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Effects of the enzymatic hydrolysis treatment on functional and antioxidant properties of quinoa protein acid-induced gels

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Abstract

Partial enzymatic hydrolysis is frequently used as strategy to improve the functional and nutritional properties of vegetable proteins. The aim of this work was to evaluate the hydrolysis of quinoa proteins, as well as the functional and antioxidant properties of their acid-induced gels. In order to fulfil this purpose, quinoa protein hydrolysates were obtained using a fungal serin protease. The hydrolysis degree, surface hydrophobicity, sulphydryl group content and the electrophoretic profile of hydrolysates were assayed. Hydrolyzed quinoa protein acid-induced gels were carried out and gels obtained were tested for their textural characteristics, water holding capacity, appearance (color and microstructural properties) as well as for their *in vitro* antioxidant activity. The changes occurring during the enzymatic hydrolysis affected the gel-forming ability of quinoa proteins and therefore the characteristics of gels. After 3h of proteolysis, protein hydrolysates with 17±2% hydrolysis degree and low surface hydrophobicity were obtained. Gels obtained of these hydrolysates presented less interconnected protein network and thus, lower textural parameters and lower water holding capacity than control gels. In conclusion, even though the hydrolysis treatment negatively affects the gelling properties of the quinoa proteins, limited hydrolysis enables us to obtain gels with antioxidant capacities which present differential characteristics.

1.Introduction

The steady and rapid increase in the world's population implies a growing demand for foods based on plant proteins. The right combination of vegetable proteins may ensure the supply of enough amounts of nutrients to cater for human health requirements. However, plant proteins are still underutilized as human food for both nutritional and functional reasons. Fortunately, there has been considerable research and development focused on improving plant protein use as food ingredients (Day, 2013).

Quinoa (*Chenopodium quinoa* Willd.) belongs to the Chenopodiaceae family that grows mainly in Ecuador, Peru, Bolivia, Argentina, and Chile. Quinoa proteins (QP) draw attention due to their well-balanced content of essential amino acids and their functional properties making them a promising food ingredient (Elsohaimy, Refaay & Zaytoun, 2015; Mäkinen, Zannini & Arendt, 2015; Ruiz, Xiao, van Boekel, Minor & Stieger, 2016; Kaspchak et al., 2017). Moreover, as quinoa is gluten-free QP have the potential to be used as food materials for celiac patients (Navruz-Varli & Sanlier, 2016).

Partial enzymatic hydrolysis is frequently used to improve the functional and nutritional properties of proteins. Generally, it produces by three distinct effects: (1) a decrease in molecular weight; (2) an increase in the number of ionizable groups; and (3) exposure of hydrophobic groups (Panyam & Kilara, 1996). These effects can effectively modify the protein conformation and structure so as to improve their techno-functional properties (solubility, emulsifying and foaming properties). In addition, protein enzymatic hydrolysates are potential sources of bioactive molecules. Recently, interest has emerged in identifying and characterizing bioactive peptides from plant protein hydrolysates since they are rich sources of pharmacologically and biologically active compounds (Sarmadi & Ismail, 2010). The antioxidant capability of these compounds is one of the most studied biological activities since the oxidation of biomolecules plays a

crucial role in all living organisms. Dietary antioxidants provide a valuable help in delaying or inhibiting the cellular oxidation process and the deterioration of food quality. Studies have focused on characterizing natural antioxidants from food resources for their potential health benefit with no or little side effects (Sarmadi & Ismail, 2010).

Several articles have reported the radical-scavenging activities in QP. Aluko and Monu (2003) obtained antioxidant peptides from quinoa by enzymatic hydrolysis with alcalase. They found that low-molecular-weight peptides possess higher potential than high-molecular-weight peptides to act as antioxidant compounds that reduce the number of free radicals. Nongonierma et al. (2015) demonstrated that the antioxidant activity of the quinoa protein hydrolysates (QPH) obtained with papain and a microbial papain-like enzyme was approximately twice higher than that of the quinoa protein isolate control. This proved the benefits of utilizing exogenous enzyme preparations to release bioactive peptides from QP.

Gel formation ability is important for the development of textured protein foods and required to produce yogurt and tofu-type products. A gel structure is formed when intermolecular cross-linking occurs in such a way that leads to the development of a continuous network that exhibits elastic behavior (Foegeding, 2007). The protein gelation process can occur when molecules are denatured by factors such as, heat, pressure or pH, causing to aggregation (Tarone, Fasolin, de Assis Perrechil, Hubinger & da Cunha, 2013; Kaspchak et al., 2017). Plant proteins are considered less effective than animal proteins in terms of gelling properties. Partial enzymatic hydrolysis of vegetable proteins was reported to improve gelation properties by increasing the availability of hydrophobic regions and ionizable groups (Hou & Zhao, 2011; Zhao, Liu, Zhao, Ren & Yang, 2011; Nieto-Nieto, Wang, Ozimek & Chen, 2014).

The aim of this work was to evaluate the properties of acid-induced gel of quinoa protein hydrolysates. The QP enzymatic hydrolysates were obtained by using a serin protease from *Aspergillus niger*. The relationship between the hydrolysis treatment and the functional and antioxidant properties acid-induced gels was identified as well.

2. Materials and methods

2.1 Materials

Quinoa flour from Sturla, (Argentina) was purchased in local market; 1-anilino-8-naphthalene sulfonate (ANS), 2,2´-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyil-picrylhydrazyl (DPPH), 5,5´-dithio-bis 2-nitrobenzoic acid (DTNB), glucono delta-lactone (GDL), rodhamine B, sodium dodecyl sulfate (SDS), trinitrobenzenesulfonic acid (TNBS) and Trolox were purchased in Sigma – Aldrich, (Argentina).

2.2 Enzyme extraction

The enzymatic extract used in this work was obtained from the solid-state fermentation of *Aspergillus niger*. The production of the extracellular serin peptidase was carried out according to the protocol detailed by López et al. (2018). The enzymatic activity was determined using casein as substrate, according to Cupp-Enyard method described by López et al. (2018).

2.3 Quinoa Protein isolation

The QP isolation was carried out according to the method proposed by Abugoch et al. (2008) with some modifications. Quinoa flour was solubilized in water (10%w/v) at pH 8. The pH was adjusted to the required value with NaOH. The suspension was stirred for 40 min at room temperature and then centrifuged at 1000g for 20 min. The supernatant was adjusted to pH 4.5 adding HCl 0.1 M and then centrifuged for 20 min at 1000g. The precipitates were resuspended in water, adjusted to pH 8 and stored at

8°C until use. Soluble proteins were quantified by the Bradford method (1976). A 50 g/L QP suspension was prepared and heated at 100°C for 15 min, in order to increase the protein unfolding.

2.4 Quinoa protein hydrolysates (QPH)

QP (50 g/L) was incubated at 40°C for 0, 0.5, 1, 1.5, 2, 3 h with the peptidase from *Aspergillus niger* at a ratio of 500AU/g QP in order to obtain the QPH samples QPH0, QPH0.5, QPH1, QPH1.5, QPH2, QPH3 respectively.

2.5 Degree of hydrolysis determination

In this work, the degree of hydrolysis (DH) of QPH was determined by the Adler-Nissen (1979) method. QPH samples were mixed with a sodium phosphate buffer (0.2 M, SDS 1% w/v, pH 8.2). Then, TNBS reagent (0.1% w/v) was added. Test tubes were mixed and incubated at 50°C for 60 min. Reaction was stopped after incubation by addition of 0.1N HCl. Absorbance was measured at 340 nm. The standard solution (100% DH) was obtained by complete hydrolysis of QP in HCl 6N for 12h at 100ºC.

2.6 Determination of exposed free sulfhydryl contents

The sulphydryl groups of QPH were determined according to the method of Beveridge, Toma & Nakai (1974) with some modifications. Ellman's reagent was prepared according to the protocol detailed by Yin, Tang, Wen, and Yang (2010). Then, 500 µL of the Ellman's reagent was added to 500 µL of each QPH sample. The resultant suspension was incubated for 1h at 25 ºC. Finally, the absorbance of the samples was determined at 412 nm. The contents of sulfhydryl groups was determined by using the extinction coefficient of 2-nitro-5-thiobenzoateat 412 nm (13600L mol $^{-1}$cm^{-1}) and expressed as µmol/g of protein.

2.7 Surface hydrophobicity

The Surface hydrophobicity (S_0) was determinate according to the Kato & Nakai (1980) method, using ANS as a hydrophobic probe (Kato & Nakai, 1980; Fan et al., 2005). Measurements were carried out with a spectrofluorometer (Aminco Bowman Series 2, Japan) using an excitation and emission wavelength of 380 and 484 nm respectively, previously determined from the excitation and emission spectra of the protein-ANS complex. The fluorescence intensity was measured in samples containing ANS 0.04 mM in phosphate buffer 5 mM (pH 7) and with consecutive aggregates of QPH samples. The slope of the curve of relative fluorescence intensity vs. protein concentration was used as a measure of S_0.

2.8 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

The QPH samples were analyzed using SDS-PAGE. Gel electrophoresis was performed under reducing conditions using 8% and 15% of acrylamide-bisacrylamide for the stacking and resolving gel respectively on a Mini-PROTEAN 3 Cell system (Bio-Rad Laboratories, USA), according to the manufacturer's instructions. Samples were mixed 1:1 (v/v) with loading buffer under reducing conditions (with β-mercaptoethanol). Proteins were visualized by staining with Coomassie brilliant blue 0.025% (w/v). A wide–range molecular weight calibration kit (6,500 to 66,000 Da, Sigma-Aldrich) was used as molecular weight standard.

2.9 Gelation process

The cold gelation of QPH was induced by reducing the electrostatic repulsion after lowering the pH towards the isoelectric point (Duran, Galante, Spelzini & Boeris, 2018). The addition of GDL, which slowly hydrolyzes to gluconic acid, causes a gradual reduction in pH and formation of a regular gel (Alting et al., 2000). The final pH of the system is a function of the amount of GDL added (Braga et al., 2006). GDL (16.7 g/L) was added to QPH (50g/L) to obtain the acid-induced gels. The gelation

process was carried out at 25ºC in a proper container and concluded after 15 min of the addition of GDL. QPH gels (QPHG) obtained from the samples with different hydrolysis times were prepared and named as QPHG0, QPHG0.5, QPHG1, QPHG1.5, QPHG2, and QPHG3.

2.10 Radical scavenging determinations

The antioxidant activity in the QPHG samples was measured with the ABTS and DPPH* assay that quantifies an antioxidant's suppression of the radical cation $ABTS^{\bullet+}$ and $DPPH^{\bullet+}$ respectively, based on a single-electron reduction of the relatively stable radical cations. In order to determine the antioxidant activity, the QPHG were solubilized by the addition of NaOH 0.5 M before the antioxidant determination protocols.

2.10.1 DPPH radical scavenging activity

The scavenging effect on DPPH free radical was measured by the method of Brand-Williams et al. (1995) with some modifications. An aliquot of 100 µL of the sample or buffer (control) was mixed with 100 µL of an ethanolic solution of DPPH (0.5 mM) and 800 µL of ethanol. The mixtures were left in the dark at 25°C for 30 min. The absorbance of the solution was measured at 517 nm in a spectrophotometer UV-VIS JascoV-550 (Berlin, Germany). Radical scavenging activity (RSA) was obtained according to:

$$RSA = Abs_C/(Abs_S-Abs_B) \text{ (Equation 1)}$$

where Abs_C is the absorbance of the control, $Abs_B$is the absorbance of the blank without DPPH and Abs_S is the absorbance corresponding to the sample. Values above the unity indicate that the sample contains scavenging activity (Aluko & Monu, 2003).

2.10.2 ABTS radical cation scavenging activity

The ABTS antioxidant assay was performed according to the method proposed by Re et al.(1999). $ABTS^{\bullet+}$ stock solution (7mM) was prepared and allowed in the dark at room temperature for 24 h before use. The antioxidant compound content in the QPHG was analyzed by diluting the $ABTS^{\bullet+}$ solution with phosphate buffer (0.100M, pH 7) to an absorbance of 0.70 ± 0.02 at 730 nm. 10 µL of the samples or Trolox standard (final concentration 0-1.2 mM) was added to 990 µL of diluted $ABTS^{\bullet+}$ solution and absorbance was read before and after sample addition. The RSA of the analyzed samples was expressed as scavenging percentages (%S) and was calculated from the following equation:

$$\%S = [(Abs_B - Abs_S)/Abs_B]*100 \quad \text{(Equation 2)}$$

where Abs_B and Abs_S are the absorbance values of the diluted $ABTS^{\bullet+}$ solution before and after the sample addition, respectively. The trolox equivalent antioxidant coefficient (TEAC) was quantified according to:

$$TEAC = (\%S - b)/m \quad \text{(Equation 3)}$$

where b is the intersection and m is the slope of the regression analysis of the %S vs. Trolox concentration graphical representation (Segura-Campos, Salazar-Vega, Chel-Guerrero & Betancur-Ancona, 2013).

2.11 Color measurement

A high-resolution digital camera (Canon EOS-Rebel T3) was used to measure color by capturing the images of the QPHG samples under proper lighting according to the method detailed by Galante *et.al*. (2018).The digital images were processed, using Photoshop software (Adobe Systems Inc., San José, California, USA) in order to obtain the L*, a* and b* parameters. The total color difference (ΔE) was calculated according to:

$$\Delta E = \sqrt{(L^*_0 - L^*)^2 + (a^*_0 - a^*)^2 + (b^*_0 - b^*)^2} \quad \text{(Equation 4)}$$

where L^*_0, a^*_0 and b^*_0 are the color parameters of the reference material (QPHG0). A larger ΔE value denotes greater color changes from the reference sample (Maskan, 2001).

2.12 Microstructure analysis with confocal scanning laser microscopy

The microstructure of QPHG was observed by using confocal scanning laser microscopy (CSLM). Rhodamine B (0.1 mg/mL) was mixed with QP or QPH solutions. After GDL addition, 300 μL of each sample was immediately placed in compartment cells, where the gelation reaction takes place at 25 °C. The images were obtained using a confocal microscope (Nikon Eclipse TE-2000-E, Japan). The digital images were acquired and analyzed according to the method detailed by Galante *et.al*. (2018).

2.13 Mechanical properties of gels

Texture measurements were performed by using the textural machine Perten TVT6700 (Hägersten, Sweden) equipped with a 5 N load cell. Gel samples were prepared in 20 mL cylindrical containers (diameter: 35 mm; height: 30 mm) according to the protocol detailed in section 2.9. Three independent repetitions were made for each sample at room temperature. The cylindrical plunger (diameter: 20 mm; height: 20 mm) penetrated 50% into the gel mesh at a speed of 1 mm/s. The textural parameters reported in this study were gel strength and firmness.

2.14 Water holding capacity

The liquid expelled from the QPHG samples was quantified after 24 h storage at 25 ºC. The percentages of the water holding capacity (%WHC) were obtained according to:

$$\%WHC = 100 * (m_1 - m_2)/m_1 \quad \text{(Equation 5)}$$

where m_1 is the initial weight and m_2 is the liquid expelled weight.

2.15 Statistical analysis

All determinations were performed at least in duplicate. The data obtained were statistically evaluated by ANOVA and a Holm-Sidak post-hoc test. The statistical analysis was made using Sigma Stat software. Differences were significant when $p < 0.05$.

3. Results and Discussion

3.1 Protein hydrolysates

3.1.1 Evaluation of the hydrolysis treatment

The extent of proteolysis was quantified by the DH. Table 1 shows the DH of the QPH samples. The DH of the QPH increased, as expected, when the hydrolysis time increased, reaching a value of 17±2% at 3 h of incubation with the serin protease.

Several authors have reported that structural factors, such as the exposition of sulfhydryl or hydrophobic residues contribute to the gel-forming ability of a protein (Fan et al., 2005; Zhao et al., 2011). Therefore, the S_0 and the amount of sulfhydryl groups content were determined and presented in Table 1. The amounts of sulfhydryl groups in QPH samples did not vary significantly ($p > 0.05$). According to this, the hydrolysis treatment did not modify the sulfhydryl group exposure. S_0 of the QPH decreased significantly when the DH increased, reaching the lowest value for the QPH3 samples ($p< 0.05$). This indicates that the higher the DH, the lower the hydrophobic surface available to favor the hydrophobic interactions among the hydrolysates. This behavior was also reported for soy protein treated with pepsin (Fan et al., 2005).

The SDS-PAGE patterns of QPH samples were shown in Figure 1. The SDS-PAGE analysis of QPH showed numerous bands of varying intensity. The most intense bands in the QPH0 profile are at about 30 kDa, 20 kDa, and 14 kDa. The 30 kDa and 20 kDa bands correspond to the acid and basic polypeptides of chenopodin in reducing condition (with β-mercaptoethanol), respectively. The band corresponding to about

14kDa could be assigned to the 2S seed storage protein (Brinegar & Goundan, 1993; Brinegar, Sine & Nwokocha, 1996; Abugoch et al., 2009; Ruiz et al., 2016). When the hydrolysis time increased, the electrophoretic patterns of the QPH showed high intensity of low-molecular-weight bands and the disappearance of high-molecular-weight bands. A considerable amount of protein was observed at the boundary between the stacking gel and the separating gel for the QPH0 sample. The intensity of this dark blur observed in the top of all lanes decreased as the hydrolysis time increased, indicating that more proteins are capable to pass into the resolving gel.

3.2 Gels´ properties

Acid-induced gels were prepared by lowering the pH of the dispersions of QPH at room temperature to a final pH of 5.4±0.2. In water, GDL hydrolyses to gluconic acid, causing a gradual reduction in pH. The acidification of the media in all cases led to a turbid gel formation. The gels obtained were characterized by colorimetric, structural and mechanical methods. Moreover, antioxidant activity was determined.

3.2.1 *In vitro* antioxidant activity

The antioxidant activities for the analyzed samples are shown in Figure 2. According to the results obtained from the DPPH assay, all the samples showed antioxidant capacity since they presented an RSA index above 1. There are no significant differences in the mean values among different hydrolysis times ($p = 0.082$). The RSA values obtained in this work for the QPHG are similar to that reported by Aluko and Monu (2003) for the QP treated with alcalase. When the antioxidant activity was tested by the ABTS assay, all the analyzed samples showed antioxidant capacity. The TEAC values obtained for QPHG0, QPHG0.5, QPHG1, QPHG1.5, and QPHG2 samples were not statistically different ($p > 0.05$). Only the QPHG3 sample, which presented the highest DH, showed an antioxidant activity statistically different from the

others ($p<0.001$). In addition, the antioxidant activities of the hydrolysates were measured before gel formation (data not shown). Although the antioxidant activity was modified by the gel formation process, the DPPH radical scavenging capability of the hydrolysates before and after gel formation were correlated ($p = 0.026$).

3.2.2 Color

Food color could be used to study the effect of a treatment or process in a food product (Pathare, Opara & Al-Said, 2013). Table 2 shows the L*, a*, b*, and ΔE color parameters obtained for the different QPHG. The QPHG0 sample showed higher L* values and lower b* values than the other QPHG ($p<0.05$) and, thus, QPHG0 was significantly brighter and less yellowish than the rest. The QPHG0 was used as reference to obtain the ΔE parameter, which indicates the total color difference. The ΔE parameter showed an increase as the DH of the QPHG increased. L* values decreased and b* values increased when the hydrolysis time of the QP increased, while a* value was not significantly different among the different hydrolysis times assayed ($p>0.05$). Thus, enzymatic hydrolysis reactions are assumed to have contributed to the reduction in the luminosity, making QPHG look darker. Furthermore, the significant increase in the b* parameter of the QPHG compared to the control sample (QPHG0) indicated an increase in the yellowness of the hydrolysates. These results are in agreement with the ones reported by Kotlar et al. (2013) for the color parameters of the barley proteins after the hydrolysis treatment with an extracellular protease from *B. cereus* spp.

3.2.3 Microstructure analysis

The microstructure of QPHG was visualized by CLSM. An image of each gel sample is shown in Figure 3. The protein mesh (which is stained with rhodamine B), is seen as bright areas while the black areas represent the non-protein phase. A continuous

protein network was observed in the QPHG0. On the other hand, the protein network from QPHG became less interconnected when the hydrolysis time increased.

Figure 4 shows the pore size distributions of QPHG samples. The pore size distribution for each sample confirms the previous observation of the CLSM images. When the hydrolysis time of the QP samples used to form the acid gels increased, an increase in the average pore size value was obtained for the gel since the microstructure changed from a continuous protein matrix to an isolated protein sector in a continuous non-protein phase. Finally, a bimodal pore size distribution was observed for QPHG3.

3.2.4 Textural analysis

The mechanical properties of the QPHG were studied. The maximum force observed called "gel strength" is reached just before the gel breaks. The gel firmness is defined as the initial slope of the penetration curve. Figure 5A shows the comparative textural profile of the different QPHG. All force-displacement curves showed a sharp increase in the force over a short distance as the probe moved into the samples. Figure 5B shows the gel strength and the gel firmness of the different samples. The gel strength decreased when the DH increased, reaching a minimum of 0.4±0.01N for QPHG3 samples. In addition, a maximum value of gel strength of 1.64±0.05N was obtained for QPHG0. These results indicate that a lower force is needed to break the gel mesh when the hydrolysis time increased. The gel firmness was significantly decreased by the hydrolysis of the QP. Although the firmness did not change significantly between QPHG1 and QPHG2 samples, QPHG3 presented the lowest value of firmness. In conclusion, both analyzed textural parameters were affected by QP hydrolysis since this treatment affected the protein-protein interaction capability of the samples. These results agree with those reported by Fan et al. (2005) for gels obtained from soy protein hydrolysates.

3.2.5 Water-holding capacity

WHC is an important property of food gels since the separation of liquid from the gel network affects the perceived texture. Thus, a high WHC is required in gels used for food applications (Nieto-Nieto et al., 2014). Figure 6 shows the WHC results of QPHG. Although all analyzed gels demonstrated excellent WHC (87.7–91.7%), the WHC of QPHG, obtained from QP treated at least for 1h with the enzyme, decreased significantly ($p<0.001$). There is no statistical difference between the WHC of QPH0 and QPHG0.5 ($p>0.05$). The minimum WHC value (87±1%) was reached by the QPHG2 and QPHG3 samples. These WHC values are related to the microstructure found for the QPHG at different hydrolysis time since a less interconnected protein network (large size pore) leads to a less water retention in the gel mesh.

4. Conclusion

The QPH obtained from the enzymatic hydrolysis of QP with an *Aspergillus niger* serin peptidase has the potential to be used as food materials in the production of healthy food. Even though partial hydrolysis treatment does not improve the gel properties of QP, the results show the possibility of integrating enzymatic hydrolysis and cross-linking for the preparation of gels with potential antioxidant activity. The lower gel-forming ability of the hydrolysates was shown to be related to a decrease in the surface hydrophobicity of the protein samples. Gels obtained from QPH with lower DH values could be used to prepare semi-solid foods that combine both antioxidant and gelling capabilities**.** Otherwise, QPH could be used in combination with a gelling polymer that enhances the gel formation capability of QPH to form a strong gelled network. Finally, future research needs to be focused on finding such applications for QPHG in order to develop new products to fulfill the consumers' needs.

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Figure captions

Figure 1. SDS-PAGE profiles of QPH samples obtained by enzymatic hydrolysis at different incubation times.

Figure 2. Vertical bar chart of the mean DPPH and ABTS antioxidant activity assays. The error bars indicate the standard deviation of 3 replicates. Mean values with different letters are significantly different ($p<0.05$).

Figure3. Representative digital images obtained by CLSM of QPHG samples at different hydrolysis time 0, 0.5, 1, 1.5, 2 and 3 hs (named QPHG0, QPHG0.5, QPHG1, QPHG1.5, QPHG2 and QPHG3 respectively) and 3D images of the QPHG0 and QPHG3. The scale bars represent 20 μm.

Figure 4. Pore size distributions of QPHG samples.

Figure 5. A) Force-displacement curve obtained from the penetration test determinations. **B)** Vertical bar chart of the mean gel strength and firmness of the different samples. The error bars indicate the standard deviation of 3 replicates. Mean values with different letters are significantly different ($p<0.05$).

Figure 6. Vertical bar chart of the mean water holding capacity (WHC) values of samples. The error bars indicate the standard deviation of 3 replicates. Mean values with different letters are significantly different ($p<0.05$).

Table 1: Degree of hydrolysis **(**DH), sulphydryl groups (SH) exposed content and surface hydrophobicity (S_0) of the QPH samples.

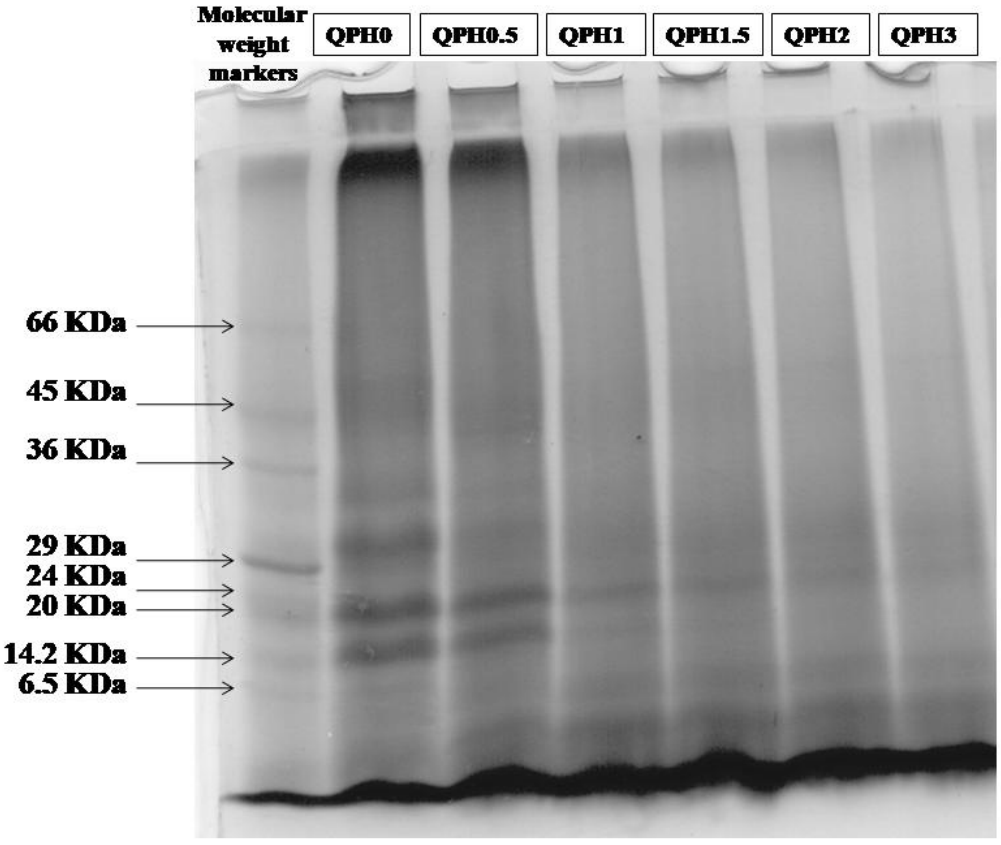
SAMPLE	DH (%)	SH(µmol/g QPH)	S_0
QPH0	-	0.6 ± 0.4^{a}	1.81 ± 0.08^{a}
QPH0.5	8 ± 2^{b}	0.9 ± 0.2^{a}	1.67 ± 0.01^{b}
QPH1	8 ± 3^{b}	0.7 ± 0.3^{a}	1.540 ± 0.001^{bc}
QPH1.5	10 ± 2^{b}	-	1.47 ± 0.07^{c}
QPH2	11 ± 2^{b}	0.5 ± 0.1^{a}	1.42 ± 0.03^{c}
QPH3	17 ± 2^{a}	0.5 ± 0.4^{a}	1.20 ± 0.01^{d}

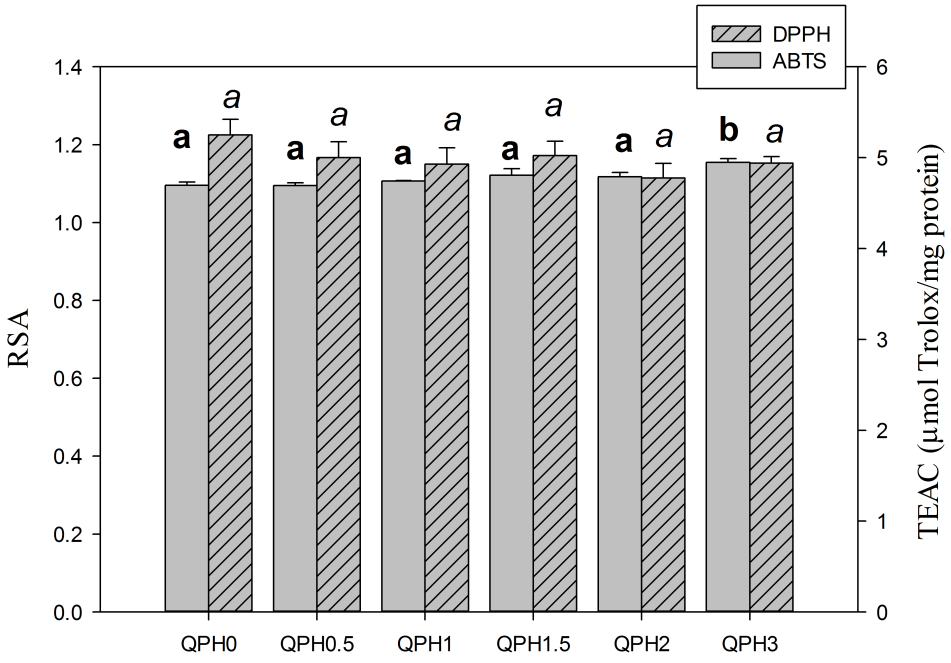
Different letters in the same column indicated that the analyzed samples are significantly different ($P< 0.001$).

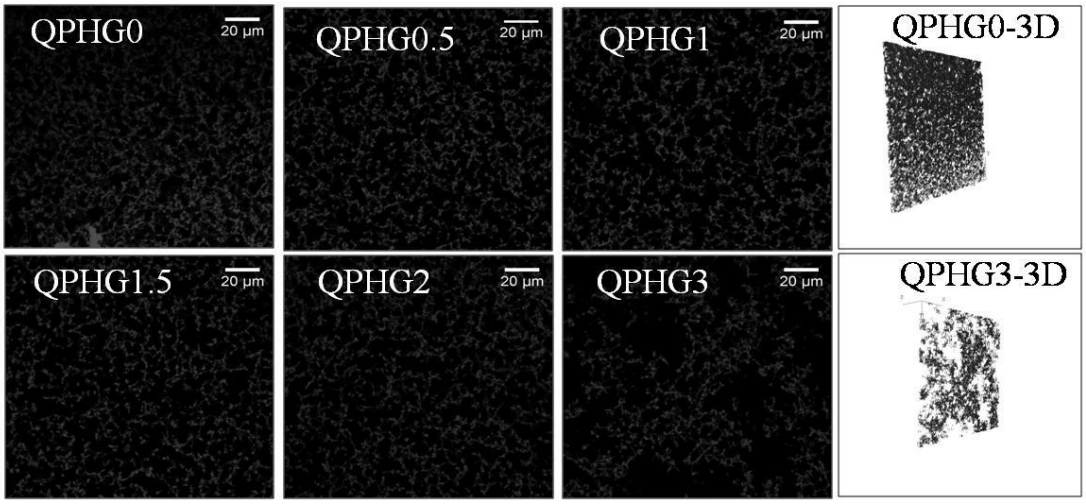
Table 2. L*, a*, b* values, and total color difference (ΔE) for QPHG samples. L* values are a measure of lightness; a* values are a measure of redness; b* values are measure of yellowness.

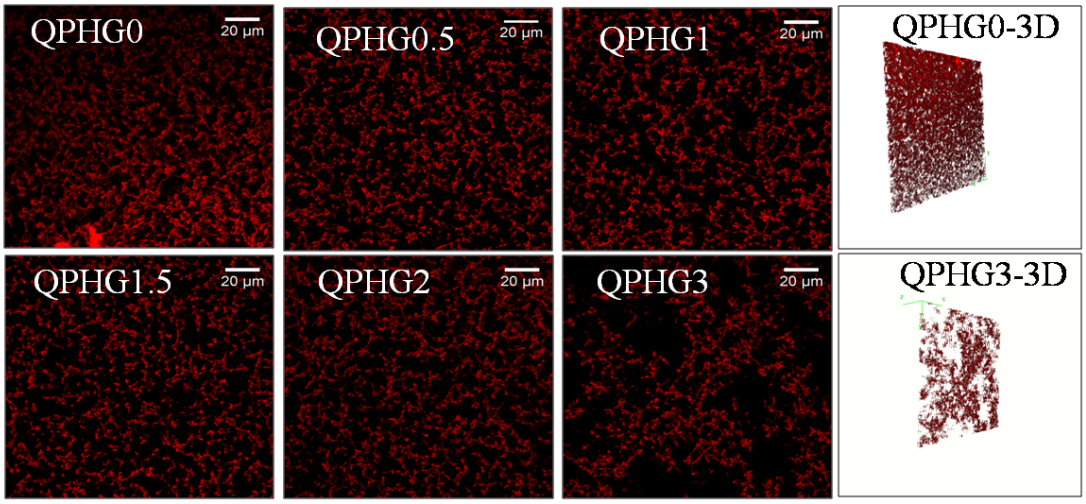
Sample	L*	a*	b*	ΔE
QPHG0	73.8±0.6^{a}	5.6±0.8^{a}	22±1^{a}	-
QPHG0.5	71.3±0.4^{b}	6.6±0.5^{a}	24.7±0.8^{ab}	3.6±0.8^{a}
QPHG1	72.1±0.5^{b}	6.4±0.1^{a}	24.8±0.1^{bc}	3.1±0.2^{ab}
QPHG1.5	71.7±0.8^{b}	6.7±0.8^{a}	26±1^{c}	4±1^{b}
QPHG2	71.0±0.4^{b}	6.8±0.5^{a}	25.7±0.7^{c}	4.6±0.9^{b}
QPHG3	70.4±0.7^{b}	6.7±0.5^{a}	26.7±0.7^{c}	6±1^{b}

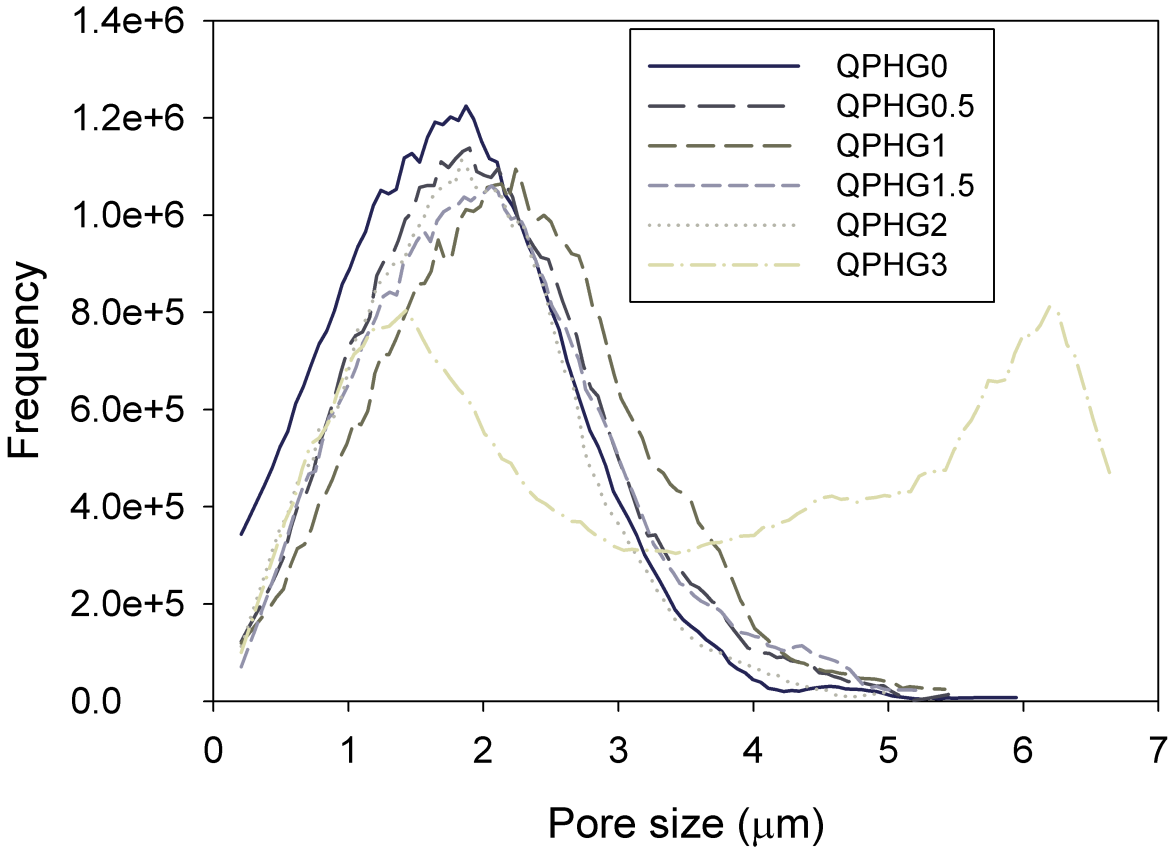
Mean values with different letters in a same column are significantly different ($p < 0.05$).

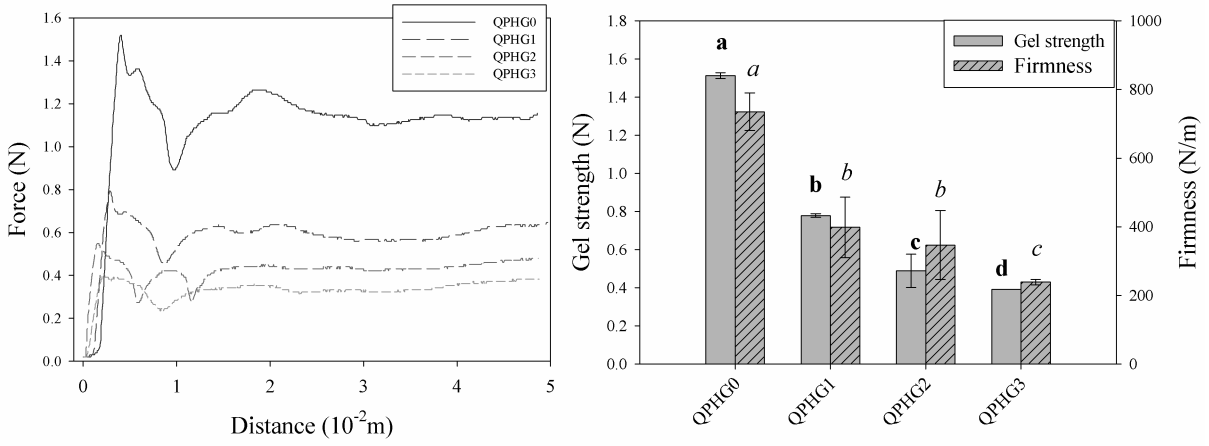

Molecular weight markers
QPH0
QPH0.5
QPH1
QPH1.5
QPH2
QPH3
66 KDa
45 KDa
36 KDa
29 KDa
24 KDa
20 KDa
14.2 KDa
6.5 KDa

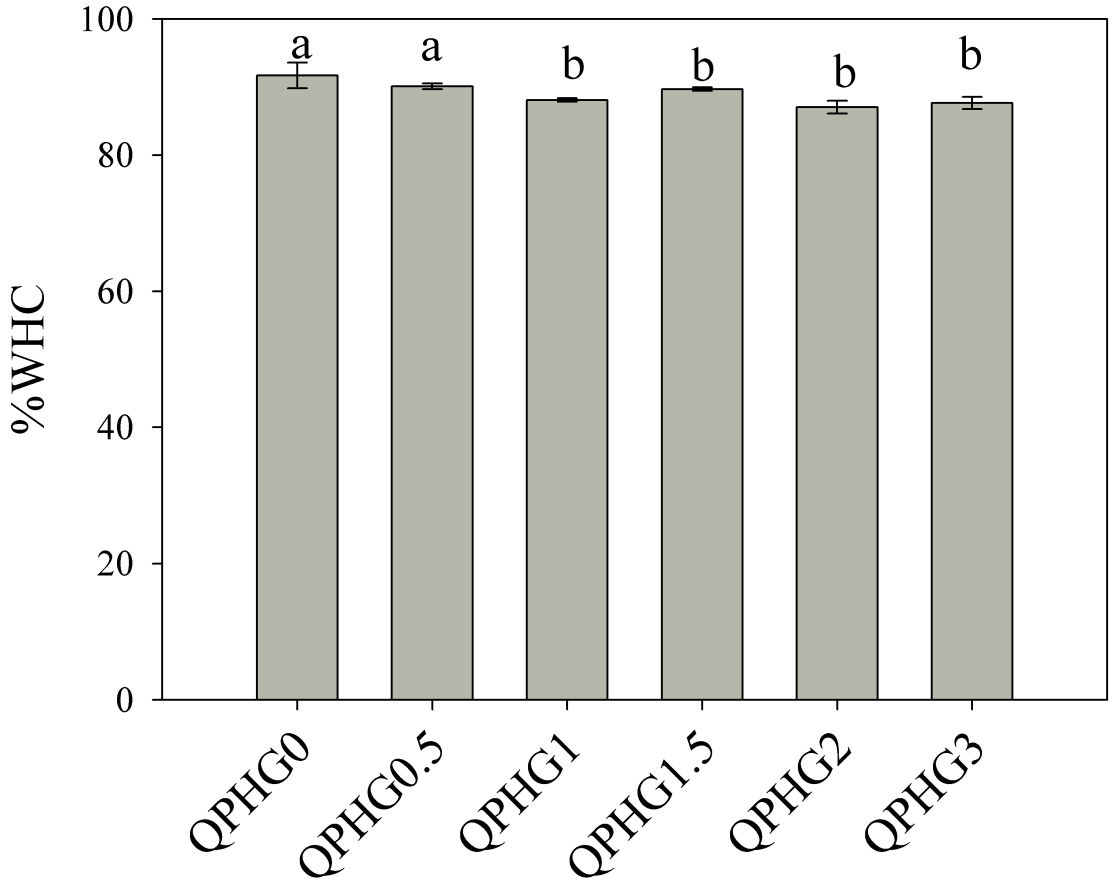

DPPH
ABTS
RSA
TEAC (μmol Trolox/mg protein)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
6
5
4
3
2
1
0
a
a
a
a
a
a
a
a
a
a
b
a
QPH0
QPH0.5
QPH1
QPH1.5
QPH2
QPH3






1.4e+6
1.2e+6
1.0e+6
8.0e+5
6.0e+5
4.0e+5
2.0e+5
0.0
Frequency
0
1
2
3
4
5
6
7
Pore size (μm)
QPHG0
QPHG0.5
QPHG1
QPHG1.5
QPHG2
QPHG3




100
80
60
40
20
0
%WHC
a
a
b
b
b
b
QPHG0
QPHG0.5
QPHG1
QPHG1.5
QPHG2
QPHG3

Highlights

- Quinoa protein hydrolysates were obtained using a fungal serin protease
- Quinoa protein gels were obtained from the hydrolysates
- Gels obtained from the hydrolysates presented a less interconnected protein network
- Hydrolysis enables us to obtain gels with differential characteristics and antioxidant capacities