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Effect of precooking on antinutritional factors and mineral bioaccessibility in kiwicha grains

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ABSTRACT

Kiwicha is the most important genus of *Amaranthus* and has a high mineral content. However, it contains antinutritional factors that may influence their bioaccessibility. Thus, the aim of our study was to evaluate the effect of precooking on antinutritional factors and mineral bioaccessibility of iron, calcium and zinc in kiwicha grains. Puffed kiwicha (PK) had the highest bioaccessibility and potential contribution (PC) of iron. The bioaccessibility of zinc was reduced in PK and laminated kiwicha (LK). The heat treatment decreased the content of: total dietary fiber (11–14%), bound fraction (15–36%) and total polyphenols (15–16%) and inositol hexaphosphate (19–27%). Phytic acid free fraction and total polyphenols, values exhibited a high negative correlation with iron, calcium and zinc bioaccessibility. On the other hand, iron bioaccessibility improved with puffing and therefore PK had the highest PC to requirements for children (4–8 years old), pregnant women and aging adults. Puffing and lamination processes increased the calcium bioaccessibility but decreased that of zinc. We concluded that the precooking of kiwicha grain influenced on antinutritional factors and, consequently, the minerals bioaccessibility. It would be important to study the effect of other processes and the use of enhancers to improve mineral bioaccessibility and potential contribution.

1. Introduction

Amaranthus caudatus is the most important Andean species, known as “kiwicha”. It is grown in Perú, Bolivia, Ecuador and Argentina (Repo-Carrasco-Valencia et al., 2009). Kiwicha is a very good source of iron, calcium and zinc. It contains more zinc and iron than conventional maize and beans (Repo-Carrasco-Valencia et al., 2010). Also, it is commonly used as a puffed product, but there is little research on the lamination process (Burgos and Armada, 2015).

Mineral absorption depends on the mineral content, food components, amounts of the trace element in the diet or food, the chemical form and properties, physical properties (solubility, adsorption capacity on inert components of the food), ability to react with other components of the food matrix or drug and biochemical properties, and the ability to compete with other elements for organism active sites (Hurrell and Egli, 2010). In this regard, the food matrix may contain substances which act as promoters or inhibitors of absorption. Phytate, phenolic compounds and fiber are antinutritional factors that reduce bioaccessibility of iron and zinc in plant foods (Raes et al., 2014).

Bioaccessibility has been defined as the soluble fraction of a compound that is released from the food on the gastrointestinal tract and so becomes available to the intestinal absorption (typically based on *in vitro* procedures) (Fernández-García et al., 2009). If the amount of recovered nutrients after digestion is of relevance, then the term to use is bioaccessibility (Faulks and Southon, 2005). It is well-established that although the total amount of a nutrient is potentially bioaccessible, very few nutrients are actually totally converted in soluble form during digestion to its absorption. The *in vitro* dialysis method is based on the simulation of

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gastrointestinal digestion of food followed by the determination of the amount of nutrient that crosses a semipermeable membrane that simulates the intestinal wall (Kiskini et al., 2007).

The aim of our study was to evaluate the effect of precooking on antinutritional factors and mineral bioaccessibility of iron, calcium and zinc in kiwicha grains.

2. Experimental

2.1. Reagents

PIPES (piperazine-N,N-bis[2-ethanesulfonic acid] disodium salt) buffer; digestive enzymes: α-amylase type VI-B (A-3176) and pepsin (P-7000); extract of bilis (B-8631) and pancreatin (P-1750) were purchased from Sigma Chemical Co. (St Louis, MO, USA). These reagents were used to prepare simulated gastric and intestinal juices. Spectra/Pore dialysis tubing (cut-off 6000–8000) was purchased from Fischer Scientific (Fairlawn, NJ, USA). All glass materials and polyethylene bottles were washed with deionized water, kept in HNO_3 (20%) for 24 h, and washed again with deionized water before use.

2.2. Samples

2.2.1. Raw grain

Kiwicha grain (*Amaranthus caudatus*) was obtained from an agricultural field in the local town of Cachi (Salta, Argentina). Commercial puffed amaranth (CPA) was purchased from a local market. It was used as a reference standard.

2.2.2. Precooked products

Precooked products of kiwicha were obtained by the method of Burgos and Armada (2015). Puffed kiwicha (PK) was made using an aluminum pan which consists of a lid with a propeller that allows the grains to be mixed, while they pop. A gas cooker Tivoli 500 G.E. V/S 50CM (Argentina) was also used. After the puffing process, popped grains were passed through a 16 mesh (1.190 mm ASTM) to be selected for analysis. The process variables were: 7.20% of initial moisture, 30 s of expansion time and 160 °C ± 2 °C of temperature.

Laminated kiwicha (LK) was obtained using a double drum roller. The sample was placed in the feed zone and then passed to the outer surface of the heated rollers, which rotate slowly on their horizontal axis. Prior to the lamination process, kiwicha grains were moisturized with distilled water, mass:volume ratio of 1:0.8. The process variables were: 18% of initial humidity content, roller temperature at 100 °C and residence time of 11 s at 5 rpm.

2.3. Analyses

2.3.1. Total concentration of minerals

The total concentration of minerals was assessed using atomic spectroscopy (Perkin Elmer®AAnalyst 400®) after mineralizing 1 g of each sample with 10 mL of a HNO_3-$HClO_4$ (50:50) mixture at 140 °C for 4 h. Atomic absorption was made using a hollow cathode lamp for calcium iron and zinc. Lanthanum chloride was added to all samples and standards analyzed for Ca to reach a final concentration of 0.65% (Dyner et al., 2007).

2.3.2. Determination of bioaccessibility and potential contribution of iron, zinc and calcium (BFe%, BZn% and BCa%)

Previously milled samples were suspended in deionized water, ratio 1:5 (sample:water) at room temperature and homogenized with agitation in a shaking bath (Vicking, Dubnoff model, Germany). Aliquots of homogenized samples (50 g) were incubated with 5 mL of a 3% α-amylase solution at 37 °C in a shaking water bath (Vicking, Dubnoff model, Germany) with movement of agitation, 60 rpm of speed for 30 min, then adjusted to pH 2.0 with 6 mol/L HCl and, after adding 1.6 mL pepsin digestion mixture (16% pepsin solution in 0.1 mol/L HCl), were incubated at 37 °C in a shaking water bath for 2 h. At the end of pepsin digestion, two aliquots of digest (15 g) were weighed in 100 mL beakers. Dialysis bags (Spectral/Por®, Spectrum Laboratories Inc., CA, USA) of 14.6 mm diameter, 20 cm length and 6000–8000 Da cut-off were used. They were immersed in deionized water for at least 1 h before use. Dialysis bags contained 18.75 mL 0.15 mol/L PIPES (piperazine-1,4-bis (2- ethanesulfonic acid) buffer were placed in each beaker. Buffer pH used for each food matrix was calculated in order to obtain a final pH of 6.5 ± 0.2 for digest–dialysate (Drago et al., 2005).

Aliquots of each pepsin digest with dialysis bags containing PIPES buffer were incubated at 37 °C in a shaking water bath for 50 min. Pancreatin–bile mixture (3.75 mL of 2.5% bile, 0.4% pancreatin solution in 0.1 mol/L $NaHCO_3$) was then added to each beaker, and the incubation continued at 37 °C for another 2 h. At the end of the pancreatin–bile incubation, dialysis bags were removed and rinsed with water.

Bag contents were transferred to tared flasks, weighed, then analyzed for their iron, zinc and calcium content by flame atomic absorption spectroscopy (AAS).

Mineral bioaccessibility (Fe, Zn, Ca) was calculated as the percentage of the mineral dialyzed (mg) with regard to the total concentration of each mineral in pepsin digest (mg), using the following formula:

$$\text{Mineral Bioaccesibility (BFe\% – BCa\% – BZn\%)} = \frac{\text{concentration of each mineral in the dialysate x 100}}{\text{concentration of each mineral in pepsin digest}}$$

The potential contribution (PC) of each mineral was calculated as each mineral concentration (mg%) multiplied by its bioaccessibility (Dyner et al., 2007):

$$\text{Mineral PC} = \frac{\text{concentration of each mineral in the sample x B\%}}{100}$$

2.3.3. Potential contribution to requirements of grain, puffed, laminated kiwicha and commercial puffed amaranth

The Potential Contribution (PC) of each mineral (mg/100 g) of the grain, PK, LK and CPA, the percentage provided per serving was calculated according to the minimum requirements (R) for children 4–8 years old, pregnant women and aging adults (FAO/OMS, 2004). Serving sizes used were 15 g of kiwicha grains and 25 g of precooked products.

2.3.4. Determination of inhibitory factors

2.3.4.1. Total dietary fiber. The total (TDF), insoluble (IDF) and soluble dietary fiber (SDF) was analyzed by an enzymatic–gravimetric method according to the AOAC official method (1996). Triplicate samples were subjected to a sequential enzymatic digestion by heat stable α-amylase, protease and amyloglycosidase. The enzyme digestate was treated with alcohol before filtering for TDF, and TDF residue was washed with alcohol and acetone. The enzyme digestate was filtered for IDF, and residue IDF was washed with warm water, both fractions were dried and weighed. SDF was calculated for difference between TDF and IDF.

2.3.4.2. Total, free and bound phenolic compounds

2.3.4.2.1. Extraction. Total phenolic compound extraction was

developed by (Chlopicka et al., 2012) with some modifications. Forty mL of solvent: methanol, hydrochloric acid (0.16 mol/L) and water mixed in an 8:1:1 ratio, respectively, were added to the samples (2 g) and let stand for 2 h. Then, the extracts were centrifuged at 1500xg (centrifugal Gelec 142, Argentina) and filtered. The residues were extracted with 40 mL of acetone for 2 h, centrifuged at 1500xg and filtered with filter paper. Both extracts were combined and concentrated to dryness by using a rotary evaporator (Decalab-FBR, Argentina) at 40 °C. The dried extract was redissolved in 5 mL of 50% methanol and used as crude extract, and stored away from light in a freezer in temperature of −18 °C until analysis.

2.3.4.2.2. Alkaline hydrolysis. The crude extract and the residue remaining from the extraction separately were hydrolyzed with 40 mL of 4 M NaOH on a shaking water bath (Vicking, Dubnoff model, Germany). Then, the solution was adjusted to a pH 1.5–2.0 with 6 M HCl and extracted with 70 mL of ethyl acetate. The ethyl acetate were evaporated to dryness and reconstituted in 2 mL of 50% methanol. The alkaline hydrolyzed derived of the crude extract was called the free phenol fraction, and that which was obtained from the residue was called the bound phenol fraction. Both fractions were directly subjected to total phenolic determination (Yang et al., 2010).

2.3.4.2.3. Determination of total phenolic content. The total phenolic compounds were measured according to the method described by Singleton et al. (1999). Ten μL of the extracts were added to 800 μL of an aqueous solution of sodium carbonate 15.9%, stirred vigorously in vortex (Vicking, Argentina) and allowed to stand for 20 min at room temperature. After two minutes, 200 μL of Folin-Ciocalteu reagent dissolved in water (1:1) were added. Absorbance at 725 nm was measured against the reagent blank in a spectrophotometer (Spectronic Unicam Model Genesys 10 UV, England). The calibration curve was made with gallic acid (1 mg/mL) at concentrations of 0–90 mg/mL. The results were expressed in gallic acid equivalents (GAE mg/100 g dry matter).

2.3.4.3. Inositol phosphates (IPs). The IP methodology was developed by Dyner (2011). Inositols hexa, penta, tetra and tri phosphates (IP6, IP5, IP4 and IP3) were measured. We used an HPLC system comprising a 515 Waters pump, a refraction index detector (temperature 30 °C), a Rheodyne injector with a 50 mL loop, 0.9 mL/min flow and a C18 column (XBridge®; C18; 5 mm; 4.6 × 150 mm; Waters). The mobile phase consisted of methanol: aqueous solution (51:49) pH = 4.30. Each 100 mL of the aqueous solution contained: 89.6 mL of 0.05 mol/L formic acid, 4.5 mL of 0.05 mol/L Na_2EDTA, 4.7 mL of 20% tetrabutyl ammonium hydroxide and 0.2 mL of 0.6 g/100 mL phytic acid (hydrolyzed in an autoclave for 40 min, 121 °C and 1 atm). Data acquisition was done using Chromatography Station CSW from Data Apex Ltd. All reagents used were HPLC quality (J.T. Baker) and ultrapure water (EASY pure RF, Barnstead). One g of the sample was mixed with 20 mL of an aqueous solution of 0.5 mol/L HCl for IPs extraction, and it was stirred at room temperature for 2 h. The mixture was centrifuged for 20 min at 2000 rpm and the supernatant was filtered through a 0.22 mm nylon membrane. The residue was dried and reconstituted with 15 mL of 25 mM/L HCl. Then, IPs were purified and concentrated using a 0.70 g anion Exchange resin column (AG® 1-X4, 100–200 mesh, chloride form, BIO-RAD®) washed with 25 mL of 25 mM/L HCl. For the elution of IPs, 15 mL of 2 mol/L HCl was used and the collected fraction was dried out. Finally, the sample was reconstituted with 1 mL of ultrapure water (EASY pure RF, Barnstead) and injected into the chromatographic system. Many authors relate the total content of phytic acid (IP6) with mineral bioavailability. Thus, IPs were converted to IP6 by adding the mols of phosphorous contributed by each of the different IPs and transforming them into IP6 using the molecular weight.

2.3.5. Molar ratio

Molar ratios of Phy/Fe, Phy/Ca and Phy/Zn were calculated using a molecular mass unit of 660.03 for phytic acid, an atomic mass unit of 55.845 for iron, 40.078 for calcium and 65.38 for zinc, respectively. Corresponding molar ratios were then obtained using these values.

2.4. Statistical analysis

All determinations were done in triplicate except in the case of bioaccessibility, which was done in sextuplicate. Results were analyzed using analysis of variance (ANOVA). Tukey's test was used to separate means. Significance was accepted at $p < .05$. Multiple regression analysis was used to explain the influence of the inhibitory factors on the mineral bioaccessibility. All statistical analyses were carried out using Infostat software 2016p.

3. Results and discussion

3.1. Total mineral contents

Table 1 shows the total mineral contents of the samples studied. The iron and calcium values decreased in PK and LK, respectively ($p < .05$). The content of zinc was similar in raw grain and PK, while LK showed the highest value ($p < .05$).

Other researchers found higher mineral contents than those obtained in this study: iron (12.11 ± 0.73 mg/100 g), calcium (205.98 ± 4.48 mg/100 g) and zinc (3.33 ± 0.04 mg/100 g) in whole amaranth flour (Dyner et al., 2007), which is most likely due to the variety and genotype of amaranth grain. The mineral values of raw grain kiwicha were similar to established by the USDA (2016) (iron: 7.61 mg/100 g, zinc: 2.87 mg/100 g) and lower in calcium (159 mg/100 g). Repo-Carrasco-Valencia et al. (2010) observed a reduction of iron in the roasted grain, whereas no changes were presented in calcium and zinc, findings which are consistent with the present study.

3.2. Bioaccessibility (B%) and potential contribution (PC) of iron, zinc and calcium

Table 1 shows the bioaccessibility and potential contribution of iron, zinc and calcium of the samples studied. CPA had the lowest bioaccessibility and PC of iron values (0.79% and 0.06 mg%) respectively. On the other hand, PK had the highest bioaccessibility of iron (1.94%) and its PC of iron was significantly ($p < .05$) higher than laminated and CPA samples (0.14 mg %).

Regarding calcium, PK presented the highest bioaccessibility (12.10%) and CPA had the highest PC value ($p < .05$). We found that the calcium bioaccessibility improved during the precooking process. When grains go through heat treatment, they lose their integrity due to different effects such as, the temperature and time combination, different locations of the minerals in the grain matrix and the inhibitors present in them, may cause to an interaction between these minerals and inhibitor factors (Repo-Carrasco-Valencia et al., 2010; Raes et al., 2014). The iron and zinc bioaccessibility of the samples (except PK) was affected by the heat treatment, which led to a reduction in the bioaccessibility of these minerals.

PK had higher zinc bioaccessibility than other precooked samples. However, the zinc bioaccessibility was reduced in PK and LK ($p < .05$). Galán et al. (2013) obtained lower values in the Zn bioaccessibility of *Amaranthus caudatus* (1.64%) than in the present study, they developed a mix of extruded maize:amaranth (50:50),

Table 1
Total, bioaccessible and potential contribution of calcium, iron and zinc of raw, puffed, laminated kiwicha and commercial puffed amaranth.

Samples	Fe			Ca			Zn		
	Total (mg/100 g)	B%	PC (mg%)	Total (mg/100 g)	B%	PC (mg%)	Total (mg/100 g)	B%	PC (mg%)
Raw Grain	7.65 ± 0.8^{ab}	1.30 ± 0.4^{b}	0.12 ± 0.1^{b}	99.53 ± 4.9^{b}	10.03 ± 1.2^{a}	10.06 ± 0.6^{a}	2.10 ± 0.1^{a}	5.00 ± 0.8^{d}	0.10 ± 0.0^{b}
Puffed	6.95 ± 0.5^{a}	1.94 ± 0.4^{c}	0.14 ± 0.0^{b}	101.21 ± 0.5^{b}	12.10 ± 1.1^{b}	10.60 ± 0.7^{a}	2.33 ± 0.1^{a}	3.09 ± 0.1^{c}	0.07 ± 0.0^{a}
Laminated	8.33 ± 0.1^{b}	0.89 ± 0.1^{a}	0.07 ± 0.0^{a}	92.75 ± 0.8^{a}	11.91 ± 0.5^{ab}	11.05 ± 0.5^{a}	3.10 ± 0.6^{c}	0.68 ± 0.1^{a}	0.07 ± 0.0^{a}
CPA	8.04 ± 0.2^{b}	0.79 ± 0.1^{a}	0.06 ± 0.0^{a}	129.78 ± 2.7^{c}	11.00 ± 1.4^{ab}	13.10 ± 0.5^{b}	3.05 ± 0.0^{b}	1.92 ± 0.5^{b}	0.06 ± 0.0^{a}

Means ± standard deviation. Mean values with different letters for a particular column differ significantly ($p < .05$). All data are expressed on a dry basis. PC: Potential contribution; B%: Percent bioaccessibility; CPA: commercial puffed amaranth.

in which the Zn bioaccessibility decreased by about 80%, due the presence of absorption inhibitors, especially the phytates. Also, a decrease in phytate content does not implicate into an increase in zinc bioaccessibility, may be the inhibitory effect of other fiber components on solubility (Riera Sanchez, 2011).

Repo-Carrasco-Valencia et al. (2010) reported higher values of calcium, iron and zinc dialyzability in grain and precooked kiwicha than those in the present study. Also, they obtained lower values PC of iron (0.05 mg%-0.11 mg%), calcium (7.50 mg%-7.85 mg%) and zinc (0.06 mg%-0.07 mg%) in raw grain, roasted and boiled kiwicha.

Dyner et al. (2007) reported lower values of the dialyzability of whole amaranth flour (Fe: 0.61%; Ca: 7.25%, Zn: 1.32%) than this study. Regarding the PC of calcium, it was lower (14.93 mg%), while the iron (0.07 mg%) and zinc (0.04 mg%) were higher than in this study. Riera Sanchez (2011) reported lower values of calcium, iron and zinc bioaccessibility (0–0.9%), which was attributed to a higher content of phytic acid in amaranth grain. Also, this difference may be due to that the composition depend the variety, type of species and there are other antinutritional factors such as oxalates, which influence on mineral bioaccessibility in amaranth grains.

Values obtained from mineral dialyzability of kiwicha grain were lower than wheat flour (BFe% 9.9; BZn% 10.0; BCa% 44.1) (Dyner et al., 2007). However, the potential contribution of wheat flour (mg%) (Fe: 0.07; Ca: 8.14; Zn: 0.10) was lower than that of kiwicha grain presented in this study.

3.3. Contributions of grain, puffed, laminated kiwicha and commercial puffed amaranth to requirements of iron, calcium and zinc toward vulnerable populations

Iron deficiency is currently the most prevalent micronutrient deficiency in the world. It primarily affects children, pregnant women and aging adults. Calcium, iron and zinc are essential minerals required for diverse physiological and biochemical functions (Organización Panamericana de la Salud, 2002). The mineral bioaccessibility was used to estimate the contribution each per serving sample could make toward the minimum requirements for calcium, iron and zinc (these include requirements for growth, basal losses, and, in women, menstrual losses). This was done to guarantee that the contributions displayed would be the minimum within each vulnerable population and without overestimating the contribution.

Fig. 1 (A, B, C) shows the Potential Contribution (PC) per serving of iron, zinc and calcium (respectively) of kiwicha grain, PK, LK and CPA compared with the minimum requirements for the population of children 4–8 years old, pregnant women and aging adults.

The product that presented the highest contribution to iron requirements for all vulnerable populations was PK (0.29% pregnant woman; 1.5% aging adult; 2.1% children) ($p < .05$) (Fig. 1A).

Galán et al. (2013) estimated that an amaranth extruded portion (25 g) provided 0.72% in relation to iron daily requirements for adult and adolescent females both sexes (1.8 mg/day).

The wheat flour studied by Dyner et al. (2007) had half the percentage with respect to that of PK obtained in this study.

According to contribution for calcium (Fig. 1B), the highest percentage per serving for all vulnerable populations was provided by CPA ($p < .05$), which presented values for 0.5%–4.5%, due to the greater bioaccessibility of this mineral. On the other hand, values of PK and LK were higher than those reported by other authors (Galán et al., 2013; Repo-Carrasco-Valencia et al., 2010).

According to the requirements of zinc per serving, raw grain kiwicha represented the best contribution (0.9% children; 1.3% pregnant; 3.2% in aging adults) ($p < .05$). However, no significant differences were reported in PK, LK and CPA (Fig. 1C). Galán et al. (2013) obtained lower percentages than those in this study.

The bioaccessibility and potential contribution of minerals are very important because they are directly linked in the nutritional use, promote and revalorization of amaranth grains in our country. Although the potential contribution in our study was low, it is very important to study the incorporation of mineral absorption enhancers to improve bioavailability and the contribution to mineral requirements in vulnerable populations.

3.4. Inhibitory factors

3.4.1. Total, soluble and insoluble fiber

Table 2 shows the content of soluble, insoluble and dietary fiber of the samples studied. The content of insoluble fiber was similar among the studied samples ($p > .05$), except in the CPA. The dietary fiber content in both raw grain and precooked products was high. The samples can be considered a good source of insoluble fiber, especially for CPA, which was significantly different ($p<0.05$). The insoluble/soluble fiber ratio was 2.16. Tosi et al. (2001), obtained values of insoluble and soluble fiber of 8.1 g% and 6.1 g% for the whole grain of *Amaranthus cruentus*. Consistent with other authors, insoluble fiber represents 75–88% of dietary fiber (Repo-Carrasco-Valencia et al., 2009; Tosi et al., 2001). Glorio et al. (2006) obtained lower values of soluble (3.2%) and insoluble fiber (5.7%) than those obtained in this study. This depends mainly on the species and the variety. As a result, several species and varieties of amaranth present values of dietary fiber from 9.0 to 16.5%.

The heat treatment decreased the total dietary fiber content: 11% and 14% for the puffed and laminated kiwicha, respectively. This loss was statistically significant ($p < .05$). Capriles et al. (2008) found no significant differences for levels of total dietary fiber and their fractions in the processes of puffing, roasting, boiling and lamination. The content of total dietary and insoluble fiber decreased in extruded products of *A. caudatus*, in another study (Repo-Carrasco-Valencia et al., 2009).

3.4.2. Phenolic compounds

Table 2 shows the free, bound fraction and total polyphenol content of the samples studied. The bound fractions contained more phenolic compounds than the free fractions for the all samples. Raw grain had the highest and laminated the lowest value in the bound fraction ($p < .05$). The changes in bound fraction were

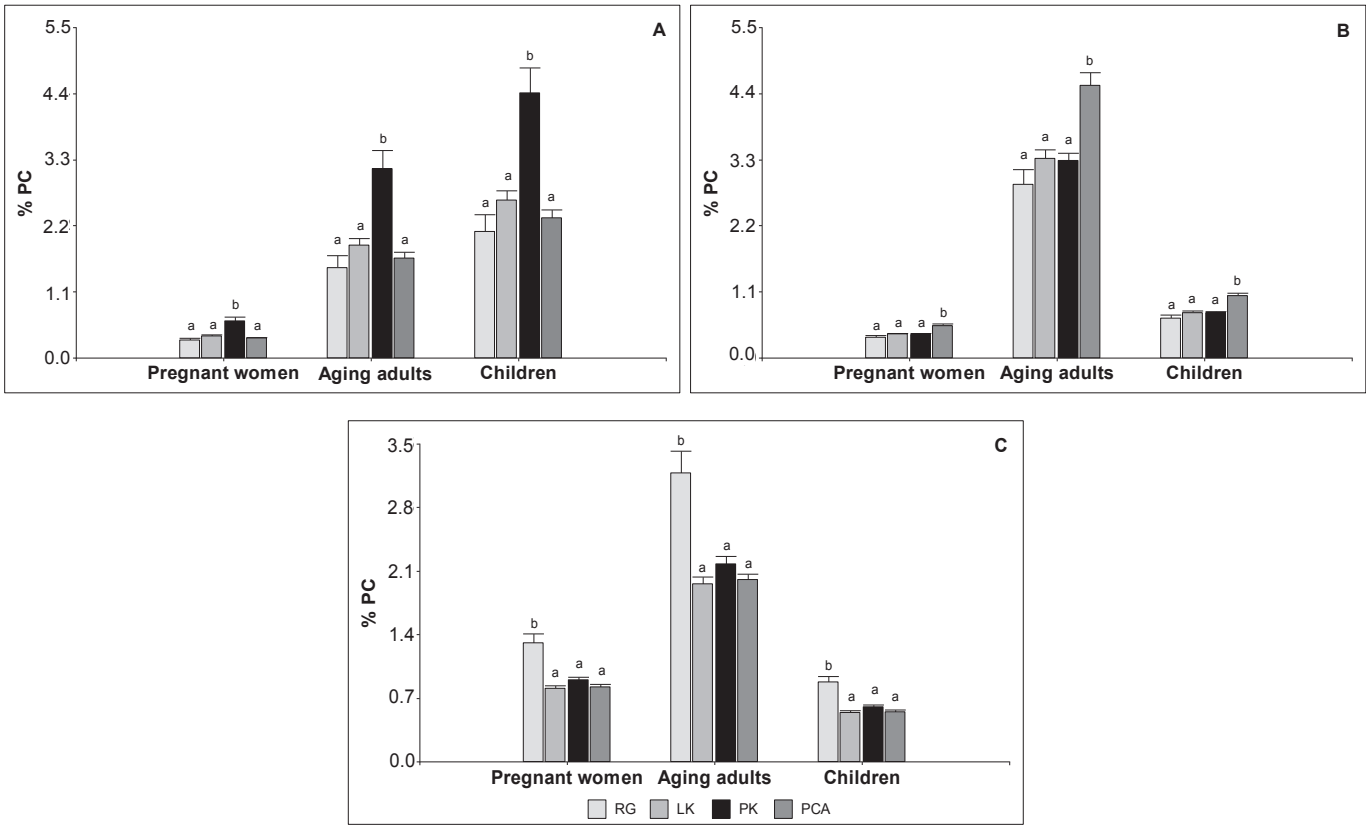

Fig. 1. Potential contribution (% PC) per serving raw grain, puffed, laminated kiwicha and puffed commercial amaranth to the daily iron (A), calcium (B) and zinc (C) requirements of children (4–8 years of age), pregnant women and aging adults. Means ± standard deviation. Statistical comparison was among samples. For each population, means with different letters differ significantly ($p < .05$). RG: raw grain; LK: laminated kiwicha; PK: puffed kiwicha; CPA: commercial puffed amaranth. Daily iron requirements (mg/d): children 4–8 years old, 0.70; pregnant women: 5.00; aging adults: 0.98. Daily calcium requirements (mg/d): children 4–8 years old, 440; pregnant women: 420; aging adults: 520. Daily zinc requirements (mg/d): children 4–8 years old, 1.8; pregnant women: 8.0; aging adults: 3.3.

Table 2
Polyphenols (free and bound fraction) and dietary fiber contents in raw grain and processed products of kiwicha (dry matter).

Samples	Polyphenols (mg GAE/100 g sample)			Dietary Fiber (g/100 g sample)		
	Free Fraction	Bound Fraction	Total	Soluble[1]	Insoluble	Total
Raw Grain	161.47 ± 0.1^{b}	317.07 ± 0.2^{d}	439.78 ± 2.1^{d}	4.84	10.46 ± 2.6^{a}	15.30 ± 4.6^{c}
Puffed	160.50 ± 0.3^{a}	269.79 ± 0.2^{c}	424.55 ± 0.8^{c}	2.85	10.27 ± 0.2^{a}	13.12 ± 0.3^{a}
Laminated	171.85 ± 0.1^{c}	201.97 ± 0.1^{a}	369.88 ± 0.7^{a}	2.98	10.58 ± 2.2^{a}	13.56 ± 0.8^{ab}
CPA	202.98 ± 0.1^{d}	238.51 ± 0.3^{b}	401.79 ± 5.5^{b}	1.65	12.57 ± 0.4^{b}	14.22 ± 0.8^{b}

Mean ± SD. Mean values with different letters for a particular column differ significantly ($p < .05$). All data are expressed on a dry basis. CPA: commercial puffed amaranth.
[1]Calculated for difference between Total Dietary Fiber and Insoluble Fiber.

evident moving from raw to precooked grain of kiwicha, where was observed a significantly reduction ($p < .05$). The destruction or transformation of phenolic chemical structures during heat treatment may also be responsible for the reduced levels of bound phenolic compounds.

The highest and lowest values were respectively for CPA and puffed kiwicha in the free fractions ($p < .05$). Interestingly, laminated products showed a significant increase of free phenolic after the heat treatment. Likely, a specific fraction of phenolic tends to degrade after the heat treatment by lamination, but these portions could not be detected by the Folin Ciocalteu approach used.

The sum of total phenolic contents present in free and bound fraction for each sample (data not reported) was higher than the corresponding value of crude extract due that in this study was released the polyphenols are covalently bound to polymers by hydrolyses alkaline, thus produced a better extraction and quantification of bound polyphenols.

The values obtained for the total polyphenols were higher than those reported by other authors, who recorded ranges of 96.68–112.89 mg GAE/100 g in amaranth grain (Repo-Carrasco-Valencia et al., 2009). This difference is due to the fact that in our study a mixture of different solvents were used, which allow extracting higher polyphenol content.

The precooking process reduced significantly the total content of polyphenols of raw grain ($p < .05$) (Table 2). The process of extrusion, roasting and cooking amaranth grains reduced the polyphenol content by about 24%–30%, in other research. This degree of loss is due to processing has been shown to be dependent on factors such as substrate type and processing conditions, notably process time and temperature (De Queiroz et al., 2009). Repo-Carrasco-Valencia et al. (2009) observed that the extrusion process affected the total phenol content in the varieties of *Amaranthus*

Table 3
Inositol Phosphates and molar ratios of phytates to calcium, iron and zinc in samples of kiwicha and puffed commercial amaranth.

Samples	Inositol Phosphates (mg/100 g) of dry matter						Molar ratio		
	IP3	IP4	IP5	IP6	Total	Phytic Acid[1]	Phy/Ca	Phy/Fe	Phy/Zn
Raw Grain	31.5 ± 4.3^{b}	257.2 ± 12.7^{d}	350.0 ± 35.1^{a}	2412.6 ± 100.1^{c}	3051.3	3031.2	1.85	33.52	142.98
Puffed	29.7 ± 2.4^{b}	205.7 ± 11.9^{b}	887.2 ± 43.2^{b}	1764.0 ± 89.2^{a}	2886.7	2809.7	1.68	34.20	119.45
Laminated	3.7 ± 0.3^{a}	35.0 ± 2.1^{a}	440.2 ± 41.1^{a}	1953.2 ± 160.9^{b}	2432.2	2404.4	1.57	24.42	76.83
CPA	32.7 ± 4.4^{b}	211.3 ± 9.9^{c}	909.9 ± 38.5^{c}	1785.6 ± 93.2^{a}	2939.5	2886.4	1.35	30.37	93.74

Mean ± SD. Mean values with different letters for a particular column differ significantly ($p < .05$). All data are expressed on a dry basis. CPA: commercial puffed amaranth. IP3: inositol triphosphate, IP4: inositol tetraphosphate; IP5: inositol pentaphosphate; IP6: inositol hexaphosphate; Phy: phytic acid. [1]Was performed using the molecular weight of IP3, IP4, IP5 e IP6 and was transformed everything in phytic acid (Dyner, 2011).

caudatus, with a decrease of 64%–80%, due that the phenolic acids can experience decarboxylation during food processing.

3.4.3. Inositol phosphates (IPs) and molar ratio

Table 3 shows the values of inositol phosphates: triphosphate (IP3), tetraphosphate (IP4), pentaphosphate (IP5) and hexaphosphate (IP6) (mg/100 g) and content of phytic acid of the samples studied. The IP6 and IP5 forms of IPs account for over 90% of the total phytate content in raw grains. These forms exhibit chelating properties with minerals, which may make intestinal absorption difficult. Therefore, a reduction in the level of IP6 and IP5 forms is highly desirable in cereal products (Agte et al., 1999).

As shown in Table 3, CPA had high values of IP5, IP6 and total phytic acid, which may influence the lower bioaccessibility of iron and zinc (Table 1). This behavior coincides with the obtained values in the bioaccessibility. The heat treatment caused a significant reduction in the IP6 content ($p < .05$) in PK (27%) and LK (19%), accompanied by a marked increase in values of IP5 mainly in PK. Regarding the increase in IP5 levels in this study, it may be due to the fact that in the process of kiwicha grain, the phytate was not completely hydrolyzed by the endogenous phytases of the grains. Similar behavior was found in cereals/vegetables flours increased 43–368% of IP5 after roasting of samples (Frontela et al., 2008).

Researchers have studied the phytic acid content in the kiwicha grain and extruded product, and observed a slight decrease, without significant difference, after the application of the extrusion process (Repo-Carrasco-Valencia et al., 2009). According to Gamel et al. (2006), thermal treatments such as cooking and popping reduce the content of phytic acid.

The obtained results of phytic acid in this study were much higher than values reported by other authors (Repo-Carrasco-Valencia et al., 2009; Riera Sanchez, 2011). Some reasons for these discrepancies include the origin of the plant-food, and the different cultivars. Additionally, the quantification method used in the reference was colorimetric, whereas we used an HPLC method, known for its higher sensitivity and accuracy. Similar values of phytates were obtained by Dyner (2011) in amaranth grains, who considered this content to be very high and its possible negative effect on the minerals bioaccessibility.

The phytates/minerals molar ratios are used to predict its inhibitory effect on the minerals bioavailability. Phytate-minerals molar ratios capable of impairing mineral bioavailability have been established: calcium>0.24, iron>1 and zinc 15 (Lazarte et al., 2015). Table 3 summarizes the molar ratios of phytates-minerals of samples. The molar ratios of phytate-calcium of all samples were among 1.35 to 1.85. Ratios of phytate-iron and zinc were high above the critical values. These ratios indicate that the calcium, iron and zinc bioaccessibility would be impaired by phytate in the samples studied. There is very little recent data on neither the concentration of phytates or on molar ratios in amaranth, as well as in other Andean grains. One study showed that the content of phytate in quinoa (Andean grain) was high (1530–2280 mg %), therefore, the molar ratios were high above the critical values (Phy:Zn 56.5, Phy:Fe 33.3, Phy:Ca 0.72) (Lazarte et al., 2015).

We expected in the present study, that with lower molar ratios of Phy-minerals, their *in vitro* accessibility would increase, but there was no relationship between Phy-minerals and minerals *in vitro* accessibility. This discrepancy was also observed by authors who studied *in vitro* soluble iron of faba bean (Luo et al., 2009).

Our results suggest that, to improve mineral bioavailability, phytase treatment or the application of uptake enhancers may be included.

3.4.4. Correlation between the minerals bioaccessibility and the inhibitory factors

The influence of inhibitory factors such as phytic acid, free, bound, total polyphenols, and soluble, insoluble, total dietary fiber contents on mineral bioaccessibility was studied by multiple regression analysis (Table 4). Phytic acid of the samples exhibited a high negative correlation with iron, calcium and zinc bioaccessibility, which were explained by 98%, 93% and 100% of the variability of the data, respectively. This is coincident to the obtained value in phytate-minerals molar ratios (Table 3). The regression coefficient of the free fraction and total polyphenols indicated their negative influence on iron, calcium and zinc bioaccessibility ($p < .05$). Gallic acid, *p*-Hydroxybenzoic acid and traces vanillic acid and *p*-Coumaric acid were detected in amaranth grains. The gallic acid is one of the main phenolics which bind with iron to form a complex (Pasko et al., 2008). Thus, correlating iron bioaccessibility may be more appropriate to fit the regression model with these specific phenolic groups.

The reduction of inhibitory factors by heating is insufficient to have a major impact on mineral bioaccessibility. An increase of iron bioaccessibility was observed only in PK, in this study. However,

Table 4
Multiple regression equations of the various inhibitory factors in relation to calcium, iron and zinc bioaccessibility in grain and precooked kiwicha.

Bioaccessibility	R^2	*p-valor*	Regression Equation
Fe	0.98	<0.0001	$-43.79-0.0012x_1 - 0.22x_2 + 0.06x_3 - 0.05x_4 + 1.75x_5 + 3.32x_6 + 3.74x_7$
Zn	0.97	<0.0001	$-42.47-0.0009x_1 - 0.14x_2 + 0.06x_3 - 0.02x_4 + 1.37x_5 + 2.41x_6 + 2.48x_7$
Ca	0.88	0.005	$-436.77-0.001x_1 - 1.59x_2 + 0.70x_3 - 0.64x_4 + 21.30x_5 + 27.75x_6 + 36.20x_7$

x_1: phytic acid, x_2: free fraction polyphenols, x_3: bound fraction polyphenols, x_4: total polyphenols, x_5: soluble fiber, x_6: insoluble fiber, x_7: total dietary fiber.

these effects were not observed for zinc bioaccessibility (Table 1).

Dietary fiber may form insoluble complexes with mineral elements and thus reduce bioavailability of minerals (Luo et al., 2009). Soluble, insoluble and total dietary fiber showed a positive effect on all minerals bioaccessibility (Table 4). The complexation of fiber with minerals is ambiguous. Some authors believe that fibers complex minerals only in small amounts and that most of the complexation from fiber rich foods may be related to other impurities, e.g. phytic acid that accumulates in the outer layer of seeds (Raes et al., 2014).

According with Raes et al. (2014), it can be concluded that the effect of different heat treatments on the different antinutritional factors seems to be rather limited, and in-depth insight is missing, about the effect of heat treatments on mineral bioaccessibility.

4. Conclusions

This study demonstrates that iron bioaccessibility improves by the puffing process of kiwicha grain. Consequently, this product may be destined to vulnerable populations.

Both puffing and lamination processes increased the bioaccessibility of calcium. Precooking process significantly changed the phytic acid, free, bound and total polyphenols and total dietary fiber content. Phytic acid, free fraction and total polyphenols values, exhibited a high negative correlation on iron, calcium and zinc bioaccessibility. The influence of soluble, insoluble and total dietary fiber content on minerals bioaccessibility was positive.

Looking forward, it would be important to study the effect of other processes and the use of enhancers to improve mineral bioaccessibility. This way, it would be possible to improve the mineral bioaccessibility and potential contribution of these products in order to use them as ingredients of other products for different nutritional applications.

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