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Stability and release of an encapsulated solvent-free lycopene extract in alginate-based beads

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ABSTRACT

A free-solvent lycopene extract was obtained from a natural and non-conventional source such as pink grapefruit. Five matrices were evaluated to select the matrix for lycopene extraction, choosing freeze-dried pulp based on the high content and conservation of all-trans lycopene. The extraction was dependent on both partial preservation of the fruit cellular/tissular structure and water content. The extract was then encapsulated in alginate beads with the addition of sugars and galactomannans. The influence of beads composition was studied on stability towards isomerization, transport properties and release of lycopene. Alginate beads and those supplemented with trehalose and *vinal* gum were the ones that best preserved lycopene content and minimized isomerization changes. Transport properties measured by LF-NMR showed that lower diffusion coefficients could be related to higher lycopene content in alginate-trehalose beads. Lycopene release was strongly influenced by composition. Then, it is possible to design formulations with different release rates for particular applications.

1. Introduction

Lycopene (ψ,ψ-carotene, $C_{40}H_{56}$) is a bioactive liposoluble carotenoid found naturally in certain plants (watermelon, apricot, papaya, passion fruit, pink grapefruit, carrot, and a large number of red fruits (Roldán-Gutiérrez & Luque de Castro, 2007)) and microorganisms. Lycopene main bioactivity is its ability to inactive the reactive oxygen species (ROS) preventing or delaying the oxidative damage (Singh & Goyal, 2008), reducing risk of certain types of cancer, cardiovascular diseases and hepatic fibrogenesis (Pereira dos Santos et al., 2016). As a food ingredient, carotenoids are used as antioxidants, pigments or flavor modifiers (Jaswir, Noviendri, Hasrini, & Octavianti, 2011). In nature, lycopene is present as the all-trans-isomer which is highly susceptible to isomerization into its cis-isomer and also to oxidation and degradation processes (Regier, Mayer-Miebach, Behsnilian, Neff, & Schuchmann, 2005).

Encapsulation of bioactive substances (flavors, drugs, enzymes, vitamins, essential oils and carotenoids) is use to protect them from deteriorative processes and also to control their release (Santagapita, Mazzobre, & Buera, 2011, 2012 and; Tang, Wu, & Shi, 2015). Lycopene was encapsulated by spray-drying (Goula & Adamopoulos, 2012), and by molecular inclusion with β-cyclodextrin (β-CD) or emulsification with gelatin followed by freeze-drying (Chiu et al., 2007; Nunes & Mercadante, 2007), obtaining powders as a final product. Among the encapsulation techniques, the use of ionically cross-linked hydrogels, particularly alginate-calcium beads, has the advantages of being easy to perform, with a relative low cost and eco-friendly procedure, using a nontoxic and biocompatible polymer (Gombotz & Wee, 1998), and provides extremely mild conditions without thermal treatment. Besides, the beads could be used directly as ingredient or could be incorporated in a subsequent technological process (freeze-drying and extrusion for example), giving different alternatives of dosage. Also, bio-compounds retention, mechanical strength and release rate could be managed by the addition of other compounds (food grade sugars and biopolymers) prior to hydrogel formation (Gombotz & Wee, 1998; Santagapita, Mazzobre, & Buera, 2011; Shu, Zhang, Wu, Wang, & Li, 2010; Zhang, Zhang, & McClements, 2016). So, the potential for industrial application is considerable (Santagapita et al., 2011).

Trehalose (T) is a non-reducing disaccharide which has broad biotechnological applications as dehydro- and cryo-protectant of

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labile biomolecules (Santagapita & Buera, 2008; Santagapita et al., 2012). Guar gum (GG) is an extensively used commercial galactomannan. *Vinal* gum (VG) and *espina corona* gum (ECG) are obtained from two different non-conventional sources, *Prosopis ruscifolia* and *Gleditsia amorphoides*, respectively (Busch, Kolender, Santagapita, & Buera, 2015; Perduca et al., 2013). Each of these galactomannans presents different physicochemical properties that may determine their functionality in the encapsulation systems (Wu, Li, Cui, Eskin, & Goff, 2009). Busch et al. (2017) showed that the addition of different polymers as a second ingredient in spray dried encapsulated systems also modified the protection of biocompounds and their physicochemical properties and stability.

The purpose of the present work was to encapsulate a free-solvent extract of lycopene from pink grapefruit, using alginate with trehalose and different galactomannans. The effect of the formulation on lycopene encapsulation, stability towards isomerization and release were analyzed, as well as the physicochemical properties of the obtained systems. Molecular mobility and diffusion coefficient were related to the bead encapsulation/stabilization capacity.

2. Materials and methods

2.1. Materials

Pink grapefruits (*Citrus paradisi*, Red variety, from Jujuy, Argentina) were obtained in the local market from the same batch. Not-damaged or not-defective fruits were selected, stored at room temperature until used. Several encapsulation agents were used: sodium alginate (A) from Cargill S.A. (San Isidro, Buenos Aires, Argentina), $M_W = 1.97 \cdot 10^5$ g/mol, with mannuronate/guluronate ratio = 0.6; trehalose (α-D-glucopyranosyl-(1,1)-α-D-glucopyranoside) dihydrate from Hayashibara Co., Ltd. (Shimoishii, Okayama, Japan); guar gum from Cordis S.A. (Villa Luzuriaga, Buenos Aires, Argentina), M_W~$1{,}8 \cdot 10^6$ g/mol, protein content 21 g/kg, and mannose/galactose (M/G) = 1.8; *VG*, extracted from *Prosopis ruscifolia* as reported by Busch et al. (2015), $1{,}4 \cdot 10^6$ g/mol, protein content of 19 g/kg, and M/G = 1.6; *ECG* from Idea Supply Argentina S.A (Chaco, Argentina), M_W~$1{,}4 \cdot 10^6$, protein content of 22 g/kg, and M/G = 2.5 (Perduca et al., 2013). Extra-virgin olive oil (Molino Cañuelas SACIFIA, Mendoza, Argentina) was used for lycopene extraction.

2.2. Fruit-matrix preparation and lycopene extraction

Five matrices were studied to optimize the lycopene extraction: juice, freeze-dried juice, freeze-dried slices, pulp and freeze-dried pulp. Samples were frozen for 24 h at −18 °C prior to freeze-drying, which then was performed in an ALPHA 1–4 LD2 freeze-drier (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany), operating at −56 °C and a minimum chamber pressure of 0.04 Pa, performed without shelf temperature control. Secondary drying was performed at 25 °C.

The lycopene extraction was adapted from patent ES 2 183 471 T3 (2001). Briefly 15 g of freeze-dried pulp was placed in graduated conical test tubes of 50 mL (Kartell SpA, Noviglio, Italy) and mixed with extra-virgin olive oil in a 1:2 ratio at 25 °C by stirring with a high speed blender (5000 rpm) for 15 min (using time intervals of 5 min and 1 min pause in order to avoid heating). Then, it was centrifuged at 5000 rpm for 10 min at 5 °C (model 5804, Eppendorf AG., Hamburg, Germany) and storage at 4–8 °C and darkness for further use.

2.3. Lycopene encapsulation

10 g/kg alginate with or without 200 g/kg trehalose and/or 2.5 g/kg biopolymers suspensions were prepared until complete dissolution (up to 12 h). Emulsions were produced by mixing alginate solutions containing sugars and polymers and lycopene extract (2:1 mass ratio) in an Ultra-Turra (IKA®-Werke GMBH & CO.KG, Staufen, Germany) at 15 500 rpm, for 10 min, by using time intervals of 5 min and 1 min of pause.

Beads were prepared by ionotropic gelation according to the drop method described previously (Aguirre Calvo & Santagapita, 2016; Santagapita et al., 2011, 2012). Five systems were prepared: A; A-T, AGG-T; AVG-T and AECG-T. 25 g/kg calcium chloride was used. Water content and activity, size and shape and FT-IR spectra of the produced beads were reported by Aguirre Calvo & Santagapita (2016). Beads were stored at 4–8 °C and darkness until determinations.

2.4. Lycopene content and isomer stability

Lycopene content was measured according to Fish, Wayne, Perkins, and Collins (2002). A weighted sample (0.25–0.3 g of fruit matrices, extracts, emulsions, or beads) was mixed with 3 mL of a 50:25:25 solvent mixture (hexane:ethanol:acetone containing 0.63 g/kg of BHT) in an ice bath, and stirred for 15 min (in darkness). A Ca^{2+}-quelating agent (100 g/kg potassium citrate) was added on beads samples to allow disintegration. Then, 0.45 mL of distilled water was added, stirred for another 5 min and after phase separation, hexane phase was measured in a spectrophotometer Jasco V-630 Uv–Vis (JASCO Inc., Easton, MD, USA) in the range 300–800 nm. Darkness and cold were always maintained in order to prevent light and heat damage. Lycopene content was calculated according to equation (1) and expressed as μg per g of dried sample. An average value of three replicates was reported along with the standard deviation.

$$\text{Lycopene content}\left(\frac{\mu g}{g_{\text{dried sample}}}\right) = \frac{Abs_{503} \times MW \times DF \times 10^6 \times V_{hexane}}{\varepsilon \times l \times DM} \quad (1)$$

Where: MW is the molar mass of lycopene (536.9 g/mol) (Fish et al., 2002), DF is the dilution factor, V_{hexane} is the volume of hexane (0.0015 L), ε is the molar extinction coefficient in hexane ($17.2 \cdot 10^4$ L/(mol cm), Britton, 1992), l is the path length (1 cm) and DM is the weight of sample on dry basis.

The spectral fine structure allows to identify, to analyze and to diagnose the degree of persistence of the chromophore in a given system (Britton, 1992; Rodríguez Amaya & Kimura, 2004). Lycopene spectra show the typical carotenoid shape, with peak maxima at λ_{max} at 444, 472 and 503 nm, characteristic of this chromophore (Britton, 1992; Rodríguez Amaya & Kimura, 2004). The spectral fine structure was analyzed through the %III/II ratio. This index is the ratio of the height of the longest wavelength absorption peak (at 503 nm), designated as III, and the height of the middle absorption peak (at 472 nm), designated as II, taking the minimum between the two peaks as the baseline, multiplied by 100. A standard %III/II ratio for all-trans lycopene in hexane is 60–62%; if isomerization to its cis form occurs, lower values are obtained (Britton, 1992). The values of spectral fine structure does not give exact account of which isomer (since 13 double bonds can be isomerized) but revealed that much of the encapsulated all-trans lycopene was lost during processing.

2.5. Low-field proton nuclear magnetic resonance measurements (LF-NMR)

Proton transverse relaxation times and diffusion coefficient were evaluated by using specific pulse sequences in a Bruker Minispec mq20 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) with a 0.47 T magnetic field operating at a resonance frequency of 20 MHz. Beads were placed inside 10-mm outer diameter tubes covering 1 cm of the tubes so that they did not exceed the active region of the radio frequency coil. All samples were previously equilibrated at 25.00 ± 0.01 °C in a thermal bath (Haake, model Phoenix II C35P, Thermo Electron Corporation Gmbh, Karlsruhe, Germany).

2.5.1. Proton transverse relaxation time

Proton transverse or spin-spin relaxation time (T_2) was measured in two samples for each system by applying the Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence (Carr & Purcell, 1954; Meiboom & Gill, 1958). The parameters used were: $\tau = 1$ ms, scans = 4, recycle delay = 2 s; number of points = 600, gain = 81 dB; phase cycling. A representative sample (A-T beads) was used for tune pulse duration, detection angles, gain and magnetic field homogeneity.

A bi-exponential function (as stated in equation (2)) was found to fit the experimental data adequately, from which T_2 values were obtained.

$$y = A_{21}\exp\left(\frac{-x}{T_{21}}\right) + A_{22}\left(\frac{-x}{T_{22}}\right) + offset \quad (2)$$

where: A_{21} and A_{22} are the amplitudes and T_{21} and T_{22} are the relaxation times of the two proton populations, respectively. Offset represents the difference respect to x axis ($y = 0$).

2.5.2. Diffusion coefficient

Diffusion coefficient (D) measurements were performed through the pulsed magnetic field gradient spin echo (PGSE) sequence (Stejskal & Tanner, 1965). The applied magnetic field gradient intensity (G) was calibrated in the range between 1.4 and 2.4 T/m by employing a 1.25 g/L $CuSO_4 \cdot 5H_2O$ water solution, characterized by a known D value ($2.3 \cdot 10^{-9}$ m^2/s at 25 °C (Holz, Heil, & Sacco, 2000)). The beads samples were analyzed by setting the magnetic field gradient amplitude to 1.4 T/m, τ (the time between 90° and 180° pulses) to 7.5 ms, δ to 0.5 ms, and Δ to 7.5 ms. The number of scans, the recycle delay and gain were 16, 2 s and 77 dB, respectively. D was calculated following the procedure reported by Santagapita et al. (2013).

2.6. Lycopene release

A weighted amount of beads (0.25–0.3 g) were placed into glass vials with 3 mL of the solvent mixture (hexane:ethanol:acetone containing 0.63 g/kg BHT, 50:25:25), in duplicate. Samples were constantly stirred in an ice bath and were maintained in a dark environment. At fixed intervals (5 min, for 1 h), two vials were removed, and lycopene was determined.

2.7. Statistical analysis

The effect of matrices/beads composition on lycopene content, spectral fine structure, T_2 and D were analyzed by 1-way ANOVA with Tukey post-test using Prism 5 (GraphPad Software Inc., San Diego, CA, USA). Release curves were modeled using Peppas' equation (Coppi & Iannuccelli, 2009; Peppas, 1985; Ritger & Peppas, 1987; Santagapita et al., 2011). An F test was used to evaluate significant differences between the fitted release curves and the calculated parameters (n and k) for different beads compositions.

3. Results and discussion

3.1. Lycopene extract from pink grapefruit: fruit-matrix selection and isomer stability

Lycopene was extracted from wet (pulp and juice) and freeze-dried matrices (pulp, juice and slices) in order to assess both influence of water content and tissular/cellular structure. Lycopene concentration is shown in Fig. 1a. Among wet matrices, pulp was a better matrix than juice for lycopene extraction. This is probably related to the fact that some of the hydrophobic compounds are trapped in the cell/tissular structures of the pulp, and they are not fully released during the juice obtention. The freeze-dried (FD) pulp showed the highest concentration of lycopene. Atasoy (2012) showed that since lycopene is a highly fat-soluble organic compound, the prior removal of water from the matrix allows the concentration of the solute, thereby increasing availability and minimizing the volume of the extracting agent. FD slices showed lower values of lycopene content than the other FD matrices, probably because the extraction process from a much-conserved cellular structure is more complex, since the tissue structure is

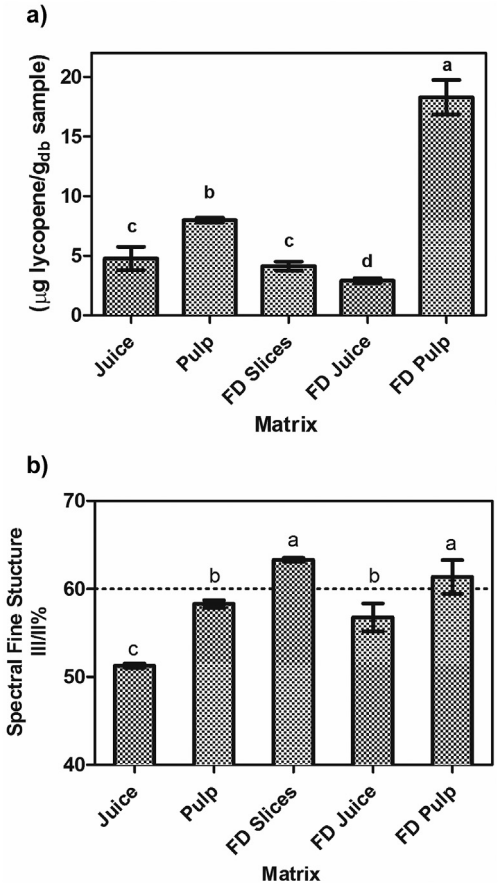

Fig. 1. Evaluation of the lycopene content per gram of dried samples (a) and lycopene spectral fine structure (%III/II) (b) of pink grapefruit matrices. The %III/II is the ratio between the height of the longest-wavelength absorption peak (503 nm, designated as III) and that of the middle absorption peak (472 nm, designated as II), taking the minimum between the two peaks as the baseline. Standard deviations are included. Different letters on the columns (a–d) indicate significant differences between values with $p < 0.05$. FD: freeze-dried material.

better conserved than in pulp. For FD slices it would be necessary to use larger extraction times, specific enzymes or ultrasound to achieve similar results than FD pulp.

The fine structure of each spectrum was further analyzed: each particular chromophore possesses a known spectral fine structure, which allows to identify it and to follow the changes associated especially to isomerization (Britton, 1992). The obtained index %III/II values for the various matrices are shown in Fig. 1b. The FD-pulp and FD-slices %III/II index correspond to all-trans lycopene isomer, which was reported by Britton (1992) to be 60–62%. Then, taking into consideration both lycopene content and fine structure conservation, FD-pulp was the selected matrix to maximize the concentration of extracted lycopene for further encapsulation. The other matrices showed lower %III/II values, revealing that a part of the lycopene was isomerized (trans → cis) during the extraction.

3.2. Encapsulation of lycopene on alginate-based beads

By using olive oil as extracting lipid (free-solvent extraction), it was possible to obtain 79± 3% of the lycopene present in the freeze-dried pulp in a single extraction from pink grapefruit. Subsequent extractions did not improve the efficiency, as the volume of oil was increased. Prior to the encapsulation, the lycopene extract was emulsified with several formulations. After the emulsification process, the lycopene content was 69± 1% of that present in the extract and the stability remained unaffected (96 ± 1%).

All the beads containing lycopene were orange colored (control beads without lycopene were white) and their pH values were between 5.5 and 6 for A, A-T and between 4 and 5 for the rest of the beads. This pH is higher than the pk_a values of alginate (3.38 and 3.65 (Santagapita et al., 2011)), being a necessary condition for alginate-Ca(II) formation. Water content of the beads ranged from 37 to 44 g/100 g on wet basis, water activity between 0.96 and 0.98. Beads showed an elliptical shape with circularity values up to 0.85, and with a size between 2.2 and 2.7 mm, as reported by Aguirre Calvo & Santagapita (2016).

Fig. 2 a. and b show the concentration and the spectral fine structure of lycopene (parameter %III/II), respectively, extracted from each bead system and normalized by the content of lycopene present in the extract. During bead generation, both lycopene content and stability were reduced between 20 and 70%. The %III/II reduction indicated that encapsulated lycopene was isomerized to its cis form (Britton, 1992), even though these values do not give exact account of which isomer was formed (13 double bonds can be isomerized). Galactomannan and trehalose addition did not improve lycopene content (Fig. 2a) nor its stability (Fig. 2b). Even more, the addition of ECG and GG produce a reduction of the lycopene content. These results clearly showed that the addition of a second excipient should be carefully conducted, since stability during alginate-Ca(II) bead generation could be even compromised.

The particular characteristics of each gum (molecular weight and polymer structure) have an impact on lycopene content and stability. As reported by Pushpamalar, Veeramachineni, Owh, and Loh (2016), slight variations in the biopolymers structure can modify their physicochemical properties and encapsulation ability. The higher viscosity of ECG and GG in comparison to VG (Busch et al., 2015; Perduca et al., 2013) could have an impact on lycopene emulsification prior to bead generation. Probably, it will be necessary to increase homogenization time to improve lycopene content in the presence of these gums. Besides, the lower %III/II values obtained with GG and VG could be related to the higher degree of substitution of these gums (M/G = 1.8 and 1.6, respectively) in comparison to ECG (M/G = 2.5), possibly affecting the alginate-Ca(II) network in a greater extent. The high substituted polymer chains reduced galactomannan-galactomannan interactions due to sterical factors, possibly promoting alginate-galactomannan associations.

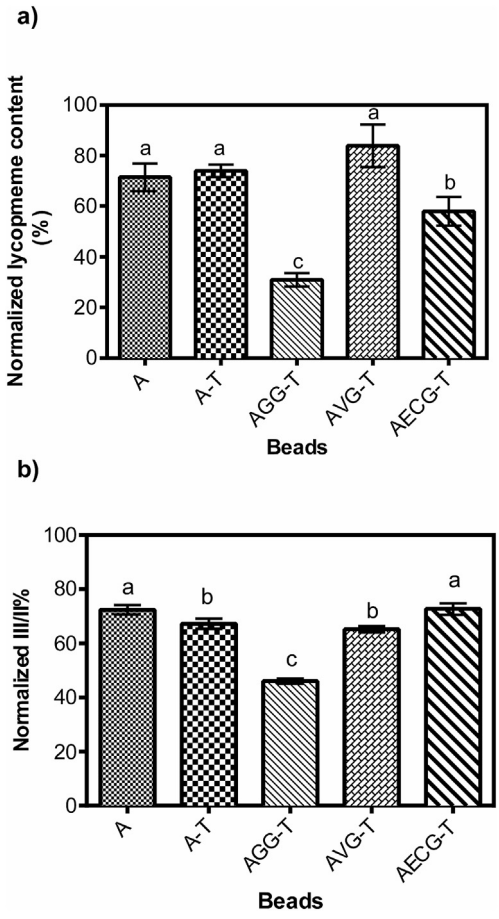

Fig. 2. Lycopene content (a) and spectral fine structure (b) normalized by their respective values of the extract used in the preparation of beads from different formulations. Standard deviations values are included. The letters above the columns indicate significant differences between values with $p < 0.05$. A: alginate; T: trehalose; GG: guar gum; VG: *vinal* gum, ECG: *espina corona* gum.

3.3. Molecular mobility and diffusion coefficients

Transverse relaxation times and diffusion coefficients obtained by LF-NMR are shown in Table 1. The CPMG sequence measurements of T_2 allow the analysis of high mobility systems (relaxation times longer than 1 ms). All beads samples containing lycopene showed two relaxation times in the 66–72 ms and 168–210 ms ranges, respectively (Table 1).

The obtained T_2 times were similar to those reported by other authors for alginate-Ca(II) beads (with a single T_2 between 40 and 80 ms) (Simpson, Grant, Blackband, & Constantinidis, 2003), between 150 and 200 m (Rayment et al., 2009), depending on the characteristics of the alginate and on the equipment. The obtained values were also coincident for the T_2 reported by Zhang, Saleh, and Shen (2013) for edible oils (two populations of approximately 80 and 220 ms). The NMR water proton response in gels or diluted polysaccharides systems is modulated by the exchange between water and biopolymers protons (Hills, Cano, & Belton, 1991; Potter, Carpenter, & Hall, 1993). As expected, the obtained T_2 values were lower than the correspondent to pure water due to the reduced flexibility of the biopolymers chains (Ablett, Lillford, Baghdadi, & Derbyshire, 1976). The magnitude of this reduction depends on

Table 1
Relaxation times (T_2) and amplitudes (A) obtained by bi-exponential fitting of the decay curves and diffusion coefficients (D) at 25 °C in beads containing lycopene determined by LF-NMR. Mean and standard deviation of (n = 2) determinations are given.

Beads	Amplitude$_1$ (%)	T_{21} (ms)	Amplitude$_2$ (%)	T_{22} (ms)	D (10^{-9} m^2/s)
A	$47 \pm 2^{(a)}$	$66 \pm 3^{(a)}$	$34 \pm 4^{(b)}$	$168 \pm 13^{(b)}$	$0.52 \pm 0.03^{(a)}$
A-T	$47 \pm 4^{(a)}$	$66 \pm 2^{(a)}$	$37 \pm 4^{(b)}$	$170 \pm 13^{(b)}$	$0.40 \pm 0.02^{(b)}$
AGG-T	$48 \pm 1^{(a)}$	$71 \pm 1^{(a)}$	$43 \pm 4^{(a)}$	$192 \pm 6^{(a,b)}$	$0.480 \pm 0.007^{(a)}$
AVG-T	$58 \pm 4^{(a)}$	$72 \pm 4^{(a)}$	$37 \pm 4^{(b)}$	$198 \pm 16^{(a,b)}$	$0.368 \pm 0.004^{(b)}$
AECG-T	$58 \pm 2^{(a)}$	$72 \pm 1^{(a)}$	$34 \pm 1^{(b)}$	$210 \pm 16^{(a)}$	$0.40 \pm 0.02^{(b)}$

$^{(a-b)}$ Different letters on the columns indicate significant differences between values with $p < 0.05$.

the aggregation state and if the system is gelled or not. Rayment et al. (2009) indicated that T_2 values are good indicators of gelation and allow to characterize the micro-structural properties of gels. In the case of the analyzed systems, not only the response obtained by NMR is from water protons but also is modulated by oil protons. Hester-Reilly and Shapley (2007) analyzed alginate beads with and without emulsions using NMR imaging, verifying that the relaxation times obtained in this study from the CPMG sequence are comparable to those obtained by these authors (about 60 ± 20 ms for the beads containing emulsions) and are much lower than those corresponding to free water (670 ± 35 ms, as reported by these authors).

The addition of trehalose (A-T) showed no significant difference with the values obtained for alginate beads (A), since the presence of a gel -alginate-Ca(II)- already causes a large decrease in the T_2 values. Higher values of T_{22} were obtained in the presence of some gums and the amplitudes of both times were kept invariant in almost all systems with a few exceptions, showing the first relaxation time higher amplitude than the second one.

Therefore, since there is an overlap of the contributions of the protons of water -exchanging with polysaccharides and sugars- and protons of oil, it is difficult to assign each of the two T_2 obtained to one or another component. Nevertheless, the diffusion coefficients results obtained at 25 °C (Table 1) presented more differences, showing different behavior between galactomannan containing systems. All beads showed diffusion coefficients values smaller than the one of water ($2.3\ 10^{-9}$ m^2/s at 25 °C (Holz et al., 2000)) due to the presence of trehalose and biopolymers. Moreover, it is probable that the presence of oil, which has lower values of D (near $9\ 10^{-12}$ m^2/s, Šmejkalová & Piccolo, 2010), provoked a further reduction of the observed values. Among the samples, beads containing VG and ECG gums showed a significant reduction in their D values in comparison to AGG-T beads. It is possible to observe that higher values of D in beads containing trehalose correspond to lower lycopene stabilities (Fig. 2b). A lower D value could be related to differences in the release/lost of lycopene from the beads during their generation, or perhaps with a greater stability of the emulsions.

It has been previously reported that sugar molecules, and particularly trehalose, can impose more local ordering to the surrounding water molecules and strengthen the hydrogen bonds between them, enhancing the tetrahedral coordinated hydrogen bond structure of water; this type of compounds are known as structure makers or kosmotropic (Barreca et al., 2014; Santagapita & Buera, 2008). In this way both T_2 and D were diminished, as revealed by LF-NMR experiments (Ekdawi-Sever, De Pablo, Feick, & Von Meerwall, 2003; Matiacevich, Santagapita, & Buera, 2010). These stronger interactions (between the trehalose and water compared to water-water interactions) are one of the reasons of its bioprotective effectiveness, by reducing the activity of the water molecules, creating a shell around biomolecules (Magazù, Migliardo, & Telling, 2008). Trehalose affects the stability of emulsions in the aqueous phase, as Álvarez Cerimedo, Iriart, Candal, & Herrera (2010) demonstrated by analyzing the volume-weighted mean diameter ($D_{4,3}$) of the particles, increasing its stability through time and towards flocculation, being this effect also content-dependent.

On the other hand, the particular characteristics of each gum impact on lycopene content and stability, possibly related to the promotion of alginate-galactomannan associations. The expected decreased of the overall mobility measured by LF-NMR depends on the reduced flexibility of the biopolymer chains with respect to water, even though the contribution of oil protons as well as the gelled state and trehalose presence are the other two main contributors. The reduced substituted polymer chains (given by M/G) of ECG in comparison to those of VG and GG provokes a more flexible structure, with a concomitant increment on T_{22} values (Table 2). Diffusion is one essential transport mechanism, and its rate in polymer hydrogels is influenced by the molecular interactions between the hydrogel and the solute/s, and by the gel microstructure (Bernin et al., 2011). Then, it could be proposed that the greater flexibility of ECG, which impact the capacity of the gum to stabilize lycopene towards isomerization (Fig. 2b), also provokes a reduced diffusion (Table 1), as a consequence of the promotion of more associations between them. Future small angle X-ray scattering (SAXS) experiments could confirm this hypothesis, by providing a detail characterization of the gel microstructure.

3.4. Lycopene release and transport mechanism modeling

The semiempirical Peppas equation was the proposed model adapted to these materials for controlled lycopene release, which simplifies the analysis by relating the fractional release of an active compound with time (Peppas, 1985; Ritger & Peppas, 1987). This model was successfully employed for different substances in alginate beads similar to the ones used in the present work (Coppi & Iannuccelli, 2009; Santagapita et al., 2011; Shu et al., 2010). Equation S(1) (see Supplementary File) adequately described the experimental data in the first part of the curves, as shown in Fig. 3. The parameters obtained by fitting Equations S1 and S2 related to transport mechanism (n, k) and diffusion coefficient (D_{eff}), respectively, are shown in Table 2. Correlation coefficients obtained for calculating n and k were greater than 0.95. D_{eff} was only calculated for Fickian diffusion cases (n ~ 0.43), as reported in Table 2.

The n value obtained for alginate beads (A) indicates that the release of lycopene is produced by diffusion, considering the lycopene size (a few nm), the average diameter of the pores in alginate-Ca(II) beads and the solvent used for extraction (Santagapita et al., 2011). Trehalose inclusion caused a change in the transport mechanism of lycopene, indicating that both the relaxation of polymeric chains and the diffusion were involved in the release of lycopene. Meanwhile, the presence of other excipients besides trehalose affected the structure of the bead by generating systems of lower n values. In the presence of guar gum, a n value close to 0.43 was obtained, while the values of the systems containing the other gums showed n values even lower. In these cases, there was a

Table 2
Parameters obtained by fitting the semi-empirical equation (Equation S(1)), number of points employed (fractional release < 0.6), transport type and diffusion coefficient (D_{eff}) calculated according to equation S(2). Mean and standard deviation of (n = 2) determinations are given.

Beads	Points	k (s^{-n})	n	Transport type	D_{eff} (10^{-11} m^2/s)
A	9	0.018 ± 0.004^{C}	0.44 ± 0.03^{B}	Fick's diffusion	2.7
A-T	8	0.0008 ± 0.0006^{D}	0.8 ± 0.1^{A}	Anomalous	–
AGG-T	5	$0.03 \pm 0.01^{B,C}$	0.43 ± 0.06^{B}	Fick's diffusion	4.8
AVG-T	8	0.23 ± 0.05^{A}	0.17 ± 0.03^{D}	Fick's diffusion [a]	–
AECG-T	5	0.06 ± 0.02^{B}	0.33 ± 0.05^{C}	Fick's diffusion [a]	–

$^{(A–D)}$ Different letters on the columns indicate significant differences between values with $p < 0.05$.
[a] Corresponds to Fick's diffusion as assigned by other authors.

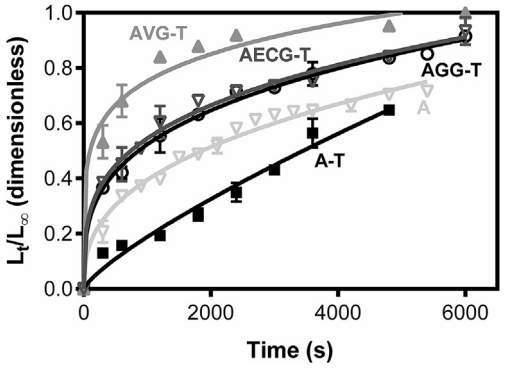

Fig. 3. Lycopene release in a solvent mixture (hexane, acetone and ethanol, 50:25:25) versus time. Lycopene release is expressed as the ratio between the lycopene released at each time (L_t) with respect to the total amount present in the beads (L_∞). Lines show the fitting obtained using Peppas equation (equation S(1)) for the experimental values. Standard deviation values are included. A: alginate; T: trehalose; GG: guar gum; VG: *vinal* gum, ECG: *espina corona* gum.

very rapid release of lycopene, as observed in Fig. 3. This behavior is consistent with partially combined mechanisms of diffusion through the matrix and also to through pores filled with solvent (Peppas, 1985). Coppi and Iannuccelli (2009) also proposed that this behavior correspond to Fickian transport. Independently of the mechanism, it is clear that the presence of some gums (*espina corona* and *vinal* gums) strongly affected the microstructure of alginate beads, provoking larger pores which allows a rapidly release of lycopene.

4. Conclusions

Pink-grapefruit freeze-dried pulp matrix was selected for lycopene extraction, showing high levels of lycopene and all-trans lycopene spectral structure. A partial preservation of tissular structure and the reduced water content were the two key points to improve lycopene extraction from this matrix, allowing to obtain a high lycopene extract by a free solvent process, using extra virgin olive oil as extracting agent, adequate for applications in the food industry. Emulsions made with sugars and hydrocolloids were successfully encapsulated in alginate beads, giving as a result a good strategy to get high lycopene formulations. Among encapsulation systems, alginate beads and those supplemented with trehalose and *vinal* gum were the ones that best preserved the lycopene content, minimizing structural changes. Alginate beads containing emulsified lycopene showed two proton populations assigned for protons corresponding to both water-polysaccharides and oil. The determination of diffusion coefficient values showed that lower diffusion coefficient leads to higher lycopene content and stability towards isomerization processes in alginate beads containing trehalose. The composition of the beads strongly affects lycopene release making possible to manage controlled kinetics. The results of this work can be used as a starting point for the selection of excipients to obtain alginate beads for stabilization and release of labile encapsulated biomolecules.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2016.11.074.

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