Phenolic compounds in native potato (*Solanum tuberosum* L.) cooking water, with potential antioxidant activity
ABSTRACT

Objective: This research objective was to identify and quantify phenolic compounds in potato cooking water from freeze-dried slices with peel and from the whole potato stored for 20 days at 18.5 - 21.5 °C, 68 - 70% Relative Humidity. Methods: Extracts were obtained with an aqueous solution composed of 50% methanol and 0.5% acetic acid. Fifteen secondary metabolites were monitored using the Ultra Performance Liquid Chromatography system coupled to mass spectrometry (UPLC-MS / MS). A calibration curve (from 0.1ng to 100 μg) was generated, and the data were analyzed using the software “MassHunter Workstation” VB 06.00. The results were expressed as mg/100 g of sliced potato or raw potato. Principal Components Analysis (PCA) was performed using XLSTAT 2015 Software. Results: The main phenolic compounds (mg / 100 g of freeze-dried sliced potato) are chlorogenic acid (46.18), vanillin (16.03), p-coumaric acid (9.73), caffeic acid (9.41), 4-hydroxy -3-methoxy cinnamaldehyde (7.83), ferulic acid (5.97), neochlorogenic acid (2.07). The metabolite content in the cooking water of the Huagalina native potato is directly related to the freshness of the product before cooking. Conclusions: Potato cooking water could be considered a nutraceutical food. However, further research is required to identify any other substances harmful to health, depending on the amount consumed.

Keywords: Native Potato, Phytochemicals, Boiled Potato, Chlorogenic Acid, Cancer.
OBJECTIVE

Identify and quantify phenolic compounds found in potato cooking water to demonstrate that it contains bioactive antioxidant compounds with a potentially beneficial effect on human health.

METHODS

Chemical materials

HPLC grade standards were obtained from Sigma-Aldrich (USA): 3,5-dihydroxy benzoic acid, neochlorogenic acid, chlorogenic acid, 4-hydroxybenzoic acid, caffeic acid, syringic acid, vanillin, p-coumaric acid, ferulic acid, sinapic acid, 4-hydroxy-3-methoxy-cinnamaldehyde, coumarin, daidzein, genistein, 7-hydroxyflavone.

Plant materials

Fields trials were carried out in Santiago de Chuco - La Libertad at two experimental sites: El Zuro (EZ) and Huayatan Alto (HA).

El Zuro (3 750 m.a.s.l.), sandy loam texture soil, with good drainage, moderate and deep slope, acid with a pH of 5.3, electrical conductivity of 0.3 dS/m (no salt), organic matter: 6.4% (very high), phosphorus 17 ppm (high) and potassium 477 ppm (very high), annual average temperature 9 °C (dry cold climate) and organic manuring (Guano de Islas 2 MT/ha) under drought conditions.

Huayatan Alto (3 450 m.a.s.l.), loamy soil with good drainage, with slightly pronounced and moderately deep slope, acid with a pH of 5.0, 1.5 dS/m (no salt) electrical conductivity, organic matter 5.2% (high), phosphorus 14 ppm (average) and potassium 80 ppm (low), with average annual temperature of 10 °C (temperate climate with presence of drizzle) and inorganic fertilization (180-200-160 kg of NPK/ha, under drought conditions.

Potatoes from EZ and HA were classified in two samples:

a. Whole fresh potatoes were washed with tap water and the surface allowed to dry, manually cut into slices of 5 mm thickness, freeze-dried and packaged with liquid nitrogen in sealed jars. These samples will be called throughout the article as SP-EZ and SP-HA.

b. Whole potatoes were stored as raw material at 18.5 - 21.5 °C, 68 - 70% Relative Humidity for 20 days. These samples will be called throughout the articles as RP-EZ and RP-HA.
Sample preparation

The following treatments were used prior to extraction:

a. SP-EZ and SP-HA were unfreezed and then boiled in water 1/3 w/w (slices / water) for 20 min from boiling point. We analyzed phenolic compounds in SP water.

b. RP-EZ and RP-HA were boiled in water 1/3 w/w (potato/ water) for 20 min from start point. We analyzed phenolic compounds in RP water.

Extraction

Extraction solution (ES) used was an aqueous solution of 50% methanol (HPLC grade-Sigma Aldrich) and 0.5% acetic acid (Fluka Analytical HPLC-grade), the same ES used by Narváez-Cuenca et al., (2012). An aliquot solution of boiling water of each two treatment (SP and RP) was placed with 500 μL of the ES in vortex (VWR Analog Vortex Mixer, USA) 4 times for 10 seconds, sonicated (Branson Ultrasonic bath 3800, USA) for 10 min at 4 °C and kept in ice (30 minutes). After centrifugation (Eppendorf 5424, Germany) at 15000 g for 10 minutes at 4°C, 350 μL of the supernatant was removed and put in a new tube. The process was repeated two more times with the resultant pellet, and the supernatants were pooled together. A final centrifugation (Eppendorf 5424, Germany) at 20000 g for 10 min at 4°C was performed to remove any remaining tissue suspension, and stored at -80°C until further analysis. Each treatment was repeated three times.

UPLC – MS/MS analysis

Extracts were automatically injected (5 μl) in the system using UPLC (Agilent 1200 Infinity Series, USA) coupled to a Mass Spectrometry type triple Quadrupole (QqQ), (model 6430 Agilent Technologies, USA). Chromatographic separation was carried out on a column Zorbax Eclipse Plus C18, 1.8 μm, 2.1 x 50 mm (Agilent, USA) at 30°C, in series with a guard column Zorbax SB-C18, 1.8 μm (Agilent, USA). The solvents used were: (A) acetic acid 0.02% in water and (B) acetic acid 0.02 % in acetonitrile (LC-MS grade, Fluka Analytical). The solvent flow rate was 0.3 mL/min. A linear gradient with the following proportions of solvent B was used: gradient from 0 to 11 minutes, 5 to 60% B; from 11 to 13 minutes, 60 to 95% B; from 13 to 17 minutes, 95% B; from 17 to 19 minutes, 95 to 5% B; from 19 to 20 minutes, 5% B. The ionization method used in the mass spectrometry was an ESI (Electrospray Ionization) following these conditions: gas temperature of 300°C, nitrogen flow rate of 10 L/min, nebulizer pressure of 35 psi and capillary voltage of 4000 V. The equipment was operated on mode MRM (Multiple Reaction Monitoring). The mass of the precursor ion/fragment (m/z) established was monitored by fragmentation tests of each molecule: 3,5 dihydroxybenzoic acid (155.02/137.01), chlorogenic acid (355.00/163.00),
4-hydroxybenzoic acid (139.12/121.00), 4- hydroxy-3-methoxy cinnamaldehyde (179.10/147.04),
coumarin (147.06/91.00), daidzein (255.00/199.00), genistein (271.00/243.00), 7-hydroxyflavone
(239.07/137.00), neochlorogenic acid (353.10/179.00), caffeic acid (179.00/135.00), syringic
acid (197.00/121.200), vanillin (151.00/92.00), \( p \)-coumaric acid (163.04/119.00), ferulic acid
(193.00/134.00), sinapic acid (223.00/164.10). The first eight were scanned in positive mode and
the last in negative mode. A calibration curve (0.1 ng a 100μg) using the respective standards
of each metabolite was generated to determine the absolute quantification. The generated data
were analyzed in the software “MassHunter Workstation” VB 06.00, which obtained the peak
areas for each phenolic metabolite and the results were expressed in mg/100 g of dry tissue.

**Statistical analysis**

Principal Components Analysis (PCA) was performed using Software XLSTAT 2015
(Digital license).

**RESULTS**

In Figure 1, the phenolic compounds have been identified and quantified in the cooking
water of the Huagalina native potato. The main phenolic compounds (mg / 100 g of freeze-
dried sliced potato) are chlorogenic acid (46.18), vanillin (16.03), \( p \)-coumaric acid (9.73),
caffeic acid (9.41), 4-hydroxy -3-methoxy cinnamaldehyde (7.83), ferulic acid (5.97), neochlo-
rogenic acid (2.07). In RP water, the main phenolic compound (mg/100 g of raw potato) is
coumarin (9.31).

![Figure 1. Phenolic compounds in potato cooking water](image)
The results are shown in Figure 2; principal component analysis (PCA), indicates principal component F1 (72.01%) values are on the x-axis and principal component F2 (16.20%) values are on the y-axis. The total variance accounts for 88.22%. F1 component shows that the metabolite content in the cooking water of the Huagalina potato is directly related to the freshness of the product (SP).

**Figure 2. PCA of phenolic compounds in potato cooking water**

**DISCUSSION**

The higher concentration of metabolites in SP- potato cooking water (Figure 1) could be explained by potatoes boiling in water. This contact surface between potatoes and water is higher. Therefore it is an excellent transfer of analytes to water that increases the concentration of phenolic compounds. Also, phenolic compounds are leached into the hot water and are often supplemented by the heat that extracts them and improves their bioavailability. They are highly reactive species that participate in the reactions that occur during the cooking process and are related to the cultivar: agro-technical processes, climatic conditions, maturity during the harvest, post-harvest manipulations (STRATIL et al., 2006; PALERMO et al., 2014).

It has been found in this research. that the boiling water of potato contains phenolic compounds (phytonutrients) whose human health benefits have been demonstrated in several
studies in vivo or in vitro: Chlorogenic acid (CHO et al., 2010; SATO et al., 2011; SHI et al., 2016), vanillin (LIRDPRAPAMONGKOL et al., 2005), caffeic acid, coumarin, and ferulic acid (NASR BOUZAIENEA et al., 2015; SERAFIM et al., 2015) sinapic acid (ROY & PRINCE, 2012).

According to the World Health Report (WORLD HEALTH ORGANIZATION, 2002), low fruit and vegetable consumption reflects the population’s economic, cultural, and agricultural environment. It has become one of the ten main risk factors attributed to mortality from non-communicable diseases. It depends on whether many people with low consumption of fruit and vegetables result in approximately 19% of gastrointestinal cancers, 31% of ischemic heart disease, and 11% of cerebrovascular accidents. In total, 2.7 million deaths (4.9%) and 26.7 million lost years of healthy life (1.8%) were attributed to the low consumption of fruit and vegetables.

Food and Agricultural Organization of the United Nations (2015) launched The Global Initiative on Food Loss and Waste Reduction. It focuses on sustainable food production and diets and sustainable consumption (for example, reducing food waste). In his sense, potato cooking water contains phytonutrients for disease prevention as it is not currently a habit to consume it if we encouraged its consumption instead of discarding it. It would help complete the recommended intake of antioxidants that, according to the World Health Organization (2004), should be five fruits and vegetables a day (approx. 400 g) for the diversity of antioxidants they provide.

By Figure 2, the metabolite content in the cooking water of the Huagalina potato is directly related to the freshness of the product because the sample was freeze-dried and packaged with liquid nitrogen in sealed jars until analysis (SP), with what was mentioned by Carrillo et al., (2012). Some studies report that cold storage (< 5 °C) of potatoes either leads to an increase in the phenolic compounds or keeps it constant (MQNDY et al., 1966; LEWIS et al., 1999; STUSNHNOFF et al., 2008).

In the cooking water of potatoes stored at 18.5 - 21.5 °C, 68 - 70% Relative Humidity for 20 days (RP-EZ), only two phenolic compounds (daidzein and coumarin) were found with low correlation and significance (p>0.05).

F2 component shows a differentiation in the distribution of phenolic compounds due to climatic differences in crops (REYES et al., 2004) and according to the conventional and organic fertilization (HAJSLOVÁ et al., 2005). Eleven metabolites were distributed in SP cooking water. Still, the highest correlation was found in SP-EZ, as can be seen on the Biplot. Chlorogenic acid and its isomer neochlorogenic acid showed similar vector directions implying a high relationship between them. The same as vanillin- syringic acid; p-cumaric acid; syringic acid; ferulic acid-syringic acid; sinapic acid- vanillin (Pearson correlation coefficient >0.9724 and p<0.05).

For the results in this research, potato cooking water could be considered as a nutraceutical food. This potato water is traditional medicine in the Peruvian Andes for illness and wellness purposes in humans. However, further research is required to identify any other substances harmful to health, depending on the amount consumed. The thirteen metabolites
found in potato cooking water are the same as those found in raw potatoes of the same variety and origin (ROJAS -PADILLA & VÁSQUEZ VILLALOBOS, 2016).

**CONCLUSION**

The cooking water of the Huagalina native potato SP-EZ and SP-HA contains phytonutrients with potential antioxidant activity to prevent non-transmissible degenerative diseases. The phenolic compounds with the higher concentrations were (mg/100 g freeze-dried SP): Chlorogenic acid (46.18), vanillin (16.03), p-coumaric acid (9.73), caffeic acid (9.41), coumarin (9.31), 4-hydroxy-3-methoxy-cinnamaldehyde (7.83), ferulic acid (5.97), neochlorogenic acid (2.07). According to F1 (72.01%), metabolite content in the cooking water of Huagalina potato is directly related to the freshness of the product before cooking. F2 (16.29 %) demonstrates that the highest correlations of phenolic compounds were found in SP-EZ. Chlorogenic acid-neochlorogenic acid, with a high degree of relationship between them. The same as vanillin-syringic acid; p-cumaric acid –syringic acid; ferulic acid-syringic acid; sinapic acid- vanillin (Pearson correlation coefficient >0.9724 and p<0.05). These metabolites have been studied, and the health benefits have been demonstrated *in vivo or in vitro*.

It is vital to continue studying the environmental conditions of potato cultivation, which are responsible for the different distribution of the phenolic compounds in both locations studied, highlighting the predominance of the genetics of the Huagalina variety in the profile of the phytonutrients found. Potato cooking water could be considered a nutraceutical food. However, further research is required to identify any other substances harmful to health, depending on the amount consumed.

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