DETERMINATION OF TOTAL PHENOLIC COMPOUNDS CONTENT AND THE ANTIOXIDANT CAPACITY OF ANDEAN TUBERS AND ROOTS (ISAÑO, OCA, ULLUCO AND ARRACACHA)

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Key Words: Andean tubers, isaño, oca, ulluco, roots of arracacha, Total Antioxidant Capacity, ABTS, FRAP, Total Phenolic Compounds.

ABSTRACT

Four species of Andean tubers, isaño (*Tropaeolum tuberosum*), oca (*Oxalis tuberosa*), ulluco (*Ullucus tuberosus*) and roots of arracacha (*Arracacia xanthorrhiza*), were studied for the quantification of the Total Antioxidant Capacity (TAC) and determination of Total Phenolic Compounds (TPH). The total antioxidant capacity was measured using ABTS and FRAP methods, and the total phenolics compounds were measured using the Folin & Ciocalteu reagent. The antioxidant capacity found in the tubers and root studied ranged from 0.35 to 11.8 µmol trolox equiv/g dry sample, and phenolics ranged from 0.002 to 0.02 µmol gallic acid equiv./g dry sample. In general black isaño tubers showed the highest values of antioxidant capacity and phenolic compounds compared with the other tubers./ *Cuatro especies de tubérculos andinos, isaño (Tropaeolum tuberosum), oca (Oxalis tuberosa), ulluco (Ullucus tuberosus) y raíces de arracacha (Arracacia xanthorrhiza) fueron estudiados para la cuantificación de la capacidad antioxidante total (TAC) y la determinación de compuestos fenólicos totales (TPH). La capacidad antioxidante fue medida mediante el uso de los métodos ABTS y FRAP, los compuestos fenólicos fueron medidos mediante el uso del reactivo de Folin & Ciocalteau.La capacidad antioxidante encontrada en los tubérculos estudiados tienen un rango de 0.35 a 11.8 µmol equiv. de trolox/g de muestra seca, el rango de compuestos fenolicos es de 0.002 a 0.02 µmol equiv. de ácido gálico/g de muestra seca. En general el isaño oscuro muestra los valores mas altos de capacidad antioxidante y compuestos fenólicos comparados con los otros tubérculos.*

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INTRODUCTION

The interest in natural antioxidants has increased considerably in recent years because many antioxidants exhibit beneficial biological effects, including antibacterial, antiviral, antiallergic, antithrombotic and because they are linked to lower incidence of cardiovascular disease and certain types of cancer disease [1]. The Andean habitants have been using tubers and roots such as isaño, oca, ulluco and arracacha for their medicinal and nutritional properties since remote times. The diversity of tubers growing in the Andean region at altitudes between 2000 and 4200 m above sea level shows a large variability in size, color, form, primary nutrient constituents and secondary metabolites [2, 3, 4]. Bolivia is well known for its ample diversity of genetic recourses of potential utility for the humanity. The traditions and the diversity of cultivation constitutes an important source of knowledge that must be systematized and utilized by means of different actions. Andean tubers have a big importance for the food supply and economy of the people living in the Andean region who eat isaño, oca, ulluco and arracacha both fresh and dry. The unfavorable climate at high altitudes only permits a limited number of cultivars. They are also used in folk medicine [5]. These mostly unproven medicinal properties could in part be attributed to the antioxidants present in these tubers. The objective of this study was to provide new data regarding Andean tubers and roots from Bolivia as a source of antioxidant compounds

RESULTS AND DISCUSSION

Total Antioxidant Capacity of Andean tubers

The TAC values measured in the polar and non-polar extracts from seven samples by the ABTS and FRAP methods are shown in Table 1and 2. The highest TAC value was observed in black isaño in the methanol extract.

Intermediate values were found in yellow isaño, yellow oca, arracacha and dappled ulluco, and lower TAC values were demonstrated in pink oca and pink ulluco. For black isaño higher TAC values were obtained in the polar fractions (methanol and ethyl acetate) than in the non-polar fractions (dichloromethane and petroleum ether) by the ABTS and FRAP methods. The TAC values of the calibration curves showed good reproducibility (Figure 1,2) by both methods. Data from FRAP displayed a linear correlation with TPH data for the 28 extracts (r =0.96; Figure 3). Similar linear correlations have also been observed in previous studies [6]. The TAC values obtained for Andean tuber samples using the FRAP method were in most, but not in all cases, higher than those obtained with the ABTS method. In black isaño the highest values was found by the ABTS method in the methanol extract as well as in the total value of TAC.

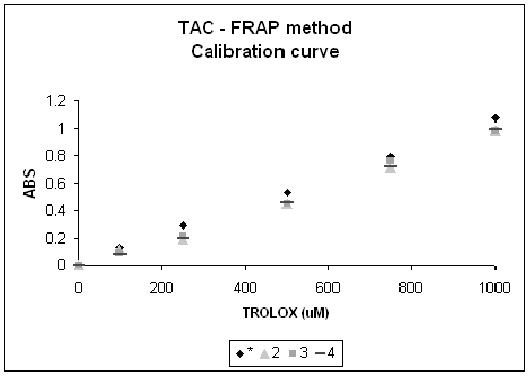

Figure 1: Calibration curve obtained by FRAP method on different days for the measure of TAC expressed as Trolox Equivalents/L (TE)

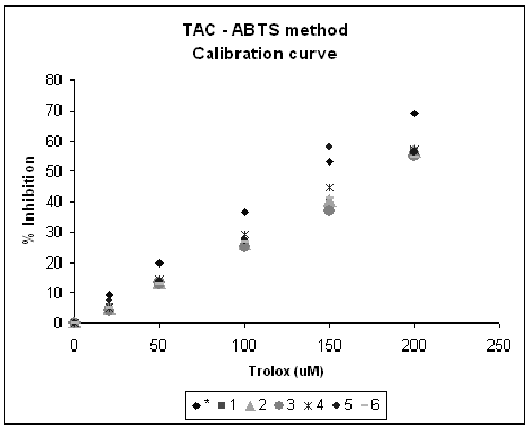

Figure 2: Calibration curve obtained by ABTS method on different days for the measure of TAC expressed as trolox equivalents/L (TE)

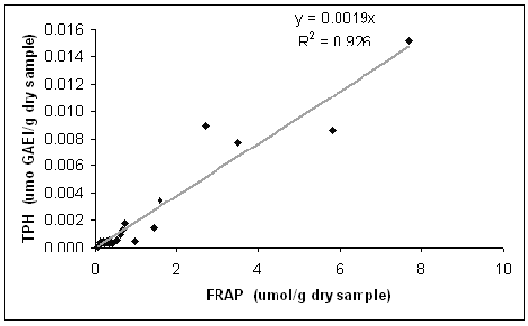

Figure 3: Relation between TAC-FRAP and TPH measurements in the 28 extracts of 7 andean tubers.

FRAP and ABTS methods have been used for analysis of TAC in different kinds of foods and some data on tubers and roots are available [7, 8]. Previous TAC values on tubers were approx. 0.27 – 3.03 µmol/g fresh sample and 0.9 – 14.7 µmol/g dry sample using ABTS and 0.32 – 1.57 µmol/g fw and 1.0 - 7.6 µmol/g dw by FRAP.

The content of Total Phenolic Compounds of the seven samples and twenty-eight extracts measured using the Folin & Ciocalteau method is shown in Table 3. The highest value was obtained in black isaño in both the methanol and ethyl acetate extracts. In the present study the range of TPH determined in tuber pulp was 0.002-0.02 μmol GAE/g dm expressed as the sum of the polar and non polar fractions. The values of Total Phenolic Compounds of the extracts showed a good linear correlation (Figure 3) between TAC values obtained by FRAP (r =0.96) and by ABTS (r =0.78) methods.

EXPERIMENTAL SECTION

Chemicals

Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid, 97%.), TPTZ (2,4,6-tripyridyl-s-triazine), ABTS (2,2'-azinobis-3-ethyl benzotiazoline-6-sulphonic acid, 98%), potassium persulfate, sodium acetate, Folin-Ciocalteau reagent, gallic acid (98%) and sodium carbonate were purchased from Sigma-Aldrich (St. Louis, USA). The solvents used were: methanol, ethanol, dichloromethane, ethylacetate and petroleum ether.

Plant material

Seven samples of andean tubers were purchased in the markets of La Paz, Bolivia in June 2006. The selected plants were ulluco (*Ullucus tuberosus*), arracacha (*Arracacia xanthorrhiza*), oca (*Oxalis tuberosa)* and isaño (*Tropaeolum tuberosum*). The seven varieties of samples were: two samples of oca (pink and yellow), two samples of Isaño (yellow with blach eyes and black), two samples of Ulluco (pink and dappled) and a sample of Arracha (yellow).

Sample preparation

The collected tubers and root were washed and peeled and 800 g of fresh sample was dried at 30°C during 24 hours. The dry samples (only dry pulp) were ground and sequentially soaked with petroleum ether, dichloromethane, ethyl acetate and methanol during 24 hrs for each solvent. TAC and TPH were measured in the seventy-eight concentrated extracts.

Measurement of TAC

TAC was assessed by using the ABTS [2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)] method described by Re *et al.* [9] and a modification of the FRAP (Ferric Reducing Antioxidant Power) method described by Benzie & Strain [10]. Both are spectrophotometric methods and the absorbance readings were performed on a double beam UV-Visible spectrophotometer Perkin Elmer model lambda 25 at 25°C. As a standard compound Trolox (6-hydroxy-2,5,7,8–tetramethyl-chroman-2-carboxylic acid) was used, The stock solution contained 5 mmol/L of Trolox in ethanol, and was stored at 4°C.

ABTS method. The ABTS method is based on the oxidation of ABTS to the green ABTS•+ radical cation after the addition of potassium persulphate (2.42 mmol/L) [11] for 12-16 hours at room temperature in the dark. On the day of analysis the ABTS•+ solution was diluted with acetate buffer pH 5.0 to an absorbance of 0.70 (±0.02) at 734 nm. After the addition of 1.0 of ABTS•+ solution to 100 μl of sample the mixture was stirred for 30 s and the absorbance at 734 nm and 25°C was recorded for 6 min. The decrease in absorbance caused by the addition of sample was compared with that of a standard curve by use of Trolox (20-200 μmol/L).

FRAP (ferric reducing/antioxidant power) method. The FRAP method was used for the determination of total antioxidant capacity, based on the reduction of yellow Fe3+-TPTZ complex to the blue Fe2+ -TPTZ complex by electron donating substance under acidic condition. The FRAP reagent (900 μl) containing TPTZ, FeCl3, and acetate buffer, was mixed with 90 μl of distilled water and 30 μl of the test sample or the blank (solvents used for extraction). Maximum absorbance values at 593 nm were recorded for 10 min at 25°C. The final absorbance was compared with the standard curve (100 – 1000 μmol/L). The data were expressed as μmol trolox equivalents per gram of dry matter.

Measurement of total phenolic compounds

The phenolic compounds were determined using the Folin-Ciocalteu method, based on the reduction of phosphor-wolframate-phosphomolybdate complex by phenolics to a blue reaction product [12, 13, 14]. The Folin-Ciocalteu reagent, diluted 10 times (2.5 ml) was mixed with 2 ml of saturated sodium carbonate (75 g/L) and

50 µl of sample (supernatant) and homogenized for 10 s and heated for 30 min at 45°C. The absorbance was measured at 765 nm after cooling at room temperature. The data were calculated by comparison between a standard curve (212-1062 µmol gallic acid /L) and the absorbance of each sample. The data were expressed as µmol gallic acid equivalents per gram of dry matter.

Statistical Analysis.
Results were expressed as mean values (standard deviation) of six replicates measured over three days of one extract. Linear correlation coefficient was calculated.

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