



## Encapsulation of a lycopene-rich watermelon concentrate in alginate and pectin beads: Characterization and stability

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### ABSTRACT

The aim of this work was to evaluate the influence of the type of polymer and the effect of drying on the stability of lycopene-rich beads obtained by ionic gelation of a watermelon concentrate. Sodium alginate and pectin were used as polymers for encapsulation. The wet and the dried beads were characterized for lycopene retention when exposed to different thermal treatments (60 °C and 90 °C) and pHs (2, 5 and 8), as well as for particle size distribution, morphology and thermogravimetric analysis. All the particles showed high lycopene protection against the evaluated conditions of temperature and pH. Storage stability at different temperatures was also evaluated. The wet beads produced with alginate and pectin showed lycopene retention of 29 and 21%, respectively, after 8 weeks of refrigerated storage, while the dried beads were showed retentions higher than 80% above 25 °C. Ionic gelation showed to be a promising alternative to obtain stable lycopene-rich dried ingredients.

### 1. Introduction

Watermelon (*Citrullus lanatus*) is an economically important fruit and one of the main vegetable sources of lycopene, its main bioactive component (Kong et al., 2017). Lycopene is known for its antioxidant properties and thus, its consumption has been associated to a prevention against some types of cancer and degenerative diseases (Costa-Rodrigues, Pinho, & Monteiro, 2018; Zu et al., 2014). Moreover, lycopene is responsible for the red color of watermelon pulp and thus can be considered a potential natural colorant for use in the food industry.

However, due to the presence of double bonds in its structure, lycopene is very unstable to factors such as oxygen, light, heat and humidity, which can affect its stability during processing and storage, since oxidation may promote losses in its coloration and functional activity (Rodríguez-Amaya, 2001). In this sense, some studies have shown that encapsulation can increase lycopene stability when exposed to adverse processing conditions (Aguirre Calvo, Busch, & Santagapita, 2017; Rocha, Fávoro-Trindade, & Grosso, 2012) and enhance carotenoids stability and bioaccessibility (Liu, Wang, McClements, & Zou, 2018).

Various methods of encapsulation have been employed in food-related research, such as spray drying, spray cooling/chilling, ionic gelation, fluidized bed coating, coacervation, liposome entrapment,

inclusion complexation and others (Ray, Raychaudhuri, & Chakraborty, 2016; Rutz, Borges, Zambiasi, Rosa, & Silva, 2016). Ionic gelation is a method that has been increasingly used to encapsulate bioactive compounds, due to the simplicity of operation, besides being performed at mild conditions, i. e., at room temperature, without the use of organic solvents. In this technique, anionic polymers such as alginate and pectin form insoluble gels in the presence of divalent ions, such as calcium (McClements, 2017).

Alginates are the polymers most commonly used for encapsulation by ionic gelation. They are composed by units of β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues, which form homopolymeric blocks chains. For gel formation, the divalent ions (Ca<sup>2+</sup>) settle in the cavities between two or more G block chains, connecting the carboxyl groups of the guluronic residues, resulting in a three-dimensional network arrangement commonly referred to as the “egg-box”. Pectin is another polymer used for beads production by ionic gelation, which consists of a linear chain of galacturonic acid units negatively charged. Its ability to form gel is associated to the size of the galacturonic acid chain and the degree of esterification of its carboxylic groups. Low methoxyl pectins, when in contact with divalent ions such as Ca<sup>2+</sup>, which “reticulates” the chains, form a gel network by the similar “egg-box” model (Burey, Bhandari, Howes, & Gidley, 2008).

Several works have reported the encapsulation by ionic gelation of

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carotenoids such as lycopene (Aguirre Calvo & Santagapita, 2019; Celli, Teixeira, Duke, & Brooks, 2016) and  $\beta$ -carotene (Rutz et al., 2016), showing high encapsulation efficiencies and good carotenoid protection against adverse conditions. Most of them used a commercial purified carotenoid or made a lipid extraction of carotenoids from some fruits.

In a previous work of our research group, a lycopene-rich concentrate was developed directly from watermelon juice, using membrane technology, with potential for use as natural colorants or antioxidants in food products (Oliveira et al., 2016). However, further studies revealed that the lycopene content considerably decreased throughout the storage under refrigeration. Therefore, the objective of this work was to encapsulate by ionic gelation the lycopene-rich concentrate obtained by Oliveira et al. (2016), aiming at obtaining stable particles that can be applied as natural additives in foods.

## 2. Material and methods

### 2.1. Material

Fresh watermelons were purchased in the local market (Rio de Janeiro, Brazil).

The polymers used for encapsulation were sodium alginate (Dinâmica, Diadema, Brazil) and low methoxyl partially amidated pectin LM-101 AS Genu® (CP Kelco, Limeira, Brazil).

### 2.2. Preparation of the watermelon concentrate

The watermelon concentrate was produced by coupling microfiltration and diafiltration processes, as described by Oliveira et al. (2016). Briefly, cross-flow microfiltration was performed in a pilot unit comprising four tubular modules of  $\alpha$ -Al<sub>2</sub>O<sub>3</sub> membranes T1-70 with a mean pore size of 0.2  $\mu$ m (Pall Corporation, Membralox® Ceramic Membrane Products, Port Washington, NY, USA), at 35 °C, with transmembrane pressure of 2 bar. After reaching a concentration factor of 6, the microfiltration process was conducted at the same conditions, in diafiltration mode, in order to purify the pre-concentrated extract, using distilled as the washing fluid.

The concentrate was characterized for pH, soluble solid content and total titratable acidity (A.O.A.C., 2006) and lycopene content (Sadler, Davis, & Dezman, 1990, modified by; Perkins-Veazie, Collins, Pair, & Roberts, 2001).

### 2.3. Encapsulation by ionic gelation

The lycopene-rich concentrate was added of 2% (w/v) of sodium alginate or 4% (w/v) of pectin and the solution was kept in a water bath at 50 °C until dissolution. These polymer concentrations were selected in preliminary tests as the minimum concentrations required for obtaining spherical beads (visually observed).

Encapsulation was performed according to the methodology proposed by Belscak-Cvitanovic et al. (2015) with some modifications. The mixture (concentrate + polymer) was dripped using a 22G gauge syringe coupled to a universal holder, at 20 cm height over a 2% (w/v) CaCl<sub>2</sub> bath. After formed, the beads were kept under magnetic stirring for 30 min. Then, the liquid was drained and the beads were washed with distilled water to remove the calcium excess.

Process performance was evaluated by the encapsulation efficiency (EE), which was calculated according to Equation (2):

$$EE(\%) = \frac{\text{Lycopene in the initial solution} \times 100}{\text{Lycopene in the initial solution} - \text{Lycopene in the CaCl}_2 \text{ bath}} \quad (2)$$

Where the initial solution corresponded to the watermelon concentrate added of alginate or pectin.

Part of the wet beads was transferred to a vacuum oven drying at 60 °C for 24 h, in order to obtain dried beads.

## 2.4. Beads characterization

### 2.4.1. Particle size

For the wet beads, images containing around 100 beads were taken from a Nikon D7200 camera (Nikon Corporation, Thailand) equipped with Sigma F2.8 Ex Macro lens, focal length of 105 mm, ISO-160. Particles size was measured using the image processing software Image-J®. The smallest and largest diameters of each particle were measured, and the diameter was expressed as a mean of them (Kumara, Hayano, & Ogiwara, 2012).

The particle size distribution of the dried beads was analyzed in a SDC - Microtrac S3500 (Microtrac, Montgomery Ville, USA) laser diffraction equipment using isopropanol as dispersing medium, with 6 readings for each sample.

In both cases, results were expressed as the median diameter D<sub>50</sub>. The width distribution of droplet sizes was determined by the span value, calculated according to Equation (3):

$$\text{Span} = \frac{D_{90} - D_{10}}{D_{50}} \quad (3)$$

Where D<sub>10</sub>, D<sub>50</sub>, and D<sub>90</sub> are the diameters at 10, 50, and 90% cumulative volume, respectively.

### 2.4.2. Morphology

As mentioned, for the wet beads, morphology was observed by images taken from a Nikon D7200 camera (Nikon Corporation, Thailand) equipped with Sigma F2.8 Ex Macro lens, focal length of 105 mm, ISO-160.

For the dried particles, morphology was evaluated by scanning electron microscopy (SEM). The samples were fixed to a metal stub with a conventional double-sided adhesive tape and observed in a TM 3000 tabletop scanning electron microscope (Hitachi Ltd., Tokyo, Japan), operating at 15 kV.

### 2.4.3. Thermogravimetric analysis (TGA)

The thermal degradation profile of the beads was evaluated by thermogravimetric analysis under controlled atmosphere of N<sub>2</sub> in a Pyris 1 TGA equipment (PerkinElmer, Norwalk, USA). About 5 mg of each sample were inserted into the equipment. Mass loss measurements were performed within the temperature range of 25 °C–700 °C, at a heating rate of 10 °C/min.

### 2.4.4. Lycopene content

The beads were dissolved in sodium citrate 10% (w/v) and kept under agitation at 100 rpm in a bath at 37 °C for 20 min (Deladino, Anbinder, Navarro, & Martino, 2008).

Lycopene was determined according to the methodology described by Sadler et al. (1990) and modified by Perkins-Veazie et al. (2001), consisting in extraction with a hexane, acetone and ethanol solution (2:1:1, v:v:v) followed by reading of the hexane phase in spectrophotometer at 503 nm. Results were expressed in  $\mu$ g lycopene/g of sample.

### 2.4.5. Stability to temperature and pH

The effect of thermal treatment on lycopene stability was evaluated by placing the wet and dried beads in a water bath, at 65 °C or 90 °C, for 30 min, using a Ma093 shaker (Marconi, Piracicaba, SP) at 60 rpm.

The influence of different pHs on lycopene stability was evaluated by adding the beads to three different aqueous solutions at pH 2.0, 5.0 and 8.0, under magnetic stirring, for 1 h. The adjustment of pH was made with solutions of 0.1 M HCl or 0.1 M NaOH.

Lycopene retention was calculated by measuring the lycopene content before and after each treatment. Results were expressed as the ratio between the final and the initial lycopene content (C/C<sub>0</sub>).

**Table 1**Physicochemical characterization of *in natura* and concentrated watermelon juice.

Analysis	<i>In natura</i> juice	Concentrate
Titration acidity (g/100 g)	2.7 ± 0.02	1.94 ± 0.07
pH	5.6 ± 0.02	5.05 ± 0.01
Soluble solids (°Brix)	8.9 ± 0.05	1.5 ± 0.05
Total lycopene content (µg/g)	61.0 ± 0.8	220.6 ± 8.4

#### 2.4.6. Storage stability

For evaluation of storage stability, the dried particles were placed in metallized polyester + polyethylene bags and stored at four different temperatures: 10 °C, 7 °C, 25 °C and 40 °C. The wet beads were evaluated only at 7 °C, since freezing temperatures promoted structural losses, while storage at 25 and 40 °C led to fast microbiological deterioration. Lycopene content was evaluated for a period of 56 days. Results were expressed as the ratio between the lycopene content at each time and the initial lycopene content ( $C/C_0$ ).

#### 2.5. Statistical analysis

Results were subjected to Analysis of Variance (ANOVA) and Fisher test, with a significance level of 5% ( $p \leq 0.05$ ), using the Statistica 10.0 software (Statsoft, Tulsa, USA).

### 3. Results and discussion

#### 3.1. Characterization of the concentrated watermelon juice

Table 1 shows the characterization of the watermelon juice concentrated by microfiltration followed by diafiltration.

Microfiltration promoted an increase of 3.6 times in the lycopene content, with respect to the *in natura* watermelon juice, resulting in a concentrate with  $220.6 \pm 8.4$  µg/g lycopene. Diafiltration promoted a considerable reduction on the soluble solid content (about 6 times), which can be interesting regarding the development of an ingredient

**Table 2**Median diameter ( $D_{50}$ ) and *Span* values of the wet and dried beads produced with alginate and pectin.

Sample	$D_{50}$ (mm)	<i>Span</i>
Alginate (wet)	2.09 <sup>b</sup>	0.11 <sup>c</sup>
Pectin (wet)	2.93 <sup>a</sup>	0.13 <sup>c</sup>
Alginate (dried)	0.92 <sup>d</sup>	0.63 <sup>b</sup>
Pectin (dried)	1.24 <sup>c</sup>	0.81 <sup>a</sup>

Different letters in the same column indicates significant difference between samples ( $p \leq 0.05$ ).

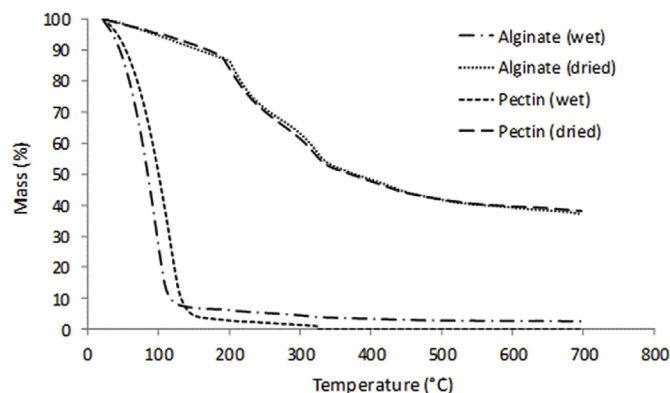


Fig. 2. Thermogravimetric curves for wet and dried alginate and pectin beads.

with coloring and/or antioxidant properties, with low sugar content. As expected, these results are similar to those found by Oliveira et al. (2016).

#### 3.2. Encapsulation by ionic gelation

The encapsulation efficiency, which was measured considering the initial lycopene content and the lycopene that was lost in the gelation bath, was around 100%, since no lycopene was detected in the  $\text{CaCl}_2$

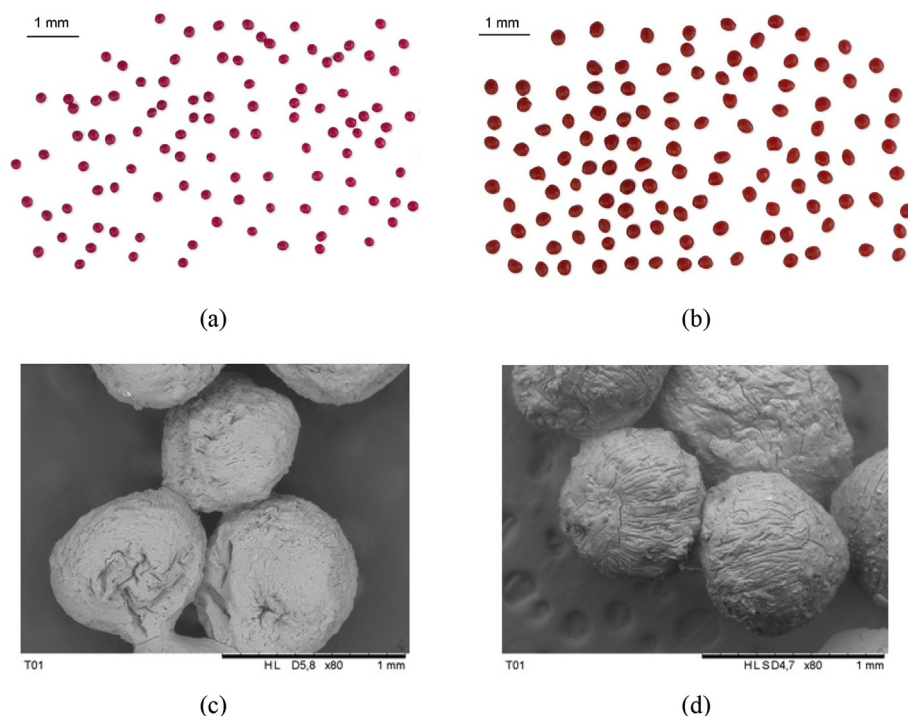


Fig. 1. Morphology of: (a) wet alginate beads, (b) wet pectin beads, (c) dried alginate beads and (d) dried pectin beads.

**Table 3**  
Thermal parameters from thermogravimetric analysis curves.

Sample	Events	$T_{onset}$ (°C)	$T_{max}$ (°C)
Alginate (wet)	1 <sup>a</sup>	58	95
Pectin (wet)	1 <sup>a</sup>	67	113
Alginate (dried) <sup>a</sup>	1 <sup>a</sup>	199	209
	2 <sup>a</sup>	299	322
Pectin (dried) <sup>a</sup>	1 <sup>a</sup>	188	199
	2 <sup>a</sup>	319	336

<sup>a</sup> Thermal events disregarding the water loss temperature.

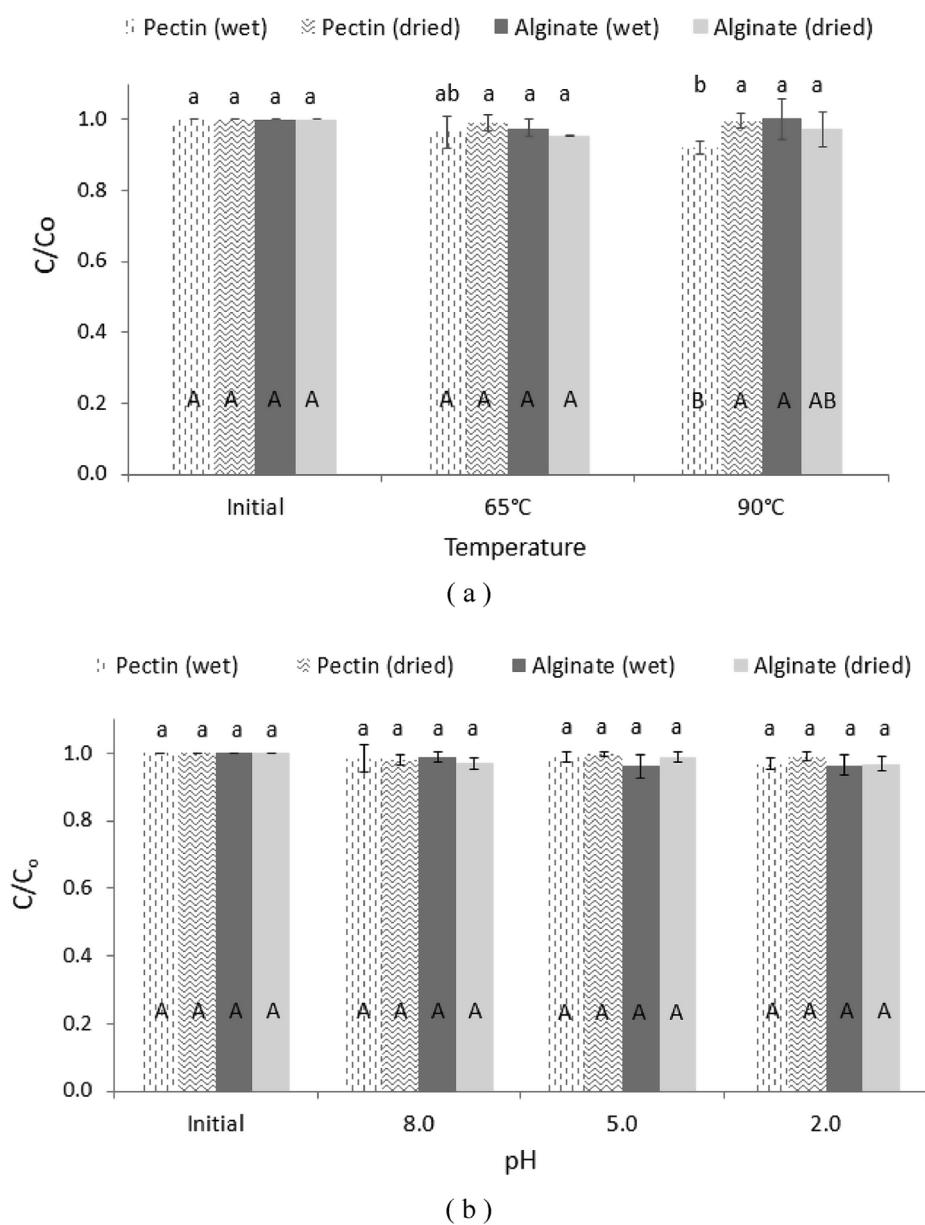
bath. This high retention can be attributed to the lipophilic nature of carotenoids and confirms that ionic gelation is a promising method to encapsulate this type of compound. High encapsulation efficiency for lipophilic substances was also reported by [Menin et al. \(2018\)](#) for flaxseed oil encapsulated in pectin beads, and by [Chan \(2011\)](#) for palm oil encapsulated in calcium alginate beads.

The lycopene content in the alginate and pectin beads, calculated in a dry basis, was  $6375.6 \pm 10.6$  and  $4007.3 \pm 6.8$   $\mu\text{g/g}$ , respectively, and no significant ( $p \leq 0.05$ ) losses were observed after drying. The higher lycopene content in the alginate beads is related to the amount of polymer used for these samples (2%), which was lower in comparison to the pectin beads (4%), since they were determined on the basis of dry weight.

### 3.2.1. Morphology and particle size

[Fig. 1](#) shows the morphology of the wet and dried beads produced with alginate and pectin.

The wet alginate beads were more round-shaped when compared to the pectin beads, which were more elongated, as shown in [Fig. 1](#) (a) and (b). This visual observation was corroborated by the circularity values calculated by the Image-J<sup>®</sup> software, which were  $0.86 \pm 0.05$  and  $0.73 \pm 0.04$  for the beads produced with alginate and pectin, respectively. Similar trend was reported by [Díaz-Rojas et al. \(2004\)](#) in the encapsulation of shark liver oil by ionic gelation using linseed pectin



**Fig. 3.** Relative lycopene content in wet and dried beads: (a) after 30 min of exposure to 65 °C and 90 °C; (b) after 60 min of exposure to different pHs. Different lower case letters indicate significant difference ( $p \leq 0.05$ ) between different samples. Different upper case letters indicate significant difference ( $p \leq 0.05$ ) between different treatments. C = Lycopene concentration after exposition to pre-determined temperatures or pHs and  $C_0$  = Initial lycopene concentration.

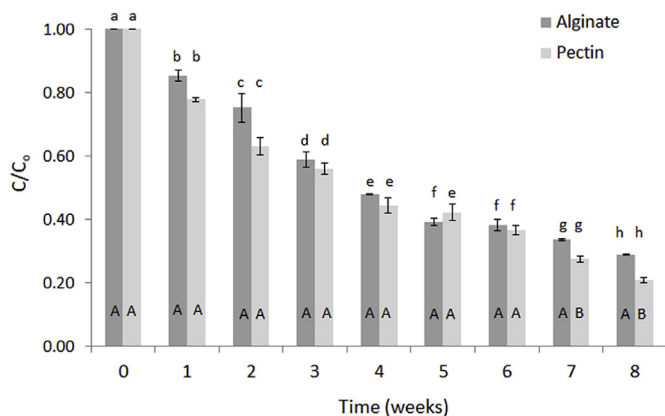


Fig. 4. Relative lycopene content in alginate and pectin wet beads along storage at 7 °C. Different lower case letters indicate significant difference ( $p \leq 0.05$ ) between different storage times, for the same sample. Different upper case letters indicate significant difference ( $p \leq 0.05$ ) between different samples, in the same storage time. C = Lycopene concentration after storage in pre-determined times, and  $C_0$  = Initial lycopene concentration.

and alginate, coated by chitosan. The authors observed that the alginate beads had regular spherical shape, while the addition of pectin in proportions of 28% and 71% led to the production of irregular spheres and irregular ellipsoids, respectively, due to the weaker mechanical stability of the pectin-calcium network compared to that from alginate-calcium, which was corroborated by measurements of mechanical strength and swelling.

In general, the dried beads showed spherical shape and rough surface, with some cracks observed especially in the alginate ones. The drying process promotes the beads deformity due to water loss, which weakens the matrix gel-structure, causing the shrinking and collapses in the beads surface. Arriola et al. (2019) observed heterogeneous surface morphologies and a wrinkled spongy shape in freeze-dried alginate beads containing stevia extracts and suggested that the addition of polysaccharides such as starch and inulin as “filler-ingredients” in the beads formulation could improve their morphological properties. Belscak-Cvitanovic et al. (2016) reported that the addition of whey proteins or hydroxypropyl methylcellulose (HPMC) in the formulation of alginate and pectin hydrogels improved the surface appearance and particles morphology of freeze-dried beads. According to the authors, these polymers, when added, are embedded in the gel matrix, acting as “structural supports” and thus controlling the beads shrinking and fractures after drying.

The median Diameter ( $D_{50}$ ) and the *Span* value of the wet and dried beads produced by ionic gelation using sodium alginate and pectin are shown in Table 2.

Both the alginate and pectin wet beads showed a narrow distribution range, confirmed by the low *Span* values, which indicates that samples were highly homogeneous with respect to size. Alginate beads diameters varied from 1.9 to 2.3 mm, while pectin beads were bigger, with diameters varying between 2.6 and 3.3 mm. This difference could be attributed to the higher amount of wall material used in the particles produced with pectin, in comparison to those produced with alginate.

This difference between the size of beads produced with the two polymers was also reported by other authors, who stated that alginate forms a more reticulated structure, which promotes higher shrinkage of the polymer gel, resulting in lower diameters, when compared to pectin (Sandoval-Castilla, Lobato-Calleros, García-Galindo, Alvarez-Ramírez, & Vernon-Carter, 2010).

As expected, particles size decreased after drying, due to shrinkage promoted by water evaporation. The dried particles showed higher polydispersity, but could still be considered homogeneous, since *Span* values were lower than 1 (Ismail et al., 2016). This is an important parameter regarding the beads application in different kinds of food

products.

### 3.2.2. Thermogravimetric analysis (TGA)

The thermogravimetric curves obtained for the alginate and pectin beads are shown in Fig. 2, and the identified peaks and main thermal events are summarized in Table 3.

As expected, the dried beads showed higher thermal stability than the wet ones. The alginate and pectin wet beads showed a progressive mass loss from 58 to 95 °C and from 67 to 113 °C, resulting in mass losses of 92% and 96%, respectively, which could be associated to the evaporation of the high water content present in the beads.

In the case of the dried alginate beads, a mass loss of 14% was observed up to 190 °C, which could be related to the desorption of the sample residual moisture. Then, a second event with  $T_{onset}$  of 199 °C was observed, while the final event of mass loss occurred at  $T_{max}$  of 322 °C. These two last events in the alginate beads could be attributed to the formation of sodium carbonate and the carbonization of polymeric chains, respectively (Paula et al., 2010).

Regarding the dried pectin beads, the initial mass loss, relative to water evaporation, was 12% and occurred up to 180 °C. The highest mass loss (36%) occurred between 188 and 199 °C, and the last event ended at 336 °C, promoting 11% of mass loss, resulting in a residual mass similar to the observed for the alginate beads. Assifaoui, Bouyer, Chambin, and Cayot (2013) studied the thermal decomposition of calcium pectinate beads produced by ionic gelation using amidated low methoxyl pectin and also observed two stages of mass loss in the intervals of 22–298 °C and 300–463 °C, which were attributed to a primary and a secondary decarboxylation involving the acid lateral group and a carbon of the pyranosidic ring.

Pu and Tang (2017) evaluated the thermal stability of lycopene and observed only 17% of residual mass after thermal decomposition at 600 °C. In addition, an endothermic peak at 173 °C and an exothermic peak at 237 °C were identified by differential scanning calorimetry, which were related to its melting point and thermal degradation, respectively. Faisal, Ruane-O’Hora, O’Driscoll, and Griffin (2013) observed a similar behavior for lycopene, with an endothermic peak at 133 °C, which could indicate a lycopene *cis*-isomer. Thus, the thermal events occurring in the present work could also be related to the thermal degradation of this carotenoid.

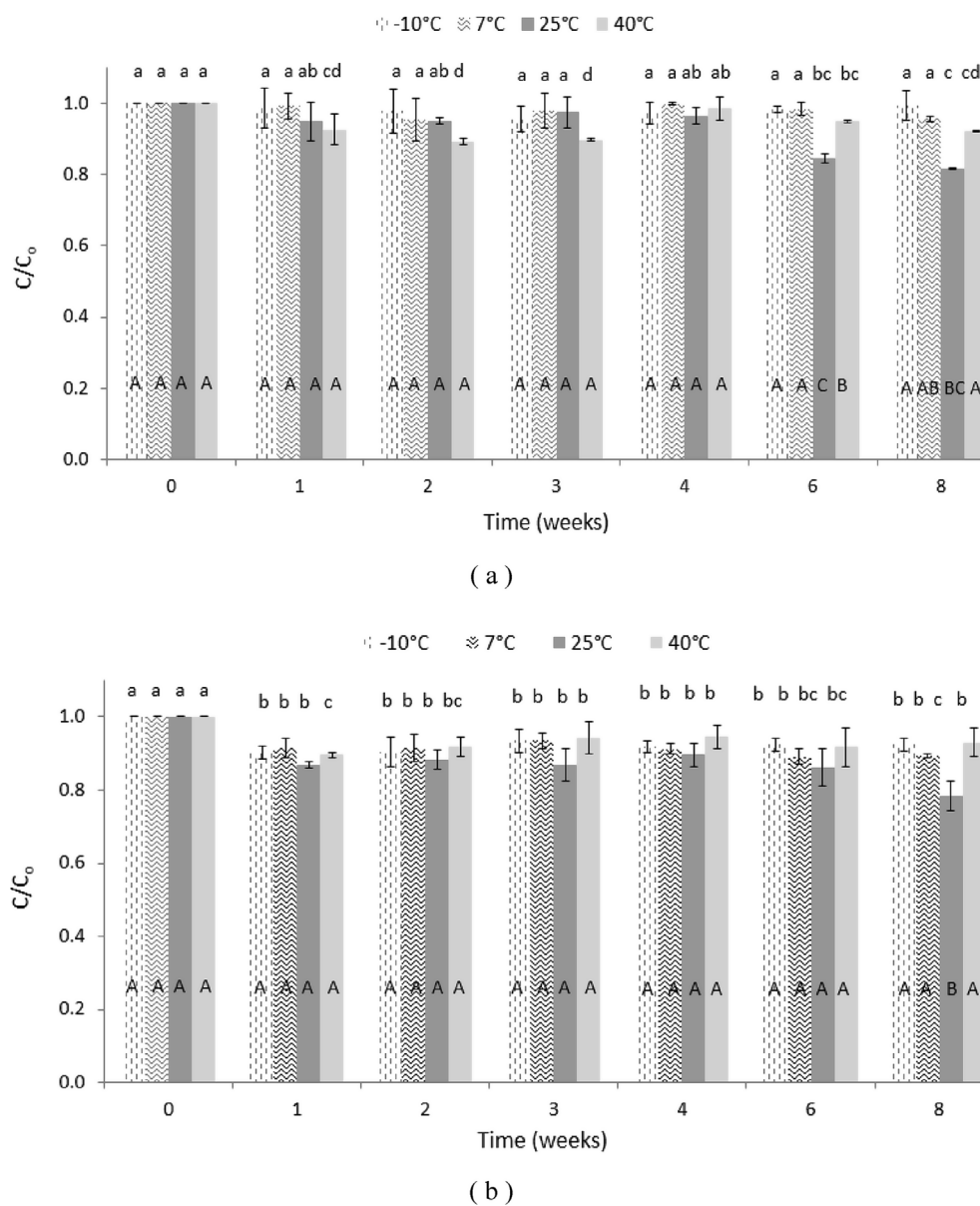
### 3.2.3. Particles stability to temperature and pH

Fig. 3 shows the relative lycopene concentration in the wet and dried beads, when exposed to different thermal treatments (65 and 90 °C, for 30 min) and pHs (2, 5 and 8).

All the lycopene beads were highly stable to thermal processing at the studied temperatures. The wet pectin beads showed a slight reduction of 8% on the lycopene content at 90 °C, which can be due to a possible isomerization or degradation, reactions that can occur at different intensities, depending on the time and temperature to which the product is exposed (Rodríguez-Amaya, 2001).

Lycopene is predominantly found in nature in the *all-trans* configuration, which is the most thermodynamically stable geometric isomer in fruits and vegetables. However, high temperatures, as well as exposition to acids, light and chemical reactions, promote isomerization of *trans* carotenoids to the *cis* forms, which leads to a decrease of color intensity. On the other hand, carotenoids can also undergo to oxidative degradation, the main cause of extensive carotenoid losses, which depends on the oxygen availability and is stimulated by light, enzymes, metals, and co-oxidation with lipid hydroperoxides (Rodríguez-Amaya, 2001).

Regarding lycopene stability to different pHs, no lycopene degradation was observed after 60 min of exposure. Low pHs are favorable to the formation of lycopene *cis*-isomers (Khoo, Prasad, Kong, Jiang, & Ismail, 2011). In this sense, considering that lycopene is highly suitable to isomerization, the polymers used for encapsulation showed satisfactory results in the protection of lycopene against the stress



**Fig. 5.** Relative lycopene content in (a) alginate dried beads and (b) pectin dried beads along storage at  $-10^\circ\text{C}$ ,  $7^\circ\text{C}$ ,  $25^\circ\text{C}$  and  $40^\circ\text{C}$ . Different lower case letters indicate significant difference ( $p \leq 0.05$ ) between different storage times, for the same temperature. Different upper case letters indicate significant difference ( $p \leq 0.05$ ) between different temperatures, in the same storage time. C = Lycopene concentration after storage in pre-determined times, and  $C_0$  = Initial lycopene concentration.

conditions applied.

Considering that lycopene is more easily absorbed when associated with the intake of a small amount of oil or fat, since it is highly hydrophobic (Bailey, 2015), the obtained results suggest that the beads produced with alginate and pectin could be applied in fat-containing products such as yogurts, fermented milks, cookies and others, in either acidic or alkaline conditions.

#### 3.2.4. Storage stability

Fig. 4 shows the lycopene stability in the wet beads stored at  $7^\circ\text{C}$ . All the wet beads showed a continuous reduction on lycopene content along time. The alginate beads showed a slightly higher lycopene retention, mainly in the first two weeks. At the end of 8 weeks of storage, lycopene retention in the alginate and pectin beads were 29% and 21%, respectively. The lower lycopene retention in the pectin beads can be attributed to the weaker mechanical stability of the pectin-calcium network when compared to the alginate-calcium gel, as previously

reported by Díaz-Rojas et al. (2004). In addition, although the viscosity of the polymer solutions was not measured, it was possible to visually observe that the alginate solution was clearly more viscous than the pectin solution, which could also explain the better performance of alginate beads.

Sodium alginate presents a pattern of guluronic acid blocks distribution that can vary according to the algae species. In the case of pectin, a de-esterification of high-methoxyl pectin is carried out to obtain low methoxyl pectin, which is able to form a gel with calcium ions. This procedure promotes chemical destabilization in its structure. In addition, pectin amidation methods result in a fairly random distribution of free galacturonic acid residues along the polymer chain. For alginate, dimers generally grow in a more sequenced mode, whereas for pectin in a more casual mode (Fang et al., 2008; Winning, Viereck, Nørgaard, Larsen, & Engelsen, 2007). In this regard, comparing the two polymers used for encapsulation, the particles produced with 2% (w/v) sodium alginate, in smaller proportions, were more effective than those

produced with 4% (w/v) pectin. This can possibly be attributed to the structural characteristics of pectin, which may cause “defects” in the formation of dimers of the “egg-box” model that occurs in the ionic gelation process.

The poor stability of the wet beads under refrigerated storage could be explained by their high moisture content (around 95%), since water is directly related to the occurrence of degradation chemical reactions. Otálora, Carriazo, Iturriaga, Osorio, and Nazareno (2016) evaluated the stability of betalain encapsulated in calcium alginate beads, stored at different conditions of relative humidity, and observed that betalain stability decreased with the increase of moisture content in the environment, which probably affected the beads moisture content.

In addition, one of the limitations of encapsulation by ionic gelation is the formation of porous particles, which enables rapid and easy diffusion of water and/or other substances into and out of the particle matrix, making easier the release of the encapsulated compound and possibly increasing the contact with atmospheric oxygen, depending on the size of the pores (Gouin, 2004). In this sense, two alternatives could be suggested to overcome this problem: (a) the addition of other polymers, such as maltodextrin or gelatin, in order to modify the gel structure and reduce its porosity, or (b) coating with another substance of opposite charge, such as chitosan or some proteins, in order to promote an electrostatic interaction after the ionic gelation, forming a complex on the beads surface. These results suggest that application and even consumption of these wet beads should be made in a short time after their production, considering the fast lycopene degradation even under refrigeration.

As mentioned before, in nature, lycopene, which mostly exists as the all-*trans*-isomer, can undergo degradation via isomerization (into its *cis*-isomer) or via oxidation during processing and storage. Heat, light and oxygen differently affect lycopene stability, since each treatment produces a different form of energy. In the present work, the mild conditions during storage (refrigerated temperature, light protection) suggests the *cis*-*trans* isomerization as the main degradation pathway of lycopene in the alginate wet beads (Shi, Wu, Brian, & Maguer, 2002).

Lycopene stability of the dried beads stored at different temperatures are shown in Fig. 5. The dried alginate beads stored at  $-10^{\circ}\text{C}$  and  $7^{\circ}\text{C}$  showed no significant differences in the lycopene content throughout time, indicating that storage under these conditions was satisfactory for preservation of this carotenoid. The dried pectin beads also had good stability, although they showed a decrease in lycopene content of approximately 10% after the first week of storage, for all the evaluated temperatures. When stored at  $25^{\circ}\text{C}$  and  $40^{\circ}\text{C}$ , the beads produced with both polymers showed a slight decrease at the end of storage, varying from 8 to 20%, which may be attributed to a possible lycopene degradation.

#### 4. Conclusions

Encapsulation by ionic gelation using sodium alginate and pectin as polymers resulted in lycopene-rich beads highly stable to different pHs and temperatures. Alginate beads were predominantly round-shaped, while pectin beads were more elongated, probably due to the weaker gel network. Lycopene retention in the wet beads were lower than 30% at the end of 8 weeks of storage, suggesting that they should be applied/consumed soon after preparation. The particles produced with sodium alginate at 2% (w/v), were more effective than those produced with 4% (w/v) pectin regarding lycopene stability. Drying considerably improved the lycopene stability during storage at temperatures varying from  $-10$  to  $40^{\circ}\text{C}$ . These results allows to conclude that dried particles produced with alginate were the best option to produce lycopene-rich beads, which could be applied as natural colorants/antioxidants in different kinds of food products.

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