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Iron, zinc and calcium dialyzability from extruded product based on whole grain amaranth (*Amaranthus caudatus and Amaranthus cruentus*) and amaranth/*Zea mays* blends

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Abstract

Amaranth is a Native American grain appreciated for its high nutritional properties including high mineral content. The aim of this study was to evaluate the availability of Fe, Zn and Ca from extruded products made with two varieties of amaranth and their mixtures with maize at two levels of replacement. Mineral availability was estimated using dialyzability method. The contents of Fe (64.0–84.0 mg/kg), Ca (1977.5–2348.8 mg/kg) and Zn (30.0–32.1 mg/kg) were higher in amaranth than in maize products (6.2, 19.1, 9.7 mg/kg, respectively). Mineral availability was in the range of (2.0–3.6%), (3.3–11.1%) and (1.6–11.4%) for Fe, Ca and Zn, respectively. Extruded amaranth and amaranth/maize products provide higher amount of Fe and Ca than extruded maize. Extruded amaranth products and amaranth addition to maize could be an interesting way to increase nutritional value of extruded products.

Keywords: *amaranth, iron, calcium, zinc, dialyzability, extrusion*

Abbreviations: *AC,* Amaranthus cruentus*, AC:M 30:70,* Amaranthus cruentus *–Maize blend (30:70), AC:M 50:50,* Amaranthus cruentus *–Maize blend (50:50),* DCa%, *Dialyzability of Ca,* DFe%, *Dialyzability of Fe,* DZn%, *Dialyzability of Zn, LSD, least significant difference test, M, Maize,* PS_{Ca}, *Potential supply Ca,* PS_{Fe}, *Potential supply of Fe,* PS_{Zn}, *Potential supply of Zn, Q, Quiwicha (*Amaranthus caudatus*), Q:M 30:70, Quiwicha –Maize blend (30:70), Q:M 50:50, Quiwicha – Maize blend (50:50)*

Introduction

Both amaranth and maize are present in America since 4000 years BC. Mayans and Aztecs used amaranth in their religious ceremonies, which was prohibited by Spanish conquerors. As a consequence of that, a big decay of amaranth production occurred, and only recently their remarkable nutritional properties have been recognized (González et al. 2007).

The family *Amaranthacea* includes more than 800 species and 60 genera. Grains of *Amaranthus* such as *hypochondriacus* and *cruentus* are harvested in Mexico and Guatemala, and *Amaranthus caudatus* are harvested in Peru (Saunders and Becker 1984).

Amaranth is not a cereal since it is dicotyledonous, and cereals are monocotyledonous. However, because of the similarities with cereal composition, it is considered as a pseudo-cereal.

Amaranth grains are an important source of minerals. It was reported that the amount of calcium, magnesium, iron and zinc in amaranth seeds is highest than that of cereals (Alvarez-Jubete et al. 2010). Protein content of grains is around 16%, which is much higher than that of other cereals such rice (7–10%), maize (9–10%) and wheat (11–14%). Moreover, amaranth proteins are considered as

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high-quality proteins because of their high content of lysine and sulphur amino acids (Bertoni 1997). Its high lysine content makes it particularly attractive for use as a blending food source to increase the biological value of processed foods (Pedersen et al. 1987). The lipids are rich in tocotrienols and squalene, which are natural organic compounds positively involved in lowering low-density lipoprotein blood cholesterol (Bodroza-Solarov et al. 2008). However, whole grains contain significant amounts of phytic acid, a well-known inhibitor of Fe absorption and other minerals (Sandberg et al. 1999; Hurrel 2003).

One of the alternatives for using such small size grains in food formulation is obtaining whole grain flour, with the advantage of the mineral contributions made by embryo and bran (Dyner et al. 2007). However, mineral absorption through the intestine wall depends not only on the content and chemical form themselves, but also on the presence of other components in the formulation, individual physiological factors and chemical interaction with some components (Benito and Miller 1998). Thus, to estimate the mineral supply by a food, it is not enough knowing the particular mineral content, but also the amount of mineral really absorbed and utilized, known as bioavailability (O'Dell 1984).

One of the *in vitro* techniques that may be used as an estimator of availability or accessibility is the dialyzability of a particular element, which is the proportion of the element diffusing through a semi-permeable membrane during a simulation of gastrointestinal digestion, after the equilibrium time (Ummadi et al. 1995).

Although none of the *in vitro* methods reproduce physiological conditions (Schricker et al. 1981), iron dialyzability has been shown to predict iron bioavailability (Luten et al. 1996). This technique has been also used to estimate bioavailability of other minerals such as Zn, Ca, Mg and Cu. With respect to Zn and Ca, several authors have observed a good correlation between results of dialyzability and those obtained *in vivo* (Ayorinde et al. 1989; Becker 1989; Singhal and Kulkarni 1990; Berganza et al. 2003). With respect to that, Kernefick y Cashman (Han-Ping et al. 2002) have suggested that dialyzability is a screening technique to evaluate the effect of dietary factors such as phytates, oxalates, fiber, lactose and casein-phosphopeptides affecting Ca absorption.

Snacks are a kind of food that is not part of the main meals. They are usually used to temporarily satisfy hunger, to provide a minimum amount of energy for the body or simply for pleasure. However, snacks are often questioned for their low nutritional value (Pérez Navarrete et al. 2006). Cereals, particularly maize, are most suitable for extrusion because its high starch content gives excellent expansion, a feature that is essential in this type of product (Chen et al. 1991). Extrusion cooking has been used to obtain pre-cooked flours from cereals, legume grains and also from their mixtures (Hurtado et al. 2001), which improve nutritional value of cereal-based products.

Mineral dialyzability (D%), as predictor of mineral availability of cereal-based products, was studied by Pastor Cavada et al. (2011). They observed that extruded whole corn and extruded whole rice presented values of 6.4% and 7.4% for DFe% and 14.5% and 10% for DZn%, respectively. When whole corn or rice was blended with 15% of wild legumes (*Lathyrus*) from the south of Spain, DFe% was in the range of 7.1–12.1% for corn and approximately 10% for rice and DZn% was in the range of 16.1–18.6% for corn and approximately 14% for rice.

Drago et al. (2007) analysed DFe% and DZn% from corn blended in an 80:15 proportion with cowpea flour treated to inactivate lipoxygenase. The sample extruded at 165°C and 17% moisture presented values of 20.6 ± 1.2% for DFe% and 43.5 ± 1.0% for DZn%. However, there is scarce information about the effects of extrusion on mineral availability of pre-cooked amaranth flours and their mixtures with maize.

The aim of this work was to evaluate Fe, Zn and Ca dialyzability as predictors of mineral availability from extruded snack type product, based on amaranth (*A. caudatus and Amaranthus cruentus)* and amaranth/*Zea mays* blends.

Materials and methods

Materials

Distilled, deionized water was used throughout the experiments. All glassware was washed with detergent, rinsed with water, soaked overnight in 20% HNO_3, rinsed again and dried. All chemicals were of analytical grade.

Raw materials

Degermed and dehulled commercial maize grits (M) and two amaranth samples *A. cruentus* (AC) and *A. caudatus*, known as Quiwicha (Q), were used. Qiwicha (Ecotipo Fuerte Alto) from Departamento de Cachi, Salta, and *A. cruentus* from an Argentinean grower (La Pampa, Argentina) were used.

Grains of both amaranth samples were ground with a roller mill (Vario Miag, Bad Homburg, Germany). Care was taken for not to produce too many fine fractions (below 250 μm). Final grits particle size was between 420 and 250 μm, and less than 4% of particle size was below 250 μm.

Flour blend preparation

Flour moisture content was determined to establish the amount of water to be added to adjust moisture content to required levels. The amaranth was blended with maize grits in a 50:50 or 30:70 proportions in sufficient quantities to produce 500 g flour blend per treatment. The blends were placed in a planetary mixer (Brabender P600; Duisburg, Germany), homogenized for 10 min and water added until the required moisture content was attained. Blend preparation was done 1 h before extrusion.

Extrusion process

The extrusion process was carried out with a Brabender 10 DN single screw extruder (South Hackensack, NJ, USA), using a 3:1 compression ratio screw, a 3/20-mm (diameter/length) die and a screw speed of 175 rpm. Extrusion temperature and grits moisture were selected according to previous trials and they were 180°C and 16%, respectively. The feeding rate of the extruder was at full capacity. While the extruder feeding section was maintained cool by circulating water through the jacketed device, the metering and die sections were both kept at 180°C by using the heat control device of the extruder.

Seven different extruded samples were obtained:

(a) *A. cruentus*: Maize blend (30:70): AC:M 30:70
(b) *A. cruentus:* Maize blend (50:50): AC:M 50:50
(c) *Quiwicha:* Maize blend (30:70): Q:M 30:70
(d) *Quiwicha:* Maize blend (50:50): Q:M 50:50
(e) Extruded maize: M
(f) *A. cruentus*: AC
(g) *A. caudatus:* Q

The samples were then allowed to dry in the ambient air and kept in plastic bags. Flour was used for different analysis. For that, 100 g of each sample was first ground with a laboratory hammer mill (Retsch GmbH & Co., KG, Haan, Germany) with a 2-mm sieve, and then with a Cyclotec (FOSS, Hillerød, Denmark) mill through a 1-mm sieve.

Composition

Centesimal composition (protein, lipids, moisture, ash, dietary fibre) was assessed according to the AOAC methods (2000).

Mineral composition

Assessment of minerals (iron, zinc and calcium) in pre-cooked flour samples was made by Flame Atomic Absorption Spectroscopy after dry ashing (AOAC 2000), using an Atomic Absorption spectrophotometer Analyst 300 (Perkin-Elmer, Norwalk, CT, USA).

Determination of mineral dialyzability (DFe%, DZn%, DCa%)

All measurements were performed using the pre-cooked flour. A modification of the widespread *in vitro* Miller et al. (1981) method according to Drago et al. (2005) was followed. Aliquots coming from 500 g of each extruded sample were prepared to 10% solid concentration (w/w) using deionized water. Aliquots (25 g) of homogenized samples were adjusted to pH 2.0 with 4 mol/l HCl, and after addition of 0.8 ml pepsin digestion mixture (16% pepsin (Sigma P-7000) solution in 0.1 mol/l HCl) was incubated at 37°C for 2 h in a shaking water bath. At the end of pepsin digestion, dialysis bags containing 20 ml of 0.15 mol/l PIPES (piperazine-*N*,*N*′-bis[2-ethane-sulfonic acid] disodium salt) buffer (Sigma P-3768) were placed in each flask and were incubated for 50 min in a shaking water bath at 37°C. Pancreatin–bile mixture (6.25 ml of 2.5% bile (Sigma B-8631) and 0.4% pancreatin (Sigma P-1750) solution in 0.1 mol/l $NaHCO_3$) was then added to each flask and the incubation continued for another 2 h. Then, bag contents were weighed and analysed for its mineral content by flame atomic absorption spectroscopy. Mineral dialyzability was calculated from the amount of each dialyzed mineral expressed as a percentage of the total amount present in each sample:

$$\text{Dialyzable mineral (DM\%)} = \left[\frac{D}{(W \times A)}\right] \times 100,$$

where D is the total amount of dialyzed mineral (μg), W is the weight of sample (g) and A is the concentration of each mineral in the sample (μg/g).

The potential supply (PS) for each mineral (M) was calculated according the following equation and considering a 25 g serving. This serving size was considered according to 47/03 MERCOSUR technical ruler:

$$\text{PS} = \text{M concentration} \times \text{DM\%} \times \text{serving (25 g)}.$$

Statistical analysis

All analyses (proximal composition, mineral content and dialyzability) were performed in triplicate. Analysis of variance was carried out using the software Statgraphics Plus 5.1, and the statistical differences among samples were determined using the least significant difference (LSD) test.

Results and discussion

Centesimal and mineral composition of samples

Table I shows centesimal composition in dry weight of the different raw materials. The values are similar to those reported in bibliography (Teutonico and Knorr 1985; Bressani et al. 1987; Bressani 1989; Tosi et al. 2001; Dyner et al. 2007; Roda and Bressani 2009).

Table II shows Fe, Ca and Zn contents corresponding to each extruded sample. There is an agreement with results found in literature for maize and amaranth grains (Bertoni et al 1984; Teutonico

Table I. Centesimal composition (dry basis) of raw materials.

Components (g/100 g), dry base	Quiwicha *A. caudatus*	*A. cruentus*	Maize (*Zea mays*)
Protein	14.32 ± 0.30^b	16.67 ± 0.34^c	8.53 ± 0.32^a
Ether extract	6.2 ± 0.22^b	6.4 ± 0.27^b	0.44 ± 0.19^a
Moisture	11.54 ± 0.65^a	10.59 ± 0.69^a	11.27 ± 0.58^a
Ash	2.05 ± 0.01^b	2.62 ± 0.01^c	0.37 ± 0.02^a
Dietary fibre	14.40 ± 1.10^b	14.50 ± 1.18^b	6.24 ± 0.90^a

Note: Different letters in the same row mean significant differences ($p < 0.05$). Values were expressed as x ± SD.

and Knorr 1985; Moreno 1993; Bertoni 1997). Regarding non-extruded flours from maize and amaranth samples, the values of Fe, Zn and Ca content did not show significant differences from extruded flours (data not shown).

Samples containing amaranth had significantly higher mineral content than that from maize alone, particularly for Ca and Fe, whose values were several times higher. The mixtures containing only 30% amaranth had five times more Fe and 31.6 times more Ca than the corresponding from maize.

On the other hand, Q showed higher values of Fe and Ca and lower value of Zn, than the corresponding values of AC, although the differences reported in the literature for both amaranth species are smaller than the ones we obtained.

Dialyzability of Fe (DFe%), Ca (DCa%) and Zn (DZn%):

Table III shows mineral dialyzability from different extruded samples.

With respect to DFe%, no detection of Fe was verified on maize sample dialyzates, probably because the small amount of Fe contained in degermed and dehulled maize (Maldonado and Samman 2000). It was observed that DFe% of AC samples was higher than that of Q. In the case of the blends, dialyzability corresponding to AC samples remained approximately constant as the percentage of amaranth added increased, while DFe% from Q-containing mixture increased as addition of Q increased.

With respect to Ca, it was found that maize-containing samples had good dialyzability value (100%), although the content of Ca was very low. However, AC sample had higher DCa% value than Q sample, while in the case of maize–amaranth mixtures, no difference in DCa% values was observed between AC and Q containing samples.

Samples containing maize had DZn% values higher than those containing amaranth, while no difference was found between AC and Q samples.

In the case of AC:M mixture, DZn% did not decrease as the addition of AC increased, while for the Q:M mixture, this value decreased as Q increased.

When AC was used, no differences in mineral availability were observed as the percentage of amaranth replacement increased, but in the case of Q, DZn% decreased by 80%. This could be explained by taking into account that when Q is added to maize, mineral content increases, as well as absorption inhibitors, mainly phytates.

Sanz-Penella et al. (2012) observed that bread supplemented with whole amaranth flour (40%) showed significant increase in soluble phytates levels in comparison with controls, which do not have detectable values. Phytic acid, or myo-inositol (1,2,3,4,5,6)-hexakisphosphate (InsP6), is the major storage form of phosphorous in plants. It exists in the form of mixed salts, as phytates, and occurs in many locations within the kernel. Phytic acid has a strong ability to form complexes with multivalent metal ions, especially iron, calcium and zinc. This binding can result in very insoluble salts (varying with the pH) with poor bioavailability of minerals (Pedersen et al. 1987; Zhou and Erdman 1995; Martinez Dominguez et al. 2002).

PS of Fe, Ca and Zn:

Whole grain extruded amaranth products present with higher PS_{Fe} (48–57 μg) compared with mixtures made with amaranth: maize 30:70 (13–22 μg) and 50:50 (27–31 μg) or maize (not detected Fe in dialyzate). In addition, the blends have higher PS_{Fe} at higher percentage of replacement.

It is estimated that 1.8 mg of Fe daily is necessary absorb to meet the needs of 80–90% of adult women and adolescents of both sexes (Monsen et al. 1978). Taking into account the values of PS_{Fe}, amaranth-

Table II. Fe, Zn and Ca contents (dry basis) corresponding to each extruded sample.

Samples	Fe (mg/kg)*	Zn (mg/kg)*	Ca (mg/kg)*
AC:M 30:70	34.88 ± 0.27^c	12.57 ± 0.29^b	630.65 ± 0.25^c
AC:M 50:50	42.53 ± 1.22^d	15.18 ± 0.48^c	753.22 ± 4.49^d
Q:M 30:70	30.50 ± 0.52^b	10.62 ± 0.35^a	585.71 ± 21.80^b
Q:M 50:50	52.04 ± 1.45^e	14.42 ± 0.39^d	840.75 ± 73.18^e
M	6.18 ± 0.06^a	9.67 ± 0.66^a	19.09 ± 0.38^a
AC	64.05 ± 1.29^f	32.09 ± 0.64^f	1977.49 ± 42.75^f
Q	84.05 ± 1.51^g	30.05 ± 0.39^e	2348.84 ± 34.87^g

Notes: AC:M 30:70: *A. cruentus:* maize (30:70); AC:M 50:50: *A. cruentus*: maize (50:50); Q:M 30:70: Quiwicha: maize (30:70); Q:M 50:50: Quiwicha: maize (50:50); M: extruded maize; AC: *A. cruentus*; Q: Quiwicha *(A. caudatus)*. Different letters in the same column mean significant differences ($p < 0.05$). *x ± SD.

Table III. % Dialyzability of Fe (%DFe), Ca (%DCa) and Zn (%DZn).

Samples	%DFe*	%DZn*	%DCa*
AC:M 30:70	2.80 ± 0.25^{d}	4.90 ± 0.79^{b}	$9.68 \pm 0.95^{c,d}$
AC:M 50:50	2.90 ± 0.24^{d}	$3.52 \pm 0.85^{a,b}$	11.13 ± 0.35^{d}
Q:M 30:70	1.98 ± 0.08^{b}	11.44 ± 0.75^{c}	8.28 ± 0.32^{c}
Q:M 50:50	$2.72 \pm 0.26^{c,d}$	2.30 ± 0.81^{a}	9.24 ± 0.89^{c}
M	Nd	21.75 ± 2.51^{d}	100.00^{e}
AC	3.56 ± 0.53^{e}	2.35 ± 0.20^{a}	5.94 ± 0.86^{b}
Q	$2.28 \pm 0.17^{b,c}$	1.64 ± 0.22^{a}	3.29 ± 0.18^{a}

Notes: Nd: Non-detected iron in dialyzate; AC:M 30:70: *A. cruentus:* maize (30:70); AC:M 50:50: *A. cruentus*: maize (50:50); Q:M 30:70: Quiwicha: maize (30:70); Q:M 50:50: Quiwicha: maize (50:50); M: extruded maize; AC: *A. cruentus*; Q: Quiwicha *(A. caudatus)*. Different letters in the same column mean significant differences ($p < 0.05$). *$x \pm$ SD.

extruded products and its blends provide from 0.72% to 3.16% of these requirements by 25 g of serving.

The PS of Ca (PS_{Ca}) followed a similar trend than PS_{Fe}. PS_{Ca} was higher in amaranth (1.72–2.62 μg) compared with maize (0.42 μg); and higher the amaranth percentage in blends, higher the PS_{Ca} was (1.71 to 1.87 μg). Taking into account the fact that the inevitable losses in the adult of this mineral is found around 300 mg/day (Martín de Portela 1993), products of amaranth and blends would supply from 0.57% to 0.87% of such losses.

With respect to the PS of Zn (PS_{Zn}), it was higher in maize products (47 μg) than in amaranth (11–17 μg). Even though the former has less zinc content, the availability was higher. The PS_{Zn} was not improved by increasing the percentage of amaranth replacement. In the case of this mineral, the daily requirement for adults is 2.2 mg/day (Martín de Portela 1993). A portion of amaranth snacks or amaranth blends supply about 0.5–0.8% of those recommendations.

The recommended daily intake of a nutrient is always above of its actual requirement, since the nutritional recommendation is calculated using factors related with environmental factors, the individual variability and the bioavailability of such nutrient in the diet (Ziegler and Filer 1990). The expression of the PS of a nutrient takes into account the availability and thus the contribution from a particular food.

Considering the high mineral content of amaranth and amaranth/maize blends, it could be interesting to use enhancers, such as Na_2EDTA or sodium citrate to improve mineral absorption and PS. Considering that, Bernardi et al. (2006) used ascorbic acid (AA) and citric acid (CA) at different molar AA:Fe and CA:Fe ratios, in order to increase mineral availability from cookies made with wheat flour and 30% of *Prosopis alba* (Algarrobo) pulp. The ratios were 5:1 and 10:1 for AA:Fe, whereas 50:1 and 100:1 and combinations of them were for CA:Fe. The best AA:Fe and CA:Fe ratios were 5:1 and 50:1, respectively, which allow obtaining values of 4% for DFe% and 26.6% for DCa%. Also, Drago et al. (2011) used mineral enhancers to increase mineral supply of extruded maize:soy blends (88:12). Molar ratios of Fe:AA (1:8), Fe:citrate (1:50) and Fe:EDTA (1:1) were used. Na_2EDTA or sodium citrate increased the dialysis of Fe, Zn and Ca with respect to the sample without enhancers, but this was not observed for AA addition. They concluded that the use of Na_2EDTA may be an appropriate strategy to enhance the intrinsic mineral supply of these products.

CONCLUSIONS

Samples added with amaranth had higher contents of Fe, Ca and Zn than samples added with maize. Although, in general, dialyzability of Fe, Zn and Ca was not high, samples added with amaranth supply higher amount of Fe and Ca than maize. Moreover, the use of absorption enhancers could contribute to promote mineral absorption. Addition of amaranth to maize may be an interesting way to increase nutritional value of maize-extruded products not only for enhanced mineral supply but also for providing higher amount of good quality protein.

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