mayo-F	1.0 $\pm$ 0.3 ^a	0.6 $\pm$ 0.1 ^{a,ns}	16.2 $\pm$ 2.7 ^b	11.7 $\pm$ 0.3 ^{b,*}	16.3 $\pm$ 0.0 ^b	19.5 $\pm$ 0.1 ^{b,*}

Different letters (a, b) indicate statistical significant differences ($P < 0.05$) between different types of mayonnaise. “*” indicates statistical significant differences ($p < 0.05$), and “ns” no statistical significant differences between the same sample at days 0 and 21.

Fig. 2. Peroxide value (PV) of zein electrospayed capsules loaded with fish oil and neat fish oil during 35 days of storage at dark and 20°C.

Letters (a-d) indicate statistical significant differences ($P < 0.05$) for each sample (neat fish oil or capsules) during storage.

“ns” indicates no statistical significant differences ($P > 0.05$) between capsules and neat fish oil at 35 days.

Fig. 3. Images obtained via cryo-SEM, showing a freeze fractured surface of a) mayonnaise containing electrospayed capsules, and b) mayonnaise with neat fish oil. The black arrows in a) highlight individual capsules while the white arrows show fish oil droplets and spherical voids where fish oil was encapsulated within the capsules prior to freeze fracture. Scale bars = 5 µm

Fig. 4. Viscosity of mayonnaise enriched with capsules (Mayo-C), and viscosity of mayonnaise with neat fish oil (Mayo-F) at day 0 ($\blacktriangle$) and at day 21 ($\bullet$) (storage 25 °C and in the dark).

*Mayonnaise with neat fish oil was analysed in duplicate, however only one replicate was carried out for mayonnaise enriched with capsules since the amount of sample was limited.

Fig. 5. a) Peroxide value (PV), and b) tocopherol content of mayonnaises enriched with zein electrosprayed capsules containing fish oil (Mayo-C) or with neat fish oil (Mayo-F) during storage at 25 °C in the dark.

Different letters (a,d) denote significant statistical differences ($P < 0.05$) between mayonnaise samples at each sampling time. “*” indicates statistical significant differences ($P < 0.05$) between samples at day 21.

*The duration of the storage period was reduced to 21 days since fungal growth was detected in Mayo-C.

Fig. 6. Secondary volatile oxidation products of mayonnaise samples during storage (25 °C in the dark): a) 2-ethyl-furan, b) 1-penten-3-one, c) 1-penten-3-ol, d) (E,E)-2,4-heptadienal, e) hexanal, f) 3-methyl-butanal, g) 3-methyl-1-butanol, and h) 1-pentanol.

Mayo-C: mayonnaise enriched with zein electrosprayed capsules loaded with fish oil. Mayo-F: mayonnaise enriched with neat fish oil.

Fig. 1. SEM images (a, b) and particle size distribution of zein electrospayed capsules loaded with fish oil, and (c) histogram of capsule diameter distribution.

Highlights

- Zein capsules remained intact when incorporated into a water-based food matrix
- Capsules-enriched mayonnaise had increased viscosity, droplet size and yellowness
- Zein electrosprayed capsules loaded with fish oil were oxidized after production
- Capsules-enriched mayonnaise showed high content of volatile oxidation compound

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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