ANDEAN INDIGENOUS FOOD CROPS: NUTRITIONAL VALUE AND BIOACTIVE COMPOUNDS

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ABSTRACT

The Andean area of South America is a very important center for the domestication of food crops. This area is the botanical origin of potato, peanut and tomato. Less well- known crops, such as quinoa (*Chenopodium quinoa*), kañiwa (*Chenopodium pallidicaule)* and kiwicha *(Amaranthus caudatus*), were also domesticated by ancient Andean farmers. These crops have a long history of safe use with the local populations and they have contributed to the nutrition and wellbeing of the people for centuries. Several studies have reported the nutritional value of Andean grains. They contain proteins with a balanced essential amino acid composition that are of high biological value, good quality oil and essential minerals, for example iron, calcium and zinc. They are potential sources of bioactive compounds such as polyphenols and dietary fiber.

The main objective of the practical work was to assess the nutritional value of Andean native grains with a special emphasis on the bioactive components and the impact of processing. The compounds studied were phenolic acids, flavonoids, betalains and dietary fiber. The radical scavenging activity was measured as well. Iron, calcium and zinc content and their bioavailability were analyzed as well. The grains were processed by extrusion with the aim to study the effect of processing on the chemical composition.

Quinoa, kañiwa and kiwicha are very good sources of dietary fiber, especially of insoluble dietary fiber. The phenolic acid content in Andean crops was low compared with common cereals like wheat and rye, but was similar to levels found in oat, barley, corn and rice. The flavonoid content of quinoa and kañiwa was exceptionally high. Kiwicha did not contain quantifiable amounts of these compounds. Only one variety of kiwicha contained low amounts of betalains. These compounds were not detected in kañiwa or quinoa.

Quinoa, kañiwa and kiwicha are good sources of minerals. Their calcium, zinc and iron content are higher than the content of these minerals in common cereals. In general, roasting did not affect significantly mineral bioavailability. On the contrary, in cooked grains, there was an increase in bioavailability of zinc and, in the case of kañiwa, also in iron and calcium bioavailability.

In all cases, the contents of total and insoluble dietary fiber decreased during the extrusion process. At the same time, the content of soluble dietary fiber increased. The content of total

phenolics, phytic acid and the antioxidant activity decreased in kiwicha varieties during the extrusion process. In the case of quinoa, the content of total phenolic compounds and the radical scavenging activity increased during the extrusion process in all varieties.

Taken together, the studies presented here demonstrate that the Andean indigenous crops have excellent potential as sources of minerals, flavonoids and dietary fiber. Further studies should be conducted to characterize the phenolic compound and antioxidant composition in processed grains and end products. Quinoa, kañiwa and kiwicha grains are consumed widely in Andean countries but they also have a significant, worldwide potential as a new cultivated crop species and as an imported commodity from South America. Their inclusion in the diet has the potential to improve the intake of minerals and health-promoting bioactive compounds. They may also be interesting raw materials for special dietary foods and functional foods offering natural sources of specific health-promoting components.

ABBREVIATIONS

AAS	atomic absorption spectroscopy
ABTS	2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
ANOVA	analysis of variance
AOAC	Assosiation of Official Analytical Chemists
ARs	alkylresorcinols
BHA	botyl-4-hydroxyanisole
BV	Biological Value
Ca	calcium
CMC	carboxymethylcellulose
CEC	cationic exchange capacity
Cu	copper
DG	degree of gelatinization
DPPH	2,2-diphenyl-1-picrylhydrazyl
EU	European Union
Fe	iron
GAE	gallic acid equivalent
HPLC	high performance liquid chromatography
IDF	insoluble dietary fiber
K	potassium
LDL	low-density lipoproteins
LEC	low-cost extruder cooker
Mn	manganese
MOAI	monoamineoxidase inhibitors
Na	sodimu
NFR	Novel Food Regulation
NPR	Net Protein Ratio
NPU	Net Protein Utilization
PC	potential contribution
PER	Protein Efficiency Ratio
QP	quinoa protein isolate
SD	standard deviation
SDF	soluble dietary fiber
SEI	sectional expansion index
TDF	total dietary fiber
UV	ultraviolet
WAI	water absorption index
WSI	water solubility index

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles referred to in the text by Roman numerals I-V:

I Repo-Carrasco-Valencia, R., Peña, J., Kallio, H. and Salminen, S. 2009. Dietary fiber and other functional components in two varieties of crude and extruded kiwicha (Amaranthus caudatus). Journal of Cereal Science. 49: 219-224.

II Repo-Carrasco-Valencia, R., Acevedo De La Cruz, A., Icochea Alvarez, J. and Kallio, H. 2009. Chemical and Functional Characterization of Kañiwa (Chenopodium pallidicaule) Grain, Extrudate and Bran. Plant Foods for Human Nutrition. 64:94-101.

III Repo-Carrasco-Valencia, R. and Astuhuaman Serna, L. 2011. Quinoa (Chenopodium quinoa, Willd.) as a Source of Dietary Fiber and other Functional Components. Ciencia y Tecnologia de Alimentos. Campinas 31(1): 000-000.

IV Repo-Carrasco-Valencia, R., Hellstrom, J.K., Pihlava, J.-M. and Mattila, P.H. 2010. Flavonoids and other phenolic compounds in Andean indigenous grains: Quinoa (Chenopodium quinoa), kañiwa (Chenopodium pallidicaule) and kiwicha (Amaranthus caudatus). Food Chemistry 120: 128-133.

V Repo-Carrasco-Valencia, R. Encina, C., Binaghi, M. Greco, C. and Ronayne de Ferrer, P. 2010. Effects of roasting and boiling of quinoa, kiwicha and kaniwa on composition and availability of minerals *in vitro*. Journal of Science of Food and Agriculture. 90: 2068–2073.

1. INTRODUCTION

The Andean region of South America is an important center of domestication of food crops. This region has a great diversity of landscapes, or agroecological zones, due to several climates and altitude differences (1500-4200 m). It is different than other regions in the world where crops were domesticated. Here, there are no vast, unending plains of uniformly fertile, well-watered land as in Asia, Europe and the Middle East. Instead, there is almost total lack of flat, fertile, well-watered soil. The Andean people have always cultivated their crops on tiny plots one above another up mountainsides rising thousands of meters (1).

At the time of the Spanish invasion, the Incas cultivated almost as many species of plants as the farmers of all Asia or Europe. It has been estimated that Andean natives domesticated as many as 70 separate crop species (1). On mountainsides up to four kilometres high along the whole continent and in climates varying from tropical to polar, they grew roots, grains, legumes, vegetables, fruits and nuts. During the time of the Incas, Peru was a very prosperous farming country with a population of 10 million people, where according to researchers, malnutrition was practically unknown. The use and cultivation of many of these plants was reduced dramatically after the arrival of the conquerors. Until recently, these plants have received little scientific interest, respect, research or commercial advancement. Yet they include some widely adaptable, extremely nutritious and remarkably tasty components for foods.

Andean indigenous food products, like quinoa (*Chenopodium quinoa*) and kiwicha (*Amaranthus caudatus*) are rich in high quality proteins. They contain also dietary fiber and oil with polyunsaturated fatty acids. Dietary fiber is especially important in diets designated for disease risk reduction and the prevention of diabetes and heart disease. Some components of soluble dietary fiber act like pre-biotics which are beneficial for gut health. Yacon (*Smallanthus sonchifolius*), an Andean root, is rich in this kind of fiber. Some varieties are good sources of phenolic compounds which could act as natural antioxidants, preventing specific diseases such as cancer. Native potatoes are rich in pigments which could also be considered as natural antioxidants, further having an impact on nutrition and human health.

The Andean indigenous food crops have an enormous potential to be used as functional foods in the prevention of chronic diseases, such as cardiovascular diseases, cancer and diabetes. High variability, not only in colors and shapes, but also in primary nutrient constituents and bioactive compounds, has recently been reported. The health-related properties of Andean crops claimed by local people could be partially attributed to the presence of these bioactive compounds.

Traditionally, quinoa, kañiwa and kiwicha are mainly consumed in rural areas and in cities among the immigrants of mountain area. The consumption of quinoa in Lima, among the immigrants from the Andes, is about 30 kg/family/year in 2000 (2) and in the Andes it is about 80 kg/family/year (3). However, the consumption of quinoa and also of kiwicha increased in 2010 thanks to program "Sierra Exportadora" of Peruvian government. This program is using Andean grains in food aid program which offers breakfasts to children in shanty towns and poor rural areas. In recent years, these crops have been "rediscovered" and their status as neglected crops is changing. Trends in nutraceuticals and functional foods containing biologically active natural substances are oriented towards exotic plants or plant extracts. Functional foods with bioactive compounds are becoming indispensable dietary constituents for all population groups for the reduction, prevention or remedial treatment of many chronic diseases. Bioactive compounds are constituents that typically occur in small quantities in foods. They are being intensively studied to evaluate their effects on health. Many epidemiologic studies have shown protective effects of plant-based diets on cardiovascular disease (CVD) and cancer. Many bioactive compounds have been discovered and the Andean crops are potential soureces of theses compounds.

Besides, there exists a growing commercial interest in "exotic" and ethnic foods**.** Several factors are behind the rising interest in diverse foods from the developing world. Demographic change, especially aging and immigrant populations have led to a previously unseen demand for new health, functional and ethnic food. Many of the traditional food species of the developing countries meet the changing needs of developed country markets. Interesting attributes of these foods include particular nutritional value (high contents of vitamins or functional nutrients, good quality proteins, the absence of known allergens such as gluten, etc.), aesthetic appeal, and sourcing from environmentally sustainable and ethically managed production systems (often certified as "organic" or "fair trade" produce) (4). Recently, Peruvian cuisine and cooking

traditions have become more widely known. This has contributed to the increased demand of Peruvian foods and food ingredients on international markets.

The main objective of the experimental part of this thesis was to assess the nutritional value of Andean native grains with a special emphasis on bioactive components and the impact of processing. The literature review collects information about neglected Andean food crops with the aim to disseminate to a wider audience the most up-to-date scientific information on their nutritional value and uses.

2. REVIEW OF THE LITERATURE

2.1 ANDEAN SEED CROPS

In the Andean area of South America, three native seed crops are cultivated. These are quinoa, kañiwa and kiwicha.

2.1.1 QUINOA

2.1.1.1 General information

Quinoa (*Chenopodium quinoa* Willd) is a seed crop of the Chenopodiaceae family. It was a very important crop to the Incas who called quinoa "mother grain", *chisiya mama* in Quechua, the native language of the Incas (1). Domestication of quinoa took place at least some 8000 years ago on the high plateau of the Andes near Lake Titicaca (5). Nowadays, quinoa is cultivated mainly in the Andean region from Colombia to the north of Argentina, with Peru and Bolivia as the most important producers. There are different types of quinoa (landraces) which have adapted 6). The main survival mechanism of quinoa to frost is avoidance of ice formation by moderate supercooling. Quinoa has a high soluble sugar content, which may cause lowering of the freezing point and therefore contributing to lower the lethal temperature of the leaf tissue (7). It is suggested that the level of soluble sugars may be used as an indicator of frost resistance (8). In the under different environmental conditions. Quinoa can be cultivated at sea level, Andean valleys, altiplano (high plateau) and places like the Bolivian salt flats. The varieties which grow on the Bolivian high plateau resist low temperatures (-8 °C), alkaline soils (pH 8) and salinity of 52 mS/cm (Andean highlands, various quinoa production systems are referred to as aynokas (communal fields), waru warus (high beds), canchas (fields surrounded by stonewalls), kochas (fields around small lakes) and andenes (terraces). Quinoa is sown in crop rotations after potato, sometimes in fields of different cultivars. In the valleys, quinoa is sown in association with bean, potato, barley and Andean roots and tubers, mainly to avoid the risks of adverse climatic factors such as drought, frost, hail, high salinity and flooding (9).

Quinoa is an annual dicotyledonous plant usually about 1-2 m high. The plant can be branched or unbranched depending on variety and may be green, yellow, red or purple (see Figure 1). The inflorescence (panicle) can be amaranthiform or glomeruliform (10). The small flowers are generally monecious and self-fertil.

Figure 1. Quinoa field in Cusco

An example of classification of quinoas into five principal types according to their ecological adaptation is presented in Table 1 (2).

Table 1.The main quinoa types accordting to ecological adaptation

Quinoa type
Quinoas of valleys
Quinoas of the high plateau (altiplano)
Quinoas of the salt plateau (salares)
Quinoas of sea level
Quinoas of the jungle

The seeds of quinoa are small, varying in diameter from 1-2.5 mm. The fruit is surrounded by perianth which can be easily removed. The grain is enveloped in a two-layered pericarp. This layer contains saponins, bitter substances which must be removed before quinoa can be consumed. The color of the pericarp can be transparent, white, yellow, orange, pink, red, gray, violet or black. Beneath the pericarp is the seed coat (episperm) which can be transparent, white, brown or black. Episperm covers the starchy perisperm. The embryo of the quinoa seed forms a coil around the perisperm and its proportion of the weight of the whole grain is about 30%. It should be pointed out that the proportion of the embryo of wheat is only 1% of the whole grain. In cereals, like maize and wheat, the starch reserves for embryo development are stored in endosperm tissue, but in quinoa the endosperm tissue is reduced to one or two layers surrounding the hypocotyl-radicle axis. Quinoa starch is stored in the non-living, thin-walled perisperm that occupies about 40% of the volume of the seed (11). According to Mujica *et al.* (9), quinoa has a high yield potential; it is possible to increase production and productivity through the use of improved varieties, higher-quality seed and more appropriate agronomic practices, even without increasing the production area.

2.1.1.2 Composition and nutritional value

Table 2 shows the chemical composition of quinoa grain. Quinoa is an excellent source of proteins, lipids and carbohydrates. The embryo occupies a greater proportion of the seed than in common cereals, so the protein and oil content are relatively high**Table 2**. Chemical composition of quinoa grain.

Component	Ref. (12)	Ref. (13)	Ref. (14)	Ref. (15)
Protein g/100 g	11.2	14.4	14.1	14.5
Crude fat g/100 g	4.0	6.0	9.7	5.2
Crude fiber g/100 g	n.d.	4.0	n.d.	14.2**
Ash g/100 g	3.0	2.9	3.4	2.7
Carbohydrates g/100 g	32.6*	72.6	72.5	64.2

*starch content
** dietary fiber
n.d. = not determined

There is a significant difference between the values of fiber of references 13 and 15. This difference is partly due to the different analysis techniques, the first one is analysis of crude fiber and the second one analysis of dietary fiber. The values for dietary fiber are always higher than the values for crude fiber. On the other hand, Repo-Carrasco (13) analyzed quinoa without saponins. This quinoa has lost its outer layers during the process of eliminating saponins and thus the fiber content is decreaesed.

Proteins

The highest concentration of proteins in quinoa is located in the embryo. They are mainly albumins and globulins with 11 S globulin (also called chenopodin) as the major protein group, with apparent molecular weights of the A and B subunits of 22-23 kDa and 32-39 kDa, respectively, and similar amino acid composition to legume globulin (11, 16,).

There are very few studies on the amino acid composition of Andean indigenous grains. Table 3 shows the amino acid composition of quinoa, kañiwa, kiwicha, rice and wheat. The importance of quinoa's proteins rests on the fact that the lysine content is high. Lysine is the first limiting amino acid in common cereals, and as can be seen in Table 3, the content of lysine is double compared to its content in wheat.

Table 3. Amino acid content of Andean grains, rice and wheat (g amino acid/16 g N) (13)

Amino acid	Quinoa	Kañiwa	Kiwicha	Rice	Wheat
Aspartic acid	7.8	7.9	7.4	8.0	4.7
Threonine*	3.4	3.3	3.3	3.2	2.9
Serine	3.9	3.9	5.0	4.5	4.6
Glutamic acid	13.2	13.6	15.6	16.9	31.3
Proline	3.4	3.2	3.4	4.0	10.4
Glycine	5.0	5.2	7.4	4.1	6.1
Alanine	4.1	4.1	3.6	5.2	3.5
Valine*	4.2	4.2	3.8	5.1	4.6
Isoleucine*	3.4	3.4	3.2	3.5	4.3
Leucine*	6.1	6.1	5.4	7.5	6.7
Tyrosine *	2.5	2.3	2.7	2.6	3.7
Phenylalanine*	3.7	3.7	3.7	4.8	4.9
Lysine*	5.6	5.3	6.0	3.2	2.8
Histidine *	2.7	2.7	2.4	2.2	2.0
Arginine	8.1	8.3	8.2	6.3	4.8
Methionine*	3.1	3.0	3.8	3.6	1.3
Cysteine*	1.7	1.6	2.3	2.5	2.2
Tryptophan*	1.1	0.9	1.1	1.1	1.2
% N of the grain	2.05	2.51	2.15	1.52	2.24
% protein	12.8	15.7	13.4	9.5	14.0

* essential amino acids

Essential amino acids, found in abundance in high-quality dietary protein, are needed daily. One method for assessing dietary protein quality is by determining the chemical score, ie, the ratio of a gram of the limiting amino acid in a test diet to the same amount of the corresponding amino acid in a reference pattern. The international recommendations for an amino acid reference pattern are given separately for infants and pre-school children (17). Amino-acid scores provide a useful estimate of the protein quality of foods and are acceptable substitute of the biological assays (18). Leucine and threonine are the first limiting amino acids for some

quinoa varieties, whilst some varieties, like "Amarilla de Marangani", do not have any limiting amino acids (see Table 4).

Table 4. Content of essential amino acids and chemical score of three types of quinoa (g/16 g N) (19)

Amino acid	Nariño	Amarilla de Marangani	Commercial sample	FAO/WHO/UNU Reference pattern 1985 for pre-school child(17)
Histidine	2.6	2.8	2.7	1.9
Isoleucine	3.7	3.9	3.4	2.8
Leucine	6.4	6.9	6.1	6.6
Lysine	6.4	6.3	5.6	5.8
Methionine + cysteine	3.9	3.7	4.8	2.5
Phenylalanine + tyrosine	6.8	7.2	6.2	6.3
Threonine	3.3	3.4	3.4	3.4
Tryptophan	1.2	1.1	1.1	1.1
Valine	4.5	4.6	4.2	3.5
Chemical score	0.97	1.00	0.92	
Limiting amino acid	*Leucine*	-	*Leucine and lysine*	

Quinoa protein meets the FAO/WHO/UNU protein reference pattern for children (17). The protein quality of quinoa has been studied by biological assays. The Protein Efficiency Ratio (PER) of quinoa is similar to that of casein (20, 21). Protein digestibility is remarkably high (92 %) while net protein utilization and protein biological value appear to be moderate to high (76% and 83 %, respectively (22).

Lipids

The fat content in quinoa is higher than in common cereals (see Table 2). The fat is mainly located in the embryo. Quinoa oil is rich in polyunsaturated fatty acids (linoleic and linolenic) but also in oleic acid (see Table 5). The level of unsaturated fatty acids is excellent with respect to nutrition: the essential fatty acid, linoleic acid, delivers 10% of the energy. According to the American Society of Pediatricians, infant food should contain at least 2.7% energy in the form of linoleic acid (23). In addition, quinoa's linolenic/linoleic acid ratio is adequate. A diet with a high n-6/n-3 ratio (linoleic/linolenic acid ratio) promotes the pathogenesis of many degenerative diseases such as cardiovascular disease, cancer, osteoporosis, as well as inflammatory and autoimmune diseases. An increased n-3 fatty acid intake reduces the biological markers associated with the abovementioned diseases. The current n-6/n-3 ratio in Western countries has been estimated to be in the range 14:1-20:1 and is far from the recommended levels of 5:1-10:1. Quinoa's n-6/n-3 ratio, at 6.2, falls within the recommended values (15).

Table 5. Characteristics and fatty acid composition of quinoa oil

Characteristic	Ref. (24)	Ref. 11)	Ref. (25)	Ref. (15)
Fatty acids (% of lipid fraction)				
Myristic (C14:0)	-	0.1	0.2	
Palmitic (C16:0)	9.59	9.7	9.9	11.0
Palmitoleic (C16:1)	-	0.2	0.1	
Stearic (C18:0)	0.1	0.6	0.8	1.1
Oleic (C18:1)	26.04	24.5	24.5	26.7
Linoleic (C18:2)	50.24	50.2	50.2	48.2
Linolenic (C18:3)	4.77	3.9	5.4	8.3
Arachidic (C20:0)	-	0.4	2.7*	0.6
Cis-11,14-Eicosadienoic (C20:2)	-	-	-	1.4
Docosanoics (all C22s)	-	-	2.7	-
Tetrasanoics (all C24s)	-	-	0.7	-
Specific gravity	0.930	0.891	n.d.	n.d.
Iodine value (Wijs) (g I_2/100 g)	128	129	n.d.	n.d.
Unsaponifiable matter (%)	5.01	5.2	n.d.	n.d.

*all C20s
n.d. = not determined

One application of the iodine number is the determination of the amount of unsaturation contained in fatty acids. This unsaturation is in the form of double bonds which react with iodine compounds. The higher the iodine number, the more unsaturated fatty acid bonds are present in a fat. The determination of non-fat materials other than water is done by saponifying the fat by heating with strong caustic soda or potash solution until all the triglycerids have been decomposed into glycerin and soap. This is called the unsaponifiable matter and it is an indicator of the presence of compounds such as tocopherols, squalene and sterols in oil. This value for quinoa oil is high and it is known that quinoa oil is rich in tocopherols and this makes it stable against oxidation (11).

Carbohydrates

The main carbohydrate in quinoa is starch. Quinoa starch is located mainly in the perisperm and it occurs both as small individual granules and larger compound granules composed of hundreds of individual granules (11). The individual granules are polygonal with a diameter of 1.0-2.5 µm and the compound granules are oval, with a diameter of 6.4-32 µm (26). Quinoa starch has a low amylose content as compared to common starches (11-12.2 %) (26, 27). Quinoa starch is rich in amylopectin and it gelatinizes at relatively low temperatures (57-71 °C) (26, 27). The starch has a high pasting viscosity and single-stage starch swelling in the temperature range 65-95°C (28). Quinoa starch has excellent freeze-thaw stability which is related to the fact that it is rich in amylopectin (11).

Dietary Fiber

The content of dietary fiber in quinoa is similar to that of common cereals. There are varietal differences in content of dietary fiber in quinoa (Table 6). This is common in grains, Gebruest et al. (2008) (29) found substantial variation in the content of dietary fiber between different wheat types and varieties. Similar results were obtained for oat and barley types and varieties, as well (30, 31). Some of this variation may relate to environmental conditions, such as soil nutrient status and water availability Furthermore, interactions between the genotype and environment may occur, resulting in different impacts on the concentrations of components (32).

Table 6. Dietary fiber content in Andean grains and cereals

Species/variety	IDF %	SDF %	TDF %	Ref.
Quinoa				
La Molina 89	14.4	2.5	16.9	33
Blanca de Juli	12.2	2.4	14.6	33
Sajama	12.0	2.5	14.5	33
Kcancolla	12.7	2.3	15.0	33
Salcedo INIA	23.5	3.1	26.5	33
Kiwicha				
Centenario	14.9	2.4	17.3	33
Kañiwa				
Cupi	23.5	4.1	27.6	33
LP1	21.9	4.4	26.3	33
Ramis	23.1	4.2	27.3	33
Oat	n.d.	n.d.	10-23	32
Barley	n.d.	n.d.	15-24	31
Wheat	n.d.	n.d.	10-18	29

n.d. = not determined, IDF = insoluble dietary fiber, SDF = soluble dietary fiber, TDF = total dietary fiber

Some authors report relatively low dietary fiber content for quinoa, 13-14% (15, 22). This could be due to the different varieties and also differences in handling and processing of the seed. Eliminating the bitter substances, saponins, of quinoa, decreases the fiber content. Quinoa grains analyzed in the study of Alvarez-Jubete et al. (15) were pre-processed by washing, centrifuging and drying.

Vitamins and minerals

The minerals of quinoa are concentrated in the outer bran layers, as in cereals (11). Quinoa is rich in calcium, magnesium, iron and phosphorus (see Table 7). The availability of these minerals can be affected by some components of quinoa, mainly by saponins and phytic acid.

Konishi *et al.* (34) studied the distribution of minerals in quinoa grain. They found that phosphorus, potassium and magnesium were localized mainly in the embryonic tissue. Calcium and potassium were present in the pericarp, probably associated with pectin. They also studied the effect of dehulling of the grain to the mineral content and discovered that calcium content was decreased after dehulling.

Table 7. Mineral content of quinoa

Mineral	Ref. (25) (mg/kg dry basis)	Ref. (12) (%)	Ref. (35) (mg/kg sample)	Ref. (34) (mg/100g)	Ref. (15) (mg/100 g dry basis)	Ref. (36) (mg/100 g)
Calcium	200-3900	0.1020	860	86.3	32.9	56.5
Magnesium	1300-4600	n.d.	2320	502	206.8	176.0
Sodium	12-425	0.06125	930		n.d.	26.6
Phosphorus	1290-6300	0.140	220	411	n.d.	468.9
Iron	5-321	0.01050	26	15	5.5	14.0
Copper	6-87	n.d.	76		n.d.	0.2
Zinc	12-99	n.d.	38	4	1.8	2.8
Potassium	5000-19800	0.82250	7140	732	n.d.	1193.0

n.d. = not determined

Thiamin and riboflavin content of quinoa is similar to that of common cereals (37). In comparison with common cereals, quinoa appears to be among the best sources of vitamin E. Quinoa is an excellent source of γ-tocopherol, containing about 5 mg/100 g, (23). The content of γ-tocopherol is of particular biological relevance because of its potential anticarcinogenic and anti-inflammatory activities (38). Quinoa contains significant amounts of vitamin C, which is not common in cereals. The vitamin content of quinoa is presented in Table 8.

Table 8. Vitamin content of quinoa

Vitamin	Ref. (23)	Ref. (39)	Ref. (15)
Thiamine (mg/100 g grain)	0.4	n.d.	n.d.
Riboflavin (mg/100 g grain)	0.2	n.d.	n.d.
Folic acid (µg/100 g)	78.1	n.d.	n.d.
Vitamin C (mg/100 g grain)	16.4	12-13	n.d.
α-tocopherol (mg/100 g grain)	2.6	n.d.	24.7*
Vitamin A (mg RE/100 g)	0.2	n.d.	n.d.

*total tocopherol content

Recently, Schönelechner *et al*. (40) analyzed folate content in quinoa and quinoa products. They found that the content of this vitamin in quinoa was 132.7 mg/100 g dm, about ten times as much as in wheat. The bran fractions contained on average 124% of total folate, while only 57% on average was present in the flour fractions. Staple foods (bread, pasta and cookies) were produced from pseudocereals and total folate content and its losses were determined in these products. Quinoa products were characterized by the highest total folate content. According to this study, quinoa based products offer a substantial alternative for folate intake.

Antinutrients

Quinoa contains saponins which are glycosylated secondary metabolites found in many plants. Quinoa saponins are soluble in methanol or water. They produce stable foams in aqueous solutions, and hemolyze red blood cells (41). Quinoa contains three or four different sapogenins: oleanolic acid, hederagenin, phytolaccagenic acid and sometimes deoxyphytolaccagenic acid, depending on the variety (42, 43). Glucose, arabinose and occasionally galactose are the sugars bound to the sapogenins.

The triterpene saponins can be classified into three main groups: monodesmosidic saponins with one carbohydrate chain, bidesmosidic saponins carrying two carbohydrate chains and tridesmosidic saponins with three carbohydrate chains. Quinoa saponins carry one or more carbohydrate chains, which basically consist of arabinose, glucose, galactose, glucuronic acid, xylose and rhamnose glycosidically linked to a hydrophobic aglycone, mainly oleanane- and

phytolaccagenanetype (44). At least 16 types of saponins have been detected in quinoa seeds (45).

The quantity of saponins is variable between the quinoa types. The quinoas can be classified as bitters or sweet according to their saponin content. The bitter genotypes contain 4.7 to 11.3g /kg of sapogenins whereas sweet genotypes contain 0.2 to 0.4g /kg sapogenins (46).

Saponins are concentrated in the pericarp-seed coat and they must be removed before the use of the quinoa grain. Saponins are reported to be toxic for cold-blooded
animals and they have been used as fish poison by the inhabitants of South America (47). Saponins have some adverse physiological effects, since they are membranolytic against cells of the small intestine and possess hemolytic activity. Monodesmoside saponins have higher hemolytic activity than the bidesmoside saponins (45). Saponins form complexes with iron and may reduce its absorption. Besides the negative effects of saponins, they also have possible positive effects by reducing serum cholesterol levels, possessing anti-inflammatory and antioxidant activity and exhibiting insecticidal, antibiotic and fungicidal properties (11, 44, 45, 47). Saponin containing additives in small quantities (100–400 ppm) have shown positive effects on the performance of ruminants, monogastric animals and fish (48, 49, 50).

Like most grains, quinoa contains phytic acid. Phytate forms complexes with multivalent metal ions such as iron, calcium, magnesium and zinc, reducing their bioavailability. According to Ruales and Nair (41) the content of phytic acid in quinoa seeds is about 1% of the dry matter. Scrubbing and washing reduce the phytic acid content of the seeds by about 30%. These authors detected neither protease inhibitors nor tannins in quinoa grains.

2.1.1.3 Traditional uses, processing and actual situation

Before the consumption of quinoa, the bitter substances, saponins, must be removed. Traditionally the grain is washed in running water. The problem with this method is that it causes pollution of rivers and lakes. Another traditional method is physically removing the saponin-rich pericarp. This method has the advantage of not causing pollution of rivers but its disadvantage is that some nutrients are lost. Torres and Minaya (51) developed an abrasive dehulling machine for quinoa. With this device, it was possible to reduce the content of

saponins without important loss of nutrients. Ridout *et al*. (42) compared the removal of saponins by washing and abrasion and found that similar levels of saponin reduction can be achieved by both methods.

A combination of the two methods for the removal of saponins from quinoa seems to be the most adequate. Using a short period of abrasive polishing in the first step and washing in the second step, it is possible reduce the content of saponins to an acceptable level without the loss of nutrients. In the future, it may be possible to apply an enzymatic method utilizing enzymes of *Eurysacca quinoae* Povolny. However, this method is not yet commercialized (52).

Traditional uses of quinoa are as whole seeds in soups, *quispiño*, *tactte* and *pesqhe*. *Quispiño* is a cooked quinoa bread made from raw flour and animal fat. It is to be used on long trips and may be kept preserved for at least six months without cooling, maintaining its consistency. *Tactte* is a small cake made from quinoa flour and fried in animal fat. It has a crunchy consistency and maintains its flavor for a long time. *Pesqhe* is a salted porridge of whole, sweet quinoa seed (52). Quinoa is used in traditional medicine for its anti-inflammatory and disinfectant properties and also as an insect repellent. In Bolivia, the plant of quinoa is used for contusions and luxations (53). Mapuche Indians in Chile use quinoa as a diuretic, to treat catarrhal infections and externally to treat wounds and cuts (54).

Quinoa is generally used as a cooked grain in Peru and Bolivia. It is used in soups or as a replacement for rice. Another traditional processing method of quinoa is puffing. This product, “quinoa pop”, is produced by gun puffing. The grain is heated in high pressure vessel with a small amount of water. The pressure is then released using a valve. Water in the grain is vaporized and this causes expansion of the grain and partial gelatinization of starch.

Quinoa grain can be milled into flour. Dry milling of common grains, such as wheat and rye, separates the anatomical parts of the grain. Pericarp and germ are removed and the endosperm is milled into flour which is rich in starch and some protein. Quinoa is generally washed and pearled and then milled whole without separating pericarp and germ because of the very small size of the grain.

Extrusion cooking has been successfully used to process quinoa (55, 56, 57, 58). Ruales and Nair (59) investigated the effect of extrusion cooking on quinoa. They found that extrusion cooking increased the *in vitro* digestibility of starch, and that there was no adverse effect on *in vitro* protein digestibility, indicating that the damage to sensitive amino acids, such as lysine, was minimal.

Quinoa does not contain gluten and therefore is not suitable for traditional breadmaking alone. However, it can be used as a composite flour with wheat flour. The use of quinoa flour in yeast-leavened bread results in a reduction in loaf volume. Lorenz and Coulter (60) studied the performance of quinoa-wheat flour blends (5/95, 10/90, 20/80, 30/70) in breads, cakes and cookies. Breads baked with 5% and 10% quinoa flour were of good quality. Loaf volume decreased, crumb grain became more open and the texture slightly harsh at higher usage levels of quinoa flour. A bitter aftertaste was noted at the 30% level. Cake quality was acceptable with 5% and 10% of quinoa flour. Cookie spread and top grain scores decreased with increasing levels of quinoa flour blended with high-spread cookie flour. The cookies with 30 % of quinoa flour did not have an acceptable flavor.

Quinoa flour has been used to substitute wheat flour (61, 62). In these studies the chemical, rheological and breadmaking characteristics were investigated. An increase in the amount of substitution of quinoa flours (10, 20, and 30%) for wheat flour negatively affected bread quality, but the addition of lipase to quinoa substitution flours improved it. Germinated and milled quinoa was mixed with wheat flour (10/90) to study the physical properties of dough and baking quality (63). These properties of dough and bread made from germinated quinoa were no significantly different with control (wheat bread).

The use of quinoa in gluten-free breads has been investigated recently (38, 64). Bread volumes were found to be significantly increased for the quinoa breads in comparison with the control breads. Quinoa breads had a softer crumb texture in comparison with control (64). The authors found that the replacement of potato starch with a pseudocereal flour resulted in gluten-free breads with an increased content of important nutrients such as protein, fiber, calcium, iron and vitamin E. The resultant breads also had a significantly higher content of polyphenol compounds and their *in vitro* antioxidant activity was also increased (65). The quality of gluten-free breads could be improved using sourdough fermentation. There are several positive effects

on sensorial and nutritional properties of gluten-free breads when sourdough fermentation is used: improved dough softening, increased bread volume, improved gas retention, improved palatability, improved mineral bioavailability and decreased glycemic index (66). Recently, quinoa and amaranth has been used successfully in gluten-free breads using sourdough fermentation (67). Quinoa flour can be considered a feasible ingredient in the preparation of healthy and nutritional gluten-free breads.

Quinoa can be used in gluten-free pasta (68,69). Pasta from quinoa showed increased cooking loss. By combination quinoa with other no cereal grain flours (buckwheat, amaranth) the dough matrix was improved. Using structuring agents, such as carboxymethylcellulose (CMC) and pregelatinized starch, it was possible to manufacture gluten-free spaghetti only with quinoa (70). CMC and pregelatinized starch influenced both the rheological and mechanical properties of quinoa doughs and spaghetti samples. In particular, CMC and pregelatinized starch had an effect on the elongational and shear viscosity. The elongational and shear viscosity declined, for the quinoa dough, with the addition of CMC.

Quinoa milk, which may have potential for consumption directly as milk or in dairy products, may in the near future be of significant consumption. It will be necessary to extend the production areas of quinoa cultivars for milk production, and to build processing plants for milk in areas of quinoa production, for the benefit of farmers, consumers, and rural agroindustries (71). An aspect of great importance will be the dissemination of the use of this highly nutritive and tasty product among consumers, who may be people unable to ingest animal lactose or casein. Quinoa milk might be an alternative to the most common vegetable milk, from soybean (52).

There is a need for obtaining high-quality protein concentrates to solve problems of chronic malnutrition affecting rural and urban populations of the Andean region. Quinoa, in addition to having a high protein content (from 10 to 22% depending on genotype), has an adequate balance of the essential amino acids. The protein is mainly found in the embryo of the seed, which contains up to 45% protein. The embryo can be separated from the rest of the seed through processes of pregermination or abrasive dehulling, which is also used for the removal of saponins. The concentrated embryo can then be utilized directly in food for children, for instance, to obtain a quick recovery of the nutritional level of children suffering from

malnutrition, and in adults, such as pregnant and breast-feeding women, in a diversity of dishes (52).

Quinoa has a very interesting potential to be used in the preparation of biodegradable films. Quinoa contains significant amounts of starch, which has an amylose content of ~10–21% (depending on the variety) and a small starch granule size (~1 μm), characteristics that allow for easier dispersion, which make this starch a promising material for film production. Araujo-Farro *et al*. (72) developed biodegradable films based on quinoa starch. The process developed in this study produced colorless films with good mechanical properties and excellent barrier properties. Edible blend films based on quinoa protein and chitosan were prepared by Abugoch *et al.* (73). These films presented good mechanical and gas barrier properties without the use of auxiliary plasticizers.

Quinoa has recently received worldwide attention. It is considered as one of the most nutritive grains used as human food and has been selected by FAO as one of the crops destined to offer food security in this century. In recent years, there is a growing interest concerning quinoa and the products derived from it. Commercially it is been highly demanded by markets such as Japan and USA. In Europe it can be found in most markets. Its successful introduction in new markest can also help the introduction of other less known Andean crops such as kiwicha and kañiwa, which have an enormous nutritional potential. Most important, the growing demand of Andean crops would benefit the small scale farmers that produce them, creating opportunities to sell their products.

2.1.2 KAÑIWA

2.1.2.1 General information

Kañiwa (*Chenopodium pallidiaule* Aellen) is closely related to quinoa, in fact it was considered a variety of quinoa until 1929 when it was classified as distinct species (72). Kañiwa grows under very harsh environmental conditions, mainly in Peruvian and Bolivian altiplano. It is more resistant than quinoa against frost. In its native area, year-round temperatures average less than 10°C and frost occurs during at least nine months of the year. The frost resistance of

kañiwa is probably due to its special anatomical structure which protects kañiwa's flowers from damage at low temperatures (1, 10). Kañiwa is a very important crop for highland farmers: when other crops fail because of frost, kañiwa still provides food. The most intensive production of kañiwa occurs north of Lake Titicaca in the department of Puno in Peru. The department of La Paz is the main producer of kañiwa in Bolivia.

Kañiwa's origins are uncertain, but it is almost certainly an Andean native. Archaeological evidence of kañiwa is unavailable and it is not known for how long before the coming of Spaniards kañiwa may have been in cultivation (74). At the time of Conquest, it was cultivated over a much wider area than at present (1). As quinoa, kañiwa produces cereal-like seed but it is not a true cereal. The "seed" is in fact a hard-walled fruit (achene) containing the seed (1). The plant is not completely domesticated; it grows almost like a weed and reseeds itself, which is typical for wild species (10). Other adverse characteristics of kañiwa as a semidomesticated plant are: great variation in appearance and time to maturity and failure of plants from the same seed to ripen at the same time. But it also exhibits favorable characteristics: self-sufficiency and adaptation to widely varying habitats. According to the National Research Council (1), kañiwa could prove to be a valuable "life-support crop" in extreme highlands throughout the world.

Kañiwa is weedy annual between 20 and 70 cm high. It can be erect or semiprostrate and branched. It is much smaller than quinoa (Figure 2). The stalks and leaves can be red, yellow or green. The stems and leaves as well as flowers are covered by white or pink vesicles which protect the plant against frost. The tiny flowers are covered by leaves, thus protecting them against the low temperatures of the altiplano. The flowers are hermaphroditic and self-pollinating. The seeds are about 1-1.2 mm in diameter, half the size of quinoa grains. The grains are covered by a perigone, usually gray in color.

Four principal kañiwa ecotypes can be distinguished: 1. Saigua qañiwa: erect, brown grain 2. Saigua ccoito: erect, dark brown or black grain 3. Lasta qañiwa: branched, brown grain 4. Lasta ccoito: branched, dark brown or black grain (10). "Saigua" means erect and "lasta" branched.

At the Agronomical Experimentation Station Illpa/INIAE, in Puno, Peru, the varieties Ramis, Cupi and Lampa have been selected. However, the peasants recognize additional landraces, such as Chilliwa, Kello (yellow), Puka (red) and Airampo (violet) (10).

Figure 2. Kañiwa and quinoa in Puno.

2.1.2.2 Composition and nutritional value

The nutritional value of kañiwa is excellent. The proximate composition of kañiwa grain is presented in Table 9.

Table 9. Chemical composition of kañiwa

Component (g/100 g dry basis)	Ref. (75)	Ref. (20)	Ref. (76)
Protein	14.1	15.3	16.7
Crude fat	4.1	7.8	6.8
Crude fiber	10.7	7.0	5.4
Ash	4.6	3.5	3.7
Carbohydrates	n.d.	66.4	56.4

n.d. = not determined

Kañiwa has higher protein, fiber and fat content than quinoa.

Proteins

Kañiwa's small grain contains more proteins than common cereals and even more than quinoa. Its composition of essential amino acids is very good (see Table 10). Kañiwa, as well as quinoa, is rich in lysine, the first limiting amino acid in common cereals.

Table 10. Content of essential amino acids and chemical score of kañiwa (g/16 g N) (76)

Amino acid	
Isoleucine	3.2
Leucine	6.5
Lysine	5.7
Methionine + cysteine	2.9
Phenylalanine + tyrosine	6.8
Threonine	3.1
Tryptophane	1.2
Valine	4.0
Chemical score	0.91
Limiting amino acid	Threonine

Lipids

The fat content of kañiwa is relatively high, 6.4-7.6 % (77). The fatty acid composition of kañiwa oil is presented in Table 11. Kañiwa oil is rich in unsaturated fatty acids, the principal fatty acid being linoleic acid (42.6 %). Oleic acid is the second most common fatty acid, in quantities of 23.5%. The content of linolenic acid is 6.0 % and the content of palmitic acid is 17.94 %. 72.9 % of the fatty acids of kañiwa oil are unsaturated (76).

Table 11. Characteristics and fatty acid composition of kañiwa oil (24)

Characteristic	
Fatty acids (% of lipid fraction)	
Myristic (C14:0)	-
Palmitic (C16:0)	17.9
Palmitoleic (C16:1)	-
Stearic (C18:0)	0.4
Oleic (C18:1)	23.5
Linoleic (C18:2)	42.6
Linolenic (C18:3)	6.0
Arachidic (C20:0)	-
Specific gravity	0.936
Iodine value (Wijs)	121
Unsaponifiable matter (%)	4.2
Acidity (% free fatty acids)	0.14

Repo-Carrasco *et al.* (76) analyzed the tocopherol content in kañiwa oil. The content of γ-tocopherol and α-tocopherol was 788.4 ppm and 726 ppm, respectively. The tocopherols exist as four different isomers with antioxidant power that is in decreasing order: δ, γ, β, α. the concentration of γ-tocopherol in kañiwa oil is higher than in corn germ oil, which has 251 ppm α-tocopherol and 558 ppm γ-tocopherol. The high content of γ-tocopherol in kañiwa oil guarantees a long shelf life, due to the antioxidant power of this tocopherol. In addition, the content of α-tocopherol in kañiwa is important because of its vitamin E activity.

Carbohydrates

Like quinoa, kañiwa is rich in starch. There are no studies on the rheological properties or the content of amylose and amylopectin in kañiwa starch. Repo-Carrasco (13) analyzed the content of free sugars in Andean grains. The content of total sugars, glucose, fructose, maltose and sacharose for kañiwa was 6.50, 1.80, 0.40, 1.70 and 2.60 %, respectively. The content of free sugars in kañiwa is higher than in common cereals (78).

Dietary fiber

Kañiwa is an excellent source of dietary fiber. The content of total, soluble and insoluble dietary fiber in kañiwa is about 26-27, 4.1-4.4 and 22-24%, respectively (33). It has more dietary fiber than common cereals and other Andean grains. The perigone, which covers the grain, contains mainly cellulose contributing to the high dietary fiber content of kañiwa. The content of dietary fiber of kañiwa, quinoa, kiwicha and some common cereals is presented in Table 6.

Minerals

Kañiwa is a very good source of minerals, especially of iron (Table 12). It also has a relatively high content of B vitamins. With respect to these nutrients, kañiwa compares favorably with common cereals such as wheat, barley, oats and rice. The content of riboflavin is higher in kañiwa than in common cereals (37).

Table 12. Content of minerals and vitamins in kañiwa

Component (mg/100 g)	Ref. (75)	Ref. (77)
Calcium	126	141
Phosphorus	461	387
Iron	18.8	12
Thiamine	0.78	0.67
Riboflavin	0.55	0.30
Niacin	1.34	1.45

2.1.2.3 Traditional uses and processing

The traditional preparation of kañiwa seed as food is a rather laborious process. First, the pericarp must be removed. Pericarp gives to kañiwa bitter taste and hard texture. Kañiwa contains small levels of saponins and other bitter susbtances. This is done by soaking the grain in water. The seed is then dried in the sun. Most kañiwa is made into *kañiwaco*, toasted and

milled grain. This product is consumed with milk, water or broth as breakfast. Kañiwa flour is also made into small cookies, *quispiño* (74). Kañiwa is not a commercial product, as most of it is consumed by the family that grows it. Local people use kañiwa when they must walk long distances because of its high food value. Kañiwa in the form of *kañiwaco* is used in traditional Andean medicine to counteract high altitude disease, against dysentery, and the ashes of the stems are used to repel insects. Of the three Andean cereals, kañiwa is less known outside of its place of origin. However, it is the most nutritional and the best adapted cereal in the Andean environment. It has a great potential to be used in Europe and the United States by health-conscious consumers.

2.1.3. AMARANTH

2.1.3.1 General information

Domestication of amaranths as grain crops took place only in tropical America. Three species of domesticated grain crops were developed in pre-Columbian America: *Amaranthus caudatus*, *Amaranthus cruentus* and *Amaranthus hypochondriacus*. A crucial step in the evaluation of the domesticated grain amaranths was selection by unknown ancient farmers of mutant forms in which the normal, wild-type dark seeds had been altered to white seeds. This resulted in better-flavored grain with superior popping quality. Artificial selection changed domestication in the direction of larger plant size and more seed production and also plants with red colors. This red coloration had magical and ceremonial connotations. Some Indian groups, for example the Hopi and Zuñi, grow amaranths primarily as sources of pigment for coloring ceremonial maize bread used in their traditional dances (79). In Peru, the amaranth has also been used as source of pigment in some traditional ceremonies.

The most important Andean species is *Amaranthus caudatus* Linnaeus. In the Quechua language, the local language in Peru, it is called "kiwicha". In Ecuador, it is known as "sangoracha" and "ataco" and in Bolivia as "coimi" and "millmi". In this thesis word "kiwicha" will be used for the Andean amaranth, *Amaranthus caudatus*. Kiwicha is cultivated in the Andes of Peru, Bolivia, Ecuador and Argentina. The word "amaranth" in Greek means "everlasting" and despite the suppression of its cultivation, amaranth has endured and spread

all over the world (11). *A. caudatus* originated in the same region in the Andean highlands as the common potato. The Spanish conquerors called it Inca wheat, but it was known long before the Incas. In ancient tombs seeds have been found which are more than 4000 years old (80). Kiwicha was one of the basic foods in the Incan Empire, nearly as important as maize and potatoes.

A. cruentus was domesticated in Central America and *A. hypochondriacus* in Mexico (79). *A. cruentus* is one of the most ancient crops cultivated in America. In the famous Tehuacan caves in Mexico, archaeologists have found grains of this plant. The dark-seeded forms of this species are used mainly as vegetables. Like corn, sweet potato and also other American Indian crops, *A. cruentus* was introduced to Africa by Europeans and it became one of the most important green vegetable (81).

A. hypochondriacus is the most robust and heaviest-yielding grain amaranth. It was probably domesticated in central Mexico, later than *A. cruentus.* Like corn and beans, *A. hypochondriacus* was grown in Arizona, the United States, in prehistoric times. It was a very important crop in ancient Mexico where it was used in pagan rituals. The tribute list of Montezuma, the last Aztec emperor, shows that at the time of the Spanish conquest of Mexico, grain amaranth was one of the most important crops. The Aztecs made idols from milled seeds of amaranth and kneaded with the blood of human sacrifices which were eaten in a kind of Aztec Holy Communion. This caused the catholic Spanish to complain against its use. This was one of the causes for the drastic decline in the cultivation of amaranth after the arrival of the Europeans (79). It was introduced to Europe before 1600 where it was used as ornamental plant. It was also introduced to Asia by 1800. There, the amaranth has become a regular food grain.

There are some wild species of amaranth with excellent food potential. *A. pumilus* is endemic to the barrier island beaches of the Atlantic coast of the United States. This species offers plant breeders a much larger and more desirable seed size and weight for improved biomass production in addition to lower levels of free carbohydrate compared with cultivated amaranth species (82). In North American deserts, the leaves of *A. palmeri* have been utilized as salad or potherb by Indians. *A. tricolour* and *A. dubius* are cultivated for their leaves in many countries (80).

Amaranth belongs to a family of dicotyledonous plants, Amarantanthaceae. It is an annual broad-leaved plant with height between 0.8 and 2.5 m (2) (see Figure 3). It produces cereal-like, starch-rich seeds, but it is not a true cereal. The cereals are, from botanical view, monocotyledonous grasses. The grains of amaranth are very small but they occur in huge numbers, sometimes more than 100,000 to a plant (1). The grain is lenticular in shape and measures approximately 0.8-1.0 mm in diameter, and weighs approximately 1 mg (83). A relatively large embryo surrounds the perisperm in the form of a ring. The color of the seeds varies from black to red, usually cream or ebony. Although selection has been made over the years for pale seeds (the wild species all have black seeds), large inflorescences and more seeds per plant, there has apparently been little selection for larger seed size. Amaranth germ and bran constitute 26 percent of the seed (80).

Amaranth is drought-resistant and grows from sea level to 3600 m, mainly in inter-Andean valleys (1). *Amaranthus* species are grown successfully worldwide in the tropics and semiarid regions because of their hardiness and their ability to grow in places where other more common grains cannot. This successful revival of amaranth can be ascribed to its C4 photosynthetic metabolic capacity, which endows it with a number of agronomic attributes, for example environmental hardiness and a capacity for efficient water utilization (83). C4 photosynthesis is especially efficient at high temperatures and under brilliant sunlight and under moisture stress (79). Amaranths have been cultivated from the tropics to semiarid lands and from sea level to high mountains. There are ecotypes which tolerate alkaline soils with pH as high as 8.5 as well as the acidic clays of hillsides (80).

In the Andes, there are various ecotypes of kiwicha, generally distinguished by the shape of the ear and by the color of the leaves and seeds. The oldest varieties in Peru are Noel Vietmayer and Oscar Blanco. Recently, some new varieties have been developed, such as Consuelo, Ayacuchana, San Luis, Otusco and Roja de Cajamarca. In Bolivia the Cahuayuma, Pairumani 1 and Pairumani 2 have been selected (10).

Figure 3. Andean amaranth, kiwicha in the Sacred Valley of the Incas, Cusco.

2.1.3.2 Composition and nutritional value

The chemical composition of three Amaranth species is presented in Table 13. The content of nutrients depends on the species, variety, growth location and cultivation practices. These species have not been compared under similar conditions.

Table 13. Chemical composition of amaranth species

Component/*A.* species	*A.caudatus* % Ref. (20)	*A.hypochondriacus* % Ref. (83)	*A.cruentus* % Ref. (11)	*A.hybridus* % Ref. (84)
Protein	15.5	17.9	15.5	13.1
Crude fat	7.6	7.9	7.7	7.5
Crude fiber	4.7	n.d.	4.4	6.8
Ash	3.4	3.3	3.3	2.0
Carbohydrates	68.8	63.6	58.3[a]	n.d.

n.d. = not determined

[a] Starch content

Proteins

Amaranth is often called the "small giant" because of its small grains with high nutritive value. The most important issue, in a nutritional aspect, is its high protein content and, in addition, its very favorable amino acid composition. Among the essential amino acids, the high content of lysine in amaranth protein must be emphasized. Lysine is one of the limiting amino acids in common cereals. Not only the seeds, but also the leaves of amaranth, are sources of protein of very high quality.

Of the proteins, 65% are located in the germ and seed coat, and 35% in the perisperm (11). Zheleznov *et al*. (85) found a variation between 13 and 21% in protein content in different amaranth species. They studied as well the wild as cultivated forms of amaranth. According to these authors, the proteins are highly digestible due to the presence of albumins and globulins.

Several studies have been carried out on the composition of amaranth proteins. Gorinstein *et al.* (86) studied the alcohol-soluble and total proteins of amaranth seeds and compared them with other cereals. The *Amaranthus* species studied were *A. cruentus*, *A. caudatus, A. flavus* and *A. hypochondriacus*. They found the albumins, globulins and glutelins as major protein fractions. They found also that amaranth contains much less prolamins than the common grains. Marcone *et al*. (87) carried out a research on the albumin protein fraction of *A. hypochondriacus*. They found that the albumin has a molecular mass of 133400 Da and a pI of 7.50. Secondary structure analysis revealed that albumin has a high proportion of β-sheet structures. According to their sedimentation coefficient, the globulins of amaranth are 7S and 12S globulins (88, 89). The globulins are particularly widespread in leguminous species while common cereals (wheat, rye) contain mainly prolamins. Amaranth globulins, in addition to their high nutritional value, also present some good functional properties, like emulsifying property (90).

In another study, Gorinstein and Moshe (91) evaluated four amaranth species through protein electrophoretic patterns and amino acid composition. They fractionated the seed proteins of *A. cruentus, A. flavus, A. caudatus* and *A. hypochondriacus* as albumins, globulins, alcohol soluble proteins A1 and A2 and glutelins G2 and G3. Albumins, globulins and G3 proteins had much higher lysine content than the alcohol soluble and G2 fractions.

The nutritional value of the proteins of amaranth has been studied by various researchers. To evaluate protein quality, several indexes are used, like protein efficiency ratio (PER), net protein ratio (NPR), net protein utilization (NPU), true protein digestibility and protein biological value. Afolabi *et al.* (92) found that the nutritional value of the protein of *A. hybridus* was low. They got a negative protein efficiency ratio, PER-value (-0.4). This was due to the high tannin content. However, Osuntogun and Oke (84) found a very high PER-value for the same species of amaranth. It was comparable to the value of PER of casein, 2.3 and 2.5, respectively. This variety had very low tannin content, so it can be concluded that the nutritional value of the proteins of amaranth depends on the presence or absence of antinutritional factors in the seed. When amaranth seeds are processed, the nutritional value of the protein increases (22). Morales *et al.* (93) evaluated the effect of toasted, popped and flaked products of amaranth (kiwicha) in convalescent and malnourished infants and young children. Their results indicated that the apparent nitrogen retention from kiwicha was superior to that from most cereals studied and was similar to that from rice or high-lysine maize.

The protein quality of amaranth has been studied *in vitro, in vivo* and in human experiment. *In vitro* protein digestibility for raw flour of different amaranth varieties was between 73 and 76%. Heat processing significantly improved the digestibility of the flour. Amino acid profile of amaranth was also studied (94) and leucine was detected as the first limiting amino acid in wholemeal flour. The overall amino acid profile of *Amaranthus* protein was extremely favorable. This attribute and its fairly good digestibility showed that *Amaranthus* is indeed a source of high quality proteins. *In vivo* studies with growing rats, addition of amaranth to cereal flours improved the protein quality without affecting energy utilization. Amaranth substitution also resulted in remarkable increases in weight gain of the experimental animals. Amaranth seems to be an effective source of protein to combine with other cereals (95).

The nutritional value of kiwicha processed by popping and extrusion was compared with cheese in a human experiment (96). True digestibility results of the protein were 101.4, 89.8 and 85.5% for cheese, extruded amaranth and popped amaranth, respectively. The statistical analysis showed that the true digestibility of the protein was the same for the two products of amaranth and different than the digestibility of cheese. The calculation of nitrogen intake for nitrogen equilibrium indicated that amaranth protein is among the highest in nutritive quality of vegetable origin and close to those of animal origin products.

Lipids

The lipid content of amaranth seed is relatively high, 7.6% (20). The fat of amaranth is characterized by a high content of unsaturated fatty acids. Becker *et al*. (97) determined the fatty acid composition of three amaranth species, *A. cruentus*, *A. hypochondriacus* and *A. caudatus*. The main fatty acid for the three species was linoleic acid. The content of oleic acid was high in all species (19.4-25.3%). .

The amaranth (*A. caudatus*, *A. cruentus*) oil has been studied to determinate its fatty acid, tocopherols and squalene content (98, 99, 100). The oil samples of *A. caudatus* from Ecuador showed interesting chemical characteristics for potential use as nutraceuticals. With regard to the alimentary potential of amaranth seeds, the Ecuadorian ones in particular, it can be pointed out that the total amount of vitamin E isomers in *A. caudatus* was slightly lower than that in wheat germ oil and significantly higher than that found in other edible oils. With a degree of unsaturation of 77.6% recorded for the extracts and an average saturation/unsaturation (S/U) ratio of 0.3, Ecuadorian *A. caudatus* oil is on a dietary par with rice bran oil and just slightly lower than soybean oil, known for their dietary properties. The squalene content was about 6 % in both amaranth species (96. 99). This reveals that there is a potential for the use of amaranth as a renewable source of squalene and as an alternative to animal sources. This triterpene is found in shark oil in abundance and in lesser amounts in vegetable oils. It is an ingredient in cosmetics, pharmaceuticals and lubricants. The physiological importance of squalene is due to it being the real precursor of steroidal terpenoids, and it is a key intermediate of cholesterol synthesis in humans. Regulation of cholesterol metabolism by dietary squalene in man has been studied, since the consumers of Mediterranean olive oil – where squalene is also found in high amounts – have low serum cholesterol levels

Carbohydrates

Starch is the most abundant component in amaranth seed, like in all cereals and pseudocereals. Its content is reported to range from 48 to 68% (11. The starch of amaranth has extremely small granules (0.75-3 μm) and high water-absorption capacity. The gelatinization temperature

of the starch of *A. hypochondriacus* is between 62 and 68°C (101). The starch of amaranth is mainly constituted by amylopectin, with a very low amylose content, 5-7% (97).

Physical properties of amaranth starch of different species were studied by Wu and Corke (102). This study indicated broad diversity in the properties of starches of different species. They pointed out that assessing the utilization of *Amaranthus*, it should be borne in mind that there is no generic or typical *Amaranthus* starch from a functional point of view.

Amaranth starch has been studied by several researchers (103, 104, 105, 106, 107). Amaranth starch can be isolated removing the proteins (104). The thermal properties, intrinsic viscosity, apparent viscosity and clarity of cold pastes of amaranth (*A. cruentus)* were similar to that of waxy corn starch (103). Starch-rich fractions can be obtained by differential milling. These fractions can be considered as an interesting raw material for the production of precooked amaranth high-starch flours having a wide range of hydration properties (105). Freeze-thaw stability of amaranth starch has been studied (106). Based on DSC (Differential Scanning Calorimetry) and centrifugation methods, amaranth starch had better stability after freezing and thawing through four cycles than did corn, wheat and rice starches. Amaranth starch shows slower retrogradation rates than corn, wheat and rice starches (107). The stability of starch gels during freeze-thaw cycling and its slow retrogradation rate enhance amaranth´s potential in different food products.

Dietary Fiber

There are very few studies on amaranth fiber. Repo-Carrasco (13) studied the dietary fiber content of three Andean crops: kiwicha (*A. caudatus)*, quinoa and kañiwa. In this study, it was found that all three crops are rich sources of dietary fiber, especially the insoluble fraction.

The effect of amaranth and oat bran on the lipids of blood serum and liver in rats was studied by Grajeta (108). Amaranth and oat bran added to the diet provided 4–4.5% of dietary fiber. Amaranth significantly decreased the level of total cholesterol in rat blood serum (by 10.7% in the case of a diet containing lard and by 14% with sunflower oil) and in the liver (by 20% in the case of diet with lard and by 23% with sunflower oil). Similarly, oat bran decreased the level of total cholesterol in the blood serum by 19% in the case of a diet containing lard and by 22%

with sunflower oil; and in the liver by 22 and 27%, respectively. Amaranth and oat bran did not influence HDL-cholesterol in the blood of rats. High fiber fractions can be obtained by differential milling of amaranth (109). These fractions contained 21.7 and 37.2% dietary fiber.

Vitamins, minerals and minor components

Vitamin and mineral components of amaranth grain are presented in Table 14. Niacin and thiamine contents are lower than in common cereal grains (37). In difference of common cereals, amaranth contains ascorbic acid (vitamin C). Amaranth seed is a good source of tocopherols and tocotrienols, and also of biotin and folic acid. It is also high in calcium and iron. Schönelechner *et al.* (40) analyzed folate content in amaranth and amaranth products. The content of folate in four amaranth varieties ranged from 52.8 to 73.0 µg/100 g. The amaranth samples showed only small differences between the fractions (wholemeal flour, flour and bran), which was probably due to the fact that, in amaranth, the embryo is located in the form of a ring outside the starch-rich kernel.

Amaranth can fully satisfy the recommended daily intake of iron and one gram of amaranth seed may contribute 46% of the recommended daily intake of calcium (22). The calcium/phosphorus ratio is very good, 1:1.9-2.7 (11). Nutritionists recommend a Ca:P ratio around 1:1.5 (11).

Table 14. Vitamin and mineral content of amaranth seeds

Component (mg/100 g)	Ref. (77)	Ref. (22)	Ref. (83)	Ref. (15)
Calcium	236	217-800	327	180
Phosphorus	453	556-600	170	n.d.
Iron	7.5	21-104	6.5	9.2
Thiamin	0.3	0.1-0.14	n.d.	n.d.
Riboflavin	0.01	0.19-0.32	n.d.	n.d.
Niacin	0.4	1.0-1.5	n.d.	n.d.
Ascorbic acid	1.3	3.0-7.1	n.d.	n.d.
Alphatocopherol	n.d.	1.57	1.84	n.d.
Biotin	n.d.	43-51	n.d.	n.d.
Folic acid	n.d.	42-44	n.d.	n.d.

n.d. = not determined

Amaranth species contain red pigments in leaves, inflorescences, stems and seeds. These pigments are water-soluble betacyanins. Betacyanins occur in plants of families of the order Centrospermae. It was once thought that betalains were related to anthocyanins, the reddish pigments found in most plants. Both betalains and anthocyanins are water-soluble pigments found in the vacuoles of plant cells. However, betalains are structurally and chemically unlike anthocyanins and the two have never been found in the same plant together. The amaranth betacyanins have been identified as amaranthine and isoamaranthine. Amaranthine has the same basic structure as the betacyanins from red beet. Cai *et al.* (110) found an extensive variability in betacyanin content among and within different amaranth species. They also concluded that grain amaranth species which have higher biomass and more betacyanins over a longer growth period than vegetable amaranth species, have a greater commercial potential for the development of natural pigments.

Cai *et al*. (111) studied colorant properties and the stability of amaranth pigments. Wide variation in color characteristics and significant differences in pigment stability were found as much within as between *Amaranthus* species. *Amaranthus* betacyanins, like red beet pigments, were susceptible to temperature and also affected by pH, light, air, and water activity, with better pigment stability at lower temperatures in the dark and in the absence of air.

Amaranth contains 0.3-0.6 % phytic acid (11). Phytic acid serves the plant as a form of phosphorus storage. Cereals and legumes contain 1-3 % of phytic acid (37). On a dry weight basis, corn contains 0.9% phytate, soft wheat 1.1%, brown rice 0.9%, barley 1.0% and oats 0.8% (112) Phytate can bind minerals such as iron, calcium and zinc, and there is some evidence showing decreased absorption of these minerals in the presence of phytate. However, phytic acid has also positive effects, for example a blood cholesterol lowering effect.

Cereals and legumes contain also tannins. They are bound mainly in the hulls of grains. Like phytic acid, tannins influence the bioavailability of some nutrients (proteins, minerals). The dark seeds of amaranth contain more tannins than the light ones (113).

2.1.3.3 Traditional uses, processing and actual situation

In ancient Mexico, the Aztec women ground the seed of amaranth, mixed it with honey or human blood and shaped it into forms of snakes, birds and gods that were eaten in ceremonies in temples or at home. More recently, the popped seeds are eaten as well in Mexico as in Peru. In Mexico the popped seeds are used to prepare "alegrias", a snack made with molasses and honey. In Peru, popped kiwicha is used to make "turrones", which are kind of snack bars. Flour of toasted seeds is also used both in Mexico and Peru. The flour can be used in soups, porridges, breads, cookies, etc. The popped grains are suitable for snack bars, confections etc. In Mexico "pinole" is made of toasted ground amaranth seeds. It is a flour sweetened with sugar and can be eaten as a sweet or be used in the preparation of beverages and bakery products. "Chuales" are sweet tamales, prepared only on the most sacred fiesta days: All Souls Day and Holy Week. They can be made of flour of amaranth or blue corn. The flour is mixed with molasses and the tamales are wrapped in corn husks and steamed (114). In some places in Mexico and Central America, "atole" or amaranth milk is prepared popping and grinding the seeds. Boiling water is added and the liquid is boiled. It can be sweetened and it is usually drunk warm. It is used to combat minor stomach problems.

In Peru, the most usual way of processing of kiwicha is popping. This is a very easy method of processing the amaranth and the product is ready to eat directly or to be incorporated in food formulations. The traditional way of popping amaranth is using direct heat contact on a hot surface. The cleaned seeds are laid on a hotplate (180-190 °C) and after a short time (20-30 s), expansion occurs. However, this method is very slow and inefficient and some modifications have been made to achieve an industrial method (115). Amaranth grains can be popped using a fluidized bed system (116). Using this method, higher yield (% of popped grains) can be achieved compared to the traditional method.

Kiwicha flour can be used in breads, substituting wheat flour. Blends of 80% wheat and 20% kiwicha can be used to produce normal leavening breads. These breads have a better nutritional quality than breads made of wheat flour alone. The kiwicha flour can also be used in cookies, crackers, soups, pancakes, porridges, rolls and muffins. Sindhuja *et al*. (117) developed and studied cookies with amaranth flour. They found that the substitution of 25% of the wheat flour by amaranth flour was optimum, considering taste, color, flavor and surface appearance.

Amaranth does not contain gluten and is suitable for persons with celiac disease. Recently, the use of amaranth flour in gluten-free breads has been investigated (38, 64). Calderon de la Barca *et al.* (118) used raw and popped amaranth flour in preparation of gluten-free breads and cookies. The best formulation for bread included 60–70% popped amaranth flour and 30–40% raw amaranth flour which produced loaves with homogeneous crumb and higher specific volume (3.5 ml/g) than with other gluten-free breads. The best cookies recipe had 20% popped amaranth flour and 13% whole-grain popped amaranth.

Extruded kiwicha can be used in salted snacks and as breakfast cereals. Mendoza and Bressani (119) studied nutritional and functional properties of extruded amaranth flour. The results indicated that the extrusion process improved the protein quality of amaranth. They developed a drink which could be used in the diets of preschool children with nutritional benefits for its high protein content.

The leaves are used as vegetables, like spinach. Usually, young leaves and stems are boiled as greens. The flowers of red varieties are used as colorants in traditional beverages in Peru and Ecuador. After the grain has been threshed, the amaranth residue can be used as a source of cattle feed. Andean farmers traditionally maintain their livestock on residues of crops during the dry season.

Amaranth is nowadays a relatively well-known grain in European and American health food markets. It is mainly exported from Mexico and Peru and used as a whole grain. In health food stores, it can be found in other products which contain amaranth, such as muesli and snackbars.

2.2 PULSES

Pulses are rich in protein, carbohydrates, dietary fiber and also in minerals and vitamins. They are cultivated and consumed worldwide. The main pulses used for human nutrition include peas, beans, lentils, chickpeas and fava beans. Pulses are a good source of minor compounds which may have important metabolic and/or physiological effects. These compounds have been considered as antinutritional factors. More recent
evidence, however, provides potential information on their impact on health, so these secondary metabolites are currently marketed as functional foods and nutraceutical ingredients (120).

2.2.1 TARWI

2.2.1.1 General information

Tarwi (*Lupinus mutabilis* Sweet) belongs to the *Lupinus*-family which is very diverse with more than 100 different species in the New World and a smaller number in the Mediterranean region (1). The Mediterranean species are agriculturally important and are used mainly as feed in Europe, the United States, Australia and South Africa. These lupines (*L. angustifolius, L. luteus, L. albus)* are sweet, and their content of bitter alkaloids is low compared with tarwi. The white flowering *L. albus* was cultivated by ancient Romans, Egyptians and Mesopotamians and is still an important crop in the Mediterranean region, North Africa and Australia.

Tarwi was domesticated by pre-Inca cultures more than 4000 years ago. Tarwi seeds have been found in the tombs of the Nazca culture (100-500 BC) on the coast of Peru (121). In the southern Andes, paintings in ceremonial vessels of the Tiahuanaco culture indicate the wide distribution and use of tarwi (10). Because of its high protein content (about 40%), tarwi was a very important crop for pre-Incan and Incan cultures. Tarwi is also an important component in the crop rotations of the Andes because of its capacity to fix nitrogen to the soil. Tarwi is reported to be able to fix as much as 400 kg of nitrogen per hectare. On the traditional Andean terraces, tarwi was planted on the upper levels in order to allow the soluble nitrogen to flow towards the lower levels. In addition to this, tarwi's strong roots loosen the soil; all of these abilities benefit the soil in which tarwi is grown (1). Tarwi adapts well in marginal soils and

resists high salinity and acidity (10). It also tolerates frost, drought and many pests. Since the Spanish colonial period, however, with the changing of food habits, tarwi was largely replaced by other crops.

Tarwi is an annual crop, 1-2.5 m tall. The flowers are purplish-blue and the pods are 5-15 cm long containing 2-6 ovoid seeds (Figure 4). The seeds can be white, gray, brown, black or more commonly white with black spots. In the Andes, many ecotypes and landraces of tarwi can be found. In Peru, there are five selected varieties of tarwi from the Cusco, Mantaro and La Libertad regions (10). Tarwi is cultivated in the Andes above 1500 m from Venezuela to Chile and Argentina.

Figure 4. Tarwi field in Cusco.

2.2.1.2 Composition and nutritional value

The chemical composition of tarwi is presented in Table 15. With its two-fold advantage, which includes high amounts of both protein and oil, tarwi represents a valuable food crop to combat malnutrition and a promising cash crop for edible oil production.

Table 15. Chemical composition of tarwi

Component	Ref. (122)		Ref. (123)
	Raw % dry matter	Cooked and debittered % dry matter	%
Protein	41.4	48.6	44.3
Crude fat	20.1	25.7	16.5
Crude fiber	6.5	10.7	7.1
Ash	3.6	2.5	3.3
Carbohydrates	28.4	12.5	28.2

Proteins

Tarwi has an exceptionally high protein content (more than 40%). It has more protein than soybean and even more than other lupine species (see Table 16).

Table 16. Chemical composition of the grain of four cultivated lupine species (124)

Composition of whole seed (% dry weight)	Crude protein	Ether extract	Crude fiber	Ash	N-free extract
L. albus	36.7	11.5	9.8	3.4	37.8
L. angustifolius	31.1	5.0	14.7	3.5	43.1
L. luteus	41.8	5.4	15.8	4.1	35.0
L. mutabilis	42.6	18.7	7.3	3.7	27.3

The essential amino acid content of raw and processed tarwi is shown in Table 17.

The limiting amino acids of tarwi are the S-containing amino acids, methionine and cysteine, as is the case in other legumes. Tryptophan and valine are other limiting amino acids in tarwi. However, tarwi contains sufficient amounts of lysine and threonine which are limiting in cereal proteins. Thus, tarwi protein is an excellent supplement for cereal proteins.

Table 17. Essential amino acid content of raw, water- or alcohol-debittered tarwi seeds (g amino acid/16 g N). (125)

Amino acid	Raw tarwi seeds	Cooked, water-debittered tarwi seeds	Oil-cake, alcohol-debittered	FAO Reference Protein
Isoleucine	4.8	5.5	5.0	4.0
Leucine	7.0	7.9	7.9	7.0
Lysine	5.9	5.6	6.4	5.5
Methionine/Cysteine	1.6	1.9	2.4	3.5
Phenylalanine/Tyrosine	7.9	8.1	6.3	6.0
Threonine	3.8	3.6	4.2	4.0
Tryptophan	0.7	0.7	0.6	1.0
Valine	4.2	4.5	4.5	5.0

The quality of tarwi protein assayed by animal trials (PER) is relatively low because of the low content of S-containing amino acids but it can be improved by methionine supplementation or by the combination of tarwi with cereals (125). To obtain more details about the protein quality of water-extracted lupines, Schöneberger *et al*. (125) determined the true digestibility, the net protein utilization (NPU) and the biological value (BV). The results of those studies showed surprisingly very high "true digestibility", that is, the N digestibility considering the endogenous N losses: the value of 92% was equivalent to that of casein. MacLean *et al*. (126) carried out clinical studies in order to study the protein quality of tarwi. These studies confirmed that methionine is the first-limiting amino acid in tarwi and suggested that threonine becomes limiting when methionine is provided in adequate amounts.

Lipids

Tarwi is an excellent source of oil (about 20%). Tarwi oil is of nutritionally good quality, main fatty acids being oleic and linoleic acids (see Table 18.). The composition of fatty acids in tarwi oil is similar to that of soybean oil. The low linolenic acid content gives to tarwi oil good stability against oxidation.

Table 18. Fatty acid composition of tarwi

Fatty acid (% of total fatty acids)	Ref. (127)	Ref. (128)
Myristic acid	n.d.	0.32
Palmitic acid	13.4-13.9	9.83
Palmitoleic acid	n.d.	0.43
Stearic acid	2.8-3.0	7.83
Oleic acid	41.2-41.7	53.87
Linoleic acid	38.8-39.6	25.89
Linolenic acid	2.6-3.0	2.57
Arachidic acid	n.d.	0.62
Behenic acid	n.d.	0.50

n.d. = not determined

Carbohydrates

The main carbohydrates in tarwi are oligosaccharides (see Table 19.)

Table 19. Oligosaccharide composition of two tarwi varieties (129)

Oligosaccharide (% dry weight)	Inti	Line 2150
Sucrose	9.0	9.9
Raffinose	16.9	16.6
Stachyose	68.3	67.7
Verbascose	5.8	5.8
α-galactosides	13.5	13.9

The stachyose content in tarwi is relatively high. This is interesting because there exists substantial evidence that high stachyose content reduces the digestibility of carbohydrates and thus of total energy.

Vitamins and minerals

The content of vitamins and minerals in tarwi can be observed in Table 20. Tarwi is a good source of calcium, iron and zinc.

Table 20. Content of some minerals and vitamins of tarwi (129)

Component	content
β-carotene (mg/100 g)	0.09
Thiamin (mg/100 g)	0.51
Riboflavine (mg/100 g)	0.42
Niacine (mg/100 g)	4.1
Potassium %	1.63
Sodium %	1.21
Phosphorus %	0.88
Magensium %	0.43
Calcium %	0.18
Iron (ppm)	76
Zinc (ppm)	59
Manganese (ppm)	57
Copper (ppm)	6.6

Antinutrients

A major hindrance to the wider use of lupines as food and animal feed has been their content of toxic, bitter quinolizidine alkaloids. Lupanin, spartein, 4-hydroxylupanin and 13-hydroxylupanin are the principal alkaloids found in *Lupinus mutabilis*. Spartein and lupanin are the most toxic, while hydroxylupanin is about ten times less toxic. The content of total alkaloids is about 3-4 % in tarwi seeds. Amounts of 10-25 mg per kg are toxic for small children, 25-45 mg per kg are toxic for adults (130). Poisonings by the bitter substances are rare because, on the one hand, the bitter taste prevents consumption and, on the other hand, the removal of the bitter substances is relatively easy.

Common legumes contain several antinutrients such as trypsin inhibitors, cyanogenetic glucosides and hemagglutinins. In tarwi, as in the other lupines, the content of these substances is low. Tarwi has an advantage over other lupines: it doesn't contain the toxic erucic acid.

2.2.1.3 Traditional uses and processing

The removal of alkaloids is the critical step in the processing of tarwi. The content of alkaloids must be reduced to about 0.05% to assure the safety of the final product (131). The traditional process of debittering the lupine begins with the cleaning and selection of the grains. Then the seeds are soaked in water for several hours to bring about their hydration, or else they are cooked directly for 45 to 60 minutes. The thermal process is essential to destroy the seed's germinative capacity, to inhibit enzymatic and bacterial decomposition, to reduce the loss of proteins through their coagulation and to facilitate the physical washing away of the alkaloids. Sometimes, lime is also added to the cooking water. This helps the debittering process by making it easier to remove the alkaloids, because the cellulose hull of the grain is dissolved by the lime. Finally, the lupine is placed in running water, to completely wash away the remaining alkaloids. The easiest way to do this is to put seeds into sacks, and place the sacks into a stream. This process takes from three to five days. Care must be taken not to exceed this period of time because the loss in the dry mass could be too great (132).

The final product is normally eaten fresh or used in traditional preparations. The lupines that the peasant does not use or sell are dried and sometimes processed to make lupine flour. After drying, which can be carried out in the sun in the arid atmosphere at an altitude of 3000 to 4000 m, the lupines can be stored without losses for long periods of time. Recipes for the proper mixture of lupine flour with wheat, yuca (cassava), bananas and other products have been developed. An especially successful recipe is that of a flatbread: 40% wheat flour, 40% yuca flour and 20% lupine flour (130).

In the traditional debittering process, unfortunately not only the alkaloids are washed out, but also other water-soluble components such as proteins and carbohydrates. This can be partially avoided using acidified water (pH 4.5) in the hydration step. Solvents other than water have been tested with the aim to improve the debittering process of tarwi. De la Cruz Flores (131)

compared the use of ethyl ether, ethanol and hexane and found out that the most promising solvent was ethanol.

In an effort to promote the use of lupines as a protein and oil crop in Peru, feasibility studies have been undertaken by the German Agency for Technical Cooperation (GTZ)
in the late 1980s and early 1990s. Tarwi oil has many advantages: it doesn't contain erucic acid and its low linolenic acid content (about 2%) makes it more stable against oxidation. Tarwi oil has also a relatively high content of γ-tocopherol which acts as natural antioxidant. Lupine oil production is similar to that of soybean, however due to the high alkaloid content, one more step has to be added. The remaining oil cake is rich in protein (about 70%) and can be utilized as a protein concentrate. In spite of these efforts, the cultivation and use of tarwi in Peru is still incipient.

In Ecuador tarwi is commercialized and used widely. INIAP (Instituto Nacional Autónomo de Investigaciones Agropecuarias) has recently presented new varieties of tarwi and investigated the use of alcaloids as fungicide. New products, such as yogur and soft cheese have been developed.

In Peru, tarwi is used in traditional medicine. It is used to heal diabetes, kidney problems and external parasites. In the Aymara culture, in the Puno area, tarwi is also used in religious rituals (123). The water which has been used to eliminate the alkaloids is used as a biocide for pest control. Tarwi is not known in Europe, but there are other *Lupinus* species cultivated in the Mediterranean area. They are mainly used as animal feed.

2.2.2 OTHER NATIVE PULSES

The common bean (*Phaseolus vulgaris)* was grown by the Incas in low elevations. At high elevations, the common bean grows slowly. Basul, nuñas, lima bean and tarwi are well adapted to grow in high mountains.

Lima bean (*Phaseolus lunatus*) was probably first encountered by Europeans some 400 years ago near by the city of Lima. It was cultivated mainly in the river valleys of coastal Peru. Lima

beans have been found in archaeological excavations dated to 6000-5000 B.C. (1). The Lima bean was distributed throughout the world and named by the place where it was first cultivated. These beans are one of the most widely distributed pulses, especially in tropical areas. The protein content of lima bean is relatively high (20-24 %). Lima bean is deficient in methionine and cysteine, like most legumes. Lima bean is rich in potassium, phosphorus and magnesium (133).

One of the least known pulses from the Andean area is basul (*Erythrina edulis*). Basul is a tree of the Leguminosae family which is native to the Andean region and grows between 1100 and 2700 m. It is grown in Andean towns for both decorative and nutritive purposes. It is an important crop because it grows in areas where seasonal food deficits often occur. Its dried seeds are used particularly in the months just before field crops are ready to harvest and the food supply is scarce (1).

Basul is not only used as food but also as lumber for construction, for fences and for fuel. The leaves are given to animals and as mulch in gardens. The flowers are edible as well and are used to decorate and season foods. The trees of basul are important themselves. Basul is very easy to grow and can be used in agroforestry systems. In this system, trees are grown together with food and cash crops. Shaded plantation systems are probably the most important agroforestry systems of the Americas. Cacao has been grown under shade since its domestication. When coffee was introduced into the
Americas in the 18th century, it invariably was grown as a shaded crop. Other crops frequently grown under shade or with leguminous trees for support are vanilla, black pepper, tropical yam, various spice trees, and tobacco (134). This system is highly productive and sustainable. In addition, basul as a leguminous tree, supplies nitrogen that fertilizes the soil around it (1).

Seeds must be boiled for at least 45 minutes or fried thoroughly before being eaten. Research indicates that uncooked *E. edulis* seeds can be toxic if consumed over a long period (135). The seeds of basul are usually boiled in water and served as a side dish. Sometimes, they are mashed with cheese and also fried. The seeds contain about 20% protein and are rich in lysine. The limiting amino acids are methionine and tryptophan (135). Leterme *et al*. (136) analyzed the mineral content of basul. They found the following values for calcium, phosphorus, potassium,

magnesium, sodium, chloride and sulfur: 20, 60, 584, 38, 8, 6 and 16 mg/100 g edible portion, respectively.

Nuñas are a type of common beans (*Phaseolus vulgaris*). They were cultivated by Incas and other pre-Columbian cultures. Nuñas are also called "popping beans" because they can be prepared as popcorn. When heated with a little oil, nuñas burst out of their seed coats. The resulting product is soft and has a nutty taste. Nuñas look like common beans, and their color varies between white, red or black spotted. Nuñas are grown in Ecuador, Peru and Bolivia above 2500 m altitude. They are produced mainly for home consumption but also sometimes sold as part of a mixture of beans to be used in soups.
In Peru, nuñas are prepared by toasting for 5-10 minutes in a hot pan with vegetable oil. The seed coat splits and the product is served as a side dish or eaten as a snack. In some places, for example in Cusco, popped nuña is often sold to tourists. Nuñas have about 22% protein (1).

2.3 TUBERS

2.3.1 NATIVE POTATOES

Potatoes have been cultivated for approximately 8000 years in the Andes. The ancient farmers selected ecotypes to meet their local needs and preferences and to adapt them at different ecological levels. This selection process has resulted in thousands of distinct types and nowadays many peasants grow up to 200 ecotypes of potatoes in a single field (1).

The centre of domestication of the potato is the southern Andes of Peru. The island of Chiloe, in southern Chile, is a secondary center of domestication of this crop. Potatoes belong to nine different species: *Solanum goniocalyx, S. phureja, S. stenotomum, S. tuberosum, S. ajanhuiri, S. chaucha, S. juzepczukii*, *S. curtilobum* and *S. tuberosum* ssp. *andigenum* (137). Each of these species has its own morphological characteristics and adaptation to altitude.

Andean native potatoes look quite different compared with common potatoes. Their skin and flesh can be colored, for ex. yellow or purple (see Figure 5). The shapes can be elongated, thin and wrinkled. Their flavor is rich and many have appealing culinary qualities. They are often

less watery than common potatoes because of their high dry matter content. These potatoes are well adapted to marginal growing conditions and are very resistant to disease, insects, nematodes and frost.

The native potatoes have some limitations, too. Many of them can be grown only on high mountains and they are less vigorous and yield fewer and smaller tubers than modern commercial potatoes. Most of them have deep eyes and irregular shapes that make them harder to process and handled than regular potatoes. In addition, many of them need short days to introduce tuberization (1).

The peasants of the Andes classify the potatoes into two types: the sweets and the bitters. There are some 2000 varieties of native potatoes in the Andes of Peru and Bolivia. The sweet varieties are consumed directly whereas the bitter ones are used to prepare the traditional dehydrated product, *chuño.* Cultivars belonging to the bitter species are *S. curtilobum , S. juzepczukii* and *S. ajanhuiri* . Their high glycoalkaloid content restricts their use for fresh consumption. However, these cultivars are consumed fresh in some communities because of their medicinal properties.

Chuño can be stored for up 10 years. Archaeological and linguistic evidence suggest that this process was common among pre-Inca and Inca cultures (137). Depending on the process followed and cultivars used, different kind of *chuño* are recognized (138). Black and white *chuño* are processed at altitudes form 3600 up to 4300 m. These two types of *chuño* are processed in slightly different ways. White *chuño*, called also *moraya* or *tunta*, is usually commercialized while the black one is mainly consumed at home. The elaboration of both types of *chuños* takes advantage of frosts at night alternating with daytime solar radiation and low levels of relative humidity during the months of June and July (139). The principal difference between the processes of elaborating black or white *chuño* relates to the prolonged exposure of tubers to running water. White chuño is always washed or soaked, in part to remove glycoalkaloids . Bitter *chuño* is not exposed to water and its preparing is simpler, basically consisting of tending, treading, freezing and drying (140). *Chuño* is an important commodity for peasants in the high mountains for its long-term storability and high food value.

Figure 5. Native potatoes

2.3.2 OCA

Together with potato, oca (*Oxalis tuberosa*) is a very important root crop in the Andean highlands Its cultivation and consumption in the Andean region occupies the second position after potato. Oca is cultivated in the Andean region form Venezuela to Argentina. Besides South American countries, oca is also grown commercially in New Zealand. Oca looks like a wrinkled carrot, and can be white, yellow, red or spotted in color. Some varieties have a slightly acid taste, some are sweet. Oca is very resistant and can prosper in poor soils and in harsh climates. It can be grown at altitudes between 3000 and 4000 m. Its yields can be twice that of the potato (1).

Oca can be prepared in many ways: boiled, baked, fried, in salads and pickled in vinegar. In Puno, Peru, a jam is prepared with the red variety. In the Andes, oca tubers are often placed in the sun for a few days. During this time, the tubers become sweet because the content of glucose increases. Bitter varieties of oca are transformed into dehydrated products (*caya*). The process is similar to that of preparing *chuño:* tubers are first soaked in water, then frozen during

the night and squeezed by stamping on them to remove the water. This product can be stored for long periods without refrigeration.

The nutritional value of oca is similar to that of potato. On average, the tubers of oca contain 70-80% moisture, 11-22% carbohydrate and 1% each of fat, fiber and ash. Some varieties can have more than 9% protein on a dry-weight basis (1). Oca can be considered as good source of calcium and iron (141).The bitter varieties contain oxalic acid, 80-194 mg/100 g wet matter (142). High-oxalate foods can cause negative effects on calcium and iron absorption. Since oxalic acid is soluble in water, cooking is likely to diminish its concentration by leaching into the cooking water. In the case of oca, this doesn't happen because the tubers are normally cooked with the skin on and the skin appears to prevent the leaching of oxalates into the cooking water (142).

2.3.3 OLLUCO

Olluco or "ulluco" (*Ullucus tuberosus*) is a completely domesticated crop. Its tubers have been found in 4250-year-old ruins in coastal Peru (1). Nowadays, olluco is grown in mid-to high-altitudes in the Andes from Venezuela to Chile. It has also been experimentaly grown at sea level in Canada, the UK and Finland(1). The plant of olluco is easy to grow and frost resistant and can give good yields in marginal soils. An additional advantage of olluco is that it has few pest and disease problems.

Ollucos can be round, elongated (5-15 cm) and curved. Their color varies from yellow to orange, pink, red or purple and the tubers have a waxy skin. The skin is thin and soft and needs no peeling before consumption. The flesh of the olluco tubers is mostly yellow and its texture is smooth and the taste is pleasant.

The tubers have high water content (85%) . The main component in dry matter is starch (about 14%). Olluco is a good source of vitamin C, containing 23 mg per 100 g fresh weight and also of vitamin A (1). Olluco is traditionally processed by washing and rubbing, removing the mucosa. The natural colorants of some genotypes, whose pigments are stable, are used traditionally, but not commercially, in food preparation and for staining cloth (52). The tubers of olluco are most often boiled rather than baked, because of their high moisture content. One

typical dish in Peru is the *olluquito con charqui* which is an olluco stew with dried llama meat. The tubers have a good shelf life, they can be stored up to a year in the cool areas of the Andes. Sometimes, the tubers are freeze-dried (like potatoes to make *chuño*). This long-lasting product is called *llingli*. Peru has exported canned olluco to the United States, mainly for Hispanic markets.

2.3.4 MASHUA

Mashua, or "añu" or "isaño" (*Tropaeolum tuberosum*), is the fourth most important root crop in the Andean region. Mashua is closely related to the garden nasturtium, an ornamental plant well-known worldwide. Mashua is one of the highest yielding and fasest growing tubers in the Andean region. It is very cold resistant and it repels many insects, nematodes and other pathogens. It is often grown together with potato, oca and olluco with the aim to control pests and diseases.

The tubers are relatively small and can be conical or elongated. They are commonly yellow, but varieties with purple coloration surrounding the skin and dispersed throughout the tuber also exist. Mashua is usually boiled with meat to form a stew. Most varieties have a sharp flavor due to isothiocyanates, but boiling diminishes this sharp taste. Like all tubers, mashua has a high water content, about 80%. Solids contain about 75% carbohydrates and 11% protein. Mashua is rich in C vitamin, 476 mg/ 100 g dry matter (1). Vitamin C can be preserved in tubers when boiled with unpeeled. In addition, carotenoids have been found in mashua, 70 to 132 μg β-carotene equivalents g^{-1} DM (143).

Recently, it was found that purple mashua possesses a high content of antioxidant compounds comparable with those present in already recognized antioxidant sources. According to Chirinos *et al.* (144), purple mashua contains phenolic contents in the range of 14–24 mg/g dry matter (DM), comparable to raspberry and blackberry. Chirinos *et al.* (144) found that the phenolic compounds present in purple mashua are anthocyanins. The major anthocyanins found in the different genotypes investigated were delphinidin di- and triglycosides acylated with acetic acid.

Mashua has several folk-medicine uses. It is considered to be anti-aphrodisiac, which is why it is mainly eaten by women and children. The Incas gave mashua to warriors when they had to travel far away from their homes. Mashua would make them to forget their wives while on military operations. It is believed that mashua tubers suppress sexual appetite and decrease fertility in men, but in women they enhance reproduction. This belief continues to the present day. The compound probably responsible for these effects is the p-methoxybenzylglucosinolate, which is the main secondary metabolite of mashua. Jones *et al.* (145) studied the anti-reproductive effect of mashua in male rats. Experimental animals and controls showed equal capability in impregnating females, although animals fed mashua showed a 45% drop in their blood levels of testosterone/dihydrotestosterone.

In folk medicine, mashua has other uses as well. In Bolivia, mashua is used for prostate disorders (53). In Peru, the tubers are widely used to treat kidney and related ailments and also skin ulcers and to kill lice. In their research, Jones *et al*. (145) showed the positive antibiotic, nematocidal and diuretic effects of mashua. According to these studies, the medicinal traditional uses of mashua by the peoples of the Andes Mountains appear to have biological basis.

2.4 ROOTS

2.4.1 MACA

Maca (*Lepidium meyenii* Walp.) belongs to the family of Brassicaceae. It is a relative of the radish. The edible part is derived from the tuberous hypocotyl. There are eight or more different ecotypes in the cultivation area, distinguished according to the color of their roots, such as yellow, purple, white, grey, black, yellow/purple and white/purple (146). It is an annual or biennial herbaceous plant and it grows at higher altitudes than perhaps any other crop in the world. It was domesticated in the central Andes of Peru at elevations of 3500–4500 m above sea level. The area where maca is grown has extreme conditions: intense sunlight, strong winds and low humidity and temperatures. Daily temperature fluctuations are great, such that during the daytime, the temperature can be 20°C and at night it can sink to -10°C. Maca survives in areas where even bitter potatoes cannot grow. Domesticated maca has been grown in Peru for at least 2000 years, but little is known about its origin. Maca is an Andean crop of narrow

distribution. It is restricted to the suni and puna ecosystems of the Departments of Junín and Cerro de Pasco of Peru at elevations above 3500 m and often reaching 4450 m in the central Andes of Peru (147). Maca's cultivation has declined; in 1982, it was declared to be in danger of extinction as a domesticated plant. The main reason for the decline has been maca's image as "poor man's food". Maca has been displaced by imported foods. However, in the last decades, maca has received worldwide attention for its medicinal properties. In Peru, a product "Maca Andina" has been developed. This product is marketed as "Andean Viagra" and it became extremely popular, not only in Peru but also abroad. This caused an expansion on the cultivated area of maca in last decates.

Fresh maca roots have more than 80% water content. Dehydrated powdered maca root contains 8.87–11.6% protein, 1.09–2.2% lipid, 54.6–60.0% carbohydrate (148). The roots are rich in micronutrients, especially in iron and iodine. These nutrients are often deficient in the highland diet. The mineral contents of maca reported in Dini *et al.* (149) were Fe 16.6, Mn 0.8, Cu 5.9, Zn 3.8, Na 18.7, K 2050 and Ca 150 (mg/100 g dry weight).

Maca can be consumed fresh but is commonly dried. In the Andes, the roots are sun-dried to get a long-lasting product. Maca has a strong and peculiar flavor which is not acceptable to many people. In most cases, this is disguised by other components used in the preparation of juices and beverages. Flour is also prepared from the dried roots for making bread and cookies. In Andean towns, a beverage of maca is sold for breakfast. Liquor of maca is prepared as well.

However, maca is not only used as a foodstuff. It plays a very important role in traditional medicine, especially as an aphrodisiac to enhance sexual drive and female fertility in human beings and domesticated animals (148). According to Spanish chroniclers, soon after the conquest, the Spanish found that their livestock were reproducing poorly in the highlands, and the Indians recommended maca. The results were remarkable (1). Inca warriors were said to be fed with maca to increase their energy and vitality; however, they were prohibited from consuming it after the conquest of a city as a measure to protect women from their sexual impulses. These effects of maca has been scientifically examined using rat models and also in humans. Maca was shown to improve sexual performance of male rats (150, 151). Administration of maca was found to prevent the reduction in body weight and epididymal sperm count induced by high altitude (152). In humans, it was found that oral administration of

maca tablets to normal adult men over a period of four months led to seminal volume increase and also to increased number of sperm count per ejaculum (153). In the study of Gonzales *et al*. (154), the sexual desire of healthy men was assessed while taking a maca supplement. The result showed a widespread increase in sexual desire in the test group. The mechanisms of maca's improvement of sexual performance have not yet been clarified. Maca has also been shown to improve fertility in females (148). Some researchers have found that maca has estrogenic activity (155), but others studies showed that maca does not possess estrogen-like effects to improve fertility (153, 157). There should be more studies to clarify the mechanism of action of maca in improving fertility.

Maca also has other claimed medicinal properties, according to traditional beliefs, such the capacity to cure or relieve rheumatism, ameliorate respiratory ailments, as a laxative, to regulate hormonal secretion, to stimulate metabolism, in memory improvement. Additionally, maca is said to possess antidepressant activity and to be effective in combating anemia, leukemia, AIDS, cancer and alcoholism, among others .

Maca is used to improve vitality and stress tolerance in the Andean region. Maca powder has been shown to exhibit an anti-tiredness effect (148). The reason behind maca's effect on stress tolerance is unclear. It is hypothesized that maca may lead to activation of the hypothalamic-pituitary-adrenal axis to increase adaptogens and by this means increase resistance to various noxious and stressful stimuli (158).

Many kinds of secondary metabolites have been found in maca root. The typical markers for maca are macaene and macamide, the novel polyunsaturated fatty acids and
their amides which are not found in other plants (148). The content of glucosinolates in fresh maca is about 1% , which is about 100 times higher than the level found in other cruciferous crops such as cabbage, cauliflower and broccoli (159). In recent years, glucosinolates have received scientific attention because of their biological activities, especially their ability to combat pathogens and cancer (160). These compounds are considered to be responsible for the pungent flavor of maca. Glucosinolates can be hydrolyzed to a series of different compounds, such as isothiocyanate, thiocyanate and nitriles by the endogenous enzyme, myrosinase (160). When maca is processed, the glucosinolates can be easily broken down by this enzyme. This is why the content of glucosinolates in processed products is much lower than in fresh roots.

Maca has the ability to scavenge free radicals and provide cytoprotection under oxidative stress (161). Reactive oxygen and nitrogen species are implicated in the etiology of degenerative diseases, including cardiovascular disease, diabetes, cancer and aging. Maca can help protect cells from pathological changes.

Other biologically active compounds in maca are alkaloids, sterols and monoamineoxidase inhibitors. Three alkaloids have been isolated from maca roots: two imidazole alkaloids and one benzylated derivative of 1,2-dihydro-N-hydroxypyridine (148). Many types of phytosterols have been found in maca (149, 162). Phytosterols are reducers of plasma cholesterol levels and also exhibit anti-cancer, anti-inflammatory and anti-oxidant properties (163). 1R,3,S-1-methyltetrahydro-β-carboline-3-carbolylic acid has been identified in maca. This compound acts as an inhibitor of the enzyme monoamineoxidase (148). Monoamineoxidase inhibitors (MOAI) are used in psychiatry for the treatment of depressive disorders and in neurology for alleviation of the symptoms of Parkinson's disease (164). Recently, the occurrence of tetrahydro-β-carbolines in chocolate and cocoa has been reported and it has been hypothesized that, on the basis of their activity as mild inhibitors of MOA, they can potentiate the effect of amines (phenylethylamine, tryptamine, serotonin, and others) in chocolate. Furthermore, chocolate is considered to be a craved food, and, although the hedonic appeal of chocolate is the predominant factor, it has also been supposed that chocolate contains pharmacologically active substances responsible for the craving. Tetrahydro-β-carbolines, with their neuroactive properties, could play a role in craving (165).

2.4.2 YACON

Yacon (*Smallanthus sonchifolius*) is a relative of sunflower. Yacon is grown in many localities the Andes, from Ecuador to north-western Argentina. It has been found in pre-Incan tombs in Peru. Yacon is grown for its edible roots. The yacon is a perennial herb, and stands1.5-3 m tall. The root system is composed of 4-20 fleshy tuberous storage roots that can reach a length of 25 cm by 10 cm in diameter, and an extensive system of thin fibrous roots (147).

Yacon is mainly a source of carbohydrates. Fresh tubers contain 69-83% moisture, 0.4-2.2% protein and 20% sugars. The sugars are fructose, glucose, sucrose and inulin type oligofructans and thus may be prospective prebiotics as they are fermented by beneficial species of gut

bacteria. Yacon roots could also be used as a source of natural sweeteners and syrups suitable for persons suffering from digestive problems. The inulin type oligofructans of yacon are β-(2→1) oligofructans. These oligfructans play a role in the biological activity of yacon tubers. They are not digested in the upper gastrointestinal tract and are therefore fermented by gut bacteria and thus promote the growth of beneficial bacterial species from the *Lactobacillus* and *Bifidobacterium* species, which exert a range of positive physiological effects (166). For people suffering from metabolic disorders of glucose metabolism (metabolic syndrome, diabetes etc.), a low content of glucose and a high content of β-(2→1) fructooligosaccharides is desirable. Yacon root has favorable composition of these sugars (166).

Yacon is usually eaten raw after a period of exposure to the sun. This procedure increases the sweetness of the roots, and they are consumed as fruits. Yacon has a crunchy texture and mild, sweet taste. It is often added to salads. The tubers are also consumed boiled and baked. In the Andes, they are often grated and squeezed through a cloth to yield a sweet, refreshing drink. Sometimes this is concentrated to form a dark-brown syrup. Another promising processing technique is the production of dry chips. In this case, yacon tuberous roots are peeled and cut in thin slices which are oven-dried (147).

Yacon is used for medicinal purposes as well. Traditionally, yacon roots and dried leaves are recommended to people suffering from diabetes, digestive or renal disorders (168). Yacon syrup is a very good source of fructooligosaccharides and its long term consumption produce beneficial health effects on persons with insulin resistance (169). Medicinal (antidiabetic) properties have been attributed to yacon leaves in Brazil, where the dried leaves are used to prepare a medicinal tea. Dried yacon leaves are used in Japan, mixed with common tea leaves for their hypoglycemic activity (147).

2.4.3 ARRACACHA

Arracacha (*Arracacia xanthorrhiza*) is a relative of carrots and celery. It produces roots that resemble white carrots. It is a perennial and both the root and leaves are used as food. The cylindrical central root bears numerous lateral roots that are 5-25 cm long and 2-6 cm in diameter (1). The flesh of the root is white, yellow or purple. Arracacha is cultivated from 600

m to 3200 m in Peru, Ecuador, Brazil, Venezuela, Bolivia and Colombia. Usually, it is grown in small gardens for local use.

Young arracacha roots are eaten boiled, baked or fried and also added to stews and soups. They have a crisp texture and delicate flavor. In Brazil, arracacha is made into dried chips to use in dehydrated soups. In Peru, fried chips are made of arracacha. Another traditional food in Peru is "dulce de arracacha", a sweet snack made with grated arracacha and molasses. The young stems are used in salads or as a cooked vegetable.

Arracacha root dry matter can range from 17 to 34% of the fresh weight The overwhelming part of arracacha root dry matter is carbohydrates, of which about 95% is starch and 5% is sugars (mainly sucrose) Arracacha roots have a starch content ranging from 10 to 25% (1). The starch granules are small and the starch is easily digested and it is used in baby foods. The starch of arracacha has some interesting properties, for instance, it has very low syneresis. Arracacha has high calcium content (45-128 mg/100 g) and the yellow colored roots are good sources of vitamin A (255-6879 IU). Arracacha is also a good source of ascorbic acid (18.3-28.4 mg/100 g) (147).

2.4.4 MAUKA

Mauka (*Mirabilis expansa* Ruiz & Pavon), also known as *chago* is a little-known plant grown in the high valleys of Bolivia, the uplands of Ecuador and in the northern Andes of Peru. It is a low, compact plant, not exceeding 1 m in height. Mauka survives in wet, cold areas as well as in arid regions. It is cultivated at altitudes from 2200 to 3500 m. Commonly, mauka is interplanted with crops such as corn (1).

The edible parts of mauka are the upper part of the root and the lower part of the stem. They are commonly smooth and fleshy, about 5 cm in diameter and 50 cm in length. When freshly harvested, the mauka roots grown in Bolivia have an astringent taste. Exposing the roots to the sun replaces the bitterness with a pleasant sweet flavor. Interestingly, it is said that the mauka grown in Ecuador is not astringent (1).

Mauka is usually boiled or fried and served as a vegetable. It is prepared in two ways: salty or sweet. Traditionally, the sun-sweetened roots are chopped, boiled and mixed with honey or brown sugar. For salty mauka, the roots are cleaned, cooked and peeled.

Mauka is rich in carbohydrates (87% on a dry-weight basis) and has a relatively high protein content (7%). Mauka is richer than other Andean tubers in calcium, phosphorus and potassium. The leaves contain about 17 % protein (1).

2.4.5 AHIPA

The ahipa (*Pachyrhizus ahipa*) is a leguminous plant but it is grown for its roots. It is cultivated mainly in the Andean valleys of Peru and Bolivia, between 1500 and 3000 m in elevation. This plant has the legume family advantage: rhizobia bacteria in its root nodule make nitrogenous compounds that nourish the plant and the soil. Ahipa is an erect or semierect herb about 30-60 cm in height. Each plant has a single swollen root. The roots may be 15 cm or more in length and usually weight 500-800 g. The pale yellow or tan skin encloses a white pulp (1).

The tuberous root is the part used as food. The seeds are not used because of their rotenone/rotenoid content (170). The tubers have a protein content of 8-18% (dry weight). In addition to the interesting and valuable protein content, the tuber is very rich in carbohydrates, which provides energy. The range of the starch content is between 45 and 55% and the sugar content is between 8 and 24%, whereas the lipid content is below 1% (170). Ahipa has received interest because of its high starch content and the high proportional amount of amylopectin. Therefore, the Andean ahipa starch would represent a very promising raw material for the starch industry. Ahipa roots are mainly eaten raw; it has a sweet and refreshing taste. It is used in green salads and fruit salads. Ahipa can also be cooked.

2.5. NATIVE FRUITS

In the Andean region, there are several native fruits which were used by Incas and other pre-Columbian cultures. Few of them are cultivated on a large commercial scale; they are mainly cultivated in home gardens. Fruits are generally good sources of vitamins, for example of vitamin A and C. Some varieties have also a high content of minerals. Fruits are used by the

Andean peasants both as food and as a cash crop. Nowadays, they have received growing interest by the food industry with the aim to develop new, exotic products for local markets and for exportation, as well.

2.5.1. GOLDENBERRY

Also known as cape gooseberry and as *aguaymanto* in Peru, goldenberry (*Physalis peruviana*) is nowadays grown outside of its centre of origin, the Andes. Goldenberry is a relative of potatoes and tomatoes. This perennial herb presents fuzzy, heart-shaped leaves and yellowish flowers. It is about 1-2 meters high and it is strongly branched. Its fruits are aromatic with pleasant taste and resemble a small, golden tomato, measuring about 1.2-2 cm in diameter. The fruit is covered by papery husk (calyx) which looks like a Chinese lantern (Figure 6). This gives the fruit a very exotic and eye-catching look. In Andean villages, goldenberries are cultivated in home gardens for domestic use but also for commercial purposes. The plant of goldenberry tolerates poor soils and does not need much care when growing. The plant is good ground cover for protecting land form erosion (1). A single plant can yield 300 fruits and carefully cultivated plants can provide 20-33 tons per hectare (171).

Goldenberry is an excellent source of provitamin A (3000 I.U. of carotene per 100 g) and 58-68 mg of vitamin C per 100 g (1, 172). The content of phenolic compounds and antioxidant activity of goldenberry was determined by Repo de Carrasco and Encina (173). According to this study, goldenberry is a good source of phenolic and other antioxidant compounds. They also found that goldenberry has a relatively high content of minerals, such as iron, potassium and zinc. The fruit contains about 2% oil on a fresh weight basis and the main fatty acid of the oil is linoleic acid. Goldenberry also contains a relatively high proportion of oleic acid and γ-linoleic acid (156).

Fruits have a long storage-life since the calyx takes over a certain defensive
function: berries with an intact expanded calyx, containing air that reduces the specific weight, are less prone to handling damage and have a longer storage life, probably due to both mechanical and chemical protection (174). Goldenberry is mainly eaten fresh but it also processed to obtain jam, juice and dehydrated to get “raisins”. In restaurants, it is used in sauces for meat and seafood. It is also used in desserts and ice cream.

Many medicinal properties have been attributed to goldenberry, for example anti-asthmatic, diuretic and antiseptic properties (171). It has been used in folk medicine to treat cancer, leukemia, hepatitis, rheumatism and other diseases (175, 176).

Figure 6. Goldenberry

2.5.2 HIGHLAND PAPAYAS

Highland papayas are *Carica* species, like the common tropical papaya. They are smaller and less succulent than their well-known tropical cousin. These mountain papayas incorporate a wide range of flavors and qualities (1). They resemble the tropical papaya plant in appearance and in cultivation requirements. The fruits look like tropical papaya and they contain the enzyme papain. The most common and widespread species of highland papaya is *Carica pubescens*, which is cultivated from Panama to Chile and Argentina. In Peru it is called *papaya arequipeña*. *Papaya arequipeña* is grown in Peru in the highlands between 1500 and 3000 m.

The fruit of *papaya arequipeña* is five-sided, turning yellow or orange at maturity with firm flesh and with an aromatic and pleasant fragrance. They are 15-20 cm long and weigh about

130 g. The interior cavity contains many seeds (1). The flesh is yellow and tart, even when fully ripe. The fruit of this highland papaya is rich in vitamins, such as vitamin A (100 I.U./100 g) and vitamin C (70 mg/100 g) (177). The highland papaya is also a very important source of phosphorus and potassium and phenolic compounds (173).

Fruits can be eaten fresh, mainly as juice. It is used in jams and desserts. It has medicinal and ornamental uses as well. Its leaves are used to tenderize meat because of their papain content. In traditional medicine *papaya arequipeña* is used to treat tuberculosis and dysentery. It is also used as hypotensive and muscle relaxant (177).

2.5.3 TREE TOMATO

The tree tomato or tamarillo (*Cyphomandra betacea*) is native to the Andes. Today, it is grown in gardens from Chile to Venezuela and it is one of the most popular fruits in the Andean region (1). In Peru it is known as *sacha tomato*. It is cultivated between 1000 and 3000 m, and does not resist frost.

The plant of the tree tomato is a fast-growing herbaceous bush reaching a height of 1-7 m. It forms a single trunk with lateral branches. The leaves are large, fuzzy, veined and have a pungent smell. The fruits hang from the natural branches. Tree tomatoes are egg shaped, 5-10 cm long and have red or golden skin. Inside, they look like a tomato but the taste is quite different. The flesh is juicy and flavorful.

Tree tomato is a good source of provitamin A (carotene-150 I.U. per 100 g), vitamin B_6, vitamin C (25 mg per 100 g), vitamin E , iron, zinc and potassium (1, 173). It is a good source of phenolic compounds and has relatively high antioxidant activity (173).

Tree tomatoes can be eaten fresh. The skin is easily removed and the seeds are soft and edible. The fruits can be used in desserts, in fruit salads and in green salads. The whole fruit is used in drinks. Cooked tree tomatoes are used in stews, soups and sauces, especially hot sauces with chili pepper (*aji).* Tree tomatoes make good jellies, jams and chutneys because of their high pectin content. The fruit can be frozen and stored for a long period.

2.5.4. OTHER NATIVE ANDEAN FRUITS

Sauco (*Sambucus peruviana* H.B.K.), the parent of European Elderberry (*Sambucus nigra*), is a small tree growing 3-6 m tall. The fruit is black, 5-6 mm in diameter and grows in clusters. Sauco is originally from the Andes, and in Peru it grows in inter-Andean valleys. (1200-3500 m) (177). Sauco can be consumed crude, but most commonly it is prepared to make jam or fruit wine. Sauco has also several medicinal uses in Peru; the leaves are used to cure bronchitis, cough, fever and kidney inflammation (78).

Capuli is large-fruited subspecies of North American black cherry, designated as *Prunus serotina* subsp. *capuli* (1). It is a common tree in Andean villages from Venezuela to southern Peru. It is grown in cool upland areas between 2000 and 3000 m. At harvest time, capuli fruits can be found in Andean markets. The fruits of capuli are round, about 2.5 cm diameter and are black in color. The flesh is pale green and juicy. The skin is thin and firm and it has a trace of bitterness. However, in the best varieties it is so thin that it doesn't cause an unpleasant taste. Apart from bearing fruit, capuli tree is a fast-growing timber and reforestation species. It produces well in poor soils. It is suitable for agroforestry systems; its deep roots help prevent erosion. It can be interplanted with field crops such as alfalfa, corn and potatoes. It protects the plants form wind and acts like a biological barrier: the birds eat the fruits and leave nearby crops alone (1). Capuli is mostly eaten as fresh fruit but sometimes is made into jam or wine.

At least 40 *Passiflora* species produce fruits, of which 11 are cultivated on at least a small scale (1). Several of them occur in the Andes, while others are tropical species. All these plants are vines that produce round or ellipsoid fruits. When the Spanish came to South America, they discovered that passion fruit was used as a sedative by native people. When the Spanish brought the passion fruit to Europe, the leaves were used as a sleep-inducing medicine. The name "Passion" was given by Catholic missionaries in South America. The corona threads of the passion flower were seen as a symbol of the crown of thorns, the five stamens for wounds, the five petals and five sepals as the ten apostles (excluding Judas and Peter) and the three stigmas for the nails on the cross.

Passiflora mollissima is often called the banana passionfruit in English and is grown in Andean valleys from Venezuela to Bolivia and Peru. In Peru it is known as *tumbo.* It is cultivated in

home gardens and commercial orchards and can be purchased in local markets in Peru. *Tumbo* prospers at elevations up to 3400 m and tolerates low temperatures (-5°C) (1). The fruits are oblong, up to 15 cm long, and golden-yellow in color. *Tumbo* has very pleasing aroma and flavor and is preferably prepared as juice but can be eaten as fresh fruit as well. The fruits are used in jams, desserts and ice cream. Sweet granadilla (*Passiflora ligularis*) is commonly cultivated in Ecuador, Peru, Bolivia and Colombia between 800 and 3000 m. Its fruits are round with relatively hard rind and the pulp inside is translucent and almost liquid. They are eaten mainly as fresh fruit or prepared in juice.

2.6 PEPPERS

Peppers, genus *Capsicum*, are the most commonly used condiment in almost every country in the world; nowadays indeed they are popular in countries which have not traditionally used them. The number of species in this genus is approximately 27, consisting of 5 domesticated and 22 wild species. *Capsicum* species have also been divided into three complexes based on morphological characteristics, chromosome banding and hybridization studies. The *C. annuum* complex (CA) contains *C. annuum, C. chinense, C. frutescens*, and *C. galapagoense*. The *C. baccatum* complex (CB) contains *C. baccatum, C. praetermissum*, and *C. tovarii*. The *C. pubescens* complex (CP) consists of *C. pubescens, C. cardenasii*, and *C. eximium* (179).

Capsicum peppers can be hot or mild and their colors vary from red, yellow, green and brown. They are used in several kinds of dishes, for example in sauces, meat, chicken, bean, rice, and even in jams, jellies and in cocktails. In Figure 7, the biodiversity of Andean peppers can be observed.

The South American Indians were probably the first to use peppers, perhaps more than 7000 years ago (1). It is believed that the genus *Capsicum* came from the Andes and the Yungas of Bolivia. By the time Columbus arrived in the Americas, peppers were widely used by Incas and Aztecs. Columbus came to New World looking for the black pepper of Asia and found this much more tasty spice. From here came the name “pepper”. Pepper was very quickly spread around the world thereafter. The foods from India, China, Thailand, West Africa and Hungary are spiced with hot peppers or paprika. The species used in those countries is mainly *Capsicum*

annuum, but there are also two other species used; *C. frutescens* and *C. chinese*. In the Andes, there remain other promising species that are not very well known outside the region.

The pepper was called *uchu* by Incas, but this name was quickly replaced with aji, a word of Caribbean origin. However, *uchu* is still used by Andean and Amazonian people to name the different types of peppers and also in designation of dishes that are seasoned by aji peppers.

The most common cultivated pepper in Southern Andean area is the yellow *aji amarillo*, *Capsicum baccatum*. Andean aji is today cultivated in Peru, Bolivia, Ecuador, Argentina and Brazil. It is mainly cultivated in lowland areas but it is found up to around 1100 m in elevation (1). The fruit of Andean aji are most commonly shiny orange but there are also yellow and brown variations. They are very hot and used mainly in sauces. *Aji amarillo* has a distinctive, fruity flavor and is used fresh in ceviche (lime-marinated fish) in South America. They are also used in fresh salsas and the small yellow varieties are prized for their lemony aroma. The fruits of all ajís are also dried in the sun and then crushed into colorful powders.

The rocoto (*Capsicum pubescens*) is cultivated in the high Andes. It is also known as tree pepper since because of its compact erect growing habit, it can grow up to 1.5 m tall. It produces fruits which resemble the common pepper, paprika, but are normally smaller and very pungent. The plant is the most cold tolerant of the cultivated peppers, and grows at higher altitudes than other species, generally from 1500 to 2900 m (1). It is a perennial that grows for 10 years or more.

Rocoto is used in sauces and often eaten stuffed with meat. The fruit can be dried and ground into a powder for use as a pepperlike condiment. Rocoto is used in folk medicine to cure several ailments. The fruit has antihemorrhoidal, antirheumatic, antiseptic, diaphoretic, digestive, irritant, rubefacient and tonic effects when taken in small amounts. It is taken internally for fevers during a cold, debility in convalescence or old age, varicose veins, asthma and digestive problems. Externally, it is used in the treatment of sprains, unbroken chilblains, neuralgia, pleurisy, etc. (180).

Like all peppers, rocoto is rich in vitamin C (23.15 mg/100g). It is also a good source of phenolic compounds. This pepper is a potent inhibitor of lipid peroxidation; this is probably due

to its high phenol and vitamin C content and reducing power (181). In addition, the antioxidant activity of rocoto is probably linked to some non-phenolic substances, such as carotenoids (181).

The aji preferred for use in ceviche, a typical Peruvian seafood dish, is the aji limo (*Capsicum sinense/Capsicum annuum)*. It is a small pepper but it is very spicy and aromatic. It comes in different colors: red, yellow, green, orange, white and purple.
In the Amazonian area, there are several ajies which are very hot. The main Amazonian ajies are *Charapita, Ayuyo* and *Pucuna*.

The piquant, pungent taste of peppers is caused by capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), an odorless and colorless compound which causes a burning sensation when it comes in contact with mucous membranes. Biting into a pepper stimulates nerve receptors in the mouth and causes increased salivation and gastric flow. Capsaicin is concentrated in the inner part of pepper, in the placental tissue which holds the seeds. There is a rough correlation between the amount of capsaicin and the amount of carotenoid pigment. Thus, the stronger the flavor, the deeper the color of the fruits (1). Capsaicin demonstrates a high degree of biological activity affecting the nervous, cardiovascular and digestive systems. Furthermore, clinical trials have shown that capsaicin may have potential value in the management of painful conditions such as rheumatoid arthritis and cluster headaches (182). Based on a recent investigation by Galvez-Ranilla *et al.* (181), Peruvian peppers could have the potential to manage hyperglycemia-induced hypertension and oxidation-linked vascular complications. Further, peppers exhibit high free radical scavenging-linked antioxidant activities and a significant correlation between their total phenolic contents and ACE (hypertension relevant angiotensin I-converting enzyme) inhibitory activities were found in this study.

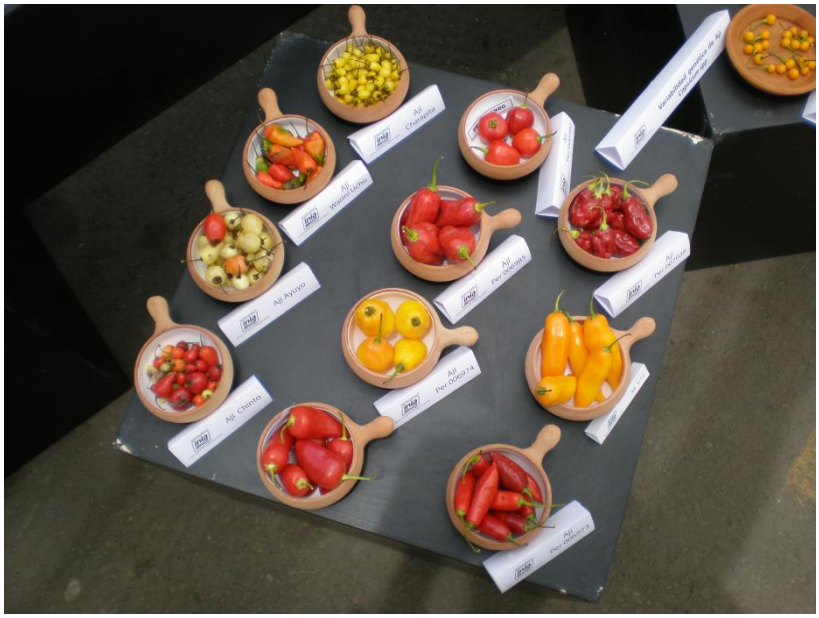


Figure 7. Andean peppers.

2.7. HEALTH-PROMOTING BIOACTIVE COMPOUNDS IN ANDEAN INDIGENOUS CROPS

2.7.1 DIETARY FIBER

Dietary fiber is the edible parts of plants or analogous carbohydrates that are resitant to digestion and absorption in the human smaal intestine and are completely or partially fermented in the large intestine (183). For whole grains, such components include cellulose, hemicellulose,lignin, inulin, resistant starch, and other constituents
distributed in the bran and starchy endosperm parts of the grain. It is generally accepted that the consumption of food naturally rich in dietary fiber is beneficial to the maintenance of health. Epidemiological studies have shown that whole grain foods have protective role against the risk of many chronic diseases, especially those related to metabolic syndrome, that is type 2 diabetes and cardiovascular diseases. Increasing the consumption by two servings per day can decrease the risk of type 2 diabetes and also the cardiovascular events (184, 185). Whole grain dietary fibre may have these effects through multiple physiological mechanisms that include binding

and eliminating cholesterol, binding bile acids, modulation of hormonal activity, stimulation of the immune system, facilitating toxicant transit through the digestive tract, production of short chain fatty acids in the colon, dilution of gut substances, lowering caloric content and glycemic index of foods, improving insulin response, providing bulk in foods, and scavenging free radicals (186).

However, the intake of fiber in many diets is inadequate and nutritionists recommend a higher intake of fiber-rich whole grain cereals as opposed to refined grains. Studies have shown that the Andean cereals amaranth, quinoa and kañiwa represent good sources of total dietary fiber (15, 22, 33). However, there exists no information about the components of dietary fiber in Andean crops. This kind of information would be very important to understand the potential health effects of Andean grains.

2.7.2 OIL

An important feature of the composition of the Andean cereals is their fat content. Lipid content in amaranth and quinoa is between two and three times higher than in buckwheat and common cereals such as wheat (15). Amaranth, quinoa and kañiwa lipids are characterized by a high degree of unsaturation, which is desirable from a nutritional point of view. Linoleic acid is the most abundant fatty acid (50% of the total fatty acids in quinoa, kañiwa and kiwicha) followed by oleic acid (25%) and palmitic acid.

In quinoa seeds, a high α-linolenic acid (C18:3 n-3) content is found, with values ranging from 3.8% (23) to 8.3% (15). A high content of α-linolenic acid is beneficial, as studies have shown that an increased intake of n-3 fatty acids reduces biological markers associated with many degenerative diseases such as cardiovascular disease, cancer, osteoporosis, as well as inflammatory and autoimmune diseases (183). Also, the current estimated n-6/n-3 ratio in Western countries at 14:1-20:1 is far from the recommended levels of 5:1-10:1 (188) which suggests the need for increased intake of foods high in n-3 fatty acids, such as α-linolenic acid (187).

Plant sterols (phytosterols) are another group of biologically active components present in pseudocereal lipids. Phytosterols, which cannot be absorbed in the human intestine,

have a very similar structure to cholesterol and inhibit intestinal cholesterol absorption, thereby lowering plasma total and low-density lipoprotein (LDL) cholesterol levels (187). Phytosterols have also shown antiviral and antitumor activity (189). Total sterols in amaranth lipids can represent approximately 20% of the unsaponifiable fraction with the predominant sterol present being chondrillasterol (98); quinoa oil has been reported to contain 1.5% sterols (25).

The three major phytosterols have been found in amaranth, β-sitosterol being the principal (Marcone *et al*. (190) . In one study, it was found that animal diets supplemented with amaranth oil lowered total serum cholesterol and LDL cholesterol levels while increasing HDL cholesterol (191).

Another interesting component of amaranth oil is squalene, a terpenoid compound ubiquitous in the unsaponifiable fraction of cereal grains. Amaranth seed oil contains
approximately 6% squalene, a considerably higher amount than usually found in oils from other cereal grains (187). Its importance as a food constituent resides in its ability to lower cholesterol levels by inhibiting its synthesis in the liver (191,192). In addition, it is hypothesized that the decreased risk for various cancers associated with high olive oil consumption could be due to the presences of squalene (193).

2.7.3 OTHER BIOACTIVE COMPOUNDS

Polyphenol compounds have been extensively researched in the last decade for health promoting properties such as their role in the prevention of degenerative diseases which include cancer and cardiovascular disease. The most important phenolic compounds in cereals are phenolic acids, alkylresorcinols and flavonoids. These phytochemicals in whole grains are complementary to those in fruits and vegetables when consumed together (194). Quinoa and kañiwa seeds are abundant sources of flavonoids, which consist mainly of glycosides of the flavonols kaempferol and quercetin (65, 195). Kañiwa is exceptionally rich in resorcinols, compounds not very common in plants (175). Of the major cereals, resorcinols have been reported to be present in high levels in wheat, rye and triticale and in low amounts in barley, millet and maize. Cereal alkylresorcinols (ARs) have been reported to have anticancer and antimicrobial effects, as well as an ability to inhibit some metabolic enzymes *in vitro*. ARs have also been reported to have antioxidant activity (196).

The antioxidant activity of amaranth betalains was determined by Cai *et al*. (197). They also investigated the relationship between the chemical structure and the activity of betalains. This study demonstrated that the amaranth betalains have very strong antioxidant activity, as compared to typical antioxidants (ascorbic acid, rutin and catechin), suggesting that these betalains may become a useful source of both natural antioxidants and natural colorants. This study also revealed that antioxidant activity of different betalains generally depended on their chemical structures. The free radical scavenging activity of the betalains increased with the number of hydroxyl groups and imino groups in the molecule. The C-5 position of the hydroxyl group on aglycones in the betalain molecules significantly improved activity, and more glycosylation of aglycones clearly reduced activity.

Antioxidant activity and total phenolic content of ethanolic extracts of *A. caudatus* and *A. paniculatus* seeds have been studied (198). The total phenolic content was 107 µg/g of seed for *A. caudatus* and 296 µg/g of seed for *A. paniculatus*. Both species showed appreciable antioxidant activity in the model system compared to β-carotene and linoleate. Gorinstein *et al*. (199) studied the effect of phenolic substances on the antioxidant potentials of some cereals and pseudocereals, such as buckwheat, amaranth and quinoa. They concluded that based on high contents of polyphenols, flavonoids and antioxidant activities, these pseudocereals can be a substitute for cereals for common and atherosclerotic diets and also in the allergic cases.

The health beneficial phytochemicals of whole grains are uniquely distributed as free, soluble-conjugated, and bound forms. Most of these phytochemicals are in the insoluble form, bound to cell wall materials. Cell wall materials are difficult to digest, may survive upper gastrointestinal digestion, and finally reach the colon. Colonic digestion of such materials by microflora may release the bulk of the bound phytochemicals to exert their health benefits locally and beyond absorption. (186).

According to Chirinos *et al*. (200), mashua extract can inhibit LDL oxidation in both radical-induced (AAPH) and metal-induced (Cu2+) systems. Inhibition of AAPH-induced oxidative hemolysis of erythrocytes has also been found. These results indicate that mashua phenolics are capable of scavenging peroxyl radicals, as well as chelating redox metal ions *in vitro*. The authors presume that these protective effects of mashua extracts are dependent on their phenolic composition in terms of their lipophilicity/hydrophilicity partition and the molecular structures

that define their reactivity against free radicals, as well as on phenolic concentrations. This study suggests that phenolic compounds of mashua provide a good source of dietary antioxidants that could offer potential protective effects against lipid oxidation and which could be exploited by the food or cosmetics industry.

Amaranth has several minor constituents that may possess positive or negative effects. The phytic acid content of amaranth (0.34-0.61%) is higher than that found in rice, but lower than those reported in maize and wheat. The content of polyphenols in amaranth is between 2 and 4 mg/g (22). These compounds have been reported to exhibit several biological effects, for example anti-inflammatory and vasodilatory activity. They can also inhibit lipid peroxidation.

Trypsin and chymotrypsin inhibitors have been found in amaranth seeds. Tamir *et al.* (201) isolated and characterized the thermostable protease inhibitor from amaranth seeds and studied its effect on trypsin- and chymotrypsin-like proteases and its possible role in modulating tumorigenic behavior in human breast cancer cells *in vitro*. They found that this inhibitor, AmI, inhibits trypsin and chymotrypsin from the digestive system of insects such as *Tribolium castaneum* and *Locusta migratoria*, supporting the hypothesis that inhibitors may have evolved as defense mechanisms of seeds against insects. AmI was found to inhibit the anchorage-independent growth of MCF-7 breast cancer cells, suggesting that AmI may have anticarcinogenic activity.

Other minor components found in amaranth are the lectins. One lectin, called amaranthin, has been isolated from *A. cruentus*. According to Guzman-Maldonado and Paredes-Lopez (22) its concentration ranges from 1.6 to 1.7 mg/g. Amaranthin has been used as a histochemical probe for proliferating cells in sections of human colonic tissues. Experimental data suggest that amaranthin may be useful for identifying abnormal proliferation in colorectal cancer syndromes (202).

Quinoa saponins have some health-promoting properties. For instance, saponins can reduce cholesterol levels and they exhibit insecticidal, antibiotic and fungicidal properties (47). There is also some evidence that quinoa saponins possess anti-inflammatory activity (203). The research of Guclu-Ustundag and Mazza
(204) has shown that saponins may have anticarcinogenic and cholesterol lowering properties.

Chilean researchers recently detected isoflavones, particularly daidzein and genistein, in quinoa seeds (205). These hormones are implicated in plant physiology (protection from pathogens, from UV light and nitrogen-limited soils) and can be recognized by α and β estrogen receptors in humans. These endoplasmic reticulum-linked receptors are implicated as inhibitors of tyrosine kinase enzymes, and as antagonists of vessel contraction. They also reduce arterial resistance, improve bone density and stimulate osteoprogerin secretion by osteoblasts, in addition to its antioxidant properties (205).

2.7.4 HYPOGLYCEMIC AND -CHOLESTROLEMIC EFFECTS

Quinoa has shown to be effective in plasma cholesterol reduction. In a study by Takao *et al.* (206), quinoa protein isolate (QP) significantly reduced plasma cholesterol concentration even though the mice were fed a cholesterol-supplemented diet. According to these authors, QP was thought to inhibit both bile acid re-absorption in the intestine and cholesterol synthesis and to promote cholesterol catabolism.

Konishi *et al.* (207) reported that a 3% quinoa pericarp supplemented diet significantly decreased serum and liver cholesterol levels in mice. It has been suggested that the hypocholesterolemic effect of the quinoa pericarp can be attributed to the water-soluble dietary fiber content, as in oat, rice bran or other fibers. Several studies to date have shown that amaranth grain, extruded amaranth, amaranth protein concentrate or amaranth oil can lower serum and hepatic cholesterol as well as triglycerides (191, 192,208, 209).

Berti *et al*. (210) demonstrated that quinoa has hypoglycemic effects *in vivo* and can be recommended as an alternative to traditional ingredients in the production of cereal-based gluten-free products with a low glycemic index. In addition, amaranth grain has been shown to effectively reduce serum glucose levels and increase serum insulin levels in diabetic rats, which suggests that amaranth grain could be beneficial in the correction of hyperglycemia and preventing diabetic complications (211).

2.8 NOVEL VS. TRADITIONAL FOODS; CHANGING THE RULES?

According to current EU regulations, novel foods are foods that are new in relation to existing “normal” foods. Two basic groups of novel foods are foods containing new synthetic

ingredients and foods containing new biological ingredients (212). The current definition of a novel food is: "Food that has not been used for human consumption to a significant degree within the Community before 15 May 1997." When a food is considered as a novel food, the current legislation has established that it is subject to strict safety assessments before reaching the market. This is a problem for developing countries because a crop may have been used safely for a long time in the countries of origin, but may still be considered "novel" in Europe, thus requiring controls. According to the rules, a food is novel in the EU regardless of its use in third world countries. These limits established by the of European novel food regulations have hindered food innovation based on tropical biodiversity and curtailed the income opportunities for poor producing countries (4). This is a problem particularly for Andean native crops which have been used for centuries and are considered safe in their countries of origin.

The Novel Food Regulation (NFR) is a regulatory framework for foods derived from novel processes and molecules. As such, GMO foods were originally included in the NFR. On the other side, exotic traditional foods, such as Andean indigenous crops, including a vast variety of food items of growing importance to the diet diversification desired by EU consumers, are also considered within the same category. As can be seen, novel foods category includes a very diverse group of products. Taking this fact into account, it is unreasonable to subject them all to a single safety assessment. A separate novel food category for exotic traditional foods is needed as opposed to innovative products with no history of long-term consumption outside the EU (4).

However, in recent times, the novel food regulations are changing. In the new proposal, tropical traditional plants not used in Europe are still considered novel but a simplified procedure is created for them. According to the proposed Novel Food Regulation, "traditional food from a third country" means "novel food with history of food use in a third country, meaning that the food in question has been and continues to be part of the normal diet for at least one generation in a large part of the population of the country". Some of the Andean crops reviewed in this thesis are listed in the EU commissions Novel Food Catalogue as species not restricted by Novel Food Regulation. Others would fulfill the new proposed definition. In what follows, the status of the reviewed Andean crops as novel food is discussed.

Quinoa, kiwicha, maca and oca are not considered as novel foods because they have been on the market as a food or food ingredient and consumed to a significant degree before 15 May 1997.

However, some cases are confusing. In January 2003, maca was not considered a novel food because the Belgian authority issued a statement that it has been on the market in Belgium before 1997. However, in May 2003, maca appeared as a "non-authorized novel food". Finally, in December 2008, maca was listed in the Novel Food Catalogue (4).

In the case of yacon, there was a request as to whether this product requires authorization under the Novel Food Regulation, or not. According to the information available to the competent authorities of Member States, this product was not used as a food or food ingredient before 15 May 1997. Therefore, before it may be placed on the market in the EU as a food or food ingredient, a safety assessment under the Novel Food Regulation is required. However, yacon has a long history of safe use and it fulfils the requirements of the recently proposed regulation as having a history of consumption in the normal diet, as a primary product and in products derived from its simple processing. Yacon and yacon products appear to be ready for evaluation as a novel food with a long history of safe use (213). Kañiwa, tarwi, olluco, mashua, arracacha, mauka and ahipa are not mentioned in the Novel Food Catalogue of the European Commission. They have a long history of safe use in Peru and other Andean countries and could fulfill the requirements of the recently proposed regulation.

3. AIMS OF THE STUDIES

The main objective of the research was to characterize and assess the nutritional value of Andean native grains with a special emphasis on bioactive components and the impact of processing. For this purpose, a series of studies was planned to achieve the following targets:

1. To characterize differences in the chemical composition, dietary fiber and content of some bioactive compounds such as phenolic acids, flavonoids and betalains in different varieties of the Andean native grains quinoa, kañiwa and kiwicha.
2. To assess the nutritional value of Andean native grains with specific reference to iron, calcium and zinc content and their bioavailability in raw and processed grains.
3. To show the effect of extrusion processing on properties of Andean grains.

4. MATERIALS AND METHODS

4.1 MATERIALS

Grain species and varieties used in the present studies were collected in Peru in (Table 21). The samples of studies I-III were from growing season 2004-2005 and of the studies IV-V from growing season 2007-2008. For the studies I, II, III and V 5 kg of each variety was obtained. These samples were collected in the experimental field of La Molina University or of the INIA. For the study IV, 250 g of each variety were obtained of INIA or private farmers. The commercial samples were purchased in local market in Cusco. These samples were milled and subsamples were taken for chemical analysis. The composition of components in crops can vary considerably between regions. Such differences can be caused by variation in temperature, rainfall and access to water, use of fertilizer, nutrient content of the soil. Thus, the samples in this study only represent the crops cultivated in specific location and growing season.

Table 21. Grains used in the present studies

Grain	Scientific name	Variety/ecotype	Origin/	**Study/**
Kiwicha	*Amaranthus caudatus*	Centenario	National Agrarian University La Molina/	**I, V**
		Oscar Blanco	National Agrarian University La Molina, Lima	**I**
		Black ecotype	Mollepata, Cusco	**IV**
		Pink ecotype	Mollepata, Cusco	**IV**
		Cream ecotype	Mollepata, Cusco	**IV**
		Black ecotype	San Salvador, Cusco	**IV**
Kañiwa	*Chenopodium pallidicaule*	Cupi	Agrarian Experimental Station Illpa, department of Puno	**II, V**
		Ramis	Agrarian Experimental Station Illpa, department of Puno	**II**
		Kello	Agrarian Experimental Station Salcedo, department of Puno	**IV**
		Wila	Agrarian Experimental Station Salcedo, department of Puno	**IV**

		Guinda	Agrarian Experimental Station Salcedo, department of Puno	**IV**
		Ayara	Agrarian Experimental Station Salcedo, department of Puno	**IV**
		Commercial sample	Cusco	**IV**
Quinoa	*Chenopodium quinoa*	La Molina 89	National Agrarian University La Molina	**III**
		Kancolla	Agrarian Experimental Station Salcedo, department of Puno	**III**
		Blanca de Juli	Agrarian Experimental Station Salcedo, department of Puno	**III**
		Sajama	Agrarian Experimental Station Salcedo, department of Puno	**III**
		Ccoito	Agrarian Experimental Station Salcedo, department of Puno	**IV**
		INIA-415 Pasankalla	Agrarian Experimental Station Salcedo, department of Puno	**IV, V**
		Roja de Coporaque	Agrarian Experimental Station Salcedo, department of Puno	**IV**
		Witulla	Agrarian Experimental Station Salcedo, department of Puno	**IV**
		03-21-0093	Agrarian Experimental Station Salcedo, department of Puno	**IV**
		Salcedo INIA	Agrarian Experimental Station Salcedo, department of Puno	**IV**
		Commercial 1.	Cusco	**IV**
		Commercial 2.	Cusco	**IV**
		Huaripongo	Agrarian Experimental Station Salcedo, department of Puno	**IV**
		03-21-1181	Agrarian Experimental Station Salcedo, department of Puno	**IV**

4.2 METHODS OF ANALYSIS

Chemical analyses are subjected to different type of errors. U The method used should have accuracy and precision. Another feature of an analytical method is specifity. That means that the method detects only the component of interest. Some methdos used in these thesis, are not specific, for ex. the method for total phenolic compounds. This method not only measures total phenols, it measures any reducing substance present in sample as well.

4.2.1 EXTRACTION OF POLYPHENOLS

All the samples were ground through a Foss cyclotec mill before extraction.
Five grams of milled raw or extruded quinoa were mixed with 25 mL methanol and homogenized using an Ultra-Turrax homogenizer. The homogenates were allowed to stand for 12-24 hours under refrigeration (4°C) and then centrifuged for 15 minutes. The supernatants were recovered and stored until analysis (I, II, III).

4.2.2 CHEMICAL COMPOSITION

Water content, proteins (N x 6.25), fat, crude fiber and ashes were determined according to AOAC Methods: 923.02, 920.39, 930.15, 960.52 (214). Carbohydrates were calculated by the difference using the formula:

CHO = 100 - (fat+protein+crude fiber+ash)
(I, II, III, IV, V)

4.2.3 DIETARY FIBER

The total, soluble and insoluble dietary fiber were analyzed by an enzymatic-gravimetric method according to the Approved Method 32-21 (215) using the TDF-100 kit from Sigma Chemical Company (St. Louis, MO) (I, II, III, V).

4.2.4 LIGNIN

Lignin was determined according to the Approved Method 32-25 (216). The method includes selective, enzymatic removal of starch, using a thermostable α-amylase and an amyloglucosidase; precipitation of soluble polysaccharides with 80% ethanol; hydrolysis of amylase-resistant polysaccharides (precipitated and insoluble) with sulfuric acid. Klason lignin (sulfuric acid lignin) was calculated gravimetrically as the acid-insoluble residue after correction for ash (I, II).

4.2.5 BETA-GLUCANS

The content of β-glucans was determined according to the AOAC (214), method 995.16. Extracts were hydrolyzed with lichenase and betaglucosidase. Reducing sugars in the aliquots were determined using 3,5-dinitrosalicylic acid reagent. Anhydrous glucose was used as the reference compound (I, II).

4.2.6 RESISTANT STARCH

Resistant starch (RS) content was analyzed using methodology according to the Approved Method 32-40 (216) (I, II).

4.2.7 RADICAL SCAVENGING ACTIVITY

Radical scavenging activity was determined according to the method of Brand-Williams *et al*. (217) based on the decrease of absorbance at 515 nm produced by reduction of DPPH (2, 2-Diphenyl-1-picrylhydrazyl) by an antioxidant. Trolox was used as the reference compound. Radical scavenging activity was also determined according to Re *et al*. (218) based on the decrease of absorbance at 734 nm produced by reduction of ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) by an antioxidant. Trolox was used as the reference compound. The percentage inhibition of absorbance at 734 nm was calculated using the formula according to Katalinic *et al*. (219):

% inhibition = $[(A_{C(0)} - A_{A(t)})/ A_{C(0)}] \times 100$

where $A_{C(0)}$ is the absorbance of the control at t = 0 min and $A_{A(t)}$ is the absorbance of the antioxidant at t = 16 min.
(I, II, III)

4.2.8 PHENOLIC COMPOUNDS

The content of total phenolics was analyzed according to the method of Swain and Hillis (220). The phenolic compounds were extracted with methanol and the extract was allowed to react with the Folin-Ciocalteau phenol reagent. Absorbance was measured at 725 nm. Gallic acid equivalents were determined from a standard concentration curve (I, II, III, V).

4.2.9 PHYTATE

Phytic acid was determined according to Schmidt-Hebbel (221). This method is based on indirect iron (III) complexometry (I, II).

4.2.10 PROTEIN *IN VITRO* DIGESTIBILITY

The digestibility of proteins was determined using an *in vitro* method according to Hsu *et al*. (222). The multi-enzymatic method is based on the decrease in pH over 10 minutes. The percentage of digestibility was calculated using a formula: Y = 210.464 – 18.103 X

Where:
X = pH of the protein suspension after 10 minutes of digestion
Y = percentage of protein hydrolysis
(I, III)

4.2.11 STARCH *IN VITRO* DIGESTIBILITY

The digestibility of starch was determined by an *in vitro* method according to Holm *et al.* (223). Five hundred milligrams of starch were mixed with phosphate buffer (pH 6.9) and incubated with α-amylase at 37ºC for 1 hour. The sugars released were determined by spectrophotometry (I, III).

4.2.12 PHYSICOCHEMICAL PROPERTIES

The following indices and properties were determined for extruded kañiwa (II):

$$\text{Water absorption index (WAI)} = \frac{\text{g water absorbed}}{\text{g dry sample (1-soluble fraction)}}$$

$$\text{Water solubility index (WSI)} = \frac{\text{g water soluble matter}}{\text{g dry sample}}$$

(58)

The sectional expansion index (SEI) was measured as the ratio of the diameter of the extrudate to that of the die (58). The degree of gelatinization (DG) was determined according to Birch and Priesty (224) and density according to Muller (225). Water-holding capacity was measured according to Robertson and Eastwood (226).Swelling and oil absorption capacity were measured using the methods proposed by Tamayo and Bermudez (227). Cationic exchange capacity (CEC) was tested by the methodology of McConnel *et al*. (228).

4.2.13 FLAVONOIDS

Flavonoids were analyzed as aglycones according to the method explained by Mattila et al. (229) (IV). Briefly, a sample (0.3–1 g) was weighed into a 100 mL Erlenmeyer flask and dispersed in 40 mL of 62.5% aqueous methanol containing 2 g/L of 2,(3)-tert-butyl-4-hydroxyanisole (BHA). To this extract, 10 mL of 6 M HCl was added. Hydrolysis was carried out in a shaking water bath at 90ºC for 2 h. After hydrolysis, the sample was allowed to cool. Then, it was filtered and made up to 100 mL with methanol. Before quantification by HPLC, the sample was filtered through a 0.45 µm membrane filter.

The analytical HPLC system consisted of an Agilent 1100 Series high-performance liquid chromatograph equipped with a diode array detector. The HPLC pumps, autosampler, column oven, and diode array system were monitored and controlled using the HP Chem Station compute program. The wavelengths used for identification and quantification of flavonoids with the diode array detector were 280 nm for eriodictyol, naringenin, and hesperetin, 329 nm for luteolin and apigenin and 370 nm for myricetin, kaempferol, quercetin and isorhamnetin. Flavonoid separation was performed on an Inertsil (GL Sciences, Inc, Japan) ODS-3 (4.0 x 150 mm, 3 µm) column with a C-18 guard column. The temperature of the column oven was set at 35ºC. Gradient elution was employed for flavonoids with a mobile phase consisting of 50 mM H_3PO_4, pH 2.5 (solution A) and acetonitrile (solution B) as follows: isocratic elution 95% A, 0–5 min; linear gradient from 95% A to 50% A, 5–55 min; isocratic elution 50% A, 55–65 min; linear gradient from 50% A to 95% A, 65–67 min; post-time 6 min before next injection. The flow rate of the mobile phase was 0.7 mL/min, and the injection volumes were 10 µL of the standards and sample extracts. All flavonoids were quantified using the external standard method. All samples were analyzed in triplicate.

4.2.14 PHENOLIC ACIDS

Phenolic acids were analyzed according to the method of Mattila and Hellström (230) (IV). Briefly, a 0.5 g sample was homogenized in 7 mL of a mixture of methanol, containing 2 g/L of butylated hydroxyanisole (BHA) and 10% acetic acid (85:15) using a Heidolph Diax 900 homogenizer. The homogenized extract was ultrasonicated for 30 min and made up to a volume of 10 mL with distilled water. After mixing, 1 mL was filtered for HPLC analysis of soluble phenolic acids. Next, 12 mL of distilled water containing 1% ascorbic acid and 0.415% EDTA and 5 mL of 10 M NaOH were added into the test tube, sealed, and stirred overnight (about 16 h) at 20°C using a magnetic stirrer. The solution was then adjusted to pH 2 with concentrated HCl, and the liberated phenolic acids were extracted with 15 mL of a mixture of cold diethyl ether and ethyl acetate (1:1), centrifuged at 620 g (Rotofix 32, Hettich Zentrifugen, Germany); the organic layer was then recovered. The extraction was repeated twice and the organic layers were combined. After alkaline hydrolysis, an acid hydrolysis was performed by adding 2.5 mL of concentrated HCl into the test tube and incubating in a water bath at 85ºC for 30 min. The sample was then cooled, and further sample handling was performed in the same manner following alkaline hydrolysis. The organic layers from alkaline and acid hydrolysis were combined, evaporated to dryness, dissolved into 2 mL of methanol, filtered and analyzed for total phenolic acids by HPLC.

The analytical HPLC system was the same for phenolic acids as for flavonoids except for a modification in gradient elution: isocratic elution 95% A, 0–5 min; linear gradient from 95% A to 85% A, 5–17 min; linear gradient from 85% A to 80% A, 17–40 min; linear gradient from 80% A to 50% A, 40–60 min; isocratic elution 50% A, 60–65 min; linear gradient from 50% A to 95% A, 65–67 min; post-time 6 min before the next injection. The wavelengths used for the quantification of phenolic acids with the diode array detector were: 254 nm for protocatechuic acid, p-hydroxybenzoic acid and vanillic acid; 280 nm for syringic acid, p-coumaric acid, m-coumaric acid, o-coumaric acid and *E*-cinnamic acid; and 329 nm for caffeic acid, ferulic acid, sinapic acid and chlorogenic
acid. All samples were analyzed in triplicate. Both total and soluble forms were quantified as aglycones. Phenolic acids obtained after hydrolysis were identified according to their retention times and UV spectra (190–600 nm) consistent with commercial reference compounds while soluble forms were identified solely by their UV spectra.

4.2.15 BETALAINS IN KIWICHA SPECIES

Finely ground material (0.5 g) was weighed in a test tube and made up to 5 mL with acidified water (pH 3–4). The test tube was carefully flushed with argon, sealed and extracted overnight in a magnetic stirrer. After extraction, 5 mL of methanol was added and the sample was centrifuged. The supernatant was transferred to another test tube. To the solid residue, 2 mL of acetone was added and after vortexing the sample was centrifuged again. Acetone supernatants were combined and the sample was evaporated to near dryness with a stream of nitrogen. After evaporation, the volume was adjusted to 1 ml with methanol, filtered through a 0.45 µm membrane filter and analyzed by HPLC-DAD (Hewlett-Packard 1100 series). Nova Pak C18 (3.9 x 150 mm, 4 µm, Waters, Massachusetts, USA) was used as an analytical column protected with the same manufacturer's precolumn. The mobile phase consisted of 0.05 M phosphate buffer (A) pH 2.4 and methanol (B). The gradient elution used was: 5–60% B in 50 min followed by 60–90% B in 6 min, hold at 90% B for 12 min, and finally to 100% B within 32 min. The HPLC method was basically the same as described by Mattila *et al.* (231) for avenanthramides except that the quantification of betalains was done at 535 nm. For identification purposes UV spectra were recorded at 190–600 nm. Three compounds were detected in one kiwicha sample at 535 nm and tentatively identified as betacyanin amaranthine, iso-amaranthine and betanin according to the elution order and UV spectra presented in the literature (232). Amaranthine, previously isolated at MTT, was used as a reference compound for quantification (IV).

4.2.16 IRON, ZINC, AND CALCIUM BIOAVAILABILITY

A modification of the *in vitro* method (233) introduced by Wolfgor *et al*. (234) was used (V). Aliquots of homogenized samples (50 g) were incubated with 5 mL of a 3% α-amylase solution for 30 minutes at 37°C in a shaking water bath, then adjusted to pH 2.0 with 6 *N* HCl and, after addition of 1.6 mL pepsin digestion mixture (16% pepsin solution in 0.1 *N* 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) containing 18.75 mL 0.15 *M* 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 the digest-dialysate (235).

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 *N* $NaHCO_3$) 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). An assessment of minerals in the pepsin digests was made by AAS after wet washing with HNO_3-$HClO_4$ (50:50). Lanthanum was added to all samples and standards were analyzed for Ca to reach a 0.5% final concentration to prevent possible phosphate interference. Mineral bioavailability (FeD%, ZnD%, CaD%) was calculated from the amount of each dialyzed mineral and 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 bioavailability (236).

PCFe = ([Fe] x FeD%)/100; PCCa = ([Ca] x CaD%)/100; PCZn = ([Zn] x ZnD%)/100

4.3 STATISTICAL ANALYSIS

Each analysis was done in duplicate or in triplicate and expressed as means and standard deviation (SD).The data was analyzed by analysis of variance and Tukey's test (significance of differences $p<0.05$) was used to find significant differences between the samples and treatments (I, II, III, IV). In the mineral bioavailability study, 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 and p values <0.05 were considered significant.

4.4 PROCESSING METHODS

4.4.1 EXTRUSION (I, II, III)

The extrusion process of quinoa, kañiwa and kiwicha was carried out using a low-cost extruder cooker (LEC) simulating the local processing conditions. The equipment was manufactured by Jarcon del Peru, Huancayo, Peru. This equipment is a single screw extruder having the following parameters: 254.5 rpm, resident time 10-13 sec, work temperature 180°C and two orifices on a die. No external heat was transferred to the barrel or the screw during extrusion. The aim was to produce results applicable to local conditions where this technology is used widely.

4.4.2 PREPARATION OF KAÑIWA BRAN (II)

The grains of kañiwa were cleaned using a sifting machine with sieves sized 1.40 mm and 0.85 mm. After cleaning, the grains were milled in a laboratory mill, Cyclotec 1093 (FOSS Inc. Denmark), using 1.00 mm mesh. The meal was then sieved with sieves sized 0.425 mm and 0.212 mm with the purpose of obtaining bran of two different particle sizes (II).

4.4.3 ELIMINATING OF SAPONINS OF QUINOA (III)

Quinoa was washed for 20 min running with tap water (23°C) with the aim of eliminating bitter tasting and toxic saponins. Washed grains were dried at 45ºC for 12 h. Dried seeds were packed in polyethylene bags and stored at 4ºC until they were used in analysis and processing (III).

4.4.4 PREPARATION OF SAMPLES FOR BIOAVAILABILITY STUDY (V)

Roasting: 500 g of the grains of quinoa, kañiwa and kiwicha were roasted by a traditional method using a hot plate at a temperature about 190°C for three minutes.

Cooking: grains of quinoa, kañiwa and kiwicha were cooked with tap water for 20 min (°C) in a proportion of 250 g grains/1000 mL water.

5. RESULTS AND DISCUSSION

5.1 CHEMICAL COMPOSITION AND BIOACTIVE COMPOUNDS

5.1.1 CHEMICAL COMPOSITION

All Andean grains and their different varieties had relatively high protein and fat content. In reference to common cereals, the values were higher (237). The two kiwicha varieties were especially rich in fat, about 10 % and could be potential sources of oil. Kañiwa varieties had the highest fiber content of all samples. A detailed proximate composition of the grains and their varieties can be observed in Table 22 (I, II, III).

Table 22. The chemical composition of Andean grains and their varieties. All the contents g/100 g dry weight except moisture g/100 g fresh weight. (I, II, III)

Crop/variety	Moisture	Protein	Ash	Fat	Crude fiber	Carbohydrates
Kiwicha/ Centenario	9.80±0.10	14.55±0.17	2.39±0.05	10.08±0.16	7.43±0.10	65.55
Kiwicha/ Oscar Blanco	9.44±-0.09	14.70±0.12	2.61±0.01	10.15±0.03	7.27±0.11	65.27
Kañiwa/Cupi	10.37±0.19	14.41 ± 0.26	5.03 ± 0.21	5.68 ± 0.02	11.24 ± 1.15	63.64
Kañiwa/Ramis	11.79±0.10	14.88 ± 0.46	4.33 ± 0.26	6.96 ± 0.24	8.18 ± 0.02	65.65
Quinoa/Blanca de Juli	11.39	13.96	3.38	5.51	2.00	75.15
Quinoa/ Kcancolla	10.78	15.17	3.52	5.77	3.07	72.47
Quinoa/La Molina 89	12.03	15.47	5.46	6.85	3.38	68.84
Quinoa/Sajama	12.62	14.53	3.04	4.69	1.92	75.82

All data are the mean +/- SD of three replicates except for quinoa which are the mean of two replicates

5.1.2 DIETARY FIBER AND RELATED COMPOUNDS

Many definitions of dietary fiber exist worldwide, some based on analytical methods and others physiologically based. In 2001, the Food and Nutrition Board (238), Institute of Medicine of the National Academy of Sciences released proposed definitions for dietary fiber developed by a panel of experts. Based on the Panel's deliberations, the following definitions were proposed: Dietary Fiber consists of nondigestible carbohydrates and lignin that are intrinsic and intact in plants. Functional Fiber consists of isolated, nondigestible carbohydrates that have beneficial physiological effects in humans (239). Total Fiber is the sum of Dietary Fiber and Functional Fiber. The generally used clasfication for dietary fiber is: total, soluble and insoluble fiber. There has been a trend to assign specific physiological effects either to soluble or insoluble fibers. This approach makes it difficult to evaluate the effects of fiber provided by mixed diets. Dietary fiber provided by mixed diets is two-thirds to three-quarters insoluble, although the exact distribution between soluble and insoluble is very dependent on the method of analysis. Further, some fibers are placed in one category or another, when in fact, they may have major benefits attributable to both soluble and insoluble fibers; psyllium seed husk, oats and oat bran are examples (Slavin 2003). The recommendation is to discontinue use of the terms soluble and insoluble fiber and this will make current fiber values in nutrient databases obsolete. Since these changes are still in the proposed stage, the existing dietary fiber data, including total dietary fiber, soluble fiber, and insoluble fiber is still consistent (239).

Total, insoluble and soluble dietary fiber content of Andean grains and their varieties are presented in Table 23. The content of insoluble and total dietary fiber in both kañiwa varieties was very high (II). In the case of kiwicha, the Centenario variety was higher than in the Oscar Blanco variety in both types of fiber (I). The quinoa varieties contained between 14 and16% of total dietary fiber (III). Nyman *et al.* (240) reported a total dietary fiber content of 12.1%, 16.1% and 18.8% for wheat, rye and barley, respectively.The concentration of dietary fiber in most Andean grain varieties is higher than the values for wheat, oats, triticale, corn and sorghum (241). This result is important, especially when considering the application of the studied cereals to improve the diets of persons with problems of high cholesterol level.

Table 23. Dietary fiber constituents of Andean grains and their varieties expressed as % of dry basis (I, II, III)

Crop/variety	IDF	SDF	TDF
Kiwicha/Centenario	13.92±0.14	2.45±0.24	16.37
Kiwicha/Oscar Blanco	12.15±0.72	1.65±0.52	13.80
Kañiwa/Cupi	22.27±2.30	2.98±0.42	25.24
Kañiwa/Ramis	23.16±0.89	2.79±0.57	25.95
Quinoa/ Blanca de Juli	12.18±1.65	1.54±0.01	13.72
Quinoa/Kcancolla	12.70±1.15	1.41±0.13	14.11
Quinoa/ La Molina 89	14.39±0.81	1.60±0.18	15.99
Quinoa/Sajama	11.99±0.28	1.58±0.05	13.56

IDF = insoluble dietary fiber SDF = soluble dietary fiber TDF = total dietary fiber

All data are the mean +/- SD of three replicates, except for quinoa

The content of resistant starch, lignin and betaglucans is presented in Table 24 (I, II). According to this study, kañiwa cannot be considered a good source of betaglucans, as the content of this compound was very low (0.04-0.07%). Oat has about 3-7% betaglucans (242). The lignin content was 6.88% for Cupi and 7.98% for Ramis which is considerably higher as compared to other cereals; 2.0%, 2.1%, 3.5%, 2.5%, 3.9% and 1.4 % for wheat, rye, barley, sorghum, rice and corn, respectively (240). This probably due to the fact that these kañiwa varieties were used with their pericarp which is rich in lignin.

Lignin content in the two kiwicha varieties was 3.95 and 3.97% (Centenario and Oscar Blanco, respectively), see Table 24. It was higher than the lignin content in rye found by Glitsot and Bach Knudsen (221), and 1.5% and this could be of nutritional importance. Betaglucan content in the Centenario variety was 0.97% and in the Oscar Blanco variety it was 0.63%. These values are low compared with rye at 1.5% (243), oat at 3.9-6.8 (244) and barley at 3.9-6.5 % (245), but are very similar to that found in some wheat varieties, up to 1% .

Resistant starch content in the Centenario variety was 0.12% and in the Oscar Blanco variety it was 0.10%. These values are lower than the values found for rice and corn, 2.63% and 2.85%, respectively (246). Vasanthan *et al.* (245) found a resistant starch content of 0-0.83% for barley.

Table 24. Resistant starch, lignin, betaglucans and phytic acid in two kañiwa and kiwicha varieties. All the contents g/100 g dry weight (I, II)

Component/ Crop variety	Kañiwa/Cupi	Kañiwa/Ramis	Kiwicha/Centenario	Kiwicha/ Oscar Blanco
Resistant starch	0.24 ± 0.03	0.43 ± 0.01	0.12 ± 0.01	0.10 ± 0.01
Lignin	6.88±0.34	6.30±0.27	3.95 ± 0.45	3.97 ± 0.42
Betaglucans	0.07 ± 0.02	0.07 ± 0.01	0.97 ± 0.07	0.63 ± 0.19
Phytic acid	0.83 ± 0.03	0.84 ± 0.04	0.31 ± 0.02	0.35 ± 0.01

All data are the mean +/-SD of three replicates

Table 24 shows the phytic acid content of kiwicha (I). Raw kiwicha contained 0.28-0.31% phytic acid. This value coincides with the values reported by Guzman-Maldonado and Paredes-Lopez (22). They reported a content of phytic acid for amaranth between 0.34 and 0.61. Kiwicha contains less phytates than some common cereals, such as corn and wheat, (22). Gualberto *et al*. (247) found 1.42, 4.32 and 5.27% of phytate in oat, rice and wheat bran, respectively. The phytate content for the two kañiwa varieties was very similar, about 8.0 mg/g, which is higher than in kiwicha but lower than in common cereals. Gualberto *et al*. (247) found 14.2 mg/g, 43.2 mg/g and 52.7 mg/g of phytate in oat, rice and wheat bran, respectively. Phytic acid has long been considered an antinutrient because it chelates minerals and trace elements. However, its antioxidant potential is now recognized (248).

5.1.3 TOTAL PHENOLIC COMPOUNDS

Table 25 presents the content of total phenolic compounds, as gallic acid equivalents (GAE) in Andean grains and their varieties (I, II, III). The phenolic compounds were measured by the Folin-Ciocalteu reagent (FCR) or Folin's phenol reagent. It works by measuring the amount of the substance being tested needed to inhibit the oxidation of the reagent. However, this reagent does not only measure total phenols and will react with any reducing substance. It will also react with some nitrogen-containing compounds such as amino acids The reagent has also been shown to be reactive towards thiols and many vitamins (249)

Raw kiwicha contained 0.99 and 1.13 mg gallic acid/g of sample, dry basis. Guzman-Maldonado and Paredes-Lopez (22) reported levels of 2-4 mg/g of total phenolic compounds in

amaranth which is higher than the content found in this study. This difference could be due to different amaranth species and to different growing conditions. Kiwicha has more total phenolic compounds than oat. Emmons et al. (250) analyzed the total phenolic compounds in different milling fractions of oat and they found the content of these compounds between 8.9 and 34.2 mg GAE/100 g of sample. The content of total phenolic compounds was lower than in bran-enriched wheat milling fractions, 130-530 mg/100g found by Trust *et al*. (251). In any case, if bran-enriched kiwicha milling fractions could be used, the content of total phenolic compounds would probably be higher than that of wheat. Del Pozo-Insfran *et al*. (252) analyzed the content of total phenolic compounds in three genotypes of corn, two blue genotypes and one white. The content of total phenolic compounds was between 410 and 3430 mg/100 g calculated as gallic acid equivalents. Dykes *et al*. (253) determined the total phenolic compounds in sorghum varieties. Grain sorghum is very high in these compounds (201-910 mg gallic acid/100 g). Generally, the content of total phenolic compounds in kiwicha is lower than that of most common cereals.

The content of phenolic compounds was 2.54 and 2.43 mg of gallic acid equivalents (GAE)/g for Cupi and Ramis, respectively (Table 24.). This content is higher than in oat (199), buckwheat, quinoa and rice (251). Yawadio *et al*. (254) analyzed the total phenolic compounds in quinoa and amaranth (*A. hypochondriacus*, *A. cruentus*) and found a content between 94.3 and 148 mg/g of tannic acid equivalents.

There were significant differences between the quinoa varieties in the contents of total polyphenols (Table 24.) (III). The contents of total polyphenols in the four quinoa varieties ranged from 1.42 to 1.97 mg GAE/g. Pasko et al. (255) defined the content of total polyphenols in quinoa to be 3.75 mg GAE/g by using a two-step extraction process, first with methanol and then with acetone. As we used methanol only, some polyphenols may not have been included in the extract.

Table 25. Total phenolic compounds in Andean grains (I, II, III).

Crop/variety	mg GAE/ g dry basis
Kiwicha/Centenario	1.13 ± 0.1
Kiwicha/Oscar Blanco	0.99 ± 0.0
Kañiwa/Cupi	2.54 ± 1.2
Kañiwa/Ramis	2.43 ± 1.4
Quinoa/Blanca de Juli	1.42 ± 0.5
Quinoa/Kcancolla	1.57 ± 0.3
Quinoa/La Molina 89	1.97 ± 0.2
Quinoa/Sajama	1.63 ± 0.1

GAE = gallic acid equivalents

5.1.4 PHENOLIC ACIDS

Both the soluble and total phenolic acid contents in the Andean cereals were quantified as aglycones (Table 26) (IV). Soluble phenolic acids (free and bound soluble forms) were extracted with methanolic acetic acid whereas the total phenolic acid content (the sum of bound soluble, insoluble and free phenolic acids) was obtained after alkaline and acid hydrolyses. Due to a lack of reference standards for soluble bound phenolic acids, the results are to be considered tentative and are reported only as percentage shares of total phenolic acids in Table 25. However, this information may be of interest because the bioavailability of soluble phenolic acids may differ from that of insoluble ones.

The total content of phenolic acids varied from 16.8 to 59.7 mg/100 g in the samples analyzed and the percentage share of soluble phenolic acids varied from 7% to 61%.

There were several differences in the phenolic acid composition of the three different grains (quinoa, kañiwa and kiwicha) (Table 25). The samples of *Chenopodium* species contained caffeic acid, ferulic acid, *p*-coumaric acid, *p*-OH-benzoic acid and vanillic acid. In addition to these sinapinic acid and protocatechuic acid were detected in *Amaranthu*s samples (Table 26). There was a statistically significant difference in the

content of ferulic acid in quinoa, kañiwa and kiwicha, kañiwa having the highest and kiwicha the lowest. Of the *Chenopodium* species kañiwa samples contained less vanillic acid but more caffeic and ferulic acids than quinoa samples. The content of total phenolic acids was higher in

quinoa than in kiwicha but much variation existed between samples. In quinoa varieties, the proportion of soluble phenolic acids was high
(mean 39±11%). In kañiwa and amaranthus varieties, these mean values were 21±9% and 10±3%, respectively.

Very little information has been published concerning the phenolic acid content of *Chenopodium* and *Amaranthus* seeds. Peñarrieta *et al*. (195) identified vanillic and ferulic acids in whole plants of *Chenopodium pallidicaule*. Their result for vanillic acid was of the same magnitude, whereas a lower level of ferulic acid was found compared with the present study. This discordance probably arises from sample differences (seeds vs. whole plants) as well as different methodologies. However, Peñarrieta *et al*. (195) also found much variation between samples (ecotypes). Klimczak *et al*. (198) analyzed the free phenolic acid content of *Amaranthus caudatus* seeds and found the same phenolic acids as in the present study except for sinapinic and vanillic acids. However, in our study soluble (or free) caffeic, ferulic, p-coumaric and protocatechuic acids were not found. Recently, Barba de la Rosa *et al*. (256) published information concerning the phenolic acid content of a different amaranth species (*Amaranthus hypochondriacus*). According to their data, amaranth seed flour contained soluble 4-hydroxybenzoic acid 0.17–0.22 mg/100 g, vanillic acid 0.15–0.18 mg/100 g and syringic acid 0–0.08 mg/100 g. These figures are much lower than those obtained in our study. This is probably due to the different methodology as well as the different species studied.

The Andean cereals contained lower levels of phenolic acids compared with common cereals like wheat (*Triticum spp*.) and rye (*Secale cereale*). In these cereals, the phenolic acids accumulate in bran where their levels are as high as 419 and 453 mg/100 g in rye and wheat bran while the whole grain flours of these grains contain 137 and 134 mg/100 g, respectively (231). However, according to Mattila *et al*. (231) the phenolic acid content of other cereals like oat (*Avena sativa*), barley (*Hordeum vulgare)*, corn (*Zea mays*), rice (*Oryza sativa*), millet (*Panicum miliaceum*) and buckwheat (*Fagopyrum esculentum*) is of the same magnitude (25–60 mg/100 g) as in the Andean grains studied here.

Table 26. Total contents (mg/100 g) phenolic acids in quinoa, kaniwa and kiwicha grains.

Sample	***caffeic acid***	***ferulic acid***	***p-coumaric acid***	***p-OH-benzoic acid***	***vanillic acid***	***sinapic acid***	***protocatachuic acid***	***total***
Quinoa samples								
Ccoito	0.95 ± 0.04	15.3 ± 0.5	6.46 ± 0.18	3.87 ± 0.07	8.97 ± 0.01	n.d.	n.d.	35.6 ± 0.4
INIA-415 Pasankalla	0.61 ± 0.03	20.0 ± 0.2	27.5 ± 0.4	2.44 ± 0.02	9.19 ± 0.36	n.d.	n.d.	59.7 ± 0.5
Roja de Coporaque	0.50 ± 0.03	13.9 ± 0.6	4.07 ± 0.01	2.60 ± 0.08	11.0 ± 0.3	n.d.	n.d.	32.1 ± 1.0
Witulla	1.47 ± 0.21	14.9 ± 0.7	2.26 ± 0.08	2.46 ± 0.09	9.20 ± 0.28	n.d.	n.d.	30.3 ± 0.6
03-21-0093	0.86 ± 0.02	16.6 ± 0.5	8.72 ± 0.02	2.80 ± 0.13	10.7 ± 0.5	n.d.	n.d.	39.7 ± 1.1
Salcedo INIA	0.25 ± 0.01	12.3 ± 0.9	8.02 ± 0.36	3.17 ± 0.02	14.6 ± 0.2	n.d.	n.d.	38.4 ± 1.5
Commercial 1.	0.57 ± 0.02	18.6 ± 1.7	2.84 ± 0.14	3.38 ± 0.24	11.9 ± 0.3	n.d.	n.d.	37.2 ± 1.9
Commercial 2.	0.87 ± 0.03	14.3 ± 0.1	2.60 ± 0.03	3.88 ± 0.04	10.3 ± 0.1	n.d.	n.d.	32.0 ± 0.1
Huaripongo	0.37 ± 0.04	12.0 ± 0.1	4.01 ± 0.06	2.65 ± 0.02	12.4 ± 0.1	n.d.	n.d.	31.4 ± 0.2
03-21-1181	0.59 ± 0.07	13.7 ± 0.7	9.50 ± 0.36	1.92 ± 0.08	10.7 ± 0.5	n.d.	n.d.	36.3 ± 1.2

Kañiwa samples							
Kello	1.10 ± 0.01	26.1 ± 1.9	1.34 ± 0.12	1.77 ± 0.09	4.34 ± 0.30	n.d.	n.d.	34.7 ± 2.4
Wila	2.16 ± 0.02	29.8 ± 0.2	1.00 ± 0.01	1.77 ± 0.04	3.61 ± 0.08	n.d.	n.d.	38.3 ± 0.3
Guinda	2.37 ± 0.12	26.0 ± 0.8	1.74 ± 0.19	1.55 ± 0.08	3.04 ± 0.18	n.d.	n.d.	34.7 ± 1.3
Ayara	7.04 ± 0.11	23.4 ± 1.2	0.70 ± 0.04	1.97 ± 0.19	6.95 ± 0.21	n.d.	n.d.	40.1 ± 1.7
Commercial sample	1.10 ± 0.09	12.0 ± 0.4	0.37 ± 0.02	1.54 ± 0.13	3.23 ± 0.38	n.d.	n.d.	18.3 ± 0.8
Kiwicha samples							
1	0.85 ± 0.01	8.32 ± 0.70	0.81 ± 0.04	3.16 ± 0.02	6.67 ± 0.03	0.32 ± 0.04	12.8 ± 0.4 (0%)	32.9 ± 1.3
2	0.87 ± 0.02	6.46 ± 0.64	0.99 ± 0.09	1.97 ± 0.15	4.28 ± 0.42	0.09 ± 0.01	6.28 ± 0.42	20.9 ± 1.4
3	0.70 ± 0.07	6.21 ± 0.09	0.80 ± 0.05	3.19 ± 0.02	6.38 ± 0.40	0.09 ± 0.01	n.d.	17.4 ± 0.6
4	1.13 ± 0.04	6.57 ± 0.01	0.98 ± 0.02	3.68 ± 0.10	4.35 ± 0.26	0.09 ± 0.01	n.d.	16.8 ± 0.4

n.d. =not detected

5.1.5 FLAVONOIDS

The flavonoid content of *Chenopodium* species was exceptionally high, varying from 36.2 to 144.3 mg/100 g (Table 27) (IV). The predominant flavonoids in quinoa samples were quercetin and kaempferol while in some varieties myricetin and isorhamnetin were also found. Kañiwa samples contained mostly quercetin and isorhamnetin with smaller
amounts of myricetin, kaempferol and rhamnetin in some varieties. As in the case of phenolic acids, much variation was found between different samples. There were no statistically significant differences in the content of quercetin, rhamnetin and total flavonoids in quinoa and kañiwa. The content of isorhamnetin was significantly higher in kañiwa compared with quinoa. In the case of kaempferol, the content in kañiwa was
significantly lower than in quinoa.

Berries have been considered as an excellent source of flavonols, especially quercetin and myricetin. For example, lingonberry contains 10 mg/100 g fw of quercetin and cranberry contains 10.4 and 6.9 mg/100 g fw quercetin and myricetin, respectively (229). The levels in these flavonoid-rich berries are 5–10 times lower than those found in *Chenopodium* seed samples. When compared on a dry weight basis. the flavonoid contents in berries and *Chenopodium* samples are of the same magnitude. Quinoa and kañiwa seeds can thus be considered very good sources of flavonoids. Common cereals (wheat, rye, oat, barley etc.) do not contain any flavonols (257).

This is the one of the first studies reporting the total content of flavonoids in quinoa and kañiwa seeds. Peñarrieta *et al.* (195) analyzed extractable flavonoids in the whole plant of *Chenopodium pallicaule* and found quercetin and kaempferol. The levels of quercetin were much lower than those obtained in the present study. De Simone *et al.* (258) and Zhu *et al*. (259) characterized flavonol glycosides in quinoa (*Chenopodium quinoa* Willd) seeds. Zhu *et al*. (259) isolated and characterized six flavonol glycosides: four kaempferol glycosides and two quercetin glycosides. In a recent study, Hirose *et al.* (260) found large amounts of quercetin and kaempferol glycosides in quinoa grains. Kaempferol and quercetin were also the main flavonoid aglycones found in the present study.

There were no quantifiable amounts of flavonoids in amaranth samples: only traces of quercetin were found. Barba de la Rosa *et al.* (256) also detected low levels of quercetin-glycoside, rutin (4.0–10.2 µg/g) in *Amanranthus hypochondriacus* seeds.

Table 27. Contents of flavonoids in quinoa and kaniwa grains (mg/100 g) (IV).

Sample	*myricetin*	*quercetin*	*kaempferol*	*isorhamnetin*	*rhamnetin*	*total*
Quinoa samples						
Ccoito	n.d.	38.1 ± 2.3	16.3 ± 1.6	n.d.	n.d.	54.5 ± 4.0
INIA-415 Pasankalla	n.d.	35.7 ± 0.2	0.45 ± 0.11	n.d.	n.d.	36.2 ± 0.3
Roja de Coporaque	0.22 ± 0.04	55.5 ± 4.2	16.9 ± 1.1	n.d.	n.d.	72.6 ± 5.3
Witulla	0.86 ± 0.11	23.5 ± 0.8	44.7 ± 1.2	n.d.	n.d.	69.0 ± 2.1
03-21-0093	0.90 ± 0.13	32.6 ± 0.1	14.2 ± 0.7	n.d.	n.d.	47.7 ± 1.0
Salcedo INIA	n.d.	11.6 ± 0.1	54.2 ± 0.5	n.d.	n.d.	65.8 ± 0.6
Commercial 1.	1.24 ± 0.07	36.8 ± 0.6	10.2 ± 0.3	2.08 ± 0.06	n.d.	50.3 ± 1.0
Commercial 2.	0.51 ± 0.08	47.1 ± 2.4	21.5 ± 1.1	n.d.	n.d.	69.2 ± 3.6
Huaripongo	0.88 ± 0.20	53.2 ± 4.1	14.2 ± 0.8	0.89 ± 0.11	n.d.	69.2 ± 5.2
03-21-1181	0.67 ± 0.12	28.5 ± 2.7	11.5 ± 0.3	1.02 ± 0.10	n.d.	41.7 ± 3.2
Kañiwa samples						
Kello	n.d.	84.3 ± 1.2	n.d.	60.0 ± 1.3	n.d.	144.3 ± 2.5
Wila	n.d.	68.7 ± 5.8	n.d.	14.2 ± 0.8	n.d.	83.0 ± 6.6
Guinda	n.d.	25.1 ± 2.0	n.d.	29.5 ± 1.3	n.d.	54.6 ± 3.3
Ayara	n.d.	21.4 ± 1.4	5.97 ± 0.02	n.d.	18.7 ± 2.0	46.1 ± 3.5
Commercial sample	0.18 ± 0.01	78.6 ± 6.6	2.24 ± 0.33	24.8 ± 2.4	n.d.	105.8 ± 9.3

n.d. =not detected

5.1.6 BETALAINS

Of the analyzed kiwicha samples, only the pink variety contained betacyanins above 0.1 mg/100 g. The total amount of betacyanins was low (1.9 ± 0.4 mg/100 g dw) compared to the values (mean 91.4 ± 4.0 mg/100 g fresh weight) reported in different vegetative parts, i.e. seedlings, leaves and inflorescences, of *Amaranthus* (232). Amaranthine, iso-amaranthine and betanin contents were 1.0, 0.8 and 0.1 mg/100 g, respectively. No data on betacyanins in kiwicha seeds have previously been reported in the literature.

5.2 MINERAL CONTENT AND BIOAVAILABILITY

5.2.1 MINERAL CONTENT IN RAW AND PROCESSED GRAINS

Iron, calcium and zinc content of raw and processed quinoa, kañiwa and kiwicha are presented in Table 28 (V). Iron content was similar in raw kañiwa and kiwicha. Regarding zinc and calcium, quinoa grains contained the highest levels of both minerals. There was a significant decrease in iron content during the cooking process in all samples. Wet processing procedures in general cause a loss of dry matter and iron (261). In the case of kiwicha, roasting also reduced the content of this mineral. Cooking reduced the content of zinc in quinoa and kañiwa, but not in kiwicha. Roasting negatively affected the content of calcium in quinoa but not in kañiwa and kiwicha.

Compared with unenriched wheat flour which is commonly used in Peru and Andean countries (iron, 0.68 mg/100 g; zinc, 0.98mg/100 g; and calcium, 18.46 mg/100 g) (236), 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) and finger millet (2.13 mg/ 100 g) (262). Quinoa is sometimes used to replace rice in some culinary preparations in Peru. Pachon *et al*. (263) 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.

Table 28. Mineral composition of raw, toasted and cooked Andean grains (V).

Sample	Iron mg/100 g	Zinc mg/100 g	Calcium mg/100 g
Quinoa, raw	2.95 ± 0.16 a	2.95 ± 0.38 a	68.55 ± 3.68 a
Quinoa, toasted	3.15 ± 0.08 a	3.18 ± 0.42 a	59.29 ± 4.99 b
Quinoa, cooked	1.08 ± 0.17 b	1.79 ± 0.39 b	67.03 ± 4.63 a
Kañiwa, raw	4.91 ± 0.24 $_{1}$	2.15 ± 0.23 $_{1}$	29.76 ± 4.09 $_{1}$
Kañiwa, toasted	5.44 ± 0.60 $_{2}$	2.72 ± 0.21 $_{2}$	32.33 ± 3.95 $_{1\,2}$
Kañiwa, cooked	1.89 ± 0.05 $_{3}$	1.48 ± 0.42 $_{3}$	37.56 ± 2.07 $_{2}$
Kiwicha, raw	5.00 ± 0.92 $_{*}$	1.25 ± 0.16 $_{*}$	27.90 ± 1.43 $_{*}$
Kiwicha, toasted	3.75 ± 0.63 $_{**}$	1.33 ± 0.19 $_{*}$	29.73 ± 3.16 $_{*}$
Kiwicha, cooked	3.55 ± 0.41 $_{**}$	1.05 ± 0.32 $_{*}$	25.06 ± 4.30 $_{*}$

All data are the mean +/- SD of three replicates

Means within columns not sharing a common superscript differ ($P < 0.05$)

5.2.2 BIOAVAILABILITY AND POTENTIAL CONTRIBUTION OF IRON, ZINC AND CALCIUM IN RAW AND PROCESSED GRAINS

Several approaches have been used to estimate iron bioavailability including in vitro digestion to measure mineral solubility or bioavailability and animal studies. *In vitro* testing with dialysis in a simulated gastrointestinal digestion system proved to be a promising technique (234). Iron, zinc and calcium bioavailability is presented in Figures 8, 9 and 10, respectively. The potential contribution (PC) is shown in Table 29. The potential contribution of each mineral (PC) was calculated as each mineral concentration times its bioavailability.

In the case of kañiwa, the cooked samples had higher mineral bioavailability than the raw and toasted samples. In toasted samples of kañiwa, mineral bioavailability 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 bioavailability between raw and roasted grains, although the cooked grains showed lower values ($p<0.05$). Consequently, the PC of iron diminished in cooked grains.

With respect to zinc, cooked quinoa, kañiwa and kiwicha showed significantly higher zinc bioavailability regarding both raw and roasted samples ($p<0.01$). Accordingly, the 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, toasted and cooked quinoa had lower calcium bioavailability than raw while cooked kañiwa had higher values than raw. In the case of kiwicha, there were no significant differences between raw, toasted and cooked samples. The bioavailability of calcium in raw grains was between 23 and 28%. In processed products, it was 22 to 30%.

According to research by Kamchan *et a*l (264), amaranth leaves are rich in calcium. The bioavailability of calcium, however, is low (4.1%). Whole milk power was used as the reference food for calcium bioavailability comparison in a study by Kamchan *et al*. (264). Drago and Valencia (265) analyzed the bioavailability of calcium in dairy products. The bioavailability of calcium for fresh milk was 35%. Skibniewska et al. (266) analyzed *in vitro* availability of minerals in oat products. The *in vitro* availability of calcium was 27-40 % for different oat

products. In our study, the bioavailability of calcium was high in all samples (22 to 30%), comparable to the calcium bioavailability of milk power (25%).

The calcium, zinc and iron bioavailability of kiwicha was considerably higher in our study than in the research carried out by Dyner *et al*. (236). They analyzed the bioavailability 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.* (236) The potential contribution (PC) of calcium was lower and the PC of iron and zinc higher in our study in comparison with the study by Dyner *et al.(*236).

Iron bioavailability was relatively low in all samples (1-6%). These values are lower than values for oat products (7-30%) (266) but similar the values for cooked and extruded legumes (1-5%) (267). In cooked quinoa, kañiwa and kiwicha there was an increase in the bioavailability 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.* (268) found that cooking 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 bioavailability than kiwicha and kañiwa samples. This could be due to the presence of saponins and phytic acid in quinoa seeds (23). It is well known that phytic acid and saponins lower the bioavailability of zinc and iron (269, 270).

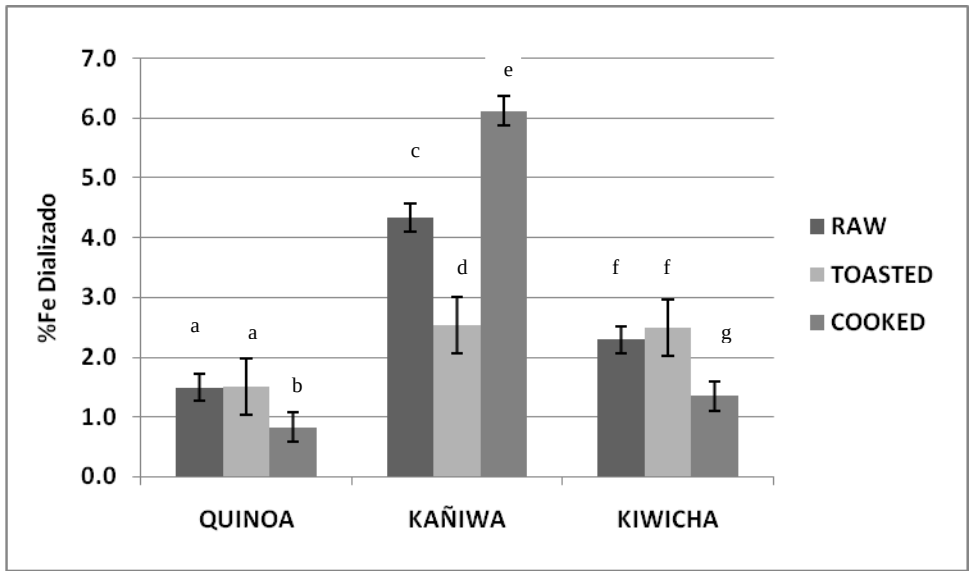


Figure 8. Iron bioavailability (FeD %) of raw, toasted and cooked quinoa, kañiwa and kiwicha. Means +/- standard deviation for six analyses. Statistical comparison was between raw, toasted and cooked grain. For each grain type, means with same letter are not significantly different (V).

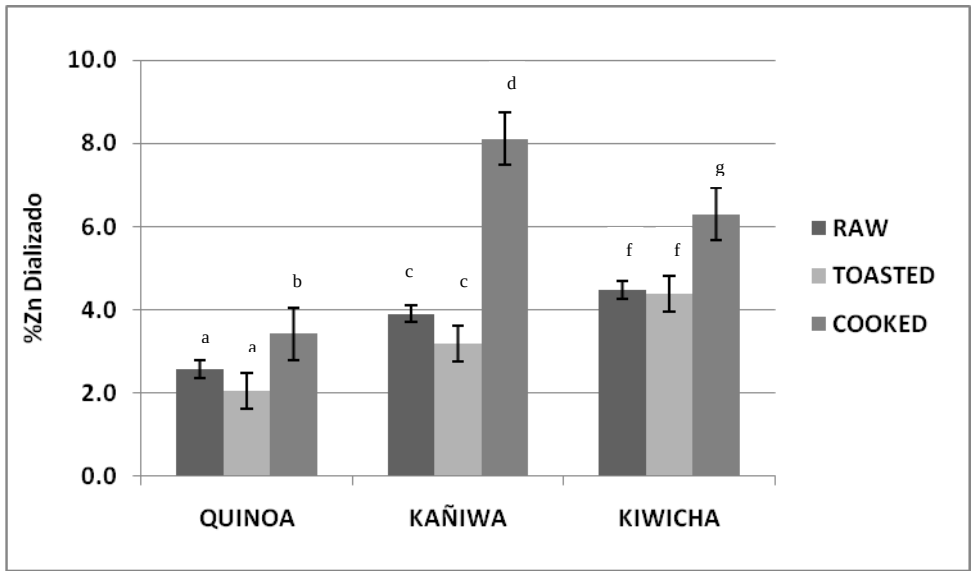


Figure 9. Zinc bioavailability (ZnD %) of raw, toasted and cooked quinoa, kañiwa and kiwicha. Means +/- standard deviation for six analyses. Statistical comparison was between raw, toasted and cooked grain. For each grain type, means with same letter are not significantly different (V).

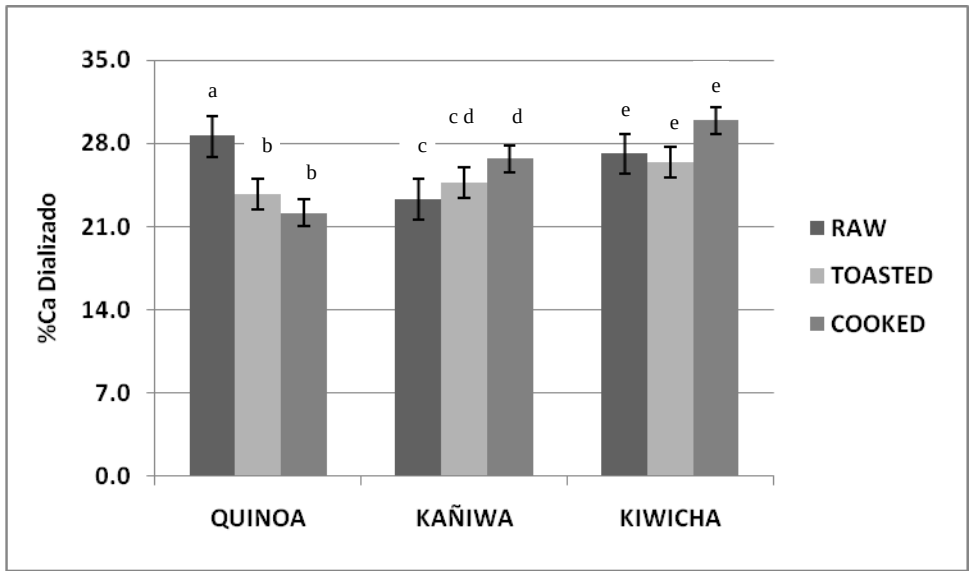


Figure 10. Calcium bioavailability (CaD %) of raw, toasted and cooked quinoa, kañiwa and kiwicha. Means +/- standard deviation for six analyses. Statistical comparison was between raw, toasted and cooked grain. For each grain type, means with same letter are not significantly different (V).

Table 29. Potential contribution (PC) of iron, zinc and calcium in raw, toasted and cooked quinoa, kañiwa and kiwicha grains (V).

Sample	PC iron mg%	PC zinc mg%	PC calcium mg%
Quinoa, raw	0.04 a	0.08 a	19.63 a
Quinoa, toasted	0.05 a	0.06 b	14.00 b
Quinoa, cooked	0.01 b	0.06 b	14.91 b
Kañiwa, raw	0.21 $_{1}$	0.08 $_{1}$	6.95 $_{1}$
Kañiwa, toasted	0.14 $_{2}$	0.09 $_{1}$	8.00 $_{1}$
Kañiwa, cooked	0.12 $_{3}$	0.12 $_{2}$	10.02 $_{2}$
Kiwicha, raw	0.11 $_{*}$	0.06 $_{*}$	7.56 $_{*}$
Kiwicha, toasted	0.09 $_{**}$	0.06 $_{*}$	7.85 $_{*}$
Kiwicha, cooked	0.05 $_{***}$	0.07 $_{*}$	7.50 $_{*}$

All data are the mean +/- SD of three replicates

Means within columns not sharing a common superscript differ ($P < 0.05$)

Roasting and cooking 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*. (267) studied the effect of high and low impact extrusion processes on mineral bioavailability in legumes. The major differences in these processes include: screw configuration, screw speed, moisture content and barrel zone temperatures. They found that low impact extrusion increased the bioavailability of iron in legumes.

On the other hand, if we compare mineral bioavailability values in these grains with those in wheat flour (FeD% 9.8; ZnD% 10.1; CaD% 44.1) (236) 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 (263). It is possible to enhance mineral availability in cereals using, for example, cereal and sourdough fermentation. These processes show to be effective in solubilizing minerals. This is especially beneficial in products rich in bran to deliver minerals and potentially protective compounds in the blood circulation (271).

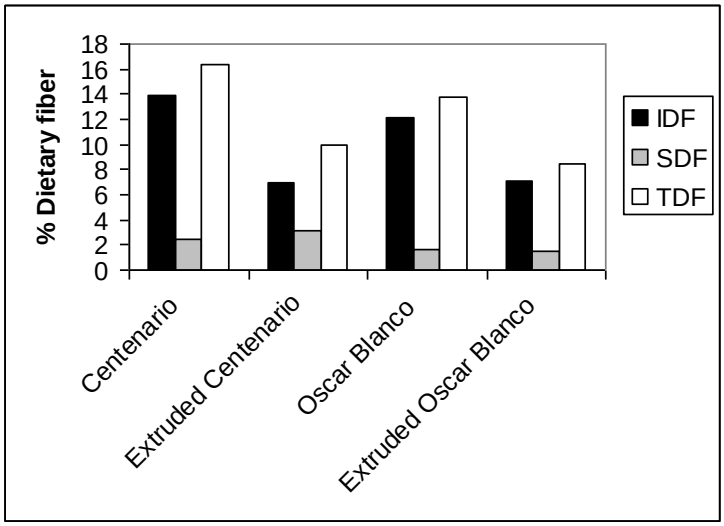
5.3 EFFECT OF PROCESSING ON COMPOSITION OF ANDEAN GRAINS

5.3.1 EFFECT OF EXTRUSION ON CHEMICAL COMPOSITION OF KIWICHA

The effect of extrusion on the dietary fiber, phytates, phenolic compounds and the radical scavenging capacity in Andean grains was studied. The content of total dietary fiber in the extruded kiwicha was similar to the content found by Plate and Areas (209), 8.20% for *A. caudatus*. In both varieties, the content of total and insoluble dietary fiber decreased during the extrusion process. In the case of the Centenario variety, the content of soluble dietary fiber increased from 2.45 to 3.06% during the extrusion process (I). However, in the Oscar Blanco variety, the amount of soluble dietary fiber decreased slightly (from 1.65 to 1.46%) (Figure 11).

Gualberto et al. (247) investigated the effect of extrusion on the dietary fiber and phytic acid in cereal brans. They found also a decrease in the content of insoluble dietary fiber during extrusion cooking and an increase in the content of soluble fiber. This could be due to shear stress caused by high screw speed and also to high temperature. The exposure to shear stress and high temperature causes chemical bond breakage creating smaller particles which are

soluble. There is a transformation of some insoluble fiber components into soluble fiber during extrusion.



IDF= insoluble dietary fiber SDF = soluble dietary fiber TDF = total dietary fiber
Figure 11. Dietary fiber content in raw and extruded kiwicha varieties (I)

The raw kiwicha contained 0.28-0.31% of phytic acid. This value coincides with the values reported by Guzman-Maldonado and Paredes-Lopez (22). They reported the content of phytic acid for amaranth to be between 0.34 and 0.61%. There was a slight decrease in the phytic acid content for both varieties during the extrusion process. However, this decrease was not significant. This coincides with the results of Gualberto *et al*. (247) who did not find a significant decrease of phytate content during the extrusion process. Kiwicha contains less phytates than some common cereals, such as corn and wheat, (22). Gualberto *et al*. (247) found 1.42, 4.32 and 5.27% phytate in oat, rice and wheat bran, respectively.

Figure 12 presents the content of total phenolic compounds, as gallic acid equivalents (GAE), in raw and extruded kiwicha. The extrusion process affected the content of total phenolic compounds in both varieties, decreasing it by 80.3% and 64.4% for Centenario and Oscar Blanco, respectively. Del Pozo-Insfran *et al*. (252) also studied the effect of processing to the total phenolic compounds and found that Mexican white corn lost 90% of its initial total phenolic content, while the Mexican and American blue corns lost 61 and 78% during lime-cooking. Phenolic acids can suffer decarboxylation during processing of food. Ferulic acid, for

example, forms *p*-vinylguaiacol during storage of orange juice (272). *P*-coumaric acid can form *p*-hydroxybenzaldehyde (273).

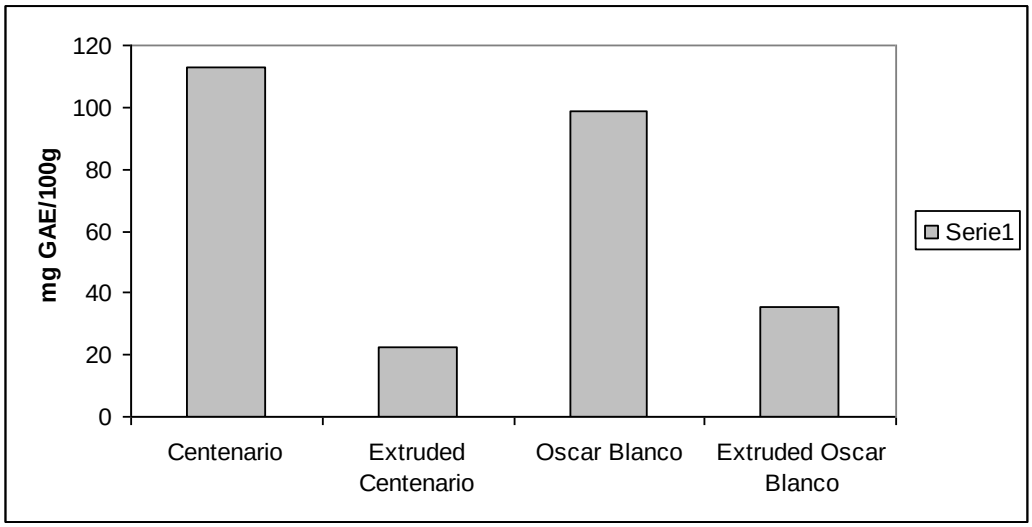
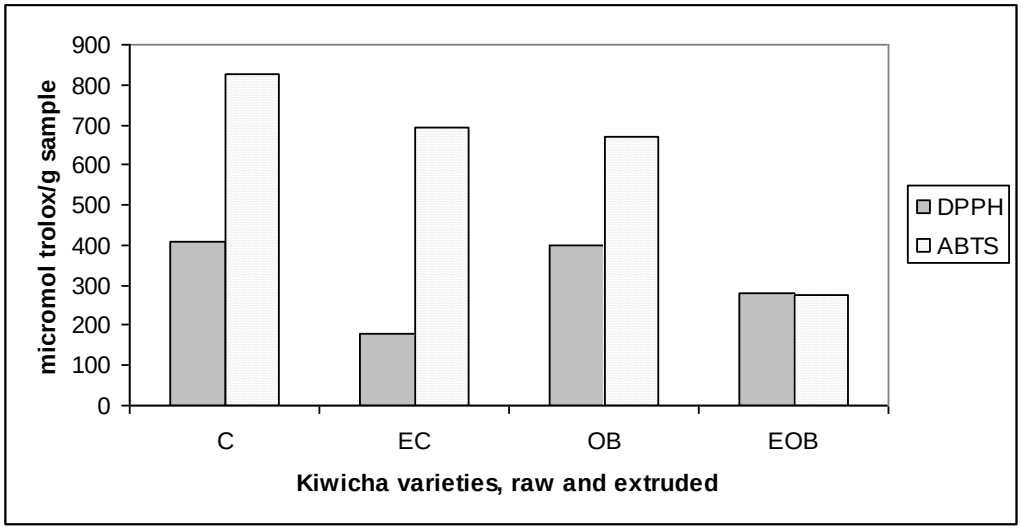


Figure 12. Content of total phenolic compounds in the Centenario and Oscar Blanco varieties of kiwicha (I).

The radical scavenging activity of the two varieties of kiwicha is presented in Figure 13. Radical scavenging activity for the raw kiwicha of the two varieties was 410 µmol trolox/g sample for Centenario and 398 µmol trolox/g sample for Oscar Blanco measured by the DPPH method. Using the ABTS method, these values were 827 and 670 µmol trolox/g sample for Centenario and Oscar Blanco, respectively. These values are high compared with other cereals. Awika *et al*. (274) determined the antioxidant activity of sorghum and sorghum products using the DPPH and ABTS methods. They found antioxidant activities with DPPH for different sorghum varieties between 6 and 202 µmol trolox/g sample and with ABTS these values were between 6 and 226 µmol trolox/g. The brans of red variety and the high tannin variety demonstrated higher values of antioxidant activity, 21-716 µmol trolox/g sample (DPPH) and 28-768 µmol trolox/g sample (ABTS), similar to this study. They found that the ABTS values were higher than the DPPH values for black sorghum brans. In our study, the ABTS values were higher than the DPPH which coincides with the results of Awika *et al.* (274). Zielinska *et al*. (275) studied the antioxidant capacity of buckwheat sprouts using the DPPH method. They found antioxidant capacity between 41.55 and 218.36 µmol trolox/g sample for the sprouts. For

different grain products lower values than those found in this study are reported: whole wheat flakes 35, whole wheat biscuit 30, whole grain oat flake 27, whole grain puffed oat 26, corn flakes 20 µmol trolox/g sample (276). In our study we found moderate correlation between phenolic compounds and antioxidant activity with the ABTS method (R=0.584) and a high correlation between the phenolic compounds and DPPH method (R= 0.959). This observation is in agreement with Katsube *et al.* (277) who reported that there was a high correlation between the DPPH and Folin-Ciocalteau assays (R=0.969). In Andean grains, the phenolic compounds are the principal contributors to the antioxidant capacity. They are not very rich in vitamin C and there is evidence that the contribution of vitamin C to the antioxidant activity is lower than that of phenolic compounds (278).



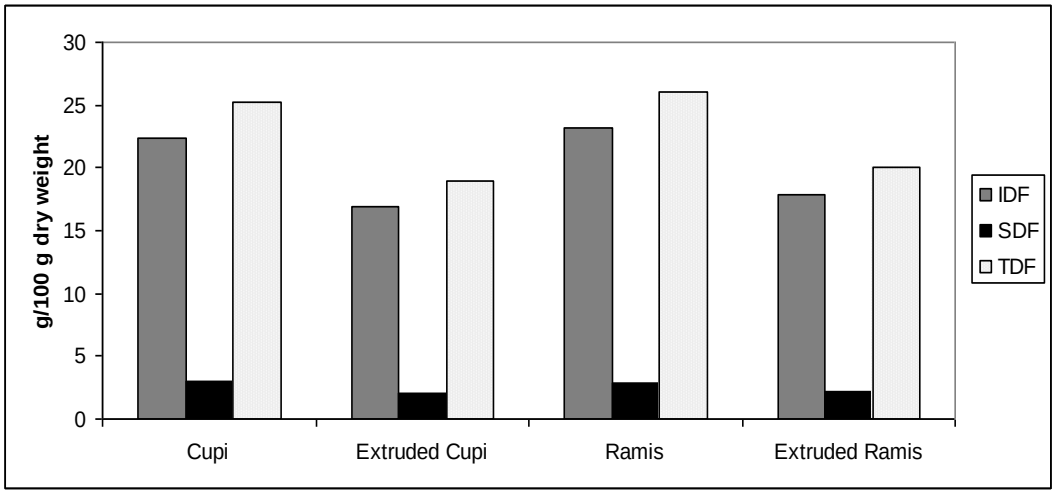
C = Centenario, EC = extruded Centenario, OB = Oscar Blanco, EOB = extruded Oscar Blanco

Figure 13. Radical scavenging activity of the two varieties of kiwicha, raw and extruded (I)

The cooking extrusion process affected the antioxidant activity of the two varieties of kiwicha, decreasing this value. In the case of the Centenario variety, there was a reduction of 16 -56% of the original value and in the case of the Oscar Blanco variety, a decrease of 29-58% of the original value. Del Pozo-Insfran *et al.* (252) found a difference in the loss of antioxidant capacity for different varieties of corn in the processing of nixtamals, tortillas and chips. The blue varieties suffered a higher loss than the white varieties. The average loss for nixtamals, tortillas and chips was 42, 49 and 62% of the initial antioxidant capacity, respectively.

5.3.2 EFFECT OF EXTRUSION ON CHEMICAL COMPOSITION OF KAÑIWA

The content of fat and crude fiber was reduced during the extrusion process. There was a significant decrease on the total and insoluble dietary fiber content of both varieties of kañiwa (Figure 14) (II). Frolich and Hestangen (279) analyzed the total dietary fiber content in rye grain and in extruded rye. They observed a decrease of total dietary fiber from 16.8% to 12.7%. The content of soluble dietary fiber was also significantly decreased in both varieties according to analysis of variance. Björck *et al.* (280) obtained similar results in the extrusion of wheat flour: the content of soluble dietary fiber was decreased from 2.3% to 1.7%.



IDF= insoluble dietary fiber SDF = soluble dietary fiber TDF = total dietary fiber

Figure 14. Dietary fiber content in raw and extruded kañiwa varieties (II).

The content of resistant starch increased during the process of extrusion, from 0.24% to 0.43% in the Cupi variety and from 0.26% to 0.31% for the Ramis variety. Huth *et al*. (281) also found an increase in resistant starch in barley during the extrusion process, especially in high temperatures (170ºC). The increase of resistant starch during the extrusion process can be explained by modification of the amylase structure.

Gonzalez-Soto *et al*. (282) studied the effect of extrusion on the resistant starch content of corn. The content of resistant starch in corn was between 1.97% and 2.05%. It was reported that the content of resistant starch decreased when the screw velocity was increased. This is probable

due to the increase in shear stress which causes rupture of the structure of resistant starch. Resistant starch acts as soluble fiber in the colon. It is fermented by the intestinal microflora, resulting in the formation of short chain fatty acids which are protective to the colonic mucosa (283). The lignin content of the Ramis and Cupi varieties decreased in both cases. Benchaar et al. (269) also found a decrease in lignin content from 2.3% to 1.1% for raw and extruded horse beans, respectively. The content of betaglucans in extruded kañiwa was insignificant.

The three extrudates with different initial moisture were evaluated by the degree of gelatinization (DG), sectional expansion index (SEI), water absorption index (WAI), water solubility index (WSI) and density g/mL aiming to choose the best treatment. The results of this evaluation are presented in Table 30.

Table 30. Effect of extrusion on functional properties of two varieties of kañiwa (II).

Variety/moisture %	DG %	SEI	Density g/ml	WAI	WSI
Cupi					
12	98.35±1.19	1.98±0.27	0.10±0.00	2.88±0.41	0.48±0.06
14	96.61±0.97	1.77±0.28	0.20±0.02	3.84±0.48	0.36±0.08
16	88.33±1.16	1.61±0.29	0.30±0.01	3.96±0.33	0.32±0.05
Ramis					
12	98.19±0.86	1.87±0.27	0.14±0.01	3.20±0.41	0.45±0.05
14	97.14±1.27	1.63±0.05	0.22±0.01	3.48±0.19	0.39±0.02
16	96.27±2.30	1.39±0.04	0.39±0.01	3.83±0.28	0.32±0.02

All data are the mean +/-SD of three replicates

DG= degree of gelatinization, SEI = Sectional expansion index, WAI = water absorption index (g H_2O/g dry sample) , WSI = water solubility index (g water soluble matter/g dry sample)

Table 31 shows that the degree of gelatinization decreases as the initial moisture of grains increases. According to Harper (284), not enough shear stress for gelatinization of starch is achieved in raw materials with high moisture contents. The highest DG was reached with 12% of initial moisture, for both varieties. The DG was 98.4% and 98.2% for Cupi and Ramis, respectively. Dogan and Karwe (58) studied the physicochemical properties of quinoa (*Chenopodium quinoa*) extrudates. They found a maximum DG of 84.4% which is lower than the values found in the current study. They used higher initial moistures (16-24%) and lower temperatures (130-170ºC) than we used (180ºC). In general, starchy materials need low feed

moisture and high product temperature to reach high DG. Materials with high lipid content, like kañiwa, also need elevated shear stress for effective extrusion cooking (58).

The sectional expansion index was decreased with more initial moisture content. The highest SEI was achieved with the initial moisture of 12%. It was 1.98 for Cupi and 1.87 for Ramis. The expansion of cereals in extrusion depends on the degree of gelatinization of the starch. This fact was revealed in this study since with the highest degree of gelatinization, the highest expansion index was reached. Dogan and Karwe (58) measured SEI in whole meal quinoa extrudates and found values from 0.92 to 3.58. In their study, SEI was significantly affected by temperature, feed moisture content and screw speed. A high expansion ratio at a low feed moisture content for extruded products is typical for cereals.

The density of the extrudate increased when the initial moisture was increased. Huth *et al.* (281) discovered that the density of barley extrudate increased when the initial moisture content was high. Similar results were shown also in the research of Gambus *et al.* (285) for corn and wheat extrudates. Lee *et al.* (286) mentioned that the extruded products generally have a density of between 0.1 g/ml and 0.2 g/ml. The extrudates of kañiwa with an initial moisture content of 12% had a density of 0.10 and 0.14 g/ml, for Cupi and Ramis varieties, respectively. Gambus *et al*. (285) found the following values of density for corn and wheat starch extrudates: 0.23-0.30 and 0.12-0.28 g/ml. They used a higher initial moisture than we used in our study.

The water absorption index (WAI) increased when the initial moisture increased. WAI depends on the availability of hydrophilic groups and the gel formation capacity of the macromolecules (287). It is a measure of denaturated starch together with protein denaturation and new macromolecular complex formations (58). Extruded products should have a low WAI to maintain the crispiness of the final product. The lowest WAI was achieved at the initial moisture content of 12% for both varieties. The WAI was 2.88 and 3.20 for Cupi and Ramis, respectively. These values were lower than the values found by Dogan and Karwe (58) for quinoa extrudates.

The water solubility index (WSI) decreased in higher initial moisture contents. This fact can be explained by the greater rupture of starch granules in lower initial humidity. The highest WSI was obtained using an initial moisture level of 12% for both varieties. These values were 0.48

and 0.45 for Cupi and Ramis, respectively. There is a direct correlation between the degree of gelatinization and WSI. Low moisture content of the raw material in extrusion enhances the friction and the energy dissipation to the product causing dextrinization of the starch and, at the same time, improving the WSI. According to Gutkoski and El-Dash (288) WSI is a parameter which indicates the degradation of starch granules. WSI and WAI are used as parameters for the degree of cooking of cereal products.

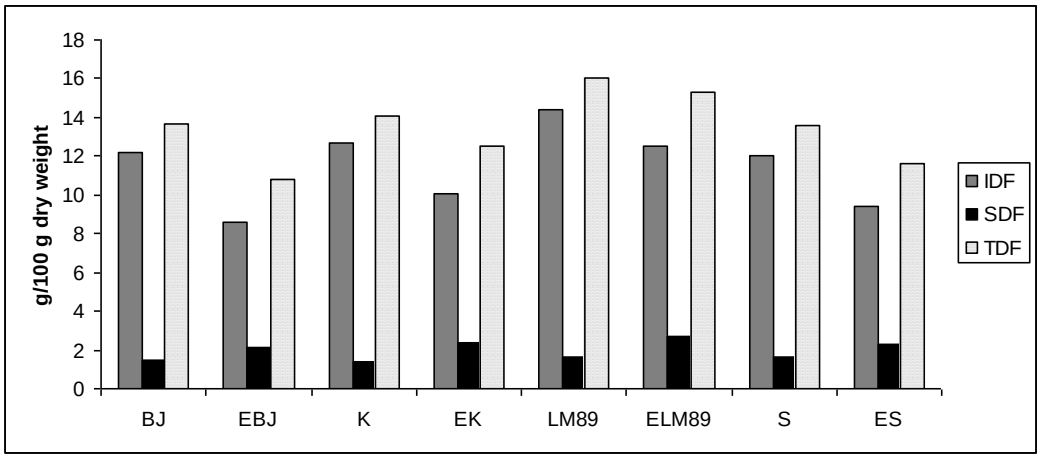
As regards the degree of gelatinization, sectional expansion index, water absorption index, water solubility index and density, the results demonstrated that the initial moisture content of 12% was the optimum to obtain an extrudate of good physicochemical characteristics.

5.3.3 EFFECT OF EXTRUSION ON CHEMICAL COMPOSITION OF QUINOA

The contents of moisture, protein, ash and crude fiber were reduced during the extrusion process in all varieties. In Figure 15, the contents of total (TDF), insoluble (IDF) and soluble dietary fiber (SDF) are presented for raw and extruded quinoa varieties (III).

There were no significant differences in the contents of TDF, IDF and SDF between the varieties. In all cases, the contents of total and insoluble dietary fiber decreased during the extrusion process; however, this decrease was significant only in the case of the Sajama variety. At the same time, the content of soluble dietary fiber increased during the extrusion process. The increase in the content of soluble dietary fiber was statistically significant in the case of the Blanca de Juli, Kcancolla and La Molina 89 varieties. Gualberto *et al.* (247) also found a decrease in the content of insoluble dietary fiber and an increase in the content of soluble fiber during extrusion-cooking. This could be due to shear stress caused by high screw speed as well as high temperature. The exposure to shear stress and high temperature causes chemical bond breakage creating smaller particles which are soluble. There is a transformation of some insoluble fiber components into soluble fiber during extrusion. Rinaldi *et al*. (289) studied the effect of extrusion on dietary fiber of wheat extrudates enriched with wet okara and their results coincide with ours. Extrusion of the formulations resulted in decreased insoluble fiber and increased soluble fiber contents of the products. Extrusion-cooking of white heat flour has also been found to cause a redistribution of insoluble to soluble
dietary fiber (280).

The extrusion-cooking process has been investigated by Lue *et al*. (290) with the expectation that mechanic rupture of the glycosidic bonds will lead to an increase of soluble fiber. In some cases, an increase of insoluble fiber was observed (291). Esposito *et al.* (292) studied the effect of extrusion on the dietary fiber of durum wheat. The data showed that the extrusion-cooking process did not have an effect on the amount of soluble dietary fiber, independent of the fiber typology of the different samples. This difference in fiber solubilization during processing could be explained by the variability in the raw material composition but also by different experimental conditions, for example screw share forces and pressure in the extrusion. The high mechanical stress during extrusion may cause breakdown of polysaccharide glycosidic bonds releasing oligosaccharides and therefore end up with an increase in soluble dietary fiber (292).



IDF= insoluble dietary fiber SDF = soluble dietary fiber TDF = total dietary fiber

Quinoa varieties: BJ = Blanca de Juli, EBJ = extruded Blanca de Juli, K= Kcancolla, EK = extruded Kcancolla, LM89 = La Molina 89, ELM89 = extruded La Molina 89, S= Sajama, ES = extruded Sajama

Figure 15. Dietary fiber in raw and extruded quinoa varieties (III).

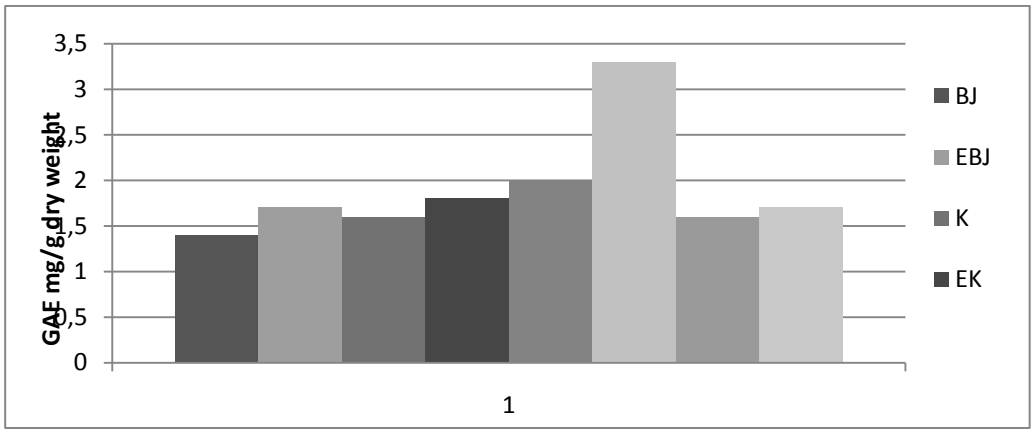
Ruales and Nair (59) determined the contents of dietary fiber in raw and processed quinoa samples. They found 13.4% of total dietary fiber for raw quinoa. This value is comparable to our values for the Blanca de Juli and Sajama varieties. The content of total dietary fiber was decreased only in cooked quinoa, while in autoclaved and drum-dried samples it remained the

same. Some soluble fiber was lost during cooking, and in autoclaved samples it was lost probably due to depolymerization of fiber components.

The content of the total phenolic compounds and the radical scavenging activity increased during the extrusion process in the case of all four varieties (Figures 16 and 17). There were significant differences between the varieties and the contents of total polyphenols.

Figure 9 presents the radical scavenging activity of raw and extruded quinoa. This increased during the extrusion process. The finding is in agreement with the report of Dewanto *et al.* (293) who discovered that the antioxidant activity and the content of total phenolics of sweet corn increased during thermal processing. An increase of the total antioxidant activity in processed grains could be explained by the increase of soluble phenolic compounds released by thermal processing. In cereals, the phenolic acids are in free, esterified and insoluble bound forms. Dewanto *et al*. (293) found that heat treatment increased the free and conjugated ferulic acid contents in sweet corn due to the release of bound ferulic acid across both the heating time and heating temperature parameters. Miranda *et al*. (36) found that air-drying quinoa had an important effect on the total phenolic content, leading to a notable reduction in these components, especially at high temperatures (e.g. 60, 70 and 80°C). However, these authors observed a higher antioxidant capacity in dehydrated quinoa compared with fresh grain.

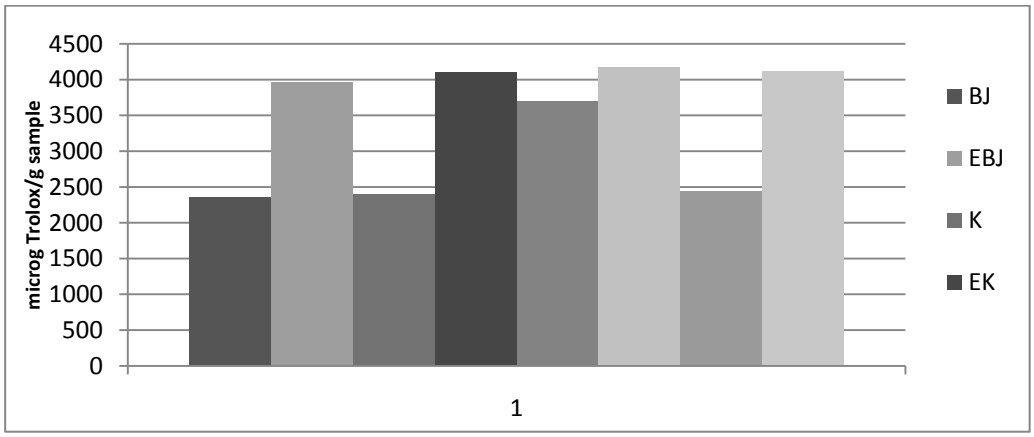
Processing methods are known to have variable effects on total phenolic compounds and antioxidant activity of food samples. Effects include little or no change, significant losses, or enhancement in antioxidant properties. Food processing can improve the properties of naturally occurring antioxidants or induce the formation of new compounds with antioxidant capacity, so that the overall antioxidant activity increases or remains unchanged (36).



Quinoa varieties: BJ = Blanca de Juli, EBJ = extruded Blanca de Juli, K= Kcancolla, EK = extruded Kcancolla, LM89 = La Molina 89, ELM89 = extruded La Molina 89, S= Sajama, ES = extruded Sajama

Figure 16. Content of total phenolic compounds in raw and extruded quinoa varieties (III).

Xu and Chang (294) studied the effect of thermal processing on total phenolics and specific phenolic compounds in yellow and black soybeans. They found that the content of phenolics increased in yellow varieties and decreased in black varieties during heat treatment. In yellow soybean varieties, thermal processing caused more free gallic acid to be released, leading to higher total phenolic content and antioxidant activity compared with raw beans.



Quinoa varieties: BJ = Blanca de Juli, EBJ = extruded Blanca de Juli, K= Kcancolla, EK = extruded Kcancolla, LM89 = La Molina 89, ELM89 = extruded La Molina 89, S= Sajama, ES = extruded Sajama

Figure 17. Radical scavenging activity of four quinoa varieties, raw and extruded (III).

The values of degree of gelatinization (DG), as well as the *in vitro* digestibility of starch and protein are presented in Table 31. The values of DG of the four quinoa varieties was between 79.9% and 89.0%. These values are lower than those found by Ruales and Nair (59) for drum-dried (96%) and cooked (97%) quinoa samples, but higher than for the autoclaved (27%) samples. Dogan and Karwe (58) investigated the physicochemical properties of quinoa extrudates. They found that the starch was only partially gelatinized to a maximum of 84.4%, depending on the extrusion conditions (feed moisture, screw speed and die temperature). During extrusion-cooking, both temperature and shear are responsible for starch gelatinization.

Table 31. Degree of gelatinization of starch, *in vitro* digestibility of starch and protein of four varieties of extruded quinoa (III).

Variety	Degree of gelatinization %	*In vitro* digestibility of starch %	*In vitro* digestibility of protein %
Blanca de Juli	79.85	68.53	80.54
Kcancolla	86.15	65.11	79.34
La Molina 89	86.74	68.42	76.80
Sajama	89.02	68.69	76.32

All data are the mean of two replicates.

Ruales and Nair (59) reported the *in vitro* digestibility of starch in raw, autoclaved, cooked and drum-dried quinoa. Their values for raw, autoclaved and cooked quinoa were lower than ours (22%, 32% and 45%, respectively), but the value for drum-dried quinoa was higher (73%). As the starch granules of quinoa are surrounded by a protein matrix, they are not very easily hydrolyzable by α-amylase. The degree of starch hydrolysis could be improved by treating quinoa flour with proteolytic enzymes prior to hydrolysis with α-amylase.

The *in vitro* digestibility of protein of the four extruded quinoa varieties was between 76.3% and 80.5%. Zia-Ur-Rehman and Shah (295) studied the protein *in vitro* digestibility of black grams after soaking and cooking. They obtained values of digestibility like our studies, between 75% and 84% for cooked black grams. Dahlin and Lorenz (296) studied protein in *vitro* digestibility of extruded cereal grains, including quinoa. The effect of extrusion on *in vitro* protein digestibility was similar in all cereals investigated.

6. CONCLUSIONS

Quinoa, kañiwa and kiwicha are used in the Andean region, but little is known of the nutritional properties and the content of bioactive components of these crops. This work provides a basic characterization of the chemical composition and scientific information for the basis of their nutritional and functional properties and potential uses. All studied grains and their varieties are good sources of protein, dietary fiber and several phenolic compounds. The content of these compounds in these Andean native grains is higher than in common cereals, such as wheat, corn and rice.

Very little information has been published concerning the phenolic acid content of *Chenopodium* and *Amaranthus* seeds. The total content of phenolic acids varied from 16.8 to 59.7 mg/100 g in different varieties of quinoa, kañiwa and kiwicha and the percentage share of soluble phenolic acids varied from 7% to 61%. There were several differences in the phenolic acid composition of the three different grains. The samples of *Chenopodium* species contained caffeic acid, ferulic acid, *p*-coumaric acid, *p*-OH-benzoic acid and vanillic acid. In addition to these, sinapic acid and protocatehuic acid were detected in *Amaranthus* samples. In quinoa varieties, the proportion of soluble phenolic acids was high. In general, the Andean grains contained lower levels of phenolic acids compared with common cereals, like wheat and rye, but the phenolic acid content of other cereals, like oat, barley, corn, rice, millet and buckwheat is of the same magnitude as in quinoa, kañiwa and kiwicha. Thus, this thesis provides a basis for nutritional information in these grains.

The flavonoid content of *Chenpodium* species was exceptionally high, varying from 36.2 to 144.3 mg/100 g. The predominant flavonoids in quinoa samples were quercetin and kaempferol, while in some varieties myrcetin and isorhamnetin were also found. Kañiwa samples contained mostly quercetin and isorhamnetin with smaller amounts of myricetin, kaempferol and rhamnetin in some varieties. As in the case of phenolic acids, much variation was found between different samples. The levels of flavonoids in quinoa and kañiwa seeds were superior to those in flavonoid-rich berries such as lingonberry and cranberry and thus these grains can be considered very good sources of flavonoids. There were no quantifiable amounts of flavonoids in amaranth (kiwicha) samples. Of the analyzed kiwicha, samples only the pink variety

contained betacyanins. No data on betacyanins in kiwicha seeds have been previously reported in the literature.

According to this thesis, the Andean grains quinoa, kañiwa and kiwicha are very good sources of iron, calcium and zinc. Compared with unenriched wheat flour, the concentration of these minerals is considerably higher in Andean grains. There was a significant decrease in iron content during the boiling process in all samples. Boiling enhanced iron, zinc and calcium bioavailability in kañiwa. Zinc bioavailability was improved in boiled quinoa and kiwicha as well. All samples demonstrated high calcium bioavailability but the iron bioavailability was relatively low in all grains. If we compare mineral bioavailability values in Andean grains with those in wheat flour, they are much lower. However, given the high content of minerals in quinoa, kañiwa and kiwicha, the potential contribution of iron, zinc and calcium would not differ greatly from that in wheat flour. In order to increase the potential contribution of minerals in Andean grains, it would be important to study the effect of different ways of processing, for example sourdough fermentation, and the use of enhancers on mineral availability.

The cooking extrusion affected the content of dietary fiber and total phenolic compounds in quinoa, kañiwa and kiwicha. The content of total and insoluble dietary fiber was decreased in all grains and varieties. The content of soluble dietary fiber was increased in the case of all four quinoa varieties and in one kiwicha variety. In quinoa, the content of total phenolic compounds and the radical scavenging activity increased during the extrusion process. In kiwicha, the content of these compounds decreased during extrusion. This provides the first information on the impact of processing on bioactive components and fiber after processing through extrusion.

Taken together, the studies presented here demonstrate that Andean indigenous crops have excellent potential as sources of flavonoids and dietary fiber. Further studies should be conducted to characterize the phenolic compounds and antioxidant composition in processed grains. Quinoa, kañiwa and kiwicha grains are consumed widely in Andean countries but they also have 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 exported to Europe and North America from Peru, Bolivia and Ecuador. Their consumption is constantly growing outside of South America. Their inclusion in the diet has the potential to improve the intake of minerals and health-promoting bioactive compounds. They may also be interesting raw

materials for special dietary foods and functional foods offering natural sources of specific health-promoting components.

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Lima, April 2011

Ritva Repo-Carrasco-Valencia

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Dietary fiber and other functional components in two varieties of crude and extruded kiwicha (*Amaranthus caudatus*)

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ABSTRACT

Two varieties (Centenario and Oscar Blanco) of Andean native grain, kiwicha (*Amaranthus caudatus*), were evaluated as sources of dietary fiber and of some bioactive compounds. The impact of low-cost extrusion on the content of these components was studied for technological applications. The content of total dietary fiber in Centenario was higher (16.4%) than in Oscar Blanco (13.8%). In both varieties, the content of total and insoluble dietary fiber decreased during the extrusion process. In Centenario, the content of soluble dietary fiber increased, from 2.5 to 3.1% during the extrusion process. The content of phytic acid in raw kiwicha was 0.3% for both varieties, and the content of total phenolic compounds was 98.7 and 112.9 mg GAE/100 g of sample, for Centenario and Oscar Blanco, respectively.
Antioxidant activity with the DPPH method for the raw kiwicha of the two varieties was 410.0 μmol trolox/g sample for Centenario and 398.1 μmol trolox/g sample for Oscar Blanco. With ABTS method those values were 827.6 and 670.1 μmol trolox/g sample for Centenario and Oscar Blanco, respectively. The content of total phenolics, phytic acid and the antioxidant activity decreased in both varieties during the extrusion process. The *in vitro* digestibility of protein and starch was improved after the extrusion process in both varieties, demonstrating potential for nutritional applications.

1. Introduction

Amaranth is an ancient and very nutritious food crop cultivated mainly in South America and Mexico, but grows also very well in different regions all over the world. Domestication of grain amaranth crops took place in tropical America. Three species of domesticated grain crops were developed in pre-Columbian America: *Amaranthus caudatus*, *Amaranthus cruentus* and *Amaranthus hypochondriacus*.

The most important Andean species is *A. caudatus* Linnaeus. In Quechua, the local language, it is called "kiwicha". It is cultivated in the Andes of Peru, Bolivia, Ecuador and Argentina. *A. caudatus* originated in the same region in the Andean highlands as the common potato. The Spanish conquerors called it Inca wheat, but it was known long before the Incas. Seeds more than 2000 years old have been found in ancient tombs (Anon, 1984).

In Peru popped kiwicha is used to make "turrones", which are kinds of snack bars. Flour of toasted seeds is used in soups, porridges and cookies. Kiwicha flour can be used in breads, substituting for wheat flour. Blends of 80% of wheat and 20% of kiwicha can be used to produce normal leavening breads. These breads have a better nutritional quality than the breads made of wheat flour.

The leaves are used as vegetables, like spinach. Usually young leaves and stems are boiled as greens. The flowers of red varieties are used as colorants in traditional beverages in Peru and Ecuador. After the grain has been threshed, the amaranth residue can be used as a source for feeding cattle. Andean farmers traditionally maintain their livestock on residues of crops during the dry season.

Edible, polymeric plant tissues resistant to digestion and absorption in the human small intestine but susceptible for complete or partial fermentation in the large intestine, is called dietary fiber (AACC, 2001). This definition includes polysaccharides, oligosaccharides, lignin and plant substances (waxes, cutin and suberin).

Cellulose is the major building block of the cell wall structure of cereals (Shelton and Jong Lee, 2000). It is insoluble in water and usually it is associated with the structural components such as hemicellulose and pectin. These three chemical components are referred to as the plant structural polysaccharides.

Arabinoxylans are pentosan heteropolysaccharides consisting mainly of arabinose and xylose residues. Pentosans form the major

Abbreviations: ABTS, 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GAE, gallic acid equivalent; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; SD, standard deviation; TDF, total dietary fiber.

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parts of hemicelluloses (Shelton and Jong Lee, 2000). Lignin and cutin are the main noncarbohydrate components of the plant cell wall.

Cereals are important sources of dietary fiber, especially of the insoluble fraction. Total dietary fiber is divided into two fractions, one of which is soluble in water and another which is insoluble. The latter is mainly related to intestinal regulation, including an increase in fecal bulk, reduced transit time of fecal material through the large intestine and other benefits, whereas the soluble fraction is involved in lowering effects on blood cholesterol and glucose intestinal absorption.

The properties of dietary fiber are affected by food processing. Chang and Morris (1990) investigated the effect of heat treatments on the components of dietary fiber in various food samples. They found that heat treatment (autoclave) reduced the content of total and insoluble dietary fiber in apples and in oat bran. In the case of corn, heat treatment (autoclave and microwaves) increased the content of soluble dietary fiber.

The composition of dietary fiber in common cereals, like wheat, rye and oat, is well known. However, there exists very little information about the dietary fiber of the native Andean crops, like amaranth or kiwicha. The common cereals contain phenolic antioxidant compounds (Yu et al., 2002). These natural antioxidants may protect DNA, protein and membrane lipids from oxidative damage in biological systems and may provide health benefits for disease prevention and health promotion (Halliwell, 1996). There is no information about the content of antioxidants in kiwicha.

Extrusion cooking is a popular food-processing technique, especially for cereals. It has many advantages: versatility, high efficiency, low cost and good product quality. Extrusion conditions (high shear, elevated temperature, and low moisture) may cause compositional and nutritional changes in the end product. Some beneficial nutritional effects of extrusion are the increased starch and protein digestibility, and destruction of anti-nutritional factors, for example trypsin inhibitors of soybeans (Cheftel, 1989). However, nutritional damage (e.g. loss of available lysine) may also occur during extrusion when very high temperature and shear forces are used. Modification in particle size, solubility and chemical structure of various fiber components may occur and cause changes in their bacterial degradation in the intestine and in their physiological properties (Cheftel, 1986). Extrusion cooking may also cause a shift from insoluble fiber to soluble fiber (Asp and Björck, 1989; Gualberto et al., 1997).

The aim of this study was to evaluate two commonly cultivated varieties, Oscar Blanco and Centenario, of the Andean native grain, kiwicha (*A. caudatus*), as sources of dietary fiber and other nutritionally bioactive compounds and the effect of low-cost extrusion on the content of dietary fiber and other components.

2. Experimental

2.1. Materials

Amaranth (*A. caudatus*) grains, 2 varieties: Centenario and Oscar Blanco, were obtained from the experimental field of the Agrarian University, Lima, Peru, in the growing season 2004–2005. The Oscar Blanco variety was chosen because it is the major commercial variety and has excellent processing properties. Centenario is a new variety developed by the National Agrarian University and specific studies on its composition are needed for future assessment.

2.2. Analysis

2.2.1. Proximate analysis

Water content, protein ($N \times 6.25$), fat, crude fiber and ash were determined according to AOAC (1995). The carbohydrates were calculated by difference using the formula

$$\mathrm{CHO} = 100 - (\mathrm{fat} + \mathrm{protein} + \mathrm{crude\ fiber} + \mathrm{ash})$$

2.2.2. Dietary fiber

The total, soluble and insoluble dietary fiber were analyzed by an enzymatic–gravimetric method according to the Approved Method 32-21 (AACC, 1995) using the TDF-100 kit from Sigma Chemical Company (St. Louis, MO).

2.2.3. Cellulose

The content of cellulose was determined according to the method of Van Soest and Wine (1968). The acid–detergent fiber analysis (AOAC method 973.18, 1995) was used to estimate indirectly the cellulose content.

2.2.4. Lignin

Lignin was determined according to the Approved Method 32-25 (AACC, 2000). The method includes selective, enzymatic removal of starch, using a thermostable α-amylase and an amyloglucosidase; precipitation of soluble polysaccharides with 80% ethanol; hydrolysis of amylase-resistant polysaccharides (precipitated and insoluble) with sulfuric acid. Klason lignin (sulfuric acid lignin) was calculated gravimetrically as the acid-insoluble residue after correction for ash.

2.2.5. Beta-glucans

The content of beta-glucans was determined according to the AOAC (1995), method 995.16. Extracts were hydrolyzed with lichenase and betaglucosidase.

Reducing sugars in the aliquots were determined using 3,5-dinitrosalicylic acid reagent. Anhydrous glucose was used as the reference compound.

2.2.6. Resistant starch

Resistant starch (RS) content was analyzed using the methodology according to the Approved Method 32-40 (AACC, 2000).

2.2.7. Pentosans

The content of pentosans was analyzed using a spectrophotometric method and the measurement was made at 552 and 512 nm according to Douglas (1981). Xylose was used as the reference compound.

2.2.8. Radical scavenging activity

Radical scavenging activity was determined according to the method of Brand-Williams et al. (1995) based on the decrease of absorbance at 515 nm produced by reduction of DPPH (2,2-Diphenyl-1-picrylhydrazyl) by an antioxidant. The Trolox was used as the reference compound.

Radical scavenging activity was also determined according to Re et al. (1999) based on the decrease of absorbance at 734 nm produced by reduction of ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) by an antioxidant. The Trolox was used as the reference compound.

2.2.9. Phenolic compounds

The content of total phenolics was analyzed according to the method of Swain and Hillis (1959). The phenolic compounds were extracted with methanol and the extract was allowed to react with the Folin–Ciocalteau phenol reagent. The absorbance was measured at 725 nm. Gallic acid equivalents were determined from a standard concentration curve.

2.2.10. Phytate

Phytic acid was determined according to Schmidt-Hebbel (1986). This method is based on indirect iron (III) complexometry.

2.2.11. Protein in vitro *digestibility*

The digestibility of proteins was determined by an *in vitro*-method according to Hsu et al. (1977). The multi-enzymatic method is based on decrease of pH during 10 min. The percentage of digestibility was calculated using a formula: $Y = 210.464 - 18.103X$, where X = pH of the protein suspension after 10 min of digestion and Y = percentage of protein hydrolysis.

2.2.12. Starch in vitro *digestibility*

The digestibility of starch was determined by an *in vitro*-method according to Holm et al. (1985). 500 mg of starch was mixed with phosphate buffer (pH 6.9) and incubated with α-amylase at 37 °C during 1 h. The sugars released were determined by spectrophotometry.

2.2.13. Statistical analysis

Each analysis was done in triplicate and expressed as means and standard deviation (SD).

2.2.14. Extrusion

The extrusion process was carried out using a low-cost extruder simulating the local processing conditions. The equipment was manufactured by Jarcon del Peru, Huancayo, Peru. This equipment is a single screw extruder having the following parameters: 254.5 rpm, resident time 10–13 s, work temperature 180 °C, 2 orifices on die. No external heat was transferred to the barrel on the screw during extrusion. The aim was to produce results applicable to local conditions where the low-cost technology is used widely.

3. Results and discussion

This study resulted in previously unreported data on the nutrient composition of kiwicha. Both varieties had relatively high protein content, 14.6% and 14.7% for Centenario and Oscar Blanco, respectively. With reference to common cereals the values were higher (Kent, 1983). The fat content was very similar for both varieties, 10.1 and 10.2%, for Centenario and Oscar Blanco, respectively. The main component was carbohydrate, 82.8 and 82.0%, for Centenario and Oscar Blanco, respectively. Detailed proximate composition of the two varieties of kiwicha can be observed in Table 1.

The results of dietary fiber determinations are shown in Table 2. The content of total dietary fiber in Centenario was higher (16.37%) than in Oscar Blanco (13.80%). The concentration of fiber of Centenario was higher than the values for wheat, oats, triticale, corn and sorghum but lower than those for barley and rye found by Picolli da Silva and Santorio Ciocca (2005). The total dietary fiber content of the Oscar Blanco variety was similar to that of corn and triticale. This result is important especially when considering the application of the study to cereals in western diets.

The content of total dietary fiber in the extruded kiwicha was similar to the content found by Plate and Areas (2002), 8.20% for *A. caudatus*. In both varieties, the content of total and insoluble dietary fiber decreased during the extrusion process. In the case of Centenario, the content of soluble dietary fiber increased from 2.45 to 3.06% during the extrusion process. However, in Oscar Blanco variety the amount of soluble dietary fiber decreased slightly (from 1.65 to 1.46%).

Table 2
Dietary fiber constituents of Oscar Blanco and Centenario varieties of kiwicha expressed as % of dry basis.

Variety	IDF	SDF	TDF
Centenario	13.92 ± 0.14	2.45 ± 0.24	16.37 ± 0.10
Extruded Centenario	6.91 ± 0.50	3.06 ± 0.25	9.97 ± 0.74
Oscar Blanco	12.15 ± 0.72	1.65 ± 0.52	13.80 ± 0.20
Extruded Oscar Blanco	7.06 ± 0.18	1.46 ± 0.16	8.51 ± 0.34

IDF = insoluble dietary fiber; SDF = soluble dietary fiber; and TDF = total dietary fiber. All data are the mean ± SD of three replicates.

Gualberto et al. (1997) investigated the effect of extrusion on the dietary fiber and phytic acid in cereal brans. They also found a decrease in the content of insoluble dietary fiber during extrusion cooking and an increase in the content of soluble fiber. This could be due to shear stress caused by high screw speed and also to high temperature. The exposure to shear stress and high temperature causes chemical bond breakage, creating smaller particles which are soluble.

There is a transformation of some insoluble fiber components into soluble fiber during extrusion.

Table 3 shows phytic acid content of raw and extruded kiwicha. The raw kiwicha contained 0.28–0.31% of phytic acid. This value coincides with the values reported by Guzman-Maldonado and Paredes-Lopez (1998). They reported the content of phytic acid for amaranth to be between 0.34 and 0.61. There was a slight decrease in the phytic acid content for both varieties during the extrusion process. However, this decrease was not significant. This coincides with the results of Gualberto et al. (1997) who did not find a significant decrease of phytate content during the extrusion process. Kiwicha contains less phytates than some common cereals, such as corn and wheat (Guzman-Maldonado and Paredes-Lopez, 1998). Gualberto et al. (1997) found 1.42, 4.32 and 5.27% of phytate in oat, rice and wheat bran, respectively.

Table 4 presents the content of total phenolic compounds, as gallic acid equivalent (GAE), in raw and extruded kiwicha.

The raw kiwicha contained 0.10 and 0.11 g gallic acid/100 g of sample, dry basis. Guzman-Maldonado and Paredes-Lopez (1998) reported levels of 2–4 mg/g 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. Raw kiwicha has more total phenolic compounds than oat. Emmons et al. (1999) analyzed the total phenolic compounds in different milling fractions of oat and they found that the content of these compounds was between 8.9 and 34.2 mg GAE/100 g of sample. The content of total phenolic compounds was lower than in bran-enriched wheat milling fractions, 130–530 mg/100 g found by Trust et al. (2005). In any case, if bran-enriched kiwicha milling fractions would be used, the content of total phenolic compounds would probably be higher than that of wheat. Del Pozo-Insfran et al. (2007) analyzed the content of total phenolic compounds in three genotypes of corn, two blue genotypes and one white. The content of total phenolic compounds was between 410 and 3430 mg/100 g, calculated as gallic acid equivalents. Dykes et al. (2005) determined the total phenolic compounds in sorghum varieties. Grain sorghum is very high in these compounds (201–910 mg gallic acid/100 g). Generally, the content of total phenolic compounds in kiwicha was lower than that of most common cereals.

Table 1
The proximate composition of kiwicha varieties Centenario and Oscar Blanco expressed as % of dry basis.

Variety	Moisture (%)	Protein (%)	Ash (%)	Fat (%)	Crude fiber (%)	Carbohydrates (%)
Centenario	9.80 ± 0.10	14.55 ± 0.17	2.39 ± 0.05	10.08 ± 0.16	7.43 ± 0.10	65.55
Oscar Blanco	9.44 ± 0.09	14.70 ± 0.12	2.61 ± 0.01	10.15 ± 0.03	7.27 ± 0.11	65.27

All data are the mean ± SD of three replicates.

Table 3
Phytic acid content in Centenario and Oscar Blanco varieties of kiwicha as % of wet basis.

Variety	% Phytic acid
Centenario	0.31 ± 0.02
Extruded Centenario	0.23 ± 0.00
Oscar Blanco	0.35 ± 0.01
Extruded Oscar Blanco	0.28 ± 0.02

All data are the mean ± SD of three replicates.

Table 5
Radical scavenging activity of two varieties of raw and extruded kiwicha expressed as μmol trolox/g sample dry basis.

Variety	Radical scavenging activity μmol trolox/g sample, DPPH method	Radical scavenging activity μmol trolox/g sample, ABTS method
Centenario	410.19 ± 0.01	827.61 ± 0.08
Extruded Centenario	180.32 ± 0.00	692.78 ± 0.01
Oscar Blanco	398.09 ± 0.03	670.06 ± 0.03
Extruded Oscar Blanco	281.30 ± 0.01	276.40 ± 0.02

All data are the mean ± SD of three replicates.

The extrusion process affected the content of total phenolic compounds in both varieties, decreasing it 80.3% and 64.4% for Centenario and Oscar Blanco, respectively. Del Pozo-Insfran et al. (2007) also studied the effect of processing on the total phenolic compounds and found that Mexican white corn lost 90% of its initial total phenolic content, while the Mexican and American blue corns lost 61 and 78% during lime-cooking. Phenolic acids can suffer decarboxylation during processing of food. Ferulic acid, for example, forms *p*-vinylguaiacol during storage of orange juice (Maillard and Berset, 1995). P-coumaric acid can form *p*-hydroxybenzaldehyde (Dimberg et al., 1996).

The radical scavenging activity of the two varieties of kiwicha is presented in Table 5.

Radical scavenging activity for the raw kiwicha of the two varieties was 410 μmol trolox/g sample for Centenario and 398 μmol trolox/g sample for Oscar Blanco measured by the DPPH method. Using the ABTS method these values were 827 and 670 μmol trolox/g sample for Centenario and Oscar Blanco, respectively. These values are high compared with other cereals. Awika et al. (2003) determined the antioxidant activity of sorghum and sorghum products using the DPPH and ABTS methods. They found antioxidant activities with DPPH for different sorghum varieties between 6 and 202 μmol trolox/g sample. With ABTS, these values were between 6 and 226 μmol trolox/g. The brans of the red variety and the high tannin variety demonstrated higher values of antioxidant activity, 21–716 μmol trolox/g sample (DPPH) and 28–768 μmol trolox/g sample (ABTS), similar to this study. They found that the ABTS values were higher than the DPPH values for black sorghum brans. In our study, the ABTS values were higher than the DPPH which coincides with the results of Awika et al. (2003). Zielinska et al. (2007) studied the antioxidant capacity of buckwheat sprouts using the DPPH method. They found antioxidant capacity between 41.55 and 218.36 μmol trolox/g sample for the sprouts. For different grain products lower values than those found in this study are reported: whole wheat flakes 35, whole wheat biscuit 30, whole grain oat flake 27, whole grain puffed oat 26, corn flakes 20 μmol trolox/g sample (Miller et al., 2000). In our study we found moderate correlation between phenolic compounds and antioxidant activity with the ABTS method ($R = 0.584$) and high correlation between the phenolic compounds and the DPPH method ($R = 0.959$). This observation is in agreement with Katsube et al. (2004) who reported that there was a high correlation between DPPH and Folin–Ciocalteau assays ($R = 0.969$). In Andean grains, the phenolic compounds are the principal contributors to the antioxidant capacity. They are not very rich in vitamin C and there is evidence that the contribution of vitamin C to the antioxidant activity is lower than that of phenolic compounds (Proteggente et al., 2002).

The cooking extrusion process affected antioxidant activity of the two varieties of kiwicha, decreasing their values. In the case of Centenario, there was a reduction of 16–56% of the original value and in the case of Oscar Blanco, a decrease of 29–58% from the original value. Del Pozo-Insfran et al. (2007) found a difference in loss of antioxidant capacity for different varieties of corn in the processing of nixtamals, tortillas and chips. The blue varieties suffered a higher loss than the white varieties. The average loss for nixtamals, tortillas and chips was 42, 49 and 62% of the initial antioxidant capacity, respectively.

Table 6 summarizes the results of the digestibility evaluation of protein *in vitro* of crude and extruded kiwicha. The digestibility of the two varieties of kiwicha was improved by the extrusion process. Oscar Blanco had a higher value of digestibility *in vitro* than Centenario. Both varieties had lower digestibility than the casein, used as the reference material. Zamora (2003) found a digestibility *in vitro* for proteins of 89.46% for extruded Jack bean (*Canavalia ensiformis*).

The results of the digestibility of starch *in vitro* for crude and extruded kiwicha are presented in Table 7. In both cases the digestibility was clearly improved during the extrusion process. As in the case of protein digestibility, Oscar Blanco had higher values of digestibility than Centenario. Zamora (2003) also found that the extrusion process improved the digestibility of starch of Jack bean, from 37.7% to 53%. The cooking extrusion process causes gelatinization of starch which makes it available to the digestive enzymes.

In Table 8, the content of nonstarch polysaccharides, lignin and resistant starch are presented.

The content of cellulose for the two varieties of kiwicha was 3.88 and 2.51%, for Centenario and Oscar Blanco, respectively. These values are similar to the values of whole meal wheat flour, 2.46% (Lineback and Rasper, 1988) and superior to the content of cellulose in whole rye, 1.3% (Glitsot and Bach Knudsen, 1999). Kiwicha has also more cellulose than corn, millet, rice and sorghum (Wang et al., 1991).

Lignin contents in the two kiwicha varieties were 3.95 and 3.97% (Centenario and Oscar Blanco, respectively). It was higher than

Table 4
Total phenolic compounds in Centenario and Oscar Blanco varieties of kiwicha, expressed as mg GAE/100 g dry basis.

Variety	mg GAE/100 g sample
Centenario	112.89 ± 0.09
Extruded Centenario	22.22 ± 0.01
Oscar Blanco	98.68 ± 0.00
Extruded Oscar Blanco	35.15 ± 0.00

All data are the mean ± SD of three replicates.

Table 6
Digestibility of protein *in vitro* of two varieties of raw and extruded kiwicha.

Sample	%
Centenario, raw	78.78 ± 1.64
Centenario, extruded	83.40 ± 2.30
Oscar Blanco, raw	80.05 ± 0.31
Oscar Blanco, extruded	86.02 ± 1.02
Casein	91.22 ± 1.02

All data are the mean ± SD of three replicates.

Table 7
Digestibility of starch *in vitro* of two varieties of raw and extruded kiwicha.

Sample	%
Centenario, raw	31.29
Centenario, extruded	62.78
Oscar Blanco, raw	33.45
Oscar Blanco, extruded	68.78

lignin content in rye found by Glitsot and Bach Knudsen (1999), 1.5% and this could be of nutritional importance.

Beta-glucan content in Centenario was 0.97% and in Oscar Blanco was 0.63%. These values are low compared with rye, 1.5% (Glitsot and Bach Knudsen, 1999), oat, 3.9–6.8 (Wood, 1984) and barley, 3.9–6.5% (Vasanthan et al., 2002) but very similar found in some wheat varieties, up to 1% (Lineback and Rasper, 1988).

The content of pentosans (arabinoxylans) in Centenario was 0.72% and in Oscar Blanco it was 0.98%. Rye is very rich in pentosans, 8.5% (Henry, 1985) and they are very important for the baking properties of rye flour. All common cereals have higher content of pentosans than kiwicha, for example wheat has 6.6%, barley 5.9%, rice 1.2%, oat 7.7% (Henry, 1985).

Resistant starch content in Centenario was 0.12% and in Oscar Blanco it was 0.10%. These values are lower than the values found for rice and corn, 2.63% and 2.85%, respectively by Rosin et al. (2002). Vasanthan et al. (2002) found a resistant starch content of 0–0.83% for barley.

Kiwicha is a good source of protein, dietary fiber and good quality fat. The content of these components in kiwicha is higher than in cereals like wheat, corn and rice, which are commonly consumed in Peru and all over the world. The two varieties of kiwicha had high dietary fiber content, especially the insoluble fraction when compared with common cereals.

Cooking extrusion decreased the content of total and insoluble dietary fiber in both varieties. At the same time, there was a slight increase of the soluble portion of dietary fiber in one variety. Centenario had more dietary fiber, total phenolics, beta-glucans and higher antioxidant activity than the Oscar Blanco variety. Oscar Blanco was richer in pentosans and starch and protein digestibility *in vitro* was higher than that of Centenario. The content of beta-glucans, pentosans and resistant starch in the two varieties of kiwicha was lower than in common cereals. The content of total phenolics, phytic acid and antioxidant activity was decreased in both varieties during the extrusion process. The digestibility of protein and starch was improved after the extrusion process. These results show that extrusion cooking has similar effects on the dietary fiber, phenolic compounds and phytates of kiwicha as on these compounds in common cereals. Our study adds to previous works (Dimberg et al., 1996; Gualberto et al., 1997; Vasanthan et al., 2002) that gives information on specific species having better nutritional properties than the common cereals.

Taken together, this study demonstrates that kiwicha can be considered a very nutritive cereal when compared with commonly used cereals such as wheat, barley and corn. It has a relatively high content of good quality protein and can be considered as a good source of dietary fiber and other bioactive compounds such as phenolics. Further studies should be conducted to characterize phenolic compound composition and antioxidant content and activity of specially colored varieties of kiwicha.

Table 8
Cellulose, lignin, beta-glucan, pentosan and resistant starch content in Centenario and Oscar Blanco variety of kiwicha (g/100 g on dry basis).

Variety	Cellulose	Lignin	Beta-glucans	Pentosans	Resistant starch
Centenario	3.88 ± 1.69	3.95 ± 0.45	0.97 ± 0.07	0.72 ± 0.05	0.12 ± 0.01
Oscar Blanco	2.51 ± 1.98	3.97 ± 0.42	0.63 ± 0.19	0.98 ± 0.06	0.10 ± 0.01

All data are the mean ± SD of three replicates.

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ORIGINAL PAPER

Chemical and Functional Characterization of Kañiwa (*Chenopodium pallidicaule*) Grain, Extrudate and Bran

II

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Abstract Cereals provide a good source of dietary fibre and other important compounds with nutritional potential, such as phenolic compounds, antioxidants, minerals and vitamins. Although native Andean cereals are known to have high nutritional value, their minor components have not been studied thoroughly. In this study, two varieties of a native Andean crop, kañiwa (*Chenopodium pallidicaule*), were investigated as sources of dietary fibre and specific antioxidant compounds. Two products, an extrudate and bran, were also prepared and their functional properties and bioactive compounds were determined. Both varieties were rich in total dietary fibre and lignin, and the phenolic components analyzed had high antioxidant activity. The extrudates had good functional properties, such as degree of gelatinization, sectional expansion index and water solubility index; the bran was high in bioactive compounds, such as total phenolics. In conclusion, kañiwa may offer an alternative to traditional cereals as a health-promoting food ingredient.

Keywords *Chenopodium pallidicaule* · Dietary fibre · Extrusion · kañiwa

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Abbreviations

CEC	cation exchange capacity
d.b.	dry basis
DG	degree of gelatinization
DPPH	2,2-diphenyl-1-picrylhydrazyl
IIF	insoluble indigestible fraction
SD	standard deviation
SEI	sectional expansion index
SIF	soluble indigestible fraction
TIF	total indigestible fraction
WAI	water absorption index
WSI	water solubility index

Introduction

Kañiwa (*Chenopodium pallidicaule*) is a remarkably nutritious grain from the Andean highlands. Its protein content and quality is exceptional and it is also rich in micronutrients such as iron and calcium. It is grown on Andean highland plateaus at over 4,000 m.a.s.l. For people who live on subsistence agriculture in the *altiplano*, kañiwa is extremely important as a main source of calories and good quality proteins. It is a resistant plant that flourishes in poor and rocky soil and can survive frosts and drought. Kañiwa is usually unaffected by snowstorms and strong winds that destroy fields of barley and even its parent, quinoa (*Chenopodium quinoa*) [1]. When all other crops fail, kañiwa still provides food for highland farmers, thus securing their survival.

Kañiwa is not a true cereal equivalent to its parent crop quinoa. This annual, herbaceous plant is 0.2–0.7 m high and its seeds are 1.0–1.2 mm long. Red, yellow or green colour variation occurs in the stalks and leaves of kañiwa. It

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also varies in precocity: one kind matures within only 95 days from the sowing date, although most varieties require about 150 days before they can be harvested [2]. At the time of the Spanish conquest, both kañiwa and quinoa were considered very important foods in high Andean highlands. At present, kañiwa is grown mainly in the Peruvian and Bolivian *altiplano*. Kañiwa is mainly grown by families for their own consumption. Kañiwa is normally prepared as *kañiwaco*, toasted kañiwa flour. This nutty-tasting flour is mixed with water or milk and eaten as breakfast. It is taken by local people on their long travels, because of its high caloric and protein value. Kañiwa flour can be used in bread, noodles and pastry. Some varieties of kañiwa expand when toasted and can be included in sweets and snacks. Kañiwa can also be used in weaning food mixtures.

Kañiwa is used in traditional medicine in the Andes, its stem ash, *llipta,* is used when chewing coca leaves. *Llipta* is rich in calcium and provides this essential nutrient to the diet of highland people. The nutritional value of kañiwa has been studied by White et al. [3], deBruin [4] and Gross et al. [5]. White et al. [3] discovered that the nutritional value of kañiwa proteins is equivalent to that of milk proteins. Gross et al. [5] reported that kañiwa contains 15.3% protein and a nutritionally balanced amino acid composition. The chemical score of amino acids in kañiwa is high [6]. Unlike its parent quinoa, which contains bitter tasting saponins, kañiwa can be used directly as food without washing. Although it was thought that it did not contain saponins, Rastrelli et al. [7] found seven triterpene saponins in kañiwa seed; however, the content is very low and the saponins do not give the product a bitter taste. Kañiwa is relatively rich in oil containing mainly unsaturated fatty acids. The content of tocopherols in kañiwa oil is higher than that of corn oil [6].

There are several studies that confirm that whole grain cereals protect the body against age-related diseases such as diabetes, cardiovascular diseases and some cancers [8]. The components responsible for this protective effect include dietary fibre, polyphenols, vitamins and minerals. In general, there are very few studies on the minor components of kañiwa. In one of them, Rastrelli et al. [9] isolated and characterized two new flavanol glycosides in kañiwa. Peñarrieta et al. [10] determined the total antioxidant capacity and the content of phenolic compounds in Bolivian kañiwa varieties. We did not find any study on dietary fibre in kañiwa. Furthermore, there are no studies on processed kañiwa products, their nutritional value and functional properties.

Consequently, the aims of this study were to evaluate two varieties of kañiwa (*Chenopodium pallidicaule*) grain, as sources of dietary fibre and other nutritionally bioactive compounds, and to obtain kañiwa extrudate and bran. The functional properties of these products were determined as well as their content of dietary fibre and bioactive compounds.

Experimental

Materials

Two varieties of kañiwa (*Chenopodium pallidicaule*)—'Cupi' and 'Ramis'—were obtained from the Agrarian Experimental Station Illpa, department of Puno, Peru.

Preparation of Kañiwa Bran The kañiwa grain was cleaned using a sifting machine with sieves of 1.40 and 0.85 mm. After the cleaning the grain was milled in a laboratory mill, Cyclotec 1093 (FOSS Inc. Denmark), using 1.00 mm mesh. The meal was then sieved with sieves of 0.425 and 0.212 mm to obtain bran of two different particle sizes.

Extrusion Process The kañiwa grain was cleaned by sifting with sieve No. 16 US Standard Sieves ASTM. Five hundred grams of cleaned grain were placed in plastic bags for the humectation process. The grain was moistened to three different humidity levels (12, 14, 16%) for extrusion. Three replicates were run for each humidity level.

The extrusion process was carried out at 180 °C, with a screw speed of 254.5 rpm and a residence time of 10–13 s. The extruder was a single screw extruder manufactured by Jarcon del Peru, Huancayo, Peru. No external heat was transferred to the barrel of the screw during extrusion. The aim was to produce results applicable to local conditions where this low cost technology is widely used.

Analysis

Chemical Analysis

Proximate Analysis Water content, proteins (N ×6.25), fat, crude fibre and ashes were determined according to the AOAC [11]. Carbohydrates (CHO) were calculated by difference using the formula $\mathrm{CHO} = 100 - (\text{moisture} + \textit{fat} + \textit{protein} + \textit{crude fibre} + \textit{ash})$

Dietary Fibre The total, soluble and insoluble dietary fibre were analyzed by an enzymatic-gravimetric method according to the Approved Method 32–21 [12] using the TDF-100 kit from Sigma Chemical Company (St. Louis, MO, USA).

Lignin Lignin was determined in accordance with the Approved Method 32–25 [13]. The method includes selective enzymatic removal of starch using a thermostable alpha-amylase and an amyloglucosidase; precipitation of soluble polysaccharides with 80% ethanol; and hydrolysis of amylase-resistant polysaccharides (precipitated and insoluble) with sulphuric acid. Klason lignin (sulphuric acid lignin) was calculated gravimetrically as the acid-insoluble residue after correction for ash.

Beta-Glucans The content of beta-glucans was determined according to the AOAC [11], method 995.16. Extracts were hydrolyzed with lichenase and betaglucosidase. Reducing sugars in the aliquots were determined using 3,5-dinitrosalicylic acid reagent. Anhydrous glucose was used as the reference compound.

Resistant Starch Resistant starch (RS) content was analyzed using the methodology according to the Approved Method 32–40, AACC [13].

Phytate Phytic acid was determined according to Schmidt-Hebbel [14]. This method is based on indirect iron (III) complexometry.

Antioxidant Activity Antioxidant activity was determined according to the method of Brand-Williams et al. [15] based on the decrease of absorbance at 515 nm produced by reduction of DPPH (2, 2-diphenyl-1-picrylhydrazyl) by an antioxidant. Trolox was used as the reference compound.

Total Phenolics The content of total phenolics was analyzed according to the method of Swain and Hillis [16]. The phenolic compounds were extracted with methanol and the extract was allowed to react with the Folin–Ciocalteau phenol reagent. The absorbance was measured at 725 nm. Gallic acid equivalents were determined from a standard concentration curve.

Fraction The indigestible fraction was determined according to the *in vitro* method of Saura-Calixto et al. [17]. This is an alternative method to dietary fibre analysis.

Physicochemical Properties

Physicochemical Properties of Extruded Kañiwa

The following indexes and properties were determined:

$$\text{Water absorption index (WAI)} = \frac{\text{g water absorbed}}{\text{g dry sample } (1 - \text{soluble fraction})}$$

$$\text{Water solubility index(WSI)} = \frac{\text{g water soluble matter}}{\text{g dry sample}}$$

The sectional expansion index (SEI) was measured as the ratio of the diameter of the extrudate to that of the die. SEI is dimensionless [18].

Degree of gelatinization (DG) [19]

Density [20]

Physicochemical Properties of Kañiwa Bran

Water-holding capacity was measured according to Robertson and Eastwood [21].

Swelling and oil absorption capacity were measured using the methods proposed by Tamayo and Bermudez [22].

Cationic exchange capacity (CEC) was tested using the methodology of McConnel et al. [23].

Statistical Analysis

Each analysis was done in triplicate and the results are expressed as means and standard deviation (SD).

The data were analyzed by analysis of variance, and Tukey's test (significance of differences $p<0.05$) was used to find significant differences between the samples and treatments.

Results and Discussion

Characterization of Two Varieties of Kañiwa Grain

Table 1 presents the proximate composition and the components of dietary fibre of raw and extruded kañiwa. Both varieties of kañiwa are good sources of protein, fat and especially of dietary fibre. Kañiwa has a higher dietary fibre content than common cereals such as wheat, rye and barley. Nyman et al. [24] reported a total dietary fibre content of 12.1%, 16.1% and 18.8% for wheat, rye and barley, respectively. The content of insoluble dietary fibre was very high for both varieties.

According to this study, kañiwa cannot be considered a good source of betaglucans because the content of this compound was very low (0.04–0.07%). Oat has about 3–7% betaglucans [25]. The lignin content was 6.88% for Cupi and 7.98% for Ramis. This content is relatively high compared to other cereals: 2.0%, 2.1%, 3.5%, 2.5%, 3.9% and 1.4%, for wheat, rye, barley, sorghum, rice and corn, respectively [24].

There were statistically significant differences between the two varieties of kañiwa in moisture, fat, crude fibre and ash content.

The content of phenolic compounds was 2.54 and 2.43 mg of gallic acid equivalent (GAE)/g for Cupi and Ramis, respectively (Table 2). This content is higher than in oat [26], buckwheat, quinoa and rice [27]. Yawadio et al. [27] analyzed the total phenolic compounds in quinoa and amaranth (*A. hypochondriacus*, *A. cruentus*) and found a content between 94.3 and 148 mg/g tannic acid equivalent. Phytate content for the two kañiwa varieties was very similar, at about 8.0 mg/g, which is higher than in amaranth

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Table 1 Composition of raw and extruded kañiwa varieties 'Cupi' and 'Ramis'

Component	Raw grain		Extruded	
	Cupi	Ramis	Cupi	Ramis
Moisture %	10.37±0.19	11.79±0.10	4.08±0.56	3.67±0.18
Proteins %	14.41±0.26	14.88±0.46	14.33±0.26	13.93±0.55
Fat %	5.68±0.02	6.96±0.24	5.47±0.08	5.79±0.03
Crude fibre %	11.24±1.15	8.18±0.02	4.33±0.08	4.79±0.42
Ash %	5.03±0.21	4.33±0.26	4.48±0.10	3.98±0.39
Carbohydrates %	63.64	65.65	71.39	71.51
Total dietary fibre %	25.24	25.95	18.93	20.12
Soluble dietary fibre%	2.98±0.42	2.79±0.57	2.00±1.06	2.24±1.59
Insoluble dietary fibre %	22.27±2.30	23.16±0.89	16.93±0.99	17.88±2.40
Resistant starch %	0.24±0.03	0.26±0.04	0.43±0.01	0.31±0.03
Lignin %	6.88±0.34	7.98±1.04	6.30±0.27	5.61±0.22
Betaglucans %	0.07±0.02	0.04±0.04	0.07±0.01	0.01±0.01

All data are the mean ±SD of three replicates. All the contents are shown in g/100 g dry weight except moisture, which is shown in g/100 g fresh weight. Extrusion conditions: temperature 180 °C, moisture 12 g H_2O/100 g dry weight

(3.4–6.1 mg/g [28]) but lower than common cereals. Gualberto et al. [29] found 14.2, 43.2 and 52.7 mg/g of phytate in oat, rice and wheat bran, respectively. Phytic acid has long been considered as an anti-nutrient because it chelates minerals and trace elements. However, its antioxidant potential is now recognized [8].

Antioxidant activities were 4,200 and 4,050, expressed as microgrammes of trolox equivalent (TE)/g for Cupi and Ramis, respectively. These values are high compared with some other products, such as red cabbage (2,500 mcg trolox equivalent/g), potatoes (800 mcg trolox equivalent/g) and sweet potatoes (800 mcg trolox equivalent/g) [30]. Villarreal-Lozoya et al. [31] studied the antioxidant activity of different pecan cultivars using the DPPH method. They found an average antioxidant activity of 97,000 microgrammes of trolox equivalent/g. Lower values than those found in this study have been reported for other grain products: whole wheat flakes 35, whole wheat biscuit 30, whole grain oat flake 27, whole grain puffed oat 26, and corn flakes 20 μmol trolox/g sample [32]. The antioxidant activity of kañiwa is lower than that of blueberries and purple corn. Cevallos-Casals and Cisneros-Zevallos [33] used the same method as us, resulting in a value of 35,232 microgrammes trolox eq./g (dry basis) for blueberries and 23,132 microgrammes trolox eq./g (dry basis) for purple corn. Campos et al. [34] studied the antioxidant activity of Andean native potatoes and other less well-known Andean tubers, mashua, oca and ulluco. They obtained values from 483 to 9,800 mcg trolox eq./g. Peñarrieta et al. [10] analyzed the total antioxidant capacity in ten samples of Bolivian kañiwa using the ABTS and FRAP method; their values were lower than our values. This could be due to the different extraction procedure used.

Table 2 Phenolic compounds, phytates and antioxidant activity in two varieties of kañiwa grain

Variety	Phenolic compounds, mg gallic acid equivalent/g d.b.	Phytate % d.b.	Antioxidant activity microgrammes trolox eq./g d.b.
Cupi	2.54±1.20	0.83±0.03	4200±5.50
Ramis	2.43±1.35	0.84±0.04	4050±5.33

All data are the mean ±SD of three replicates

Characterization of Extruded Kañiwa

The content of fat and crude fibre was reduced during the extrusion process. There was a significant decrease in the total and insoluble dietary fibre content of both varieties of kañiwa. Frolich and Hestangen [35] analyzed the total dietary fibre content in rye grain and extruded rye. They observed a decrease in total dietary fibre from 16.8% to 12.7%. The content of soluble dietary fibre was also significantly decreased in both varieties according to analysis of variance. Björck et al. [36] obtained similar results in extrusion of wheat flour: the content of soluble dietary fibre decreased from 2.3% to 1.7%.

The content of resistant starch was increased during the process of extrusion, from 0.24% to 0.43% in Cupi and from 0.26% to 0.31% in Ramis. Huth et al. [37] also found an increase in resistant starch in barley during the extrusion process, especially at high temperatures (170 °C). The increase in resistant starch during the extrusion process can be explained by the modification of the amylase structure.

Gonzalez-Soto et al. [38] studied the effect of extrusion on the resistant starch content of corn and found between 1.97% and 2.05%, with a decrease as the screw velocity was increased. This is probably due to the increase in shear stress, which causes rupture in the structure of resistant

Table 3 Functional properties of two varieties of kañiwa extrudate

Variety/moisture %	DG %	SEI	Density g/ml	WAI	WSI
Cupi					
12	98.35±1.19	1.98±0.27	0.10±0.00	2.88±0.41	0.48±0.06
14	96.61±0.97	1.77±0.28	0.20±0.02	3.84±0.48	0.36±0.08
16	88.33±1.16	1.61±0.29	0.30±0.01	3.96±0.33	0.32±0.05
Ramis					
12	98.19±0.86	1.87±0.27	0.14±0.01	3.20±0.41	0.45±0.05
14	97.14±1.27	1.63±0.05	0.22±0.01	3.48±0.19	0.39±0.02
16	96.27±2.30	1.39±0.04	0.39±0.01	3.83±0.28	0.32±0.02

All data are the mean + /-SD of three replicates

DG degree of gelatinization, *SEI* sectional expansion index, *WAI* water absorption index (g H_2O/g dry sample). *WSI* water solubility index (g water soluble matter/g dry sample)

starch. Resistant starch acts as soluble fibre in the colon. It is fermented by the intestinal microflora resulting in formation of short-chain fatty acids that are protective to the colon mucosa [37]. The lignin content of Ramis and Cupi varieties was decreased in both cases.

Benchaar et al. [39] also found a decrease in lignin content from 2.3% to 1.1% for raw and extruded horse beans, respectively. The content of betaglucans in extruded kañiwa was not significant.

The three extrudates with different initial moisture were evaluated by degree of gelatinization (DG), sectional expansion index (SEI), water absorption index (WAI), water solubility index (WSI) and density g/ml, with the aim of choosing the best treatment. The results of this evaluation are presented in Table 3.

Table 3 shows that the degree of gelatinization decreased as the initial moisture of grains increased. According to Harper [40], insufficient shear stress for gelatinization of starch is achieved in raw materials with a high moisture content. The highest DG was reached at 12% of initial moisture for both varieties, with a value of 98.4% and 98.2% for Cupi and Ramis, respectively. Dogan and Karwe [18] studied the physicochemical properties of quinoa (*Chenopodium quinoa*) extrudates. They found a maximum DG of 84.4%, which is lower than the values found in our study. They used higher initial moistures (16–24%) and lower temperatures (130–170 °C) than we used (180 °C). In general, starchy materials need low feed moisture and high product temperature to reach a high DG. Materials with a high lipid content, like kañiwa, also need elevated shear stress for effective extrusion cooking [18].

The sectional expansion index decreased as initial moisture content increased. The highest SEI was achieved with the initial moisture of 12%, with values of 1.98 for Cupi and 1.87 for Ramis. The expansion of cereals in extrusion depends on the degree of gelatinization of the starch, as demonstrated here, the highest expansion index was reached at the highest degree of gelatinization. Dogan and Karwe [18] measured SEI in whole meal quinoa extrudates and found values from 0.92 to 3.58. In their study SEI was significantly affected by temperature, feed moisture content and screw speed. A high expansion ratio at low feed moisture content for extruded products is typical for cereals.

The density of the extrudate increased when the initial moisture was increased. Huth et al. [37] discovered that the density of barley extrudate increased when the initial moisture content was high. Similar results were shown by Gambus et al. [41] for corn and wheat extrudates. Lee et al. [42] mentioned that the extruded products generally have a density of between 0.1 and 0.2 g/ml. The extrudates of kañiwa with an initial moisture content of 12% had a density of 0.10 and 0.14 g/ml, for Cupi and Ramis, respectively. Gambus et al. [41] found the following values of density for corn and wheat starch extrudates: 0.23–0.30 and 0.12–0.28 g/ml. They used a higher initial moisture than we used in our study.

The water absorption index (WAI) increased when the initial moisture increased. The WAI depends on the availability of hydrophilic groups and the gel formation capacity of the macromolecules [43]. It is a measure of denatured starch together with protein denaturation and new macromolecular complex formation [18]. Extruded products should have a low WAI to maintain the crispiness of the final product. The lowest WAI was achieved at the initial moisture content of 12% for both varieties. The WAI was 2.88 and 3.20 for Cupi and Ramis, respectively. These values were lower than the values found by Dogan and Karwe [18] for quinoa extrudates.

The water solubility index (WSI) decreased as initial moisture content increased. This fact can be explained by the greater rupture of starch granules at the lower initial humidity. The highest WSI was obtained using an initial moisture of 12% for both varieties. These values were 0.48 and 0.45 for Cupi and Ramis, respectively. There is a direct correlation between the degree of gelatinization and WSI. Low moisture content in the raw material in extrusion enhances the friction and the energy dissipation to the product, causing the dextrinization of the starch and, at the same time, improving the WSI. According to Gutkoski and

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Table 4 Physicochemical properties (water-holding capacity, swelling capacity, oil absorption capacity, cation exchange capacity) of kañiwa bran

Properties	Cupi		Ramis	
	Particle size mm		Particle size mm	
	0.425	0.212	0.425	0.212
Water-holding capacity g H_2O/g fibre d.b.	5.42±0.15 a,x	3.54±0.07 a,y	4.66±0.26 b,x	3.30±0.09 b,y
Swelling capacity ml H_2O/g fibre d.b.	4.19±0.28 a,x	3.19±0.02 a,y	0.70±0.14 b,y	2.20±0.28 b,x
Oil absorption capacity g oil/g fibre d.b.	3.57±0.15 a,x	2.36±0.11 a,y	2.09±0.25 b,x	1.61±0.09 b,y
Cation exchange capacity meq H + /g fibre d.b.	0.57±0.05 a,x	0.37±0.01 b,y	0.44±0.04 b,y	0.42±0.04 b,y

All data are the mean ±SD of three replicates

a,*b* significant differences between the varieties; *x*,*y* significant differences between the particle size

El-Dash [44], *WSI* is a parameter that indicates the degradation of starch granules. WSI and WAI are used as parameters for the degree of cooking of cereal products.

Regarding the degree of gelatinization, sectional expansion index, water absorption index, water solubility index and density, the results demonstrated that the initial moisture content of 12% was the optimum to obtain an extrudate with good physicochemical characteristics.

Characterization of Kañiwa Bran

Table 4 presents the physicochemical properties of kañiwa bran of two different particle sizes. The water holding capacity was between 3.30 and 5.42 g H_2O/g. The effect of two factors, variety and particle size, were significant according to analysis of variance. Cupi had a higher water-holding capacity than Ramis, and the fibres of larger particle size also demonstrated a higher capacity than the fibres of smaller particle size. This can be partly explained by the reduction of pores or intercellular spaces in the smaller particles [45]. This property is important for dietary fibre from a technological and physiological point of view. Fibre-rich fractions with high water-holding capacity can be used as functional ingredients to avoid syneresis and to modify viscosity and texture in food products. Physiologically this kind of fibre is related to an increase in faecal bulk and fermentability of fibre in the gut [46]. The hydration properties of fibre depend on the physicochemical nature of the fibre constituents, especially of soluble fibre. Generally cereal fibre has a water-holding capacity between 5 and 10 g H_2O/g of fibre [46].

Cupi had a higher swelling capacity than Ramis. Particle size affected this property differently in the two varieties. In the case of Cupi the swelling capacity decreased with decreasing particle size. In Ramis the effect was the opposite: there was an increase in swelling capacity with smaller particle size.

In both varieties, the oil absorption capacity decreased when the particle size of the bran decreased. Cupi had higher values than Ramis. Generally, these differences were statistically significant. This property is important from the physiological point of view. Fibre with a high oil absorption capacity can have a beneficial effect in intestinal absorption of lipids. Dietary fibre decreases the absorption of lipids due to its effect on increasing intestinal bulk and decreasing the transit time, which hinders the action of digestive enzymes and absorption in the upper gastrointestinal tract [47].

The cation exchange capacity (CEC) decreased with decreasing particle size in Cupi. This variety had a significantly higher capacity than Ramis in the case of particles of 0.44 mm. The CEC of kañiwa bran was higher than the CEC of bran in wheat and similar to that of corn [48]. This property is related to the capacity of some fibres to form insoluble complexes with inorganic ions. This may cause an increase in faecal excretion of some nutritionally important minerals and electrolytes.

Table 5 Indigestible fraction in kañiwa bran of kañiwa varieties Cupi and Ramis

Variety	IIF (% d.b.)	SIF (% d.b.)	TIF (% d.b.)
Cupi	60.91±3.36	0.75±0.15	61.66±3.51
Ramis	54.34±0.54	1.00±0.11	55.34±0.43

All data are the mean ±SD of three replicates

IIF insoluble indigestible fraction, *SIF* soluble indigestible fraction, *TIF* total indigestible fraction

Table 6 Phenolic compounds, phytates and antioxidant activity in kañiwa bran (varieties Cupi and Ramis)

Variety	Phenolic compounds, mg gallic acid equivalent/g d.b.	Phytates % d.b.	Antioxidant activity micrograms trolox/g d.b.
Cupi	2.18±1.33	1.70±0.00	3109±176
Ramis	2.21±0.74	1.26±0.01	2909±174

All data are the mean ±SD of three replicates

The bran of both varieties of kañiwa presented better physicochemical properties at larger particle sizes than at smaller particle sizes. The former was selected for the analysis of the indigestible fraction, total polyphenols, phytates and antioxidant activity.

The results of the evaluation of the indigestible fraction in kañiwa bran are presented in Table 5. Both varieties had a high proportion of total and insoluble indigestible fractions. The soluble portion was small. The IF is defined as the part of vegetable foods that is neither digested nor absorbed in the small intestine, but reaches the colon, where it is a substrate for the fermentative microflora. It comprises not only dietary fibre, but also other compounds of proven resistance to the action of digestive enzymes, such as a fraction of dietary starch, protein, certain polyphenols, and other associated compounds [17]. Saura-Calixto et al. [17] determined the indigestible fraction in different food samples and found values ranging from 10.28 to 43.67% for the total indigestible fraction. Our values were higher because we used kañiwa bran in this analysis.

Table 6 presents the content of total polyphenols, phytates and antioxidant activity in the bran of the two varieties of kañiwa. The antioxidant activity was reduced in kañiwa bran in comparison to grain in both varieties (Table 2); however, the content of these compounds was still high. The kañiwa bran contained more phytate than the kañiwa grain. This is probably due to the concentration of phytates in the bran fraction of the grain.

Conclusions

Kañiwa is a little known crop and this paper provides scientific information on its nutritional and functional properties and potential uses. Both studied varieties of kañiwa had a high content of protein and dietary fibre, especially of the insoluble fraction. Kañiwa grain is also an excellent source of phenolic compounds and has very high antioxidant activity. The extrudate with a 12% initial moisture level showed the best functional properties, such as the degree of gelatinization, density, sectional expansion index and water solubility index. Kañiwa bran of large particle size presented better functional properties than the bran with small particle size. This bran is an important source of the indigestible fraction, and can be used as an ingredient in healthy foods because of these antioxidant nutrients and dietary fibre. The extrudate of kañiwa also offers an ingredient that should be assessed by the food industry because of its functional properties and high nutrient content.

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Quinoa (*Chenopodium quinoa*, Willd.) as a source of dietary fiber and other functional components

Quinoa (Chenopodium quinoa, Willd.) como fonte de fibra alimentar e de outros componentes funcionais

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Abstract

Four varieties of an Andean indigenous crop, quinoa (*Chenopodium quinoa* Willd.), were evaluated as a source of dietary fiber, phenolic compounds and antioxidant activity. The crops were processed by extrusion-cooking and the final products were analyzed to determine the dietary fiber, total polyphenols, radical scavenging activity, and in vitro digestibility of starch and protein. There were no significant differences in the contents of total dietary fiber between varieties of quinoa. In all cases, the contents of total and insoluble dietary fiber decreased during the extrusion process. At the same time, the content of soluble dietary fiber increased. The content of total phenolic compounds and the radical scavenging activity increased during the extrusion process in the case of all 4 varieties. There were significant differences between the varieties and the content of total polyphenols. The in vitro protein digestibility of quinoa varieties was between 76.3 and 80.5% and the in vitro starch digestibility was between 65.1 and 68.7%. Our study demonstrates that quinoa can be considered a good source of dietary fiber, polyphenols and other antioxidant compounds and that extrusion improves the nutritional value.
Keywords: *quinoa; Chenopodium quinoa Willd.; dietary fiber; bioactive compounds; extrusion.*

Resumo

Quatro variedades de quinoa (*Chenopodium quinoa* Willd.), cultura de origem andina, foram avaliados como fonte de fibra dietética, de compostos fenólicos e atividade antioxidante. As quinoas foram processadas por extrusão e os produtos finais foram analisados para determinar a fibra alimentar, o total de polifenóis, atividade de ligar os radicais livres e digestibilidade in vitro do amido e proteínas. Não houve diferença significativa no conteúdo de fibra dietética total entre as variedades de quinoa. Em todos os casos, o teor de fibra alimentar insolúvel e total diminuiu durante o processo de extrusão. Ao mesmo tempo, o teor de fibra alimentar solúvel teve um incremento. O teor de compostos fenólicos totais e a atividade de ligar os radicais livres foram aumentados durante o processo de extrusão, no caso das quatro variedades. Houve diferenças significativas entre o conteúdo total de polifenóis por variedades. A digestibilidade proteica in vitro das variedades de quinoa ficou entre 76,3 e 80,5%, e a digestibilidade in vitro do amido situou-se entre 65,1 e 68,7%. Nosso estudo demonstra que a quinoa pode ser considerada como uma boa fonte de fibra dietética, polifenóis e outros compostos antioxidantes e que o processo de extrusão – cocção pode melhorar o valor nutricional dos grãos.
Palavras-chave: *quinoa; Chenopodium quinoa Willd.; fibra dietética; componentes funcionais; processo de extrusão – cocção.*

1 Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a crop used by pre-Columbian cultures in South America for centuries. There is a long history of safe use of the grain in South America. Cultivated and collected Chenopodium species have been part of the Tiahuanacotan and Incan cultures. Quinoa has fulfilled various roles in these ancestral cultures, in addition to its role in human and animal nutrition, quinoa had a sacred importance (BONIFACIO, 2003). Archaeological studies have shown that quinoa was already known in 5000 B.C. (TAPIA et al., 1979). For the Incas, quinoa was a very important crop together with corn and potato. Quinoa is currently grown for its grain in the South American countries of Peru, Bolivia, Ecuador, Argentina, Chile and Colombia. The plant is cold and drought tolerant and it can be cultivated in high altitudes in the mountain areas. Quinoa can be grown in a wide range of pH of the soil, including acidic soils, and it can tolerate poor and rough environments. This crop grows perfectly at high altitudes, where it is not possible to grow maize, and it matures in 4 to 7 months, depending on the variety or ecotype (CARMEN, 1984). The genetic variability of quinoa is great, with cultivars of quinoa being adapted to growth from sea level to an altitude of 4,000 m, from 40° S to 2° N latitude, and from the cold highland climate to subtropical conditions. This makes it possible to select, adapt, and breed cultivars for a wide range of environmental conditions, providing basic nutrition in demanding environmental conditions (JACOBSEN, 2003).

Quinoa is usually referred to as a pseudo-cereal since it is not a member of the Gramineae family, but it produces seeds that can be milled in to flour and used as a cereal crop. It is an annual dicotyledonous plant usually standing 0.5-2.0 m high

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with large panicles of 1.8-2.2 mm long seeds produced at the end of the stem. The seed is usually pale yellow, but it may vary from almost white through pink, orange or red to brown and black. The embryo can hold 60% of the seed weight and it forms a ring around the endosperm that loosens when the seed is cooked (NATIONAL RESEARCH COUNCIL, 1989). Grain yields vary from 1 to 3 $t.ha^{-1}$, depending on the variety and the level of cultivation technology (CARMEN, 1984).

Most of the varieties of quinoa contain saponins, bitter-tasting triterpenoid glycosides, which are concentrated in the seed coat and must be removed before consumption. The most popular method for removing saponins involves washing the grains with water in the ratio of 1:8 quinoa:water, although there are several other traditional methods (ANTUNEZ DE MAYOLO, 1981). Large-scale commercial methods haven been developed in Peru and Bolivia.

Quinoa is one of the most nutritive grains used as human food and it has been selected by FAO as one of the crops destined to offer food security in this century (FOOD..., 1998). Its protein content is remarkable and the composition of the essential amino acids is excellent. The nutritional value of quinoa protein is comparable to that of milk protein (KOZIOL, 1992; RANHOTRA et al., 1993). The content of lysine, methionine and cysteine in quinoa is higher than in common cereals and legumes, making it complementary to these crops. Quinoa is rich in oil, containing beneficial fatty acids and a high content of tocopherols (REPO-CARRASCO-VALÊNCIA; ESPINOZA; JACOBSEN, 2003). Based on the high quality of the oil, and on the fact that some varieties show oil concentrations of up to 9.5%, quinoa could be considered as a potentially valuable new oil crop (KOZIOL, 1992).

Actually, quinoa is widely used in many South American countries, especially in Peru, Bolivia, Ecuador, Chile, and Argentina. In Peru, the population of Lima is aware of the nutritive qualities of quinoa and other Andean crops. They consume quinoa once a day on average. In rural areas of southern Peru, the population is accustomed to eating quinoa every day in different preparations (AYALA, 2003).

Quinoa grains do not contain gluten and thus, they cannot be used alone for bread- making. However, they can be mixed with wheat flour in the preparation of bread with high nutritional value (MORITA et al., 2001). The fact that quinoa contains no gluten, makes it an interesting ingredient for the diets of persons who have celiac disease. Quinoa flour is commonly used in infant foods. Flakes, similar to oat flakes, have also been prepared from quinoa. Puffed grains of quinoa are produced commercially in Peru and Bolivia. The plant is sometimes grown as a green vegetable and its leaves are eaten fresh or cooked (NATIONAL RESEARCH COUNCIL, 1989). The saponins obtained as a by-product in the processing of quinoa can be utilized by the cosmetics and pharmaceutical industries (TAPIA et al., 1979).

Cereals are commonly known as good sources of dietary fiber, phenolic compounds, and natural antioxidants. Some studies on dietary fiber, phenolic compounds, and antioxidant activity of quinoa have been published (RUALES; NAIR, 1994; GORINSTEIN et al., 2007; NSIMBA; KIKUZAKI; KONISHI, 2008). These investigations have shown that quinoa is a very good source of antioxidants and it can be a substitute for common cereals. However, we did not find any reports on the content of phenolic compounds and antioxidant activity of processed quinoa. Thus, the aim of this study was to analyze the content of dietary fiber, phenolic compounds, and antioxidant activity in 4 varieties of raw and extruded quinoa.

2 Materials and methods

2.1 Raw material

The following 4 varieties of quinoa (*Chenopodium quinoa,* Willd.) were used in this study: ´La Molina 89´, of the leguminous and cereal program of National Agrarian University La Molina, Lima, Peru; ´Kcancolla´, 'Blanca de Juli´ and ´Sajama´ from the Agronomical Experimentation Center of Altiplano University, Puno, Peru.

2.2 Preparation of raw material

Quinoa was washed for 20 minutes with tap water with the aim to eliminate bitter taste and toxic saponins. Washed grains were dried at 45 °C for 12 hours. Dried seeds were packed in polyethylene bags and stored at 4 °C until they used in analysis and processing.

2.3 Extrusion

Washed and dried quinoa seeds were moistened to 12% humidity for the extrusion process. The extrusion process was carried out using a low-cost extruder simulating local processing conditions (Jarcon del Peru, Huancayo, Peru). The single-screw extruder was operated with the following parameters: 389.4 rpm, 10-13 seconds resident time, 200 °C work temperature, 2 orifices on the die. No external heat was transferred to the barrel on the screw during extrusion. The aim was to produce results applicable to local conditions where this technology is widely used.

2.4 Extraction of polyphenols and radical scavenging analysis

All samples were ground through a Foss cyclotec mill before extraction. Five grams of milled quinoa or extrudate were mixed with 25 mL methanol and homogenized using the Ultra-Turrax homogenizer. The homogenates were allowed to stand for 12-24 hours under refrigeration (4 °C) and then centrifuged for 15 minutes. The supernatant was recovered and stored until analysis.

2.5 Analysis

Proximate analysis. Water content, proteins (N × 6.25), fat, crude fiber and ash were determined according to AOAC (ASSOCIATION..., 1995). The carbohydrates (CHO) were calculated by difference, using the Equation 1:

$$CHO = 100 - (fat + protein + crude\ fiber + ash) \quad (1)$$

Dietary fiber. The total, soluble and insoluble dietary fiber were determined by an enzymatic-gravimetric method according to the Approved Method 32-21 (AMERICAN..., 1995) using the TDF-100 kit from Sigma Chemical Company (St. Louis, MO, U.S.A.). Radical scavenging activity. Radical scavenging activity was determined according to Re et al. (1999) based on the decrease of absorbance at 734 nm produced by reduction of ABTS (2,2´-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) by an antioxidant. Trolox was used as the reference compound for calibration curve. The percentage inhibition of absorbance at 734 nm was calculated using the formula according to Katalinic et al. (2006), Equation 2:

$$\%\ inhibition = \left[\frac{\left(A_{C(0)} - A_{A(t)}\right)}{A_{C(0)}}\right] \times 100 \quad (2)$$

where $A_{C(0)}$ is the absorbance of the control at t = 0 minute and $A_{A(t)}$ is the absorbance of the antioxidant at t = 16 minutes.

Total polyphenols. The content of total polyphenols was analyzed according to the method of Swain and Hillis (1959). The phenolic compounds were extracted with methanol and the extract was allowed to react with the Folin- Ciocalteau phenol reagent. The absorbance was measured at 725 nm. Gallic acid equivalents (GAE) were determined from a standard concentration curve.

Protein in vitro digestibility. Digestibility of proteins was determined by an in vitro method according to Hsu et al. (1977). The multi-enzymatic method is based on the decrease of pH during 10 minutes. The percentage of digestibility was calculated using the Equation 3:

$$Y = 210.464 - 18.103\,X \quad (3)$$

where: X = pH of the protein suspension after 10 minutes of digestion and Y = percentage of protein hydrolysis.

Starch in vitro digestibility. The digestibility of starch was determined by an in vitro method according to Holm et al. (1985). Starch (500 mg) was mixed with phosphate buffer (pH 6.9) and incubated with α-amylase at 37 °C for 1 hour. The sugars released were determined by spectrophotometry.

Degree of gelatinization was based on the method of Wooton; Weeden and Munk (1971).

2.6 Statistical analysis

Each analysis was done at least in duplicate and expressed as means and standard deviation (SD). The data were analyzed by analysis of variance and Tukey´s test (significance of differences $p < 0.05$) was used to find significant differences between the samples and treatments.

3 Results and Discussion

The results of analysis of the proximate composition of 4 quinoa varieties and their extrudates are presented in Table 1. Then, protein content of the grain of the 4 varieties was between 14.0 and 15.5%. This coincides with values by Guzman-Maldonado and Paredes-Lopez (1998). According to these authors, the protein content of quinoa is between 11.0 and 15.0%. There were differences between the 4 varieties of quinoa in protein content, La Molina 89 having the highest and Blanca de Juli the lowest protein contents. Extrusion affected the protein content, decreasing it. La Molina 89 had the highest crude fat content. The contents of moisture, ash, and crude fiber were reduced during the extrusion process in all varieties.

In Table 2, the contents of total (TDF), insoluble (IDF), and soluble dietary fiber (SDF) are presented for raw and extruded quinoa varieties. There were no significant differences in the contents of TDF, IDF, and SDF between the varieties. In all cases, the contents of total and insoluble dietary fiber decreased during the extrusion process, however, this decrease was significant only in the case of the Sajama variety. At the same time, the content of soluble dietary fiber increased during the extrusion process. The increase of content of soluble dietary fiber was statistically significant in the case of Blanca de Juli, Kcancolla,

Table 1. Proximate composition of 4 quinoa varieties (% dry basis).

Component	Blanca de Juli	Kcancolla	La Molina 89	Sajama
Raw				
Moisture	11.39	10.78	12.03	12.62
Ash	3.38	3.52	5.46	3.04
Protein	13.96	15.17	15.47	14.53
Crude fat	5.51	5.77	6.85	4.69
Crude fiber	2.00	3.07	3.38	1.92
Carbohydrates	75.15	72.47	68.84	75.82
Extruded				
Moisture	7.46	8.98	7.31	7.58
Ash	2.61	2.61	2.45	2.55
Protein	13.41	14.19	15.45	14.08
Crude fat	3.48	3.75	4.21	4.13
Crude fiber	1.64	2.60	2.13	1.62
Carbohydrates	78.86	76.85	75.76	77.62

All data are the means of 2 replicates. All contents g.100 g^{-1} dry weight except moisture g.100 g^{-1} fresh weight.

Table 2. Total, insoluble, and soluble dietary fiber content in 4 quinoa varieties, raw and extruded, g.100 g^{-1} dry basis.

Variety	TDF	IDF	SDF
Raw			
Blanca de Juli	$13.72 \pm 1.63^{a,x}$	$12.18 \pm 1.65^{a,x}$	$1.54 \pm 0.01^{a,y}$
Kcancolla	$14.11 \pm 1.02^{a,x}$	$12.70 \pm 1.15^{a,x}$	$1.41 \pm 0.13^{a,y}$
La Molina 89	$15.99 \pm 0.63^{a,x}$	$14.39 \pm 0.81^{a,x}$	$1.60 \pm 0.18^{a,y}$
Sajama	$13.56 \pm 0.23^{a,x}$	$11.99 \pm 0.28^{a,x}$	$1.58 \pm 0.05^{a,x}$
Extruded			
Blanca de Juli	$10.77 \pm 0.32^{b,x}$	$8.64 \pm 0.17^{b,x}$	$2.13 \pm 0.15^{a,x}$
Kcancolla	$12.52 \pm 0.64^{b,x}$	$10.13 \pm 0.88^{b,x}$	$2.39 \pm 0.23^{a,x}$
La Molina 89	$15.27 \pm 0.49^{a,x}$	$12.54 \pm 0.59^{a,x}$	$2.73 \pm 0.10^{a,x}$
Sajama	$11.64 \pm 0.30^{b,y}$	$9.38 \pm 0.71^{b,y}$	$2.26 \pm 0.41^{a,x}$

TDF = total dietary fiber, IDF = insoluble dietary fiber, SDF = soluble dietary fiber. All data are the means ± SD of three replicates. $^{a-d}$Varieties are compared. Means in the same row followed by same letter are not significantly different ($p < 0.05$). ^{x,y}Raw and extruded quinoa are compared. Means in the same column followed by same letter are not significantly different ($p < \pm 0.05$).

and La Molina 89 varieties. Gualberto et al. (1997) also found a decrease in the content of insoluble dietary fiber and an increase in the content of soluble fiber during extrusion-cooking. This could be due to shear stress caused by high screw speed and also to high temperature. The exposure to shear stress and high temperature causes chemical bond breakage creating smaller particles, which are soluble. There is a transformation of some insoluble fiber components into soluble fiber during extrusion. Rinaldi; NG and Bennink (2000) studied the effect of extrusion on dietary fiber of wheat extrudates enriched with wet okara and his results coincide with ours. Extrusion of the formulations resulted in decreased insoluble fiber and increased soluble fiber contents of the products. Extrusion-cooking of white heat flour has also been found to cause a redistribution of insoluble to soluble dietary fiber (BJORCK; NYMAN; ASP, 1984).

The extrusion-cooking process was investigated by Lue, Hsieh and Huff (1991) with the expectancy that mechanic rupture of the glycosidic bonds would lead to an increase of soluble fiber. In some cases, an increase of insoluble fiber was observed (UNLU; FALLER, 1998). Esposito et al. (2005) studied the effect of extrusion on dietary fiber of durum wheat. The data showed that the extrusion-cooking process did not have an effect on the amount of soluble dietary fiber, independently from the fiber typology of the different samples. This difference in fiber solubilization during processing could be explained by the variability in the raw material composition, but also by different experimental conditions, for example, screw share forces and pressure in extrusion. The high mechanical stress during extrusion may cause breakdown of polysaccharide glycosidic bonds releasing oligosaccharides and, therefore, end up with an increase of soluble dietary fiber (ESPOSITO et al., 2005).

Ruales and Nair (1994) determined the contents of dietary fiber in raw and processed quinoa samples. They found 13.4% of total dietary fiber for raw quinoa. This value is comparable to our values for Blanca de Juli and Sajama varieties. The content of total dietary fiber was decreased only in cooked quinoa, while in autoclaved and drum-dried samples it remained the same. Some soluble fiber was lost during cooking, and in autoclaved samples, it was lost probably due to depolymerization of fiber components.

Content of the total phenolic compounds and the radical scavenging activity increased during the extrusion process in the case of all 4 varieties (Table 3.). There were significant differences between the varieties and the contents of total polyphenols. The contents of total polyphenols in the 4 quinoa varieties ranged from 1.43 to 1.97 mg.GAE.g^{-1}. Pasko et al. (2009) defined the content of total polyphenols in quinoa to be 3.75 mg.GAE.g^{-1} by using a 2-step extraction process, first with methanol and then with acetone. As we used methanol only, some polyphenols may not have been included in the extract.

Figure 1 presents the radical scavenging activities of raw and extruded quinoa. These increased during the extrusion process. The finding is in agreement with the report of Dewanto, Wu and Hai Liu (2002) who discovered that the antioxidant activity and the content of total phenolics of sweet corn increased during thermal processing. Increase of the total antioxidant activity in processed grains could be explained by the increase of soluble phenolic compounds released by thermal processing. In cereals, the phenolic acids are in free, esterified and insoluble bound forms. Dewanto, Wu and Hai Liu (2002) found that heat treatment increased the free and conjugated ferulic acid contents in sweet corn due to the release of bound ferulic acid across both the heating time and heating temperature parameters.

Xu and Chang (2008) studied the effect of thermal processing on total phenolics and specific phenolic compounds in yellow and black soybeans. They found that the content of phenolics increased in yellow varieties and decreased in black varieties during the heat treatment. In yellow soybean varieties, thermal processing caused more free gallic acid to be released leading to higher total phenolic content and antioxidant activity compared to raw beans.

The values of degree of gelatinization (DG), as well as the in vitro digestibility of starch and protein are presented in Table 4. DG of the 4 quinoa varieties was between 79.9 and 89.0%. These values are lower than those found by Ruales and Nair (1994) for drum-dried (96%) and cooked (97%) quinoa samples, but higher than for the autoclaved (27%) samples. Dogan and Karwe (2003) investigated the physicochemical properties of quinoa extrudates. They found that the starch was only partially gelatinized with a maximum of 84.4%, depending on the extrusion conditions (feed moisture, screw speed and die temperature). During extrusion-cooking, both temperature and shear are responsible for starch gelatinization.

Ruales and Nair (1994) reported the in vitro digestibility of starch in raw, autoclaved, cooked, and drum-dried quinoa. Their values for raw, autoclaved and cooked quinoa were lower than ours (22, 32 and 45%, respectively), but the value for drum-dried quinoa was higher (73%). As the starch granules of quinoa are surrounded by a protein matrix, they are not very easily

Table 3. Total polyphenols and radical scavenging activity of 4 varieties of quinoa, raw and extruded.

Variety	Raw quinoa total polyphenols mg GAE/g d.b.*	Extruded quinoa total phenolics mg gallic acid/g d.b.*	Raw quinoa radical scavenging activity microg trolox/g sample**	Extruded quinoa radical scavenging activity microg trolox/g sample**
Blanca de Juli	$1.42 \pm 0.5^{c,y}$	$1.70 \pm 0.1^{c,x}$	2351.9	3960.8
Kcancolla	$1.57 \pm 0.3^{b,y}$	$1.82 \pm 0.4^{b,x}$	2389.9	4095.4
La Molina 89	$1.97 \pm 0.2^{a,y}$	$3.28 \pm 0.3^{a,x}$	3689.5	4165.6
Sajama	$1.63 \pm 0.1^{b,x}$	$1.66 \pm 0.2^{c,x}$	2440.3	4118.8

*Data are the means ± SD of 3 replicates. **Data are means of duplicate analyses. $^{a-d}$ Varieties are compared. Means in the same column followed by same letter are not significantly different ($p < 0.05$). ^{x,y}Raw and extruded quinoa are compared. Means in the same row followed by same letter are not significantly different ($p < 0.05$). d.b. = dry basis.

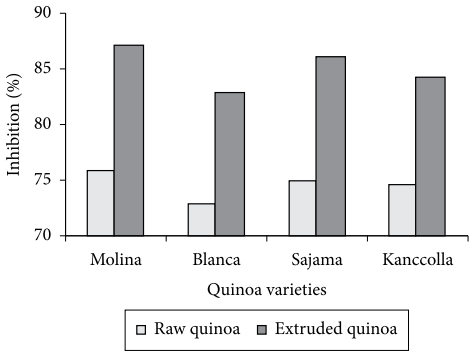

Figure 1. Radical scavenging activity of raw and extruded quinoa The results given here are means values of two separate experiments.

Table 4. Degree of gelatinization of starch, in vitro digestibility of starch and protein of 4 varieties of extruded quinoa.

Variety	Degree of gelatinization%	In vitro digestibility of starch%	In vitro digestibility of protein%
Blanca de Juli	79.85	68.53	80.54
Kcancolla	86.15	65.11	79.34
La Molina 89	86.74	68.42	76.80
Sajama	89.02	68.69	76.32

All data are the means of two replicates.

hydrolysable by α-amylase. The degree of starch hydrolysis could be improved by treating quinoa flour with proteolytic enzymes prior to hydrolysis with α-amylase.

The in vitro digestibility of protein of the 4 extruded quinoa varieties was between 76.3 and 80.5%. Zia-Ur-Rehman and Shah (2001) studied the protein in vitro digestibility of black grams after soaking and cooking. They obtained values of digestibility as in our study, between 75 and 84% for cooked black grams. Dahlin and Lorenz (1993) studied protein in vitro digestibility of extruded cereal grains, including quinoa. The effect of extrusion on in vitro protein digestibility was similar in all cereals investigated. The optimum extrusion process conditions for cereals used in this study were 15% feed moisture, 100/150 °C product temperatures and screw speed of 100 rpm.

4 Conclusions

Altogether, this study demonstrates that quinoa can be considered a very nutritive cereal when compared to commonly consumed cereals such as wheat, barley, and corn. It has a relatively high content of good-quality protein and it can be considered a good source of dietary fiber and other bioactive compounds such as phenolics. It has a long history of safe use in South America, especially in low-income areas. Therefore, it may present a new viable crop option for low-income areas and also provide a new ingredient for specific foods for particular target populations with potential health benefits. Thus, further studies should be conducted to characterize the phenolic compound composition and the antioxidant content and activity, especially of colored varieties of quinoa.

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Flavonoids and other phenolic compounds in Andean indigenous grains: Quinoa (*Chenopodium quinoa*), kañiwa (*Chenopodium pallidicaule*) and kiwicha (*Amaranthus caudatus*)

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IV

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ABSTRACT

The amount of phenolic acids, flavonoids and betalains in Andean indigenous grains, quinoa (*Chenopodium quinoa*), kañiwa (*Chenopodium pallidicaule*) and kiwicha (*Amaranthus caudatus*), was determined. The total amount of phenolic acids varied from 16.8 to 59.7 mg/100 g and the proportion of soluble phenolic acids varied from 7% to 61%. The phenolic acid content in Andean crops was low compared with common cereals like wheat and rye, but was similar to levels found in oat, barley, corn and rice. The flavonoid content of quinoa and kañiwa was exceptionally high, varying from 36.2 to 144.3 mg/100 g. Kiwicha did not contain quantifiable amounts of these compounds. Only one variety of kiwicha contained low amounts of betalains. These compounds were not detected in kañiwa or quinoa. Our study demonstrates that Andean indigenous crops have excellent potential as sources of health-promoting bioactive compounds such as flavonoids.

1. Introduction

Quinoa (*Chenopodium quinoa*), kañiwa (*Chenopodium pallidicaule*) and kiwicha (*Amaranthus caudatus*) are nutritious grains that are grown in the Andean highlands. These crops were used by pre-Colombian cultures in South America for centuries. They were very important for the Incas together with corn and potatoes. These plants are cold- and drought-tolerant and can be cultivated in high mountains, particularly kañiwa, which can be grown at over 4000 masl. The genetic variability of quinoa, kañiwa and kiwicha is huge, with cultivars being adapted to growth from sea level to high mountains, and from cold, highland climates to subtropical conditions.

Quinoa, kañiwa and kiwicha are usually referred to as pseudocereals since they are not members of the grass family, but produce seeds that can be milled into flour and used like a cereal crop. Quinoa is mainly used in soups and also instead of rice in main courses. Kañiwa is usually toasted and milled and consumed as meal (*kañiwako*). Kiwicha is toasted to obtain "pop-kiwicha", a puffed product. It is consumed directly or used to make "turrones", a kind of snack bar. All these grains are gluten-free and can be used by persons who suffer from coeliac disease. They are also used in baby foods to make porridge.

Several studies have reported the nutritional value of quinoa. This crop contains proteins with a balanced essential amino acid composition that are of high biological value (Koziol, 1992; Ranhotra, Gelroth, Glaser, Lorenz, & Johnson, 1993; Repo-Carrasco, Espinoza, & Jacobsen, 2003). A close relative of quinoa, kañiwa, also has a relatively high protein content with adequate levels of essential amino acids (Repo-Carrasco et al., 2003). The high nutritional value of amaranth proteins was demonstrated by Bressani, De Martell, and Godinez (1993). Among the essential amino acids, the content of lysine in quinoa, kañiwa and kiwicha is notable. The consumption of quinoa and kañiwa has compensated the lack of animal protein, and they are still principal protein sources in many areas (Tapia, 1997). These crops are also very good sources of good quality edible oil (Berganza et al., 2003; Repo-Carrasco et al., 2003) and minerals, such as calcium and iron (Bressani, 1994). Starch is the most abundant component in quinoa, kañiwa and kiwicha seed, as in all cereal crops.

Polyphenols are bioactive secondary plant metabolites that are widely present in commonly consumed foods of plant origin. The three main types of polyphenols are flavonoids, phenolic acids and tannins, which act as powerful anti-oxidants *in vitro*. These compounds are considered to carry many potential beneficial health effects, e.g. in reduction of the risk of cardiovascular diseases, cancers, neurodegenerative diseases, diabetes, and osteoporosis. In food, polyphenols may contribute to bitterness,

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astringency, colour, flavour, and oxidative stability of products (Han, Shen, & Lou, 2007; Scalbert, Manach, Morand, & Rémésy, 2005; Shahidi & Naczk, 1995). Very little information exists concerning polyphenols in Andean cereals such as quinoa, kañiwa and kiwicha. A few articles concerning the isolation and characterisation of flavonoids in quinoa and kañiwa seeds have been published (De Simone, Dini, Pizza, Saturnino, & Scettino, 1990; Rastrelli, Saturnino, Schettino, & Dini, 1995; Zhu et al., 2001).

In addition, Peñarrieta, Alvarado, Åkesson, and Bergenståhl (2008) analysed levels of flavonoids and other phenolic compounds in *Chenopodium pallicaule* (edible part of the plant). Repo-Carrasco-Valencia, Peña, Kallio, and Salminen (2009) analysed the content of total phenolic compounds, phytic acid and anti-oxidant activity in two varieties of raw and extruded kiwicha. Anti-oxidant activity with the DPPH method for the raw kiwicha of the two varieties was 410.0 μmol trolox/g sample for Centenario and 398.1 μmol trolox/g sample for Oscar Blanco. With ABTS method those values were 827.6 and 670.1 μmol trolox/g sample for Centenario and Oscar Blanco, respectively. The content of total phenolics, phytic acid and the anti-oxidant activity decreased during the extrusion process.

To our knowledge, there are no previous data on the phenolic acid and flavonoid content in the seeds of *Chenopodium* species. Klimczak, Malecka, and Pacholek (2002) published results concerning the phenolic acid content of amaranth seeds. Another article concerning the phenolic and flavonoid content in amaranth (*Amaranthus hypochondriacus*) has also recently been published (Barba de la Rosa et al., 2009). Pasko et al. (2009) analysed the total polyphenol content and anti-oxidant activity in two amaranth varieties (*Amaranthus cruentus*) and quinoa seeds and sprouts. Anti-oxidant activity of the investigated seeds decreased in the following order: quinoa, amaranth v. Rawa, amaranth v. Aztek for FRAP and quinoa, amaranth v. Aztek, amaranth v. Rawa for both ABTS and DPPH. The data obtained by the three methods showed significant correlation between total polyphenols content in seed and sprouts.

Betalains are yellow and red compounds that are found in few selected plants such as beetroot, cactus pears and amaranthus. Chemically they can be divided into betaxanthins, which are condensation products of betalamic acid and various amino compounds, and betacyanins, which are conjugates of betalamic acid and *cyclo*-Dopa with various substitutions (Stintzing & Carle, 2004). The betalains from red beet have been extensively used as colourants in the modern food industry. Recently, several studies on the antiradical and anti-oxidant activity of betalains (mainly betanin) from beetroot (*Beta vulgaris*) have been published (Kanner, Harel, & Granit, 2001; Pedreno & Escribano, 2000). Cai, Sun, and Corke (2003) studied the anti-oxidant activity of betalains from plants of *Amaranthaceae*. They found that plants of the *Amaranthaceae*, containing betacyanins and betaxanthins, demonstrated very strong anti-oxidant activity.

The aim of this study was to determine the levels of flavonoids, phenolic acids and betalains in the Andean grains quinoa, kañiwa and kiwicha. In addition, the basic composition of these pseudocereals was analysed.

2. Materials and methods

2.1. Samples

Six ecotypes of Quinoa (*C. quinoa*) were obtained from the Agronomical Experimental Station-INIA Salcedo, Puno, Peru (03-21-1181, Witulla, Roja Coporaque, 03-21-0093, Huaripongo, Ccoito) and two varieties (INIA-415 Pasankalla, Salcedo INIA), and two commercial samples from Cusco were purchased for the study.

Table 1
Description of the quinoa, kañiwa and kiwicha samples.

Sample	Colour	Place cultivated
Quinoa		
Ccoito	Grey	Puno
INIA-415 Pasankalla	Grey/red	Puno
Roja de Coporaque	Red	Puno
Witulla	Red	Puno
03-21-0093	Red	Puno
Salcedo INIA	Cream	Puno
Commercial 1.	Red	Cusco
Commercial 2.	Black	Cusco
Huaripongo	Yellow	Puno
03-21-1181	Yellow	Puno
Kañiwa		
Kello	Yellow	Puno
Wila	Brown	Puno
Guinda	Brown	Puno
Ayara	Grey	Puno
Commercial sample	Brown	Cusco
Kiwicha		
1.	Black	Mollepata, Cusco
2.	Black	San Salvador, Cusco
3.	Pink	Mollepata, Cusco
4.	Cream	San Salvador, Cusco

Four ecotypes of kañiwa (*C. pallidicaule*) were obtained from the Agronomical Experimental Station-INIA Salcedo, Puno, Peru (Kello, Wila, Guinda and Ayara) and one commercial sample from Cusco was purchased. Black, pink and white grains of kiwicha (*A. caudatus*) were collected from Mollepata, Cusco and one black sample from San Salvador, Cusco.

See Table 1 for more details. All grain was from 2007 to 2008 growing season.

2.2. Proximate analysis

Water content, proteins, fat, crude fibre and ash were determined according to AOAC methods (1995). The carbohydrates were calculated by difference.

2.3. Flavonoids

Flavonoids were analysed as aglycones according to the method explained by Mattila, Astola, and Kumpulainen (2000). Briefly, a sample (0.3–1 g) was weighed into a 100-ml Erlenmeyer flask and dispersed in 40 ml of 62.5% aqueous methanol containing 2 g/l of 2,3-tert-butyl-4-hydroxyanisole (BHA). To this extract 10 ml of 6 M HCl was added. Hydrolysis was carried out in a shaking water bath at 90 °C for 2 h. After hydrolysis the sample was allowed to cool. Then it was filtered and made up to 100 ml with methanol. Before quantification by HPLC the sample was filtered through a 0.45 μm membrane filter.

The analytical HPLC system consisted of an Agilent 1100 Series high-performance liquid chromatograph equipped with a diode array detector. The HPLC pumps, autosampler, column oven, and diode array system were monitored and controlled using the HP Chem Station computer programme. Wavelengths used for identification and quantification of flavonoids with the diode array detector were 280 nm for eriodictyol, naringenin, and hesperetin, 329 nm for luteolin and apigenin and 370 nm for myricetin, kaempferol, quercetin and isorhamnetin. Flavonoid separation was done by an Inertsil (GL Sciences, Inc., Japan) ODS-3 (4.0 × 150 mm, 3 μm) column with a C-18 guard column. The temperature of the column oven was set at 35 °C. Gradient elution was employed for flavonoids with a mobile phase consisting of 50 mM H_3PO_4, pH 2.5 (solution A) and acetonitrile (solution B) as follows: isocratic elution 95% A, 0–5 min; linear gradient from 95% A to 50%

A, 5–55 min; isocratic elution 50% A, 55–65 min; linear gradient from 50% A to 95% A, 65–67 min; post-time 6 min before next injection. The flow rate of the mobile phase was 0.7 ml/min, and the injection volumes were 10 μl of the standards and sample extracts. All flavonoids were quantified using the external standard method. The samples were analysed in triplicate.

2.4. Phenolic acids

Phenolic acids were analysed according to the method of Mattila and Hellström (2007). Briefly, a 0.5 g sample was homogenised in 7 ml of a mixture of methanol, containing 2 g/l of butylated hydroxyanisole (BHA) and 10% acetic acid (85:15) using a Heidolph Diax 900 homogenizer. The homogenised extract was ultrasonicated for 30 min and made up to a volume of 10 ml with distilled water. After mixing, 1 ml was filtered for HPLC analysis of soluble phenolic acids. Next, 12 ml of distilled water containing 1% ascorbic acid and 0.415% EDTA and 5 ml of 10 M NaOH were added into the test tube, sealed, and stirred overnight (about 16 h) at 20 °C using a magnetic stirrer. The solution was then adjusted to pH 2 with concentrated HCl, and the liberated phenolic acids were extracted with 15 ml of a mixture of cold diethyl ether and ethyl acetate (1:1), centrifuged at 620*g* (Rotofix 32, Hettich Zentrifugen, Germany) and the organic layer was recovered. The extraction was repeated twice and the organic layers were combined. After alkaline hydrolysis, an acid hydrolysis was performed by adding 2.5 ml of concentrated HCl into the test tube and incubating in a water bath at 85 °C for 30 min. The sample was then cooled, and further sample handling was performed in the same manner following alkaline hydrolysis.

The organic layers from alkaline and acid hydrolyses were combined, evaporated to dryness, dissolved into 2 ml of methanol, filtered and analysed for total phenolic acids by HPLC.

The analytical HPLC system was the same for phenolic acids as for flavonoids except for a modification in gradient elution: isocratic elution 95% A, 0–5 min; linear gradient from 95% A to 85% A, 5–17 min; linear gradient from 85% A to 80% A, 17–40 min; linear gradient from 80% A to 50% A, 40–60 min; isocratic elution 50% A, 60–65 min; linear gradient from 50% A to 95% A, 65–67 min; post-time 6 min before the next injection. The wavelengths used for the quantification of phenolic acids with the diode array detector were: 254 nm for protocatechuic acid, *p*-hydroxybenzoic acid and vanillic acid; 280 nm for syringic acid, *p*-coumaric acid, *m*-coumaric acid, *o*-coumaric acid, and *E*-cinnamic acid; and 329 nm for caffeic acid, ferulic acid, sinapic acid and chlorogenic acid. The samples were analysed in triplicate. Both total and soluble forms were quantified as aglycones. Phenolic acids obtained after hydrolysis were identified according to their retention times and UV spectra (190–600 nm) consistent with commercial reference compounds while soluble forms were identified solely by their UV spectra.

2.5. Betalains in kiwicha species

Finely ground material (0.5 g) was weighed in a test tube and made up to 5 ml with acidified water (pH 3–4). The test tube was carefully flushed with argon, sealed and extracted overnight in a magnetic stirrer. After extraction 5 ml of methanol was added and the sample was centrifuged (10 min at 1500 rpm). The supernatant was transferred to another test tube. To the solid residue 2 ml of acetone was added and after vortexing the sample was centrifuged again. Acetone supernatants were combined and the sample was evaporated to near dryness with a stream of nitrogen. After evaporation the volume was adjusted to 1 ml with methanol, filtered through a 0.45 μm membrane filter and analysed by HPLC-DAD (Hewlett–Packard 1100 series). Nova Pak C18 (3.9 × 150 mm, 4 μm, Waters, Milford, USA) was used as an analytical column protected with the same manufacturer's precolumn. The mobile phase consisted of 0.05 M phosphate buffer (A) pH 2.4 and methanol (B). Gradient elution was used: 5–60% B in 50 min followed by 60–90% B in 6 min, hold at 90% B for 12 min, and finally to 100% B within 32 min. The HPLC method was basically the same as described by Mattila, Pihlava, and Hellström (2005) for avenanthramides except that the quantification of betalains was done at 535 nm. For identification purposes UV spectra were recorded at 190–600 nm. Three compounds were detected in one kiwicha sample at 535 nm and tentatively identified as betacyanin amaranthine, iso-amaranthine and betanin according to the elution order and UV spectra presented in the literature (Cai, Sun, & Corke, 2001; Cai, Sun, Wu, Huang, & Corke, 1998). Amaranthine, previously isolated at MTT, was used as a reference compound for quantification.

2.6. Statistical analysis

Each analysis was done at least in duplicate and the results are expressed as mean and standard deviation (SD). The data were analysed by analysis of variance, and Tukey's test (significance of differences $p < 0.05$) was used to find significant differences between the species.

3. Results and discussion

The results of the proximate analysis of the Andean grains are presented in Table 2. The protein content of quinoa varieties was 12.61% on average. This value is similar to that reported by Guzman-Maldonado and Paredes-Lopez (1998), at between 11.0% and 15.0%. The protein content of kañiwa grains was significantly higher than that of quinoa and kiwicha. There were no statistically significant differences in the fat content of quinoa, kiwicha and kañiwa. In general, quinoa, kañiwa and kiwicha seeds are good sources of protein and fat. The main component of all three grain species was carbohydrates.

Both the soluble and total phenolic acid contents in the Andean cereals were quantified as aglycones (Table 3). Soluble phenolic acids (free and bound soluble forms) were extracted with methanolic acetic acid whereas the total phenolic acid content (the sum of bound soluble, insoluble and free phenolic acids) was obtained after alkaline and acid hydrolyses. Due to a lack of reference standards for soluble bound phenolic acids, the results are to be considered tentative and are reported only as percentage shares of total phenolic acids in Table 3. However, this information may be of interest because the bioavailability of soluble phenolic acids may differ from that of insoluble ones.

The total content of phenolic acids varied from 16.8 to 59.7 mg/100 g in the samples analysed and the percentage share of soluble phenolic acids varied from 7% to 61%.

There were several differences in the phenolic acid composition of the three different grains (quinoa, kañiwa and kiwicha) (Table 3). The samples of *Chenopodium* species contained caffeic acid, ferulic acid, *p*-coumaric acid, *p*-OH-benzoic acid and vanillic acid. In addition to these sinapinic acid and protocatechuic acid were detected in *Amaranthus* samples (Table 3). There was a statistically significant difference in the content of ferulic acid in quinoa, kañiwa and kiwicha, kañiwa having the highest and kiwicha the lowest. Of the *Chenopodium* species kañiwa samples contained less vanillic acid but more caffeic and ferulic acids than quinoa samples. The content of total phenolic acids was higher in quinoa than in kiwicha but much variation existed between samples. In quinoa varieties the proportion of soluble phenolic acids was high (mean 39 ± 11%). In kañiwa and amaranthus varieties these mean values were 21 ± 9% and 10 ± 3%, respectively.

To our knowledge, very little information has been published concerning the phenolic acid content of *Chenopodium* and

Table 2
Proximate composition of Andean grains (g/100 g).

Sample	Moisture	Protein	Fat	Crude fiber	Ash	Carbohydrates
Quinoa samples						
Ccoito	8.47 ± 0.08	14.72 ± 0.11	5.33 ± 0.06	1.81 ± 0.02	2.83 ± 0.00	68.1
INIA-415 Pasankalla	9.76 ± 0.07	12.69 ± 0.06	6.85 ± 0.10	2.20 ± 0.02	2.49 ± 0.03	67.0
Roja de Coporaque	8.30 ± 0.07	11.51 ± 0.10	5.22 ± 0.08	2.26 ± 0.02	2.93 ± 0.05	70.8
Witulla	8.81 ± 0.08	12.28 ± 0.00	5.32 ± 0.01	2.62 ± 0.02	2.57 ± 0.04	69.5
03-21-0093	8.47 ± 0.07	11.79 ± 0.11	nd	nd	2.76 ± 0.02	nd
Salcedo INIA	8.26 ± 0.05	13.23 ± 0.01	5.30 ± 0.09	1.84 ± 0.20	2.37 ± 0.05	70.0
Commercial 1	10.13 ± 0.05	13.18 ± 0.01	6.51 ± 0.04	4.23 ± 0.03	2.34 ± 0.10	63.6
Commercial 2	11.51 ± 0.04	13.48 ± 0.06	6.34 ± 0.07	7.04 ± 0.03	2.27 ± 0.10	59.4
Huaripongo	10.34 ± 0.02	11.32 ± 0.01	6.14 ± 0.01	2.51 ± 0.01	2.92 ± 0.04	67.8
03-21-1181	9.37 ± 0.06	11.89 ± 0.02	3.95 ± 0.03	2.88 ± 0.01	3.12 ± 0.02	69.8
Mean ± SD	*9.34 ± 1.1*^{a}	*12.61 ± 1.1*^{a}	*5.66 ± 0.9*^{a}	*3.04 ± 1.7*^{a}	*2.66 ± 0.3*^{a}	*67.3 ± 3.7*^{a}
Kañiwa samples						
Kello	10.37 ± 0.04	15.38 ± 0.03	7.36 ± 0.08	5.33 ± 0.04	3.56 ± 0.19	59.4
Wila	9.61 ± 0.07	13.29 ± 0.09	6.87 ± 0.08	7.52 ± 0.01	3.67 ± 0.08	60.3
Guinda	9.79 ± 0.07	14.72 ± 0.11	4.46 ± 0.00	7.46 ± 0.05	3.38 ± 0.04	61.5
Ayara	10.39 ± 0.09	14.38 ± 0.01	6.66 ± 0.07	14.37 ± 0.20	3.13±	52.4
Commercial sample	9.38 ± 0.09	18.28 ± 0.12	7.92 ± 0.03	4.79 ± 0.06	2.73 ± 1.16	58.5
Mean ± SD	*9.91 ± 0.5*^{a}	*15.21 ± 1.9*^{b}	*6.65 ± 1.3*^{a}	*7.89 ± 3.8*^{b}	*3.31 ± 0.4*^{b}	*58.39 ± 3.5*^{b}
Kiwicha samples						
1	12.07 ± 0.17	15.88 ± 0.10	6.54 ± 0.02	7.49 ± 0.09	2.50 ± 0.06	55.5
2	11.52 ± 0.04	12.80 ± 0.11	6.74 ± 0.10	3.07 ± 0.07	2.23 ± 0.13	63.7
3	11.40 ± 0.08	14.54 ± 0.10	7.56 ± 0.20	2.68 ± 0.03	2.16 ± 0.09	61.7
4	11.09 ± 0.08	13.69 ± 0.11	6.31 ± 0.02	6.73 ± 0.05	2.77 ± 0.08	59.4
Mean ± SD	*11.52 ± 0.4*^{b}	*14.23 ± 1.3*^{a,b}	*6.79 ± 0.5*^{a}	*4.99 ± 2.5*^{a,b}	*2.42 ± 0.3*^{a}	*60.06±3.5*$^{b\pm}$

Means within a column with the same superscript letter are not significantly different ($\alpha = 0.05$).
nd = not determined.

Amaranthus seeds. Peñarrieta, Alvarado, Åkesson, and Bergenståhl (2008) identified vanillic and ferulic acids in whole plants of *C. pallicaule*. Their result for vanillic acid was of the same magnitude, whereas a lower level of ferulic acid was found compared with the present study. This discrepancy probably arises from the sample differences (seeds vs. whole plants) as well as different methodology. Peñarrieta et al. (2008) only analysed extractable phenolic acids, and according to our study a large proportion of vanillic acid, unlike ferulic acid, is present in soluble forms in *C. pallicaule* samples. However, Peñarrieta et al. (2008) also found much variation between samples. Klimczak et al. (2002) analysed the free phenolic acid content of *A. caudatus* seeds and found the same phenolic acids as in the present study except for sinapinic and vanillic acids. However, in our study soluble (or free) caffeic, ferulic, *p*-coumaric and protocatechuic acids were not found. Recently, Barba de la Rosa et al. (2009) published information concerning the phenolic acid content of a different amaranth species (*A. hypochondriacus*). According to their data amaranth seed flour contained soluble 4-hydroxybenzoic acid 0.17–0.22 mg/100 g, vanillic acid 0.15–0.18 mg/100 g and syringic acid 0–0.08 mg/100 g. These figures are much lower than those obtained in our study. This is probably due to the different methodology as well as the different species studied.

The Andean cereals contained lower levels of phenolic acids compared with common cereals like wheat (*Triticum* spp.) and rye (*Hordeum vulgare*). In these cereals the phenolic acids accumulate in bran where their levels are as high as 419 and 453 mg/100 g in rye and wheat bran while whole grain flours of these grains contain 137 and 134 mg/100 g, respectively (Mattila et al., 2005). However, according to Mattila et al. (2005) the phenolic acid content of other cereals like oat (*Avena sativa*), barley (*H. vulgare*), corn (*Zea mays*), rice (*Oryza sativa*), millet (*Panicum miliaceum*) and buckwheat (*Fagopyrum esculentum*) is of the same magnitude (25–60 mg/100 g) as in the Andean grains studied here.

The flavonoid content of *Chenopodium* species was exceptionally high, varying from 36.2 to 144.3 mg/100 g (Table 4). The predominant flavonoids in quinoa samples were quercetin and kaempferol while in some varieties myricetin and isorhamnetin were also found. Kañiwa samples contained mostly quercetin and isorhamnetin with smaller amounts of myricetin, kaempferol and rhamnetin in some varieties. As in the case of phenolic acids much variation was found between different samples. There were no statistically significant differences in the content of quercetin, rhamnetin and total flavonoids in quinoa and kañiwa. The content of isorhamnetin was significantly higher in kañiwa compared with quinoa. In the case of kaempferol, the content in kañiwa was significantly lower than in quinoa.

Berries have been considered as an excellent source of flavonols, especially quercetin and myricetin. For example, lingonberry contains 10 mg/100 g fw of quercetin and cranberry contains 10.4 and 6.9 mg/100 g fw quercetin and myricetin, respectively (Mattila et al., 2000). The levels in these flavonoid-rich berries are 5–10-fold lower than those found in *Chenopodium* seed samples. When compared on a dry weight basis the flavonoid contents in berries and *Chenopodium* samples are of the same magnitude. Quinoa and kañiwa seeds can thus be considered very good source of flavonoids. Common cereals (wheat, rye, oat, barley, etc.) do not contain any flavonols (Shahidi & Naczk, 1995).

To our knowledge, this is the first paper reporting the total content of flavonoids in quinoa and kañiwa seeds. Peñarrieta et al. (2008) analysed extractable flavonoids in the whole plant of *C. pallicaule* and found quercetin and kaempferol. The levels of quercetin were much lower than those obtained in the present study. De Simone, Dini, Pizza, Saturnino, and Scettino (1990) and Zhu et al. (2001) characterised flavonol glycosides in quinoa (*C. quinoa* Willd) seeds. Zhu et al. (2001) isolated and characterised six flavonol glycosides: four kaempferol glycosides and two quercetin glycosides. Among them kaempferol 3-O-[β-D-apiofuranosyl(1‴-2″)]-β-D-galactopyranoside, kaempferol 3-O-[2,6-di-α-L-rhamnopyranosyl)-β-D-galactopyranoside and quercetin 3-O-[2,6-di-α-L-rhamnopyranosyl)-β-D-galactopyranoside were the main flavonoid glycosides found in quinoa seeds. Kaempferol and quercetin were also the main flavonoid aglycones found in the present study.

There were no quantifiable amounts of flavonoids in amaranth samples: only traces of quercetin were found. Barba de la Rosa et al. (2009) also detected low levels of quercetin glycoside, rutin (4.0–10.2 μg/g) in *A. hypochondriacus* seeds.

Table 3
Total contents (mg/100 g) and percentual shares of soluble phenolic acids in quinoa, kaniwa and kiwicha grains.

Sample	Caffeic acid	Ferulic acid	*p*-Coumaric acid	*p*-OH-benzoic acid	Vanillic acid	Sinapic acid	Protocatechuic acid	Total
Quinoa samples								
Ccoito	0.95 ± 0.04 (63%)	15.3 ± 0.5 (3%)	6.46 ± 0.18 (48%)	3.87 ± 0.07 (66%)	8.97 ± 0.01 (53%)			35.6 ± 0.4 (40%)
INIA-415 Pasankalla	0.61 ± 0.03 (84%)	20.0 ± 0.2 (36%)	27.5 ± 0.4 (72%)	2.44 ± 0.02 (71%)	9.19 ± 0.36 (56%)			59.7 ± 0.5 (61%)
Roja de Coporaque	0.50 ± 0.03 (98%)	13.9 ± 0.6 (50%)	4.07 ± 0.01 (49%)	2.60 ± 0.08 (60%)	11.0 ± 0.3 (42%)			32.1 ± 1.0 (49%)
Witulla	1.47 ± 0.21 (33%)	14.9 ± 0.7 (21%)	2.26 ± 0.08 (39%)	2.46 ± 0.09 (68%)	9.20 ± 0.28 (57%)			30.3 ± 0.6 (38%)
03-21-0093	0.86 ± 0.02 (61%)	16.6 ± 0.5 (39%)	8.72 ± 0.02 (46%)	2.80 ± 0.13 (92%)	10.7 ± 0.5 (44%)			39.7 ± 1.1 (46%)
Salcedo INIA	0.25 ± 0.01 (92%)	12.3 ± 0.9 (17%)	8.02 ± 0.36 (46%)	3.17 ± 0.02 (83%)	14.6 ± 0.2 (39%)			38.4 ± 1.5 (37%)
Commercial 1	0.57 ± 0.02 (39%)	18.6 ± 1.7 (19%)	2.84 ± 0.14 (18%)	3.38 ± 0.24 (63%)	11.9 ± 0.3 (41%)			37.2 ± 1.9 (30%)
Commercial 2	0.87 ± 0.03 (16%)	14.3 ± 0.1 (15%)	2.60 ± 0.03 (27%)	3.88 ± 0.04 (55%)	10.3 ± 0.1 (39%)			32.0 ± 0.1 (29%)
Huaripongo	0.37 ± 0.04 (95%)	12.0 ± 0.1 (19%)	4.01 ± 0.06 (0%)	2.65 ± 0.02 (98%)	12.4 ± 0.1 (10%)			31.4 ± 0.2 (21%)
03-21-1181	0.59 ± 0.07 (0%)	13.7 ± 0.7 (52%)	9.50 ± 0.36 (0%)	1.92 ± 0.08 (56%)	10.7 ± 0.5 (60%)			36.3 ± 1.2 (40%)
Mean ± SD	*0.7 ± 0.4*^{a}	*15 ± 3*^{a}	*8 ± 7*^{a}	*2.9 ± 0.6*^{a}	*11 ± 2*^{a}	*0 ± 0*^{a}	*0 ± 0*^{a}	*37 ± 9*^{a}
Kañiwa samples								
Kello	1.10 ± 0.01 (63%)	26.1 ± 1.9 (4%)	1.34 ± 0.12 (0%)	1.77 ± 0.09 (37%)	4.34 ± 0.30 (20%)			34.7 ± 2.4 (10%)
Wila	2.16 ± 0.02 (69%)	29.8 ± 0.2 (18%)	1.00 ± 0.01 (0%)	1.77 ± 0.04 (30%)	3.61 ± 0.08 (92%)			38.3 ± 0.3 (28%)
Guinda	2.37 ± 0.12 (8%)	26.0 ± 0.8 (10%)	1.74 ± 0.19 (0%)	1.55 ± 0.08 (18%)	3.04 ± 0.18 (89%)			34.7 ± 1.3 (17%)
Ayara	7.04 ± 0.11 (15%)	23.4 ± 1.2 (10%)	0.70 ± 0.04 (0%)	1.97 ± 0.19 (25%)	6.95 ± 0.21 (56%)			40.1 ± 1.7 (19%)
Commercial sample	1.10 ± 0.09 (83%)	12.0 ± 0.4 (6%)	0.37 ± 0.02 (0%)	1.54 ± 0.13 (72%)	3.23 ± 0.38 (97%)			18.3 ± 0.8 (32%)
Mean ± SD	*3 ± 2*^{b}	*23 ± 7*^{b}	*1.0 ± 0.5*^{a}	*1.7 ± 0.2*^{b}	*4 ± 2*^{b}	*0 ± 0*^{a}	*0 ± 0*^{a}	*33 ± 9*^{a,b}
Kiwicha samples								
1	0.85 ± 0.01 (0%)	8.32 ± 0.70 (0%)	0.81 ± 0.04 (0%)	3.16 ± 0.02 (71%)	6.67 ± 0.03 (9%)	0.32 ± 0.04 (0%)	12.8 ± 0.4 (0%)	32.9 ± 1.3 (9%)
2	0.87 ± 0.02 (0%)	6.46 ± 0.64 (0%)	0.99 ± 0.09 (0%)	1.97 ± 0.15 (65%)	4.28 ± 0.42 (5%)	0.09 ± 0.01 (0%)	6.28 ± 0.42 (0%)	20.9 ± 1.4 (7%)
3	0.70 ± 0.07 (0%)	6.21 ± 0.09 (0%)	0.80 ± 0.05 (0%)	3.19 ± 0.02 (47%)	6.38 ± 0.40 (15%)	0.09 ± 0.01 (0%)		17.4 ± 0.6 (14%)
4	1.13 ± 0.04 (0%)	6.57 ± 0.01 (0%)	0.98 ± 0.02 (0%)	3.68 ± 0.10 (15%)	4.35 ± 0.26 (25%)	0.09 ± 0.01 (0%)		16.8 ± 0.4 (10%)
Mean ± SD	*0.9 ± 0.2*^{a,b}	*6.9 ± 1.0*^{c}	*0.89 ± 0.11*^{a}	*3.0 ± 0.7*^{a}	*5.4 ± 1.3*^{b}	*0.15 ± 0.11*^{a}	*5 ± 6*^{b}	*22 ± 7*^{b}

Means within a column with the same superscript letter are not significantly different ($\alpha = 0.05$).

Table 4
Contents of flavonoids in quinoa and kaniwa grains (mg/100 g).

Sample	Myricetin	Quercetin	Kaempferol	Isorhamnetin	Rhamnetin	Total
Quinoa samples						
Ccoito		38.1 ± 2.3	16.3 ± 1.6			54.5 ± 4.0
INIA-415 Pasankalla		35.7 ± 0.2	0.45 ± 0.11			36.2 ± 0.3
Roja de Coporaque	0.22 ± 0.04	55.5 ± 4.2	16.9 ± 1.1			72.6 ± 5.3
Witulla	0.86 ± 0.11	23.5 ± 0.8	44.7 ± 1.2			69.0 ± 2.1
03-21-0093	0.90 ± 0.13	32.6 ± 0.1	14.2 ± 0.7			47.7 ± 1.0
Salcedo INIA		11.6 ± 0.1	54.2 ± 0.5			65.8 ± 0.6
Commercial 1	1.24 ± 0.07	36.8 ± 0.6	10.2 ± 0.3	2.08 ± 0.06		50.3 ± 1.0
Commercial 2	0.51 ± 0.08	47.1 ± 2.4	21.5 ± 1.1			69.2 ± 3.6
Huaripongo	0.88 ± 0.20	53.2 ± 4.1	14.2 ± 0.8	0.89 ± 0.11		69.2 ± 5.2
03-21-1181	0.67 ± 0.12	28.5 ± 2.7	11.5 ± 0.3	1.02 ± 0.10		41.7 ± 3.2
Mean ± SD	*0.5 ± 0.5*^{a}	*36 ± 13*^{a}	*20 ± 20*^{a}	*0.4 ± 0.7*^{a}	*0 ± 0*^{a}	*58 ± 13*^{a}
Kañiwa samples						
Kello		84.3 ± 1.2		60.0 ± 1.3		144.3 ± 2.5
Wila		68.7 ± 5.8		14.2 ± 0.8		83.0 ± 6.6
Guinda		25.1 ± 2.0		29.5 ± 1.3		54.6 ± 3.3
Ayara		21.4 ± 1.4	5.97 ± 0.02		18.7 ± 2.0	46.1 ± 3.5
Commercial sample	0.18 ± 0.01	78.6 ± 6.6	2.24 ± 0.33	24.8 ± 2.4		105.8 ± 9.3
Mean ± SD	*0.04 ± 0.08*^{b}	*60 ± 30*^{a}	*2 ± 3*^{b}	*30 ± 20*^{b}	*4 ± 8*^{a}	*90 ± 40*^{a}

Means within a column with the same superscript letter are not significantly different ($\alpha = 0.05$).

Table 5
Contents of betacyanins in kiwicha grains (mg/100 g).

Sample	Amaranthine	Iso-amaranthine	Betanin	Total
1	nd[a]	nd	nd	nd
2	nd	nd	nd	nd
3	1.0 ± 0.2	0.8 ± 0.2	0.1 ± 0.2	1.9 ± 0.4
4	nd	nd	nd	nd

[a] nd ⩽ 0.1 mg/100 g.

The betacyanin content is presented in Table 5. Of the analysed kiwicha samples only the pink variety contained betacyanins above LOQ (0.1 mg/100 g). The total amount of betacyanins was low (1.9 ± 0.4 mg/100 g dw) compared to the values (mean 91.4 ± 4.0 mg/100 g fresh weight) reported in different vegetative parts, i.e. seedlings, leaves and inflorescences, of *Amaranthus* (Cai et al., 2001). To our knowledge no data on betacyanins in kiwicha seeds have previously been reported in the literature.

Our results indicate that the Andean indigenous crops, quinoa and kañiwa, are very good sources of flavonoids. Kiwicha does not contain quantifiable amounts of these compounds. The levels of flavonoids in quinoa and kañiwa were superior to those in flavonoid-rich berries such as lingonberry and cranberry. The phenolic acid content of Andean indigenous crops is comparable to the content of these substances in oat, barley, corn and rice. Overall, our study demonstrates that Andean indigenous crops have excellent potential as sources of health-promoting bioactive compounds such as flavonoids. More studies are required with the aim of identifying the most promising varieties in this respect.

Further studies should be conducted to determine phenolic compound composition and anti-oxidant content and activity in processed Andean grains.

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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.

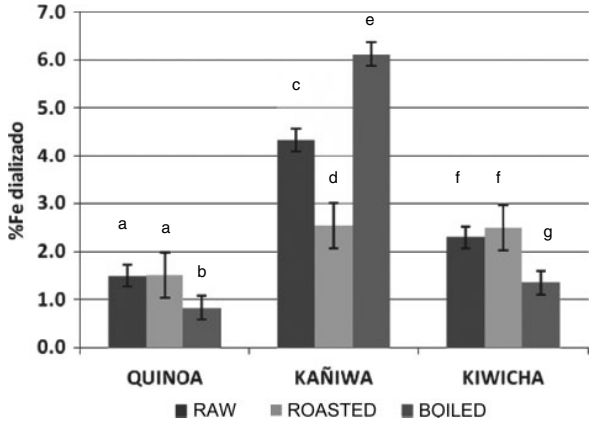

Figure 1. Iron dialyzability (FeD%) of raw, roasted and boiled quinoa, kañiwa and kiwicha. Means ± standard deviation for six analyses. Statistical comparison was between raw, roasted and boiled grain. For each grain type, means not sharing a common letter differ.

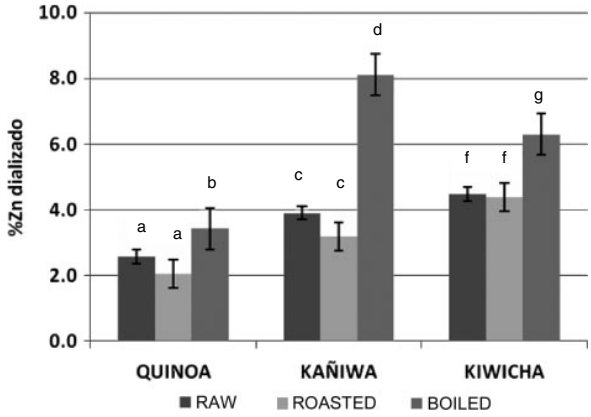

Figure 2. Zinc dialyzability (ZnD%) of raw, roasted and boiled quinoa, kañiwa and kiwicha. Means ± standard deviation for six analyses. Statistical comparison was between raw, roasted and boiled grain. For each grain type, means not sharing a common letter differ.

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%.

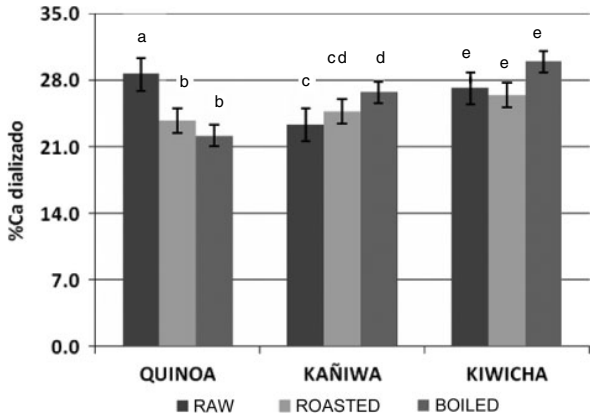

Figure 3. Calcium dialyzability (CaD%) of raw, roasted and boiled quinoa, kañiwa and kiwicha. Means ± standard deviation for six analyses. Statistical comparison was between raw, roasted and boiled grain. For each grain type, means not sharing a common letter differ.

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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