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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.

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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, & McClemments, 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 is (since 13 double bonds can be isomerized) but revealed that much of the encapsulated all-trans lycopene was lost during processing.

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