Hindawi
Journal of Chemistry
Volume 2019, Article ID 7125169, 8 pages
https://doi.org/10.1155/2019/7125169

Research Article

Influence of Drying Temperature on Phenolic Acids Composition and Antioxidant Activity of Sprouts and Leaves of White and Red Quinoa

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Received 30 September 2018; Revised 8 December 2018; Accepted 16 December 2018; Published 12 February 2019

Academic Editor: Davide Barreca

The aim of this study was to evaluate the influence of drying temperature on the phenolic acids profile and antioxidant activity of sprouts and leaves red and white *Chenopodium quinoa* (RQ and WQ, respectively). Four-day sprouts and leaves dried at 30, 45, and 60°C were tested. All sprouts contained significant amounts of phenolic compounds; however, higher content was determined in the RQ sprouts. Phenolic compounds from WQ sprouts seem to be thermostable in the studied temperature range, whereas total phenolics content in RQ sprouts decreased significantly after drying in the 60°C. Content of vanillic and *p*-coumaric acids did not differ significantly between sprouts dried at the same conditions; however, their level decreased in the high temperature. Irrespective of the drying temperature, higher activity against ABTS free radicals and reducing power was observed in the case of RQ sprouts extracts. Sprouts dried at 30°C had a higher ability to scavenge hydroxyl radicals. RQ sprouts were characterized by about two times higher antioxidant activity regardless of the method used. No significant differences between total phenolics (TPC) and flavonoids content in RQ and LQ leaves were found. In the both cases, decrease of TPC was observed after drying in the highest temperature. The leaves do not differ too much in terms of the phenolic acids profile, whereas the differentiating factor is thermal processing. Leaves of both quinoa contained thermostable compounds able to scavenge hydroxyl radicals. Reducing power and ability to scavenge OH radicals were correlated with all components of quinoa sprouts which suggest synergism between them and does not indicate the key role of a particular compound in creating antioxidant capacity. Germination and subsequent oven-drying at 30°C of quinoa seeds significantly increased the antioxidant properties compared with raw seeds. Also, in the case of leaves, the best results were obtained after drying at 30°C.

1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) has been cultivated for thousand years in South America. This plant is the main food crop in the Andean mountains of Bolivia and Peru but recently has increased interest for the product in other regions of the world like United States, Europe, and Asia [1].

In the human diet, especially the seed of this pseudocereal has been popular. The nutritional value of quinoa seeds is in particular related to the high protein content (with all essential amino acids), unsaturated fatty acids, and fiber, although the content of vitamins, minerals, and other phytochemicals and the gluten-free nature of these products should also be mentioned [2–4]. The main uses of quinoa seeds are similar to rice, for cooking or baking [5].

An increased interest in nutrition, in recent years, is functional food, therefore growing interest in new products whose consumption reduces the risk of disease and exerts health-promoting effects. The use of quinoa as a source of bioactive compounds can be included as such trends in the research. Most of the recent researches are focused mainly on the studies of nutritional and prohealth properties of quinoa seeds [6, 7]. There are some examples of using quinoa leaves or sprouts as functional food, but such research is not enough. Some authors suggest that quinoa leaves have been eaten similarly to spinach or as salads components [5]. In [8], Gawlik-Dziki et al. studied the antioxidant and anticancer potential of *Chenopodium quinoa* leaves' phenolic compounds that has been demonstrated. Our previous research has also indicated using quinoa leaves as source of bioactive compounds in fortified food-like bread [9]. The sprouts of quinoa have also attracted scientists interest in recent years due to their composition and prohealth properties [1, 4, 10, 11]. The ancient source of quinoa genotypes is found in Chile; however, quinoa has gained worldwide attention due to its nutritional value and functional properties [1], as well as its ability to grow in a wide range of climates, showing a good potential as a grain crop even in new areas outside of its native region [12]. Agricultural practices and cultural and climate changes also resulted in differentiation of quinoa cultivars. Recent studies on local ecotypes of quinoa in Chile have reported a wide genetic diversity, which have been related to the variation in their nutritional properties as well as total phenolic content and thus antioxidant potential [13]. However, all ecotypes showed an outstanding nutritional quality, higher than that of most traditional cereals. Such nutritional and nutraceutical properties of quinoa from very diverse geographical areas offer opportunities for genetic improvement trials and the creation of new varieties with unique properties [13].

Most varieties of quinoa commonly differ in the morphology, phenology, and the chemical composition of the tissues. Additionally, due to the fact that there are attempts to adapt different types of quinoa to other climatic conditions, need for additional research regarding their composition and biological activity seems to be appropriate [14]. The aim of this study was to evaluate the drying temperature on the phenolic acids profile and antioxidant activity of sprouts and leaves red and white quinoa.

2. Materials and Methods

2.1. Chemicals. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), potassium, ferricyanide, sodium salicylate, quercetin, gallic, *p*-hydroxybenzoic, vanillic, *p*-coumaric, salicylic, and ferulic acids were purchased from Sigma-Aldrich Company (Poznan, Poland). All other chemicals were of analytical grade.

2.2. Plant Material. Bolivian red quinoa seeds (*Chenopodium quinoa*) and seeds of white quinoa, la Regalona variety derived from Chile (quinoa real-mix of grains of similar colour (white) and of similar grain size (say 2-3 mm in seed diameter)), were used.

Before germination, quinoa seeds were sterilized in 1% (v/v) sodium hypochlorite for 10 min. At the end of the disinfection process, the seeds were drained and washed repeatedly with distilled water until the pH became neutral. After that, the seeds were soaked in distilled water by 6 h at 25°C and put into in a growth chamber. The germination process was performed in darkness during 4 days on the Petri dishes (/125 mm) lined with the absorbent paper. Sprouts were watered with 5 mL of Milli-Q water daily.

Four-day red sprouts (sprouted at 20°C) and leaves of quinoa collected after 90 days of vegetation were tested. Quinoa was harvested on an experimental farm belonging to the University of Life Sciences in Lublin, Poland. The soil was characterized by very low level of phosphorus, potassium, and magnesium content and mean content of humus and was acidic in reaction. During vegetation, the inter-rows were cultivated, and plants were weeded by hand three times. Sprouts and leaves were air-dried at the three levels of temperature: 30, 45, and 60°C. A UN single convection dryer type Single DISPLAY was used for drying. The sprouts and leaves were dried to a moisture content of 12%.

2.3. Determination of Total Phenolics Content (TPC). Total phenolics content was determined according to the Folin–Ciocalteu method [15]. To 0.5 mL of the extract 0.5 mL of H_2O, 2 mL of the Folin reagent (1 : 5 H_2O) were added, and after 3 min, the reaction mixture was mixed with 10 mL of 10% Na_2CO_3. After 30 min of incubation at room temperature, the absorbance of samples was measured at 725 nm. The amount of phenolic compounds was expressed as gallic acid equivalents (GAE) per gram of dry weight (DW).

2.4. Determination of Total Flavonoids Content (TFC). Total flavonoids content (TFC) was estimated according to Bahorun et al. [16] and expressed as quercetin (QE) equivalent (mg/g DW).

2.5. Phenolic Acids Analysis. A portion (2 g) of dried and powdered plant material was placed in a stainless steel cell of ASE 100 accelerated solvent extractor (Dionex; Sunnyvale, CA). Three cycles of extraction (15 min, methanol concentration 4 : 1 (v/v), temperature 80°C) were performed. The obtained extracts were collected, concentrated under reduced pressure, and dissolved in 10 mL of 80% methanol [17].

2.5.1. Sample Preparation. About 10 ml of extract previously diluted with water (1 : 1, v/v) was applied to Sep-Pak C18 Cartridges (500 mg; Waters, Milford, MA) previously activated with 10 mL of methanol and 10 mL of water. The cartridge was eluted with 10 mL of 80% methanol. The eluates were combined and concentrated under reduced pressure and then dissolved in 2 mL of 50% methanol. Prior to further analysis, the extracts were filtered again through 45 lm nylon filters. Liquid Chromatography and Electrospray Ionization Mass Spectrometry Conditions of Analysis of Phenolic Acids

2.6. Antioxidant Activity. Phenolic acids profile was determined according to [18]. Agilent 1200 Series HPLC system (Agilent Technologies, USA) equipped with a binary gradient solvent pump, a degasser, an autosampler, and column oven connected to 3200 QTRAP Mass spectrometer (AB Sciex, USA) was used. For chromatographic separations (at 25°C), the Zorbax SB-C18 column (2.1 × 50 mm, 1.8 μm particle size; Agilent Technologies, USA) with a mobile phase consisting of water containing 0.1% HCOOH (solvent A) and methanol containing 0.1% HCOOH (solvent B), using 3 mL injections. The flow rate was 400 mL·min^{-1}, and the gradient was as follows: 0-1 min, 5% B; 2–4 min, 20% B; 8–9.5 min, 70% B; 11.5–15 min, 5% B. The QTRAP-MS system was equipped with an electrospray ionization source (ESI) operated in the negative-ion mode. Operating conditions of ESI are as follows: capillary temperature 400°C, curtain gas at 30 psi, nebulizer gas at 60 psi, negative ionisation mode source voltage −4500 V. Nitrogen was used as curtain and collision gas. For each compound, the optimum conditions of multiple reaction mode (MRM) were determined in the infusion mode. The data were acquired and processed using Analyst 1.5 software (AB Sciex, USA). Triplicate injections were made for each standard solution and sample. The limits of detection (LOD) and quantification (LOQ) for phenolic compounds were determined at a signal-to-noise ratio of 3 : 1 and 10 : 1, respectively, by injecting a series of dilute solutions with known concentrations.

2.6.1. Determination of ABTS Radicals Scavenging Activity. Antiradical activity was determined as the ability to scavenge free ABTS^{+} radicals was carried out according to [19]. The radical cation was prepared by mixing 7 mM ABTS and 2.45 mM potassium per sulfate and leaving the mixture in the dark at room temperature for 16 h until the reaction was complete. The solution was diluted to reach absorbance measures around 0.70–0.72 at 734 nm. 1.8 mL ABTS^{+} solution was mixed with 0.04 mL of the tested sample. The absorbance was measured in even minute of reaction during 10 min at 734 nm. Percentage inhibition of the ABTS^{+} radical was calculated by using the following equation:

$$\text{scavenging}\,(\%) = \left[1 - \left(\frac{A_s}{A_c}\right)\right] \times 100, \quad (1)$$

where A_s is the absorbance of sample and A_c is the absorbance of control (ABTS solution).

Antiradical activity was expressed as EC50—extract concentration (mg·DW/mL) that provided 50% of activity based on a dose-dependent mode of action.

2.6.2. Ferric Reducing Antioxidant Power (FRAP). Reducing power was determined according to Oyaizu [20]. 2.5 mL of extracts were mixed with 2.5 mL of 200 mmol/L phosphate buffer (pH 6.6) and 2.5 mL of 1% aqueous solution of potassium ferricyanide $K_3[Fe(CN_6)]$. The mixtures were incubated at 50°C for 20 min. At the end of the incubation, 0.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 25 × g for 10 min. The upper layer of solution (2.5 mL) was mixed with 2.5 mL of deionised water and 0.5 mL of 0.1% ferric chloride. The colored solution was read at 700 nm.

Reducing power was determined as EC50—the effective concentration at which the absorbance was 0.5.

2.6.3. Determination of Hydroxyl Radicals Scavenging Ability. Hydroxyl radicals scavenging activity was determined according to Su et al. [21]. Hydroxyl radicals were generated by the Fenton reaction in the system of $FeSO_4$ and H_2O_2. The reaction mixture contained 0.5 mL of $FeSO_4$ (8 mM), 0.8 mL of H_2O_2 (6 mM), 0.5 mL distilled water, 1.0 mL of extract, and 0.2 mL of sodium salicylate (20 mM). The mixture (3.0 mL) was incubated at 37°C for 1 h. The intensity of the purple color formed was measured at 562 nm against a reagent blank. The scavenging activity was calculated using the following equation:

$$\text{scavenging activity}\,(\%) = \left[1 - \left(\frac{A_1 - A_2}{A_0}\right)\right] \times 100, \quad (2)$$

where A_0 is the absorbance of the control (without extract), A_1 is the absorbance of the extract addition, and A_2 is the absorbance without sodium salicylate.

Antiradical activity was expressed as EC50—extract concentration (mg DW/mL) provided 50% of activity based on a dose-dependent mode of action.

2.7. Statistical Analysis. Three parallel experiments were performed ($n = 9$), and the results were expressed as mean ± S.D. The data were subjected to statistical analysis by using STATISTICA 7.0. The analysis of variance (ANOVA) was performed, and significant differences between means were evaluated using Tukey`s test ($\alpha = 0.05$).

3. Results

3.1. Phenolics Profile. All tested sprouts contained significant amounts of TPC (determined with the Folin–Ciocalteu reagent); however, higher content was determined in the sprouts from red quinoa. Importantly, phenolic compounds from white quinoa sprouts seem to be thermostable in the studied temperature range, whereas TPC content in red quinoa sprouts decreased significantly after drying in the highest temperature (Table 1). Red quinoa sprouts contained also higher amounts of flavonoids; however, their content in the both (white and red quinoa sprouts) was relatively low and decreased with increasing drying temperature. Phenolic acids are the main phenols of cereals and pseudocereals, and thus we decided to examine their profile. Both sprouts (from white and red quinoa) dried at the lowest temperature contained comparable amounts of PHB acid. Significant decrease of these compounds was observed in the sprouts dried at 60°C. Content of vanillic and *p*-coumaric acids did not differ significantly between sprouts dried at the same conditions; however, their level decreased in the high temperature. Sprouts from red quinoa contained a higher level of ferulic acid; however, their significant decrease after drying at the highest temperature was observed. Only trace

Table 1: Total phenolics content (TPC), total flavonoids content (TFC), and phenolic acids profile of red and white quinoa sprouts.

Sample	TPC (mg GAE/g·DW)	TFC (mg QE/g·DW)	Phenolic acid (μg/g·DW)				
			p-Hydroxybenzoic	Vanillic	*p*-Coumaric	Salicylic	Ferulic
RQ	13.61 ± 0.45^{a}	2.38 ± 0.08^{a}	3.26 ± 0.11^{a}	0.14 ± 0.004^{a}	0.68 ± 0.013^{a}	0.03 ± 0.0011^{a}	1.89 ± 0.019^{a}
RQS30	27.92 ± 0.98^{b}	4.49 ± 0.11^{b}	1.82 ± 0.033^{b}	13.08 ± 0.55^{b}	7.51 ± 0.31^{b}	0.08 ± 0.002^{b}	8.82 ± 0.31^{b}
RQS45	28.79 ± 1.02^{b}	3.34 ± 0.125^{c}	1.55 ± 0.023^{b}	12.95 ± 0.47^{b}	6.1 ± 0.26^{c}	0.05 ± 0.002^{c}	7.88 ± 0.26^{c}
RQS60	17.57 ± 0.68^{c}	2.65 ± 0.09^{a}	1.41 ± 0.045^{b}	11.38 ± 0.25^{c}	4.77 ± 0.18^{d}	0.09 ± 0.003^{b}	7.35 ± 0.29^{c}
WQ	7.23 ± 0.22^{d}	0.72 ± 0.026^{d}	3.06 ± 0.12^{c}	0.18 ± 0.006^{a}	0.34 ± 0.012^{e}	nd	1.37 ± 0.016^{d}
WQS30	14.82 ± 0.58^{e}	1.13 ± 0.052^{e}	1.91 ± 0.015^{b}	13.95 ± 0.41^{b}	7.83 ± 0.31^{b}	nd	7.52 ± 0.24^{c}
WQS45	15.15 ± 0.46^{e}	0.63 ± 0.021^{f}	1.64 ± 0.017^{b}	11.33 ± 0.31^{c}	6.75 ± 0.28^{c}	nd	6.38 ± 0.19^{e}
WQS60	13.94 ± 0.39^{f}	0.18 ± 0.007^{g}	1.47 ± 0.019^{b}	10.33 ± 0.22^{d}	4.28 ± 0.16^{d}	nd	6.11 ± 0.21^{e}

RQ, red quinoa seeds; RQS30, red quinoa sprouts dried at 30°C; RQS45, red quinoa sprouts dried at 45°C; RQS60, red quinoa sprouts dried at 60°C; WQ, white quinoa seeds, WQS30, white quinoa sprouts dried at 30°C; WQS45, white quinoa sprouts dried at 45°C; WQS60, white quinoa sprouts dried at 60°C. The values designated by the different letters in the columns of the table are significantly different ($\alpha = 0.05$).

amount of salicylic acid was detected in the sprouts from red quinoa (Table 1).

Taking into account leaves of studied quinoa varieties, no significant differences between total phenolics and flavonoids content were found. In the both cases, decrease of TPC was observed after drying in the highest temperature. Leaves of both quinoa contained comparable amounts of *p*–hydroxybenzoic acid. Additionally, this compound seems to be thermostable (during drying conditions). Similar relationship was observed in the cases of vanillic and salicylic acids, whereas amount of ferulic acid decreased as the drying temperature increased. In conclusion, the leaves do not differ too much in terms of the phenolic acids profile, whereas the differentiating factor is thermal processing (Table 2).

3.2. Antioxidant Activity. Irrespective of the drying temperature, higher activity against ABTS free radicals was observed in the case of red quinoa sprouts extracts. Their activity expressed as EC50 was about 50% higher than activity of white quinoa sprouts samples. Importantly, only slight effect of the drying temperature was observed. The lowest activity was determined in the case of sprouts dried at 60°C (Figure 1).

The same effect was observed taking into account reducing activity (RED). Although the differences between red and white quinoa were significantly lower than in the case of antiradical activity, again higher activity was determined for red quinoa sprouts. Similarly to ABTS, the highest reducing power was determined for sprouts dried in the lower temperature (Figure 2).

Sprouts of red quinoa dried at the lower temperature had higher ability to scavenge hydroxyl radicals. The similar relationship was observed in the case of white quinoa sprouts (Figure 3.). Also, in this case, activity of red quinoa sprouts was higher than activity of those obtained from white quinoa.

Red quinoa sprouts were characterized by about two times higher antioxidant activity regardless of the method used. Drying temperature had little, however, statistically significant influence which allows you to choose the optimal conditions—drying at 30°C both in terms of energy consumption and bioactivity.

In the case of leaves from white quinoa, temperature of drying did not affect the antiradical (ABTS) potential, while in the case of red quinoa leaves, the highest activity was determined for samples dried at 30°C (Figure 4). Thus, it may be supposed that antiradical compounds from RQL were not thermostable.

The same relationship was observed when reducing power was analyzed. Activity of both (red and white) leaves dried at 45°C was comparable, while activity of red leaves dried at 30°C was more than 50% higher than the white quinoa sample (Figure 5).

Most importantly, leaves of both quinoa contained compounds able to scavenge hydroxyl radicals. Additionally, active compounds seem to be thermostable—any difference between samples was determined (Figure 6).

Based on statistical analysis, it can be concluded that, in the white quinoa sprouts case, antiradical activity (expressed as the ABTS scavenging potential) was strongly correlated with total phenolics content, whereas significant dependence was found only in the case of *p*-hydroxybenzoic acid and *p*-coumaric acid. Reducing power and ability to scavenge OH radicals were correlated with all components which suggest synergism between them and does not indicate the key role of a particular compound in creating antioxidant capacity. The analysis of red quinoa sprouts extracts confirms this thesis because also in this case, it is impossible to point to a specific compounds responsible for the antioxidant potential (Table 3). In the case of white quinoa leaves, the key role of *p*-hydroxybenzoic acid, ferulic acid, and flavonoid compounds in antiradical creating antiradical potential (against ABTS) was confirmed. Lower correlation coefficient ABTS/TPC may indicate antagonistic interactions of extracts components, whereas in the cases of RED and OH, the positive interactions may occur. These relationships were not confirmed when red quinoa leaves were analyzed. In this case, the positive correlation between all extract components and all activities was found. It worth noting that ABTS/TPC, RED/TPC, and OH/TPC coefficients were lower than others, which can indicate antagonistic interactions; however, explanation of this results needs more studies.

4. Discussion

Health benefits of quinoa have been a consequence of unique composition of proteins, fiber, vitamins and minerals, fatty

Table 2: Total phenolics content (TPC), total flavonoids content (TFC), and phenolic acids profile of red and white quinoa leaves dried in different temperatures.

Sample	TPC (mg GAE/g·DW)	TFC (mg QE/g·DW)	Phenolic acid (μg/g·DW)				
			p-Hydroxybenzoic	Vanillic	*p*-Coumaric	Salicylic	Ferulic
RQL	16.02 ± 0.95^{a}	2.58 ± 0.11^{a}	11.28 ± 0.55^{a}	24.82 ± 0.98^{a}	58.41 ± 2.04^{a}	0.48 ± 0.015^{a}	785.42 ± 25.82^{a}
RQL30	15.91 ± 0.88^{a}	2.40 ± 0.09^{a}	9.85 ± 0.65^{b}	24.11 ± 1.02^{a}	52.63 ± 2.25^{b}	0.32 ± 0.012^{b}	676.25 ± 22.38^{b}
RQL45	16.09 ± 0.72^{a}	2.02 ± 0.14^{b}	8.12 ± 0.49^{c}	24.02 ± 0.89^{a}	45.12 ± 1.87^{c}	0.28 ± 0.009^{c}	658.32 ± 22.45^{c}
RQL60	12.61 ± 0.61^{b}	1.94 ± 0.12^{b}	7.99 ± 0.38^{c}	23.64 ± 1.01^{b}	38.66 ± 1.55^{d}	0.25 ± 0.011^{c}	627.11 ± 21.58^{d}
WQL	16.58 ± 0.55^{a}	3.07 ± 0.17^{c}	12.85 ± 0.82^{d}	23.78 ± 0.75^{b}	55.32 ± 2.05^{e}	0.42 ± 0.019^{a}	734.95 ± 33.35^{e}
WQL30	16.05 ± 0.86^{a}	2.87 ± 0.11^{c}	11.96 ± 0.66^{d}	23.41 ± 0.86^{b}	51.07 ± 2.11^{b}	0.34 ± 0.015^{b}	689.47 ± 29.87^{f}
WQL45	16.03 ± 0.67^{a}	2.54 ± 0.12^{a}	10.88 ± 0.99^{de}	23.21 ± 0.79^{b}	42.43 ± 1.98^{c}	0.36 ± 0.014^{b}	662.38 ± 28.66^{c}
WQL60	12.21 ± 0.88^{b}	1.92 ± 0.13^{a}	9.58 ± 0.87^{e}	23.75 ± 0.88^{b}	37.25 ± 1.32^{d}	0.29 ± 0.014^{c}	648.27 ± 25.45^{g}

RQL, fresh red quinoa leaves; RQL30, red quinoa sprouts dried at 30°C; RQS45, red quinoa sprouts dried at 45°C; RQS60, red quinoa sprouts dried at 60°C; WQL, fresh white quinoa laves; WQS30, white quinoa sprouts dried at 30°C; WQS45, white quinoa sprouts dried at 45°C; WQS60, white quinoa sprouts dried at 60°C. The values designated by the different letters in the columns of the table are significantly different ($\alpha = 0.05$).

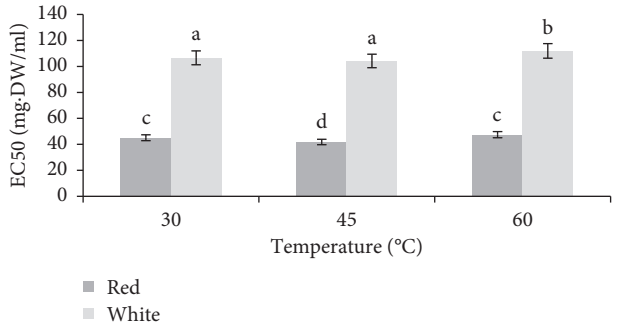

Figure 1: Comparison of ABTS radicals scavenging activity of red and white quinoa sprouts extracts.

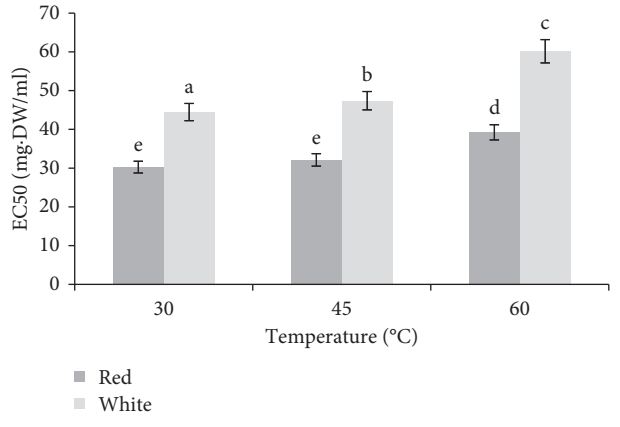

Figure 2: Comparison of reducing power of red and white quinoa sprouts extracts.

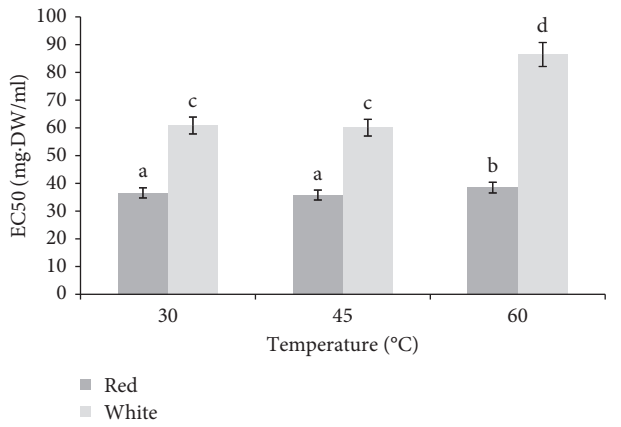

Figure 3: Comparison of OH radicals scavenging activity of red and white quinoa sprouts extracts.

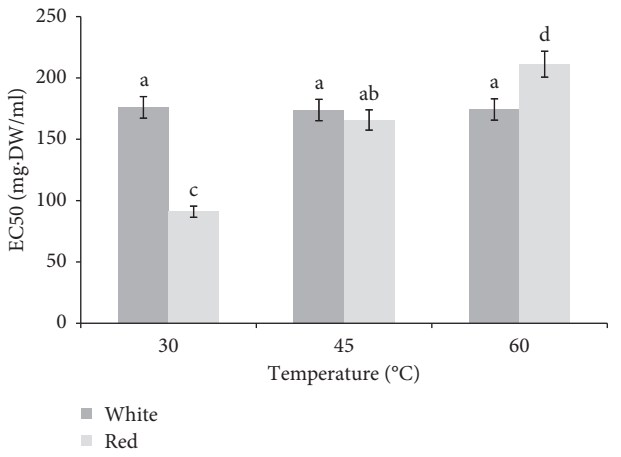

Figure 4: Comparison of ABTS radicals scavenging activity of red and white quinoa leaves extracts.

acids, and secondary metabolites such as phenolics. High content of phenolic compounds were associated with antioxidant capacity determined for quinoa seeds [5].

More than 20 phenolic compounds in either free or conjugated forms were determined. Mostly, they are phenolic acids (vanillic and ferulic acids and their derivatives) as well as the flavonoids: quercetin and kaempferol (free and conjugated with sugars) [22, 23].

The data presented in Table 1 are consistent with the cited literature; however, it is important to mention that the amount of phenolic compounds in the seed samples strongly influences the genotype (cultivar/variety), soil, environmental conditions, level of maturity at harvest, and their storage in postharvest conditions.

Biochemical processes occurring during seeds germination lead to radical changes in their composition and

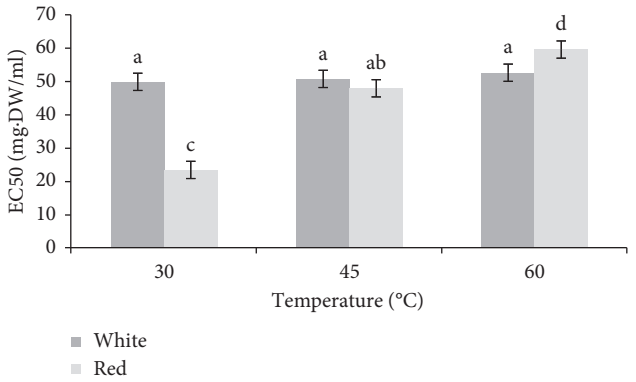

Figure 5: Comparison of reducing power of red and white quinoa leaves extracts.

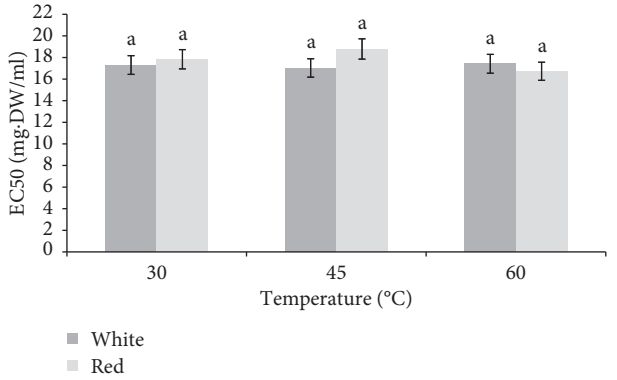

Figure 6: Comparison of free OH radicals scavenging activity of red and white quinoa leaves extracts.

proportions of primary and secondary metabolites, which could result in a change in the profiles of phenolic compounds and antioxidant activity [11]. Our study confirms this fact (Table 1). As presented, total phenolics and flavonoids content significantly increased in the sprouted seeds. Also, Alvarez-Jubete et al. [10] confirmed the changes in the level of phenolic compounds in quinoa seeds after 82 h of germination.

Carciochi et al. [11] reported that quinoa sprouts contain benzoic acid derivatives (*p*-hydroxybenzoic, vanillic, and gallic acids) and cinnamic acid derivatives (ferulic, *p*-coumaric, and caffeic acids). In addition, raw and germinated seeds of quinoa contained various concentrations of *p*-hydroxybenzoic, vanillic, *p*-coumaric, and ferulic acids.

At the end of germination (72 h), an 8.57-fold increase in the content of phenolic acids was observed. The dominant compounds were vanillic and ferulic acids (51.2 and 21.6% of the total concentration of phenolic compounds, respectively). According to Alvarez-Jubete et al. [10], the main phenolic acid in *C. quinoa* sprouts was vanillic acid. Among the cinnamic acid derivatives, the most abundant were ferulic and *p*-coumaric acids. Similar results were obtained in our laboratory (Table 1).

A significant increase in the content of *p*-coumaric acid during germination should be emphasized. Its 21-fold increase was found in relation to the content in raw seeds. Other authors also confirmed an increase in the content of hydroxycinnamic acids during germination of pseudocereals [4, 24]. The main role of hydroxycinnamic compounds is to build cell walls, most often as esters on the side chains of arabinose, arabinoxylans, and lignin [25].

Table 3: Correlation coefficients between phenolic compounds level and antioxidant activities.

Sample	Activity	TPC	TFC	PHB	Val	PCA	SAL	FE
Sprouts								
	ABTS	0.999	0.652	0.573	0.469	0.822	0.388	0.388
White	RED	0.903	0.929	0.888	0.825	0.992	0.771	0.771
	OH	0.971	0.836	0.777	0.694	0.946	0.627	0.627
	ABTS	0.854	0.283	0.247	0.774	0.402	0.267	0.267
Red	RED	0.964	0.891	0.874	0.991	0.941	0.884	0.884
	OH	0.976	0.578	0.547	0.937	0.677	0.565	0.565
Leaves								
	ABTS	0.764	0.937	0.972	0.191	0.551	0.551	0.997
White	RED	0.931	0.999	0.995	0.499	0.792	0.792	0.946
	OH	0.724	0.449	0.338	0.996	0.889	0.889	0.111
	ABTS	0.758	0.975	0.948	0.886	0.998	0.998	0.956
Red	RED	0.716	0.987	0.966	0.855	0.992	0.992	0.937
	OH	0.874	0.912	0.865	0.961	0.989	0.989	0.995

TPC, total phenolics content; TFC, total flavonoids content; PHB, *p*-hydroxybenzoic acid; Val, vanillic acid; PCA, *p*-coumaric acid; SAL, salicylic acid; FE, ferulic acid; ABTS, antiradical activity against ABTS free radicals; RED, reducing power; OH, hydroxyl radicals scavenging activity. Correlation coefficients above 0.551 are statistically significant ($p < 0.05$).

During germination, endogenous esterases are activated, which can lead to the release of phenolic compounds associated with cell walls or for their synthesis de novo [26]. This explains the increase in TPC content observed during seed germination. An evaluation of total antioxidant activity using only one method is not possible because different antioxidant compounds can act in the plant food matrix through different mechanisms and potential synergistic interactions that can take place among these molecules [27].

Thus, it is generally accepted to use a combination of different methods to determine antioxidant activity [28]. In the present study, a combination of assays was used, which included two methods for determining the antiradical ability (against synthetic ABTS free radicals and physiologically occurring hydroxyl radicals) and reducing power to evaluate antioxidant activity.

In the sprouts case, extracts from red quinoa sprouts were characterized by about two times higher antioxidant activity regardless of the method used. Additionally, antioxidants contained in RQL demonstrated comparable activities (expressed as EC50) regardless of methods used. Drying temperature had little, however, statistically significant influence which allows you to choose the optimal conditions both in terms of energy consumption and bioactivity. Considerable differences were observed in the case of leaves. Regardless of quinoa variety, relatively low activity against free ABTS radicals was observed, whereas their activity towards OH radicals and reducing power was significantly higher. Most importantly, the highest activity against the OH radical was observed. It is very important

because ABTS is synthetic free radical commonly used in food analysis, whereas very active hydroxyl radicals occur in the living systems. Thus, reducing power and anti-OH activity have a reference to the conditions prevailing *in vivo*.

It is widely known that germination significantly improved the antioxidant potential of quinoa seeds. Paśko et al. [4] found that antiradical potential was maximal at the sixth day of germination. This activity was significantly higher in quinoa seeds compared to amaranth seeds. However, Alvarez-Jubete et al. [10] reported no statistical differences in antiradical capacity (determined as ability to scavenge DPPH free radicals) among quinoa, amaranth, and wheat sprouts after 82, 98, and 110 h of germination, respectively.

Leaves of red quinoa (especially dried in low temperature) were more active than leaves of white quinoa. In the case of RQL, higher temperature of drying significantly decreased ABTS and RED activities, whereas no effect on anti-OH activity was observed, and in turn no effect on OH activity was found. Thus, the recommended drying temperature of quinoa leaves is 30°C, both without the loss of biological activity and for ergonomic reasons.

This results obtained by Carciochi et al. [11] indicated that phenolic compounds are closely related to antioxidant activity of quinoa sprouts. Cited authors indicated phenolics acids as a main compound affecting their antioxidant potential. Among the identified phenolic acids, ferulic acid was the compound that most influenced the antioxidant activity in quinoa seeds, followed by vanillic acid. These results were in accordance with those obtained in our lab.

Most reports concern the nutraceutical potential of quinoa seeds. On the contrary, little information is available on the antioxidant potential of the green parts of *C. quinoa*. In particular, this applies to pseudocereal leaves, previously treated as worthless waste. It should be emphasized that they are edible and can be eaten in salads and also used as a valuable addition to functional foods [8].

Quinoa leaves contain a significant amount of ash (3.3%), fiber (1.9%), nitrates (0.4%), vitamin E (2.9 mga-TE/100 g), vitamin C (1.2–2.3 g/kg), and 27–30 g/kg of proteins [12]. However, little is known about the content and biological activity of phenolic compounds in *C. quinoa* leaves. According to Gawlik-Dziki et al. [8], ethanolic quinoa leaf extract prevented lipid oxidation, which may be the result of high metal chelating capacity. In addition, ethanolic quinoa leaf extract significantly restricted the proliferation of prostate cancer cells (MAT-LyLu and AT-2). The results obtained by Chen et al. [29] indicated that 95% of EQL contained a high concentration of phenolic compounds which translated into high scavenging capacity of DPPH radicals as well as inhibition of NO production in LPS-induced RAW 264.7 cells. According to Lu et al. [30], the polyphenols extracted from quinoa leaves exhibited a strong scavenging effect on DPPH and OH free radicals, and its IC50 (half inhibitory concentration) was, respectively, up to 1.876 μg/mL and 6.520 μg/mL. Meanwhile, great variation in polyphenol content has been found in quinoa leaves among different varieties.

5. Conclusion

The germination process can be a good strategy to improve nutritional potential of quinoa sprouts resulting from, *inter alia*, an increase in polyphenols concentration.

In the present work, it was found that germination and subsequent oven-drying at 30°C of quinoa seeds significantly increased the antioxidant properties compared with raw seeds. Also, in the case of leaves, the best results were obtained after drying at 30°C. The phenolic compounds, especially phenolic acids, were found to have strong positive correlation on antioxidant activity.

Therefore, not only fresh but also dried sprouts and leaves of quinoa can be used as an food additive and/or raw material for functional foods production.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The study was financed by the University of Life Sciences in Lublin, Poland (Project VKB/DS/2 in 2013–2017).

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