

## Immunochemical and Molecular Properties of Proteins in *Chenopodium quinoa*

C. Berti,<sup>1</sup> C. Ballabio,<sup>2</sup> P. Restani,<sup>2</sup> M. Porrini,<sup>1</sup> F. Bonomi,<sup>3</sup> and S. Iametti<sup>3,4</sup>

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Quinoa (*Chenopodium quinoa* Willd) is an indigenous crop grown in the Andean regions and is used either boiled as the whole grain or ground into flour. Quinoa is nutritionally interesting because it contains high levels of lysine-rich proteins (Coulter and Lorenz 1990), polyunsaturated fatty acids, micronutrients, and vitamins C and E (Chauhan et al 1992; Ranhotra et al 1993; Gutzmán-Maldonado and Paredes-Lopez 1999). Mixtures of cereal grains and quinoa were processed to prepare foods with enhanced nutritional value (Coulter and Lorenz 1990). Bread or cakes made from a blend of 5–10% quinoa flour and wheat flour were reported as being of good quality (Lorenz and Coulter 1991). Also, blends of corn grits and quinoa have been extruded successfully (Coulter 1989). However, there is a lack of information as to those biochemical properties of quinoa proteins that may be relevant to processing this material into foods without using it in mixtures with gluten-containing cereals.

Quinoa is evolutionarily very distant from those cereals that contain gluten fractions toxic for celiacs (*Graminaceae*). In quinoa flours, very low amounts of protein are found in the prolamin fraction (0.8%), whereas albumins (31%) and globulins (37%) are predominant (Fairbanks et al 1990; Prakash and Pal 1998). However, there is no general consensus about quinoa being suitable for the production of gluten-free foods. For these reasons, we assessed the content of gliadin-like proteins in quinoa flours by immunochemical approaches, and we also investigated some biochemical properties of quinoa proteins that may represent useful indicators for processing purposes.

### MATERIALS AND METHODS

#### Sample Preparation

Quinoa grains (*Chenopodium quinoa* Willd) were supplied by the Asociación Nacional de Productores de Quinoa (Anapqui, Bolivia). The seeds were washed vigorously in cold, running water to remove bitter saponins, oven-dried overnight, and ground in a coffee mill. The resulting flour was sieved through a 60-mesh sieve.

#### SDS-PAGE and Immunoblotting

Quinoa proteins were fractionated into albumins, globulins, prolamins, and alkali-soluble proteins by sequential flour extraction (Prakash and Pal 1998). The protein profile of each extract was analyzed by SDS-PAGE under reducing conditions either on a fixed porosity 12% gel (Berti et al 2002) or a 9–19% gradient gel (Restani et al 1995). Immunoblotting was performed on the gradient gels as described by Restani et al (1998). Polyvinylidene difluoride

(PVDF) membranes were incubated overnight in 10 mL of a 0.25% gelatin solution, to which either 20  $\mu$ L of serum from celiac subject or 10  $\mu$ L of a rabbit antigliadin polyclonal antibody (Sigma, St. Louis, MO) were added. Alkaline phosphatase-labeled goat anti-human IgA or antihuman IgG, and murine antirabbit IgG antibodies (Sigma) were used as secondary antibodies and diluted 1:1000 (v/v) in 0.25% gelatin. Stained gels and membranes were analyzed with Image Master 1D software from Bio-Rad (Hercules, CA).

#### Determination of Gliadins by ELISA

Gliadin content was measured by a Transia Plate Gluten ELISA kit, (Diffchamb Italia, San Giuliano Milanese, Italy). The assay was performed according to the kit instructions. Quinoa flour (1 g) was suspended in 10 mL of 40% (v/v) aqueous ethanol. The clear supernatant obtained by centrifugating the suspension at 10,000  $\times$  g for 30 min at 20°C was used for serial dilution. Plates were read in a computerized 3550 microplate reader (Bio-Rad). Two different ELISA kits were used for duplicate analysis, so that each gluten content figure represents the average of quadruplicate determinations.

#### Aggregation State and Thiol Content of Quinoa Proteins

Proteins were extracted by suspending 500 mg of quinoa flour in 10 mL of 50 mM phosphate buffer, 0.1M NaCl, pH 7.0, at 25°C for 30 min. Where indicated, 8M urea and 10 mM dithiothreitol (DTT) were added to the buffer. The amount of soluble protein was determined according to Bradford (1976). Accessible -SH groups were measured directly on suspensions of flour in 50 mM phosphate buffer, pH 7.0, containing 0.2 mM 5,5'-dithiobis-(2-nitrobenzoate) (DTNB) (Ellman 1959) and 8M urea where indicated.

### RESULTS AND DISCUSSION

#### Properties of Quinoa Relevant to Use in Gluten-Free Products

The immunochemical reactivity of the protein fractions solubilized by specific solvents (Prakash and Pal 1998) was verified by immunoblotting. The electrophoretic patterns of quinoa fractions extracted by various solvents are shown in Fig. 1, which also includes protein patterns for a gluten-free flour and for commercial gluten. The immunoreactivity of the quinoa proteins extracted by the various solvent systems was very low with either commercial antigliadin antibody (Fig. 1B) or serum of a celiac subject (Fig. 2), and was comparable to that of proteins in gluten-free flour. The only weak positive band detected by human serum antibodies (either IgA or IgG) could be ascribed to cross-reactivity toward a protein that was present also in gluten-free flours.

TABLE I  
Solubility of Quinoa Proteins and Accessible Thiol Content  
in Quinoa Flours

Additions to 50 mM Phosphate pH 7.0	Soluble Proteins (mg/g of original flour)	Accessible SH (nmol/mg of original flour)	Accessible SH (nmol/mg of protein)
None	49 $\pm$ 1	2.88 $\pm$ 0.2	59
8M urea	98 $\pm$ 3	3.99 $\pm$ 0.3	41
8M urea, 10 mM DTT	100 $\pm$ 2	na	na

<sup>1</sup> Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, Milan, Italy.

<sup>2</sup> Dipartimento di Scienze Farmacologiche, Università degli Studi di Milano, Milan, Italy.

<sup>3</sup> Dipartimento di Scienze Molecolari Agroalimentari and Centro Studi Celiachia, Università degli Studi di Milano, Milan, Italy.

<sup>4</sup> Corresponding author. Phone: (+39)02-5031-6819. Fax: (+39)02-5031-6801. E-mail: stefania.iametti@unimi.it

The possibility of accidental contamination of gluten-free pseudo-cereals with gluten proteins either during storage or transport or in the milling process impairs the use of pseudo-cereals as an alternative to gluten-containing cereals in gluten-free diets. Accidental contamination with gluten proteins is a common problem when oats or buckwheat are milled or processed in commercial plants (Iametti et al 2002). The gluten content in quinoa flour was measured using a commercial ELISA kit based on monoclonal antibodies against heat-resistant  $\alpha$ -gliadins (Skerrit and Hill 1991). The content of gliadin-like proteins was evaluated also on flours obtained from other grains (soybeans, buckwheat, oats, and maize). A wheat sample was the positive control. Gliadin standards covered the range of 2–20 ng of gliadin. Soybeans and maize showed a gluten content below the assay detection limits. The gluten content in quinoa ( $1.6 \pm 0.6$  mg/kg) was less than half that of buckwheat ( $4.2 \pm 0.2$  mg/kg), a pseudo-cereal that already has gained acceptance for use in gluten-free diets.

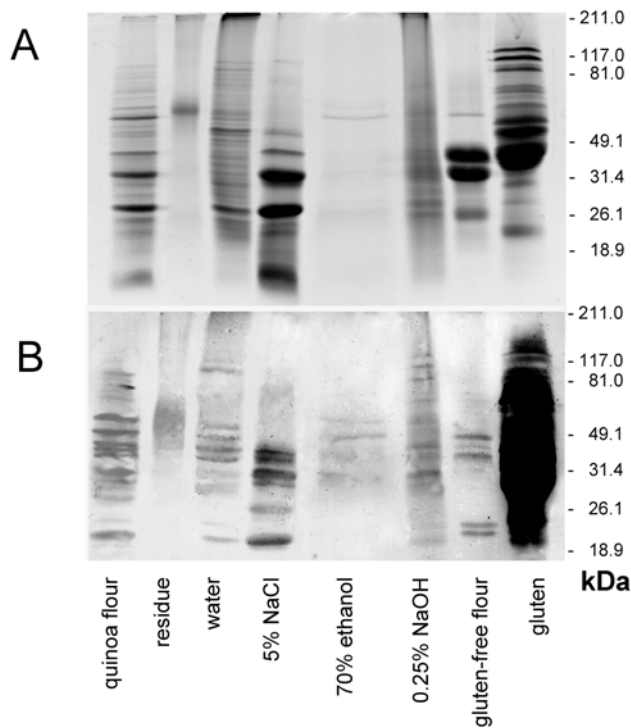
### Molecular Characterization of Proteins in Quinoa Flours

The solubility of proteins in quinoa flour was evaluated by extraction in buffers with different dissociating ability. A coarse correlation was established between the physicochemical properties of proteins, including their aggregation state, and their behavior during food processing (Ciaffi et al 1996).

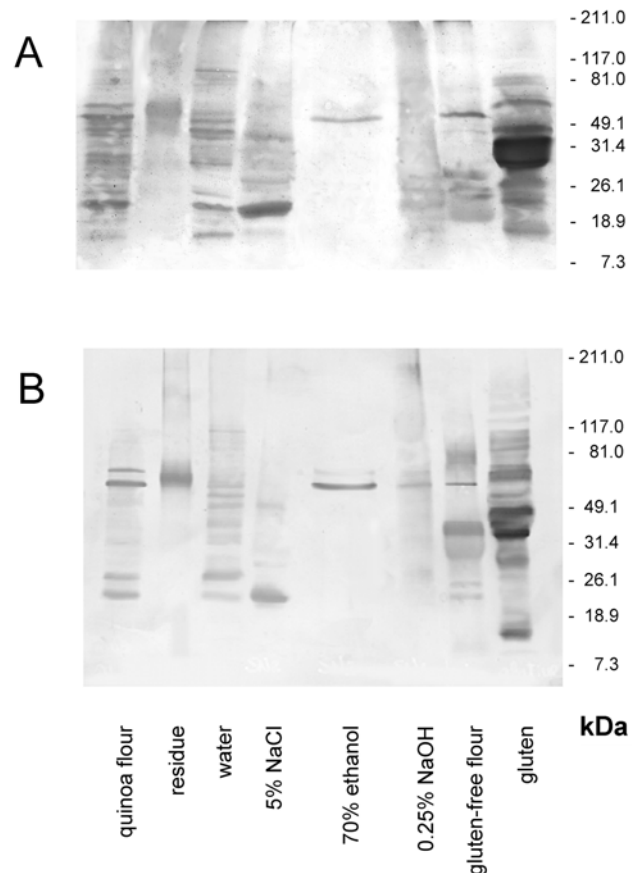
Solubility of quinoa proteins in phosphate buffer in the presence and in the absence of denaturing agents and DTT is reported

in Table I. Addition of urea sensibly increased the amount of extracted protein, with only a marginal further increase in the presence of DTT. This suggests the absence of significant reticulation of quinoa proteins by interprotein disulfide bridges.

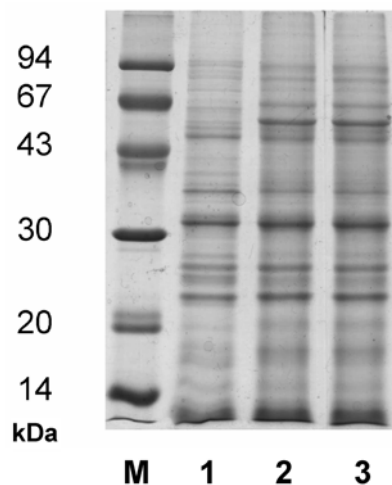
Analysis of the electrophoretic patterns for quinoa proteins extracted in these buffers (Fig. 3) indicates that proteins extracted by adding urea or urea-DTT have a polypeptide composition quite



**Fig. 1.** A, Gradient-gel SDS-PAGE of proteins solubilized from various materials in different solvent systems. A 0.5-g aliquot of each original material was suspended in 10 mL of the given solvent, followed by stirring for 30 min at 25°C and by removal of insoluble materials by centrifugation. A fixed volume (10  $\mu$ L) of the resulting solution was loaded in each sample lane. Lanes: 1, quinoa flour in denaturing buffer (50 mM phosphate, 0.1M NaCl, 8M urea, 10 mM DTT, pH 7.0); 2, insoluble material after performing the sequential water-NaCl-ethanol-NaOH extraction steps on quinoa flour, in denaturing buffer; 3, quinoa flour in water; 4, quinoa flour in 5% NaCl; 5, quinoa flour in 70% ethanol; 6, quinoa flour in 0.25% NaOH; 7, gluten-free flour in denaturing buffer; 8, commercial gluten in denaturing buffer. B, Immunoblotting of different protein extracts from quinoa incubated with a commercial antigliadin monoclonal antibody. Murine antirabbit IgG antibodies were used as secondary antibodies. Sample lanes as in A.



**Fig. 2.** Immunoblotting of different protein extracts from quinoa incubated with serum of a celiac subject. Alkaline phosphatase-labeled goat anti-human IgA (A) or antihuman IgG (B) were used as secondary antibodies. Sample lanes as in A.



**Fig. 3.** Fixed porosity SDS-PAGE of quinoa proteins soluble in different buffer systems. Lanes: 1, buffer (50 mM sodium phosphate, 0.1M NaCl, pH 7.0); 2, 8M urea in buffer; 3, 8M urea and 10 mM DTT in buffer. A fixed volume (10  $\mu$ L) of a protein solution was prepared and loaded in each sample lane. M, molecular weight markers.

similar to the proteins extracted in phosphate buffer, if not for an urea-solubilized protein with MW  $\approx$ 60,000. By combining the solubility data and the information obtained from electrophoresis, it is evident that noncovalently bound protein aggregates are present in quinoa flours and that some polypeptides play a significant role in the stabilization of these aggregates.

Sulfhydryls (-SH) and disulfides (-S-S-) play an important role in the structure and reactivity of food proteins and in establishing the technological properties of flours (Pomeranz 1994; Schofield 1994). The arrangement of thiols in native quinoa flour was studied by assessing the amount of -SH groups reactive toward the bulky thiol reagent DTNB under the different conditions reported in Table I.

The number of exposed -SH groups was similar in the presence or in the absence of urea, suggesting that protein denaturation of individual polypeptides and dissociation of noncovalent aggregates did not lead to an increase in the number of accessible thiols. Thus, it appears that acquisition of a compact structure and formation of a protein network depending on the presence or formation of intra- and intermolecular S-S bonds may be difficult to achieve with quinoa proteins.

In conclusion, quinoa could be a safe choice for the production of gluten-free products, at least from an immunochemical point of view. However, from a technological standpoint, the low content of interprotein disulfides in quinoa proteins may make it difficult the straightforward transformation of quinoa flour into textured foods by standard processes. Indeed, reticulation of quinoa proteins into a stable network such as that typical of gluten proteins seems hard to achieve by simple mechanical denaturation, and other processes (or combination of processes) must be developed if quinoa is to be used alone in the production of highly structured foods such as bread or pasta.

#### LITERATURE CITED

- Berti, C., Iametti, S., Porrini, M., and Bonomi, F. 2002. *Chenopodium quinoa* may represent a viable alternative for gluten-free products. *Pol. J. Food Nutr. Sci.* 11:138-140.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. *Anal. Biochem.* 72:248-254.
- Chauhan, G. S., Eskin, N. A. M., and Tkachuk, R. 1992. Nutrients and antinutrients in quinoa seed. *Cereal Chem.* 69:85-88.
- Ciaffi, M., Tozzi, L., and La Fiandra, D. 1996. Relationship between flour protein composition determined by size-exclusion high performance liquid chromatography and dough rheological parameters. *Cereal Chem.* 73:346-351.
- Coulter, L. 1989. Extrusion of corn grits-quinoa blends. MS thesis. Colorado State University: Fort Collins, CO.
- Coulter, L., and Lorenz, K. 1990. Quinoa—Composition, nutritional value, food applications. *Lebensm. Wiss. Technol.* 23:203-207.
- Ellman, G. L. 1959. Tissue sulphhydryl groups. *Arch. Biochem. Biophys.* 82:70-77.
- Fairbanks, D. J., Burgener, K. W., Robinson, L. R., Andersen, W. R., and Ballon, E. 1990. Electrophoretic characterization of quinoa seed proteins. *Plant Breed.* 104:190-195.
- Gutzmán-Maldonado, S. H., and Paredes-Lopez, O. 1999. Functional products of plants indigenous to Latin America: Amaranth, quinoa, common beans, and botanicals. Pages 293-328 in: *Functional Foods. Biochemical and Processing Aspects.* G. Mazza, ed. Technomic Publishing: Lancaster, PA.
- Iametti, S., Bonomi, F., Mariotti, M., Pagani, A. M., and Scafuri, L. 2002. Gluten contents in oat and buckwheat flours from different technological treatments. *Tec. Molitoria Intl.* 53:120-125.
- Li, M., and Lee, T.-C. 1996. Effect of cysteine of the functional properties and microstructures of wheat flour extrudates. *J. Agric. Food Chem.* 44:1871-1880.
- Lorenz, K., and Coulter, L. 1991. Quinoa flour in baked products. *Plant Foods Human Nutr.* 41:213-223.
- Pagani, M. A., Iametti, S., D'Egidio, M. G., and Bonomi, F. 1998. The evaluation of protein surface hydrophobicity as a quality index for semolina. Pages 225-228 in: *Plant Proteins from European Crops: Food and Non-Food Applications.* INRA: Nantes.
- Pomeranz, Y. 1994. Composition and functionality of wheat flour components. Chapter 7 in: *Wheat—Production, Properties, and Quality.* W. Buschuk and V. F. Rasper, eds. Blackie Academic and Professional: Glasgow.
- Prakash, D., and Pal, M. 1998. *Chenopodium*: Seed protein, fractionation and amino acid composition. *Int. J. Food Sci. Nutr.* 49:271-275.
- Ranhotra, G. S., Gelroth, J. A., Glaser, B. K., Lorenz, K. J., and Johnson, D. L. 1993. Composition and protein nutritional quality of quinoa. *Cereal Chem.* 70:303-305.
- Restani, P., Velonà, T., Plebani, A., Ugazio, A. G., Poiesi, C., Muraro, A., and Galli, C. L. 1995. Evaluation by SDS-PAGE and immunoblotting of residual antigenicity in hydrolysed protein formulas. *Clin. Exp. Allergy* 25:651-658.
- Restani, P., Fiocchi, A., Beretta, B., Velonà, T., Giovannini, M., and Galli, C. L. 1998. Effect of structure modifications on IgE binding properties of serum albumins. *Int. Arch. Allergy Immunol.* 117:113-119.
- Rondanini, A., Bonomi, F., Iametti, S., Lucisano, M., Pagani, M. A., and Rovere, P. 2000. On the nature and the practical relevance of the molecular modifications induced in wheat proteins by high pressure treatment. *High Pressure Res.* 19:167-173.
- Schofield, J. D. 1994. Wheat proteins: Structure and functionality in milling and breadmaking. Chapter 7 in: *Wheat-Production. Properties and Quality.* W. Buschuk and V. F. Rasper, eds. Blackie Academic and Professional: Glasgow.
- Shewry, P. R., Tatham, A. S., and Kasarda, D. D. 1992. Cereal proteins and coeliac disease. Pages 305-348 in: *Coeliac Disease.* M. N. Marsh, ed. Blackwell Scientific Publications: Oxford.
- Skerrit, J. H., and Hill, A. S. 1991. Enzyme immunoassays for determination of gluten in foods: A collaborative study. *J. Assoc. Off. Anal. Chem.* 71:257-264.
- Wieser, H. 1996. Relation between gliadin structure and coeliac toxicity. *Acta Paediatr. Suppl.* 412:3-9.

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