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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-

formed in a Synergy HT plate reader (Bio-Tek Instruments, Winooski, USA). All experiments were performed in triplicate and mean values and standard deviations were plotted. Data analysis was carried out using linear regression fitting under Sigma Plot Scientific Graphing for Windows version 8.0 (2001; SPSS, Chicago, USA).

2.7. Standard betalains

Betanin was obtained from roots of *Beta vulgaris,* dopaxanthin was extracted and purified from yellow flowers of *Lampranthus productus*, betanidin was obtained from violet flowers of the same plant (Gandía-Herrero, Escribano, & García-Carmona, 2007), and amaranthin was extracted from *Celosia argentea* (Guadarrama-Flores, Rodríguez-Monroy, Cruz-Sosa, García-Carmona, & Gandía-Herrero, 2015). Other pigments were obtained by a combined procedure for the release of betalamic acid from purified betanin and condensation with amines and amino acids, following a previously described method (Gandía-Herrero, García-Carmona, & Escribano, 2006). All compounds were characterized spectrophotometrically, chromatographically, and by electrospray ionization mass spectrometry (ESI-MS).

2.8. Electrospray ionization mass spectrometry

A VL 1100 apparatus with LC/MSD Trap (Agilent Technologies, Palo Alto, CA) was used for HPLC-ESI-MS/MS analyses. Elution conditions were as described above using the same column and decreasing the flow rate to 0.8 mL/min. Vaporizer temperature was 350 °C, and voltage was maintained at 3.5 kV. The sheath gas was nitrogen, operated at a pressure of 45 psi. Samples were ionized in positive mode. Ion monitoring mode was full scan in the range *m*/*z* 50–1200. The electron multiplier voltage for detection was 1350 V.

Accurate mass determination for saponins was carried out after the same chromatographic separation with a TOF/Q-TOF MS spectrometer Agilent 6220, equipped with a dual ESI-APCI interface (Gómez-Caravaca, Segura-Carretero, Fernández-Gutiérrez, & Caboni, 2011). Samples were ionized in negative mode, using a capillary voltage of 3.5 kV. The gas temperature was 350 °C, nitrogen drying gas was set at 11 L·min^{-1} and the nebulizer pressure was 40 psi. Data were processed through the software MassHunter (Agilent Technologies).

2.9. Fluorescence imaging

Brightfield and fluorescence images were obtained in a Leica Z6 APO macroscope with incident light beam (Leica Microsystems, Wetzlar, Germany) attached to a digital camera Leica DC500. In the case of fluorescence imaging, the filtercube I3 (Leica Microsystems) was used limiting excitation to the range λ = 450–490 nm and emission to λ = 510–515 nm.

3. Results and discussion

3.1. Pigment analysis in Chenopodium quinoa grains

Multiple varieties of *Chenopodium quinoa* exhibiting different colors and hues in their grains were selected for this study in order to cover all the possibilities of coloration present in nature. Grains of plants grown in different areas of the Peruvian altiplano and valleys were collected by the local growers and stored at the Peruvian germplasm bank located in Lima. 29 varieties exhibiting different colors were finally selected as shown in Fig. 1A and listed in Table 1.

The recent determination of the betacyanin betanin and its isomer isobetanin in red quinoa grains (Abderrahim et al., 2015; Tang et al., 2015) opened the possibility of the existence of betalains in this edible part of the plants. In the present work the application of a HPLC method able to separate multiple betalains (Gandía-Herrero et al., 2005a) attached to a photodiode detector in order to follow both betacyanins (536 nm) and betaxanthins (480 nm) to different quinoa samples revealed the existence of multiple pigments. The betacyanins betanin (**2**) (Rt = 11.6 min) and its isomer isobetanin (Rt = 12.32 min) were detected and corroborated the previous determinations. However the main pigment detected in these samples was not betanin. All red-violet quinoa grains showed the presence of amaranthin (**1**) (Rt = 10.27 min) and its corresponding isomer isoamaranthin (Rt = 10.78 min) in greater amounts, thus expanding the number of possible betacyanins present in the grains of *C. quinoa*. Table 1 summarizes the results for pigment identification and quantitation by HPLC using individual pigment standards. Furthermore previously unconsidered betaxanthins were detected in red-violet grains. The main peak accounted for DOPA (dihydroxyphenylalanine) derived betaxanthin (**3**), also known as dopaxanthin (Rt = 13.0 min) and first described in the yellow flowers of *Glottiphyllum longum*

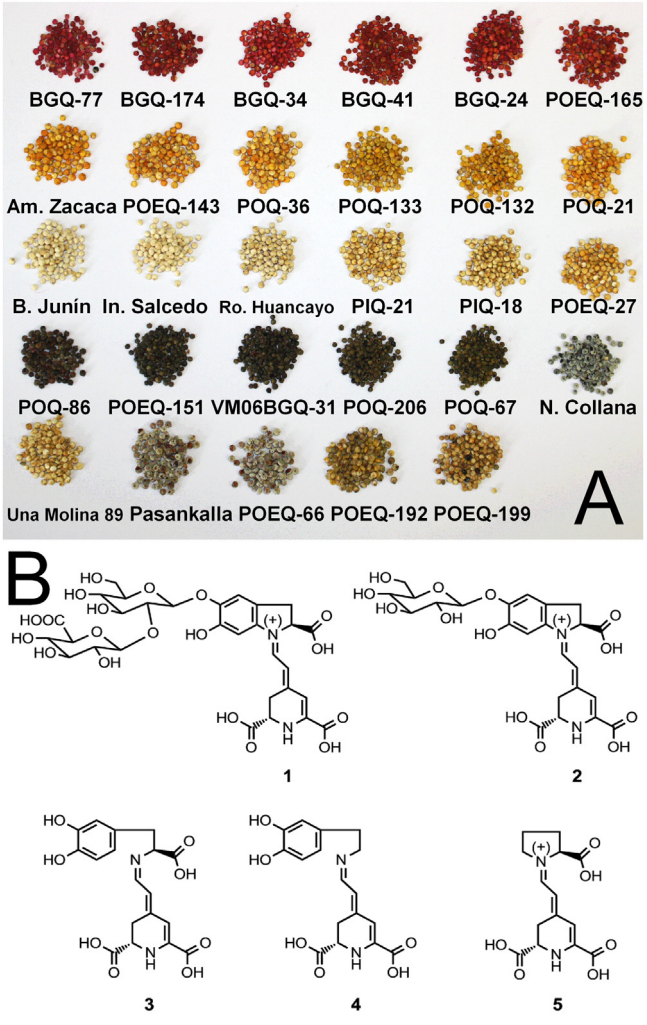

Fig. 1. A: Macroscopic image of all the different varieties of *Chenopodium quinoa* grains considered in this study. **B:** Betalains structures identified in the colored quinoa grains: Amaranthin (**1**), betanin (**2**), dopaxanthin (**3**), miraxanthin V (**4**), and indicaxanthin (**5**).

Table 1
Varieties of *C. quinoa* with differently colored grains considered in this study and pigment identification. HPLC retention times, molecular ions as determined by ESI-MS, and PDA maximum wavelengths are shown together with the varieties geographical origin.

	Betalains	Amaranthin	iso-Amaranthin	Betanin	iso-Betanin	Dopaxanthin	Dopamine-BX	Proline-BX	Unknown-BX
	Rt (min)	10.3	10.8	11.6	12.3	13	14.9	12.4	14.1
	$[M+H]^+$ (m/z)	727	727	551	551	391	347	309	347
	daughter ion (m/z)	551	551	389	389	347	303	265	303
	λ_m (nm)	535	535	535	535	471	462	481	471
Quinoa variety	Origin	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
BGQ-77	Ancash	144.1 ± 6.2	108.3 ± 4.5	5.1 ± 0.2	2.8 ± 0.1	17.6 ± 1.0	n.d.[a]	n.d.	n.d.
BGQ-174	Apurimac	124.6 ± 5.9	132.9 ± 5.2	9.7 ± 0.4	7.7 ± 0.3	85.3 ± 4.7	7.8 ± 0.3	n.d.	n.d.
BGQ-34	Ancash	141.0 ± 6.6	118.8 ± 5.0	6.8 ± 0.3	3.1 ± 0.1	17.9 ± 1.1	5.7 ± 0.2	n.d.	n.d.
BGQ-41	Ancash	121.9 ± 4.8	109.0 ± 4.1	7.1 ± 0.3	n.d.	79.5 ± 3.8	7.3 ± 0.3	n.d.	n.d.
BGQ-24	Ancash	148.6 ± 7.0	145.5 ± 8.3	8.2 ± 0.5	n.d.	63.1 ± 3.7	10.5 ± 0.6	n.d.	n.d.
POEQ-165	Ancash	89.6 ± 5.3	80.7 ± 3.7	5.7 ± 0.4	n.d.	35.9 ± 2.1	8.0 ± 0.3	n.d.	n.d.
Amarilla Zacaca	Cusco	1.2 ± 0.1	1.3 ± 0.1	0.8 ± 0.1	n.d.	1.0 ± 0.1	3.6 ± 0.1	3.7 ± 0.2	n.d.
POEQ-143	Arequipa	0.9 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	n.d.	2.6 ± 0.2	1.6 ± 0.1	2.1 ± 0.1	n.d.
POQ-36	Puno	0.8 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	n.d.	0.4 ± 0.1	0.5 ± 0.1	1.8 ± 0.1	n.d.
POQ-133	Puno	n.d.	n.d.	n.d.	n.d.	33.1 ± 1.8	3.8 ± 0.2	n.d.	7.7 ± 0.3
POQ-132	Puno	n.d.	n.d.	n.d.	n.d.	54.1 ± 2.3	2.5 ± 0.1	n.d.	8.6 ± 0.3
POQ-21	Cusco	n.d.	n.d.	n.d.	n.d.	1.1 ± 0.1	n.d.	7.5 ± 0.3	n.d.
Blanca de Junín	Junín	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Inia Salcedo	Puno	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Rosada de Huancayo	Junin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PIQ-21	Cusco	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.3 ± 0.1	n.d.
PIQ-18	Cusco	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.3 ± 0.1	n.d.
POEQ-27	Cajamarca	n.d.	n.d.	n.d.	n.d.	0.4 ± 0.1	n.d.	0.3 ± 0.1	n.d.
POQ-86	Puno	6.9 ± 0.3	3.9 ± 0.2	1.2 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	n.d.	n.d.	n.d.
POEQ-151	Cusco	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
VM06BGQ-31	Unknown	0.8 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	n.d.	n.d.	n.d.
POQ-206	Puno	0.9 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	n.d.	5.5 ± 0.3	0.7 ± 0.1	n.d.	n.d.
POQ-67	Puno	n.d.	n.d.	n.d.	n.d.	1.3 ± 0.1	1.2 ± 0.1	1.6 ± 0.1	n.d.
Negra Collana	Puno	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Una la Molina 89	Puno	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pasankalla	Puno	0.8 ± 0.2	0.8 ± 0.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
POEQ-66	Puno	0.8 ± 0.2	0.8 ± 0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
POEQ-192	Apurimac	n.d.	n.d.	n.d.	n.d.	5.9 ± 0.4	0.7 ± 0.1	n.d.	0.9 ± 0.1
POEQ-199	Apurimac	n.d.	n.d.	n.d.	n.d.	0.4 ± 0.1	n.d.	n.d.	n.d.

[a] Not detected.

(Impellizzeri, Piattelli, & Sciuto, 1973). Dopamine derived betaxanthin (**4**) (Rt = 14.9 min), known as miraxanthin V (Piattelli, Minale, & Nicolaus, 1965) was also present in lower quantities in most of the red-violet samples. Yellow-orange quinoa grains were analyzed in this work for the first time ever and presented a variety of betaxanthins. The main pigments corresponded to dopaxanthin (**3**) and dopamine-betaxanthin (**4**) (Table 1). The presence of the dihydroxylated betaxanthins both in red-violet and in yellow orange grains is of special relevance due to the high antioxidant and free radical scavenging activities described for these betalains (Gandía-Herrero, Escribano, & García-Carmona, 2009). Proline-derived betaxanthin (**5**), a pigment also known as indicaxanthin was detected in high amounts in the yellow variety POQ-21. A minor pigment identified as a conjugate of betalamic acid with a m/z for the parent ion $[M+H]^+$ of 347 and Rt = 14.1 min was present mainly in the varieties POQ-133 and POQ-132. This betaxanthin did not match to any known pigment or synthetic alternatives although standards of the same mass were prepared for conjugates of dopamine, octopamine and 2-(2,5-dihydrophenyl)glycine. HPLC profiles for pigment extracts of representative varieties of *C. quinoa* of different colors are shown in Fig. 2. As can be seen in Table 1, no pigment could be detected in the white varieties "Blanca de Junín", "Inia Salcedo" and "Rosada de Huancayo". However low quantities of indicaxanthin and dopaxanthin were detected in PIQ-21, PIQ-18, and POEQ-27. The lack of extractable pigments was shared with the black varieties POEQ-151 and "Negra Collana". Black varieties POQ-86, VM06BGQ-31, POQ-206, and POQ-67 showed the presence of variable amounts of violet or yellow betalains, which were of special relevance in the case of variety POQ-206 with the main presence of dopaxanthin (Table 1). Pigments were identified by electrospray ionization mass spectrometry (ESI-MS) analyses and comparison with real standards in terms of HPLC retention times and PDA spectra. For all pigments the mass values determined for the parent ions were as expected for the corresponding protonated molecular ions $[M+H]^+$ of the betalains, thus confirming the proposed structures (Fig. 1B). For the betacyanin betanin (**2**), the main daughter ion was generated by the cleavage of the glucose unit at the level of the *O*-glucosidic bond (m/z $[M+H]^+$ - 162), being the ion detected at m/z 389, corresponding to the mass for betanidin. Amaranthin (**1**) yielded a molecular ion of m/z 727 that was fragmented into m/z 551, corresponding to the loss of a glucuronic acid moiety m/z $[M+H]^+$ - 176 (betanin) and into m/z 389, corresponding to an additional loss of glucose m/z $[M+H]^+$ - 176 - 162 (betanidin). For betaxanthins, a daughter ion corresponding to m/z $[M+H]^+$ - 44 was detected in all cases, accounting for the loss of a carboxylic acid group, in addition to the corresponding protonated molecular ions $[M+H]^+$. The existence of betaxanthins justifies the color exhibited by the yellow grains and its mixture with betacyanins generates the red shades shown by the varieties BGQ-174, BGQ-41, BGQ-24 and POEQ-165 (Fig. 1).

3.2. Color analysis of quinoa grains

The color of the different varieties of *C. quinoa* grains was quantitated by the results of color measurements in a spectrophotometer equipped with an integrating sphere and analyzed in terms of the parameters corresponding to the uniform CIELAB color space. Whole grains that were not submitted to any grounding process or treatment were directly placed in the sphere cell. Thus all the measurements corresponded to the colored outer layer of the

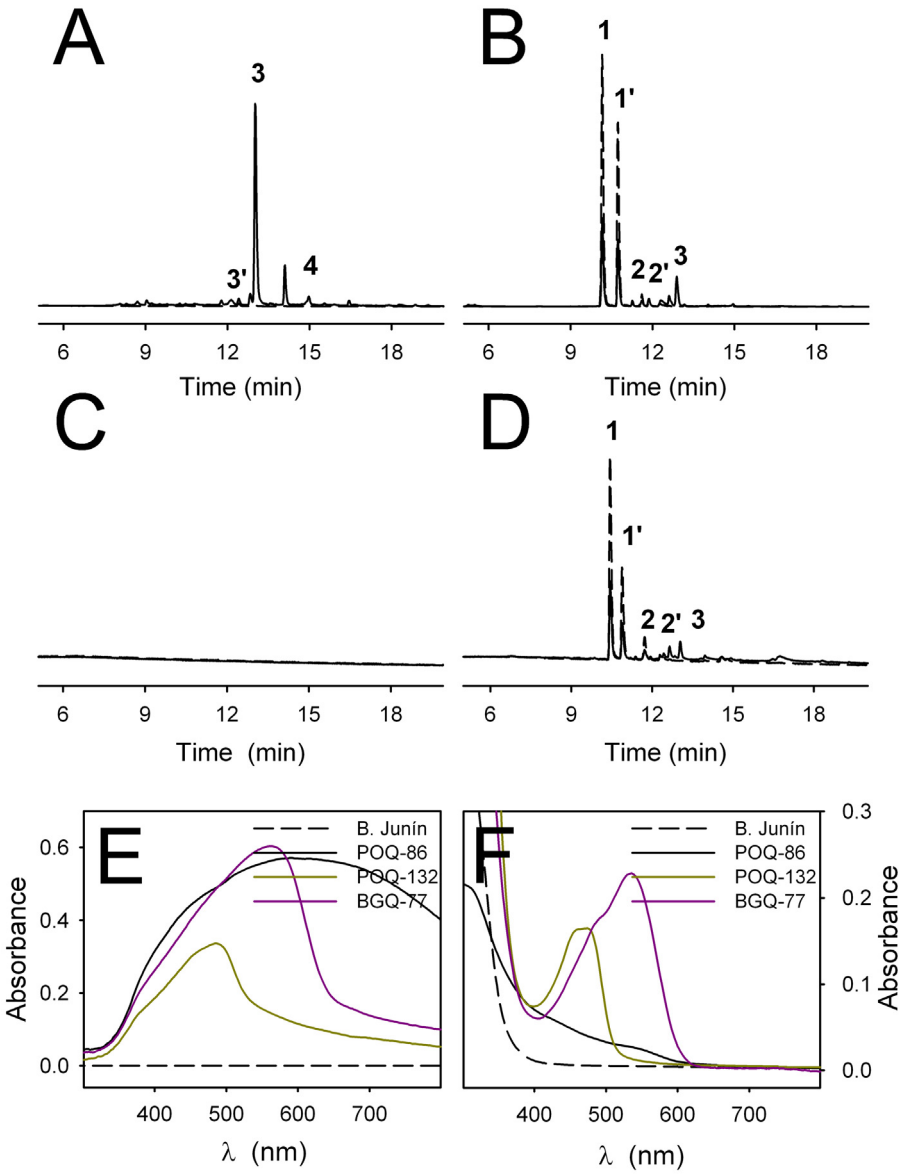

Fig. 2. Pigment analysis of differently colored quinoa varieties. HPLC recordings for extracts of POQ-132 (yellow) (**A**), BGQ-77 (violet) (**B**), "Blanca de Junín" (white) (**C**), and POQ-86 (black) (**D**). Continuous line shows signal registered at λ = 480 nm, dashed line at λ = 536 nm. Full scale is 300 mAU for panel **A**, 600 mAU for **B** and 30 mAU for **C** and **D**. Pigments shown are amaranthin (**1**), isoamaranthin (**1′**), betanin (**2**), isobetanin (**2′**), dopaxanthin (**3**), isodopaxanthin (**3′**), and miraxanthin V (**4**). **E:** UV/Vis spectra obtained with an integrating sphere for whole grains of varieties POQ-132 (yellow), BGQ-77 (violet), "Blanca de Junín" (white), and POQ-86 (black) after subtraction of the spectrum for the basal absorbance of white grains. **F:** Same varieties analyzed after aqueous extraction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

grains as visible under normal physiological conditions. Supplementary Table 1 summarizes the results obtained for all the considered varieties of quinoa including white, black, yellow and red-violet colors.

The lack of pigments in the white varieties of quinoa makes these grains appropriate controls in terms of the influence of betalains in the color perception of colored ones. White grains (*C. quinoa* varieties "Blanca de Junín", "Inia Salcedo" and "Rosada de Huancayo") are characterized by values of the parameter L∗ in the range 72.04–75.6. This value indicates the high luminosity of these samples. The color is characterized by low values of a∗ indicating the absence of red-green intensities and by b∗ values that relate to a cream pale yellow background even in the absence of betalains (Supplementary Table 1). In contrast the black varieties (POQ-86, POEQ-151, VM06BGQ-31, POQ-206, and POQ-67) exhibit CIELAB parameters that range among 41.57 and 45.26 for the L∗ values, and very low values for a∗ (0.89–2.26) and b∗ (2.80–8.51). As expected, these values clearly indicate a reduced luminosity of the black grains and the absence of a predominant color. There is no apparent difference among the black betalain containing grains and those that showed no extractable pigments (Table 1 and Supplementary Table 1). In the case of yellow grains the CIELAB parameters show L∗ values in the range 60.76–67.01, a∗ values among 6.41 and 12.94, and b∗ values among 26.81 and 28.78. These parameters demonstrate a contribution of betaxanthins to the yellow color (b∗) of the grains above the basal level measured for the white varieties (Supplementary Table 1). For those red-violet grains containing both betaxanthins and betacyanins it is shown that as the betaxanthin proportion is reduced, the value of the *b*∗ parameter, which measures the yellow color, decreases and, at the same time, the value of the *a*∗ parameter increases due to the higher betacyanins content. In general for the colored

grains the $L*$ parameter decreases when the contribution of betacyanins is higher, indicating a reduction in the clarity of the grains. A change of the hue angle (h^o) is also linked to the lower proportion of betaxanthins being decreased from the yellow varieties towards those containing more betacyanins and thus exhibiting color from red to violet (Supplementary Table 1). In this sense, purple quinoa grains are those containing mainly violet betacyanins (varieties BGQ-77 and BGQ-34) and showed CIELAB parameters characterized by the lowest hue angle (h^o) with values of 14.67 (BGQ-77) and 19.19 (BGQ-34).

Fig. 2E shows the spectra obtained using an integrating sphere for the four types of quinoa colored grains. The varieties "Blanca de Junín", POQ-86, POQ-132, and BGQ-77 were selected as representatives of the behavior of the white, black, yellow, and violet grains respectively. For the yellow whole grains the maximum wavelength was determined as λ_{max} 484 nm while for the violet quinoa the maximum wavelength was measured as λ_{max} 552 nm. Both the shape and maximum wavelengths corresponded to the typical results for betaxanthins and betacyanins thus indicating that the detected pigments (Table 1) were responsible for the grains colorations. Spectra for the aqueous extracts where pigments were identified are shown for comparison in Fig. 2F. A range of colors from yellow to violet was obtained with the quinoa grains used in this study. Maximum wavelengths obtained ranged from 467 nm to 542 nm. For the betacyanin containing varieties with limited contribution of betaxanthins (varieties BGQ-77 and BGQ-34), the maximum wavelength is restricted to the range 530–536 nm. Those containing betaxanthins in a higher extent (varieties BGQ-174, BGQ-41, BGQ-24, and POEQ-165) present in addition another maximum wavelength placed in the range 480–484 nm and appeared red in color. The yellow varieties ("Amarilla Zacaca", POEQ-143, POQ-36, POQ-133, POQ-132 and POQ-21) containing mainly betaxanthins possess maximum wavelengths ranging from 454 nm to 472 nm.

The characterized diversity of colors present in quinoa grains, including the yellow varieties characterized here for the first time, offers multiple possibilities for obtaining vegetable colored powders or flours suitable for food applications.

3.3. Fluorescent quinoa

Betaxanthins are molecules that exhibit natural fluorescence in the visible range of the electromagnetic spectrum (Gandía-Herrero, García-Carmona, & Escribano, 2005b). Excitation with blue light promotes the emission of green light. This phenomenon is general to all betaxanthins and relies in the electron resonance system present in the betalamic acid moiety with a limited contribution of the amine substructure (Gandía-Herrero et al., 2010).

The possible fluorescence of the betaxanthin containing extracts derived from the yellow varieties of quinoa grains was analyzed by 2D and 3D fluorescence spectroscopy. Fig. 3 shows the results obtained for the variety POQ-132, taken as a model of the yellow quinoa. The yellow quinoa extract possesses a maximum excitation wavelength of 473 nm, and emission maximum occurred at 551 nm. This implies a difference of 78 nm for the Stokes shift. Spectral widths measured at half the maximum intensity were 58 nm for excitation, and 38 nm for emission. The spectral shapes are shown in Fig. 3A and are in accordance with previously reported spectra of individual betaxanthins (Gandía-Herrero et al., 2010). All betaxanthins present maximum excitation wavelengths between 471 nm and 474 nm, while emission maxima are found in the range 548–551 nm. The entire range of possible wavelengths for both excitation and emission was recorded in three-dimensional fluorescence spectral analysis. Fig. 3B graphically shows the excitation-emission matrix for the fluorescence spectroscopic data. The analysis provides full characterization of the fluorescence properties of yellow quinoa, and shows a fluorescence peak centered at the maximum excitation and emission wavelengths.

A specific filter system was used in order to test if the presence of betaxanthins makes quinoa grains fluorescent in the visible range of the electromagnetic spectrum. Supplementary Fig. 1A shows close pictures for the four possible types of quinoa grains under normal white light. When illuminated by white light, the grains containing betaxanthins appear yellow due to the main contribution of reflectance of the non-absorbed radiation combined with the light emitted by means of fluorescence. Supplementary Fig. 1B exemplifies the results derived from the betaxanthins autofluorescence and was obtained under a filter system able to irradiate the sample with blue light and to record the emitted light. Only the yellow grains of quinoa present light emission under blue light stimulation in the fluorescence image. During the excitation process, UV light is filtered and neither UV excitation nor emission is possible. Red-violet grains do not present light emission and they appear dark in the fluorescence photograph, as do white and black varieties. The white and black phenotypes can be considered as controls in the fluorescence exhibited by the yellow one. Betacyanins do not significantly fluoresce, and due to the overlapping between the betacyanin absorbance and the betaxanthin emission spectra, the former are able to absorb the light emitted by the fluorophores thus making the structures where they are found together non-fluorescent as seen in Supplementary Fig. 1B and previously described in flowers (Gandía-Herrero et al., 2005b). A closer view of the fluorescent quinoa grain is shown under white light and blue light stimulation in Fig. 3C and D respectively.

The identified betaxanthins give the yellow coloration to quinoa grains and make it possible for the entire structure to glow. Fluorescence of betaxanthins has previously been used to visualize petal cells (Gandía-Herrero et al., 2005b), to detect the presence of *Plasmodium falciparum* (malaria) in erythrocytes (Gonçalves et al., 2013) and to follow the accumulation of betaxanthins in *C. argentea* cell cultures (Guadarrama-Flores et al., 2015). This is the first report of betalains fluorescence in plant tissues other than flowers and demonstrates the existence of light emission by visible fluorescence in intact viable grains. Although the relevance of light emission in flowers for the attraction of pollinators is a matter of debate (García-Plazaola et al., 2015; Rao & Ostroverkhova, 2015), the possible influence of fluorescence in seed dispersal has not been considered and these new data may open the discussion.

3.4. Determination of saponins in multiple varieties of quinoa grains

Preliminary assays performed for the analysis of the quinoa betalains revealed the existence of thick foam formed on some varieties during extraction. Foam was tentatively associated with the presence of saponins and thus the extraction procedure was adapted to consider extraction and analysis of these molecules. Saponins are bitter tasting, water-soluble triterpenoids found in the outer seed coat layer of quinoa. The quinoa saponins have been shown to have anti-inflammatory activity (Yao, Yang, Shi, & Ren, 2014) and are involved in cytostatic effects against cancer cells and in the reduction of cholesterol level in serum (Francis, Kerem, Makkar, & Becker, 2002). The extraction procedure followed a well established afrosimetric protocol that allowed a semi-quantitative determination of the amount of foam forming saponins (Koziol, 1991). The presence of saponins in quinoa grains is visible after vigorous shaking in aqueous buffered solutions and can be reproducibly measured by following exact timing, amounts and tubes sizes. Inherent limitations of the method are derived from the different properties of specific saponins. However it allows a quick estimation of the overall saponins content and the

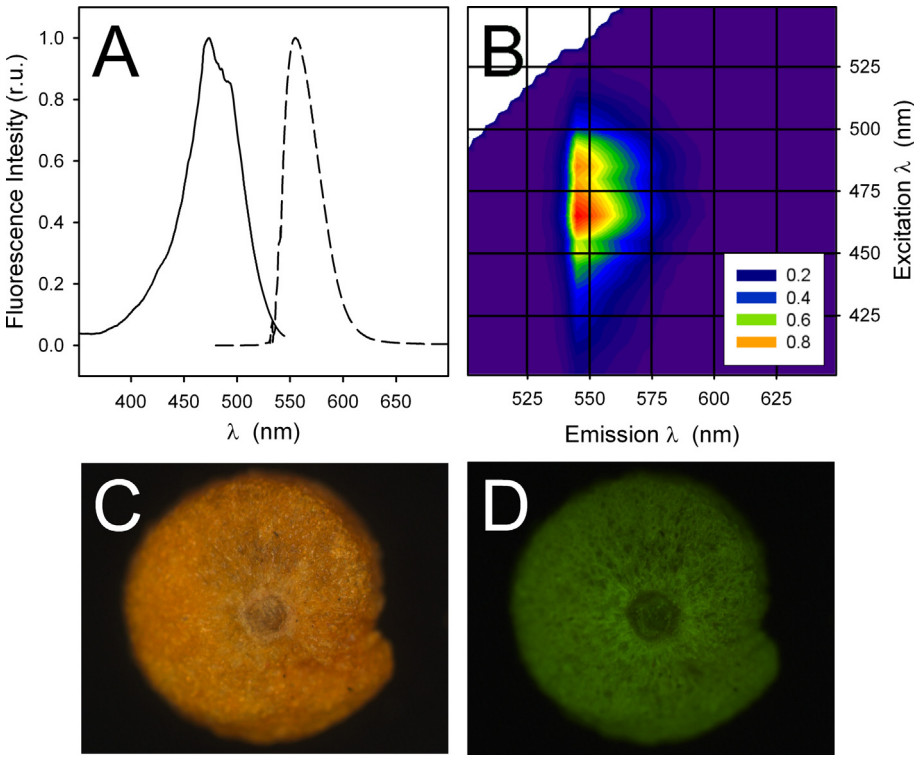

Fig. 3. Fluorescent properties of yellow quinoa grains. **A:** Excitation (continuous line) and emission (dashed line) fluorescence spectra for a betaxanthin-containing quinoa extract (POQ-132). **B:** Contour plot and 3D fluorescence spectrum for the same extract. **C:** Close view of a yellow quinoa grain obtained under normal white light illumination. **D:** Same yellow quinoa grain recorded by fluorescence under blue light stimulation. Quinoa images shown correspond to variety POQ-132. Spectra were obtained in water at 25 °C (total pigment concentration 1 μM).

classification of the grains as sweet (low saponins content) or bitter (high saponins content) (Koziol, 1991). Supplementary Table 2 shows the results for the afrosimetric method. Proteins' contribution to foam height is minimal due to the high foam forming capacity of saponins and sweet varieties present reduced afrosimetric values. In order to identify the nature of individual saponins in the multiple varieties used, leachates were submitted to HPLC analysis coupled to a TOF/Q-TOF MS spectrometer for exact mass determinations. Saponins successfully determined were the bidesmosidic compounds 3-*O*-*β*-D-glucuronopyranosyl oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester (Rt = 39.5 min) (**I**), 3-*O*-α-L-arabinopyranosyl phytolaccagenic acid 28-*O*-*β*-D-glucopyranosyl ester (Rt = 35.7 min) (**II**), 3-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucuronopyranosyl oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester (Rt = 38.9) (**III**), 3-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucuronopyranosyl hederagenin 28-*O*-*β*-D-glucopyranosyl ester (Rt = 35.9 min) (**IV**), and 3-*O*-*β*-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl serjanic acid 28-*O*-*β*-D-glucopyranosyl ester (Rt = 37.6 min) (**V**) (Fig. 4). All these saponins were detected through their ESI mass spectra showing an accurate mass (experimental mass) corresponding to their respective molecular formula (Supplementary Table 2). In all cases the experimental mass matched the calculated values with errors bellow the accepted accuracy threshold for elemental composition analysis, established at 5 ppm (Ferrer, García-Reyes, & Fernandez-Alba, 2005). The detected saponins have been previously reported as molecules contributing to the bitter taste of quinoa grains (Gómez-Caravaca et al., 2011). Fig. 4B graphically shows the overall results for saponin content in *C. quinoa* grains identifying the varieties with higher amount of saponins. These are PIQ-18, POQ-21, POEQ-27, POEQ-143, POQ-36, and "Amarilla Zacaca". All of them were identified as bitter varieties by the afrosimetric method. Thus the identified molecules support the foam forming properties of the extracts and the bitter taste of these varieties.

Saponins have been found to possess anti-inflammatory and anti-cancer activities (Liu et al., 2013; Yao et al., 2014) and thus they are components of quinoa with potential pharmacological interest. However their bitter taste may limit the nutritional application of the varieties where they are found in higher quantities. Washing the grains releases the saponins but also eliminates the bioactive betalains. Thus the identification of quinoa varieties with high betalain content and a reduced level of saponins is a matter of potential agronomic interest.

3.5. Free radical scavenging and antioxidant activities

Extracts of quinoa grains of multiple colors were assayed for antioxidant and free radical scavenging activities using the FRAP and ABTS radical assays respectively. Although the FRAP and ABTS assays have both electron transfer mechanisms, FRAP analyses are based on the direct capacity of extracts to reduce Fe (III) to Fe (II) as a measure of the antioxidant power (Benzie & Strain, 1996) and the ABTS assay is based on the capacity of extracts to reduce the stable $ABTS^{\cdot+}$ radical in aqueous solutions (Re et al., 1999). Both activities were recorded and compared with that of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble derivative of vitamin E. TEAC (Trolox equivalent antioxidant activity) values of the extracts were then calculated and referred to the starting amount of dry grains.

FRAP results indicate a very high antioxidant capacity for the pigmented quinoa samples (Fig. 5). All violet, red and yellow quinoa grains extracts show remarkable antioxidant activity in comparison with the white and black ones. This is as high as 18.4 ± 1.7 mmol Trolox/kg for the variety BGQ-174. The highest activity was observed for the red-violet varieties containing both betacyanins and betaxanthins, with remarkable activity also exhibited by yellow varieties POQ-133 (12.5 ± 2.3 mmol Trolox/kg) and POQ-132 (15.2 ± 1.4 mmol Trolox/kg). These two varieties are

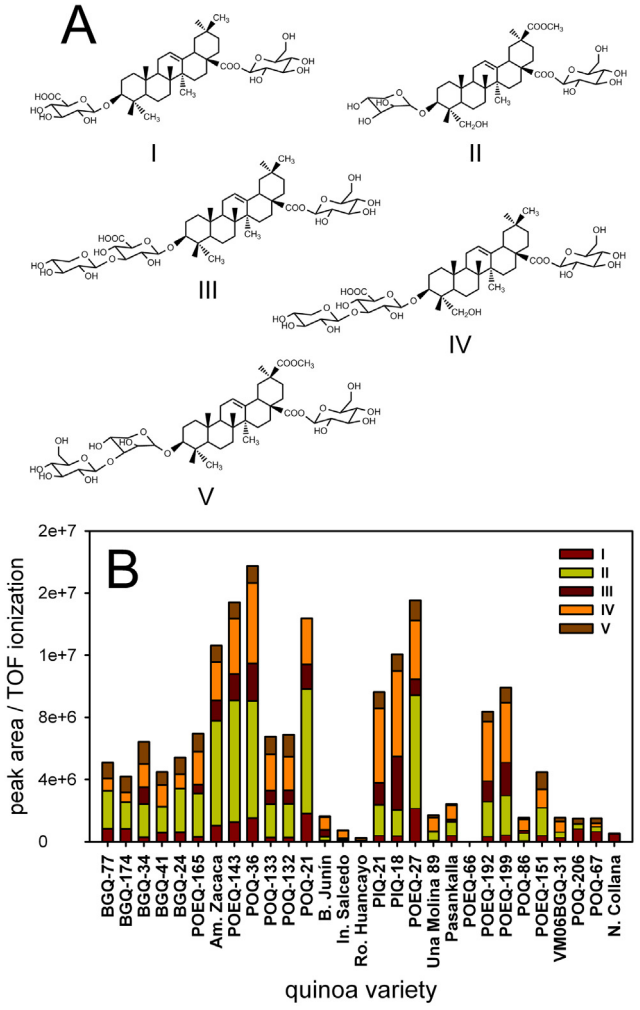

Fig. 4. Saponin content in quinoa grains of different varieties. **A:** Structures for the saponins 3-*O*-*β*-D-glucuronopyranosyl oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester (**I**), 3-*O*-*α*-L-arabinopyranosyl phytolaccagenic acid 28-*O*-*β*-D-glucopyranosyl ester (**II**), 3-*O*-*β*-D-xylopyranosyl-(1 → 3)-*β*-D-glucuronopyranosyl oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester (**III**), 3-*O*-*β*-D-xylopyranosyl-(1 → 3)-*β*-D-glucuronopyranosyl hederagenin 28-*O*-*β*-D-glucopyranosyl ester (**IV**), and 3-*O*-*β*-D-glucopyranosyl-(1 → 3)-*α*-L-arabinopyranosyl serjanic acid 28-*O*-*β*-D-glucopyranosyl ester (**V**). **B:** Overall and individual content of identified saponins in quinoa varieties determined by HPLC-TOF/Q-TOF analysis.

characterized by a high content of dopaxanthin (**3**) (Table 1), whose dihydroxylated substructure demonstrated high antioxidant capacity (Gandía-Herrero et al., 2010).

Regarding the $ABTS^{\cdot+}$ radical assay, a decrease in the concentration of the radical was observed in all samples but this was higher for the red-violet and yellow varieties. In general it is observed that those varieties containing more betalains present higher TEAC values (Fig. 5). The highest antiradical capacity was determined for the red-violet variety BGQ-34 (TEAC value 47.4 ± 1.5 mmol Trolox/kg). The highest value determined for yellow quinoa grains was 44.1 ± 0.8 mmol Trolox/kg.

The values determined for the Trolox equivalence of the colored quinoa extracts indicate high antioxidant and free radical scavenging activities. These results are higher than those of fruits' extracts of recognized antioxidant capacity as blackberry or raspberry that exhibit TEAC values under the ABTS assay of 20.2 and 16.8 mmol Trolox/kg respectively (Pellegrini et al., 2003). The white varieties of quinoa lacking betalains yielded TEAC values of 8.3 ± 1.5 (variety "Blanca de Junín"), 8.2 ± 1.9 ("Inia Salcedo"), and 6.0 ± 1.5 ("Rosada de Huancayo") mmol Trolox/kg. Although the general trend among quinoa varieties is maintained for the FRAP and ABTS assays the relative values across the different varieties present divergences. The lack of correlation among the two electron transfer assays is of relevance in order to establish the actual ranking of varieties as a function of their bioactive potential. In addition the values for the ABTS assay were higher than those for the FRAP assay, thus ambiguously quantifying the strength of the antioxidant-antiradical effect. The different TEAC values calculated for the same grains depending on the assay is explained by the different pH at which each assay is performed. FRAP assay is performed at pH 3.6 while the standard ABTS assay uses buffered solutions at pH 7.0. For betalains it has been described a strong pH dependence on the measured activity (Gandía-Herrero et al., 2010). A protonation equilibrium makes betalains better electron donors and more active antioxidants at pH values above pH 6.0 (Gliszczyńska-Świgło et al., 2006). Taking into account that FRAP is performed at pH 3.6, the ABTS assay was repeated at this pH in order to examine if the mentioned observations on quinoa antioxidant activity are related to pH. Fig. 5 shows how the use of the same pH for both assays involves an analogous trend across all the varieties. The values obtained for ABTS assay at acidic pH (Fig. 5C) are closer to those obtained for the FRAP assay (Fig. 5A) and implies a reduction in the previous estimation (Fig. 5B). However it is still high and above well documented extracts of antioxidant fruits and vegetables (Pellegrini et al., 2003). In addition there is a linear tendency among the TEAC values determined for quinoa grains under ABTS and FRAP assays performed under equivalent conditions (Fig. 5D) with a linear correlation coefficient of r = 0.973. Data correlation implies analogous relative differences among extracts and the same ranking for the different varieties thus supporting the previous observations on colored quinoa varieties. The fact that a pH value of 7.0, around the more common physiological conditions, increases the free radical scavenging activity of extracts is in relation to the presence of betalains and implies a high bioactive potential. In order to test the free radical scavenging capacity of the extracts under these conditions with an assay based on the alternative hydrogen atom transfer mechanism (HAT), the ORAC (Oxygen Radicals Absorbance Capacity) assay was performed on all the 29 quinoa varieties. ORAC assay is based on the capacity of the extracts to protect the fluorescent probe fluorescein from peroxyl radicals, resulting in longer fluorescence decay curves that can be integrated (Ou, Hampsch-Woodill, & Prior, 2001). Supplementary Fig. 2A shows the curves recorded for the fluorescence decay of the probe in the absence of extracts and in the presence of varieties POQ-132 (yellow), BGQ-77 (violet), "Blanca de Junín" (white), and POQ-86 (black). Yellow and violet quinoa varieties present a strong protective effect of the probe while the white and black varieties are closer to control measurements. Overall results for all quinoa grains are presented on Supplementary Fig. 2B. The tendency marked by the ABTS assay at pH 7 is maintained in the hydrogen transfer ORAC assay. Violet and yellow quinoa samples identified as the strongest scavengers of free radicals in the ABTS assay and containing betacyanins and dihydroxylated betaxanthins are also the most active varieties under the ORAC assay. On the other hand, samples with the lowest activity respond analogously in the two assays independently on the electron transfer or hydrogen atom transfer mechanism. The results on the antioxidant/free radical scavenging activity of quinoa extracts indicate an important contribution of betalains to the extraordinary capacity shown by the analyzed extracts. The use of multiple assays, based on different mechanisms, strongly support the bioactive potential of the varieties used.

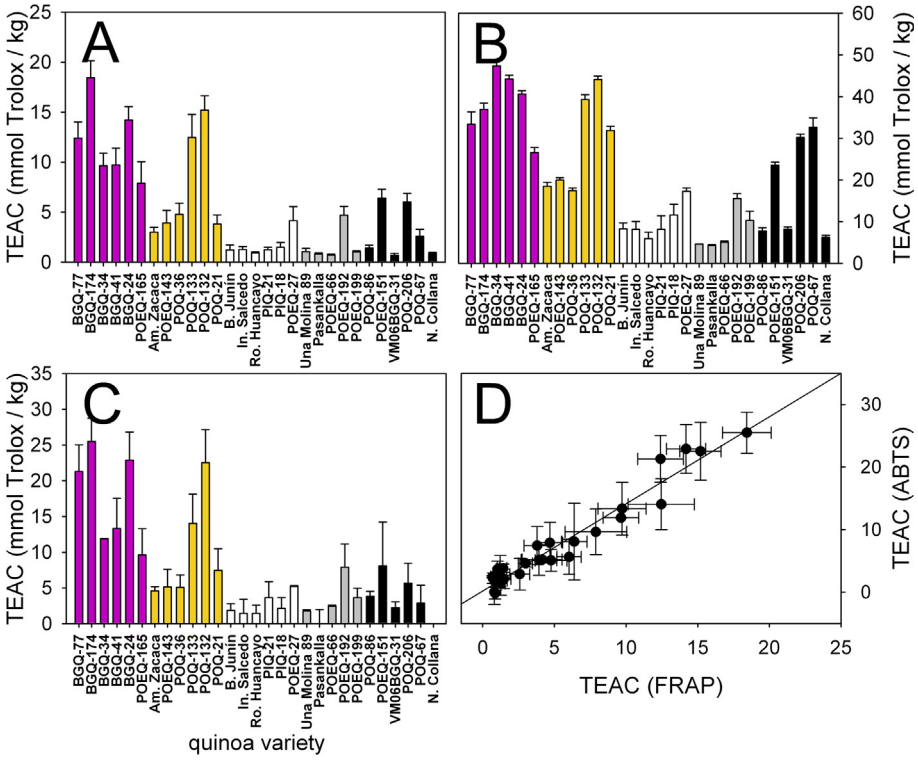

Fig. 5. TEAC values determined for extracts of *C. quinoa* grains under FRAP (**A**) and ABTS^{+} radical assays at pH 7.0 (**B**) and pH 3.6 (**C**). Correlation between the TEAC values obtained under the FRAP and ABTS assays measured at pH 3.6 (**D**).

4. Conclusions

The discovery of betaxanthins and the identification of novel betacyanins in quinoa grains explain the diversity of colors present in this edible pseudo-cereal used as a traditional agronomic crop in the Andean region. Co-existence of pigments generates different shades that could be used to fit specific color requirements of products for the pharmaceutical, cosmetic or food industries. Not only color and the novel fluorescent properties described here are modulated by the presence of betalains in the grains but also the high antioxidant and free radical scavenging activities characterized for these varieties, with the identification of dopaxanthin as a significant constituent.

Conflict of interest

The authors declare no competing financial interest. This work was performed attending to the principles of cooperation with third countries set by the European Research Council.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.04.187.

References

Abderrahim, F., Huanatico, E., Segura, R., Arribas, S., Gonzalez, M. C., & Condezo-Hoyos, L. (2015). Physical features, phenolic compounds, betalains and total antioxidant capacity of coloured quinoa seeds (*Chenopodium quinoa* Willd.) from Peruvian altiplano. *Food Chemistry, 183*, 83–90.

Amin, I., Norazaidah, Y., & Hainida, K. I. E. (2006). Antioxidant activity and phenolic content of raw and blanched *Amaranthus* species. *Food Chemistry, 94*, 47–52.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry, 239*, 70–76.

Brockington, S. F., Yang, Y., Gandia-Herrero, F., Covshoff, S., Hibberd, J. M., Sage, R. F., et al. (2015). Lineage-specific gene radiations underlie the evolution of novel betalain pigmentation in Caryophyllales. *New Phytologist, 207*, 1170–1180.

Capparelli, A., Pochettino, M. L., Lema, V., López, M. L., Andreoni, D., Ciampagna, M. L., et al. (2015). The contribution of ethnobotany and experimental archaeology to interpretation of ancient food processing: Methodological proposals based on the discussion of several case studies on *Prosopis* spp., *Chenopodium* spp. and *Cucurbita* spp. from Argentina. *Vegetation History and Archaeobotany, 24*, 151–163.

Escribano, J., Pedreño, M. A., García-Carmona, F., & Muñoz, R. (1998). Characterization of the antiradical activity of betalains from *Beta vulgaris* L. roots. *Phytochemical Analysis, 9*, 124–127.

FAO-ALADI (2014). Tendencias y perspectivas del comercio internacional de quinua. FAO-ALADI joint report. ISBN 978-92-5-308135-6.

Ferrer, I., García-Reyes, J. F., & Fernandez-Alba, A. (2005). Identification and quantitation of pesticides in vegetables by liquid chromatography time-of-flight mass spectrometry. *Trends in Analytical Chemistry, 24*, 671–682.

Francis, G., Kerem, Z., Makkar, H. P. S., & Becker, K. (2002). The biological action of saponins in animal systems: A review. *British Journal of Nutrition, 88*, 587–605.

Fuentes, F., & Paredes-Gonzales, X. (2015). Nutraceutical perspectives of quinoa: biological properties and functional applications. Chapter 3.5. In FAO & CIRAD. State of the Art Report of Quinoa in the World in 2013 (pp. 286–299). Rome.

Gandía-Herrero, F., Cabanes, J., Escribano, J., García-Carmona, F., & Jiménez-Atiénzar, M. (2013). Encapsulation of the most potent antioxidant betalains in edible matrixes as powders of different colors. *Journal of Agricultural and Food Chemistry, 61*, 4294–4302.

Gandía-Herrero, F., Escribano, J., & García-Carmona, F. (2007). Characterization of the activity of tyrosinase on betanidin. *Journal of Agricultural and Food Chemistry, 55*, 1546–1551.

Gandía-Herrero, F., Escribano, J., & García-Carmona, F. (2009). The role of phenolic hydroxy groups in the free radical scavenging activity of betalains. *Journal of Natural Products, 72*, 1142–1146.

Gandía-Herrero, F., Escribano, J., & García-Carmona, F. (2010). Structural implications on color, fluorescence, and antiradical activity in betalains. *Planta, 232*, 449–460.

Gandía-Herrero, F., Escribano, J., & García-Carmona, F. (2016). Biological activities of plant pigments betalains. *Critical Reviews in Food Science and Nutrition, 56*, 937–945.

Gandía-Herrero, F., & García-Carmona, F. (2013). Biosynthesis of betalains: Yellow and violet plant pigments. *Trends in Plant Science, 18*, 334–343.

Gandía-Herrero, F., García-Carmona, F., & Escribano, J. (2005a). A novel method using high-performance liquid chromatography with fluorescence detection for the determination of betaxanthins. *Journal of Chromatography A, 1078*, 83–89.

Gandía-Herrero, F., García-Carmona, F., & Escribano, J. (2005b). Floral fluorescence effect. *Nature, 437*. 334-334.

Gandía-Herrero, F., García-Carmona, F., & Escribano, J. (2006). Development of a protocol for the semi-synthesis and purification of betaxanthins. *Phytochemical Analysis, 17*, 262–269.

García-García, M. I., Hernández-García, S., Sánchez-Ferrer, A., & García-Carmona, F. (2013). Kinetic study of hydroxytyrosol oxidation and its related compounds by red globe grape polyphenol oxidase. *Journal of Agricultural and Food Chemistry, 61*, 6050–6055.

García-Plazaola, J. I., Fernández-Marín, B., Duke, S. O., Hernández, A., López-Arbeloa, F., & Becerril, J. M. (2015). Autofluorescence: Biological functions and technical applications. *Plant Science, 236*, 136–145.

Gliszczyńska-Świgło, A., Szymusiak, H., & Malinowska, P. (2006). Betanin, the main pigment of red beet: Molecular origin of its exceptionally high free radical-scavenging activity. *Food Additives & Contaminants, 23*, 1079–1087.

Gómez-Caravaca, A. M., Segura-Carretero, A., Fernández-Gutiérrez, A., & Caboni, M. F. (2011). Simultaneous determination of phenolic compounds and saponins in quinoa (*Chenopodium quinoa* Willd) by a liquid chromatography-diode array detection-electrospray ionization-time-of-flight mass spectrometry methodology. *Journal of Agricultural and Food Chemistry, 59*, 10815–10825.

Gómez-Pando, L. R., Álvarez-Castro, R., & Eguiluz-de la Barra, A. (2010). Effect of salt stress on Peruvian germplasm of *Chenopodium quinoa* Willd.: A promising crop. *Journal of Agronomy and Crop Science, 196*, 391–396.

Gonçalves, L. C. P., Tonelli, R. R., Bagnaresi, P., Mortara, R. A., Ferreira, A. G., et al. (2013). A nature-inspired betalainic probe for live-cell imaging of *Plasmodium*-infected erythrocytes. *PLoS One, 8*, e53874.

Guadarrama-Flores, B., Rodríguez-Monroy, M., Cruz-Sosa, F., García-Carmona, F., & Gandía-Herrero, F. (2015). Production of dihydroxylated betalains and dopamine in cell suspension cultures of *Celosia argentea* var. plumosa. *Journal of Agricultural and Food Chemistry, 63*, 2741–2749.

Impellizzeri, G., Piattelli, M., & Sciuto, S. (1973). A new betaxanthin from *Glottiphyllum longum*. *Phytochemistry, 12*, 2293–2294.

Khan, M. I., Harsha, P. S. C. S., Giridhar, P., & Ravishankar, G. A. (2012). Pigment identification, nutritional composition, bioactivity, and in vitro cancer cell cytotoxicity of *Rivina humilis* L. berries, potential source of betalains. *LWT - Food Science and Technology, 47*, 315–323.

Koziol, M. J. (1991). Afrosimetric estimation of threshold saponin concentration for bitterness in quinoa (*Chenopodium quinoa* Willd). *Journal of the Science of Food and Agriculture, 54*, 211–219.

Liu, Q., Liu, H., Zhang, L., Guo, T., Wang, P., Geng, M., et al. (2013). Synthesis and antitumor activities of naturally occurring oleanolic acid triterpenoid saponins and their derivatives. *European Journal of Medicinal Chemistry, 64*, 1–15.

Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry, 49*, 4619–4626.

Pellegrini, N., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S., Bianchi, M., et al. (2003). Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *Journal of Nutrition, 133*, 2812–2819.

Piattelli, M., Minale, L., & Nicolaus, R. A. (1965). Betaxanthins from *Mirabilis jalapa* L.. *Phytochemistry, 4*, 817–823.

Rao, S., & Ostroverkhova, O. (2015). Visual outdoor response of multiple wild bee species: Highly selective stimulation of a single photoreceptor type by sunlight–induced fluorescence. *Journal of Comparative Physiology A, 201*, 705–716.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine, 26*, 1231–1237.

Tang, Y., Li, X., Zhang, B., Chen, P. X., Liu, R., & Tsao, R. (2015). Characterisation of phenolics, betanins and antioxidant activities in seeds of three *Chenopodium quinoa* Willd. genotypes. *Food Chemistry, 166*, 380–388.

Trezzini, G. F., & Zrÿd, J.-P. (1991). Characterization of some natural and semi-synthetic betaxanthins. *Phytochemistry, 30*, 1901–1904.

Yao, Y., Yang, X., Shi, Z., & Ren, G. (2014). Anti-inflammatory activity of saponins from quinoa (*Chenopodium quinoa* Willd.) seeds in lipopolysaccharide-stimulated RAW 264.7 macrophages cells. *Journal of Food Science, 79*, 1018–1023.