Supplementary Material

Multifactorial characterization of quinoa varieties based on chemical, functional, and morphological parameters

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Materials and Methods

Sample preparation

Quinoa seeds were ground and sieved through a 500 μm mesh to obtain a fine flour. Then, samples were stored in airtight containers at room temperature until analysis.

Nutritional Composition

Composition analysis was carried out in accordance with the Official Methods of Analysis of the Association of Official Analytical Chemists [1].

Moisture content was determined by gravimetric method, using a dry air oven (MCH Modelo SL30S, Argentina), at 100± 2 °C during 4 h, until constant weight (AOAC 952.08) [1]. Total ash analysis was carried out in a muffle furnace, (332 model, Indef, Argentina), at 550 ± 5 °C for 240 min, according to AOAC 923.03 [1].

Total fat determination was performed by extraction using a Soxhlet apparatus (J.P. SELECTA, S.A. Spain) for 1 h 30 min with petroleum ether (40–60 °C), as the extraction solvent [2].

Total nitrogen was analyzed by the Kjeldahl method (AOAC 991.20) [1] with a copper catalyst using a block digestion system (Gerhardt TTA Turbotherm Block Digester - Gemini BV, Germany) and a Distillation unit (K-355, Buchi). The protein content was calculated by using the 6.25 factor.

The content of total dietary fiber (TDF) was measured by the enzymatic–gravimetric method (AOAC, 985.29) [1]. This method quantifies soluble, insoluble, and total dietary fiber. Briefly, 1 g dried food sample (in duplicate) is subjected to sequential enzymatic digestion with heat-stable α-amylase, protease and amyloglucosidase. One residue from each fiber type is analyzed for protein, while the second residue from the duplicate is analyzed for ash. A duplicate blank assay was conducted using the same procedure as the digested samples (AOAC 991.43) [1].

Analyses were performed in triplicate, except for fiber content. Results are expressed as mean ± standard deviation.

Techno-functional properties

The water absorption index (WAI), water solubility index (WSI), and swelling power (SP) of various quinoa flour were determined following the method informed by Cornejo and Rosell [3], with slight modification. Briefly, a flour sample (1.00 g ± 0.01 mg) was dispersed in 10 mL of distilled water and heated at 90 °C for 15 min in a water bath. After heating, the paste was cooled to room temperature and centrifuged at 3000 g at 4°C for 10 minutes (Hanil, COMBI-514R model, Korea). The supernatant was decanted for solid content analysis into an evaporating dish, while the sediment was weighed. The dry solids recovered by evaporating the supernatant overnight at 100 °C were measured.

Five replicates were made for each sample. WSI, WAI and SP were calculated by the Equations (1-3):

WAI (g/g) = Weight of sediment/Sample weight (Eq.1)

WSI (g/g) =100*Weight of dissolved solids in supernatant/Sample weight (Eq.2)

SP (g/g) =Weight of sediment/(Sample weight - Weight of dissolved solids in supernatant) (Eq. 3)

Antioxidant content and activity

Antioxidant compounds from quinoa seeds were extracted using the Park et al. procedure [4], with some modifications. Five grams of ground quinoa seeds were mixed with 50 mL of 80% (v/v) ethanol. Total polyphenol contents in samples were determined by using the Folin–Ciocalteu method and expressed as gallic acid equivalents in mg/g sample [5]. Total flavonoid content was determined using the aluminum chloride colorimetric method at λ= 765 nm and expressed as quercetin equivalents in mg /g sample.

The antiradical activity was determined by the methods of free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) and acid 2,2'-azino-bis (3-etilbenzotiazolina-6-sulfónico (ABTS) at λ = 517 and 734 nm, respectively. DPPH antiradical activity was expressed as IC50, defined as the concentration of sample (mg/mL) required inhibiting 50% of the DPPH radicals.

Ferric-Reducing Antioxidant Power (FRAP) Assay was carried out to evaluate the total antioxidant capacity. Reaction was monitored at λ = 593 nm.

FRAP and ABTS results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC). All determinations were performed in triplicate.

Digital analysis

Quinoa seeds were analyzed using a combined approach of epifluorescence microscopy and digital image processing. Structural observations were conducted using a Confocal Laser Scanning Microscope (CLSM) Zeiss LSM 980 (Carl Zeiss, Germany), equipped

with a DAPI fluorescence filter (excitation: 335–383 nm; emission: 420–4095 nm) and a 2.5× objective, selected according to the optical characteristics of the plant tissues. Image capture was performed using the Axiocam 712 camera.

Images were subsequently digitized and processed using Fiji software (version 2.9.0, USA), an open-source platform widely validated for morphological analyses in plant studies [6].

Image segmentation was performed using the Red-Green-Blue (RGB) color model, followed by conversion to gray scale to facilitate the separation of seeds (objects) from the background. Thresholding was consistently applied across all samples to ensure uniformity in the analysis. Under controlled lighting conditions, pixel values corresponding to seeds exhibited gray scale intensities below 230, while the background exceeded this threshold, allowing for clear and efficient object discrimination.

Basic measurements included area (mm^2), perimeter (mm), Feret diameter (mm), and minimum Feret diameter (mm). Derived morphometric descriptors, such as circularity (C), roundness (R), solidity (S), and aspect ratio (AR) were also calculated. These parameters allowed for precise characterization of the shape, symmetry, and compactness of quinoa seeds.

Color determination

The Color of flour samples was determined with a ColorQuest XE spectrophotometer (Hunter Associates Laboratory, Inc.). Color characteristics were also analyzed using the CIE L* a* b* method. The L* component, luminance or lightness, range from 0 to 100, while the two colour coordinates a* and b* range from green to red and from blue to yellow, respectively. The instrument was calibrated using the white and black standards supplied with the equipment. Analyses of flour samples were performed in quintuplicate.

Statistical analysis

Means and standard deviations were calculated for all measured parameters. Differences between samples were assessed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test ($p < 0.05$). Pearson correlation coefficients were calculated to evaluate relationships between variables. Additionally, principal component analysis (PCA) and cluster analysis were used as exploratory tools to identify associations and differentiation patterns among quinoa samples. All analyses were performed using XLStat software (Copyright © 2018Addinsoft).

References

1. AOAC (2000) Official Methods of Analysis of AOAC International. AOAC, Arlington, VA, USA
2. Nascimento AC, Mota C, Coelho I, Gueifão S, Santos M, Matos AS, Castanheira I (2014) Characterisation of nutrient profile of quinoa (Chenopodium quinoa), amaranth (Amaranthus caudatus), and purple corn (Zea mays L.) consumed in the North of Argentina: Proximates, minerals and trace elements. Food Chem 148:420–6. https://doi.org/10.1016/j.foodchem.2013.10.133
3. Cornejo F, Rosell CM (2015) Physicochemical properties of long rice grain varieties in relation to gluten free bread quality. LWT Food Sci Technol 62(2):1203–10. https://doi.org/10.1016/j.lwt.2014.10.040
4. Park JH, Lee YJ, Kim YH, Yoon KS (2017) Antioxidant and antimicrobial activities of quinoa (Chenopodium quinoa Willd.) seeds cultivated in Korea. Prev Nutr Food Sci 22(3):195–202. https://doi.org/10.3746/pnf.2017.22.3.195

5. Rios F, Lobo M, Samman N (2018) Acceptability of beehive products as ingredients in quinoa bars. J Sci Food Agric 98(1):174–82. https://doi.org/10.1002/jsfa.8454

6. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al (2012) Fiji: an open-source platform for biological-image analysis. Nat Methods 9:676–682. https://doi.org/10.1038/nmeth.201

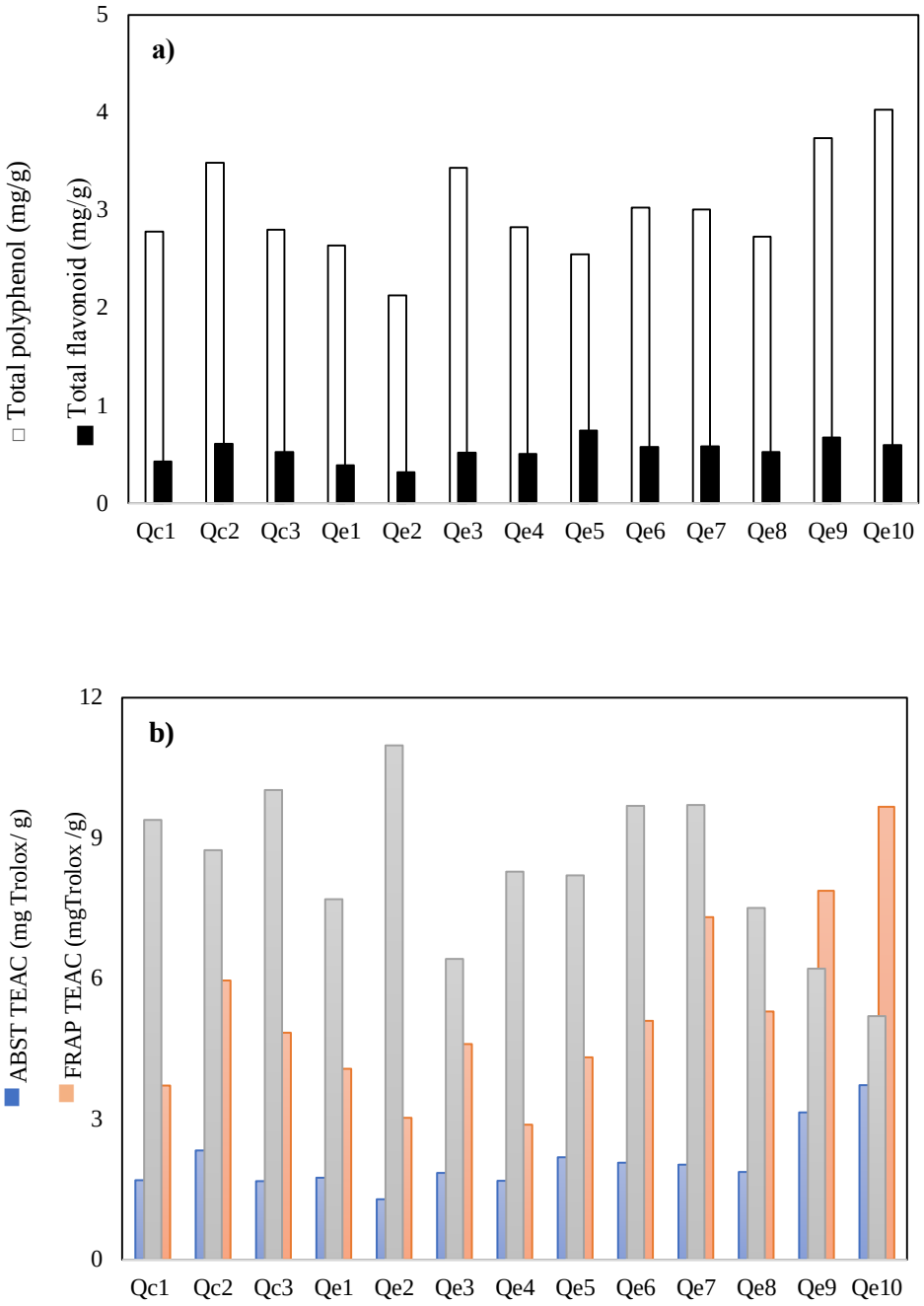

Figure 1. a) Total polyphenol and flavonoid content in quinoa samples. b) TEAC values determined for quinoa samples using FRAP, ABTS and DPPH radical scavenging assays.