**Stabilization of a saffron extract through its encapsulation within
electrospun/electrosprayed zein structures**

Ali Alehosseini^{1,2}, Laura G. Gómez-Mascaraque³, Behrouz Ghorani¹, Amparo López-
Rubio^{2*}

¹Department of Food Nanotechnology, Research Institute of Food Science & Technology
(RIFST), Km 12 Mashhad-Quchan Highway, P.O. Box: 91895/157/356 Mashhad, Iran.

²Food Safety and Preservation Department, IATA-CSIC, Avda. Agustin Escardino 7,
46980 Paterna (Valencia), Spain.

³Food Chemistry and Technology Department, Teagasc Food Research Centre,
Moorepark, Fermoy, Co. Cork, Ireland.

***Corresponding author:** E-mail address: amparo.lopez@iata.csic.es (A. López-Rubio)

Tel.: +34 963900022; fax: +34 963636301

ABSTRACT

In this work, electrospinning and electrospraying techniques were used to encapsulate the sensitive bioactive compounds of a saffron extract (i.e. picrocrocin, safranal, and crocin) using zein as protective matrix. Initially, a dried saffron extract (DSE) was obtained and characterized and was subsequently incorporated within zein solutions prepared at two concentrations (10% w/v for capsules and 20% for fiber development). The greatest encapsulation efficiency was observed for the fiber samples, with retention values for picrocrocin, safranal, and crocin of up to 97, 88, and 97%, respectively. The stability of the free and encapsulated extract when exposed to UV light, different pH (2, 7.4) and temperatures (25, 75 °C) was also studied. The photostability of crocin and safranal was significantly ($p<0.05$) increased upon encapsulation. Similarly, while about 98% of the non-encapsulated crocin degraded after 15 hours of exposure to acetic acid at 75 °C, only 67.23% of the encapsulated compound was lost under the same conditions. The release profiles of crocin, safranal and picrocrocin from the encapsulation structures were fitted using the Peppas-Sahlin model ($R^2=0.98$), showing that the dominant release mechanism was diffusion. Finally, the encapsulation structures retained their integrity upon contact with water, highlighting their potential use as food packaging coatings.

Keywords: Microencapsulation; electrohydrodynamic process; photostability; coating; crocin.

1. INTRODUCTION

Saffron, the dried stigmas of *Crocus sativus* L. flowers, is considered the world's most expensive spice (Maggi et al., 2010), often referred to as “red gold” or “golden spice” due to its great value (Bolandi & Ghoddusi, 2006; Melnyk, Wang, & Marcone, 2010; Shahi,

Assadpour, & Jafari, 2016). Saffron is highly appreciated for its unique color, bitter taste and aroma, which are the main characteristics determining its quality (Bolandi & Ghoddusi, 2006). Due to its distinctive organoleptic properties, it has been traditionally used as a natural flavoring and coloring ingredient (Melnyk et al., 2010) and it is intensively used in the food industry for the manufacture of a wide range of products, including dairy, bakery, sauces, soups, chicken, rice, and beverages (Selim, Tsimidou, & Biliaderis, 2000; Shahi et al., 2016). Although currently the main use of saffron is culinary, recent research has demonstrated the potential of its consumption to promote health (Melnyk et al., 2010). In this regard, antioxidant, antitumorigenic, antidepressant, cardioprotective and neuroprotective properties are some of the numerous therapeutic benefits which have been attributed to saffron (Alavizadeh & Hosseinzadeh, 2014; Rahaiee, Moini, Hashemi, & Shojaosadati, 2015; Shahi et al., 2016). In fact, despite its high price, the use of saffron has gradually increased in recent years due to the growing consumers' demand for food products with functional properties (Selim et al., 2000). The main compounds of interest found in saffron are crocin, picrocrocin and safranal, which are responsible for its color, bitter taste and aroma, respectively (Bolandi & Ghoddusi, 2006). In particular, crocin (or crocetin digentiobiose ester), which is a water-soluble carotenoid (Alavizadeh & Hosseinzadeh, 2014), is considered one of its major bioactive components, exhibiting a wide spectrum of biological activities (Melnyk et al., 2010). It represents about 6-16% of saffron's dry weight (Gregory, Menary, & Davies, 2005) and contents of up to 30% of crocin have been reported for good quality saffron (Rahaiee, Hashemi, Shojaosadati, Moini, & Razavi, 2017). However, like other carotenoids, crocin has a highly unsaturated chemical structure and is thus susceptible to degradation during processing and storage (Selim et al., 2000). Crocin is especially sensitive to light, heat, and oxidative stress (Bolandi & Ghoddusi, 2006; Rahaiee et al.,

2017) and its stability is also affected by the water activity and the pH (Ordoudi et al., 2015; Selim et al., 2000). The rapid degradation of saffron pigments in aqueous solution has been reported even at mild temperatures (40 °C) (Orfanou & Tsimidou, 1995). Given that some food products have high moisture contents, varying pHs and are frequently processed at high temperatures, the bioactivity of crocin may be negatively affected during the manufacture of saffron-containing food products.

Microencapsulation, which consists of coating or embedding an ingredient of interest within a micron-sized protective matrix (Gomez-Mascaraque, Ambrosio-Martín, Fabra, Rocío, & López-Rubio, 2016), is a promising approach for the stabilization of sensitive bioactive compounds during food processing, storage and consumption (Gomez-Mascaraque et al., 2016). Particularly, encapsulation of saffron extracts within polymeric matrices has shown to improve their stability during storage (Chranioti, Nikoloudaki, & Tzia, 2015; Selim et al., 2000) and in simulated gastric conditions (Esfanjani, Jafari, & Assadpour, 2017). At present, there are a number of technologies which can be used for the microencapsulation of bioactive ingredients, being spray-drying and freeze-drying the most widely used techniques in the food industry (Kwak, 2014). In fact, most of the recent works addressing the microencapsulation of saffron extract to date have been focused on these drying technologies (Chranioti et al., 2015; Esfanjani, Jafari, Assadpour, & Mohammadi, 2015; Rajabi, Ghorbani, Jafari, Mahoonak, & Rajabzadeh, 2015; Selim et al., 2000). However, spray-drying involves the use of high temperatures for processing, which may be detrimental for thermo-sensitive bioactive molecules like carotenoids (Pérez-Masiá, Lagaron, & Lopez-Rubio, 2015), and freeze-drying is a very time-consuming and expensive technique which yields highly porous materials, generally resulting in poor barrier properties (Cook, Tzortzis, Charalampopoulos, & Khutoryanskiy, 2012; Zuidam & Shimoni, 2010).

Electrohydrodynamic (EHD) processing is an alternative to conventional microencapsulation techniques which has been recently applied for the design of functional foods (Gómez-Mascaraque, Hernández-Rojas, et al., 2017). This technology allows the production of dry nano- or microstructures in a one-step process and at mild temperature conditions by subjecting a polymeric fluid, which flows through a conductive capillary, to a high voltage electric field (Bhushani & Anandharamakrishnan, 2014). As a result, a charged polymer jet is ejected towards the opposite electrode, where the collector is placed. During the flight, the jet is elongated and the solvent evaporates, producing dry ultrathin fibers (electrospinning process), or fine particles if jet fragmentation takes place (electrospraying process) (Alehosseini, Ghorani, Sarabi-Jamab, & Tucker, 2018; Kriegel, Arrechi, Kit, McClements, & Weiss, 2008). This offers the unique opportunity to tailor the morphology of the obtained microencapsulation structures in order to obtain powdery ingredients (Alehosseini, Sarabi-Jamab, Ghorani, & Kadkhodae, 2019; Gómez-Mascaraque, Sipoli, de La Torre, & López-Rubio, 2017) or edible functional coatings (Blanco-Padilla, López-Rubio, Loarca-Piña, Gómez-Mascaraque, & Mendoza, 2015; Gómez-Mascaraque, Tordera, Fabra, Martínez-Sanz, & Lopez-Rubio, 2018).

In this work, EHD processing was used to produce microencapsulated saffron extract with the aim of developing new ingredients that would not only provide food products with the unique culinary benefits of saffron, but would also potentially preserve its health-promoting properties by improving the stability of its bioactive compounds. For this purpose, a dried saffron extract (DSE) was prepared and incorporated within zein matrices by EHD processing to obtain DSE-enriched powders (electrosprayed microparticles) and edible food coatings (electrospun fiber mats). The obtained materials were characterized in terms of physicochemical properties and encapsulation efficiency. Moreover, their

ability to stabilize the main saffron compounds under different stress conditions was assessed. The release of crocin, picrocrocin and safranal from the zein microstructures was also assessed.

2. MATERIALS AND METHODS

2.1. Materials

Premium saffron stigma (all red saffron-Sargool) was purchased from Esfedan Saffron Trading Co. (Iran). Prior to their use, stigmas were kept in an air-tight plastic bag in the fridge to protect them from light and moisture sorption. Zein from maize (grade Z3625), with reported molecular weight of 22-24 kDa, was purchased from Sigma-Aldrich (Spain). Soybean oil (SBO) and phosphate buffered saline system (PBS, pH = 7.4) were obtained from Sigma-Aldrich (Spain). Acetic acid 96% (v/v) was supplied by Scharlab (Spain). Absolute ethanol (>99.9%) was purchased from VWR (UK).

2.2. Preparation of saffron extract

Saffron extract was prepared by using a solvent extraction method based on the protocol described in a previous work, with some modifications (Ghorani, Kadkhodai, & Alehosseini, 2017). Briefly, stigmas were ground, sieved (50 μ m mesh) and dispersed in aqueous ethanol 60% (v/v) at 25 °C for 16 h, at a solvent to saffron powder weight ratio of 10:1. Prior to pre-concentration using a rotary evaporator (IKA, RV 3 FLEX, Germany), the ethanolic extract was filtered (Whatman filter paper, Grade 41). A freeze-dryer (OPERON CO., FDO-8606, Korea) was used to produce dried saffron extract (DSE) powder at -80 °C, and pressure of 0.370 mbar for 36 h. The freeze-dried extract was collected, packed in air-tight plastic bottles, and stored at +5 °C until further analysis.

The drying efficiency (%) and moisture content (wet basis) of the freeze-dried saffron extract were also determined according to ISO-3632 (2010).

2.3. Characterization of the saffron extract

The content of the most relevant saffron compounds, i.e. picrocrocin, safranal, and crocin, in the DSE was estimated by calculating the characteristic indices of saffron ($E_{1\text{cm}}^{1\%}$) at 257 nm (flavor strength), 330 nm (aroma strength), and 440 nm (coloring strength), respectively, according to ISO-3632 (2010) (Eq. 1). The absorbance of DSE aqueous solutions (0.01 mg/mL) at the three wavelengths was measured using a NanoDrop spectrophotometer (ND1000-Thermo Fisher Scientific, USA).

$$E_{1\text{cm}}^{1\%} = [D \times 10000] / [m \times (100-H)] \quad (\text{Eq. 1})$$

where D is the specific absorbance; m is the mass of the saffron sample (g), and H is the moisture content of the samples that for the saffron extract was 6.45% and almost negligible for the EHD processed samples.

2.4. Preparation of feed solutions

Zein solutions (10% and 20% w/w) were prepared by dissolving the protein in aqueous ethanol 80% (v/v) under magnetic agitation (IKA, C-MAG HS 4, Germany), as previously described (Alehosseini, Gómez-Mascaraque, Martínez-Sanz, & López-Rubio, 2019). These concentrations were selected in preliminary trials to obtain two different systems, i.e. microparticles or nanofibers, respectively. 2 or 4% of DSE with respect to the mass of zein was then added to the solutions at room temperature under magnetic stirring. Table 1 summarizes the assayed compositions.

2.5. Electrohydrodynamic processing of DSE-containing zein solutions

The polymer solutions were loaded in 5 mL plastic syringes connected to an 18-gauge stainless steel nozzle and processed using an electrohydrodynamic apparatus assembled in a horizontal configuration, equipped with a variable high-voltage 0-30 kV power supply (Acopian, USA), and a syringe pump (KD Scientific, USA). The flow rate and applied voltage were set to 0.15-0.20 mL/h and 15 kV, respectively, based on a preliminary optimization. The tip-to-collector distance was 10 cm. Electrohydrodynamic processing was carried out at ambient conditions.

2.6. Characterization of the electrospun/electrosprayed materials

2.6.1. Morphological characterization

The morphology of the samples was analyzed using scanning electron microscopy (SEM) (Hitachi S-4800, Japan). Samples were placed on stainless steel sample holders and sputter coated (SC7620, Quorum Technologies, UK) with a gold-platinum mixture under vacuum. SEM was performed at 20 kV acceleration voltage and a working distance of ~9.5 mm (Gómez-Mascaraque, Casagrande Sipoli, de La Torre, & López-Rubio, 2017).

2.6.2. Fourier transform infrared (FT-IR) analysis

The infrared spectra of the electrospun fibers, electrosprayed particles, and DSE were obtained using a Bruker (Rheinstetten, Germany) FT-IR Tensor 37 equipment in ATR mode in a range of 650 to 4000 cm^{-1} . Each recorded spectrum was determined from an average of 15-16 scans at 4 cm^{-1} resolution (Gómez-Mascaraque, Casagrande Sipoli, et al., 2017).

2.6.3. Thermogravimetric analysis (TGA)

The thermal stability of the samples was analyzed by TGA using a TA Instruments model Q500 TGA. Samples (ca. 10 mg) were heated from 25 °C to 850 °C at a heating rate of 10 °C/min under dynamic nitrogen flow. Differential thermogravimetric curves express the weight loss rate (dm/dT) as a function of temperature (Gómez-Mascaraque & López-Rubio, 2016).

2.7. Encapsulation efficiency (EE)

To estimate the encapsulation efficiency of the main bioactive compounds from saffron, i.e. picrocrocin, safranal and crocin, in the zein structures, 20 mg of each sample were dissolved in ethanol 80% (v/v), and the absorbance of the resulting solutions was measured at 257, 330, and 440 nm as described in Section 2.3. The amount of DSE on the surface of the produced zein structures was initially quantified by rinsing the encapsulation structures with distilled water and measuring the absorbance of this aqueous solution and it was found to be negligible. Calibration curves for DSE were previously obtained at each wavelength, taking into account the contribution of zein to the total absorbance of the samples. The EE for each compound was then calculated according to Eq. (2) (Alehosseini, Gómez-Mascaraque, et al., 2019):

$$EE (\%) = \frac{T_E}{T_T} \times 100 \quad (\text{Eq. 2})$$

where T_E is the experimental content of crocin, safranal or picrocrocin incorporated within the zein structures, as estimated by UV-Vis spectroscopy, and T_T is their corresponding theoretical content.

2.8. Photostability test by UV-irradiation

The photostability of saffron compounds in the encapsulation structures was compared to that of free DSE using a modified version of a previously published protocol (López-Rubio & Lagaron, 2011). All samples were placed in polystyrene petri dishes (60 mm × 15 mm, Fisher, USA), lacking lid, and exposed to UV light ($\lambda=350\text{--}400$ nm, 15 W, Actinic BL, Philips, Germany). The distance between the UV lamp and the samples was set at 10 cm. At selected time intervals, samples (~10 mg) were collected, and completely dissolved in 80% ethanol (1 mL) using a vortex mixer (ISG, Westlab, Canada). The absorbance of the resulted solutions was then measured at 257, 330, and 440 nm as described in section 2.3.

2.9. Saffron degradation assays

In order to investigate the protective effect of the protein structures on the bioactive compounds from the DSE in different environments and food processing conditions, degradation assays were performed at two different pHs and temperatures, i.e. in PBS (pH = 7.4) and in acetic acid 20% (v/v) (pH = 2), and both at 25 °C and 75 °C. For this purpose, a method adapted from a previously published protocol was used (Alehosseini, Gómez-Mascaraque, et al., 2019; Gómez-Mascaraque, Casagrande Sipoli, et al., 2017). Briefly, DSE-loaded protein fibers/particles and free DSE, as a control, were dispersed in PBS or acetic acid 20% to achieve a theoretical DSE concentration of 0.01 mg/mL. The dispersions were then stored either at room temperature (25 °C) or in a hot bath (75 °C). After selected time intervals, samples were diluted 3-fold with ethanol 80% (v/v) to release the SDE. The absorbance of the final solutions was then measured at 440 nm as described in section 2.3, prior calibration in both media.

2.10. *In-vitro* release assays

Ethanol 50% (v/v) and a soybean oil were selected as food simulants, according to the Commission-regulation10/2011EU (2011). Briefly, the DSE-loaded materials were immersed in the release media at a concentration of ca. 15 mg/mL. Periodically, the concentration of saffron components released to the selected media was determined by measuring the absorbance of the supernatant at 440 nm as described in section 2.3. Release data were fitted to empirical models including Kopcha (Eq. 3), Ritger–Peppas (Eq. 4), and Peppas–Sahlin (Eq. 5) models (Bruschi, 2015).

$$M_t = (A \times t^{0.5}) + (B \times t) \quad (\text{Eq. 3})$$

where M_t is the amount of released saffron ingredients at time t , A and B are the diffusion and erosion rate constants, respectively.

$$\frac{M_t}{M_\infty} = K \times t^n \quad (\text{Eq. 4})$$

where M_∞ is the initial content of saffron ingredients-loaded zein structures, K and n are the release kinetic constant and release exponent, respectively.

$$\frac{M_t}{M_\infty} = K_1 t^m + K_2 t^{2m} \quad (\text{Eq. 5})$$

where K_1 and K_2 are diffusion and erosion rate constants, respectively and m is the diffusion exponent.

2.11. Microstructural stability assessment of the materials

The microstructural stability of the produced materials was investigated based on a protocol published by Alehosseini, Gómez-Mascaraque, et al. (2019), with some modifications. Briefly, samples were electrospun or electrosprayed on aluminum foil and cut into 15 mm × 50 mm pieces. Each piece was immersed in a separate tube containing

40 mL of ethanol 80% (v/v) and/or distilled water. At the various time intervals, the pieces of aluminum foil were removed from the tubes, and dried in a desiccator at 0% RH. The morphology of the structures was then explored by SEM as described in Section 2.6.1.

2.12. Statistical analysis

One-way analysis of variance (ANOVA) was applied to estimate the significant differences between sample means at a significance level of $\alpha=0.05$ based on Duncan's Test. IBM SPSS Statistics software (version 20, IBM Corp., USA) was applied for statistical analysis of experimental data. The release kinetics in food simulants were fitted to some empirical models using SigmaPlot software (version 14.0, Systat Software, Inc., USA). All experiments were performed in triplicate, and data are presented as mean $\pm$ standard deviation (SD) values.

3. RESULTS AND DISCUSSION

3.1. Characterization of the saffron extract

The drying efficiency, moisture content (wet basis), and indexes of crocin, safranal and picrocrocin of the DSE were determined to be $55.3\pm4.4\%$, $6.4\pm1.0\%$, 255.3 ± 23.3 , 45.5 ± 6.4 , and 73.5 ± 8.1 , respectively. Sánchez, Carmona, del Campo, and Alonso (2009) developed a solid phase extraction technique and compared the characteristics of twenty different saffron spice samples. Moisture content of some selected Iranian spices, and their crocin, safranal, and picrocrocin index, were reported to be $8.24\pm1.15\%$, 220.76 ± 36.82 , 39.20 ± 1.72 , and 86.5 ± 9.72 , respectively (Sánchez et al., 2009). Similar amounts of crocin, safranal, and picrocrocin in other saffron extracts have been reported (Caballero-Ortega, Pereda-Miranda, & Abdullaev, 2007; Esfanjani et al., 2017). Differences between the indexes reported in the mentioned studies and the present one

are expected to come from inherent variations in the original spice biomass and also can depend on the extraction conditions.

3.2.Characterization of the electrospun/sprayed structures

3.2.1. Morphological characterization

Zein has been reported as a promising carrier to encapsulate a wide spectrum of bioactive compounds through EHD processing (Nieuwland et al., 2014). Hence, zein-based nanofiber mats (produced by electrospinning) and microparticles (produced by electrospraying), both containing the saffron extract, were obtained by EHD processing from zein/DSE solutions. The saffron extract was soluble in both ethanol and water (Serrano-Díaz, Sánchez, Maggi, Carmona, & Alonso, 2011), so it could be readily dissolved in the zein solutions prior to the EHD process. Zein concentrations of 10 and 20 wt.% were selected to produce microparticles and nanofibers, respectively, based on previous works (Alehosseini, Gómez-Mascaraque, et al., 2019; Costamagna et al., 2017). The EHD process was adjusted in preliminary assays in order to obtain a stable cone-jet throughout this process. SEM images of the obtained zein particles and fibers together with the size distribution of the obtained structures are shown in Fig. 1.

Figure 1. SEM images of DSE-loaded zein structures obtained through EHD processing from A) Z10S2; B) Z10S4; C) Z20S2; D) Z20S4. Scale bars correspond to 5 μm .

The obtained morphologies were similar to those previously reported for zein electrospun/electrosprayed structures produced from the highest and lowest protein concentrations, respectively (Gómez-Mascaraque, Casagrande Sipoli, et al., 2017; Li, Lim, & Kakuda, 2009). While the former yielded ultrathin fibrillar structures with ribbon-like morphologies, the latter exhibited a particulate shape. Higher biopolymer concentrations favor a greater extent of chain entanglements, which tend to yield more fibrillar structures through EHD processing (Alehosseini et al., 2018; Ghorani & Tucker, 2015). Samples containing 20% zein showed a higher production yield (related to the greater protein content of the solutions) with smaller average diameter than those of zein 10%.

Interestingly, the diameter of both the fibers and the particles significantly decreased when the DSE content increased (specifically a decrease from 590 ± 298 nm to 330 ± 217 nm was observed for the electrosprayed particles and from 290 ± 128 nm to 210 ± 87 nm for the electrospun fibers) ($p<0.05$), most probably explained by the lower flow rate used in the cases of Z10S4 and Z20S4 in order to keep the jet-cone mode during processing. It has been previously demonstrated that reducing the flow rate leads to a decrease in size of electrospun/electrosprayed structures (Alehosseini et al., 2018; Ghorani & Tucker, 2015). Apart from that, this phenomenon could be also attributed to changes in solution properties caused by the greater extract content. This is confirmed as, in the case of the 10% zein solution, an increase in extract content (Z10S4) led to the formation of thin fibers apart from the electrosprayed particles observed for the materials with just 2% of extract (Z10S2).

3.2.2. Encapsulation efficiency of DSE

To determine the amount of DSE effectively incorporated into the electrospun/electrosprayed structures, the encapsulation efficiency was calculated according to Eq. (2). Table 1 shows the summarized results:

Table 1. Description of the different samples produced and DSE encapsulation efficiency (%).

Sample code	Zein concentration (wt.%)	DSE loading (wt.%)	Electrohydrodynamic processing mode	Picrocrocin	Safranal	Crocin
Z10S2	10	2	Electrospraying	94±2 ^{a c}	89±4 ^{a c}	94±5 ^{a c}
Z10S4	10	4	Electrospraying	82±5 ^{b c}	82±8 ^{ab c}	85±3 ^{b c}
Z20S2	20	2	Electrospinning	97±5 ^{a c}	88±1 ^{a d}	97±1 ^{a c}
Z20S4	20	4	Electrospinning	80±7 ^{d c}	74±5 ^{b c}	81±3 ^{b c}

*Means in same column with different superscripts (a-b) differ significantly ($p<0.05$). Means in same row with different superscripts (c-d) differ significantly ($p<0.05$).

Very high encapsulation efficiencies were achieved in all cases, being the extract content on the encapsulation structures' surface negligible. These values were higher than those previously reported for the microencapsulation of saffron bioactive compounds within biopolymers through spray-drying and gelation methods (Rahaiee, Shojaosadati, Hashemi, Moini, & Razavi, 2015; Rajabi, Ghorbani, Jafari, Sadeghi Mahoonak, & Rajabzadeh, 2015), emphasizing the suitability of the EHD process for the encapsulation of sensitive bioactive compounds (Alehosseini et al., 2018; Gómez-Estaca, Balaguer, López-Carballo, Gavara, & Hernández-Muñoz, 2017). The encapsulation efficiency significantly decreased as the theoretical DSE loading of the zein structures increased, as generally occurs when the ingredient/wall material ratio increases in encapsulation systems (Gómez-Mascaraque, Casagrande Sipoli, et al., 2017; Khoshakhlagh, Koocheki, Mohebbi, & Allafchian, 2017). On the other hand, the encapsulation efficiency of safranal was in general lower than for crocin or picrocrocin, although the differences were only found to be statistically significant for sample Z20S2. This may be attributed to the higher

volatility of safranal as compared to the crocin and picrocrocin (Caballero-Ortega et al., 2007; Kyriakoudi, Ordoudi, Roldán-Medina, & Tsimidou, 2015).

3.2.3. FT-IR spectroscopy analysis

FT-IR spectra of zein fibers and particles, both in the presence and absence of different amount of saffron extract are shown in Figure 2. Typical bands from zein were observed in the FTIR spectra from electrospun and electrosprayed structures, being the main representative ones the amide A or N-H stretching band at $\sim 3300\text{ cm}^{-1}$, the amide I mainly ascribed to C=O stretching vibrations at $\sim 1650\text{ cm}^{-1}$, the amide II mainly coming from N-H bending and stretching vibrations at $\sim 1540\text{ cm}^{-1}$ and the amide III band ascribed to C-N stretching at $\sim 1250\text{ cm}^{-1}$ (Alehosseini, Gómez-Mascaraque, et al., 2019; Gómez-Mascaraque, Perez-Masiá, González-Barrio, Periago, & López-Rubio, 2017). In contrast with previous studies, no significant shifts were observed in these spectral bands independently of the final morphology of the encapsulation structures and suggesting (from the position of the amide I band) that the conformation of the zein in both capsule and fiber structures consisted on α -helices and unfolded protein chains (Aceituno-Medina, López-Rubio, Mendoza, & Lagaron, 2013).

The characteristic bands of pure saffron appeared at 1022 cm^{-1} (corresponding to C-O-C stretching), 1227 cm^{-1} (attributed to the C-O stretching vibration of ester groups), 1610 cm^{-1} (asymmetric and symmetric stretching peaks of carboxylic groups), 1700 cm^{-1} (C=O stretching from carbonyl groups, $2898\text{-}2937\text{ cm}^{-1}$ (corresponding to asymmetric -CH₂-, symmetric -CH₃ and -CH₂- stretching vibrations), and $\sim 3335\text{ cm}^{-1}$ (assigned to the O-H sugar groups attached to crocin) (Ordoudi, de los Mozos Pascual, & Tsimidou, 2014; Tarantilis, Beljebbar, Manfait, & Polissiou, 1998).

No remarkable changes were observed among the FTIR spectra of the DSE-loaded zein structures, probably due to the relatively low extract content of the structures, thus being predominant the zein bands, which moreover overlap with most of the bands from the saffron extract. The presence of saffron compounds within the fibers was evident by the relative increase in intensity of some specific bands from saffron, like the one at 1700 cm^{-1} and the one at 1022 cm^{-1} , which in the structures was shifted to 1111 , 1118 , 1087 , and 1106 cm^{-1} for Z10S2, Z10S4, Z20S2, and, Z20S4, respectively, thus suggesting some interactions between the extract and the encapsulation matrices. These interactions between the DSE and zein were also evidenced by the displacement of some characteristic bands from the protein when the extract was incorporated, e.g., the amide III band shifted to higher wavenumbers (from 1250 to 1264 cm^{-1}).

Figure 2. FT-IR spectra of DSE-loaded electrospun/sprayed zein structures; Zein 10% (A), Zein 20% (B). The spectrum of non-encapsulated DSE is also shown.

3.2.4. Thermal properties

Figure 3 shows the thermograms of zein-based particles (A) and zein-based fiber mats (B) in comparison with that of the non-encapsulated extract. The thermogram of the saffron extract displays a two-step degradation profile with maximum degradation temperatures around 120 °C and 200 °C. Given the small amounts of saffron extract incorporated within the structures, its thermal degradation could not be unequivocally detected, but previous studies have shown an effective thermal stabilization of bioactive compounds encapsulated within electrohydrodynamically processed structures (Brahatheeswaran et al., 2012; Gómez-Mascaraque, Lagarón, & López-Rubio, 2015).

Figure 3. Thermograms of zein-based beads particles (A), and zein-based fiber mats (B) in comparison with the thermogram of the non-encapsulated saffron extract.

It is interesting to note that the morphology of the structures did not have an impact on their thermal stability.

3.3. Photostability test by UV-irradiation

It has been demonstrated that light degradation of bioactive compounds leads to a reduction in their antioxidant activity, strength coloring, and therapeutic attributes (Duncan & Chang, 2012). Accordingly, it is important to understand how encapsulation modifies the stability of the extract when exposed to irradiation, as a protective shielding effect is expected when embedding the extract within the zein matrix. Saffron is normally used as a food additive because of its attributes (e.g. colorants agent), but the molecules responsible for these attributes are susceptible to oxidation and degradation by light exposure as a consequence of the presence of conjugated double bonds in their chemical structures (Duncan & Chang, 2012). Crocin, as a sensitive carotenoid, is a glucosyl ester of a polyene dicarboxylic acid, which main unit consists of seven conjugated double bonds (Tarantilis, Tsoupras, & Polissiou, 1995). Degradation of conjugated double bonds and production of free radicals upon exposure to UV light have been previously investigated (Frank et al., 1997; López-Rubio & Lagaron, 2011).

Previous studies have shown that encapsulation can effectively protect different bioactive compounds against UV irradiation such as vitamin C and gallic acid encapsulated in a whey protein concentrate (Tandale, 2007), β -carotene in protein structures (de Freitas Zômpero, López-Rubio, de Pinho, Lagaron, & de la Torre, 2015; López-Rubio & Lagaron, 2011), or vitamin D₃ in soy protein isolate (Lee et al., 2016). It has also been reported that the oxidation rate of a crocin-containing beverage was accelerated by increasing light intensity (Manzocco, Kravina, Calligaris, & Nicoli, 2008).

However, to the best of our knowledge, there is no available information about the ability of zein matrices for protecting saffron against UV exposure.

Therefore, the zein structures containing the saffron extract were exposed to UV irradiation and compared with the UV stability of the free saffron extract. Figure 4 shows the degradation profile of picrocrocin, safranal, and crocin, respectively, as a function of UV irradiation time. According to Fig. 4A, picrocrocin was rather stable upon UV irradiation and no remarkable reduction was observed during exposure (even for the non-encapsulated compound). In contrast, a significant reduction in safranal (Fig. 4B) and crocin (Fig. 4C) was observed after 6 h of UV irradiation in the non-encapsulated extract. From the results it is also evident that amongst the studied compounds, the most UV-sensitive one was safranal, fact which is not surprising as it is known to be the main volatile compound present in saffron (Caballero-Ortega et al., 2007), also having the greatest antioxidant capacity. It has also been demonstrated that the intensity of the spectral peak related to crocin (440 nm) decreases as a consequence of its degradation giving raise to less colored isomers and/or hydroperoxy-crocin which is also a non-colored compound (Manzocco et al., 2008).

All the structures were efficient in preventing UV degradation, but from the results it appears that the electrosprayed structures showed a better protective effect when compared to the fiber morphologies. This is most probably due to the larger diameter of the particles which may have contributed to a greater shielding effect. In fact, it has been previously demonstrated that the oxidation of encapsulated compounds highly depends on capsule size (Fernandez, Torres-Giner, & Lagaron, 2009).

Summarizing, while UV exposure promoted the degradation of non-encapsulated picrocrocin, safranal, and crocin reducing their contents around 5.5, 60, and 20%, respectively, encapsulation of the extract in zein fibers/particles significantly prevented their photo degradation ($p < 0.05$). Moreover, zein is a slightly colored polymer, and it is known to limit UV penetration (Fernandez et al., 2009).

Figure 4. Degradation profiles of free and encapsulated DSE exposed UV irradiation; picrocrocin (A), safranal (B), and crocin (C). Different letters (a-e) indicate different statistical groups with significant differences ($p < 0.05$); Z10S2 (◆), Z10S4 (■), Z20S2 (▲), Z20S4 (●), and DSE (×). Means with different superscripts (a-e) differ significantly ($p < 0.05$).

3.4. Stabilization of crocin in aqueous environments

Crocin, the main bioactive compound in saffron (Melnyk et al., 2010), has been reported to rapidly degrade in aqueous environments (Orfanou & Tsimidou, 1995), being its stability dependent on the temperature and pH (Ordoudi et al., 2015; Selim et al., 2000). Similarly, decomposition of picocrocin into safranal may be also facilitated by heating (Lozano, Castellar, Simancas, & Iborra, 1999; Sarfarazi, Jafari, & Rajabzadeh, 2015). It has been shown that incorporation of bioactive compounds within electrospun/electrosprayed protein structures effectively improves their stability (Gómez-Mascaraque, Casagrande Sipoli, et al., 2017; Gómez-Mascaraque et al., 2018). To assess the stabilization effect of the zein matrices on crocin, the most bioactive compound from saffron (Melnyk et al., 2010), the degradation of this compound at different pH and temperatures simulating common food processing conditions (Bhat, Hamdani, & Masoodi, 2018; Licón, Carmona, Rubio, Molina, & Berruga, 2012; Tarantilis, Polissiou, & Manfait, 1994) was evaluated. For this purpose, free and encapsulated DSE were dispersed in PBS (pH=7.4) and acetic acid 20% (v/v) (pH=2) at both 25 and 75 °C, conditions which have been reported to trigger rapid degradation of the bioactive compounds found in saffron (Sánchez, Carmona, A. Ordoudi, Z. Tsimidou, & Alonso, 2008; Serrano-Díaz et al., 2011) and the degradation of crocin was monitored for 15 h. The percentage of crocin degradation in the various conditions was estimated by measuring its absorbance at 440 nm and the results are compiled in Table 2 and Supplementary Figure 1. From the results in this table it can be observed that free crocin quickly degraded in acetic acid at both temperatures, being degradation more extensive at the higher temperature as expected.

Table 2. Degradation (%) of free crocin and encapsulated after 15 hours in selected conditions.

Sample code	Degradation (%)			
	PBS (pH=7.4)		Acetic acid (pH=2)	
	T=25 °C	T=75 °C	T=25 °C	T=75 °C
Z10S2	28.15 ^a	63.14 ^b	58.39 ^a	66.95 ^a
Z10S4	31.33 ^{ab}	63.10 ^b	64.00 ^a	65.49 ^a
Z20S2	39.35 ^b	62.71 ^b	57.19 ^a	77.23 ^b
Z20S4	34.49 ^{ab}	49.64 ^a	55.42 ^a	59.27 ^a
Pure DSE	58.49 ^c	67.12 ^b	91.1 ^b	98.09 ^c

*Means in same column with different superscripts (a-c) differ significantly ($p<0.05$). Statistical analysis has been performed for the last time point.

PBS also promoted some degradation of free crocin, both at 25 and 75 °C, with reductions of about 60 and 68% in 15 h, respectively. In contrast, encapsulated crocin showed enhanced stability at room temperature in both pH conditions, while only a slight improvement was observed in PBS at 75 °C.

Overall, encapsulation of the DSE within the electrospun/sprayed protein structures significantly enhanced the stability of crocin ($p<0.05$), especially in acidic conditions, in which the saffron bioactive compound (i.e. crocin) was more unstable.

3.5. Saffron release from the electrospun/electrosprayed zein structures

Encapsulated structures should not only have a protective effect on the bioactive ingredients incorporated within them but should also be able to eventually release them so that they can exert their biological activities. Accordingly, the release of crocin, safranal and picrocrocin from the electrospun/electrosprayed structures was assessed in 50% ethanol and in soybean oil, as two simulants for oil-in-water emulsions and fatty foods, respectively (Commission-regulation10/2011EU, 2011).

Figure 5 shows the release profiles of the bioactive compounds in ethanol 50%. Their release in the oil was negligible over the 6 days of experiment (data not shown), most

likely due to the poor solubility of the DSE in this food simulant, suggesting that the developed materials would find a more suitable application in aqueous food products.

Figure 5. DSE release profiles from the electrospun/electrosprayed structures in ethanol 50% (v/v) fitted to Peppas–Sahlin model: Safranal (A), picrocrocin (B), and crocin (C); Z10S2 (♦), Z10S4 (■), Z20S2 (▲), and Z20S4 (●).

Similar release profiles were observed independently of the compound considered and also of the encapsulation structure and the release during the experimental time was in all cases greater than 60% of the total bioactive compound content. It has been reported that reducing the effective interactions between core and wall materials leads to an increase in the release of bioactive compounds (Gómez-Estaca et al., 2017). In this case, although a complete release was not observed in any of the structures, most of the encapsulated bioactive compounds were released after 6 days in ethanol.

Several mechanisms have been proposed to explain the release of encapsulated bioactive compounds from encapsulation structures such as erosion, diffusion, disintegration, fragmentation, etc. (McClements, 2012). The release data were fitted to different empirical models to better understand the release behavior of encapsulated saffron compounds from the electrospun/sprayed zein structures. Table 3 shows the coefficient of determination (R^2) and constant values from the Kopcha, Ritger–Peppas, and Peppas–Sahlin models. Considering the R^2 values, Peppas–Sahlin model was chosen as the best model to explain the release kinetics of crocin, safranal, and picrocrocin in 50% ethanol. Fitting to other models such as zero-, first-order, and Higuchi kinetics was also attempted (data not shown). However, the obtained R^2 values were lower than 0.4, indicating that these models were not suitable to explain the experimental data. The Peppas–Sahlin model is considered appropriate to evaluate the erosion and diffusion release mechanisms through the K_1/K_2 ratio. This study revealed that the dominant release mechanism was diffusion for both electrospun fibers and electrosprayed particles, with K_1/K_2 ratios greater than 1 in all cases (Table 3) (Peppas & Sahlin, 1989). This was also confirmed by the ratios $A/B > 1$ and $n \leq 0.4$ obtained for Kopcha and Ritger–Peppas models, respectively (Kopcha, Lordi, & Tojo, 1991; Rezaeinia, Ghorani, Emadzadeh, & Tucker, 2019).

Table 3. Kinetic constants of the crocin, safranal, and picrocrocin release profile in 50% ethanol.

Empirical release models	Kopcha model			Ritger–Peppas model			Peppas–Sahlin model			
	<i>A</i>	<i>B</i>	<i>R</i> ²	<i>K</i>	<i>n</i>	<i>R</i> ²	<i>K</i> ₁	<i>K</i> ₂	<i>m</i>	<i>R</i> ²
Crocin										
Z10S2	24.95	-1.65	0.75	0.39	0.13	0.92	0.46	-0.07	0.28	0.98
Z10S4	22.96	-1.42	0.70	0.39	0.15	0.97	0.44	-0.06	0.21	0.98
Z20S2	23.08	-1.52	0.77	0.36	0.14	0.92	0.42	-0.06	0.29	0.98
Z20S4	24.00	-1.48	0.60	0.42	0.14	0.98	0.48	-0.06	0.18	0.98
Safranal										
Z10S2	30.31	-1.98	0.88	0.44	0.16	0.89	0.48	-0.06	0.34	0.98
Z10S4	20.39	-1.25	0.86	0.31	0.17	0.94	0.34	-0.04	0.30	0.98
Z20S2	22.83	-1.45	0.98	0.30	0.20	0.85	0.29	-0.02	0.44	0.98
Z20S4	20.86	-1.22	0.80	0.34	0.17	0.98	0.37	-0.04	0.23	0.98
Picrocrocin										
Z10S2	30.52	-1.98	0.93	0.43	0.18	0.87	0.44	-0.05	0.37	0.98
Z10S4	19.83	-1.26	0.89	0.29	0.17	0.90	0.30	-0.03	0.34	0.97
Z20S2	21.75	-1.35	0.96	0.29	0.29	0.90	0.28	-0.02	0.40	0.98
Z20S4	18.97	-1.15	0.75	0.31	0.16	0.97	0.57	-0.05	0.01	0.98

3.6. Structural stability of the materials in aqueous media

Since the encapsulated structures are intended to be in contact with food materials, the structure stability and morphological changes of electrospun fibers and electrosprayed particles upon immersion in water were estimated. Morphological changes were recorded by SEM images at selected time intervals after immersion (Fig. 6).

Figure 6. SEM images of electrospun/electrosprayed structures upon immersion in water.

Due to the inherent properties of zein, as a hydrophobic protein, both electrospun and electrosprayed structures although considerably swelled in water, they were able to keep their integrity in this medium. However, some merging of the structures was seen, especially in the case of electrosprayed particles. Similarly, the fibers were partially fused together, although, as previously mentioned, no dissolution of the structures was observed in water, in agreement with the findings of Alehosseini, Gómez-Mascaraque, et al. (2019).

4. CONCLUSIONS

In this work, encapsulation of a saffron extract was carried out through electrohydrodynamic processing. Encapsulation efficiency, release behavior, thermal

stability, and photostability of DSE-loaded nanofibers/microparticles were assessed. Zein concentrations of 10 and 20 wt.% were selected to produce microparticles and nanofibers, respectively. The results showed that as the DSE content was increased, the diameter of both the fibers and the particles decreased. Very high encapsulation efficiencies were achieved in all cases and the thermal stability of the structures was similar independently of their morphology. Moreover, the zein structures developed were also capable of enhancing the stability of the saffron-derived bioactive compounds at different pH values (pH 2 and 7.4) and different storage temperatures (20 and 75 °C) and upon UV exposure. A negligible extract release was observed in soybean oil after 6 days of experiment, most probably due to the poor solubility of the DSE in this media. In contrast, an almost complete extract release was observed after the same time period in aqueous ethanol, due to the progressive dissolution of the structures in this media. To better understand the release behavior of encapsulated saffron compounds from the produced zein structures, different empirical models were also fitted. This study revealed that Peppas-Sahlin model was the best model to explain the release kinetics of crocin, safranal, and picrocrocin in 50% ethanol. The dominant release mechanism was diffusion for both electrospun fibers and electrosprayed particles. This work demonstrates the potential of the developed encapsulation structures as bioactive ingredients enhancing the stability of saffron compounds to be used either as food packaging coatings for active packaging applications, or in food formulations.

Acknowledgements

A. Alehosseini received a scholarship by the Ministry of Science, Research and Technology (MSRT) of Iran. Dr. M.J. Fabra is acknowledged for fruitful discussions. The authors would also like to thank Research Institute of Food Science and Technology

(RIFST) for technical support. The authors would like to thank the Spanish MINECO project AGL2015-63855-C2-1 for financial support. Central Support Service for Experimental Research (SCSIE) of the University of Valencia should be acknowledged for the electronic microscopy service.

References

- Aceituno-Medina, M., López-Rubio, A., Mendoza, S., & Lagaron, J. M. (2013). Development of novel ultrathin structures based in amaranth (*Amaranthus hypochondriacus*) protein isolate through electrospinning. *Food hydrocolloids*, 31, 289-298. doi: <https://doi.org/10.1016/j.foodhyd.2012.11.009>
- Alavizadeh, S. H., & Hosseinzadeh, H. (2014). Bioactivity assessment and toxicity of crocin: a comprehensive review. *Food and Chemical Toxicology*, 64, 65-80. doi: <https://doi.org/10.1016/j.fct.2013.11.016>
- Alehosseini, A., Ghorani, B., Sarabi-Jamab, M., & Tucker, N. (2018). Principles of electrospraying: A new approach in protection of bioactive compounds in foods. *Critical Reviews in Food Science and Nutrition*, 58(14), 2346-2363. doi: <https://doi.org/10.1080/10408398.2017.1323723>
- Alehosseini, A., Gómez-Mascaraque, L. G., Martínez-Sanz, M., & López-Rubio, A. (2019). Electrospun curcumin-loaded protein nanofiber mats as active/bioactive coatings for food packaging applications. *Food Hydrocolloids*, 87, 758-771. doi: <https://doi.org/10.1016/j.foodhyd.2018.08.056>
- Alehosseini, A., Sarabi-Jamab, M., Ghorani, B., & Kadkhodae, R. (2019). Electro-encapsulation of *Lactobacillus casei* in high-resistant capsules of whey protein containing transglutaminase enzyme. *LWT-Food Science and Technology*, 102, 150-158. doi: <https://doi.org/10.1016/j.lwt.2018.12.022>
- Bhat, N. A., Hamdani, A. M., & Masoodi, F. (2018). Development of functional cookies using saffron extract. *Journal of Food Science and Technology*, 1-10. doi: <https://doi.org/10.1007/s13197-018-3426-1>
- Bhushani, J. A., & Anandharamakrishnan, C. (2014). Electrospinning and electrospraying techniques: Potential food based applications. *Trends in food science & technology*, 38(1), 21-33. doi: <https://doi.org/10.1016/j.tifs.2014.03.004>
- Blanco-Padilla, A., López-Rubio, A., Loarca-Piña, G., Gómez-Mascaraque, L. G., & Mendoza, S. (2015). Characterization, release and antioxidant activity of curcumin-loaded amaranth-pullulan electrospun fibers. *LWT - Food Science and Technology*, 63(2), 1137-1144. doi: <https://doi.org/10.1016/j.lwt.2015.03.081>
- Bolandi, M., & Ghoddusi, H. (2006). Flavour and colour changes during processing and storage of saffron (*Crocus sativus* L.). *Developments in food science*, 43, 323-326. doi: [https://doi.org/10.1016/S0167-4501\(06\)80077-4](https://doi.org/10.1016/S0167-4501(06)80077-4)
- Brahatheeswaran, D., Mathew, A., Aswathy, R. G., Nagaoka, Y., Venugopal, K., Yoshida, Y., . . . Sakthikumar, D. (2012). Hybrid fluorescent curcumin loaded zein electrospun nanofibrous scaffold for biomedical applications. *Biomedical Materials*, 7(4), 1-16. doi: <https://doi.org/10.1088/1748-6041/7/4/045001>
- Bruschi, M. L. (2015). Mathematical models of drug release. In M. L. Bruschi (Ed.), *Strategies to Modify the Drug Release from Pharmaceutical Systems* (pp. 63-86): Elsevier.
- Caballero-Ortega, H., Pereda-Miranda, R., & Abdullaev, F. I. (2007). HPLC quantification of major active components from 11 different saffron (*Crocus sativus* L.) sources. *Food Chemistry*, 100, 1126-1131. doi: <https://doi.org/10.1016/j.foodchem.2005.11.020>

- Chranioti, C., Nikoloudaki, A., & Tzia, C. (2015). Saffron and beetroot extracts encapsulated in maltodextrin, gum Arabic, modified starch and chitosan: Incorporation in a chewing gum system. *Carbohydrate polymers*, 127, 252-263. doi: <https://doi.org/10.1016/j.carbpol.2015.03.049>
- Commission-regulation10/2011EU. (2011). Commission regulation (EU) on plastic materials and articles intended to come into contact with food. *No 10/2011*.
- Cook, M. T., Tzortzis, G., Charalampopoulos, D., & Khutoryanskiy, V. V. (2012). Microencapsulation of probiotics for gastrointestinal delivery. *Journal of Controlled Release*, 162(1), 56-67. doi: <https://doi.org/10.1016/j.jconrel.2012.06.003>
- Costamagna, M. S., Gómez-Mascaraque, L. G., Zampini, I. C., Alberto, M. R., Pérez, J., López-Rubio, A., & Isla, M. I. (2017). Microencapsulated chañar phenolics: A potential ingredient for functional foods development. *Journal of Functional Foods*, 37, 523-530. doi: <https://doi.org/10.1016/j.jff.2017.08.018>
- de Freitas Zômpero, R. H., López-Rubio, A., de Pinho, S. C., Lagaron, J. M., & de la Torre, L. G. (2015). Hybrid encapsulation structures based on β -carotene-loaded nanoliposomes within electrospun fibers. *Colloids and surfaces. B, Biointerfaces*, 134, 475-482. doi: <https://doi.org/10.1016/j.colsurfb.2015.03.015>
- Duncan, S. E., & Chang, H.-H. (2012). Implications of light energy on food quality and packaging selection. In J. Henry (Ed.), *Advances in food and nutrition research* (Vol. 67, pp. 25-73): Elsevier.
- Esfanjan, A. F., Jafari, S. M., Assadpour, E., & Mohammadi, A. (2015). Nano-encapsulation of saffron extract through double-layered multiple emulsions of pectin and whey protein concentrate. *Journal of Food Engineering*, 165(Supplement C), 149-155. doi: <https://doi.org/10.1016/j.jfoodeng.2015.06.022>
- Esfanjan, A. F., Jafari, S. M., & Assadpour, E. (2017). Preparation of a multiple emulsion based on pectin-whey protein complex for encapsulation of saffron extract nanodroplets. *Food chemistry*, 221, 1962-1969. doi: <https://doi.org/10.1016/j.foodchem.2016.11.149>
- Fernandez, A., Torres-Giner, S., & Lagaron, J. M. (2009). Novel route to stabilization of bioactive antioxidants by encapsulation in electrospun fibers of zein prolamine. *Food Hydrocolloids*, 23, 1427-1432. doi: <https://doi.org/10.1016/j.foodhyd.2008.10.011>
- Frank, H. A., Chynwat, V., Desamero, R. Z., Farhoosh, R., Erickson, J., & Bautista, J. (1997). On the photophysics and photochemical properties of carotenoids and their role as light-harvesting pigments in photosynthesis. *Pure and applied chemistry*, 69(10), 2117-2124. doi: <https://doi.org/10.1351/pac199769102117>
- Ghorani, B., Kadkhodai, R., & Alehosseini, A. (2017). The effect of biopolymer type, temperature and relative humidity on the physicochemical characteristics and stability of microencapsulated bioactive compounds of saffron. *Iranian Journal of Food Science and Technology*, 14, 127-142 (In Persian).
- Ghorani, B., & Tucker, N. (2015). Fundamentals of electrospinning as a novel delivery vehicle for bioactive compounds in food nanotechnology. *Food Hydrocolloids*, 51, 227-240. doi: <https://doi.org/10.1016/j.foodhyd.2015.05.024>
- Gómez-Estaca, J., Balaguer, M., López-Carballo, G., Gavara, R., & Hernández-Muñoz, P. (2017). Improving antioxidant and antimicrobial properties of curcumin by means of encapsulation in gelatin through electrohydrodynamic atomization. *Food Hydrocolloids*, 70, 313-320. doi: <https://doi.org/10.1016/j.foodhyd.2017.04.019>
- Gomez-Mascaraque, L. G., Ambrosio-Martín, J., Fabra, M. J., Rocío, P.-M., & López-Rubio, A. (2016). Novel nanoencapsulation structures for functional foods and nutraceutical applications In S. Sen & Y. Pathak (Eds.), *Nanotechnology in Nutraceuticals: Production to Consumption*: CRC Press.
- Gómez-Mascaraque, L. G., Casagrande Sipoli, C., de La Torre, L. G., & López-Rubio, A. (2017). Microencapsulation structures based on protein-coated liposomes obtained through electrospraying for the stabilization and improved bioaccessibility of curcumin. *Food chemistry*, 233, 343-350. doi: <https://doi.org/10.1016/j.foodchem.2017.04.133>
- Gómez-Mascaraque, L. G., Hernández-Rojas, M., Tarancón, P., Tenon, M., Feuillère, N., Ruiz, J. F. V., . . . López-Rubio, A. (2017). Impact of microencapsulation within electrosprayed

- proteins on the formulation of green tea extract-enriched biscuits. *LWT-Food Science and Technology*, 81, 77-86. doi: <https://doi.org/10.1016/j.lwt.2017.03.041>
- Gómez-Mascaraque, L. G., Lagarón, J. M., & López-Rubio, A. (2015). Electrospayed gelatin submicroparticles as edible carriers for the encapsulation of polyphenols of interest in functional foods. *Food Hydrocolloids*, 49, 42-52. doi: <https://doi.org/10.1016/j.foodhyd.2015.03.006>
- Gómez-Mascaraque, L. G., & López-Rubio, A. (2016). Protein-based emulsion electrospayed micro-and submicroparticles for the encapsulation and stabilization of thermosensitive hydrophobic bioactives. *Journal of colloid and interface science*, 465, 259-270. doi: <https://doi.org/10.1016/j.jcis.2015.11.061>
- Gómez-Mascaraque, L. G., Perez-Masiá, R., González-Barrio, R., Periago, M. J., & López-Rubio, A. (2017). Potential of microencapsulation through emulsion-electrospaying to improve the bioaccessibility of β -carotene. *Food hydrocolloids*, 73, 1-12. doi: <https://doi.org/10.1016/j.foodhyd.2017.06.019>
- Gómez-Mascaraque, L. G., Sipoli, C. C., de La Torre, L. G., & López-Rubio, A. (2017). Microencapsulation structures based on protein-coated liposomes obtained through electrospaying for the stabilization and improved bioaccessibility of curcumin. *Food chemistry*, 233, 343-350. doi: <https://doi.org/10.1016/j.foodchem.2017.04.133>
- Gómez-Mascaraque, L. G., Tordera, F., Fabra, M. J., Martínez-Sanz, M., & Lopez-Rubio, A. (2018). Coaxial electrospaying of biopolymers as a strategy to improve protection of bioactive food ingredients. *Innovative Food Science & Emerging Technologies*. doi: <https://doi.org/10.1016/j.ifset.2018.03.023>
- Gregory, M. J., Menary, R. C., & Davies, N. W. (2005). Effect of drying temperature and air flow on the production and retention of secondary metabolites in saffron. *Journal of Agricultural and Food Chemistry*, 53(15), 5969-5975. doi: <https://doi.org/10.1021/jf047989j>
- ISO-3632. (2010). Saffron (*Crocus sativus* L.) Part 1: Specifications, Part 2: Test Methods, TS 3632-1, 2. Geneva, Switzerland: International Organization for Standardization.
- Khoshakhlagh, K., Koocheki, A., Mohebbi, M., & Allafchian, A. (2017). Development and characterization of electrospayed *Alyssum homolocarpum* seed gum nanoparticles for encapsulation of d-limonene. *Journal of Colloid and Interface Science*, 490, 562-575. doi: <https://doi.org/10.1016/j.jcis.2016.11.067>
- Kopcha, M., Lordi, N. G., & Tojo, K. J. (1991). Evaluation of release from selected thermosoftening vehicles. *Journal of pharmacy and pharmacology*, 43(6), 382-387. doi: <https://doi.org/10.1111/j.2042-7158.1991.tb03493.x>
- Kriegel, C., Arrechi, A., Kit, K., McClements, D., & Weiss, J. (2008). Fabrication, functionalization, and application of electrospun biopolymer nanofibers. *Critical reviews in food science and nutrition*, 48(8), 775-797. doi: <https://doi.org/10.1080/10408390802241325>
- Kwak, H.-S. (2014). Overview of nano-and microencapsulation for foods. *Nano-and Microencapsulation for Foods*, ed. H.-S. Kwak, South Korea, 1-14.
- Kyriakoudi, A., Ordoudi, S. A., Roldán-Medina, M., & Tsimidou, M. Z. (2015). Saffron, A functional spice. *Austin Journal of Nutrition and Food Sciences*, 3(1), 1-5.
- Lee, H., Yildiz, G., Dos Santos, L., Jiang, S., Andrade, J., Engeseth, N., & Feng, H. (2016). Soy protein nano-aggregates with improved functional properties prepared by sequential pH treatment and ultrasonication. *Food Hydrocolloids*, 55, 200-209. doi: <https://doi.org/10.1016/j.foodhyd.2015.11.022>
- Li, Y., Lim, L. T., & Kakuda, Y. (2009). Electrospun zein fibers as carriers to stabilize (-)-epigallocatechin gallate. *Journal of food science*, 74(3). doi: <https://doi.org/10.1111/j.1750-3841.2009.01093.x>
- Licón, C., Carmona, M., Rubio, R., Molina, A., & Berruga, M. (2012). Preliminary study of saffron (*Crocus sativus* L. stigmas) color extraction in a dairy matrix. *Dyes and Pigments*, 92(3), 1355-1360. doi: <https://doi.org/10.1016/j.dyepig.2011.09.022>

- López-Rubio, A., & Lagaron, J. M. (2011). Improved incorporation and stabilisation of β -carotene in hydrocolloids using glycerol. *Food Chemistry*, 125, 997-1004. doi: <https://doi.org/10.1016/j.foodchem.2010.09.106>
- Lozano, P., Castellar, M., Simancas, M., & Iborra, J. (1999). A quantitative high-performance liquid chromatographic method to analyse commercial saffron (*Crocus sativus* L.) products. *Journal of Chromatography A*, 830(2), 477-483. doi: [https://doi.org/10.1016/S0021-9673\(98\)00938-8](https://doi.org/10.1016/S0021-9673(98)00938-8)
- Maggi, L., Carmona, M., Zalacain, A., Kanakis, C. D., Anastasaki, E., Tarantilis, P. A., . . . Alonso, G. L. (2010). Changes in saffron volatile profile according to its storage time. *Food Research International*, 43(5), 1329-1334. doi: <https://doi.org/10.1016/j.foodres.2010.03.025>
- Manzocco, L., Kravina, G., Calligaris, S., & Nicoli, M. C. (2008). Shelf life modeling of photosensitive food: the case of colored beverages. *Journal of agricultural and food chemistry*, 56(13), 5158-5164. doi: <https://doi.org/10.1021/jf800072u>
- McClements, D. J. (2012). Requirements for food ingredient and nutraceutical delivery systems *Encapsulation technologies and delivery systems for food ingredients and nutraceuticals* (pp. 3-18): Elsevier.
- Melnyk, J. P., Wang, S., & Marcone, M. F. (2010). Chemical and biological properties of the world's most expensive spice: Saffron. *Food Research International*, 43(8), 1981-1989. doi: <https://doi.org/10.1016/j.foodres.2010.07.033>
- Nieuwland, M., Geerdink, P., Brier, P., van den Eijnden, P., Henket, J. T. M. M., Langelaan, M. L. P., . . . Martin, A. H. (2014). Reprint of "Food-grade electrospinning of proteins". *Innovative Food Science & Emerging Technologies*, 24, 138-144. doi: <https://doi.org/10.1016/j.ifset.2014.07.006>
- Ordoudi, S. A., Cagliani, L. R., Lalou, S., Naziri, E., Tsimidou, M. Z., & Consonni, R. (2015). 1 H NMR-based metabolomics of saffron reveals markers for its quality deterioration. *Food Research International*, 70, 1-6. doi: <https://doi.org/10.1016/j.foodres.2015.01.021>
- Ordoudi, S. A., de los Mozos Pascual, M., & Tsimidou, M. Z. (2014). On the quality control of traded saffron by means of transmission Fourier-transform mid-infrared (FT-MIR) spectroscopy and chemometrics. *Food Chemistry*, 150, 414-421. doi: <https://doi.org/10.1016/j.foodchem.2013.11.014>
- Orfanou, O., & Tsimidou, M. (1995). Influence of selected additives on the stability of saffron pigments in aqueous extracts. *Developments in Food Science*, 37, 881-894. doi: [https://doi.org/10.1016/S0167-4501\(06\)80204-9](https://doi.org/10.1016/S0167-4501(06)80204-9)
- Peppas, N. A., & Sahlin, J. J. (1989). A simple equation for the description of solute release. III. Coupling of diffusion and relaxation. *International journal of pharmaceuticals*, 57(2), 169-172. doi: [https://doi.org/10.1016/0378-5173\(89\)90306-2](https://doi.org/10.1016/0378-5173(89)90306-2)
- Pérez-Masiá, R., Lagaron, J. M., & Lopez-Rubio, A. (2015). Morphology and stability of edible lycopene-containing micro-and nanocapsules produced through electrospraying and spray drying. *Food and bioprocess technology*, 8(2), 459-470. doi: <https://doi.org/10.1007/s11947-014-1422-7>
- Rahaiee, S., Hashemi, M., Shojaosadati, S. A., Moini, S., & Razavi, S. H. (2017). Nanoparticles based on crocin loaded chitosan-alginate biopolymers: Antioxidant activities, bioavailability and anticancer properties. *International Journal of Biological Macromolecules*, 99, 401-408. doi: <https://doi.org/10.1016/j.ijbiomac.2017.02.095>
- Rahaiee, S., Moini, S., Hashemi, M., & Shojaosadati, S. A. (2015). Evaluation of antioxidant activities of bioactive compounds and various extracts obtained from saffron (*Crocus sativus* L.): a review. *Journal of food science and technology*, 52(4), 1881-1888. doi: <https://doi.org/10.1007/s13197-013-1238-x>
- Rahaiee, S., Shojaosadati, S. A., Hashemi, M., Moini, S., & Razavi, S. H. (2015). Improvement of crocin stability by biodegradable nanoparticles of chitosan-alginate. *International journal of biological macromolecules*, 79, 423-432. doi: <https://doi.org/10.1016/j.ijbiomac.2015.04.041>
- Rajabi, H., Ghorbani, M., Jafari, S. M., Mahoonak, A. S., & Rajabzadeh, G. (2015). Retention of saffron bioactive components by spray drying encapsulation using maltodextrin, gum

- Arabic and gelatin as wall materials. *Food hydrocolloids*, 51, 327-337. doi: <https://doi.org/10.1016/j.foodhyd.2015.05.033>
- Rajabi, H., Ghorbani, M., Jafari, S. M., Sadeghi Mahoonak, A., & Rajabzadeh, G. (2015). Retention of saffron bioactive components by spray drying encapsulation using maltodextrin, gum Arabic and gelatin as wall materials. *Food Hydrocolloids*, 51, 327-337. doi: <https://doi.org/10.1016/j.foodhyd.2015.05.033>
- Rezaeinia, H., Ghorani, B., Emadzadeh, B., & Tucker, N. (2019). Electrohydrodynamic atomization of Balangu (*Lallemantia royleana*) seed gum for the fast-release of *Mentha longifolia* L. essential oil: Characterization of nano-capsules and modeling the kinetics of release. *Food Hydrocolloids*, 93, 374-385. doi: <https://doi.org/10.1016/j.foodhyd.2019.02.018>
- Sánchez, A. M., Carmona, M., A. Ordoudi, S., Z. Tsimidou, M., & Alonso, G. L. (2008). Kinetics of individual crocetin ester degradation in aqueous extracts of saffron (*Crocus sativus* L.) upon thermal treatment in the dark. *Journal of agricultural and food chemistry*, 56(5), 1627-1637. doi: <https://doi.org/10.1021/jf0730993>
- Sánchez, A. M., Carmona, M., del Campo, C. P., & Alonso, G. L. (2009). Solid-phase extraction for picrocrocin determination in the quality control of saffron spice (*Crocus sativus* L.). *Food Chemistry*, 116(3), 792-798. doi: <https://doi.org/10.1016/j.foodchem.2009.03.039>
- Sarfarazi, M., Jafari, S. M., & Rajabzadeh, G. (2015). Extraction optimization of saffron nutraceuticals through response surface methodology. *Food analytical methods*, 8(9), 2273-2285. doi: <https://doi.org/10.1007/s12161-014-9995-3>
- Selim, K., Tsimidou, M., & Biliaderis, C. (2000). Kinetic studies of degradation of saffron carotenoids encapsulated in amorphous polymer matrices. *Food Chemistry*, 71(2), 199-206. doi: [https://doi.org/10.1016/S0308-8146\(00\)00156-4](https://doi.org/10.1016/S0308-8146(00)00156-4)
- Serrano-Díaz, J., Sánchez, A. M., Maggi, L., Carmona, M., & Alonso, G. L. (2011). Synergic effect of water-soluble components on the coloring strength of saffron spice. *Journal of Food Composition and Analysis*, 24, 873-879. doi: <https://doi.org/10.1016/j.jfca.2011.03.014>
- Shahi, T., Assadpour, E., & Jafari, S. M. (2016). Main chemical compounds and pharmacological activities of stigmas and tepals of 'red gold'; saffron. *Trends in Food Science & Technology*, 58, 69-78. doi: <https://doi.org/10.1016/j.tifs.2016.10.010>
- Tandale, S. R. (2007). *Microencapsulation of vitamin C and gallic acid in whey protein concentrate by spray and freeze drying: Characterization and degradation kinetics*. University of Georgia.
- Tarantilis, P. A., Beljebbar, A., Manfait, M., & Polissiou, M. (1998). FT-IR, FT-Raman spectroscopic study of carotenoids from saffron (*Crocus sativus* L.) and some derivatives. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 54(4), 651-657. doi: [https://doi.org/10.1016/S1386-1425\(98\)00024-9](https://doi.org/10.1016/S1386-1425(98)00024-9)
- Tarantilis, P. A., Polissiou, M., & Manfait, M. (1994). Separation of picrocrocin, cis-trans-crocins and safranin of saffron using high-performance liquid chromatography with photodiode-array detection. *Journal of Chromatography A*, 664(1), 55-61. doi: [https://doi.org/10.1016/0021-9673\(94\)80628-4](https://doi.org/10.1016/0021-9673(94)80628-4)
- Tarantilis, P. A., Tsoupras, G., & Polissiou, M. (1995). Determination of saffron (*Crocus sativus* L.) components in crude plant extract using high-performance liquid chromatography-UV-visible photodiode-array detection-mass spectrometry. *Journal of Chromatography A*, 699(1-2), 107-118. doi: [https://doi.org/10.1016/0021-9673\(95\)00044-N](https://doi.org/10.1016/0021-9673(95)00044-N)
- Zuidam, N. J., & Shimoni, E. (2010). Overview of microencapsulates for use in food products or processes and methods to make them *Encapsulation technologies for active food ingredients and food processing* (pp. 3-29): Springer.

858 Graphical abstract

859

860

861

862

863

Supplementary Material

864

865

866

Supplementary Figure 1.

867

Highlights

- A saffron extract was produced and encapsulated by electrohydrodynamic processing
- High encapsulation efficiencies (74 - 97%) were achieved
- Stability of crocin upon UV exposure was significantly increased ($p<0.05$)
- The release of bioactive components in a food simulant was modeled
- The encapsulation structures kept their integrity upon water contact