

RESEARCH

Open Access



# Encapsulation of a free-solvent extract of lycopene in alginate-Ca(II) beads containing sugars and biopolymers

Tatiana Rocío Aguirre Calvo<sup>1,2</sup> and Patricio Román Santagapita<sup>1,2\*</sup> 

## Abstract

**Background:** The purpose of the present study was to enhance the stability toward isomerization and control the release of an encapsulated free-solvent extract of lycopene, obtained from a nonconventional natural source, by means of alginate beads containing sugar (trehalose) and biopolymers (chitosan, low methoxyl pectin, and arabic gum).

**Methods:** Lycopene was extracted from freeze-dried pulp of pink grapefruit obtaining a free solvent extract. Lycopene encapsulation was conducted by a double procedure consisting of emulsification and ionotropic gelation in alginate-Ca(II) beads, modified by the addition of sugar and biopolymers. The influence of beads' composition was studied on lycopene stability and release, as well as molecular mobility and diffusion in the beads.

**Results and Conclusions:** The addition of a second excipient (besides alginate) in the formulation should be carefully conducted, since stability during alginate-Ca(II) bead generation could be even compromised, leading to high lycopene losses. Beads containing trehalose and chitosan were the ones that best preserved the lycopene content and minimized isomerization changes. This could be related to the reduced molecular mobility and lower diffusion coefficient of this system. Lycopene release was severely affected by the composition of the beads, allowing to modulate its release depending on a desired application. Then, a good strategy to obtain high lycopene formulations ready to use or for their incorporation in a subsequent technological process (such as freeze-drying or extrusion) was reported in the present study.

**Keywords:** Isomerization, Molecular mobility, Carotenoids, Transport properties

## Background

In the recent years, the search of antioxidants sources has been increased due to its direct influence on human health. Different diseases such as cancer, diabetes, cataract, and cardiovascular diseases were related to free radicals' generation and cellular redox imbalance [1]. Then, the regular intake of antioxidants becomes a need in order to accomplish the prevention (or even treatment) of some diseases [2]. Hence, finding a way to obtain natural and effective delivery to the human body has become one of the top goals in the food industry.

Lycopene is a natural compound of high-valuable nutritional and medical properties; its structure consists of a long chain of conjugated carbon-carbon (11 conjugated double bonds) [3]. Lycopene is an antioxidant with a singlet-oxygen-quenching ability twice as high as that of  $\beta$ -carotene and ten times higher than that of  $\alpha$ -tocopherol [4–6].

Encapsulation techniques have been studied for their remarkable effects in protection and preservation of bio-compounds against oxidation, isomerization, and degradation during storage of healthy foods for an extended period of time [7]. Encapsulation in alginate-calcium beads, a particular type of crosslinked hydrogels, is one of the available methods. It has several advantages: (i) it is a relatively low-cost and eco-friendly procedure; and (ii) it is nontoxic, biocompatible, and thermally and chemically stable [8, 9].

\*Correspondence: prs@di.fcen.uba.ar

<sup>1</sup> Departamentos de Industrias y Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Intendente Güiraldes 2160, C1428EGA Buenos Aires, Argentina  
Full list of author information is available at the end of the article

Also, biocompounds' retention, mechanical strength, and release rate could be managed by the addition of other compounds (food grade sugars and biopolymers) prior to hydrogel formation [8, 10, 11]. Lycopene was previously encapsulated in alginate-Ca(II) beads, by combining trehalose and galactomannans as secondary excipients [10], obtaining the lycopene content similar to or lower than that of alginate-Ca(II) beads containing trehalose, as well as higher releasing rates from beads. However, neither of the analyzed formulations increased the conservation of lycopene during beads' generation, nor reduced the releasing rates, offering the possibility to manage the release, which is very valuable for technological applications. In order to achieve these tasks, the search for new combinations was performed and analyzed in the present paper. Instead of using galactomannans, chitosan, low methoxyl pectin, and arabic gum were considered, based on their surface properties (charged). Chitosan, a chitin-derived biopolymer, has positive charges, which are able to interact with alginate affecting beads' permeability by generation of an alginate-chitosan layer [12]. Besides, chitosan is antimicrobial, biocompatible, and biodegradable [12]. Instead, low methoxyl pectin can form gels with divalent cations by ionotropic gelation like alginate, but modifies the properties of beads wall, changing both loading efficiency and release properties [13]. Its inclusion could lead to a modification of the hydrogel network, affecting release. Arabic gum, a commercial and widely used gum, is a complex mixture of a polysaccharide (an arabinogalactan) and proteins [14], which conferred optimal emulsion properties. Then, the inclusion of any of these biopolymers could deeply affect both stability and release of lycopene in the beads. Trehalose, a dehydro- and cryo-protectant of labile biomolecules, was also included since its presence allowed for increasing the loading efficiency of alginate beads containing invertase, besides providing further protection of the enzyme upon thermal treatment and dehydration [11].

The purpose of the present study was to enhance the stability toward isomerization and control the release of an encapsulated free-solvent extract of lycopene, obtained from a nonconventional natural source such as pink grapefruits, by means of alginate beads containing sugar and biopolymers as encapsulation systems. The effects of the formulation on lycopene encapsulation, stability, and release were analyzed, as well as the physicochemical properties of the obtained systems. Molecular mobility and diffusion coefficient were related to the beads' encapsulation/stabilization capacity.

## Methods

### Materials

Pink grapefruits (*Citrus paradisi*, Red variety, from Jujuy, Argentina) were obtained from the local market

from the same batch, selecting non-damaged fruits. The used encapsulation agents were sodium alginate (A) from Cargill S.A. (San Isidro, Buenos Aires, Argentina),  $M_w = 1.97 \times 10^5$  g/mol, with M/G (mannuronate/guluronate) ratio of 0.6; trehalose (T) ( $\alpha$ -D-glucopyranosyl-(1,1)- $\alpha$ -D-glucopyranoside) dihydrate from Hayashibara Co., Ltd. (Shimoishii, Okayama, Japan); low methoxyl pectin (P) from Degusta Meath & Nutrition Argentina S.A. (San Isidro, Buenos Aires, Argentina), with a degree of esterification and amidation between 26 and 31% and 16–19%, respectively; arabic gum (AG) from Biopack S.A. (Zárate, Buenos Aires, Argentina), MW  $\sim 2.5 \times 10^5$  g/mol, purity: 99%; and chitosan (Ch) medium molecular weight from Sigma-Aldrich Chemie GmbH (Steinheim, Germany), with a degree of deacetylation of 75–85% and viscosity of 200–800 cP [1% w/v in 1% acetic acid (25 °C, Brookfield)]. Extra-virgin olive oil (Molino Cañuelas SACIFIA, Mendoza, Argentina) was used for lycopene extraction.

### Lycopene extraction and encapsulation

Lycopene was extracted from freeze-dried pulp following the procedure described in [10], obtaining a free-solvent extract of lycopene.

Lycopene encapsulation was conducted by a double procedure consisting of emulsification and ionotropic gelation. For encapsulation, 10 g/kg of sodium alginate with or without 200 g/kg trehalose and/or 2.5 g/kg of P and AG were prepared until complete suspension. Emulsions were prepared using a Ultra-Turrax T18B (IKA<sup>®</sup>-Werke GMBH & CO.KG, Staufen, Germany) at 15,500 rpm, mixing lycopene extract with the biopolymer solutions (1:2 mass ratio) for 10 min, using time intervals of 5 min and 1 min of pause.

Beads were prepared by ionotropic gelation according to the drop method described previously [10, 11, 13]. Table 1 summarizes the used emulsions and the composition of each gelling solution. 25 g/kg calcium chloride was used for preparing A, supplemented with 200 g/kg trehalose for A-T, AP-T, and AAG-T beads and with 5 g/kg of chitosan for A-TCh. Chitosan was first hydrated for 3 h, and then, after adding 1% (w/v) acetic acid, was kept in storage overnight with agitation until complete suspension.

Beads were stored at 4–8 °C under darkness.

### Lycopene content and stability

Lycopene content was measured according to Fish et al. [15], following the modifications described in [10]. An average value of three replicates was reported along with the standard deviation.

Stability was studied through the analysis of the spectral fine structure. Lycopene spectra show the typical carotenoid shape, with peak maxima at  $\lambda_{max}$  at 444, 472,

**Table 1 Compositions of emulsions and of external media used for beads' preparation**

Beads						System
Emulsions			Gelling solutions			
Alginate 10 g/kg	Trehalose 200 g/kg	Biopolymer 2.5 g/kg	Calcium chloride 25 g/kg	Trehalose 200 g/kg	Biopolymer	
Initial solution of encapsulant agents:lycopene extract (2:1)						
X	–	–	X	–	–	A
X	X	–	X	X	–	A-T
X	X	–	X	X	Chitosan 5 g/kg	A-TCh
X	X	Pectin	X	X	–	AP-T
X	X	Arabic gum	X	X	–	AAG-T

The abbreviation employed for each bead system was also indicated

and 503 nm, characteristic of this chromophore [16, 17]. 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 [16]. The values of spectral fine structure do not indicate an exact account of which isomer is formed (since 13 double bonds can be isomerized), but revealed that much of the encapsulated all-*trans* lycopene was lost during processing.

Lycopene content and spectral fine structure of lycopene values were normalized by the content of lycopene and the %III/II, respectively, of the corresponding extract used in the preparation of beads of different formulations.

#### Proton transverse relaxation times and diffusion coefficient

A Bruker Minispec mq20 low-field proton nuclear magnetic resonance (LF-NMR) spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) was used to evaluate proton transverse relaxation times and diffusion coefficient. The equipment generates a 0.47 T magnetic field at a resonance frequency of 20 MHz. Bead samples equilibrated at  $25.00 \pm 0.01$  °C in a thermal bath (Haake, model Phoenix II C35P, Thermo Electron Corporation GmbH, Karlsruhe, Germany) were placed inside 10-mm outer diameter tubes covering 1 cm of the tubes.

Proton transverse relaxation times ( $T_2$ ) and diffusion coefficients ( $D$ ) were measured in duplicate and analyzed as described in [10]. Carr–Purcell–Meiboom–Gill (CPMG) and pulsed magnetic field gradient spin echo (PGSE) sequences were used, respectively.

A bi-exponential function [10] was found to fit the experimental data adequately, from which two  $T_2$  values (with their corresponding amplitudes) were obtained.  $D$

was calculated following the procedure as reported by Santagapita et al. [18].

#### Size through digital image analysis

The size of the beads was analyzed through digital images captured by a digital camera coupled to a binocular microscope and analyzed by the free license software ImageJ, as described in [19]. The Feret's diameter (size) corresponds to the longest distance between any two points along the bead boundary. At least 60 beads were analyzed by applying the “analyze particle” command of the software.

#### pH measurement

pH was measured by means of a pH meter (Mettler Delta 320, Mettler Toledo AG, Greifensee, Switzerland), calibrated between 4 and 7. All the prepared emulsions and solutions were measured in triplicate.

#### Water content and water activity

The total water content of the beads was determined gravimetrically according to [19], in triplicate. Water activity ( $a_w$ ) was determined by means of an Aqualab instrument (Decagon Devices, Inc., USA), in triplicate.

#### Lycopene release

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

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 ( $L_\infty$ ). The release curves were modeled using the Peppas equation [10, 13, 20–22] using Prism 6 (GraphPad Software Inc., San Diego, CA, USA), and the corresponding parameters ( $n$  and  $k$ ) were

calculated. The release media was selected as the optimal solubilization media which favors lycopene release from beads, in order to obtain important microstructural characteristics by analyzing transport mechanisms.

### Statistical analysis

The effects of beads' composition on size reduction, lycopene content, %III/II,  $T_2$ , and  $D$  were analyzed by one-way ANOVA with Tukey post-test using Prism 6 (GraphPad Software Inc.).

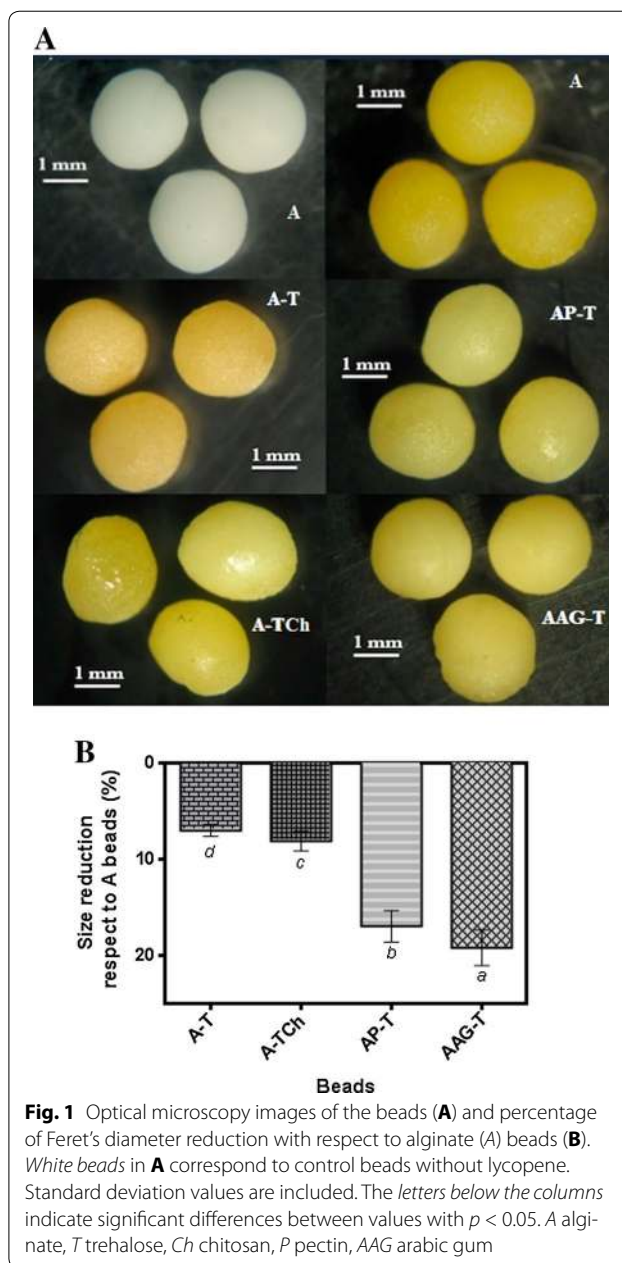
## Results and discussion

### Alginate-Ca(II)-based beads containing lycopene

Lycopene was extracted by means of olive oil as extracting lipid (free-solvent extraction) from pink grapefruit freeze-dried pulp. This matrix was previously selected to maximize the concentration of extracted all-*trans* lycopene among other matrices [10].

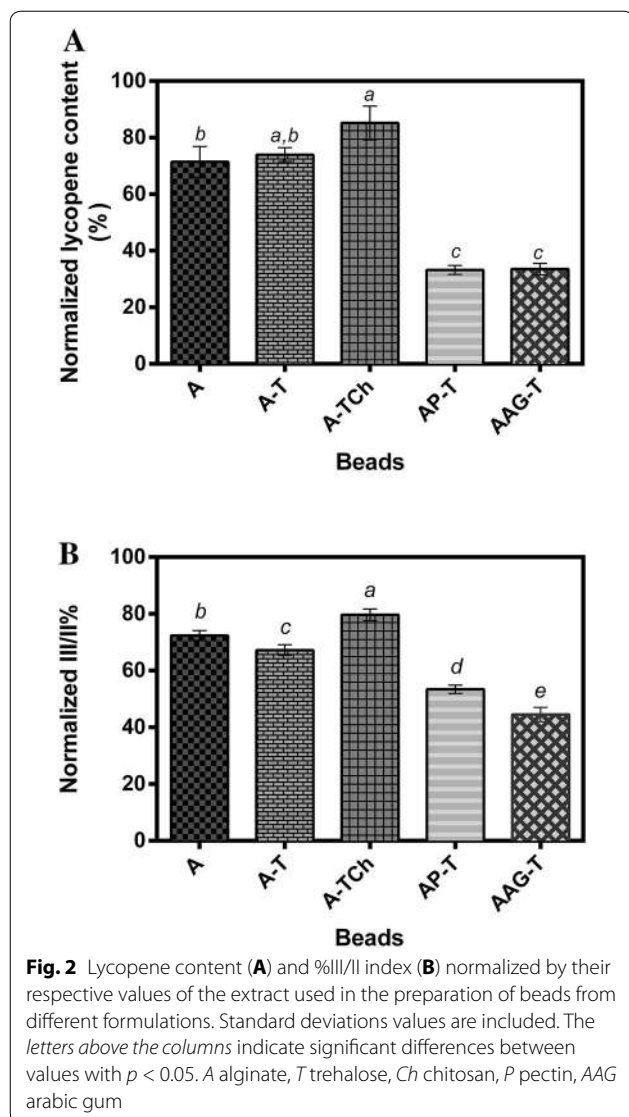
Beads containing lycopene were orange colored (control beads without lycopene were white), as showed in Fig. 1A. Beads showed an elliptical shape with circularity values up to 0.85 as reported in [19], and with a size between 2.2 and 2.7 mm. Beads with trehalose and biopolymers showed a reduction of their size in comparison with A, as reported in Fig. 1B. This reduction was probably related to changes in the surface tension/viscosity of the droplets containing the initial emulsions, which were further used to form alginate beads. The pH values of the emulsions were between 5.5 and 6 for A and A-T and between 4 and 5 for the rest of the systems. pH should be higher than the  $pK_a$  values of alginate (3.38 and 3.65 [13]) for alginate-Ca(II) formation. Water content of the beads ranged from 37 to 44 g/100 g on wet basis, and water activity between 0.96 and 0.98, in agreement with the previously published values [19].

Figure 2A, B shows the content and the spectral fine structure of lycopene, respectively, normalized by the content of lycopene present in the extract. More than 80% of the available lycopene in the extract was retained in the A-TCh beads (Fig. 2A) and about 70% for the A and A-T beads. AP-T and AAG-T beads showed the lowest values (<40%). The spectral fine structure preservation of the encapsulated extract showed two types of general behaviors (Fig. 2B): values between 67 and 80% for the A-TCh, A, and A-T beads, and lower than 55% for the AP-T and AAG-T beads. These %III/II reductions indicated that the encapsulated lycopene was isomerized to its *cis* form [17]. The values of spectral fine structure do not indicate the exact account of which isomer was formed since 13 double bonds can be isomerized, but revealed that much of the encapsulated all-*trans* lycopene was lost during processing. These results showed that both lycopene content and stability strongly depend on the used formulation.



**Fig. 1** Optical microscopy images of the beads (A) and percentage of Feret's diameter reduction with respect to alginate (A) beads (B). White beads in A correspond to control beads without lycopene. Standard deviation values are included. The letters below the columns indicate significant differences between values with  $p < 0.05$ . A alginate, T trehalose, Ch chitosan, P pectin, AAG arabic gum

Trehalose or biopolymer addition to the initial emulsion did not improve (or even reduce!) lycopene content (Fig. 2A) as well as its stability (Fig. 2B). Only chitosan inclusion provokes higher lycopene content and stability than A beads. This could be due to the well-documented chitosan's ability to interact with alginate [11, 13, 20], forming a complex alginate-chitosan network. This change on beads wall provokes (i) a higher mechanical resistance provided by the interaction between alginate and chitosan [11, 23, 24], resulting in higher lycopene retention during bead



generation (it is interesting to analyze that this higher retention was observed for different types of compounds, such as enzymes and antioxidants); and (ii) a reduction of  $O_2$  permeability [25], which will explain the reduction of lycopene isomerization in these beads, as well as degradation.

On the other hand, the inclusion of anionic biopolymers (pectin and Arabic gum) leads to higher lycopene loss and isomerization. This could be due to the competence for Ca(II) between alginate and pectin, or by the alginate–arabic gum interaction, reducing the ability of alginate to interact with Ca(II), affecting the network. Then, the reduced size of these beads could be a manifestation of a less extent of the alginate–Ca(II) network. Future small-angle X-ray scattering (SAXS) experiments could confirm this hypothesis, by providing a detailed characterization of the gel's microstructure.

### Transport properties and mobility by low-field nuclear magnetic resonance

Transverse relaxation times and diffusion coefficients are shown in Table 2.  $T_2$  values were obtained applying the CPMG sequence, which allows for the analysis of high-mobility systems of relaxation times longer than 1 ms. In concordance with Aguirre Calvo et al. [10], all beads' samples containing lycopene showed two relaxation times in the 52–69 and 164–179 ms ranges, respectively (Table 2).

The addition of trehalose and biopolymers slightly affects the mobility properties of the analyzed systems. In general, the amplitudes of both relaxation times were kept invariant in all systems, showing the first relaxation time having higher amplitude than the second one. Besides,  $T_2$  times were not modified by the addition of trehalose and pectin or arabic gum biopolymers. Only chitosan provokes a reduction of  $T_{21}$  revealing the reduced mobility of the system. This fact could explain the higher conservation and stability of lycopene observed in Fig. 2, since a reduced mobility is often linked with a more solid-like environment with higher interactions among the components [26].

It is important to keep in mind that the responses obtained by NMR in gels or diluted polysaccharides systems are from both water protons (modulated by the exchange between water and biopolymers protons [27, 28] but also from oil protons, as previously discussed in [10]. The obtained  $T_2$  times were similar to those reported by several authors for alginate–Ca(II) beads [10, 26, 29], considering the characteristics of the alginate and of the equipment, as well as those of edible oils [30]. Therefore, the overlap of the contributions of water protons (exchanging with polysaccharides and sugars) and oil protons makes the assignment of the two  $T_2$  to one or the other component difficult.

The mobility reduction observed for A-TCh beads was confirmed by diffusion coefficients at 25 °C (Table 2),

**Table 2** Transport properties and mobility by low-field nuclear magnetic resonance

Beads	Amplitude <sub>1</sub> (%)	$T_{21}$ (ms)	Amplitude <sub>2</sub> (%)	$T_{22}$ (ms)	$D$ ( $10^{-9}$ m <sup>2</sup> /s)
A	58 ± 2 <sup>a</sup>	66 ± 3 <sup>a</sup>	42 ± 5 <sup>a</sup>	168 ± 13 <sup>a</sup>	0.52 ± 0.03 <sup>a</sup>
A-T	56 ± 5 <sup>a</sup>	66 ± 2 <sup>a</sup>	44 ± 5 <sup>a</sup>	170 ± 13 <sup>a</sup>	0.40 ± 0.02 <sup>b</sup>
A-TCh	63 ± 10 <sup>a</sup>	52 ± 1 <sup>b</sup>	37 ± 5 <sup>a</sup>	164 ± 3 <sup>a</sup>	0.37 ± 0.02 <sup>b</sup>
AP-T	59 ± 11 <sup>a</sup>	67 ± 1 <sup>a</sup>	41 ± 3 <sup>a</sup>	174 ± 7 <sup>a</sup>	0.450 ± 0.001 <sup>a</sup>
AAG-T	63 ± 3 <sup>a</sup>	69 ± 1 <sup>a</sup>	38 ± 3 <sup>a</sup>	179 ± 3 <sup>a</sup>	0.51 ± 0.02 <sup>a</sup>

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. Mean and standard deviation of duplicate determinations are informed

Different letters in superscript on the columns indicate significant differences between values with  $p < 0.05$

showing a significant reduction in its  $D$  value in comparison to the rest of the systems. The obtained  $D$  values were in agreement with those reported in [10]. As expected, all beads showed diffusion coefficient values smaller than water ( $2.3 \cdot 10^{-9} \text{ m}^2/\text{s}$  at  $25 \text{ }^\circ\text{C}$  [31]) due to the presence of trehalose and biopolymers, as well as by the presence of oil ( $9 \times 10^{-12} \text{ m}^2/\text{s}$ , [32]), which provoked a further reduction of the observed values.

Taking into consideration this study and the previously published one [10], it is possible to state that higher values of  $D$  in beads containing trehalose correspond to lower lycopene contents (Fig. 2A). A lower  $D$  value would promote a slower loss of lycopene during beads' generation, even though greater emulsion stability could also play a role on this higher conservation. 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 itself [33]. Besides, the ability of trehalose to impose more local ordering to the surrounding water molecules and strengthen the hydrogen bonds [34, 35] affects also the stability of emulsions in the aqueous phase by reducing its size [36]. Chitosan also can act as an emulsifier and emulsion stabilizer through adsorption on the protective layer at oil–water interfaces, viscosity enhancement, or by facilitating the interaction with other surface-active agents [37]. These mechanisms should be further analyzed, as well as the gel's microstructure.

#### Release of lycopene: Fickian diffusion and anomalous transport

Lycopene release from alginate beads was successfully modeled [10] by means of a semiempirical equation (Peppas equation), which simplifies the analysis by relating the fractional release of an active compound with time [21, 22], and was adapted to our material in the following way:

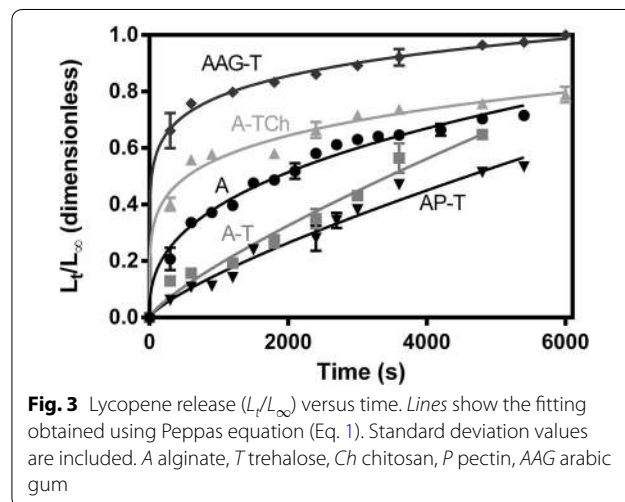
$$\frac{L_t}{L_\infty} = kt^n \quad (1)$$

where  $L_t$  is the lycopene content released at time  $t$ ;  $L_\infty$  is the maximum content corresponding to the total loaded lycopene;  $k$  (in  $\text{s}^{-n}$ ) is a constant related to geometric and structural characteristics, to the macromolecular network of the system and to the agent released from the bead;  $t$  is the time (in s); and  $n$  is indicative of the type of release mechanism (dimensionless).

The general expression of the Eq. 1 was previously employed to study the release of several substances from dry and wet beads [13, 20, 23]. The application of this equation assumes that diffusion is concentration independent, and volume changes are less than 25% of the initial volume during the agent release [21, 38]. Figure 3

shows the release profiles obtained for each bead system, modeled by Eq. 1. Table 3 includes the parameters related to transport mechanism ( $n$ ,  $k$ ). Correlation coefficients obtained for calculating  $n$  and  $k$  were greater than 0.95. The limits of  $n$  are defined for each geometry [22]. In the case of spheres, for polymers that do not swell when wetting, the Fick's diffusion (or "case transport I" according to Alfrey et al. [39]) is defined for values of  $n = 0.43$ . This case corresponds to situations in which polymer relaxation is much lower than diffusion.  $n$  values between 0.43 and 1 correspond to a mixed transport mechanism (often called "anomalous"), in which both the rate of diffusion and the polymer relaxation process are comparable. For polymer chains that swell when in contact with water,  $n$  values range between 0.43 and 0.85, when the upper limit corresponds to a transport limited by the rate of the relaxation of the polymer chains during swelling [40].

As already observed by Aguirre et al. [10], the release of lycopene is produced by diffusion in alginate beads (A), which is logical considering the size of lycopene (a few nm), the average pore diameter of alginate-Ca(II) beads, and the used solvent [13]. Trehalose inclusion



**Fig. 3** Lycopene release ( $L_t/L_\infty$ ) versus time. Lines show the fitting obtained using Peppas equation (Eq. 1). Standard deviation values are included. A alginate, T trehalose, Ch chitosan, P pectin, AAG arabic gum

**Table 3** Lycopene release and transport mechanism

Beads	Points	$k$ ( $\text{s}^{-n}$ )	$n$	Transport type
A	9	$0.018 \pm 0.004^c$	$0.44 \pm 0.03^b$	Fick's diffusion
A-T	8	$0.0008 \pm 0.0006^d$	$0.8 \pm 0.1^a$	Anomalous
A-TCh	5	$0.10 \pm 0.05^b$	$0.25 \pm 0.07^c$	Fick's diffusion*
AP-T	12	$0.0011 \pm 0.0005^e$	$0.73 \pm 0.05^a$	Anomalous
AAG-T	11	$0.32 \pm 0.01^a$	$0.130 \pm 0.005^c$	Fick's diffusion*

Parameters obtained by fitting the semi-empirical equation (Eq. 1), number of points employed (fractional release  $<0.6$ ), and transport type

Different letters in superscript on the columns indicate significant differences between values with  $p < 0.05$

\* Corresponds to Fick's diffusion as assigned by other authors

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. A similar situation also occurred in AP-T beads, which contains pectin, another anionic polyelectrolyte as alginate, which also participates in the gel network. However,  $k$  values showed differences among these two systems (they could be compared since no significant differences were obtained among  $n$  values), which could arise due to a combination of factors: the addition of pectin which affected the gel network and the geometric and structural characteristics of the beads (there are differences in sizes as shown in Fig. 1A, and AP-T showed higher circularity [19], which in turn could be affected by the network).

Meanwhile, the values of the systems containing arabic gum or chitosan showed  $n$  values much lower than 0.43, revealing that the structure of the beads is severely affected. In these cases, there was a very rapid release of lycopene, as observed in Fig. 3. As discussed in a previous study [10], this behavior is consistent with partially combined mechanisms of diffusion through the matrix and also that through pores filled with solvent [21]. Coppi and Iannuccelli [20] also proposed that this behavior corresponds to Fickian transport. Regardless of the mechanism, it is clear that the presence of some biopolymers (arabic gum and chitosan) strongly affected the microstructure of alginate beads, provoking larger pores which allows for a rapid release of lycopene.

## Conclusions

Emulsified lycopene (extracted from pink grapefruit freeze-dried pulp matrix) with sugars and hydrocolloids were successfully encapsulated in alginate beads, being a good strategy to obtain high lycopene formulations ready to use or for their incorporation in a subsequent technological process (such as freeze-drying or extrusion). Alginate-Ca(II) beads could then be incorporated in a variety of products, from dairy products to desserts, as well as snacks and candies, for the production of the so-called functional foods.

The inclusion of chitosan-generating beads, with higher lycopene content relative to alginate-Ca(II) ones, minimizes structural changes. However, the addition of a second excipient in the formulation should be carefully conducted, since stability during alginate-Ca(II) beads' generation could be even compromised, as would occur in the presence of pectin or arabic gum. Chitosan-containing beads showed reduced molecular mobility and lower diffusion coefficient, both being related to lycopene content conservation during bead generation.

Lycopene release was severely affected by the composition of the beads, allowing to reduce or enhance the

release, which is of high technological value, depending on the desired application.

## Authors' contributions

ACT conducted all the reported experiments. She processed and analyzed the obtained data. She also participated during the preparation of the manuscript. SP was Tatiana's supervisor during her Master thesis. He contributed through planning the experiments, supervising data analysis, discussing results, and providing guidance in the preparation and editing of the manuscript. Both the authors read and approved the final manuscript.

## Author details

<sup>1</sup> Departamentos de Industrias y Química Orgánica, Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires, Ciudad Universitaria, Intendente Güiraldes 2160, C1428EGA Buenos Aires, Argentina. <sup>2</sup> Instituto de Tecnología de Alimentos y Procesos Químicos (ITAPROQ), CONICET-Universidad de Buenos Aires, Buenos Aires, Argentina.

## Acknowledgements

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## Availability of data and materials

The dataset supporting the conclusions of this article is available in the Zenodo repository [DOI: 10.5281/zenodo.212116 and hyperlink to dataset in <https://doi.org/10.5281/zenodo.212116>].

## Funding

The authors acknowledge the financial support of ANPCYT (PICT 2013 no 0434, PICT 2013-1331), CIN-CONICET (PDTs 2015 no 196), CONICET, and UBA (Project UBACyT 20020130100443BA). PRS is member of CONICET.

Received: 20 December 2016 Accepted: 17 March 2017

Published online: 04 July 2017

## References

1. Labat-Robert J, Robert L. Longevity and aging. Role of free radicals and xanthine oxidase. A review. *Pathol Biol.* 2014;62:61–6.
2. Sen L, Chen G, Zhang C, Wu M, Wu S, Liu Q. Research progress of natural antioxidants in foods for the treatment of diseases. *Food Sci Hum Wellness.* 2014;3:110–6.
3. Clinton SK, Emenhiser C, Schwartz SJ, Bostwick DG, Williams AW, Moore BJ, et al. *cis-trans* lycopene isomers, carotenoids, and retinol in the human prostate. *Cancer Epidemiol Biomark Prev.* 1996;5:823–33.
4. Miller NJ, Sampson J, Candeias LP, Bramley PM, Rice-Evans CA. Antioxidant activities of carotenes and xanthophylls. *FEBS Lett.* 1996;384:240–6.
5. Woodall AA, Lee SWM, Weesie RJ, Jackson MJ, Britton G. Oxidation of carotenoids by free radicals: relationship between structure and reactivity. *Biochim Biophys Acta.* 1997;1336:33–42.
6. DiMascio P, Kaiser S, Sies H. Lycopene as the most effective biological carotenoid singlet oxygen quencher. *Arch Biochem Biophys.* 1989;274:532–8.
7. Zeller BL, Saleeb FZ, Ludescher RD. Trends in development of porous carbohydrate food ingredients for use in flavor encapsulation. *Trends Food Sci Technol.* 1999;9:389–94.
8. Gombotz WR, Wee SF. Protein release from alginate matrices. *Adv Drug Del Rev.* 1998;31:267–85.
9. Fang Z, Bhandari B. Encapsulation of polyphenols—a review. *Trends Food Sci Technol.* 2010;21:510–23.
10. Aguirre Calvo TR, Busch VM, Santagapita PR. Stability and release of an encapsulated solvent-free lycopene extract in alginate-based beads. *LWT Food Sci Technol.* 2017;77:406–12.
11. Santagapita PR, Mazzobre MF, Buera MP. Invertase stability in alginate beads. Effect of trehalose and chitosan inclusion and of drying methods. *Food Res Int.* 2012;47:321–30.

12. Z-d Zhou, G-y Li, Y-j Li. Immobilization of *Saccharomyces cerevisiae* alcohol dehydrogenase on hybrid alginate–chitosan beads. *Int J Biol Macromol.* 2010;47:21–6.
13. Santagapita PR, Mazzobre MF, Buera MP. Formulation and drying of alginate beads for controlled release and stabilization of invertase. *Bio-macromol.* 2011;12:3147–55.
14. Patel S, Goyal A. Applications of natural polymer gum arabic: a review. *Int J Food Prop.* 2015;18:986–98.
15. Fish P, Wayne W, Perkins V, Collins JK. A quantitative assay for lycopene that utilizes reduced volumes of organic solvent. *J Food Comp Anal.* 2002;15:309–17.
16. Britton G. Structure and properties of carotenoids in relation to function. *FASEB J.* 1992;9:1551–8.
17. Rodríguez Amaya DB, Kimura M. Harvestplus handbook for carotenoid analysis. In: HarvestPlus Technical Monograph 2. Washington: HarvestPlus; 2004.
18. Santagapita PR, Laghi L, Panarese V, Tylewicz U, Rocculi P, Dalla Rosa M. Modification of transverse NMR relaxation times and water diffusion coefficients of kiwifruit pericarp tissue subjected to osmotic dehydration. *Food Bioprocess Technol.* 2013;6:1434–43.
19. Aguirre Calvo TR, Santagapita PR. Physicochemical characterization of alginate beads containing sugars and biopolymers. *J Qual Reliab Eng.* 2016; Article ID: 9184039.
20. Coppi G, Iannuccelli V. Alginate/chitosan microparticles for tamoxifen delivery to the lymphatic system. *Int J Pharm.* 2009;367:127–32.
21. Peppas NA. Analysis of Fickian and non-Fickian drug release from polymers. *Pharm Acta Helvetiae.* 1985;60:110–1.
22. Ritger PL, Peppas NA. A simple equation for description of solute release. I. Fickian and non-Fickian release from non-swelling devices in the form of slabs, spheres, cylinders or discs. *J Control Release.* 1987;5:23–36.
23. Deladino L, Anbinder PS, Navarro AS, Martino MN. Encapsulation of natural antioxidants extracted from *Ilex paraguariensis*. *Carbohydr Pol.* 2008;71:126–34.
24. Rha CK, Rodríguez-Sánchez D. Process for encapsulation and encapsulated active material system. US patent. 1988;4(744):933.
25. Gasserod O, Sannes A, Skjask-Braek G. Microcapsules of alginate–chitosan. II. A study of capsule stability and permeability. *Biomaterials.* 1999;20:773–83.
26. Simpson NE, Grant SC, Blackband SJ, Constantinidis I. NMR properties of alginate microbeads. *Biomaterials.* 2003;24:4941–8.
27. Hills BP, Cano C, Belton PS. Proton NMR relaxation studies of aqueous polysaccharide systems. *Macromolecules.* 1991;24:2944–50.
28. Potter K, Carpenter TA, Hall LD. Mapping of the spatial variation in alginate concentration in calcium alginate gels by magnetic-resonance-imaging (MRI). *Carbohydr Res.* 1993;246:43–9.
29. Rayment P, Wright P, Hoad C, Ciampi E, Haydock D, Gowland P, Butler MF. Investigation of alginate beads for gastro-intestinal functionality, Part 1: In vitro characterization. *Food Hydrocoll.* 2009;23:816–22.
30. Zhang Q, Saleh ASM, Shen Q. Discrimination of edible vegetable oil adulteration with used frying oil by low field nuclear magnetic resonance. *Food Bioprocess Technol.* 2013;6:2562–70.
31. Holz M, Heil SR, Sacco A. Temperature-dependent self-diffusion coefficients of water and six selected molecular liquids for calibration in accurate  $^1\text{H}$  NMR PFG measurements. *Phys Chem.* 2000;2:4740–2.
32. Šmejkalová D, Piccolo A. High-power gradient diffusion NMR spectroscopy for the rapid assessment of extra-virgin olive oil adulteration. *Food Chem.* 2010;118:153–8.
33. Bernin D, Goudappel G-J, Van Ruijven M, Altskär A, Ström A, Rudemo M, Hermansson A-M, Nydén M. Microstructure of polymer hydrogels studied by pulsed field gradient NMR diffusion and TEM methods. *Soft Matter.* 2011;7:5711–6.
34. Barreca D, Laganà G, Magazù S, Migliardo F, Gattuso G, Bellocchio E. FTIR, ESI-MS, VT-NMR and SANS study of trehalose thermal stabilization of lysozyme. *Int J Biol Macromol.* 2014;63:225–32.
35. Santagapita PR, Buera MP. Electrolyte effects on amorphous and super-cooled sugar systems. *J Non Crystall Sol.* 2008;354:1760–7.
36. Álvarez Cerimedo MS, Iriart CH, Candal RJ, Herrera ML. Stability of emulsions formulated with high concentrations of sodium caseinate and trehalose. *Food Res Int.* 2010;43(5):1482–93.
37. Klinkesorn U. The role of chitosan in emulsion formation and stabilization. *Food Rev Int.* 2013;29:371–93.
38. Peppas NA, Korsmeyer RW. Dynamically swelling hydrogels in controlled release applications. In: Peppas NA, editor. *Hydrogels in medicine and pharmacy.* Boca Raton: CRC Press; 1986. p. 109–36.
39. Alfrey E, Gurnee EF, Lloyd WGJ. Diffusion in glassy polymers. *J Pol Sci.* 1966;12:249–61.
40. Llabot JM, Manzo RH, Allemandi DA. Drug release from carbomer: carbomer sodium salt matrices with potential use as mucoadhesive drug delivery system. *Int J Pharm.* 2004;276:59–66.

Submit your manuscript to a SpringerOpen<sup>®</sup> journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Immediate publication on acceptance
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

---

Submit your next manuscript at ► [springeropen.com](http://springeropen.com)

---