Effect of Sprouting on Proteins and Starch in Quinoa (Chenopodium quinoa Willd.)

Effect of sprouting on proteins and starch in quinoa (*Chenopodium quinoa* Willd.)

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ABSTRACT: This study aims at understanding the relation among sprouting time (from 12 up to 72 h), changes in protein and starch components, and flour functionality in quinoa. Changes related to the activity of sprouting-related proteases were observed after 48 h of sprouting in all protein fractions. Progressive proteolysis resulted in relevant modification in the organization of quinoa storage proteins, with a concomitant increase in the availability of biologically relevant metals such as copper and zinc. Changes in the protein profile upon sprouting resulted in improved foam stability, but in impaired foaming capacity. The increased levels of amylolytic enzymes upon sprouting also affected starch, making it less prompt to gelatinize upon heating. Consequently, starch re-association in a more ordered structure upon cooling was less effective, resulting in low setback viscosity. The nature and the intensity of these modifications suggest various possibilities as for using flour from sprouted

quinoa as an ingredient in food formulation to improve some technological and nutritional properties of baked products.

Keywords: quinoa; sprouting; protein features; functional properties; pasting properties

Introduction

Quinoa (Chenopodium quinoa Willd.) - a dicotyledonous plant of the Amaranthaceae family - is grown all over Central and Southern America. Quinoa production and consumption have been increasing constantly worldwide, due to its interesting agronomic features and nutritional value. Quinoa has a high protein content, a good balance of essential amino acids, a low glycemic index, and is rich in vitamins and minerals [1, 2]. Quinoa also does not have coeliac-related sequences in its proteins, and thus is a suitable raw material for gluten-free products [3]. The consumption of quinoa is mainly limited by its bitterness, primarily caused by saponins in the seed pericarp. Among the main approaches adopted to reduce the content in saponins, washing of seeds is still the most used approach in Andean countries, whereas pearling technology is preferred in Western ones [4], although pearling decreases the content in minerals, vitamins, fiber, and other bioactive compounds [5].

Sprouting (or germination) has been widely and successfully used to enhance both sensory and nutritional features of grains, including quinoa. Sprouting decreases the levels of antinutritional compounds in grains [6], due to the activities of ex-novo synthesized enzymes, and has been shown to have positive effects on the technological performance of either cereal [7-9] or legume flours [10]. The development of enzymatic activities in quinoa has been studied at different sprouting times [11, 12]. However, there is very little information on how sprouting may affect the structural features and the organization of proteins and starch, and how these changes may impact on the functional properties of flours from germinated seeds. The present work aims at understanding: 1) the effects of sprouting on proteins and starch in quinoa seeds; 2) how changes in structural and chemical features of either starch or proteins may affect the potential applications of flour from sprouted quinoa.

Materials and Methods

Raw materials. Seeds of whole and pearled quinoa (Chenopodium quinoa Willd. var. Titicaca), were purchased from Quinoa Marche s.r.l. (Ancona, Italy). For sprouting experiments, five aliquots of whole seeds (1 kg each) were soaked in water (1:1 w/w) for 14 h at 22 °C. After removing excess water, the soaked seeds were sprouted for 12, 24, 48 and 72 h at 22 °C and 90% of relative humidity in a climate chamber (HPP, Memmert GmbH+Co. KG, Schwabach, Germany). Soaked and sprouted samples were dried (55 °C for 6 h, Self Cooking Center, Rational International AG, Landsberg am Lech, Germany) to a final moisture content from 7% to 10%. All samples to be used for assessment of enzyme activity were treated with liquid nitrogen, immediately frozen and then freeze-dried (-80°C for 72h; Alpha 1-2 LD plus; Deltek s.r.l., Naples, Italy). Prior to analysis, all samples were milled to a particle size lower than 250 µm (99% of total flour) (Cyclotec 1093 Foss Sample Mill, Höganäs, Sweden).

Chemical composition. Protein content of quinoa samples was determined by the AACC 46-12.01 method [13] using 6.25 as a conversion factor. Starch was measured according to the AACC 76-13.01 method [13]. Soluble sugars were measured using by the K-MASUG assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland). The saponin content was assessed by methods reported elsewhere [17, 18], and is expressed as mg g^{-1} (db). All analytical measurements were carried out in triplicate.

Enzymatic activities. Enzymatic activities were assessed on freeze-dried samples, to avoid enzyme inactivation during drying at 55°C. Alpha-amylase activity was measured according to the AACC method 22-02.01 [13]. β-amylase activity was determined by the K-BETA3 kit (Megazyme International Ireland Ltd., Wicklow, Ireland). Proteolytic activity was measured as described by Marengo et al. [14].

Protein aggregation state and accessibility of protein thiols. Protein aggregation was assessed by differential solubility as described by Marengo et al. [14]. The amount of solubilized proteins was assessed by a dye-binding method [15]. Accessible thiols in both soluble and insoluble proteins were

assessed on buffered suspensions of flour samples, prepared in the presence/absence of 6M urea, following the procedure reported by Marengo et al. [10].

SDS-PAGE. The proteins solubilized from flours by various media were separated by SDS-PAGE in the presence of 1% (v/v) 2-mercaptoethanol, according to Marengo et al. [10], in a MiniProtein apparatus (BioRad, Richmond, VA, USA), by loading about 0.015 mg protein per lane.

Metal determination. Metal content was assessed by ICP-MS (AURORA M90, Bruker, Milan, Italy) after mineralization with nitric acid, as described in [10]. Unbound metals were assessed by ultrafiltration through a Centricon device (5 kDa MWCO, Merck Millipore, Vimodrone, Italy).

Foaming capacity and stability. Foaming capacity and stability were measured as in [16], using 2 g of flour in 50 mL of distilled water in a 100 mL cylinder. After vortexing for 1 min at room temperature, the height of foam was measured by Image ProPlus v.6.0 software (Media Cybernetics Inc., Rockville, USA). Foam stability was based on the foam height after 1 h of resting at room temperature.

Pasting properties. Pasting profiles were assessed in a Brabender Micro Visco-Amylo-Graph (Brabender OHG, Duisburg, Germany), as reported by Marti et al. [8], with a 3 min pre-treatment at 30 °C.

Statistics. Analysis of variance was carried out using Statgraphics Plus 5.1 (StatPoint Inc., Warrenton, VA, USA), considering different treatments (i.e., pearling, soaking, sprouting) and sprouting time as factors. Significant differences among the samples were assessed by the Tukey HSD test ($p<0.05$).

Results and Discussion

Chemical composition. Sprouting significantly decreased starch content after 48 h (Table 1), confirming that starch is the primary source of energy for seedling growth. No further modifications were noticed up to 72 h. The hydrolysis of starch by endogenous amylases led to an increase in the content of both glucose and maltose, in agreement with previous studies [11]. In this frame, it should also be noted that high levels of α-amylase activity over sprouting time do not necessarily translate into increased content of mono- or disaccharides in the sprouted grains, as most of the released sugars supposedly are used

for metabolic or biosynthetic purposes by the sprouting seeds. Sucrose content decreased during the first 24 h of sprouting, when it is used as an energy source [11], and then it increased up to 72 h, an event attributed to increased amount of enzymes which synthesize sucrose in late sprouting steps. The increase in starch content observed in pearled sample is only apparent and related to the removal of external layers, where starch is absent.

Table 1 Compositional data and enzymatic activities in treated and untreated quinoa grains

		sugars, g/100 g db				enzymatic activities, U/g db		
		Starch	Sucrose	D-Glucose	Maltose	α – amylase	β – amylase	Protease
Whole		60.6±1.7^{b}	2.33±0.06^{b}	0.42±0.05^{a}	0.31±0.06^{a}	0.22±0.03^{a}	1.65±0.12^{a}	1.4 ±0.4^{a}
Soaked		62.0±1.8^{b}	1.41±0.10^{a}	1.61±0.06^{b}	0.31±0.04^{a}	0.65±0.22^{a}	2.08±0.09^{b}	1.7±0.4^{ab}
Pearled		66.3±1.6^{c}	2.04±0.10^{b}	0.50±0.07^{a}	0.23±0.04^{a}	0.21±0.01^{a}	1.56±0.11^{a}	1.6±0.2^{ab}
Sprouted	12 h	60.6±1.9^{b}	1.43±0.11^{a}	1.75±0.04^{b}	2.34±0.39^{b}	2.07±0.16^{b}	1.9 ±0.2^{b}	
	24 h	59.1±2.4^{b}	2.06±0.04^{b}	2.06±0.15^{c}	3.87±0.77^{c}	2.02±0.14^{b}	2.9±0.5^{c}	
	48 h	52.6±1.4^{a}	2.18±0.12^{b}	2.20±0.13^{c}	5.89±0.79^{d}	1.99±0.12^{b}	2.9±0.4^{c}	
	72 h	50.7±1.9^{a}	3.09±0.21^{c}	2.23±0.09^{c}	5.34±0.51^{d}	2.00±0.06^{b}	2.9±0.6^{c}	

Mean ± standard deviation (n=3). Different letters in the same column indicate significant differences (Tukey HSD; $p<0.05$).

Enzymatic activities. Untreated whole quinoa seeds showed low levels of hydrolytic enzymatic activities, that were not affected by pearling (Table 1). Soaking resulted in a modest increase in the activity of proteases and of β-amylase but gave a three-fold increase in α-amylase levels, consistent with previous reports on biochemical changes occurring in quinoa at short soaking times (4-6 h) [20]. The activity of β-amylase did not change during subsequent sprouting of the soaked grains, whereas a progressive increase in the levels of proteolytic activity was evident up to 24 h of sprouting. The increase in β–amylase activity upon soaking and the absence of further changes during sprouting are similar to what reported for wheat [21], where the increase activity in the soaked seeds was attributed to the release of the free form of β–amylase enzyme by intracellular proteases [22]. A remarkable increase upon sprouting was observed for α-amylase activity, that at 12 h was four-fold higher than in the soaked

seeds and progressively increased its activity up to 48 h of sprouting, consistent with previous reports [11], that suggested a progressive migration of enzymes from the embryo to the starchy perisperm.

Sprouting-related changes in protein features

Association state of proteins. Fig. 1 reports the results of a differential solubility study carried out on quinoa flour from variously treated seeds. There were no relevant differences among whole, pearled, and soaked samples, and the present data confirm previous reports on the presence of equivalent amounts of free and disulfide-linked hydrophobic proteins in quinoa [3]. Data in Fig. 1 indicate that sprouting for at least 24 hours decreased the amount of proteins solubilized in phosphate buffer-saline (i.e., albumins and globulins, down to 47.5% of the initial value after 48 h), as detected by the dye-binding method used in these studies. This suggests that proteins not associated into large aggregates are an easy target for breakdown by endogenous proteases.

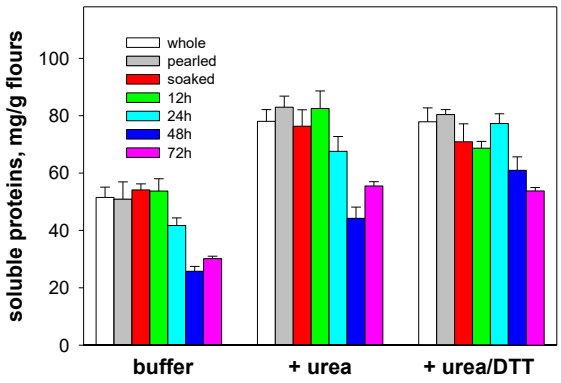

Fig. 1 Amount of quinoa proteins solubilized in various media

Endogenous proteases acting during sprouting were not very efficient in breaking down protein aggregates stabilized by hydrophobic interactions (i.e. soluble only in the presence of chaotropes), even when they were not cross-linked by disulfide bonds. Indeed, breakdown of proteins in hydrophobic-only aggregates was lower than what observed for proteins soluble in buffer-saline (32.2% vs 47. 5% at 48h). Proteolytic breakdown was even more limited for those proteins that formed aggregates stabilized through a combination of hydrophobic interactions and intermolecular disulfides (i.e., being

soluble only when urea and DTT were present). Proteins in the covalently-bound aggregates decreased only by 22% after 48 h sprouting.

In this frame, it is noteworthy that the breakdown of proteins linked through both hydrophobic interactions and/or disulfide bonds did not result in an increase in proteins soluble in buffer-saline. This implies that the products of proteolysis either remained associated to the original peptides or that the hydrolytic products were too small to be detected by the dye-binding method used for protein quantification. Indeed, the dye-binding method used for these studies requires peptides with a minimal size around 3-4 kDa. Unfortunately, peptides of this size also escape detection in SDS-PAGE (see below).

Protein profiling by SDS-PAGE. In accordance with previous reports on untreated quinoa [3], the overall protein patterns in the SDS tracings presented in Fig. 2 indicate that similar protein families are solubilized by the solvent systems used in conditional solubility experiments. However, some large polypeptides at approximately 60 and 70 kDa are solubilized only in the presence of urea in soaked grains (i.e., prior to any proteolysis) both in the presence and in the absence of a disufide-reducing agent, suggesting that these large proteins may represent nucleation centers for the aggregation of the majority of the other proteins in quinoa seeds into aggregates made up of coarsely similar amounts of the same individual polypeptides, interacting through both covalent disulfide bonds and non-covalent hydrophobic interactions.

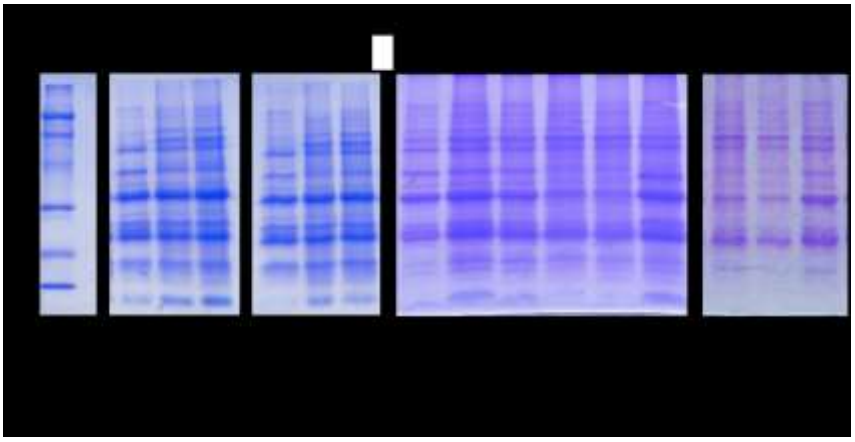

Fig. 2 SDS-PAGE of quinoa proteins solubilized in various media

As for sprouting-related changes, the tracings in Fig. 2 indicate that protein modifications were most evident after 48 h, confirming the observations provided by solubility studies (Fig. 1). The breakdown of proteins at 48 and 72 h is time-progressive, and targets most effectively proteins that are not participating to a disulfide-stabilized network (i.e., solubilized in saline buffer +/- urea in the absence of disulfide-reducing agents) supporting evidence from solubility studies. The relative intensity of individual bands confirms that protein bands at 25 and 32 kDa (the most abundant species among quinoa proteins) are a favorite target for endogenous proteases, that degrade them to fragments small enough to escape from the gel (or anyway undetectable by Coomassie Blue staining).

At 48 h sprouting (i.e., when proteolysis was highest, see Fig. 1) breakdown of components at 48 and 25 kDa was almost complete, concomitantly with transient appearance of a component at 35 kDa - most evident in the material solubilized by urea/DTT - that was subsequently degraded. Interestingly, the 60 kDa component that was only observed in the presence of chaotropes in the soaked grains appeared to be released in the buffer-soluble fraction after sprouting for 24 h, along with its 70 kDa companion. Both were degraded slowly at the longest sprouting times. This offers additional evidence that the 60 and 70 kDa components may represent nucleation sites for the aggregation of smaller “storage” proteins in quinoa seeds, and become buffer-soluble when endogenous proteases break down some of its aggregation partners. This results in their exposure to proteolytic action and to their degradation at sprouting times exceeding 48h.

Features of the protein network of relevance to binding of micronutrients and to techno-functional properties. The molecular changes in the protein fraction as a consequence of sprouting presented and discussed above may result in a change in the overall compactness of the protein network formed by quinoa protein. This – in turn – may impact their ability to interact with other ingredients in food formulations (by either covalent or non-covalent interactions), as well as their techno-functional properties, and possibly their digestibility when consumed by humans. A convenient approach for

assessing the compactness of a protein network even in the presence of insoluble and/or aggregated proteins relies on measuring the accessibility of protein thiols to suitable water-soluble reagents in the presence/absence of chaotropes [23]. In the case of quinoa, the content of readily-accessible protein thiols was not significantly affected by pearling, soaking, and sprouting, and remained in all cases in the range from 3.2 to 2.7 micromol g^{-1} flour. Treatment with 6M urea increased the content in accessible thiols to 4-6 micromol g^{-1} flour, again with no-clear-cut dependence on the previous treatments of the grain. Thus, although the sprouting process may have altered the spatial relationships among individual storage proteins in quinoa as a consequence of their significant breakdown by endogenous proteases, it does not lead to an increased exposure of thiols, suggesting that the structure of the individual proteins is somewhat preserved. Of course, it is also possible that the cysteine derived from proteolysis of storage proteins ends up – to some extent at least – in newly synthesized proteins, so that the overall accessibility remains unaltered during sprouting. However, both solubility and peptide profiling data (Figures 1 and 2) seem to indicate that protein neogenesis is quantitatively limited in the time frame (up to 72 h sprouting) considered in this study. Since the methodology used for these studies detects thiols in aggregates as well as in soluble materials, the absence of an increase in the number of accessible thiols in spite of extensive sprouting-related proteolysis suggests that cysteine-rich regions in quinoa proteins are located in regions shielded form the action of sprouting-related proteases, and become exposed only upon protein unfolding by chaotropes.

To assess whether the proteolytic events that accompany sprouting had an impact on the speciation of metals of biological relevance – and therefore on their bioavailability – the total metal content in aqueous extracts of the various flours was assessed by ICP-MS before and after separation of the metals bound to micromolecular species through an ultrafiltration membrane. The total content in the individual metals investigated in this study did not significantly change upon soaking or sprouting (not shown) but sprouting modified the speciation state of specific metal species. As shown

in Table 1S in the supplementary materials, sprouting may increase the fraction of some metals present as water-soluble species, making them capable of permeating an ultrafiltration membrane with a nominal cutoff around 3 kDa. For instance, the permeable fraction of Mg^{2+} increased about 20% upon sprouting, but no effects were observed for Ca^{2+} or manganese. Sprouting slightly improved the permeability of iron, whereas the largest sprouting-related improvement in permeability was observed for the nutritionally relevant copper (from 8.7% to about 16%) and zinc ions (from 8.3 to about 24%). The observation that sprouting effects are larger for metal ions that are preferentially bound to histidine-type ligands (copper and zinc), as opposed to metals that bind to generic negative charges (calcium and magnesium, that may be sequestered also by a number of non-protein ligands) suggests that proteolysis may play a significant role in the transition from the bound to the free form of these elemental micronutrients.

Sprouting-related molecular changes in quinoa proteins may also impact their techno-functional proteins. Among them, we focused on foaming capacity and stability, as these properties have been shown to be very sensitive to proteolytic modification. Proteolysis alters the interfacial properties of proteins, that stem from the availability of amino acid sequences capable to interact with either the aqueous or the gas phase (and with other food components). Protein unfolding and/or limited proteolysis have often been used to modulate foaming capacity in food-related systems [24]. Also, foam (and emulsion) stability is almost invariably improved when proteins – included those of plant origin - are present as aggregates of appropriate size [25]. Guo et al. [26] proposed that medium-size protein aggregates may improve the integrity of the air/water interface (and thus foam stability) by filling in the pores between large-size aggregates and/or by improving the viscosity of the interface protein film.

As shown in Table 2, there was a marked decrease in foaming capacity and stability upon soaking, likely due to the removal of part of the saponins [4]. The foaming capacity of quinoa flour improved slightly in the earliest phases of sprouting, but decreased to pre-sprouting figures after 48 h

or 72 h. A similar result was reported for soybeans [27] and attributed to an overall modification of the protein fraction upon sprouting. The foaming capacity of quinoa flours might be mainly attributed to proteins that are soluble under non-dissociating conditions, i.e., albumins and globulins, that decreased most sensibly upon sprouting (Fig. 1). The decrease in foaming capacity as affected by sprouting was only in part attributable to a decrease in the saponin content (from 3.6 mg g^{-1} in soaked seeds to 3.6, 3.3, 2.9, and 2.8 mg g^{-1} after 12, 24, 48, and 72 h, respectively). The relevance of proteins to foam formation and stability is made evident by the figures obtained for flour from pearled quinoa grains, having a very low saponins content (0.7 mg g^{-1}).

Table 2. Foaming capacity and foaming stability of quinoa flours

		Foaming capacity (%)	Foaming stability (%)
Whole		30.5±1.3^{c}	46.9±2.0^{ab}
Soaked		18.3±0.4^{a}	35.1±2.9^{a}
Sprouted	12 h	26.6±3.2^{ab}	42.4±4.8^{ab}
	24 h	24.5±3.5^{b}	62.9±7.3^{cd}
	48 h	18.3±0.5^{a}	55.4±2.2^{bc}
	72 h	18.6±0.7^{a}	74.1±3.9^{e}
Pearled		17.3±1.7^{a}	53.8±6.8^{bc}

Mean ± standard deviation (n=3). Different letters in the same column indicate significant differences (Tukey HSD; $p<0.05$).

Conversely, sprouting increased foam stability, with a maximum value attained at 72 h (i.e., when proteolysis was almost complete, see Figures 1 and 2). This may indicate that aggregates of quinoa proteins escaping the action of endogenous proteases may indeed play a significant role in foam stabilization, as suggested for other plant-derived proteins [25, 26].

Pasting properties. Sprouting was responsible for remarkable changes in maximum and final viscosity as detected in viscoamylographic measurements (Fig. 3 and Table 3). Both viscoamylographic parameters decreased in time-progressive fashion up to 48 h of sprouting. The observed changes were insensitive to the addition of 1 mM $AgNO_3$, a chemical inactivating agent for α-amylase (data not shown). This observation rules out the possibility that any endogenous amylase activity surviving the 55°C drying step used to stabilize the sprouted material could interfere with pasting profiles.

A comparison of the pasting data in Fig. 3 with the composition and enzymatic activity data in Table 1 offers some hints as for structural changes occurring in quinoa starch upon sprouting. The high levels of the endo-hydrolytic activity of α-amylase in the soaked and sprouted samples result in "nicking" of starch components. In turn, this results in formation of polymers, smaller than the original macromolecule, but still measured as starch by the standard total starch assay. The relatively small glucose polymers formed upon enzymatic hydrolysis are not likely to contribute to the intermolecular interactions relevant to viscosity measurements in pasting tests.

Table 3. Micro-visco-amylograph indices of quinoa flours

		Pasting temperature, °C	Maximum viscosity, BU	Final viscosity, BU	Setback, BU
Whole		67.3±1.2^{ab}	218.8±6.5^{e}	277.8±4.7^{c}	80.0±8.6^{a}
Soaked		66.4±1.3^{ab}	186.0±5.3^{d}	310.7±7.5^{d}	127.0±3.5^{c}
Sprouted	12 h	65.7±0.5^{ab}	160.0±1.7^{c}	267.7±1.5^{c}	109.0±1.0^{b}
	24 h	66.1±0.6^{ab}	135.0±3.6^{b}	244.7±4.9^{b}	114.7±7.1^{bc}
	48 h	65.2±0.2^{a}	80.7±6.1^{a}	155.0±3.0^{a}	76.3±2.1^{a}
	72 h	65.7±1.5^{ab}	84.7±2.3^{a}	166.3±3.1^{a}	81.0±6.1^{a}
Pearled		68.2±1.1^{b}	235.0±1.7^{f}	305.7±6.8^{d}	73.7±5.5^{a}

Mean ± standard deviation (n=3). Different letters in the same column indicate significant differences (Tukey HSD; p<0.05).

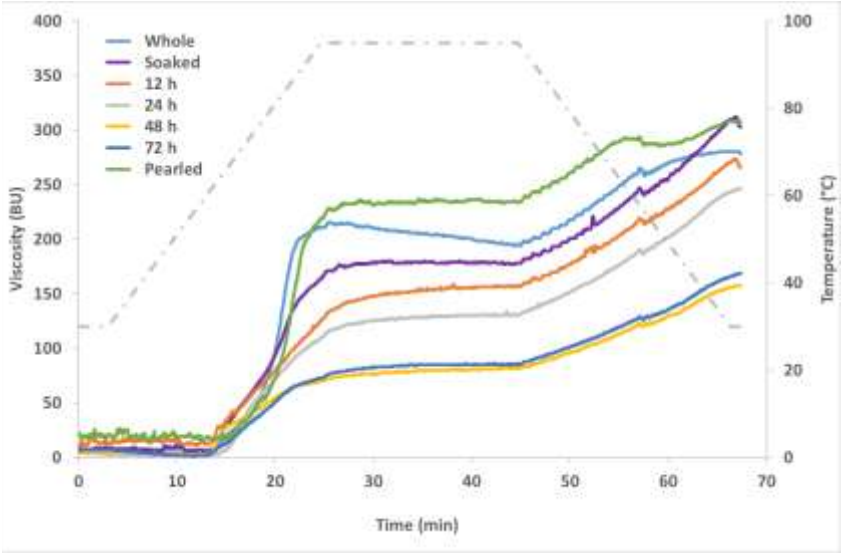

Fig. 3 Pasting properties of quinoa flour

Conclusions

Quinoa undergoes important physical and chemical changes during sprouting. The maximum intensity of the macromolecular modifications can be seen after 48 h of sprouting, although the activities of the hydrolytic enzymes responsible for most of the observed molecular changes are already present at 12 h. Endogenous proteases seem to act mostly on proteins not present as intermolecular aggregates and have an impact on the capacity of proteins to interact with nutritionally relevant metals, including copper and zinc. In the sprouted materials, both micronutrients were associated with small-sized species, suggesting an improvement in their bioavailability. The impressive increase in α-amylase activity occurring in the early stages of sprouting results in progressive nicking of starch molecules, with a very noticeable impact on the pasting properties of residual starch fractions in the sprouted material. Thus, the nature and the intensity of grain modifications occurring upon sprouting may improve the functional and technological properties of quinoa, as well as some of its nutritional features, encouraging the use of flour from sprouted quinoa as an ingredient in food formulations. Sprouting may be regarded also as a simple and economic bio-process suitable for further increasing the variety of food products in which incorporation of quinoa may be tested as a way of improving specific traits of potential interest for consumers.

Acknowledgments: Diego Suárez is grateful recipient of a PhD fellowship from Secretaría de Educación Superior, Ciencia, Tecnología e Innovación (SENESCYT), Ecuador. The Authors thank professor Francesco Bonomi for fruitful discussion and for constructive criticism.

References

[1] Bastidas EG, Roura R, Rizzolo DAD, Massanés T, Gomis R (2016) Quinoa (Chenopodium quinoa Willd), from nutritional value to potential health benefits: an integrative review. J Nutr Food Sci 6: 1-10. https://doi.org/10.4172/2155-9600.1000497

[2] Friedman M (1996) Nutritional value of proteins from different food sources. A review. J Agric Food Chem 44: 6-29. https://doi.org/10.1021/jf9400167

[3] Berti C, Ballabio C, Restani P, Porrini M, Bonomi F, Iametti S (2004) Immunochemical and molecular properties of proteins in Chenopodium quinoa. Cereal Chem 81: 275-277. http://dx.doi.org/10.1094/CCHEM.2004.81.2.275

[4] Suárez-Estrella D, Torri L, Pagani MA, Marti A (2018) Quinoa bitterness: Causes and solutions for improving product acceptability. J Sci Food Agric 98: 4033-4041. https://doi.org/10.1002/jsfa.8980

[5] Gómez-Caravaca AM, Iafelice G, Verardo V, Marconi E, Caboni MF (2014) Influence of pearling process on phenolic and saponin content in quinoa (Chenopodium quinoa Willd). Food Chem 157: 174-178. https://doi.org/10.1016/j.foodchem.2014.02.023

[6] Omary MB, Fong C, Rothschild J, Finney P (2012) Effects of germination on the nutritional profile of gluten-free cereals and pseudocereals: a review. Cereal Chem 89: 1-14. https://doi.org/10.1094/CCHEM-01-11-0008

[7] Elkhalifa AEO, Bernhardt R, Cardone G, Marti A, Iametti S, Marengo M (2017) Physicochemical properties of sorghum flour are selectively modified by combined germination-fermentation. J Food Sci Tech 54: 3307-3313. https://doi.org/10.1007/s13197-017-2781-7

[8] Marti A, Cardone G, Nicolodi A, Quaglia L, Pagani MA (2017) Sprouted wheat as an alternative to conventional flour improvers in bread-making. LWT-Food Sci Technol 80: 230-236. https://doi.org/10.1016/j.lwt.2017.02.028

[9] Cardone G, D'Incecco P, Pagani MA, Marti A (2020) Sprouting improves the bread-making performance of whole wheat flour (Triticum aestivum L.). J Sci Food Agric 100: 2453-2459

[10] Marengo M, Carpen A, Bonomi F, Casiraghi MC, Meroni E, Quaglia L, Iametti S, Pagani MA, Marti A (2017) Macromolecular and micronutrient profiles of sprouted chickpeas to be used for integrating cereal-based food. Cereal Chem 94: 82-88. https://doi.org/10.1094/CCHEM-04-16-0108-FI

[11] Hager AS, Mäkinen OE, Arendt EK (2014) Amylolytic activities and starch reserve mobilization during the germination of quinoa. Eur Food Res Technol 239: 621-627. https://doi.org/10.1007/s00217-014-2258-0

[12] Mäkinen OE, Hager AS, Arendt EK (2014) Localisation and development of proteolytic activities in quinoa (Chenopodium quinoa) seeds during germination and early seedling growth. J Cereal Sci 60: 484-489. https://doi.org/10.1016/j.jcs.2014.08.009

[13] AACC Approved Methods of Analysis, 2001. Cereals & Grains Association, St. Paul, MN, U.S.A.

[14] Marengo M, Bonomi F, Marti A, Pagani MA, Elkhalifa AEO, Iametti S (2015) Molecular features of fermented and sprouted sorghum flours relate to their suitability as components of enriched gluten-free pasta. LWT-Food Sci Technol 63: 511-518. https://doi.org/10.1016/j.lwt.2015.03.070

[15] 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. https://doi.org/10.1016/0003-2697(76)90527-3

[16] Coffmann CW, Garciaj VV (1977) Functional properties and amino acid content of a protein isolate from mung bean flour. Int J Food Sci 12: 473-484. https://doi.org/10.1111/j.1365-2621.1977.tb00132.x

[17] INEN (1988) Quinua. Determinación del contenido de saponinas por medio del método espumoso (método de rutina) (NTE INEN 1672). In Registro oficial del Ecuador n° 978 de 1988.

[18] Koziol MJ (1991) Afrosimetric estimation of threshold saponin concentration for bitterness in quinoa (Chenopodium quinoa Willd). J Sci Food Agric 54: 211-219. https://doi.org/10.1002/jsfa.2740540206

[19] Rosa M, Hilal M, González JA, Prado FE (2004) Changes in soluble carbohydrates and related enzymes induced by low temperature during early developmental stages of quinoa (Chenopodium quinoa) seedlings. J Plant Physiol 161: 683-689. https://doi.org/10.1078/0176-1617-01257

[20] Moreno C, Seal CE, Papenbrock J (2018) Seed priming improves germination in saline conditions for Chenopodium quinoa and Amaranthus caudatus. J Agron Crop Sci 204: 40-48. https://doi.org/10.1111/jac.12242

[21] Grassi S, Cardone G, Bigagnoli D, Marti A (2018) Monitoring the sprouting process of wheat by non-conventional approaches. J Cereal Sci 83: 180-187. https://doi.org/10.1016/j.jcs.2018.08.007

[22] Ziegler P (1995) Carbohydrate degradation during seed development. In Kigel J, Galili G (eds) Seed development and germination, Marcel Dekker Inc, New York, pp 447-474

[23] Iametti S, Bonomi F, Pagani MA, Zardi M, Cecchini C, D'Egidio MG (2006) Properties of the protein and carbohydrate fractions in immature wheat kernels. J Agric Food Chem 54: 10239-10244. https://doi.org/10.1021/jf062269t

[24] Uluko H, Liu L, Lv JP, Zhang SW (2016) Functional characteristics of milk protein concentrates and their modification. Crit Rev Food Sci Nutr 56: 1193-1208. https://doi.org/10.1080/10408398.2012.758625

[25] Amagliani L, Scmitt C (2017) Globular plant protein aggregates for stabilization of food foams and emulsions. Trends Food Sci Tech 67: 248-259. https://doi.org/10.1016/j.tifs.2017.07.013

[26] Guo F, Xiong YL, Qin F, Jian H, Huang X, Chen J (2015) Surface properties of heat-induced soluble soy protein aggregates of different molecular masses. J Food Sci 80: C279-C287. https://doi.org/10.1111/1750-3841.12761

[27] Mostafa MM, Rahma EH, Rady AH (1987) Chemical and nutritional changes in soybean during germination. Food Chem 23: 257-275. https://doi.org/10.1016/0308-8146(87)90113-0

Declarations: Funding: Not applicable**; Conflicts of interest:** The authors declare no conflict of interest. **Ethics approval:** Not applicable**; Consent to participate:** Not applicable**; Consent for publication:** Not applicable**; Availability of data and material:** Not applicable**; Code availability:** Not applicable