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Encapsulation of ginger oil in alginate-based shell materials

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Abstract:	Hydrogels consisting of a blend of sodium alginate and k -carrageenan aqueous solutions were prepared using Ca 2+ as the gelling agent in a shell material formulation for encapsulation of food-grade ginger oil. A preliminary study on the rheological and textural behavior of two hydrogels prepared from (1) alginate water solution and (2) alginate blended with k -carrageenan in water showed that the latter produced gels with higher values of elastic and viscous moduli and gel strength, related to added mechanical rigidity. In the encapsulation of ginger oil, 4 formulations of shell material were prepared from 1% w/w alginate solution and from the blend of 1% w/w alginate solution with 1.5% w/w k -carrageenan (at 80:20 v/v ratio) with and without incubation in a 0.1 w/w chitosan solution as a final coating in a two-stage capsule hardening procedure. The ability of the formulated shell materials to protect the encapsulated ginger oil from oxidative degradation was measured using both primary and secondary oxidation products using peroxide value, p -anisidine value, and thiobarbituric acid reactive substances. Encapsulated ginger oil gave lower values of the oxidation products compared to unencapsulated. Moreover, the ginger oil extracted from capsules with alginate and k -carrageenan, along with chitosan as the final coating, showed the lowest content of oxidation products throughout the storage period, suggesting a better protection of ginger oil.
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Cover Letter

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Dear Prof.,

I enclose a new revision of the manuscript FBIO_2019_1368_R6, accepted with conditions, to be reconsidered for publication in Food Bioscience. I have taken into account the comments enclosed in the manuscript. I hope it fits the Journal now.

Sincerely,

Alicia Maestro

Manuscript title: **Encapsulation of ginger oil in alginate-based shell materials**

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This article reports the encapsulation of ginger oil to prevent its oxidation through gelation of polysaccharides with calcium, in core-shell capsules. Mixtures of alginate and kappa carrageenan offer a harder gel with higher viscoelastic and Bloom values than alginate alone, although before gelation similar rheological functions are obtained. An improvement of preservation against oxidation

is obtained when ginger oil is encapsulated with alginate-kappa carrageenan and an external film of chitosan.

The content of this manuscript has not been published previously by any of the authors and is not under consideration for publication in another journal at the time of submission.

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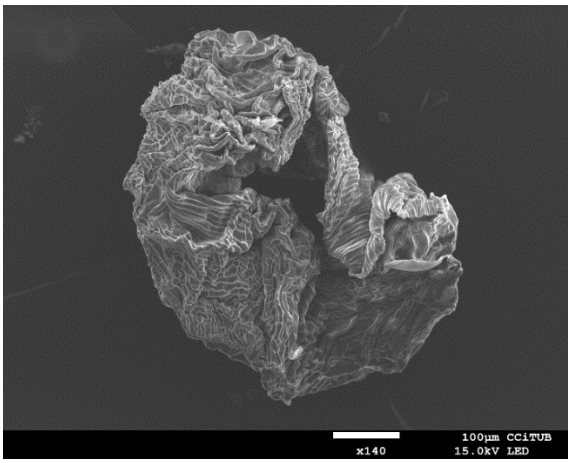
Response to editor

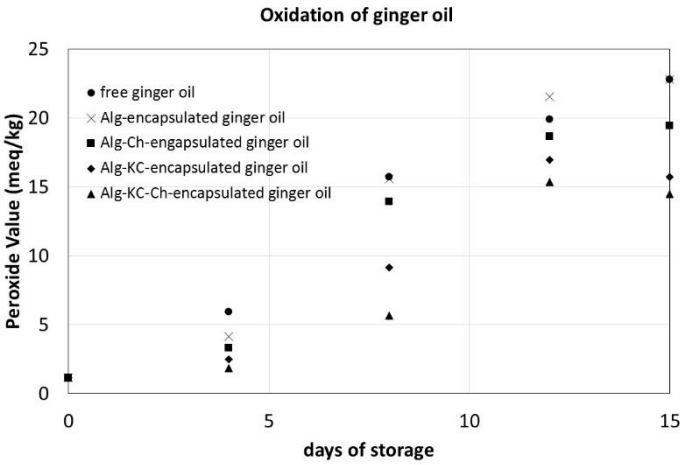
Changes in the manuscript have been done.

Highlights

- Ginger oil was encapsulated in alginate (Alg), *kappa*-carrageenan (KC), and chitosan (Ch).
- Alg-KC gels had better mechanical behavior than Alg gels, using rheology and ~~bloom~~ gel strength tests.
- Encapsulation of ginger oil offered protection against oxidation.
- Protection against oxidation was improved when the Alg-KC shell was covered with Ch.

Graphical Abstract





Encapsulation of ginger oil in alginate-based shell materials

Running title: Ginger oil in alginate-based shell materials

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ABSTRACT

Hydrogels consisting of a blend of sodium alginate and κ-carrageenan aqueous solutions were prepared using Ca^{2+} as the gelling agent in a shell material formulation for encapsulation of food-grade ginger oil. A preliminary study on the rheological and textural behavior of two hydrogels prepared from (1) alginate water solution and (2) alginate blended with κ-carrageenan in water showed that the latter produced gels with higher values of elastic and viscous moduli and gel strength, related to added mechanical rigidity. In the encapsulation of ginger oil, 4 formulations of shell material were prepared from 1% w/w alginate solution and from the blend of 1% w/w alginate solution with 1.5% w/w κ-carrageenan (at 80:20 v/v ratio) with and without incubation in a 0.1 w/w chitosan solution as a final coating in a two-stage capsule hardening procedure. The ability of the formulated shell materials to protect the encapsulated ginger oil from oxidative degradation was measured using both primary and secondary oxidation products using peroxide value, *p*-anisidine value, and thiobarbituric acid reactive substances. Encapsulated ginger oil gave lower values of the oxidation products compared to unencapsulated. Moreover, the ginger oil extracted from capsules with alginate and κ-carrageenan, along with chitosan as the final coating, showed the lowest content of oxidation products throughout the storage period, suggesting a better protection of ginger oil.

Keywords: Ginger oil, *Zingiber officinale*, Encapsulation, Alginate, Kappa-carrageenan, Chitosan

1. Introduction

The increasing number of health conscious consumers and their demands for natural and extract-based products is the major factor driving the growth of the ginger essential oil (EO) market (Future Market Insights, 2018). Ginger (*Zingiber officinale* Roscoe) is used in the food and pharmacy industry because of its culinary and medicinal properties. Its rhizomes and its extracts have a high content of sesquiterpenes and, to a lesser extent, of monoterpenes, and have strong antioxidant, anti-inflammatory, antimicrobial and antinociceptive activities, which have been confirmed (Kejing et al., 2015; Kottarapat et al., 2013; Sharma et al., 2016; Tisserand & Young, 2014). Moreover, it is valued due to the volatile components, which give a spicy and pungent characteristic aroma (Purnomo et al., 2010).

Ginger oil oxidizes quickly in contact with the atmosphere, decreasing its health-promoting benefits. Touré et al. (2007) noted that attention should be paid to preventing the oxidative deterioration of ginger oil when it is prepared, stored and added to food preparations. Encapsulation of ginger oil has the potential to improve its oxidative stability, increase its consumption despite its strong characteristic odor and taste, and improve its handling and use (Touré et al., 2011). Encapsulation in the food industry is a technique whereby an ingredient can be incorporated within a shell coating, mainly for protection against undesirable degradation and/or to control release, while maintaining its bioavailability or functionality (Sobel et al., 2014; Whelehan & Marison, 2011).

Little information is available on encapsulation of ginger oil and only a few of the published studies report the influence of shell materials on the oxidative stability of this EO. Touré et al. (2011) microencapsulated ginger oil using spray drying with maltodextrin (MD) and whey protein isolate (WPI) as wall materials and reported a good storage life with MD:WPI (1:1) and core:wall (1:4). Fernandes et al. (2017) reported that moderate wall material concentrations

(22.3%) and high inlet air temperature (170°C) were the best conditions for ginger oil encapsulation using spray drying, with WPI and inulin as shell materials. Motlagh et al. (2016) reported that 2:1 and 1:1 blends of gum Arabic:maltodextrin as shell material were feasible for the encapsulation of ginger oil in green tea extract using spray drying. Wang et al. (2016) studied the coacervation between gelatin and sodium alginate (Alg) for ginger oil microencapsulation as a function of mass ratio, pH and concentration of wall and core material load and observed a successful encapsulation that improved the EO stability.

Stability of encapsulated materials is influenced by the composition of the shell material (Peanparkdee et al., 2016; Touré et al., 2011). Alg, a naturally occurring linear anionic hydrocolloid extracted from brown seaweed, has become the most common shell material for encapsulation, due to its biocompatibility, low toxicity, relatively low cost, and mild gelation with the addition of divalent cations (Albuquerque et al., 2016; Belščak-Cvitanović et al., 2015). Lee and Mooney (2012) provided an overview of the general properties of Alg and its hydrogels, biomedical applications, and perspectives for future studies. Alg forms gels in the presence of various divalent cations such as Ca^{2+}, by cross-linking the carboxylate groups of the glucuronate on the polymer backbone. However, two major concerns about Ca^{2+}-Alg beads are: i) instability in the release media due to leaching of Ca when it complexes with other molecules and/or due to salt exchange; and ii) the high porosity of the beads leading to a burst effect or to uncontrolled active principle release (Matricardi et al., 2008). Because aqueous solutions of Alg have high viscosities even at low concentrations, concentration of Alg is limited to concentrations that produce spherical and relatively monodisperse well-shaped capsules. Therefore, the gel network has a low density of crosslinking points and does not provide the necessary barrier effect for the encapsulated material. In addition, Ca^{2+}-Alg capsules or beads are chemically susceptible to

disintegration in the presence of excess monovalent ions, Ca^{2+}-chelating agents and harsh conditions such as low pH (Zanjani et al., 2015).

Because of these challenges, there is an increasing interest to improve the barrier properties and reduce the porosity of Alg-based capsules combining Alg with complementary biopolymers, which can increase gel mechanical properties and reduce permeability (Belščˇak-Cvitanovic´ et al., 2015; Matricardi et al., 2008; Wittaya-areekul et al., 2008). Combinations of Alg with carrageenan, pectin or chitosan (Ch) have been described for immobilization of drugs and active principles (Krishnamoorthy & Basu, 2013; Mohamadnia et al., 2008; Satar et al., 2008; Zanjani et al., 2015). Carrageenan and Ch are two natural biopolymers whose synergistic action with Alg has already been used for immobilization of various drugs and active compounds (Belščˇak-Cvitanovic´ et al., 2015). Carrageenan is a family of hydrophilic linear sulfated galactanes extracted from marine red algae. It forms elastic, dry gels especially in the presence of Ca salts (Popa et al., 2011). Mixed gels of Alg and carrageenan seem to be synergistic due to their similar gelling mechanism, providing better mechanical properties. Gelation occurs due to interaction of cations such as Ca^{2+} with specific segments of carrageenan and Alg.

Ch, a natural polysaccharide obtained by extensive deacetylation of chitin, is formed by β (1–4) linked glucosamine units and some proportion of N-acetylglucosamine. The strong interaction of the cationic amine groups of Ch with the carboxylic groups of Alg forms a polyelectrolyte complex (Jayanudin et al., 2015). It can result in the formation of a membrane coating for the Alg capsule, thereby increasing its physical and chemical stability and reducing permeability (Chavarri et al., 2010; Zanjani et al., 2014). Wittaya-areekul et al. (2008) described the formation of Ch-coated Alg capsules using either a one-stage or two-stage process. In a one-stage process, the membrane coating is formed when the Alg solution is dripped directly into a

114 $CaCl_2$ solution containing Ch. The two-stage process comprises a primary production of Alg
115 capsules in a Ca^{2+} bath followed by suspension in a Ch solution for the formation of the Ch-Alg
116 complex as an external membrane.

117 Considering the promising characteristics of ginger oil and the lack of studies on its
118 encapsulation, this study focused on the development of ginger oil capsules using co-extrusion
119 technology with Alg and κ-carrageenan as shell materials, along with Ch as an external coating.
120 The protective ability of the shell materials for the EO was measured in terms of the oxidative
121 stability of the ginger oil extracted from the capsules, quantifying the content of primary and
122 secondary oxidation products during a 15 day study. A complimentary study of rheological and
123 mechanical properties of the shell gels was also done.
124

125 **2. Materials and Methods**

126 *2.1. Materials*
127 Technical grade sodium Alg with a ratio β-D-mannuronic acid:α-L-guluronic acid = 58.9:41.1,
128 measured using nuclear magnetic resonance (DMX-500, 500 MHz, Bruker, Billerica, MA,
129 USA), and $M_n \approx 668{,}000$, $M_w \approx 1{,}750{,}000$, obtained using size exclusion chromatography (see
130 below for methods details), and potato starch with an amylose content of 20.5% according to the
131 manufacturer were purchased from Panreac AppliChem ITW Reagents, Barcelona, Spain. Food-
132 grade κ-carrageenan, a linear polysaccharide formed with α(1-3)-D-galactose-4-sulfate and ß(1-
133 4)-3,6-anhydro-D-galactose, i.e., one sulfate group/disaccharide, was purchased from Sosa
134 Ingredients, Moià, Spain. Its average MW was ~690,000 according to the manufacturer.
135 Technical grade Ch from deacetylation of chitin of shrimp shells, with a degree of deacetylation
136 ~70% and MW ~11,000, and food-grade ginger oil with ~67% sesquiterpenes and 17%

monoterpenes were purchased from Sigma-Aldrich, St. Louis, MO, USA. Ginger oil was stored at 4ºC. See below for determination of oxidation state.

Technical grade Tween 20 and analytical grade NaOH, $CaCl_2$, $NaNO_3$, methanol, hexane, chloroform, glacial acetic acid, isooctane, KI, sodium thiosulfate, thiobarbituric acid, 1,1,3,3-tetraethoxypropane (TEP), trichloroacetic acid and p-anisidine reagent were purchased from Sigma-Aldrich.

2.2. Determination of Alg MW

Alg MW was obtained using size exclusion chromatography, with a Waters 2695 separation module, using a Waters 2414 refractive index detector and two hydrogel columns 7.8 x 300 mm of 2000 and 1000 *Å* (Waters Corp., Milford, MA, USA). Dextran calibration solutions of 2 mg/~~mL~~ ml in the range of 80,900 – 1,800,000 were used for calibration. The dextran and Alg solutions were eluted with 0.1 M $NaNO_3$, with a flow rate of 0.6 ~~mL~~ml/min at 30ºC. ~~Obtained~~ ~~d~~Data were processed using Empower 3 software (Waters Corp.).

2.3. Preparation of Alg-based shell material solutions

Two Alg-based hydrogel carrier systems were formulated: 1% w/w ~~Na~~Alg ~~solution (hereafter Alg) ,~~ and 1% w/w Alg blended with 1.5 or 2% w/w *κ*-carrageenan (KC)~~solution (designated as Alg-KC)~~. Single solutions of Alg and ~~*κ*-carrageenan~~KC ~~(KC)~~ were prepared by dissolving the polysaccharide in deionized water (treated in a coupled strong sulfonated acid and strong basic polystyrene ion exchange column – 8% divinylbenzene crosslinking degree, Hidro-Water, Aldaya, Valencia, Spain) at room temperature (20 – 25ºC) followed by 24 hr at 4ºC for hydration. The Alg-KC blend was prepared by combining both hydrocolloid solutions at several

158 v/v ratios, based on the previous study of Belščak-Cvitanovic´ et al. (2015). NaOH (0.1 M) was
159 used to adjust the pH of all solutions to 7.2. The Alg-KC proportions were chosen to obtain an
160 equilibrium viscosity of the blend before gelation in the range of viscosities recommended for the
161 use of the Buchi encapsulator B-390 (Büchi Labortechnik AG, Flawil, Switzerland) to obtain well-
162 shaped spheres. This range was determined using preliminary tests (data not shown).

163 For the rheological and textural studies, the preparation of the hydrogels was carried out
164 using an ionic gelation technique, adding Ca^{2+} ions, using the method described by Belščak-
165 Cvitanovic´ et al. (2015), with modifications. To prepare each hydrogel, 10 g of shell material
166 solution was weighed into a cut syringe and stirred with a Vortex mixer (Heidolph Instruments,
167 Schwabach, Germany) for 1 min with 1 g of $CaCl_2$ solutions of varying concentrations to obtain a
168 final [Ca^{2+}] in the range of 0.01 - 0.18 M. The syringe was covered with Parafilm (Heathrow
169 Scientific, Madrid, Spain), and the hydrogel was left for 24 hr at 4ºC. The gel formed in the syringe
170 could be unmold without breaking by pushing off the plunger.

171 *2.4. Rheological measurements*

172 The rheological characterization of Alg and Alg-KC solutions and hydrogels was carried
173 out as a function of polysaccharides and Ca^{2+} concentrations. Steady state viscosity measurements,
174 oscillatory shear stress sweeps and frequency sweeps were done using a HAAKE-MARS III
175 rheometer (ThermoElectron GmbH, Karlsruhe, Germany), and a serrated parallel plate measuring
176 geometry (35 mm diameter, 1 mm gap) to avoid slippage. Temperature was controlled at
177 25.0 ± 0.1ºC. The instrument allowed data processing using HAAKE RheoWin Data Manager
178 software Version 3.12 (ThermoElectron GmbH). After loading, a resting time of 5 min was used
179 before measurement to allow stress and temperature equilibration.

Oscillatory stress sweeps at a fixed frequency of 1 Hz were used as a preliminary test to determine the proper stress amplitude inside the linear viscoelastic range (LVR) for the subsequent frequency sweep tests, i.e., a stress small enough to not modify microstructure and, therefore, to obtain viscoelastic measurements independent of imposed stress amplitude. A stress amplitude of 1 Pa was chosen for the subsequent frequency sweep tests, carried out in the range of 0.01-10 Hz. Solid/elastic (G') and viscous/loss (G") moduli were measured to characterize linear viscoelasticity.

The stationary viscosity of samples was measured for different shear rates in the range of 1-1000 sec^{-1}. The rheometer was programmed to fix a shear rate and monitor the viscosity *vs*. time, and the viscosity value was taken only when it remained constant with time (equilibrium viscosity). After a constant value of viscosity was reached, a new shear rate was subsequently established along with a new steady state.

2.5. Gel strength

The determination of gel strength as a modification of Bloom value of the hydrogels was carried out using the Standard Testing Methods for Edible Gelatin of the Gelatin Manufacturers Institute of America, Inc. (2013) with modifications, using a TA-XT2i, Stable Micro Systems, Godalming, UK, with a cylindrical probe (P/0.5R, 12.5 mm) at room temperature ~~(20–25ºC)~~. Samples ~100 ml each of the Alg and Alg-KC hydrogels were prepared by mixing polysaccharide solutions with three Ca^{2+} concentrations (0.04, 0.09 and 0.13 M) and filling separate Bloom jars (Stable Micro Systems). Jars were stored for 24 h at ~~room temperature~~4ºC for maturation. The test involved a controlled compression of the sample's surface at a rate of 1.0 mm/sec to a depth of 4 mm where the maximum force reading (i.e., the resistance to penetration) was obtained. The

viscosity of the solutions did not allow to prepare gels at 6.67%, therefore gel strength was used as an alternative to Bloom values, using the Bloom test with modifications.

2.6. Encapsulation of ginger oil using co-extrusion

The encapsulation of ginger oil using co-extrusion technology was done using the Encapsulator B-390. Four different shell formulations were prepared, two of them being hydrogel solutions, i.e., 1% w/w Alg, and 1% w/w Alg blended with 1.5% w/w KC at an 80:20 v:v ratio. The other two shell materials had the hydrogel solutions previously mentioned, but capsules were put in a 0.1% w/w Ch solution to form the final coating. During encapsulation, the core material (ginger oil) and each one of the shell materials were simultaneously pumped into concentric nozzles (450 μm inner and 900 μm outer diameters) with an air pressure of 400 mbar to give a core-shell fluid stream which was sprayed out with a vibration frequency of 40 Hz with an electrostatic field of 350 V to avoid potential aggregation of capsules. This core-shell fluid was dropped into a 1.0% (w/v) $CaCl_2$ solution (corresponding to 0.09 M) for gelation and formation of capsules and maintained for 10 min with 200 rpm stirring for hardening. The dropping flow rate was ~0.9 ml/s, which gave a necklace-shaped uninterrupted flow, as recommended for the Encapsulator (a ~~snapshot~~ picture showed the flow as individual falling drops are close to each other). For the Ch-coated capsules, the capsules were hardened using the previously described method of Chew et al. (2015), involving a two-stage procedure in which the capsules were incubated in the $CaCl_2$ solution for 10 min, as described above, followed by 5 min incubation in 0.1% w/w Ch solution. Four different batches of ginger oil-loaded capsules were produced, varying the shell material formulation, each replicated 3 times. The protective ability of these shell materials against undesirable oxidative degradation of the EO was then measured.

2.7. Bead size determination

Four hundred Alg beads produced from each experimental run were measured under the Optika Microscopes ST-40-2LR optical microscope (Optika S.r.l., Ponteranica, Italy) previously calibrated using a micrometer slide for software calibration, 1 mm/100 µm, 10 mm/1000 µm (Optika Vision Pro, Ponteranica, Italy). Three runs were independently carried out for measurements to obtain a mean value and confidence interval.

2.8. Measurement of oxidative stability of ginger oil

2.8.1. Extraction of ginger oil from capsules

Ginger oil was extracted from each of the 4 samples using a modified procedure from Sun-Waterhouse et al. (2011) and Leong et al. (2016). A weighed fraction of capsules from each batch was ground and homogenized with methanol (Sonicator Sonopuls HD2070.2, 40 W, 20 kHz, Bandeling Electronic, Lichterfelde, Berlin, Germany) to solubilize the ginger oil. After filtration (Fisherbrand Grade 600 cellulose filter paper, ThermoFisher Scientific, Barcelona, Spain), hexane was added as the extraction solvent and the immiscible mixture was placed into a decanting funnel. The methanol phase was discarded, and ginger oil was recovered from the hexane using a rotatory evaporator (IKA Industrie-GmbH, Königswinter, Germany) at 55ºC and 250 mbar increasing the rotational speed from 20 to 250 rpm until no condensation was observed.

2.8.2. Encapsulation efficiency

After hardening was completed, a thin supernatant was observed, corresponding to free ginger oil. The core and shell were probably not perfectly concentric for all the drops, so that in some cases the core was not completely covered by the shell, or the shell was too thin in some parts of the capsule, and as a consequence ginger oil was released. To evaluate the encapsulation efficiency, once all the capsules were separated from the hardening bath, they were washed with

water and extraction of ginger oil was carried out as described in section 2.8.1 for all the capsules prepared in the same batch. The recovered ginger oil was weighed. The amount of ginger oil used was calculated using the weight difference in the feed bottle. Then encapsulation efficiency was defined as the ratio of recovered oil:oil used. On the other hand, to corroborate results, hexane was added to the mixture from the wash after hardening and the residual hardening bath in such a way that free, non-encapsulated ginger oil was recovered using the rotatory evaporator, similar to that described in section 2.8.1. Encapsulation efficiency was then calculated as (oil used-recovered free oil)/oil used.

2.8.3. Protection against oxidation

To study the ability of the different shells to protect ginger oil from oxidation, monolayers of the 4 types of capsules were placed in separated Petri dishes, and a small amount of free, non-encapsulated ginger oil was put in another dish as a control. All dishes were stored open in contact with the atmosphere at 4ºC for 15 days. Samples were taken at various times.

2.*8.3.1. Peroxide value (PV)*

Peroxide values (PV) of the samples were determined using the procedure of Touré et al. (2007). Samples (5.0 g) were dissolved in 10 ml of chloroform and mixed with 15 ml of glacial acetic acid and 1 ml of saturated potassium iodide (KI) solution. After 5 min, 75 ml of deionized water was added and the solution was titrated with a standardized 0.01 N sodium thiosulfate solution. Starch solution (1% w/w) was used as the indicator and the titration was continued to liberate all iodine from the solvent layer. PV was calculated in terms of meq of active oxygen/kg of ginger oil as shown in ~~Equation~~ equation 1,

$$PV = \frac{1000\,(V - V_o)c}{m} \qquad (1)$$

where V was the volume of sodium thiosulfate solution used for the determination, in ml; V_o was the volume of sodium thiosulfate solution used for the blank determination, in ml; c was the concentration of the sodium thiosulfate solution, in moles/l; and m was the mass of the sample, in g.

2.8.3.2. p-Anisidine value (p-AnV)

The determination of p-AnV was done adapting the ISO 6885:2016-Animal and vegetable fats and oils - Determination of anisidine value with some modifications using the method of the International Association of Fish Meal Manufacturers (1981). About 0.5 g of ginger oil sample was dissolved in isooctane. The solution was allowed to stand for 10 min and the absorbance was measured (UV-VIS Lambda 265 spectrophotometer, PerkinElmer, Madrid, Spain) at 350 nm and denoted as A_0, using isooctane as the blank solution (A_2). Then, 5 ml was mixed with 1 ml of the 0.25% p-AnV reagent. After 10 min, the absorbance of the reacted solution was read at 350 nm and denoted as A_1. The p-AnV of the sample was then calculated using ~~Equation~~ equation 2,

$$p - AnV = \frac{100\,QV}{m}\,[1.2(A_1 - A_2) - A_0] \qquad (2)$$

where V was the volume in which the test sample was dissolved, in ml (V = 25 ml); m was the mass of the test portion, in g; Q was the sample content of the measured solution, in g/ml (Q = 0.01 g/ml); A_2 was the absorbance of the blank solution.

2.8.3.3. Thiobarbituric Acid Reactive Substances (TBARS) Assay

The TBARS assay was done in accordance with a modified procedure of Wen (2013) and Papastergiadis et al. (2012). A standard solution of 20 mM thiobarbituric acid (TBA) reagent in deionized water was prepared. ~~1,1,3,3-Tetraethoxypropane (~~TEP~~)~~ was used as a standard precursor

of malonaldehyde (MDA). The standard curve was prepared using TEP dilutions ranging from 2.0 to 10 µM. Each TEP dilution was mixed with 1 ml of 7.5% (v/v) trichloroacetic acid (TCA) brought to 5 ml and mixed with 5 ml of the 20 mM TBA solution, and with deionized water for the blank solution. All tubes were Vortexed and placed in a water bath at 85°C for 45 min. The absorbance values of the MDA-TBA complexes were measured at 530 nm. For sample measurements, 1 g of ginger oil sample was mixed with 15 ml of 7.5% (v/v) TCA and 15 µl of Tween 20. The resulting mixture was sonicated for 10 min and then centrifuged at 3,000 g (Eppendorf 5804, Eppendorf, Hamburg, Germany) at room temperature for 15 min. The lower phase containing the MDA extract was collected using a syringe and 2 ml of the sample transferred into separated screw cap glass tubes, after which 2 ml of 20 mM 2-thiobarbituric acid (TBA) was added. The tube was Vortexed and placed in a water bath at 85ºC for 45 min. The absorbance of the solution was measured at 530 nm. MDA concentration was expressed as mg of MDA/kg of ginger oil.

2.9. Scanning electron microscopy (SEM) analysis

SEM was done using a FEI Quanta Environmental SEM (ESEM Quanta-200, ThermoFisher Scientific, Munich, Germany) and evaporator (Emitech K-950X, Quorum Technologies, Ashford, UK) in high vacuum conditions, with a voltage magnification of 3 kV. Freeze-dried Alg capsules, cut into halves with a razor blade, were attached to stubs using a two-sided adhesive tape, then coated with carbon to improve conductivity (EM ACE200, Leica, Wetzlar, Germany). Alternatively, fresh capsules were cut once frozen and later defrosted and stored for 3 days, before SEM images were taken.

2.10. Statistical treatment

Measurements of bead diameters and the different degradation determinations were made in triplicate and the mean value and confidence interval were calculated for a 95% confidence level. Statgraphics Centurion XV, Stategraphics Technologies Inc., The Plains, ~~Virginia~~VA, USA, was used for calculations.

3. Results and Discussion

3.1. Rheological behavior of hydrogels

3.1.1. Steady-state viscosity of Alg-based shell material solutions

Alg hydrogels were reported as fast gelation systems, hard to control, and often the resulting structure was not uniform nor mechanically strong (Popa et al., 2011). Preliminary tests showed that solutions of non-gelled 1% Alg flowed across the nozzle of the Encapsulator. The viscosity of these solutions with flow led to nearly spherical and monodisperse beads once dropped into the $CaCl_2$ solutions. A blend of Alg-KC with rheological behavior similar to Alg before gelation was obtained (data not shown). The viscosity of the extruded mixture in the Encapsulator was one of the process parameters that influenced the encapsulation process, as it influenced the flow rate and the sphericity of beads. A shear thinning behavior was required as it enabled the hydrogel to be extruded when shear stress was applied during encapsulation (Saha & Bhattacharya, 2010). Various blends of the two hydrocolloid solutions were prepared at different volume ratios of 1% w/w Alg and 1.5 or 2% w/w KC. Steady state viscosity as a function of shear rate was measured. As seen in Figure 1, samples showed non-Newtonian, shear thinning behavior. An Alg-KC 80:20 volume ratio of 1% (w/w) Alg solution to 1.5% (w/w) KC (triangles in Figure 1) showed a nearly identical flow curve as 1% Alg. Therefore, this mixture was used for further studies. The

337 % w/w of total polymer was 1.1%, similar to 1 % Alg shown as diamonds in Figure 1. The volume
338 ratio used was consistent with the results of Belščak-Cvitanović et al. (2015).

339 *3.1.2. Gel point of Alg-based shell material*

340 The gelation process, i.e., the change of a viscoelastic solution into an elastic solid (gel)
341 was studied for 1% Alg and 1% Alg-1.5-KC (80:20) using frequency sweep tests, with Ca^{2+} as
342 the gelling agent. Viscoelastic functions, G' and G", of both solutions in the presence of several
343 [Ca^{2+}] were plotted *vs.* frequency. As an example, results in the absence of Ca and with 1% w/v
344 $CaCl_2$ (0.09 M) are shown in Figure 2. Before gelation (without Ca), the viscoelastic functions of
345 Alg and Alg-KC were similar, as occurred with steady state viscosity (see Figure 1). Both solutions
346 showed poor elasticity, with a predominance of viscous behavior. However, when 0.09 M $CaCl_2$
347 was present, a strong increase of elastic and loss moduli occurred, with a predominance of elastic
348 modulus and with the viscoelastic functions nearly independent of frequency. This behavior is
349 typical of a gel. However, G' and G" values were higher for Alg-KC than for Alg, although
350 functions before gelation were similar, for both stationary flow (Figure 1) and oscillatory tests
351 (Figure 2) and total concentration of polymer was similar. Therefore, synergistic effects between
352 Alg and KC were observed during gelation that resulted in the formation of a stronger, more
353 compact gel, consistent with other authors (Belščak-Cvitanović et al., 2015; Mohamadnia et al.,
354 2008).

355 The gel point, GP, defined as the minimum [Ca^{2+}] for gelation, was determined for Alg and
356 Alg-KC. At the GP a macroscopic three-dimensional network was observed, due to the Ca^{2+}
357 induced intermolecular junctions extending to the whole bulk, resulting in infinite viscosity and a
358 drastic increase of elasticity (Djabourov et al., 1988; Saha and Bhattacharya, 2010). For GP
359 determination, tan δ = G"/G' for several frequencies was plotted *vs.* [Ca^{2+}] in Figure 3 for Alg and

Alg-KC. When approaching the GP, tan δ decreased abruptly as the moduli started to increase and G' became larger than G" as a result of the transition from a 'fluid' into a 'gel' (Saha and Bhattacharya, 2010). Consistent with Winter and Chambon (1986), at the GP tan δ was independent of frequency and, as a result, GP could be calculated as the $[Ca^{2+}]$ where curves at all frequencies collapsed, as GP is strictly dependent on the material.

Figures 3a) and b) show that the GP was around 0.04 M Ca^{2+} for both hydrogels, Alg and Alg-KC, although Figure 2 showed that once formed, the Alg-KC gel was stronger (for the same $[Ca^{2+}]$ = 0.09 M, higher values of G' and G" were obtained for Alg-KC than for Alg). Amici et al. (2001) reported a related study involving Alg-gellan mixtures and noted that the rheological behavior of Alg-blended hydrogels were governed by the polysaccharide present in higher concentration. The results showed that the addition of KC to the Alg prior to forming the hydrogel had an effect in the gelation behaviour. Consistent with Hermansson et al. (1991), the pure KC-Ca^{2+} gave rise to weak gels in the range 0.03 - 0.10 M Ca^{2+}. KC gels did not form at 0.02 M Ca^{2+} or below, which was consistent with the requirements of 0.02 to 0.03 M Ca^{2+} for the helix-coil transition needed for KC gelation. In their study, syneresis effects were observed, i.e., they reported moisture on the surface and release of water from the gel over time, attributed to the formation of a too highly crosslinked gel and separation of excess of water, at higher concentrations of Ca^{2+}. This behaviour was described for Alg (Lupo et al., (2014), where, above a certain $[Ca^{2+}]$, water was expelled from capsules with time. It was attributed to a too tight and compact structure when too many junction points appeared, although the $[Ca^{2+}]$ from which syneresis occurred depended on the particular formula of polymer (for example MW and mannuronic:guluronic ratio). In the present study syneresis that increased with time was observed

above [Ca^{2+}] = 0.13 M. Therefore, subsequent stability experiments were carried out at [Ca^{2+}] = 0.09 M to obtain a developed but not too tight gel and avoid any loss of water.

3.2. Gel strength

The gel strength is essentially a measure of the rigidity or stiffness of a gel measured using standard condition (Lai, 2009). The Bloom value refers to the maximum force, expressed in g, necessary to depress by 4 mm the surface of a gel with a standard 0.5" diameter cylinder probe (Gelatin Manufacturers Institute of America, Inc., 2013). For proteins it is done at 6.67% w/w. For Alg-based gels this concentration could not be used as the viscosity was too high for the Encapsulator, so the gel strengthht will be used as a modification of the Bloom test. The force *vs.* time for 1% Alg and 1% Alg-1.5-KC (80:20) with [Ca^{2+}] = 0.09 M can be seen in Figure 4. The gel strength of the hydrogel samples for three [Ca^{2+}] above the GP but below observation of syneresis are shown in Table 1.

As shown in Figure 4, a higher force was needed to penetrate the Alg-KC hydrogel compared to Alg with the same [Ca^{2+}], indicating that a harder gel was formed. These results were consistent with those shown in Figure 2, where higher G' and G" functions obtained for Alg-KC suggested a stronger, more crosslinked gel than that obtained with Alg, as discussed above. Table 1 shows that gel strength values were higher for Alg-KC for the three [Ca^{2+}] tested, one at the GP, the other at [Ca^{2+}] = 0.09 M (1% w/v Ca^{2+}) and the third at the highest [Ca^{2+}] before syneresis was observed. This can be related to the added rigidity of the gel by the combination of Alg and KC. An increase in gel strength can be related to improved mechanical properties, consistent with Lai (2009). Gel strength increased as [Ca^{2+}] was increased from 0.04 to 0.13 M, indicating a stronger, more crosslinked gel due to the presence of more crosslinking points in the range tested. Those

404 results were consistent with the <50 g gel strength values reported by Freile-Pelegrin and Robledo
405 (2008) for 1.5% KC gels at room temperature after overnight maturation.

406 *3.3. Encapsulation and oxidative stability of ginger oil*

407 The stability of an encapsulated material is mainly influenced by the composition and
408 structure of the shell materials. Alg, KC, and Ch were expected to be a good combination for
409 ginger oil encapsulation. Rheological and gel strength tests showed that the combination of Alg
410 with KC improved mechanical properties of the gels formed, compared to that of Alg alone. The
411 use of a Ch external coating has been reported to decrease permeability of beads and decrease
412 degradation of encapsulated active principles (Belšcˇak-Cvitanovic´ et al., 2015; Chew et al.,
413 2015). Beads were prepared and bead size measured. Encapsulation efficiency was calculated to
414 be 76%, when the data of recovered ginger oil from capsules was used, and 85% if the data used
415 the recovered ginger oil from the hardening and washing bath, indicating a slight loss of oil during
416 the extraction procedure.

417 Figure 5 shows a microscopic image of beads, which visually seemed spherical and relatively
418 monodisperse. The mean diameter was determined to be ~1600 ± 100 μm ($p<0.05$). No significant
419 differences between mean values were observed for uncoated and Ch-coated capsules ($p<0.05$).
420 To test oxidative protection for ginger oil, 4 sample groups of encapsulated ginger oil were
421 prepared, varying in shell material formulation, as described previously, and oxidation results are
422 found in section 3.3.2.

423 *3.3.1. Scanning Electron Microscopy (SEM) of the beads*

424 The structure of the freeze-dried beads prepared with the Encapsulator in the absence of
425 oil was observed for differences between the 4 formulations of bead formers. All fresh hydrogel

426 beads, regardless of their formulation, were spherical; however, upon freeze-drying the shape of
427 the beads became wrinkled and showed some degree of collapse in the core and a creased surface.
428 Similar observations of the structure of Alg-based beads were reported by Fundueanu et al. (1999).
429 This was likely due to sublimation of water originally trapped within the hydrogel matrix. Figure
430 6 shows the beads showing an internal hollow, which could be related to a limited advance of the
431 Ca^{2+} through the Alg structure when external gelation was used, as discussed by Lupo et al. (2015).
432 However, the hole could also be related to the collapse of the structure when sublimation occurred
433 due to its weakness, as a large cavity was more evident in beads with Alg bead-former (Figure 6.a)
434 than in beads with Alg-KC (Figure 6.b), where some structured network was preserved. It
435 suggested a stronger network, related to the presence of KC, as pointed out by Daniel-da-Silva et
436 al., 2012. The weaker mechanically stabilized beads (Alg beads in this case) generally had
437 smoother morphologies (Mohamadnia et al., 2008). As shown in sections above, the mechanical
438 properties of Alg-KC were stronger, and it could represent more resistance to the collapse during
439 freeze-drying.

440 To avoid the collapse caused by freeze-drying, fresh beads without ginger oil and fresh
441 core-shell capsules with oil in the core were frozen to facilitate handling and then cut. They were
442 stored for three days at room temperature, and later SEM images were obtained with defrosted
443 samples. All the beads were prepared with Alg-KC and coated with Ch. Images are shown in
444 Figure 7. Figures 7.a and 7.b are halves of beads without oil, so only the Alg-KC matrix is present.
445 In Figure 7.a the sphericity was slightly lost due to some water loss and the resulting slight
446 shrinkage was probably due to the freeze-defrost cycle and the three days of storage. However, no
447 collapse was observed, unlike the freeze-dried beads (Figure 6), and a network structure was
448 present, indicating that the gel network was strong enough to be retained. A detail of the core is

shown in Figure 7.b, where the structure of the remaining gel can be seen. The slight water loss could produce the small holes. It is a porous structure. Although it can protect oil against oxidation (Belščak-Cvitanović et al., 2015), it could probably be improved. Figure 7.c shows the bead surface, where a film of Ch is layered. Some wrinkles appear, but no holes are present, indicating that the film of Ch was probably a more compact barrier and could improve resistance to oxidation, which is discussed in the next section. Figure 7.d shows a capsule that initially had trapped ginger oil inside. It appears void as oil was lost when the bead was cut and defrosted. The same wrinkled outer surface was observed.

3.3.2. Oxidation stability of encapsulated ginger oil

Oxidation of ginger oil with time was determined for free, unencapsulated ginger oil (control), encapsulated ginger oil in an Alg shell with and without a Ch coating, and encapsulated in Alg-KC with and without a Ch coating.

The ability of the shell materials to protect the EO was measured in terms of the oxidation stability of the ginger oil. Oil was extracted and PV, p-AnV, and TBARS were measured during a 15-day storage at 4ºC (Annamalai et al., 2015; Rossel, 1994). The initial oxidation level of ginger oil was 1.2 ± 0.3 meq peroxide/kg oil for the PV; 5.4 ± 0.6 for the p-AnV, and 1.08 ± 0.05 mg MDA/kg oil for the TBARS. These values suggested that the oil had initially low values of oxidation.

The values showed the extent of oxidative deterioration of ginger oil throughout the storage period. The Codex Alimentarius Commission (2015) set a standard of 10 meq peroxide/kg oil for good quality of oils. Although ginger oil is not an oil, PV values after 5 days significantly increased >10 for all samples, indicating that the samples were prone to oxidative degradation. Control

unencapsulated oil showed an increase in PV to 23 ± 1 meq/kg on the 15th day. This is consistent with the results of Abitogun and Badejo (2010) who reported a PV of 82 ± 2 meq peroxide/kg of ginger oil extracted from rhizomes previously sun-dried.

Table 2 shows the PV of the control and of ginger oil extracted from capsules varying in shell formulation. In general, the PV of control oil was significantly higher ($p<0.05$) than the PV of oil extracted from capsules, especially in the first 8 days, indicating that encapsulation was able to slow down the formation of primary oxidation products. Touré *et al*. (2007) encapsulated ginger oil in maltodextrin:WPI using spray drying and reported that microencapsulation of the oil provided stability against oxidation, monitored as low PV values. The PV of control and of oil encapsulated in Alg did not vary significantly ($p<0.05$) at the end of the storage period. It may suggest that Alg was not able to protect the oil from oxidation for relatively long periods of time, due to its high permeability, as already reported by Belščak-Cvitanović et al. (2015). Due to the high viscosity of aqueous Alg solutions even at low concentrations, higher concentrations of Alg could not be used as they could not be managed in the Encapsulator, resulting in an open gel network with low crosslinking density that did not provide the necessary barrier effect (Crittenden et al., 2006). Hence, combining Alg with complementary plant-derived biopolymers, specifically KC, along with Ch as a coating, can serve as a successful strategy to increase the gel mechanical properties of the Alg beads and decrease permeability without increasing the viscosity of solutions, as already shown in Figure 1, and therefore allowing preparation of capsules. The oil extracted from the Alg-KC capsules showed lower values of PV than that extracted from Alg capsules. Alg-KC wall material provided a more effective barrier for the oil against environmental factors that promote oxidation. KC has a similar gelation mechanism to Alg, and both of them are polyelectrolytes tending to form physical hydrogels with uni/polyvalent metallic cations (Paşcalău

et al., 2013). The addition of KC increased the gel mechanical properties of the Alg and reduced the permeability of the Alg beads due to their synergistic action (Belščak-Cvitanović et al., 2015). On the other hand, Table 2 shows that the addition of an external Ch layer to Alg-KC capsules improved the results, indicating that it acts as an extra barrier to degradation, partially sealing pores due to its ionic combination with Alg and KC. Oil oxidation processes also contributed to an increase in the amount of secondary oxidation products, as seen from the increase of p-AnV (Table 3). The p-AnV measures the secondary oxidation compounds, primarily 2-alkenals and 2,4-alkadienals produced by hydroperoxide decomposition (Yang & Boyle, 2016).

The p-AnV levels for the 15-days were consistent with PV data, confirming that free ginger oil showed low resistance to oxidation if it was stored outside its original packaging. However, oil extracted from the Ch-coated Alg capsules showed significantly lower values ($p<0.05$) than the unencapsulated oil and the oil encapsulated with Alg alone. Therefore, addition of Ch as a final coating had a positive effect on protecting the encapsulated oil due to an increase of the barrier properties. Lower p-AnV values were constantly observed for the oil extracted from Alg-KC and Ch-coated Alg-KC blended capsules, with the latter having significantly lower values ($p<0.05$). These results were consistent with PV results. However, secondary oxidation was delayed even more than primary oxidation using Ch-coated Alg-KC capsules, as shown in Table 3. This suggested that oil encapsulated in Alg-KC wall materials showed slower progress of hydroperoxide decomposition.

Results of TBARS (Table 4) showed that the concentration of TBARS of the control oil sample increased for the 15 days and values were significantly higher ($p<0.05$) than those obtained for the oils extracted from the capsules, especially for Ch-coated Alg-KC ones, consistent with the results for PV and p-AnV.

The lower values of TBARS of the oil extracted from these capsules suggested the effectiveness of encapsulation as a barrier of protection against oxidative stress. That was consistent with the study of Annamalai et al. (2015) who reported that oxidative stability of fish oil was improved when encapsulated and its shelf life could be extended when stored in a refrigerated environment.

Tables 2, 3 and 4 show that there was a good correspondence among the three methods of measuring the stability of ginger oil, therefore any of these methods, developed to measure stability of oils, can be used to measure the stability of EO such as ginger oil, as observed by Touré et al. (2007) who used PV for ginger oil.

4. Conclusions

The addition of KC to Alg prior to gelling with Ca^{2+} affected the rheological and textural properties of the resulting hydrogels, mainly producing harder, more crosslinked gels, without significantly affecting the viscosity of precursor solutions and, therefore, the use of the Encapsulator. Ginger oil extracted from capsules made from 4 different shell formulations gave lower values of oil oxidation products as compared to that of the control, unencapsulated oil sample, indicating that encapsulation was able to protect the oil from oxidative deterioration. However, Alg alone did not seem to provide a significant protection. The combination of Alg with other natural hydrocolloids as shell materials, i.e., KC and Ch, increased the gel mechanical properties and reduced the permeability and porosity of Alg gels, thereby improving its ability as shell material for encapsulation. The Ch film formed by ionic interaction with Alg reduced porosity and permeability. The best results to avoid oil degradation were obtained using encapsulation with

a combination of KC-Alg mixed gel and an external Ch film formed when oil-containing polysaccharide mixtures were gelled using a Ca^{2+} solution and then incubated in a Ch solution, thus using a two-stage hardening procedure for the capsules.

Declaration of ~~competing~~ conflict of interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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Figure legends

Figure 1. Steady state viscosity *vs*. shear rate for 1% w/w alginate (Alg) and alginate-*kappa*-carrageenan blends . T = 25ºC. pH = 7.2.

Figure 2. Frequency sweep tests of 1% w/w alginate (Alg) and alginate-*kappa*-carrageenan blends (Alg-KC), 1% w/w-1.5% w/w, 80:20 volume ratio, in the absence of Ca^{2+} and gelled with 0.09 M of $CaCl_2$. T = 25ºC.

Figure 3. Frequency sweep results: G"/G' = tan δ *vs*. [Ca^{2+}] at different frequencies for (a) 1% w/w alginate hydrogel; (b) Alginate-*kappa*-carrageenan (1% w/w-1.5% w/w, 80:20 volume ratio) hydrogel. T = 25ºC.

Figure 4. Force *vs.* time to determine the gel strength for 1% w/w alginate , Alg (crosses) and alginate-*kappa*-carrageenan, Alg-KC (1% w/w-1.5% w/w, 80:20 volume ratio) (~~cercles~~circles). [Ca^{2+}] = 0.09 M. T = 25ºC.

Figure 5. Microscopic image of alginate-*kappa*-carrageenan-chitosan beads.

Figure 6. Scanning electron micrographs of a cut (a) chitosan-coated alginate bead; and (b) chitosan-coated alginate-*kappa*-carrageenan bead. Beads were freeze-dried and did not contain ginger oil.

Figure 7. Scanning electron micrographs of (a) a cut chitosan-coated alginate-*kappa*-carrageenan bead; (b) the core section of a chitosan-coated alginate-*kappa*-carrageenan bead; c) surface of the chitosan film of a bead; (d) a cut core-shell bead. Beads of images (a), (b) and (c) did not contain ginger oil. All the beads were frozen, cut and defrosted.

Figure 1

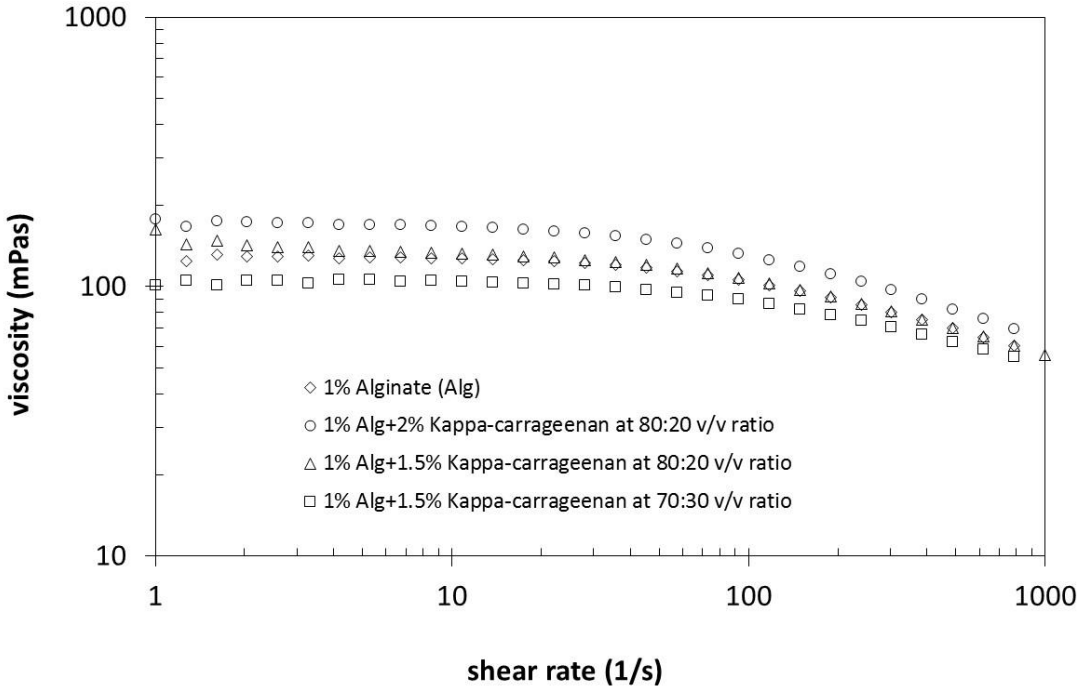
1000
100
10
viscosity (mPas)
1
10
100
1000
shear rate (1/s)
◇ 1% Alginate (Alg)
○ 1% Alg+2% Kappa-carrageenan at 80:20 v/v ratio
△ 1% Alg+1.5% Kappa-carrageenan at 80:20 v/v ratio
□ 1% Alg+1.5% Kappa-carrageenan at 70:30 v/v ratio

Figure 2

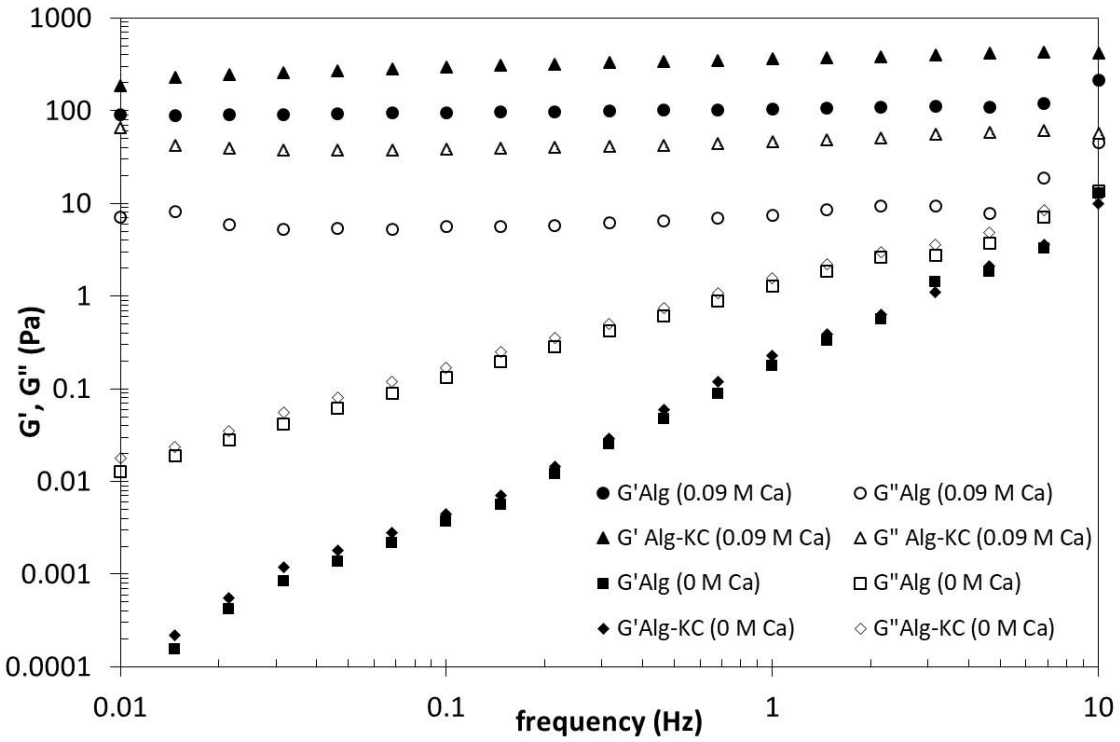
1000
100
10
1
0.1
0.01
0.001
0.0001
G', G'' (Pa)
0.01
0.1
1
10
frequency (Hz)
G'Alg (0.09 M Ca)
G''Alg (0.09 M Ca)
G' Alg-KC (0.09 M Ca)
G'' Alg-KC (0.09 M Ca)
G'Alg (0 M Ca)
G''Alg (0 M Ca)
G'Alg-KC (0 M Ca)
G''Alg-KC (0 M Ca)

Figure 3

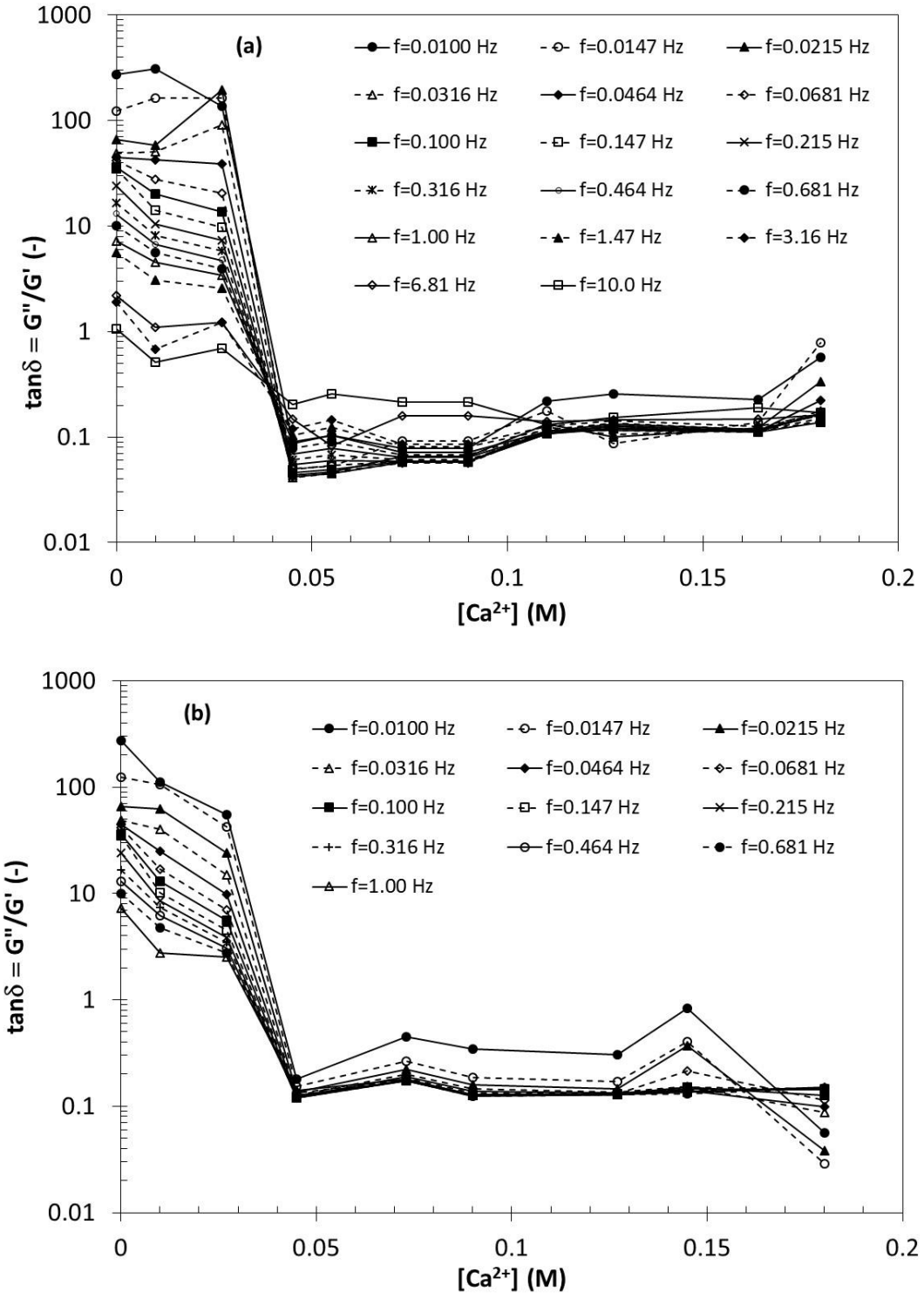
(a)
f=0.0100 Hz
f=0.0147 Hz
f=0.0215 Hz
f=0.0316 Hz
f=0.0464 Hz
f=0.0681 Hz
f=0.100 Hz
f=0.147 Hz
f=0.215 Hz
f=0.316 Hz
f=0.464 Hz
f=0.681 Hz
f=1.00 Hz
f=1.47 Hz
f=3.16 Hz
f=6.81 Hz
f=10.0 Hz
tanδ = G''/G' (-)
[Ca2+] (M)
(b)
f=0.0100 Hz
f=0.0147 Hz
f=0.0215 Hz
f=0.0316 Hz
f=0.0464 Hz
f=0.0681 Hz
f=0.100 Hz
f=0.147 Hz
f=0.215 Hz
f=0.316 Hz
f=0.464 Hz
f=0.681 Hz
f=1.00 Hz
tanδ = G''/G' (-)
[Ca2+] (M)

Figure 4

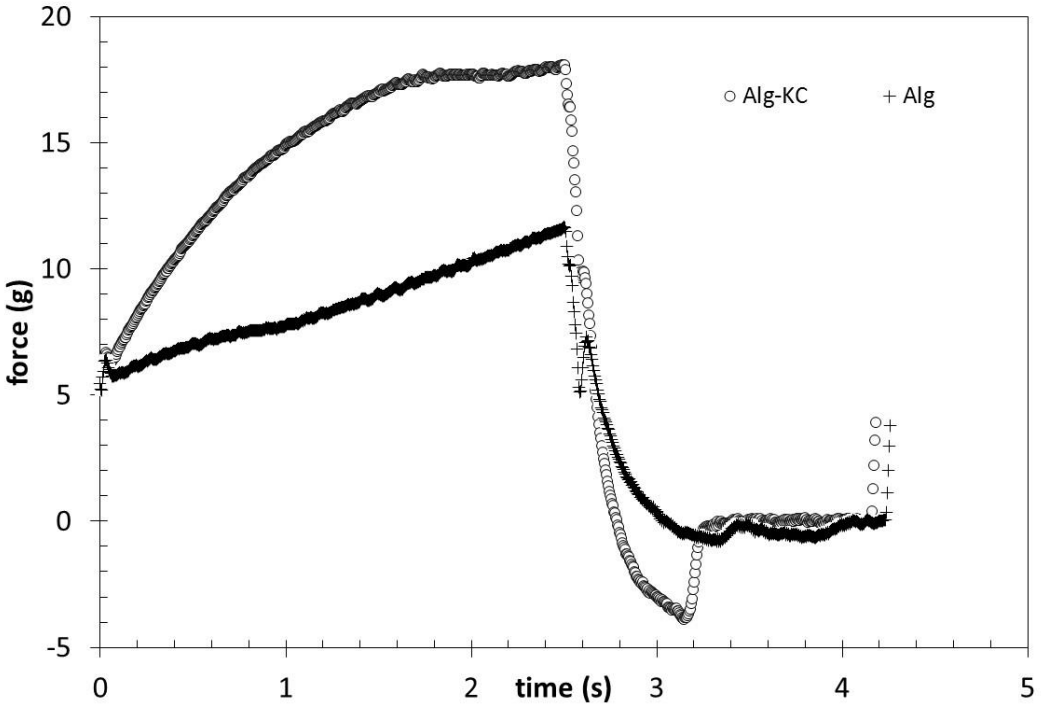

Figure 5

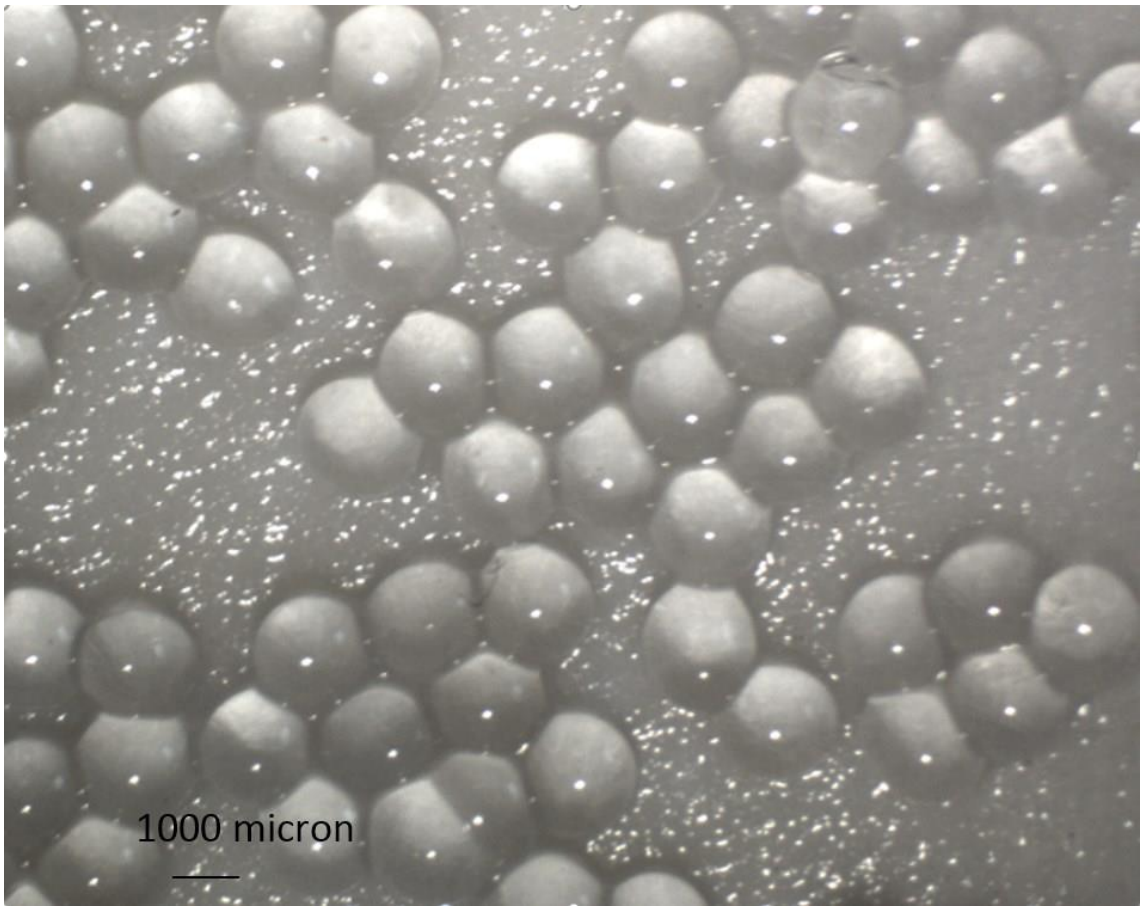

Figure 6

Fig. 6.a.

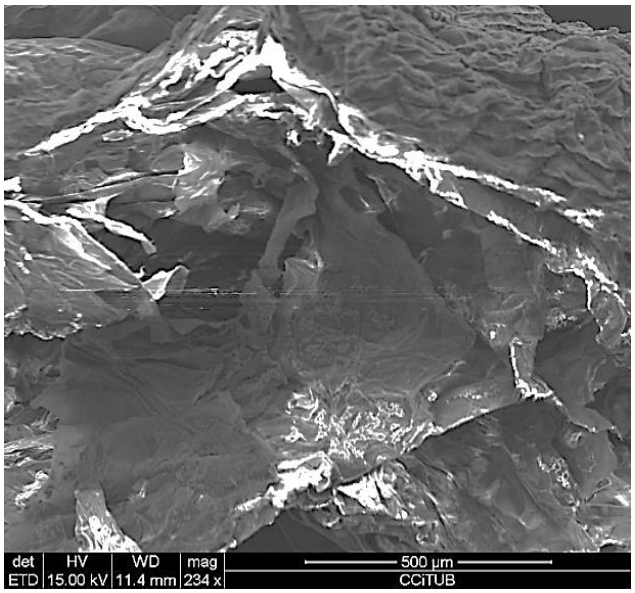

Fig. 6.b.

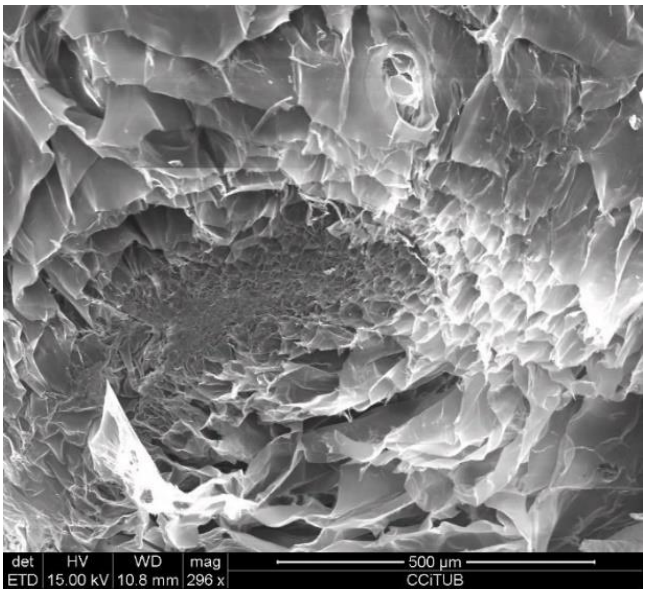

Figure 7

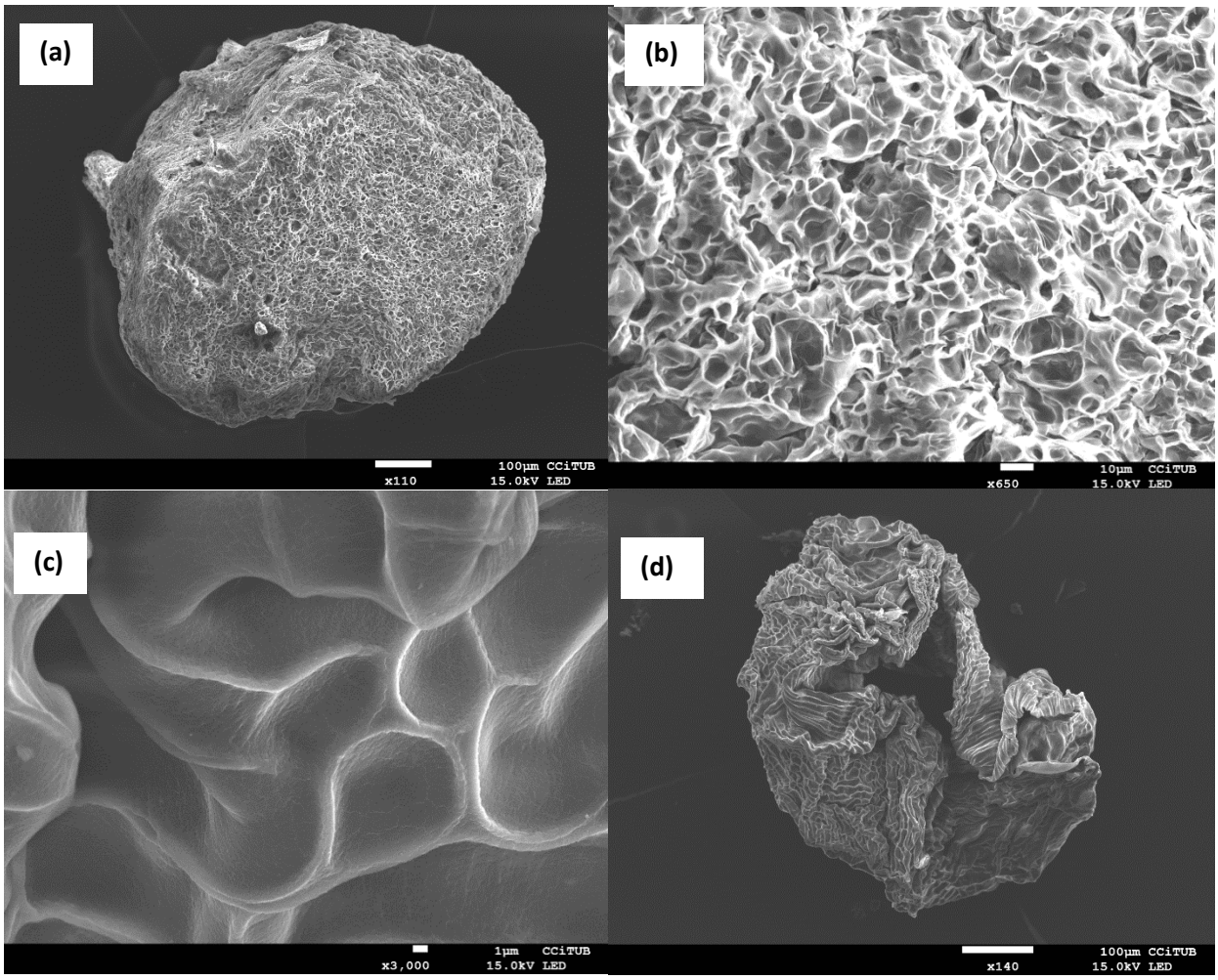
(a)
x110
100µm CCiTUB
15.0kV LED
(b)
x650
10µm CCiTUB
15.0kV LED
(c)
x3,000
1µm CCiTUB
15.0kV LED
(d)
x140
100µm CCiTUB
15.0kV LED

Table 1

Gel strength of the hydrogel samples

Ca^{2+} concentration, M	Gel strength of hydrogel, g	
	Alginate	Alginate-*Kappa*-carrageenan
0.04	11±2	18±4
0.09	12±2	18±4
0.13	14±3	21±4

Table 2

Peroxide values, PV, of the control free ginger oil sample and of ginger oil extracted from the capsules (meq/kg)

Shell material of capsule	**Days of storage after opening the container**			
	4	8	12	15
Control	6.0 ± 0.5^{d}	16 ± 1^{d}	19.9 ± 0.02^{c}	23 ± 1^{c}
Alginate	4.2 ± 0.3^{c}	15.6 ± 0.3^{d}	22 ± 1^{d}	$21 \pm 1^{b,c}$
Chitosan-coated alginate	3.3 ± 0.3^{b}	13.9 ± 0.01^{c}	18.7 ± 0.03^{c}	19 ± 3^{b}
Alginate-κ-carrageenan	2.5 ± 0.5^{a}	9.1 ± 0.3^{b}	17 ± 1^{b}	16 ± 1^{a}
Chitosan-coated alginate-κ-carrageenan	1.8 ± 0.3^{a}	5.6 ± 0.3^{a}	15 ± 1^{a}	15 ± 1^{a}

Means $\pm$ SD with different superscripts in each column were significantly different ($p<0.05$).

Table 3

p-Anisidine number of the control free ginger oil sample and of ginger oil extracted from the capsules

Shell material of capsule	Days of storage after opening the container			
	4	8	12	15
Control	8.6 ± 0.5^{d}	19 ± 1^{e}	30.5 ± 0.3^{e}	34 ± 3^{c}
Alginate	8.6 ± 0.5^{d}	17.9 ± 0.2^{d}	24 ± 1^{d}	40 ± 3^{c}
Chitosan-coated alginate	7.3 ± 0.3^{c}	16.0 ± 0.2^{c}	22.8 ± 0.4^{c}	26 ± 6^{b}
Alginate-Kappa-carrageenan	5.9 ± 0.4^{b}	14 ± 1^{b}	20 ± 1^{b}	25 ± 6^{b}
Chitosan-coated alginate-kappa-carrageenan	4.6 ± 0.4^{a}	10 ± 1^{a}	14 ± 1^{a}	16 ± 1^{a}

Means $\pm$ SD with different superscripts in each column were significantly different ($p<0.05$).

Table 4

TBARS values of the control oil sample and of oil extracted from the capsules (mg MDA/ kg)

Shell material of capsule	**Days of storage after opening the container**			
	4	8	12	15
Control	2.23 ± 0.03^{b}	4.3 ± 0.1^{e}	4.2 ± 0.4^{c}	5.8 ± 0.2^{d}
Alginate	2.2 ± 0.2^{b}	3.86 ± 0.04^{d}	4.7 ± 0.1^{d}	5.80 ± 0.05^{d}
Chitosan-coated alginate	$2.0 \pm 0.3^{a,b}$	3.4 ± 0.1^{c}	$4.0 \pm 0.2^{b,c}$	5.2 ± 0.1^{c}
Alginate-Kappa-carrageenan	1.8 ± 0.1^{a}	3.2 ± 0.1^{b}	3.8 ± 0.1^{b}	4.86 ± 0.04^{b}
Chitosan-coated alginate-kappa-carrageenan	1.75 ± 0.04^{a}	2.9 ± 0.1^{a}	3.2 ± 0.2^{a}	4.44 ± 0.02^{a}

Means $\pm$ SD with different superscripts in each column were significantly different ($p<0.05$).

Declarations of interest: none

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Esther Santamaria: Investigation

José M Gutiérrez: Methodology, Writing - Review & Editing

Carmen González: Conceptualization, Supervision, Project administration, Writing - Review & Editing