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Research Article

Anthocyanins, Antioxidant Capacity and Content of Flavonoids and Other Phenolic Compounds Oca (*Oxalis Tuberosum*) an Andean Tuber

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Abstract

To investigate the content of bioactive compounds and chemical composition in food plants growing at high altitude. The content of the Total Antioxidant Capacity (TAC), Total Phenolic Compounds (TPH), Total Flavonoids (TF) and individual phenolic compounds was studied in a tuber specie, oca (*Oxalis tuberosa)* collected in La Paz Bolivia at 3600 meters above sea level. The TAC values were 4-7 in by the FRAP method, and 2-6by the ABTS method, expressed as µmol of Trolox equivalents/g fw. The content of TPH content was 3-7(µmol gallic acid equivalents/g fw) and that of TF was 0.5-2 µmol of catechin equivalents/g fw. Prior to analysis by HPLC the samples were subjected to acid hydrolysis and in both the water-soluble and water-insoluble extracts this led to an increase (up to 3.5-fold in oca) in the TAC values in comparison with the values of the non-hydrolyzed samples. HPLC analysis showed the presence of, cyanidin, malvidin, delphinidinand kaempferol and quercetin and these compounds were also confirmed by HPLC-MS analysis. The results show that oca (*Oxalis tuberosa*) an Andean tuber growing at high altitude above sea level can be consider a good dietary source of phenolic compounds. More studies are necessary regarding its chemical composition in oca in order to know more about their active properties and it nutritional contribution to add scientific value that can contribute to the revalorization of this crop.

Keywords: Oca; Bolivia; Antioxidant capacity; Phenolic compounds; Anthocyanins; HPLC-MS

Abbreviations

ABTS: 2,2´-Azino-Bis(3-ethylbenzothiazoline-6-sulphonic acid); CE: Catechin Equivalent; FRAP: Ferric Reduction Antioxidant Power; GAE: Gallic Acid Equivalent; HPLC-DAD: High Performance Liquid Chromatography/Diode Array Detector; HPLC-MS: High Performance Liquid Chromatography with Mass Spectrometry; TAC: Total Antioxidant Capacity; TE: Trolox Equivalent; TF: Total Flavonoids; TPH: Total Phenolic Compounds

Introduction

In the Andean highlands between 2000 and 3000 years ago during the pre-tiwanakan Chiripa culture period (aprox. 400 B.C.) the production of endemic species was the base of the agriculture in the Lake Titicaca western region (presently Bolivia and Perú) and it expanded to the South American continent. The production of endemic tubers (more than 30 endemic species) was one the most important targets for the "sukakollos", a system of irrigation trenches surrounding the flat cultivation beds providing them with a mild micro-climate during cold nights providing frost protection [1,2]. Among them several tubers were common products, and potato (*Solanum tuberosum*) which is one of the most popular tubers around the world is a typical example of the contribution of this region. However, most Andean tubers are still not very well known outside their home countries and also due to the adoption of Western diet there is a decrement of their traditional use as foods within their countries. As an example of one of these crops, oca (*Oxalis tuberosa*) is specie growing at 2000-4500 meters above sea level (m.a.s.l.) in the Andean region being today part of traditional dishes in Bolivia, Peru, Colombia and Ecuador. *Oxalis tuberosa* is also found in Venezuela and the northern parts of Chile and Argentina. A relevant curiosity is that at the end of the 19th century *Oxalis tuberose* was introduced in New Zealand where nowadays it is a popular plant food under the name of New Zealand yam but a part of these examples oca is still unknown in the world.

As it was mentioned before oca is an important ingredient in the diet of many people in South America and can be consider a good source of active compounds. For instance, previous studies showed high antioxidant capacity in oca in comparison with other Andean tubers and the presence of anthocyanins especially init peels, which are also associated with a potential antioxidant capacity [3-5].

Much information indicates that consumption of antioxidants and other bioactive compounds from plant foods may have health promoting effects and there is a scientific interest in the investigation of new plant food sources of antioxidants. As a part of a programme focussing on the content of these compounds in Bolivian foods [3,6,7], the Total Antioxidant Capacity (TAC), phenolic and flavonoid compounds have been measured in oca (*Oxalis tuberosa*) growing in the Andean region of Bolivia.

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Table 1: Total Antioxidant Capacity (TAC), and content of Total Phenolic Compounds (TPH) and Total Flavonoids (TF) obtained in oca fractions (medians and range; µmol Trolox equivalents per gram of fresh matter).

Sample	ABTS						FRAP						TPH			TF		
	WS Before[a]	WS After[a]	WIS Before	WIS After[a]	WS+ WIS Before	WS+ WIS After[a]	WS Before	WS After[a]	WIS Before	WIS After[a]	WS+ WIS Before	WS+ WIS After[a]	WS	WIS	WS+ WIS	WS	WIS	WS + WIS
Oca Median	1.7	7.e6	0.4	1.8	2.8	9.6	4.2	7.4	0.8	1.6	5.2	8.8	3.8	0.9	5.2	0.5	0.6	1.2
Range	1.4-3.8	6.4-9.8	0.2-2.1	0.7-3.6	1.8-5.5	7.1-13.4	3.5-4.6	5.3-9.9	0.3-2.2	0.8-3.1	3.8-6.8	8.3-13	2.5-5.7	0.1-1.9	2.8-6.4	0.1-0.9	0.3-1	0.4-1.9

WS: Water-Soluble; WIS: Water-Insoluble

[a]Significantly different from the values obtained before hydrolysis ($p<0.001$).

Materials and Methods

Plant material

Seven samples of oca (*Oxalis tuberosa*) were collected in the markets of La Paz- Bolivia. For the determination of the antioxidant capacity, total flavonoids, total phenolic compounds and individual phenolics by High performance Liquid Chromatography (HPLC) analysis, the samples were cleaned and after that stored cool in plastic bags for 24-48 h and then transferred to a freezer (-80ºC) until being extracted with buffer (water-soluble fraction) and acetone (water-insoluble fraction).

Sample preparation

The samples were mixed with sodium acetate buffer (0.1 M, pH 5.0) in a liquid: sample ratio of 25:1 and homogenized in a mixer. The samples were centrifuged in a Thermo IEC Multi/RF with an 8850 rotor at 20000 *g* during 30 min at 4°C. The supernatant liquids were aspirated and stored at –80°C before being analyzed. One gram of the remaining pulp was homogenized with 8 ml of acetone and was stirred during 30 min at room temperature. Then the mixture was centrifuged for 10 min at 1200 *g* and room temperature. The supernatant solution was separated and stored at – 80ºC before being analysed [6,7].

Chemicals

The Folin-Ciocalteu reagent, gallic acid, sodium carbonate, sodium nitrite, aluminium chloride hexahydrate, acetone (p.a.) and methanol (HPLC grade) were purchased from Merck (Darmstadt, Germany), ABTS [2,2'-azino-bis(3-ethylbenzotiazoline-6-sulphonic acid)], baicalein (98 %), kaempferol (99%), quercetin (99%), potassium persulfate, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 97%) and TPTZ (2,4,6-tripyridyl-*s*-triazine) were obtained from Sigma-Aldrich (St. Louis, USA), ferric chloride from ICN Biomedicals Inc. (Costa Mesa, CA, USA), cyanidin Cl, delphinidin Cland malvidin Cl were obtained from Extrasynthèse (Genay, France),acetic acid (glacial p.a.), and sodium acetate from BDH Chemicals Ltd. (Poole, UK.)

Measurement of total antioxidant capacity (TAC), total phenolic compounds (TPH) and total flavonoids (TF)

TAC was measured by the ABTS method and the FRAP method as described elsewhere [6,7] and the data were expressed as µmol Trolox equivalents per gram of fresh matter. TPH was determined using the Folin-Ciocalteu reagent as described elsewhere [6,7] and the data were expressed as µmol gallic acid equivalents per gram of fresh matter.TF was measured as described elsewhere by [6,7] and the data were expressed as µmol catechin equivalents per gram of fresh matter.

High performance liquid chromatography

The extracts were refluxed in 1.5 M HCl in methanol for 2 h at 90°C after the addition of baicalein as an internal standard. The phenolic compounds were separated using an Agilent liquid chromatographic system (Palo Alto, CA, USA) series 1000, equipped with a quaternary Pump with Degasser (G1354A), an auto-injector, a column oven and a diode-array detector (DAD). The column was a 3.5 times 150 mm Kromasil C18 reversed-phase type and protected by a 10 mm pre-column (Eka Chemicals, Separation Products, Bohus, Sweden). The flow rate was 0.8 ml/min and the injection volume was 20 µl. The mobile phase was a binary solvent system consisting of (A) 1% acetic acid/water and (B) methanol and the gradient used was 40% B at 0 min 65% B after, 5 min, 90% B after 10 min and 40% B after 15 min until 17 min. The UV absorbance of the elute was recorded using a multiple diode array detector (190-550nm). Retention times and absorbance spectrum profiles were compared with standards. Pure standards were also added to the samples as a control and peak splitting was used as an indication of a potential misinterpretation [7].

HPLC-MS

HPLC–MS analysis was carried out on a Crhom RP C18 (3.5 µm, 2.0 mm × 250 mm) column mounted on a Modelo 1090 Serie II Waters Separation Module. A binary solvent gradient Solvent A: H_2O, Solvent B: methanol and 1% formic acid in water. Flow rate: 0.10 mL min−1. Initial conditions were 80 A/20B (0 to 32 min), to 100% B (32 to 41 min.) with a return to initial set-up conditions by 42min. A 10-mL injection of each sample collected was analyzed, the temperature of the column held steady at 25 °C. The HPLC was equipped with a diode array UV/Visible light detector monitoring A210 and A500 with continuous scanning of each peak from 190 to 600 nm. The phenolic compounds were separated by isocratic elution using A/B from= 20/80 (v/v) in 21 min. The compound was detected with a Waters Quattro micro API mass spectrometer operated in positive mode, using MRM (ES+ 125.0 > 109.0).

Statistical analysis

The results were expressed as mean values (SD) of six measurements made over three days for TAC, FRAP, TPH and TF. The significance of differences between groups was assessed by the paired t-test and the Wilcoxon's signed ranks test (SPSS, version 11.0).

Results

The total antioxidant capacity TAC values of the oca measured in the water-soluble and water-insoluble fractions are summarised in Table 1. Higher TAC values were observed in oca in both fractions

Table 2: Content of individual phenolic compounds in oca (median and range; expressed as µmol per 1 kilogram of fresh weight).

Sample		Quercetin	Kaempferol	Cyanidin	Delphinidin	Malvidin
Oca	Median	2	4	20	30	1
	Range	1-20	3-8	10-30	1-90	1-30

Table 3: HPLC-MS and Molecular weight.

Compounds	Retention time (min)	MW (g/mol)	3 (+H)
Kaempherol	21,5	283	286
Cyaniding	14	287	289,9
Quercetina	6,5	302	305
Malvidin	36	331	334
Ddelphinin	5	338	341

and the same tendency was observed regarding the content of TPH and TF (Table 1).The TAC values ranged from 4 to 7 in oca expressed as µmol of Trolox equivalents/g fw by the FRAP method. By the ABTS method, TAC was 1.8-5.5 in oca µmol /g fw. Thecontent of TPH ranged from2.8 to 6.4 in oca expressed as µmol gallic acid equivalents/g fw and the TF values were 0.4-1.9 of catechin equivalents/g fw. The TAC values differed significantly among samples and extracts. Prior to analysis by HPLC the samples were subjected to acid hydrolysis, and in both the water-soluble and water-insoluble extracts this led to an up to 3.5-fold in oca increases ($p<0.001$, paired-t test) in the TAC values in comparison with the values of the non-hydrolyzed samples (Table 1).

As regards individual compounds cyanidin, malvidin, delphinidin, kaempferol and quercetin were found in oca (Table 2) and their presence was confirmed by HPLC-MS (Figure 1)(Table 3).

Discussion

In comparison with data in the literature, the TAC values by the ABTS method were somewhat lower than those obtained in the same tuber species collected in Peru [4,8] but the data were in the same range regarding TPH values. The values obtained in the present investigation for oca were higher than those data obtained from literature for sweet potatoes, Andean potatoes and potatoes collected in different parts of the world and lower than those obtained for vegetables and tubers from the Mediterranean region [9-12]. The increase in the TAC values after acid hydrolysis suggested that antioxidant compounds were released by glycoside hydrolysis as also observed in a previous investigation [7], indicating that TAC values from this tuber can be underestimated if they are measured in unhydrolysed samples and this is a relevant information fron the nutritional point of view since during digestion phenolic compounds will be hydrolised.

As regards the anthocyanins identification, the compounds malvidin and delphinidin and their derivatives were demonstrated in oca by Alcalde-Eon et al [5] However, Cyanin was not found in oca in previous studies. In another investigation [8], the phenolic acids vanillic, caffeic and cinnamic acids were identified in oca and in addition several unknown compounds probablyflavan-3-ols and flavones were observed, with absorbance spectra similar to those of quercetin and kaempferol, which were identified in the present study.To confirm the presence of the phenolic compounds in oca, the extracts were analysed by HPLC–MS using Multiple Reaction Monitoring (MRM) which provided the high sensitivity required [13,14].

The present study shows that the tuber oca (*Oxalis tuberosa*) can

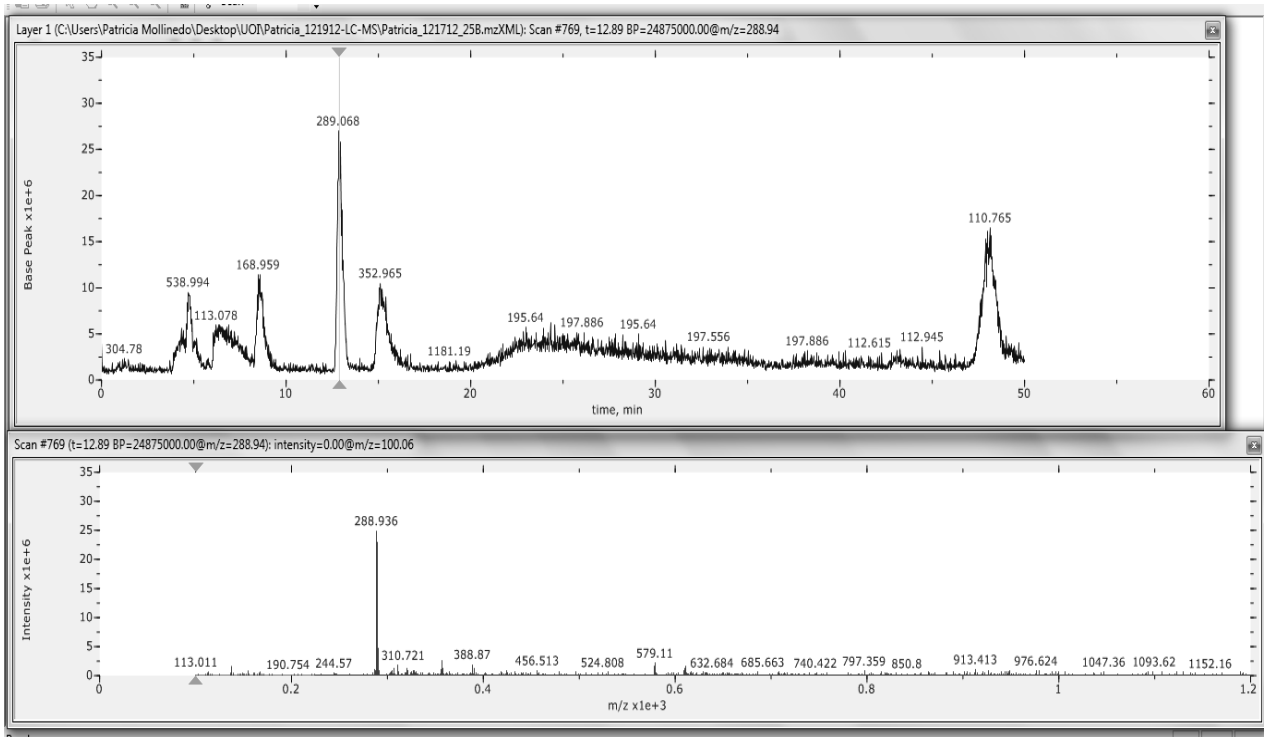

Figure 1: HPLC – MS of cynidin.

be important sources of antioxidants in the Andean diet. Therefore, it is important to get more information about the chemical and nutritional properties of this Andean crop such as carotenoids composition and starch properties, etc. It is also important to study in the future the bioavailability of the phenolic compounds and their antioxidant potential after consumption. This needs to be related tothe mechanism of action of flavonoids and other phenolic compounds in human tissue including the concept that mixtures of such compounds may have other effects than the individual compounds [15,16]. This will be important for the future evaluation of the possible health effects of oca and other Andean crops. So far the effects of only few polyphenols have been studied in human intervention studies [17].

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