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Effect of quinoa protein concentration and oil volume fraction on the physicochemical and mechanical properties of alginate-based emulsion gels

Nadia Lingiardi [1,2,3] | Micaela Galante [1,2,4] | Darío Spelzini [1,2]

[1]Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina

[2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Rosario, Argentina

[3]Facultad de Química, Universidad del Centro Educativo Latinoamericano, Rosario, Argentina

[4]Facultad de Química e Ingeniería del Rosario, Universidad Católica Argentina, Rosario, Argentina

Correspondence
Darío Spelzini, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, CP C1008AAL, Suipacha 570, Rosario, Santa Fe, Argentina.
Email: dspelzini@fbioyf.unr.edu.ar

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Abstract

Background: This work studies emulsion gel (EG) formulation with quinoa proteins (QP), high-oleic sunflower oil and alginate, and the effect of QP concentration (0.5–1–2%) and the oil volume fraction (10–30–50%) on the physicochemical and mechanical properties of EG systems. EGs were tested for their microstructural and textural attributes, color, and water holding capacity as well as for their thermal, physical, and oxidative stability.

Results: The microstructure of EG showed that with increasing QP concentrations, the gel structure tended to be much denser, with oil droplets entrapped within the network. A significant decrease in droplet diameter with increasing QP concentration ($p = 0.015$) and oil volume fraction ($p < 0.000$) was observed. Hardness mean value was 2.8 N ± 0.5, reaching the highest value with 1 and 2% QP and 30% oil ($p < 0.000$). Cohesiveness shows a similar trend to that observed for hardness, while springiness showed the opposite behavior. As for adhesiveness, there were no significant differences between samples. EG have high lightness with slight yellow and green contributions. The mean water holding capacity was 88 ± 4%, and after heat treatment all samples exhibited a good fluid retention, significantly lower for the lower oil volume fraction ($p = 0.001$). EG, also proved to be highly stable against creaming and oxidative damage.

Conclusion: Results suggest that EG could be useful to create a new generation of healthier and innovative products that could substitute animal fat and deliver nutrients and biological compounds, thus improving food quality.

KEYWORDS
alginate, emulsion gels, monounsaturated fatty acids, vegetable proteins

INTRODUCTION

Nowadays emulsions (E) are being studied in order to create a new generation of healthier and innovative foods.[1] Composition (type and content of oils, biopolymers, stabilizers, thickeners, among others) and processing conditions (such as homogenization, pH, among others) of the emulsion-based food impact upon their physicochemical properties and sensory attributes.[2]

In the last few years, there has been increasing interest in the study of emulsion gels (EG) mainly focused on their application in product design as fat replacers as well as delivery systems to encapsulate and release food nutrients in the gastrointestinal tract.[3] EG are complex colloids with polymeric gel matrices where oil droplets are incorporated and entrapped. EG offer interesting features compared with the emulsion systems: high thermodynamic stability and the ability to solubilize both hydrophilic and lipophilic compounds.[4]

Recently, plant-derived proteins are being used not only as a possible option to substitute animal sources in the human diet, but also as value-added ingredients for food development.[5] Even though vegetable proteins from soy, chia, and pea have been used in EG formulation[6–8] quinoa proteins (QP) have been sparsely applied in this kind of systems.[9,10] Despite not being as common and widespread as wheat or corn, quinoa (*Chenopodium quinoa* Willd.) has been the focus of attention as its consumption has increased progressively due to its attractive nutritional quality. Quinoa provides around 15% of proteins with a well-balanced amino acid profile, unsaturated fatty acids, and tocopherols, among other micronutrients.[11]

Concerning gelling agents, alginate is a linear unbranched polysaccharide isolated from brown seaweed and used in a wide range of applications, particularly in the food field as a thickener. Sodium alginate is able to gel by ionic crosslinking through a mechanism known as egg-box model, where sodium is replaced by calcium.[12] EG based on alginate and vegetable sources, like soy and chia, have received special attention in recent years.[4,13,14] In alginate-based EG, some studies demonstrated that protein and alginate can interact through electrostatic attractions, hydrogen bonds, and hydrophobic forces, modifying the morphological and mechanical properties of these systems. Lin et al.[15] described the interaction of whey and soy protein isolates in alginate-based EG and stated that this interaction is driven by electrostatic attraction between positively charged amino acids and alginate, at pH 7.0. As QP have similar positive amino acid content to soy, 178 and 180.2 mg/g, respectively, they can interact with alginate in a similar way.[16] Besides, QP can form soluble complexes with alginate through hydrophobic interactions and hydrogen bonds.[17]

Regarding the dispersed phase of EG, proper choice of a constituent oil is key to formulating products with an improved nutritional profile. In many industrialized countries, there is a markedly unbalanced diet toward ω6 fatty acid consumption probably related to high sunflower oil intake. As a consequence, the ω6:ω3 ratio is far from the internationally recommended standard values.[18] To improve this fatty acid ratio, ω3 intakes should be increased from other sources like canola, chia, and non-hydrogenated soy oils. However, it is important to notice that these oils are highly unstable and confer a particular flavor that could be rejected by consumers of products where they are incorporated.[19] To get around these limitations, partial substitution of high-linoleic oils for high-oleic oils (olive/high-oleic sunflower oil) would help to restore the desirable essential fatty acids balance with the additional advantage of being more stable against oxidation.[20] Within this group, high-oleic sunflower oil shows significant advantages over olive oil-related to their lower cost and neutral aroma and flavor, which allow the replacement of animal fat and conventional oils in both sweet and savory products.[21,22]

To sum up, the structure and functional properties of EG are determined by the characteristics of their components and the complex interactions between them. In this regard, the aim of this work was to develop oil-in-water (O/W) EG formulated with QP, high-oleic sunflower oil, and alginate as a gelling agent, and to study the effect of QP concentration and oil volume fraction on the physicochemical and mechanical properties of these systems.

MATERIALS AND METHODS

Materials

Quinoa flour from Sturla (Buenos Aires, Argentina) and high-oleic sunflower oil was purchased in a local market. Sodium alginate was purchased from Sigma-Aldrich (Saint Louis, MO, United States). All other reagents were of analytical grade.

QP concentrates

The obtention of QP concentrates was carried out as previously reported by Montellano Duran et al.[23] with some modifications. Quinoa flour was dispersed in distilled water (10% w/v) adjusting the pH to 9 with 2 N NaOH. The suspension was stirred for 30 min at room temperature and then centrifuged at 2500 rpm for 20 min. The supernatant was adjusted to pH 4.5 by adding 2 N HCl and centrifuged for 20 min at 2500 rpm. Precipitates were resuspended in distilled water, adjusted to pH 8 with 0.2 N NaOH, lyophilized, and stored at 4°C until analysis. The protein content of concentrates was 56 ± 2% determined according to the Micro Kjeldahl method (IRAM 15852-1).

Emulsion formulation

For emulsion formulation, a 4% (w/v) QP dispersion in distilled water was stirred for 30 min and preheated at 100°C for 15 min in a thermal bath, and then cooled until room temperature. A stock solution of 2% (w/v) sodium alginate in distilled water was prepared by stirring at 400 rpm until complete hydration. To obtain the continuous phase, the alginate stock solution was mixed with the pretreated QP dispersion with the addition of distilled water until final concentrations of 0.5% (w/v) for alginate and 0.5, 1, and 2% (w/v) for QP. A 0.04% (w/v) sodium azide was added. Finally, O/W emulsions were obtained by adding high-oleic sunflower oil (10–30–50%) to the continuous phase mixing for 2 min with a homogenizer (Ultra-Turrax, IKA-Labortechnik, Germany).

Gelation of mixed systems

The E samples were transferred into dialysis membranes 15 cm in length and 1 cm in diameter, with a syringe. These membranes were then plunged into a $CaCl_2$ solution (5% w/v) at 4°C for 24 h until EG were formed. EG were formulated with the same concentrations of the E detailed in Section 2.3. Table 1 shows the composition of E and EG systems.

Characterization of emulsions and EGs

Optical microscopy

Microscopic images of E were obtained using an optical microscope (Olympus 118 microscope CX31, Tokyo, Japan) equipped with a

TABLE 1 Composition of emulsions (E) and emulsion gels (EG) based on quinoa proteins (QP), high-oleic sunflower oil and alginate.

	Continuous phase		
System	QP (%)	Sodium alginate (%)	Disperse phase (%)
E/EG0.5-10	0.5	0.5	10
E/EG1-10	1	0.5	10
E/EG2-10	2	0.5	10
E/EG0.5–30	0.5	0.5	30
E/EG1-30	1	0.5	30
E/EG2-30	2	0.5	30
E/EG0.5-50	0.5	0.5	50
E/EG1-50	1	0.5	50
E/EG2-50	2	0.5	50

digital camera (Olympus 117 Camedia D-580, Tokyo, Japan), and a 10× magnification objective. In order to obtain oil droplet size distribution, images were taken immediately after E homogenization and were processed with Image J software.

Confocal laser scanning microscopy

Microstructure of the EG systems was determined in a confocal laser scanning microscope (CLSM) (ZEISS LSM880, Germany). Nile blue (oil phase) and Rhodamine B (protein phase) were used as fluorescence dyes, with excitation wavelengths at 488 and 543 nm, respectively. Then, 10 µL Nile blue solution (0.1 g/100 mL methanol) was added per mL of oil phase, and 10 µL Rhodamine B solution (0.2 g/100 mL distilled water) was added per mL of QP dispersion. Several CLSM images were obtained at 20× magnification; those included in this paper are representative of the rest. To obtain oil droplet size distribution, EG images were processed with Image J software.

Color measurements

A high-resolution digital camera (Canon EOS-Rebel T3) was used to measure color by capturing images of the E and EG samples under proper and uniform lighting according to the method detailed by Galante et al.[24] Digital images were processed using Image J software plugin Color Space Converter in order to obtain the L* (lightness), a* (greenness/redness), and b* (blueness/yellowness) parameters and then to calculate whiteness index (WI)[25] and yellowness index (YI),[26] according to following equations:

$$\mathrm{WI}: 100 - \left[(100 - \mathrm{L}^*)^2 + \mathrm{a}^{*2} + \mathrm{b}^{*2}\right]^{1/2} \quad (1)$$

$$\mathrm{YI}: 142{,}86\,\mathrm{b}^*/\mathrm{L}^*. \quad (2)$$

Creaming index

Creaming stability measurements were carried out on the E and EG systems according to Surh et al.[27] with slight modifications. Approximately 5 mL of the samples were transferred into a test tube and stored at 4°C for 7 and 30 days. The total height of the E or EG (H_e) and the height of the serum layer (H_s) were measured. The creaming index was determined by the following equation:

$$\mathrm{CI}\% = (H_s/H_e) * 100. \quad (3)$$

Water holding capacity and oil holding capacity

Water holding capacity (WHC) and oil holding capacity (OHC) were determined according to Muñoz-González et al.[13] with slight modifications. Approximately 5 g of EG samples were precisely weighed, transferred to tubes, and centrifuged at 500 rpm for 30 min at room temperature. Tubes were inverted to drain the residue liquid (water and oil) on dry filter paper. Water loss was determined as percentage weight loss after heating the total released fluid (oil and water) for 24 h in an oven at 100°C. Oil loss was determined as the difference between total fluid exudate and water loss. WHC and OHC were expressed as a percentage of the initial sample weight, by the following equations:

$$\%\mathrm{WHC} = 100 - ((w_1 - w_2)/w_3) * 100 \quad (4)$$

$$\%\mathrm{OHC} = 100 - ((w_2 - w_4)/w_3) * 100 \quad (5)$$

where w_1 is the weight of the filter paper with the total exudate (water and oil), w_2 is the paper filter weight after heating in the oven, w_3 is the initial sample weight, and w_4 is the paper filter weight.

Thermal treatment

Thermal treatment of EG was determined according to Muñoz-González et al.[13] with slight modifications. Approximately 5 g of EG samples were transferred to hermetically sealed tubes, heated in a water bath for 30 min at 100°C, and then centrifuged at 500 rpm for 5 min at room temperature. After the thermal treatment tubes were left to stand upside down over a plate for 1 h and the total liquid (water and oil) was weighed. WHC and OHC after thermal treatment were determined as in Section 2.5.5.

Lipid oxidation

Lipid oxidation was determined by the TBARS method according to Pintado et al.[14] with modifications. A mass of 5 g of crushed

EG0.5-50, EG1-50, and EG2-50 was blended with 35 mL of 10% trichloroacetic acid for 1 min in a homogenizer (Ultra-Turrax, IKA-Labortechnik, Germany) and stirred 1 h. Next, 1 mL of this suspension was mixed with 1 mL of 20 mM thiobarbituric acid and the mixture was placed in a water bath at 100°C for 30 min until pink color emerged. The lower phase was carefully collected after centrifugation at 3500 rpm for 10 min, and the absorbance was measured in a spectrophotometer (Paralwall PWL 3100-UV, China) at 532 nm. The calibration curve was carried out with 1,1,3,3-tetraethoxypropane (Sigma Chemical Co., St. Louis, MO, USA) to determine the malonaldehyde (MDA) concentration and the results were expressed as mg MDA/kg of the sample.

Textural properties

Textural properties of EG were determined using a texturometer Perten TVT 6700 (Perten Instruments, Hägersten, Sweden) at room temperature. Double compression tests at 50% of their initial height at an initial velocity of 1 mm/s and retraction of 10 mm/s were carried out on samples 10-mm thick and 10 mm in diameter. A stainless steel cylindrical probe with 25-mm diameter was used. Hardness (N), cohesiveness (dimensionless), adhesiveness (J), and springiness (dimensionless) were evaluated.

Statistical analysis

All determinations were performed at least in triplicate and results were expressed as mean and standard deviation (SD). Data were analyzed using two-way ANOVA and post hoc Tukey test; differences at $p < 0.05$ were significant. Normality and homoscedasticity assumptions were tested before performing parametric tests. All statistical analyses and correlations were made using statistic R 3.6.0 software.

RESULTS AND DISCUSSION

Optical microscopy of emulsions

The size of the E droplets obtained during homogenization is relevant because droplet size influences the stability, appearance, and texture of products where the E will be incorporated.[28] Droplet size normally decreases with increasing homogenization intensity and time, as well as with increasing surfactant agent concentration.[29] The oil droplet diameter of E was 17.78–32.31 µm. Figure 1a shows the optical microscopy images of E, where a significant reduction in oil droplet size was observed when QP concentration ($p = 0.006$) increases. This could be attributed to the fact that an increase in QP concentration enhances surface coverage of oil droplets and protein adsorption at the oil–water interface, reducing interfacial tension and thus improving the emulsification process with decreasing droplet size.[8] This behavior was observed in wheat gluten-stabilized E as mayonnaise replacers: results indicated that the size of E droplets decreased with increasing wheat gluten concentrations.[30] Besides, Figure 1b shows a slight but significant reduction in droplet size when the oil volume fraction increases ($p = 0.010$). Similar results were reported by Anvari and Joyner[31] who studied the effects of fish gelatin concentration and oil volume fraction on the rheological and microstructural properties of E. These authors found that as the oil volume fraction increased from 50% to 80% at each fish gelatin concentration, the size of the oil droplets showed a decreasing trend.

Resistance of an interfacial layer to coalescence depends not only on the emulsifier type and concentration but also on the structural and physicochemical characteristics of the interface. Alginate could increase the thickness of the interfacial layer, increasing steric repulsion and reducing van der Waals attractions between droplets and subsequently the rate of droplet collisions and coalescence when the oil volume fraction increases.[28]

CLSM of EGs

Microstructure of EG systems was evaluated using CLSM, as shown in Figure 2a. Substantial differences were observed among the EG samples. With increasing QP concentration, the gel structure tended to be much denser with oil droplets entrapped within the network. Images show a significant decrease in droplet diameter with increasing QP concentration ($p = 0.015$) and oil volume fraction ($p < 0.000$) (Figure 2b); similar to the tendency observed for the E with the same composition (Figure 1b). Liu et al.[32] also studied E and EG stabilized by whey protein isolates and they found that the mean droplet diameter of E and EG with the addition of glucono-δ-lactone as a gelling agent progressively decreased ~2 µm and ~1 µm, respectively, as protein concentration increased (2.5%–10% w/v).

At a low oil volume fraction, most droplets seem to be in a non-flocculated state (Figure 2a), possibly because the amount of QP was sufficient to be absorbed in the interfacial area, thus conferring high stability during the gelling process. However, when the oil volume percentage increases and QP remain at 0.5%, the protein cannot cover all the oil droplet surface area. Thus, QP possibly acted as bridges among the oil droplets and led to droplet flocculation and coalescence.[32] At 1% QP, bridging flocculation may occur to a lesser extent, while this phenomenon was not observed with an increase in QP up to 2%. Moreover, at 2% QP and 50% oil volume fraction, the smaller droplets have a larger surface area surrounded by proteins, thereby resulting in a denser structure[33] with oil droplets homogeneously distributed across the surface, as can be observed in Figure 2a.

Color parameters of emulsions and EGs

Surface color assays are an important measure of sensory quality attributes of food systems and can be correlated with the presence of certain pigments and some physicochemical properties.[26] Color parameters of E (data not shown) and EG were influenced by the

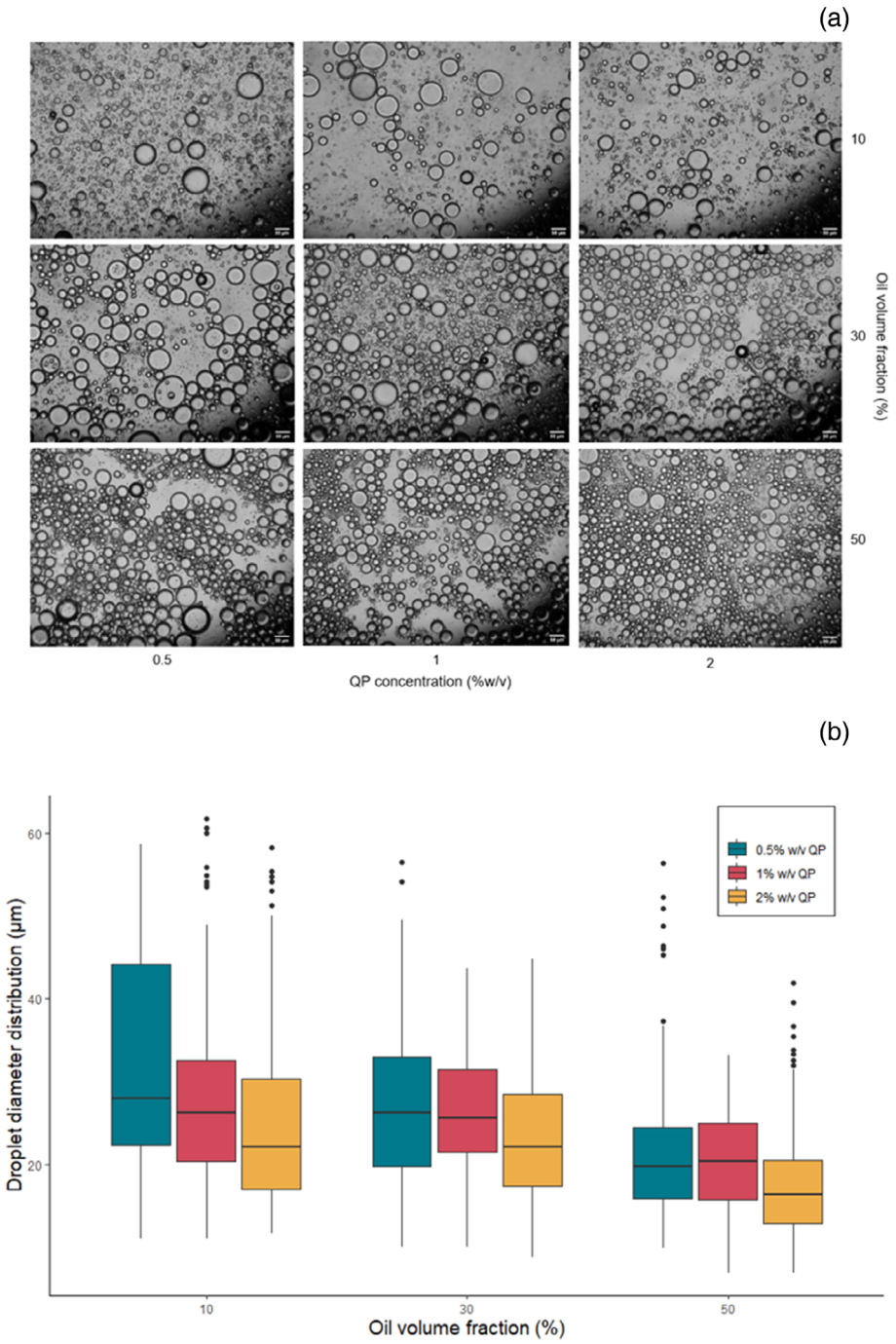

FIGURE 1 (a) Representative micrographs of emulsions with quinoa protein (QP) concentration of 0.5, 1, and 2% w/v, alginate 0.5% w/v and oil volume fraction of 10, 30, and 50%, respectively. The bars indicate 50 µm in length. (b) Droplet diameter (µm) of emulsions with QP concentration of 0.5, 1, and 2% w/v, alginate 0.5% w/v and oil volume fraction of 10, 30, and 50%.

composition and are shown in Table 2. These results indicate that E and EG systems have high lightness with slight yellow and green contributions. Color measurements of EG could be of interest for the development of fat analogs or substitutes since the appearance of a product as judged by its color can often determine its acceptance by consumers. The high value of lightness observed in EG could be a desirable attribute for its successful applications in different food products.[34] Our results show that the higher the QP content and the oil volume fraction, the higher the lightness reaching the highest value at 2% QP and 50% oil volume fraction. This could be related to a larger light reflection, due to the smaller diameter of the oil droplets when the sample oil volume fraction increases. Pearson's correlation coefficient was determined and a negative correlation was observed between EG droplet diameter and L* (−0.772, $p = 0.000$). It was previously stated that EG with bigger oil droplets had larger network voids, which made it easier for the oil droplets to move to the surface,

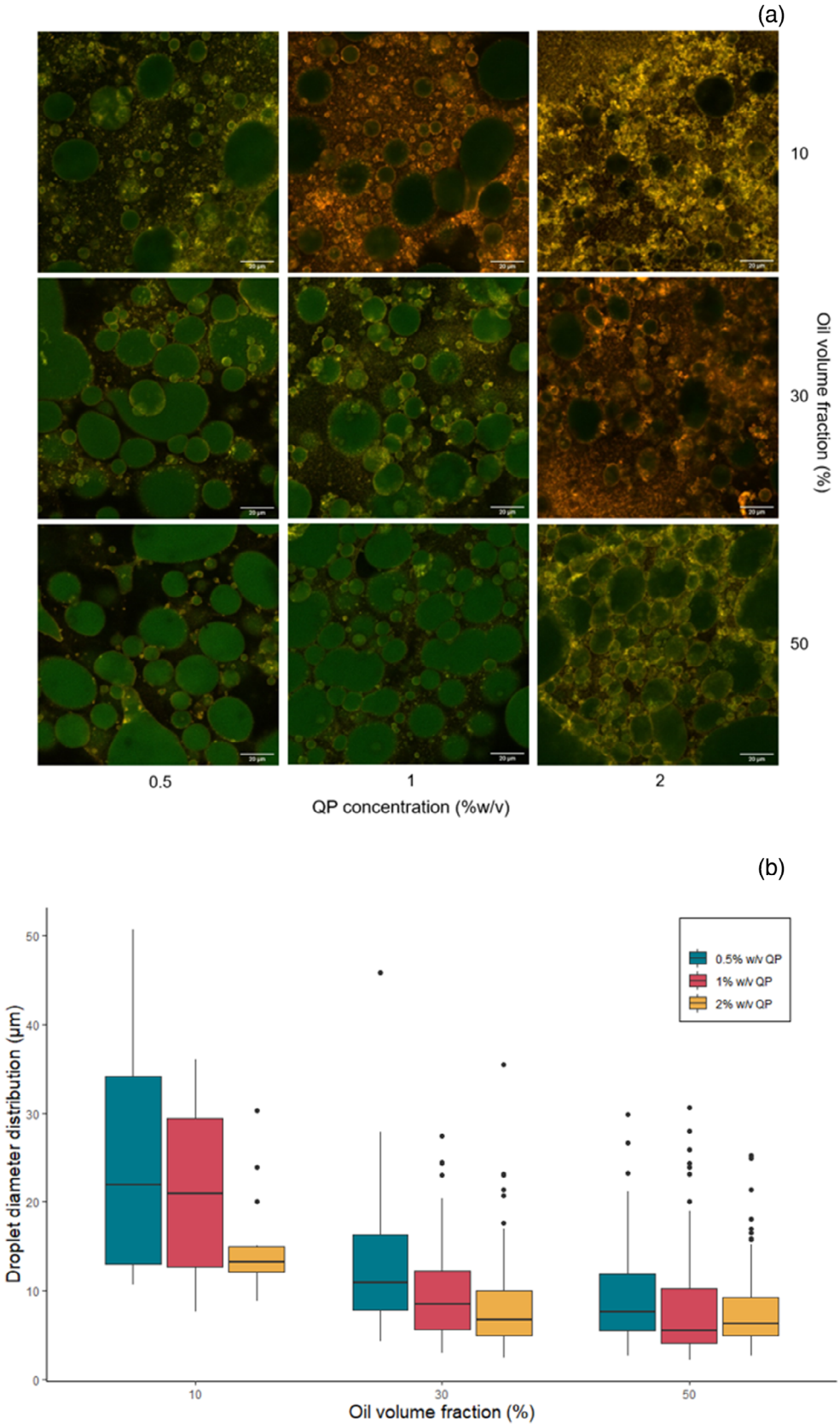

FIGURE 2 (a) Representative confocal laser scanning microscope images of emulsion gels with quinoa protein (QP) concentration of 0.5, 1, and 2% w/v, alginate 0.5% w/v and oil volume fraction of 10%, 30%, and 50%. All the images were obtained with the dyes Rhodamine B (red for protein) and Nile blue (green for oil phase), excited at 488 and 633 nm, respectively. The bars indicate 20 µm in length. (b) Droplet diameter (µm) of emulsion gels with QP concentration of 0.5%, 1%, and 2% w/v, alginate 0.5% w/v and oil volume fraction of 10%, 30%, and 50%, respectively.

TABLE 2 Color parameters of emulsion gel (EG) based on quinoa proteins, high-oleic sunflower oil, and alginate.

Samples	L*	a*	b*	WI	YI
EG0.5-10	81.8 ± 0.5^{e}	-19.3 ± 0.1^{a}	31.8 ± 0.3^{a}	58.5 ± 0.6^{b}	55.6 ± 0.9^{a}
EG1-10	84 ± 1^{de}	-18.4 ± 0.2^{b}	31.9 ± 0.6^{a}	59.8 ± 0.4^{b}	54 ± 2^{a}
EG2-10	85 ± 3^{cd}	-18.5 ± 0.2^{b}	31.7 ± 0.1^{a}	60 ± 1^{b}	53 ± 2^{a}
EG0.5-30	86.4 ± 0.4^{bcd}	-15.9 ± 0.2^{cd}	19.8 ± 0.9^{d}	71 ± 1^{a}	32 ± 2^{b}
EG1-30	87.9 ± 0.4^{bc}	-15.8 ± 0.2^{cd}	20.2 ± 0.2^{d}	71.7 ± 0.4^{a}	32.7 ± 0.5^{b}
EG2-30	88.6 ± 0.5^{b}	-15.6 ± 0.1^{cde}	20.8 ± 0.1^{cd}	71.55 ± 0.01^{a}	33.70 ± 0.07^{b}
EG0.5-50	92.3 ± 0.9^{a}	-16.14 ± 0.06^{c}	22.2 ± 0.2^{bc}	71.4 ± 0.2^{a}	34.5 ± 0.2^{b}
EG1-50	93.0 ± 0.3^{a}	-15.36 ± 0.08^{de}	22.6 ± 0.5^{bc}	71.8 ± 0.3^{a}	34.6 ± 0.5^{b}
EG2-50	93.4 ± 0.3^{a}	-15.2 ± 0.2^{e}	22.7 ± 0.1^{b}	71.9 ± 0.2^{a}	34.6 ± 0.3^{b}

Note: Means ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$). For sample denominations, see Table 1. Abbreviations: WI, whiteness index; YI, yellowness index.

darkening the EG.[35] Pintado et al.[14] reported high values of L* (between 72% and 78%) in EG based on chia and olive oil where the highest values correspond to samples with alginate as a gelling agent. Other researchers found L* values (around 60% and 70%) similar to those for pork fat in EG formulated with konjac and olive oil and chia flour and soybean oil, as fat replacers in frankfurters and Bologna sausages, respectively.[36,37] The a* parameter values determined were negative in all cases, indicating a slight tendency toward a greenish coloration and it was significantly higher with 10% oil content ($p < 0.000$). The b* parameter values were positive in all samples, indicating a yellowish coloration, and were significantly higher with 10% oil ($p < 0.000$). These color tendencies could be explained due to the higher proportion of aqueous phase with QP in the EG formulated with less oil content, which could confer their color. QP color could possibly be related to the presence of different types and concentrations of coloring compounds, like polyphenols, present in the flours that might have interacted with the proteins and extracted along with them.[34] The color of vegetable protein constituents has been correlated with colored polymer synthesis that occurs during protein-polyphenol interaction.[38]

It must be emphasized that compared with other fat sources, such as margarine[39] and butter,[40] EG developed here showed similar L* and b* values but with a slightly higher greenish contribution. This could promote or better position EG systems as versatile fat analogs in different food systems.

Values of WI present a high correlation with the consumers' preferences for white colors; while YI is often associated with general product degradation by light exposure, chemical agents, and processing.[26] The WI of EG was significantly lower with 10% oil ($p < 0.000$); while YI, at the same oil volume fraction, was significantly higher ($p < 0.000$). The larger content of QP in these samples could be related to these findings.

After the gelling process, only L* and b* values significantly increased ($p = 0.012$; $p = 0.028$). a*, WI, and YI parameters did not significantly differ between E samples and gelled ones ($p = 0.811$; $p = 0.699$; $p = 0.088$).

Creaming index of emulsions and EGs

Stability of E and EG systems was evaluated according to their phase separation. The creaming index of E significantly varied with QP concentration ($p < 0.000$) and oil volume fraction ($p < 0.000$) (Figure 3a). The creaming index showed the lowest value with the highest QP concentration (2%). A significant reduction of the creaming index with 50% oil volume fraction was also observed. This may be related to droplet packaging, as at a high dispersed phase's volume fraction, droplets are prevented from moving because they are too closely packed together and thus increase the E stability against creaming.[28] Moreover, E viscosity increases with increasing dispersed phase's volume fraction, which increases their stability to gravitational separation.[41] Nevertheless, it should be noticed that it is not always feasible to increase the oil volume fraction to prevent creaming because of the particularities of products where they will be incorporated. Another way to increase the effective volume fraction without increasing the overall oil content could be the use of multiple E (W/O/W) instead of conventional ones (O/W).[37] Besides, the greatest stability of the E could be correlated with the small droplet diameter. Pearson's correlation coefficient was determined, and a negative correlation was observed between E droplet diameter and creaming index (-0.741, $p = 0.020$). The stability of E systems is enhanced by reducing the size of the droplets.[28] Our results indicate that between the 7th and 30th day of storage, there was no significant increase in destabilization ($p = 0.968$).

On the other hand, EG systems showed to be highly stable against creaming. After 7–30 days of storage at 4°C, none of them formed a bottom aqueous phase, and the creaming index value was zero in all the samples (Figure 3b). The simultaneous use of QP and alginate could be an interesting way to stabilize oil droplets by entrapping them into a dense gelled network which improves mechanical and steric stability of EG systems. Similar results were reported in gelled double E formulated with canola oil, whey proteins, chia mucilage, and different biopolymers: after 35 days of storage, there was no phase separation.[7]

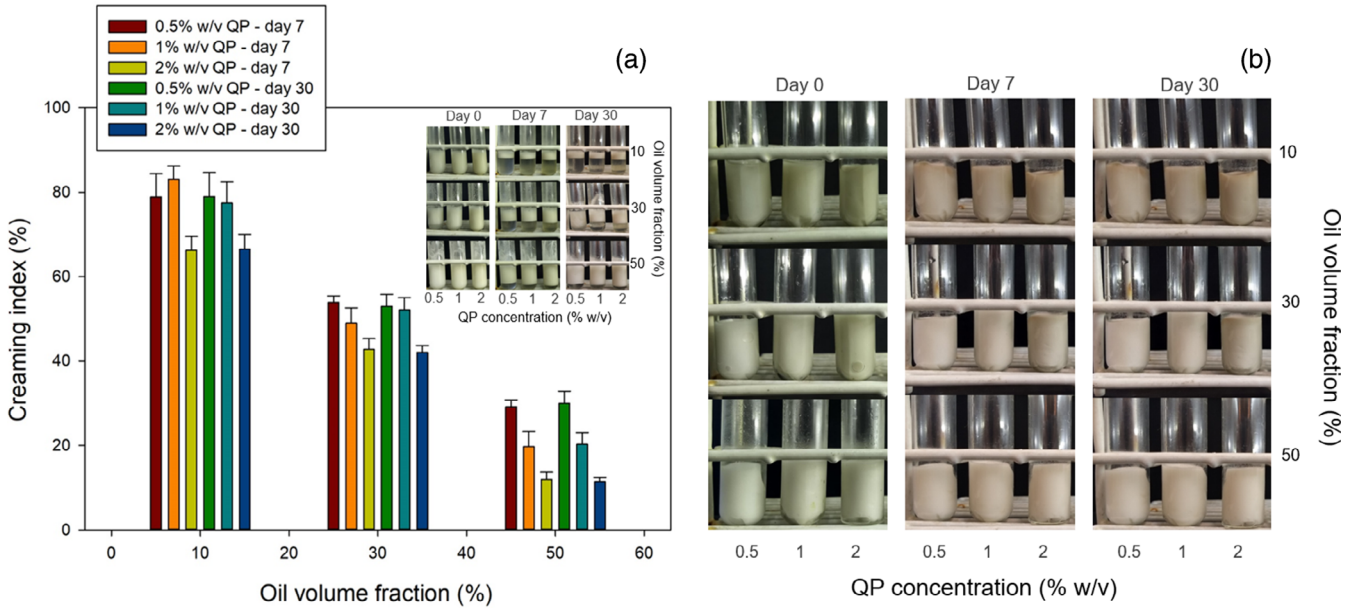

FIGURE 3 Creaming index of emulsions (inset: aspect of emulsion) (a) and aspect of emulsion gels (b) with quinoa protein (QP) concentration of 0.5%, 1%, and 2% w/v, alginate 0.5% w/v and oil volume fraction of 10%, 30%, and 50%, respectively; at 0, 7, and 30 days of storage.

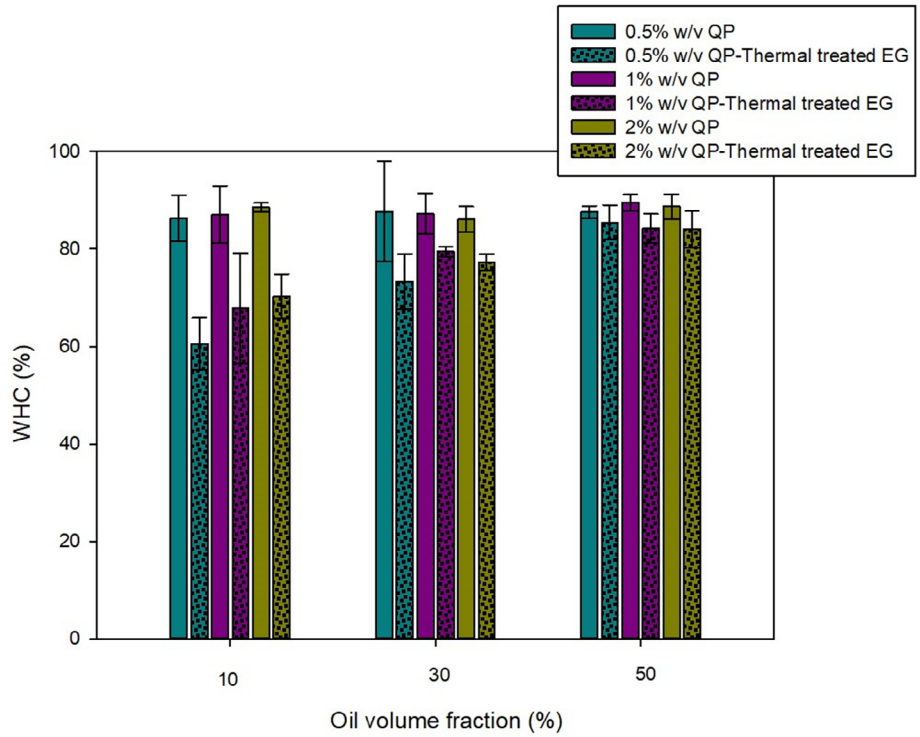

FIGURE 4 Water holding capacity (WHC) of emulsion gels with quinoa protein (QP) concentration of 0.5%, 1%, and 2% w/v, alginate 0.5% w/v and oil volume fraction of 10%, 30%, and 50%; with and without thermal treatment.

WHC and OHC

WHC and OHC are measures of the gel capacity to effectively immobilize water and oil within its matrices through capillary force, and it is related to gel microstructure.[42] Figure 4 shows WHC as a function of oil volume fraction and QP content in the presence and in the absence of thermal treatment. The mean WHC value of the EG, in the absence of thermal treatment, was 88 ± 4%. Without heat treatment, no significant differences in WHC between QP concentration ($p = 0.962$) and oil volume fraction ($p = 0.825$) were observed. de Souza Paglarini

et al.[43] reported similar values of WHC (84.2%) for EG formulated with soy protein isolate and different gelling agents (inulin and carrageenan). These researchers found that soy protein isolate and carrageenan concentration had a positive and significant effect on the WHC of gels. These high WHC levels could probably be associated with the hydrocolloid's ability to bind water. In their review, Farjami and Madadlou[44] state that hydrocolloids such as pectin, agar, gelatin, gellan gum, and alginate make up fine-stranded gel matrices with small and homogeneous pore sizes which hold water to a larger extent, preventing it to be released.

In the absence of heat treatment, no oil release was detected in the EG samples, which represents an advantage since fluid separation from the gel affects food texture.[45]

In terms of water and oil retention after heat treatment, all EG systems exhibited lower values of WHC than without thermal treatment ($p < 0.000$); and around 5% of the total loss was oil. There was a significantly lower oil loss with 10% of oil volume fraction ($p = 0.001$), and no differences in QP concentration were found ($p = 0.609$). As Figure 4 shows, WHC after thermal treatment was significantly lower for the lowest oil volume fraction ($p = 0.001$). At a low oil volume fraction, the continuous phase becomes larger. Thus, the protein network in EG systems could be affected to a greater extent by heat treatment. The long heat treatment (30 min at 100°C) here applied to EG could lead to exacerbating protein denaturation and aggregation in the continuous phase, probably promoting protein–protein interactions and decreasing water-protein associations.[46] Wu et al.[47] studied the WHC of soy protein gels and they found a positive correlation between aggregate size and water loss of the gels, where larger protein aggregates increase the coarseness of the gel networks, causing lower fluid retention. On the contrary, other researchers reported different findings: in $CaSO_4$-induced EG, WHC values were between ~62–80% and ~ 60–75%. The higher values were for the EG with larger content and/or size of soy protein aggregates and for those which had received a pre-aggregation with Ca^{+2} before the gelling process.[48,49]

Lipid oxidation

Lipid oxidation consists of a sequence of chained reactions which cause undesirable effects on the nutritional and organoleptic quality of foods. This phenomenon is mainly dependent on the degree of unsaturation of fatty acids and the exposure to some environmental factors that promote oxidation.[50]

MDA is an aldehyde obtained from the rupture of unsaturated fatty acids chains, and it is the most widely used marker in the production of secondary lipid oxidation substances.[51] As Figure 5 shows, MDA concentrations were undetectable until the 15th day of storage. After that period of time, lipid oxidation started to increase at a relatively low rate, while the increase was more pronounced after the 45th day. Nevertheless, for all samples, MDA values were low, as was expected, since the oxidative stability of high-oleic sunflower oil is influenced by its monounsaturated fatty acids content. Moreover,

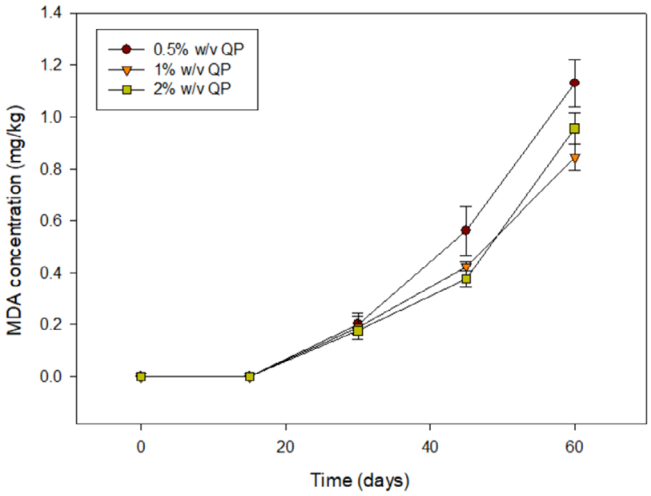

FIGURE 5 Malondialdehyde (MDA) concentration of emulsion gels with quinoa protein (QP) concentration of 0.5%, 1%, and 2% w/v, alginate 0.5% w/v and oil volume fraction of 50% as a function of time (days).

lower values may be linked to the presence of alginate in these systems, which increases the viscosity of the continuous phase and hinders oxygen diffusion within EG, as was previously stated by Sato et al.[52] for alginate-based EG. However, other researchers found MDA levels between 1.5 and 1.8 mg/kg of sample for EG stabilized with chia flour or seed and 2% alginate.[14] This would indicate that another mechanism may be involved in reducing lipid oxidation. Our results show that at the same alginate concentration (0.5%), lipid oxidation was highest with 0.5% QP ($p = 0.001$). Probably, protein interfacial film surrounding oil droplets may be able to act as a physical barrier between lipid substrates and oxygen-active species or other pro-oxidants molecules in the aqueous phase.[53]

Textural properties

Textural parameters are shown in Table 3. Hardness mean value, defined as the maximal force required to compress the sample,[54] was 2.8 N ± 0.5; this value was similar to other gelled emulsion systems based on soy proteins.[43,55]

At constant oil volume fractions of 30% and 50%, hardness significantly increased with the increase in QP from 0.5% to 2% ($p = 0.011$). This probably could be explained because of the reduction in droplet diameter. Oil droplets with smaller diameters were more effective in reinforcing the gel structure since a larger interfacial area around particles allowed more interactions with the gelled matrix.[56-58] Hardness also increased when the dispersed phase increased from 10% to 30%, reaching the highest values with 1% and 2% QP and 30% oil ($p < 0.000$). At these concentrations, the amount of QP would be enough to cover all droplet surfaces and thus entrap them in the gelled matrix. Similar results were obtained by Zhang et al.[59] in egg-soy protein EG, where the increase in hardness was

TABLE 3 Textural parameters of emulsion gels (EG) based on quinoa proteins, high-oleic sunflower oil, and alginate.

Samples	Hardness (N)	Cohesiveness	Springiness	Adhesiveness (J)
EG0.5-10	2.937 ± 0.007^{bc}	0.72 ± 0.02^{ab}	0.50 ± 0.01^{d}	3.9 ± 0.6^{b}
EG1-10	2.2 ± 0.2^{e}	0.695 ± 0.007^{ab}	0.54 ± 0.01^{cd}	4 ± 2^{ab}
EG2-10	2.4 ± 0.1^{cde}	0.74 ± 0.01^{a}	0.575 ± 0.007^{bc}	4.21 ± 0.05^{ab}
EG0.5-30	2.65 ± 0.01^{cde}	0.715 ± 0.007^{ab}	0.59 ± 0.01^{bc}	6.7 ± 0.9^{ab}
EG1-30	3.5 ± 0.1^{ab}	0.72 ± 0.02^{ab}	0.60 ± 0.01^{b}	5 ± 3^{ab}
EG2-30	3.61 ± 0.09^{a}	0.74 ± 0.03^{a}	0.595 ± 0.007^{b}	8.1 ± 0.8^{ab}
EG0.5-50	2.3 ± 0.2^{de}	0.65 ± 0.03^{b}	0.615 ± 0.007^{b}	6.1 ± 0.9^{ab}
EG1-50	2.8 ± 0.2^{cd}	0.70 ± 0.01^{ab}	0.67 ± 0.02^{a}	7.9 ± 0.5^{ab}
EG2-50	2.9 ± 0.1^{cd}	0.71 ± 0.03^{ab}	0.67 ± 0.01^{a}	10.2 ± 0.5^{a}

Note: Means ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$). For sample denominations, see Table 1.

correlated with the increase in oil content from 0 to 20%, with 10% protein. However, when the oil volume fraction reaches 50%, hardness decreases probably due to the fact that 0.5% and 1% QP would not completely cover the increased surface area of the droplets. Thus, droplet–droplet interactions appear to prevail, creating a network of aggregated droplets less bound to the matrix, therefore decreasing the EG hardness. It has been reported that gel strength decreases with increasing filler concentration of droplets unbound to the matrix, i.e. inactive droplets. However, due to the complexity of food systems, these EG can be considered a hybrid with active and inactive fillers.[60] Besides, at 50% oil, the high content of oil involved in emulsification induced a decrease in the amount of protein taking part in the gel structure of the EG, weakening the gelled network structure. This was also observed in EG formulated with peanut proteins and fish oil.[61] Feng et al.[62] demonstrated that gels with emulsion concentration above 20 wt% had a substantial decline in hardness and even appeared partially collapsed on the surface, which may be explained by the fact that the presence of excess emulsion restricted the crosslinking degree of polymers. Similar findings were reported by Lv et al.[63] in wheat bran arabinoxylan-soy protein isolate EG, where textural properties improved as the E concentration increased from 0 to 15 g/100 g, while above this value they deteriorated.

Considering the above assumptions, in EG systems where the major structure consists of an aggregated particle gel, the textural properties are mainly determined by interactions among oil droplets, while in EG with the main structure of particle-filled biopolymer gel, the textural characteristics are mostly influenced by those of the bulk phase biopolymer network.[64]

In addition to hardness, other texture parameters are important since they also influence the sensory attributes of the EG. Cohesiveness, a measure of the degree of difficulty in breaking down the gel's internal structure[65] shows a similar trend to that observed for hardness. Lower cohesion was obtained in samples with the highest oil volume fraction and the lowest QP concentration. An oil content above 30% did not translate into an increase in EG cohesiveness since a maximum crosslinking was reached, and at 50% oil content, droplet–droplet interactions started to prevail. These results were also observed in EG stabilized by egg and soy proteins.[59]

Springiness refers to the ability of the EG to spring back after deformation during compression, and it showed the opposite behavior. It reached the highest values with 50% oil volume fraction and 1 and 2% of QP, which could probably be related to a more compact gel structure as previously discussed, and also reported by Yu et al.[61] in pea protein-based EG as a fat substitute in surimi gels.

Concerning adhesiveness, the negative area under the curve obtained between cycles, there were no significant differences between QP concentrations ($p = 0.094$). The lowest adhesiveness was for EG with the lowest oil volume fraction studied here ($p = 0.005$).

CONCLUSIONS

The findings of this study highlight the role of QP and the oil volume fraction in the physicochemical and mechanical properties of alginate-based E and EG systems. The results showed that an increase in QP concentration or an increase in the oil volume fraction causes a decrease in oil droplet size. In EG systems, hardness increases when QP increases at 30% and 50% oil volume fraction; this behavior could be due to the droplets behaving as active fillers. At 10% oil, the increase in QP concentration did not produce an increase in hardness, probably due to an increase in protein–protein interactions to the detriment of protein-droplet interactions. The increase in QP concentration and oil volume fraction did not have an effect on either WHC or OHC without thermal treatment. However, the effect of the thermal treatment on WHC was observed mainly at 10% oil volume fraction and may be related to the fact that heating of the EG could exacerbate denaturation and aggregation of QP, decreasing protein-water associations.

EG systems also proved to be highly stable against creaming, and greater lipid protection against oxidation was observed mainly at 1% and 2% QP.

The high lightness and slight yellow and green contribution of the samples may position EG as versatile ingredients to be included in different food formulations without changing their original color.

Our results suggest that EG have the potential to create a new generation of healthier and innovative products with the capacity to act as fat replacers and to deliver nutrients and biological compounds, thus improving food quality.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ORCID

Nadia Lingiardi https://orcid.org/0000-0003-1968-8637
Micaela Galante https://orcid.org/0000-0001-7958-6056
Darío Spelzini https://orcid.org/0000-0002-5023-9330

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