



Effect of Cooking on the Content and Bioaccessibility of Minerals in Pseudocereals [†]

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Abstract: The cooked Andean cereals can be considered a good source of minerals, contributing to the recommended daily intakes as observed in previous works. This study evaluated quinoa, amaranth, and buckwheat's chemical and nutritional compositions and their bioaccessibility through an in vitro gastric digestion simulation to understand their dietary changes. ICP-OES was used to quantify the mineral profile, and the impact of cooking on bioaccessibility was evaluated using multivariate statistical analysis. In this context, the contents of some essential minerals (potassium, magnesium, calcium, zinc, copper, iron and manganese) were evaluated. The lowest cooking losses were noted for calcium in quinoa (67%), and the highest was found for zinc in buckwheat (73%). The calcium and manganese concentration varied considerably with boiling among Andean cereals. For copper, magnesium, iron and manganese, was observed a higher bioaccessibility in cooked quinoa and amaranth. The lowest bioaccessibility was detected for phosphorus in the boiled quinoa fraction (36%). The results highlight the need to consider the losses in bioavailability for minerals during digestion and the related influence on the estimation of proper nutrient intake. These results contribute to understanding the bioaccessibility of minerals in cooked Andean cereals and the changes in these nutrient contents through the boiling process. Other ongoing cooking processes lead to a scientific recommendation of the best cooking method for boosting nutrient intake.

Keywords: amaranth; bioaccessibility; buckwheat; quinoa; minerals; nutrient intake

1. Introduction

Dietary Guidelines recommend Andean cereal consumption worldwide, recognising their favourable nutrient profile. Moreover, international organisations such as the Food and Agriculture Organization recommend Andean Cereals as staple foods to fulfil the human diet's essential protein and energy requirements [1]. The growing interest of Western Diets in Andean cereals, especially Quinoa Amaranth and Buckwheat, as ingredients to improve the nutritional value of plant-based pathways and to create novel foodstuffs to replace meat. The value of Quinoa and Amaranth as nutritious food is due to the highest combined amino acid profile with dietary fibre and minerals [2,3] quinoa and amaranth

have a significant amount of anti-nutrients like saponins and phytates, which can have contradictory effects depending on the amount. Saponins have been shown to interfere with the absorption of micronutrients by displaying anti-enzymatic activities. Phytates can have a chelating impact at higher doses, decreasing minerals' bioavailability [4]. Published Data on Andean Cereal does not provide sufficient information to highlight the relevance of Andean Cereals mainly due to the unknown effect of anti-nutrients on cooked foods and their impact on the human body after digestion.

The standardisation of analytical and in vitro digestion procedures is a strategy accepted by the scientific community to define the influence of food processing on food composition data. Household processing such as boiling reduces the anti-nutrients effects of saponins and phytates, improving the mineral bioavailability [5]. Therefore, accurate determination of the component values (of foods as consumed) is achieved using a combination of analytical determination with in vitro studies [6,7].

The present study addresses the question by comprehensively describing the mineral profile of the buckwheat, quinoa and amaranth, comparing the bioavailability of raw material with boiled under traceable measurements procedures to guarantee a deep inside estimation of mineral nutrient intakes.

2. Materials and Methods

2.1. Chemicals

All reagents are of standard analytical grade. For mineral analysis, hydrogen peroxide (30%) and supra pure nitric acid (65%), ultrapure grade, were purchased from Merck (Darmstadt, Germany). Standards of each mineral, with a concentration of 1000 mg/L, in trace element grade nitric acid 2–3%, were purchased from SCP SCIENCE (Courtaboeuf, France). All calibration curves, diluted in nitric acid at 2%, ranged from 0.02–0.2 mg/L for Cu and Mn, 0.05–0.5 for Fe and Zn, 1–10 mg/L for Mg, 2–20 mg/L for Ca, P, Na and 2.5–25 mg/L for K. For bioaccessibility assessment all reagents are standard analytical grade. The different enzymes (Human salivary α -amylase; Pancreatin; Porcine pepsin, and Bile) and Pefabloc SC (4-(2-Aminoethyl) benenesulfonyl fluoride) were provided by Sigma Aldrich (St Louis, MO, USA). Salts such as calcium chloride anhydrous; sodium hydroxide; sodium chloride; magnesium chloride; sodium bicarbonate; ammonium carbonate; potassium phosphate monobasic; potassium chloride, and hydrochloric acid, used to perform the different digestion phases, were provided by Merck (Darmstadt, Germany).

2.2. Sampling and Sample Preparation

White amaranth (*Amaranthus* sp.), buckwheat (*Fagopyrum esculentum* Moench) and quinoa (*Chenopodium* sp.) are from biological agriculture collected in Lisbon markets. The sampling plan, including cooking methods and samples, was defined according to the protocol described in Motta et al. (2019) [2]. All samples were washed before any procedure. Boiling was performed in 50 g of raw seeds with 150 g of ultrapure water in a Termomix® TM31 food processor (Vorwerk, Wuppertal, Germany), set to 100 °C during 15 min. All raw and cooked grains were ground in a GRINDOMIX GM 200 high-speed grinder (Retsch, Düsseldorf, Germany) and stored in vacuum bags at 4 °C until use.

2.3. Moisture and Mineral Analysis

Moisture Content Was Determined According to AOAC (AOAC 952.08, 2000) in a Dry Air oven (102 °C $\pm$ 2 °C) until Constant Weight as described in our previous work [2]. Briefly, each sample was tested in quadruplicate for mineral quantification, using 0.5 g of sample to digest in a closed vessel microwave digestion system (Milestone ETHOS 1 Series, Shelton, CT, USA) under acid hydrolysis with nitric acid and hydrogen peroxide. Minerals were analysed by an inductively coupled plasma optical emission spectrometer, ICP-OES iCAP 6000 series (Thermo Fisher Scientific, Waltham, MA, USA), with radial and axial configuration and quantified by an external calibration curve. As part of the quality control,

two independent samples were analysed in each group of 10 samples with an acceptance criterion of $\pm 10\%$.

2.4. In Vitro Gastrointestinal Digestion

To access minerals bioaccessibility, a portion of one gram of each sample was weight. All experiments were conducted in triplicate, and a reagent blank was performed in every batch of samples. Bioaccessibility studies were performed according to the harmonised static in vitro digestion (IVD) model described by [6,7]. This model includes three sequential phases: oral phase (simulated saliva fluid with amylase—75 U/mL, pH 7), gastric phase (simulated gastric fluid with pepsin—2000 U/mL, pH 3) and intestinal phase (simulated intestinal fluid containing a pancreatin-bile mixture—100 U/mL and 10 mM, respectively, pH 7). After intestinal phase incubation, the reaction was stopped by adding 1 mM of Pefabloc® (Sigma-Aldrich, St. Louis, MO, USA). Digests of sample seeds and blanks were immediately placed in liquid nitrogen, and after that, samples were kept at -80°C . The mineral content was quantified in the blank, and seed samples digest fluids. Blank quantification was deducted from the seed sample digested extract quantification.

Bioaccessibility values were determined according to Equation (1):

$$\text{Bioaccessibility (\%)} = ([\text{mineral}] \text{ in digestion extract}) / ([\text{mineral}] \text{ in non-digested sample}) \quad (1)$$

2.5. Statistical Analysis

Results were expressed as mean and standard deviation (SD). The comparison of means was analysed by analysis of variance (ANOVA) and Tukey's test ($p < 0.05$) using Statistica v. 8 software (Statsoft Ibérica, Lisboa, Portugal).

3. Results

3.1. Moisture and Mineral Content

Mineral content for raw and cooked pseudocereals is present in Table 1. The water content was increased from 55% to 63% in boiled amaranth buckwheat and quinoa, where amaranth was the seed with the higher water retention. Raw amaranth also presents the highest content in all minerals except copper and potassium. In all cases, as expected, the mineral content was reduced after the seeds were cooked. Considering pseudocereals as consumed, the highest concentration was obtained for potassium in boiled quinoa (182 mg/100 g) and for phosphorus in boiled amaranth (175 mg/100 g), while the lowest mineral activity was found for copper in boiled buckwheat.

Table 1. Mineral and moisture content in raw and boiled pseudocereals mg/100 g (fresh weight) ¹.

Food	Process	Cu	Mn	Fe	Zn	Mg	Ca	P	K	Moisture
Amaranth	Raw	0.485 $\pm$ 0.018	2.93 $\pm$ 0.288	6.81 $\pm$ 0.487	3.85 $\pm$ 0.184	267 $\pm$ 23.0	158 $\pm$ 19.4	505 $\pm$ 55.6	455 $\pm$ 32.5	10.4 $\pm$ 0.96
	Boiled	0.172 $\pm$ 0.004	1.20 $\pm$ 0.016	1.96 $\pm$ 0.018	1.25 $\pm$ 0.030	80.8 $\pm$ 1.01	54.5 $\pm$ 0.388	173 $\pm$ 1.56	142 $\pm$ 0.745	73.9 $\pm$ 0.83
Buckwheat	Raw	0.427 $\pm$ 0.021	1.11 $\pm$ 0.098	2.55 $\pm$ 0.161	1.98 $\pm$ 0.336	216 $\pm$ 12.9	15.3 $\pm$ 0.889	387 $\pm$ 35.6	460 $\pm$ 26.7	13.4 $\pm$ 0.15
	Boiled	0.138 $\pm$ 0.001	0.355 $\pm$ 0.011	0.830 $\pm$ 0.038	0.535 $\pm$ 0.011	66.6 $\pm$ 2.43	5.22 $\pm$ 0.148	118 $\pm$ 4.05	146 $\pm$ 2.97	68.5 $\pm$ 0.17
Quinoa	Raw	0.534 $\pm$ 0.057	2.24 $\pm$ 0.323	4.30 $\pm$ 0.461	2.97 $\pm$ 0.327	224 $\pm$ 33.0	57.9 $\pm$ 6.54	444 $\pm$ 42.1	506 $\pm$ 18.4	11.7 $\pm$ 0.17
	Boiled	0.154 $\pm$ 0.005	0.638 $\pm$ 0.052	1.49 $\pm$ 0.097	0.979 $\pm$ 0.080	64.0 $\pm$ 2.65	24.8 $\pm$ 3.39	149 $\pm$ 3.10	179 $\pm$ 2.61	66.6 $\pm$ 0.16

¹ Values as mean and standard deviation ($n = 4$).

3.2. Bioaccessibility of Minerals

The elemental bioaccessibility (%) of pseudocereals is presented in Table 2. The table shows the variation of the mineral bioaccessibility (%) in raw and boiled seeds.

Raw quinoa presented higher bioaccessibility for potassium (87–100%), while boiled quinoa's highest values appear in copper and calcium (79–100%). The bioaccessibility of the different minerals only increases after cooking, especially in amaranth. In that case, amaranth presents a higher bioaccessibility for phosphorus and potassium (78–89%). Buckwheat shows the higher bioaccessibility for zinc, either raw or boiled (61–100%), for magnesium and calcium before cooking (42–58%).

Table 2. Bioaccessibility (%) of minerals in pseudocereals after digestion (%) ^{1,2}.

Food	Process	Cu	Mn	Fe	Zn	Mg	Ca	P	K
Amaranth	Raw	25.1 ± 4.66	13.9 ± 2.21	10.9 ± 1.75	62.4 ± 8.67	27.0 ± 6.49	50.8 ± 5.01	47.0 ± 5.67	59.2 ± 13.57
	Boiled	60.6 ± 2.20	31.3 ± 1.54	13.8 ± 0.70	45.9 ± 1.91	40.4 ± 1.64	54.4 ± 1.65	82.0 ± 3.28	83.3 ± 5.84
Buckwheat	Raw	48.9 ± 8.10	12.8 ± 1.59	21.5 ± 1.75	75.8 ± 14.08	49.6 ± 8.12	98.0 ± 8.61	54.2 ± 6.58	79.5 ± 5.43
	Boiled	53.7 ± 2.20	50.5 ± 3.59	20.5 ± 2.43	88.5 ± 5.67	63.8 ± 4.92	77.3 ± 13.5	40.0 ± 4.65	79.3 ± 0.79
Quinoa	Raw	33.9 ± 8.14	14.0 ± 2.95	23.0 ± 4.07	35.5 ± 6.59	41.1 ± 4.24	36.8 ± 7.48	52.6 ± 9.59	96.4 ± 8.71
	Boiled	85.8 ± 6.66	31.2 ± 1.08	34.7 ± 2.37	37.1 ± 1.90	63.7 ± 2.69	97.0 ± 5.64	36.0 ± 0.44	39.7 ± 2.01

¹ Values as mean and standard deviation ($n = 4$). ² Values of bioaccessibility (%) were obtained by Equation (1).

Nutrient retention (NR) is related to the food matrix complexity that occurs naturally with the cooking processes. Using the bioaccessibility (%) for each nutrient in the cooked food and the correspondent bioaccessibility (%) in the raw food, we can easily, by a ratio, evaluate the impact of the boiling process on bioaccessibility. Data regarding NR impact on bioaccessibility for amaranth, buckwheat and quinoa, as shown in Figure 1. Comparing the effect of boiling on the bioaccessibility of the seeds, we can conclude that boiling can increase to 3.5 times the bioaccessibility of manganese and zinc in buckwheat, calcium and copper in quinoa and phosphorus, copper, and potassium in amaranth.

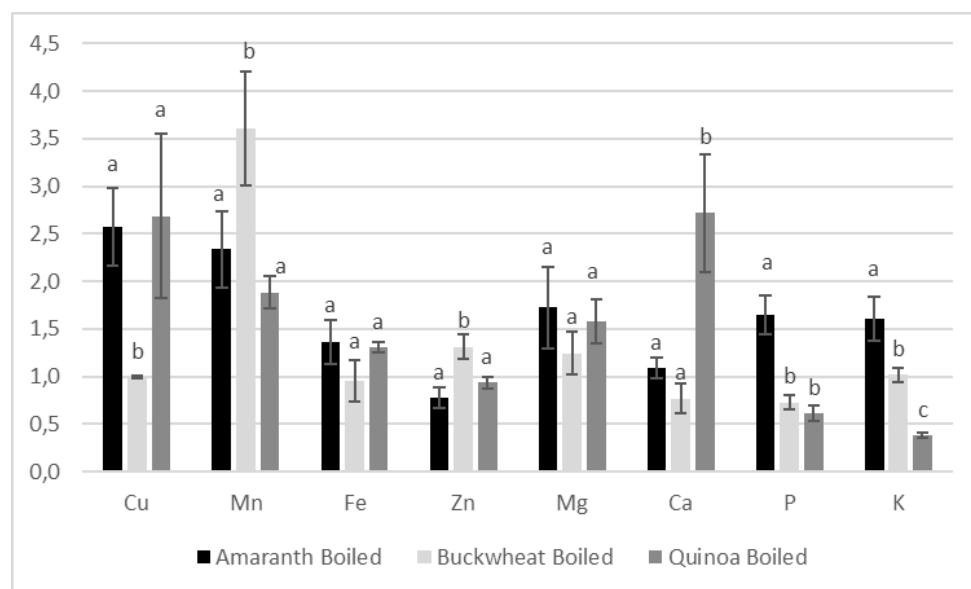


Figure 1. Effect of boiling on bioaccessibility concerning the nutrient retention (NR), using the ratio of bioaccessible portion on raw and boiled seeds. Bioaccessibility % raw seed/Bioaccessibility % boiled seed. Note: Different letters show statistically significantly ($p < 0.05$) differences in mineral content after boiling when compared with raw.

3.3. Contribution of Pseudocereals to the Population Intake

To evaluate the pseudocereals nutrient contribution to the Recommended Nutrient Intake (RNI) or Adequate Intakes (AI) of raw and boiled seeds, we defined, under nutritional portion/day recommendations, that one equivalent portion corresponds to two tablespoons of raw seeds (70 g), that after cooking represent 166 g of quinoa and 200 g of amaranth and buckwheat. RNI has been assessed by the World Health Organization and Food and Agriculture Organization of the United Nations WHO/FAO, for adults, males and females [8]. European Food Safety Authority [7,9] reports the AI for manganese and phosphorus for adults. Results for the bioaccessible fraction and composition are presented in Table 3.

Table 3. Contribution of one equivalent portion of pseudocereals to the population reference intakes (PRI) and adequate intakes (AI), for males and females aged over 18 years, for minerals before and after digestion.

		Cu		Mn		Fe		Zn		Mg		Ca		P		K		
PRI */AI Rference Value		M	1.6	3.0		11 *		16.3 *		350		950 *		550		3500		
		F	1.5			16 *		12.7 *		300						4000		
% Contribution			Bioac ^a	Comp ^b	Bioac ^a	Comp ^b	Bioac ^a	Comp ^b	Bioac ^a	Comp ^b	Bioac ^a	Comp ^b	Bioac ^a	Comp ^b	Bioac ^a	Comp ^b	Bioac ^a	Comp ^b
Amaranto	Raw	M	5.3	21	9.4	68	4.7	44	10	16	14	53	5.9	11	30	64	5.3	9.0
		F	5.7	23			3.2	30	13	21	17	62					4.7	7.9
	Boiled	M	13	22	25	80	4.9	36	7.0	15	19	46	6.3	11	52	63	6.8	8.1
		F	14	23			3.4	25	9.0	20	22	54					5.9	7.1
Buckwheat	Raw	M	9.1	17	3.3	26	3.5	16	6.4	8.6	21	43	1.1	1.1	27	50	7.3	9.2
		F	9.7	20			2.4	11	8.2	11	25	51					6.4	8.1
	Boiled	M	9.3	17	12	24	3.1	15	5.8	6.6	24	38	0.9	1.1	17	43	6.6	8.3
		F	9.9	18			2.1	10	7.5	8.4	28	44					5.8	7.3
Quinoa	Raw	M	7.8	23	7.1	52	6.2	27	4.5	13	18	45	1.6	4.3	29	57	9.7	10
		F	8.3	25			4.3	19	5.8	16	21	52					8.5	8.9
	Boiled	M	14	16	10.4	35	7.9	22	4.0	10	19	30	4.6	4.3	16.2	45	3.4	8.5
		F	15	17			5.4	15	5.1	13	22	35					2.9	7.4

^a—Bioaccessible fraction. ^b—Composition data. M—Male; F—Female. Intakes expressed in mg/day; * Population reference intakes.

Considering the bioaccessible portion between raw and cooked seeds, all minerals except for zinc and potassium in quinoa present a higher contribution to RNI or AI. Especially in amaranth, the boiling process promotes a significant increase in bioaccessibility, between 41% and 62% for manganese, copper, and phosphorus. In opposition, boiling seems to decrease in mean, around 23%, the zinc bioaccessibility for all seeds.

Regarding the percentage of intake supplied by one portion of cooked seeds, amaranth contributes to 52% of phosphorus and buckwheat with 26% of magnesium intake. However, pseudocereals are not good sources of calcium (3%) or iron (4%) of the RNI.

Suppose we use the mineral composition to calculate de RNI or AI of a consumer without knowledge about the bioaccessible fraction. In that case, and using the presented results, we can induce an error in the calculations of intakes for copper, zinc, magnesium, calcium, phosphorus, and potassium, around 45% below the corrected intake, then if we consider the bioaccessible fraction. For manganese and iron, the error can be higher than 70%.

4. Discussion

The obtained results considering mineral content are aligned with previous work published by Motta et al. [10] and in agreement with retention factors for quinoa obtained in USDA [3] for pools of boiled quinoa from different sources. Low bioaccessibility of some minerals, especially in raw seeds, can occur due to phytates, known as anti-nutrients, that may strongly connect with Zn, reducing absorption by metal precipitation. Ovca et al. (2011) results in studies with pumpkin seeds report that 50–80% of phosphorus in seeds occurs as phytic acid, which may cause complex nutrients such as K, Mg, Ca, Fe, Zn and Mn reducing their bioavailability in gastrointestinal conditions. Rousseau et al. [4] also conclude that although cooking can have a positive effect, it can also reduce mineral bioaccessibility, induced by insoluble and non-absorbable mineral chelates formed with dietary fibre, phytic acid, which cannot be destroyed during the digestion process. Our work converged with concepts postulated by Rousseau et al. [4]. Knowing that cooking is relevant in enhancing bioavailability patterns in the intestine, we continue working on other processing methods to evaluate its effects on mineral bioaccessibility.

5. Conclusions

This research represents the first study for understanding the mineral bioaccessibility in boiled amaranth quinoa and buckwheat. Furthermore, we developed a standardisation in vitro method allowing the comparability of bioaccessibility fraction. This achievement is relevant for accurately estimating mineral uptake and highlighting the contribution of pseudocereals to a healthy diet.

Although our experimental data based on retention rate demonstrated that boiled pseudocereals might accumulate a considerable amount of minerals in bioaccessible fraction, further research into the mineral release mechanisms is needed to understand the effect of the anti-nutrient and membrane brake on each pseudocereal at a cellular level.

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