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Comparative Evaluation of Phenolic and Antioxidant Properties of Red and White Quinoa (*Chenopodium quinoa* Willd.) Seeds

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Abstract

Quinoa seed has obtained a great interest in recent years due to its high nutritional value and phytochemical content including natural antioxidants with beneficial biological activities. The present study was designed to determine the phenolic compounds and antioxidant properties of red and white quinoa seeds. High-performance liquid chromatography/electrospray ionization tandem mass spectrometry (LC–DAD-ESI–MS/MS) method was used for the analysis of the phenolic compounds. Twenty-two phenolic compounds were identified and quantified in the quinoa cultivars. The total concentrations of phenolic compounds of red quinoa was higher than that of the white cultivar. Ferulic acid 4-glucoside, vanillic acid, ferulic acid, quercetin 3-rutinoside and quercetin 3- arabinoside were the most abundant phenolics in both cultivars. Antioxidant activities of quinoa were also measured using the ABTS and DPPH assays, and the data obtained were in agreement with total concentrations of phenolics.

Keywords: *Quinoa, Chenopodium quinoa, phenolic compounds, antioxidant capacity, DPPH, ABTS.*

1. INTRODUCTION

Quinoa (*Chenopodium quinoa*) is one of the seeds considered as pseudocereals; it is a broadleaf plant that has been used like the cereals. It, botanically, belongs to the class Dicotyledoneae, family *Chenopodiaceae*, genus Chenopodium, and species quinoa. The full name is *Chenopodium quinoa* Willd. Quinoa is one of a species native of South America and was domesticated for thousands of years by the people living the Andes, mostly in Peru, Chile and Bolivia. The plant produces flat, oval-shaped seeds that are usually the outer layer of the fruit, i.e. pericarp which maybe translucent or highly colored (e.g. white, yellow, orange, pink, red, brown, grey or black) (James, 2009). Quinoa is a very valuable grain due to its complete nutritional properties both as a human food and as an animal feedstuff. It is rich in essential amino acid, proteins, lipids, polyphenols, dietary fibers, minerals (iron, magnesium, copper, phosphorus, potassium, and zinc) and vitamins (including E and B vitamins). In addition to presenting high nutritional characteristics, quinoa is also described by being gluten-free cereal, a type that enables greater offer and variety of more nutritious and appropriate food products for patients who suffer from celiac disease (Filho et al., 2017; Repo-Carrasco et al., 2003).

Phenolic compounds are bioactive secondary metabolites that are extensively present in commonly consumed food products of plant based origin. The three principal types of these compounds are flavonoids, phenolic acids and tannins (Repo-Carrasco et al., 2010). Quinoa is rich in phenolic compounds that are potentially responsible for a wide range of biological and

physiological properties such as powerful antimicrobial, antioxidant, anti-inflammatory, antitumor and anti-carcinogenic effects. Major phenolic compounds in quinoa include vanillic acid, ferulic acid, rutin, their derivatives and certain flavonoids such as kaempferol and quercetin (Hirose et al., 2010; Tang et al., 2015). Published papers on the polyphenol composition of the pseudocereals like quinoa are limited; however, substantially more has been reported on the antioxidant properties and high performance liquid chromatography (HPLC) determination of phenolic compounds. Zhu et al. (2001) have identified six flavonol glycosides from quinoa seed samples; these compounds exhibited antioxidant capacity, suggesting that samples can serve as a powerful source of free radical scavenging agents. Hemalatha et al. (2016) elucidated the distribution of phenolic components in different milled fractions and whole grain quinoa (*Chenopodium quinoa* Willd) in relation to their antioxidant properties and inhibitory properties against α-amylase enzyme. In this paper, HPLC–DAD showed that the distribution of phenolic compounds in quinoa was not completely localized in the outer layers of the kernel. Authors reported that extracts of bran and hull fractions displayed strong inhibition towards α-amylase [IC50, 108.68 µg/ml (bran) and 148.23 µg/ml (hulls)] and α-glucosidase [IC50, 62.1 µg/ml (bran) and 68.14 µg/ml (hulls)]. Han et al. (2019) investigated the effect of milling degree of quinoa seeds on the content of saponins, free and bound phenolic compounds, and their antioxidant activity evaluated through oxygen radical absorbance capacity and ferric reducing power. Authors found that the contents of phenolics including both free and bound forms decreased significantly with increasing degree of milling; gallic and ferulic acids were determined to be the principal compounds present in the free and bound forms, respectively.

The scientific literature contains several reviews that summarizes the different information about chemistry, composition, functional and nutritional properties of quinoa as well as the antioxidant properties of quinoa seeds. Thus, the aim of this work was to elucidate the antioxidant properties and phenolic compounds of white and red quinoa samples using DPPH-ABTS assays and LC-DAD-ESI-MS.

2. MATERIALS AND METHODS

2.1. Chemicals

The water used in all analyses of this study had been previously deionized (resistivity about 18.2 MΩ cm) by a Millipore Q (Millipore Corp., Saint-Quentin, France) water purification system. Caffeic acid, vanillic acid, epicatechin, epigallocatechin, p-coumaric acid, ferulic acid, quercetin, isoferulic acid, quercetin 3-rutinoside, kaempferol 3-glucoside, kaempferol were obtained from Sigma-Aldrich (Steinheim, Germany). Moreover, ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH (2,2-Diphenyl-1-picrylhydrazyl) reagents for antioxidant analysis were purchased from Merck (Darmstadt, Germany).

2.2. Preparation of quinoa samples

Quinoa seeds were purchased from a local market in 2019 in Adana. The material (500g) was ground in Retsch ZM 200 grinder before analysis. The ground material was then passed through a standard 20-mesh sieve (particle size <0.825 mm). The analytical conditions were based on those described by Tang et al. (2015).

2.3. Extraction and analysis of phenolics by LC-DAD-ESI-MS/MS

The phenolic extraction of quinoa samples were carried out with 100 mL of 80 % ethanol-water mixture (v/v) using a magnetic stirrer (Isolab, Istanbul – Turkey) for 2 hours. The decision of a suitable solvent was made with respect to an earlier optimization study performed on red peppers with different solvents (Kelebek, 2016). The aqueous phase was subjected to a 0.45µm membrane filter and directly injected into a HPLC

system (Agilent 1100, Agilent Technologies, Palo Alto, CA, USA) connected with the software system Windows NT-based ChemStation. The LC-MS system utilized in the present work consisted of a binary pump, degasser and auto sampler. A Phenomenex reversed-phase C-18 column (4.6 mm × 250 mm, 5 μm) was used (Torrance, CA, USA) combined with a diode array detector (DAD). During the elucidation of the phenolics, two different solvents: water/formic acid (99:1; v/v) as solvent A and acetonitrile/solvent A (60:40; v/v) as solvent B were used (Kelebek, 2016; Keser et al. 2020). Phenolic compounds were eluted under the following conditions: setting to 0.5 mL/min flow rate at 25 °C; isocratic conditions from 0 to 5 min with 0% B; gradient conditions for all the following steps: from 0% to 5% B in 20 min; from 5% to 15% B in 18 min; from 15% to 25% B in 14 min; from 25% to 50% B in 31 min; from 50% to 100% B in 3 min; followed by washing and reconditioning of the column (Kelebek et al., 2019). All peaks were detected in the UV-VIS (ultra-violet-visible) spectra (between 200 and 600 nm). In order to identify and assign which phenolic compounds existed in the extracts, the retention times and UV spectra to authentic standards were compared and subsequently approved by an Agilent 6430 LC-MS/MS spectrometer having an electrospray ionization source. The negative ionization mode was performed with the following optimized parameters: capillary temperature 400 °C, N2 12 L/min nebulizer pressure, 45 psi electrospray ionization mass spectrometry detection. Each phenolic compound was quantified by using calibration curves of the standard phenolic compounds. The standard curves were acquired utilizing the commercial standards at concentrations normally exist in extracts (nearly 1–100 mg/L) and getting regression coefficients (r2) above 0.995 in all cases. In the absence of reference compounds, the calibration of structurally related substances was used, taking into account the molecular weight correction factor. The concentrations were provided as a mean of three repetitions. The limit of quantification (LOQ) and the limit of detection (LOD) were calculated via signal-to-noise ratios (S/N) of 10 and 3, respectively (Kelebek et al., 2019; Sen and Sonmezdag, 2020).

2.4. Antioxidant assays

2.4.1. DPPH assay:

An aliquot of 0.1 mL of diluted extracts was added to 3.9 mL of DPPH solution in methanol (6×10−5 M). The mixture was shaken vigorously and left standing at room temperature for 30 minutes. The absorbance of the resulting solution was then measured at 515 nm by a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, California, USA) (Kelebek et al., 2020).

2.4.2. ABTS assay:

The ABTS radical cation was prepared by the reaction of 7 mM ABTS with 2.54 mM potassium persulfate, after incubation at room temperature for 12-16 hours. Prior to the assay, the ABTS solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. A total of 3.9 mL of the diluted ABTS solution was added to 0.1 mL of each sample. The reaction mixture was allowed to stand at room temperature for 30 minutes and the absorbance at 734 nm was immediately recorded (Zlotek et al., 2019).

3. RESULTS AND DISCUSSION

The data revealed by LC-DAD-ESI-MS/MS concerning the phenolic profiles of white and red quinoa cultivars are given in Table 1. The chromatograms of some identified compounds and total ion chromatograms are presented in Figure 1. In the current work, a total of 22 phenolic compounds were identified and quantified. It was observed that the phenolic profiles of both cultivars were quite similar. However, the total concentration of identified compounds

Table 1. Phenolic compounds identified in quinoa samples by LC-ESI-MS/MS, including: retention time (rt), molecular ion [M-H], main fragment ions (MS2), and tentative identification (mg/kg)

Peak	Phenolic compounds	UV max (nm)	Molecular weight	$[M-H]^-$ (m/z)	MS^2 (m/z)	White Quinoa	Red Quinoa
1	3,4-Dihydroxybenzoic acid	260, 295	154	153	109	1,27±0,01	34,78±0,31
2	*p*-Coumaric acid 4-glucoside	280, 320	326	325	163	0,89±0,01	30,38±0,27
3	*p*-Hydroxybenzoic acid	260, 300	138	137	113	16,32±0,15	18,34±0,16
4	Vanillic acid 4-glucoside	262, 290	330	329	167	18,56±0,17	26,03±0,23
5	2,5-Dihydroxybenzoic acid	260, 295	154	153	153	0,41±0,00	0,86±0,01
6	Caffeic acid	270	180	179	135	4,93±0,04	21,00±0,19
7	Vanillic acid	260, 295	168	167	151	51,76±0,46	67,47±0,60
8	Epigallocatechin	279	305	304	233	2,67±0,02	3,58±0,03
9	Epicatechin	279	290	289	245	3,95±0,04	4,37±0,04
10	Vanillin	262, 290	152	151	136	6,60±0,06	7,49±0,07
11	*p*-Coumaric acid	320	164	163	119	21,47±0,19	28,30±0,25
12	Ferulic acid	330, 290	194	193	149	47,39±0,42	65,54±0,58
13	Ferulic acid 4-glucoside	330, 290	356	355	193	125,92±1,12	161,79±1,44
14	Isoferulic acid	330, 290	194	193	149	12,62±0,11	15,14±0,13
15	Kaempferol 3,7-dirhamnoside	260, 355	578	577	285	25,93±0,23	28,97±0,26
16	Kaempferol 3-galactoside	256, 355	448	447	285	28,52±0,25	32,37±0,29
17	Quercetin-3-rutinoside	256, 355	610	609	301	41,19±0,37	56,59±0,50
18	Kaempferol 3-glucoside	256, 355	448	447	285	15,36±0,14	23,42±0,21
19	Quercetin 3-arabinoside	266, 350	434	433	301, 179	25,81±0,23	44,34±0,39
20	Quercetin	260, 365	302	301	151	11,98±0,11	14,65±0,13
21	Kaempferol	265, 365	286	285	193	1,24±0,01	3,36±0,03
22	Biochanin A	262	284	283	283	0,65±0,01	5,36±0,05
	Total Phenolics					465,43±4,14	694,13±6,18
	Antioxidant analysis						
	DPPH (µmol Trolox/g)					1,38±0,22	2,57±0,52
	ABTS (µmol Trolox/g)					2,07±0,16	2,88±0,12

in red quinoa (694,13 mg/kg) was clearly higher than the white (465,43 mg/kg) cultivar. The profiles of phenolic compounds coincide with the previous report on the identification of phenolics in *Chenopodium quinoa* Willd. genotypes made by Tang et al. (2015). In that study, an increase of the total phenolic contents was reported with colour intensity at 467, 635 and 682 mg/kg, respectively in the white, red and black quinoa.

Ferulic acid 4-glucoside (Peak 13, [M – H]- ion at m/z 193 in negative ionization mode) was the most dominant compound in red and white quinoa cultivars, as it accounted for the largest proportion of the total phenolic compounds (Table 1) in agreement with the literature (Tang et al. 2015). The total amount of ferulic acid 4-glucoside was between 125.92 and 161.79 mg/kg in white and red quinoa cultivars, respectively. Based on the similarity of UV data to ferulic acid and its MS and MS/MS daughter ions, it is confirmed to be ferulic acid-glucoside (Gómez-Caravaca et al., 2011). Peak 7 (m/z 167) was confirmed to be vanillic acid and it was one of the major phenolic compounds common to both cultivars. Red quinoa cultivar had a slightly greater amount of this compounds compared to white cultivar (Table 1). Peak 17 was identified as quercetin-3-rutinoside ([M – H]- ion at m/z 609, MS at 301, 179 and 151) by co-elution with an authentic standard. The concentration of peak 17

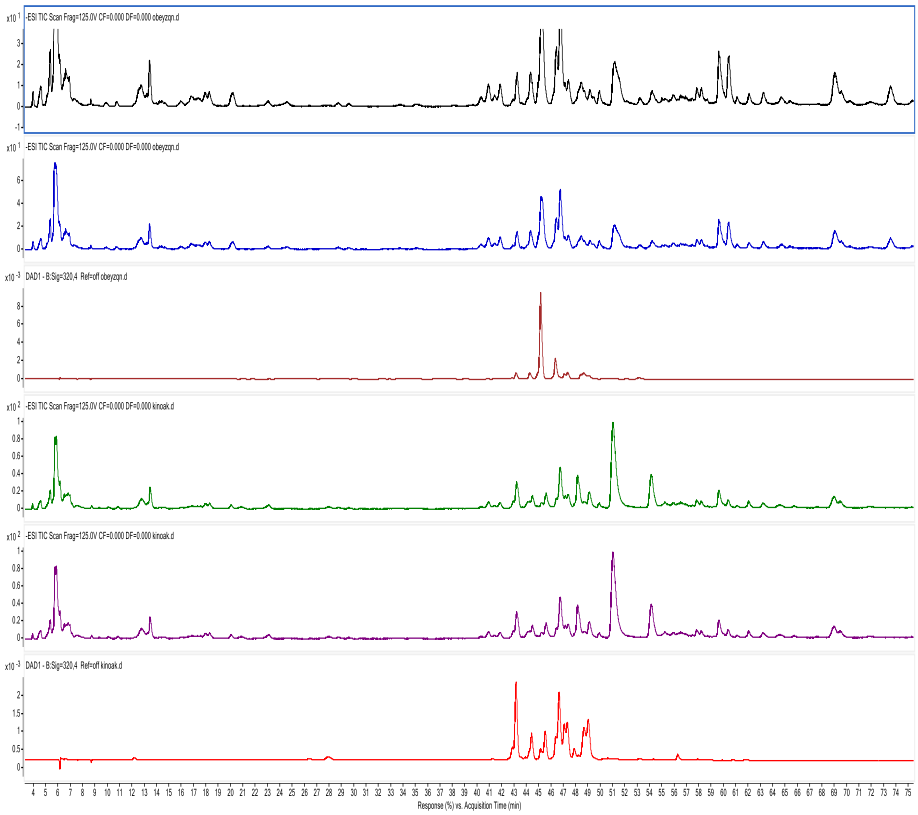

Figure 1. LC-DAD and LC-ESI-MS/MS chromatograms of some of phenolic compounds identified in quinoa cultivars.

was higher in red quinoa (56.59 mg/kg) than white quinoa (41.19 mg/kg). The flavonoid quercetin-3-rutinoside (rutin) is a flavonol glycoside comprised of the flavonol quercetin and the disaccharide rutinoside. This plant-derived phenolic pigment has some established pharmacological effects thanks to its antioxidant and anti-inflammatory properties, as well as cytoprotective actions connected with anti-aging and anti-cancer properties. Therefore, it has been used clinically as a therapeutic medicine (La Casa, Villegas, Alarcon, Motilva, & Martin Calero, 2000). Four peaks were identified as kaempferol derivatives according to their UV spectra and MS fragmentation leading to the kaempferol aglycone at m/z 285 in negative mode. The flavonols identified in this manner were kaempferol 3,7-dirhamnoside (Peak 15), kaempferol 3-galactoside (Peak 16), kaempferol 3-glucoside, (Peak 18), and kaempferol (Peak 21). Within the kaempferol derivatives, the major peak detected in the quinoa cultivars was kaempferol 3-galactoside ([M-H]- at m/z 447). The molecular ion fragmentation yielded fragment ions corresponding to this peak following the loss of the rhamnosyl moiety (146 amu), and finally to kaempferol (m/z 285) after losing a hexose moiety (162 amu). Peak 18 had [M-H]- at m/z 447 with fragment at m/z 285 (loss 162 amu, hexose moiety), and was identified as kaempferol-3-O-glucoside. Peaks 16 and 18 were also confirmed by comparison of their absorbance spectrums and retention times with those of an authentic standards. As can be seen in Table 1, Peak 16 has the same MS spectra as Peak 18. Galactosides elute earlier than corresponding glucosides, and a kaempferol 3-galactoside was detected this way (Del Rio et al., 2004; Kelebek 2016).

The antioxidant capacity of red quinoa was clearly greater than the white quinoa. The phenolic contents and antioxidant capacity as measured by DPPH and ABTS assays were compared. Table 1 presents the results of the antioxidant activities obtained by the quinoa cultivars. It was previously reported that the antioxidant activity of the black quinoa grain samples was approximately 5.6 μM

Trolox/g, while for the white and red samples it was between 2.0 and 12.0 μM Trolox/g (Tang et al. 2015), respectively. Our results showed a similar trend, being the coloured grains with the highest antioxidant activities. However, an unidentified Bolivian quinoa sample presented 38.84 μM Trolox/g (Paśko et al. 2009), a value much higher than ours.

4. CONCLUSIONS

Our results showed that the majority of the extractable phenolics were in conjugated forms. The red quinoa cultivars had higher phenolic concentration and antioxidant activity than the white quinoa. Further analysis of the individual phenolic compounds revealed that at least 22 phenolic compounds were found in both cultivars, among them ferulic acid 4-glucoside and vanillic acid and ferulic acid were the main phenolic acids, and quercetin 3-rutinoside, quercetin-3-arabinoside and kaempferol-3-glucosides the main flavonoids. It was observed that the phenolic profiles of both cultivars were similar. However, the total phenolic content in red quinoa was higher than the white quinoa cultivar. The antioxidant capacity of red quinoa was found higher than the white quinoa.

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