EFFECT OF WHOLE AMARANTH FLOUR ON BREAD PROPERTIES AND NUTRITIVE VALUE

J.M. Sanz-Penella[a,b], M. Wronkowska[b], M. Soral-Smietana[b], M. Haros[a*]

[a]Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC),
Av. Agustín Escardino 7. Parque Científico, 46980 Paterna-Valencia, Spain

[b]Institute of Animal Reproduction of Food Research of the Polish Academy of Sciences, Division of Food Science, Department of Functional Properties of Food, 10 Tuwima Str., 10-747 Olsztyn, Poland.

*Corresponding Author. Tel.: +34 96 390 00 22; Fax.: +34 96 363 63 01
e-mail: mharos@iata.csic.es (Monika Haros)

Abbreviations: *a** and *b**, colour-opponent dimensions; DRIs, dietary reference intakes; E_1, energy during first compression; E_2, energy during second compression; F_1, force during first compression; F_2, force during second compression; InsP_6, phytic acid or *myo*-inositol hexakisphosphate; InsP_5, *myo*-inositol pentakisphosphate; InsP_4, *myo*-inositol tetrakisphosphate; InsP_3, *myo*-inositol triphosphate; InsP_2, *myo*-inositol diphosphate; InsP_1, *myo*-inositol monophosphate; L*, Lightness; VRC, volume recovery coefficient; WAF: whole amaranth flour.

Abstract

This study investigated the effect of replacing wheat flour by whole *Amaranthus cruentus* flour (up to 40 g/100g) to evaluate its potential utility as a nutritious breadmaking ingredient. The incorporation of amaranth flour significantly increased protein, lipid, ash, dietary fibre and mineral contents. Breads with amaranth have significantly higher amounts of phytates and lower *myo*-inositol phosphates, which could predict low mineral bioavailability at high levels of substitution (30–40 g/100g). An increase in crumb hardness and elasticity was observed, and tristimulus colour values were significantly affected when the amaranth concentration was raised. Mineral contents, both micro- and macroelements, were increased significantly by the wheat flour substitution. Whole amaranth flour could be used as a partial replacement for wheat flour in bread formulations, increasing the product's nutritional value and raising dietary fibre, mineral and protein levels, with a significant slight depreciation in bread quality when used in proportions between 10 and 20 g/100g. Thus, the inclusion of amaranth flour could be limited to a maximum proportion of 20 g/100g, thereby maintaining both product quality as well as the nutritional benefit of this ingredient.

Key words: whole amaranth flour, bread, minerals, phytate, bread performance.

1. Introduction

Whole grain may increase the nutritional value of bakery products made with refined wheat flour (Marquart, Asp & Richardson, 2004; Sanz-Penella, Collar & Haros, 2008; Miller Jones, 2009). One possibility would be to include whole amaranth grain in bread formulations or bakery products. Amaranth is one of the most important pre-Hispanic crops and was part of the diet of the Aztecs, Mayas, Incas and other pre-Colombian civilizations. It belongs to the family of pseudocereals as it has similar properties to those of cereals but botanically does not belong to that family. The genus *Amaranthus* includes more than 60 species that are grown in various parts of the world, such as Central and South America, India, Africa and China (Budin, Breene, & Putnam, 1996). There is increasing interest in the consumption of this genus in Europe, the USA and Japan, and it is already grown in some parts of these regions. Most species are considered as opportunistic weeds and only three of them, *A. caudatus*, *A. cruentus and A. hypochondriacus*, are commonly consumed by humans as a seed or used as a functional ingredient in foods (Gamel, Linssen, Mesallam, Damir, & Shekib, 2006). The amaranth grain can be toasted, popped, extruded or milled into flour and can therefore be consumed as such or included in other cereal products such as bread, cakes, muffins, pancakes, cookies, dumplings, crepes, noodles and crackers. The nutritional quality of amaranth seed is higher than that of most cereal grains, owing to its high protein content and balanced essential amino acid composition (Oszvald, Tamás, Rakszegi, Tömösközi, Békés & Tamás, 2009). Moreover, amaranth grain protein is rich in lysine, which is usually deficient in cereal grains. The total mineral content has been reported to be generally higher than that observed in cereal grains, especially calcium and magnesium (Alvarez-Jubete, Auty, Arendt, & Gallagher, 2010). On the other hand, it is characterized by higher dietary fibre and lipid content than most cereals and also contains between 50 and 60 g of starch per 100g of grains (Alvarez-Jubete et al., 2010).

Amaranth oil is reported to have high levels of tocotrienols and squalene, which are natural organic compounds that are involved in the metabolism of cholesterol and that could play an important role in lowering LDL-cholesterol in blood (Bodroza-Solarov, Filiocev, Kevresan, Mandic & Simurina, 2008; Budin et al., 1996). The optimal nutritive composition of this seed has made its use attractive as a blending food source to improve the nutritional value of some cereal by-products. Protein content was significantly increased by up to 4.4 g/100g by using popped amaranth grain or amaranth flour in bread, with maximum levels of substitution of 20 g/100g (Bodroza-Solarov et al., 2008; Tosi, Re, Masciarelli, Sanchez, Osella, & de la Torre, 2002). Mineral and dietary fibre contents in bread and pasta were also significantly increased by flour substitution at levels up to 20 g/100g (Dyner et al., 2007). With regard to sensory appreciation, bakery products incorporating amaranth have been accepted at levels up to 15–25 g/100g (Bodroza-Solarov et al., 2008; Sindhuja, Sudha, & Rahim, 2005). Despite all the virtues attributed to amaranth grain, there have been reports of the presence of some anti-nutritional factors, such as phenolic compounds, trypsin inhibitors and phytic acid (*myo*-inositol hexakisphosphate, InsP_6) or its salts, the phytates (Gamel et al., 2006). Phenols and trypsin inhibitors are at such low levels that they do not present a risk to the nutritional status (Bodroza-Solarov et al., 2008). Phytate content in various whole grains of the *Amaranthus* genus has been published, ranging from 4.8 to 9.4 μmol/g (Lorenz & Wright, 1984; Teutonico & Knorr, 1985; Colmenares de Ruiz & Bressani, 1990). Phytic acid intake has been reported to have favourable effects, such as antioxidant function, prevention of heart diseases and anticarcinogen effect, which it performs through its hydrolysis products (Haros, Carlsson, Almgrem, Larsson Alminger, Sandberg, & Andlid, 2009; Kumar, Sinha, Makkar & Becker, 2010). Phytic acid is strongly negatively charged and thus has a great potential for complexing positively charged multivalent cations such as calcium, magnesium, zinc, copper and

iron. This has adverse effects on mineral bioavailability, owing to the formation at physiological pH values of insoluble complexes which are non-absorbable in the human gastrointestinal tract (Sandberg, Hulthen & Türk, 1996; Lopez, Krespine, Guy, Messager, Demigne & Remesy, 2001). The negative health effects of phytates are more significant in developing countries and in risk populations owing to their higher incidence of undergoing mineral deficiencies (Hurrell, Reddy, Juillerat & Cook, 2003). During the breadmaking process phytate is sequentially hydrolysed by the action of the cereal's own phytate-degrading enzymes. However, wholegrain breads still contain high phytate levels owing to a slow and inefficient enzymatic dephosphorylation (Türk & Sandberg, 1992; Haros, Rosell & Benedito, 2001). Some strategies to reduce or eliminate phytate in breadmaking processes include increasing fermentation time, lowering process pH by the inclusion of sourdough, or adding exogenous phytase (Türk et al., 1992; Lopez et al., 2001; Sanz-Penella et al., 2008; Sanz-Penella, Tamayo-Ramos, Sanz & Haros, 2009; Sanz-Penella, Tamayo-Ramos, Wronkowska, Soral-Smietana, & Haros, 2010).

Much of the published research on phytate content in baking products has focused on wholegrain breads made from wheat, rye, rice or mixtures of them, but no data are available for the amount of phytate in bread made with amaranth flour and there is a lack of scientific reports regarding this field. Therefore the purpose of the present work was to provide further information on how replacing wheat flour by whole amaranth flour from *Amaranthus cruentus* (up to 40 g/100g) affects the phytate content of bread and its performance, and to evaluate its potential utility as a nutritious breadmaking ingredient.

2. Materials and methods

2.1. Materials

The commercial grain amaranth and flour were purchased from the local Spanish market. *Amaranthus cruentus* was used in this research, whose colour was yellow-gold. The characteristics of the commercial wheat and amaranth flours used were (g/100g): moisture 15.28±0.01 and 11.04±0.01; protein (Nx5.70) 11.70±0.06 and (Nx5.85) 14.04±0.01 dry matter (d.m.); fat content 1.11±0.01 and 6.04±0.01 d.m.; and ash 0.53±0.01 and 2.44±0.08 d.m., respectively. Mineral content and the amount of *myo*-inositol phosphates are summarized in Table 1. Compressed yeast (*Saccharomyces cerevisiae,* Lesaffre, Wołczyn, Poland) was used as starter.

2.2. Breadmaking procedure

The bread dough formula consisted of commercial wheat flour (500 g) with replacement by different concentrations of amaranth flour, 0, 10, 20, 30 and 40 g/100g (Control, 10WAF, 20WAF, 30WAF and 40WAF, respectively), compressed yeast (15 g), sodium salt (5 g) and tap water up to optimum absorption (500 Brabender Units), between 51.0 and 58.4 g of water/100g of flour, conditioned by the formula. The ingredients were mixed (Kitchen Aid, Long Beach, USA) for 4.5 to 5.5 min, depending on the formulation, and the doughs were fermented (ZBPP, Bydgoszcz, Poland) for 60 min at 30 ºC and 65% relative humidity. The doughs were then kneaded, divided into three pieces of 250 g, put into pans and proofed under the above-mentioned conditions for 60 min. After the fermentation step, the doughs were baked in an electric oven with an incorporated proofing chamber (ZBPP, Bydgoszcz, Poland) at 225 ºC for 20 min. Finally, the bread loaves were cooled at room temperature for 60 min for their subsequent analysis. The experiments were done in triplicate.

2.3. Bread composition

Starch content was measured by the total starch assay procedure (AOAC, 1996). The resistant starch, considered as the starch fraction not hydrolysed *in vitro* by pancreatic α-amylase, EC 3.2.1.1, from porcine pancreas (Sigma, A-3176, St. Louis, USA), was determined in dried bread crumb according to the Champ, Martin, Noah & Gratas method (1999). The products of hydrolysis were extracted with 80 g/100g (v/v) ethanol and the non-digested material was solubilised in 2 mol/L KOH, and then hydrolysed with amyloglucosidase EC 3.2.1.3 (Novozymes, AMG 300L, Bagsvaerd, Denmark) into glucose. The free glucose was finally quantified with a glucose oxidase/peroxidase analysis kit (Liquick Cor-Glucose 120, Cormay, Lublin, Poland) and measured spectrophotometrically at 500 nm. Protein determination was carried out by the Kjeldahl technique. Lipid content was extracted with ethylic ether under reflux conditions in a Soxhlet. Ash content was determined in a furnace by incineration at 910 ºC. The dietary fibre content was measured by the total dietary fibre assay procedure (AOAC, 1991). Mineral contents were quantified using the atomic absorption spectroscopy method with a Unicam 939 spectrometer (Labexchange**,** Burladingen, Germany) equipped with ADAX data base, background correction and cathode lamps (Wronkowska, Troszynska, Soral-Smietana & Wolejszo, 2008). All samples were wet mineralized with a mixture of acids: nitric and perchloric (3:1). Potassium was assayed with the photometric flame method and phosphorus was investigated with the colorimetric method by molybdate with hydroquinonate and sodium sulphate (IV). For the validation of calcium measurement, a solution of lanthanum chloride was added to all samples in amounts ensuring a 0.5 g of La^{3+}/ml (Whiteside & Miner, 1984). The residual concentration of InsP_6 in the bread and the lower *myo*-inositol phosphates generated were measured following the high pressure liquid chromatographic method described by Türk et al. (1992), later modified by Sanz-Penella et al. (2008).

2.4. Technological parameters

Technological parameters analysed were: moisture content (g/100g) of whole bread, loaf specific volume (cm^3/g) and crumb texture using an Instron 1011 compression device (Instron Ltd., High Wycombe, England). The crumb samples of fresh bread (2.0 × 2.0 × 2.0 cm) were twice compressed to 70% strain at a crosshead speed of 20 mm/min (Sadowska, Błaszczak, Fornal, Vidal-Valverde & Frias, 2003). Hardness expressed as maximum force during first compression, F_1 (kPa, $Pa=N/m^2$), elasticity and cohesiveness expressed as ratios of maximum forces, F_2/F_1, and energies, E_2/E_1, determined in both compressions, and gumminess, characterized by the expression $E_2 \times F_1/E_1$ (kPa). Additionally, crumb springiness was described by volume recovery coefficient (VRC), expressed as the ratio of sample volumes before second and first compression, V_1/V_2, according to Sadowska et al. (2003). At least eight replicates were made; two loaves per baking were used in the analysis. Digital image analysis was used to measure bread crumb structure. Images were previously squared at 80 pixels per cm with a flatbed scanner (Epson Perfection V200 Photo, Nagano, Japan) supported by Epson Creativity Suite Software. Two 20 mm x 20 mm square fields of view of the central slice (20 mm thick) of each of two loaves were used, thereby yielding four digital images per each baking. Data was processed using Sigma Scan Pro Image Analysis Software (version 5.0.0, SPSS Inc., San Jose, USA). The crumb features chosen were cell area/total area (cm^2/cm^2), wall area/total area (cm^2/cm^2), number of cells per cm^2 and mean cell area (mm^2) (Sanz-Penella et al., 2009, 2010). The instrumental measurement of the bread crust and crumb colour was carried out with a HunterLab ColorFlex (Reston, USA), and the results were expressed in accordance with the CIELab system with reference to illuminant D65 and a visual angle of 10º. The measurements were performed through a 3 cm diameter diaphragm containing an

optical glass. The parameters determined were L* (Lightness, L* = 0 [black] and L* = 100 [white]), a* and b* (colour-opponent dimensions, [–a* = greenness and +a* = redness], [–b* = blueness and +b* = yellowness]). Five replicates were made (one loaf per baking was used in the analysis, so 15 replications were made in all). Each bread was cut in two halves to measure the crumb colour. All the measurements were made by placing the sample directly on the colorimeter diaphragm.

2.5. Statistical analysis

Results were expressed as the mean values of at least 3 replications. Multiple sample comparison of the means and Fisher's least significant differences (LSD) were applied to establish statistical significant 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 chemical composition of the breads supplemented with different percentages of whole amaranth flour is presented in Table 2. The incorporation of amaranth flour to the formulation, whatever percentage was incorporated, gradually and significantly increased proteins, lipids and ash content and decreased the starch content with regard to the control sample. The greater levels of proteins, lipids and ash registered in the raw amaranth flour with regard to the wheat flour directly affected the increase of these parameters, as expected. These results are in agreement with other studies on breads incorporating different types of amaranth (Diner et al., 2007; Bodroza-Solarov et al., 2008). The same tendency was observed for the loaf moisture content and total dietary fibre, modifying significantly from 38.79 to 41.94 g/100g and from 3.79 to 5.90 g/100g,

respectively, with the replacement of wheat flour by amaranth flour. The moisture increase was fundamentally due to the inclusion of a greater amount of insoluble dietary fibre with the amaranth flour, whereas the soluble fibre remained almost constant without significant changes. The resistant starch content registered slight modifications among samples, being higher in samples with the pseudocereal. The mineral content increased significantly as a result of the replacement of wheat flour as was expected, owing to the flour composition (Table 1). The substitution of 40 g/100g increased the amount of Cu from 2.25 to 4.21 μg/g, Mn from 6.39 to 19.41 μg/g, Zn from 11.65 to 24.91 μg/g, Fe from 18.85 to 43.74 μg/g, Ca from 0.31 to 0.99 mg/g, Mg from 0.29 to 1.32 mg/g and K from 1.88 to 3.21 mg/g, respectively. However, the Na level decreased or remained unchanged by the substitution because it was included in the formulation as an ingredient (3.72–4.08 mg/g). In general, white bread has a low mineral content and should be supplemented to meet the daily requirements for different elements (Dyner et al., 2007; Skrbic & Filipcev, 2008). In this context, whole grain breads are known to be richer sources of macro- and microelements than breads made of refined flours. The amounts of Cu, Zn, Fe and Mg in whole wheat bread are similar to the content in bread with 30–40 g/100g amaranth flour, while the amounts of Mn, Ca and K are close to half (Skrbic et al., 2008). Table 3 shows the contributions of mineral intake from bread with or without amaranth to the dietary reference intakes (DRIs) given by the Food and Nutrition Board of the Institute of Medicine, National Academy of Science (NAS, 2004), taking into account the World Health Organization's recommendation of a daily intake of 250 g of bread per person. When expressed in terms of DRIs, the control bread contributes 38.3% of the Cu recommended for adults, whereas the breads incorporating amaranth contribute significantly increased intakes of this mineral, ranging from 43.0 to 69.6% (10WAF and 40WAF, respectively). Moreover, consumption of the control bread satisfies 42.5 or 54.3% of the Mn recommendation, whereas bread with 20-30

g/100g amaranth flour could cover the requirements of this microelement in adults (Table 3). Regarding Zn, consumption of the control bread would provide only a fifth (or less) of the daily requirement in adults, while the bread made with amaranth flour could provide nearly 50% of these daily requirements in females. The same tendency was observed with Fe, where 20% flour substitution could supply more than 50% of the daily requirement of this mineral in males. The macronutrients followed the same trend. About 50% of the requirements of Mg and P could be covered by the inclusion of amaranth in the bread formulation (30–40 g/100g). However, the P in cereal and pseudocereal whole flours corresponds almost exclusively to phytic phosphorus; it might not be bioavailable unless it is hydrolysed during the fermentation by the action of the endogenous phytase in the cereal. Moreover, it is known that the bioavailability of minerals depends on the presence of certain anti-nutrients, including phytic acid, which act as inhibitors of mineral uptake and have adverse effects on their bioavailability, owing to the formation of insoluble complexes (Sandstrom & Sandberg, 1992; Lopez et al., 2001). Solubility in the gastrointestinal media (bioaccessibility) is a pre-requisite for absorption by enterocytes in the intestine. In this context, it is assumed that the predicted intakes that are derived from DRIs for the minerals analysed in this study are almost certainly overestimated. It is therefore necessary to find out the content of this anti-nutritional compound in the bread samples. In order to determine how the inclusion of amaranth flour affected phytate and lower *myo*-inositol phosphate concentrations, the amount of InsP_6 and its hydrolysis products were measured (Table 2). The amount of phytates in the amaranth seed was 21.1 μmol/g in dry matter (Table1), which was higher than in previous investigations. The phytate content reported in amaranth from *A. cruentus*, *A. hypochondriacus* and *A. hybridus* showed a wide variation between 4.8 and 9.4 μmol/g (Lorenz et al., 1984; Teutonico et al., 1985; Colmenares de Ruiz et al., 1990), taking into account the fact that the InsP_6 content in

grain depends on many factors (Bohn, Meyer & Rasmussen, 2008). The inclusion of whole amaranth flour in the bread formulation significantly increased the amount of phytate from non-detectable values to 2.35 μmol/g (d.m.) for the control sample and 40 g/100g WAF, respectively. The same tendency was observed in InsP_5, and even more in InsP_4 and InsP_3, which increased significantly with the inclusion of amaranth flour. Phytates are mainly present in outer layers of the grain, and during the breadmaking process endogenous phytate-degrading enzymes with the potential to hydrolyse phytates to InsP_5–InsP_3 could be active (Sanz-Penella et al., 2008; 2009). The fermentation stage used in the breadmaking process in this study was maintained for two hours. Consequently, the endogenous phytase could have had enough time to significantly reduce the phytate content present in the amaranth flour. Even so, the amount of InsP_6 and lower *myo*-inositol phosphates increased as the whole amaranth flour was introduced in the formulation. 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/calcium molar ratio could impair calcium bioavailability in humans at values higher than 0.24. In the case of iron, if the molar ratio is more 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 sample made with 40 g/100g whole amaranth flour had a phytate/Zn molar ratio value higher than 5 (phytate/Zn: 6.17). This sample and 30WAF both showed phytate/Ca molar ratio values higher than 0.24 (0.95 and 0.43, respectively). The phytate/Fe molar ratio showed values higher than 1 for the 30WAF (1.42) and 40WAF (3.00) samples. The high phytate concentration resulting from the inclusion of a high proportion of whole amaranth flour in the bread formula (30–40 g/100g) could lead to a mineral bioavailability that is deficient, or at least reduced in the cases of zinc, calcium and iron. Studies carried out with the Caco-2 cell line supported this hypothesis, showing

inhibition of the iron bioavailability of samples with 40% flour substitution. Nevertheless, the use of up to 20 g/100g amaranth flour allowed an increase in iron uptake with regard to the control sample (Sanz-Penella, Laparra, Sanz & Haros, 2011). Moreover, it must be emphasized that some lower *myo*-inositol phosphates are considered compounds that could perform positive biological functions in the human body such as second messenger, bringing about a range of cellular functions including cell proliferation via intracellular Ca^{2+} mobilization, particularly InsP_3 (Shi, Azab, Thompson & Greenberg, 2006; Haros et al., 2009).

3.2. Technological parameters

The parameters that describe the quality of bread are shown in Table 4. The loaf volume slightly decreased with the addition of amaranth, while the weight of breads remained almost constant among samples. Consequently, the loaf specific volume showed a slight tendency to decrease with the inclusion of amaranth flour up to 40 g/100g in the formulation, from 2.74 to 2.51 ml/g, with significant differences for the highest level of addition. The presence of amaranth did not produce meaningful changes in crumb hardness; only the sample with 40 g/100g substitution showed a value significantly higher than the control bread. In the case of crumb elasticity, a significant increase was observed in breads with between 30 and 40 g/100g with regard to the control sample. Opposite behaviour was shown in cohesiveness, gumminess and VRC parameters, and a significant decrease with regard to the control sample was recorded with the inclusion of amaranth flour. Morita, Kang, Hamazu and Sugimoto (1999) showed a regular increase in hardness of breads with an increase of amaranth flour from 5 to 20 g/100g. Other researchers observed a significant decrease in loaf specific volume, with harder breads produced when increased levels of replacement with amaranth were used (Bodroza-Solarov et al., 2008). Gluten content is diluted by the inclusion of amaranth

flour, which usually results in slight hardening of the crumb structure. However, Oszvald et al. (2009) found that amaranth albumin proteins are capable of interacting with gluten proteins in wheat flour, showing similar effects to gluten subunits. This could lead to little change in crumb hardness, as the present study shows (Table 4). On the other hand, the lipid content in amaranth flour, which is 6 times higher than in wheat flour, may act as a surface active agent. The high polar lipid content in amaranth, in general about 10 g/100g of total lipids, may have functionality as a gas stabilising agent during breadmaking, which probably improves bread elasticity (Alvarez-Jubete et al., 2010).

The effect of the inclusion of amaranth flour on crumb structure showed no significant changes in nearly all of the parameters analysed, although some slight variations were observed. Only a significant increase in the mean cell area was recorded for the samples with a substitution percentage between 20 and 40 g/100g. Moreover, the samples with amaranth flour showed a tendency to increase cell area, but without significant differences with regard to the control sample. There were changes in crumb and crust colour due to the inclusion of amaranth flour, which were estimated by the CIELab system (Table 4). In general, the tristimulus colour values in both crumb and crust were affected when the amaranth concentration was raised (Table 4). The crust redness was statistically higher in breads with amaranth, whereas the yellowness showed an opposite behaviour in comparison with the control sample. The crust lightness showed slight changes and was significantly lower than the control bread when a high concentration of amaranth flour was used in the formulation. Crumb tristimulus colour parameters were more affected than the crust with the inclusion of amaranth flour. Lightness was significantly lower than the control, with darker, more strongly coloured crumbs, with greater red and yellow components. The typical darker colour of amaranth flour in

comparison with wheat flour affected the colour parameters of the bread, particularly in the crumb section.

Preliminary sensory evaluation studies of breads made with whole amaranth flour showed that they did not achieve greater acceptability than the control bread. They were described as having a different flavour, a slightly bitter nutty taste (results not shown). The consumers also concluded that if bread with amaranth (in proportions between 10 and 20 g/100g) is more nutritious they would choose to consume it even though its taste and aroma are different from those of traditional bread.

4. Conclusions

Whole amaranth flour from *Amaranthus cruentus* could be used as a replacement for wheat flour in bread formulations, increasing the product's nutritional value and providing an increase in dietary fibre (with the resistant starch level remaining constant) and also mineral and protein levels, with a slight depreciation in bread performance when used in proportions between 10 and 20 g/100g. High levels of phytates were found in amaranth flour and this contributed to similarly high phytate levels in bread containing high proportion of amaranth (30-40 g/100g), which could affect the mineral bioavailability of zinc, calcium and iron, as was predicted by phytate/mineral ratios. The inclusion of amaranth flour in bakery products could be limited to a maximum proportion of 20 g/100g, not only for maintaining product quality but also for preserving the principal nutritional benefit of this ingredient.

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13

1 **Table 1.** Mineral and *myo*-inositol phosphates content of flours

Sample[a]	**Units**[b]	**Wheat flour**	**Whole amaranth flour**
Ash	g/100g	0.53±0.01	2.44±0.08
Microelements			
Cu	μg/g	1.83±0.03	6.94±0.01
Mn	μg/g	5.82±0.01	36.55±0.12
Zn	μg/g	7.35±0.10	42.08±0.32
Fe	μg/g	12.66±0.04	82.13±0.17
Macroelements			
Ca	mg/g	0.22±0.01	2.04±0.01
Mg	mg/g	0.25±0.01	2.69±0.01
P	mg/g	1.11±0.02	5.30±0.02
Na	μg/g	112.4±1.4	8.21±0.27
K	mg/g	1.56±0.01	4.70±0.03
***Myo*-inositol phosphates**			
InsP_6	μmol/g	n.d.	21.1±2.1
InsP_5	μmol/g	n.d.	2.3±0.5
InsP_4	μmol/g	n.d.	0.86±0.08
InsP_3	μmol/g	n.d.	n.d.

2 [a]Mean±SD, n=3; InsP_3 to InsP_6: *myo*-inositol containing 3-6 phosohates per inositol residue; not
3 detected (n.d.). [b]Units expressed in dry matter.

4

Table 2. Effect of different amount of whole amaranth flour on chemical composition of bread[ab]

Sample	Control	10WAF	20WAF	30WAF	40WAF
Main components (g/100g d.m.)					
Moisture[c]	38.79±0.03a	39.94±0.08c	39.55±0.03b	41.94±0.03e	40.51±0.10d
Starch	68.21±0.03d	68.78±0.19d	66.61±0.16c	65.52±0.06b	63.78±0.03a
Proteins	14.29±0.05a	14.66±0.17ab	14.96±0.35b	16.14±0.31c	16.30±0.05c
Lipids	0.67±0.03a	0.97±0.05b	1.29±0.08c	1.36±0.03d	1.75±0.07e
Ash	1.35±0.01a	1.54±0.01c	1.50±0.02b	1.87±0.04d	2.06±0.02e
Dietary fibre (g/100g d.m.)					
Insoluble	1.91±0.13a	2.35±0.02ab	2.96±0.10bc	3.44±0.04c	4.17±0.14d
Soluble	1.88±0.13a	1.84±0.02a	1.67±0.04a	1.62±0.07a	1.73±0.15a
Total	3.79±0.23a	4.19±0.01b	4.63±0.06c	5.06±0.03d	5.90±0.01e
Resistant starch	1.81±0.08a	2.04±0.04bc	2.10±0.10c	1.94±0.11ab	1.90±0.06ab
Mineral content					
Microelements (μg/g d.m.)					
Cu	2.25±0.01a	2.58±0.02b	3.77±0.02d	3.98±0.04c	4.21±0.01e
Mn	6.39±0.02a	9.99±.0.09b	13.02±0.04c	16.39±0.04d	19.41±0.11e
Zn	11.65±0.25a	15.75±0.04b	18.55±0.20c	21.67±0.15d	24.91±0.04e
Fe	18.85±0.11a	22.66±0.13b	30.05±0.27c	35.91±0.43d	43.74±0.28e
Macroelements (mg/g d.m.)					
Ca	0.31±0.01a	0.48±0.01b	0.64±0.01c	0.85±0.02d	0.99±0.04e
Mg	0.29±0.01a	0.53±0.01b	0.75±0.01c	1.04±0.01d	1.32±0.02e
P	1.27±0.01a	1.81±0.01b	2.12±0.02c	2.60±0.01d	3.05±0.03e
K	1.88±0.02a	2.22±0.05b	2.42±0.01c	2.86±0.01d	3.21±0.02e
***Myo*-inositol phosphates** (μmol/g d.m.)					
InsP_6	n.d.	n.d.	0.11±0.01a	0.91±0.01b	2.35±0.02c
InsP_5	n.d.	n.d.	0.24±0.06a	0.65±0.03b	0.83±0.04c
InsP_4	n.d.	0.65±0.02a	2.06±0.14b	2.65±0.02c	2.51±0.01c
InsP_3	0.31±0.08a	2.29±0.08b	2.35±0.14b	2.88±0.10c	3.07±0.06c

[a]Codes: Control, 10WAF, 20WAF, 30WAF and 40WAF: amount of amaranth flour 0, 10, 20, 30 and 40 g/100g of flour, respectively. Dry matter (d.m.); InsP_3 to InsP_6: myo-inositol containing 3-6 phosohates per inositol residue; not detected (n.d.). [b]Mean±SD, n=3. Values followed by the same letter in the same row are not significantly different ($p < 0.05$). [c]Wet basis.

Table 3. Contribution of micro- and macroelement intake to the relevant dietary reference intakes (DRIs) for consumption of a daily average portion of 250 g of bread incorporating whole amaranth flour

Nutrient	Gender	DRIs[a]	Contribution to DRIs (%)[b,c]				
		mg/day	Control	10WAF	20WAF	30WAF	40WAF
Microelements							
Cu	Adults	0.9	38.3	43.0	60.8	66.8	69.6
Mn	Male	2.3	42.5	65.2	85.5	103.4	125.5
	Female	1.8	54.3	83.3	109.3	132.2	160.4
Zn	Male	11	16.2	21.5	25.5	28.6	33.7
	Female	8	22.3	29.6	35.0	39.3	46.3
Fe	Male	8	36.1	42.5	57.8	65.2	81.3
	Female	18*	16.0	18.9	25.2	29.0	36.1
Macroelements							
Ca	Adults	1000**	4.7	7.2	9.7	12.3	14.7
Mg	Male	420*	10.6	18.9	27.0	35.9	46.7
	Female	320*	13.9	24.9	35.4	47.2	61.3
P	Adults	700	27.8	38.8	45.8	53.9	64.8
K	Adults	4700	6.1	7.1	7.8	8.8	10.2

[a]DRIs: Dietary Reference Intakes: recommended dietary allowances and adequate intakes, Elements. Life stage group: between 19 and >70 years; *between 31 and >70 years, **males between 19 and 70 years, females between 19 and 50 years. Food and Nutrition Board, Institute of Medicine, National Academy of Science (NAS, 2004). [b]Data adapted from the National Academy of Science (NAS, 2004). [c]Codes: Control, 10WAF, 20WAF, 30WAF and 40WAF: amount of amaranth flour 0, 10, 20, 30 and 40 g/100g of flour, respectively.

Table 4. Effect of different amount of whole amaranth flour on bread quality

Sample[a]	Control	10WAF	20WAF	30WAF	40WAF
Technological parameters[b]					
Loaf volume (cm^3)	620±26b	619±23b	606±26b	598±30b	553±21a
Loaf weight (g)	226±5b	225.7±2.8b	225.0±3.9b	226.5±2.3b	220.4±3.5a
Specific volume (cm^3/g)	2.74±0.16b	2.74±0.11b	2.70±0.15b	2.64±0.14ab	2.51±0.10a
Textural parameters[c]					
Hardness (KPa)	24.9±4.0a	25.1±5.2a	26.9±4.8a	29.5±5.2ab	31.9±3.0b
Elasticity	0.83±0.03a	0.83±0.03a	0.85±0.02a	0.90±0.02c	0.90±0.02b
Cohesiveness	0.45±0.03d	0.44±0.05d	0.36±0.02c	0.31±0.02b	0.30±0.01a
Gumminess (KPa)	11.0±1.2c	10.9±1.2b	9.8±1.8ab	9.2±1.4ab	9.4±0.8a
VRC	0.70±0.07d	0.69±0.07c	0.59±0.04b	0.47±0.02a	0.48±0.02a
Crust Colour parameters[d]					
L*	47.0±2.2a	48.7±2.8a	48.6±2.8a	47.6±4.3a	42.7±3.3b
a*	13.2±1.9a	13.8±1.2ab	14.9±0.7c	14.1±0.7bc	14.3±0.6c
b*	31.1±1.9a	30.5±1.6ab	28.2±2.4c	30.5±3.3ab	28.6±1.6bc
Crumb Colour parameters[d]					
L*	58.9±1.6a	56.5±1.6b	54.8±1.3c	53.0±1.6d	54.2±2.5cd
a*	1.89±0.16a	2.70±0.15b	3.30±0.14c	4.88±0.16d	5.84±0.37e
b*	21.4±0.4a	22.0±0.5ab	22.3±0.5b	25.5±0.4c	27.4±0.8d

[a]Codes: Control, 10WAF, 20WAF, 30WAF and 40WAF: amount of amaranth flour 0, 10, 20, 30 and 40 g/100g of flour, respectively. Volume Recovery Coefficient or Springiness (VRC). Mean±SD, [b]n=6; [c]n=8; [d]n=15. Values followed by the same letter in the same row are not significantly different ($p < 0.05$).