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Review

Encapsulation of betacyanins and polyphenols extracted from leaves and stems of beetroot in Ca(II)-alginate beads: A structural study

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ABSTRACT

The aim of this work was to use the recovery of antioxidant compounds (betacyanin and polyphenols) derived from beetroot industrial wastes (stems and leaves), and their subsequent encapsulation in Ca(II)-alginate beads containing sugars, providing a detailed structural characterization of these systems determined by SAXS from the molecular (arrange of Ca(II)-alginate dimers) to the supramolecular (interconnection of the rods composing the hydrogel microstructure). Water extract contained significant quantities of betacyanin and polyphenol, which were retained in Ca(II)-alginate beads between 15 and 60%, depending on the formulation, retaining also the antioxidant activity. Both the inclusion of sugars as synthesis additives and beetroot extracts induced main structural changes, which can have counteracting effects. We revealed that, though being overlooked in most alginate encapsulation research, the presence of natural extracts prompts important structural changes in the alginate network, affecting key parameters which define the encapsulation performance in most of industrial and environmental applications.

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1. Introduction

Beetroot (*Beta vulgaris*) has been cataloged among the ten vegetables with greater antioxidant activity (Halvorsen et al., 2002) and its commercially exploited commodities are growing increasing interest. Recent studies have provided compelling evidence that beetroot ingestion offers beneficial physiological effects that may translate to improved clinical outcomes for several pathologies, such as hypertension, atherosclerosis, type II diabetes and dementia (Clifford et al., 2015). In addition, antioxidants from beetroot are bioavailable for the human organism as they permeate from the alimentary tract to the bloodstream (Netzel et al., 2005). The growing demand in the consumption of this vegetable has generated diverse studies of its health benefits. The bulb (root), being by far the most consumed, has become the main object of study, leaving the leaves and the stems as waste, even if the shoot have also nutritional value and important benefits for human health. Red beets (including root, leaves and stems) contain betalain pigments, one of the most important natural colorants (FDA and EFSA approved) and also one of the earliest natural colorants developed for use in food systems (Francis., 1999), belonging to the group of cation antioxidants (Kanner et al., 2001). Betalains are water-soluble nitrogen compounds, found in the cell sap; they show antioxidant and radical scavenging activities (Pedreño and Escribano, 2000; Gliszczyńska-Świgło et al., 2006; Allegra et al., 2005). Betalains can be divided into two groups: red-violet betacyanins and yellow-orange betaxanthins. Its concentration is decreasing in the following order: peel, crown and flesh (Kujala et al., 2000, 2002). The betacyanin and betaxanthin concentration ratio usually ranges between 1 and 3. It depends on beetroot varieties, part of the plant and applied extraction technology (Nemzer et al., 2011). Polyphenols are naturally occurring compounds largely found in fruits, vegetables, cereals and beverages (Pandey and Rizvi, 2009). Studies suggest that a diet based on rich-polyphenols provides significant protection against the development and progression of many chronic pathological conditions, thus developing great scientific interest from their beneficial effects on human health (Jastrebova et al., 2003; Georgiev et al., 2010; Graf et al., 2005; Arts and Hollman, 2005). Current tendency leads to recover target compounds from what is commonly known as food waste, as an alternative and cheap source of several bioactive compounds with technological and healthy properties. In this scenario, no effort should be spared in developing low-cost methods for recovering these valuable components of food by-products and recycling them inside the food chain (Galanakis, 2012, 2015).

Encapsulation is being used to improve stability and bioavailability of several bioactive compounds due to the interest in developing more efficient and selective methods for their protection and preservation (Aguirre Calvo and Santagapita, 2016; Traffano-Schiffo et al., 2017a, 2017b; Lupo et al., 2014). The incorporation of bioactives into food products provides many advantages in food preservation and contributes to the development of functional foods promoted by the application of emerging technologies (Galanakis, 2013). Thus, in the food industry, encapsulation not only allows adding value to a product food and generating a source of new additives with specific properties (Campañone et al., 2014), but it is also characterized, in addition to scalability, by the ease of operation, cost effectiveness, and broad regulatory acceptance (Abubakr et al., 2010). The study of alginates has generated many research due to their renewability, biodegradability, biocompatibility, and nontoxicity characteristics (Messaoud et al., 2015; Santagapita et al., 2012). In particular, much research is focused on the design and development of encapsulation systems capable of producing beads with the desired characteristics, among them the rapid, reproducible and controlled formation of uniform drops (Traffano-Schiffo et al., 2018; Santagapita et al., 2012; Deladino et al., 2008. Balanč et al., 2015). Studies have shown that interaction of dextran and alginate during the formation of beads improves encapsulation efficiency of bioactive compounds while increasing the stability of the system at typical gastric tract pH conditions (Martins et al., 2007). Sucrose is highly used for the generation of alginate beads and films due to its cryo-protective properties (De Giulio et al., 2005). The advantage of its addition during the external gelation procedure and its ability to modify the crosslinking reaction to generate uniform films without the appearance of localized gelling areas at the surface (Al-Remawi; 2012) are also to be noted.

In order to explore the nanoscale morphology and microstructure of the Ca(II)-alginate, small angle X-ray scattering (SAXS) affords information in the scale 1–100 nm. The most recognized mechanism of Ca^{2+} serving as the cross-linker for alginate through the G-blocks where specific Ca-mediated interactions involve two polymer chains was named the egg-box model, which describes the local cage conformation adopted by adjacent L-guluronate residues involved in coordination to the divalent cations (Wang et al., 1995; Sikorski et al., 2007a,b; Waters et al., 2010). The gelation of Ca(II)-alginate is explained by the cooperative association of these polymer dimers, to form rod like structures and the subsequent ramification of these rods, generating a percolating network (Traffano-Schiffo et al., 2018). Thus, this technique gives insight into different scales, providing information of the cross-sectional radius of gyration of the rods, which allows assessing the “junction zone multiplicity” or the size of the junction zone domains (Stokke et al., 2000), but also at larger scale indicating the degree of ramification of rods, and at a smaller scale, giving information on the polymer dimers formation. Being their functional properties highly dependent on the nano and microstructure, a detailed structural characterization of these systems becomes of great scientific and technological interest.

The aim of this work was to emphasize in the extraction of antioxidant compounds (betacyanins and polyphenols) derived from two industrial wastes (stems and leaves) of beetroot, and their subsequent encapsulation in Ca(II)-alginate beads, providing a

detailed structural characterization of these systems in order to assess the changes produced by the presence of the extracts in the microstructure of the alginate network.

2. Materials and methods

2.1. Extraction

Stems/leaves of *Beta vulgaris*, obtained from the local market, were scald and then mixed in a blender; weighted samples were then mixed with solvent (1:2) (water, ethanol or methanol) for 45 min at 20 °C and then centrifuged at 10,000 rpm during 30 min. Samples were kept in the dark at 4±1 °C until used.

2.2. Beads generation

Ca(II)-alginate beads were prepared by ionotropic gelation according to the drop method described previously (Traffano-Schiffo et al., 2018, Aguirre Calvo and Santagapita, 2016, Aguirre Calvo et al., 2017, Santagapita et al., 2011, 2012). Three different formulations containing extracts (leaf or stem) were prepared, with the following composition: alginate (A); alginate-sucrose (AS); alginate-sucrose-dextran (ASD). For beads preparation, 10 g kg^{-1} alginate solution containing the extracts (leaf or stem) was dropped with a peristaltic pump model BT50-1J-JY10 (Boading Longer Precision Pump Co, Ltd, China) on 25 g kg^{-1} $CaCl_2$ solution (Cicarelli S.A., Argentina) in 0.1 M acetate buffer pH 5.5. Sucrose (200 g kg^{-1}) was incorporated for AS and ASD formulations, both in alginate and calcium chloride solutions. Dextran (Sigma; average molecular weight 69,000) was added (2.5 g kg^{-1}) in alginate solution for ASD preparations. Control beads were made without extract for comparative purposes.

2.3. Extract characterization

Protein concentration in samples was determined by the method of Bradford (1976), using bovine serum albumin as the standard, with some modifications. Briefly, 10.0 μL of extract was mixed with 490 μL of Bradford reagent. After 5 min of incubation at 25 °C, 200 μL were placed in a microplate and read it at 600 nm in a GloMax Promega multiplus lector (Promega Corporation, Madison, Wisconsin, USA). A calibration curve with BSA was made in the range (0–1 g.L^{-1}).

The water content of the extract and of the beads was obtained gravimetrically by the difference in weight before and after drying in a vacuum oven for 48 h at 70 ± 2 °C. The ashes content was determined for gravimetry as the official method 940.26 (ash of fruits and fruits products). Soluble sugar content (°Brix) was obtained by a Portable Full-Range Digital Automatic Refractometer (Reichert, Depew, New York, USA). All determinations were made in triplicate.

2.3.1. Betacyanin content

Quantification of betacyanins was performed spectrophotometrically following the method reported by Wruss et al. (2015) and Stintzing and Carle (2004). Spectra of extracts was recorded using a Jasco V-630 UV-VIS spectrophotomer (JASCO Inc., Maryland, USA) from wavelengths of 300–800 nm. To determine the content in the beads, 30 beads were mix with 0.175 mL of 100 g kg^{-1} sodium citrate. The percentage of betacyanin was calculated from the following equation:

$$Betacyanin\left(\frac{mg}{mL}\right) = \frac{A*DF*MW*1000}{\varepsilon*b}$$

where A = absorbance, DF = dilution factor; MW (molecular weight) = 550 g mol^{-1}, ε = 60,000 (molar extinction coefficient in L.mol^{-1}.cm^{-1}), b = path length (cm). Absorbance of the extracts was maintained at values < 1.0, by performing volumetric dilutions when needed, to ensure that the linear relationship between absorbance and concentration holds true.

The loading efficiency was calculated:

$$L.E(bc) = \left(\frac{[BC]_{beads}}{[BC]_{extract}}\right)*100 \quad (1)$$

where [BC] = betacyanin content, either in the beads or in the extract. The $[BC]_{beads}$ was normalized by the size and number of beads, considering the volume $Vol_{bead} = \frac{4}{3}\pi(r^3)$ for each of the systems, where [r] is the half of the Feret's diameter.

2.3.2. Total polyphenols

Total polyphenols for extracts and beads were determined using the technique of Singleton et al. (1998). Briefly, 125 μL of a solution of Na_2CO_3 (200 g/Kg), 800 μL of distilled water and 50 μL of sample were added to 125 μL of the Folin Ciocalteau reagent (Biopack®, Zárate, Buenos Aires, Argentina). The absorbance at 765 nm was measured after 30 min of reaction at 40 °C in the dark. Total polyphenols (TP) were expressed as mg gallic acid/mL through a calibration curve.

The loading efficiency was calculated:

$$L.E(polyphenols) = \left(\frac{[TP]_{beads}}{[TP]_{extract}}\right)*100 \quad (2)$$

where [TP] = total polyphenols content, either in the beads or in the extract. The $[TP]_{beads}$ was normalized by the size and number of beads, considering the volume $vol_{bead} = \frac{4}{3}\pi(r^3)$ for each of the systems, where [r] is the half of the Feret's diameter.

2.3.3. ABTS radical cation scavenging activity

Antioxidant activities of the samples before and after encapsulation were analyzed by detecting their ability to scavenge the $ABTS^{\bullet+}$ free radical using technique by Re et al. (1999). ABTS (7 mM) reacts with potassium persulfate (2.45 mM) after 12–16 h of incubation in the dark. The solution was then diluted to an absorbance with 0.01 M buffer phosphate pH 7.4 until absorbance of 0.90 ± 0.01 at 734 nm to form the $ABTS^{\bullet+}$ reagent. Reaction mixtures containing 100 μL of sample and 1.9 mL of reagent were incubated in a water bath at 30 °C for 30 min. The percentage inhibition was calculated against a control and compared to a gallic acid standard curve and expressed as mg eq gallic acid (GAE)/mL (Wootton-Beard et al., 2011).

The $ABTS^{\bullet+}$ remaining activity was calculated:

$$R.A.A.\left(ABTS^{\bullet+}\right) = \left(\frac{[AnA]_{beads}}{[AnA]_{extract}}\right)*100 \quad (3)$$

where [AnA] = antioxidant activity, either in the beads or in the extract. The $[AnA]_{beads}$ was normalized by the size and number of beads, considering the volume $vol_{bead} = \frac{4}{3}\pi(r^3)$ for each of the systems, where [r] is the half of the Feret's diameter.

2.4. Beads characterization

2.4.1. Digital image analysis

Size and shape of the beads were analyzed through digital

analysis of images captured by a digital camera coupled to a binocular microscope and analyzed by the free license software ImageJ (http://rsbweb.nih.gov/ij/), as described by Aguirre Calvo and Santagapita (2016). Area, perimeter, circularity and Feret's diameter were analyzed for 30 beads by applying the "analyze particle" command of the software. The ImageJ software was calibrated to transform the measured pixels to length units (mm).

2.4.2. Microstructure characterization

The microstructure characterization was performed by small-angle X-ray scattering (SAXS) at the LNLS SAXS2 beamline in Campinas, Brazil, working at $\lambda = 0.1488$ nm. The wave vector (q) was selected in the range $0.096\ nm^{-1} < q < 2.856\ nm^{-1}$. Five parameters were analyzed: (i) α_1, the fractal dimension at distances greater than the characteristic size of the rods that integrate the structure (R_1), which describes the degree of interconnection of the rods, at $q < 0.28$; (ii) α_2, the fractal dimension at distances lower than R_1, at $q > 0.55$, describing the degree of compactness within the rods; (iii) α_3, related to the connectivity between the associated polymer chains that blend dimers (basic units that constitute the rods) at $q > 1.5$; (iv) R_1, the outer radius of the rods, related to the multiplicity of the domains of the junction zone in the alginate rods, this parameter is given by $R_1 = R_g\sqrt{2}$, with R_g being the mean gyration radius in the cross-section of the rods; and (v) R_2 indicating the size related to the outer radius of the basic units of polymer dimers. The first three parameters were evaluated from the slope of the intensity averaged along azimuthal angles versus the scattering vector q in the log–log scale. The last two parameters were evaluated from the Kratky plot, scattering intensity multiplied by the square modulus of the scattering vector, $I(q)\cdot q^2$, as a function of the modulus of the scattering vector, q, which gives a maximum value at the intersection of power-law regions at $q \approx 1/R_g$. All measurements were made in triplicate.

2.5. Statistical analyses

The statistical analyses were performed by one-way ANOVA with Tukey's post-test by using Prism 6.01 (GraphPad Software Inc., San Diego, CA, USA) in order to determine significant differences between the mean values of beads of different compositions on the measured parameters. When the analysis of variance indicates differences among means, a *t*-test was used to differentiate means with 95% of confidence ($p < 0.05$).

3. Results and discussion

3.1. Effect of solvent extraction of bioactive compounds from beetroot residues

Betacyanin (BC), one of the main pigments obtained from beetroot, is currently used as a main production of dyes in food industry (Sawicki et al., 2016; Ng and Sulaiman, 2018), being obtained from the root or bulb. BC is one of the main constituent of beetroot, belonging to the betalains and categorized as group of phenolic secondary plant metabolites (Esatbeyoglu et al., 2014).

Stems and leaves are often being considered as waste in most of markets, but they show a significant content of BC in their composition, as derived from data shown in Table 1. Regardless of the extraction solvent, BC concentration is higher in the stem extracts than in leave ones (between 7 and 11 times more). A different behavior is observed in the case of total polyphenols (TP) in which leaf extracts are between 1.5 and 2.5 times higher in concentration than stem ones. Among the analyzed solvents, the increasing order of extraction for both leaves and stems for BC content were water > methanol > ethanol. Analyzing the TP content of leaf extracts, the tendency was ethanol > methanol = water, while for stem extracts, the observed tendency was ethanol = methanol > water. These results agree with the study by Sanchez-Gonzalez et al. (2013), in which aqueous extraction of BC from beetroot bulb was more efficient than in methanol or ethanol, resulting in a higher concentration of pigments in the extracts.

Table 1
Betacyanin (BC) content and total polyphenols (TP) content of leaf and stem extracts of beetroot in different extracting solvents. Standard deviation values are included. Different letters on the columns (a–c) indicate significant differences between columns ($p < 0.05$).

	Leaf extracts		Stem extracts	
	BC ($g.L^{-1}$)	GAE[a] ($g.L^{-1}$)	BC ($g.L^{-1}$)	GAE[a] ($g.L^{-1}$)
Water	6.0 ± 0.2^{a}	0.43 ± 0.02^{b}	40.6 ± 0.9^{a}	0.17 ± 0.03^{b}
Ethanol	1.6 ± 0.3^{b}	0.55 ± 0.02^{a}	13.4 ± 0.3^{c}	0.32 ± 0.03^{a}
Methanol	2.7 ± 0.1^{c}	0.42 ± 0.06^{b}	29.5 ± 0.4^{b}	0.28 ± 0.02^{a}

[a] GAE: gallic acid equivalents.

Given that an ethanolic or methanolic extraction would carry on some troubles compared with the aqueous extraction that is a simple, efficient and low-cost method for crude BC and TP extractions and promotes better stability for the pigments (Azeredo, 2009; Cai et al., 2005), all further assays were performed with the water-extracts. It is worth mentioning that water extraction procedures showed to be effective in the extraction of polyphenols from olive mill for sunscreens applications (Galanakis et al., 2018).

Soluble protein content in aqueous extracts of leaves and stems were not significantly different from each another, as well as the water content of each extract, as shown in Table 2. The soluble solids content of leaf extract was significantly higher than that of the stem one (21%), but showed a slightly lower ash content. Since the nutritional and health properties of beetroot are associated with their content of antioxidant compounds (Albano et al., 2015), the antioxidant capacity of the extracts allow a closer look at their added value in food industry applications. These results show that the leaf extract has a much higher capacity than the stem extract (more than threefold higher).

3.2. Encapsulation of betacyanins and polyphenols on Ca(II)-alginate-based beads

Leaf and stem extracts were encapsulated in Ca(II)-alginate beads with and without sucrose and dextran. BC and TP contents after beads generation were evaluated as loading efficiency, which was determined as the amount of BC and TP in the beads with different composition normalized by the content of the extract (eqs (1) and (2)). On the other hand, the remaining antioxidant activity of ABTS (RAA_{ABTS}) is the antioxidant capacity (ABTS) of the beads normalized by that of each extract (eq. (3)). Fig. 1 shows the loading efficiency for BC and TP contents as well as the RAA for each extract encapsulation system.

Although the loading efficiency of BC is significantly higher for beads containing the leaf extracts than for the stem extracts ones (Fig. 1A), the absolute amount of BC for leaf extract beads was between 0.82 and 1.00 $g.L^{-1}$ while it was significantly higher for stem extract systems, 1.7–3.2 $g.L^{-1}$. These results are confirmed by naked eye observation of the beads, as the latter systems presented a more intense reddish coloration. The addition of sucrose as synthesis additive slightly increases the loading of BC in the case of leaf extract encapsulations, but generates an important decrease in the loading of BC for the stem extract systems. Nonetheless, the advantages of including sucrose in formulations are well documented, especially when beads are subjected to freezing and drying processes (Traffano-Schiffo et al., 2018, 2017b; Santagapita et al., 2012).

Table 2
Water content (x_w), sucrose content obtained from °Brix (x_{suc}), soluble proteins by Bradford (x_{prot}), and ashes content (x_{ash}), as well as antioxidant capacity evaluated from ABTS.$^+$ analysis of stem and leaf extracts of beetroot. Different letters on the columns (a–b) indicate significant differences between rows ($p < 0.05$).

	x_w ($kg_w.kg_T^{-1}$)	x_{suc} ($kg_{suc}.kg_T^{-1}$)	x_{prot} ($kg_{prot}.kg_T^{-1}$)	x_{ash} ($kg_{ash}.kg_T^{-1}$)	Antioxidant capacity, ABTS (g_{GAE}[a].kg_T^{-1})
Stem	0.988 ± 0.002^a	0.00994 ± 0.00001^a	0.00013 ± 0.00001^a	0.0044 ± 0.0002^a	0.034 ± 0.004^b
Leaf	0.991 ± 0.001^a	0.01294 ± 0.00001^b	0.00016 ± 0.00003^a	0.0040 ± 0.0001^b	0.113 ± 0.008^a

[a] GAE: gallic acid equivalents.

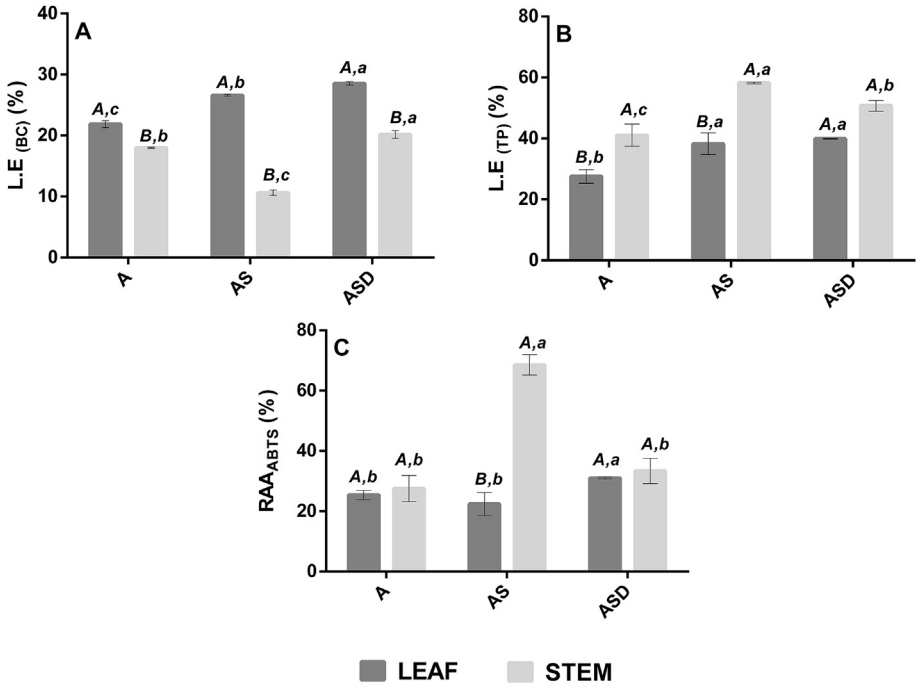

Fig. 1. Loading efficiency of betacyanin ($L.E._{BC}$) and polyphenols ($L.E._{TP}$) and remaining antioxidant activity of ABTS of different alginate beads, for leave and stem extracts. A: alginate; S: sucrose; D: dextran. Different capital letters on the columns (A–C) indicate significant differences between extracts on each system ($p < 0.05$). Different lowercase letters on the columns (a–c) indicate significant differences between systems of the same extract ($p < 0.05$).

On the other hand, and according to our hypothesis based on the incorporation of dextran as additive in bare Ca(II)-alginate systems (Martins et al., 2007), the incorporation of dextran in Ca(II)-alginate beads containing sucrose also promotes a higher loading efficiency for both the leaf and stem extracts (7.5% and 90% higher, in leaf and stem extracts, respectively).

The TP loading efficiency (Fig. 1B) is significantly higher for the stem than for the leaf extracts, and as well as for the BC contents, the incorporation of dextran as synthesis additive had a beneficial effect increasing the TP loading efficiency. In addition, absolute TP content for leaf and stem extracts were in the range 0.13–0.19 $g_{GAE}.L^{-1}$ and 0.028–0.063 $g_{GAE}.L^{-1}$, respectively, where beads containing leaf extracts had higher values than the stem extract ones. For the RAA_{ABTS} (Fig. 1C) except for the beads that had stem extract in the sucrose system, that presented a 70% of activity, the RAA was between 20 and 40% for all the systems. The antioxidant activity (AA) absolute values present a good correlation with the absolute TP content of the systems (see Supp. File, Fig. S1), which indicates that the antioxidant capacity can be attributed to total polyphenols. Regardless of the encapsulation system, all formulations present an important antioxidant activity with a view to functional food applications.

3.3. Ca(II)-alginate-based beads structural characterization

The log–log SAXS profile plots of representative samples of Ca(II)-alginate beads synthesized at pH 5.5, containing or not beetroot extracts and with or without the addition of sugars, are shown below in Fig. 5. The microstructure of beads was characterized by five parameters, related with different scales. Ca(II)-alginate interaction produce rods of defined size (R_1) and compactness (α_2), which are interconnected forming a network that can be characterized by a fractal density (α_1). Each rod is formed by several Ca(II)-alginate dimers of characteristic size and density (R_2 and α_3, respectively).

The influence of pH in the microstructural parameters was assessed between pH 5.0 and 7.0 since, although the calcium chloride solution was buffered at pH 5.5, the pH of each beetroot extract was slightly different (pH 6.9 and pH 5.2 for leaf and stem extracts, respectively). However, no significant differences were observed due to pH within this range in any of the microstructural parameters, as reported in the Suppl. File, Fig. S2, S3 and S4.

Considering the smaller basic units, it was observed that the addition of sucrose in any system generated a significant decrease in α_3, the compactness of the dimers forming the *egg box*. No trend was seen for the different extracts at this point (as shown in Fig. 2A). Regarding the size of these basic units (R_2), dependences with respect to the excipients as well as with respect to the extracts were observed (as shown in Fig. 2B). As a general trend, the presence of sucrose and dextran modulated the nanostructure of the dimers generating an increase in their size (R_2) and a decrease in their compactness (α_3) (Fig. 2B and A, respectively), while the

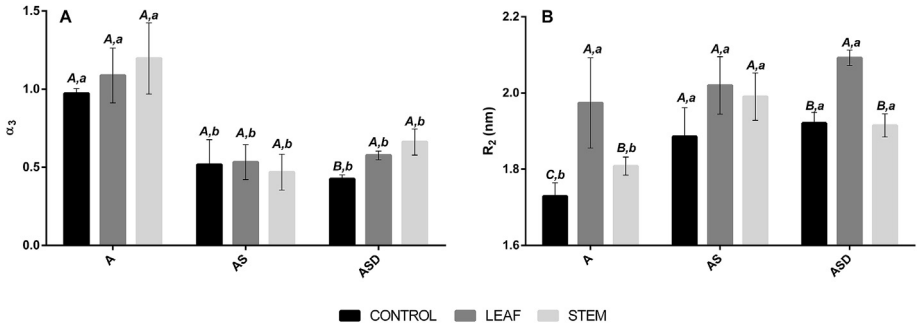

Fig. 2. Microstructure parameters of Ca(II)-alginate beads with and without extract. b. Characteristic size of the Ca(II)-alginate dimers (R_2) deduced from the minima obtained on Kratky plots. a. Fractal dimension at distances lower than R_2 or parameter α_3 of the microstructure derived from log–log SAXS pro-files. Standard deviations values are included. A, alginate; S, sucrose; D, dextran. Different capital letters on the columns (A–C) indicate significant differences between extracts on each system ($p < 0.05$). Different lowercase letters on the columns (a–c) indicate significant differences between systems of the same extract ($p < 0.05$).

extracts had no important influence in the compactness yet increasing the characteristic size of the dimers (R_2) in the case of leaf extract.

In a larger scale, microstructure parameters related to the size and compactness of the rods (R_1 and α_2, respectively) with or without extract in the different systems are shown in Fig. 3A–B. Both parameters α_2 and R_1 were affected with the addition of sucrose in the formulations, presenting a strong decrease in α_2 values which is indicative of a lower packing of the Ca(II)-alginate dimers within the rods, and a slight decrease in R_1, radius of the rods. In addition, the characteristics of the rods in bare Ca(II)-alginate systems (A) were also affected by the presence of the extracts, showing in this case an increase in both parameters, α_2 and R_1. As the presence of sucrose and extracts are in opposite fashions, for the density of rods (α_2) the effect of sucrose seemed to prevail, while for the radius of the rods (R_1), the prevailing effect seemed to be that of the extracts.

Further augmenting the scale of analysis, α_1 reflects the fractal dimension at distances greater than the characteristic size of the rods that build the structure. As it was described on Traffano-Schiffo et al. (2018) this parameter is between 1 and 2. A value of $\alpha_1 = 1$, can be related to randomly oriented rods, and a value of $\alpha_1 = 2$, can be attributed to a network of well interconnected rods. Fig. 4 shows the α_1 parameter for all the systems, for both extracts and control beads. Contrary to what is observed at a smaller scale in the systems structure, the sole addition of sucrose showed no effects in the degree of interconnection of rods, but the presence of dextran significantly increased parameter α_1 in the systems without extracts (from 1.45 in both A and AS systems to 1.77 in ASD system). On the other hand, the presence of leaf and stem extracts

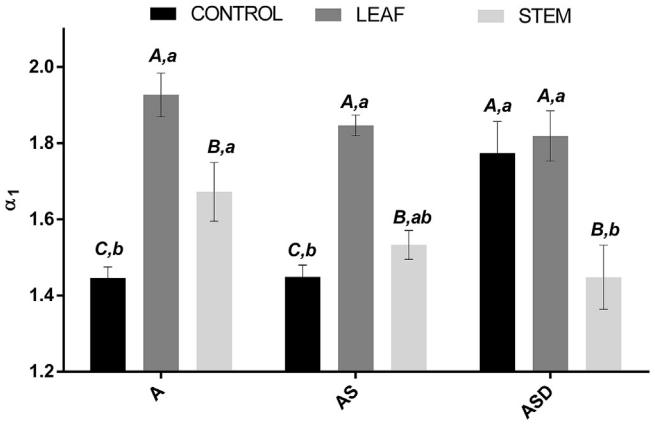

Fig. 4. Microstructure parameter of Ca(II)-alginate beads with and without extract. Fractal dimension of the rod network or parameter α_1 of the microstructure derived from log–log SAXS profiles. Standard deviation values are included. A, alginate; AS, alginate-sucrose; ASD, alginate-sucrose-dextran. Different capital letters on the columns (A–C) indicate significant differences between extracts on each system ($p < 0.05$). Different lowercase letters on the columns (a–c) indicate significant differences between systems of the same extract ($p < 0.05$).

also increased the degree of interconnection of rods in the Ca(II)-alginate without sugar additives, presenting a remarkable effect in the case of the leaf extract: the parameter α_1 changed from 1.45 in the control system to 1.67 and 1.93 in the systems with stem and leaf extracts, respectively. However, the presence of sugars and beetroot extracts did not show a synergic effect, but on the contrary

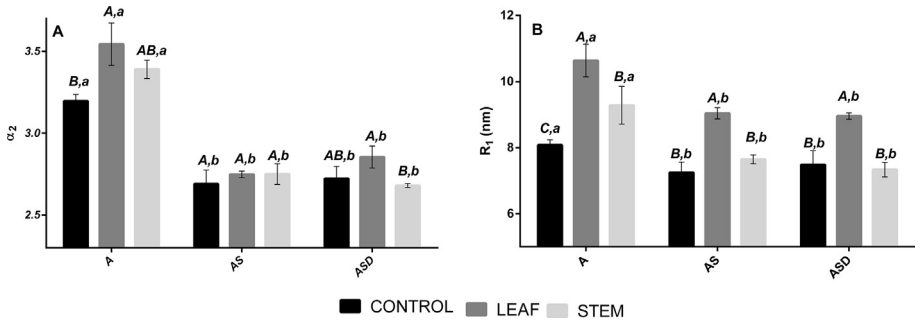

Fig. 3. Microstructure parameters of Ca(II)-alginate beads with and without extract. A. Fractal dimension at distances lower than the characteristic size of the rods or parameter α_2 of the microstructure derived from log–log SAXS profiles. B. Rod cross-sectional radius (R_1) deduced from the maxima obtained on Kratky plots. Standard deviation values are included. A, alginate; AS, alginate-sucrose; ASD, alginate-sucrose-dextran. Different capital letters on the columns (A–C) indicate significant differences between extracts on each system ($p < 0.05$). Different lowercase letters on the columns (a–c) indicate significant differences between systems of the same extract ($p < 0.05$).

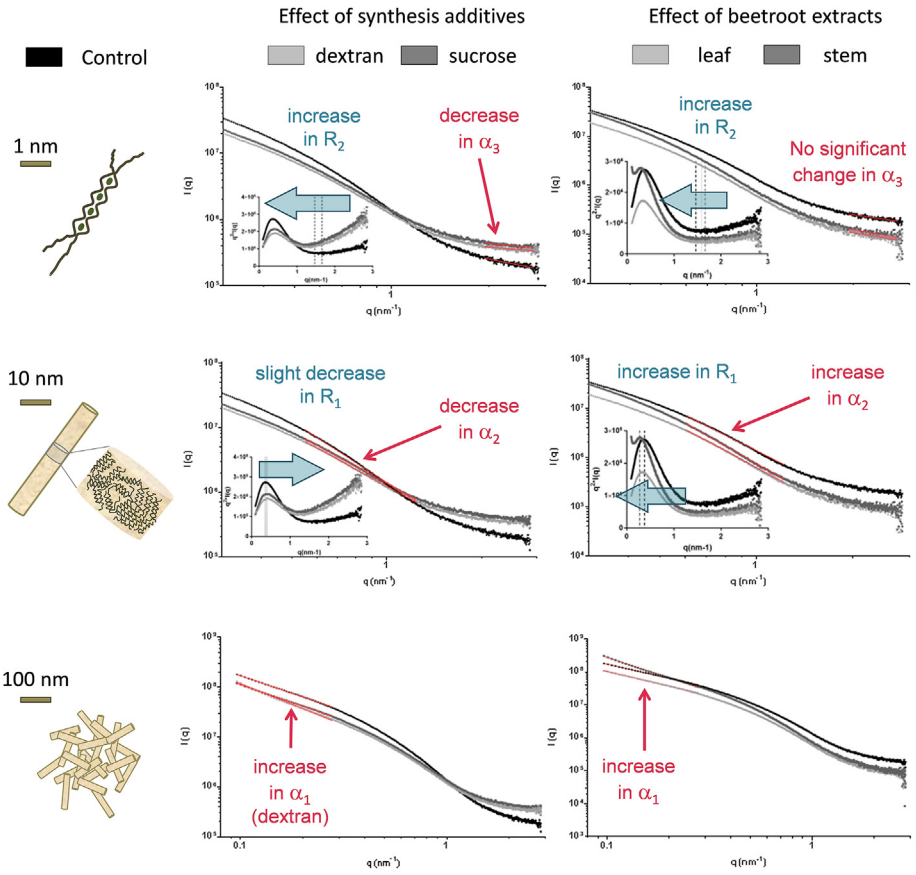

Fig. 5. Log–log SAXS profile plots of representative samples of Ca(II)-alginate hydrogels with and without beetroot extracts (right) and Ca(II)-alginate hydrogels with or without the addition of sucrose and dextran (left). From top to bottom, the structural parameters at the different scales derived from SAXS analysis. The insets show the Kratky plots from which the radii of gyration of the rods or the polymer dimers composing the rods (parameters R_1 and R_2, respectively) were obtained.

for the particular case of the stem extract they seemed to counteract each other, as the simultaneous addition of dextran and stem extract, the fractal dimension of the network presented no significant differences with the bare Ca(II)-alginate.

From a macroscopic point of view, the area, perimeter, Feret's diameter and circularity of the beads were analyzed by a digital image processing technique. Table 3 shows Feret's diameter and circularity obtained for all the systems analyzed. As expected, changes in perimeter and area were in the same trend as the Feret's diameter parameter (as is shown in Supp. File, Fig. S5), as reported in similar systems by Santagapita et al. (2011, 2012).

The addition of sugar additives to the control beads in the absence of beetroot extracts did not modify the size or circularity of the beads. On the other hand, when Ca(II)-alginate system without sugar additives contain stem extract, a slight increase in circularity is verified while maintaining the Feret's diameter. The addition of leaf extract generated a higher increase in circularity as well as an increase in the diameter of the bead. Once again, as observed above for the microstructure parameter α_1, the presence of sugars and beetroot extracts did not show a synergic effect with respect to the macroscopic parameters of the beads, diameter and circularity. In general terms, the systems containing both sugars and beetroot extracts give beads with almost the same diameter and circularity as the bare Ca(II)-alginate ones.

Fig. 5 summarizes the main structural changes introduced by the inclusion of sugars as synthesis additives, on one hand, and the incorporation of beetroot extracts, on the other hand. Log-log SAXS profile plots of representative samples with and without beetroot extracts (right) and with or without the addition of sucrose and dextran (left) are shown together with the structural parameters derived from them at the different scales (from top to bottom). As discussed above, the sugar additives and the beetroot extracts have opposite effects in the structure at a lower scale (1–10 nm), while causing a similar change in the structure at the larger scale (~100 nm), both increasing the interconnectivity of rods (i. e. the fractal density of the alginate rod's network).

Coming from natural sources, a complete chemical analysis of the composition of the leaf and stem extracts is not only difficult and time consuming, but also impracticable as different varieties of beetroot, harvested at different moments and/or from different cultivars would greatly differ from one another. However, the structural alterations caused by both extracts match with those

Table 3
Circularity and Feret's diameter of beads of different compositions (A, alginate; AS, alginate-sucrose; ASD, alginate-sucrose-dextran), containing or not beetroot extracts (control, leaf and stem extracts). Standard deviation values are indicated. At least, 30 beads of each system were analyzed. Different capital letters on the columns (A-C) indicate significant differences between extracts on each system ($p < 0.05$). Different lowercase letters on the columns (a–c) indicate significant differences between systems of the same extract ($p < 0.05$).

		Control	Leaf extract	Stem extract
Circularity	A	$0.6 \pm 0.1^{B,a}$	$0.7 \pm 0.1^{A,a}$	$0.78 \pm 0.06^{A,a}$
	AS	$0.6 \pm 0.1^{C,a}$	$0.79 \pm 0.08^{B,a}$	$0.7 \pm 0.1^{A,b}$
	ASD	$0.59 \pm 0.09^{B,a}$	$0.78 \pm 0.08^{A,a}$	$0.79 \pm 0.08^{A,a}$
Feret's diameter	A	$1.4 \pm 0.2^{B,a}$	$1.4 \pm 0.1^{B,a}$	$1.85 \pm 0.07^{A,a}$
	AS	$1.40 \pm 0.09^{A,a}$	$1.4 \pm 0.1^{A,a}$	$1.4 \pm 0.1^{A,b}$
	ASD	$1.47 \pm 0.06^{A,a}$	$1.4 \pm 0.1^{B,a}$	$1.5 \pm 0.1^{A,c}$

generated by the addition of trivalent cations. Compared to divalent cations, trivalent ones offer a more versatile and larger coordination environment, expanding the possible chain arrays within the crosslinked hydrogel, and resulting in a more ramified network as previously reported (Yang et al., 2013; Sonego et al., 2016). According to bibliography typical concentrations of Fe^{3+} in beetroot extracts are ~0.004 $g.L^{-1}$ (Wruss et al., 2015). Although in a low concentration, it is worth noting that the trivalent cations are incorporated in the sodium alginate solution before the addition of the main crosslinking cation, Ca^{2+}. In this sense, all the Fe^{3+} present is expected to coordinate triple alginate polymer centers and establish a highly ramified pre-connected polymer network before the addition of Ca^{2+}. Not only an increase in the rods connectivity (α_1) but also at a lower scale the increase in the characteristic size of the rods (R_1) as well as of the polymer dimers composing them (R_2) and the degree of interconnectivity of these dimers (α_2), can also be explained from these events of coordination of carboxylic groups from three different alginate chains and the incidence of this "tripartite junction nodes".

It has been observed that in similar Ca(II)-alginate hydrogel systems the addition of trehalose affected the extent of the rod formation, reducing the radius of the rods as well as their compactness, parameters R_1 and α_2, respectively (Traffano-Schiffo et al., 2018). As expected, similar structural modifications were observed here with the addition of sucrose, possibly due to the erratic intercalation of disaccharides in between alginate polymer chains, reducing the strength of the alginate-alginate interactions. At the larger scale, the addition of this disaccharide does not promote significant changes in the microstructure. However, the presence of dextran, a high molecular weight polysaccharide, induced a significant increase in the degree of interconnection of rods (parameter α_1). Interestingly, although causing similar effects to the microstructure (increase in α_1), no synergy is observed between dextran and beetroot extracts, which goes to show that their mechanisms of action are different. Our hypothesis is that dextran polymers present strong interactions with alginate polymers, partially hindering alginate diffusion during the crosslinking process. Thus, the higher incidence of ramifications would be related to the increased difficulty of fast pairing of alginate polymers to form dimers. In this scenario, the simultaneous presence of dextran and trivalent cations coming from beetroot extracts would have counteracting effects, as the third coordination position could be easily occupied by a −OH group from the polysaccharide, preventing the formation of alginate tripartite junctions.

4. Conclusion

Here we show that, though being overlooked in most alginate encapsulation research, the presence of natural extracts prompts important structural changes in the alginate network, from the molecular (arrange of alginate polymer dimers) to the supramolecular (interconnection of the rods composing the microstructure of the hydrogel). These natural extracts contained significant quantities of betacyanin and polyphenol and were water-based for both red beet leaf and stem, being a simple, efficient and low-cost method for crude BC and TP extractions, promoting better stability for the pigments. Both BC and TP were successfully encapsulated in Ca(II)-alginate beads, which retained between 15 and 60% of them, depending on the formulation, with a good conservation of the antioxidant activity (up to 70%). However, we also show that certain synthesis additives generally used in these systems to improve their properties (cryo-preservation, preservation and/or release of encapsulated bioactives under certain conditions, and so on) can also modulate nano and microstructure modifications when beetroot extracts are present in the formulation.

Analyzing the structural modifications can shed light in the understanding of the nature of interactions between alginate polymers and additives and/or encapsulated species. From a technological point of view, these structural changes are expected to affect relevant parameters as transport and dissolution kinetics, size exclusion and sorption properties of the obtained hydrogels. These physicochemical parameters define the performance of these phases in most of industrial and environmental applications.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jfoodeng.2018.04.015.

References

Abubakr, N., Jayemanne, A., Audrey, N., Xuqi, L.S., Dong, C.X., 2010. Effects of encapsulation process parameters of calcium alginate beads on Vitamin B12 drug release kinetics. Asia-Pacific. J. Chem. Eng. 5, 804–810.
Aguirre Calvo, T.R., Santagapita, P.R., 2016. Physicochemical characterization of alginate beads containing sugars and biopolymers. J. Qual. Reliab. Eng. 9184039.
Aguirre Calvo, T.R., Busch, V.M., Santagapita, P.R., 2017. Stability and release of an encapsulated solvent-free lycopene extract in alginate-based beads. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 77, 406–412.
Al-Remawi, M., 2012. Sucrose as a crosslinking modifier for the preparation of calcium alginate films via external gelation. J. Appl. Sci. 12 (8), 727–735.
Albano, C., Negro, C., Tommasi, N., Gerardi, C., Mita, G., Miceli, A., Blando, F., 2015. Betalains, phenols and antioxidant capacity in cactus pear [*Opuntia ficus-indica (L.) mill.*] fruits from apulia (south Italy) genotypes. Antioxidants 4 (2), 269–280.
Allegra, M., Furtmüller, P.G., Jantschko, W., et al., 2005. Mechanism of interaction of betanin and indicaxanthin with human myeloper- oxidase and hypoclorous acid. Biochem. Biophys. Res. Commun. 332, 837–844.
Arts, I.C.W., Hollman, P.C.H., 2005. Polyphenols and disease risk in epidemiologic studies. Am. J. Clin. Nutr. 81, 317–325.
Azeredo, H.M.C., 2009. Betalains: properties, sources, applications, and stability - a review. Int. J. Food Sci. Technol. 44 (12), 2365–2376.
Balanč, B., Kalušević, A., Drvenica, I., Coelho, M.T., Djordjević, V., Alves, V.D., Sousa, I., Moldão-Martins, M., Rakić, V., Nedović, V., Bugarski, B., 2015. Calcium–alginate–inulin microbeads as carriers for aqueous carqueja extract. J. Food Sci. 81 (1), E65–E75.
Bradford, M.M., 1976. Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein dye binding. Anal. Biochem. 72, 248–254.
Cai, Y.Z., Sun, M., Corke, H., 2005. Characterization and application of betalain pigments from plants of the *Amaranthaceae*. Trends Food Sci. Technol. 16 (9), 370–376.
Campañone, L., Bruno, E., Martino, M., 2014. Effect of microwave treatment on metal-alginate beads. J. Food Eng. 135, 26–30.
Clifford, T., Howatson, G., West, D.J., Stevenson, E.J., 2015. The potential benefits of red beetroot supplementation in health and disease. Nutrients 7 (4), 2801–2822.
De Giulio, B., Orlando, P., Barba, G., Coppola, R., De Rosa, M., Sada, A., De Prisco, P.P., Nazzaro, F., 2005. Use of alginate and cryo-protective sugars to improve the viability of lactic acid bacteria after freezing and freeze-drying. World J. Microbiol. Biotechnol. 21, 739–746.
Deladino, L., Anbinder, P.S., Navarro, A.S., Martino, M.N., 2008. Encapsulation of natural antioxidants extracted from *Ilex paraguariensis*. Carbohydr. Polym. 71 (1), 126–134.
Esatbeyoglu, T., Wagner, A.E., Motafakkerazad, R., Nakajima, Y., Matsugo, S., Rimbach, G., 2014. Free radical scavenging and antioxidant activity of betanin: electron spin resonance spectroscopy studies and studies in cultured cells. Food Chem. Toxicol. 73, 119–126.
Francis, F.J., 1999. Anthocyanins & Betalains. 1999. Chap 7 in Colorants. Eagan Press, St. Paul, MN, pp. 55–66.
Galanakis, C.M., 2012. Recovery of high added-value components from food wastes: conventional, emerging technologies and commercialized applications. Trends

Food Sci. Technol. 26, 68–87.
Galanakis, C.M., 2013. Emerging technologies for the production of nutraceuticals from agricultural by-products: a viewpoint of opportunities and challenges. Food Bioprod. Process. 91, 575–579.
Galanakis, C. M. The Universal Recovery Strategy. 2015. Chap 3 in Food Waste Recovery. Processing Technologies and Industrial Techniques, Academic Press, San Diego, pp. 59–81.
Galanakis, C.M., Tsatalas, Ph., Galanakis, I.M., 2018. Phenols from olive mill wastewater and other natural antioxidants as UV filters in sunscreens. Environ. Technol. Innovat. 9, 160–168.
Georgiev, V.G., Weber, J., Kneschke, E.M., Denev, P.N., Bley, T., Pavlov, A.I., 2010. Antioxidant activity and phenolic content of betalain extracts from intact plants and hairy root cultures of the red beetroot *Beta vulgaris* cv. detroit dark red. Plant Foods Hum. Nutr. 65, 105–111.
Gliszczyńska-Świgło, A., Szymusiak, H., Malinowska, P., 2006. Betanin, the main pigment of red beet: molecular origin of its exceptionally high free radical-scavenging activity. Food Addit. Contam. 23 (11), 1079–1087.
Graf, B.A., Milbury, P.E., Blumberg, J.B., 2005. Flavonols, flavonones,flavanones and human health: epidemological evidence. J. Med. Food 8, 281–290.
Halvorsen, B.L., Holte, K., Myhrstad, M.C., Barikmo, I., Hvattum, E., Remberg, S.F., Wold, A.B., Haffner, K., Baugerød, H., Andersen, L.F., Moskaug, Ø., Jacobs Jr., D.R., Blomhoff, R., 2002. A systematic screening of total antioxidants in dietary plants. J. Nutr. 132, 461–471.
Jastrebova, J., Witthoäft, C., Grahn, A., Svensson, U., Jaägerstad, M., 2003. HPLC determination of folates in raw and processed beetroots. Food Chem. 80, 579–588.
Kanner, J., Harel, S., Granit, R., 2001. Betalains-A new class of dietary cationized antioxidants. J. Agric. Food Chem. 49, 5178–5185.
Kujala, T.S., Loponen, J.M., Klika, K.D., Pihlaja, K., 2000. Phenolics and betacyanins in red beetroot (*Beta vulgaris*) root: distribution and effect of cold storage on the content of total phenolics and three individual compounds. J. Agric. Food Chem. 48 (11), 5338–5342.
Kujala, T.S., Vienola, M.S., Klika, K.D., Loponen, J.M., Pihlaja, K., 2002. Betalain and phenolic compositions of four beetroot (*Beta vulgaris*) cultivars. Eur. Food Res. Technol. 214 (6), 505–510.
Lupo, B., Maestro, A., Porras, M., Gutiérrez, J.M., González, C., 2014. Preparation of alginate microspheres by emulsification/internal gelation to encapsulate cocoa polyphenols. Food Hydrocolloids 38, 56–65.
Martins, S., Sarmento, B., Souto, E.B., Ferreira, D.C., 2007. Insulin-loaded alginate microspheres for oral delivery – effect of polysaccharide reinforcement on physicochemical properties and release profile. Carbohydr. Polym. 69, 725–731.
Messaoud, G.B., Sánchez-González, L., Jacquot, A., Probst, L., Desobry, S., 2015. Alginate/sodium caseinate aqueous-core capsules: a pH-responsive matrix. J. Colloid Interface Sci. 440, 1–8.
Nemzer, B., Pietrzkowski, Z., Spórna, A., Stalica, P., Thresher, W., Michałowski, T., Wybraniec, S., 2011. Betalainic and nutritional profiles of pigment-enriched red beet root (*Beta vulgaris* L.) dried extracts. Food Chem. 127 (1), 42–53.
Netzel, M., Stintzing, F.C., Quaas, D., Strass, G., Carle, R., Bitsch, R., Bitsch, I., Frank, T., 2005. Renal excretion of antioxidative constituents from red beet in humans. Food Res. Int. 38, 1051–1058.
Ng, M.L., Sulaiman, R., 2018. Development of beetroot (Beta vulgaris) powder using foam mat drying. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 88, 80–86.
Pandey, K.B., Rizvi, S.I., 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity 2 (5), 270–278.
Pedreño, M.A., Escribano, J., 2000. Studying the oxidation and the antiradical activity of betalain from beetroot. J. Biol. Educ. 35, 49–51.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization Assay. Free Radic. Biol. Med. 26 (98), 1231–1237.
Sanchez-Gonzalez, N., Jaime-Fonseca, M.R., Martin-Martinez, E.S., Zepeda, L.G., 2013. Extraction, stability, and separation of betalains from Opuntia joconostle cv. using response surface methodology. J. Agric. Food Chem. 61, 11995–12004.
Santagapita, P.R., Mazzobre, M.F., Buera, M.P., 2011. Formulation and drying of alginate beads for controlled release and stabilization of invertase. Biomacromolecules 12 (9), 3147–3155.
Santagapita, P.R., Mazzobre, M.F., Buera, M.P., 2012. Invertase stability in alginate beads. Effect of trehalose and chitosan inclusion and of drying methods. Food Res. Int. 47 (2), 321–330.
Sawicki, T., Bączek, N., Wiczkowski, W., 2016. Betalain profile, content and antioxidant capacity of red beetroot dependent on the genotype and root part. J. Funct. Foods 27, 249–261.
Sikorski, P., Mo, F., Skjåk-Bræk, G., Stokke, B.T., 2007a. Evidence for egg-box-compatible interactions in Calcium–Alginate gels from fiber X-ray diffraction. Biomacromolecules 8 (7), 2098–2103.
Sikorski, P., Mo, F., Skjåk-Bræk, G., Stokke, B.T., 2007b. Evidence for egg-boxcompatible interactions in calcium-alginate gels from fiber X-ray diffraction. Biomacromolecules 8 (7), 2098–2103.
Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1998. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Meth. Enzymol. 299 (197), 152–178.
Sonego, J.M., Santagapita, P.R., Perullini, M., Jobbágy, M., 2016. Ca(II) and Ce(III) homogeneous alginate hydrogels from the parent alginic acid precursor: a structural study. Dalton Trans. 45 (24), 10050–10057.
Stintzing, F.C., Carle, R., 2004. Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. Trends Food Sci. Technol. 15 (1), 19–38.
Stokke, B.T., Draget, K.I., Olav, Smidsrød, Yuguchi, O., Urakawa, H., Kajiwara, K., 2000. Small-angle X-ray scattering and rheological characterization of alginate gels. 1. Ca–Alginate gels. Macromolecules 33 (5), 1853–1863.
Traffano-Schiffo, M.V., Aguirre Calvo, T.R., Castro-Giraldez, M., Fito, P.J., Santagapita, P.R., 2017a. Alginate beads containing lactase: stability and microstructure. Biomacromolecules 18 (6), 1785–1792.
Traffano-Schiffo, M.V., Castro-Giraldez, M., Fito, P.J., Santagapita, P.R., 2017b. Encapsulation of lactase in Ca(II)-alginate beads: effect of stabilizers and drying methods. Food Res. Int. 100, 296–303.
Traffano-Schiffo, M.V., Castro-Giraldez, M., Fito, P.J., Perullini, M., Santagapita, P.R., 2018. Gums induced microstructure stability in Ca(II)-alginate beads containing lactase analyzed by SAXS. Carbohydr. Polym. 179, 402–407.
Wang, Z.Y., White, J.W., Konno, M., Saito, S., Nozawa, T., 1995. A small-angle X-ray scattering study of alginate solution and its sol-gel transition by addition of divalent cations. Biopolymers 35, 227–238.
Waters, D.J., Engberg, K., Parke-Houben, R., Hartmann, L., Ta, C.N., Toney, M.F., Frank, C.W., 2010. Morphology of photopolymerized end-linked poly(ethylene glycol) hydrogels by small-angle X-ray scattering. Macromolecules 43, 6861–6870.
Wootton-Beard, P.C., Moran, A., Ryan, L., 2011. Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after in vitro digestion measured by FRAP, DPPH, ABTS and Folin-Ciocalteu methods. Food Res. Int. 44 (1), 217–224.
Wruss, J., Waldenberger, G., Huemer, S., Uygun, P., Lanzerstorfer, P., Müller, U., Weghuber, J., 2015. Compositional characteristics of commercial beetroot products and beetroot juice prepared from seven beetroot varieties grown in Upper Austria. J. Food Compos. Anal. 42 (3), 46–55.
Yang, C.H., Wang, M.X., Haider, H., Yang, J.H., Sun, J.S., Chen, Y.M., Zhou, J., Suo, Z., 2013. Strengthening alginate/polyacrylamide hydrogels using various multivalent cations. ACS Appl. Mater. Interfaces 5 (21), 10418–10422.