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Wafa Taktak[a], Rim Nasri[a,e,*], Amparo Lopez-Rubio[b], Marwa Hamdi[a], Laura G. Gomez-Mascaraque[b], Nadia Ben Amor[d], Ahlem Kabadou[d], Suming Li[c], Moncef Nasri[a], Maha Karra-chaâbouni[a]

[a] *Laboratory of Enzyme Engineering and Microbiology, University of Sfax, National Engineering School of Sfax, B.P. 1173, 3038 Sfax, Tunisia*
[b] *Food Quality and Preservation Department, IATA-CSIC, Avda. Agustin Escardino 7, 46980 Paterna, Valencia, Spain*
[c] *European Institute of Membranes, UMR CNRS 5635, University of Montpellier, Place Eugene Bataillon, 34095 Montpellier Cedex 5, France*
[d] *Laboratory of Material Sciences and Environment, University of Sfax, Faculty of Science of Sfax, B.P. 1173, 3038 Sfax, Tunisia*
[e] *Higher Institute of Biotechnology of Monastir, University of Monastir, Monastir, Tunisia*

Keywords:
European eel protein isolate
Microencapsulation
Ultrasonication
Oxidative stability
ATG
SEM

A B S T R A C T

The objective of this study was to prepare European eel oil (EO) microcapsules using European eel protein isolate (EPI) as a wall material and investigate its oxidative stability. The EPI emulsions were obtained at different EO: EPI ratios (1:1, 1:2 and 1:4, w/w) and using two emulsification procedures: Homogenization (H) and homogenization followed by ultrasonication (HU) treatments. The microcapsules prepared by combining the two emulsification processes (HU) and at core and wall ratio of was 1:4 presented the smallest particles size and the greatest encapsulation efficiency (68.50%) and oxidative stability. Scanning electron microscopy (SEM) images proved the spherical shape of all microcapsules without fissure on the surface. The capsules exhibited an interesting antioxidant activity depending on the EO:EPI ratio, especially for the metal chelating potential. Thus, the effect of ultrasonication process and the EPI concentration on the characteristic, the stability and the antioxidant activity of the encapsulated EO has been proved.

1. Introduction

Actually, there is a growing interest in food with health benefit by consumers. As a consequence, the food industry initiated, among others, an innovation program for the development of functional foods [1]. This includes the concept of encapsulation enabling the isolation, protection, transport and release of basic nutrients required for good health such us the polyunsaturated fatty acids [2]. At present, fish oil is the major source of omega-3 fatty acids (ω-3 PUFAs), especially long-chain eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids, which exert a strong positive influence on human health such as prevention of cardiovascular disease [3], some types of cancer [4], reduction the levels of blood pressure and cholesterol [5], and treatment of major depression and Alzheimer's disease [6]. In contrast, the omega-3 fatty acids oxidation limit their use in food formulation as a result of decrease of their nutritional and biological properties [7].

Meanwhile the incorporation of fish oil into a variety of foodstuffs has been attempted. Advanced technologies for the protection of bioactive molecules in food formulations require encapsulation processes. In fact, microencapsulation is a technology that allows sensitive ingredients to be enveloped as a 'core' material within a polymer matrix or wall. Several studies have employed microencapsulation methods to provide protection to fish oil using various biolpolymers, as wall matrices, such as krill protein isolate [7], whey protein isolates and carbohydrates [8], vanillic acid grafted chitosan [9]. Furthermore, this technique provides a means to mask the taste and smell of the oils within food formulations to maintain the product's sensory appeal and consumer satisfaction [10,11]. Borneo, Kocer, Ghai, Tepper, and Karwe [12] found that the DHA and EPA entrapped in starch and gelatin, as wall material, and incorporated into cookies, had good sensory effect. Overall, several food products can be fortified with loaded- fish oil [7,13].

* Corresponding author at: Laboratory of Enzyme Engineering and Microbiology, University of Sfax, National Engineering School of Sfax, B.P. 1173, 3038 Sfax, Tunisia.
E-mail address: rymnasri2@gmail.com (R. Nasri).

Protein-based microencapsulation in food and pharmaceutical industries is widely used due to the interesting emulsifying and gel formation properties of proteins [14]. Proteins have been claimed to be a good encapsulating agent for oils/fats and volatiles, exhibiting effective microencapsulating properties [15]. The evaluation of the encapsulation of oil with fish proteins (gelatin or myofibrillar proteins) has not been properly studied, despite their interesting emulsifying properties.

This study aimed to evaluate the effect of emulsification process (homogenization and/or ultrasonication) and the core/wall ratio on physicochemical characteristics of the emulsions and the microcapsules obtained by spray drying process. The oxidative stability and antioxidant activity of the encapsulated oil were evaluated and compared to the free oil.

2. Materials and methods

2.1. Materials

1,1-Diphenyl-2-picrylhydrazyl (DPPH), Ferrozine, butylated hydroxyanisole (BHA), linoleic acid and other chemicals, such as potassium ferricyanide, trichloroacetic acid (TCA), $FaCl_3$, $FeCl_2$, EDTA, sodium hydroxide were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Solvents such us hexane, methanol, chloroform were of analytical grade, were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Extraction of European eel proteins

The protein isolate was extracted from European eel muscle according to the method used by Taktak et al. [16]. The fish muscle was minced (1:9; w/v) with cold water (4 °C), followed by pH adjustment to 11.5 using 2 N NaOH solution. The homogenate was centrifuged at 9500*g* for 20 min at 4 °C. Three layers were obtained: i: the upper layer was neutral lipid; ii: the middle layer was soluble proteins; iii: the bottom layer was insoluble materials. The soluble proteins were then precipitated at their nominal isoelectric point (pH 5.5) using 2 N HCl and centrifuged (9500*g* for 20 min). Finally, the obtained pellet was resuspended in distilled water, followed by pH adjustment to 7.0 using 2 N NaOH solution. European eel protein isolate (EPI) was freeze-dried using freeze-dryer lab (Moduloyd-230, Thermo Fisher Scientific, USA) at a temperature of −50 °C and a pressure of about 121 mbar.

2.3. European eel oil extraction and fatty acids composition

European eel oil (EO) was extracted from the upper layer, obtained in the first step of pH-shifting process, reported by Folch et al. [17] and the fatty acids composition was determined using gas chromatography (GC) analysis. After EO extraction, 0.2 mL of potassium hydroxide in methanol (2 N) was added for the fatty acid (FA) methylation process. The mixture was vortexed then centrifuged and the upper phase containing FA methyl esters (FAMEs) was subjected to gas chromatography (GC) analysis. FAMEs were analyzed by gas chromatography using a Shimadzu gas chromatograph (GC-17A) equipped with polar capillary column (DB-WAX, 3.0 m length, 0.25 mm I.D, 0.25 µm film thicknesses; Supelco). The initial temperature of the column was 150 °C and then reached progressively 250 °C. Nitrogen was used as a carrier gas at a flow rate of 1 mL/min. The injection port and the flame ionization detector were maintained at 250 °C. Identification was made by comparison of retention times to those of authentic standards. FAs were identified by matching their retention times with those of their respective standards; the results were expressed as a percentage of the area (%).

2.4. Emulsifying properties of EPI

The emulsifying capacity (EC) and stabilty (ES) of EPI (10 mg/mL) was estimated at different pHs (2 to 11). as described by Pearce and Kinsella [18]. The microscopic observation of each emulsion (20 µL) was carried out after 24 h using a CX31-12C04 microscope (Motic 2048 × 1536 pixels, Olympus Co., Tokyo, Japan).

Table 1
Processes for developing EO emulsions at different EPI:EO ratios and using homogenization (H) and homogenization followed by ultrasonication (HU) treatments.

	Feed emulsion (g 100 g^{-1})		Emulsification processes	
	EPI	EO	Homogenization (H)	Ultrasonication (U)
E1:1-H	10	10	x	
E1:1-HU	10	10	x	x
E1:2-H	10	5	x	
E1:2-HU	10	5	x	x
E1:4-H	10	2.5	x	
E1:4-HU	10	2.5	x	x

EO: European eel oil. EPI: European eel protein isolate. $E_{1:1}$, $E_{1:2}$ and $E_{1:4}$ are emulsions prepared at EO:EPI ratios of 1:1, 1:2 and 1:4, respectively.

2.5. Preparation of European eel protein isolate/oil emulsions

The experiments were conducted according to the Table 1. EPI powder was initially dispersed in distilled water at a concentration of 10 wt%. Then, the pH of EPI solution was adjusted to 10.0 with 2 N NaOH and stirred overnight. After that, EO was slowly incorporated into the EPI solution by stirring at 6000*g* for 5 min, using Ultra-Turrax (IKA T18 basic, Wilmington, USA), to form the emulsions. Three ratios of EO to EPI, 1:1, 1:2 and 1:4 (w/w), were prepared by homogenization (H) and were named $E_{1:1}$-H, $E_{1:2}$-H and $E_{1:4}$-H, respectively (Table 1). For the emulsions prepared applying ultrasound after the homogenization step for the process (HU), a nominal power of 160 W, 20 kHz and 2 min of treatment were used using Branson Digital Sonifier® (Model S-450D, Branson Ultrasonics Corporation, Dan-bury, USA). The height contact between the ultrasonic probe and the emulsions was standardized to 35 mm. Emulsions obtained by combining both processes were named $E_{1:1}$-HU, $E_{1:2}$-HU and $E_{1:4}$-HU.

2.6. Characterization of emulsions

2.6.1. Optical microscopy images

Optical microscopy images were taken using a digital microscopy system (Nikon Eclipse 90i) fitted with a 12 V, 100 W halogen lamp and equipped with a digital camera head (Nikon DS-5Mc). Nis Elements software was used for image capturing.

2.6.2. Zeta potential and size distribution

The zeta potential analysis of the different emulsions was determined using a particle Zetasizer instrument (Malvern Zetasizer Nano ZS, Worcestershire, UK) at 25 °C in triplicate.

2.6.3. Creaming stability

The stability of the emulsions was assessed following the creaming index method of Surh, Decker, & McClements [19].

2.7. Production of microencapsulation matrices by spray drying

The emulsions were spray dried using a Mini Spray Dryer apparatus (Büchi® Mini Spray Dryer, B-290, Switzerland). The inlet air temperature was set at 175 °C, as it proved to be enough to achieve complete drying of the particles under these conditions, the outlet air temperature was 80 °C. The atomizing air flow and the compressed air pressure were kept in 35 L min^{-1} and 6 bars, respectively. The dried powder was collected and stored at 4 °C for further analysis.

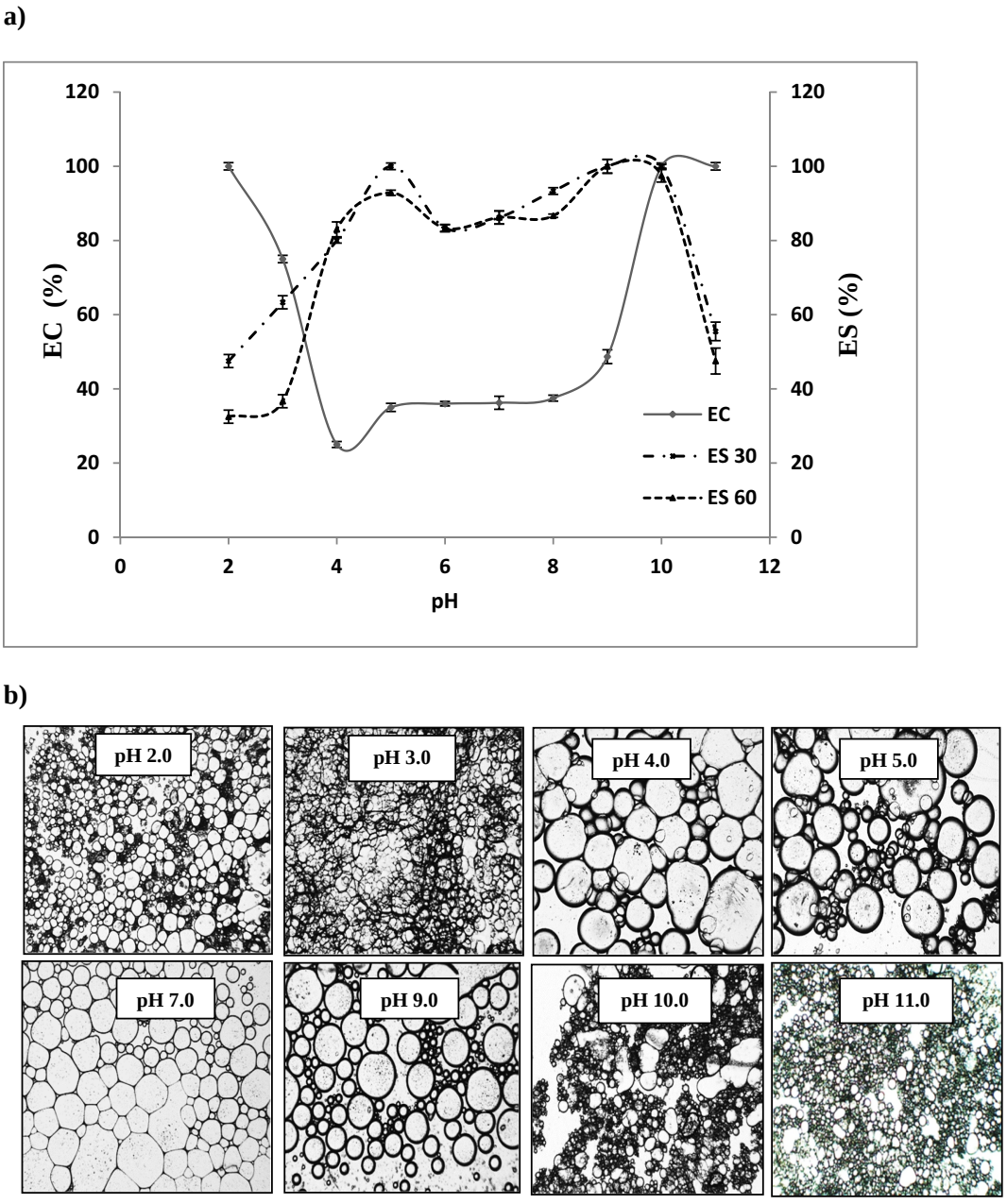


Fig. 1. a) Emulsifying capacity (EC) and stability (ES) at 30 and 60 min of EPI at different pH (2 − 11); b) microscopy optic observation of emulsions stabilized using European eel protein isolate (EPI) prepared at different pHs (2–11) with optical microscope (Motic, ME 2000) (× 400).

2.8. Microcapsule properties

2.8.1. Moisture content and water activity

The water activity (aw) of microcapsules was determined using an AquaLab 4TE water activity meter (Decagon Devices, Inc., Pullman, WA, USA) with a 0.001 sensitivity at 22 °C.

2.8.2. Microencapsulation efficiency

The microencapsulation efficiency (MEE) of the EO-loaded capsules was measured using FT-IR assay as reported by Gómez-Mascaraque and López-Rubio [20].

2.8.3. Fourier-transform infrared (FT-IR)

FT-IR analysis of the EPI powder and free and encapsulated EO was performed in transmission mode using a Bruker (Rheinstetten, Germany) FT-IR Tensor 37 equipment. These measurements were achieved at ambient temperature and were recorded in the range of 4000–450 cm^{-1}.

2.8.4. Microstructure of the particles

Scanning electron microscopy (SEM) was conducted on a Hitachi microscope (Hitachi S-4800) at an accelerating voltage of 10 kV and a working distance of 8–9 mm.

2.9. Accelerated oxidation assay

The evaluation of the oxidative stability against thermal treatment of free and encapsulated European eel oil ($C_{1:4}$-H and $C_{1:4}$-UH) was conducted as described by Torres-Giner, Martinez-Abad, Ocio, and Lagaron [21].

Table 2
Fatty acid composition of European Eel oil (EO).

Fatty acid (FA)		FA content (%)
SFAs	C 14:0	3.52
	C16:0	22.07
	C18:0	3.12
MUFAs	C16:1 (ω7)	11.52
	C18:1 (ω9)	42.45
	C20:1 (ω9)	0.37
PUFAs	C18:2 (ω6)	1.82
	C18:3 (ω6, ALA)	3.77
	C20:2 (ω6)	0.41
	C20:4 (ω6)	1.59
	C20:4 (ω3)	0.74
	C20:5 (ω3, EPA)	2.51
	C22:0	0.36
	C23:0	0.32
	C24:0	1.50
	C22:6 (ω3, DHA)	3.84
Σ PUFA ω3		10.12
Σ PUFA ω6		7.66
PUFA/SFA		0.62

FA: fatty acid. EPA: eicosapentaenoic acid; DHA: docosahexaenoic acidALA: alpha-Linolenic acid. MUFAs: monounsaturated fatty acids. SFAs: saturated fatty acids. PUFAs: poly-unsaturated fatty acids.

2.10. Thermogravimetric analysis

The thermal stability of the free EO, EPI powder and the microparticles was determined by thermogravimetric analysis (TGA) using TGA50H thermobalance (Corporation Shimadzu, Kyoto, Japan). The analysis was conducted under the following operating conditions: alumina pan; dynamic nitrogen atmosphere with flow of 100 mL/min; heating rate: 10 °C min^{-1}; temperature range: 50–550 °C.

2.11. Determination of antioxidant activities

The DPPH free radical-scavenging activity of EPI, EO and capsules was determined as described by Bersuder, Hole, and Smith [22]. The chelating activity of samples towards ferrous ion (Fe^{2+}) was determined according to the method of Decker and Welch [23]. Total antioxidant capacity is based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH [24].

2.12. Statistical analysis

Data were expressed in mean ± SD (standard deviation) and analyzed using SPSS package ver. 17.0 for Windows, professional edition (SPSS Inc., Chicago, USA) using ANOVA analysis. Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Physicochemical characterization of EPI and EO

3.1.1. EPI characterization

European eel protein (EPI) was isolated by isoelectric solubilization/precipitation processing [16]. As described previously, the recovered EPI contained 94 g/100 g crude protein and 3.41 g/100 g total lipid (based on the dry weight matter). It is important to note that the minimum of solubility of EPI was obtained at 5.5 which corresponds to its pHi. Additionally, SDS-PAGE protein pattern of EPI showed a predominance of heavy chain myosin and actin [16].

The emulsifying capacity (EC) and stability (ES) of EPI at different pH values (2.0–11.0) are also investigated and the results are shown in Fig. 1a. Results revealed that the EC of EPI was excellent at both acidic and alkaline pH values. However, at pH values ranging from 4.0 to 9.0, the EC decreased to 20% and 40%, respectively. Thus, at pH values close to the isoelectric point, proteins become poor emulsifiers, because of the minimization of electrostatic repulsive forces [25]. In the other hand, resultats showed that the emulsions obtained with EPI at pH 5.0, 9.0 and 10.0 were stable after 60 min of incubation. This is in line with previous work of Felix et al. [26] who proved the positive correlation between solubility and emulsifying capacity of proteins. Taktak et al. [16] also revealed that EPI was totally soluble at extremely acid and alkaline pHs.

In order to confirm these results, microscopy images of emulsions, prepared using only ultra-turrax, were taken. The emulsions prepared at different pHs showed significant structural changes (Fig. 1b). The smallest droplets were obtained for the emulsions prepared at extreme pH values (pH 2.0, 3.0, 10.0 and 11.0), whereas, the emulsions produced at pHs ranging from 4.0 to pH 9.0, gave rise to large and flocculated droplets. Thus, at pH values close to their isoelectric point, proteins can become poor emulsifiers, because of the minimization of electrostatic repulsive forces [25].

Therefore, for the second part of the study focused on the encapsulation and protection of European eel oil using EPI solution as wall material, the emulsions were prepared at pH 10.0, as at this pH value, the solubility of the protein was optimal and the properties of the emulsions obtained (in terms of EC, ES and droplet size) were also adequate.

3.1.2. Fatty acid composition of EO

The fatty acid composition of European eel oil, presented in Table 2, was 54.34% monounsaturated fatty acids (MUFAs), 28.71% saturated fatty acids (SFAs) and 16.86% poly-unsaturated fatty acids (PUFAs). In fact, oleic acid (C18:1), which represented 42.45% of total fatty acids, was the primary MUFA, followed by palmitoleic acid (C16:1) (11.52%). In addition, palmitic acid (C16:0) is the most predominant SFA, accounting for 22.07% of the total fatty acids. These findings were in line with previous work of Derouiche Aoun, and Kraiem [27] who found that the contents of C16:1 and C18:1 of European eel oil muscle, collected from three lagoons in the north of Tunisia, ranged between 21 and 29% and from 32.63 to 45.02%, respectively. Furthermore, from the 16.86% of ω3-PUFA contained in EO, 3.84% corresponded to docosahexaenoic acid (DHA, C22:6), 3.77% was α-linoleic acid (ALA, C18:3) and eicosapentaenoic acid (EPA, C20:5) was present at a concentration of 2.51%. The results reported in Table 2 show that the PUFA/SFA ratio was 0.58, higher than the minimum recommended threshold ratio (0.45) by HMSO [28]. Thus, results revealed that *A. anguilla* is an important source of ω3-PUFA with reported health beneficial activities such as anti-hypertensive, anti-inflammatory and hypocholesterolemic effects [5]. In addition, the EPA and DHA are extremely important for the development and functioning of the brain and retina [6,29].

3.2. Characterization of emulsions

The emulsification process and the biopolymer concentration, used as an emulsion stabilizer, can influence the characteristics of emulsions. In this context, the present study assessed the effects of emulsification process and the European eel oil and protein isolate ratio on the stability and the physicochemical properties of the emulsions. With this aim, different emulsions were prepared by applying only homogenization (H) or homogenization followed by ultrasonication (HU) at different ratios of EO to EPI (1:1, 1:2 and 1:4). The stability of the emulsion formulations was evaluated by determining the creaming index (CI) and Zeta potential.

Regarding the CI, which reflects the stability of oil emulsions to gravitational separation, results showed that all emulsions were gravitationally stable after 24 h of incubation (Table 3). The total stability of emulsions mainly depends on fish protein emulsifying properties, lipid

Table 3
Zeta potential, and creaming index (CI) values of emulsions prepared at different EO:EPI ratios and by homogenization (H) and ultrasonication (HU) processes.

	Emulsification process	Zeta potential (mV)	CI (%)
E1:1	H	−26.70 ± 0.20[a]	100[a]
E1:2		−25.43 ± 0.25[a]	100[a]
E1:4		−26.53 ± 0.57[a]	100[a]
E1:1	HU	−26.15 ± 0.47[a]	100[a]
E1:2		−26.57 ± 0.70[a]	100[a]
E1:4		−27.20 ± 0.61[a]	100[a]

Values within a column with different superscript letters indicate significant differences ($p < 0.05$). EO: European eel oil. EPI: European eel protein isolate. $E_{1:1}$, $E_{1:2}$ and $E_{1:4}$ are emulsions prepared at EO:EPI ratios of 1:1, 1:2 and 1:4, respectively.

Table 4
Moisture content, water activity (WA) and microencapsulation efficiency (MEE) of microcapsules prepared using different EO:EPI ratios and using homogenization (H) and/or ultrasonication (HU) processes.

	Emulsification processes	Moisture content (%)	WA	MEE (%)
C1:1	H	3.44 ± 0.21[a]	0.563 ± 0.001[a]	58.64 ± 1.50
C1:2		2.72 ± 0.08[b]	0.482 ± 0.002[c]	64.17 ± 0.56
C1:4		3.90 ± 0.91[a]	0.525 ± 0.009[b]	68.50 ± 1.78
C1:1	HU	1.53 ± 0.07[c]	0.524 ± 0.005[b]	60.78 ± 1.77
C1:2		2.65 ± 0.16[b]	0.539 ± 0.009[b]	61.26 ± 3.87
C1:4		2.85 ± 0.48[b]	0.558 ± 0.009[a]	64.44 ± 1.84

Values within a column with different superscript letters indicate significant differences ($p < 0.05$). EO: European eel oil. EPI: European eel protein isolate. $C_{1:1}$, $C_{1:2}$ and $C_{1:4}$ are microcapsules prepared at EO:EPI ratios of 1:1, 1:2 and 1:4, respectively.

composition (PLs vs. TAGs), and the selected emulsification process. Shi et al. [7] reported that the krill oil emulsion was stabilized with a krill protein isolate and showed no phase separation over 1-week storage at 4 °C.

Furthermore, the zeta potential, defined as the charge developed at the interface between a solid surface and its liquid medium, was determined for each sample (Table 3). The emulsification processes and the different EO:EPI ratios produced emulsions with Z-potential values ranging from −25.43 to −27.20 mV (Table 3). These results explain the gravitational stability of all emulsions, as it is widely reported that emulsions with high absolute value of zeta potential (negative or positive) are electrically stabilized against aggregation and flocculation, possibly leading to higher physical stability [30].

Furthermore, droplets morphology and average droplet diameters were investigated (data not shown). Optical micrograph images showed that, for the same emulsification method, the droplet size of emulsions decreased with increasing of EPI concentration. In addition, the combination of the ultrasonication process with the homogenization at high speed produced particles with smaller droplet size compared to those obtained by only (H), especially for emulsion prepared with oil to protein ration of 1:1. These findings are in line with the studies of McClements [31] and De Barros Fernandes et al. [32] who found that the ultrasonication procedure reduced droplets size. This can have an impact on emulsion properties, enhancing the stability of the emulsions and improving the bioavailability of the active ingredients.

Consequently, the decrease of emulsion size depends on the concentration and the properties of the wall material as well as the energy provided during the process. De Barros Fernandes et al. [32] showed that processing of blends, homogenization followed by ultrasonication treatment, provided emulsions with smaller sizes, indicating that the level and the type of the energy provided during blend steps influenced the disruption of the droplets during preparation. Silva, Zabot, and Meireles [33] reported that the emulsion droplet size is related to the functional properties and concentration of wall material which, in turn, influence the viscosity of the emulsion.

3.3. Characterization of the microcapsules

3.3.1. Moisture content, water activity (WA) and microencapsulation efficiency (MEE)

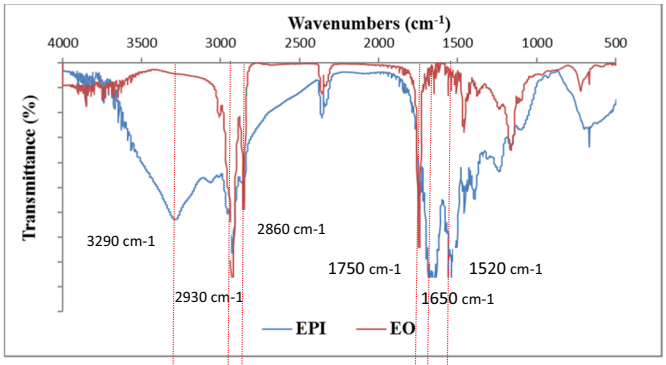
The moisture content is one of the crucial parameters influencing the storage stability of spray-dried microcapsules. Results, illustrated in Table 4, show that the moisture content of the microcapsules varied from 1.53% to 3.9%. These findings were similar to that obtained by De Barros Fernandes et al. [32] who found that the moisture contents of ginger oil encapsulated using gum Arabic almond was 2.48%.

In another aspect of the study, the water activity is an indicator of the amount of free water available in the polymer structure and provides supplement data for the shelf life of microparticles [34]. The water activity of the microcapsules prepared using EPI did not significantly change among all samples and was around 0.482–0.563, which is in accordance with the previous study of Beristain, Azuara, and Vernon-Carter [35]. A powder, with a WA index lower than 0.6, is microbiologically stable [34]. Therefore, EPI-based microcapsules could be considered an appropriate stable matrix against microorganisms' proliferation.

Furthermore, focusing on the microencapsulation efficiency (MEE) of EO after spray-drying (Table 4), there was a significant difference among the oil retention capacity among all samples. The microcapsules prepared with EO:EPI ratio of 1:4 contained the highest level of retained oil which was 68.50% for $C_{1:4}$-H and 64.44% for $C_{1:4}$-UH. It seems that the entrapment efficiency of microcapsules increased with increasing wall material concentration, i.e. a greater protein content was required for efficient oil encapsulation [30,36]. Similarly, Shi et al. [7] found that the loading efficiency decreased with the increase of krill oil content. In other side, the results, shown in Table 4, revealed that a significant decrease in MEE values was observed with emulsions produced using UH processes. In fact, the MEE values of $C_{1:4}$-H and $C_{1:2}$-H were 64.17% and 68.50% respectively, while these values decreased to 61.26% and 64.44% for $C_{1:4}$-UH and $C_{1:2}$-UH, respectively. These results could be explained by the increase of temperature during the ultrasonication treatment, which may have degraded the thermosensitive fatty acids. Gómez-Mascaraque & López-Rubio [20] reported that despite the short duration (i.e. 30 s) of the treatment intervals and the use of an ice bath to cool down the samples, the temperature of the emulsions after the ultrasonication treatment raised up to 45 °C. In summary, microcapsules stabilized with EPI at a ratio of 1:4 (w/w) and prepared by homogenization process, had the smallest oil droplet size and the highest encapsulated oil content.

The spray-dried EPI, EO and microcapsules were also characterized using FT-IR spectroscopy. The infrared spectra of EPI powder and free EO, shown in Fig. 2a, presented the characteristic bands of fish oil at 1740, 1711 and 1640 cm^{-1}, attributed to C=C, C=O and C=C-C=C stretching, respectively [37]. In addition, the specific absorption band of omega-3 PUFA at 3012 cm^{-1}, which corresponds to the C–H stretching of cis-alkene (-HC=CH-) groups, was also detected [38]. Moreover, the spectra of EPI revealed characteristic bands at 3273 cm^{-1} (Amide A, N–H or O–H), 2935–2962 cm^{-1} (C–H stretching bands), 1643 cm^{-1} (Amide I, C=O and C=N), 1533 cm^{-1} (Amide II, C–N and N–H), 1398 cm^{-1} (C–H bending), and 1236 cm^{-1} (Amide III) [14]. Regarding the spectra of the different microcapsules, the characteristic bands of both oil and protein isolate were observed (Fig. 2b). In addition, a reduction in the intensity of Amide A and C–H stretching bands at 3273 cm^{-1} and 2935–2962 cm^{-1}, respectively, was observed, which may be correlated to the formation of hydrogen bonding interactions among the hydroxyl groups of amide A of EPI.

a)



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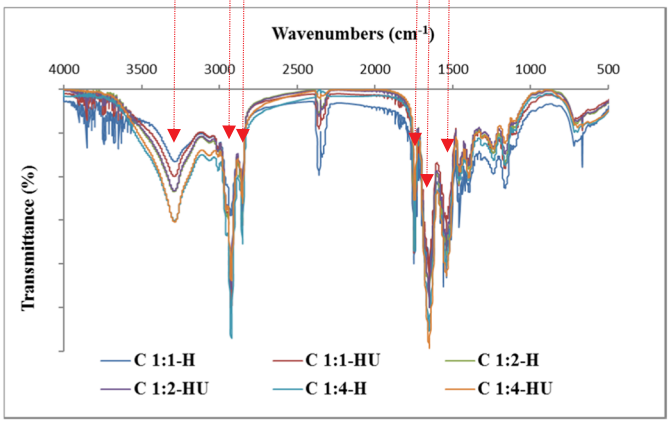

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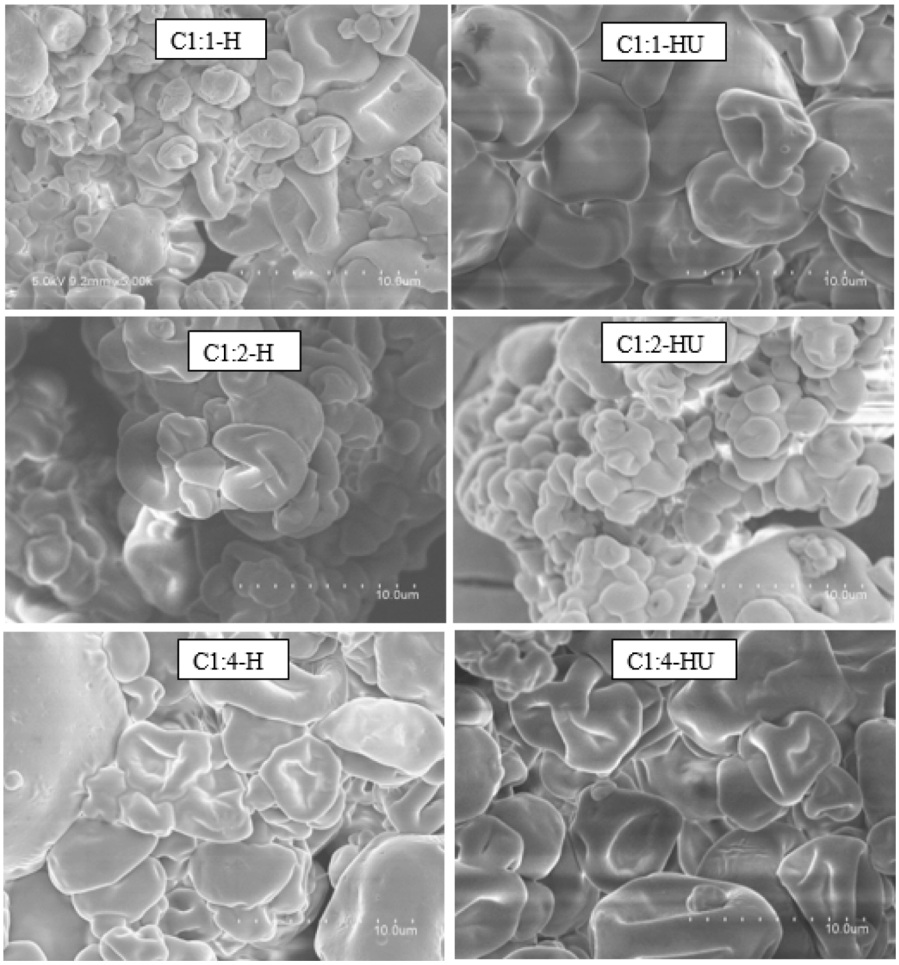

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Fig. 2. FTIR spectra of EPI powder and free EO (a), and EO-loaded microcapsules prepared at different EPI:EO ratios and using homogenization (H) and homogenization followed by ultrasonication (HU) steps (b). Microstructure of microcapsules prepared at different EO:EPI ratios and using homogenization (H) and homogenization followed by ultrasonication (HU) process (c). $C_{1:1}$, $C_{1:2}$ and $C_{1:4}$ are microcapsules prepared at EO:EPI ratios of 1:1, 1:2 and 1:4, respectively.

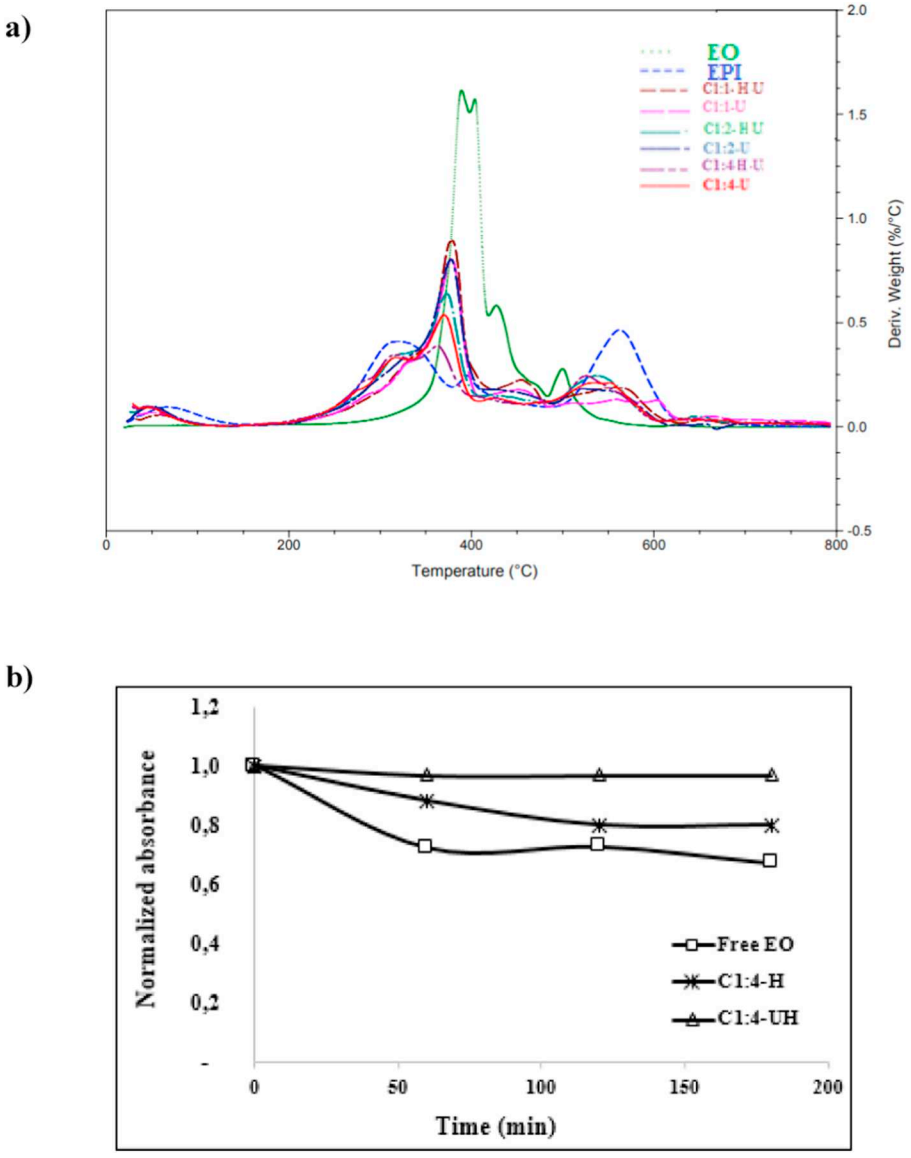

Fig. 3. a) DTG; b) oxidative stability of free EO, C1:4-H and C1:4-HU during storage at 80 °C during 180 min.

3.3.2. *Microstructure of the microcapsules*

Fig. 2c shows the scanning electron microscopy (SEM) images of the various microcapsules obtained through spray-drying, in which individual pseudo-spherical particles with a corrugated surface were observed, which are typically described in spray-dried particles [39,40]. In addition, the surface morphology of the particles depended on the initial fish oil concentration. Empty particles had a smoother surface, whereas the presence of high amounts of fish oil resulted in the formation of more wrinkled surfaces, which is in agreement with the previous study of Yang & Ciftci [41].

3.4. *Thermogravimetric analysis*

Thermogravimetric (TG) analysis was used to assess the thermal stability of the microcapsules developed. As seen in Fig. 3a, DTG curves showed the thermal decomposition of EO, EPI and microcapsules. In the case of EPI powder, several degradation steps were clearly distinguished. The first one, around 100 °C, is normally attributed to the moisture loss from the samples (Fig. 3a). The main degradation stage for the EPI protein took place in the range of 360–420 °C and in the range of 550–620 °C.

The DTG curves of the EO-loaded encapsulation structures showed degradation profiles corresponding to both components, although the positions of the main peaks varied. A decrease in the maximum temperature of degradation from the protein was observed in the encapsulations systems, a fact which has been previously observed and attributed to the structural changes caused by the encapsulation process and the reduction of the particle size, which results in an increase of the specific area and, consequently, of their susceptibility to thermal degradation [42]. Regarding the peaks corresponding to the oil degradation, after the loss of water, the second step of thermal degradation in general was slightly delayed with respect to the unprotected oil, thus confirming the ability of the protein matrix to slightly protect the bioactive from temperature. In contrast, the last degradation step of the

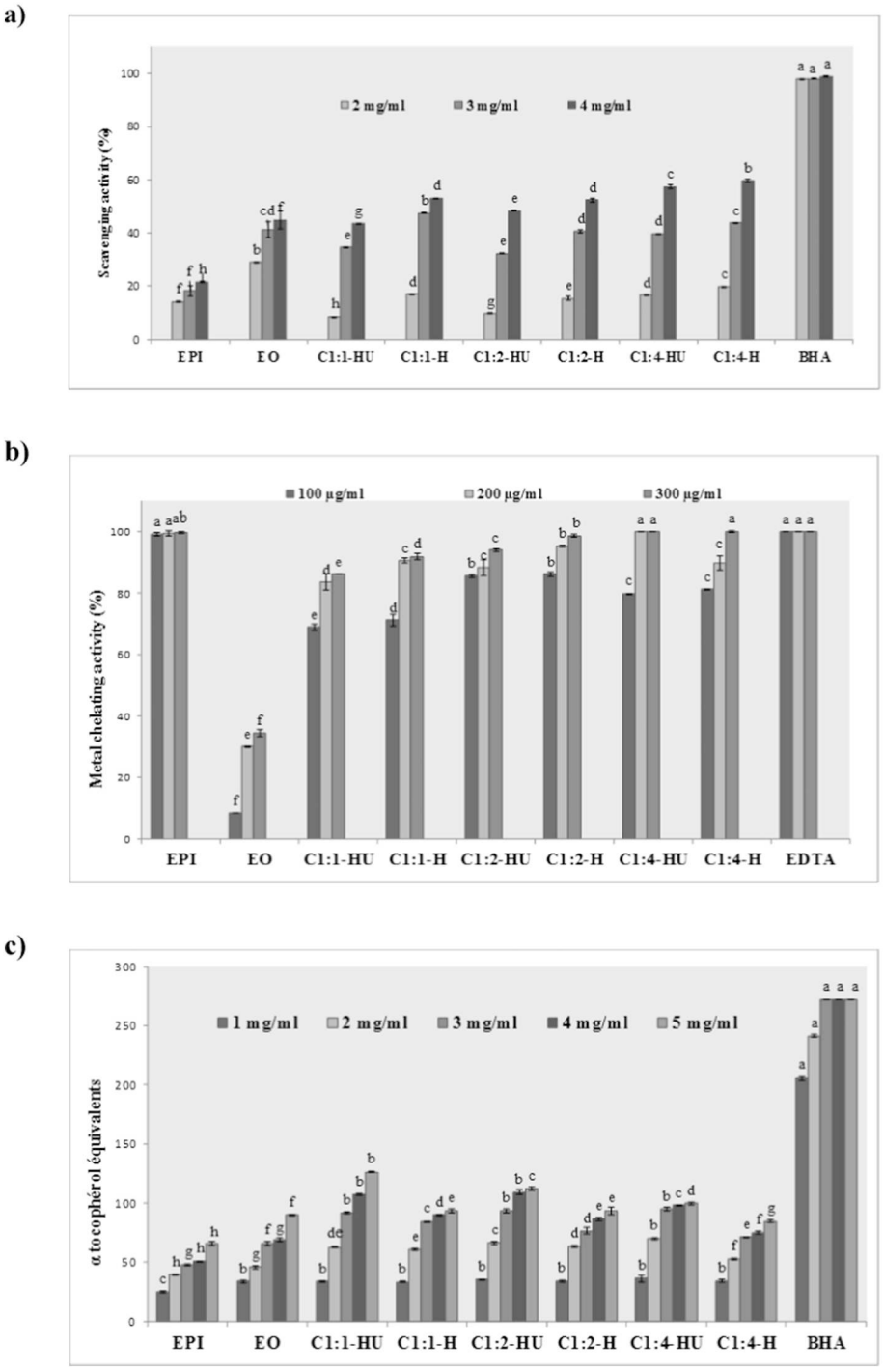

Fig. 4. Antioxidant activity: DPPH radical scavenging (a), iron chelating (b), total antioxidant activities (c) of EPI powder, free EO and microcapsules C1:1, C1:2 and C1:4 obtained with H and HU. Different subscripts indicate significant differences for the different samples at the same concentration ($p < 0.05$).

entrapped oil slightly decreased to 387.5 °C compared to the free oil (397.63–417.50 °C). This finding may indicate a certain EO oxidation during spray-drying process. It is interesting to note that the relative intensity of the degradation bands from the protein and the fish oil do not correspond to the actual ratios used to prepare the structures. Instead, they are related to the actual oil effectively incorporated and it can be clearly observed that, increasing the protein content in the formulations, led to greater oil content in the capsules as demonstrated before through FTIR.

3.5. Accelerated oil oxidation

Oxidative stability of free and encapsulated EO (at a ratio of 1:4) was studied using an accelerated degradation test at 80 °C during 60, 120 and 180 min. The assessment of EO oxidation was monitored by the measurement of cis double (-HC = CH-) groups intensity at 3012 cm^{-1}. Fig. 3b shows that the normalized absorbance obtained for free EO significantly decreased by 33% during heat treatment, suggesting the oxidation of PUFA ($p < 0.05$). Interestingly, the encapsulation of EO

by EPI, at a ratio of 1:4, significantly protected the oil against degradation. In fact, the normalized absorbance obtained for C1:4-HU capsules was constant during exposure at 80 °C, while, the intensity band at 3012 cm^{-1} from the capsules obtained from the emulsion obtained using only homogenization (C1:4-H) was reduced by 20%. Therefore, the results seem to indicate that the emulsions prepared by the combination of ultrasonication and homogenization (HU) steps, subsequently generated spray-dried microcapsules which were more efficient for oil protection, probably due to better integration of smaller oil droplets in the microcapsule structure, thus having less surface oil content.

3.6. Antioxidant activities of encapsulated and free EO

Lipid oxidation of fish oil and other PUFA-rich foods is a serious problem that leads to loss of shelf-life, consumer acceptability, functionality, nutritional value, and safety [43]. In fact, the microencapsulation process using protein isolate as wall matrix, is mainly aimed at protecting the fish oil against oxidation. Antioxidants are able to retard the oxidation process by interacting with free radicals. The results of the DPPH free radical-scavenging activity, presented in Fig. 4a, indicated that microcapsules exhibited an interesting scavenging activity, at 4 mg/mL, compared to those obtained by free EO and EPI powder. A positive correlation between the capsule concentrations and their radical-scavenging potential was observed. On the other hand, the evaluation of the chelating potential showed that microcapsules had an interesting metal chelating effects (100% at 0.3 mg/mL C1:4-H and C1:4-HU) than EPI, but higher than the EO (34%, at the same concentration) (Fig. 4b). Furthermore, as seen in Fig. 4c, the total antioxidant activity of the samples, expressed as α-tocopherol equivalents, showed that encapsulated EO, at the different ratios, showed a total antioxidant activity which corresponded to the contributions from both the protein and the oil, thus having an additive effect. Therefore, spray-drying did not seem to affect the antioxidant potential of the constituent materials and could potentially broaden EO industrial applications.

4. Conclusion

European eel oil was extracted and encapsulated at different EO to EPI ratios and using two emulsification (homogenization or homogenization followed by ultrasonication) steps. The present study showed that the decrease of EO content in the emulsions (EO:EPI ratio = 1:4) resulted in the reduction of the mean diameters of droplets and the amelioration of the MEE of the core material. Furthermore, after the spray-drying process, spherical shape capsules were obtained. The spectrum of FTIR and DTG of all components applied in the microencapsulation process indicated the presence characteristic bands of both EO and EPI and approved the results of MEE. Although, results revealed that the particles prepared using the combination of two processes presented the high oxidative stability after incubation at 80 °C during 3 h, compared to that obtained with only homogenization at the same ratio. In summary, the selected EPI, as wall matrix, was effective to enhance the oxidative stability, thermostability, antioxidant activity of EO. Additionally, the impact of the ultrasonication step and the amount of wall matrix (EPI) on the characteristic, the stability and the antioxidant activity of the encapsulated EO was proved.

Acknowledgements

This study was funded by the Ministry of Higher Education and Scientific Research, Tunisia. Authors would also like to thank the Central Support Service for Experimental Research (SCSIE) of the University of Valencia for the electronic microscopy service.

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