Stability of quinoa flour proteins (*Chenopodium quinoa* Willd.) during storage

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Summary The amino acid composition and the physicochemical and functional properties of quinoa flour proteins (QFP) were evaluated during storage (at 20, 30 and 40 °C). Quinoa flour showed a protein content of 14.2 ± 0.1 g 100 g^{-1} and high levels of essential amino acids as lysine. SDS–PAGE of the QFP presented ten major band, and native-PAGE of the QFP showed similar banding; there was a little variation due to time-temperature. TCA-protein solubility variation (%) was small and the values of water activity were low, a non-significant endogenous hydrolysis was observed. Differential scanning calorimetry flour analysis allowed determining two endotherms, starch and protein. Important structural changes of protein soluble fractions were not detected by UV and fluorescence spectroscopy due to temperature and time of storage. It was found during storage time loss of protein solubility and water absorption. These changes could be to influence in the manufacture of quinoa flour based products. For avoid changes in these functional properties (solubility and water holding capacity), quinoa flour can be stored at ambient temperature (between 20 and 30 °C) and packed in double kraft paper bags (2 months).

Keywords Composition, functionality, protein stability, proteins, Quinoa flour.

Introduction

Quinoa (*Chenopodium quinoa* Willd.), a dicotyledonous indigenous plant in the Andean region, is considered an excellent pseudocereal for its nutritional characteristics; it is widely cultivated in Peru, Bolivia, Ecuador, Chile and Argentina (Bhargava *et al*., 2006). This plant has been investigated extensively because of its high protein content, 12–23% (Koziol, 1992; Ruales & Nair, 1992; Ando *et al*., 2002), and in particular its amino acid composition, which is close to the ideal protein balance recommended by FAO (FAO, 1973; Oshodi *et al*., 1999), even though the proportions vary depending on the location (Prakash & Pal, 1998). Due to its great food potential, quinoa is being introduced in many other countries (Comai *et al*., 2007) and it is considered a potential crop for National Academy of Sciences (NASA 1975). Because of its high protein content (Koziol, 1992), it can be used as an alternative protein resource for the development of blends for end products. Its protein fractionation shows the presence of albumin as well as a globulin called chenopodin (Brinegar & Goundan, 1993; Brinegar *et al*., 1996; Abugoch *et al*., 2008). Although quinoa seeds contain bitter tasting saponins, these can be removed either by washing the seeds in cold water or by mechanical dehulling (Reichert *et al*., 1986), because the saponin is concentrated in the outer seed layer, adhered to the pericarp covering two seed coat layers (Varriano-Marston & De-Francisco, 1984).

Even though amino acid composition and agronomical aspects have been studied extensively, only very limited information is available on quinoa flour stability and protein quality during storage, particularly on quinoa flour proteins, and this is very important for quality assurance in industrial use, where low storage temperatures are required (20–40 °C) (Becker & Hanners, 1990; Coulter & Lorenz, 1990). Quinoa is used to make flour, soup, cereal breakfast and beer. Quinoa flour is used with wheat flour or corn meal to make biscuits, bread and processed food such as spaghetti (Bhargava *et al*., 2006). Oshodi *et al*. (1999) reported on the good gelation, water-absorption, emulsifying properties of quinoa flour. Determining the appropriate functional properties related to protein structure and their changes during storage are of major importance for the use of quinoa flour in the food industry (Kinsella & Phillips, 1989; Oshodi & Ekperigin, 1989). The objective of this research was to determine some

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structural and functional properties of quinoa flour proteins and their stability during storage.

Materials and methods

Raw materials and flour preparation

Two 50-kg bags of three different batches of organic dehulled quinoa seeds were supplied by Mr Pablo Jara from Chile's VI Region. From these bags, 2-kg samples were obtained and the seeds were washed with cold water to remove possible saponin residues. The samples were oven-dried at 50 °C until the moisture content reached 15 ± 1 g 100 g^{-1} and were then ground in an impact mill (Retsh Muhle GmbH, Dreieich, West-Germany) to sixty mesh.

Proximate composition

Sample moisture (method 945.15), fat (method 945.16) and ash (method 920.153) were determined using AOAC methods (AOAC, 1996). Protein content was determined by Kjeldahl (AOAC, 1996) (method 945.18), using a factor of 5.85 (Becker *et al*., 1981; Segura-Nieto *et al*., 1994). Determinations were made in duplicate.

Storage of quinoa flour

Quinoa flour was packed and stored in double kraft paper bags at 20, 30 and 40 °C for periods of 1–5 months. While 20 ± 2 °C was reached in a box at room temperature, the 30 ± 1 °C and 40 ± 1 °C were obtained in ovens. Each batch was analysed separately in duplicate or triplicate.

Amino acid assay

Amino acids were separated by precolumn derivatisation with diethyl ethoxymethylene malonate followed by reversed-phase high-performance liquid chromatography (HPLC) with spectrophotometric detection at 280 nm according to Alaiz *et al*. (1992). The HPLC system consisted of a Merck-Hitachi L-6200A pump (Merck, Darmstadt, Germany) with a Rheodyne 7725i injector and a 20 μL sample loop, a Merck-Hitachi L-4250 UV–Vis detector, and a Merck-Hitachi D-2500 chroma-to-integrator. The derivatives were separated on a Nova-Pack C18 (300 × 3.9 mm i.d., 4 μm particle size; Waters, Milford, USA). Sample preparation and chromatographic conditions were according to Abugoch *et al*. (2008).

Total proteolytic endogenous activity and determination of TCA-protein solubility

Quinoa flour was dispersed at 1% (w/v) in 0.1 m phosphate buffer pH 5.92 and stirred in a bath at 37 °C for 90 min. Untreated samples were used as controls. The reaction was stopped by addition of 100 μL of 5% TCA, and the sample was centrifuged at 10 000 ***g*** for 15 min at 15 °C. Soluble peptides were determined in the supernatant according to Bradford (1976) and TCA-solubility (TCAS) was calculated as percent of total protein. The increase in solubility ($\%\Delta S_{TCA}$) was calculated with respect to the corresponding control using the following equation (Molina & Wagner, 2002):

$$\%\Delta S_{TCA} = 100[(S_{TCA})_t - (S_{TCA})t/(S_{TCA})_0]$$

where t stands for the hydrolysis time of 90 min.

Determinations were made in duplicate and the storage period was 4 months.

Water activity (a_w)

Water activity was measurement at 25 °C with a Novasina Thermoconstanter electric hygrometer according to Pollio *et al*. (1986). Determinations in each of the batches were made in duplicate and the storage period was 5 months.

Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) was ran on gel minislabs (Mini Protean III Model, Bio-Rad Laboratories, CA, USA). SDS–PAGE and non-denaturing PAGE (5%) were performed according to Laemmli (1970). SDS–PAGE gels contained 12% (w/v) acrylamide (5% acrylamide stacking gels) and native gels contained 6% (w/v) acrylamide (5% acrylamide stacking gels). The molecular mass of the polypeptides were calculated using the following protein standards (Bio-Rad Kaleidoscope): myosin (202 kDa), β-galactosidase (133 kDa), bovine serum albumin (70 kDa), carbonic anhydrase (30.6 kDa), trypsin inhibitor (20.1 kDa), aprotin (6.9 kDa). To 20 mg of sample was added 200 μL of sample loading buffer (native or denaturant). The sample loading buffer contained 0.124 M Tris–HCl (pH 6.8), 15% (v/v) glycerol, and (for SDS–PAGE only) 2% (w/v) SDS. For reducing conditions, 5% (v/v) β-mercaptoethanol (2-ME) was added, and the samples were heated (100 °C, 1 min). Protein bands were stained with Coomassie Brilliant Blue R according to Weber & Osborn (1969).

Fluorescence spectroscopy

Quinoa flour was dispersed in 0.08 M phosphate buffer pH 8.5, the dispersions were stirred gently for 1 h at room temperature, centrifuged at 8500 ***g*** for 30 min at 15 °C, and the concentration of the soluble fraction of protein was normalised at 0.2 mg mL^{-1} by the Warburg

method (Warburg & Christian, 1941). Fluorescence spectra were recorded on an LS 50 B Perkin-Elmer luminescence spectrometer at room temperature with an excitation wavelength of 290 nm. The emission spectra were recorded as the average of three spectra from 310 to 500 nm using a scan speed of 30 m min^{-1}. Determinations were made in duplicate and the storage period of the sample was in the range of 0–4 months.

UV spectra

Soluble proteins (0.02 mg mL^{-1}) were prepared as described above. UV spectra of the samples were measured with a UNICAM Type UV3-200 (Cambridge, UK) UV–Vis spectrometer at room temperature in the 250–350 nm wavelength range. Determinations were made in duplicate and the storage period analysed was 5 months.

Differential scanning calorimetry

Measurements were made in a Mettler Toledo 822 calorimeter with Mettler Toledo Star System software (Schwerzenbach, Switzerland). Suspensions of quinoa flour (20% w/w) were prepared in 0.08 M phosphate buffer, pH 8.5. Differential scanning calorimetry (DSC) samples consisted of hermetically sealed aluminium pans filled with 12–14 mg of quinoa flour suspensions. Analyses were ran at a rate of 10 °C min^{-1} from 300 K (27 °C) to 393 K (120 °C), and a double empty pan was used as reference. After each run, the dry matter content was determined by puncturing the pans and exposing them overnight at 107 °C. The denaturation parameters calculated were temperature (T_d) and transition enthalpy (ΔH). Determinations were made in duplicate at zero time and at month five of storage.

Solubility

Quinoa flour was dispersed in 0.08 M phosphate buffer, pH 8.5, at 1% w/v, the dispersions were gently stirred for 1 h at room temperature and centrifuged at 8500 ***g*** for 30 min at 15 °C. Soluble proteins (S_p) in the supernatant were determined by the Bradford (1976) method and solubility (S) was expressed as percentage as follows:

$$\%S = (0.142 \times 100 \times \text{mg}S_p)/\text{mg total protein}$$

Determinations were made in triplicate and the storage period analysed was 5 month.

Water holding capacity

The flour was dispersed in distilled water (1% w/v) with a vortex mixer and was then stirred every 10 min for 30 min at 25 °C. After the mixture was thoroughly wetted, the samples were centrifuged (8500 ***g*** for 30 min). The amount of supernatant in the test tube was recorded and soluble proteins were determined by Bradford method (1976). The water holding capacity (WHC) (grams of water per gram of sample) was calculated as

$$\text{WHC} = [m2 - (m1 - m3)]/m1d$$

where m_1 is the weight of dry sample (g); m_2 is the weight of sediment (g); m_3 is the weight of soluble protein from the supernatant (g), and d is the density of water (mL g^{-1}). In this case triplicate samples were analysed for each sample and the storage period analysed was 4 months.

Kinetic analyses

Changes in 'C' (solubility, WHC, a_w) under isothermal conditions can be represented by:

$$dC/dt = -k(C)^n$$

where k is the rate constant, C is the quantitative indicator at time t, and n is the of reaction order. The integrated form for the zero order kinetic model is

$$Ct = C_0 - k \cdot t$$

where C_0 stands for the initial value at zero time, C_t is the value at time t, and k is the rate constant.

The Arrhenius equation is often used to describe the temperature dependence of reaction rates:

$$k = k_0 E^{-\text{Ea}/RT\text{abs}}$$

A semi-logarithmic plot of the rate constant as a function of reciprocal absolute temperature ($1/T_{abs}$) should give a straight line, and the activation energy can be determined as the slope of the line multiplied by the gas constant R (Kong *et al.*, 2007).

Statistical analysis

The data were analysed by multifactor ANOVA and the significance of differences by the tukey multiple-range test (Statgraphics Plus 5, Rockville, MD, USA). A P value of 0.05 was used to determine significance. The influence of independent variables (time and temperature) over dependent variables was evaluated.

Results and discussion

Proximate and amino acid composition of raw quinoa flour

The results presented in this section correspond to the starting quinoa flour. The moisture content was 11.8 g 100 g^{-1}. The flour had a carbohydrate content (66.1 g 100 g^{-1} by difference) comparable to that of amaranth (Bressani, 1994) and low ash (1.5 ±

Table 1 Essential amino acid pattern and score for all age groups except infants*

Amino acid	Amino acids (mg g^{-1} protein)	Amino acid score
Phenylalanine + Tyrosine	394	0.8
Histidine	119	1.4
Isoleucine	175	1.2
Leucine	413	0.9
Lysine	363	0.9
Methionine + cysteine	156	1.1
Threonine	213	1.4
Tryptophan	n.d.	n.d.
Valine	219	1.3

*FAO/WHO/UNU, 1985, Conversion Factor N: 5.85.

0 g 100 g^{-1}). Protein content was 14.2 g 100 g^{-1} and fat content 6.4 g 100 g^{-1}. Protein content was higher than that of cereals like millet (Chauhan *et al*., 1992; Oshodi *et al*., 1999), oat, and barley (Comai *et al*., 2007). The essential amino acid profile (mg g^{-1} of protein) of quinoa flour is given in Table 1 along with the human needs for all age groups except infants. Compared to common cereals, quinoa is higher in protein, lysine, fat and fiber, and protein quality is high. The protein score reflects its amino acid content compared an ideal protein (FAO/OMS, 1973). The chemical scores of methionine + cystine, isoleucine, histidine, threonine, phenylalanine + tyrosine leucine, lysine and valine were higher or almost equivalent to the FAO/WHO requirement pattern in adults (FAO/OMS, 1973). Amino acid analysis showed that quinoa is an excellent source of lysine, methionine, cystine in addition to other essential amino acids, and it meets or exceeds the recommendations for proper amino acid nutrition, in close agreement with previous observations made by Ruales & Nair (1992) and Koziol (1992).

Electrophoretic polypeptide profile

The extracted proteins were analysed by native-PAGE (Fig. 1) and showed a similar and predominant band (marked with an arrow in the figure) at different storage times and temperatures, implying no variation at the molecular level. The profile of extracted proteins (Fig. 1) shows globulins, a result comparable to amaranth globulins (Martínez & Añón, 1996).

Total extractable protein was also analysed by SDS–PAGE, (Fig. 2) providing different polypeptide groups ranging from 8 to 84 kDa, as shown in Fig. 3 by the right-hand arrows. The 8–11 kDa polypeptides group was similar in size to the 2S type of proteins in quinoa seeds (Brinegar *et al*., 1996). The 11S protein type of quinoa seed (Fig. 3) is characterised by two heterogeneous sets of polypeptides, showing size ranges of 30–40 kDa (acidic subunits) and 20–25 kDa (basic subunits) (Fig. 3, right-hand arrows) linked by disulfide bonds (Brinegar & Goundan, 1993). The polypeptide band intensity decreases slightly at various times and temperatures (Figs 2 and 3). Although temperature seems to have more influence than time on storage, it was found that time and temperature may affect the proteins, since the decreasing band intensity found can be attributed to partial denaturation of quinoa proteins due to storage conditions. There appear to be much greater differences between temperatures than between different times at a given temperature.

TCA-protein solubility – endogenous hydrolysis

TCA-solubility was studied to follow peptide liberation by endogenous hydrolysis (Fig. 4). By the first month, endogenous hydrolysis, determined by the TCAS value, increased at all storage temperatures ($P > 0.05$) (Fig. 4). Such values were found to be low compared to those obtained by Molina & Wagner (2002). The results indicated that the effect of the endogenous hydrolytic enzymes was not significant under the storage conditions.

Fluorescence spectra

Tryptophan residues are the most sensitive markers of the protein environment (Chen & Barkley, 1998), since the fluorescence properties of tryptophan residues in

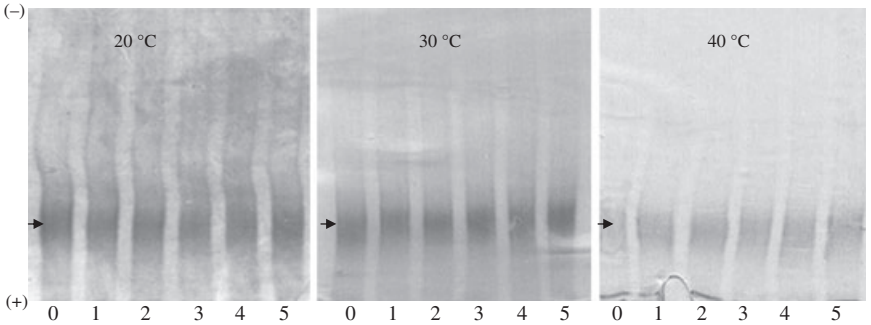

Figure 1 Electrophoretic analysis (Native-PAGE) of total extractable quinoa flour protein. Stored at different temperatures from 0 to 5 months.

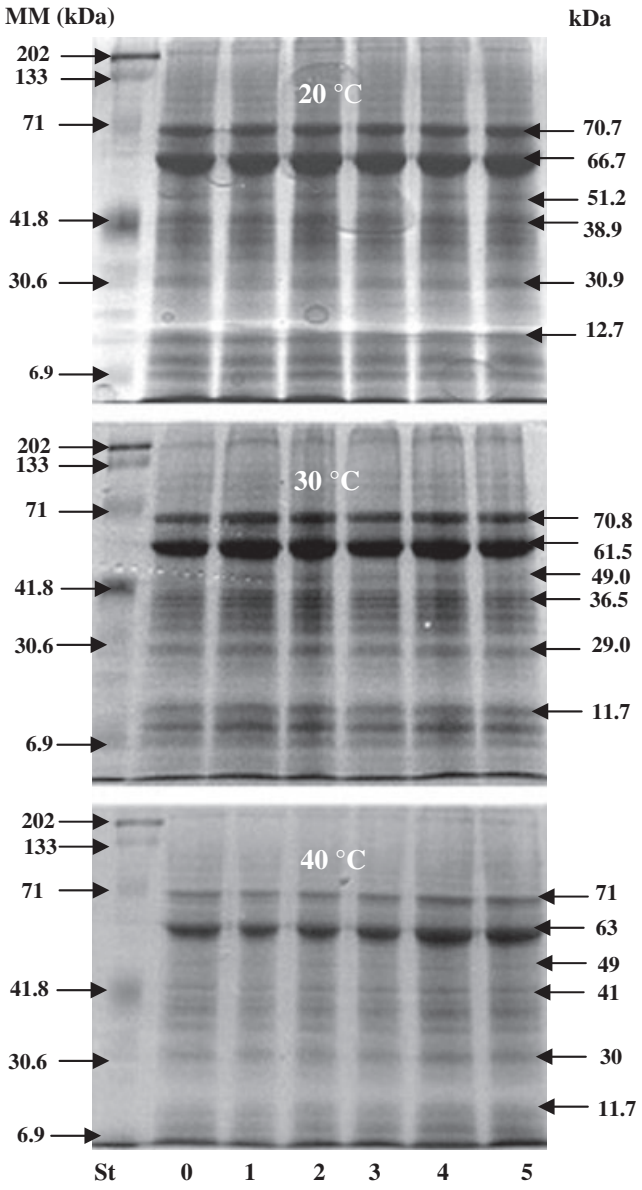

Figure 2 Electrophoretic analysis (12% SDS–PAGE) of total extractable quinoa flour protein. Stored at different temperatures from 0 to 5 months. Lane St, molecular mass markers.

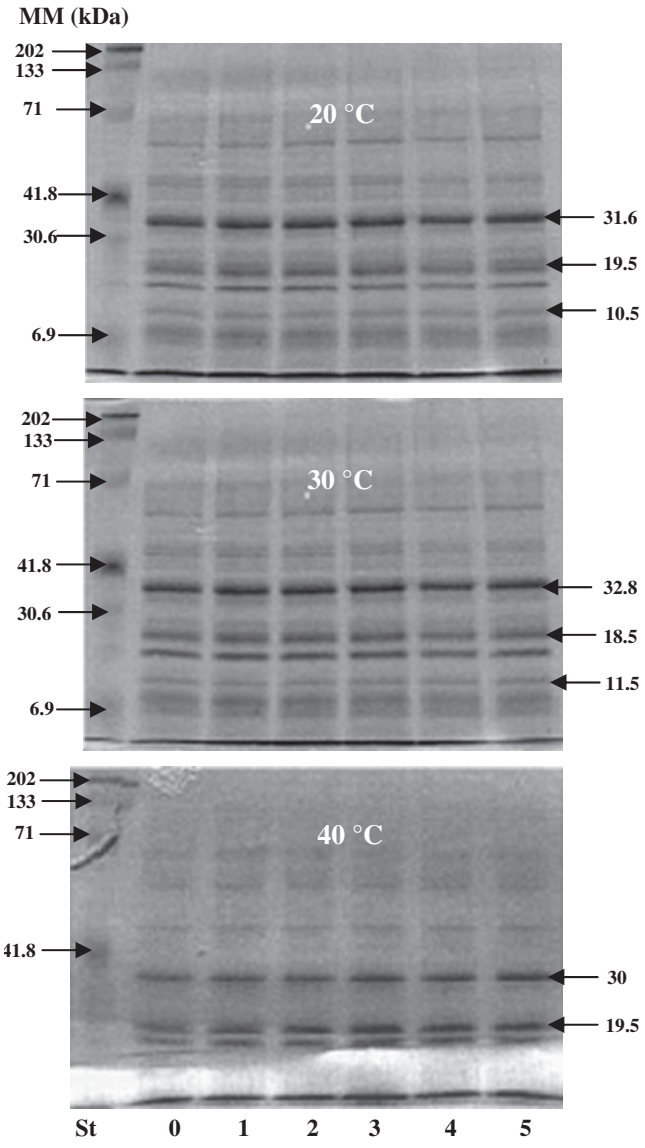

Figure 3 Electrophoretic analysis (12% SDS–PAGE with 2-ME) of total extractable quinoa flour protein. Stored at different temperatures from 0 to 5 months. Lane St, molecular mass markers.

proteins change on denaturation of the protein (Royer, 1995). Quinoa flour has shown a proteic tryptophan value of 187 mg 100 g^{-1} dry weight (Comai *et al.*, 2007). In the present study, fluorescence intensity was plotted as a function of storage time and temperature; Gaussian curves were obtained in all cases according to Abugoch *et al.* (2008). Only data corresponding to fluorescence spectra at 20 °C (Fig. 5a) are provide, since similar curves were obtained at other temperatures. Fluorescence measurements showed a decreasing fluorescence intensity (FI) at the end of the storage time (Fig. 5a),. The quenching of fluorescence intensity may be caused by the exposure of tryptophan residues to the environment from the internal hydrophobic environment, suggesting a denaturation (Sripad & Narasinga Rao, 1987).

UV spectra

The absorption spectra of the supernatants were obtained at different time-temperature conditions. Only

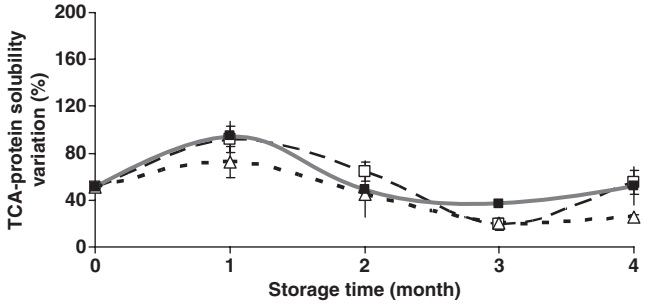

Figure 4 Effect of storage time and temperature (□, 20 °C; Δ, 30 °C and ■, 40 °C) on endogenous proteolytic activity of quinoa flour. *Means value of three determinations. Standard error is indicated by bars.

data corresponding to UV spectra at 20 °C (Fig. 5b) are provided, since similar curves were obtained at other temperatures. These spectra showed a decrease in absorbance with storage time, which agrees with a solubility decrease, but with no change in the curve shapes. These spectra were similar to those for amaranth protein isolate (Avanza & Añón, 2006).

Water activity (a_w)

Water activity has particular relevance in food chemistry and preservation, due to its relationship with enzymatic activity (Blandamer *et al.*, 2005). Control of water activity in quinoa flour did not show significant differences with storage temperature ($P > 0.05$), but with storage time ($P < 0.05$) aw was about 0.488 ± 0.005 and its value decreased to 0.261 ± 0.083 ($P < 0.05$) at 40 °C. These values show water migration from quinoa flour to its surrounding, which can be explained by the low endogenous hydrolysis development found. Furthermore, this low a_w prevents microbial growth.

DSC measurements of quinoa flour

By DSC, two temperature and enthalpy peaks were obtained, related to gelatinization temperature, T_g = 65.7 °C (for starch), and the other denaturalization temperature, T_d = 98.9 °C (for proteins). The enthalpy value obtained was 5.9 J g^{-1} (starch) and 2.2 J g^{-1} (proteins). The high denaturation temperature of quinoa flour proteins was found comparable to globulins and to most cereal and legume proteins such as field pea and faba bean, and oat and soy proteins (Arntfield & Murray, 1981; Abugoch *et al.*, 2003). On the other hand, the lower gelatinisation temperature, was similar to that of corn starch (Li *et al.*, 2007). The denaturation temperature of quinoa proteins suffered significant changes at month five of storage under all temperature conditions. The final (after month 5) gelatinisation temperature was 63.7 °C and the enthalpy was 1.1 J g^{-1}. The denaturation value at month 5 agrees with the fluorescence spectra results.

Water holding capacity

Water holding capacity (WHC) is an important physical characteristic affecting the quality of manufactured foods. WHC of quinoa meal was 4.5 mL of water g^{-1} of flour (Table 2) and this property showed an important decrease during storage ($P < 0.05$) (Table 2). For all the temperatures tested, after 4 months of storage WHC decreased at least 45% with respect to the WHC of the starting material. The WHC of quinoa meal was higher than the values reported for quinoa meal (Ogungbenle, 2003), amaranth meal (Mahajan & Dua, 2002), sunflower flour and protein concentrates (Venktesh & Prakash, 1993), and were similar to soy protein isolate (Tang *et al.*, 2006). Only for this property an Arrhenius equation dependency was found. Data were normalised to zero order kinetics for WHC versus temperature; an activation energy (E_a) of 3961.4 cal mol^{-1} was obtained.

Protein solubility

Protein solubility (Fig. 6) of quinoa flour was about 14% at time zero, a value similar to that obtained by Ogungbenle (2003) and lower than that for amaranth meal (Mahajan & Dua, 2002). A significant decrease of solubility ($P < 0.05$) was found during storage time

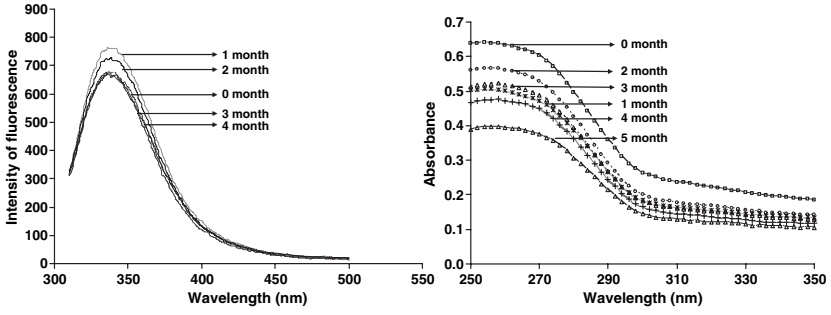

Figure 5 (a) Fluorescence emission spectra of protein soluble fraction of quinoa flour (2 mg mL^{-1}) in 0.08 M phosphate buffer pH 8.5 stored at 20 °C. An excitation wavelength of 290 nm was used. (b) UV spectrum from soluble protein fraction of quinoa flour (2 mg mL^{-1}) in 0.08 M phosphate buffer pH 8.5 stored at 20 °C.

Table 2 Effect of storage time and temperature on water holding capacity (mL of water g^{-1} of flour)

	Temperature (ºC)		
Time (months)	**20**	**30**	**40**
0	4.5 ± 0.2^{a}	4.5 ± 0.2^{a}	4.5 ± 0.2^{a}
1	3.5 ± 0.3^{b}	$4.1 \pm 0.2^{a,b}$	$3.9 \pm 0.8^{a,b}$
2	3.4 ± 0.2^{b}	$3.7 \pm 0.3^{b,c}$	3.1 ± 0.8^{b}
3	2.8 ± 0.5^{c}	$3.1 \pm 0.5^{c,d}$	3.8 ± 0.8^{b}
4	2.3 ± 0.2^{c}	2.7 ± 0.3^{d}	2.9 ± 0^{c}

*Values in the same column followed by different superscripts are statistically different ($P < 0.05$).
Standard errors are indicated.

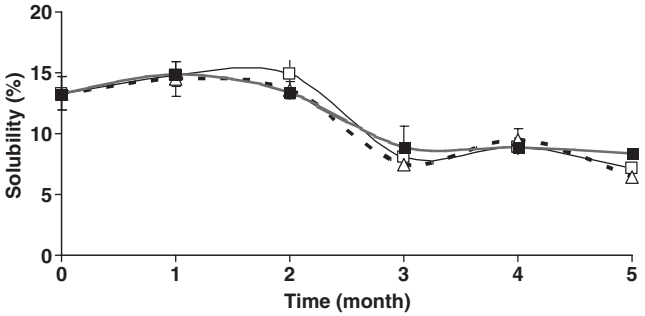

Figure 6 Effect of storage time and temperature (□, 20 °C; Δ, 30 °C and ■, 40 °C) on protein solubility. * Mean value of three determinations. Standard error is indicated by bars.

(Fig. 6). For this parameter, the effect of storage temperature was not significant ($P > 0.05$).

Conclusions

Quinoa flour is an excellent contribution to the diet according to its protein content and amino acid composition. Loss of protein solubility and water absorption occurred during storage. These changes must influence the manufacture of products based on quinoa flour. To avoid changes in these functional properties (solubility and WHC), quinoa flour can be stored at ambient temperature (between 20 and 30 °C) and packed in double kraft paper bags (2 months). Under those conditions its technological properties remain without significant changes and can guarantee the quality of the flour for manufacturing. On the other hand, quinoa flour has potential use in processed food, such as in drink formulations and soups due to good hydration properties.

Acknowledgment

This work was supported by the FIA SUB-ES-C-2004-1-A-015.

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