BREAD WITH WHOLE QUINOA FLOUR AND BIFIDOBACTERIAL PHYTASES INCREASES DIETARY MINERAL INTAKE AND BIOAVAILABILITY

Esther Iglesias-Puig[a], Vicente Monedero[b] and Monika Haros[a*]

[a]Cereal Group, [b]Lactic Acid Bacteria and Probiotics Laboratory, Institute of Agrochemistry and Food Technology (IATA-CSIC), Valencia, Spain.

*Corresponding autor. Mailing address: Institute of Agrochemistry and Food Technology (IATA-CSIC), Av. Agustín Escardino 7, Parque Científico, 46980 Paterna-Valencia, Spain. Phone: +34 96 390 00 22; Fax: +34 96 363 63 01; e-mail: mharos@iata.csic.es (Monika Haros)

Abstract

The purpose of the present work was to provide further information on how replacing wheat flour by whole quinoa flour (at 25 and 50 g/100g of flour) affects bread performance and to assess its potential as a nutritious ingredient. Bread with quinoa resulted in a depreciation in quality in terms of loaf specific volume (from 4.48 to 3.46/2.63 cm^3/g), crumb firmness (from 0.77 to 1.55/2.64 N) and acceptability (from 7.94 to 7.58/5.94). Quinoa increased the bread nutritional value, raising fibre (from 5.5 to 7.2 g/100g) and minerals (Ca from 0.35 to 1.28 mg/g, Fe from 17 to 34 μg/g, and Zn from 23 to 48 μg/g). The phytates were controlled by bifidobacterial phytase treatment during breadmaking (from 4.7 μmol/g to below the detection limit), which decreased phytate/mineral molar ratios to values lower than the threshold for inhibition of Fe and Zn absorption.

Quinoa could partially replace wheat flour in bread, increasing its nutritional value in terms of dietary fibre, minerals, proteins of high biological value and healthy fats, with only a small depreciation in bread quality at 25 g/100g of flour substitution. The high phytate contents were efficiently removed by phytase treatment and the breads were accepted by consumers.

KEY WORDS: quinoa, minerals, DRIs, phytase, mineral availability

Abbreviations

25Q, 25 g whole quinoa flour/75 g wheat flour; 50Q, 50 g whole quinoa flour/50 g wheat flour; ΔE^*, total color difference; a^*, redness to greenness; ATCC, American Type Culture Collection; b^*, yellowness to blueness; C^*, chroma**;** d.m., dry matter; DRIs, Dietary Reference Intakes; DSC, differential scanning calorimetry; h^*, hue angle; HPLC, high-performance liquid chromatography; InsP_6, phytic acid, *myo*-inositol (1,2,3,4,5,6)-hexakisphosphate or phytate; InsP_5, *myo*-inositol pentakisphosphate; InsP_4, *myo*-inositol tetrakisphosphate; InsP_3, *myo*-inositol triphosphate; L^*, lightness; LSD, Fisher's least significant differences; n.d., not detected; SD, Standard deviation; T_o, onset temperature, T_p, peak temperature, and T_c conclusion temperature of gelatinization and retrogradation transitions.

1. Introduction

The year 2013 was declared the "International Year of the Quinoa" by the United Nations Food and Agriculture Organization in recognition of the indigenous peoples of the Andes, recognizing the high value of this pseudocereal crop (FAO, 2011). Pseudocereals are grown for the same purpose as cereals, but they are no members of the monocotyledoneous *Gramineae*. They are dicotyledoneous plants not closely related to each other and they comprise three crops: buckwheat (*Fagoryrum esculentum*, *Polygonaceae*), which is thought to have arisen in China, amaranth (*Amaranthus* spp., *Amaranthaceae*) and quinoa (*Chenopodium quinoa* Willd., *Chenopodiaceae*), which are natives of Meso-America and South America (Shewry, 2002). Quinoa seeds are traditionally used for human and livestock consumption in the Andean region and have exceptional nutritional qualities (Repo-Carrasco-Valencia, Espinoza & Jacobsen, 2003; Ruiz, Biondi, Oses, Acuña-Rodríguez, Antognoni, Martinez-Mosqueira et al., 2013). Its

nutritional value, its adaptability to different agro-ecological soils and its potential contribution to the fight against hunger and malnutrition are characteristics to highlight (Koziol, 1992). Quinoa is endemic in all countries of the Andean region, ranging from Colombia to northern Argentina and southern Chile. FAOSTAT (2013) reports that, in the period 1992–2010, the cultivated area and total production of quinoa almost doubled and tripled, respectively, in the main producer countries (Bolivia, Peru and Ecuador). Quinoa cultivation has crossed continental boundaries to reach Europe. It is cultivated in France, England, Sweden, Spain, Denmark, Finland, Holland and Italy (Medina, Skurtys & Aguilera, 2010; FAOSTAT, 2013). It is grown in the United States and in Canada, as well as in Kenya, in the Himalayas and India (FAOSTAT, 2013).

From the nutritional point of view, quinoa represents a significant source of dietary fibre and vitamins as folate (Schoenlechner, Wendner, Siebenhandl-Ehn & Berghofer, 2010) or vitamin E (Ryan, Galvin, O`Connor, Maguire & O´Brien, 2007), which has a protective effect against lipid oxidation (Dini, Tenore & Dini, 2010), and, minerals such as Ca, Mg, Zn and Fe (Alvarez-Juvete, Arendt & Gallagher, 2009). However, usually whole grains, especially pseudocereals, contain significant amounts of phytic acid or its salts (phytates), a well-known inhibitor of mineral, proteins and trace elements bioavailability (Hurrell, 2003; Hager, Wolter, Jacob, Zannini & Arendt, 2012; Sanz-Penella, Wronkowska, Soral-Smietana & Haros, 2013). The negative effects of phytates in human nutrition are more relevant in developing countries, in risk populations such as pregnant women or those who follow an unbalanced diet and also in animal feed (Fretzdorff & Brümmer, 1992; Nielsen, Damstrup, Dal Thomsen, Rasmussen & Hansen, 2007). Under conventional processing conditions such as pasta or bread making, optimal conditions for the degradation of phytate are rarely reached (Hager et al., 2012). Thereby, the use of exogenous phytases has been suggested and proven as an

efficient practice to eliminate phytates in cereals/pseudocereals processing (Sanz-Penella, Frontela, Ros, Martinez, Monedero & Haros, 2012; García-Mantrana, Monedero & Haros, 2014).

Quinoa is also noteworthy for its high protein content with a balanced composition of essential amino acids (Repo-Carrasco-Valencia et al., 2003; Comai, Bertazzo, Bailoni, Zancato, Costa & Allegri et al., 2007). It shows a high content of essential fatty acids such as oleic and linoleic acids (Alvarez- Jubete et al., 2009). Polyphenolic compounds as flavonols, with antioxidant activity and linked to the prevention of various diseases are also present in this pseudocereal (Repo-Carrasco-Valencia & Astuhuaman-Serna, 2011). The different functional properties of starch from quinoa, makes it suitable as ingredient in bread formulation by replacing flour (Berti, Riso, Monti & Porrini., 2004). This adds value to this crop to be included in the diet of populations with nutritional risk (Schoenlechner, Drausinger, Ottenschlaeger, Jurackova & Berghofer, 2010; Alvarez-Jubete, Arendt & Gallagher 2010).

The purpose of the present work was to provide further information on how replacing wheat flour by whole quinoa flour at different levels (25 and 50 g/100 g) affects the bread performance and to assess its function as a nutritious ingredient. Also, a strategy to increase the mineral availability by using different phytase treatments was assayed.

2. Materials and Methods

2.1. Materials

Commercial Spanish wheat flour and quinoa kernels (*Chenopodium quinoa*) were purchased from the local market (La Meta, S.A. and Ecobasic – Bio, S.L., Spain, respectively). The characteristics of the raw materials are shown in Table 1. Compressed yeast (*Saccharomyces cerevisiae*, Levamax, Spain) was used as a starter

for the breadmaking process. Phytases from *Bifidobacterium longum* spp. *infantis* ATCC 15697 and *Bifidobacterium pseudocatenulatum* ATCC 27919 were overexpressed in *Escherichia coli* strains carrying the bifidobacterial phytase genes and purified by affinity chromatography (Tamayo-Ramos, Sanz-Penella, Yebra, Monedero & Haros, 2012).

2.2. Breadmaking procedure

The control bread dough formula consisted of wheat flour (500 g), compressed yeast (5 g/100 g flour basis), sodium chloride (1.6 g/100 g flour basis) and tap water (up to optimum absorption, 500 Brabender Units, 60 g/100 g flour basis, AACC Method 54–21, 1995).

The whole quinoa flour was added at 25 g/100 g (25Q samples) or 50 g/100 g (50Q samples) on flour basis to the bread dough formula (water absorption 62.5 and 64.0 g/100 g, respectively). A sponge method mixing dough in a two stage was used. The first stage involved mixing half water and flour amount together with the total yeast amount and fermenting for 24 h at 4 ºC. The sponge is then mixed for 4.3-5.3 min with the rest of ingredients in a second stage. Later, doughs were divided into 100 g pieces, kneaded and then rested for 15 min. Doughs were manually sheeted and rolled, proofed (up to optimum volume increase, at 28 °C, 85 % relative humidity). Fermentation was monitored by measuring pH, temperature and volume increase of the dough at regular intervals.

After the fermentation step, the doughs were baked in an electric oven at 160 °C-180 ºC during 27-20 min, according to the formulation. Later, the obtained breads were cooled at room temperature for 75 min for subsequent analysis (Sanz-Penella, Tamayo-Ramos, Sanz & Haros, 2009). The experiments were done in duplicate. In experiments made

153 with addition of exogenous phytase, the enzyme was added during mixing stage. The
154 total phytase activity in the doughs was doubled by adding the same units of phytase
155 that were present in the flour mixtures. Phytase activity was determined for the purified
156 enzymes and in flours as previously described (Haros, Rosell & Benedito, 2001).
157
158 *2.3. Composition of raw materials and bread*
159 Protein determination was carried out by the Kjeldahl technique (f: N×5.7) (AACC
160 Method 46-13, 1995). Lipid content was extracted with petroleum ether under reflux
161 conditions by the Soxhlet technique (AACC Method 30-20, 1995), whereas ash content
162 was determined in a muffle furnace by incineration at 910 °C. The dietary fibre content
163 was measured by the total dietary fibre assay procedure of AOAC Method 991.43 (Lee,
164 Prosky, & De Vries 1992), and *myo*-inositol phosphates (InsP_6, InsP_5, InsP_4 and InsP_3)
165 were determined by HPLC, being the detection limit at 0.01 μmol/g (Sanz-Penella et al.,
166 2009). The total Fe, Ca and Zn concentrations in bread samples were determined using
167 a flame atomic absorption spectrometer at the Servei Central d'Instrumentació
168 Científica from the University of Jaume I (Garcia-Mantrana et al., 2014).
169
170 *2.4. Technological parameters of bread*
171 The technological parameters analysed were: weight (g), volume (cm^3) (seed
172 displacement), loaf specific volume (cm^3/g), width of central slice/height of central slice
173 ratio (cm/cm), moisture content of AACC Method 44-15A (1995) (g/100 g) and the
174 crumb firmness using a TA.XT Plus Texture Analyser (Stable Micro Systems,
175 Godalming, United Kingdom) (Sanz-Penella et al., 2009). Bread slices of 1 cm
176 thickness were compressed twice by using a stainless steel 1.0 cm diameter plunger,
177 moving at 1.0 mm/s to a penetration distance of 50 %, with an interval of 50 s between

compressions. Each parameter was measured at least in triplicate. The tristimulus colour parameters L^* (lightness), a^* (redness to greenness) and b^* (yellowness to blueness) of the baked loaves (crumb and crust) were determined using a digital colorimeter (Chroma Meter CR-400, Konika Minolta Sensing, Japan), previously calibrated with the white plate supplied by the manufacturer. The instrument settings were: illuminant C, display L^* a^* b^*, and observer angle 10°. From the parameters determined, hue angle (h^*), chroma (C^*) and total colour difference (ΔE^*) were calculated by the equations: $h^*_{ab} = \arctan(b^*/a^*)$; $C^*_{ab} = (a^{*2} + b^{*2})^{1/2}$; $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ (Iglesias-Puig & Haros, 2013). Each sample was measured 18 times at different points to minimize the heterogeneity produced by the quinoa ingredient.

Preliminary sensory analysis of the fresh breads was performed by a panel of 50 untrained tasters who usually consume wheat bread, using a hedonic scale of global 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).

2.5. Differential scanning calorimetry (DSC) analysis

The thermal properties of starch flour during baking of the fermented dough (gelatinization) and changes induced during the bread storage (amylopectin retrogradation) were measured on a calorimeter (DSC-7, Perkin-Elmer). Indium (enthalpy of fusion 28.41 J/g, melting point 156.4 °C) was used to calibrate the calorimeter. Fermented dough samples (30–40 mg) were weighed directly in DSC stainless steel pans (LVC 0319-0218, Perkin-Elmer) and hermetically sealed (Quick-Press, 0990-8467, Perkin-Elmer). The calorimeter scan conditions used were those of the methodology described by Iglesias-Puig & Haros (2013). Briefly, to simulate the

temperature profile in the centre of the bread crumb during baking, the samples were kept at 30 °C for 1 min, heated from 30 to 110°C at 11.7 °C/min, kept at this temperature for 5 min, and cooled to 30°C at 50 °C/min. To analyse amylopectin retrogradation, heated-cooled pans were stored at 20°C for 3 days and heated again in the calorimeter from 30 to 130°C, at 10 °C/min (Iglesias-Puig & Haros, 2013). An empty pan was used as a reference and three replicates of each sample were analysed. The parameters recorded were onset temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c) of gelatinization and retrogradation transitions. Straight lines were drawn between T_o and T_c and the enthalpies associated with starch gelatinization and amylopectin retrogradation were calculated as the area enclosed between the straight line and the endotherm curve. The enthalpies were expressed in Joules per grams of dough in dry matter.

2.6. Statistical analysis

Multiple sample comparison of the means and Fisher's least significant differences (LSD) were applied to establish significant statistical differences between treatments. All statistical analyses were carried out with the Statgraphics Plus 7.1 software (Bitstream, Cambridge, MN) and differences were considered significant at $p<0.05$.

3. Results and Discussion

3.1. Bread composition

The raw quinoa flour displayed higher levels of lipids, dietary fibre and ash with regard to the wheat flour (Table 1). The chemical composition of the breads supplemented with different percentages of whole quinoa flour is presented in Table 2. As expected, the incorporation of quinoa flour to the formulation, whatever percentage was incorporated,

gradually and significantly increased lipids, total dietary fibre and ash content compared to the control sample. The moisture remained without significant differences despite the inclusion of a greater amount of insoluble dietary fibre derived from the quinoa flour. In this study a strong wheat flour with deformation energy (W) 315.10^{-4} J was used. The substitution of this strong flour by whole quinoa flour (with bran but without gluten), only resulted in small changes in the water absorption of the flour mix and thus the bread moisture did not change significantly. The soluble fibre remained almost constant without significant changes. The mineral content increased significantly as a result of the replacement of wheat flour, as was expected from the flour composition (Table 1). The substitution of 50 g/100 g of flour increased the amount of Ca from 0.35 to 1.28 mg/g, Fe from 17 to 34 μg/g, and Zn from 23 to 48 μg/g. In general, white bread has a low mineral content and should be supplemented to meet the daily requirements for different elements. In this context, whole grain breads are known to be richer sources of macro- and microelements than breads made of refined flours. Table 2 shows the contributions of Ca, Fe and Zn intake from bread with or without quinoa to the dietary reference intakes (DRIs) given by the Food and Nutrition Board of the Institute of Medicine, National Academy of Science (National Research Council, 2001; Ross, Taylor, Yaktine, & Del Valle, 2011), taking into account the World Health Organization's recommendation of a daily intake of 250 g of bread per person (Sanz-Penella et al., 2013). When expressed in terms of DRIs, the control bread contributes 7 % of the Ca recommended for adults, whereas the breads incorporating quinoa contribute almost 26 % of the daily requirement of this mineral at 50 % level of wheat flour substitution (Table 2). Regarding Fe, consumption of the control bread would provide less than 20 % of the daily requirement in women, while the bread made with quinoa flour could provide nearly 40 % of these daily requirements. The same tendency

was observed with Zn, where 50 % flour substitution could supply the whole daily requirement of this mineral in women. Moreover, consumption of 250 g of control bread satisfies around 40 % of the Fe and Zn recommendation in men, whereas bread with 50 g/100g quinoa flour could cover more than 85 % of the requirements of these microelements (Table 2). However, the pseudocereal whole flours show high concentration of phytates (*myo*-inositol hexakisphosphate or InsP_6), so the minerals are not bioavailable unless they are efficiently hydrolysed during the fermentation by the action of the endogenous phytases (Garcia-Mantrana et al., 2014). In this context, it is assumed that the contribution of daily mineral intakes of bread with quinoa are overestimated. It is therefore necessary to determine the content of phytate in bread samples. The amount of InsP_6+InsP_5 (*myo*-inositol hexakis and pentakisphosphate, respectively) in the quinoa seed was 9.75 μmol/g in dry matter (0.64 g/100 g expressed as phytic acid/100 g of quinoa in dry matter). The phytate content reported in quinoa showed a wide variation between 0.76 and 1.34 g of phytic acid/100 g (Ruales & Nair, 1993; Cook, Reddv, Burn, Juillerat & Hurrell, 1997; Hager et al., 2012), which can be explained by the fact that the InsP_6 content in grain depends on many factors (Bohn, Meyer & Rasmussen, 2008). The inclusion of whole quinoa flour in the bread formulation significantly increased the amount of phytate from non-detectable values to 4.7 μmol/g (d.m.) for control sample and formulation with 50 g of quinoa/100 g, respectively. The same trend was observed in InsP_5, and other lower *myo*-inositol phosphates (InsP_4 and InsP_3), which increased significantly with the inclusion of quinoa flour. Phytates in quinoa are mainly present in protein bodies of embryonic cells of the grain (~60 g/100 g of the total phytate), and during the breadmaking process endogenous phytases could be active (Ando, Chen, Tang, Shimizu, Watanabe & Mitsunaga, 2002; Sanz-Penella et al., 2009). The fact that all fermentation stages used

in the breadmaking process in this study lasted for approximately two hours, allows endogenous phytase to act on phytate levels. Even so, the amount of InsP_6 and lower *myo*-inositol phosphates increased as the whole quinoa flour was introduced in the formulation, with the exception of InsP_3 (Table 2), and no reduction in their levels was observed compared to those present in flours, indicating a negligible contribution of the endogenous phytase activity to phytate hydrolysis.

3.2. Phytate levels and mineral availability

The phytate/minerals molar ratios are used to predict the inhibitory effect of InsP_6 on the bioavailability of minerals (Ma, Jin, Plao, Kok, Guusie & Jacobsen, 2005). The phytate/Ca molar ratio could impair calcium bioavailability in humans at values higher than 0.24. In the case of iron, bioavailability is compromised if the phytate/Fe molar ratio is higher than 1; whereas if the phytate/Zn molar ratio is higher than 5 the bioavailability of Zn could be less than 50 % (Ma et al., 2005). The breads made with whole quinoa flour had a phytate/Zn molar ratio value higher than 5 (phytate/Zn: 5.8 and 6.4; Table 2). However, these samples showed phytate/Ca molar ratio values lower than the threshold value for Ca availability inhibition (0.15 and 0.21). On the other hand, samples with quinoa showed phytate/Fe molar ratio values higher than 1.0 (5.4 and 7.7 for samples with 25 and 50 g of quinoa flour/100 g, respectively). Therefore, the high phytate concentration resulting from the inclusion of a high proportion of whole quinoa flour lead to a predicted deficient mineral bioavailability. In general, quinoa appeared to be a good source of minerals (Ando et al., 2002), but the absorption of minerals such as Fe and Zn could be adversely affected by phytates as was predicted by the phytate/minerals molar ratios. In this sense, the strategy of including an exogenous phytase in cereal products is broadly used in feed production for monogastric animals

303 and it has also been explored in the production of cereal and legume foods for human
304 consumption (Maez, 2001; Haros et al., 2001; Sanz-Penella et al., 2012; Garcia-
305 Mantrana et al., 2014). We investigated whether addition of bifidobacterial phytases
306 was effective in the reduction of InsP_6 levels in breads made with quinoa flour. For this
307 aim doughs were prepared with the addition of purified phytases from *Bifidobacterium*
308 *pseudocatenulatum* and *Bifidobacterium longum* spp *infantis* and the InsP_6 contents
309 were determined in the resulting breads (Table 3). As earlier reported for the preparation
310 of breads with other whole flours, the bifidobacterial phytases reduced InsP_6 levels,
311 with the *B. pseudocatenulatum* enzyme being superior to the *B. longum* spp. *infantis*
312 enzyme (Garcia-Mantrana et al., 2014). Thus, the *B. pseudocatenulatum* phytase
313 lowered InsP_6 below the detection limit in breads made with 25 g of quinoa flour/100 g,
314 whereas the *B. longum* enzyme performance was lower and could not reduce InsP_6/Fe
315 ratios below the inhibition threshold. Treatment with the *B. pseudocatenulatum* enzyme
316 reduced the ratios for Zn and Fe far below the threshold values in breads made with the
317 two percentages of quinoa flour (Table 3). Therefore, phytases from these probiotic
318 microorganisms could be an attractive strategy to enhance mineral bioavailability in
319 these breads.
320
321 *3.3. Technological parameters of breads*
322 The loaf specific volume significantly decreased with the addition of quinoa (Figure 1).
323 The presence of quinoa also produced important changes in crumb hardness, showing a
324 regular increase in this parameter which reached 2.6 N at 50 % of flour substitution
325 (Table 4). Gluten content is diluted by the inclusion of an ingredient with bran and
326 without gluten, which usually results in slight hardening of the crumb structure. During
327 the simulation of baking in the differential scanning calorimeter, we observed the peak

corresponding to the process of partial gelatinization of starch, between 65.8 °C and 98.7 °C, with an enthalpy of 3.2 J/g of control dough (Table 4). The range of gelatinization temperatures underwent significant changes with the incorporation of quinoa in the formulation (Table 3). The gelatinization enthalpy of the quinoa samples showed significant differences with respect to the control. This is probably due to the presence of quinoa starch that could change the thermal parameters, as well as to the presence of a higher concentration of lipids which generally interferes with the gelatinization process (Iglesias-Puig & Haros, 2013). Our results are in the line to those from Morita, Hirata, Park & Mitsunaga (2001), who found that the substitution of wheat flour with quinoa flour resulted in a markedly higher gelatinization temperature and gelatinization enthalpy compared with control samples.

In the second heating cycle, after 3 days of storage at 20 °C, the amylopectin retrogradation peak was observed (between 42.1 and 73.0 °C) with an enthalpy of 2.24 J/g in control dough (Table 4). The quinoa ingredient produced practically no alteration in the transition temperature range, showing a tendency to reduce the final temperature. The retrogradation enthalpy tended to be significantly lower in samples with quinoa (Table 4). Whole quinoa flour has compounds such as fibre which could affect the stabilization of the water balance in the dough and may inhibit retrogradation. This situation, in addition to the lower interaction with a reduced gluten network, would make the recrystallization of amylopectin more difficult in samples containing quinoa.

In general, the tristimulus colour values in both crumb and crust were affected. The colour analysis of the crust and crumb of the products developed showed significant differences with regard to lightness, chroma, hue and ΔE^* when the quinoa concentration was raised (Table 3 and Figure 1). Greater differences were observed in the parameters that describe crust colour. The typical colour of quinoa flour in

353 comparison with wheat flour affected the colour parameters of the bread, particularly in
354 the crumb section, more strongly coloured with greater yellow components (Figure 1).
355 Quinoa contains pigments as carotenoids, chlorophyll and lignin which give the seeds
356 their colour (Ruffino, Rosa, Hilal, Gonzalez & Prado, 2010). The total colour difference
357 between samples (crust and crumb colour), ΔE^*, were higher than 5 units, indicating
358 that significant differences are perceptible to consumers by visual observation (Gilabert,
359 2002).
360 Sensory evaluation of breads made with whole quinoa flour at 25 g/100 g of flour did
361 not show significant differences with control sample (Table 4). The samples with
362 quinoa were described as having different flavour. The consumers found the crumb
363 more dense and compact in breads with 50 g of quinoa/100 g of flour comparing to the
364 control sample (Figure 1).
365
366 **4. Conclusions**
367 We have shown that bread parameters such as loaf specific volume, shape ratio of the
368 central slice, crust and crumb colour and firmness were affected by the incorporation of
369 quinoa, especially at a 50 g/100 g of addition, whereas the nutritional quality was
370 increased. These new bread products were still accepted by the consumers and the high
371 phytate contents were easily avoided by the use of exogenous phytases.
372 In conclusion, whole quinoa flour could be a good replacement for wheat flour in bread
373 formulations, increasing the product's nutritional value in terms of dietary fibre,
374 minerals, proteins and healthy fats, with only a small depreciation in bread quality at 25
375 g/100 g of flour substitution
376

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Figure captions

Figure 1. Effect of the inclusion of quinoa on loaf shape, central slice and crumb structure. Bread formulations: **a.** White Bread; **b.** Bread with 25 g of whole quinoa flour/100 g; **c.** Bread with 50 g of quinoa whole flour/100 g.

521 **Table 1.** Composition of raw materials in dry matter.[a]

Parameter	Units	Flour	
		Wheat[b]	Whole Quinoa
Moisture	g/100 g	14.5 ± 0.0	10.3 ± 0.03
Protein	g/100 g	13.50 ± 0.10	11.00 ± 0.04
Ash	g/100 g	0.63 ± 0.01	2.69 ± 0.00
Total dietary fibre	g/100 g	3.39 ± 0.23	6.72 ± 0.28
Soluble dietary fibre	g/100 g	1.72 ± 0.07	2.88 ± 0.02
Insoluble dietary fibre	g/100 g	1.68 ± 0.15	3.85 ± 0.27
Lipids	g/100 g	1.37 ± 0.01	7.45 ± 0.12
InsP_6	μmoles/g	n.d.	9.28±0.19
InsP_5	μmoles/g	n.d.	0.47±0.02
Ca	mg/100g	15.3 ±0.5	32.7±0.7
Fe	mg/100g	1.29 ±0.09	4.65±0.11
Zn	mg/100g	1.61±0.23	5.03±0.07

522 [a]Mean, n=3, [b]strong flour W: 308.10^{-4} J, InsP_6: phytic acid or phytates; InsP_5: pentakisphosphate of *myo*-

523 inositol; n.d.: not detectable

524

525 **Table 2.** Effect of whole quinoa flour on chemical composition of bread, mineral
526 dietary reference intake contribution and mineral availability prediction.

Parameter[a]	Units	Control	Quinoa Flour, g/100 g	
			25	50
Main Components				
Moisture	g/100 g d.m.	33.4±0.3 a	33.5±0.1 a	33.0±0.0 a
Protein	g/100 g d.m.	13.60±0.00 c	12.31±0.01 b	12.24±0.02 a
Ash	g/100 g d.m.	1.42±0.10 a	1.82±0.01 b	2.19±0.05 c
Lipids	g/100 g d.m.	0.86±0.02 a	1.04± 0.02 b	1.90±0.03 c
Dietary Fibre				
Total	g/100 g d.m.	5.5±0.2 a	6.3±0.1b	7.2± 0.2 c
Soluble	g/100 g d.m.	2.4±0.5 a	2.6±0.3 a	2.8 ± 0.1 a
Insoluble	g/100 g d.m.	3.1±0.4 a	3.7±0.1ab	4.4 ± 0.2 b
***myo*-Inositol Phosphates**				
InsP_3	μmoles/g d.m.	n.d.	0.34±0.03 b	0.24±0.04 a
InsP_4	μmoles/g d.m.	n.d.	0.25±0.02 a	0.31±0.04 b
InsP_5	μmoles/g d.m.	n.d.	0.48±0.01 a	0.97±0.07 b
InsP_6	μmoles/g d.m.	n.d.	2.4±0.2 a	4.7±0.4 b
Minerals				
Ca	mg/100g d.m.	35.0±0.8 a	40.5±0.7 a	128.2±0.8 b
Fe	mg/100g d.m.	1.7±0.1 a	2.5±0.1 b	3.4±0.0 c
Zn	mg/100g d.m.	2.3±0.8 a	2.7±0.1 a	4.8±0.2 b
Contribution to DRIs[b]				
Ca Adults (1000)**	%	7.0	8.0	25.7
Fe Man (8)	%	42.5	62.5	85.1
Woman (18)*		18.9	27.8	37.8
Zn Man (11)	%	41.8	49.1	87.4
Woman (8)		57.5	67.4	120.2
Threshold ratios[c]				
InsP_6/Ca > 0.24	mol/mol	0.0	0.21	0.15
InsP_6/Fe > 1.0	mol/mol	0.0	5.4	7.7
InsP_6/Zn > 5.0	mol/mol	0.0	5.8	6.4

527 [a]Mean±SD, n=3. Values followed by the same letter in the same row are not significantly different at 95
528 % confidence level; d.m., dry matter; n.d., not detected; Ins$P_{6\text{-}3}$: hexakis, pentakis, tetrakis and tri
529 phosphate of *myo*-inositol, respectively.
530 [b]DRI (Dietary Reference Intakes) contribution (%) for a daily average intake of 250 g of bread if the
531 mineral absorption inhibitors are absent. The values in parenthesis are recommended dietary allowances
532 and adequate intakes for individuals between 19 and >70 years, except for: *(between 31 and >70 years),
533 and **(men between 19 and 70 years, women between 19 and 50 years) (National Research Council,
534 2001; Ross et al., 2011).
535 [c]Threshold ratios (InsP_6/mineral) for mineral availability inhibition (Ma et al., 2005); InsP_6: *myo*-inositol
536 hexakisphosphate; minerals: Ca, Fe or Zn.

Table 3. Effect of phytase addition to breads made with whole quinoa flour on InsP_6 levels and mineral availability prediction[a].

Phytase[b]	**Sample[a]**	**InsP_6[b]**	**InsP_6/Ca[c]**	**InsP_6/Fe[c]**	**InsP_6/Zn[c]**
		μmol/g d.m.	**>0.24 mol/mol**	**>1.0 mol/mol**	**>5.0 mol/mol**
B. pseudocatenulatum	25Q	n.d.	0.0	0.0	0.0
	50Q	0.1±0.0a	0.003	0.16	0.13
B. longun spp. *infantis*	25Q	2.5±0.1b	0.25	5.6	6.01
	50Q	3.2±0.1b	0.10	5.3	4.29

[a]25Q and 50Q: bread dough formula with whole quinoa flour at 25 or 50 g/100 g of flour basis, respectively d.m., dry matter; n.d., not detected; InsP_6: hexakisphosphate of *myo*-inositol or phytate.

[b]Mean±SD, n=3. Values followed by the same letter in the same column are not significantly different at 95 % confidence level.

[c]Threshold ratios (InsP_6/mineral) for mineral availability inhibition (Ma et al., 2005), minerals: Ca, Fe or Zn.

546 **Table 4.** Effect of whole quinoa flour on bread performance

Parameter	Units	Control	Quinoa Flour, g/100 g	
			25	50
Technological Parameters[a]				
Specific Volume[b]	ml/g	4.48±0.46 c	3.46±0.04 b	2.63±0.05 a
Shape Ratio[b]	cm/cm	1.65±0.14 b	1.54±0.03 a	1.62±0.05ab
Crumb Firmness[b]	N	0.77±0.09 a	1.55±0.16 b	2.64±0.10 c
Starch Gelatinization[b]				
Onset Temperature	ºC	65.8±0.7 a	66.9±0.4 b	67.3±0.3 b
Peak Temperature	ºC	74.3±0.5 a	76.0±0.1 b	75.3±0.6 b
Conclusion Temperature	ºC	98.7±0.5 b	91.2±0.5 a	95.0±0.5 a
Gelatinization Enthalpy	J/g d.m.	3.2±0.3 a	3.7±0.1 b	3.6±0.4 b
Amylopectin retrogradation[b]**, 3 days**				
Onset Temperature	ºC	42.1±0.2 a	42.0±0.6 a	41.1±0.3 a
Peak Temperature	ºC	57.9±0.4 a	57.0±0.5 a	57.5±0.3 a
Conclusion temperature	ºC	73.0±0.6 a	72.5±0.2 a	71.6±0.6 a
Retrogradation Enthalpy	J/g d.m.	2.24±0.06 c	1.70±0.03 b	1.36±0.08 a
Crust Colour Parameters[c]				
*L**		66.3±0.4 c	52.4±1.1 b	51.5±0.9 a
*C**		37.9±0.3 c	34.6±0.8 b	32.7±0.6 a
*h**		75.0±0.2 c	66.3±0.3 b	63.1±0.5 a
*ΔE**		---	13.6±1.5 a	17.1±1.6 b
Crumb Colour Parameters[c]				
*L**		72.0±0.5 b	72.4±0.6 b	69.2±0.7 a
*C**		15.2±0.5 a	18.4±0.5 b	21.0±0.3c
*h**		95.3±0.4 c	94.7±0.6 b	93.6±0.3 a
*ΔE**		---	7.2±1.3a	8.3±0.9b
Sensory Evaluation (Hedonic Scale)[d]				
Overall Acceptability		7.94 b	7.58 b	5.94 a

547 [a]Mean, n=6, [b]n=3, [c]n=18, [d]n=50, values followed by the same letter in the same row are not significantly
548 different at 95 % confidence level; d.m. dry matter.
549

550 **Figure 1**