RHEOLOGICAL AND THERMAL PROPERTIES OF ROYAL QUINOA AND WHEAT FLOUR BLENDS FOR BREADMAKING

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Abstract

The increasing interest in quinoa in Europe has generated a large number of studies with this seed as a partial substitute for refined wheat flour in bakery products as a strategy to improve their nutritional value. However, the wide genetic diversity of this seed offers very different compositions in different varieties, which would lead to different technological behaviours in the breadmaking process. The aim of this work was to make a comparative study of the protein profile and rheological and thermal properties of three varieties of quinoa widely available commercially in Europe in order to study their technological potential as breadmaking ingredients with 25% replacement of wheat flour by whole quinoa flour. The results obtained during the analysis offered a view of the proteins present in the various quinoas, and of the processes of hydrolysis and generation of new bonds between wheat and quinoa proteins during the breadmaking process. The changes in the thermal and pasting properties of the bread doughs that included whole quinoa flour led to the development of baked products with different physico-chemical and textural properties, producing an increase on crumb staling. However, replacement of 25% of the wheat flour with whole quinoa flour produced only a slight decrease in the technological quality of the products. A significant increase (p<0.05) in dietary fibre, minerals, lipids and proteins in comparison with a whole wheat product, together with the overall consumer acceptance of the products that were developed, was conclusive for proposing replacement with quinoa flour as a strategy for nutritional improvement in the manufacture of bakery products.

Keywords: Quinoa; bread characteristics; protein profile; thermal parameters; pasting properties

Introduction

Bread is one of the most common foods made with cereals in the world. However, the main cereal used for breadmaking is flour obtained by dry milling of wheat grain, which removes valuable nutrients and bioactive compounds [1]. Whole cereal and pseudocereal flours can be included in bakery products as a strategy to improve their nutritional profile without needing to use whole products completely [2,3,4]. Among the pseudocereals, quinoa (*Chenopodium quinoa*) is a dicotyledon originally from South America, although, because of its adaptation characteristics and wide genetic diversity, it is now grown in nearly every continent in the world, including Europe [5]. Because its composition is similar to that of cereals, it has a suitable balance of carbohydrates, proteins, lipids and minerals, and it can be sold without restrictions in Europe in accordance with Regulation (EU) 2015/2283 [6], which means that a large number of varieties are marketed in countries of the European Union, all of which has created increasing interest in society. Moreover, unlike wheat, which contains gluten-forming proteins (gliadins and glutenins), the main proteins in quinoa are albumins and globulins, bound together by disulfide bridges [7]. The most abundant of these proteins are of type 11S, also known as globular chenopodin, with a molecular size of 30–40 kDa [8], followed by those of type 2S albumin, which are polypeptides of a relatively small size, about 9–10 kDa [9,10]. The predominance of globulins and albumins in quinoa is technologically significant because they have foaming, emulsifying and gelling properties, which in some cases are similar to the techno-functional properties of soya or casein proteins [11].

Various studies show that the incorporation of whole quinoa flour in bread formulations causes technological changes produced by the dilution of gluten, inclusion of fibre and/or lipids, or its starch characteristics [1,12]. However, marked

differences between varieties have been reported in recent years, regarding their chemical composition and physical properties, size of starch granules and amylose/amylopectin ratio, polyphenol content and antioxidant capacity, among other things [13–16].

Accordingly, the aim of this work was to make a comparative study of the protein profile and rheological and thermal properties of three varieties of quinoa widely available commercially in Europe in order to study their technological potential as breadmaking ingredients with 25% replacement of wheat flour by whole quinoa flour.

Materials & Methods

Materials

Three types of commercial Bolivian quinoa seeds (*Chenopodium quinoa*) grown by members of ANAPQUI (La Paz, Bolivia) were purchased from Ekologikoak (Ondarroa-Bizkaia, Spain). Organic “quinoa real” (royal quinoa) (white, red and black) was used to produce flour in a mill (Aromatic, Taurus, Oliana, Spain). The chemical composition of the white, red and black quinoa flours according to the labelling was: 12.0, 11.0 and 11.2 g/100 g of moisture; 64.0, 56.7 and 57.2 g/100 g of carbohydrates; 6.0, 5.4 and 5.1 g/100 g of lipids; 4.0, 11.8 and 12.8 g/100 g of fibre; and 14.0, 15.1 and 13.7 g/100 g of proteins, respectively. Dehydrated yeast (*Saccharomyces cerevisiae*, Maizena, Spain) was used as starter for the breadmaking process. Commercial strong wheat flour (Carrefour, Madrid, Spain) was used for the bread formulation. The chemical composition of the wheat flour was: 12.6 g/100 g of moisture; 71 g/100 g of carbohydrates; 1.4 g/100 g of lipids; 3 g/100 g of fibre; and 12 g/100 g of proteins.

Breadmaking procedure

The control bread dough formula consisted of wheat flour (500 g), dehydrated yeast (1.0 g/100 g flour basis), sodium chloride (1.6 g/100 g flour basis) and distilled water (70.8 g/100 g flour basis). Whole quinoa flour was incorporated in the bread dough formula at 25 g/100 g on flour basis. The breadmaking procedure was performed in a breadmaker (BM 3989, Severin, Germany). The process variables consisted of the following steps: a. kneading phase and rising phase for 9 min and 20 min, respectively; b. kneading phase and rising phase for 14 min and 20 min, respectively; short stirring for 30 sec; c. rising phase for 4 min and 30 sec; d. rising phase for 45 min, and lastly baking for 60 min. The breads obtained were cooled at room temperature for 75 min for subsequent analysis. The breadmaking process was performed in triplicate.

Chemical composition

Moisture content was determined by an official assay procedure [17]. Starch content was measured by an enzymatic procedure according to Method 996.11 [17]. Protein determination was carried out by the Dumas Combustion method (N conversion factor 5.7) according to ISO/TS 16634-2 (2016)[18]. Lipid content was extracted with petroleum ether under reflux conditions by the Soxhlet technique [19], whereas ash content was determined in a muffle furnace by incineration at 900 °C [19]. The dietary fibre content was measured by an enzymatic and gravimetric method [17]. The analyses were performed in triplicate.

Technological parameters

The technological parameters analysed were as follows: the height of the bread piece (cm) and the texture profile analysis using the TA.XT Plus Texture Analyser (Stable Micro Systems, Godalming, United Kingdom) with a 35 mm flat-end aluminium compression disc [20]. Each parameter was measured at least in triplicate in crumb of fresh bread and after 24 and 48 hours of storage at room temperature in polyethylene bags. The experiments were conducted in triplicate.

Digital image analysis was used to measure the bread crumb structure. Images were taken at 600 pixels per cm with a scanner (HP Scanjet G2410, Hewlett Packard, USA) supported by HP Photosmart Essential 3.5 software. Data were processed using Fiji Image J (version 1.49q, National Institute of Health, USA) and NIS-Elements (Basic Research version, Nikon Instruments Inc., Amsterdam). The analysis was performed in triplicate.

Preliminary sensory analysis of the fresh breads was performed by a panel of 50 untrained tasters who usually consume bread, using a nine-point hedonic scale of overall acceptance (9. Like extremely; 8. Like very much; 7. Like moderately; 6. Like slightly; 5. Neither like nor dislike; 4. Dislike slightly; 3. Dislike moderately; 2. Dislike very much; 1. Dislike extremely).

Protein profile

The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method was performed, based on the original procedure of Laemmli [21] modified by Fu & Sapirstein [22]. In order to obtain equal concentrations of proteins, the quinoa flours, wheat flour, wheat bread and wheat and quinoa bread samples were weighed on the basis of their dry weight protein contents and mixed with 1 mL of sample buffer solution (pH 6.8) containing 0.063 mol/L Tris-HCl, 2% (w/v) SDS, 20% (w/v) glycerol

(Merck, Germany) and 0.01% (w/v) Pyronine Y (Sigma-Aldrich, USA). The reduced samples were prepared by using 7% (v/v) *β*-mercaptoethanol (2-ME, Sigma-Aldrich, USA) included in sample buffer. The blend was vortexed (Reax Top model, Heidolph, Germany) for 1 min every 10 min during 2 hr. Extracted and dissolved samples were heated in a dry block heating thermostat (Bio TDB-120 model, BIOSAN, Latvia) for 3 min in order to denature proteins before analysing, and then applied (10 µL) to the SDS-PAGE, which was carried out in a cooled slab gel unit (Protean II xi Cell, Bio-Rad, CA, USA). The acrylamide concentrations of resolving gel and stacking gel were 12.5% and 5%, respectively. After concluding the electrophoresis, the gels were rinsed in rinsing solution (57% (v/v) water + 33% (v/v) methanol + 10% (v/v) trichloroacetic acid (100% w/v)) overnight in order to remove excess SDS from the surface of the gels. Then the gels were stained overnight with Coomassie Brilliant Blue G-250 (Merck, Darmstadt, Germany) according to Ng and Bushuk [23]. Apparent molecular weights were determined using wide-range molecular weight protein markers (S8445, Sigma, MO, USA) as standards. The determination of the molecular weights of the protein bands in the quinoa flours, wheat flour, wheat bread and wheat and quinoa breads were carried out by using Bio-Rad Image Lab 5.0 software after scanning from the gel imager (ChemiDoc MP Imaging System, Bio-Rad, USA).

Differential scanning calorimetry (DSC)

The thermal properties of the raw materials and during baking of the fermented dough as well as the amylopectin retrogradation induced during the bread storage were measured on a differential scanning calorimeter (DSC-7, PerkinElmer) according to the methodology described by Iglesias-Puig et al. (2015) with

modifications. The calorimeter was calibrated with indium (enthalpy of fusion 28.4 J/g, melting point 156.4 °C). Flours were weighed into DSC pans and mixed with Milli-Q water to obtain a water:flour ratio of 3:1. Samples were scanned at a rate of 10 °C/min from 25 °C to 110 °C. Fermented dough samples (30–40 mg) were weighed directly in DSC stainless steel pans (LVC 0319-0218, PerkinElmer). After sealing, the pans were kept at 25 °C for 1 min, scanned at a rate of 10 °C/min from 25 °C to 110 °C, kept at this temperature for 5 min, and cooled to 25 °C at 50 °C/min. Afterwards, the pans were stored at 4 °C for 24 and 48 hours and heated again in the calorimeter from 25 to 130 °C at 10 °C/min to analyse amylopectin retrogradation. An empty pan was used as a reference, and three replicates of each sample were analysed. The parameters recorded were onset (T_o), peak (T_p), and conclusion (T_c) temperatures of gelatinization and retrogradation transitions. The starch gelatinization and amylopectin retrogradation (ΔH_G and ΔH_R, respectively) were calculated as the area enclosed between the straight line and the endotherm curve between T_o and T_c. They were expressed in joules per gram of starch and the experiments were conducted in triplicate.

Rapid Visco Analyser (RVA)

The pasting properties of samples were measured using a Rapid Visco Analyser (RVA-4; Newport Scientific, Warriewood, Australia) according to AACC Method 76-21 (1995) [19]. Distilled water (25 mL) was added to 3.0–3.5 g of sample placed into the aluminium RVA canister. The suspensions were stirred thoroughly at 160 rpm. The temperature was first maintained at 50 °C for 1 min to obtain a uniform temperature and then raised to 95 °C at a rate of 12 °C/min, held at 95 °C for 2.5 min, cooled to 50 °C at a rate of 12 °C/min, and finally held at 50 °C for 2 min. Pasting parameters

evaluated included: pasting temperature (P_{temp}), peak viscosity (PV), hot paste viscosity (HPV), final or cool paste viscosity (CPV), breakdown (PV – HPV) and setback (CPV – HPV). The RVA experiments were conducted in triplicate.

Statistical analysis

The data generated were analysed by ANOVA using SPSS Statistics Version 22 (International Business Machines Corporation, USA). Fisher's least significant difference (LSD) test was used to determine statistically significant differences (p<0.05) between mean values for different samples, at a 95% confidence level.

Results & Discussion

SDS-PAGE protein profiles in reduced and unreduced forms

Total extractable proteins of whole quinoa flours, wheat flour, wheat bread and wheat and quinoa breads in reduced form are shown in Figure 1. There were a few differences among the protein patterns of the quinoa flours, such as a noticeable protein band with a molecular weight (MW) of 102 kDa in white quinoa flour, whereas red quinoa flour and black quinoa flour did not have this protein band (Lanes 1, 2 and 3); there was also a clear protein band with 38 kDa MW (Lane 3). Otherwise, the protein band profiles of the quinoa flours were generally very similar in reduced form (Figure 1).

The main protein fractions in quinoa grain are albumins and globulins (chenopodin) which are stabilized by disulfide bonds. The globulins, also called chenopodin or 11S-type proteins, consist of two subunits which are acidic subunits (30–40 kDa MW) and basic subunits (20–25 kDa MW). Lower MW (8–11 kDa) proteins of quinoa grain are called 2S-type proteins [8,9,24,25]. These proteins are also indicated in Figure 1

and Figure 2. The effects of the breadmaking process on quinoa flour proteins were also investigated in reduced form. The composition of individual proteins in the quinoa flours was significantly modified during both fermentation and baking processes. It was found that, during the breadmaking process, the mixing, fermentation and baking processes caused some changes in quinoa flour proteins, such as protein hydrolysis by proteases that caused breaking of proteins [26] or disulfide formation through oxidation causing polymerization of proteins which could not enter into the gel. These changes are mainly responsible for the flavour during the fermentation and baking stages [27,28]. Ingredients notably influence aromatic compounds, and flours usually have distinct aromatic characteristics [29]. In contrast, a small number of protein bands were observed in wheat and quinoa bread samples when compared with those found in the corresponding flours. In all the quinoa flours, a double protein band around 79 kDa MW seemed to be hydrolysed and then smaller fragments may have been polymerized with other wheat proteins (Lanes 1, 2 and 3; Lanes 6, 7 and 8). The intensities of the protein bands with MW of 50, 52, 58 and 62 kDa decreased considerably after the breadmaking process (Lanes 1, 2 and 3; Lanes 6, 7 and 8). These protein bands might be hydrolysed and then polymerized with wheat proteins, and conclusively an intense protein band around 41 kDa MW appeared in wheat and quinoa bread samples (Lanes 6, 7 and 8). Similarly, the protein bands at 35 and 37 kDa in the quinoa flours were hydrolysed via protease attack and then accumulated as a protein band at 34 kDa that appeared very intensely on gel. Also, the intensity of the binary protein band around 30 kDa in the quinoa flours (Lanes 1, 2 and 3) decreased substantially after the breadmaking process (Lanes 6, 7 and 8). The protein bands located below 25 kDa MW in all the

quinoa flours also did not appear after the bread-making process, owing to protein hydrolysis or polymerization with higher MW wheat proteins.

A protein band that did not appear in the protein profile of wheat flour (Lane 4) was detected at 110 kDa MW in the profile of wheat bread (Lane 5). The protein bands detected in wheat flour at 13, 28 and 58 kDa did not appear after breadmaking owing to protein hydrolysis and subsequent polymerization with other wheat proteins by formation of cross-linking via disulfide linkages.

Total extractable proteins of quinoa flours, wheat flour, wheat bread and wheat and quinoa bread samples were investigated without using reducing agent (*2*-ME), and the SDS-PAGE results of the unreduced samples are shown in Figure 2. The protein patterns of the quinoa flours in unreduced form were generally found to be similar (Lanes 1, 2 and 3). However, some changes were observed that were due to varietal differences in the quinoa flours. For example; white quinoa flour and red quinoa flour had a thin protein band at 103 kDa MW, whereas the black quinoa flour did not have this protein band in the unreduced form (Lanes 1, 2 and 3). Similarly, intense protein bands between 34 and 37 kDa MW were observed in the white quinoa flour and red quinoa flour, but these protein bands were not detected in the black quinoa flour. Furthermore, protein bands around 21.5 kDa and 30 kDa were detected in the white and red quinoa flours but were not detected in the black quinoa flour. Double protein bands around 84 kDa in the white quinoa flour were also not detected in the red and black quinoa flours in unreduced form (Figure 2).

After the breadmaking process, a few faint bands of proteins were detected in the wheat bread and wheat and quinoa breads in unreduced form (Figure 2). The higher MW protein bands above 49 kDa in the quinoa flours did not appear in unreduced form, probably owing to protein polymerization and because they could not enter into

the gel. The intense protein bands at 49, 57 and 60 kDa MW were probably hydrolysed by proteases or may have been polymerized with other proteins and finally they did not appear on gel after breadmaking. Similarly, the protein bands between 30 and 37 kDa MW and the protein bands lower than 29 kDa MW did not appear on gel in unreduced form after breadmaking (Lanes 6, 7 and 8; Figure 2). When the protein profiles of the wheat flour and its bread were examined (Lanes 4 and 5 in Figure 2) it was seen that the intensities of the protein bands between 42 and 62 kDa decreased after breadmaking. In addition, the intensity of the protein band at 28 kDa decreased in unreduced form as well (Lanes 4 and 5).

The results presented in Figure 1 indicated that during thermal processing, owing to Maillard and protein cross-linking reactions, the structure of the dough proteins might have changed. This could cause formation of aggregates or protein cross-linking through the formation of disulfide bonds, resulting in the creation of high MW insoluble proteins. Since MWs higher than 200 kDa could not enter into the gel, they could not be detected on the gel. Similar findings have been reported previously in several studies [26,30,31]. Singh [30] explained that a low degree of protein extraction from bread samples was due to differences in rate of temperature change and in moisture content in different parts of the bread, and disulfide bonds were the major cross-links formed in bread crusts during baking and they were responsible for protein insolubility.

Thermal properties

The thermal properties of the raw materials, analysed in the differential scanning calorimeter (DSC), are shown in Table 1. These properties are influenced by the protein and lipid contents, the granule structure (amorphous/crystalline structure

299 relationship) and the molecular structure of the amylopectin, such as its branching,
300 chain length and molecular weight, among other things [32]. The starch gelatinization
301 onset temperature (T_o) of the quinoa flours presented lower values than those of the
302 wheat flour, and this difference was significantly lower (p<0.05) in black quinoa. Also,
303 lower peak temperature (T_p) values were observed in the white quinoa flour than in
304 the wheat flour (p<0.05). Lower gelatinization temperatures indicate shorter
305 amylopectin chains, because they need lower temperatures to dissociate completely
306 [33,34]. The conclusion temperature (T_c) and gelatinization enthalpy (ΔH_G) were
307 significantly higher (p<0.05) in the red and black quinoa flours than in the wheat and
308 white quinoa flours, owing to the high crystallinity of the starch granules in the quinoa
309 [35].
310 In varieties from Peru, Repo-Carrasco-Valencia and Valdez-Arana [51] reported ΔH_G
311 values similar to those observed in the present work, but the gelatinization
312 temperatures were slightly higher. These differences are basically due to the
313 variability between cultivars.
314 The thermal properties of the bread doughs during the simulation of baking are
315 shown in Table 1. With regard to gelatinization, a general increase in the T_o and T_p
316 temperatures was observed in the formulations with quinoa in comparison with the
317 control sample, but this increase was only significant (p<0.05) in the formulations with
318 white or black quinoa. Furthermore, there was a general decrease in the T_c and ΔH_G
319 values in comparison with the control dough, and they were significantly lower
320 (p<0.05) in the doughs with white quinoa. This behaviour is due to the inclusion of
321 fibre from the whole quinoa flour. During the cooking stage, when the gelatinization of
322 the starch takes place the water is less available in the formulations with quinoa,
323 basically because of the presence of fibre, so the ungelatinized granules would need

higher temperatures and less energy to gelatinize, producing increases in T_o and T_p and decreases in T_c and ΔH_G [37].

A significant increase (p<0.05) in the enthalpy of the amylopectin retrogradation (ΔH_R) was observed during storage in all the formulations (Figure 4.a), as reported by other authors in studies on retrogradation kinetics [38,39]. No significant changes in ΔH_R were observed during the first 24 hours of storage. However, the incorporation of quinoa in the doughs produced a significant reduction (p<0.05) of this parameter with respect to the control after 48 hours. The replacement of wheat flour with red or black quinoa caused a significant increase (p<0.05) in the retrogradation temperatures with respect to the control and the formulation with white quinoa during storage (data not shown).

Pasting properties

The pasting properties of the raw materials and the bread mixtures were analysed (Table 2). The pasting temperature (P_{temp}) of the quinoa flours was significantly higher (p<0.05) than that of the control flour, which might lead to poor cooking characteristics [40], although the inclusion of 25% of whole quinoa flour did not alter this parameter significantly. The quinoa flours presented significantly higher (p<0.05) peak time (P_{time}) values than the control (Table 2). However, the inclusion of these flours in the formulation produced a significant decrease (p<0.05) in the time needed for peak formation, denoting a non-additive behaviour and suggesting the appearance of physico-chemical interactions between the components of the flours. The differences in size and structure of the starch granules cause unequal distribution of moisture during heating, and therefore the behaviour of the doughs is different from that of the individual flours [41]. On the other hand, it is worth noting

that the peak viscosity (PV) and breakdown values were significantly lower (p<0.05) in the quinoa flours than in the wheat flour, which caused a corresponding decrease in these parameters in the analysis of the breadmaking mixtures. Hot paste viscosity (HPV) is related to the final volume of the loaf after baking, owing to its effect on the incorporation and capacity of movement of CO_2 in the dough [42,43]. This might indicate that the lower HPV shown by the quinoa flours with respect to the wheat flour might lead to an increase in the volume of the final product [44,45]. However, the incorporation of quinoa flours in the breadmaking mixtures led to a general increase in HPV, which was significant (p<0.05) in the mixtures with white or red quinoa. Setback is the stage in which there is a regrouping and/or reordering of starch molecules and it is associated with the texture of bakery products [46]. The analysis of the raw materials showed significantly lower (p<0.05) setback values in the quinoa flours than in the control sample. However, the only significant reduction (p<0.05) in the breadmaking mixtures was in the one with black quinoa.

In general, the values of the pasting properties of the quinoa flours were lower than those of the wheat flour. This can be explained by the characteristics of the starch granules of the various raw materials with regard to their degree of crystallinity and amylopectin chain length and by the higher fibre content in the quinoa flours, reducing the availability of water in the breadmaking mixtures and consequently affecting the pasting properties [47]. In general, the results obtained for the royal quinoa flours in the present study fit within the results reported by Wu et al. [48] after analysing 13 varieties of quinoa.

Effect of incorporation of quinoa on bread performance

373 The physico-chemical parameters of the wheat bread and the bakery products
374 incorporating whole quinoa flour are shown in Table 3. A significant decrease
375 (p<0.05) in loaf height was observed in the breads made with black quinoa in
376 comparison with the control sample (~6.5%). Although the incorporation of white or
377 red quinoa did not lead to significant differences with respect to the control, the value
378 of this parameter tended to decrease. The reduction in loaf height was similar to the
379 loss of volume reported by other authors [12,49], basically affected by the dilution of
380 gluten and the higher fibre concentration in the quinoa flours. However, there were
381 no significant changes in loaf weight between the breads that incorporated quinoa
382 and the control bread (Table 3). The moisture content of the samples with quinoa,
383 except the one with red quinoa, increased significantly (p<0.05), basically owing to
384 the use of whole quinoa flours. The protein content tended to increase, and this
385 increase was statistically significant (p<0.05) in the formulations with white and red
386 quinoa. It is worth noting that the replacement of wheat flour with whole quinoa flour
387 not only increases the protein content but also produces an improvement in the
388 biological value of the proteins in these formulations, because quinoa proteins are
389 more digestible than wheat proteins and they provide essential amino acids that are
390 limiting in wheat flours [50,51]. There was also a significant increase (p<0.05) in the
391 dietary fibre and mineral contents in the formulations with white and red quinoa in
392 comparison with the control, thus contributing to a suitable intake of fibre and
393 minerals such as Ca, Fe and Zn in the diet [42,52].
394 The results of the digital image analysis of the crumb of the products developed are
395 shown in Table 3. There was a significant increase (p<0.05) in the value of the cell
396 area/total area parameter in the crumb of breads that included red or black quinoa in
397 comparison with the bread with white quinoa and the control (Figure 3). Although

398 significant changes were not seen in the cells/cm^2 parameter, a decreasing tendency
399 was observed in the sample with black quinoa. It is worth noting that there was a very
400 significant increase (p<0.05) in the maximum cell area in the crumb of the breads
401 with various varieties of quinoa in comparison with the control bread. These
402 differences may be due to greater α-amylase activity in the quinoa, leading to an
403 increase in the quantity of fermentable sugars produced from the starch [53,54].
404 Although the maximum cell area increased in the crumb of the breads with quinoa,
405 there was a decrease in the median cell area of those breads, most probably due to
406 the formation of large gas cells which compressed the other gas cells, reducing the
407 median cell area.
408 With regard to texture, the parameters analysed are shown in Table 3. A significant
409 increase (p<0.05) was observed in the firmness parameter of the breads with white
410 or black quinoa in comparison with the control, basically due to the reduction in the
411 percentage of gluten. The incorporation of quinoa in the bread formulations also led
412 to significant increases (p<0.05) in the gumminess and chewiness parameters,
413 whereas there was a significant decrease (p<0.05) in cohesiveness with respect to
414 the control sample. In general, during storage there were significant changes in all
415 the texture parameters of the products developed (data not shown). However, a very
416 marked increase was observed in the firmness values of the products formulated with
417 quinoa in comparison with the control sample during two days of storage (Fig. 4.b).
418 This crumb hardening can be explained partly by the phenomenon of amylopectin
419 retrogradation (Fig. 4.a). Retrogradation is a complex phenomenon that depends on
420 many factors, such as the size and structure of the starch granules, and it involves
421 phenomena such as the formation of bonds with proteins and/or the presence of
422 lipids with surfactant properties that can cause differences in the migration of water

molecules between gluten and starch during storage [55]. Accordingly, the significant increase (p<0.05) in the crumb firmness during storage of the products with quinoa may be due to a greater loss of moisture generated by an irregular dough, with layers of gluten surrounding conglomerates of starch granules [56].

The preliminary sensory analysis indicated that partial replacement of wheat flour with 25% of whole quinoa flour did not significantly affect the general acceptability of the products developed. However, the breads with quinoa were given slightly better scores than the control sample, with the exception of the bread with red quinoa, which received slightly less acceptance. The acceptance of products made with quinoa might be due, among other things, to the formation of aromatic compounds, such as pyridines, characteristic of quinoa flours, generating flavours accepted by consumers [28].

Conclusions

The global proteomic approach offered a general view of the various proteins in the different quinoas and the changes that took place during the breadmaking process, which included hydrolysis and formation of bonds between quinoa proteins and wheat proteins, modifying the protein structure of the doughs formulated. In general, the three varieties of quinoa presented a similar behaviour in terms of pasting properties, thermal characteristics and proximal composition that were different if comparing to wheat flour. The gelatinization thermal transition of starch from red and black quinoa flours appeared in a greater temperature range than white quinoa flour. The replacement of 25% of the wheat flour with whole quinoa flour in making bakery products caused a change in the thermal and pasting properties of the bread doughs, which led to the development of baked products with different physico-chemical and

448 textural characteristics. However, a significant increase (p<0.05) in the nutritional
449 profile together with the overall consumer acceptance of the products developed was
450 conclusive for proposing replacement with quinoa flour as a strategy for nutritional
451 improvement in the manufacture of bread with refined wheat despite the slight
452 decrease in the technological quality of the products developed. Therefore black
453 quinoa bread presented a higher amount of dietary fibre/ash and a lower amount of
454 starch compared to white and red quinoa breads. These differences produced breads
455 with a lower loaf height and higher crumb firmness, chewiness and resilience with a
456 similar acceptability by consumers regardless the different formulations.
457
458 **Compliance with ethical standards**
459 **Conflict of interest** None
460 **Compliance with Ethics Requirements** This article does not contain any studies
461 with human or animal subjects.
462
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Figure captions

Figure 1. SDS-PAGE patterns of the total extractable proteins of quinoa flours and wheat and quinoa bread samples. All samples were reduced with 7% *β*-mercaptoethanol. Lane **M:** Wide-range protein markers (Sigma S8445). Lane **1:** White Quinoa Flour. **2:** Red Quinoa Flour. **3:** Black Quinoa Flour. **4:** Wheat Flour. **5:** Wheat Bread. **6:** Wheat Bread with White Quinoa. **7:** Wheat Bread with Red Quinoa. **8:** Wheat Bread with Black Quinoa. (MW: Molecular Weight).

Figure 2. SDS-PAGE patterns of the total extractable proteins of quinoa flours and wheat and quinoa bread samples prepared without using any reducing agent. Lane **M:** Wide-range protein markers (Sigma S8445). Lane **1:** White Quinoa Flour. **2:** Red Quinoa Flour. **3:** Black Quinoa Flour. **4:** Wheat Flour. **5:** Wheat Bread. **6:** Wheat Bread with White Quinoa. **7:** Wheat Bread with Red Quinoa. **8:** Wheat Bread with Black Quinoa. (MW: Molecular Weight).

Figure 3. Effect of the inclusion of quinoa on crumb structure. Bread formulations: (a) Wheat bread; (b) White quinoa bread; (c) Red quinoa bread; (d) Black quinoa bread.

Figure 4. Firmness and amylopectin retrogradation of control and wheat and quinoa bread samples (n = 3): □ day 1 ▒ day 2 ■ day 3. Mean ± Standard Deviation, n=3. Values followed by the same letter in the same line are not significantly different at 95% confidence level.

638 **Table 1.** Thermal properties of raw materials and doughs[a]

639

Parameter[a]		Flours				Doughs			
Starch gelatinization	Units	Control	White	Red	Black	Control	White	Red	Black
T_o	^oC	56.7±0.6b	56.7±0.6b	55.4±0.8a,b	53.9±0.7a	62.3±0.6a	64.4±0.8b	62.5±0.9a	63.9±0.7b
T_p	^oC	62.9±0.1b	61.8±0.3a	62.6±0.1a,b	62.0±0.7a,b	69.5±0.8a	70.3±0.8a,b	69.8±0.7a	71.1±0.6b
T_c	^oC	69.7±0.4a	69.8±0.5a	71.8±0.2b	73±1b	80.5±0.7b	77.3±0.4a	79.8±0.4b	80.2±0.4b
ΔH_G	J/g of starch	8.1±0.1a	8.20±0.08a	9.28±0.02c	8.57±0.06b	0.67±0.05b	0.42±0.08a	0.9±0.1c	0.6±0.4b

640

641 [a]Mean ± Standard Deviation, n=3. Values followed by the same letter in the same line are not significantly different at 95% confidence level. DSC: Differential

642 Scanning Calorimetry; T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH_G, enthalpy of gelatinization.

643 **Table 2.** Pasting properties of raw materials and quinoa/wheat blends[a]

644

Sample	Units	Flours				Quinoa/wheat blends		
		Control	White	Red	Black	White	Red	Black
P_{temp}	°C	68.0±0.6a	84.4±0.5c	81.42±0.03b	80.3±0.6b	68.47±0.03a	68.4±0.1a	68.1±0.6a
P_{time}	min	5.87±0.00b	7.00±0.00c	7.00±0.00c	7.00±0.00c	5.67±0.09a	5.73±0.00a	5.73±0.00a
PV	cP	2271±21d	909±3a	1084±24b	942±2a	2062±81c	2086±37c	2001±11c
HPV	cP	1320±7c	782±23a	1018±6b	811±4a	1382±40d	1381±30d	1325±13d
CPV	cP	2725±14c,d	1467±4a	1706±16b	1666±38b	2743±85c,d	2805±56d	2663±13c
Breakdown	cP	951±14d	127±19b	66±18a	131±2b	680±41c	705±7c	676±1c
Setback	cP	1405±7d,e	685±27a	687±9a	855±35b	1361±45c,d	1424±25e	1338±1c

645

646 [a]Mean ± Standard Deviation, n=3. Values followed by the same letter in the same line are not significantly different at 95% confidence level. RVA, Rapid Visco
647 Analyser; P_{temp}, pasting temperature; P_{time}, peak time; PV, peak viscosity; HPV, hot paste viscosity; CPV, final or cool paste viscosity; Breakdown: PV – HPV;
648 Setback: CPV – HPV; cP, centipoises.

649 **Table 3.** Effect of whole quinoa flour on bread performance

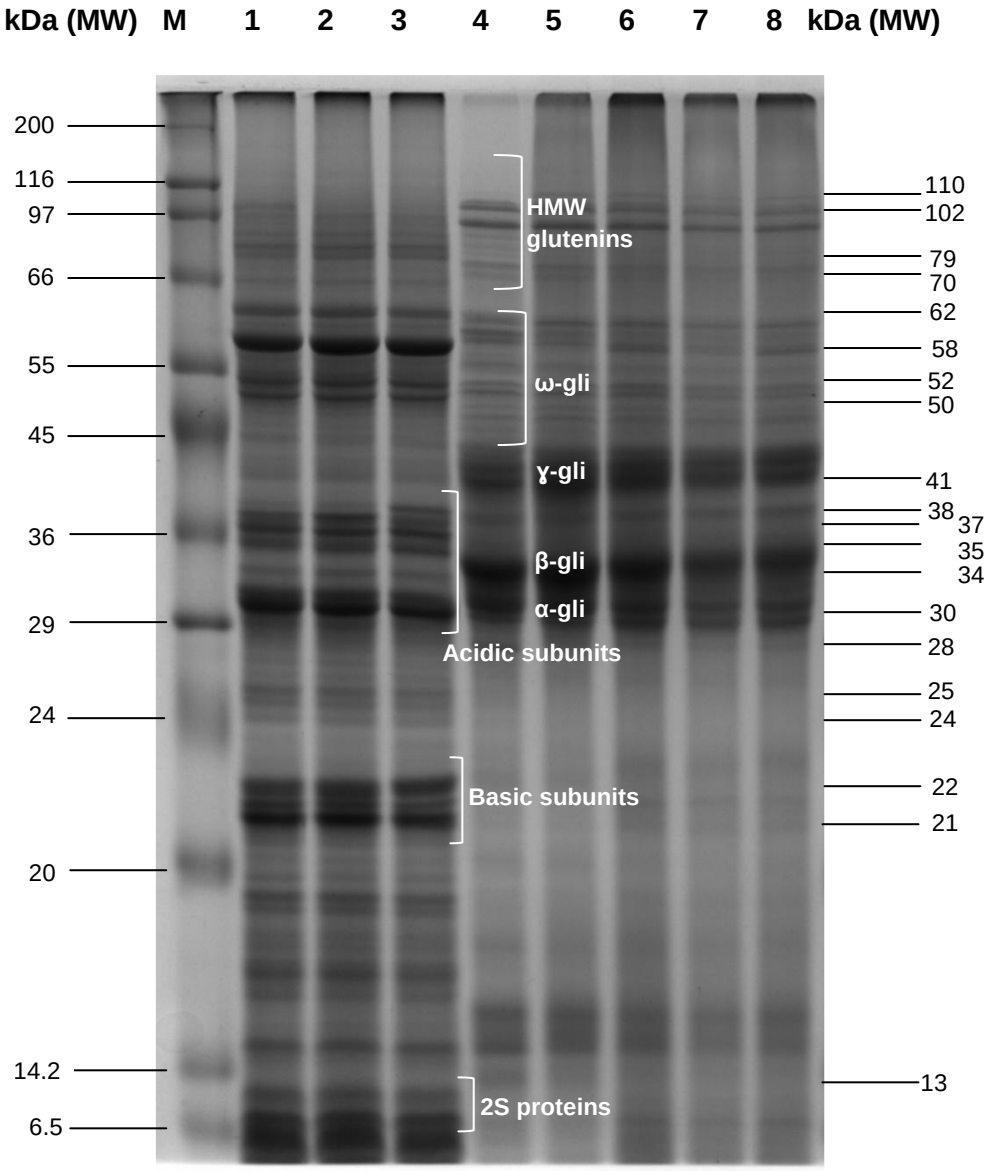
Sample	Units	Control	Quinoa		
			White	Red	Black
Physico-chemical parameters[a]					
Moisture	%, w.m.	36.6±0.1b	38.6±0.1c	35.6±0.1a	38.49±0.01c
Loaf weight	g	638±1a	641±3a	647±17a	639±3a
Loaf height	cm	12.4±0.3b	12.3±0.4b	12.0±0.2a,b	11.6±0.3a
Starch	%, d.m.	60±3b	60±1b	59±1b	56±1a
Proteins	%, d.m.	11.00±0.06a	11.5±0.1b	11.5±0.2b	11.16±0.05a
Total dietary fibre	%, d.m.	5.9±0.5a	8.51±0.01b	9±1b	10.66±0.00b,c
Lipids	%, d.m.	0.25±0.03a	0.7±0.1b	0.79±0.02c	0.78±0.05c
Ash	%, d.m.	1.06±0.04a	1.48±0.02b	1.50±0.03b	1.61±0.01c
Textural Parameters[a]					
Firmness	N	0.70±0.04a	1.08±0.07b	1.03±0.09a,b	1.3±0.4b
Springiness		1.72±0.08a	1.70±0.05a	1.73±0.02a	1.7±0.1a
Cohesiveness		0.93±0.02b	0.87±0.01a	0.87±0.01a	0.87±0.08a
Gumminess	N	0.65±0.04a	0.97±0.03b	0.90±0.09b	1.5±0.3c
Chewiness	N	1.12±0.02a	1.66±0.00b	1.6±0.2b	2.5±0.2c
Resilience		0.49±0.01a,b	0.47±0.01a	0.48±0.01a,b	1.20±0.04b
Crumb Structure[a]					
Cell area/total area	cm^2/cm^2	0.45±0.00a	0.44±0.00a	0.46±0.01b	0.47±0.00b
Wall area/total area	cm^2/cm^2	0.55±0.00b	0.56±0.00b	0.54±0.01a	0.53±0.01a
$Cells/cm^2$		17.6±0.8a	18±2a	17.85±0.05a	16.8±0.5a
Median cell area	mm^2	0.67±0.02d	0.57±0.01c	0.38±0.01b	0.31±0.01a
Maximum cell area	mm^2	73±9a	75±7ab	98±9b	176±8c
Sensory Analysis[b]					
Overall acceptability		7.1 ±1.3a	7.4 ±1.1a	6.9 ±1.5a	7.1 ±1.5a

650

651 [a]Mean ± Standard Deviation, n=3; [b]n=50. Values followed by the same letter in the same line

652 are not significantly different at 95% confidence level; d.m., dry matter; w.m., wet matter.

Fig. 1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kDa (MW)
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6.5
HMW glutenins
ω-gli
γ-gli
β-gli
α-gli
Acidic subunits
Basic subunits
2S proteins
110
102
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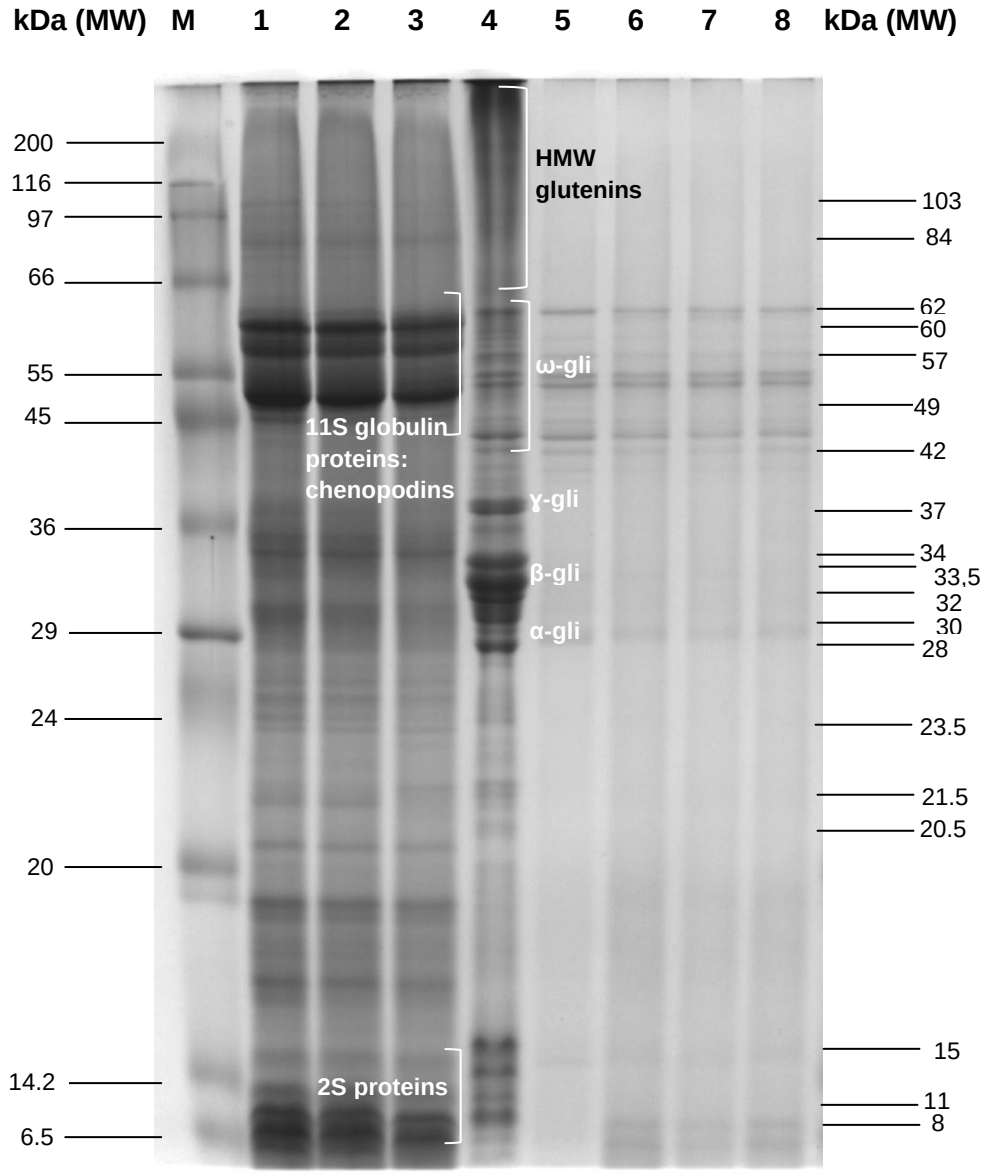
Fig. 2


kDa (MW)
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HMW glutenins
ω-gli
11S globulin proteins: chenopodins
γ-gli
β-gli
α-gli
2S proteins
103
84
62
60
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23.5
21.5
20.5
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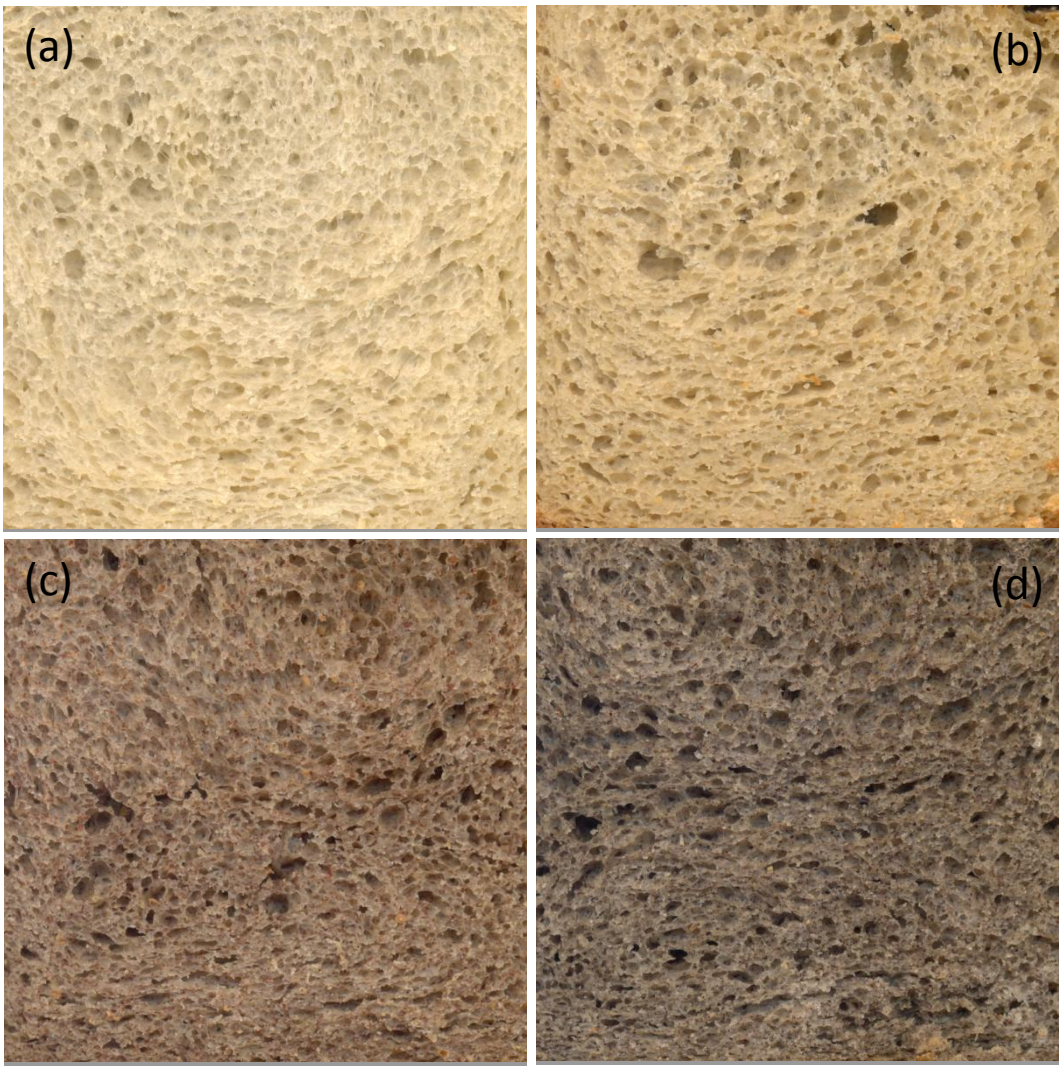
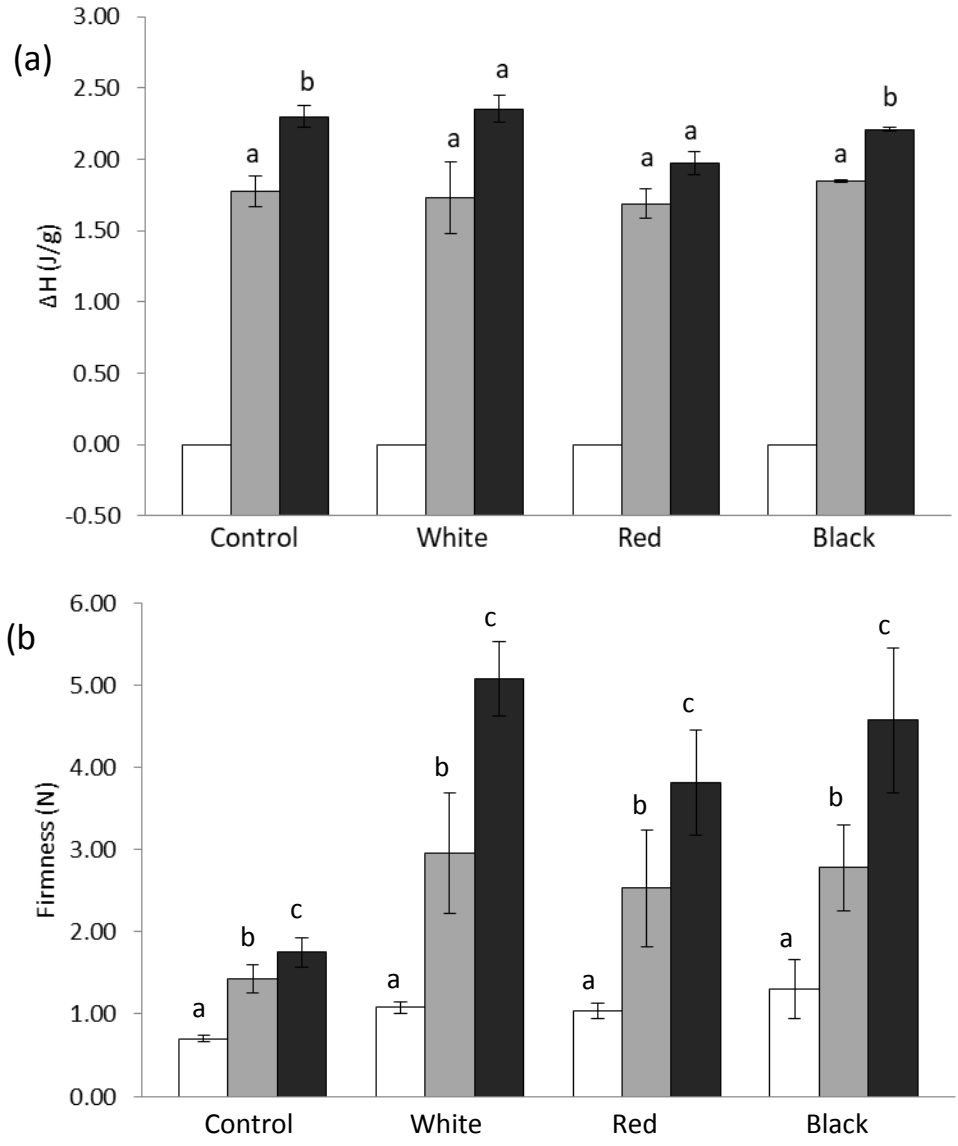
Fig. 3




(a)
ΔH (J/g)
3.00
2.50
2.00
1.50
1.00
0.50
0.00
-0.50
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b
a
a
a
a
a
b
Control
White
Red
Black
(b
Firmness (N)
6.00
5.00
4.00
3.00
2.00
1.00
0.00
a
b
c
a
b
c
a
b
c
a
b
c
Control
White
Red
Black

Fig. 4