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## Bread with whole quinoa flour and bifidobacterial phytases increases dietary mineral intake and bioavailability

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#### 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/100 g 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  $3.46/2.63 \text{ cm}^3/\text{g}$ ), 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/100 g) 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/100 g of flour substitution. The high phytate contents were efficiently removed by phytase treatment and the breads were accepted by consumers.

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#### 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

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three crops: buckwheat (Fagoryrum esculentum, Polygonaceae), which is thought to have arisen in China, amaranth (Amaranthus spp., Amaranthaceae) and guinoa (Chenopodium guinoa 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 et al., 2014). 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 (FAOSTAT, 2013; Medina, Skurtys, & Aguilera, 2010). 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

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Abbreviations: 25Q, 25 g whole quinoa flour/75 g wheat flour; 50Q, 50 g whole quinoa flour/50 g wheat flour;  $\Delta E^*$ , 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<sub>6</sub>, phytic acid, *myo*-inositol (1,2,3,4,5,6)-hexakisphosphate or phytate; InsP<sub>5</sub>, *myo*-inositol triphosphate; InsP<sub>4</sub>, *myo*-inositol tetrakisphosphate; InsP<sub>3</sub>, *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;  $T_c$ , conclusion temperature of gelatinization and retrogradation transitions.

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(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-Jubete, 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 (Hager, Wolter, Jacob, Zannini, & Arendt, 2012; Hurrell, 2003; 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 (García-Mantrana, Monedero & Haros, 2014; Sanz-Penella et al., 2012).

Quinoa is also noteworthy for its high protein content with a balanced composition of essential amino acids (Comai et al., 2007; Repo-Carrasco-Valencia et al., 2003). 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 (Alvarez-Jubete, Arendt, & Gallagher, 2010; Schoenlechner, Drausinger, Ottenschlaeger, Jurackova & Berghofer, 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 (*C. 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).

#### Table 1

Composition of raw materials in dry matter.<sup>a</sup>

Parameter	Units	Flour	
		Wheat <sup>b</sup>	Whole quinoa
Moisture	g/100 g	14.5 ± 0.0	10.3 ± 0.03
Protein	g/100 g	$13.50 \pm 0.10$	$11.00 \pm 0.04$
Ash	g/100 g	$0.63 \pm 0.01$	$2.69 \pm 0.00$
Total dietary fibre	g/100 g	3.39 ± 0.23	$6.72 \pm 0.28$
Soluble dietary fibre	g/100 g	$1.72 \pm 0.07$	$2.88 \pm 0.02$
Insoluble dietary fibre	g/100 g	$1.68 \pm 0.15$	$3.85 \pm 0.27$
Lipids	g/100 g	$1.37 \pm 0.01$	$7.45 \pm 0.12$
InsP <sub>6</sub>	μmoles/g	n.d.	9.28 ± 0.19
InsP <sub>5</sub>	μmoles/g	n.d.	$0.47 \pm 0.02$
Ca	mg/100 g	$15.3 \pm 0.5$	$32.7 \pm 0.7$
Fe	mg/100 g	$1.29 \pm 0.09$	$4.65 \pm 0.11$
Zn	mg/100 g	$1.61 \pm 0.23$	$5.03 \pm 0.07$

 $InsP_6$ : phytic acid or phytates;  $InsP_5$ : pentakisphosphate of *myo*-inositol; n.d.: not detectable.

<sup>a</sup> Mean, n = 3.

<sup>b</sup> Strong flour W: 308.10<sup>-4</sup> J.

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 °C. 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 °C 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 with addition of exogenous phytase, the enzyme was added during mixing stage. The total phytase activity in the doughs was doubled by adding the same units of phytase that were present in the flour mixtures. Phytase activity was determined for the purified enzymes and in flours as previously described (Haros, Rosell, & Benedito, 2001).

#### 2.3. Composition of raw materials and bread

Protein determination was carried out by the Kjeldahl technique (f: N × 5.7) (AACC Method 46-13, 1995). Lipid content was extracted with petroleum ether under reflux conditions by the Soxhlet technique (AACC Method 30-20, 1995), whereas ash content was determined in a muffle furnace by incineration at 910 °C. The dietary fibre content was measured by the total dietary fibre assay procedure of AOAC Method 991.43 (Lee, Prosky, & De Vries, 1992), and *myo*-inositol phosphates (InsP<sub>6</sub>, InsP<sub>5</sub>, InsP<sub>4</sub> and InsP<sub>3</sub>) were determined by HPLC, being the detection limit at 0.01 µmol/g (Sanz-Penella et al., 2009). The total Fe, Ca and Zn concentrations in bread samples were determined using a flame atomic absorption spectrometer at the Servei Central d'Instrumentació Científica from the University of Jaume I (Garcia-Mantrana, Monedero, & Haros, 2014).

#### 2.4. Technological parameters of bread

The technological parameters analysed were: weight (g), volume ( $cm^3$ ) (seed displacement), loaf specific volume ( $cm^3/g$ ),

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