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Characterization of betalains, saponins and antioxidant power in differently colored quinoa (*Chenopodium quinoa*) varieties

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ABSTRACT

Quinoa was the traditional grain crop used by the prehispanic civilizations in America. Grains are white, black, yellow, and red-violet and plants are cultivated in vast areas of Peru, Bolivia and Ecuador. The recent description of the betacyanin pigment betanin in red-violet varieties is here further analyzed detecting the presence of amaranthin not previously identified in quinoa grains. Yellow-orange grains are characterized for the first time and up to four different betaxanthins are found to be responsible for this coloration. The native fluorescence of the identified betaxanthins makes the surface of the yellow quinoa grains glow with green fluorescent light. The presence of betalains is correlated with high antioxidant and free radical scavenging activities measured under the FRAP, ABTS and ORAC assays in grain extracts of 29 Peruvian varieties. TEAC equivalence is as high as 44.1 and 47.4 mmol Trolox/kg for the yellow and red-violet varieties analyzed respectively.

1. Introduction

Quinoa (*Chenopodium quinoa*) was the traditional grain crop of the prehispanic civilizations in America, being used as a feedstock with an important economic and cultural background. It belongs to the family Amaranthaceae, subfamily Chenopodioideae, in the order Caryophyllales. Ancient quinoa remains have been found in archeological sites in multiple contexts, including storage structures, hearths and burials in addition to human digestive tracts and coprolites (Capparelli et al., 2015). In the recent years, quinoa has gained a renewed relevance as an alternative crop to cereals due to its excellent nutritional value (Fuentes & Paredes-Gonzales, 2015). It has also been introduced as a gourmet grain in international markets and their exportations have experienced a raise from 5,000 to 40,000 tons in the last ten years mainly from the main producing countries located in the Andean region: Peru, Bolivia and Ecuador (FAO-ALADI, 2014). *C. quinoa* plants are cultivated in vast areas and sustain the traditional economy of small growers that cultivate multiple varieties in these countries (FAO-ALADI, 2014). Although the main commercial varieties are white or black in color, quinoa grains may also appear as yellow or red-violet and a strong effort has been done to maintain and characterize these varieties from the agronomical point of view (Gómez-Pando, Álvarez-Castro, & Eguiluz-de la Barra, 2010).

The recent description of the presence of the pigment betanin and its isomer isobetanin in red-violet varieties added these members of the betalain family to the list of relevant phytochemicals present in quinoa grains (Tang et al., 2015; Abderrahim et al., 2015). Betalains are nitrogenous plant pigments which are characteristic of plants belonging to the order Caryophyllales. They are divided into the yellow betaxanthins and the violet betacyanins (Gandía-Herrero & García-Carmona, 2013). The joint presence of both types of pigments makes the orange and red shades that coexist in nature with the pure yellow and violet colors. Betalains substitute anthocyanins and their roles in the colored tissues of the plant families that synthesize them being both families of water soluble pigments mutually exclusive (Brockington et al., 2015). Among the Caryophyllales plants, red beet roots (*Beta vulgaris*) and the fruits of cacti belonging to the genus *Opuntia* are the best known edible sources of betalains. In addition there are betalain containing berries (*Rivina humilis*) (Khan, Harsha, Giridhar, & Ravishankar, 2012) and certain *Amaranthus* species are also consumed cooked or fresh (Amin, Norazaidah, & Hainida, 2006). Betalain-containing extracts are used as the additive 73.40 in the 21 CFR section of the Food and Drug Administration (FDA) in the USA and under the E-162 code in the European Union.

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Betalains have in recent years shown promising bioactive potential. Early investigations revealed a strong free radical scavenging capacity of betalains purified from beet root (Escribano, Pedreño, García-Carmona, & Muñoz, 1998) and subsequent research revealed the existence of an intrinsic activity present in all betalains that is modulated by structural factors (Gandía-Herrero, Escribano, & García-Carmona, 2010; Gliszczyńska-Świgło, Szymusiak, & Malinowska, 2006). Studies with different cell lines have demonstrated the potential of betalains in the chemoprevention of cancer, and experiments *in vivo* have shown that dietary pigments inhibit the formation of tumors in mice (Gandía-Herrero, Escribano, & García-Carmona, 2016). The described bioactivities are supported by the high antiradical capacity of the pigments structural unit, betalamic acid, and point out to a promising potential of betalains in tumor prevention *in vivo* and the possible role played by betalains in the diet.

This work is aimed at studying in depth the betalains content in quinoa edible grains, including the previously unconsidered yellow varieties. It is also aimed at exploring a possible correlation between the presence of the pigments and the antioxidant and free radical scavenging capacity of the grains by analyzing 29 Peruvian varieties under different assays and conditions. Native visible fluorescence of betalains in quinoa is evaluated for the first time.

2. Materials and methods

2.1. Chemicals

Amines and aminoacids to obtain semi-synthetic pigment standards from betalamic acid, including dopamine, octopamine and 2-(2,5-dihydrophenyl)glycine, trifluoroacetic acid, buffer salts, sodium ascorbate, and reagents for the FRAP, ABTS and ORAC assays were purchased from Sigma (St. Louis, MO). Solvents were from Merck Chemicals Ltd. (Dorset, England). HPLC-grade acetonitrile was purchased from Labscan Ltd. (Dublin, Ireland). Distilled water was purified using a Milli-Q system (Millipore, Bedford, MA).

2.2. Plant material and extracts preparation

Chenopodium quinoa grains (quinoa) exhibiting different colors and hues were obtained from the quinoa germplasm bank at the National Agricultural University La Molina (Lima, Peru). Grains of plants grown in different areas of the Peruvian altiplano and valley are carefully collected and unambiguously identified. Codes used in this work correspond to the bank accession numbers except those corresponding to commercial varieties named by their trivial name. Pigments and saponins for each variety to be analyzed by different techniques (HPLC, DAD, MS/MS, Q-TOF) were extracted in 10 mM sodium acetate buffer supplemented with 10 mM sodium ascorbate. 0.50 ± 0.02 g of quinoa grains were weighted into screwcap test tubes (160 mm long, 16 mm diameter) and 5 mL of the aqueous solution was added. Extraction followed a previously described standard protocol used for the extraction and afrosimetric determination of saponins (Koziol, 1991). The afrosimetric method is based on the correlation among the foam height formed under specific conditions and the total amount of saponins. Extracts used for the determination of the antioxidant and free radical scavenging activities by the FRAP, ABTS, and ORAC assays were independently repeated in the absence of sodium ascorbate. The different extractions were performed in duplicate.

2.3. Spectroscopy

A Jasco V-630 spectrophotometer (Jasco Corporation, Tokyo, Japan), attached to a Tectron thermostatic bath (JP Selecta, Barcelona, Spain) was used for absorbance spectroscopy. For the quantification of betalains, pigment concentration was evaluated taking a molar extinction coefficient of $\varepsilon = 48{,}000\ M^{-1}cm^{-1}$ at 480 nm for betaxanthins, $\varepsilon = 54{,}000\ M^{-1}\,cm^{-1}$ at 536 nm for betanidin, and $\varepsilon = 65{,}000\ M^{-1}cm^{-1}$ at 536 nm for betanin and amaranthin (Gandía-Herrero et al., 2010; Trezzini & Zrÿd, 1991). Measurements were made in water at 25 °C.

2.4. HPLC analysis

A Shimadzu LC-20AD apparatus (Kyoto, Japan) equipped with a SPD-M20A photodiode array detector (PDA) was used for analytical HPLC separations. Reversed phase chromatography was performed following a previously developed method (Gandía-Herrero, García-Carmona, & Escribano, 2005a) with some modifications. A 250 × 4.6 mm, 5 μm core-shell Kinetex C-18 column (Phenomenex, Torrance, CA, USA) was used and linear gradients were performed from 0% B to 50% B in 35 min with H_2O with 0.05% trifluoroacetic acid (TFA) (solvent A) and acetonitrile with 0.05% TFA (solvent B). The flow rate was 1 mL min^{-1}, operated at 25 °C. Injection volume was 20 μL.

2.5. Color assessment

Color determination of the surface of quinoa grains were made at 25 °C using a JASCO V-650 spectrophotometer equipped with an ISV-722 integrating sphere (Jasco Corporation, Tokyo, Japan). Untreated whole grains were directly placed in the sphere cell. Uniform CIELAB space parameters ($L*$, $a*$, $b*$, $C*$ and h^o) were obtained from the apparatus software Spectra Manager version 2.07 (Gandía-Herrero, Cabanes, Escribano, García-Carmona, & Jiménez-Atiénzar, 2013).

2.6. Antioxidant and free radical scavenging activities

2.6.1. FRAP method

The antioxidant activity of the quinoa extracts was characterized by following the ferric reducing antioxidant power (FRAP) (Benzie & Strain, 1996). $FeCl_3$ solutions in sodium acetate buffer, pH 3.6 were used and the reduction of Fe (III) to Fe (II) was observed by adding the chromogenic reagent 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) as previously described (Gandía-Herrero et al., 2013). Reaction was monitored at λ = 593 nm.

2.6.2. ABTS method

Quinoa grains extracts were assayed for antiradical capacity by following their effect on the free radical $ABTS^{\cdot+}$ [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)]. Decolorizing activity of the different quinoa extracts on $ABTS^{\cdot+}$ solutions was monitored at λ = 414 nm (Gandía-Herrero et al., 2010; Re et al., 1999) in sodium phosphate buffer, pH 7.0 in a final volume of 300 μL as previously described (Gandía-Herrero et al., 2013).

2.6.3. ORAC assay

The hydrogen atom transfer assay ORAC (oxygen radical absorbance capacity) was performed with fluorescein as fluorescent probe and 2,2′-azobis(2-amidinopropane)dihydrochloride (AAPH) as the free radical generator as previously described (García-García, Hernández-García, Sánchez-Ferrer, & García-Carmona, 2013). The latter produces the peroxyl radicals which damage the fluorescent probe, thus resulting in the loss of fluorescence. The reaction medium (200 μL) contained 37.5 nM fluorescein, 19 mM AAPH, and different concentrations of quinoa extracts in 75 mM sodium phosphate, pH 7.4.

In all cases Trolox was used as a reference antioxidant for calibration curves and measurements of 96-well plates were per-