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Characterization of acid – Induced gels of quinoa proteins and carrageenan

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ABSTRACT

In this work acid-induced gels composed of both quinoa proteins (QP, 18–42 g/L) and ι–carrageenan (Carr, 0–0.5 g/L) were characterized. The objective of this work was to characterize gels with different structures and to correlate their microstructural parameters to physical properties of gels using modeling equation. Aggregates and pores size distributions were determined using confocal laser scanning microscopy; the medians of these distributions being between 1.10–2.46 μm and 1.4–4.45 μm respectively. Digital homogeneity of the images was also estimated by the determination of the angular second moment (13–54). Water holding capacity (WHC), color, appearance and texture were measured in order to assess the macrostructure. Appearance and color (L* = 82–87, a* = 3.52–6.09, b* = 15.8–24.4) were affected by QP concentration and the glucono–δ–lactone (GDL, 0–21 g/L) to QP mass ratio (GDL/QP), probably due to the protein coloration and the final pH reached by the system. WHC (82–97%) and textural (stiffness = 12–253.2 N/mm, maximum force = 127.4–1298.5 N) properties were affected by the concentration of both biopolymers, GDL/QP and their combination. The pore size showed to be correlated to mechanical properties of the gels as well as to their WHC and appearance. Gels obtained were a result of the competition of QP–QP interaction with QP–Carr interaction.

1. Introduction

Food industry is constantly searching for novel products to satisfy and capture new consumers. Currently the most used proteins are whey and soy protein isolates due to their recognized functionality. Among others, gelation is one of the most relevant functional properties of proteins (Foegeding, 2015). Protein gels are important in food industry to obtain desirable sensory and textural structures. They are composed of three-dimensional networks of intertwined, partially associated polypeptides, with water entrapped among them. The characteristics of each gel depend not only on its components (proteins, polysaccharides) but also on the gelation conditions. Gelation is a result of denaturation (i.e., unfolding) and refolding with different interactions, so there are different types of protein gelation such as heat-induced or enzymatic or non-enzymatic hydrolysis (Van der Linden & Foegeding, 2009). Acid-induced gelation is a two – step procedure: denaturing protein structure and neutralizing protein charges (Alting, Hamer, De Kruif, & Visschers, 2000). Globular proteins can form two kinds of aggregates that, at high concentrations, give place to gel formation: microgels or strands. Microgel-type aggregates and opaque particulate gel are formed at low ionic strength and near the isoelectric point (Kharlamova, Inthavong, Nicolai, & Chassenieux, 2016).

Gels network can be modified by the incorporation of another polymer to a protein suspension, it changes the properties of the aggregates and gels formed by the mixture (Zhang & Vardhanabhuti, 2014). Different types of networks may be formed depending on the type of interaction between these polymers (de Jong & Van de Velde, 2007). Mixed protein – polysaccharide gels has been studied before (Zasypkin, Braudo, & Tolstoguzov, 1997). Food hydrocolloids mixtures may display both synergistic or antagonistic effects on the mechanical properties of the gel (Tavernier, Patel, Van der Meeren, & Dewettinck, 2016). Hydrocolloids chemistry, conformation and molecular weight, as well as the composition of their mixed solution, pH, ionic strength and temperature factors are important for the structure and properties of gels (Matia-Merino & Singh, 2007; Zhang & Vardhanabhuti, 2014).

Proteins with interesting nutritional properties such as quinoa (*Chenopodium quinoa* Willd) proteins are currently being studied to use them in the food industry. Quinoa is a pseudocereal from the Andes with a valuable protein content and an appropriate amino acid balance for human nutrition (Mäkinen, Zannini, Koehler, & Arendt, 2016). Quinoa is a new trend in industry and gastronomy due to its protein quality and also because it is a gluten-free and non-allergic grain (Nongonierma, Le Maux, Dubrulle, Barre, & FitzGerald, 2015). Moreover, the digestibility of the quinoa proteins (QP) was found to be

Abbreviations: Carr, ι-carrageenan; F_{max}, maximum force; GDL, glucono-δ-lactone; QP, quinoa proteins; St, stiffness; WHC, water holding capacity

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comparable to that of other high quality food proteins. Besides its nutritional and biological value (Navruz-Varli & Sanlier, 2016; Vilcacundo & Hernández-Ledesma, 2017), quinoa flour and QP have functional properties that introduce new functionality in food products (Abugoch, Romero, Tapia, Silva, & Rivera, 2008). QP are highly soluble at alkaline pH and most of them precipitate around pH 4.5 (Avila, Xiao, Van Boekel, Minor, & Stieger, 2016; Elsohaimy, Refaay, & Zaytoun, 2015). QP gelation has been studied recently by other authors, different heat-denaturation temperatures and different pH extraction for QP, and these procedures effect in the gelation was studied (Avila et al., 2016; Mäkinen, Zannini, & Arendt, 2015).

In order to study mixed gels composed by quinoa proteins, an edible polysaccharide, such as carrageenan, was added to the gels matrix. ι – Carrageenan (Carr) is an electrically charged polysaccharide that can lead to homogeneous microstructures with globular proteins. Carr comes from a family of sulfated polysaccharides obtained from red seaweeds species. It is used in the food industry as stabilizer, thickener and gelling agents and it contains sulfate groups in its backbone, with a pKa below 2 (Van de Jong et al., 2009a,2009b).

Acidification to prepare acid-induced gels was made with glucono–δ–lactone (GDL), an acid used in the industry to make edible protein gels such as tofu (Campbell, Gu, Dewar, & Euston, 2009). GDL hydrolyses in aqueous solution and allows the slow reduction of the pH of the system. The use of GDL as an acidifying agent enables the control of the acidification kinetics: the proton release is regulated by the amount of added GDL and the temperature. Moreover, the final pH of the system is a function of the GDL concentration. QP aggregation by acid–induced gelation using GDL is a two–step process: 1) QP are heated to denature the polypeptides, and 2) GDL is added to induce the association of the soluble aggregates into larger structures by reducing the electrostatic repulsion (Mäkinen et al., 2015).

Previously, QP – Carr interaction has been studied showing that they interact at low pH range (1–2.9) by coulombic interactions and interact around the isoelectric point (2.9–5.5) by charge regulation mechanism (Montellano Duran, Spelzini, Wayllace, Boeris, & Barroso da Silva, 2018b). In addition, the required conditions to form these gels were determined (Montellano Duran, Galante, Spelzini, & Boeris, 2018a).

The aim of this work was to study how the micro and macro structure of the QP – Carr mixed acid-induced gels are related. This study allows us to know the behavior of the macroscopic properties which is important for the industry's desire to improve the product in order to satisfy consumers or manufacturing needs. At the same time, the final gel network is formed due to the relationship between the formation of microaggregates, the pores and the density between them. In this work the micro and macro – structure of mixed acid – induced gels was studied. The effect of the presence of Carr in QP acid – induced gels and their properties was furthermore determined. The macroscopic properties were measured by color, water holding capacity and texture; and microscopic properties were measured by aggregates and pore size. Quantification of empirical data can be obtained by a comprehensive method, structural equation modeling which has been very used in similar works lately (Tan & Joyner, 2018). Because of this, it was desirable to model the behavior of the relationship effects between variables concerning patterns of statistical dependencies. The response variables are described by parameters that indicate the magnitude of each effect (direct or indirect) or their interaction.

2. Materials and methods

2.1. Materials

QP were obtained from defatted quinoa flour, purchased from Los Andes (Cochabamba, Bolivia). QP isolation was carried out as was previously reported (Montellano Duran, Galante, et al., 2018a), adjusting the solubilization pH at 8.5 and precipitation pH at 4.5. Protein content was determined by Bradford method (Bradford, 1976). ι-Carrageenan (Carr) and GDL were purchased from Sigma (Sigma Chemical, St Louis, MO, USA). All other reagents were of analytical grade and were used without further purification.

2.2. Acid-induced gelation of mixed systems

Solutions of different QP concentrations were prepared in distilled water adjusting the pH at 8.5 with 0.5 mol/L NaOH. In order to induce and improve gelation, protein dispersions were thermally treated in a water bath at 95 °C for 10 min to denature all the polypeptides and to favor interactions among them. Gelation was carried out at room temperature at different protein concentrations ([QP]: 18, 30 and 42 g/L), Carr concentrations ([Carr]: 0, 0.02, 0.04, 0.06, 0.08, 0.1 and 0.5 g/L) and GDL/QP mass ratio (GDL/QP: 0.33, 0.66 and 1.00); the gels were held overnight at room temperature to ensure the complete hydrolysis of GDL and to stabilize the gel structure. This initial conditions to form gel networks were from a first study described in (Montellano Duran, Galante, et al., 2018a).

2.3. Microstructure - confocal laser scanning microscopy

Gel samples, whose proteins were stained with Rhodamine B (in a proportion 1 mg Rhodamine B/600 mg QP). Rhodamine B was added just after sample preparation, before gelification, and incubated 1 h in darkness; then, gels were visualized by confocal laser scanning microscopy (CLSM) using the red light laser of the Confocal NIKON C1SiR PLUS microscope (Nikon Corporation, Tokyo, Japan), with a magnification of 20× and a zoom of 2, covering a square field of 250 μm of side. The thickness (aggregate size) and the pore diameter (pore size) were determined using the imaging software, ImageJ (NIH, Maryland, United States), and the plugin BoneJ (Doube et al., 2010). Pore size is defined as the space where trapped water remains in the polymer network formed by QP and Carr. Aggregate size is defined as the aggregates that form the polymer network of QP and Carr. In order to obtain the distribution of both the pore size and aggregate size images were binarized, i.e., red color was turned to white, and quantifications determined using the BoneJ plugin in ImageJ. Angular second moment, which is related to the homogeneity of the image because of the amount and position of the different intensities of color in an image, was calculated by means of the Grey Level Co – occurrence Matrix, GLCM (Zheng, Sun, & Zheng, 2006). All the samples were made in triplicate for this experiment and a single picture for each gel was analyzed.

2.4. Color measurements – visual aspects

Images from QP – Carr gels were taken with a NIKON P 7100 color digital camera (Nikon, Jakarta, Indonesia) with a resolution of 3648 × 2736 pixels. The pictures were taken on both a matte white and black background using the camera settings previously assayed by other authors (Soazo, Pérez, Rubiolo, & Verdini, 2015). The high – quality images allows to obtain a, b and L, digital imaging parameters (average values of the total picture), and to determine a*, b*, L*and ΔE (Yam & Papadakis, 2004). Yellowness (Chang, Da Silva, Sakai, Kristiansen, & Ishikawa-Nagai, 2009), whiteness (Tabilo-Munizaga & Barbosa-Casanovas, 2004) and opaqueness (Rhim, Wu, Weller, & Schnepf, 1999) were then calculated.

$$\text{yellowness} = (142.86/L^*).b^* \quad (1)$$

$$\text{whiteness} = L^* - 3b^* \quad (2)$$

$$\text{opaqueness}\ (\%) = (L^*_{black}/L^*).100 \quad (3)$$

where L*, b* are the color parameter calculated from the pictures on white background and L^*_{black} is the luminosity value obtained from the pictures on black background. All the samples were made in triplicate

for this experiment.

2.5. Water holding capacity

Water holding capacity (WHC) was determined by preparing 10 mL of each gel in falcon tubes. The systems were centrifuged at 100 g (Deeth, Yang, Pang, Bansal, & Prakash, 2017) for 10 min and the weight of the gel before ($m_{gel\ before}$) and after ($m_{gel\ after}$) the centrifugation was determined. WHC was calculated applying Equation (1). All the samples were made in quintuplicate for this experiment.

$$WHC\ (\%) = (m_{gela\ after}/m_{gel\ before}).100 \quad (4)$$

2.6. Texture measurements – mechanical properties

Twenty milliliters of each sample were gellified in cylindrical plastic containers of 2.25 cm radius and 3 cm high. Uniaxial penetration curves (force vs. distance) were performed in order to calculate the texture parameters of each gel. The Stiffness (St, Young's Modulus), defined as the initial slope of the curve, and the break force (F_{max}), defined as the maximum force reached, were calculated from the penetration curves.

The assay was performed with a Perten TVT 6700 texturometer (Perten Instruments, Hägersten, Sweden), using a cylindrical stainless steel probe P−CY25S (673025), 25 mm in diameter. A single penetration at 50% of the total gel height (18 mm) was carried out at a speed of 1 mm/s. All the samples were made in triplicate for this experiment.

2.7. Statistical analysis

The results obtained were analyzed using the trial version of the program Design Expert (Stat-Ease, Minneapolis, Minnesota, United States), which allowed analysis of the effect of each one of the three factors studied ([QP], [Carr], GDL/QP), in 3, 7 and 3 levels, respectively on every response analyzed.

All the numerical results for color, microstructure, water holding capacity, and texture were analyzed with the following process: Compute the effects produced by every experimental factor and the combinations between them with the half-normal probability. The ANOVA was checked to verify the selected model in every case, the significant effects in every model were always significant (P-value $<$ 0.05). The F tests were examined for the regression coefficients, all the terms selected were below 0.10 to maintain hierarchy. The F test was examined for the lack of fit, if it was significant a more complex model was tested until it was not significant. Then, a residual analysis by Least Significant Difference test (by Fisher) and a diagnostic plot guide were made to verify the ANOVA assumptions. In case the Normal Plot of Residuals showed some outliers, those points were not taken into account in the model. Model equations are the results of this process for every factor of interest. Parameters in each model equation are informed including their standard deviation in brackets. In the case of WHC the results are also informed in a 3D contour plot.

Linear correlation analysis with the Pearson coefficient was made for the response variables with a significance of $P < 0.05$.

3. Results and discussion

3.1. Microstructure

Gels containing different concentrations of QP and Carr, acidified with different GDL concentrations, were visualized by confocal microscopy as shown in Fig. 1. In the images, proteins were red-colored due to the binding of Rhodamine B dye.

Pores and aggregates sizes distributions were unimodal and right-tailed, i.e. none of the distributions is symmetric and in all cases the median is below the mean.

. In order to evaluate the effect of the biopolymers concentration as well as relation between GDL and QP concentration on the microstructure, the median of the pores and aggregates diameters distribution of each sample was considered as response and shown in Table 1. The significance of each factor on both responses was analyzed by ANOVA (data not shown): on the one hand, the aggregate size showed to be only significantly affected by GDL/QP (P = 0.0015), but not by QP (P = 0.4805) and Carr concentrations (P = 0.0971); on the other hand, the pore size was affected by the QP concentration ($P < 0.0001$), the Carr concentration (P = 0.0319), the interaction between both biopolymers concentration (P = 0.0065) and the interaction between [QP] and GDL/QP (P = 0.0189).

Considering the ANOVA results, data were fitted to the following model equations:

$$\text{aggregate size } (\mu m) = 1.87(3) - 0.30(9)\ (GDL/QP) \quad (5)$$

$$\text{pore size } (\mu m) = 2.35(6) - 0.011(1)\ [QP] + 4(2)\ [Carr] + 3(1)\ (GDL/QP) - 0.10(3)\ [QP][Carr] - 0.68(2)\ [QP]\ (GDL/QP) \quad (6)$$

The model equation obtained for aggregate size (Equation (5)) showed that GDL/QP affected this parameter negatively: the aggregate size decreased with an increase of GDL/QP whereas bigger aggregates were obtained with a smaller GDL/QP. This may be indicating that aggregate size only depended on the acidification rate or the final pH (Table 1), directly related to the GDL/QP ratio used. This observation about aggregate size in gels was not coincident with the previously observed when diluted QP solutions were acidified (Montellano Duran, Galante, et al., 2018a). In that case, the lowest aggregate size is obtained at pH 6 and in this case, gels with higher pH (lower GDL/QP) gave place to higher aggregate size. Taking this situation into account, the effect of GDL/QP on the aggregate size observed here may be better explained by considering that higher GDL/QP values produced faster acidification of the media and thus, the time it takes to form aggregates or to increase their size before gelation was lower. This effect is also reported as the effect of bacterial acidification producing thicker strands, while GDL leads to thinner ones (Lucey, Tamehana, Singh, & Munro, 1998). The acidification rate controls the rate of gelation, so the microstructure of the mixed gels can be modulated (de Jong et al., 2009a,2009b), a coarser microstructure can be the result of a slower acidification, since phase separation can result because of the longer time to arrange.

Pore size is a measure of the size of the cavities containing water inside the network formed by interactions between the protein aggregates. Equation (6) showed that, on the one hand, an increase in the concentration of QP produced a decrease in pore size and, on the other hand, the effect of GDL/QP and Carr concentration were dependent on the QP concentration, as the interaction terms showed. The increase in GDL/QP or in Carr concentration produced bigger pores at low protein concentration, whereas for the higher protein concentration an increase in any of these factors produced smaller pores. In acid gels of milk proteins supplemented with pectin, the increase in the polysaccharide concentration produce an increase in the pore size of the gels that is attributed to the fact that polysaccharide-polysaccharide and protein-protein interactions are favored over the polysaccharide-protein interactions (Matia-Merino & Singh, 2007). In our case, this effect was only observed for the highest protein concentrations, indicating that at low concentrations of biopolymer, QP-Carr interactions were favored whereas above a certain concentration, the exclusion effect was favored.

The angular second moment, a digital texture parameter related to the homogeneity of the images, was calculated for each image (Table 1) and the significance of each studied factor was determined by ANOVA (data not shown). Both the concentration of QP ($P < 0.0001$) as well as the concentration of Carr (P = 0.0441) resulted significant. Results were fitted to the following equation:

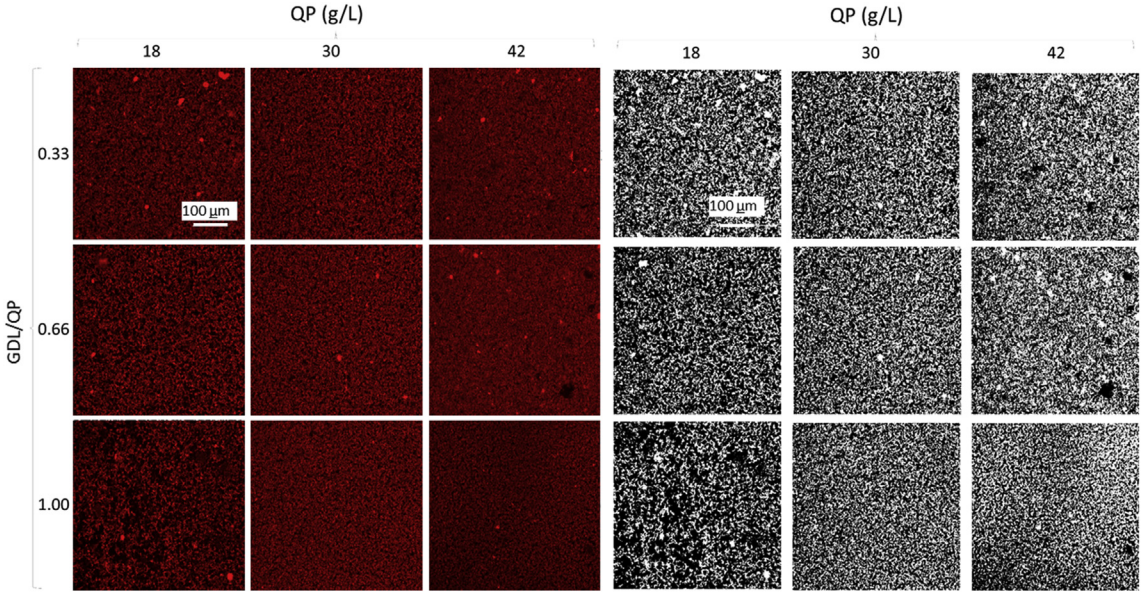

Fig. 1. Confocal microscopy images. Left: Original images (grey-scaled). Right: binarized of protein mixed gels (White: Protein network, Black: Background).

angular second moment = 32(2) – 0.7(1) [QP] + 1.02(5)*10^{-5} [Carr] (7)

As observed, an increase in protein concentration produced a decrease in homogeneity, while an increase in polysaccharide concentration caused an increase in it. The effect of each biopolymer in angular second moment may be related to its effect in the gel structure. On the one hand, high [QP] may produce the formation of compact protein aggregates, i.e., aggregates of a defined size but containing high levels of proteins. The overconcentration of QP in these aggregates were deeply red-colored when they were added with Rhodamine B and thus the amount of grey level transitions from the center of the aggregates to the pore was large. On the other hand, Carr was known to increase the QP solubility at the range of pH at which the gels were formed (from pH 3 to 5), so the higher the [Carr], the higher the increase in the solubility of the QP, explaining that there was a greater amount of soluble protein distributed more homogeneously thorough the gel. The formation of the polymeric network in these gels results from a competition between the associative interaction of QP–Carr and the QP–QP aggregation. Thus, when the [Carr] increases, more homogeneous gels were produced due to the associative interaction between QP and Carr whereas when [QP] increases, the structure was more heterogeneous due to the QP–QP interaction.

3.2. Visual aspects

By image analysis of the photographs of each mixed gel (Fig. 2), color parameters a*, b* and L* were determined, as shown in Table 1. The ranges for these color parameters of the acid gels composed by QP in the presence or in the absence of Carr were: 82 < L* < 90%, 3.5 < a* 6.0 and 13.5 < b* < 24.5. These results indicated that these gels had yellow coloration, with slight red contribution, and high luminosity. QP both solid and in suspension are yellow-colored; in fact, Steffolani et al. (Steffolani et al., 2015) determined the color of the QP isolate (powder) obtaining values of L* = 65.9%, a* = 1.5 and b* = 17.3. Comparing with amaranth and soy protein isolates (Marcone & Kakuda, 1999), the QP isolate has a lower luminosity (L*); thus, considering this, QP use is recommended for colored products.

In addition, visual inspection of gels indicates that they were quite opaque. According to all this, some specific color indexes (yellowness, whiteness, opaqueness) of these gels were calculated by applying equations (1)–(3).

The yellowness values varied between 21% and 42%, and the whiteness varied between 9% and 85%. It is to be noted that yellowness and whiteness were not negatively correlated, i.e., a decrease in whiteness was not always caused by an increase in yellowness.

The opaqueness was calculated from the luminosity parameters from the images obtained both using white and black background. The opaqueness values varied between 55% and 79%, in agreement with the fact that gels are expected to present high opacity when they are formed by acidification of suspensions of globular proteins (Brinegar & Goundan, 1993). Thermal gels compose by 160 g/L of whey proteins present an opaqueness of 44% indicating that these QP gels are particularly opaque, even considering the low protein concentration when compared with the whey protein concentration.

The effect of each factor on the calculated indexes was determined by ANOVA (data not shown). Carr concentration did not affect any of the calculated indexes (P > 0.42). On the contrary, QP concentration had the most significant effect on each calculated index (P < 0.001), whereas GDL/QP affected only whiteness and opaqueness.

Model equations for yellowness, whiteness and opaqueness were obtained from the experimental results as functions of their significant factors, as follows:

yelowness = 22.9(7) + 0.39(2) [QP] (8)

whiteness = 40.3(8) + 0.51(4) [QP] + 6.6(1) (GDL/QP)– 0.31(2) [QP] (GDL/QP) (9)

opaqueness = 52.7(3) + 0.59(4) [QP] – 4(1) (GDL/QP) (10)

Yellowness was highly affected by [QP], according to the yellow coloration of these proteins, as was previously discussed. On the other hand, whiteness decreased when [QP] increased and the effect of GDL/QP on whiteness depended on the [QP]: for the lowest assayed concentration of QP, GDL/QP had no significant effect on whiteness; however, for the highest [QP], the increase in GDL/QP produced a higher decrease in whiteness. Bolivian protein isolates present color parameters of dark yellow (Steffolani et al., 2015) in the isolation pH, assumed to be obtained from a pigment present in the protein isolated. The color of the pigment can change throughout the pH range, turning whiter in acid pH range. This assumption can explain the effect of GDL (higher GDL/QP) in the whiteness. Regarding opacity, the [QP] affected it positively and GDL/QP affected it negatively meaning that both the increase in the [QP] and the decrease in GDL/QP gave place to more

Table 1
For gels composed by different concentrations of quinoa proteins (QP) and carrageenan (Carr), and acidified by the addition of different glucono-δ-lactone to quinoa proteins ratios (GDL/QP). The median of the size distribution of both pores and aggregates was considered the PS (pore size) and AS (aggregate size) respectively. The pH of each gel was included as well. Average values of aggregate diameter, pore diameter and angular second moment (ASM). Digital imaging parameters: luminosity (L*) and chromatic components (a* and b*). Water holding capacity (WHC) and texture parameters (Stiffness, St, and maximum force, Fmax). Each parameter is informed with error numbers.

[QP] (g/L)	[GDL]/ [QP]	[Carr] (g/L)	pH	AS (μm)	PS (μm)	ASM	L*	a*	b*	%WHC	St (N/mm)	Fmax (N)
18	0.33	0	3.9 ± 0.1	1.723 ± 0.005	2.477 ± 0.002	25.4 ± 0.2	85 ± 2	5.7 ± 0.4	16.9 ± 0.4	90 ± 5	16.0 ± 0.7	152 ± 4
		0.02		1.77 ± 0.01	2.435 ± 0.005	26.0 ± 0.2	84.9 ± 0.8	5.2 ± 0.3	18.2 ± 0.3	91 ± 4	19.8 ± 0.1	147 ± 4
		0.04		1.856 ± 0.002	2.46 ± 0.01	23.2 ± 0.2	85 ± 1	5.2 ± 0.3	18.2 ± 0.4	85 ± 9	20.0 ± 0.1	181.3 ± 0.7
		0.06		1.581 ± 0.02	4.4 ± 0.5	24.5 ± 0.4	84.5 ± 0.7	5.2 ± 0.3	18.5 ± 0.4	86 ± 7	15.9 ± 0.7	167 ± 3
		0.08		1.77 ± 0.02	3.457 ± 0.005	25.6 ± 0.5	84.9 ± 0.4	5.0 ± 0.2	18.6 ± 0.5	89 ± 4	27.7 ± 0.1	176 ± 6
		0.1		2.056 ± 0.007	2.056 ± 0.003	31.2 ± 0.9	85.4 ± 0.9	4.4 ± 0.3	17.4 ± 0.3	89 ± 5	20.4 ± 0.1	181 ± 7
		0.5		1.942 ± 0.007	3.776 ± 0.003	43.8 ± 0.6	85 ± 2	5.4 ± 0.3	18.7 ± 0.4	86 ± 9	34 ± 3	352.8 ± 0.7
	0.66	0	3.8 ± 0.1	1.57 ± 0.02	3.32 ± 0.01	37.9 ± 0.3	86 ± 1	3.5 ± 0.3	15.8 ± 0.4	94.2 ± 0.7	12 ± 1	142.1 ± 0.1
		0.02		1.571 ± 0.002	3.137 ± 0.008	47.8 ± 0.9	87 ± 1	3.8 ± 0.3	16.4 ± 0.3	92 ± 1	19.1 ± 0.7	137 ± 6
		0.04		1.527 ± 0.005	3.283 ± 0.004	44.5 ± 0.2	85 ± 1	4.9 ± 0.3	18.2 ± 0.4	92 ± 2	24.4 ± 0.1	181 ± 2
		0.06		1.695 ± 0.004	4.077 ± 0.007	35.9 ± 0.5	85 ± 1	5.1 ± 0.4	18.7 ± 0.5	93 ± 3	14.5 ± 0.7	157 ± 20
		0.08		1.74 ± 0.05	5.02 ± 0.08	36 ± 1	85 ± 1	5.0 ± 0.3	18.2 ± 0.4	95 ± 6	19.0 ± 0.1	157 ± 2
		0.1		1.552 ± 0.006	4.445 ± 0.002	37.2 ± 0.2	86 ± 1	4.3 ± 0.3	16.5 ± 0.4	92 ± 2	16 ± 6	171 ± 1
		0.5		1.550 ± 0.002	4.087 ± 0.001	46.8 ± 0.4	85 ± 1	4.6 ± 0.3	17.3 ± 0.4	91 ± 9	51 ± 1	358 ± 9
	1	0	3.6 ± 0.1	1.52 ± 0.01	3.084 ± 0.006	48.6 ± 0.4	84 ± 1	4.8 ± 0.3	18.5 ± 0.4	97 ± 6	13.9 ± 0.7	137 ± 6
		0.02		1.524 ± 0.002	3.17 ± 0.02	52.3 ± 0.6	84 ± 1	4.9 ± 0.3	18.7 ± 0.4	92 ± 7	16 ± 1	127 ± 8
		0.04		1.562 ± 0.005	3.457 ± 0.005	52.8 ± 0.9	85.2 ± 0.6	4.9 ± 0.3	17.4 ± 0.4	94 ± 2	21 ± 1	137 ± 9
		0.06		1.759 ± 0.004	3.877 ± 0.006	37.6 ± 0.9	84.8 ± 0.7	4.3 ± 0.3	17.2 ± 0.3	92.7 ± 0.6	16 ± 1	137 ± 10
		0.08		1.750 ± 0.002	4.207 ± 0.004	31.9 ± 0.2	86 ± 1	5.0 ± 0.3	18.2 ± 0.3	93 ± 5	17 ± 7	132 ± 10
		0.1		1.55 ± 0.01	4.21 ± 0.02	38.2 ± 0.2	86 ± 1	4.9 ± 0.3	18.1 ± 0.3	92 ± 6	17 ± 5	142 ± 10
		0.5		1.55 ± 0.02	4.445 ± 0.005	48.4 ± 0.2	83.9 ± 0.9	5.2 ± 0.3	18.8 ± 0.3	91 ± 8	27 ± 3	274 ± 20
30	0.33	0	3.8 ± 0.1	1.557 ± 0.003	1.557 ± 0.006	34.7 ± 0.9	86 ± 1	4.5 ± 0.3	19.8 ± 0.4	86.8 ± 0.1	23.1 ± 0.7	255 ± 20
		0.02		1.549 ± 0.005	2.47 ± 0.01	32.8 ± 0.8	83 ± 2	5.9 ± 0.3	21.4 ± 0.2	86.0 ± 0.6	37.7 ± 0.7	313 ± 20
		0.04		1.716 ± 0.005	2.427 ± 0.008	30.7 ± 0.1	83.6 ± 0.8	5.3 ± .3	22.4 ± 0.4	85.5 ± 0.4	55 ± 1	421 ± 1
		0.06		1.565 ± 0.008	2.08 ± 0.02	35.1 ± 0.2	85 ± 1	5.7 ± 0.3	21.6 ± 0.4	90 ± 1	42.1 ± 0.1	260 ± 6
		0.08		1.506 ± 0.007	1.998 ± 0.008	28.3 ± 0.1	85 ± 1	3.5 ± 0.3	17.8 ± 0.4	87 ± 5	38 ± 1	279 ± 10
		0.1		1.542 ± 0.009	2.01 ± 0.02	22.7 ± 0.9	83 ± 1	4.9 ± 0.3	20.0 ± 0.5	88 ± 5	45 ± 1	343 ± 20
		0.5		2.426 ± 0.005	2.415 ± 0.005	13.0 ± 0.4	83 ± 1	4.4 ± 0.3	19.4 ± 0.4	89 ± 6	113 ± 2	637 ± 10
	0.66	0	3.6 ± 0.1	1.554 ± 0.008	1.554 ± 0.006	45.8 ± 0.2	85 ± 1	5.3 ± 0.3	21.4 ± 0.4	90 ± 4	65.2 ± 0.7	265 ± 30
		0.02		1.540 ± 0.003	2.279 ± 0.002	46.2 ± 0.5	84 ± 1	4.9 ± 0.3	21.0 ± 0.4	91 ± 3	83 ± 3	221 ± 10
		0.04		1.549 ± 0.001	1.663 ± 0.007	38.1 ± 0.4	84.7 ± 05	4.5 ± 0.3	19.7 ± 0.4	91 ± 4	63 ± 1	294 ± 5
		0.06		1.544 ± 0.003	2.431 ± 0.007	34.2 ± 0.3	84.2 ± 0.7	5.2 ± 0.3	21.3 ± 0.5	92 ± 2	60.9 ± 0.1	279 ± 3
		0.08		1.460 ± 0.005	2.103 ± .0.003	35.5 ± 0.8	83 ± 1	5.2 ± 0.3	20.8 ± 0.5	91 ± 2	69.3 ± 0.1	245 ± 4
		0.1		1.571 ± 0.004	2.2 ± 0.1	29.3 ± 0.4	82.1 ± 0.7	4.7 ± 0.3	20.9 ± 0.4	89 ± 6	54 ± 3	309 ± 20
		0.5		1.770 ± 0.002	2.43 ± 0.03	24.6 ± 0.9	83.8 ± 0.8	4.8 ± 0.3	20.6 ± 0.4	91 ± 5	113 ± 3	480 ± 20
	1	0	3.4 ± 0.1	1.515 ± 0.009	2.484 ± 0.009	53.7 ± 0.8	84 ± 1	4.7 ± 0.3	20.7 ± 0.4	93 ± 3	64.0 ± 0.7	221 ± 10
		0.02		1.483 ± 0.003	2.469 ± 0.005	47.2 ± 0.5	85.6 ± 0.8	5.0 ± 0.3	20.8 ± 0.4	90 ± 6	54 ± 2	255 ± 6
		0.04		2.458 ± 0.002	2.187 ± 0.006	41.8 ± 0.2	85.6 ± 0.8	4.2 ± 0.3	20.3 ± 0.4	93 ± 4	71.7 ± 0.7	235 ± 1
		0.06		1.474 ± 0.004	2.117 ± .0.002	43.4 ± 0.7	90.3 ± 0.9	3.7 ± 0.5	13.5 ± 0.4	93 ± 1	43.9 ± 0.7	206 ± 5
		0.08		1.096 ± 0.008	2.10 ± 0.02	37.3 ± 0.2	84.2 ± 0.6	3.7 ± 0.3	19.3 ± 0.8	91 ± 3	57 ± 6	206 ± 20
		0.1		1.531 ± 0.007	2.10 ± 0.01	34.0 ± 0.8	83.3 ± 0.6	4.4 ± 0.3	20.1 ± 0.5	89 ± 8	52 ± 9	201 ± 20
		0.5		1.549 ± 0.004	2.066 ± 0.009	28.2 ± 0.4	84 ± 1	4.6 ± 0.3	20.7 ± 0.4	93 ± 2	98 ± 8	485 ± 40
42	0.33	0	3.7 ± 0.1	2.03 ± 0.1	1.948 ± 0.003	21.4 ± 0.1	83 ± 1	5.7 ± 0.3	18 ± 3	82 ± 9	67 ± 3	485 ± 10
		0.02		1.717 ± 0.006	1.721 ± 0.005	20.2 ± 0.3	83.4 ± 0.9	5.0 ± 0.3	21.7 ± 0.4	82 ± 7	124.1 ± 0.1	564 ± 30
		0.04		1.758 ± 0.004	1.617 ± 0.004	20.8 ± 0.7	82.1 ± 0.9	5.9 ± 0.3	22.6 ± 0.5	83 ± 9	193.6 ± 0.7	632 ± 10
		0.06		1.98 ± 0.02	1.982 ± 0.002	23.4 ± 0.4	83.7 ± 0.9	4.9 ± 0.3	21.6 ± 0.5	82 ± 7	70 ± 10	671 ± 60
		0.08		1.753 ± 0.002	1.783 ± 0.009	18.6 ± 0.2	82.6 ± 0.8	5.5 ± 0.3	22.4 ± 0.5	86 ± 6	79 ± 4	696 ± 30
		0.1		2.03 ± 0.01	2.025 ± 0.005	23.1 ± 0.6	82.9 ± 0.8	5.4 ± 0.3	22.3 ± 0.4	96 ± 1	180 ± 3	706 ± 4
		0.5		1.603 ± 0.005	1.416 ± 0.003	18.1 ± 0.9	84 ± 1	5.2 ± 0.3	21.7 ± 0.4	97 ± 3	253 ± 10	1299 ± 50
	0.66	0	3.5 ± 0.1	1.534 ± 0.009	1.74 ± 0.01	25.6 ± 0.9	84.4 ± 0.9	4.6 ± 0.3	21.6 ± 0.4	93 ± 1	134 ± 10	524 ± 50
		0.02		1.744 ± 0.001	1.754 ± 0.005	32.5 ± 0.5	83 ± 1	6.0 ± 0.3	24.1 ± 0.4	91.8 ± 0.7	123 ± 4	500 ± 20
		0.04		1.622 ± 0.006	1.716 ± 0.008	22.0 ± 0.9	89 ± 2	3.8 ± 0.2	14 ± 2	91.6 ± 0.8	119 ± 2	563 ± 20
		0.06		1.740 ± 0.007	1.922 ± 0.002	24.3 ± 0.3	84 ± 1	6.1 ± 0.3	24.0 ± 0.5	91.3 ± 0.7	87 ± 9	568 ± 20
		0.08		1.929 ± 0.003	1.77 ± 0.01	19.6 ± 0.4	83.0 ± 0.8	6.0 ± 0.3	24.0 ± 0.5	90.2 ± 0.2	140.9 ± 0.1	642.0 ± 0.7
		0.1		2.018 ± 0.008	2.158 ± 0.002	16.0 ± 0.8	89 ± 1	4 ± 1	15 ± 5	90.95 ± 0.02	208.8 ± 0.7	613 ± 10
		0.5		2.03 ± 0.02	2.187 ± 0.004	21.1 ± 0.6	84.3 ± 0.9	4.6 ± 0.3	21.5 ± 0.4	86.01 ± 0.01	163 ± 3	931 ± 20
	1	0	3.4 ± 0.1	1.50 ± 0.02	1.746 ± 0.005	24.8 ± 0.5	82.5 ± 0.6	5.9 ± 0.3	24.2 ± 0.4	95.1 ± 0.6	119 ± 6	421 ± 40
		0.02		1.539 ± 0.005	1.75 ± 0.02	28.5 ± 0.2	88 ± 2	4 ± 1	15 ± 6	94 ± 2	121 ± 4	470 ± 50
		0.04		1.550 ± 0.003	1.68 ± 0.01	28.4 ± 0.1	83.3 ± 0.7	5.8 ± 0.3	23.7 ± 0.6	94.9 ± .1	190 ± 6	446 ± 20
		0.06		1.560 ± 0.008	1.77 ± 0.01	27.5 ± 0.6	83.1 ± 0.8	6.0 ± 0.3	24.3 ± 0.5	94.8 ± 0.5	109 ± 10	475 ± 40
		0.08		1.748 ± 0.002	1.995 ± 0.009	20.4 ± 0.1	83 ± 1	4.9 ± 0.3	21.7 ± 0.5	93.3 ± 0.8	129 ± 10	554 ± 50
		0.1		1.469 ± 0.006	1.566 ± 0.005	20.3 ± 0.5	82.9 ± 0.7	6.1 ± 0.3	24.4 ± 0.4	95.7 ± 0.8	155 ± 10	534 ± 30
		0.5		1.564 ± 0.002	1.399 ± 0.007	19.0 ± 0.1	82.5 ± 0.5	5.9 ± 0.5	23.9 ± 0.6	86 ± 9	190 ± 10	622 ± 60

opaque gels. The effect of GDL/QP on the opacity may be due both to the acidification rate and to the final pH of the systems.

According to equations (9) and (10), a higher GDL/QP increased the whiteness and decreased the opacity. As it was observed in the experiments, a more acid gel seems to be whiter and transparent, interesting qualities for food industry.

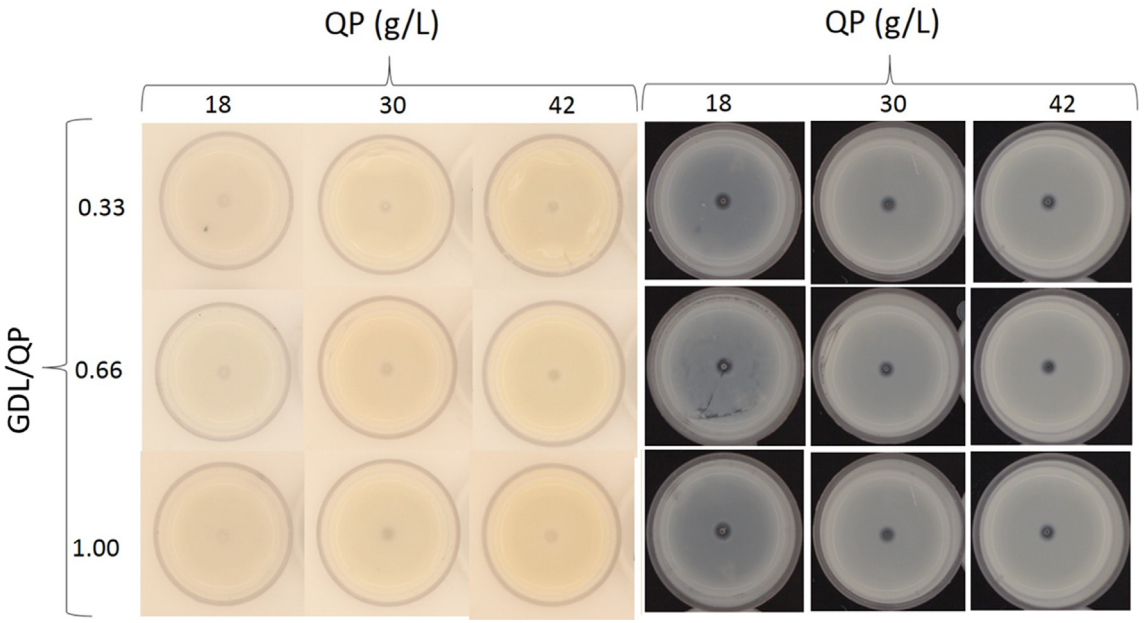

Fig. 2. Photos on a white and black background of mixed gels composed by different concentrations of quinoa proteins (QP) and acidified by different glucono-δ-lactone/quinoa proteins ratios (R).

3.3. Water holding capacity

The expelled water mass when the gel was centrifuged at a moderate speed for a short time, without breaking the protein network during this process (Quéguiner, Dumay, Cavallier, & Cheftel, 1989), was determined and shown in Table 1. The significance of each factor on WHC was determined by ANOVA (data not shown): QP concentration ($P = 0.0002$), Carr concentration ($P < 0.0001$), GDL/QP ($P < 0.0001$) as well as its quadratic term ($P = 0.0003$) were significant. In addition, the interactions between QP concentration with GDL/QP and with Carr concentration were also significant ($P < 0.0001$).

Considering the significant factors and their interactions, data was fitted to the following model equation and plotted in Fig. 3:

$$WHC(\%) = 91.5(6) - 0.5(1)[QP] + 24(3)\,(GDL/QP) - 0.21(5)[Carr] - 23(6)\,(GDL/QP)^2 + 0.6(1)[QP]\,(GDL/QP) + 0.005(1)[QP][Carr] \quad (11)$$

QP and Carr concentration terms were negative in Equation (11), which may indicate that an increase in the concentration of one or another biopolymer produced a decrease in WHC. However, there was also an interaction term involving both biopolymers. This suggests that the presence of Carr had a synergistic effect with the QP concentration on the WHC of these gels. This synergy would be related to the observed decrease in the minimum QP concentration required to form gels in the presence of Carr previously reported (Montellano Duran, Galante, et al., 2018a). On the other hand, GDL/QP had a positive effect on WHC, which meant that the acidification with a higher GDL/QP, in the range tested, allowed obtaining gels with higher WHC. This would be related to the effect of GDL/QP on the formation of the QP aggregates: the faster the acidification, the less time available for the rearrangement of the aggregates and therefore, the higher amount of water retained in the protein aggregates and not expelled during centrifugation.

Some other researchers have studied the effect of the addition of carboxymethylcellulose, an electrically charged polysaccharide, to a solution of whey proteins to form acid gels. The polysaccharide concentration differentially affects the WHC of those gels, depending on the concentration of proteins in the system (Huan, Zhang, & Vardhanabhuti, 2016), as was observed in our case. This similarity may imply the same behavior in QP–Carr mixed acid–induced gels.

3.4. Textural measurements

Penetrometry assays were carried out for every sample. The QP concentration in the systems were in the lower concentration limit, thus the conditions did not allow obtaining self-supportive gels. The probe used was smaller in diameter than the sample because it was necessary to use the cylinders. All the obtained profiles (Fig. 4) showed two stages: the first included the deformation of the gel up to its fracture and the second was the penetration of the gel. From the first stage the parameters Stiffness (St) and maximum force (F_{max}) were obtained for each sample (Table 1). The significance of the [QP], [Carr] and GDL/QP on these values was determined. The three factors under study were significant for F_{max} ($P < 0.001$) whereas only QP and Carr concentrations were significant for St ($P < 0.0001$). GDL/QP did not affected St significantly ($P = 0.0830$). The following equations were obtained by fitting data (or their proper transformations):

$$St = -88(5) + 5.0(4)\,[QP] + 110(20)\,[Carr] \quad (12)$$

$$(F_{max})^{-1/2} = 0.106(1) - 1.67(7)*10^{-3}\,[QP] - 0.083(5)[Carr] + 0.012(1)R + 1.4(2)*10^{-3}\,[QP][Carr] \quad (13)$$

St (Equation (12)) was positively affected by the concentration of both biopolymers. This meant that the increase in the concentration of [QP] or [Carr] produced an increase in St. This effect may be due to the increase in the interconnectivity of the matrix in the gel.

On the other hand, F_{max} (Equation (13)) was positively affected by the concentration of both biopolymers and negatively affected by GDL/QP. As was discussed before, the increase in [QP] or [Carr] increased the textural parameters of these gels. On the other hand, the negative effect of GDL/QP on F_{max} may be related to the final pH of the gels. Textural analysis has been previously report for gels composed by soy protein isolates (Campbell et al., 2009), showing that their hardness increase by the increase in protein concentration and the degree of protein denaturation. Moreover, the final pH of those gels is reported to affect the textural parameters: the closer the pH to the isoelectric pH of the protein, the harder and more elastic the gels. Our results were in agreement with that work: the lower GDL/QP assayed (0.33) produced harder gels which pH is close to the isoelectric pH of QP whereas higher

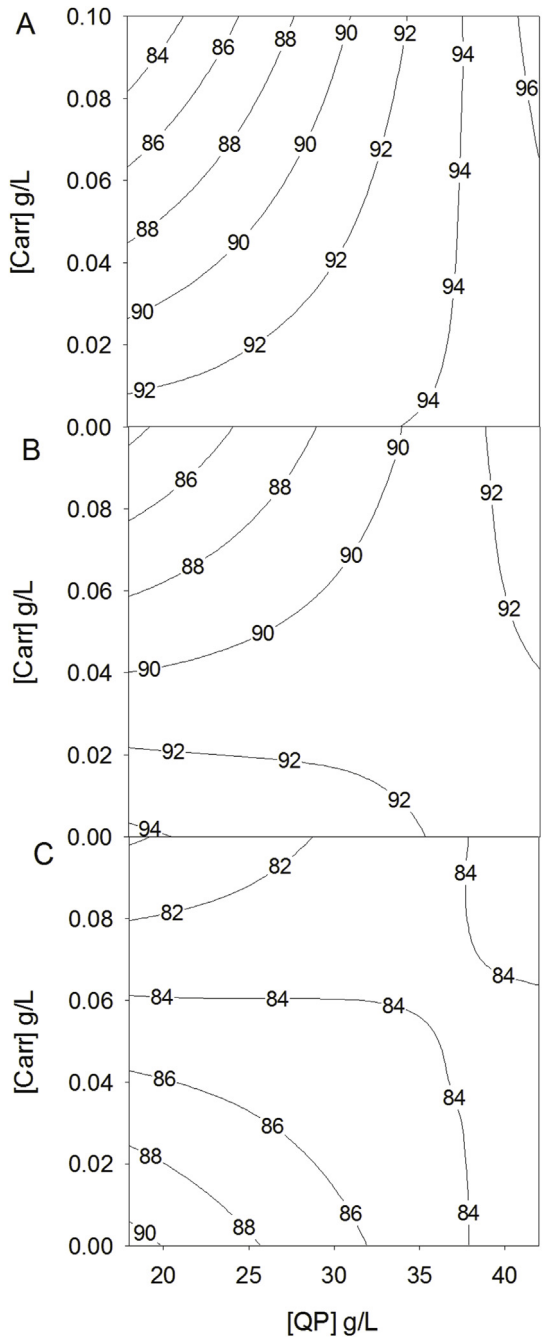

Fig. 3. Water holding capacity for gels composed by different quinoa proteins and carrageenan concentrations. Each figure represents the results obtained for gels acidified with increasing glucono–δ–lactone/quinoa protein ratios (R): A) 0.33; B) 0.66; C) 1.00.

assayed GDL/QP (0.66 and 1.00) produced more acid gels with lower F_{max}.

3.5. Relationship between micro and macro structure

The microstructure of gels was expected to be related to macroscopic characteristics such as mechanical properties, syneresis and appearance. In order to evaluate the relationship of the physical characteristics studied in this work, the correlation between the obtained parameters was assayed.

Pore size was found to be correlated to the whiteness ($\rho = 0.577$, $p < 0.005$) and to the opaqueness ($\rho = -0.832$, $p < 0.005$). This may

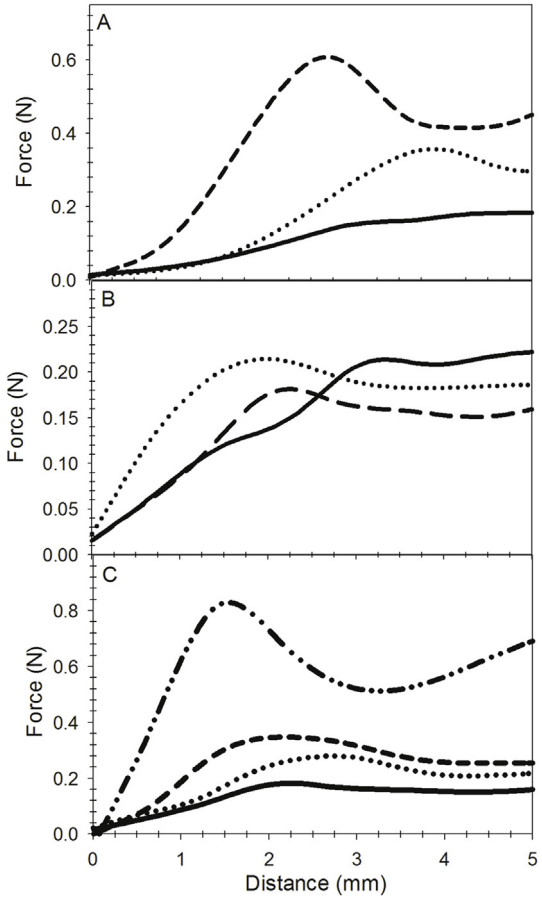

Fig. 4. Penetrometry profiles of gels composed by: A) Different quinoa proteins concentrations, [QP]: (——) [QP] = 18 g/L, (●●●) [QP] = 30 g/L, (– – –) [QP] = 42 g/L in the absence of carrageenan and acidified by a glucono-δ-lactone/quinoa proteins ratio, GDL/QP = 0.33; B) [QP] = 30 g/L, in the absence of carrageenan acidified with different R: (——) GDL/QP = 0.33, (– – –) GDL/QP = 0.66, (●●●) GDL/QP = 1, C) (——) [Carr] = 0 g/L, (●●●) [Carr] = 0.04 g/L, (– – –) [Carr] = 0.1 g/L. (—●●—) [Carr] = 0.5 g/L, quinoa proteins concentration ([QP]) is 42 g/L, glucono-δ-lactone and quinoa proteins ratio is equal to 0.66.

be due to the fact that the gels with higher pore size were more transparent because they allowed more the passage of light. However, the chromatic parameters of the gels were only related to the protein concentration and the final pH of the gels.

Both pore size and WHC were affected by all the factors studied. In the absence of Carr, pore size and WHC increased when GDL/QP increased and decreased with [QP], indicating that QP gels with higher pore size had the capacity to more efficiently retain water. However, in the mixed gels studied, no significant correlation was found between both parameters ($P = 0.0696$), which can be attributed to the effect of the presence of Carr. For the lowest [QP], the increase in the [Carr] increased the pore size, because the QP–Carr interactions give rise to more compact aggregates (Montellano Duran, Galante, et al., 2018a). In this same situation, the increase in the [Carr] produced a decrease in the WHC because the competition between the QP–QP interaction and the QP–Carr interaction weakens the protein matrix that constitutes the gel, in terms of its ability to retain water.

The concentration of both biopolymers increased the F_{max} of the gels. A negative correlation was found between pore size and F_{max} ($P = 0.00167$), indicating that gels with smaller pores were stronger, due to the greater cross-linking of the network.

4. Conclusions

The particularities of the aggregation processes that give place to gelation produce the singular characteristics of the mixed gels. In the concentration range assayed for QP, Carr and GDL, the QP–QP interactions are responsible for forming the polymer network of the gels and the presence of Carr modifies the conditions of formation of the gels, their structure and properties. The gel formation was influenced mainly by the pH and since GDL/QP regulates the rate of acidification of the systems, therefore, GDL/QP affects the structure of aggregates formed and their size. It can be seen that the microstructure can be controled by the aggregates (their size and their distribution) modifying the macrostructural properties since the matrix is changed by the pores size and distribution. The pore size was correlated with the WHC, F_{max} and color meaning that the polymer network can be changed to modify this macro properties according to the consumer's desires.

In micro scale, it is known that there is an interaction between QP and Carr in the pH range where the gel structure was formed. The Carr interacts positively in this pH range with QP by electrostatic and hydrophobic forces, this is why the gelation process is a competition between QP-QP interactions (responsible for the gel formation) and QP-Carr interaction (because of electrostatic and hydrophobic forces in this pH range, 3.4–3.9).

The potential application of the results from this work is the possibility of control and modification of the gel properties (appearance, mechanical and WHC) its formulation. The obtained model equations may contribute to the proper selection of QP and Carr concentrations as well as the amount of GDL added to the systems in order to obtain gels with desired characteristics.

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