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Characteristics of canthaxanthin-containing micro- and nanocapsules produced using electrospraying

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ABSTRACT

In this work, it showed the potential of the electrospinning (in this case electrospraying) technique to generate whey protein concentrate (WPC) and dextran micro-, submicro- and nanocapsules for the encapsulation of canthaxanthin. Furthermore, the solvent used for the development of the encapsulation morphologies was water, making these materials suitable for food applications. The effect of wall material type (WPC or dextran), wall/core ratio and wall material concentration on the morphology, stability and encapsulation efficiency (ME) was studied. The results demonstrated that the type of walls influenced the morphology, encapsulation efficiency (ME) and retention of canthaxanthin in the capsules. WPC produced the smallest emulsion droplets and electrosprayed particles. Canthaxanthin capsulated with WPC resulted in better ME and higher stability. The best wall/core ratio was found at 4 : 1, causing the capsules prepared with this ratio had the smallest capsules, the highest encapsulation efficiency and the lowest losing during process. Increase in the wall material concentration from 20 to 40% also caused an increase in the encapsulation efficiency and storage stability.

Key words : Canthaxanthin, dextran, electrospraying, encapsulation, whey protein concentrate

INTRODUCTION

Canthaxanthin (4, 4′-diketo-β-carotene) is an orange-red xanthophyll (a sub-class of carotenoids) with strong antioxidant activity (Bhosale and Bernstein, 2005). Canthaxanthin is widely used in the pharmaceutical, medical, cosmetic, fishery, poultry and food industries (Nasrabadi and Razavi, 2010). Commercial demand for canthaxanthin is mainly satisfied by chemical synthesis. However, as the application of synthetic substances is restricted in food, cosmetic and pharmaceutical industrials, production of canthaxanthin from biological sources has been attended in recent years (Ausich, 1997; Hojjati *et al.,* 2014).

For the industrial production of carotenoids, microorganisms are preferred over other natural sources, such as vegetables and fruits, owing to problems of seasonal and geographic variability in production. In addition, there are economic advantages to microbial processes that use agricultural waste and industrial wastewater as substrates (Buzzini, 2000). However, most carotenoids, including canthaxanthin, are highly unsaturated molecules and therefore highly susceptible to environmental conditions such as light, oxygen and water. Emulsification and encapsulation are among the methods that can be applied for stabilizing such components in food industry (Higuera-Ciapara *et al.,* 2004; Hojjati *et al.,* 2014).

Micro- and nanoencapsulation is the technique by which the sensitive ingredients are packed within a coating or wall material. The wall material protects the sensitive ingredient (or core) against adverse reaction,

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prevents the loss of volatile ingredient and controls release of the ingredient (Shahidi and Han, 1993; Risch, 1995). In addition, encapsulation can convert liquids into free-flowing powers, which are easy to handle. Encapsulation has found many applications in food industry. Important applications are to coat colorants, flavors, vitamins, and other sensitive food ingredients in order to increase their shelf life (Shahidi and Han, 1993). Various techniques are employed to encapsulate food ingredients (Loksuwan, 2007).

Electrospraying is a simple and highly versatile method to produce fibers and/or capsules in the sub-micron range, presenting a large surface to volume ratio, through the action of an external electric field applied between two electrodes and imposed on a polymer solution or melt. Therefore, high temperature is not required in the process. Moreover, although many polymers require organic solvents for their dissolution and subsequent electrospraying, biopolymers can be electrospun from a watery solution just by adjusting the process parameters and/or changing the solution properties through the addition of proper additives. The electrospraying technique has been widely used to generate nanofibers with applications in various fields such as regenerative medicine (tissue scaffolds), catalysis or filtration (Subbiah *et al.,* 2005), but it also has a great potential in the food science area for the development of novel functional ingredients, as it has been recently demonstrated (López-Rubio *et al.,* 2009; Torres-Giner *et al.,* 2010; López-Rubio and Lagaron, 2012).

The morphology of the structures obtained through electrospraying can be varied by adjusting the process parameters and, for a certain material, reduced size capsules can be obtained through adjusting the polymer concentration and the tip-to-collector distance. In this case, the electrospinning process is normally referred to as "electrospraying" due to the non-continuous nature of the structures obtained (López-Rubio and Lagaron, 2012).

In this work, we have studied the suitability of the electrospinning (in this case, electrospraying) technique to generate natural canthaxanthin-containing micro- and nanocapsules in various food hydrocolloid matrices for potential food applications. Specifically, whey protein concentrate (WPC) from milk and a polysaccharide (dextran) were evaluated as matrices. The use of WPC as an encapsulating matrix has barely been explored, although it has a great potential due to the excellent functional characteristics of this protein mixture and its low cost (López-Rubio and Lagaron, 2012). The morphology and the molecular organization of the capsules obtained were evaluated. Moreover, the stability of encapsulated canthaxanthin was carried out taking into account different situations that can be found in the food industry, such as the incorporation of the capsules into aqueous-based foods and exposure to thermal treatments.

MATERIALS AND METHODS

Canthaxanthin, whey protein concentrate (WPC), dextran (Mw ?70,000) and corn oil were all purchased from Sigma-Aldrich (St. Louis, MO, USA). The composition per 100 g of WPC consisted of 80 g of protein, 9 g of lactose, 8 g of lipids and 2.8 g of water, being the rest minerals like sodium and potassium. Acetonitrile, hexane, dichloromethane and methanol (all HPLC grade) were purchased from Merck (Damstadt, Germany). Pure ethanol (99.9%, v/v) was purchased from Bidestan Company (Qazvin, Iran). All the chemicals were used as received, without further purification.

Preparation of the Encapsulation Solutions

The samples of shell solutions were prepared by dissolving a variety of 20% (w/v), 25% (w/v), 30% (w/v), 35% (w/v) and 40% (w/v) of WPC or dextran in distilled water. The core solution was prepared by dissolving 1.25% (w/v) of canthaxanthin in corn oil. For the emulsion electrospraying, an aqueous surfactant solution was prepared by dissolving 10% (w/v) of Tween-20 in water. Afterwards, 16% (v/v) of canthaxanthin/soybean oil solution [1.25% (w/v)] was added to the surfactant solution to prepare an emulsion premix. The premix was ultrasonicated at 10% of amplitude and 20 kHz of process frequency for 120 s using an ultrasonic homogenizer. These conditions led to an oil-in-water (O/W) emulsion. Finally, the encapsulation solutions were prepared by dissolving the emulsion in the samples of shell solutions at ratios of 3 : 1 and 4 : 1 (Shell/Core) and then they were mechanically stirred using a laboratory mixer (Ultra Turrax T25; IKA, Staufen, Germany). This emulsion was further homogenized at 25 MPa with three recirculations by high pressure laboratory homogenizer (APV Homogenizers, Albertslund, Denmark) until homogeneous emulsions were obtained.

Characterization of the Electrospraying Solutions

The apparent viscosity of the polymeric solutions at 100 s^{-1} was determined using a rotational viscosity meter (San Feliu de Llobregat, Spain). The surface tension of the solutions was measured using the Wilhemy plate method in an EasyDyne K20 tensiometer (Krüss GmbH, Hamburg, Germany). The conductivity of the solutions was measured using a conductivity meter XS Con6 (Labbox, Barcelona, Spain). All measurements were made in triplicate at 25°C.

Encapsulation through Electrospraying

The electrospraying apparatus, equipped with a variable high voltage 0-30 kV power supply, was a Fluidnatek® L-10 assembled and supplied by Bioinicia S. L. (Valencia, Spain). Solutions were introduced in a 5-ml plastic syringe and were electrosprayed under a steady flow rate using a stainless-steel needle. For the coaxial electrospraying, two concentrical needles were used. The inner one was used for the core material and the outer one for the shell solution. The needle was connected through a PTFE wire to the syringe. The syringe was lying on a digitally controlled syringe pump, while the needle was in horizontal towards a stainless-steel plate attached to a copper grid used as collector. More details of the electrospraying equipment and the different uniaxial and coaxial configurations can be found elsewhere (Pérez-Mariá *et al.,* 2013). The flow rate, voltage and the distance between the tip and the collector were fixed at 0.15 ml/h, 15 kV and 10 cm, respectively.

Scanning Electron Microscopy

Scanning electron microscope (SEM) (JEOL JSM-6301F, Jeol Ltd., Tokyo, Japan) was used to study the morphological properties of dried encapsulated materials. Powder particles were attached to SEM strubs of 1″ diameter using a two-sided adhesive tape. The samples were then sputter coated with gold and examined at 200x and 350x magnifications. An acceleration potential of 20 kV was used during micrograph.

Stability of Encapsulated Canthaxanthin

Canthaxanthin stability in the capsules was determined based on the retained pigment quantity during the storage weekly and was compared with a control sample of non-encapsulated canthaxanthin. The powder samples were poured into 15-ml glass vials and stored in a temperature-controlled incubator at 4, 25 and 30°C for 24 weeks. Samples for the zero time were analyzed within 24 h after spraying. Other samples were analyzed over a period of 24 weeks for the total retention of canthaxanthin (defined as the ratio of the total canthaxanthin content in powder at any time to the total amount of canthaxanthin in the powder at time 0).

The loss of canthaxanthin in the electrospraying process was determined by measuring the ratio of total canthaxanthin in the powder after electrospraying to the amount of initial canthaxanthin in the feed emulsion with the following equation :

$$L_c = \frac{i_c - t_c}{t_c} \times 100 \qquad \ldots(1)$$

Where, L_c is loss of canthaxanthin, i_c is initial canthaxanthin and t_c is total canthaxanthin.

Canthaxanthin Determination

The method of Desobry *et al.* (1997) with slight modification was used to analyze the total carotene and surface carotene. Total initial carotenoids concentration was determined accordingly. Fifty mg powder was dispersed in 2.5 ml of water and 25 ml of hexane was added to test tube. The tube then was sealed and agitated at 500 rpm for 30 min. The hexane fraction was measured at 455 nm (Desobry *et al.,* 1997). Surface carotenoids were determined in the finished powders by weighting 50 mg powder into test tubes and extracted with 25 ml of hexane. After 15 sec, shaking at 100 rpm and the carotene concentration in the supernatant were measured at 455 nm. The percentage of surface carotene was determined by dividing the surface concentration by the carotenoids concentration in the powder (Desobry *et al.,* 1997; Ozcelik *et al.,* 2009).

Encapsulation Yield and Encapsulation Efficiency

Encapsulation yield (MY) in the microencapsulation process was determined as the ratio of the mass of microcapsules obtained after the process to that before the process (Nunes and Mercadante, 2007). The encapsulation efficiency (ME) was calculated using the following expression (McNamee *et al.,* 1998) :

$$ME = \frac{TC - SC}{TC} \times 100 \qquad \ldots(2)$$

Where, TC and SC are total and surface carotenoid contents of the electrospray-dried powders, respectively.

Statistical Analysis

The data reported in all the presented data are average of triplicate determinations along with their standard deviations. Analysis of the data was carried out using ANOVA (SPSS program version 10.0 for Windows). Differences between means were tested using the Duncan's multiple range tests at $P<0.05$.

RESULTS AND DISCUSSION

As briefly mentioned in the introduction, the aim of this work was to develop the electrospraying method for the encapsulation and protection of canthaxanthin, evaluating WPC and dextran as encapsulation matrices (wall). One of the strategies used to incorporate the hydrophobic bioactive within the hydrocolloidal-based matrices (WPC and dextran) was to form Oil/Water (O/W) emulsions using corn oil dispersed within the aqueous phase. Later on, the encapsulating matrices were added to the solutions and the emulsions were processed using the electrospraying technique.

In the present work, the electrospraying as a physical process was used for the formation of ultrathin fibers by subjecting the polymer solutions to high electric fields. At a critical high voltage (15 kV), the polymer solution droplet at the tip of the needle distorts and forms a Taylor cone to be ejected as a charged polymer jet. This stretches and is accelerated by the electrical field towards the grounded and oppositely charged collector. As the electrospun jet travels through the electrical field, the solvent completely evaporates resulting in the deposition of the ultrathin structures on the metallic collector. The electrospraying variation used in this work is like an atomization procedure to form ultrathin particles (instead of fibers).

The main challenge of this work was to obtain WPC or dextran capsules through electrospraying starting with aqueous solutions. There are several examples in the literature dealing with electrospinning of proteins but the strategies to generate structures (fibers or beads) involve the use of organic and alcohol-based solvents (Dror *et al.,* 2008; Regev *et al.,* 2010) or blending with synthetic spinnable polymers (Cho *et al.,* 2010; Dong *et al.,* 2010). Both strategies can pose difficulties in the use of the developed materials for functional ingredient encapsulation in food applications, either due to regulatory issues or to inactivation of the bioactive ingredients when using the organic solvents (as in the case of probiotic encapsulation).

Initially, the concentration of the WPC powder or dextran had to be adjusted so as to obtain a potentially sprayable solution. Different concentrations were tested and it was found that high concentrations (>35%) of the protein were optimum for obtaining electrosprayed capsules. For concentrations below 35% only drops of material were collected.

Physical Properties of the Electrospraying Solutions

The physical properties of the electrospraying solutions containing the biopolymers were characterized as they strongly affect the stability of the process and the successful development of structures through this technology. Moreover, capsules morphology is also influenced by solution

Table 1. Properties of the different electrospraying solutions

	Viscosity (cP)	Surface tension (mN/m)	Electrical conductivity (μS)
WPC	20.2	35.1	2037.6
Dextran	28.6	36.4	93.5
WPC/Canthaxanthin	73.8	37.8	1523.5
Dextran/Canthaxanthin	121.6	37.7	110.8

properties. Table 1 compiles the viscosity, surface tension and electrical conductivity of the electrospraying solutions assayed. These final compositions were obtained after a previous optimization process in which the concentration of the various compounds was adjusted until stable emulsions and electrospraying jets were obtained. Nevertheless, it was seen that a phase separation always occurred in dextran emulsions after a few hours. On the contrary, canthaxanthin-containing WPC emulsions were stable during days, that was in agreement with those of Pérez-Masiá *et al.* (2015).

From Table 1, it can be observed that the incorporation of canthaxanthin to the dextran and WPC solutions through the emulsion process significantly affected the solution properties. Specifically, it was seen that viscosity considerably increased for both solutions because, in this case, canthaxanthin was incorporated through corn oil which was rather viscous. It is worth noting that the viscosity increase was greater for the WPC solution, which could indicate some kind of interaction between the protein and the canthaxanthin in solution. Regarding the surface tension and electrical conductivity, it was observed that for the dextran solution these parameters slightly increased when compared to the solution without canthaxanthin. However, in the case of WPC, the presence of the corn oil/canthaxanthin in the solution significantly decreased the electrical conductivity. This again could be ascribed to the interaction between the protein and the canthaxanthin, thus, resulting in the neutralizathion of some of the protein charges (Pérez-Masiá *et al.,* 2015).

Morphology of the Canthaxanthin-containing Capsules

The scanning electron microscopy (SEM) was used in order to analyze capsules' size and morphology. The SEM images of canthaxanthin-containing capsules with matrices of dextran and WPC shown in Fig. 1, demonstrate that round, smooth canthaxanthin-containing capsules are obtained through electrospraying from a 40 wt.% WPC or dextran concentration in aqueous solution.

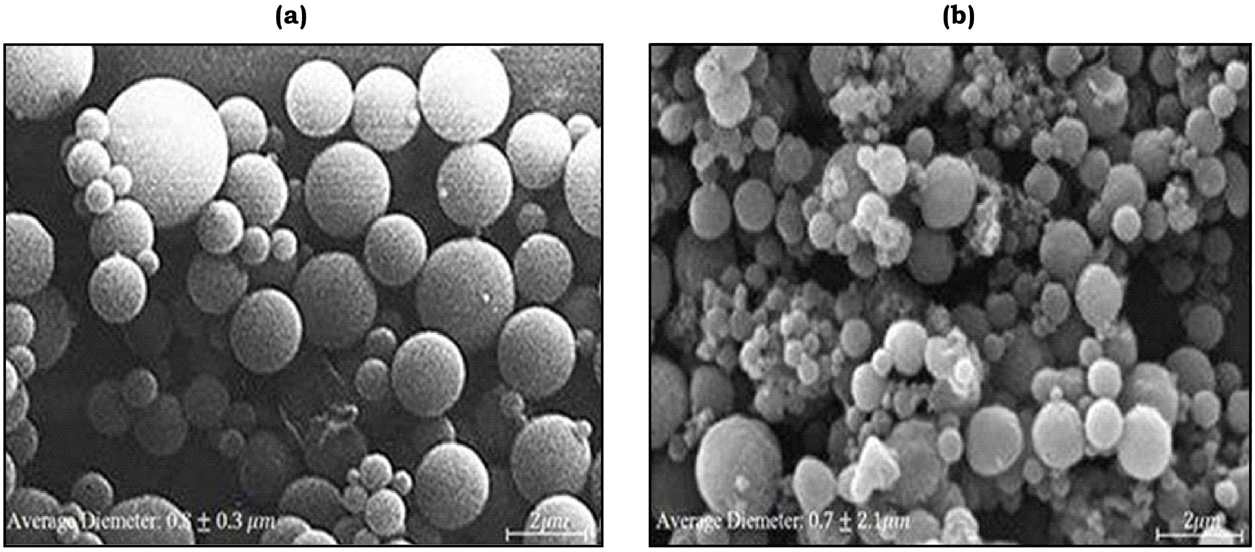

Fig. 1. Selected SEM images of dextran/canthaxanthin capsules (a) and WPC/canthaxanthin capsules (b) obtained through electro-spraying.

Emulsion electrospraying of the dextran-based solution led to very homogeneous capsule sizes, while WPC-based solution produced more aggregated particles. This result might be explained by the greater electrical conductivity of the protein solutions, which could destabilize the electrospraying jet, thus producing different particle sizes. In this case, aggregated particles were also seen when electrospraying was used, probably because of oil leakages (Pérez-Masiá *et al.,* 2015).

Capsule size is important for ingredient delivery. Larger particles generally release encapsulated compounds more slowly and over longer time periods, while particle size reduction introduces several bio-adhesive improvement factors, including increased adhesive force and prolonged gastrointestinal transit time, leading to a higher drug bioavailability (Chen *et al.,* 2006). Therefore, by changing the wall/core ratio and the wall material concentration, it is possible to control the morphology of the generated capsules depending on the specific application needs (López-Rubio and Lagaron, 2012).

Encapsulation Yield (MY)

Encapsulation yield for the electrospraying depends on the equipment configuration (Nunes and Mercadante, 2007). Fig. 2(a) shows the mean MY values of the canthaxanthin encapsulated by WPC in wall/core ratios of 3 : 1 and 4 : 1 and various concentrations of WPC. With increasing the WPC concentration from 20 to 40%, MY in the samples of wall/core ratios of 3 : 1 and 4 : 1 decreased from 80.4 to 60.2% and 78.8 to 56.7%, respectively. In all WPC concentrations, MY decreased slightly with increment of the wall/core ratio from 3 : 1 to 4 : 1. Such changes are in agreement with the results of Shu *et al.* (2006), who studied the microencapsulation of lycopene by spray-drying. In their study, when wall/core ratio decreased (from 16 : 1 to 2 : 1), MY value also decreased (from 91.3 to 76.1%). Comparing to other studies, the difference in MY values obtained in the present study could be related either to the equipments and process conditions or to the difference in the wall materials. When studying the lycopene encapsulation using spray dryer (Changzhou Equipment, Changzhou, China), they reported MY values of 74.3-91.3% when using gelatin/sucrose as a wall at 180-210°C inlet temperature. Also, Nunes and Mercadante (2007) reported a MY of 51% for lycopene microencapsulation by spray dryer (Lab plant SD-4; Keison Products, Chelmsford, UK) with β-cyclodextrin at 170°C inlet temperature.

Fig. 2(b) shows the mean MY values of the canthaxanthin encapsulated by dextran in wall/core ratios of 3 : 1 and 4 : 1 and various concentrations of dextran. Same as MY of the canthaxanthin encapsulated by WPC, MY of the canthaxanthin encapsulated by dextran

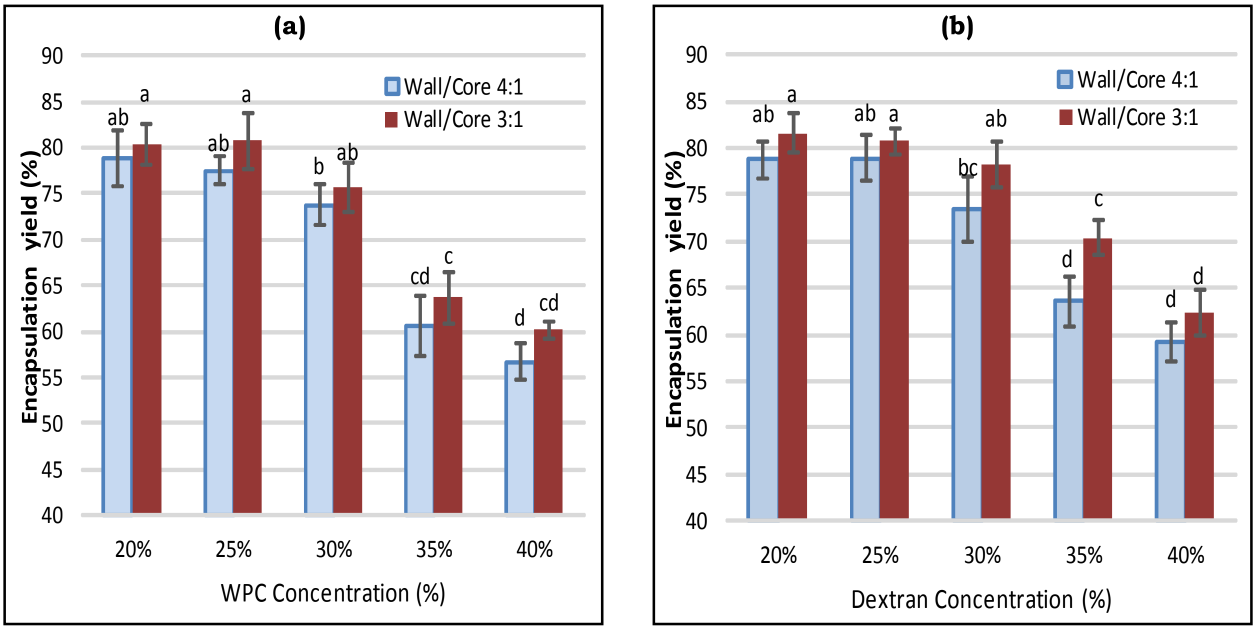

Fig. 2. Encapsulation yield of electrospraying WPC/canthaxanthin (a) and dextran/canthaxanthin (b).

decreased with increase in the concentration of dextran in both the ratios of wall/core. Moreover, increase in the wall/core ratio caused a reduction in the MY of canthaxanthin encapsulated by dextran.

Encapsulation Efficiency (ME)

ME is the fraction of encapsulated canthaxanthin in the powder. Therefore, its quantity is also dependent on the surface (un-capsulated) canthaxanthin level in the powder. Both surface and total canthaxanthin contents of electro-sprayed capsules in the studied concentrations of wall materials and wall/core ratios are shown in Table 2. The encapsulation efficiency is also included in this table and it was calculated by dividing the canthaxanthin concentration inside the capsules by the total canthaxanthin concentration. Significant differences ($P<0.05$) were found among the surface canthaxanthin contents, total canthaxanthin contents and ME levels of all the samples of this study. The highest total canthaxhanthin contents and the lowest surface canthaxanthin contents corresponding to the highest ME levels were found in the capsules prepared with WPC as wall material. However, the capsules prepared with dextran had the most surface canthaxanthin contents and therefore the lowest ME levels. These data are in agreement with those reported by Pérez-Mariá *et al.* (2013), who encapsulated lycopene with a variety of wall materials including WPC and dextran. This means that the surface and total canthaxanthin contents (or ME values) were correlated directly with the type of wall materials. It is known that the protein fraction of materials is adsorbed on the surface of the oil droplets in the oil-in-water emulsions and stabilizes such emulsions (Nakamura *et al.,* 2004). Therefore, since WPC contains proteinous residues, they could produce microcapsules with higher ME levels.

Table 2 also shows that in the case of dextran, emulsion electrospraying also led to low encapsulation efficiency, due to the unstable emulsion formed with this polymer. While in the case of WPC, it was seen that emulsion electrospraying led to proper encapsulation efficiency, since WPC and canthaxanthin solution produced a stable emulsion which did not separate during the electrospraying experiment. Regarding the electrospraying canthaxanthin with dextran, very high variability was found, probably because this technique led to a worse encapsulation process with oil leakages, which made that some capsules presented very high canthaxanthin contents, while other structures presented very low encapsulation yields.

For both the wall materials, ME increased constantly with increase in the wall material concentration from 20 to 40%. Increasing the wall/core ratio to 4 : 1 also caused a significant increase in ME, which is in good agreement with the results of other studies (McNamee *et al.,* 1998; Shu *et al.,* 2006; Hojjati *et al.,* 2011). The surface oil or carotenoid can be easily oxidized. Therefore, the amount of surface carotenoid on the capsules is quite important for a stable storage.

Table 2. Surface and total canthaxanthin and encapsulation efficiency of capsules produced using electro-spraying

Wall material concentration (%)	Surface carotenoids (µg/mg)		Total carotenoids (µg/mg)		Encapsulation efficiency (%)	
	WPC	Dextran	WPC	Dextran	WPC	Dextran
Wall/Core ratio of 4 : 1						
20%	0.35 ± 0.03^{d}	0.55 ± 0.04^{d}	0.80 ± 0.03^{g}	1.34 ± 0.04^{d}	56 ± 3.3^{cd}	59 ± 3.2^{c}
25%	0.30 ± 0.01^{de}	0.52 ± 0.03^{d}	1.00 ± 0.05^{f}	1.53 ± 0.03^{c}	70 ± 4.1^{bc}	66 ± 2.4^{b}
30%	0.29 ± 0.02^{de}	0.43 ± 0.01^{e}	1.16 ± 0.02^{e}	1.34 ± 0.04^{d}	75 ± 3.2^{b}	68 ± 1.2^{b}
35%	0.23 ± 0.01^{e}	0.43 ± 0.01^{e}	1.28 ± 0.01^{d}	1.72 ± 0.10^{b}	82 ± 1.5^{a}	75 ± 1.0^{a}
40%	0.22 ± 0.01^{e}	0.30 ± 0.03^{f}	1.47 ± 0.06^{c}	1.20 ± 0.07^{e}	85 ± 3.2^{a}	75 ± 1.7^{a}
Wall/Core ratio of 3 : 1						
20%	0.78 ± 0.02^{a}	0.95 ± 0.03^{a}	1.50 ± 0.02^{c}	1.58 ± 0.05^{c}	48 ± 2.2^{d}	40 ± 2.5^{d}
25%	0.77 ± 0.05^{a}	0.85 ± 0.01^{b}	1.83 ± 0.08^{ab}	1.60 ± 0.03^{c}	58 ± 1.4^{cd}	47 ± 3.1^{cd}
30%	0.68 ± 0.01^{b}	0.83 ± 0.02^{b}	1.48 ± 0.07^{c}	1.84 ± 0.11^{ab}	54 ± 1.4^{cd}	55 ± 2.4^{c}
35%	0.69 ± 0.04^{ab}	0.68 ± 0.01^{c}	1.82 ± 0.10^{ab}	1.79 ± 0.04^{b}	62 ± 3.6^{bc}	53 ± 3.3^{c}
40%	0.54 ± 0.03^{c}	0.68 ± 0.04^{c}	2.00 ± 0.08^{a}	2.00 ± 0.08^{a}	73 ± 2.5^{b}	66 ± 2.0^{b}

Mean±SD. In each column, means followed by different superscripts are significantly different ($P<0.05$) based on Duncan's multiple range test.

Table 2 shows that the lowest level of surface canthaxanthin was found in the samples of WPC as a wall material with the wall/core ratio of 4 : 1 and WPC concentration of 40%. Higher content of canthaxanthin was correlated with the higher surface canthaxanthin and lower ME. In the studied capsules, at the higher content of canthaxanthin, the particles would have provided larger quantities of surface canthaxanthin for the extraction resulting in low ME.

Stability

Storage treatments are commonly employed for food processing and, thus, canthaxanthin stability under different conditions is an important attribute to evaluate (Pérez-Masiá *et al.*, 2015). Specifically in this work, the stability of non-encapsulated canthaxanthin and canthaxanthin encapsulated in WPC and dextran capsules obtained through emulsion electrospraying was studied.

Encapsulated canthaxanthin was stored at 4, 25 and 30°C for 24 weeks and their storage stabilities were determined by measuring their total canthaxanthin contents during the storage period on a weekly basis (Figs. 3 and 4). Total canthaxanthin contents decreased with increase in the storage temperature. Capsules prepared with WPC exhibited higher retention values in comparison with those of dextran. Therefore, dextran was not an effective wall material for preventing the oxidation of canthaxanthin. This could be attributed to the lack of emulsification ability and low film-forming capacity for the samples prepared by dextran (Loksuwan, 2007; Hojjati *et al.*, 2011; Hojjati *et al.*, 2014).

In the previous research, a variety of wall materials including WPC and dextran were studied for lycopene encapsulation (Pérez-Mariá *et al.*, 2013). In agreement with the results of the current study, WPC in the above study was reported to be more effective wall material for the encapsulation and improvement of the stability of the lycopene cores. The hydrophobic peptides present in WPC are adsorbed on the surfaces of oil droplets and act as an anchor and consequently create a strong protective film around the oil droplets in the emulsion

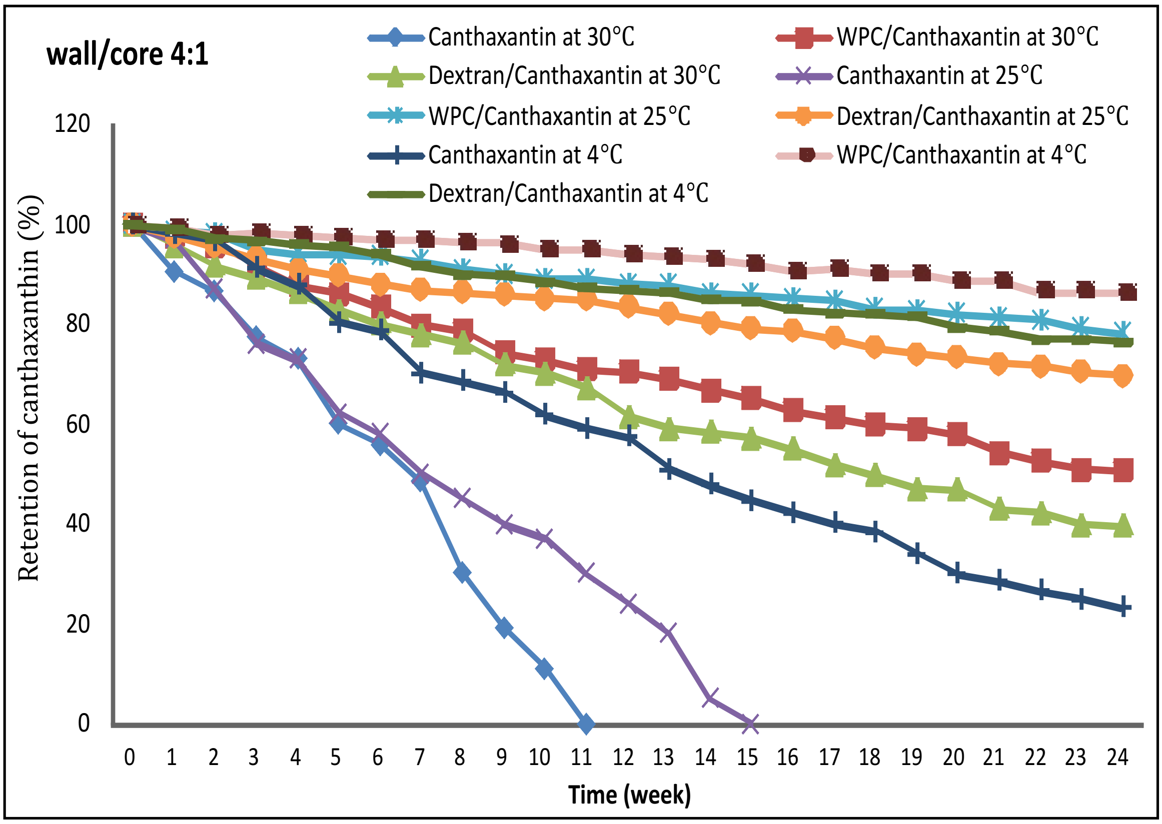

Fig. 3. Storage stability of pure canthaxanthin and encapsulated canthaxanthin with wall/core ratio of 4 : 1 at different storage temperatures.

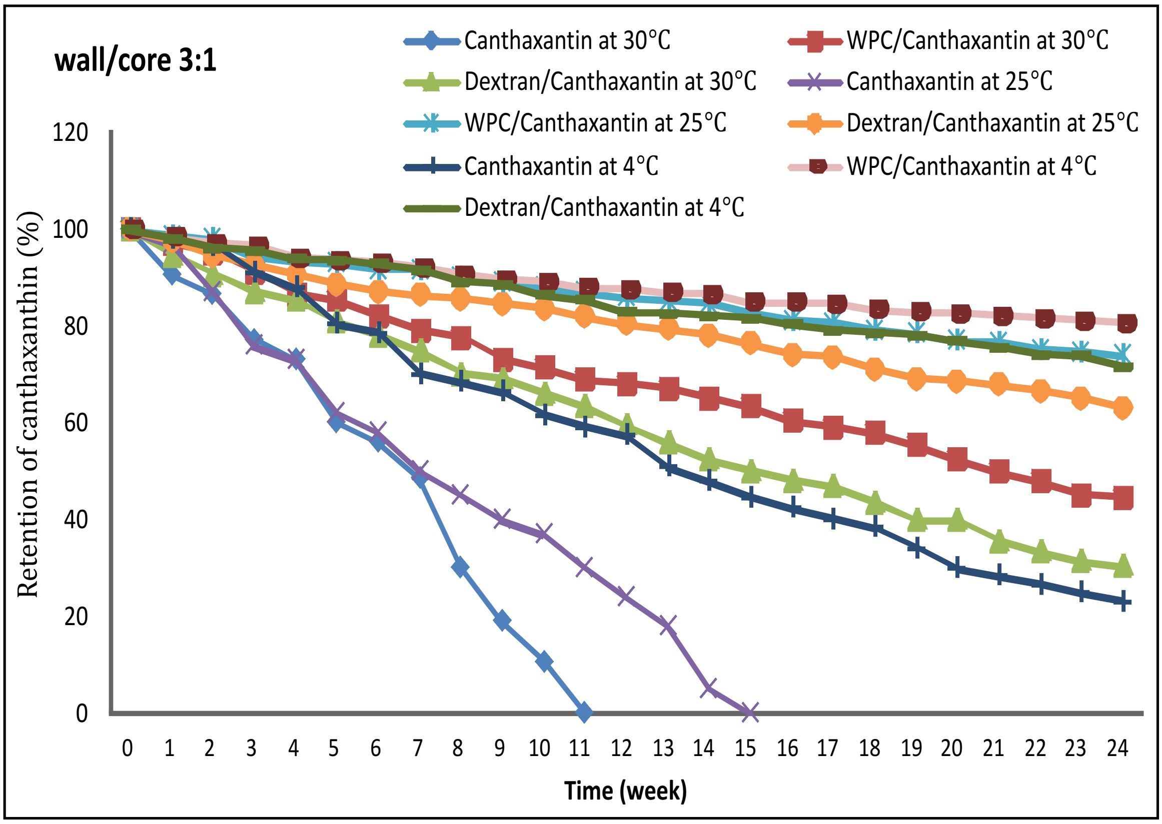

Fig. 4. Storage stability of pure canthaxanthin and encapsulated canthaxanthin with wall/core ratio of 3 :1 at different storage temperatures.

(Nakamura *et al.*, 2004, Hojjati *et al.*, 2014). The findings from the current study showed that WPC could protect canthaxanthin more than dextran. WPC contains higher hydrophobic peptide levels compared to dextran (Maeda and Nakamura, 2009; Hojjati *et al.*, 2014).

It was observed that the temperature influenced retention of canthaxanthin in the capsules during storage. Figs. 3 and 4 show that the losses of canthaxanthin in samples at 30? were more intense than those at other temperatures. In the case of wall/core ratio of 4 : 1, the retention percentage of canthaxanthin in the encapsulated samples prepared with WPC and dextran stored for 24 weeks at 25°C was 78.1 and 69.8%, respectively, and those at 30° was 50.7 and 39.6%, respectively. Fig. 3 shows that the storage of capsules at 4°C provides the minimum loss of pigments. The retention percentage of canthaxanthin at 4°C temperature for the samples prepared with WPC and dextran over the 24 weeks of storage was 86.4 and 76.6, respectively.

According to the figures, degradation of canthaxanthin in the blank samples was very fast in comparison with the encapsulated canthaxanthin samples. Canthaxanthin content of blank samples completely deteriorated after 11 weeks of storage at 30°C, while total percentage of canthaxanthin retention in the WPC capsules after 11 weeks was 71.

This result showed that the encapsulation could keep a large amount of colour of canthaxanthin and it was confirmed that WPC played a superior role in retarding of degradation process of the core. This is in agreement with the results of Pérez-Mariá *et al.* (2013) for the encapsulation of lycopene by WPC. It is evident that the WPC could protect canthaxanthin against damages of oxygen and light during the storage because of its suitable emulsifying and film forming properties (Hojjati *et al.*, 2011).

Oxidation of canthaxanthin was slower for the capsules with higher ratios of wall/core. The best storage stability and encapsulation efficiency were obtained when the ratio of wall/core was at maximum level of this study (4 : 1), which was also associated with a smaller droplet size and less surface canthaxanthin (Hojjati *et al.*, 2011).

CONCLUSION

In this work, it has been demonstrated that canthaxanthin can be properly encapsulated through electrospraying using aqueous biopolymer solutions including whey protein concentrate (WPC) and dextran. The results show the feasibility of the electrospinning technique for the development of protein-based encapsulation structures avoiding the use of organic solvents and/or high temperatures. Moreover, depending on the wall material used, the concentration and the wall/core ratio, the molecular organization and, thus, final properties of the capsules (for instance, in terms of stability) change, which have implications for the practical applications of the capsules obtained. The capsules have also demonstrated to be able to stabilize canthaxanthin, which was electrosprayed in aqueous solutions of WPC or dextran capsules with concentrations of 20-40%. The technique has proved very high encapsulation efficiency and the capsules developed successfully stabilized canthaxanthin, especially in the wall/core ratio of 4 : 1. Increasing the wall thickness and wall material concentration has significantly enhanced encapsulation efficiency and stability. It was also seen that in comparison with dextran-based capsules, WPC-based capsules were more able to protect canthaxanthin in terms of encapsulation efficiency and stability and, thus, this kind of capsules could be used to increase canthaxanthin shelf life when incorporated within different food products. Now-a-days, pilot plant and industrial-based electrospinning equipment are available which would allow the scaling up of this encapsulation process.

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