Research Article

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Effects of roasting and boiling of quinoa, kiwicha and kañiwa on composition and availability of minerals in *vitro*

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Abstract

BACKGOUND: Andean indigenous crops such as quinoa (*Chenopodium quinoa*), kiwicha (*Amaranthus caudatus*) and kañiwa (*Chenopodium pallidicaule*) seeds are good sources of minerals (calcium and iron). Little is known, however, about mineral bioavailability in these grains. Thus the aim of the present study was to determine the iron, calcium and zinc potential availability in raw, roasted and boiled quinoa, kañiwa and kiwicha seeds. Potential availability was estimated by dialyzability.

RESULTS: These seeds are good sources of phenolic compounds and kañiwa of dietary fiber. Their calcium, zinc and iron content is higher than in common cereals. In general, roasting did not significantly affect mineral dialyzability. Conversely, in boiled grains there was an increase in dialyzability of zinc and, in the case of kañiwa, also in iron and calcium dialyzability.

CONCLUSION: Taking into account the high content of minerals in Andean grains, the potential contribution of these minerals would not differ considerably from that of wheat flour. Further studies are required to research the effect of extrusion on mineral availability in Andean grains.

Keywords: quinoa; *Amaranthus*; dialyzability; mineral availability

INTRODUCTION

The Andean area of South America is a very important center of domestication of food crops. This area is the botanical origin of potato, corn, peanut and tomato. Less well- known crops, such as quinoa (*Chenopodium quinoa*), kañiwa (*Chenopodium pallidicaule)* and kiwicha (*Amaranthus caudatus*), were also cultivated by ancient Andean farmers. These crops have a long history of safe use by local populations and they have contributed to the nutrition and well-being of the people for centuries. After being neglected in many areas the Andean pseudo-cereals are now studied as good sources of several nutrients. These crops are very nutritious and well adapted to a high-mountain environment. They are not true cereals because they do not belong to the Gramineae family, but they are often called 'Andean cereals' because they produce seeds that can be milled into flour and used like a cereal crop. Quinoa is generally boiled and used in soups. Kiwicha and kañiwa are used as roasted seeds. Flours are obtained from all three crops and used in various food preparations.

Calcium, iron and zinc are essential minerals required for diverse physiological and biochemical functions. Milk and dairy products are excellent sources of calcium, and meat and meat products of iron and zinc. In many geographic areas, such as Peru, the consumption of these products is limited owing to economic and cultural factors. In South America about 65–75% of the population suffers from lactose intolerance and cannot consume dairy products.[1] In Peru the consumption of meat products is not widespread and iron deficiency anaemia is a prevalent nutritional problem.[2] Zinc is the fourth most important micronutrient after vitamin A, iron and iodine, and is receiving increasing global attention.[3]

In developing countries, iron, zinc and calcium are mainly derived from food grains. Certain vegetable foods, like seeds and pulses, are good alternative sources of these minerals. The bioavailability of the minerals in plant sources is lower than that in animal sources, however, because of the presence of certain compounds, like dietary fiber, phytate and oxalate, which have negative effects on mineral absorption. In the case of iron, this mineral is present in foods in two different forms: heme iron (HI) and non-heme iron (NHI). The absorption of these forms of iron is different: HI is high and NHI is low. The cereals contain iron in non-heme form. NHI absorption is greatly influenced by interactions with enhancers and inhibitors.[4]

The bioavailability of minerals is defined as the amount of mineral that is absorbed in the gastrointestinal tract and utilized for metabolic functions. The term '*in vitro* availability of minerals' is used to describe the amount of mineral soluble under physiological

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conditions according to a process that stimulates the human digestive system.[5] Mineral dialyzability measures soluble and ionizable mineral after digestion with pepsin and pancreatin at conditions simulating those of stomach and duodenum.[4] Mineral dialyzability has been shown to predict mineral bioavailability; however, it is important to stress that dialyzability is a relative rather than an absolute estimate of mineral absorbability.[6]

In comparison with common cereals, quinoa, kañiwa and kiwicha have relatively high protein content with excellent composition of essential amino acids.[7,8] The essential amino acid composition of these crops is close to international standards on amino acid requirements.[8] They are also good sources of dietary fiber and specific bioactive compounds.[9,10] Quinoa, kiwicha and kañiwa seeds are considered to be good sources of minerals, e.g. calcium and iron.[11,12] Little is known, however, about mineral bioavailability in these grains. Thus the aim of our study was to determine the iron, calcium and zinc potential availability of raw, roasted and boiled quinoa, kañiwa and kiwicha seeds.

MATERIALS AND METHODS

Samples

Washed and dried quinoa seeds (red quinoa, Pasankalla variety) were purchased in Cusco from a local market. Kañiwa seeds (Cupi variety) were from Puno (Agrarian Experimental Station Illpa, Department of Puno, Peru). Centenario (kiwicha variety) was obtained from the experimental field of the National Agrarian University, La Molina, Lima, Peru. All grains were from the 2007–2008 growing season. The varieties under this study are commonly used commercial varieties.

Preparation of samples for availability study

Roasting

500 g of the grains of quinoa, kañiwa and kiwicha were roasted by the traditional method using a hotplate at a temperature of about 190 °C for 3 min.

Boiling

The grains of quinoa, kañiwa and kiwicha were boiled with tap water for 20 min in a proportion of 250 g grains L^{-1} water.

Proximate composition

Moisture content, crude protein (N × 6.25), crude fat and ash were determined according to the AOAC official method.[13] The total, soluble and insoluble dietary fiber were analyzed by an enzymatic–gravimetric method according to the AACC[14] using a TDF-100 kit from Sigma Chemical Co. (St Louis, MO, USA).

Carbohydrates (CHO) were calculated by difference from the formula CHO = 100 − (moisture + fat + protein + dietary fiber + ash).

Total phenolic compounds

Total phenolic compounds were analyzed according to the method of Swain and Hillis.[15] A sample of 5 g milled grains was homogenized with 20 mL methanol to a uniform consistency and left at 4 °C for 24 h before filtration. An aliquot of extract (0.5 mL) was diluted with 8 mL water. The extract was allowed to react with the Folin–Ciocalteu phenol reagent. Absorbance was measured at 725 nm. The results were expressed in gallic acid equivalents (GAE mg 100 g^{-1} dry matter) using a gallic acid (0–0.1 mg mL^{-1}) standard curve:

$$\text{Gallic acid equivalent} = [0.005 + (0.1504 \times \text{Absorbance})] \times (20\ g^{-1}\ \text{of sample}) \times 100$$

Iron, zinc and calcium dialyzability (FeD%, ZnD%, and CaD%)

A modification of the *in vitro* method,[16] introduced by Wolfgor and others,[17] was used. Aliquots of homogenized samples (50 g) were incubated with 5 mL of a 3% α-amylase solution for 30 min at 37 °C in a shaking water bath, then adjusted to pH 2.0 with 6 mol L^{-1} HCl and, after addition of 1.6 mL pepsin digestion mixture (16% pepsin solution in 0.1 mol L^{-1} HCl), were incubated at 37 °C for 2 h in a shaking water bath. At the end of pepsin digestion, two aliquots of digest (15 g) were weighed in 100 mL beakers. Dialysis bags (Spectrapore, molecular weight cut-off 6000–8000, Fischer Scientific, Fairlawn, NJ, USA) containing 18.75 mL 0.15 mol L^{-1} PIPES (Sigma Chemical CO, St. Luis, MO, USA) (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.[18]

Aliquots of each pepsin digest with dialysis bags containing PIPES buffer were incubated for 50 min in a shaking water bath at 37 °C. Pancreatin–bile mixture (3.75 mL of 2.5% bile, 0.4% pancreatin solution in 0.1 mol L^{-1} $NaHCO_3$) was then added to each beaker, and the incubation continued for another 2 h. At the end of the pancreatin–bile incubation, the dialysis bags were removed and rinsed with water.

Bag contents were transferred to tared flasks, weighed and analyzed for their iron, zinc and calcium content by flame atomic absorption spectroscopy (AAS). Assessment of minerals in pepsin digests was made by AAS after wet ashing with HNO_3–$HClO_4$ (50 : 50). Lanthanum was added to all samples and standards analyzed for Ca to reach a 0.5% final concentration to prevent possible phosphate interference.

Mineral dialyzability (FeD%, ZnD%, CaD%) was calculated from the amount of each dialyzed mineral, expressed as a percentage of the total amount present in each pepsin digest.

The potential contribution of each mineral (PC) was calculated as each mineral concentration times its dialyzability:[19]

$$\text{PCFe} = ([\text{Fe}] \times \text{FeD\%})/100$$
$$\text{PCCa} = ([\text{Ca}] \times \text{CaD\%})/100$$
$$\text{PCZn} = ([\text{Zn}] \times \text{ZnD\%})/100$$

Statistical analysis

All analyses were performed in duplicate or triplicate. One-way ANOVA was used to calculate the differences between the constituents (proximate, dietary fiber, phenolics) of the grains. Means were compared with Tukey's multiple range test. Probability was set at $P < 0.05$.

The mineral availability data were analyzed by one-way analysis of variance (ANOVA) and Dunnet *a posteriori* test.

RESULTS AND DISCUSSION

Proximate composition and total phenolic content in uncooked grains

The results of analysis of the proximate composition of the three Andean crops are presented in Table 1. Their protein content was between 11% and 16%, kañiwa having the highest and kiwicha

Table 1. Proximate composition and total phenolic compounds in quinoa, kañiwa and kiwicha grains[a]

Component	Quinoa	Kañiwa	Kiwicha
Moisture (g kg^{-1})	101.3 ± 0.05a	101.9 ± 0.06a	113.0 ± 0.02b
Protein (g kg^{-1})	131.8 ± 0.01b	160.7 ± 0.00c	116.9 ± 0.01a
Fat (g kg^{-1})	65.1 ± 0.04a	79.6 ± 0.11c	75.7 ± 0.03b
Ash (g kg^{-1})	23.4 ± 0.03b	44.7 ± 0.03c	17.8 ± 0.01a
Digestible carbohydrates (g kg^{-1})[b]	589.7	487.5	618.6
Total dietary fiber (g kg^{-1})	88.7	125.6	58.0
Insoluble dietary fiber (g kg^{-1})	78.5 ± 0.12a	106.4 ± 0.23b	53.5 ± 0.08c
Soluble dietary fiber (g kg^{-1})	10.2 ± 0.10a	19.2 ± 0.66a	4.5 ± 0.36a
Total phenolic compounds (mg GAE 100 g^{-1} sample)	41.78 ± 0.89a	29.52 ± 0.28b	12.14 ± 0.34c

[a] All data are the mean ± SD of three replicates. Mean values with same letter within the same row are not significantly different. All data are expressed on a dry matter basis.
[b] Digestible carbohydrates = [100 − (moisture + protein + fat + ash + dietary fiber)].

the lowest protein content. The fat content of the three crops was statistically different, kañiwa having the highest value. The content of soluble, insoluble and total dietary fiber of quinoa, kañiwa and kiwicha is presented in Table 1. Kañiwa contained the highest amount of total dietary fiber (12.56%). Total dietary fiber content in kiwicha was 5.80% and in quinoa 8.87%.

The differences in protein, fat and ash content of quinoa, kañiwa and kiwicha were statistically significant. In the case of dietary fiber, there were significant differences in the insoluble dietary fiber content but there was no statistically significant difference in the content of soluble dietary fiber of these three grains. The total dietary fiber content of kañiwa (12.56%) was lower than that found by Repo-Carrasco-Valencia *et al.*[20] (25%). This is because, in this study, kañiwa was used without its outer seed coat, perigonium. This reduces the dietary fiber content significantly. Kañiwa is commonly consumed without the outer seed coat. Kañiwa can be considered a good source of dietary fiber.

Quinoa had the highest (42 mg GAE 100 g^{-1}) and the kiwicha the lowest (12 mg GAE 100 g^{-1}) content of phenolic compounds (Table 1). The differences between the content of total phenolics in quinoa, kañiwa and kiwicha were statistically significant. The content of total phenolic compounds of quinoa was similar to that of sorghum.[21] Sorghum and barley can be considered important sources of phenolic compounds.[22] The content of phenolics in kañiwa was lower than that found in previous studies by Repo-Carrasco-Valencia *et al.*[20] Peñarrieta *et al.*[9] found 1.7–7.4 mg GAE g^{-1} in different kañiwa ecotypes in Bolivia. This value is also higher than that found in our study. This can be explained by the fact that in those studies the seeds of kañiwa were used with the outer seed coat. In our study the seeds were acquired without seed coat and the process of peeling the seed probably reduces the content of total phenolic compounds. Guzman-Maldonado and Paredes-Lopez[12] reported levels of 2–4 mg g^{-1} of total phenolic compounds in amaranth, which is higher than the content found in this study. This difference could be due to the different amaranth species and to different growing conditions. Nsimba *et al.*[23] analyzed the total phenolic content in quinoa and amaranth ecotypes and found a wide range of these compounds (94–148 mg g^{-1} tannic acid equivalent). They explain the difference in the phenolic concentration by the difference in the environmental conditions or genetic background. Environmental conditions, such as temperature, injury and infections, affect the biosynthesis of phenolic compounds. Alvarez-Jubete *et al.*[24] analyzed the total phenolic content of quinoa and amaranth. The content of total phenolic compounds for amaranth was 21.2 mg GAE 100 g^{-1} and for quinoa it was 71.7 mg GAE 100 g^{-1}. These results agree with ours, demonstrating that quinoa has higher phenolic compound content than amaranth. Gorinstein *et al.*[25] also found a higher phenolic compound content in quinoa compared with amaranth. Phenolic compounds present in grains possess antioxidant properties that are associated with the health benefits of grains and grain products. Andean indigenous crops are good sources of phenolic compounds and could offer health-promoting ingredients to consumers.

Table 2. Mineral composition of raw, roasted and boiled Andean grains

Sample	Iron (mg 100 g^{-1})	Zinc (mg 100 g^{-1})	Calcium (mg 100 g^{-1})
Quinoa, raw	2.95 ± 0.16a	2.95 ± 0.38a	68.55 ± 3.68a
Quinoa, roasted	3.15 ± 0.08a	3.18 ± 0.42a	59.29 ± 4.99b
Quinoa, boiled	1.08 ± 0.17b	1.79 ± 0.39b	67.03 ± 4.63a
Kañiwa, raw	4.91 ± 0.24c	2.15 ± 0.23c	29.76 ± 4.09c
Kañiwa, roasted	5.44 ± 0.60d	2.72 ± 0.21d	32.33 ± 3.95c,d
Kañiwa, boiled	1.89 ± 0.05e	1.48 ± 0.42e	37.56 ± 2.07d
Kiwicha, raw	5.00 ± 0.92f	1.25 ± 0.16f	27.90 ± 1.43f
Kiwicha, roasted	3.75 ± 0.63g	1.33 ± 0.19f	29.73 ± 3.16f
Kiwicha, boiled	3.55 ± 0.41g	1.05 ± 0.32f	25.06 ± 4.30f

All data are the mean ± SD of three replicates.
For each grain type, means within a column not sharing a common letter differ significantly ($P < 0.05$). All data are expressed on a wet basis.

Mineral content in raw and processed grains

Iron content was similar in raw kañiwa and kiwicha. Regarding zinc and calcium, quinoa grains contained the highest levels of both minerals (Table 2).

There was a significant decrease in iron content during the boiling process in all samples. Wet processing procedures in general cause loss of dry matter and iron.[26] In the case of kiwicha, roasting also reduced the content of this mineral. Boiling reduced the content of zinc in quinoa and kañiwa, but not in kiwicha. Roasting affected negatively the content of calcium in quinoa but not in kañiwa and kiwicha.

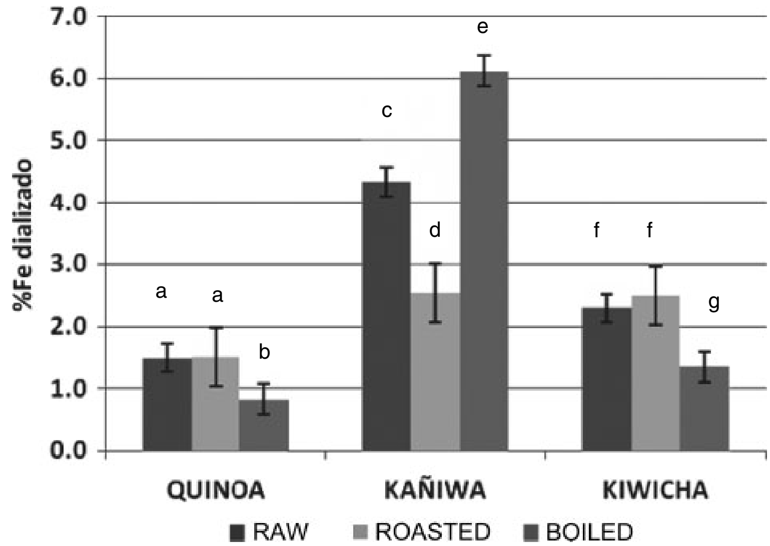

Figure 1. Iron dialyzability (FeD%) of raw, roasted and boiled quinoa, kañiwa and kiwicha. Means ± standard deviation for six analyses. Statistical comparison was between raw, roasted and boiled grain. For each grain type, means not sharing a common letter differ.

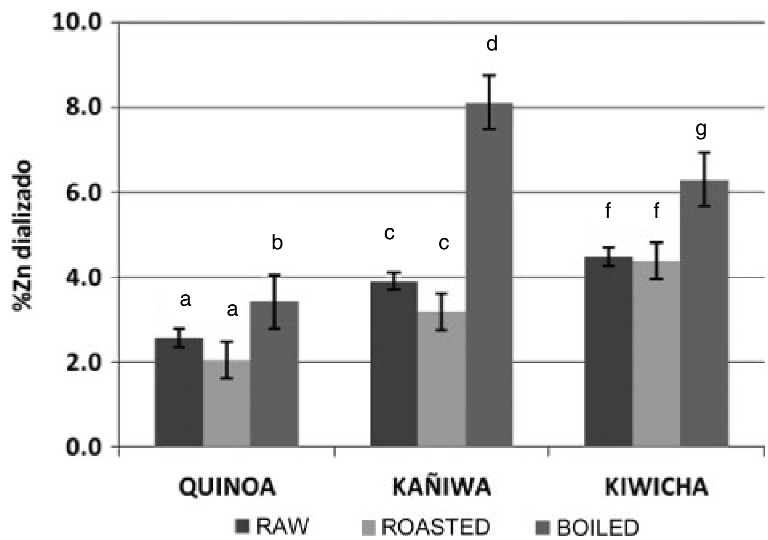

Figure 2. Zinc dialyzability (ZnD%) of raw, roasted and boiled quinoa, kañiwa and kiwicha. Means ± standard deviation for six analyses. Statistical comparison was between raw, roasted and boiled grain. For each grain type, means not sharing a common letter differ.

Compared with unenriched wheat flour (iron, 0.68 mg 100 g^{-1}; zinc, 0.98 mg 100 g^{-1}; and calcium, 18.46 mg 100 g^{-1}),[19] concentrations of these minerals are considerably higher in Andean grains. Iron content in quinoa, kañiwa and kiwicha is higher than in rice (1.32 mg 100 g^{-1}) and finger millet (2.13 mg 100 g^{-1}).[3] Pachón *et al*.[27] analyzed iron and zinc content in conventional and nutritionally enhanced beans and maize. According to our study, Andean grains contain more zinc and iron than conventional maize and beans.

Dialyzability and potential contribution of iron, zinc and calcium in raw and processed grains

Iron, zinc and calcium dialyzability is presented in Figs 1, 2 and 3, respectively. The potential contribution (PC) is shown in Table 3.

In the case of kañiwa, the boiled samples had higher mineral dialyzability than the raw and roasted samples. In roasted samples of kañiwa, mineral dialyzability tended to be similar to or lower than that in raw samples. In the case of quinoa and kiwicha, there were no differences in iron dialyzability between raw and roasted grains, although the boiled grains showed lower values ($P < 0.05$). Consequently, the PC of iron diminished in boiled grains.

With respect to zinc, boiled quinoa, kañiwa and kiwicha showed significantly higher zinc dialyzability regarding both raw and roasted samples ($P < 0.01$). Accordingly, PC of zinc in processed kañiwa and kiwicha tended to be similar to or higher than that in unprocessed grains. PC of processed quinoa was lower than in unprocessed grain. In the case of calcium, each grain showed a different behavior, with no characteristic pattern. For example, roasted and boiled quinoa had lower calcium dialyzability than raw, while boiled kañiwa had higher values than raw. In the case of kiwicha there were no significant differences between raw, roasted and boiled samples. The dialyzability of calcium in raw grains was between 23% and 28%. In processed products, it was 22–30%.

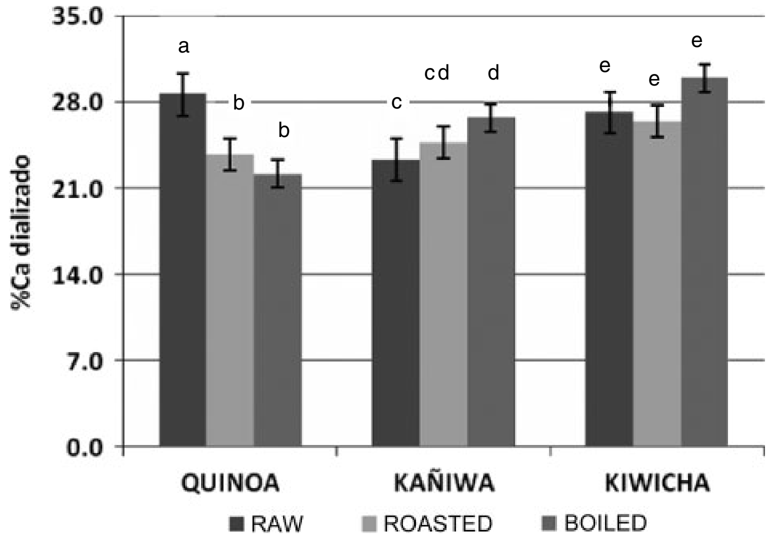

Figure 3. Calcium dialyzability (CaD%) of raw, roasted and boiled quinoa, kañiwa and kiwicha. Means ± standard deviation for six analyses. Statistical comparison was between raw, roasted and boiled grain. For each grain type, means not sharing a common letter differ.

Table 3. Potential contribution (PC) of iron, zinc and calcium in raw, roasted and boiled quinoa, kañiwa and kiwicha grains

Sample	PC iron (mg %)	PC zinc (mg %)	PC calcium (mg %)
Quinoa, raw	0.04a	0.08a	19.63a
Quinoa, roasted	0.05a	0.06b	14.00b
Quinoa, boiled	0.01b	0.06b	14.91b
Kañiwa, raw	0.21d	0.08d	6.95d
Kañiwa, roasted	0.14e	0.09d	8.00d
Kañiwa, boiled	0.12f	0.12e	10.02e
Kiwicha, raw	0.11g	0.06g	7.56g
Kiwicha, roasted	0.09h	0.06g	7.85g
Kiwicha, boiled	0.05i	0.07g	7.50g

All data are the mean ± SD of three replicates. For each grain type, means within a column not sharing a common letter differ significantly ($P < 0.05$). All data are expressed on a wet basis. The potential contribution of each mineral (PC) was calculated as each mineral concentration multiplied by its dialyzability.

According to research by Kamchan *et al.*,[28] amaranth leaves are rich in calcium. The dialyzability of calcium, however, is low (4.1%). In our study the dialyzability of calcium was high in all samples (22–30%), comparable to the calcium dialyzability of milk power (25%). Whole milk power was used as reference food for calcium bioavailability comparison in a study by Kamchan *et al.*[28] Drago and Valencia[29] analyzed the dialyzability of calcium in dairy products. The dialyzability of calcium for fresh milk was 35%. Skibniewska *et al.*[30] analyzed *in vitro* availability of minerals in oat products. The *in vitro* availability of calcium was 27–40% for different oat products.

Calcium, zinc and iron dialyzability of kiwicha was considerably higher in our study than in the research carried out by Dyner *et al.*[19] They analyzed the dialyzability of calcium and other minerals in *Amaranthus caudatus*. The content of these minerals in our study, however, was lower than in the study by Dyner *et al.*[19] The PC of calcium was lower and that of iron and zinc higher in our study in comparison with the study by Dyner *et al.*[19]

Iron dialyzability was relatively low in all samples (1–6%). These values are lower than those for oat products (7–30%)[30] but similar to the values for boiled and extruded legumes (1–5%).[31] In boiled quinoa, kañiwa and kiwicha there was an increase in dialyzability of zinc. During heat treatment the grains lose their integrity and this could lead to less interaction between these minerals and the inhibitors present in these grains, such as dietary fiber components, phytates and polyphenols, which form chelates that interfere with mineral absorption. Kayodé *et al.*[32] found that boiling drastically reduced the *in vitro* Fe and Zn solubility in sorghum porridges. Sorghum has a higher content of phenolic compounds than the Andean crops. Some phenolics can polymerize into condensed phenolics during heat treatments and be responsible for the decrease of soluble iron and zinc by chelating them. In general, the samples of quinoa had lower iron and zinc dialyzability than kiwicha and kañiwa samples. This could be due to the presence of saponins and phytic acid in quinoa seeds.[33] It is well known that phytic acid and saponins lower bioavailability of zinc and iron.[34,35]

Roasting and boiling are traditional methods of processing the Andean grains in Peru and Bolivia. Other methods, like extrusion, could improve the bioavailability of minerals in these grains. Ummadi *et al.*[31] studied the effect of high- and low-impact extrusion processes on mineral dialyzability in legumes. The major differences in these processes include screw configuration, screw speed, moisture content and barrel zone temperatures. The authors found that low-impact extrusion increased the dialyzability of iron in legumes.

On the other hand, if we compare mineral dialyzability values in these grains with those in wheat flour (FeD% 9.8; ZnD% 10.1; CaD% 44.1)[19] they are much lower. In general, the availability of calcium, iron and zinc from cereal foods is poor and the affinity of dietary fibers for different minerals varies.[26] However, given the high content of minerals in Andean grains, the potential contribution of iron, zinc and calcium would not differ greatly from that in wheat flour.

Quinoa, kañiwa and kiwicha grains are consumed widely in Andean countries but they have also a significant, worldwide potential as a new cultivated crop species and as an imported commodity from South America. In recent years, these crops have been imported to Europe and North America from Peru, Bolivia and Ecuador. Their consumption is constantly growing outside of South America. Quinoa, kañiwa and kiwicha are important sources of minerals and their inclusion in the diet would improve the intake of iron, zinc and calcium.

CONCLUSIONS

According to our study, the Andean grains quinoa, kañiwa and kiwicha are very good sources of iron, calcium and zinc. Boiling enhanced the iron, zinc and calcium dialyzability in kañiwa. Zinc dialyzability was improved also in boiled quinoa and kiwicha. All Andean grains demonstrated high calcium dialyzability but the iron dialyzability was relatively low in all samples. In order to increase the potential contribution of minerals in Andean crops, it would be important to study the effect of different ways of processing, for example extrusion, and the use of enhancers on mineral availability.

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