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Original article

Effect of roasting conditions on the antioxidant compounds of quinoa seeds

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Summary The effect of different roasting conditions on the antioxidant properties and the phenolic content of quinoa seeds was studied. Advanced and final products of the Maillard reaction were also quantified in order to evaluate the contribution to DPPH radical scavenging capacity and reducing power of samples. In general, response surface analysis showed significant increases in the phenolic content, the antioxidant activity and the level of Maillard reaction products (MRPs), mainly as processing temperature increased, while roasting time had a minor impact on these response variables. The highest antioxidant activity was achieved in extracts of quinoa seeds roasted at 190 °C for 30 min. Principal component analysis applied to the data suggested that MRPs had a greater contribution to antioxidant properties than phenolic compounds in the processed samples. These results demonstrated that roasted quinoa seeds/flour may be considered as a nontraditional ingredient with enhanced antioxidant capacity for the production of functional foods.

Keywords Antioxidant activity, *Chenopodium quinoa*, maillard reaction products, phenolic compounds, roasting.

Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a native Andean crop that has gained worldwide popularity over the last years mainly due to its outstanding nutritional properties and supply of antioxidant compounds with beneficial health properties, including phenolic acids, tocopherols, betalains and flavonoids (Abugoch James, 2009; Tang *et al.*, 2015). In addition, quinoa seed is naturally gluten-free, and thus, it is currently emerging as healthy alternative ingredient for the production of novel gluten-free products (Alvarez-Jubete *et al.*, 2010).

Like most other grains, quinoa seeds are generally processed before consumption. They may be milled to make various baked products, or also roasted or inflated to make breakfast foods and snacks (Abugoch James, 2009; Repo-Carrasco-Valencia *et al.*, 2010a,b). Between these different feasible processes, roasting is able to alter the texture, colour, flavour and appearance of grains, whereas the resulting product develops unique features of crispness and taste as compared to the raw kernels (Nicoli *et al.*, 1999; Chandrasekara & Shahidi, 2011). In addition, several studies have shown that roasting processing of different edible seeds modifies the phenolic profile, and in some cases, it improves the health benefit effects by enhancing their antioxidant capacity (Dewanto *et al.*, 2002; Jeong *et al.*, 2004; Açar *et al.*, 2009; Chandrasekara & Shahidi, 2011; Kim *et al.*, 2011), although the net effect depends on the grain considered and roasting conditions employed. The net effect of roasting on antioxidant capacity of grains depends on the balance between the thermal degradation of naturally occurring antioxidant compounds and the formation of Maillard reaction products (MRPs) that may influence the antioxidant capacity (Açar *et al.*, 2009). Thus, selection of appropriate roasting conditions for a sensorially acceptable product with an improved antioxidant capacity is essential in the roasting operation.

Roasted quinoa seeds have been previously evaluated with different purposes. Brady *et al.* (2007) reported degradation of saponins in quinoa seeds roasted at 200 °C for 10 min. Repo-Carrasco-Valencia *et al.* (2010a) measured the mineral availability of seeds after roasting quinoa at 190 °C for 3 min. A recent work evaluated the influence of roasted quinoa (177 °C, 15–45 min) on sensory and physicochemical properties in the formulation of cakes (Rothschild

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et al., 2015). To the best of our knowledge, there are no previous studies showing the effect of roasting on the phenolic content and antioxidant properties of quinoa seeds.

The objective of this study was to determine the effect of different temperatures and roasting times on the contents of phenolic compounds, MRPs and the antioxidant properties of quinoa seeds.

Materials and methods

Samples and chemicals

Quinoa seeds (*C. quinoa* Willd.) were obtained from Buenos Aires province (Argentina) during September 2012. Seeds were cleaned and stored in polyethylene containers at room temperature until use. Folin–Ciocalteau reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, ferulic acid, quercetin, kaempferol, quinine sulphate and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were supplied by Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Sodium carbonate, potassium ferricyanide, trichloroacetic acid, aluminium chloride, sodium hydroxide and ferric chloride were supplied by Merck (Darmstadt, Germany). Ethanol, methanol and hydrochloric acid were of analytical grade.

Experimental design and statistical analysis

Whole quinoa seeds (20 g) were placed in an aluminium Petri dish (7 cm diameter) and roasted in a forced hot-air convection oven (model FD 23; Binder Inc., Tuttlingen, Germany) at 100, 130, 160 or 190 °C for 15, 30, 60, 90 or 120 min. After roasting, the seeds were allowed to cool to ambient temperature in a desiccator. Then, roasted seeds were milled using a laboratory grinder (Yellow line, A10; IKA-Werke, Staufen, Germany) and sieved (0.5 mm), and the obtained flour was used for further analysis. Simultaneously, moisture determination of samples was determined gravimetrically, to refer the results on dry weight basis (DW). Each combination of temperature and time was performed in triplicate. The normality and homogeneity of the variances of the residuals were verified before applying analysis of variance (ANOVA) and Tukey post hoc tests ($P \leq 0.05$) on the experimental results. For each response (total phenolic and flavonoid content, DPPH radical scavenging activity, reducing power, fluorescent advanced and final MRPs), a quadratic equation model was used. Regression analysis was made, and the response surfaces were plotted using Statgraphics Centurion XVI (version 16.1.18; Statpoint Technologies Inc., Warrenton, VA, USA). The fit of the models to the experimental data was given by the coefficient of determination (R^2), which explains the extent of variance that can be explained by the model in each response. In addition, each model was validated by calculating the value of the lack of fit test, in which a P-value >0.05 indicates that the model is adequate to predict the response values. Principal components analysis (PCA) was also applied to experimental data using InfoStat statistical software (version 2015e; InfoStat Group, Córdoba, Argentina).

Extraction of soluble phenolic compounds

To obtain an extract rich in antioxidant compounds, raw and roasted quinoa flour was extracted twice with 80% ethanol following the optimised extraction conditions described in a previous work (Carciochi *et al*., 2014). After extraction, both supernatants were pooled, filtered (0.45 μm) and stored at −18 °C for further analysis.

Total phenolic content (TPC)

Total phenolic content in extracts was determined using Folin–Ciocalteau reagent, following the method described by Singleton *et al*. (1999). The extracts (100 μL) were mixed with 2 N Folin–Ciocalteau reagent (100 μL) and 20% sodium carbonate solution (300 μL). Then, the volume of the mixture was adjusted to 2 mL with distilled water and incubated in the dark for 2 h at room temperature (20 °C). After incubation, the absorbance of the mixture was measured at 765 nm using an UVmini 1240 spectrophotometer (Shimadzu, Noisiel, France). The results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g DW.

Total flavonoid content (TFC)

Total flavonoid content was determined by the aluminium chloride colorimetric method described by Dini *et al*. (2010). Briefly, 0.25 mL of extract was mixed with 0.15 mL of 5% sodium nitrite solution in a test tube. After 5 min, 0.15 mL of 10% aluminium chloride solution was added and mixed for 6 min, before adding 1 mL of 1 M sodium hydroxide solution. The final mixture was thoroughly mixed, and their absorbance was determined at 510 nm against a blank reaction. Total flavonoid content of extracts was expressed as milligrams of quercetin equivalents (QE) per 100 g DW.

Antioxidant activity

Antioxidant activity of extracts was evaluated by DPPH radical scavenging activity measured according to Brand-Williams *et al*. (1995). Aliquots (50 μL) of

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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. The antioxidant activity was calculated on the basis of percentage of DPPH radical scavenging and expressed as micromoles (μmol) of Trolox equivalents (TE) per 100 g DW.

Reducing power

The ability of the extracts to reduce Fe^{3+} was assayed by the method described by Chandrasekara & Shahidi (2011). Briefly, 0.25 mL of extract, 0.25 mL of 0.2 M phosphate buffer (pH 6.6) and 0.25 mL of 1% potassium ferricyanide were mixed and incubated at 50 °C for 20 min. Then, 0.25 mL of 10% trichloroacetic acid, 0.4 mL of distilled water and 0.1 mL of 0.1% ferric chloride were added to the mixture. After 10 min, the absorbance was measured at 700 nm, and the results were expressed as μg of GAE per mL of extract.

Extraction and quantification of fluorescent advanced and final MRPs

Extractions of MRPs were performed according to Michalska *et al.* (2008). Powdered samples (0.1 g) were mixed with 6% aqueous SDS solution (2 mL), kept in agitation for 30 min and centrifuged at 10 677***g*** for 5 min. The supernatant was subsequently diluted up to 10 mL with distilled water and filtered through a 0.45-μm nylon filter (Agilent Technologies, Santa Clara, CA, USA). Filtrates were employed for analysis of available fluorescence intermediary compounds (FIC) and final MRPs (melanoidins) according to the methods described by Michalska *et al.* (2008).

Fluorescence intermediary compound formed in advanced Maillard reaction stages were estimated using a LS 50B spectrofluorimeter (Perkin Elmer, Wellesley, MA, USA) with slit width set at 2 nm. Fluorescence readings were performed in quartz glass cuvettes with light path of 1 cm. Based on spectra data, FIC were measured at $\lambda_{Ex} = 353$ and $\lambda_{Em} = 438$ nm. The quantification of fluorescent compounds was made on the basis of quinine emission equivalents, using a calibration curve constructed with several concentrations of quinine sulphate in 0.2 M sulphuric acid. Results were expressed in μg of quinine equivalents per gram of sample DW (Farroni & Buera, 2012).

Development of brown pigments (melanoidins) in samples was estimated as absorbance at 420 nm using an UVmini 1240 spectrophotometer (Shimadzu). Measurements were performed after appropriate dilution with distilled water using plastic cuvettes with light path of 1 cm. Results were corrected by sample mass and expressed as arbitrary absorbance units.

HPLC analysis of phenolic compounds

Reversed-phase HPLC method for determination of phenolic acids and flavonoids was used in the conditions described in a previous work (Carciochi *et al.*, 2014). The analytical HPLC system employed was a Waters 600 high-performance liquid chromatograph equipped with a Waters 2996 diode array detector (Waters Corporation, Milford, MA, USA). Software used for data acquisition and control of HPLC pumps, autosampler, and diode array system was Empower (Waters Corporation). 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 × 4.6 mm, 3 μm) (Phenomenex, Torrance, CA, USA) maintained at 25 °C. 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, 0–5 min; linear gradient from 10% B to 35% B, 5–15 min; isocratic elution 35% B, 15–20 min, linear gradient from 35% B to 100% B, 20–30 min; isocratic elution 100% B, 30–37 min; linear gradient from 100% B to 10% B, 37–41 min; post-time 8 min before next injection. Total run time was 49 min. The flow rate of the mobile phase was 0.6 mL min^{-1}, 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. Phenolic acids and flavonoids were quantified as aglycones in triplicate using the external standard method. Standard calibration curves for *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, ferulic acid, quercetin and kaempferol were obtained in the range of 5–500 μg mL^{-1} with correlation coefficients >0.99, respectively. The amount of each compound (mg per 100 g DW) was correlated with the corresponding peak areas expressed in arbitrary units.

Results and discussion

Effect of roasting conditions on the phenolic profile of quinoa seeds

The contents of total phenolic and flavonoid compounds of quinoa seed extracts obtained under different processing conditions are shown in Fig. 1. After treatment, both responses increased significantly, principally as the roasting temperature increased. Roasting time was also significant in both cases (*P*-value of 0.0125 and 0.0299 for TPC and TFC, respectively), but with lower impact on yields. The content of total phenolic compounds obtained for raw seeds was

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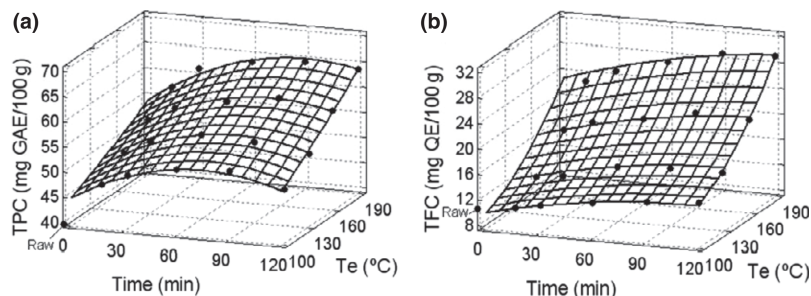

Figure 1 Influence of roasting time and temperature (Te) on total phenolic content (a) and total flavonoid content (b) of quinoa seeds. Points represent experimental data.

40.15 ± 1.08 GAE/100 g DW, whereas the observed increments after roasting ranged from 18% to 60%, the latter obtained in the samples processed at 190 °C for 30 min (Fig. 1a). Regarding TFC, the amount of total flavonoids in the extracts increased from 11.21 ± 0.34 QE/100 g DW in unroasted sample to 29.96 ± 1.27 QE/100 g DW in samples roasted at 190 °C for 90 min (Fig. 1b). According to the results obtained, thermal processing increased free TPC and TFC of quinoa seeds. These results are in agreement with similar studies conducted using other types of kernels (Dewanto *et al.*, 2002; Chandrasekara & Shahidi, 2011; Kim *et al.*, 2011). Jeong *et al.* (2004) showed that roasting (200 °C for 60 min) increased the TPC of sesame seeds by 145% compared to the raw counterpart. In another study, a heat treatment at 125 °C for 30 min increased about 100% TPC levels in barley seeds (Gallegos-Infante *et al.*, 2010).

In addition to phenolics, other compounds such as those formed during Maillard reaction of roasted seed samples could interfere with the determination of TPC by Folin–Ciocalteu's assay (Chandrasekara & Shahidi, 2011), giving overestimated results. As a way to partially explain this result, a chromatographic analysis of major phenolic compounds in quinoa was performed. Based on the results of the statistical analysis, phenolic profiles of raw and roasted quinoa seed at 100, 145 and 190 °C for 30 min were compared. The HPLC analysis detected and confirmed the presence of six phenolic compounds in quinoa seed extracts in accordance with the results obtained by Repo-Carrasco-Valencia *et al.* (2010b). Changes in the levels of *p*-hydroxybenzoic, vanillic, *p*-coumaric and ferulic acids, as well as quercetin and kaempferol, were detected between raw and roasted quinoa samples (Table 1). Analysing the phenolic compounds according to their chemical structure, the most significant increase was observed for benzoic acid derivatives (*p*-hydroxybenzoic acid and vanillic acid), which increased their content as roasting temperature increased. Cinnamic acid derivatives (*p*-coumaric acid and ferulic acid) showed a marked increase in their contents after the treatment performed at 145 °C, which resulted in a drastic decrease in the levels of both acids at higher temperature of process. Regarding flavonoids, they were not variations in their levels upon 145 °C, whereas a sharp increase was observed for both compounds at 190 °C.

These results indicated a greater thermal stability of hydroxybenzoic acids in comparison with hydroxycinnamic acids. It has been mentioned that degradation of phenolics acids depends on the number and type of substituent groups (especially, hydroxyl and methoxyl) on the ring structure (Liazid *et al.*, 2007; Ma *et al.*, 2008), which may act as an activating group that helps to thermal decarboxylation of phenolic acids (Lindquist & Yang, 2011). Thus, carboxylic group on the acrylic acid side chain in hydroxycinnamic acids might be more easily decarboxylated than carboxylic group attached to the benzene ring in hydroxybenzoic acids (Khuwijitjaru *et al.*, 2014).

These results confirmed that beyond the thermal stability of individual phenolic compounds, the levels of free phenolic acids and flavonoids after heat treatment were increased. This could be explained considering that phenolic compounds originally bounded in the seed matrix by glycosylation/esterification could be released as a consequence of roasting, making them more soluble in the extraction solvent (Dewanto *et al.*, 2002; Jeong *et al.*, 2004; Chandrasekara & Shahidi, 2011). Similarly, Craft *et al.* (2010) observed marked increases in the content of free *p*-coumaric acid after roasting peanuts at 150 °C for 15 min, whereas all *p*-coumaric acid derivative peaks decreased their intensity. These authors explained that thermal treatment caused the liberation of phenolic acid aglycones from their parent derivatives. On the other hand, some authors have mentioned that in addition to the dissociation of the phenolic conjugated forms, some subsequent polymerisation/oxidation reactions of the phenolic constituents may also occur through roasting process, thus increasing the phenolic level (Manzocco *et al.*, 1998; Ee *et al.*, 2011).

Effect of roasting conditions on MRPs formation

At advanced stages of Maillard reaction formation of fluorescence compounds as well as cross-linking

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Table 1 Contents of phenolic acids and flavonoids (mg/100 g DW) in raw and roasted quinoa seed extracts determined by HPLC analysis

Compound	Raw (0 min)	100 °C (30 min)	145 °C (30 min)	190 °C (30 min)
p-OH-benzoic acid	0.22 ± 0.02 a	0.79 ± 0.02 b	1.42 ± 0.02 c	3.31 ± 0.06 d
Vanillic acid	0.88 ± 0.11 a	3.66 ± 0.04 b	6.70 ± 0.07 c	11.35 ± 0.02 d
p-Coumaric acid	0.09 ± 0.10 a	0.18 ± 0.01 a	2.33 ± 0.04 b	0.03 ± 0.01 a
Ferulic acid	0.57 ± 0.09 a	0.54 ± 0.02 a	2.78 ± 0.01 b	0.61 ± 0.01 a
Total phenolic acids	1.75 ± 0.06 a	5.16 ± 0.03 b	13.23 ± 0.04 c	15.31 ± 0.03 d
Quercetin	0.23 ± 0.02 a	1.02 ± 0.45 a,b	1.95 ± 0.18 b	1.98 ± 0.01 b
Kaempferol	0.15 ± 0.04 a	0.16 ± 0.01 a	0.79 ± 0.02 b	2.00 ± 0.01 c
Total flavonoids	0.37 ± 0.03 a	1.19 ± 0.23 b	2.74 ± 0.10 c	3.98 ± 0.01 d
Total phenolic compounds	2.13 ± 0.06 a	6.35 ± 0.13 b	15.96 ± 0.07 c	19.28 ± 0.02 d

Values (means ± SD, $n = 3$) within a row with different letter are significantly different ($P \leq 0.05$).

products are formed (Matiacevich & Buera, 2006), which can be estimated measuring the maximal fluorescence emission at 340–370 nm excitation wavelengths of samples (Morales & Van Boekel, 1997). The final products of Maillard reaction are melanoidins, which are responsible for the brown colour development commonly estimated as absorbance value at 420 nm. Advanced MRPs as well as the resultant brown pigments have high antioxidant activities (Manzocco *et al.*, 2000; Chandrasekara & Shahidi, 2011); therefore, an estimation of their contents in roasted quinoa seeds was performed.

Figure 2a shows the results of fluorescent MRPs. Scarce formation of FIC was detected for the treatments performed at 100 and 130 °C, which showed no statistical significance ($P > 0.05$) compared to raw seed extract. The FIC level of the latter was 1.81 ± 0.08 μg of quinine equivalents per g of sample DW, which may be due to fluorescent compounds naturally occurring in quinoa grain, such as tryptophan, tyrosine and phenylalanine. Conversely, treatments carried out a 160 °C, and especially at 190 °C recorded the largest increases in FIC levels. Interestingly, at both temperatures an increasing trend in FIC values up to reach its maximum value (at 90 and 60 min for 160 and 190 °C treatments, respectively) was observed. After these points, values remained constant or even slightly decreased for further processing times. These results probably indicate that Maillard reaction progressed to the final stages, because it has been suggested that FIC formed may be precursors of brown pigments and/or final MRPs (Delgado-Andrade *et al.*, 2006; Matiacevich & Buera, 2006). This behaviour can be observed in Fig. 2b, in which the development of brown pigments increased significantly as both, temperature and processing time increased, except for the treatment carried out at 100 °C, in which no significant differences ($P > 0.05$) in brown pigments were observed for any of the time tested, respect to unroasted control extract.

Effect of roasting conditions on the antioxidant activity

The DPPH radical scavenging method is based on the measurement of the reducing ability of antioxidants towards DPPH, a stable synthetic organic radical which can be monitored by measuring the decrease in the absorbance at 517 nm (Kedare & Singh, 2011). This assay is widely used to examine the overall antioxidant activity of plant food samples because of its speed, ease and reliability.

DPPH radical scavenging activity of raw and roasted quinoa extracts is presented in Fig. 3a. The level observed for raw quinoa extract was 266.0 ± 15.9 μmol TE/100 g DW, which was comparable to those reported by other authors using the same method in quinoa seeds (Alvarez-Jubete *et al.*, 2010; Dini *et al.*, 2010; Vollmannová *et al.*, 2013). Roasting process at 100 °C or 130 °C did not change the radical scavenging activity of quinoa extracts significantly ($P > 0.05$) compared to unroasted sample; even a decrease was observed in the samples roasted up to 30 min for both temperatures. This behaviour has been observed in other heat-treated foods and can be attributed to the degradation of naturally occurring thermolabile antioxidants such as ascorbic acid (Nicoli *et al.*, 1999). Furthermore, it has been suggested that polyphenols, ascorbic acid and other carbonyl compounds can take part to the Maillard reaction itself, causing a decrease in antioxidant activity (Manzocco *et al.*, 2000; Kaur & Kapoor, 2001). Other authors have suggested that the MPRs formed at low temperatures in the early stages of browning, have pro-oxidant properties that could contribute to the decrease in antioxidant capacity observed under these conditions (Nicoli *et al.*, 1999; Kaur & Kapoor, 2001). Conversely, roasting treatments performed at 160 °C, and especially at 190 °C increased significantly the DPPH radical scavenging activities of seeds. Thus, after 60 min at 160 °C or 30 min at 190 °C, the radical scavenging activity of quinoa seed extracts increased 78% and 135% respect to unroasted sample, respectively. The maximum value reached in each case remained without significant

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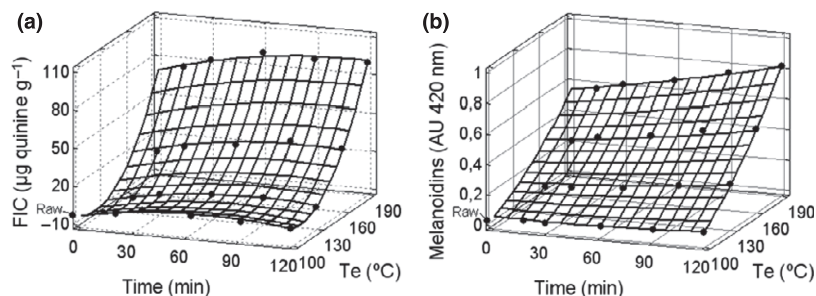

Figure 2 Influence of roasting time and temperature (Te) on FIC (a) and brown pigments (b) of quinoa seeds. Points represent experimental data. AU 420 nm, absorbance units at 420 nm.

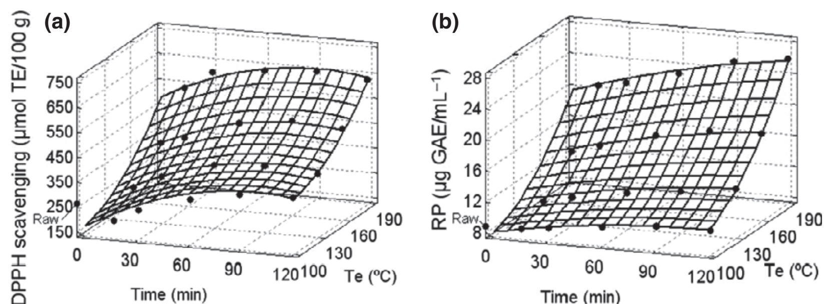

Figure 3 Influence of roasting time and temperature (Te) on DPPH radical scavenging (a) and reducing power (b) of quinoa seeds. Points represent experimental data.

changes for further processing times. These results are in agreement with other roasting studies carried out with edible common seeds, in which the antioxidant activity resulted increased with a greater intensity of heat treatment (high temperatures and/or prolonged time), mainly due to the release of phenolics from the bound fraction therein and/or the formation of new MRPs having antioxidant activity (Jeong *et al.*, 2004; Açar *et al.*, 2009; Chandrasekara & Shahidi, 2011; Kim *et al.*, 2011).

The reducing power of an extract serves as a good indicator of its antioxidative activity (Chandrasekara & Shahidi, 2011). Figure 3b shows the observed reducing power level of samples. Reducing power of extracts increased as both, temperature and roasting time increased, except for the treatment performed at 100 °C, which did not show significant differences ($P > 0.05$) respect to unroasted control extract. Thus, the reducing power in the extract increased from 8.8 ± 1.3 μg GAE mL^{-1} in unroasted control to 25.9 ± 1.7 μg GAE mL^{-1} after 120 min of roasting at 190 °C. The increased reducing power observed in high-temperature processed samples could be due to the ability to donate electrons from released phenolic compounds (as it has been confirmed in this study) or also from new reductone-like compounds formed during heat treatment (Chandrasekara & Shahidi, 2011).

Contribution of the phenolic compounds and MRPs to the antioxidant activity

This study clearly demonstrated that roasting of quinoa seeds had a significant effect on the antioxidant activity of the extracts, and this could be attributed to their phenolic contents as well as to the presence of MRPs. Thus, in an attempt to establish the relationships between the different variables analysed and their contribution on the antioxidant capacity, PCA was performed using standardised data. The obtained biplot is shown in Fig. 4. Biplot allows visualising observations (indicated with black circles) and variables (plotted as vectors from the origin) in the same space, and thus, it is possible to identify associations and to make interpretations of the joint relations between them. The observations located in the same direction of a given variable have relatively high load for that variable and a low load for variables plotted in an opposite direction. On the other hand, the angles between vectors can be interpreted in terms of correlations between variables. 90° angles between two variables indicate that both variables are not correlated, while 0° or 180° angles indicate strong positive and negative correlation, respectively. Also, similar lengths between the vectors suggest similar contributions of each variable on the representations made.

Figure 4 showed that the first two principal components (PC) accumulated 99.0% of the total variance. All evaluated variables were positively correlated to PC 1 (93.8% of the original data) with similar load values, which indicates that phenolic compounds as well as MRPs are closely related to the antioxidant activity and reducing power of samples. Interestingly, phenolic compounds were positively correlated to PC 2 (5.2% of the original data), while MRPs negatively correlated on the same PC. The fact that PC1 and

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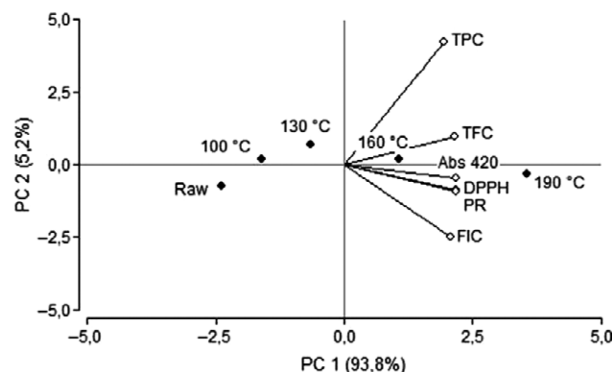

Figure 4 Biplot of PCA showing the evaluated variables and samples of quinoa seeds. TPC, total phenolic content; TFC, total flavonoid content; Abs 420, absorbance at 420 nm (melanoidins); DPPH, antioxidant activity evaluated by DPPH method; RP, reducing power; FIC, fluorescent intermediary compounds.

PC2 are orthogonal assures that information provided by PC1 about variability is different than that explained by PC2.

According to the relative contribution of each variable analysed on antioxidant activity, it was observed that MRPs were the main contributor to DPPH radical scavenging and reducing power of samples. Among the phenolic compounds, the greatest influence on the antioxidant activity was due to flavonoid compounds. Also, the location of the samples on axes showed a shift towards positive values of PC 1 as roasting temperature increased, indicating that the treatment performed at 160 °C and, especially at 190 °C were more associated with the studied variables.

Finally, it is worth mentioning the implications that the intake of dietary MRPs could have on human health. This is a topic that is gaining great interest because the consumption of thermally processed foods, and consequently of MRPs, has increased in the last years. Although some studies have presented evidence about harmful effects resulting from MRPs intake, it have also been reported that some MRPs, particularly melanoidins, have beneficial effects as anti-inflammatory, antimicrobial, prebiotic, antihypertensive and antioxidant, among others (Wang *et al*., 2011; Poulsen *et al*., 2013; Delgado-Andrade, 2014). In this context, and taking into account the diversity of compounds formed in the different food matrices, it is difficult to have conclusive information about the physiological consequences derived from MRPs intake. At this point, according to Delgado-Andrade (2014), a reasonable strategy to limit the negative effects and to preserve the positive properties of dietary MRPs seems to be maintaining a balanced and varied diet, not only in the type of food consumed, but also in the way which is processed.

Conclusion

Dry thermal processing enhanced the antioxidant capacity of quinoa seeds. Results showed that evaluated variables increased their levels mainly as processing temperature increased, while roasting time was also significant but with a minor impact on the response levels. Thus, antioxidant activity and reducing power were further improved in samples processed at higher temperatures, in which the degradation of thermolabile antioxidant compounds naturally present was overcome by the release of phenolic compounds and the formation of MRPs having antioxidant properties. Roasted quinoa seeds/flour may therefore be considered as a nontraditional ingredient with high antioxidant capacity for the production of functional foods.

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Conflict of interest

The authors declare no conflict of interests. This work does not contain any studies with human or animal subjects.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Phenolic profiles of raw (a) and roasted quinoa seed at 100 °C (b), 145 °C (c) and 190 °C (d) for 30 min. Peak identification: 1 = *p*-hydroxybenzoic acid; 2 = vanillic acid; 3 = *p*-coumaric acid; 4 = ferulic acid; 5 = quercetin; 6 = kaempferol.

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