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Original article

Effect of alkaline extraction on the structure of the protein of quinoa (*Chenopodium quinoa* Willd.) and its influence on film formation

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Summary It is important to produce hydrophobic edible protein films for use in foods. The aim of this study was to evaluate the effect of alkaline extraction of quinoa proteins (QP) on the structure and their film-forming ability without plasticiser. QP were extracted between pH 8 to 12, and their structure was evaluated by PAGE-SDS, size-exclusion HPLC light scattering, fluorescence spectroscopy and SH and SS. Film was characterised by FTIR, SEM, tensile strength, barrier and colour. Structural changes of QP showing that alkalinisation over pH 10 produce significant denaturation/aggregation/dissociation structural changes in QP. pH 12 was the condition to form a film ($film_{12}$). FTIR showed hydrogen bonds and hydrophobic film interactions. $Film_{12}$ had 16.6 ± 3.8% elongation and 15.7 ± 1.1 MPa tensile strength, and water vapour permeability was 5.18 ± 0.38 g mm m^{-2} day^{-1} kPa^{-1}. $Film_{12}$ had a brownish colour. A high degree of denaturation/aggregation/dissociation of QP structure is required to form a film without plasticiser.

Keywords Alkalinisation, edible films, mechanical barrier properties, quinoa protein, structural properties.

Introduction

An important factor that affects the structure and functional properties of proteins is pH. It changes a protein's charge and degree of denaturation, and it is possible to obtain unfolded proteins under the effect of pH, exposing functional groups like sulphydryl and hydrophobic groups and facilitating their association with water, chain-to-chain protein associations, and establishing new covalent bonds such as S-S (Damodaran & Kinsella, 1982; Mauri & Añón, 2008). Proteins are denatured at extreme pH values, and the degree of structural treatment of plant proteins at varying pH has been shown to be a function of protein yield (Arogundade *et al.*, 2004). Edible protein films have been formed from different sources, and they generally require some degree of unfolding of the film-forming protein solution prior to casting the films (Cao & Chang, 2001; Salgado *et al.*, 2010). The addition of plasticisers to the edible protein allows film formation and avoids brittle films (Gennadios *et al.*, 1994; Krochta, 2002). In general, vegetable protein films (e.g. soy, amaranth, lentil, faba proteins) are obtained using 30–60% plasticiser (McHugh *et al.*, 1994; Sothornvit & Krochta, 2001). The incorporation of plasticisers in protein films allows their formation, increasing water vapour permeability (WVP), producing edible films with poor barrier properties to water vapour (McHugh *et al.*, 1994; Sothornvit & Krochta, 2001; Cao *et al.*, 2009). In plant food preservation, edible films are important because they can help decrease respiration, reduce moisture loss and retard the ripening process. Proteins like soy and amaranth have good mechanical properties, but they are hydrophilic and are poor water vapour barriers. It is important to produce a more hydrophobic edible protein film that can be used to increase the shelf life of vegetable foods. Obtaining plant protein isolates includes their solubilisation in alkaline media and allows different structural conformations and functional properties of the proteins (Aluko & Yada, 1995). Because alkaline treatment of proteins causes denaturation, unfolding and exposure of reactive groups of the polypeptides, it could be thought that there is a range of alkaline pH values at which the quinoa protein structure will allow forming a film without the use of plasticisers. The aim

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of this study was to evaluate the effect of alkaline pH extraction of quinoa proteins on the structure of the proteins and on their film-forming ability without the use of plasticisers.

Materials and methods

Plant material

Quinoa flour (*Chenopodium quinoa* Willd) was supplied by 'Cooperativa Las Nieves', VI Region of Chile. The flour was stored at 4 °C until it was used.

Proximate composition of quinoa flour

Proximate analyses of quinoa flour were performed according to AOAC for moisture content (method 945.15), fat (method 945.16) and ash (method 920.153) methods (AOAC, 1996). Protein concentration was determined by Kjeldahl (method 945.18) (AOAC, 1996), and the percentage nitrogen was converted to crude protein multiplying it by a factor of 5.85. Total carbohydrate was estimated by difference.

Obtention of quinoa protein extracts

Flour was suspended in distilled water (25% w/v), and pH was adjusted to 8, 9, 10, 11 and 12 with 1 N NaOH. The suspensions were stirred for 60 min at room temperature and then centrifuged at 21 000 *g* for 30 min at 15 °C. The supernatants were labelled as PE-8, PE-9, PE-10, PE-11 and PE-12 according to the pH at which they were extracted. The soluble protein (SP) content of the PE was measured according to Bradford's method (Bradford, 1976). SP was expressed in mg of protein/mL of solvent. PEs were prepared and used immediately when required.

Structural analysis of protein extracts

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

Runs were carried out according to the method of Laemmli (1970). All electrophoresis analyses were performed on gel minislabs (Bio-Rad Mini Protean III Model; Bio-Rad, Hercules, CA, USA). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis gels contained 12% (w/v) acrylamide (5% acrylamide stacking gels). The molecular mass standard was from Fermentas (PageRuler™ Prestained Protein Ladder, Vilnius, Lithuania): 10, 17, 26, 34, 43, 55, 72, 95 and 130 kDa. The sample loading buffer contained 0.124 M Tris–HCl (pH 6.8), 15% (v/v) glycerol and 2% (w/v) SDS. For reducing conditions, 5% (v/v) of 2-ME was added, and the samples were heated (100 °C for 3 min). Protein bands were stained with Coomassie Brilliant Blue R.

Analysis of protein species using high-performance size-exclusion chromatography with a photodiode array detector coupled with an evaporative light scattering detector

Analysis of lyophilised PE was carried out on an Alliance® HPLC System (Waters Corp., 2695, Milford, MA, USA) with an Ultrahydrogel Linear, 7.8 mm × 300 mm column (Waters Corp., 1430) at room temperature (22 °C). UV absorbance was determined with a photodiode array system (Waters 990) consisting of a 512 diode UV/Visible detector and a stand-alone NEC personal computer. The mobile phase for the separation was 0.1 M CH_3COONH_4 adjusted to pH 8.0 with 3% NH_3 and flushed through the columns at a flow rate of 0.3 mL min^{-1}. All protein solutions in mobile phase (0.5% w/v) were filtered through a 0.45-μm membrane (Millipore, Durapore Sao Paulo, Brazil) prior to injection. The other coupled system was an ELSD system consisting of a VAREX ELSD II (Varex, Baitonsville, MD, USA) equipped with a HP 3396A integrator (Hewlett-Packard, Palo Alto, CA, USA). The LS conditions were nebuliser pressure 30 psi, drift temperature 55 °C and gain detector 20. PDA wavelength was set at 280 nm, and the chromatograms were processed using EMPOWER 2 software (Waters Corp., Milford, MA, USA). The ultrahydrogel linear column was calibrated with dextran blue (Vo) and with the following proteins of known molecular weight (kDa): amylase (200), alcohol dehydrogenase (150), bovine albumin (66), anhydrase (29) and cytochrome C (12.4). The calibration curve obtained from duplicate runs was determined from eqn 1:

$$\log MW = 6.014 - 0.3Ve \tag{1}$$

where Ve is the elution volume in mL; MW is the molecular weight.

Determination of free (SH_f), exposed (SH_e) and total (SH_t) sulphydryl groups and disulphide (SS) bonds

SH_f (without urea), SH_e (with urea) and SH_t (SH_e + reduced SS with 2-ME) groups were determined according to the procedure of Beveridge *et al.* (1974). SH_f 50 μL of PE was dissolved in 5 mL of 0.086 M Tris buffer, 0.09 M glycine and 0.004 M EDTA, pH 8.0 (GuHCl buffer). For SH_e, the same procedure was followed using GuHCl buffer and adding 8 M urea (urea-GuHCl buffer). For SH_t, 50 μL of PE was dissolved in 5 mL urea-GuHCl buffer, and 0.05 mL of 2-ME was added. The colour was developed with 40 μL of Ellman's reagent (4 mg of 5,5-dithio-bis(2-nitrobenzoic acid) mL^{-1} in methanol) added in 1-mL aliquots. Absorbance was measured at 412 nm on a Perkin Elmer Lambda 11 spectrophotometer. The extinction coefficient used to transform absorbance values into concentration values was 13 600 M^{-1} cm^{-1}. Calculations of SH and SS groups

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were performed as described by Beveridge *et al.* (1974), and SH groups were expressed as μM SH g^{-1} protein. The difference between $SH_t - SH_e$ was denoted as SS bonds. Protein concentration was determined according Bradford's method.

Fluorescence spectroscopy
Fluorescence measurements were performed on a PerkinElmer LS-50 B luminescence spectrometer at room temperature. The soluble protein fraction of PE was normalised at 0.2 mg of SP mL^{-1} of PE by Bradford's method and dispersed in pH 8 to pH 12 solutions. The excitation wavelength was 270–290 nm, and the emission spectra were recorded as the average of three spectra from 310 to 500 nm at a scan to speed of 30 nm min^{-1}.

Film preparation

Film-forming solutions were prepared from protein extracts (PE): PE-8, PE-9, PE-10, PE-11 and PE-12. With 25 mL of those extracts, were cast on a horizontal surface in plastic Petri dishes. The films were dried to constant weight at 50 °C. The dried films were removed from the plates and were conditioned at 23 °C and 60% RH for 48 h before testing.

Film properties

Fourier transform infrared spectroscopy
Full films (diameter 14 cm, 2.4 ± 0.1 g) were put on a horizontal attenuated reflectance accessory made of ZnSe to obtain the FTIR spectra on a Spectrum® 400 Fourier transform infrared spectrometer (Perkin Elmer, Model, Beaconsfield, UK). Fifty scans were recorded from 650 cm^{-1} to 4000 cm^{-1} at 1 cm^{-1} resolution on both faces of the film. Spectrum software (Perkin Elmer, version 10) was used for data acquisition.

Film microstructure
The microstructure characterisation of the films was determined by scanning electron microscopy (SEM) on an LEO Scanning Electron Microscope operating at 25 kV (SEM; LEO 1420 VP, Cambridge, UK). Prior to examination, the samples were mounted on cylindrical aluminium stubs cut like a straight chair, on which the film was fixed using double-sided tape, in such a way as to allow observation of the morphology of the cross section and the surfaces. The films were then gold-sputter-coated for 3 min at 20 kV in an argon atmosphere (PELCO 91000) to render them electrically conductive.

Colour
The colour of the film samples was determined with a colorimeter (Hunter Lab system, Model Miniscan 2.0/45, Reston, VA, USA) using the CIELab colour parameter: L^* (white = 100; black = 0), a^* (positive = red; negative = green) and b^* (positive = yellow; negative = blue) The films were cut into approximately 40-mm diameter discs that were placed at the bottom of a cylindrical container especially designed for the colorimeter.

Mechanical properties
The mechanical properties were evaluated in a universal tensile testing machine (Model LR5K, Lloyd Instruments, Fareham, UK) provided with a 5 kN load cell and controlled by the DAPMAT version 3.0 software. Five films were cut into 10 mm × 50 mm strips and were tested using a double clamp with a separation of 30 mm at a test speed of 20 mm min^{-1}. Stress–strain measurements were used to determine the tensile strength (TS max) and elongation at break (% E_{max}) values. Mechanical properties were determined by the Official Chilean Standard Method (NCh1151, 1999), equivalent to ISO R1184–1970 standard method.

Water vapour permeability
The WVP measurements were carried out according to the Official Chilean Standard Method (NCh2098, 2000), equivalent to the ASTM D1653–93 and DIN 52615 standard methods, using the wet cup method, at 23 °C and 60% RH. The weight of the cup was measured daily for 21 days. The WVP was estimated from eqn 2:

$$\mathrm{WVP} = \frac{\Delta m}{tA\Delta p} \times \varepsilon \qquad (2)$$

where WVP is the water vapour permeability in g mm m^{-2} day^{-1} kPa^{-1},
Δm is the mass change over time in g,
t is the time in days,
A is the film area in m^2,
ΔP is the partial vapour pressure difference of the atmosphere and pure water (112.353 kPa at 23 °C), and ε is the thickness in mm.

Statistical analysis

All experiments were performed at least in triplicate. Treatments were considered significantly different at $P < 0.05$ using the ANOVA procedure. The significant differences were detected using the Tukey's test.

Results and discussion

The chemical composition of quinoa flour was the following: moisture, fat and ash contents were 10.7 ± 0.2, 8.4 ± 0.1 and 2.5 ± 0.1 g per 100 g, respectively.

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Protein content was 14.4 ± 0.2 g per 100 g, and carbohydrate content was 66.1 g per 100 g by difference). These results are in agreement with the literature (Ogungbenle, 2003; Abugoch *et al.*, 2009).

Effect of alkalinisation on the extraction of quinoa protein

Protein solubility

Protein solubility (PS) increased with increasing pH, and it was highest for PE-12. PS was 7.5 ± 0.4 mg protein mL^{-1} at pH 8; 8.2 ± 0.2 mg protein mL^{-1} at pH 9; 9.1 ± 0.3 mg protein mL^{-1} at pH 10; 10.3 ± 0.3 mg protein mL^{-1} at pH 11; and 12.1 ± 0.3 mg protein mL^{-1} at pH 12. Proteins extracted at alkaline pH have a preponderance of negatively charged species due to ionisation of the carboxyl groups and deprotonation of the amine groups, a phenomenon that enhances protein–solvent interaction; the resultant electrostatic repulsion between the like-charged proteins keeps them apart and encourages their interaction with the solvent, thereby increasing the protein's solubility (Kinsella & Phillips, 1989; Lawal, 2004). Popović *et al.* (2011) also found for pumpkin protein that total soluble matter and soluble protein reached the highest value when the protein extract was prepared at pH 12.

Electrophoresis pattern (sodium dodecyl sulfate–polyacrylamide gel electrophoresis)

The SDS-PAGE profiles of PE at different pH are shown in Fig. S1, in the presence or absence of the reducing agent mercaptoethanol (2-ME). PE showed similar protein profiles; polypeptides without 2-ME were found ranging between 8.8 ± 0.8 and 72.0 ± 0.8 kDa. It was also found that the 11S protein type of quinoa at 55.0 ± 0.9 kDa (Fig. S1, denoted as Gl) corresponds to globulin (Brinegar & Goundan, 1993). This polypeptide around 55 kDa was linked by disulphide bonds, because PAGE-SDS with 2-ME showed an acidic subunit of 33.6 ± 0.7 kDa (AS Fig. S1) and a basic subunit of 21.8 ± 1.2 kDa (BS Fig. S1). Polypeptides of 9.2 ± 0.9 kDa similar to the 2S proteins were also found (Brinegar *et al.*, 1996). These results were similar to PAGE-SDS patterns of isolated quinoa protein extracted at pH 9 and 11 (Abugoch *et al.*, 2008). Between PAGE-SDS profiles at different pH, the same band intensity was seen from pH 8 to pH 11, but at pH 12, the bands were more diffuse and of lower intensity. This observation only shows that some changes can be produced under the effect of pH.

High-performance size-exclusion chromatography coupled with evaporative light scattering

Figure S2 shows the results of high-performance size-exclusion chromatography-evaporative light scattering (HPSEC-ELS) quinoa PE from pH 8 to pH 12. Similar profiles were seen for PE extracted from pH 8 to pH 10, and three species called F1 (≈ 131 kDa), F2 (≈56 kDa) and F3 (≈17 kDa) were found. F2 was the main molecular species found at pH 8, 9 and 10, at 87% (PE-10) and 94% (PE-8). The second one was F1, from 2% (PE-8) to 4% (PE-10), and the last one was F3, from 2 to 3%. When the pH was increased to 11 and 12, fractions of high molecular mass were observed (A1, A2; >1000 kDa). At pH 11, fraction A1 was 2% and A2 ≈ 3%. At pH 12, both fractions A1 (3.5%) and A2 (4.7%) were found. Fraction F3 was higher at pH 11 (11%) and 12 (25%) than at lower pH. F1 was also the main species at pH 11 (≈ 80%) and 12 (≈ 59%). The main species found in all the spectra was F2, corresponding to 11S globulins, the MM species found in SDS-PAGE without 2-ME were the same (Fig. S1, gl ≈ 55 kDa), and many authors have already described this globulin (Brinegar *et al.*, 1996; Abugoch *et al.*, 2008). F1 polypeptides correspond to soluble aggregates of globulin 11S (Castellani *et al.*, 1998), and F3 was the lowest MM species found, corresponding to albumins (F3). When the pH was increased to 11 and 12, fractions of high molecular mass were found (A1, A2 > 1000 kDa). The presence of soluble species A1 and A2 suggests that there are soluble polypeptide aggregates (Abugoch *et al.*, 2010) produced by extreme alkalinisation (pH 11 and 12). On the other hand, for the PE-11 and PE-12 species, it is possible to see a higher degree of molecular dissociation compared to the rest of the extracts (F3). The spectrum profiles of PE-12 and PE-11 were different from those of PE-8, PE-9 and PE-10, and that observation is showing the effect of pH on quinoa's polypeptide structure.

Sulphydryl and disulphide groups (SH and SS)

Cysteine content is important, not only for the nutritional aspect, but also because of the influence of these groups in establishing the protein structure, because it is responsible of forming SS bonds. Cysteine content in quinoa meal has been reported on the order of 75 μmol g^{-1} of protein (Abugoch *et al.*, 2009). Table S1 shows the free SH (SH_f), exposed SH (SH_e), disulphide (SS) and total SH (SH_t) groups of PE obtained between pH 8 and 12. It shows that SH_f increases from 2.4 ± 0.4 to 9.8 ± 0.4 μmol g^{-1} of protein, showing that some degree of protein unfolding is taking place under the effect of pH. When proteins are treated with urea, the protein structure unfolds and SH_e can be determined, with values ranging between 9.0 ± 1.4 and 19.2 ± 11.5 μmol g^{-1} of protein. SS groups also increased with alkali treatment, from 24.4 ± 3.3 to 44.3 ± 2.8 μmol g^{-1} of protein. These results showed an increase of SS formation and SH exposure, because cysteine is deprotonated under alkaline conditions, forming disulphide bonds

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and increasing SS values. Alkaline treatment generates greater exposure of SH and thiolate (S-) groups able to establish more intermolecular SS bonds. The disulphide bonds are easily reduced or oxidised; but sulphydryl–disulphide exchange can also occur under the effect of alkaline hydrolysis (Sian & Ishak, 1990). This increase of SS bonds with increasing pH can be a significant factor for the presence of aggregated polypeptides found in HPSEC-ELS (A1 and A2).

Fluorescence spectroscopy

Figure S3 shows the broad fluorescence emission spectra of the PE as a function of pH. Fluorescence analysis showed that when PEs are treated at extremely alkaline pH (11 and 12), there are substantial changes in the emission maxima and a significant decrease in the fluorescence intensity of PE-11 and PE-12. The emission maximum was $344^{a} \pm 1$ for PE-8; $347^{b} \pm 2$ for PE-9; $348^{b} \pm 2$ for PE-10; $354^{c} \pm 1$ nm for PE-11; and $357^{d} \pm 1$ nm for PE-12. Figure S3 shows that the spectrum obtained from pH 8 to 10 differed ($P < 0.05$) from those obtained at pH 11 and 12. At pH 11 and 12, fluorescence intensity was lower, and λ_{max} shifted to longer wavelengths compared to PE obtained between pH 8 to 10. Quenching of fluorescence intensity and a red shift in the maximum occurred as pH was increased. The effects were pronounced in the extreme alkaline zone (pH 11–12). The quenching of fluorescence intensity may be due to the exposure of tryptophan residues to the polar environment from the interior hydrophobic environment (Peterman & Laidler, 1979; Gorinstein *et al.*, 2001). The λ_{max} emission of tryptophan is sensitive to the pH of the medium, it varied between 334 and 355 nm for quinoa, amaranth and soy, and it is also closely related to the chromophore groups of proteins, such as tryptophan, exposed to the solvent (Sze *et al.*, 2007; Abugoch *et al.*, 2008, 2010). The red shift to higher emission maxima when the extraction pH was increased from 8–10 to 11 and 12 may be associated with a higher degree of protein denaturation (Sripad & Rao, 1987; Thomson & Ananthanarayanan, 2000). These results are in agreement with the observations found for PSGE-SDS, HPSEC-ELS and SH and SS. Alkalinisation affects quinoa protein structure significantly above pH 10; aggregated species (above 1000 kDa) and dissociated species (around 17 kDa) were found. Also, above pH 10, SH and SS increased, and these increases are probably associated with the structural changes in the proteins. Structural changes were also detected by the quenching of the fluorescence intensity and the red shift of its λ_{max}. The effect of high pH (>10) on quinoa protein extracts indicates that dissociation/denaturation/aggregation of the quinoa protein occurs. Those observations agree with other reports on plant proteins (Sripad & Rao, 1987; Thomson & Ananthanarayanan, 2000).

Effect of alkalinisation on film formation

The ability of protein extracts to form films by themselves (between pH 8 to 12) was investigated, and PE-12 was the only one that formed a film without the use of plasticisers. Film obtained from PE-12 ($film_{12}$) was self-supporting, peelable, flexible and easily handled. $Film_{12}$ formation can attributed to significant structural changes observed at pH 12 (aggregation, dissociation and denaturation), causing an unfolding of proteins due to denaturation and dissociation, allowing their reactive groups to interact with water. On the other hand, PE-12 had higher solubility than other alkaline extracts (up to 60%), which means greater negative charge on the protein molecular species present, more ionic groups exposed and greater ability to interact through hydrogen bonds with water. Water molecules acted as plasticisers, forming a film with PE-12 (Levine & Slade, 1999).

Film characterisation

Fourier transform infrared spectroscopy

Figure S4 shows the Fourier transform infrared spectroscopy (FTIR) spectrum of $film_{12}$. In the 3600–3000 cm^{-1} region, a strong band was seen at 3273 cm^{-1}, corresponding mainly to stretching vibrations of the O-H groups of adsorbed water molecules (Gorinstein *et al.*, 2005; Zhao *et al.*, 2008). Two important peaks corresponding to hydrophobic interactions are also seen at 2923 and 2854 cm^{-1} due to C–H stretching, as well as a doublet absorption with peaks attributed to the alkyl chain (CH_2) (Hsu *et al.*, 2005). The peak at 1744 cm^{-1} is related to interactions with water through C=O bonds. The peaks at 1632 and 1548 cm^{-1}, associated with –NH groups, agree with amide I and amide II bands, respectively. The same interactions were seen in films of soy protein isolates when the peptides interacted with plasticisers (Schmidt *et al.*, 2005).

Scanning electron micrographs

Micrographs of $film_{12}$ are shown in Fig. S5. The surface of $film_{12}$ had a dense and porous structure (Fig. S5a, b). This observation for protein microstructure was also reported for zein films (Lai & Padua, 1997), amaranth protein films (Colla *et al.*, 2006) and films prepared with soy protein (Denavi *et al.*, 2009).

Colour

The colour parameters of $film_{12}$ were L* 21.6 ± 2.7; a* 2.5 ± 0.3; and b* 7.0 ± 0.3. $Film_{12}$ was visually opaque, as indicated by the low lightness value and

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dark yellow to light brown in colour, related to a high yellowness value. Film prepared from aqueous extracts of soy protein at pH 12, and plasticisers had a colour similar to that of $film_{12}$ (Cao & Chang, 2001).

Mechanical properties

The thickness, tensile strength (TS) and per cent elongation at break (% E) of $film_{12}$ were 15.7 ± 1.1 MPa and 16.6 ± 3.8%, respectively. $Film_{12}$ was thicker (0.104 ± 0.010 mm) than other films based on proteins, which are generally presented in a range between 0.3 and 0.9 mm (Krochta, 1997; Rhim *et al*., 2000; Tapia-Blácido *et al*., 2005). $Film_{12}$ had high tensile strength but low elasticity compared to other films such as soy protein film (5 MPa, 86% E) (Brandenburg *et al*., 1993) and whey protein (3 MPa, 53% E) (Anker *et al*., 1999), because all the films prepared from proteins contain plasticisers that enhance their elongation properties at the expense of firmness (Sothornvit & Krochta, 2001; Cao *et al*., 2009). The high TS can also be due to the fact that the denaturing treatments of proteins promote the formation of intra- and intermolecular cross-links involving SS bonds.

Water vapour permeability

$Film_{12}$ had a WVP of 5.18 ± 0.38 g mm m^{-2} day^{-1} kPa^{-1}. This film showed better barrier properties compared to films made with other proteins (wheat gluten, corn zein protein, egg-white protein, peanut protein concentrate and soy protein isolate) (Krochta, 2002), and $film_{12}$ had WVP values several orders of magnitude lower than those of these films. The presence of hydrophilic plasticisers such as glycerol attracts additional moisture and impacts the film's WVP properties (McHugh *et al*., 1994).

Conclusions

By alkalinisation of quinoa protein between pH 8 and 12, it was possible to establish that extreme alkaline treatments above pH 10 produce significant structural changes in the protein, such as denaturation/aggregation/dissociation. These structural changes allowed defining the pH conditions under which it is possible to form a film with only water as plasticiser. PE-12 was the condition to form a film without the use of plasticisers. The structural properties of $film_{12}$ determined by FTIR showed that the main interactions were through hydrogen bonds and secondarily through hydrophobic interactions. These results confirm the existence of water/protein interactions and protein/protein interactions. $Films_{12}$ had lower WVP, which may be due to protein cross-linking by SS formation. On the other hand, the mechanical properties were better than those of soy proteins, but elongation was poor. The high TS may be due to the fact that the denaturing treatments of proteins promote the formation of intra- and intermolecular cross-linking, which involves SS bonds. Some degree of denaturation/aggregation/dissociation of the quinoa protein structure is required to form a film with water as plasticiser.

References

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

Abugoch, L., Castro, E., Tapia, C., Añón, M., Gajardo, P. & Villarroel, A. (2009). Stability of quinoa flour proteins (*Chenopodium quinoa* Willd.) during storage. *International Journal of Food Science and Technology*, **44**, 2013–2020.

Abugoch, L., Martínez, N. & Añón, M. (2010). Influence of pH on structure and function of amaranth *(Amaranthus hypochondriacus)* protein isolates. *Cereal Chemistry*, **87**, 448–453.

Aluko, R.E. & Yada, R.Y. (1995). Structure, function relationships of cowpea (Vigna unguiculata) globulin isolate. Influence of pH and NaCl on physicochemical and functional properties. *Food Chemistry*, **53**, 259–265.

Anker, M., Stading, M. & Hermansson, A. (1999). Effect of pH and the gel state on the mechanical properties, moisture contents, and glass transition temperatures of whey protein films. *Journal of Agricultural and Food Chemistry*, **47**, 1878–1886.

AOAC. (1996). *Official Methods of Analysis of AOAC International*, 16th edn. Gaithersburg, USA: AOAC International.

Arogundade, L., Akinfenwa, M. & Salawu, A. (2004). Effect of NaCl and its partial or complete replacement with KCl on some functional properties of defatted (*Colocynthis citrullus L.*) seed flour. *Food Chemistry*, **84**, 187–193.

Beveridge, T., Toma, S. & Nakai, S. (1974). Determination of SH- and SS- group in some food proteins using Ellmans reagent. *Journal of Food Science*, **39**, 49–51.

Bradford, M. (1976). Rapid and sensitive method for the quantitation of micrograms quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry*, **72**, 248–254.

Brandenburg, A., Weller, C. & Testin, R. (1993). Edible films and coatings from soy protein. *Journal of Food Science*, **58**, 1086–1089.

Brinegar, C. & Goundan, S. (1993). Isolation and characterization of chenopodin, the 11S seed storage protein of quinoa (*Chenopodium quinoa*). *Journal of Agricultural and Food Chemistry*, **41**, 182–185.

Brinegar, C., Sine, B. & Nwokocha, L. (1996). High-Cysteine 2S seed storage proteins from quinoa (*Chenopodium quinoa*). *Journal of Agricultural and Food Chemistry*, **44**, 1621–1623.

Cao, Y. & Chang, K. (2001). Edible films prepared from water extract of soybeans. *Journal of Food Science*, **67**, 1449–1454.

Cao, N., Yang, X. & Fu, Y. (2009). Effects of various plasticizers on mechanical and water vapor barrier properties of gelatin films. *Food Hydrocolloids*, **23**, 729–735.

Castellani, O., Martínez, E. & Añón, M. (1998). Structural modifications of an amaranth globulin induced by pH and NaCl. *Journal of Agricultural and Food Chemistry*, **46**, 4846–4853.

Colla, E., Sobral, P. & Menegalli, F. (2006). Amaranthus cruentus flour edible films: Influence of stearic acid addition, plasticizer concentration, and emulsion stirring speed on water vapor permeability and mechanical properties. *Journal of Agricultural and Food Chemistry*, **54**, 6645–6653.

Damodaran, S. & Kinsella, J. (1982). Effects of ions on protein conformation and functionality. In: *Food Protein Deterioration* (edited by J. Cherry) Pp. 301–357. Washington, DC: American Chemical Society.

Denavi, G., Tapia-Blácido, D., Añón, M., Sobral, P., Mauri, A. & Menegalli, F. (2009). Effects of drying conditions on some physical

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properties of soy protein films. *Journal of Food Engineering*, **90**, 341–349.

Gennadios, A., McHugh, T., Weller, C. & Krochta, J. (1994). Edible coatings and film based on proteins. In: *Edible Coatings and Films to Improve Food Quality* (edited by J.M. Krochta, E.A. Baldwin & M. Nisperos-Carriedo) Pp. 201–277 Lancaster, PA: Technomic Publishing Co., Inc.

Gorinstein, S., Delgado-Licon, E., Pawelzik, E., Permady, H., Weisz, M. & Trakhtenberg, S. (2001). Characterization of soluble amaranth and soybean proteins based on fluorescence, hydrophobicity, electrophoresis, amino acid analysis, circular dichroism, and differential scanning calorimetry measurements. *Journal of Agricultural and Food Chemistry*, **49**, 5595–5601.

Gorinstein, S., Drzewiecki, J., Delgado-Licon, E. *et al*. (2005). Relationship between dicotyledone-amaranth, quinoa, fagopyrum, soybean and monocots- sorghum and rice based on protein analyses and their use as substitution of each other. *European Food Research and Technology*, **221**, 69–77.

Hsu, B., Weng, Y., Liao, Y. & Chen, W. (2005). Structural investigation of edible zein films/coatings and directly determining their thickness by FT-Raman spectroscopy. *Journal of Agricultural and Food Chemistry*, **53**, 5089–5095.

Kinsella, J. & Phillips, L. (1989). Structure-function relationships in food proteins, film and foaming behaviour. In: *Food Proteins* (edited by J. Kinsella & W. Soucie). Pp. 52–77 Champaign, IL: Journal of the American Oil Chemists' Society.

Krochta, J. (1997). Edible protein films and coatings. In: *Food Proteins and Their Applications* (edited by S. Damodaran & A. Paraf). Pp. 529–549 New York: Marcel Dekker.

Krochta, J. (2002). Proteins as raw materials for films and coatings: definitions, current status, and opportunities. In: *Protein Based Films and Coatings* (edited by A. Gennadios). Pp. 1–41. Boca Raton, FL: CRC Press.

Laemmli, U. (1970). Cleavage of structural proteins during the assembly of head of bacteriophage T4. *Nature*, **227**, 860–865.

Lai, H. & Padua, G. (1997). Properties and microstructure of plasticized zein films. *Cereal Chemistry*, **74**, 771–775.

Lawal, O. (2004). Functionality of African locust bean (*Parkia biglobossa*) protein isolate: effects of pH, ionic strength and various protein concentrations. *Food Chemistry*, **86**, 345–355.

Levine, H. & Slade, L. (1999). Water science reviews III. In: *Water as a Plasticizer* (edited by E. Franks) Pp. 79–82. Great Bretain: Cambridge University Press.

Mauri, A. & Añón, M. (2008). Mechanical and physical properties of soy protein films with pH modified microstructures. *Food Science and Technology International*, **14**, 119–125.

McHugh, T., Aujard, J. & Krochta, J. (1994). Plasticized whey protein edible films: water vapor permeability properties. *Journal of Food Science*, **59**, 416–419.

NCh1151.Of76. (1999). Norma Chilena Oficial: Láminas y películas plásticas– Determinación de las propiedades de tracción (13 pp.).

NCh2098.Of2000. (2000). Norma Chilena Oficial: Películas de recubrimiento orgánico–Determinación de la transmisión de vapor de agua (13 pp.).

Ogungbenle, H. (2003). Nutritional evaluation and functional properties of quinoa (*Chenopodium quinoa*) flour. *International Journal of Food Science & Nutrition*, **54**, 153–158.

Peterman, B. & Laidler, K. (1979). The reactivity of tryptophan residues in proteins. Stopped-flow kinetics of fluorescence quenching. *Biochimica et Biophysica Acta*, **577**, 314–323.

Popović, S., Peričin, D., Vaštag, Ž., Popović, L. & Lazić, V. (2011). Evaluation of edible film-forming ability of pumpkin oil cake; effect of pH and temperature. *Food Hydrocolloids*, **25**, 470–476.

Rhim, J., Gennadios, A., Handa, A., Weller, C. & Hanna, M. (2000). Solubility, tensile, and color properties of modified soy protein isolate films. *Journal of Agricultural and Food Chemistry*, **48**, 4937–4941.

Salgado, P., Molina, S., Petruccelli, S. & Mauri, A. (2010). Biodegradable sunflower protein films naturally activated with antioxidant compounds. *Food Hydrocolloids*, **24**, 525–533.

Schmidt, V., Giacomelli, C. & Soldi, V. (2005). Thermal stability of films formed by soy protein isolate e sodium dodecyl sulfate. *Polymer Degradation and Stability*, **87**, 25–31.

Sian, N. & Ishak, S. (1990). Effect of pH on yield, chemical composition and boiling resistance of soybean protein-lipid film. *Cereal Foods World*, **35**, 748–752.

Sothornvit, R. & Krochta, J. (2001). Plasticizer effect on mechanical properties of beta-lactoglobulin films. *Journal of Food Engineering*, **50**, 149–155.

Sripad, G. & Rao, M. (1987). Effect of alkaline pH on sunflower 11S protein. *Journal of Biosciences*, **11**, 351–360.

Sze, C., Kshirsagar, H., Venkatachalam, M. & Sathe, S. (2007). A circular dichroism and fluorescence spectrometric assessment of effects of selected chemical denaturants on soybean (*Glycine max* L.) storage proteins glycinin (11S) and conglycinin (7S). *Journal of Agricultural and Food Chemistry*, **55**, 8745–8753.

Tapia-Blácido, D., Sobral, P. & Menegalli, F. (2005). Development and characterization of biofilms based on Amaranth flour (*Amaranthus caudatus*). *Journal of Food Engineering*, **67**, 215–223.

Thomson, C. & Ananthanarayanan, V. (2000). Structure-function studies on Hsp47: pH-dependent inhibition of collagen fibril formation in vitro. *Biochemistry Journal*, **349**, 877–883.

Zhao, X., Chen, F., Xue, W. & Lee, L. (2008). FTIR spectra studies on the secondary structures of 7S and 11S globulins from soybean proteins using AOT reverse micellar extraction. *Food Hydrocolloids*, **22**, 568–575.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. SDS-PAGE profiles of aqueous extract of quinoa protein (PE) treated at pH 8–12. No reducing conditions (left lanes);M is the protein standard; and with 2-ME (right lanes). Gl, indicate the band of globuline. AS corresponds to acidic subunit, and BS to basic subunit.

Figure S2. Size-exclusion chromatography coupled with evaporative light scattering of aqueous extract of quinoa protein (PE) treated between pH 8 to 12. A1, A2 correspond to protein aggregates, and F1, F2 and F3 indicate the protein fractions.

Figure S3. Fluorescence emission spectrum of aqueous extracts of quinoa protein (PE) treated at pH 8–12.

Figure S4. Fourier transform infrared spectroscopy spectra of quinoa protein film at pH 12.

Figure S5 Scanning electron micrographs (SEM) of surface quinoa protein film at pH 12. (a) 5009 (b) 20009 (c) 50009 and (d) 80009.

Table S1. Free (SHf), exposed (SHe), total (SHt) sulphydryl groups and disulphide bonds (SS) of aqueous extract of quinoa protein (PE) treated between pH 8–12.

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