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Physicochemical and mechanical properties of a new cold-set emulsion gel system and the effect of quinoa protein fortification

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ABSTRACT

A new approach for soft and self-sustainable oil-in-water emulsion gel systems (EGs) preparation based on a formulation with olive oil, sodium alginate, citric acid, and calcium chloride, and the combination of high-speed homogenization and gelation induction by glucono-δ-lactone addition is presented. Also, the effect of EG protein fortification by the incorporation of quinoa concentrate (QC) and quinoa flour on the physicochemical and mechanical properties of these EGs upon storage at 4 °C was investigated. Despite soft and self-sustainable EGs (10 g/kg and 20 g/kg alginate, respectively) showed higher oil droplet size increment with total biopolymer concentration, these systems presented remarkable stability during 28 days. All fortified EGs showed higher fluid retention capacity (>99%), L*, a*, and lower b* values. After one day, EGs presented pHs ranging 4.1–4.3, and longer storage times promoted an increased acidity, to a less extent for QC-fortified-EGs. Lipid oxidation degree also increased during storage (from 30 to 150 μmol/L malonaldehyde), with no significant effects upon protein fortification. Protein fortification promoted higher gel strength in all self-sustainable EGs (from 3.5 N to almost 7 N), and especially after 14 days for soft-EGs. These EGs can be used in the food industry as fat replacers to formulate healthier products.

1. Introduction

Over the last years, emulsion gels (EGs) have been generating considerable interest among several application fields i.e. pharmaceutical, cosmetic, and food (Farjami & Madadlou, 2019). An interesting way of obtaining EGs is the use of cold gelling agents, which are based on the incorporation of polysaccharides and/or proteins resulting in a continuous network responsible for the functional properties of these systems (Pintado, Ruiz-Capillas, Jiménez-Colmenero, Carmona, & Herrero, 2015). An advantage insight of this gelation method is the possibility of thermo-labile compounds incorporation into the EG system, such as bioactive ingredients, that otherwise may be inactivated in an early stage of the EG applications (Pintado, Muñoz-González, Salvador, Ruiz-Capillas, & Herrero, 2021).

Alginate is a natural anionic polysaccharide extracted from brown algae and is commonly used in the food industry to modify food properties such as rheology (thickening), water binding capacity, emulsion stabilization, and film formation (Ching, Bansal, & Bhandari, 2017). This polysaccharide offers a key strategy for the obtainment of stable EGs since it can form a gel in the presence of multivalent cations such as calcium (Roopa & Bhattacharya, 2010). The formation of gel cross-linking may occur either by external or internal gelation, based on how ions are introduced to the alginate polymer (Soazo et al., 2015). An advantage of the internal gelation method is that alginate exposure to cations is controlled to achieve a homogeneous distribution of alginate in the hydrogel, thus producing a homogeneous hydrogel final structure (Draget, 2009). In this work, citrate was incorporated in the initial aqueous dispersion with $CaCl_2$ for EG formulation to promote an early sequestering by chelation and thus inactivation of Ca^{2+} cations. Ca^{2+}-release from citrate complex and alginate gelation is then induced by acidification due to the addition of glucono-δ-lactone. This method presents several advantages when comparing with other reported methods for the preparation of EGs. First, this method does not require an energetically costly step such as heating, like other EGs based on

protein thermal gelation (Paglarini et al., 2018; Wang et al., 2017; Pintado et al., 2015). Also, when comparing with other methods, the EG preparation in this work involves just a one-step process where the oil and aqueous phases are emulsified by high-speed homogenization, whereas (internal) gelation occurs spontaneously in time. This perspective may also contribute to reduce overall costs, which is an important aspect to bear in mind when proposing an industrial application for these EGs. Also, to date, there has not been reported any alginate-based method to prepare food-grade self-sustainable EGs.

To test the incorporation of an extra component in this EG matrix and, at the same time, improve the nutritional intake, EGs were formulated with quinoa protein from two different sources. Quinoa (*Chenopodium quinoa* Willd.) has been known as the "golden grain" by the native Andean people in South America and worldwide introduced and cultivated in the last decade (Lin et al., 2019). Particularly, quinoa proteins have been considered for the last few years for protein fortification of foods since not only the existence of a gradual shift in consumers preference from meat-based foods to plant-based foods but also due to its well-balanced amino acid composition (Gupta, Sharma, & Reddy Surasani, 2021; López, Galante, Robson, Boeris, & Spelzini, 2018). In this work quinoa concentrate and quinoa flour were used as a protein source in order to exploit other secondary metabolites of interest that may be present such as minerals, dietary fiber, phenolic acids, flavonoids, etc (Lin et al., 2019).

The aim of this work was to present a novel method to prepare EG systems based on the combination of high-speed homogenization and cold-set gelation. Also, this paper seeks to address the effect of protein fortification by the incorporation of quinoa concentrate and quinoa flour on the physicochemical and mechanical properties of these EGs.

2. Materials and methods

2.1. Materials

Commercial defatted quinoa flour (QF) was purchased from Los Andes (Cochabamba, Bolivia). Quinoa concentrate (QC) was prepared from QF, following the procedures detailed by Abugoch, Romero, Tapia, Silva, and Rivera (2008) with some modifications. Briefly, quinoa proteins were extracted from a QF aqueous dispersion (100 g/L) keeping the pH at 9.0 with 2 mol/L NaOH (Cicarelli, San Lorenzo, Argentina) and stirred for 30 min. Later, the supernatant obtained after centrifugation at $1000 \times g$ for 20 min at 20 °C was acidified to pH 4.5 with HCl 1 mol/L (Cicarelli, San Lorenzo, Argentina). The precipitate was left overnight in contact with the supernatant and centrifugation was repeated at $1000 \times g$ for 20 min at 20 °C. The precipitate was then solubilized by taking the pH to 9.0 with 1 mol/L NaOH and freeze-dried. The protein content of the resultant QC was determined according to the Micro Kjeldahl method (N × 6.25) and resulted to be 570 ± 10 g/kg.

Sodium alginate and glucono-δ-lactone (GDL) were provided by Bio Vanda S.A. (Rafaela, Argentina). Sodium citrate and CaCl$_2$ were purchased from Cicarelli (San Lorenzo, Argentina). Commercial olive oil was purchased from Molinos Cañuelas (Cañuelas, Buenos Aires, Argentina). All other chemicals used were of analytical grade.

2.2. Emulsion gels (EGs) preparation

The EG system proposed in this study has resulted from several tests previously performed and represents the optimum equilibrium between emulsion destabilization, GDL hydrolysis, and calcium-induced gelation of alginate kinetics, in a context of suitable alginate, GDL, citric acid and calcium chloride concentrations. Prior to GDL addition, calcium is complexed by citrate in the initial aqueous dispersion, thus preventing alginate gelation. After GDL addition (immediately before high-speed homogenization) calcium ions are released as a consequence of citrate protonation, allowing calcium-alginate complexation and gelation (Fig. 1). Firstly, aqueous sample dispersions were obtained by mixing sodium citrate (100 mmol/L), CaCl$_2$ (75 mmol/L), and sodium alginate (10 g/kg or 20 g/kg for soft and self-sustainable EGs, respectively). When corresponded, QF or QC was also added in the mixture at 20 g/kg. Each EG system was prepared by pouring olive oil into the aqueous dispersion (ratio 1:3 in g:g, respectively), solid GDL (30 g/kg) and high-speed homogenization with an homogenizer device (OMNI GLH, Kennesaw, United States) at 25,000 rpm for 1 min. Finally, these emulsified mixtures were poured into cylindrical containers (30 mm diameter and 30 mm height), hermetically sealed, and stored 24 h at 25 °C. In order to study the chilled-storage time effect these containers were kept at 4 °C for two or four weeks before analysis.

2.3. Microstructure analysis

With the aim to study the oil droplet size distribution, emulsions were prepared according to section 2.2 without the addition of GDL. Immediately after homogenization, a drop of the emulsion was placed on a microscope slide and covered with a cover slip. Emulsion micrographs were obtained using a digital camera (Olympus Camedia D-580, Tokyo, Japan) coupled to a conventional optical microscopy (Olympus microscope CX31, Tokyo, Japan). Droplet size distribution was obtained from the acquired images after thresholding operation with Image J software (Abràmoff, Magalhães, & Ram, 2004).

In addition, to elucidate the protein distribution in fortified-EGs' microstructure, prior to high-speed homogenization (section 2.2), a small aliquot of Rhodamine B solution (10 mg/mL) was added to the dispersions to reach a final concentration of 0.03 mg/mL. A small aliquote (300 μL) of each dispersion was placed into compartments of LAB-TEK II cells. Digital images were taken with a 40.0× objective in a confocal laser scanning microscope (TE2000E Model, Nikon Instruments Inc., Melville, NY, United States). All acquired images (1024 × 1024 pixel resolution) were stored as TIFF file format.

2.4. Color

CIELAB color space coordinates (lightness, L*; redness, a*; and yellowness, b*) were obtained by digital analysis of images taken from cross-sections of each EG system with Image J software (Abràmoff et al.,

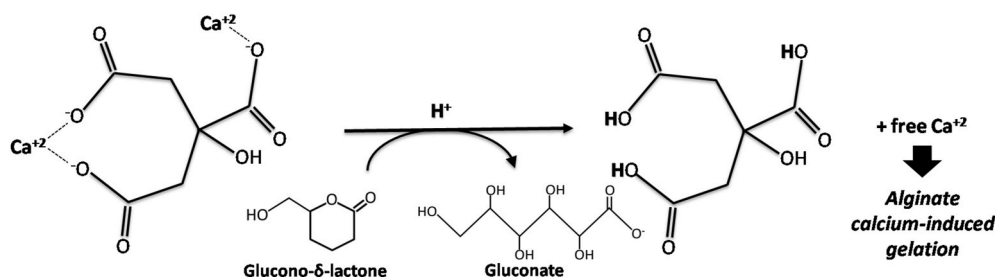

Fig. 1. Chemical reactions involved during the emulsion gel formation, based on high-speed homogenization (30,000 rpm for 1 min) of an aqueous phase containing sodium alginate, citric acid, calcium chloride, glucono-δ-lactone, and olive oil.

2004) with the Color Space Converter plugin (https://imagej.nih.gov/ij/plugins/color-space-converter.html). Samples were photographed on a matte black background with a digital camera (Canon EOS-Rebel T6, Tokyo, Japan) as described by Mendoza and Aguilera (2004).

2.5. Fluid retention capacity (FRC)

Water and lipid-binding properties were determined in each EG system following the procedure reported by Herrero, Carmona, Jiménez-Colmenero, and Ruiz-Capillas (2014) with some modifications. EGs were prepared according to section 2.2 and poured into hermetically sealed tubes, heated at 60 °C in a water bath for 30 min, and centrifuged at 800×*g* for 30 min. The amount of the released fluid was weighted so as to express the FRC as a percentage using the following equation:

$$FRC\ (\%) = [(gel\ weight–fluid\ weight)/gel\ weight]*100 \qquad (1)$$

2.6. Lipid oxidation degree

Oxidative stability of each EG system was determined by the procedure based on the change in thiobarbituric acid reactive substances (TBARs) as described by Pintado et al. (2015) with some modifications. Briefly, 3 g of each sample was homogenized with 21 mL of 75 g/L trichloroacetic acid with a high-speed homogenizer device (OMNI GLH, Kennesaw, United States) at 25,000 rpm for 1 min to precipitate proteins. This slurry was filtered and 2 mL of the supernatant was mixed with 2 mL of 20 mmol/L thiobarbituric acid. Later, this solution was kept in the dark for 12 h at room temperature. Absorbance at 532 nm was measured in a Jasco V-550 spectrophotometer (JASCO International 157 Co., Tokyo, Japan). The calibration curve was performed with 1,1,3,3-tetramethoxypropane (Sigma Chemical Co., St. Louis, MO, United States) to determine the malonaldehyde (MDA) concentration and results were expressed in μmol/L.

2.7. Mechanical properties

2.7.1. Penetration tests for cold-set EGs

Penetration tests were carried out at room temperature for EGs formulated with 10 g/kg alginate with a Perten TVT 6700 Texture Analyzer (Perten Instruments, Hägersten, Sweden) using a cylindrical probe (25 mm diameter). Each sample was penetrated axially at a constant speed of 1.0 mm/s. From each Force vs. distance plot, firmness (FN, N/mm) and fracture force (FF, N) were obtained as the initial slope of the penetration profiles and as the force at the first significant gel break, respectively.

2.7.2. Double-compression tests for self-sustainable EGs

After gently demolding self-sustainable 20 g/kg alginate-EGs from their cylindrical containers, samples were subjected to two consecutive deformation cycles to a predetermined distance at 40% compression with a Perten TVT 6700 Texture Analyzer (Perten Instruments, Hägersten, Sweden) by using a 35-mm stainless-steel compression probe at a constant speed of 1 mm/s at room temperature. Five parameters of each TPA profile were determined: Hardness (N), Springiness, Cohesiveness, Adhesiveness (J), and Chewiness (N).

2.8. Statistical analysis

Each determination was carried out in at least three independent samples, and results were expressed as mean values or mean ± standard deviation. Pooled standard deviation was calculated for each series of replicates and used as an estimator of the precision of the measurements. The statistical analysis was performed by analysis of variance (ANOVA)

and Tukey test with R software (4.0.2 version). Differences were considered statistically significant at $p < 0.05$. Principal component analysis (PCA) was performed in order to reduce dimensions of the space of physicochemical characteristics of the emulsion gels: final pH, color parameters (L*, a*, b*), FRC (%), and lipid oxidation degree (MDA concentration).

3. Results and discussion

3.1. Droplet size distribution

Fig. 2 shows droplet size distribution for emulsions and fortified emulsions with QC and QF. Droplet diameters ranged from 1.5 μm to 20 μm, which is a typical size for coarse emulsions obtained by high-speed homogenization (McClements, 2015).

It has been extensively reported that emulsion droplets coated by protein–polysaccharide interfacial layers often have improved stability. However, the successful stabilization depends on careful control of system conditions such as droplet characteristics (concentration, size, and charge), polymer characteristics (concentration and molecular weight), solution composition (pH, ionic strength), and mixing conditions (order of addition, stirring speed) to prevent bridging and depletion flocculation (McClements, 2015). Fig. 2 shows that droplet sizes increase with total biopolymer concentration. The initial pH in all aqueous dispersions before oil and GDL addition for EG formation was not significantly different among all samples assayed ($p > 0.05$), being 7.5 ± 0.7. It is important to bear in mind that at this initial dispersion pH, quinoa proteins and alginate are negatively charged (pH > pI and pH > pKa, respectively), and thus, repulsive ionic interactions are expected to occur. In fact, Romo, Abugoch, and Tapia (2020) reported quinoa protein aggregation by a segregative effect of alginate for quinoa protein-alginate systems at pH 8, which is revealed as a decrease in the surface hydrophobicity of quinoa proteins. This exclusion effect was also reported by Montellano Duran, Spelzini, Wayllace, Boeris, & Barroso da Silva (2018) for quinoa proteins and another anionic polysaccharide, carrageenan. When these biopolymers are negatively charged (at pH > 5.5), a pure Coulombic repulsion regime is observed and quinoa proteins self-associate due to the anionic polysaccharide exclusion from the
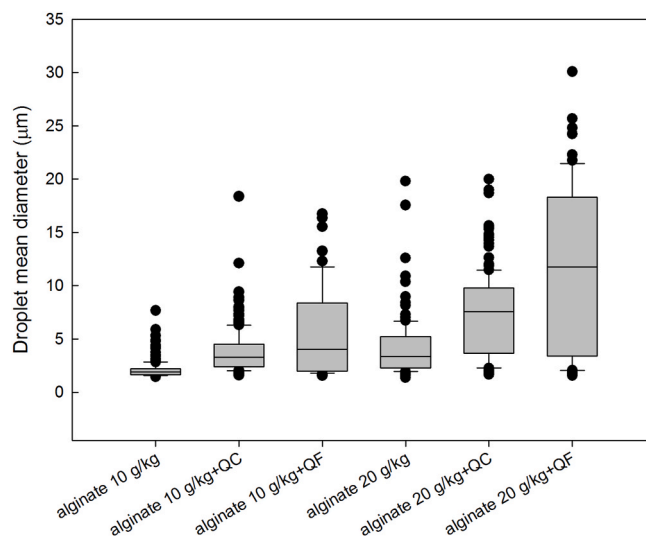
Fig. 2. Droplet diameter distribution obtained by digital analysis of non-fortified and fortified O/W emulsions' micrographs with 20 g/kg quinoa concentrate and flour (+QC and +QF, respectively) at 10 g/kg and 20 g/kg alginate. Sodium citrate and CaCl₂, were 100 mmol/L and 75 mmol/L, respectively. Emulsions were prepared by high-speed homogenization (25,000 rpm for 1 min) of the aqueous dispersion and olive oil phases in a ratio of 3 g:1 g, respectively.

protein surface. In a later work, these authors use this phenomenon as a plausible explanation for the bigger pores observed in QC-carrageenan cold-set gels (Montellano Duran, Spelzini, & Boeris, 2019).

Also, it is known that the presence of non-adsorbed polymer in the continuous phase (alginate or alginate plus quinoa proteins in this case) may lead to the generation of an osmotic attractive force between the droplets due to exclusion of the polymer molecules from the narrow region surrounding each droplet (McClements, 2004). This depletion effect may be also positively correlated to an increment of the mean droplet size (Fig. 2) since this attractive force increases as the concentration of polymer molecules increases. Pallandre, Decker, and Mc Clements (2007) reported similar results for caseinate-coated O/W microemulsions at high alginate concentrations (>4 g/kg) at neutral pH, showing also a significant increase of droplet diameter with biopolymer concentration. Moreover, Zhang et al. (2015) also reported an increase of the mean droplet size at neutral pH in the presence of alginate for coarse O/W emulsions stabilized with soy protein isolate, which is also in line with these results.

The difference observed between QC and QF emulsions for 10 g/kg and 20 g/kg alginate (Fig. 2) can be explained by the presence of insoluble components in QF and their influence in the formation of larger eddies during turbulence throughout high-speed homogenization and a concomitant and significant further increase in droplet size (McClements, 2017).

All these gelled systems after GDL addition showed micrographs with similar droplet sizes than their not-gelled emulsion counterparts, revealing a lack of significant destabilization effects by coalescence and flocculation during gelation. This remarkable emulsion stability was also reported by Li, Gong, Hou, Yang, and Guo (2020) for alginate-based EGs. These authors demonstrated no coalescence phenomena in calcium-induced mayonnaise-like EGs formulated with alginate, Tween 80, rapeseed oil, and CaCl$_2$, even after heating and/or freeze-thawing treatments. These authors also reported the same stability against these conditions in a similar EG system in the presence of konjac glucomannan (Yang et al., 2020).

Fig. 3 shows a schematic representation of the EG preparation method proposed, involving the role of each ingredient during the EG assembly process. Also, at the bottom of this Figure, a fortified-EG microstructure is depicted from the micrograph analysis obtained from CLSM and Rhodamine B-staining (QC-fortified self-sustainable EG system is shown). As previously explained, citrate is incorporated in the initial aqueous dispersion to promote Ca^{2+} sequestration by chelation. Ca^{2+}-release from citrate complex and alginate gelation is then induced by acidification due to the addition of glucono-δ-lactone. CLSM micrographs upon Rhodamine B staining showed that quinoa proteins can be found coating oil droplets in the fortified-EG systems (at the bottom of Fig. 3). The micrograph shown at the left is the same image in the light conventional microscopy mode of the confocal laser scanning microscope, which shows oil droplet distribution.

3.2. Physicochemical properties

All EG physicochemical properties are shown in Table 1. These data were analyzed by PCA in order to reduce the number of variables. The first and second principal components (PC-1 and PC-2, respectively) explained 89% of the total variability of the data. PC-1 was high and directly correlated with L*, a*, FRC (%), and to a minor extent, with final pH. PC-1 was also highly correlated with b* but in a negative way. On the other hand, PC-2 was high and directly correlated with final pH, and high and negatively correlated with MDA concentration.

PCA bi-plot of all emulsion gels assayed is shown in Fig. 4. PC-1 accounts for 64% of the variance of all data. Regardless of the storage time and alginate concentration, all non-fortified EG systems showed negative PC-1 values, whereas fortified ones showed positive PC-1 values (Fig. 4). This difference can be explained taking into account that the presence of lipophilic compounds in QC/QF i.e. proteins, fiber, and carbohydrates, promotes significantly higher values of FRC when compared to non-fortified EG. In this sense, a higher percentage of these
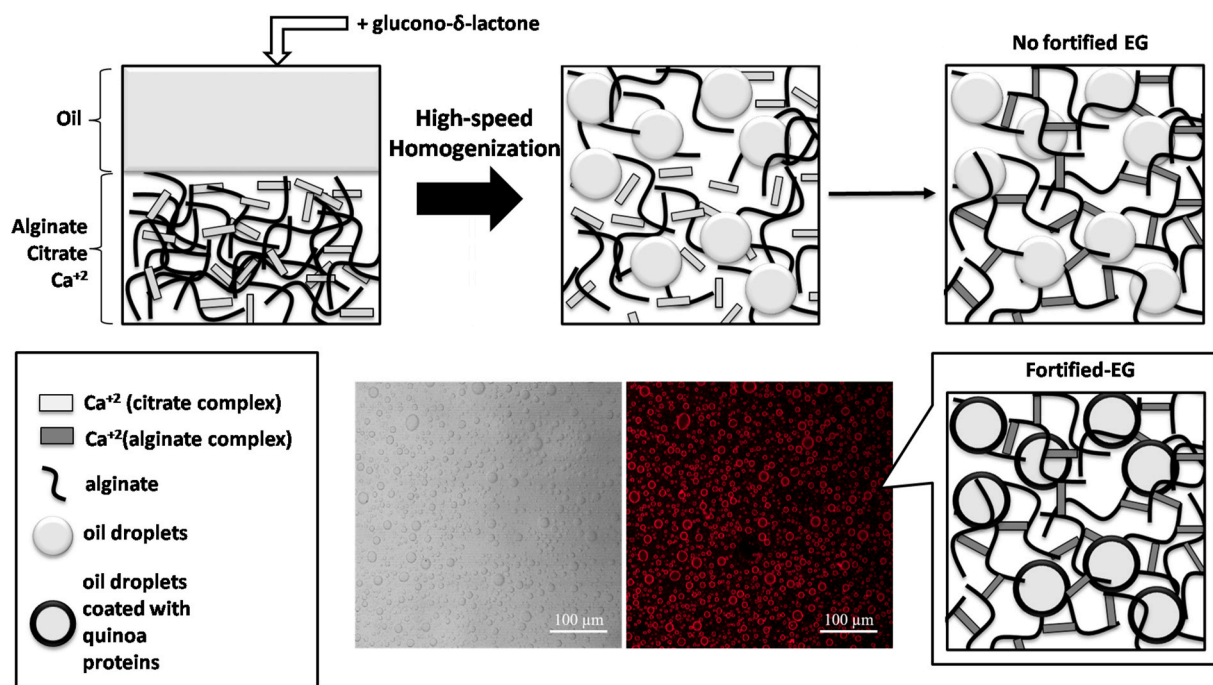

Fig. 3. Schematic representation of the microstructural processes involved in the formation of the non-fortified and fortified-emulsion gels (EGs) and micrographs obtained by confocal laser scanning microscopy and Rhodamine-B staining from 20 g/kg alginate-EG system fortified with quinoa concentrate (at the bottom). The left image corresponds to the same micrograph observed in light conventional microscopy mode. EGs were prepared in a single-step of high-speed homogenization (30,000 rpm, 1 min) and formulated as follows: 10 or 20 g/kg alginate, 100 mmol/L Sodium citrate, 75 mmol/L CaCl$_2$, and 30 g/kg glucono-δ-lactone for the aqueous dispersion, and olive oil (at a ratio of 3 g aqueous phase per 1 g oil phase). Fortified-EGs were prepared by including quinoa concentrate or quinoa flour in the aqueous phase at 20 g/kg.

Table 1
Physicochemical characteristics of non-fortified and fortified emulsion gels (QC: quinoa concentrate; QF: quinoa flour; Alg: alginate concentration; FRC: fluid retention capacity; C_{MDA}: malonaldehyde concentration).

Fortification	Alginate (g/kg)	Storage time (days)	Final pH	L*	a*	b*	FRC (%)	C_{MDA} (μmol/L)
-	10	1	4.12[e,f,g]	72[a]	−10.6[a]	64[d]	82.0[a]	35[a,b,c]
		14	4.09[c,d,e]	72[a]	−10.0[a,b]	68[d]	83.2[a,b]	48[a,b,c]
		28	4.04[a,b]	73[a,b]	−10.1[a,b,c]	69[d]	82.5[a]	160[f,g]
	20	1	4.20[i,j]	71[a]	−11.0[a]	66[d]	81.4[a]	25[a,b]
		14	4.08[b,c,d]	73[a]	−11.2[a]	67[d]	85.6[b,c]	107[e,f]
		28	4.06[b,c]	73[a]	−10.5[a,b,c]	66[d]	88.2[c]	156[f,g]
QC	10	1	4.27[k,l]	77[b]	−6.9[b,c,d]	46[a]	98.8[d]	17[a]
		14	4.25[k,l]	77[b]	−5.7[d]	41[a,b,c]	98.2[d]	50[a,b,c]
		28	4.24[j,k]	77[b]	−6.2[c,d]	45[b,c]	98.9[d]	165[g]
	20	1	4.29[l]	76[b]	−6.0[c,d]	43[a,b,c]	98.7[d]	40[a,b,c]
		14	4.18[h,i]	76[b]	−5.7[d]	40[a,b,c]	99.3[d]	111[e,f,g]
		28	4.16[h,i]	77[b]	−5.7[c,d]	37[a]	99.5[d]	123[e,f,g]
QF	10	1	4.14[f,g,h]	76[b]	−6.0[c,d]	43[a,b,c]	100.0[d]	25[a,b]
		14	4.12[e,f,g]	77[b]	−6.5[b,c,d]	43[a,b,c]	99.9[d]	75[b,c,d]
		28	4.01[a]	79[b]	−6.9[b,c,d]	40[a,b,c]	99.5[d]	148[f,g]
	20	1	4.20[i,j]	77[b]	−6.6[c,d]	41[a,b,c]	99.2[d]	43[a,b,c]
		14	4.11[d,e,f]	77[b]	−6.4[c,d]	38[a]	99.3[d]	84[c,d]
		28	4.06[b,c]	78[b]	−6.4[b,c,d]	39[a,b]	99.9[d]	152[f,g]
Pooled SD			0.1	1	0.5	2	0.9	18

All data is expressed as the mean value of the determinations of three independent samples (n = 3). Pooled standard deviation (Pooled SD) is informed as an estimator for the precision of the measurements. Means within the same column following by different letters are significantly different (p < 0.05).
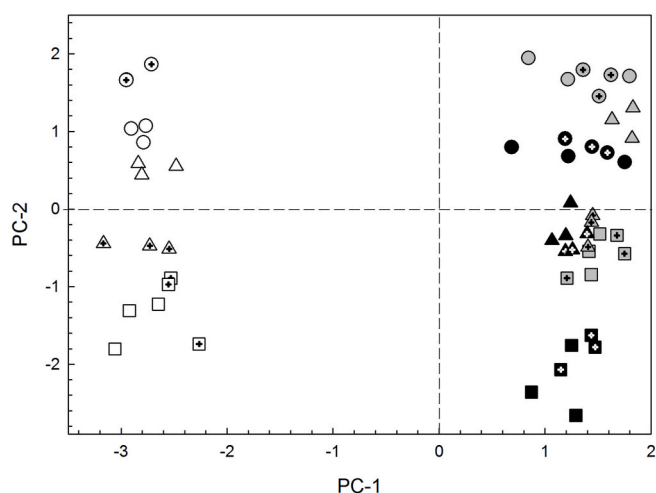
Fig. 4. Principal Component Analysis (PCA). The bi-plot represents the scores and loading of triplicates of 18 emulsion gel (EG) systems over final pH, L*, a*, b*, fluid retention capacity (FRC) (%), and malonaldehyde (MDA) concentration. The symbology used represents in the same time different storage times at 4 °C: 1-storage day (circle), 14-storage days (triangle), 28-storage days (square); protein fortification: none (white), with 20 g/kg quinoa concentrate (gray), with 20 g/kg quinoa flour (black); and alginate concentration: 10 g/kg alginate (uncrossed-symbol); 20 g/kg alginate (crossed-symbol). Sodium citrate, CaCl₂, and glucono-δ-lactone in EG systems were 100 mmol/L, 75 mmol/L, and 30 g/kg, respectively. EGs were prepared by high-speed homogenization (25,000 rpm for 1 min) of the aqueous dispersion and olive oil phases in a ratio of 3 g:1 g, respectively.

components in QC may also explain the slight increment in PC-1 values for QC-EG when comparing their counterpart QF-EG.

Also, a higher total solids content in these fortified EG promotes higher light scattering effects, dealing with significantly higher L* values (lightness ranges from 0 to 100, for black and white, respectively). These values were similar to those reported by Pintado et al. (2015) for alginate-based emulsion gels fortified with chia flour. Furthermore, the presence of flour/concentrate may conceal the yellowish color contribution of oil droplets in the entire gelled system, explaining the significantly lower positive b* values (b* ranges from −120 to +120, for blue and yellow, respectively). Moreover, a* positive

correlation with PC-1 may explain the difference between non-fortified and fortified EG (a* ranges from −120 to +120, for green to red, respectively). The brownish contribution upon quinoa concentrate/flour incorporation promotes higher a* values indicating a higher tendency to red color.

PC-2 accounts for 25% of the variance of all data. Fig. 4 shows that after 1-day of storage at 4 °C, all EG showed positive values of PC-2. In contrast, 28-days of storage promoted negative PC-2 values. This time-tendency can be explained by taking into consideration both final pH and lipid oxidation degree. Firstly, longer times promote a higher extent of GDL hydrolysis (de Kruif, 1997), and thus, significantly lower final pH values are achieved in GE systems. Secondly, the longer time, the higher exposure for oil to oxygen-dominated oxidation processes, and the concomitant increment in MDA concentration. Generally speaking, a more detailed analysis indicates that QC-EGs present higher PC-2 values when compared with the rest of EGs with the same time of chilled storage. This latter tendency can be explained by the significantly higher final pH values achieved by these systems when contrasting with their counterparts FQ-fortified and no-fortified EGs (Table 1). As described in the previous section, the initial pH in all aqueous dispersions before oil and GDL addition for EG formation was not significantly different among all samples assayed (p > 0.05), being 7.5 ± 0.7. In this context, the presence of a higher content of protein in QC-EGs may promote a significant buffer effect with a concomitant lower decrease in proton concentration after GDL hydrolysis in a certain period of storage time.

Finally, the increment of alginate concentration from 10 g/kg to 20 g/kg did not promote a significant change in the physicochemical properties shown in Table 1. This fact explains the similar PC-1-PC-2 coordinates in Fig. 4 when comparing the same fortified EG storage for identical time with a different alginate content.

3.3. Mechanical properties

As expected, the presence of a higher alginate content in EG systems promoted higher gel strength since these gelled systems presented a higher degree of inter-crossing (Ching et al., 2017). In fact, 10 g/kg-alginate containing gels resulted in non-self-sustainable ones, so-called soft gels, whereas 20 g/kg-alginate containing gels resulted in self-sustainable ones. Based on this self-sustainability and to collect as much information as possible, penetration and double-compression tests were performed for 10 g/kg alginate and 20 g/kg alginate EGs, respectively.

3.3.1. Soft emulsion gels

Fig. 5 shows texture profiles of non-fortified and QC/QF-fortified EGs stored at 4 °C for 1, 14 and 28 days. The storage time influenced significantly on FN of each EG system (p = 0.0015) and presented no significant influence on FF (p = 0.017). Regardless of storage time, protein fortification promoted significant differences among samples in FF and FN values (p < 0.0001 and p = 0.0024, respectively).

Non-fortified EGs showed similar time-profiles (Fig. 5), explaining the lack of significant differences among the corresponded FF and FN values (p > 0.05), being 1.2 ± 0.1 N and 1.4 ± 0.4 N/mm, respectively. Also, at 1-day of chilled storage, no significant changes were found in FF and FN among non-fortified and fortified samples (p > 0.05). However, changes can be easily detected when comparing plots of 14 and 28-days of chilled-storage EGs (Fig. 5). Regardless of the type of protein source, FF of fortified-EG increased more than two times after two and four weeks of storage at 4 °C in comparison with non-fortified ones (from 1.23 ± 0.03 N to 2.8 ± 0.3 N), whereas FN increased nearly three times (from 1.5 ± 0.3 N/mm to 5 ± 1 N/mm). Bearing in mind that more than 95% of pH reduction occurs before 1 day of sample storage (Table 1) it is interesting to notice these higher FF and FN values for longer periods of time. This gel strength increment with time can be partially explained in terms of water molecules restructuration due to a progressive decrease in the repulsion among protonated carboxyl of alginate residues upon acid pH (Agarwal et al., 2015; Ibrahim, Abou El Fadl, & El-Naggar, 2014; Ramdhan, Ching, Prakash, & Bhandari, 2019). On the other hand, Romo et al. (2020) demonstrated the existence of non-covalent (hydrophobic interactions and hydrogen bonds) and weak electrostatic interactions between quinoa protein and alginate. Despite the acidic final pH in EG systems (Table 1), it is still high enough to remain above the pKa of sodium alginate and nearly 4.5, the isoelectric point of chenopodins (Romo et al., 2020), and thus it may provide a context for the existence of all the types of the mentioned interactions. Also, taking into account CLSM micrographs of fortified-EGs (Fig. 3), oil droplets coated by quinoa protein might eventually interact with the alginate gel network as "active-fillers" contributing to the global gel strength (Farjami & Madadlou, 2019). Summing up, the incorporation of quinoa protein may reinforce the system structure by the formation of further attractive interactions throughout EG components with a concomitant increase in the solid-like behavior of the gel system.

These findings demonstrate the importance of the study of the storage effects on EGs formulated with proteins. For instance, Yang et al. (2020) reported a lack of solid-like properties in EGs prepared from alginate, yolk, and vinegar at a pH near or over yolk proteins pI. These authors deepen their studies and highlight the application of these systems at pHs lower than 5.0 (protein yolk pI), where negatively-charged alginate and positively-charged proteins are electrostatically attracted, which results in remarkably better viscoelastic properties. Thus, this would limit the potentiality of their EG application in food products in less acidic conditions.

3.3.2. Self-sustainable emulsion gels

Table 2 shows texture parameters obtained from TPA of self-sustainable EGs. Hardness is determined by the peak force registered during the first compression cycle (Pons & Fiszman, 1996). In general, at all storage time evaluated, fortified-EGs showed remarkably higher Hardness values (more than 50%) than their non-fortified counterparts. Once more, protein fortification may invariably lead to an increase in the EG crosslinking density by incorporating further attractive interactions throughout EG components. This behavior is in line with the results obtained in penetration tests for 14 and 28-days of chilled-storage soft EGs, as discussed in the previous section. Moreover, at this higher alginate concentration, there are significant differences between the protein source present in these self-sustainable systems: QF-EGs present significantly higher Hardness values than their QC-EGs counterparts (p < 0.05). Although QF presents a lower quinoa protein content than QC, there is an evident key role of the rest of the non-protein components that may participate in further global attractive interactions of the EG system.
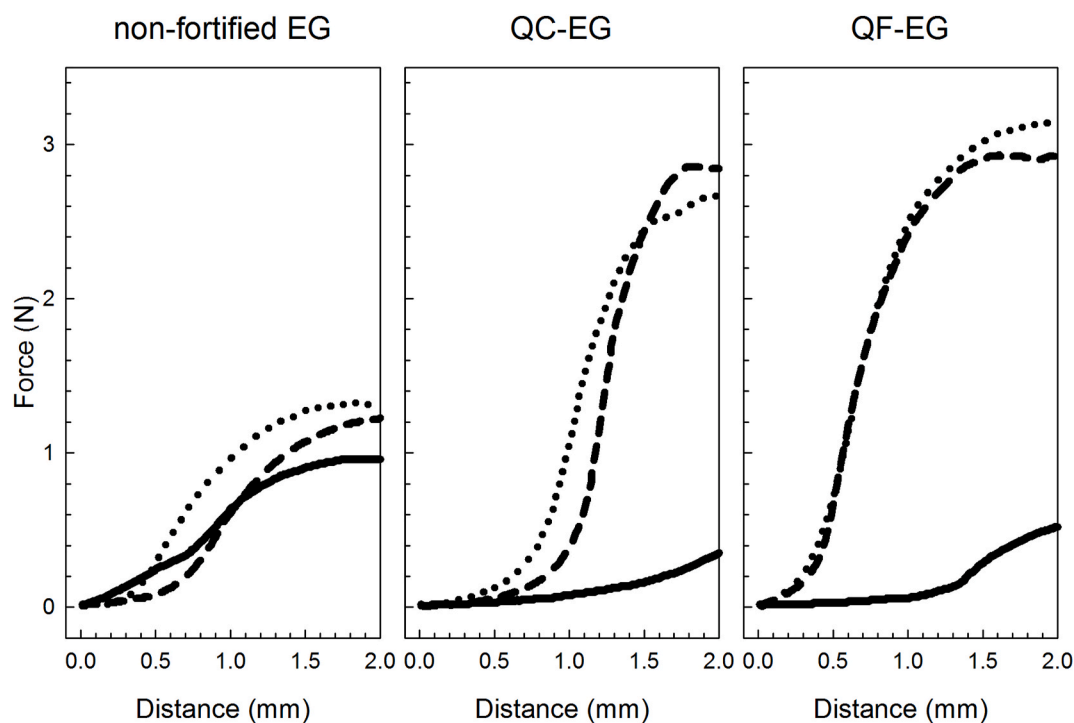

Fig. 5. Force vs. distance plots obtained from penetration tests of non-fortified- (left), QC-fortified- (middle), and QF-fortified- (right) soft emulsion gels (EGs) stored at 4 °C for 1, 14 and 28 days (solid, middle dash, and dotted line styles, respectively) (QF: quinoa flour; QC: quinoa concentrate). Sodium alginate, sodium citrate, CaCl$_2$, and glucono-δ-lactone in EG systems used were 10 g/kg, 100 mmol/L, 75 mmol/L, and 30 g/kg, respectively. EGs were prepared by high-speed homogenization (25,000 rpm for 1 min) of the aqueous dispersion and olive oil phases in a mass ratio of 3 g:1 g, respectively.

Table 2

Double-compression TPA parameters of fortified and non-fortified self-sustainable emulsion gels (QC: quinoa concentrate; QF: quinoa flour; Alg: alginate concentration).

Fortification	Storage time (days)	Hardness (N)	Springiness	Cohesiveness	Adhesiveness (J)	Chewiness (N)
-	1	3.5[a]	0.99[e]	0.90[b]	7[a]	3.2[a]
	14	4.4[b]	0.96[d,e]	0.90[b]	3[a]	3.8[b]
	28	4.4[b]	0.93[b,c,d]	0.88[b]	12[a]	3.6[a,b]
QC	1	5.8[c]	0.88[a]	0.78[a]	50[c]	4.0[b]
	14	6.7[d]	0.90[a,b,c]	0.81[a,b]	57[c]	4.9[c]
	28	7.0[d]	0.89[a,b]	0.87[a,b]	40[b,c]	5.1[c]
QF	1	6.7[d]	0.94[c,d]	0.86[a,b]	19[a,b]	5.4[c]
	14	7.8[e]	0.94[b,c,d]	0.86[a,b]	21[a,b]	6.3[d]
	28	7.6[e]	0.92[a,b,c,d]	0.82[a,b]	22[a,b]	6.1[d]
Pooled SD		0.3	0.02	0.04	8	0.3

All data is expressed as the mean value of the determinations of three independent samples (n = 3). Pooled standard deviation (Pooled SD) is informed as an estimator for the precision of the measurements. Means within the same column following by different letters are significantly different ($p < 0.05$).

On the other hand, a higher chilled-storage time promoted a slight but significant increase after 14 days in Hardness values ($p < 0.0001$). This time-dependent hardening is in agreement with the previous results for soft EGs, and can also be explained in terms of water restructuration and a concomitant increase in global attractive interactions. It is interesting to notice that this storage time effect does not impact to a higher extent than what was observed earlier for soft EGs since the higher alginate concentration in the EG system the lower number of free water molecules to participate in the restructuration. This chilled-storage effect upon fortification is in line with that reported for olive oil-in-water EGs formulated with caseinate and transglutaminase/alginate, calcium and pyrophosphate/gelatin fortified with chia-based products (Pintado et al., 2015), and even with results reported in a more complex system (frankfurters) formulated with similar EG systems used as olive oil bulking agents (Herrero, Ruiz-Capillas, Jiménez-Colmenero, & Carmona, 2014).

After one day of chilled storage Springiness decreased in a minor but in a statistically significant form in the order: no-fortified-EG > QF-EG > QC-EG ($p < 0.05$). However, these differences among fortification effects turn to not significant after 28 days ($p > 0.05$). In addition, all EGs revealed no significant differences in Cohesiveness ($p > 0.05$). Despite these scarce differences, Chewiness, which is obtained as Hardness × Cohesiveness × Springiness, followed a similar trend to Hardness as regards the protein fortification and the chilled-storage time effects. This may indicate that both effects may increase the energy requirements of swallow initiation and swift transport through the pharynx in these gelled systems (Rosenthal, 1999). These findings are in agreement with those reported by Delgado Pando, Cofrades, Ruiz-Capillas, and Jiménez-Colmenero (2010) for soy-protein-fortification in frankfurters formulated with oil-in-water emulsion prepared with caseinate and transglutaminase as pork backfat replacer.

Regardless of the storage time, EGs fortified with QC revealed significantly higher Adhesiveness values. This can be related to several factors associated with the QC-EG differential composition which may contribute to particular sticky attributes in the EG system determined by TPA assays (Adhikari, Howes, Bhandari, & Truong, 2001).

4. Conclusions

A cutting-edge solution for a one-step EG obtainment was found by confining olive oil droplets within a stable three-dimensional solid-like aqueous network by an adequate formulation of sodium alginate, citric acid, and calcium chloride concentrations. The use of a cold gelling agent such as alginate and the initial complexation of calcium by citrate have allowed the assessment of a controlled gelation process promoted by acidification with the addition of glucono-δ-lactone and a concomitant release of calcium from citrate sequestration.

This method has not only shown to be useful to obtain both soft and self-sustainable EG systems with different texture properties but also can be used to obtain EGs formulated with additional ingredients, e.g. quinoa protein concentrate and quinoa flour, to further improve the nutritional content. Thus, these EGs with a high content of unsaturated fatty acids may be used in the food industry as fat replacers in meat-based or not-meat-based products by improving the lipid profile and reducing saturated fat content.

CRediT authorship contribution statement

Romina Ingrassia: Investigation, Conceptualization, Methodology, Formal analysis, Funding acquisition, Writing – original draft. **Pablo A. Busti:** Supervision, Writing – review & editing. **Valeria Boeris:** Conceptualization, Methodology, Supervision, Project administration, Funding acquisition, Writing – review & editing.

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References

Abràmoff, M. D., Magalhães, P. J., & Ram, S. J. (2004). Image processing with ImageJ. *Biophotonics International, 11*(7), 36–42.

Abugoch, L. E., Romero, N., Tapia, C. A., Silva, J., & Rivera, M. (2008). Study of some physicochemical and functional properties of quinoa (Chenopodium quinoa Willd) protein isolates. *Journal of Agricultural and Food Chemistry, 56*(12), 4745–4750.

Adhikari, K., Howes, T., Bhandari, B., & Truong, V. (2001). Stickiness in foods: A review of mechanisms and test methods. *International Journal of Food Properties, 4*(1), 1–33.

Agarwal, T., Narayana, S. N. G. H., Pal, K., Pramanik, K., Giri, S., & Banerjee, I. (2015). Calcium alginate-carboxymethyl cellulose beads for colon-targeted drug delivery. *International Journal of Biological Macromolecules, 75*, 409–417.

Ching, S. H., Bansal, N., & Bhandari, B. (2017). Alginate gel particles–A review of production techniques and physical properties. *Critical Reviews in Food Science and Nutrition, 57*(6), 1133–1152.

Delgado Pando, G., Cofrades, S., Ruiz-Capillas, C., & Jiménez-Colmenero, F. (2010). Healthier lipid combination as functional ingredient influencing sensory and technological properties of low-fat frankfurters. *European Journal of Lipid Science and Technology, 112*, 859–870.

Draget, K. I. (2009). 29 - alginates. In G. O. Phillips, & P. A. Williams (Eds.), *Handbook of hydrocolloids* (2nd ed., pp. 807–828). Cambridge: Woodhead Publishing.

Farjami, T., & Madadlou, A. (2019). An overview on preparation of emulsion-filled gels and emulsion particulate gels. *Trends in Food Science & Technology, 86*, 85–94.

Gupta, A., Sharma, S., & Reddy Surasani, V. K. (2021). Quinoa protein isolate supplemented pasta: Nutritional, physical, textural and morphological characterization. *LWT-Food Science and Technology, 135*, 110045.

Herrero, A. M., Carmona, P., Jiménez-Colmenero, F., & Ruiz-Capillas, C. (2014). Polysaccharide gels as oil bulking agents: Technological and structural properties. *Food Hydrocolloids, 36*, 374–381.

Herrero, A. M., Ruiz-Capillas, C., Jiménez-Colmenero, F., & Carmona, P. (2014). Raman spectroscopic study of structural changes upon chilling storage of frankfurters containing olive oil bulking agents as fat replacers. *Journal of Agricultural and Food Chemistry, 62*(25), 5963–5971.

Ibrahim, S. M., Abou El Fadl, F. I., & El-Naggar, A. A. (2014). Preparation and characterization of crosslinked alginate-CMC beads for controlled release of nitrate salt. *Journal of Radioanalytical and Nuclear Chemistry, 299*(3), 1531–1537.

de Kruif, C. G. (1997). Skim milk acidification. *Journal of Colloid and Interface Science, 185*(1), 19–25.

Li, A., Gong, T., Hou, Y., Yang, X., & Guo, Y. (2020). Alginate-stabilized thixotropic emulsion gels and their applications in fabrication of low-fat mayonnaise alternatives. *International Journal of Biological Macromolecules, 146*, 821–831.

Lin, M., Han, P., Li, Y., Wang, W., Lai, D., & Zhou, L. (2019). Quinoa secondary metabolites and their biological activities or functions. *Molecules, 24*(13), 2512.

López, D. N., Galante, M., Robson, M., Boeris, V., & Spelzini, D. (2018). Amaranth, quinoa and chia protein isolates: Physicochemical and structural properties. *International Journal of Biological Macromolecules, 109*, 152–159.

McClements, D. J. (2004). Protein-stabilized emulsions. *Current Opinion in Colloid & Interface Science, 9*(5), 305–313.

McClements, D. J. (2015). *Food emulsions: Principles, practices, and techniques* (3rd ed., p. 714). Boca Raton: CRC press.

Mendoza, F., & Aguilera, J. M. (2004). Application of image analysis for classification of ripening bananas. *Journal of Food Science, 69*(9), E471–E477.

Montellano Duran, N., Spelzini, D., & Boeris, V. (2019). Characterization of acid – induced gels of quinoa proteins and carrageenan. *LWT-Food Science and Technology, 108*, 39–47.

Montellano Duran, N., Spelzini, D., Wayllace, N., Boeris, V., & Barroso da Silva, F. L. (2018). A combined experimental and molecular simulation study of factors influencing interaction of quinoa proteins–carrageenan. *International Journal of Biological Macromolecules, 107*, 949–956.

Pallandre, S., Decker, E. A., & McClements, D. J. (2007). Improvement of stability of oil-in-water emulsions containing caseinate-coated droplets by addition of sodium alginate. *Journal of Food Science, 72*(9), E518–E524.

Pintado, T., Muñoz-González, I., Salvador, M., Ruiz-Capillas, C., & Herrero, A. M. (2021). Phenolic compounds in emulsion gel-based delivery systems applied as animal fat replacers in frankfurters: Physico-chemical, structural and microbiological approach. *Food Chemistry, 340*, 128095.

Pintado, T., Ruiz-Capillas, C., Jiménez-Colmenero, F., Carmona, P., & Herrero, A. M. (2015). Oil-in-water emulsion gels stabilized with chia (Salvia hispanica L.) and cold gelling agents: Technological and infrared spectroscopic characterization. *Food Chemistry, 185*(Supplement C), 470–478.

Pons, M., & Fiszman, S. M. (1996). Instrumental texture profile analysis with particular reference to gelled systems. *Journal of Texture Studies, 27*(6), 597–624.

Ramdhan, T., Ching, S. H., Prakash, S., & Bhandari, B. (2019). Time dependent gelling properties of cuboid alginate gels made by external gelation method: Effects of alginate-CaCl$_2$ solution ratios and pH. *Food Hydrocolloids, 90*, 232–240.

Romo, I., Abugoch, L., & Tapia, C. (2020). Soluble complexes between chenopodins and alginate/chitosan: Intermolecular interactions and structural-physicochemical properties. *Carbohydrate Polymers, 227*, 115334.

Roopa, B. S., & Bhattacharya, S. (2010). Texturized alginate gels: Screening experiments to identify the important variables on gel formation and their properties. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 43*(9), 1403–1408.

Rosenthal, A. (1999). Relation between instrumental and sensory measures of food texture. In A. Rosenthal (Ed.), *Food texture: Measurement and perception* (pp. 1–17). Gaithersburg: Apen publishers.

Soazo, M., Báez, G., Barboza, A., Busti, P. A., Rubiolo, A., & Verdini, R. (2015). Heat treatment of calcium alginate films obtained by ultrasonic atomizing: Physicochemical characterization. *Food Hydrocolloids, 51*, 193–199.

Wang, X., He, Z., Zeng, M., Qin, F., Adhikari, B., & Chen, J. (2017). Effects of the size and content of protein aggregates on the rheological and structural properties of soy protein isolate emulsion gels induced by CaSO$_4$. *Food Chemistry, 221*, 130–138.

Yang, X., Gong, T., Lu, Y.-h., Li, A., Sun, L., & Guo, Y. (2020a). Compatibility of sodium alginate and konjac glucomannan and their applications in fabricating low-fat mayonnaise-like emulsion gels. *Carbohydrate Polymers, 229*, 115468. https://doi.org/10.1016/j.carbpol.2019.115468

Yang, X., Li, A., Yu, W., Li, X., Sun, L., & Xue, J. (2020b). Structuring oil-in-water emulsion by forming egg yolk/alginate complexes: Their potential application in fabricating low-fat mayonnaise-like emulsion gels and redispersible solid emulsions. *International Journal of Biological Macromolecules, 147*, 595–606.

Zhang, C., Xu, W., Jin, W., Shah, B. R., Li, Y., & Li, B. (2015). Influence of anionic alginate and cationic chitosan on physicochemical stability and carotenoids bioaccessibility of soy protein isolate-stabilized emulsions. *Food Research International, 77*, 419–425.