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Content of Free Phenolic Compounds in Cultivars of Potatoes Harvested in Tenerife (Canary Islands)

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Determination of free phenolic compounds in potato samples was optimized using a high-performance liquid chromatographic (HPLC) method with on-line diode array detection. This method was applied to samples of four cultivars of potatoes harvested in Tenerife (Canary Islands). The free phenolic compounds found in the potato samples were (+)-catechin, chlorogenic acid, caffeic acid, *p*-coumaric acid, and ferulic acid. Potato samples belonging to Colorada cultivar, ssp. *andigena*, had mean concentrations of total phenolic compounds and chlorogenic acid higher than those found for Kerr's Pink and Cara cultivars, ssp. *tuberosum*, and for Negra cultivar, *S.* × *chaucha*. In contrast, *p*-coumaric acid was not detected in any potato samples of the Colorada cultivar. Traditional potatoes presented a higher mean concentration of ferulic acid than recently imported potatoes. A significant and negative correlation was established between (+)-catechin and *p*-coumaric acid. A considerable contribution to the daily intake of flavonoids was observed with the actual consumption of potatoes.

KEYWORDS: Phenolic compounds; HPLC; potato cultivars

INTRODUCTION

Potatoes and other plant foods accumulate a great variety of secondary metabolites, including phenolic and many other phytochemical compounds, as a protection against the adverse effects of mechanical bruising, light, and injury by predators such as beetles, fungi, and insects (*1*−*3*). Therapeutic effects such as antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, antithrombotic, and vasodilatory activity have been attributed to phenolic compounds (*4*). Many of these effects result from their potent antioxidant and free radical scavenging properties (*5*). The antioxidant activity of the flavonoid components extends and sometimes synergizes the antioxidant activities of vitamin C, vitamin E, and carotenoids (*6*). Many scientists believe that flavonoid intake, along with β-carotene, vitamin C, and vitamin E, is the principal cancer chemopreventive agent and that their abundance in fruits and vegetables underlies, to a significant degree, the correlations linking high fruit and vegetable consumption with reduced cancer risk (*7*−*9*).

Because these compounds are present in the normal diet of humans, it is important to develop a better understanding of the role of these compounds and the adequate levels of intake for good health. Thus, analytical and compositional aspects of phenolic compounds in foods are key aspects. However, the data published on flavonoid concentrations in foods are variable and often overestimated. This is due to the analytical techniques for their determination; the use of new HPLC detectors, which are more sensitive and selective, has led to some improvement (*10*−*12*). This topic must be a priority if we want to quantify these compounds in different types of sample matrices. Therefore, a diode array detector (DAD) provides spectral characteristics of each peak that ensure their quantification.

As a part of studies for the characterization of several cultivars of potatoes harvested in Tenerife, chemometric studies on the chemical composition (*13*) were carried out to establish differences on the basis of the following criteria: cultivar, species/subspecies, and traditional/recently imported potatoes.

In this paper, the results on the contents of free phenolic compounds are presented to determine differences according to the above criteria. Furthermore, the contribution to the daily intake of phenolic compounds by the actual consumption of potatoes is reported.

MATERIALS AND METHODS

Reagents and Standards. Methanol of HPLC-gradient grade and acetic acid were purchased from Merck (Darmstadt, Germany), and ethanol was from Scharlau (Barcelona, Spain). Standards of phenolic compounds such as (+)-catechin, (−)-epicatechin, syringaldehyde, *p*-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, *p*-coumaric acid, and ferulic acid were obtained from Sigma (St. Louis, MO); gallic acid monohydrate was from Fluka (Buchs, Switzerland). Stock solutions at 1 g/L in methanol (Merck) were prepared and stored in darkness at 5 °C. Deionized water was purified with a Milli-Q water system (Millipore, Bedford, MA) before use.

Equipment. The analytical HPLC system comprised a Waters 2690 high-performance liquid chromatograph equipped with a Water 996

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Table 1. Descriptions of the Potato Cultivars According to Criteria Traditional/Recently Imported, Species/Subspecies, and Cultivar

	specie/subspecie	cultivar	*N*	tuber wt (g)
traditional	ssp. *andigena*	Colorada	8	62 ± 32
	S. × chaucha	Negra	7	40 ± 28
recently imported	ssp. *tuberosum*	Cara	13	125 ± 38
		Kerr's Pink	16	101 ± 39

diode array detector (Waters, Milford, MA). The separation was carried out using a Waters Nova-Pak C_{18} steel cartridge (3.9 × 150 mm), using a Waters Nova-Pack C18 guard column to protect the analytical column. The temperature of the column oven was set at 25 °C during all of the experiments. The HPLC pumps, autosampler, column oven, and diode array system were monitored and controlled using the Millennium[32] system. A wavelength of 280 nm was used for the detection of the phenolic compounds. The spectrum of each peak was superimposed after subtraction of the corresponding baseline spectrum. Peaks were considered to be pure when there was an exact correspondence among the spectra (peak purity match > 990). Similarly, peak identity was confirmed by superimposing the spectrum of each peak on the corresponding standard spectrum (peak identity match > 990) and by comparison of retention times (time window of 0.5%). Peaks were quantified only if they matched the above-mentioned criteria.

Samples. A total of 44 samples of potatoes were analyzed. The samples were purchased in local food outlets from several regions of the island of Tenerife. They were harvested between July and August of 2002, without irrigation, and the maximum time between harvest and analysis was 2 weeks. Four cultivars were analyzed, two from traditional potatoes and two from recently imported potatoes. The main characteristics of potato samples are described in **Table 1**.

Each potato was hand-rinsed under a stream of tap water for 15−20 s, and the dirt was removed by gently rubbing by hand under the water stream. After rinsing, the potatoes were shaken to remove any excess water, gently blotted with a paper towel, and placed in darkness in the laboratory for air-drying prior to processing (1−2 h).

Sample Preparation Method. About 2.5−3 g of the fresh sample was taken and weighed directly in Pyrex tubes, containing 10 mL of ethanol 80%. The samples were immediately homogenized using a model T-25 Basic Turmix (Ika-Werke, Staufen, Germany), and the mixer screw was immediately washed with another 10 mL of ethanol 80%. Afterward, the tubes were centrifuged for 10 min, and the liquid phase was stored in polyethylene tubes at −18 °C in the freezer.

An aliquot of 6 mL of this dissolution was concentrated until dryness in the water bath at 40 °C for 6−7 h in darkness. Then, 1 mL of methanol was added to the residue, and put into the ultrasound bath for 5 min. This dissolution was passed through a membrane filter of 0.45 μm (Millipore) equipped with a filter type PVDF of 13 mm (Millipore) and through a Sep-Pak cartridge (Waters), which had been previously preconditioned with 3 mL of methanol and 3 mL of ultrapure water (Milli-Q water system). The compounds were eluted by washing with 2 mL of a mixture of ultrapure water and methanol in a proportion of 1:1 (v/v). Duplicate injections were performed, and average peak areas were used for the quantification.

Chromatographic Conditions. A gradient elution was used composed of solvent A (water/methanol/acetic acid, 88:10:2, v/v/v) and solvent B (water/methanol/acetic acid, 8:90:2, v/v/v). The following gradient program was used:

time (min)	A (%)	B (%)	curve
0	100	0	
9	90	10	11
13	0	100	7
14	100	0	6

The injection volume was 20 μL for the standard solutions or for the sample extracts, at a flow rate of 1 mL/min.

Table 2. Percentage of Recovery of the Phenolic Compounds Considered ($n = 6$)

compound	recovery (%)	detection limit[a] (mg/L)
gallic acid	84.25 ± 2.16	0.060
(+)-catechin	49.60 ± 1.49	0.11
p-hydroxybenzoic acid	94.90 ± 1.21	0.011
chlorogenic acid	79.19 ± 0.76	0.050
vanillic acid	93.63 ± 2.48	0.021
caffeic acid	94.71 ± 0.45	0.079
(−)-epicatechin	44.92 ± 1.01	0.30
syringaldehyde	79.12 ± 0.52	0.040
p-coumaric acid	86.51 ± 1.16	0.050
ferulic acid	86.40 ± 0.62	0.012

[a] Calculated as that concentration giving a signal 3 times as high as the blank value.

Statistical Analysis. All statistical analyses have been performed by means of the SPSS version 10.0 software for Windows. The Kolmogorow−Smirnov−Lilliefors test was applied to verify whether the variable had a normal distribution, $p < 0.05$. The mean values obtained in the different groups were compared by one-way ANOVA and *t* test, assuming that there were significant differences between mean values when a statistical comparison gave $p < 0.05$. Simple linear and logarithmic correlation analysis was used to indicate the measure of the correlation and the strength of the relationship between two variables.

RESULTS AND DISCUSSION

The HPLC method for determining phenolic compounds previously described by Malovaná et al. (*14*) was slightly modified for its application to the potato samples. A good resolution between of all the identified peaks of the studied free phenolic compounds was obtained using the elution gradient described under Materials and Methods in a real sample of potatoes. To check the recovery of all of the compounds, six aliquots (500 μL) of a ethanolic extract of potato sample, obtained according the method described under Materials and Methods, were spiked with 500 μL of a standard solution [5 μg/mL of gallic, *p*-hydroxybenzoic, chlorogenic, vanillic, and caffeic acids, (+)-catechin, (−)-epicatechin, and syringaldehyde; and 1 μg/mL of *p*-coumaric and ferulic acids]. Another six aliquots were added with 500 μL of methanol (nonspiked). Then, the spiked and nonspiked potato samples were analyzed by calculating the recovery with the same matrix of the samples. The recovery percentage and detection limit of the 10 phenolic compounds considered are shown in **Table 2**. The recovery percentage using the proposed method was between 79 and 95%, except for (+)-catechin and (−)-epicatechin, the recoveries of which were 49.6 and 44.9%, respectively. Relatively high repeatability and reproducibility for the retention times were observed, with coefficients of variation (CV) lower than 4.5%. Both between-day precision and within-day precision of the method were determined from replicate assays of potato spiked with known concentrations of phenolic compounds, obtaining a CV lower than 4.6%. All of the peaks obtained for the real samples were checked with the standards by comparing spectra of both in the range between 190 and 400 nm.

Chlorogenic acid, (+)-catechin, caffeic acid, *p*-coumaric acid, and ferulic acid were identified in the potato samples injected, but some unidentified peaks appeared in the chromatogram. Mattila and Kumpulainen (*10*) did not detect, in cooked potatoes, syringaldehyde or vanillic, gallic, and *p*-hydroxybenzoic acids, which is in agreement with our data. Neither did these authors detect *p*-coumaric acid in any of the eight potato samples analyzed, which contrasts with the results obtained in this paper,

Table 3. Average Concentrations (Milligrams per 100 g of Wet Weight) and Standard Deviation of Free Phenolic Compounds for Different Cultivars of Potatoes[a]

cultivar	(+)-catechin	chlorogenic acid	caffeic acid	*p*-coumaric acid[b]	ferulic acid[b]	total phenols
Cara	13.2 ± 6.9a (1.8–26.1)	7.7 ± 7.1a (1.1–26.8)	1.12 ± 0.71a (0.28–2.71)	0.032 ± 0.011a (0.020–0.048)	0.19 ± 0.13a (0.061–0.50)	22.3 ± 10.3a (5.1–45.4)
Kerr's Pink	11.0 ± 7.7a (1.8–24.8)	10.2 ± 4.7a (3.5–21.4)	0.73 ± 0.56a (0.12–2.00)	0.11 ± 0.064b (0.033–0.23)	0.066 ± 0.028a (0.022–0.12)	22.1 ± 7.4a (11.2–34.1)
Colorada	9.7 ± 3.7a (3.9–16.0)	16.9 ± 4.9b (10.5–22.4)	0.85 ± 0.34a (0.46–1.50)	ND[c]	0.80 ± 0.63b (0.25–1.71)	28.3 ± 10.5a (15.2–39.8)
Negra	10.5 ± 2.9a (7.3–15.4)	9.0 ± 6.5a (1.5–20.3)	1.09 ± 0.81a (0.40–2.63)	0.068 ± 0.017ab (0.056–0.080)	0.36 ± 0.52a (0.007–1.41)	21.0 ± 8.2a (12.6–29.6)
overall	11.4 ± 6.3 (1.8–26.1)	10.2 ± 6.4 (1.1–26.8)	0.93 ± 0.65 (0.12–2.71)	0.085 ± 0.063 (0.020–0.23)	0.25 ± 0.39 (0.007–1.71)	22.9 ± 9.0 (5.1–45.4)

[a] Results in the same column with the same lettter were not significantly ($p < 0.05$) different. [b] Mean values were calculated only with the detected data. [c] Not detected.

because *p*-coumaric acid was detected in approximately half of the potato samples analyzed.

The results of the detected phenolic compounds, including the content of total phenols grouping the potato samples in the four cultivars, are presented in **Table 3**. The results of a one-way ANOVA for comparing the mean values obtained according to the cultivar are also included in this table. The sum of (+)-catechin plus chlorogenic acid represented >90% of total phenolic compounds analyzed. This agrees with studies by other authors (*1*, *10*, *15*−*19*), who have indicated that chlorogenic acid is the main phenolic acid in potatoes. However, there are no data about the content of catechin (flavonoid) in potato samples. The *p*-coumaric and ferulic acids were the compounds with the lowest concentrations. A considerable number of potato samples (47.7% of the total) with values for *p*-coumaric acid lower than the detection limit were observed (**Table 2**). In particular, the eight potato samples belonging to the Colorada cultivar presented values lower than the determination limit of *p*-coumaric acid. With respect to the results of ferulic acid, four potato samples (9.1% of the total), two belonging to the Colorada and two belonging to the Cara cultivar, presented nondetectable concentrations.

Comparing the data obtained in this paper with other values of free phenolic acids described in the literature, one can deduce that there is some controversy. The concentrations of chlorogenic acid observed in this paper were higher than some data published by Ramamurthy et al. (*19*), but similar to data reported by Dao and Friedman (*15*, *20*) and Mattila and Kumpulainen (*10*). These authors (*10*) reported levels of ferulic acid similar to ours;, however, the content of caffeic acid was lower. In contrast, Ramamurthy et al. (*19*) reported data of ferulic, caffeic, and *p*-coumaric acids higher than the data found by us. This could be due to differences in the phenolic content according to cultivar, soil, and climatic conditions as well as discrepancies associated with the analytical methods such as an erroneous quantification of phenolic compounds (matrix effect) or changes in the concentrations produced in the method for the preparation of the samples (*10*, *16*).

The mean content of the total free phenolic compounds in the potatoes of the Colorada cultivar that belongs to ssp. *andigena* was higher than in the Kerr's Pink and Cara cultivars belonging to ssp. *tuberosum* and in the Negra cultivar included in *S.* × *chaucha*, although no significant differences ($p = 0.218$) were found. The mean concentrations of ferulic, chlorogenic, and *p*-coumaric acids make it possible to distinguish the samples of the Colorada cultivar ssp. *andigena* from the rest of the cultivars. Although the *p*-coumaric acid was not detected in any of the potato samples of Colorada cultivar ssp. *andigena*, the mean concentrations of ferulic ($p = 0.001$) and chlorogenic ($p = 0.011$) acids were higher than in Kerr's Pink and Cara cultivars ssp. *tuberosum* and Negra cultivar *S.* × *chaucha*. Besides which, there were significant differences among the mean concentrations obtained by considering the detected values of *p*-coumaric acid in the rest of the potato cultivars, with the higher mean concentration being found in the Kerr's Pink cultivar with respect to the other cultivars. No significant differences ($p > 0.05$) were found between the mean concentrations of (+)-catechin and caffeic acid when the potato samples were grouped according to the cultivars. **Figure 1** shows the results obtained when the potato samples were grouped according to the criterium proposed by Gil González (*21*): traditional/recently imported. One can observe that ferulic acid makes it possible to distinguish between both types of potatoes and that traditional potatoes had ($p = 0.001$) a higher mean concentration than the recently imported potatoes. Besides which, similar results were obtained with chlorogenic acid, although no significant difference was observed ($p = 0.071$). In the rest of the phenolic compounds, as well as in the total phenolic compounds, the mean values were similar for both groups of potatoes.

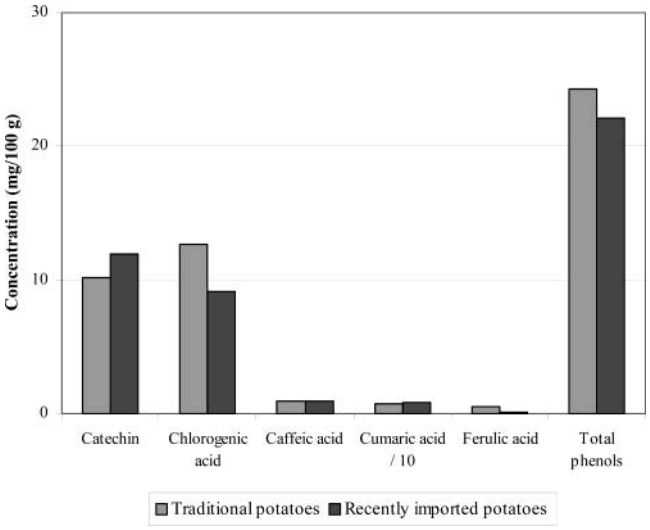

Figure 1. Mean concentrations of the free phenolic compounds for traditional potatoes and recently imported potatoes.

The double-logarithmic correlation matrix (**Table 4**) shows the presence of some significant ($p < 0.05$) correlations between the free phenolic compounds analyzed. Total free phenolic compounds presented significant and positive correlations with the concentrations of (+)-catechin ($r = 0.567$; $p = 0.000$) and

Table 4. Double-Logarithmic Correlation Matrix for All Samples

	(+)-catechin	chlorogenic acid	caffeic acid	*p*-coumaric acid	ferulic acid	total phenols
(+)-catechin		–0.187[a] (0.224)[b]	–0.078 (0.616)	**–0.770 (0.000)**	0.076 (0.640)	**0.567 (0.000)**
chlorogenic acid			–0.029 (0.850)	**0.504 (0.014)**	**0.349 (0.027)**	**0.625 (0.000)**
caffeic acid				–0.286 (0.186)	0.213 (0.187)	0.119 (0.440)
p-coumaric acid					–0.128 (0.581)	**–0.540 (0.008)**
ferulic acid						0.310 (0.052)
total phenols						

[a] Pearson correlation coefficient. [b] *p* value; significant correlation in boldface type.

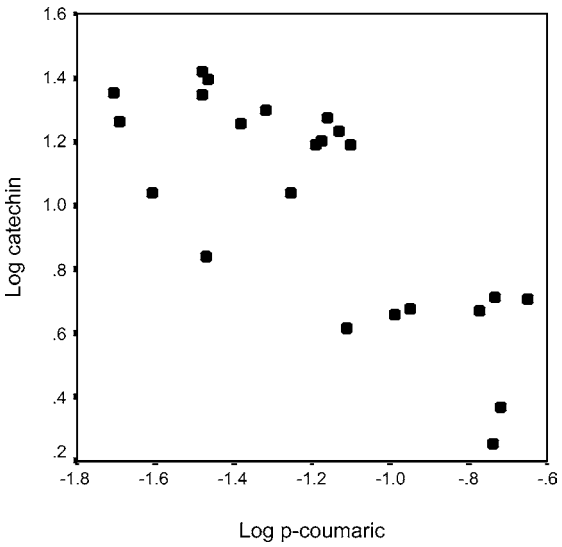

Figure 2. Double-logarithmic correlation matrix between (+)-catechin and *p*-coumaric acid.

chlorogenic acid ($r = 0.625$; $p = 0.000$) and a negative correlation with *p*-coumaric acid ($r = -0.540$; $p = 0.008$). The two first correlations are due to these two first compounds, (+)-catechin and chlorogenic acid, being the ones with the highest presence, contributing >90% of the total free phenolic compounds. The significant and negative correlation ($r = -0.770$; $p = 0.000$) between (+)-catechin and *p*-coumaric acid should be emphasized. **Figure 2** shows the graphic plot of this correlation, which defines the following regression line: log[catechin, mg/100 g] = 0.228−0.062 log[*p*-coumaric acid, mg/100 g], which allows the estimation of the content of a compound when the other is known.

Compared with data from other regions of Spain, the mean consumption of potatoes in the Canary population is high, estimated at 143.2 g/person/day (166.3 and 122.7 g/person/day for men and women, respectively) (*22*). Therefore, an important contribution to the dietary daily intake in relation to the recommended dietary intakes (RDI) for the Spanish population (*23*) of ascorbic acid and potassium and moderate contributions in the protein, fiber, and magnesium intakes are observed by the actual consumption of potatoes (*24*). Potatoes have a relatively high content of phenolic compounds, so the contribution to the intake of these compounds can also be considerable. The dietary daily intake of (+)-catechin (the only flavonoid identified and quantified in this paper) by the actual consumption of potatoes in the Canary population represents 19 and 14% of this recommendation for men and women, respectively.

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