DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY AND TOTAL PHENOLIC COMPOUNDS IN ANDEAN GRAINS (QUINUA, CAÑIHUA, AMARANTH AND QENTU)

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ABSTRACT

The nutritional habits in Bolivia are changing by the rapids adoption of new globalized diets with great influence of western civilization habits. The new habits in association with lower physical activity and the increased intake of alcohol and tobacco are risk factors for the development of chronic diseases. We performed an initial study of the antioxidant activity by FRAP and ABTS methods and Total Phenols by Folin Ciocalteau method in quinoa, cañihua, amaranth and qentu./ *Los hábitos nutricionales en Bolivia están cambiando por la adopción rápida de nuevas dietas globalizadas con una gran influencia de hábitos de la civilización occidental. Los nuevos hábitos en asociación con la baje actividad física y el aumento en el consumo de alcohol y tabaco son factores de riesgo para el desarrollo de enfermedades crónicas. Realizamos un estudio inicial de la actividad antioxidante de quinua, cañihua, amaranto y qentu por los métodos FRAP y ABTS y de Fenoles Totales por el método de Folin - Ciocalteau*

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INTRODUCTION

Bolivian diet is characterized by a great intake of vegetable foodstuff such as cereals, fruits, potatoes and others. The carbohydrates plays an excessive role in the food consume habits of Bolivian population.
The high status level population ingests a greater variety of foods but has reduced the intake of potatoes and cereals. And in another hand the low status level population bases it's consume largely on carbohydrate intake due to its economic accessibility [1]. In Latin America the nutritional habits and the style of life are dramatically changing. The Bolivian diet is changing also by the adoption of USA customs, low physic activity, increased intake of alcohol and tobacco day by day and overweight, obesity and development of chronic diseases are becoming more often. Infections, chronic or degenerative diseases, (diabetes, obesity, overweight, cardio-vascular diseases, etc.) are becoming a main problem in the Bolivian society while the malnutrition still continues affecting our population. The socioeconomic and cultural factors have a direct relation with the diseases named in the last paragraph. Recent researches found that women are more vulnerable to degenerative diseases. The present work had as goal to search the Total Antioxidant Capacity by FRAP and ABTS methods and to evaluate the Total Phenols by Folin & Ciocalteau method in Andean grains as quinoa (*Chenopodium quinoa*), cañihua (*Chenopodium pallidicaule*), amaranth (*Amaranthus caudatus*) and seeds of qentu (*Rumex acetosella*) that serves as forage for animals, as a contribution to the studies of typical Bolivian foods.

EXPERIMENTAL SECTION

Materials and methods

Chemicals

Gallic acid (99 %), ABTS, trolox (99 %), potassium persulfate, acetic acid, sodium acetate, TPTZ were obtained from Sigma Aldrich. Ferric chloride and acetone were obtained from Biopach. Hydrochloric acid, sodium carbonate and Folin - Ciocalteau reagent were purchased from Scharlam.

Sampling sites (see table 1)

Table 1. Sampling description

Sample	Site	Average height (m.o.s.l.)	Season	Month	Place´s characteristic	Sample´s characteristic
Quinoa	Ayamaya	4700	Humid	October	Very dry	Dry
Cañihua	Ayamaya	4700	Humid	October	Very dry	Dry
Qentu	Walata	3300	Dry	April	Very damp	Fresh

The amaranth's sample was collected in February of 2006 from a store in the Santa Cruz Street. According to information taken from the seller the sample came from Cochabamba city.

Plant material

The samples of quinoa and cañihua were dry with husks. The grains of both species were dehulled with a traditional technique called "soplado" i.e., blown, that consist to exert strong pressure over the grains with both hands and after this, the grains are thrown in opposite direction to the wind, action that helps to remove the impurities from the grains. The fresh qentu sample was dried with help of papers and without exposition to the light around one week. Once dried the grains were cleaned with the "blown" technique. We did not need to clean the grains of amaranth, because the sample was ready for intake. After the cleaning all the samples were ground and macerated around 24 hours in different kind of solvents (in a non polar to polar solvent sequence). We obtained four different kinds of extracts for each sample; the first extraction was with petroleum ether 20-40 ºC, methane dichloride, ethyl acetate and methanol.

Measurement of TAC

TAC was measured by ABTS and FRAP methods on a double beam UV-Visible spectrophotometer Perkin Elmer model lambda 25 at 25°C. Trolox was used liked a standard analogue of alpha – tocopherol. A stock solution containing 5 mmol/L of trolox was stored at -20 ºC.

The ABTS method

The colourless ABTS (7 mmol/L) was oxidized to the green $ABTS^{\bullet+}$ radical cation by the addition of potassium persulphate (2.42 mmol/L) and kept for 12-16 h at room temperature in the dark. On the day of analysis the $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm. After the addition of 1.0 ml of $ABTS^{\bullet+}$ solution to 100 µL of sample the mixture was stirred for 30 s and the absorbance at 734 nm and 25 ºC was recorded for 6 min. The decrease in absorbance caused by the addition of sample was compared with that of a standard curve made by use of trolox (20-200 µmol/L).

The FRAP method

The yellow Fe^{3+}-TPTZ complex by electron donating substances under acidic conditions. Any electron donating substance with a half reaction of lower red-ox potential than Fe^{3+}/Fe^{2+}-TPTZ will drive the formation of the blue complex forward. The FRAP reagent was a mixture of 0.1 mol/L sodium acetate buffer (pH 3.6), 10 mmol/L TPTZ and 20 mmol/L ferric chloride (10:1:1 v/v/v). To 900 µL of reagent 90 µL of water and 30 µL of sample were added. The absorbance readings were performed at 593 nm for 10 min. The blank consisted of 120 µL of water and 900 µL of reagent. The final absorbance of each sample was compared with that of a standard curve made using trolox (100-1000 µmol/L). The data were expressed as µmol trolox equivalents per gram of dry matter. To assess the TAC of reference compounds these compounds were dissolved in ethanol at 25-180 µmol/L.

Measurement of Total Phenolic Compounds

The Total Phenolic Compounds (TPH) were determined using the Folin - Ciocalteau reagent which oxidizes the phenolic compounds to phenolates at alkaline pH in a saturated solution of sodium carbonate resulting in a blue molybdenum-wolfram complex. The Folin Ciocalteau reagent, diluted ten times (2,5 mL) and 2 mL of saturated sodium carbonate (75g/L) and 50 µL of sample (diluted ten times) were mixed for 10 s and heated for 30 min at 45 ºC. The absorbance at 765 nm was read after cooling to the room temperature. The absorbance of each sample was compared with those obtained from the standard curve

made from gallic acid (235-1176 µmol/L). The data were expressed as µmol gallic acid equivalents (GAE) per gram of dry matter.

Statistical analysis

The results were expressed as mean values (SD) of six replicates measured over three days for TAC by FRAP and ABTS methods and TPH by Folin-Ciocalteau method. Linear correlations coefficients were calculated according to Pearson method. All calculations were done using Excel software.

RESULTS

Total Antioxidant Capacity in quinoa, cañihua, amaranth and qentu

The highest TAC values by ABTS method were observed in the sample of qentu (methanol extract) 13.02 (0.2) µmol trolox/g dw and cañihua (dichloro methane extract) 0.35 µmol trolox/g dw, respectively (Table 2). Several intermediate values 0.0001-0.22 µmol trolox/g dw were found in petroleum ether extract, 0.01-0.35 µmol trolox/g dw in dichloro methane extract, 0.008-0.32 µmol trolox/g dw in ethyl acetate extract and 0.07-13.02 µmol trolox/g dw in methanol extracts.

The lowest values were observed in cañihua (petroleum ether extract) 0.0001 µmol trolox/g dw and qentu (dichloro methane extract) 0.01 µmol trolox/g dw. Also by the FRAP method the highest TAC values were observed in sample of qentu (methanol extract) 315.9 µmol trolox/g dw and cañihua (dichloro methane extract) 1.21 µmol trolox/g dw. The range of values was found between 0.60-1.30 µmol trolox/g dw in petroleum ether extract, 0.15-1.21 µmol trolox/g dw in dichloro methane extract, 0.16-2.90 µmol trolox/g dw in ethyl acetate extract and 0.32-315.90 µmol trolox/g dw in methanol extract, while the lowest values were determined in cañihua (ethyl acetate extract) 0.16 µmol trolox/g dw and qentu (dichloro methane extract) 0.15 µmol trolox/g dw. Apparently the qentu sample (methanol extract) had a very high content of antioxidants expressed in the results obtained by both methods.

Table 2. TAC in petroleum ether (20-40ºC), dichloro methane, ethyl acetate and methanol extracts of quinoa, cañihua, amaranth and qentu samples by the ABTS and FRAP methods

Sample	ABTS				FRAP			
	Petroleum ether extract	Dichloro methane extract	Ethyl acetate extract	Methanol extract	Petroleum ether extract	Dichloro methane extract	Ethyl acetate extract	Methanol extract
Quinua	0.22(0.003)	0.04(0.03)	0.03 (0.03)	0.07 (0.007)	0.74(0.04)	0.90(0.1)	0.45(0.04)	0.74(0.08)
Canihua	0.0001(0)	0.35 (0.08)	0.008(0.008)	0.07 (0.002)	0.31(0.03)	1.21(0.06)	0.16(0.02)	0.32(0.03)
Amaranto	0.002(0.004)	0.18(0.02)	0.04(0.03)	0.05(0.002)	0.6(0.1)	1.03(0.03)	0.26(0.05)	0.33(0.04)
Qentu	0.01(0.01)	0.01(0.0002)	0.32(0.002)	13.02 (0.007)	1.3(0.2)	0.15(0.009)	2.9(0.4)	315.9(103.8)

The TAC data are expressed as µmol Trolox equivalents per gram of dry matter and are means (SD) from six measurements.

Table 3. The content of total phenolic compounds (TPH) in petroleum ether (20-40ºC), dichloro methane, ethyl acetate and methanol extracts of quinoa, cañihua, amaranth and qentu samples by Folin - Ciocalteau method.

Sample	TPH			
	Petroleum ether extract	Dichloro methane extract	Ethyl acetate extract	Methanol extract
Quinua	0.008(0.0005)	0.004(0.0002)	0.004(0.0002)	0.0007(0.00007)
Canihua	0.0006(0.00001)	0.004 (0.0002)	0.002(0.00007)	0.0002 (0.00001)
Amaranto	0.005(0.0003)	0.004(0.00002)	0.002(0.00005)	0.0004(0.00001)
Qentu	0.003(0.0001)	0.0003(0.000003)	0.0003(0.00001)	0.15(0.008)

The TPH compounds are expressed as µmol Gallic Acid equivalents (GAE) per gram of dry matter and are means (SD) from six measurements

Total Phenolic Compounds

The highest values of gallic acid equivalent were observed in the sample of qentu (methanol extract) 0.15 µmol GAE/g dw and quinoa (petroleum ether extract) 0,008 µmol GAE/g dw. While the lowest values were observed in cañihua (methanol extract) 0.0002 µmol GAE/g dw and qentu (dichloro methane extract) 0.0003 µmol GAE/g dw. Intermediate values were found between 0.0006-0.008 µmol GAE/g dw in petroleum ether extract, 0.0003-0.004 µmol GAE/g dw in dichloro methane extract, 0.0003-0.004 µmol GAE/g dw in ethyl acetate extract and 0.0002-0.15 µmol GAE/g dw in methanol extract.

Correlation among measurements

The several data obtained by ABTS, FRAP and TPH methods were correlated to each other and in this calculations the outlier qentu (methanol extract) was omitted. The statistical correlations were observed between data from ABTS method versus FRAP method, TPH versus FRAP and TPH versus ABTS (the correlations were between all values from the different kind of extracts).

Table 4. Correlation coefficient between different measurements performed in all extracts of quinoa, cañihua, amaranth and qentu.

All extracts	Correlation coefficient (r)
FRAP – ABTS	0,99929832 **
FRAP – ABTS [without the outlier qentu (methanol extract)]	0,49842535 *
FRAP - Folin & Ciocalteau	0,99184131 **
FRAP - Folin & Ciocalteau [without the outlier qentu (methanol extract)]	0,00059728
ABTS - Folin & Ciocalteau	0,99218169 **
ABTS - Folin & Ciocalteau [without the outlier qentu (methanol extract)]	0,05258337

The correlation coefficient was calculated by Pearson method. *p<0.05; **,0.001<p<0.01

DISCUSSION

Total Antioxidant Capacity in quinoa, cañihua, amaranth and qentu.

The total values of TAC (sum of the all values from different extracts) in samples of quinoa, cañihua and amaranth were lower than literature data. Could be the main reason was the store temperature, because the samples were stored at environmental temperature around 20 ºC but not at – 80 ºC as literature data,. On other hand the readings of TAC were taken ten months after the extracts were prepared. The values of FRAP found by Halvorsen et al were 0.2-5.5 µmol trolox/g dw in cereals and the highest values of buckwheat (*Fagopyrum esculentum*) were 6 and 10 µmol trolox/g dw [33]. Saura-Calixto and Goñi found that the value of TAC by FRAP method in Mediterranean cereals was 2.2 µmol trolox/g dw [34]. The total values of TAC found by FRAP method in quinoa (2,8), cañihua (2) and amaranth (2,2) were into the range 0.2 - 5.5 µmol trolox/ dw of the cereals by Halvorsen, but the highest value of qentu (320,2) was over range of the cereals and the values of buckwheat (pseudocereal). And the total values of the quinoa, cañihua and amaranth were closed to the mean value (2.2 µmol/g dw) of Mediterraen cereals by Saura-Calixto and Goñi and again the total value of qentu was over the main value. Researches made by Chandira and Fereidoon found the TAC in wheat flour was 0.6 µmol Trolox/g fw by ABTS method[35]. Saura-Calixto and Goñi published a mean value 0.2 µmol trolox/g dw in Mediterranean cereals by ABTS method [34]. The values by ABTS method in the samples of quinoa (0,4), cañihua (0,4) and amaranth (0,3) were lower than the mean value of wheat flour (0.6 µm/fw), but the qentu (13,4) was over the mean value. On another hand the values of the samples of quinoa, cañihua and amaranth compared with the main value of Mediterraen cereals by Saura-Calixto y Goñi were over the mean value and the value of qentu sample reported more activity again. Therefore, the values of quinoa, cañihua and amaranth were into the range 0.2 - 5.5 µmol/g dw in cereals by Halvorsen and were close to the mean value 2.2 µmol/g dw in Mediterranean cereals by Saura-Calixto and Goñi.

Total Phenolics Compounds in quinoa, cañihua, amaranth and qentu.

Saura-Calixto and Goñi published that the mean value in Mediterraen cereals was 6,3 µmol GAE /g dw. The values that were found in the samples of qentu (0,1), quinoa (0,02), amaranth (0,01) y cañihua (0,007) were under the main value in Mediterranean cereals by Saura-Calixto and Goñi.

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ABBREVIATIONS

ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); **FRAP**, ferric reducing antioxidant power; **GAE**, Gallic Acid Equivalents; **TAC**, Total Antioxidant Capacity; **TPH**, Total Phenolic Compounds.

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Table 5. The total sum of TAC in the different extracts from each sample by FRAP and ABTS method (µmol of trolox / g dw) and the total sum of TPH in the different extracts from each sample by Folin & Ciocalteau method (µmol of gallic acid equivalent / g dw).

Samples	FRAP	ABTS	Folin & Ciocalteau
Qentu	320,2	13,4	0,1
Quinua	2,8	0,4	0,02
Amaranto	2,2	0,3	0,01
Cañihua	2	0,4	0,007

Table 6.Comparison of experimental and published data of the TAC by FRAP and ABTS method (umol of trolox/g dw).

Samples	FRAP*		ABTS*	
Quinua	2,8	3,8	0,4	2,9
Cañihua	2	14,1	0,4	7,2
Amaranto	2,2	0,1	0,3	0,3

*Peñarrieta et al.[3]