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Research Article

Antioxidant Activity, Total Phenolic and Saponin Contents of Quinoa Seeds Having Different Hull Colors as Affected by Washing Process

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) has recently gained more interest due to its high nutritional value such as high protein quality, high phenolic and dietary fiber contents. However, saponins present in quinoa negatively impact its nutritional and sensory attributes. The aim of this study was to investigate the effects of washing process, which is commonly used to eliminate the undesired effects of saponins, on phenolic and saponin contents, and antioxidant capacities of different colored quinoa seeds. Our results show that red (55.02 mg GAE/100 g) and black (53.25 mg GAE/100 g) quinoa seeds had significantly higher total phenolic contents than their white (46.27 mg GAE/100 g) counterparts ($p<0.05$). Generally, red quinoa exhibited higher antioxidant capacity, compared to black and white seeds. The washing step caused a significant reduction in phenolic contents and antioxidant activities of all quinoa types ($p<0.05$), while the reduction in saponin contents was insignificant ($p>0.05$). Moreover, the disc diffusion assay indicated that quinoa seeds have no inhibitory activity against the tested bacteria. Consequently, red and black colored quinoa seeds have better functional properties than white counterparts. Herewith, development of improved washing methods for more efficient removal of saponin from quinoa seeds while protecting its bioactive compounds is advised.

Keywords: Red quinoa, black quinoa, white quinoa, DPPH, ABTS, antimicrobial, bioactivity

Farklı kabuk renklerine sahip kinoa tohumlarının toplam fenolik ve saponin içerikleri ile antioksidan aktiviteleri üzerine yıkama işleminin etkisi

Öz

Kinoa (*Chenopodium quinoa* Willd.), yüksek protein kalitesi, iyi bir fenolik ve lif kaynağı olmasına bağlı olarak yüksek besin değeri nedeniyle son zamanlarda daha fazla ilgi kazanmıştır. Ancak, kinoa tohumlarında bulunan saponinler kinoanın besin değerini ve duyusal özelliklerini olumsuz yönde etkilemektedir. Bu çalışmanın amacı, genellikle saponinleri uzaklaştırmak için uygulanan yıkama işleminin, farklı renkteki kinoa tohumlarının toplam fenolik madde ve saponin içerikleri ile antioksidan kapasiteye olan etkilerini belirlemektir. Elde edilen sonuçlar kırmızı (55,02 mg GAE/100 g) ve siyah (53,25 mg GAE/100 g) kinoa tohumlarının fenolik içeriklerinin beyaz olanlara (46,27 mg GAE/100 g) kıyasla anlamlı derecede yüksek olduğunu göstermiştir ($p<0,05$). Genel olarak, kırmızı renkli kinoa tohumlarının, siyah ve beyaz muadillerinden daha yüksek antioksidan aktiviteye sahip olduğu belirlenmiştir. Yıkama işlemi tüm kinoa çeşitlerinde fenolik madde içeriğinde ve antioksidan aktivitede önemli derecede azalmaya neden olurken ($p<0,05$), saponin içeriklerindeki azalmanın önemsiz olduğu görülmüştür ($p>0,05$). Ayrıca, disk difüzyon yöntemi, farklı renklerdeki kinoaların test edilen bakterilere karşı herhangi bir inhibisyon göstermediğini ortaya koymuştur. Sonuç olarak, beyaza kıyasla kırmızı ve siyah renkli kinoa tohumlarının daha iyi fonksiyonel özelliklere sahip olduğu belirlenmiştir. Bu bağlamda, kinoa tohumlarındaki mevcut biyoaktif bileşiklere zarar vermeksizin saponinin daha verimli bir şekilde uzaklaştırılabileceği ileri yıkama usullerinin geliştirilmesi tavsiye edilmektedir.

Anahtar Kelimeler: Kırmızı kinoa, siyah kinoa, beyaz kinoa, DPPH, ABTS, antimikrobiyal, biyoaktivite

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1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a pseudo-cereal originated from Andean region of South America 5000 years back. Incas considered quinoa as sacred and the "mother grain" due to its exceptional nutritional quality (Satheesh and Fanta, 2018). Quinoa came into prominence being a healthy and nutritious alternative product at a moment when the population constantly increases on the one hand and the productivity in agriculture decreases due to the changing climate conditions and global warming on the other. However, until the year of 2013, when was proclaimed as "the international year of quinoa" by Food and Agriculture Organization (FAO), quinoa was regarded as one of the "orphan" crops ironically (Arneja et al., 2015). Thereafter, quinoa has been cultivated in many countries, including Turkey, due to its high adaptability to a wide variety of climates, habitats, and stress conditions such as acidity, salinity, drought, and frost (Nowak et al., 2016; Satheesh and Fanta, 2018). However, almost all of the annual production of 150.000 tons in 2017 was supplied by only three countries; Peru, Bolivia, and Ecuador (Anonymous, 2017). Quinoa seeds have a flat surface and oval-circular shape with an average diameter of 1.5 mm (Satheesh and Fanta, 2018). They are mostly white, also black and red, but available in a wide range of intermediate colors, which has been found to affect the bioactivity of quinoa seeds (Tang et al., 2015a). The effects of seed color on the phenolic content and antioxidant activity of quinoa were investigated in different studies (Brend et al, 2012; Escribano et al., 2017; Diaz-Valencia et al., 2018; Han et al., 2019b). Brend et al (2012) found that red quinoa possessed higher antioxidant activity with higher concentrations of phenolics and flavonoids, confirming the reports of Tang et al. (2015a) which shows that the darker the color of quinoa, the higher phenolic concentration, and antioxidant activity. Besides the advantages of colored quinoa, only white quinoa is cultivated in Turkey currently.

The protein content of quinoa ranges from 13.8 and 21.9%, depending on the color, origin, and year. Moreover, the protein quality of quinoa is high due to being mainly albumin and globulin type proteins which are found in casein (FAO, 2011). Unlike other cereals such as wheat and rice, quinoa is relatively rich in lysine and other sulfur-containing amino acids with a balanced amino acid content overall (Koziol, 1992). Quinoa is considered as a good source of phenolic compounds as well as of valuable fatty acids including oleic, linoleic (ω-6), and linolenic acid (ω-3). Starch forms the majority of the carbohydrate content (52-69% dry basis, db) of quinoa, while the dietary fiber content ranges between 7-9.7% (db); significant amounts [1.3-6.1% (db of quinoa)] of which compose of soluble ones (James, 2009). Besides being rich in dietary fiber, it is gluten-free; thereby being easy to digest. Quinoa has higher calcium, magnesium, and zinc contents, compared to common cereals such as wheat, corn, rice (FAO, 2011) and contains considerable quantities of vitamin B, especially Vitamin B_6 and folic acid (James, 2009). Contrary to its rich nutritional content, quinoa also contains several anti-nutritional factors in the hull; among which, saponin is considered as the most important one due to its presence at a relatively high concentration (up to 5%) (FAO, 2011). Based on its saponin content, quinoa has been classified as either sweet (<0.11%) or bitter (>0.11%) (Koziol, 1991). Saponins not only cause the formation of the bitter taste in quinoa but also reduce the absorption of some minerals, especially iron and zinc by forming complexes with them

(Ruales and Nair, 1992; Filho et al., 2017). Wet (washing or macerating in water) and dry methods (heating, extrusion, roasting, abrasion, milling), and indirect methods of fermentation, germination and breeding have been developed for removal of saponins from bitter varieties of quinoa; among which, wet methods are more extensively used due to their easy applicability (Reichert et al., 1986; Brady et al., 2007; Filho et al., 2017; Suárez-Estrella et al., 2018; Han et al., 2019a). On the other hand, excessive processes may lead to loss of vitamins and minerals such as iron, zinc, (Jancurova et al., 2009) and calcium (Konishi et al., 2004) and bioactive compounds; i.e. phenolics and flavanoids (Han et al., 2019a).

A number of studies available regarding the composition and bioactivity of white quinoa cultivated and sold in our country; however, to the best of our knowledge, there have been no studies conducted using red and black quinoa sold in the Turkish market. Moreover, it has not been elucidated yet whether washing process, which is generally done to remove saponins, impact the bioactive properties of such quinoa. Therefore, the aim of this study was to unveil the effects of washing step on the saponin content, total phenolic content, antioxidant and antimicrobial activities of white, red, and black quinoa seeds that are commercially available in Turkey.

2. Material and Methods

2.1. Quinoa samples

Commercial white (orginated from Peru), black (orginated from Colombia), and red quinoa (orginated from Peru) (Figure 1) packed in polypropylene bags were purchased from a supermarket (Ordu, Turkey). Quinoa samples were ground using a coffee grinder (SCM 2934, Sinbo, Istanbul, Turkey) to obtain powders (these were called natural quinoa). Washed quinoa samples were obtained by washing the quinoa seeds with distilled water, as previously described by Miranda et al. (2010) with the following modifications: 700 mL distilled water were added on 70 grams of quinoa seeds, which were then incubated at 60 ºC for 60 min under constant stirring at 700 rpm, followed by draining with a strainer. The drained samples were washed one-more time with 700 mL water. The samples were then dried using fan-oven at 60 ºC for 5 hours followed by grinding using a coffee grinder.

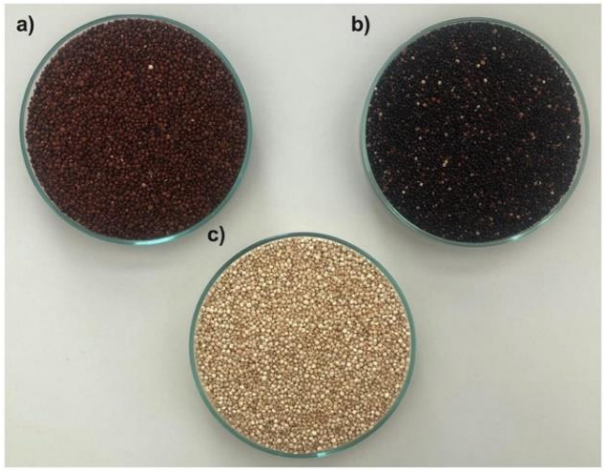

Figure 1. Quinoa seeds used in this study **a)** red quinoa, **b)** black quinoa, and **c)** white quinoa.

2.2. Proximate analysis

The moisture content was determined by drying the quinoa powders in a convection oven (Ecocell/EC 111, Germany) at 105 ± 2 ºC overnight. The protein contents of the samples were quantified by multiplying the nitrogen content with the conversion factor of 6.25 that was determined according to the Kjeldahl Method using an automatic distillation unit (UDK-149; VELP Scientifica, Italy). Ash contents were determined by weight difference after combustion of chia powders at 550 ºC in a furnace (Protherm

furnaces, Ankara, Turkey) for 14 hours (Tuncil and Celik, 2019). The total lipid content was determined by extracting the crude oil with hexane from dried quinoa powders using a solvent extractor system (SER 148; Velp Scientifica, Usmate, Italy) at 130 ºC for 150 minutes.

2.3. Extract preparation

For determination of the total phenolic contents, antioxidant activities, and antimicrobial capacities, the extracts of quinoa samples were prepared according to the method described by Pasko et al. (2009). Briefly, 4 grams of quinoa powder was dissolved in 40 mL of a solvent consisting of methanol, 0.16 M HCl and water at a ratio of 8:1:1, respectively (Solvent A). Samples were homogenized using a homogenizer (T18; IKA-Werke GmbH & Co., Staufen, Germany) in an ice water bath at 10,000 rpm for 10 minutes followed by mixing at 1,000 rpm on a stirrer (UC152D; Stuart, Cole-Parmer Instrument Co., Chicago, IL) at room temperature for 2 hours. The samples were then centrifuged at 6,000 rpm for 5 mins at 4 ºC and supernatants were collected. The remaining residue was extracted again with 40 mL of 70% acetone (Solvent B) for 2 hours. The initial methanolic extract was added to prepare a mixture, which was subsequently centrifuged at 6,000 rpm for 20 min at 4 ºC. The supernatant was collected and subsequently completed to 80 mL with the equal mixture of solvent A and solvent B.

2.4. Determination of the total phenolic content

The total phenolic content of quinoa extracts was determined using Folin–Ciocalteu reagent as previously described by Demirkol and Tarakci (2018), and Tunçil and Çelik (2019). Briefly, 20 µl of the extract was diluted with 1580 µl of distilled water, followed by addition of 100 µl of Folin–Ciocalteu reagent (2N) (Merck, Darmstadt, Germany) and 300 µl of Na2CO3 (Sigma-Aldrich Corp., St. Louis, MO, USA) solution (7.5%). The mixture was incubated for 2 hours at room temperature in the dark, followed by measuring the absorbance at 760 nm using a spectrophotometer (UVmini-1240, Shimadzu, Japan). Gallic acid (Sigma-Aldrich Corp.) was used as an external standard. The results were given as mg gallic acid equivalents (GAE) per gram of sample.

2.5. Determination of the antioxidant activity

Antioxidant activities of the samples were examined by determining their free radical scavenging activities using both 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays, as previously described by Tuncil and Celik (2019) and Celik et al., (2019), respectively. Briefly, various concentrations (0.5, 0.4, 0.3, 0.2, and 0.1 mg/mL) of the extracts were prepared with a solvent containing equal mixture of solvent A and solvent B. For DPPH analysis, 1 mL of DPPH solution (yielded an absorbance value of ~0.9 at 515 nm) was added on 50 µl of diluted extracts, followed by incubation for 30-minute at room temperature in the dark. Afterwards, the absorbance readings were recorded at 515 nm wavelength using a spectrophotometer (UVmini-1240, Shimadzu, Japan). For ABTS assay, 1 mL of ABTS solution (yielded an absorbance value of ~1.2 at 734 nm) were added on 50 µl of diluted extracts, followed by incubation for 6-minute at room temperature in the dark. Afterwards, the absorbance readings were recorded at 734 nm wavelength using a spectrophotometer. Trolox (Sigma-Aldrich

Corp.) was used to obtain the standard curve. The results were expressed as μmol Trolox equivalent (TE) per gram of sample.

The inhibition (%) values were also calculated using the following equation;

$$\% \text{ Inhibition} = [(A_0-A_1)/A_0] \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the samples tested.

A standard curve was plotted using the inhibition (%) values against different concentrations of quinoa extracts. The amount of extract (mg/mL) required for the inhibition of 50% of the DPPH (IC_{50}-DPPH) and of 50% of the ABTS (IC_{50}-ABTS) were calculated.

2.6. Saponin contents

Saponin contents of the samples were determined spectrophotometrically, as previously described by Nickel et al. (2016) with the following modifications. 10 mL of 50% ethanol was added on ~1.3 g of natural and washed quinoa samples, followed by mixing at 100 rpm (shaker) for 72 hours at room temperature. The samples were centrifuged at 6,000 rpm for 5 min at 4 ºC. The supernatant was collected and completed to 10 mL with 50% ethanol. One mL of diluted extract (1:2 dilution), or standard (Saponin, Sigma-Aldrich #47036), was mixed with 3.5 mL of Lieberman-Burchard reagent (16.7% of acetic anhydride in sulfuric acid concentred). The mixture was left to stand for 30 min at room temperature, followed by measuring in a spectrophotometer at 528 nm. Each analysis was duplicated, and the results were expressed as % of dry sample.

2.7. Antibacterial activity

Staphylococcus aureus NCTC 8530, *Escherichia coli* ATCC 8739, *Escherichia coli* BL 21, *Bacillus subtilis* NRRL-B209, and *Listeria monocytogenes* ATCC 7644, *Enterococcus faecalis* ATCC 19433, *Micrococcus luteus* NCIMB 8166, *Pseudomonas aureginosa* ATCC 9027, and *Bacillus spizizenii* ATCC 6633 were used as model bacteria to determine the antibacterial activity of the extracts of quinoa samples. All bacteria were cultivated in Tryptic Soy Broth (TSB; Merck, Darmstadt, Germany) at 37 °C and passaged in the same medium overnight prior to the assay. The concentrations of selected bacteria were adjusted to an optical density of 0.09±0.005 at 600 nm wavelength (corresponding to McFarland=0.5).

A 100 μL of bacterial culture was spread-plated onto Mueller-Hinton Agar (MHA; Merck, Darmstadt, Germany) with sterile cotton swabs. Sterile filter paper discs (Bioanalyse LLC, Ankara, Turkey) with a diameter of 6 mm were placed on the MHA and followed by impregnation of extracts (10 μL) to the discs. All plates were incubated at 37°C for 24 hours (Alzoreky and Nakahara, 2003). Following incubation, inhibition zone diameters were measured using a digital caliper. Two replicates were conducted against each bacteria for each extract.

2.8. Statistical analysis

Statistical analysis was done using Prism 7 for Mac OS X (Version 7.0d) (GraphPad Software Inc, La Jolla, CA, USA, www.graphpad.com). Analysis of variance (one-way ANOVA) was applied at a α=0.05 significance level to determine differences among the proximate results of the samples. When one-way ANOVA was applied, Tukey's multiple comparisons test at a α=0.05 was used to test whether mean differences were statistically significant. For the remaining analyses, analysis of variance (Two-way

ANOVA), where washing and color were considered as two independent variables, was performed at a α=0.05 significance level to determine differences among the samples. When Two-way ANOVA was applied, Bonferroni's multiple comparisons test at a α=0.05 was used to test whether mean differences were statistically significant. Data were expressed as mean ± standard error.

3. Results and Discussion

3.1. Proximate analysis

The moisture, protein, lipid, and ash contents of white, black, and red quinoa seeds are given in Figure 2. The protein concentrations obtained in this study are similar to the protein values reported by Villa et al. (2014). Black quinoa was found to contain significantly less protein (12.72%) than its white (13.78%) and red (13.82%) counterparts ($p<0.05$). This disagrees with the reports published by USDA (2019) and Diaz-Valencia et al. (2018) who stated that black and white quinoa samples have the highest concentration of protein. The lipid concentration of red (8.98%) and black quinoa (8.34%) was found to be significantly ($p<0.05$) higher than that of white quinoa seeds (5.90%). This agrees with the report of Pellegrini et al. (2018) who found that, among quinoa samples differing in coat color, red quinoa has the highest fat concentration. The mean ash content of quinoa seeds ranged between 2.33% and 2.71%, with black quinoa containing the the highest ash content, followed by white and red quinoas. The ash values were higher than that was determined by Ogungbenle (2003) and lower than that reported by Repo-Carrasco-Valencia and Serna (2011) and Villa et al. (2014). Pellegrini (2018) and Diaz-Valencia et al. (2018) found that white quinoa contained less ash than its red and black counterparts. The variations in these findings could be attributed to not only color but also regional, climatic, and genetic differences (FAO, 2011).

3.2. Saponin content

The saponins, mostly located in the exterior parts of quinoa, are not only anti-nutritional

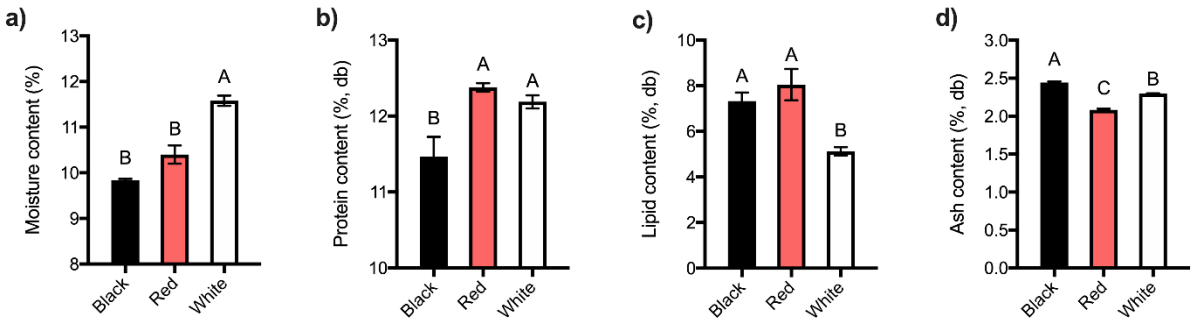

Figure 2. a) Moisture, **b)** protein, **c)** lipid, **d)** ash contents (%) of quinoa seeds having different coat color used in the study. Error bars represent the standard errors of the means of three separate replicates. Samples with different letter are significantly different ($p<0.05$). db: dry basis.

but also responsible for the bitter taste of quinoa. Their contents depend on several factors including the ecotype, irrigation level and water salinity, processing, and cooking conditions (Nowak et al., 2016). Although there is no legislation or maximum residue level set for saponin in EU and UK (Ojinnaka, 2016), washing is still the most common

method used to remove saponins from quinoa seeds in order to improve its nutritional and sensory quality (Suárez-Estrella, 2018). The saponin contents of quinoa seeds having different coat color before and after the washing step are given in Figure 3. The saponin contents of natural quinoa seeds ranged between 3.19 and 3.56%, with red one having the highest and white one having the lowest; however, these differences were statistically insignificant ($p>0.05$). The results of saponin contents obtained here are similar to the values reported by Medina-Meza et al. (2016) and Han et al. (2019b) and also in the expected limits of 0.1 to 5% (Stuardo and San Martin, 2008). Further, these results indicate that quinoa seeds used in this study can be classified as bitter (>0.11 %), according to Koziol (1991).

The washing step did not change the saponin contents of quinoa seeds significantly ($p>0.05$). After the washing process, the saponin contents of quinoa seeds ranged between 2.77 and 3.40%, with red one having the highest and its black counterpart having the lowest contents of saponins ($p>0.05$). The saponin removal rates are 4.28, 4.56, and 15.67% for white, red, and back quinoa seeds, respectively. These reduction rates obtained with washing are lower compared to the milling process (Han et al., 2019b). Saponin in quinoa is present in two major forms, namely Saponin A and Saponin B, the latter one having a lower portion (Ruales and Nair, 1993). Moreover, it was reported that after polishing and washing steps, 56% of Saponin A was recovered while Saponin B was completely removed. Considering relatively higher loss rate in saponins of black quinoa (15.67%), it can be speculated that white and red quinoa contains more Saponin B and less Saponin A fraction than their black counterparts.

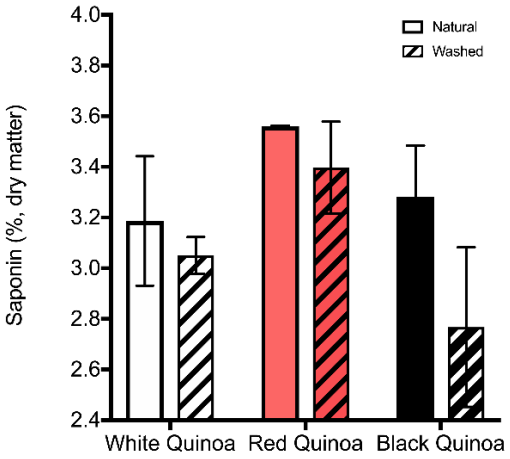

Figure 3. Saponin contents of quinoa seeds having different coat color before washing (natural) and after washing. Error bars represent the standard errors of the means of three separate replicates.

3.3. Total phenolic content

The phenolic contents of natural and washed quinoa seeds with different colors are given in Figure 4. Tang et al. (2015a) determined that black quinoa seeds had the highest phenolic content followed by its red and white counterparts, respectively. Moreover, black and red quinoa seeds were found to have higher phenolic contents than white ones in different studies confirming that the darker the color of quinoa seed, the higher the phenolic content it has (Tang et al., 2015a; Han et al., 2019b, Pellegrini et al., 2018, Diaz-Valencia et al., 2018). Similarly, Abderrahim et al. (2015) reported a negative correlation ($r=-0.619$, $p=0.024$) between phenolic content and brightness value of colored quinoa seeds suggesting that black and red quinoa seeds to have higher phenolic contents. Here, in compliance with the previous findings, natural black (55 mg GAE/100 g of extract, db) and red quinoa (53 mg GAE/100 g of extract, db) seeds had similar phenolic contents ($p>0.05$) while white one (46 mg GAE/100 g of extract, db) had significantly fewer phenolic

compounds ($p<0.05$). The concentrations of phenolic compounds are lower than the values reported in the previous studies (Diaz-Valencia et al., 2018; Pellegrini et al., 2018; Han et al., 2019a).

The washing step resulted in significant decreases in phenolic contents of all quinoa seeds tested ($p<0.05$). The highest loss of phenolic compounds was observed in black quinoa (36.14%). Among the washed ones, red quinoa (43 mg GAE/100 g of extract, db) was found to have significantly higher phenolic content than the others (36 mg GAE/100 g of extract for white and 34 mg GAE/100 g of extract for black) ($p<0.05$). Ultimately, red quinoa retained the highest phenolic content after washing, with a 77.95% recovery rate, followed by white and black quinoas with recovery rates of 77.18% and 63.86%, respectively. The phenolic compounds in quinoa are mostly acid-hydrolyzable followed by base-hydrolyzable and free ones (Tang et al., 2015a). Contrary to our findings, Nickel et al. (2016) found that the washing process under running water increased the phenolic content of quinoa grains and attributed this to the release of these conjugated phenolic compounds. The concentration of phenolic compounds depends on many factors including genetic and environmental factors, e.g. temperature, soil properties, and weather conditions. The discrepancies in the results could be explained by these factors as well as different extraction procedures applied for the determination of total phenolic content.

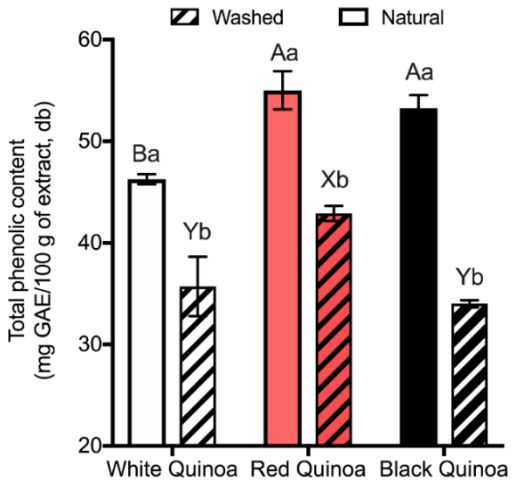

Figure 4. Total phenolic contents [mg gallic acid equivalent (GAE)/g of sample) of quinoa seeds having different coat color before washing (natural) and after washing. Error bars represent the standard errors of the means of three separate replicates. Different capital letters (AB) on the bars represent significant differences between the natural quinoa samples, different capital letters (XY) represent significant differences between the washed quinoa samples, and different small letters indicate significant differences between natural and washed quinoa samples within a quinoa type ($p<0.05$). db: dry basis.

3.4. Antioxidant activity

The antioxidant activities of red, black, and white quinoa seeds were determined using both DPPH and ABTS assays and accordingly by calculating corresponding IC_{50} values (Figures 5 and 6, respectively). Considering the natural forms of quinoa seeds, the red one showed the highest (91.5 μmol/g of extract)

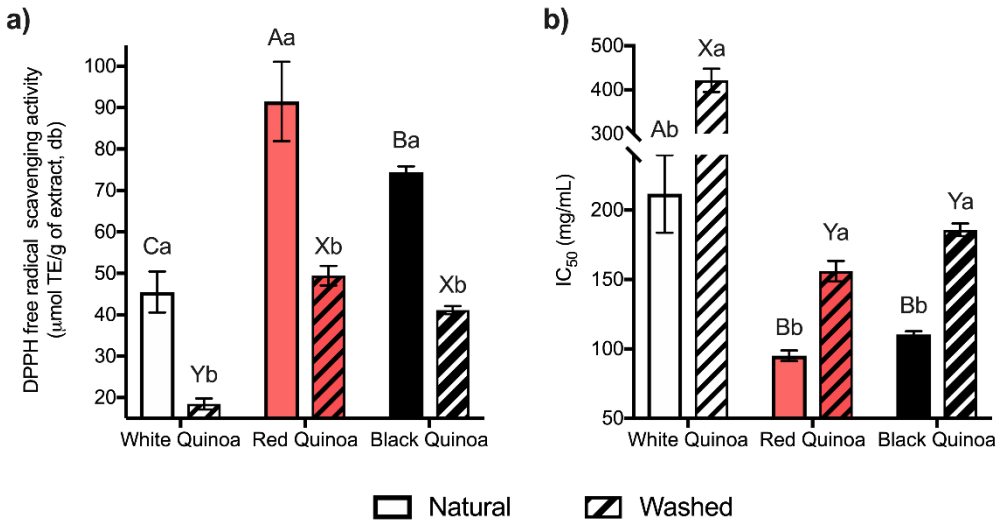

Figure 5. Total antioxidant potentials of quinoa seeds having different coat color before washing (natural) and after washing, as determined **a)** by measuring the DPPH free radical scavenging activities [µmol Trolox equivalent (TE)/g of extract] and **b)** by calculating the corresponding IC_{50} values of the extracts. The IC_{50} value is defined as the concentration of the sample required to inhibit 50% of DPPH radicals. Different capital letters (ABC) on the bars represent significant differences between the natural quinoa samples, different capital letters (XY) represent significant differences between the washed quinoa samples, and different small letters indicate significant differences between natural and washed quinoa samples within a quinoa type ($p<0.05$). db: dry basis.

DPPH free radical scavenging activity, followed by its black (74.4 µmol/g of extract) and white (45.5 µmol/g of extract) counterparts ($p<0.05$).

The washing step resulted in significant decrease in DPPH free radical scavenging activities of all quinoa seeds ($p<0.05$); after washing, DPPH activities of white, black and red quinoa samples were reduced by 59.4%, 44.7%, and 46.0%, respectively (Figure 5a).

Consistently, the IC_{50} value of the red (95 mg/mL) and black (111 mg/mL) quinoa seeds were significantly lower than white quinoa seeds (212 mg/mL) in natural forms ($p<0.05$) (Figure 5a). The washing step caused a significant increase in IC_{50} values of all quinoa samples tested ($p<0.05$). The washing step resulted in significant decrease in DPPH free radical scavenging activities of all quinoa seeds ($p<0.05$); after washing, DPPH activities of white, black and red quinoa samples were reduced by 59.4%, 44.7%, and 46.0%, respectively (Figure 5a). Consistently, the IC_{50} value of the red (95 mg/mL) and black (111 mg/mL) quinoa seeds were significantly lower than white quinoa seeds (212 mg/mL) in natural forms ($p<0.05$) (Figure 5a). The washing step caused a significant increase in IC_{50} values of all quinoa samples tested ($p<0.05$).

In general, similar trends of antioxidant activity and IC_{50} values were observed in ABTS assay (Figure 6b), in comparison to DPPH assay (Figure 5b). Although the values were quite close to each other, there were significant differences determined in ABTS

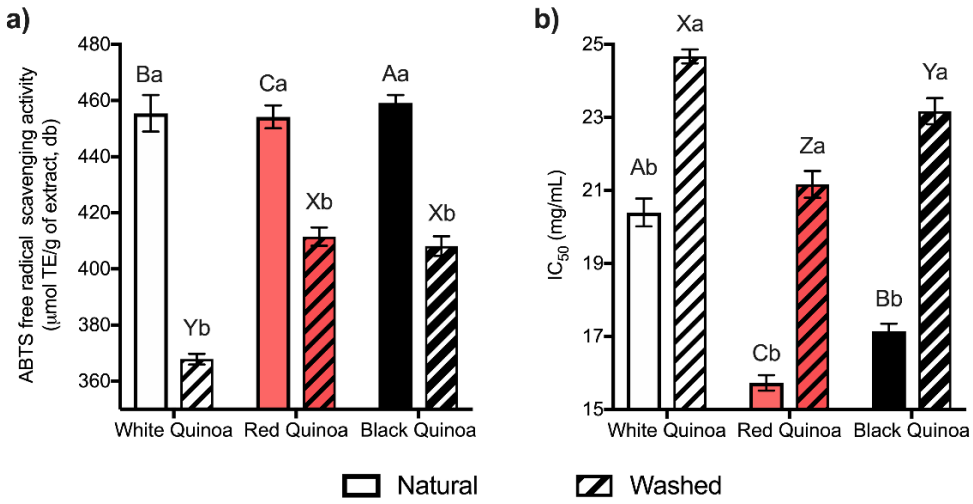

Figure 6. Total antioxidant potentials of quinoa seeds having different coat color before washing (natural) and after washing, as determined **a)** by measuring the ABTS free radical scavenging activities [µmol Trolox equivalent (TE)/g of extract] and **b)** by calculating the corresponding IC_{50} values of the extracts. The IC_{50} value is defined as the concentration of the sample required to inhibit 50% of ABTS radicals. Different capital letters (ABC) on the bars represent significant differences between the natural quinoa samples, different capital letters (XYZ) represent significant differences between the washed quinoa samples, and different small letters indicate significant differences between natural and washed quinoa samples within a quinoa type ($p<0.05$). db: dry basis.

free radical scavenging activities of black (459.1 µmol TE/g), white (455.44 µmol TE/g), and red (454.2 µmol TE/g) quinoa seeds in natural form ($p<0.05$). Same with the DPPH results, the washing step resulted in significant decreases in ABTS free radical scavenging activities of all quinoa seeds ($p<0.05$) (Figure 6a). After washing, there were significant reductions in ABTS activities of white (19.23%), black (11.09%) and red (9.40%) quinoa seeds ($p<0.05$). Considering the IC_{50}, the white seeds had higher values than its counterparts in both natural and washed forms.

Current findings of black and red-colored quinoas having higher antioxidant activity and less IC_{50} are in compliance with those reported in the literature (Tang et al., 2015a; Diaz-Valencia et al., 2018; Pellegrini et al., 2018; Han et al., 2019b). In another confirmatory study by Brend et al. (2012), it was found that red quinoa possessed higher antioxidant activity than yellow one in dry, baked, and cooked forms using the FRAP method. Moreover, Tang et al (2015b) determined that the same tendency is present in the case of antioxidant activities of quinoa lipophilic extracts. Although methods to measure antioxidant activity, quinoa types (region, ecotype, etc), and processing conditions differ, dark-colored quinoas overall have higher antioxidant activities. This behavior could be attributed to the higher concentration of phenolic compounds in colored quinoa varieties. Nickel et al. (2016) found that washing resulted in an increase in antioxidant activity of quinoa claiming that washing releases conjugated phenolic compounds. Contrarily, our results indicate that washing reduced the antioxidant activity of all quinoa

types presumably due to the reduction in the phenolic content.

3.5. Antibacterial activity

Some of the secondary metabolites, e.g. phenolic acids and benzoic acid analogs, have been reported as potential antimicrobial compounds present in the quinoa seeds (Lin et al., 2019). The quinoa seeds from different locations and ecotypes have been shown to have antimicrobial activity against foodborne bacteria and fungi in previous studies. For example, Miranda et al. (2014) prepared ethanol extracts of quinoa from three different geographical regions in Chile. *Staphylococcus aureus* was inhibited more compared to *Escherichia coli* with inhibition rates of >51% and >62% in reference to amoxicillin (100 µg/mL). In a similar study, a total of six different ecotypes of quinoa seeds from three different regions of Chile were tested using quinoa suspensions (1 g quinoa (with husk)/ 10 mL broth medium) and doing plate counts. All samples were found effective against Saccharomyces cerevisiae up to 48 hours. Only one ecotype, Regalona, was found to be effective against both *Listeria innocua* with a slight decrease of two logs and *S. cerevisiae* with almost complete inhibition (Galvez et al., 2018). The high rate of inhibition against *S. cerevisiae* could be attributed to the antifungal activity of saponins found in the husk of quinoa (Stuardo and San Martin, 2008). On the other hand, in a study by Park et al (2017), none of the quinoa samples grown in USA, Peru, and Korea were found to have strong antimicrobial activity (inhibition zone diameters<8mm) against *S. aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *E. coli*, *Salmonella typhimurium, and Campylobacter jejuni*. Similarly, none of the quinoa extracts showed any antimicrobial activity against any of the tested bacteria in this study (data not shown). Considering the earlier studies, current results confirm that the antimicrobial activity of quinoa seeds depends on many factors including quinoa cultivar, geographical region, processing conditions, extraction method, and concentration of extract as well as the species and strains of the tested microorganisms.

4. Conclusion

The results of this study show that quinoa seed coat color is an important factor affecting its total phenolic content, and antioxidant activity. Red and black quinoa seeds possessed higher phenolic contents together with higher antioxidant activities than their white counterparts, respectively. The washing step, which is commonly applied to decrease its saponins, did not cause significant reductions in the saponin content of any quinoa seeds tested; however, it decreased the total phenolic content as well as the antioxidant activity of quinoa seeds considerably. Therefore, other practical processes and washing methods with modifications should be developed and applied for more efficient removal of saponins, while maintaining its bioactive compounds and functional properties.

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