International Food Research Journal 21(2): 767-773 (2014)
Journal homepage: http://www.ifrj.upm.edu.my

Changes in phenolic composition and antioxidant activity during germination of quinoa seeds (*Chenopodium quinoa* Willd.)

[1]Carciochi, R. A., [1]Manrique, G. D. and [2]Dimitrov, K.

[1]*Faculty of Engineering, Universidad Nacional del Centro de la Provincia de Buenos Aires, Av. del Valle 5737, Olavarría, Argentina*
[2]*ProBioGEM Laboratory, Polytech'Lille, Université Lille Nord de France, Av. Paul Langevin 59655, Villeneuve d'Ascq, France*

Article history

Received: 19 September 2013
Received in revised form: 17 October 2013
Accepted: 18 October 2013

Keywords

Chenopodium quinoa
Germination
Phenolic acid
Flavonoid
Antioxidant activity

Abstract

Quinoa seed (*Chenopodium quinoa* Willd.) has obtained great interest in recent years due to its high nutritional value and its content of natural antioxidants and other phytochemicals with positive biological activity in humans. Their ability as health promoters depends strictly on the processing conditions to which the seeds are submitted. In this work, quinoa seeds were subjected to germination and subsequent oven-drying at 40ºC in order to evaluate changes on phenolic compounds composition as well as on the antioxidant activity along different germination stages. Germination resulted in a 2 fold increase in antioxidant activity measured as DPPH radical scavenging activity, after 3 days of germination. At the same time, the amounts of HPLC identified phenolic acids and flavonoids increased 8.57 fold, and 4.4 fold respectively. Germination and subsequent oven-drying was shown to be a good process to improve the phenolic content and antioxidant activity of quinoa seeds, and thereby obtain an ingredient to be used in functional food formulations.

© All Rights Reserved

Introduction

Quinoa (*Chenopodium quinoa*) is a native food plant of the Andean region of South America, that grows from sea level to an altitude of 4000 m. Quinoa can be grown under different stress conditions, such as drought, high altitudes, extreme temperatures, salinity, and poor sandy soils, conditions unsuitable for the development of other crops (Brady *et al.*, 2007). Quinoa´s aptitude to produce high-protein grains under environmental extreme conditions makes it important for the diversification of future agricultural systems. This gives it the potential to become an important industrial and food crop that could contribute to diminish the shortage of provisions in developing countries (Bhargava *et al.*, 2006). In the past decade, quinoa has been introduced in Europe, North America, Asia and Africa with high yields (Abugoch James, 2009). Nowadays, quinoa production is in a process of expansion into different geographic areas of the world due to its extraordinary adaptability. In Asia, studies in the Himalayas and the plains of Northern India have shown that the crop can successfully produce high yields with agricultural potential for other countries with similar agro-climatic conditions (Bhargava *et al.*, 2006; FAO, 2011). In addition, quinoa seeds have been cultivated in Japan, showing to have a higher content of bioactive compounds compared to others cereals and pseudo-cereals (Hirose *et al.*, 2010).

Quinoa seeds have remarkable nutritional properties, not only from its protein content (15% approximately), but also from its high amino acid balance. But, beyond their nutritional function of supplying nutrients, quinoa seeds provide compounds with promoting health properties such as phenolic acids, phytosterols and flavonoids (Abugoch James, 2009).

During seed sprouting a multitude of biochemical processes take place, leading to radical changes in primary and secondary metabolites composition, which could result in a change of intrinsic phenolic compounds profile, and antioxidant activity (Jian *et al.*, 2009). In addition, germination has shown to be a good way to enhance the nutritive value and health qualities of some seeds (Lintschinger *et al.*, 1997). Kim *et al.* (2004) have demonstrated that germinated buckwheat (*Fagopyrum esculentum* Moench) seeds notably increased the polyphenol content as sprouting progressed. For different cultivars of barley (*Hordeum vulgare*) sprouts showed significant increase in total phenolic content and antioxidant activity compared with raw seeds (Sharma and Gujral, 2010). Nevertheless, Megat Rusydi and Azrina (2012) have observed a decrease in total phenolic compounds in the case of germinated soy bean and peanuts, which means that the changes of phenolic compounds levels after germination depend on plant species and the

*Corresponding author.
Email: carciochi@fio.unicen.edu.ar
Tel/Fax: +54 2284 451055

germination conditions used. A previous study has showed the change in the level of some phenolic compounds by HPLC at fixed time of germination (82 h) in quinoa seeds (Alvarez-Jubete *et al*., 2010), without data on the changes in the profile of such compounds during the germination process.

In this study, quinoa seeds were subjected to germination and subsequent oven-drying in order to characterize the phenolic profiles of different germination periods. The antioxidant activity was also evaluated in order to point out the optimal germination conditions for obtaining flours with higher antioxidant capacity that could be used in formulations of gluten-free functional foods.

Materials and Methods

Samples and chemicals

Quinoa seeds (*Chenopodium quinoa* Willd.) were purchased from a local market and stored at 4ºC until use. Folin-Ciocalteau reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), β-carotene, linoleic acid, formic acid, gallic acid, p-hydroxybenzoic acid, vanillic acid, p-coumaric acid, ferulic acid, quercetin and kaempferol were supplied by Sigma-Aldrich Chemical Co. (St. Louis, USA). Sodium carbonate, sodium nitrite and ferric chloride were supplied by Merck (Darmstadt, Germany). Ethanol, methanol and chloroform were of analytical grade.

Germination and oven drying

Quinoa seeds (200 g) were soaked in a 2.5% sodium hypochlorite solution (5 min) for surface sterilization and then washed with distilled water to neutral pH. Then, the seeds were distributed into Petri dishes on wet filter paper and covered with the same wet paper to hydrate the seeds by capillarity. Petri dishes were incubated at 20°C (80 – 90% relative humidity) in a dark chamber. The seeds were watered every day with 2.5 ml of sterile distilled water to avoid drying and maintain the moisture content. A sample from 0 to 3 days at 24 h intervals was taken, identified hereafter as raw and stages 1, 2, 3, respectively. The duration of this germination period was based on the laboratory observation, since in longer periods sprouts overgrow.

Sprouts corresponding to each stage of germination and raw seeds were dried at 40°C in a mechanical convection oven to constant weight and were milled using a laboratory grinder (Yellow line, A10, IKA-Werke, Staufen, Germany). The obtained flour was then sieved to obtain a particle fraction smaller than 500 microns (32 mesh).

Preparation of extracts

Two g quinoa flour from germinated and raw samples was homogenized with 20 mL of 80% ethanol. The mixture was kept in agitation for 30 min at 160 rpm in an orbital shaker. Then, the homogenate was centrifuged for 10 min at 11000 rpm (Eppendorf Centrifuge 5804, Hamburg, Germany) and the supernatant was removed. The residue was extracted once again at the same conditions. Then, both supernatants were pooled, filtered (0.45 μm) and stored at -18°C for further analysis.

Total phenolic content (TPC)

TPC in extracts was determined using Folin-Ciocalteau reagent, following the method described by Singleton *et al.* (1999). The liquid extracts were diluted and mixed with Folin-Ciocalteau reagent (2 N) and 20% sodium carbonate solution. The mixture was incubated in the dark for 2 h at room temperature (20ºC). After incubation, the absorbance of the mixture was measured at 765 nm using an UVmini 1240 spectrophotometer (Shimadzu, France). The results were expressed as mg equivalent of gallic acid (GAE) per 100 g in dry weight basis (dwb).

Total flavonoid content (TFC)

TFC were measured as described Dini *et al.* (2010) with slight modifications. Briefly, 0.25 mL aliquot of extract was mixed with 2 mL of distilled water and 0.15 mL of 5% sodium nitrite solution in a test tube. After 5 min, 0.15 mL of 10% aluminum chloride solution was added. At 6 min, 1 mL 1 M sodium hydroxide solution was added to the mixture. Immediately, the solution was diluted with 1.2 mL of distilled water and thoroughly mixed. Absorbance of the final mixture was determined at 510 nm against a blank reaction. Total flavonoid content of extracts was expressed as mg of quercetin/100 g of dry matter (QE).

Chromatographic analysis of phenolic acids and flavonoids

Reversed phase HPLC method for determination of phenolic acids and flavonoids was used according to the method described by Ross *et al.* (2009) with minor modifications. A Waters 600 high performance liquid chromatograph equipped with a Waters 2996 diode array detector (Milford, MA) was employed. Software used for data acquisition and control of HPLC pumps, autosampler, and diode array system was Empower (Waters Corporation, Milford, MA). The wavelengths used for identification and quantification of phenolic acids and flavonoids were 280 and 370

nm, respectively. The separation was carried out on a reversed phase Gemini C6 – Phenyl column (250 x 4.6 mm, 3 µm) (Phenomenex, Torrance CA). The mobile phase consisted of two solvents; 0.1% formic acid aqueous solution (A) and methanol containing 0.1% formic acid (B). Gradient elution was employed as follows: isocratic elution 10% B, 0-10 min; linear gradient from 10% B to 35% B, 10-40 min; isocratic elution 35% B, 40-45 min, linear gradient from 35% B to 100% B, 45-60 min; isocratic elution 100% B, 60-65 min; linear gradient from 100% B to 10% B, 65-67 min; post-time 8 min before next injection. The flow rate of the mobile phase was 0.6 mL/min and the injection volumes for all samples and standards were 20 µL. The identification of the phenolic compounds was achieved by comparing retention times and UV spectra of the unknown compounds with standards. All phenolic acids and flavonoids were quantified as aglycones in duplicate using the external standard method and the amount of each compound was expressed as mg per 100 g of dry matter.

DPPH radical scavenging assay

The determination was carried out as described Brand-Williams *et al.* (1995). Aliquots (50 µL) of extracts were added to 1950 µL of a methanolic solution (100 µM) of DPPH radical. After agitation, the mixture was incubated in the dark for 30 min and the absorbance was measured at 517 nm in an UVmini 1240 spectrophotometer (Shimadzu, France). The radical scavenging activity was calculated according to the following equation (Liyana-Pathirana and Shahidi, 2007):

$$\% \text{ DPPH scavenging} = [(Abs_{(t=0)} - Abs_{(t=30)}) / Abs_{(t=0)}] \times 100$$

where $Abs_{(t=0)}$ = absorbance of DPPH radical + methanol at t = 0 min; $Abs_{(t=30)}$ = absorbance of DPPH radical + sample at t = 30 min.

β-Carotene bleaching method

The antioxidant activities of extracts were also evaluated by the β-carotene-linoleate model system. This method is based on the loss of the colour of β-carotene due to their reaction with radicals formed by linoleic acid oxidation in an emulsion. By means of hydrophobic repulsion, β-carotene is able to join the system of lipidic micelles and the corresponding oxidation reaction is accomplished in a lipidic environment. The rate of β-carotene bleaching can be slowed down in the presence of antioxidants. The experimental procedure was performed according to Suja *et al.* (2005). Briefly: β-carotene (0.2 mg), 20 mg of linoleic acid and 200 mg of Tween 20 were mixed in 1 mL chloroform and the solvent was evaporated in rotavapor under vacuum. The residue was diluted with 50 mL oxygenated distilled water. To 4.8 mL of the resulting emulsion, 0.2 mL of extract was added. A solution with 0.2 mL of ethanol and 4.8 mL of the above emulsion was used as control. The tubes were maintained at 50°C for 2 h in a water bath. Absorbance was measured at 470 nm in an UVmini 1240 spectrophotometer (Shimadzu, France) and the percentage inhibition of the β-carotene oxidation was calculated as follows:

$$\% \text{ inhibition of } \beta\text{-carotene oxidation} = [(A_{S(120)} - A_{C(120)}) / A_{C(0)} - A_{C(120)})] \times 100$$

where $A_{S(120)}$ and $A_{C(120)}$ is the absorbance at 120 min of the sample and control, respectively, and $A_{C(0)}$ is the absorbance of the control at zero time.

Statistical analysis

Experimental results were performed in triplicate and the data are presented as mean ± SD. Analysis of variance and comparison of treatments means (Tukey´s test, $p \leq 0.05$) as well as principal components and correlation analysis were performed using InfoStat 2011 software (InfoStat Group, Argentina).

Results and Discussion

Total phenolic and flavonoid contents of raw and germinated quinoa seeds

Values of TPC and TFC of the extracts from each germination stage and raw samples are shown in Table 1. The TPC of raw quinoa seeds in the present study (39.3 ± 0.9 mg GAE/100 g dwb) resulted comparable to those reported by other authors (Gorinstein *et al.*, 2007; Alvarez-Jubete *et al.*, 2010; Miranda *et al.*, 2010), whose results ranged from 25.0 to 71.7 mg GAE/100 g dwb. By other hand, Gorinstein *et al.* (2007) reported a TFC value of 38.6 mg catechin/100 g dwb) in raw quinoa. This value was higher compared with the found in our study (11.06 mg quercetin/100 g dwb), but these results were expressed as units of different standard compounds.

The difference between these values can be partially explained by different solvents and extraction methods used. In addition, it is important to mention that the quantity of phenolic compounds in seed samples is strongly influenced by genotype (variety/cultivar), soil, environmental conditions, maturity level at harvest and post-harvest storage conditions.

A significative increase of TPC and TFC values throughout the germination period was observed. At the end of germination (72 h) a considerable increase

Table 1. Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity of raw and germinated quinoa seeds*

Sample	TPC	TFC	Antioxidant Activity	
			DPPH	β-Carotene
raw	39.29 ± 0.92 [a]	11.06 ± 0.42 [a]	13.61 ± 1.10 [a]	71.40 ± 1.34 [a]
stage 1 (24 h)	47.04 ± 1.31 [b]	12.58 ± 0.25 [a]	16.59 ± 0.78 [a]	77.06 ± 0.89 [b]
stage 2 (48 h)	61.68 ± 1.02 [c]	15.68 ± 0.22 [b]	22.46 ± 0.67 [b]	78.33 ± 1.24 [b]
stage 3 (72 h)	79.04 ± 1.18 [d]	17.65 ± 0.45 [b]	27.39 ± 0.61 [c]	78.86 ± 0.96 [b]

* TPC in GAE (mg/100 g dwb), TFC in mg QE/100 g dwb, antioxidant activity as % DPPH radical scavenging and % inhibition of β-carotene oxidation.
Values (means ± S.D., n = 3) within a column with different superscript letter are significantly different ($p \leq 0.05$).

Table 2. Total contents (mg/100 g dwb) of phenolic acids and flavonoids in non-germinated (raw) and different germination stages of quinoa seeds

Compound	Raw	Stage 1	Stage 2	Stage 3
Gallic acid	n.d.	n.d.	n.d.	n.d.
p-OH-benzoic acid	0.22 ± 0.02 [a]	0.32 ± 0.08 [a]	0.46 ± 0.12 [a]	0.94 ± 0.04 [b]
Vanillic acid	0.88 ± 0.11 [a]	2.58 ± 0.03 [b]	5.51 ± 0.12 [c]	8.54 ± 0.09 [d]
Total Hydroxybenzoic acids	*1.09 ± 0.07* [a] (51%)	*2.91 ± 0.05* [b] (59%)	*5.97 ± 0.12* [c] (58%)	*9.48 ± 0.06* [d] (57
Chlorogenic acid	n.d.	n.d.	n.d.	n.d.
Caffeic acid	n.d.	n.d.	n.d.	n.d.
p-Coumaric acid	0.09 ± 0.10 [a]	0.27 ± 0.03 [b]	0.87 ± 0.03 [c]	1.96 ± 0.02 [d]
Ferulic acid	0.57 ± 0.09 [a]	1.23 ± 0.10 [b]	2.66 ± 0.07 [c]	3.61 ± 0.08 [d]
Total Hydroxycinnamic acids	*0.66 ± 0.09* [a] (31%)	*1.50 ± 0.07* [b] (30%)	*3.53 ± 0.05* [c] (34%)	*5.56 ± 0.05* [d] (33
Total phenolic acids	**1.75 ± 0.06** [a]	**4.40 ± 0.07** [b]	**9.50 ± 0.09** [c]	**15.0 ± 0.06** [d]
Quercetin	0.23 ± 0.02 [a]	0.33 ± 0.13 [a]	0.61 ± 0.15 [a]	1.36 ± 0.06 [b]
Kaempferol	0.15 ± 0.04 [a]	0.19 ± 0.05 [a]	0.17 ± 0.04 [a]	0.27 ± 0.05 [a]
Total flavonoids	**0.37 ± 0.03** [a] (18%)	**0.52 ± 0.09** [a] (11%)	**0.78 ± 0.10** [a] (8%)	**1.63 ± 0.06** [b] (10
Total phenolic compounds	**2.13 ± 0.06** [a] (100%)	**4.93 ± 0.07** [b] (100 %)	**10.3 ± 0.09** [c] (100 %)	**16.7 ± 0.06** [d] (100 %)

Values (means ± S.D., n = 2) within a row with different superscript letter are significantly different ($p \leq 0.05$), n.d. = not detected.

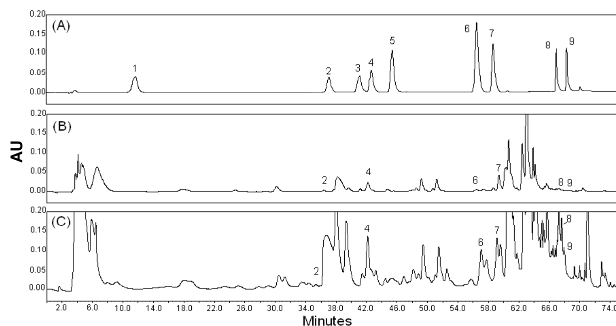

Figure 1. Representative HPLC separations of a mixture of phenolic acids and flavonoids standards recorded at 280 nm (A), an ethanolic extract of non-germinated quinoa seed (B), and 3 days germinated quinoa seed (C). Peak identification: 1 = gallic acid; 2 = p-hydroxybenzoic acid; 3 = chlorogenic acid; 4 = vanillic acid; 5 = caffeic acid; 6 = p-coumaric acid; 7 = ferulic acid; 8 = quercetin; 9 = kaempferol).

of 101.2% in the value of total phenolics was observed, respect to raw seeds. This result agrees with that reported by Alvarez-Jubete *et al.* (2010) where a 2 fold increase of total phenols in quinoa sprouts after 82 h of germination was found. Similarly, after 3 days of germination, a significant increase of the total flavonoid compounds (59.6%) with respect to raw quinoa was observed.

The TPC and TFC values by spectrophotometric methods can be used as an estimation to know the effect of different germination periods on the phenolic composition. Nonetheless, an individualized knowledge of the different phenolic acids and flavonoids is necessary to achieve a more specific conclusion.

Chromatographic analysis of phenolic acids and flavonoids

During the germination of quinoa seeds studied in this work, different profiles of flavonoids and non-flavonoids phenolic compounds were observed. Figure 1 depict the HPLC chromatograms of a representative HPLC separation of a mixture of phenolic acids and flavonoids standards (A), and an ethanolic extracts of raw (B) and germinated quinoa seeds at 72 h (C). Two classes of phenolic acids can be distinguished: derivatives of benzoic acid (*p*-hydroxybenzoic, vanillic, and gallic acid) and derivatives of cinnamic acid (ferulic, *p*-coumaric, and caffeic acid). The samples of raw and germinated quinoa seeds contained different concentrations of *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, ferulic acid, quercetin, and kaempferol. The total content of each phenolic compound during germination process is showed in Table 2. Changes in the levels of identified phenolic compounds between raw and germinated quinoa seeds were observed. At the end of the germination (72 h) an increase of 8.57 fold for phenolic acids was observed, where vanillic and ferulic acids recorded the major amounts (51.2 and 21.6%, respectively) of the total concentration of determined phenolic compounds. In accordance with other work, vanillic acid was the most abundant of identified phenolic acids in Chenopodium quinoa sprouts (Alvarez-Jubete *et al*., 2010). In the same way, ferulic acid was the most abundant phenolic acid in the group of cinnamic acid derivatives followed by p-coumaric acid. It is interesting to point out the great increased of p-coumaric acid during germination period, which increased their content 21-fold at the last germination stage, respect to raw seeds. Other authors also observed an increase of hydroxycinnamic acids during germination of pseudocereals (Pasko *et al*., 2009; Repo-Carrasco *et al*., 2010). The hydroxycinnamic compounds are constituents of the cell wall, mostly found as an ester on the arabinose side-chains of arabinoxylans and lignin (Ishii, 1997). The increases observed of TPC during germination can be explained by the action of endogenous esterases activated during germination which can lead to the release of cell wall bound phenolic compounds (Maillard *et al*., 1996) and/or the novo synthesis (Diaz-Batalla *et al*., 2006).

By other hand, the HPLC analysis of flavonoid group showed a total increased of 4.4 fold, where quercetin was the more abundant compound (1.36 ± 0.06 mg/100 g dwb). In a previous study, a moderate increase in quercetin and kaempferol glycosides

content in germinated quinoa has also been reported (Alvarez-Jubete *et al*., 2010).

Finally, Table 2 shows the composition of each extract (raw and each stage of germination) explained by groups of phenolic compounds. Hydroxybenzoic acid group represented the highest percentage of the total identified phenolic compounds in all samples: 51% in raw and between 57% and 59% in germinated quinoa seeds, while the group of hydroxycinnamic acid represented between 30% and 34% of total. The flavonoids represented the lowest percentage composition in each sample, ranged from 8 to 18%. Total phenolic contents quantified by HPLC were in accordance with those obtained by Folin-Ciocalteau method, showing an increase as sprouting day progressed. Gallic acid, chlorogenic acid and caffeic acid were not found.

Effect of germination on antioxidant activity of quinoa seeds.

Because different antioxidant compounds may act in a plant food matrix through different mechanisms, and the potential synergic interactions that could take place among these molecules, it is not possible to evaluate the total antioxidant activity by a single method in this kind of samples (Pellegrini *et al*., 2003). Thus, a generally accepted practice is to use at least two different methods for the investigation of antioxidant activity of plant food samples (Moon and Shibamoto, 2009). In the present study a combination of assays that included a free radical scavenging method and a lipid peroxidation based method for the evaluation of the antioxidant activity was carried out. In particular, DPPH radical scavenging assay monitored the capacity of phenolic compounds to scavenge free radicals in hydrophilic system, while β-carotene bleaching method monitored the early stage of lipid peroxidation in a hydrophobic system.

Antioxidant activity values of germinated quinoa seeds extracts assessed by the two methods are shown in Table 1. Germination process significantly increased the antioxidant activity, compared to control ($p \leq 0.05$) in both methods. Antioxidant activity evaluated by the lipid peroxidation based method of β-carotene bleaching, registered a slight increase of 10.4% of inhibition on β-carotene oxidation at 3 days germination period, respect to raw seeds. This value was not significantly different compared to the other stages. This small increase in antioxidant activity is due to the low lipidic content of quinoa seed (6.3%), upon which the contribution of lipophilic antioxidants on total antioxidant capacity is scarce. In the same way, due to the polarity of the extraction solvent used in the present work, it is expected that the hydrophilic antioxidants been preferentially dissolved and, as has been reported previously (Lage *et al*., 2013), the contribution of hydrophilic antioxidants will produce a low response in a lipophilic system. However, β-carotene bleaching assay can provide useful complementary information in the study of complex natural extracts containing components with variable degrees of polarity.

Table 3. Correlation coefficients (Pearson coefficient) between antioxidant activity and HPLC identified phenolic compounds

	Phenolic compound					
	p-OH-benzoic	Vanillic	*p*-Coumaric	Ferulic	Quercetin	Kaempferol
Antioxidant activity	0.95	0.99	0.97	0.99	0.95	0.83

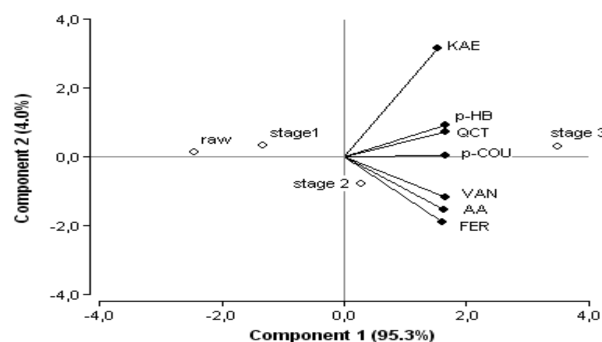

Figure 2. Plot of the principal components of each identified phenolic compound and antioxidant activity in raw and germinated quinoa seeds. AA, antioxidant activity; VAN, vanillic acid; FER, ferulic acid; *p*-COU, p-coumaric acid; QCT, quercetin; *p*-HB, *p*-hydroxybenzoic acid; KAE, kaempferol.

On contrary, DPPH radical scavenging capacity showed significant differences between germination stages, where a 2 fold increase at stage 3 of germination (72 h) was observed. The improvement of antioxidant activity during germination of quinoa seeds has also been observed by other authors. Pasko *et al.* (2008) found that DPPH value was maximal at sixth germination day and this value was significantly higher in quinoa seeds by comparison with amaranth seeds. However, Alvarez-Jubete *et al*. (2010) reported no statistical differences in antioxidant capacity (DPPH radical scavenging assay) between quinoa, amaranth and wheat sprouts after 82, 98 and 110 h of germination, respectively.

The antioxidant activity of phenolic compounds is related to their chemical structure, thus in order to analyze the influence of the identified phenolic compounds over the antioxidant activity through the germination process, a statistical analysis was applied to the data. Table 3 presents the relation between antioxidant activity (DPPH radical scavenging assay) and contents of identified phenolic compounds in raw and germinated quinoa seeds. A strong positive correlation was observed between

these variables. The observed Pearson´s coefficients suggest that there is a linear correlation between the antioxidant activity of the extracts and the content of each phenolic compound. One can conclude that all studied phenolics contribute strongly to antioxidant activity. For better illustration, principal components analysis (PCA) was applied to the data. PCA permits the evaluation of the experimental data set, developing a smaller number of artificial variables (called principal components) that will account for most of the variance of the observed variables. Thus, PCA was used to find out the relationships between the identified phenolic compounds contents and antioxidant activity, which are related to a specific principal component and associated with a particular germination stage.

The results of PCA are shown in Figure 2, where the first two components accounted the 99.3% of the total variance. The component 1 (95.3% of the original data) correlated well with all phenolic compounds contents and antioxidant activity (similar load values). This result indicated that phenolic compounds are closely related to antioxidant activity and, in particular with the stage 3 of germination (72 h) in comparison to raw quinoa. PCA has also provided information about the relative contribution of each phenolic compound to the antioxidant activity. In general, flavonoid compounds have greater antioxidant potentials than phenolic acids (Rice-Evans *et al*., 1996; von Gadow *et al*., 1997). However, in this study, quercetin and kaempferol contributed to a smaller extent (in comparison to phenolic acids) to the antioxidant activity (explained by component 2) of raw and germinated quinoa seeds. This result was also evidenced by the Pearson's coefficient obtained for these two flavonoids (Table 3) and can be explained taking into account the low levels found of these compounds in the extracts (Table 2). Among the identified phenolic acids, ferulic acid was the compound that most influenced the antioxidant activity in quinoa seeds, followed by vanillic acid. It has been reported that ferulic acid (hydroxycinnamic acid derivative) is more efficient antioxidant than vanillic acid (hydroxybenzoic acid derivative) due to a longer distance of carboxyl group to phenyl ring, which has a positive influence on its H-donating ability (Rice-Evans *et al.*, 1996).

Conclusion

Although the consumption of quinoa grain has been limited by geographical and cultural reasons to certain ethnical groups, in the last years new nutritional and culinary trends have revalued this crop as an alternative ingredient, and consequently it is being gradually introduced in diets all over the world. In the present work it was found that germination and subsequent oven-drying at 40ºC of quinoa seeds significantly increased the antioxidant properties compared with raw seeds, especially at three days of germination. The identified phenolic compounds were found to have strong positive correlation on DPPH scavenging activity. Germination process could offer a good strategy to improve the phenolic content in quinoa seeds for enhanced their antioxidant activity properties. Therefore, dried germinated quinoa seeds could be used for obtaining a dietary ingredient in the formulation of foods with functional value.

References

Abugoch James, L.E. 2009. Quinoa (*Chenopodium quinoa* Willd.): Composition, chemistry, nutritional, and functional properties. Advances in Food and Nutrition Research 58: 1-31.

Alvarez-Jubete, L., Wijngaard, H., Arendt, E. and Gallagher, E. 2010. Polyphenol composition and in vitro antioxidant activity of amaranth, quinoa, buckwheat and wheat as affected by sprouting and baking. Food Chemistry 119 (2): 770–778.

Bhargava, A., Shukla, S. and Ohri, D. 2006. Chenopodium quinoa - An Indian perspective. Industrial Crops and Products 23 (1): 73–87.

Brady, K., Ho, C., Rosen, R., Sang, S. and Karwe, M. 2007. Effects of processing on the nutraceutical profile of quinoa. Food Chemistry 100 (3): 1209–1216.

Brand-Williams, W., Cuvelier, M.E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology 28 (1): 25-30.

Diaz-Batalla, L., Widholm, J.M.., Fahey, G.C., Castaño-Tostado, E. and Paredes-López, O. 2006. Chemical components with health implications in wild and cultivated Mexican common bean seeds (*Phaseolus vulgaris* L.). Journal of Agricultural and Food Chemistry 54 (6): 2045-2052.

Dini, I., Tenore, G.C. and Dini, A. 2010. Antioxidant compound contents and antioxidant activity before and after cooking in sweet and bitter *Chenopodium quinoa* seeds. LWT - Food Science and Technology 43 (3): 447–451.

FAO, 2011. Quinoa: An ancient crop to contribute to world food security. Technical report of the 37th FAO Conference. Rome, Italy.

Gorinstein, S., Vargas, O.J.M.., Jaramillo, N.O., Salas, I.A., Ayala, A.L.M., Arancibia-Avila, P., Toledo, F., Katrich, E. and Trakhtenberg, S. 2007. The total polyphenols and the antioxidant potentials of some selected cereals and pseudocereals. European Food Research and Technology 225 (3-4): 321-328.

Hirose, Y., Fujita, T., Ishii, T. and Ueno, N. 2010. Antioxidative properties and flavonoid composition of

Chenopodium quinoa seeds cultivated in Japan. Food Chemistry 119(4):1300-1306.

Ishii, T. 1997. Structure and functions of feruloylated polysaccharides. Plant Science127 (2):111-127.

Jian, G.X., Cheng, R.T., Qing, P.H., Ji, Y.L., Xiang, D.W. and Xiang, D.T. 2009. Dynamic changes in phenolic compounds and antioxidant activity in oats (*Avena nuda* L.) during steeping and germination. Journal of Agricultural and Food Chemistry 57(21):10392-10398.

Kim, S.L., Kim, S.K. and Park, C.H. 2004. Introduction and nutritional evaluation of buckwheat sprouts as a new vegetable. Food Research International 37(4):319-327.

Lage, M.T.P., García, M.A.M., Álvarez, J.A.V., Anders,Y. and Curran, T.P. 2013. A new microplate procedure for simultaneous assessment of lipophilic and hydrophilic antioxidants and pro-oxidants, using crocin and β-carotene bleaching methods in a single combined assay: Tea extracts as a case study. Food Research International 53 (2):836-846.

Lintschinger, J., Fuchs, N., Moser, H., Jäger, R., Hlebeina, T., Markolin, G. and Gössler, W. 1997. Uptake of various trace elements during germination of wheat, buckwheat and quinoa. Plant Foods for Human Nutrition 50 (3):223-237.

Liyana-Pathirana, C. and Shahidi, F. 2005. Optimization of extraction of phenolic compounds from wheat using response surface methodology. Food Chemistry 93 (1): 47-56.

Maillard, M.N., Soum, M.H., Boivin, P. and Berset, C. 1996. Antioxidant activity of barley and malt: Relationship with phenolic content. LWT - Food Science and Technology 29 (3): 238-244.

Megat Rusydi, M.R. and Azrina, A. 2012. Effect of germination on total phenolic, tannin and phytic acid contents in soy bean and peanut. International Food Research Journal 19 (2): 673-677.

Miranda, M., Vega-Gálvez, A., López, J., Parada, G., Sanders, M., Aranda, M., Uribe, E. and Di Scala, K. 2010. Impact of air-drying temperature on nutritional properties, total phenolic content and antioxidant capacity of quinoa seeds (*Chenopodium quinoa* Willd.). Industrial Crops and Products 32 (3): 258-263.

Moon, J.K. and Shibamoto, T. 2009. Antioxidant assays for plant and food components. Journal of Agricultural and Food Chemistry 57 (5):1655-1666.

Paśko, P., Bartoń, H., Zagrodzki, P., Gorinstein, S., Fołta, M. and Zachwieja, Z. 2009. Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth. Food Chemistry 115 (3): 994-998.

Paśko, P., Sajewicz, M., Gorinstein, S. and Zachwieja, Z. 2008. Analysis of selected phenolic acids and flavonoids in *Amaranthus cruentus* and *Chenopodium quinoa* seeds and sprouts by HPLC. Acta Chromatographica 20 (4): 661-672.

Pellegrini, N., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S. Bianchi, M. and Brighenti, F. 2003. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different *in vitro* assays. Journal of Nutrition 133 (9): 2812-2819.

Repo-Carrasco-Valencia, R., Hellström, J.K., Pihlava, J.M. and Mattila, P.H. 2010. Flavonoids and other phenolic compounds in Andean indigenous grains: Quinoa (*Chenopodium quinoa*), kañiwa (*Chenopodium pallidicaule*) and kiwicha (*Amaranthus caudatus*). Food Chemistry 120 (1):128-133.

Rice-Evans, C.A., Miller, N.J. and Paganga, G. 1997. Antioxidant properties of phenolic compounds. Trends in Plant Science 2 (4):152-159.

Ross, K.A., Beta, T. and Arntfield, S.D. 2009. A comparative study on the phenolic acids identified and quantified in dry beans using HPLC as affected by different extraction and hydrolysis methods. Food Chemistry 113 (1):336-344.

Sharma, P. and Gujral, H.S. 2010. Antioxidant and polyphenol oxidase activity of germinated barley and its milling fractions. Food Chemistry 120 (3):673-678.

Singleton, V.L., Orthofer, R. and Lamuela-Raventós, R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology 299: 152-178.

Suja, K.P., Jayalekshmy, A. and Arumughan, C. 2005. Antioxidant activity of sesame cake extract. Food Chemistry 91(2): 213-219.

von Gadow, A., Joubert, E. and Hansmann, C.F. 1997. Comparison of the Antioxidant Activity of Aspalathin with That of Other Plant Phenols of Rooibos Tea (*Aspalathus linearise*), α-Tocopherol, BHT, and BHA. Journal of Agricultural and Food Chemistry 45 (3): 632-638.