**EVALUATION OF TECHNOLOGICAL AND NUTRITIONAL QUALITY OF BREAD
ENRICHED WITH AMARANTH FLOUR**

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ABSTRACT

The objective of this investigation was to develop bread, with high nutritional and technological quality, using whole flour of *Amaranthus spinosus* and *Amaranthus hypochondriacus*. Bread quality was analyzed in terms of chemical composition, loaf specific volume, width/height ratio of the central slice, crust/crumb color, crumb structure and firmness, and sensory analysis. Starch thermal properties were studied in terms of enthalpies of starch gelatinization during baking and amylopectin retrogradation during storage. Incorporation of amaranth flour significantly increased protein, lipid, fiber, ash, and *myo*-inositol phosphate contents. Bread with amaranth flours exhibited soluble/insoluble fiber ratios close to 1:2, which presents the most effective physiological action. Intake of products with high substitution of amaranth could cover the protein requirement in adults, and could contribute substantially to intake of dietary fiber, Fe, and Zn according to daily recommendations. Bread with *A. hypochondriacus* showed higher acceptability than formulations with *A. spinosus*. Inclusion of amaranth allowed delaying and decreasing crumb staling in terms of amylopectin retrogradation. The inclusion of amaranth could be limited to a maximum of 25 g/100 g, with considerable nutritional improvement and acceptable sensory and technological quality, even during the staling process.

Keywords: *Amaranth*; bread technological/nutritional qualities; dietary fiber; *myo*-inositol phosphates; starch thermal properties

1. Introduction

Wheat bread is a popular food in the category of bakery products. Refined wheat flour is the most used raw material for this product but it has a low level of essential amino acids such as lysine and threonine, and reduced fiber content compared to whole flour bread. In fact, many studies have been aimed at improving wheat flour nutritional characteristics by the incorporation of new functional ingredients and at developing safe, healthy, nutritious products (Dewettinck et al., 2008). An alternative could be bakery products enriched with a high amount of dietary fiber, amino acids and bioactive compounds from whole flours or entire grain of cereal/pseudocereal that help to prevent diseases associated with the metabolic syndrome such as cardiovascular diseases, arteriosclerosis, and colon cancer (Salas-Salvadó, Bulló, Pérez-Heras, & Ros, 2006; Motta et al., 2019). The health benefits of pseudocereals are well known, and one of the most important of them is amaranth (*Amaranthus* spp.), which is a native plant found in South and Central America, with the exception of some species that belong to tropical and subtropical regions of India, such as *Amaranthus spinosus* (Reguera & Haros, 2017). From a nutritional point of view, pseudocereals are considered better than cereals such as wheat, barley, or rice because of their content/composition of starch, oil, dietary fiber, vitamins (A, K, B₆, C, E, and B), and minerals such as calcium, magnesium, phosphorus, iron, potassium, zinc, copper, and manganese (Alvarez-Jubete, Arendt, & Gallagher, 2009; Reguera & Haros, 2017). Although many species are considered opportunistic weeds, only three of them are used for human consumption: *A. caudatus* (or *A. paniculatus*, *kiwicha*, o *quihiucha*), *A. cruentus* (or *A. flavus*, or *A. leucocarpus*), *A. hypochondriacus* (or *A. edulis*, or *A. mantegacianus*) (Gamel et al., 2006; Sangameswan & Jayakar, 2008; Reguera & Haros, 2017). However, studies have established that the seeds or plant parts of *A. spinosus* possess various biological activities such as analgesic and anti-inflammatory, anti-diabetic, anti-hyperlipidemic and spermatogenic effects (Sangameswaran

& Jayakar, 2008; Jhade et al., 2009; Rjeibi et al., 2016). Amaranth has managed to capture growing interest as a functional ingredient because of its high-quality nutritional and technological properties, especially in baking processes, and because it is very versatile for processing and industrialization (Sanz-Penella, Wronkowska, Soral-Smietana, & Haros, 2013). Amaranth flour is being used in *tortillas*, breads, cakes, muffins, pancakes, cookies, dumplings, crepes, noodles, and crackers (Alvarez-Jubete et al., 2009). The inclusion of *A. cruentus* whole flour in bread formulations by flour substitution of up to 40 g/100 g increased protein and total dietary fiber content, but this was accompanied by a marked decrease in sensory acceptability (Sanz-Penella et al., 2013). Amaranth usually contains antinutrients such as phytic acid (*myo*-inositol (1,2,3,4,5,6)-hexakisphosphate, InsP_6) or its salts, phenolic compounds, and trypsin inhibitors (D'Amico, Schoenlechner, Tömöskösi, & Langó, 2017). Phytic acid has negative health effects in cases of mineral deficiency because it inhibits mineral availability (Sanz-Penella et al., 2013). Phytate content in *Amaranthus* spp. has been found to range from 4.8 to 21.1 $\mu\text{mol/g}$ (Reguera & Haros, 2017). However, several studies have suggested that this compound has favorable effects, such as antioxidant function, prevention of heart diseases, and an anticarcinogenic effect, which it performs through its hydrolysis products (Haros et al., 2009; Kumar, Sinha, Makkar, & Becker, 2010). There are previous studies with regard to bread made with replacement of flour by whole *A. cruentus* flour (Bodroza-Solarov et al., 2008; Sanz-Penella et al., 2013; Garcia-Mantrana et al., 2014). More recently, the investigations with *A. hypochondriacus* as a bakery ingredient were substituted up to 25 % level (Kamoto et al., 2018) or in specific breads without fermentation (Banerji et al., 2018). However, there is a lack of information about the effect of replacing wheat flour with other amaranth species, such as *A. spinosus*. Accordingly, the purpose of this research was to develop bread with high nutritional, technological, and sensory quality,

replacing wheat flour with a high level (up to 50 %) of whole amaranth flour of *A. spinosus* from India, and it was compared to formulations with *A. hypochondriacus* from Mexico.

2. Materials and Methods

2.1. Materials

Commercial wheat flour (*Triticum aestivum* L.), whole *A. spinosus* grains from India, and whole *A. hypochondriacus* grains from Mexico were purchased from the local market (Corporación Proteína Americana, SCRL, and Bio Cesta, MTV, Spain, respectively). The characteristics of the wheat flour, whole *A. spinosus* flour, and whole *A. hypochondriacus* flour is shown in Table 1. *Saccharomyces cerevisiae* (Levamax, Spain) was used as starter for the breadmaking process.

2.2. Methods

2.2.1. Breadmaking process

The bread dough formula expressed on a flour basis consisted of different flour formulations (500 g), compressed yeast (5%), sodium chloride (1.8%), and water up to optimum absorption corresponding to 500 BU (Brabender Units) (between 50% and 60%, depending on the bread dough formulation). Five formulations were studied, on a flour basis: 100% refined wheat flour as Control (C), and 4 formulations in which wheat flour was replaced with 25% of whole *A. spinosus* flour (WAs-25), 50% of whole *A. spinosus* flour (WAs-50), 25% of whole *A. hypochondriacus* flour (WAh-25), and 50% of whole *A. hypochondriacus* (WAh-50). A two-stage sponge and dough method was used according to the methodology described by Iglesias-Puig, Monedero, & Haros (2015). Finally, the samples were baked at 170 °C/20 min for control sample, 165 °C/25 min for WAh-25 and WAs-25, and 165 °C/25

min for WAh-50 and WAs-50. The breads were cooled at room temperature for 2 hours for further analysis (Sanz-Penella, Tamayo-Ramos, Sanz, & Haros, 2009).

2.2.2. Composition of raw materials and bread

Moisture was determined by a gravimetric method (AACC 44-15A), ash content was determined in a muffle by incineration at 900 °C (ICC 104/1, 1990), protein determination was carried out by the Kjeldahl technique (AACC 46-13), lipids were determined by extraction with petroleum ether reflux conditions by the Soxhlet technique (AOAC Official Method 945.16), and dietary fiber content was measured by an enzymatic-gravimetric method (AOAC Official Method 991.43).

2.2.3. Determination of minerals

The total Ca, Fe and Zn concentrations in samples were determined using a flame absorption spectrometer at the Servicio de Análisis de suelos, plantas y agua from Institute of Agricultural Science (Madrid, Spain). Previously, samples (0.5 g) were placed in a Teflon perfluoroalkoxy (PFA) vessels and treated with 4 mL HNO₃ 14 M (Merck, Germany) and 1 mL of H₂O₂ 30% (v/v) (Panreac Quimica, Spain). The Teflon PFA vessels were irradiated at 800 W (15 min at 180 °C) in a Microwave Accelerated Reaction System (MARS) from CEM (Vertex, Spain). At the of the digestion program, the digests were placed in polypropylene tubes and made up to final volume with 5% HCl. Measurements were done in triplicate.

2.2.4. Determination of *myo*-inositol phosphates

InsP₆ present in the flours and the remaining InsP₆ and lower *myo*-inositol phosphates generated during the breadmaking process (InsP₅, InsP₄, and InsP₃) were purified by ion-

exchange chromatography and measured by the HPLC method described by Türk and Sandberg (1992), later modified by Sanz-Penella, Collar, & Haros (2008).

2.2.5. Technological characteristics of bread

The technological parameters analyzed were as follow: loaf specific volume (cm^3/g) by the measured of the volume (cm^3) by seed displacement (volume-meter, Chopin, France) and the weight (g), width/height ratio of the central slice (cm/cm), and color parameters (Chromameter CR-400, Konika Minolta Sensing, Japan).

Crumb texture was determined by texture profile analysis using the TA-XT Plus Texture Analyser (Stable Micro Systems, Godalming, United Kingdom). A bread slice of 2 cm thickness was compressed twice by using a stainless steel 1.0 cm diameter plunger, moving at 1.0 mm/s to a penetration distance of 50%, with an interval of 50 s between compressions. The following parameters were evaluated: relative firmness, springiness, cohesiveness, chewiness, and resilience (Haros et al., 2002).

Digital image analysis was used to measure bread crumb structure. Images were previously squared at 240 pixels per cm with a flatbed scanner (HP ScanJet 4400C, Hewlett-Packard, USA) supporting by HP PrecisianScan Pro 3.1 Software. A single 10 mm $\times$ 10 mm square field of view of two central slices (10 mm thick) of each of two loaves was used, thereby yielding 4 digital images per treatment. Data was processed using Sigma Scan Pro Image Analysis Software (version 5.0.0, SPSS Inc., USA). The crumb grain 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, μm^2 (Sanz-Penella et al., 2009).

Preliminary sensory analysis of the fresh breads was performed by a panel of 25 untrained tasters who usually consume wheat bread, using a nine point hedonic scale of global acceptance (Iglesias-Puig et al., 2015).

2.2.6. 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, PerkinElmer, USA) according the methodology described by León, Durán, & Benedito de Barber (1997), later modified by Sanz-Penella et al. (2009).

2.2.7. Statistical analysis

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

3. Results and Discussion

3.1. Bread Performance

The incorporation of increasing percentage of amaranth flour in the dough formulations progressively and significantly increased protein, lipid, and ash contents with regard to the control sample (Table 2). The higher levels of these nutrients registered in the raw amaranth flours in comparison with the wheat flour directly affected these parameters, as expected. These results agree with other studies on breads incorporating a different species of amaranth (Sanz-Penella et al., 2013). Those authors also found a higher moisture content when they increased the wheat flour substitution from 38.8 % (control sample) to 40.5 % (bread with *A. cruentus* at 40% substitution level). However, in this study only the substitution with *A. hypochondriacus* at 50% showed a significant difference in this parameter with regard to the control bread (Table 2). Despite the higher water absorption of flour blends measured by the

194 farinograph, from 55.0% for wheat flour to 57.5%–60.5% for the wheat/amaranth
195 combinations, with a higher water retention capacity of the doughs when the whole amaranth
196 flour in the formulation was increased, in general, amaranth did not significantly modify the
197 moisture of the fresh bread, with the exception of WAh-50 (Sanz-Penella et al., 2008).
198 However, it has been suggested that a higher amount of total dietary fiber is related with an
199 increase of moisture, mainly caused by the greater number of hydroxyl groups in the fiber
200 structure, which allow more water interaction through hydrogen bonding than in refined flour
201 (Sudha, Vetrimani, & Leelavathi, 2007). In the current study, it was probably because of the
202 use of a strong wheat flour in formulations with a high protein content that the water not only
203 bound to starch and fibers but also to the gluten network, both in the control sample and in
204 the samples with amaranth (Salas-Mellado & Haros, 2016). As was expected, the
205 incorporation of whole amaranth flours in the formulation progressively and significantly
206 increased the total dietary fiber (Table 2). In general, previous studies have shown that
207 pseudocereals are a good source of dietary fiber (Alvarez-Jubete et al., 2009; Iglesias-Puig et
208 al., 2015; Reguera & Haros, 2017). In the current investigation, the amount of insoluble
209 dietary fiber increased significantly with the inclusion of amaranth flours, from 3.9 to 7.9
210 g/100 g with regard to the control bread. These values are higher than the results obtained in
211 bread with whole quinoa flour at 25 and 50%, in which the amount of insoluble fiber was 3.7
212 and 4.4 g/100 g, respectively (Iglesias-Puig et al., 2015). It is noteworthy that a similar trend
213 was observed in bread formulations with up to 40% replacement with *A. cruentus* flour
214 (Sanz-Penella et al., 2013). In general, cereals and pseudocereals have more insoluble fiber,
215 mainly consisting of lignin and cellulose. However, in amaranth there is more total dietary
216 fiber than in common cereals, and a higher concentration of soluble fiber (Repo-Carrasco-
217 Valencia & Valdez, 2017). Dietary fiber exhibits the most effective physiological action at a
218 soluble/insoluble ratio of 1:2 (Jaime et al., 2001), but the diet contains a variety of foods,

such as wholegrain cereals, nuts, and vegetables, with a ratio of 1:3 (Salas-Salvadó et al., 2006). In the current investigation, an increase in the whole amaranth flour content resulted in breads with soluble/insoluble fiber ratios closer to 1:2 than 1:3, with the exception of WAs-50, which was close to the latter ratio (Table 2). On the other hand, in the formulation with 50% of *A. spinosus* the amount of 11.7 g/100 g of total dietary fiber (Table 2) is close to the minimum percentage required daily (25 grams per day according to the WHO/FAO, 2004), assuming an intake of 250 g of bread per day. The substitution of 50% of wheat flour by whole *A. spinosus* or *A. hypochondriacus* flour contributed to an increase in the intake of total dietary fiber, reaching 54/53% of the AI for adult men and 83/80% for adult women, respectively (Food and Nutrition Board, Institute of Medicine, 2005). Therefore, the compositional and structural characteristics of dietary fiber from pseudocereals such as amaranth, buckwheat, or quinoa suggest a good potential for exerting a favorable function by regulation of intestinal transit, and reduction of the risk of diabetes, hypertension, coronary heart disease, cardiovascular disease, and colon cancer (Salas-Salvadó et al., 2006).

The mineral content increased significantly as a result of the replacement of wheat flour with up to 50% of amaranth flour, with ash content increasing from 1.7% to 3.5% and 4.0% in WAs-50 and WAh-50, respectively, suggesting the presence of major minerals such as calcium, magnesium, potassium, phosphorus, iron, and zinc (Reguera & Haros, 2017). However, their bioavailability would depend on the content of antinutritional factors, such as phytic acid, which forms insoluble complexes (Sanz-Penella et al., 2009) (Table 2). Furthermore, the amaranth breads increased the protein amount to values ranging from 17.6 to 19.0 g/100 g, compared to 16.5 g/100 g in the control bread. The balanced amino acid composition of amaranth proteins, as reported, is close to the optimum protein reference pattern of the human diet according to FAO/WHO requirements (D'Amico et al., 2017). With a daily intake of 250 g of bread/day/person, bread with amaranth would cover the

requirement of adults (over eighteen, with a weight range of 55–60 kg) according to the adult protein requirements declared by FAO/WHO/UNU (2007). Sanz-Penella et al. (2013) reported that bread with *A. cruentus* (up to 40% substitution) had a protein content of 16.3 g/100 g, d.b.; in the current investigation the results were higher, even when the level of replacement was lower (25%), with both *A. hypochondriacus* and *A. spinosus* (Table 2). The same trend was observed by Iglesias-Puig et al. (2015) in bread with 50% replacement with quinoa, which had a protein content of 12.4 g/100 g. In general, the protein content in pseudocereals is usually higher in amaranth, followed by quinoa and buckwheat, and higher in pseudocereals than in common cereals such as wheat (D'Amico et al., 2017).

3.2. Effect of formulation on *myo*-inositol phosphate profile in bread

The amount of phytates in the *A. hypochondriacus* and *A. spinosus* whole flours was 18 and 21 $\mu\text{mol/g}$ dry matter, respectively. Similar values in *A. cruentus* were reported by Sanz-Penella et al. (2013), in contrast with other investigations, which reported values between 4.8 and 9.4 $\mu\text{mol/g}$ in *A. cruentus*, *A. hypochondriacus*, and *A. hybridus* (Lorenz & Wright, 1984). The inclusion of amaranth flour in the bread formulations increased the phytic acid content from negligible values in the control sample to 3.6/3.7 $\mu\text{mol/g}$ in WAs/WAh-25 and 8.5/8.8 $\mu\text{mol/g}$ in the WAh/WAs-50 formulations (Table 2). The reduction of chelating capacity (expressed as phytic acid content) in these breads was around 54–58% in bread with *A. spinosus* and *A. hypochondriacus* flour substituted at 50% and around 80–83% in bread at 25%. However, the reduction of InsP_6 is lower in the current study than in the formulations with *A. cruentus*, probably owing to the longer fermentation time used in that investigation (Sanz-Penella et al., 2013). The phytates could be hydrolyzed as a result of the action of endogenous phytase enzymes during the cereal/pseudocereal fermentation step, and as the

fermentation time increases the phytic acid content decreases (Siwatch, Yadav, & Yadav, 2019).

3.3. Effect of bread formulation on minerals and mineral dietary reference intake contribution

The mineral content increased significantly as a result of the replacement of wheat flour, as was expected, owing to the flour composition. The substitution of WAh/WAs-25 and WAh/WAs-50 contributed between two and four times more Ca, Fe, and Zn than the control bread. In addition, as a result of the incorporation of whole amaranth flour the contribution of the minerals intake increased the dietary reference intakes (DRIs) for a daily average intake of 250 g of bread if the mineral absorption inhibitors are absent, according to the Food and Nutrition Board of the Institute of Medicine, National Academy of Science (NAS, 2004). With regard to Zn, consumption of the control bread would provide only 27% or less of the daily requirement in adults, while the bread made with WAh/WAs could provide nearly 50% of the daily requirement for women. The same tendency was observed with Fe, where WAh-25/WAs-25 could supply males with more than 70% of the daily requirement of this mineral. With respect to macrominerals such as calcium, they could supply nearly four times as much as wheat bread. However, the Fe and Zn would not be bioavailable in their entirety because of the high presence of phytates, as shown by the $\text{InsP}_6/\text{mineral}$ molar ratio (Table 2).

3.3. Technological quality of fresh bread

Owing to the lack of gluten in the whole amaranth flour, the specific volume decreased from 4.05 to 2.38 mL/g as a result of the addition of amaranth flour at different levels (Figure 1 and Table 3). A similar trend was observed by Sanz-Penella et al. (2013) and by Almeida, Chang, & Steel (2013) in bread with wheat flour replaced with *A. cruentus* and *A. caudatus*,

respectively. This behavior was observed in other studies as a result of the inclusion of ingredients such as fiber in bread formulations, owing to a gluten dilution effect (Iglesias-Puig et al., 2015). On the other hand, the slice shape did not show significant differences compared to the control sample (Figure 1). The inclusion of whole amaranth flour produced a significant change in crumb firmness, reaching 2.16/2.46 for 50% substitution (Table 3). The same effect was observed in bread supplemented with other pseudocereals (Sanz-Penella et al., 2013; Iglesias-Puig et al., 2015). Whole pseudocereal flours are rich in dietary fiber and do not provide gluten, but their proteins, such as albumin, have the capacity to interact with wheat glutenin protein through disulfide bonds, which does not weaken the gluten network overmuch (Oszvald et al., 2009). The high polar lipid content in amaranth may have functionality as a gas stabilizing agent during breadmaking, which probably improves bread elasticity (D'Amico et al., 2017). In fact, Meullenet, Lyon, Carpenter, & Lyon (1998) found a direct relation between dough elasticity/crumb chewiness and crumb firmness. In the current investigation, the same tendency was observed between the chewiness and firmness parameters (Table 3).

The mean cell area for the WAs-25 and WAh-25 formulations was approximately the 50-70% of the control and WAh-50/WAs-50 areas, but they had a higher amount of cells/m² than the others. Therefore their specific volume was significantly higher than that of WAh-50/WAs-50, and lower, but not significantly lower, than that of the control sample (Table 3). Again, this effect could be due to the low amount of gluten and consequent decrease in dough elasticity in the formulations with 50% replacement (Table 3 and Figure 1). The color parameters of the crust and crumb showed a tendency to decrease in terms of lightness and hue, whereas the chroma tended to increase, especially in formulations substituted with 50% of whole amaranth flour (Table 3 and Figure 1). In general, the temperature at the center of the bread crumb does not reach 100 °C during baking, but this temperature is greatly

exceeded in the crust, so the crust color is mainly due to the effect of the Maillard reaction and sugar caramelization (Martínez et al., 2013). In the current study, it is noteworthy that, as the level of substitution of wheat flour increased, the L^* and h_{ab} parameters in the crust and crumb decreased compared to the control sample. Therefore, in all the formulations there were significant differences in crust and crumb color (ΔE^*) compared to the control sample, with values greater than 5 and clearly perceptible to the consumer according to the criteria of the International Commission on Illumination (1978) (Figure 1).

3.4. Sensory evaluation

In this study a preliminary sensory analysis was performed with a nine-point hedonic scale for the overall acceptability of the products after they had been tasted by consumers. In general, the products made with *A. hypochondriacus* flour had better acceptance than the *A. spinosus* breads. The WAh-25 sample showed 20% more acceptability than the WAh-50 sample (Table 3), and the WAs-25 and WAs-50 formulations showed 58% and 55% of acceptance, respectively. This result indicated that the consumers preferred the products made with *A. hypochondriacus* at a 25% level of substitution rather than the other amaranth formulations (Table 3). Some of the comments of the tasters who described the flavor of the bread with amaranth flour, a new flavor that they had not tasted before, were: “moisture flavor,” “mold flavor,” “strange flavor,” “sand flavor,” “indifferent flavor,” “slightly acid,” “not very pleasant,” “new flavor/different” (data not shown). The new flavor could be due to the presence of saponins, although amaranth grains have a lower amount of saponins than quinoa grains, so a product made with amaranth flour would be less bitter than one with quinoa (Reguera & Haros, 2017). Thus, the lower scores were due not only to the taste but also to the appearance and color of the slices, and their texture and palatability.

3.5. Effect of the inclusion of amaranth on starch thermal properties

The bread samples showed a peak in the thermogram, corresponding to the process of gelatinization of the amorphous phase of starch. It was observed between 62.7 and 75.9 °C, with an enthalpy of 1.6 J/g in the control sample and values significantly higher in the formulations with 25% replacement (Table 4). However, in the formulation with 50% replacement the gelatinization onset temperature did not have significant differences compared to the formulations with 25% replacement, but the enthalpy values were 1.7/1.9 J/g for WAs-25/WAh-25, significantly different from the values of 1.2/1.3 J/g for WAs-50/WAh-50, respectively (Table 4). This reduction could be due to the higher amount of water absorbed by the amaranth grain, and the greater swelling power and solubility of the amaranth starch granule compared with the wheat starch granule (Pérez-Rea & Antezana-Gómez, 2018).

In the second heating cycle, after storage at 20 °C, two peaks were observed. The first one was the amylopectin retrogradation peak, while the second one was the amylase–lipid complex melting transition. In the bread substituted with whole amaranth flour there was practically no alteration in the transition temperature range. Moreover, the retrogradation enthalpy after 4 days of storage tended to be significantly lower in the WAs-50/WAh-50 formulations compared to WAs-25/WAh-25 and the control sample. In fact, there are findings that show that amaranth starch has slower retrogradation enthalpy than other types of starches belonging to corn, wheat, and rice (Repo-Carrasco-Valencia & Valdez, 2017). It was observed that the high enthalpy retrogradation value for the control bread was directly related with the aging of the bread during storage owing to the reorganization of the amylopectin crystals. In general, the retrogradation kinetics for the bread with whole amaranth flour showed a tendency to decrease, but there were no significant differences between formulations (Table 4). However, after 10 days of storage the amylopectin retrogradation in

samples with amaranth was slightly and significantly lower than in the control bread (Fig. 2). Previous investigations concluded that the presence of the higher lipid and fiber content in bread doughs makes the recrystallization of amylopectin more difficult and reduces retrogradation enthalpy (Mondragón, Mendoza-Martínez, Bello-Pérez, & Peña, 2006). Therefore, in general, bread aging could be delayed in bread formulations with amaranth flour.

4. Conclusion

Bread formulated with *A. spinosus* and *A. hypochondriacus* flours had higher levels of protein and dietary fiber than wheat bread, and was close to the recommended intake for adults. Bread with amaranth flours exhibited soluble/insoluble fiber ratios close to 1:2, which presents the most effective physiological action. The levels of lipids, minerals, and myo-inositol phosphates were also increased significantly by the incorporation of this pseudocereal. The intake of products with high substitution with amaranth could supply a high percentage of the DRIs of Zn and Fe in adults, but the high amount of phytates in these formulations could inhibit mineral availability. Consumers preferred the bread with 25% replacement, with whole *A. hypochondriacus* rather than with *A. spinosus* at the same level of substitution. The inclusion of either amaranth species delayed aging of the bread. The level of inclusion of whole flours of the two species studied, *A. spinosus* and *A. hypochondriacus*, in bakery products could be limited to a maximum proportion of 25 g/100 g, providing acceptable technological characteristics and sensory quality with high nutritional value.

As a general conclusion, the use of *A. hypochonriacus* or *A. spinosus* as a bakery ingredient allowed to develop similar breads compared to the ones made with others Amaranth species, as *A. caudatus* or *A. cruentus*, in terms of technological and nutritional quality. However, *A. spinosus* presented a significant lower overall acceptability which is necessary to study the

minimum substitution level in bread formulations with a high acceptability and a positive impact in the human health.

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524 **Figure captions**

525

526 **Figure 1.** Appearance of the loaf bread and crumb structure. Bread formulations: C, control
527 bread; WAs-25, WAs-50, WAh-25, and WAh-50, whole *A. spinosus* and *A. hypochondriacus*
528 replaced at 25% and 50%, respectively.

529

530 **Figure 2.** Effect of the inclusion of Whole Amaranth flour on amylopectin retrogradation:

531 □, C, Bread control wheat flour; ▲, WAs-25, 25% whole *A. spinosus* flour; ▲, WAs-50,
532 50% whole *A. spinosus* flour; ×, WAh-25, 25% whole *A. hypochondriacus* flour; ×, WAh-
533 50, 50% whole *A. hypochondriacus* flour.

534

Table 1. Raw material composition

Composition	Units	Wheat flour	<i>A. spinosus</i>	<i>A. hypochondriacus</i>
Moisture	%	13.59±0.05	11.07±0.00	10.50±0.01
Proteins	% d.m.	10.2±0.3 (Nx5.7)	14.07±0.01 (Nx5.85)	14.80±0.09 (Nx5.85)
Lipids	% d.m.	1.08±0.11	6.07±0.01	5.94±0.01
Ash	% d.m.	0.09 ±0.03	2.77±08	2.62±0.02
InsP ₆	μmol/g	n.d.	21±2	18±2
Ca	mg 100 g ⁻¹ d.m.	56.2±0.1	244.3±0.7	270.3±1.0
Fe	mg 100 g ⁻¹ d.m.	1.2±0.1	8.39±0.04	8.34±0.02
Zn	mg 100 g ⁻¹ d.m.	0.65±0.01	3.6±0.1	3.81±0.01

^a Mean±SD. n=3. Values followed by the same letter in the same column are not statistically different at 95% confidence level. Codes: d.m., dry matter; InsP₆: *myo*-inositol hexakisphosphate or phytates; n.d., not detected.

Table 2. Effect of the inclusion of whole amaranth flour on bread composition

Sample	Units	Formulations				
		Control	WAs-25	WAs-50	WAh-25	WAh-50
<i>Physicochemical parameters^a</i>						
Moisture	%	27.8±0.2 a	28.6±1.7 ab	28.9±0.2 ab	28.9±0.2 ab	29.3±0.3 b
Proteins	% d.m.	16.5±1.4 a	18.1±0.6 ab	18.4±0.3 c	17.6±0.1bc	19.0±0.8 bc
Lipids	% d.m.	0.07±0.0 a	0.70±0.1b	1.45±0.1c	0.78±0.1b	1.54±0.1c
Ash	% d.m.	1.7 ± 0.3 a	3.0±0.1 b	3.5±0.2 c	3.0±0.2 b	4.0±0.1 c
<i>Dietary Fiber^a</i>						
Total Dietary Fiber	g/100 g d.m.	6.96±1.3 a	9.50±0.04 b	11.7±0.8 c	9.9±0.9 bc	11.3±0.3 bc
Soluble fiber	g/100 g d.m.	3.1±0.7 a	3.2±0.2 a	2.9±0.1 a	3.4±0.9 a	3.4±0.7 a
Insoluble fiber	g/100 g d.m.	3.9±0.5 a	6.3±0.2 b	8.7±0.6 c	6.5±0.0 b	7.9±0.4 c
Soluble/Insoluble Fiber Ratio ^b	g/g	1:1.2	1:2	1:3	1:1.9	1:2.3
AI ^c contribution	%	33/50	45/68	54/83	46/70	53/80
<i>Myo-inositol phosphates^a</i>						
InsP ₆	μmol/g	n.d.	3.6±0.3b	8.8±0.8c	3.7±0.6b	8.5±0.5c
InsP ₅	μmol/g	n.d.	0.75±0.1b	1.59±0.4c	0.87±0.2b	2.0±0.5c
InsP ₄	μmol/g	n.d.	0.40±0.1b	0.61±0.1c	0.34±0.1b	0.61±0.1c
InsP ₃	μmol/g	n.d.	0.16±0.04a	n.d.	0.27±0.04b	n.d.
InsP ₆ + InsP ₅	μmol/g	n.d.	4.4±0.4a	10.40±0.5b	4.5±0.8a	10.1±0.5b
<i>Minerals</i>						
Ca ^a	mg 100 g dm ⁻¹	37.7±0.0 a	88.2±0.8 b	139.7±0.0 d	92.7±0.5 c	152.6±0.7e
DRI contribution ^d	%**	7	16	21	16	27
InsP ₆ /Ca >0.24 ^e	mol mol ⁻¹	n.d.	0.16	0.24	0.16	0.22
Fe ^a	mg 100 g dm ⁻¹	1.5±0.0 a	3.2±0.1 b	4.9±0.3 d	3.3±0.1 c	4.9±0.3 e
DRI contribution ^d	% man/woman	35/15	72/32	108/48	74/33	108/48
InsP ₆ /Fe >1 ^e	mol mol ⁻¹	n.d.	6.2	9.7	6.2	9.7
Zn ^a	mg 100 g dm ⁻¹	1.2±0.0 a	2.1±0.2 b	2.6±0.2 c	1.9±0.0 b	2.3±0.2 bc
DRI contribution ^d	% man/woman	19/27	34/47	43/59	31/43	37/50
InsP ₆ /Zn >5 ^e	mol mol ⁻¹	n.d.	11.2	20.9	12.4	24.3

Codes: C, Control Bread; WAs-25, 25% whole *A. spinosus* flour; WAs-50, 50% whole *A. spinosus* flour; WAh-25, 25% whole *A. hypochondriacus* flour; WAh-50, 50% whole *A. hypochondriacus* flour; d.m., dry matter; InsP₃ to InsP₆: myo-inositol containing 3–6 phosphates per inositol residue; n.d., not detected.

^a Mean±SD. n=3. Values followed by the same letter in the same column are not statistically different at 95% confidence level, *n.d.* not detected

^b Ratio of soluble/insoluble fiber, 1:2 (Jaime, Mollá, Fernández, Martín-Cabrejas, López-Andreu, & Esteban, 2001); 1:3 (Salas-Salvadó, Bulló, Pérez-Heras, & Ros, 2006).

^c AI (adequate intake) contribution (%) for a daily average intake of 250 g of bread. AI in g per day for dietary fiber in adult man/woman is (38/25). The values in parentheses are recommended dietary allowances and adequate intakes for adults for each gender between 19 and 50 years; Food and Nutrition Board, Institute of Medicine (2004).

^d DRI (dietary reference intakes) contribution (%) for a daily average intake of 250 g of bread if mineral absorption inhibitors are absent. The values in parentheses are recommended dietary allowances and adequate intakes for individuals between 19 and >70 years, except for: * (between 31 and >70 years), and ** (men between 19 and 70 years, women between 19 and 50 years) (Food and Nutrition Board, 2004).

^e Threshold ratios (InsP₆/mineral) for mineral availability inhibition (Ma et al., 2005); InsP₆, myo-inositol hexakisphosphate; minerals Ca, Fe or Zn.

Table 3. Effect of inclusion of whole amaranth flour on bread quality

Sample	Units	Formulations				
<i>Technological parameters^a</i>		Control	WAs-25	WAs-50	WAh-25	WAh-50
Loaf volume	mL	334 ±13 d	305 ± 36 c	195 ± 23 a	298 ± 16 c	223 ± 17 b
Specific volume	mL/g	4.05 ± 0.16 b	3.74 ± 0.52 b	2.38 ± 0.28 a	3.63 ± 0.07 b	2.71 ± 0.20 a
Shape ratio	cm/cm	1.70 ± 0.08 a	1.61 ± 0.04 a	1.65 ± 0.08 a	1.64 ± 0.04 a	1.64 ± 0.07 a
<i>Crumb textural parameters^a</i>						
Firmness	N	0.77 ± 0.17 a	0.91 ± 0.12 a	2.16 ± 0.28 b	1.05 ± 0.19 a	2.46 ± 0.35 c
Springiness		0.95 ± 0.10 a	1.00 ± 0.01 b	0.97 ±0.02ab	1.00 ± 0.01 b	0.95 ± 0.02 a
Cohesiveness		0.89 ± 0.03 ab	0.83 ± 0.01 a	0.84 ± 0.01a	0.83 ± 0.01 a	0.84 ± 0.10 a
Chewiness		0.66 ± 0.17a	0.84 ± 0.13 ab	1.74 ± 0.12 c	0.87 ± 0.09 b	1.94 ± 0.13 c
Gumminess	N	0.68 ± 0.14 a	0.81 ± 0.12 a	1.80 ± 0.16 b	0.87 ± 0.15 a	2.06 ± 0.23 c
<i>Crust color parameters^b</i>						
<i>L*</i>		73 ± 2 d	61 ± 2 c	54 ± 2 b	58 ± 3 c	50 ± 2 a
<i>C*</i>		31 ± 3 a	37 ± 1 c	34 ± 1 b	36 ± 1 c	33 ± 1 b
<i>h_{ab}</i>		86 ± 2 c	72 ± 1 b	66 ± 1 a	70 ± 1 b	65 ± 1 a
<i>ΔE*</i>		-	16 ± 1 a	22 ± 1 c	18 ± 1 b	26 ± 1 d
<i>Crumb color parameters^b</i>						
<i>L*</i>		70 ± 3 d	68 ± 2 b	65 ± 1 b	67 ± 2 c	61 ± 1 a
<i>C*</i>		16 ± 1.1 a	21.1 ± 1.0 cd	25.1 ± 0.5 c	21.9 ± 0.6 b	25.8 ± 0.8 c
<i>h_{ab}</i>		95.1 ± 0.5 e	90.1 ± 0.4 d	85.5 ± 0.7 b	88.8 ± 0.4 c	83.7 ± 0.8 a
<i>ΔE*</i>		-	5.9 ± 0.6 a	11.1 ± 0.4 c	6.8 ± 0.2 b	14.2 ± 0.9 d
<i>Crumb grain(digital image analysis)^b</i>						
Cell Area/Total Area	cm ² /cm ²	0.53±0.04 c	0.37±0.06 a	0.46±0.05 b	0.34±0.04 a	0.45±0.06 b
Wall Area/Total Area	cm ² /cm ²	0.49±0.04 a	0.63±0.06 c	0.56±0.06 b	0.67±0.04 c	0.57±0.06 b
Cells/cm ²		110±17 a	160±46 b	129±42 ab	148±13 b	134±25 ab
Mean Cell Area	mm ²	5.13±0.01 c	2.63±0.01 a	4.00±0.02 bc	2.50±0.01 a	3.50±0.01 ab
<i>Sensory evaluation (Hedonic Scale)^c</i>						
Overall acceptability		7.5±1.4 e	5.8±0.6 b	5.6±0.7 a	6.9 ± 0.9 d	6.1 ± 0.9 c

Codes: C Bread control; WAs-25, 25% whole *Amaranth spinosus* flour, WAs-50, 50% whole *Amaranth spinosus* flour, WAh-25, 25% whole *Amaranth hypochondriacus* flour, WAh-50, 50% whole *Amaranth hypochondriacus* flour; d.m., dry matter.

Mean±SD, ^an=3. ^bn=4. ^cn=25. Values followed by the same letter in the same column are not statistically different at 95% confidence level.

Table 4. Effect of amaranth flour on starch thermal properties of dough.

Sample	Units	Control	Amaranth (%)			
			WAs-25	WAs-50	WAh-25	WAh-50
<i>Starch gelatinization^a</i>						
Onset temperature	°C	62.7±0.6 c	64.2±0.7 b	65.1±1.0 ab	64.5±1.0 ab	65.4±0.8 a
Peak temperature	°C	68.8±0.6 c	70.51±0.46 b	71.1±0.7 bc	71.04±0.9 bc	71.4±0.6 bc
Conclusion temperature	°C	75.9±1.2 b	78.6±2.4a	78.6±2.4 a	79.80±3.44a	79.1±1.5 a
Gelatinization enthalpy, ΔH _G	J/g	1.6±0.2 b	1.7±0.2 bc	1.2±0.1 a	1.9±0.2 c	1.3±0.1 a
PHI	J/g°C	0.26±0.04 a	0.27±0.05 b	0.20±0.03 c	0.29±0.04 b	0.21±0.02 c
<i>Amylopectin retrogradation, 4 days^b</i>						
Onset temperature	°C	55.2±0.4 a	55.45±0.1 a	55.4±0.4 a	55.3±0.4 a	55.2±0.7 a
Peak temperature	°C	62.9±0.1 a	63.5±0.02 a	63.2±0.1 a	63.0±0.5 a	63.7±0.4 a
Conclusion temperature	°C	76.4± 2.5 a	82.0±0.8 a	74.5±2.9 a	78.8±0.7 a	76.6±1.0 a
Retrogradation enthalpy, ΔH _R	J/g	0.62±0.02 bc	0.56±0.00 ab	0.50±0.00 a	0.63±0.06 c	0.53±0.00 a
RI		0.39±0.01 b	0.34±0.00 a	0.42±0.00 b	0.34±0.03 a	0.42±0.00 b

Codes: C, Control Bread; WAs-25, 25% whole *Amaranth spinosus* flour, WAs-50, 50% whole *Amaranth spinosus* flour, WAh-25, 25% whole *Amaranth hypochondriacus* flour, WAh-50, 50% whole *Amaranth hypochondriacus* flour; d.m., dry matter.

Mean±SD. ^an=14, ^bn=3; values followed by the same letter in the same column are not statistically different at 95% confidence level; DSC differential scanning calorimeter, PHI, Peak Height Index $\Delta H_G/(T_p-T_o)$; RI, Retrogradation Index $\Delta H_R/\Delta H_G$ (J/g).