Antioxidant Activity of Free and Bound Compounds in Quinoa (*Chenopodium quinoa* Willd.) Seeds in Comparison with Durum Wheat and Emmer

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Abstract: Antioxidant activity (AA) of quinoa (*Chenopodium quinoa* Willd.) seeds, as well as of durum wheat (*Triticum turgidum* L. ssp. *durum* Desf.) and of emmer (*T. turgidum* L. ssp. *dicoccum* Schübler) grains, was evaluated by studying hydrophilic (H), lipophilic (L), free-soluble (FSP) and insoluble-bound (IBP) phenolic extracts using the new lipoxygenase/4-nitroso-*N,N*-dimethylaniline (LOX/RNO) method, able to simultaneously detect different antioxidant mechanisms, as well as using the Oxygen Radical Absorbance Capacity (ORAC) and the Trolox Equivalent Antioxidant Capacity (TEAC) assays, which measure the scavenging activity against peroxyl and ABTS [2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonate)] radicals, respectively. The species under study were compared with respect to the sum of AA values of H, L and FSP extracts ($AA_{H+L+FSP}$), containing freely solvent-soluble antioxidants, and AA values of IBP extracts (AA_{IBP}), representing the phenolic fraction ester-linked to insoluble cell wall polymers. The LOX/RNO and ORAC methods measured in quinoa flour a remarkable $AA_{H+L+FSP}$ higher than durum wheat, although lower than emmer; according to the same assays, the IBP component of quinoa resulted less active than the durum wheat and emmer ones. The TEAC protocol also revealed a high $AA_{H+L+FSP}$ for quinoa. Interestingly, the ratio $AA_{H+L+FSP}/AA_{H+L+FSP+IBP}$, as evaluated by the LOX/RNO and ORAC assays, resulted in quinoa higher than that of both durum wheat and emmer, and much higher than durum wheat, according to the TEAC protocol. This may suggest that antioxidants from quinoa seeds may be more readily accessible with respect to that of both the examined wheat species.

Keywords: antioxidant activity, durum wheat, emmer, seed extracts, quinoa

Practical Applications: Quinoa seeds may represent an excellent source of natural antioxidant compounds and, in particular, of the free-soluble antioxidant fraction. These compounds may improve nutritive and health-beneficial properties of quinoa-based gluten-free products, thus expanding interest for quinoa utilization from celiac patients to the general population.

Introduction

Quinoa (*Chenopodium quinoa* Willd.), an Amaranthacean food plant native of Andean region, has received an increasing attention in recent years. This renewed interest is due to the excellent nutrient profile of its seeds (Abugoch 2009; Vega-Gálvez and others 2010), that are also naturally gluten-free and so currently emerging as healthy alternatives to gluten-containing grains (Alvarez-Jubete and others 2009).

Increasing appreciation of the nutritional and functional properties of quinoa has also encouraged in the last years some investigations about antioxidant properties of this species. In these reports, *in vitro* antioxidant activity (AA) of quinoa seeds was assessed in relation to phenolic content and composition (Dini and others 2010; Miranda and others 2010) and compared to that of some legumes (soybean), cereals (common wheat, rice, barley, millet) and Amaranthacean (amaranth) and Polygonacean (buckwheat) crops (Gorinstein and others 2007, 2008; Nsimba and others 2008; Paśko and others 2009; Alvarez-Jubete and others 2010; Hirose and others 2010; Chlopicka and others 2012). Furthermore, the impact of different types of processing, including boiling (Dini and others 2010), hot air-drying (Miranda and others 2010), bread-making (Alvarez-Jubete and others 2010; Chlopicka and others 2012), and sprouting (Paśko and others 2009; Alvarez-Jubete and others 2010), was also evaluated. These reports showed interesting antioxidant properties of quinoa seeds: AA values were often much higher than those of compared species and were maintained even after cooking the seeds or bread-making the flour (Alvarez-Jubete and others 2010; Dini and others 2010; Chlopicka and others 2012). From a methodological point of view, in most of these studies methanol or acidic methanol extracts were investigated (Gorinstein and others 2007; Paśko and others 2009;

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Alvarez-Jubete and others 2010; Dini and others 2010; Hirose and others 2010; Miranda and others 2010; Chlopicka and others 2012). Moreover, AA was evaluated using assays able to mainly measure scavenging or reducing capacity toward single nonbiological radical species or oxidants: the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay (Gorinstein and others 2007; Paśko and others 2009; Alvarez-Jubete and others 2010; Dini and others 2010; Hirose and others 2010; Miranda and others 2010; Chlopicka and others 2012); the ABTS [2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonate)] radical cation scavenging capacity assay or Trolox equivalent antioxidant capacity, TEAC (Gorinstein and others 2007; Paśko and others 2009) the Ferric Reducing Antioxidant Power (FRAP) assay (Paśko and others 2009; Alvarez-Jubete and others 2010; Dini and others 2010; Chlopicka and others 2012). In 2 studies, AA of various extracts and fractions obtained from quinoa seeds using solvents of different polarity was evaluated (Gorinstein and others 2008; Nsimba and others 2008). This was made by using the above mentioned DPPH (Nsimba and others 2008) and FRAP (Gorinstein and others 2008; Nsimba and others 2008) methods, as well as the β-carotene bleaching, the Total Peroxyl Radical-Trapping Antioxidant Parameter (TRAP), the Cupric-Reducing Antioxidant Capacity (CUPRAC), and the nitric oxide radical assays (Gorinstein and others 2008).

To date, AA has not been investigated taking into account the different contribution of freely soluble antioxidant fractions and of nonextractable components linked to insoluble moiety, that may have deep consequences on antioxidant bioavailability.

This study focused on this aspect. To this purpose, 4 different extraction procedures were used to obtain extracts enriched in different categories of antioxidant molecules, including hydrophilic (H) and lipophilic (L) compounds, as well as both free-soluble (FSP) and insoluble-bound phenols (IBP). The sum of AA of H, L and FSP extracts ($AA_{H+L+FSP}$), containing solvent-soluble antioxidants, was compared to AA of the IBP fraction (AA_{IBP}), representing the nonsolvent extractable phenolic component bound to insoluble cell wall polymers.

As for AA determination, the recently developed lipoxygenase/4-nitroso-*N,N*-dimethylaniline (LOX/RNO) method (Pastore and others 2009) was used for the first time on quinoa seeds. The method is based on the RNO bleaching reaction associated to linoeic hydroperoxidation catalyzed by soybean LOX-1 isoenzyme (Pastore and others 2000). This method uses physiological radical species and is able to simultaneously detect different antioxidant mechanisms and to better highlight the synergistic effects among antioxidants (Pastore and others 2009). In addition to the LOX/RNO method, 2 different well-established methodologies for AA measurement were used: the TEAC assay (Re and others 1999) and the Oxygen Radical Absorbance Capacity (ORAC) method, measuring the chain-breaking capacity against peroxyl radicals (Ou and others 2001).

Finally, because quinoa is often considered as a pseudocereal because of the use in human diet of its starchy seeds and seed-derived products, it was compared with 2 traditional cereal species, such as durum wheat (*Triticum turgidum* L. ssp. *durum* Desf.) and emmer (*T. turgidum* L. ssp. *dicoccum* Schübler). Durum wheat is basic ingredient of pasta and bread, the most widely consumed cereal-derived foods in Mediterranean areas, and it is also considered as an excellent source of natural antioxidant compounds (Liu 2007). Emmer is an ancient tetraploid hulled wheat type related to durum wheat, recently rediscovered in the light of its interesting nutritional and functional profile due to unique content in bioactive compounds (Serpen and others 2008).

Material and Methods

Chemicals and seed materials

All reagents at the highest commercially available purity were purchased from Sigma-Aldrich Corp. (St. Louis, Mo., U.S.A.).

Quinoa (*C. quinoa* Willd. cv. real) seeds (produced in Bolivia by ANAPQUI – Asociación Nacional Productores de Quinoa) were purchased from "Ctm-Altromercato" Consortium (Bolzano, Italy). Different stocks of seeds were pooled to increase the representativeness of the sample. Quinoa real is indicated as a bitter variety (San Martin and others 2008); it represents one of the varieties most widely available for consumers in Italy, where use and commercial value of quinoa flour are currently emerging. Quinoa seeds purchased by ANAPQUI had been already preprocessed by the manufacturers to partly remove the saponins, by washing with water and drying. Moreover, before use, we attempted to remove the residual saponins by further washing seeds with distilled water at 60 °C for 1 h at a (w/v) ratio equal to 1 g/10 mL, followed by centrifugation and drying. Durum wheat (*T. turgidum* L. ssp. *durum* Desf., cv. Adamello) and emmer (*T. turgidum* L. ssp. *dicoccum* Schübler, cv. Molise) seeds were kindly provided by the CRA-Cereal Research Centre (Foggia, Italy). All samples were stored at 4 °C and, before use, they were milled by means of a Cyclotec 1093 Sample Mill (1 mm sieve).

Extraction of hydrophilic (H), phenolic (P) and lipophilic (L) compounds from daily milled flour

H extracts. Extracts were prepared as described in Pastore and others (2009) and Laus and others (2012), by extracting flour samples with water at a (w/v) ratio equal to 1 g/3 mL in an ice-water bath for 1 h and centrifuging twice at 18700 × *g* for 20 min at 4 °C.

P extracts. The procedure firstly described in Sosulski and others (1982), modified as reported in Pastore and others (2009) and Laus and others (2012) was applied, properly adapted to quinoa seeds as follows.

FSP. Preliminarily, to remove free fatty acids and other lipid contaminants, quinoa flour samples were extracted 3 times with *n*-hexane at a (w/v) ratio equal to 1 g/5 mL. Then, to obtain FSP compounds, the defatted flour (1 g) was extracted twice with 10 mL of 80% (v/v) ethanol for 10 min at room temperature and centrifuged at 5000 × *g* for 10 min at 20 °C. The supernatants were pooled, then evaporated under vacuum at 40 °C to remove ethanol and concentrated to approximately 2 mL; then, they were diluted to 4 mL with water, acidified to pH 2 to 3 using HCl and centrifuged at 5000 × *g* for 10 min at 20 °C. The resultant acidic supernatant was extracted 5 times with ethyl acetate (at an ethyl acetate/water phase ratio equal to 1:1). The ethyl acetate fractions were pooled and evaporated to dryness under vacuum at 40 °C; the dry residue was reconstituted in 1.5 mL of water.

In the case of nondefatted whole flour samples of durum wheat and emmer, the procedure described in Laus and others (2012) was followed exactly. Unlike quinoa flour extraction, the acidic supernatant (obtained after acidification to pH 2 to 3 using HCl) was first subjected to 2 extractions with *n*-hexane (at a *n*-hexane/water phase ratio equal to 1:1) to remove L compounds and then to 3 ethyl acetate extractions.

IBP. Preliminary results showed that acidic rather than alkaline hydrolysis is suitable to release IBP from quinoa flour. So, the residue from ethanol extraction was digested with 20 mL of 3 M HCl at 80 °C for 2 h. The resultant hydrolysate was centrifuged at 5000 × *g* for 10 min at 20 °C and the supernatant was retained; the residue was washed twice with 10 mL of water and centrifuged at 5000 × *g* for 10 min at 20 °C. The pooled supernatants were concentrated under vacuum at 40 °C to 15 mL and extracted with ethyl acetate, as above described for the FSP. The ethyl acetate fraction was evaporated to dryness under vacuum at 40 °C and the dry residue was reconstituted in 2 mL of water.

In the case of durum wheat and emmer, alkaline hydrolysis with 20 mL of 2 M NaOH at room temperature for 1 h under nitrogen was performed, as reported in Pastore and others (2009) and Laus and others (2012). Then, the resultant hydrolysate was acidified and purified by 2 extractions with *n*-hexane and 3 extractions with ethyl acetate, as reported above.

L extracts. Extracts were obtained according to the procedure described in Panfili and others (2003). Briefly, flour samples (2 g) were treated with 2 mL of 96% (v/v) ethanol, 2 mL of 1% (w/v) NaCl and 5 mL of ethanolic 6% (w/v) pyrogallol. Then, the suspension was extracted twice with 15 mL of *n*-hexane/ethyl acetate (9:1, v/v). The organic phases were pooled, partitioned in 2 equal volumes and separately evaporated to dryness under vacuum at 40 °C. For the LOX/RNO assay, a dry residue was reconstituted in 10 mL of 80 mM sodium borate buffer pH 9.0 containing 2 mM sodium linoleate and 1.5 μL Tween 20/μmol linoleate; for TEAC and ORAC measurements, the other residue was reconstituted in 1 mL of ethanol.

Determination of antioxidant activity (AA) by the LOX/RNO, TEAC, and ORAC methods

LOX/RNO method. The LOX/RNO reaction was spectrophotometrically monitored, as described in Pastore and others (2000, 2009), by measuring the RNO absorbance decrease at 440 nm and 25 °C. The (%) decrease of the rate of RNO bleaching measured in the presence of extract (or ±-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, Trolox, used as a standard antioxidant), with respect to the rate of the control reaction, was used to quantify AA. This was made by means of a dose-response curve derived for Trolox by plotting the (%) decrease of the rate of RNO bleaching as a function of standard antioxidant concentration.

ORAC method. The ORAC protocol, described in Ou and others (2001) and modified as in Pastore and others (2009), was applied. Fluorescence intensity decay due to 3',6'-dihydroxyspiro[isobenzofuran-1[3*H*], 9'[9*H*]-xanthen]-3-one (fluorescein) oxidation by peroxyl radicals generated by AAPH [2,2′-azobis(2-amidinopropane)] thermal decomposition was continuously monitored at 37 °C at excitation and emission wavelengths of 485 and 515 nm, respectively. To quantify AA, the area under the fluorescence decay kinetic curve (area under curve, AUC) was used and, in particular, the net AUC (AUC_{net}), obtained by subtracting AUC of the blank from that of the sample. AA was calculated by means of a proper dose-response curve prepared with Trolox by plotting the AUC_{net} as a function of standard antioxidant concentration.

TEAC method. The TEAC protocol, reported in Re and others (1999) and modified as in Pastore and others (2009), was used. The colored radical cation $ABTS^{\bullet+}$ was produced by ABTS oxidation with potassium persulfate solution. Absorbance at 734 nm and 25 °C (A_{734}) was measured after a fixed time of incubation of extract (or Trolox) with the $ABTS^{\bullet+}$ diluted solution. The (%) decrease of A_{734} measured after extract (or Trolox) incubation, with respect to A_{734} of the uninhibited radical cation solution (blank), was calculated; AA was quantified by means of a proper concentration-response curve prepared with Trolox by plotting the (%) decrease of A_{734} as a function of standard antioxidant concentration.

For all 3 methods, determinations were carried out in triplicate by analyzing at least 3 different amounts of extract. A linear dependence of the inhibition on the amount of extract was verified by linear regression analysis of data. Then, AA was obtained by comparing the slope derived by linear regression analysis with that of calibration curve prepared with Trolox.

Table 1–Antioxidant activity (AA), evaluated by means of the LOX/RNO, ORAC, and TEAC methods, of hydrophilic, lipophilic, and phenolic extracts from quinoa seeds. In the columns different capital letters, reported as superscript, indicate significant differences at 0.01 *P* level, according to the Duncan's test. As for ORAC and TEAC measurements, statistical analysis was performed using ln-transformed data. All data are reported as mean value ($n = 3$).

	AA (μmol Trolox eq./g d.w.)		
Extract	LOX/RNO	ORAC	TEAC
Hydrophilic extract	138^{B}	37^{A}	12.8^{A}
Lipophilic extract	130^{B}	0.38^{C}	0.33^{D}
Free-soluble phenolic extract	81^{C}	5.75^{B}	1.67^{C}
Insoluble-bound phenolic extract	428^{A}	4.89^{B}	3.72^{B}

Statistical analysis

Data distribution was evaluated using the Shapiro-Wilk and Jarque-Bera tests, showing that normality hypothesis can be accepted. Homogeneity of variances was verified by the Bartlett's test. Where necessary, either a natural logarithmic (ln) or square root (sr) transformation was performed (see captions of figures and tables). All data were submitted to an "one-factor" analysis of variance (ANOVA) using a completely randomized block design and the mean separation was tested by the Duncan's test at 0.01 and 0.05 *P* levels of significance. Statistical analysis was performed using the JMP software (version 8.0; SAS, Cary, N.C., U.S.A.) and the MSTAT-C statistical package (version 2.1, 1991; Crop and Soil Sciences Department, Michigan State University, East Lansing, Mich., U.S.A).

Results and Discussion

AA of quinoa seed extracts as evaluated by the LOX/RNO, ORAC, and TEAC methods

In Table 1 AA values of all extracts obtained from quinoa flour are reported. The LOX/RNO method measured high AA values for all investigated antioxidant components of quinoa seeds, with the highest ones for the IBP fraction, followed by the other components according to the following rank: H = L > FSP. The ORAC and TEAC protocols also showed a remarkable peroxyl and ABTS radical scavenging activities of quinoa seeds, respectively, but the highest AA values were observed for the H extract, with the other antioxidant components distributed according to the same order: IBP ≥ FSP > L. High AA values obtained for phenolic extracts by the 3 methods are expected to be strongly associated to some polyphenolic groups, detected and identified in methanolic extracts of quinoa seeds in previous studies:

the flavonol conjugates quercetin and kaempferol oligomeric glycosides, the most abundant phenols in quinoa seeds, as well as the hydroxybenzoic (protocatechuic and vanillic) and hydroxycinnamic (ferulic and caffeic) acid derivatives (Dini and others 2004; Gorinstein and others 2008; Alvarez-Jubete and others 2010; Hirose and others 2010). High antioxidant properties of the H fraction of quinoa are in agreement with previous literature data, in which other AA assays were used (Nsimba and others 2008); this AA may be attributable to the presence of some low molecular weight water-soluble phenols, lignans, and vitamin C (Nsimba and others 2008; Dini and others 2010), and probably to proteins (Gorinstein and others 2007). As for the L extract, whereas negligible AA values were measured by the TEAC and ORAC protocols, a significant AA value was pointed out only by the LOX/RNO method; this is in accordance with the high carotenoid and tocol contents already measured in quinoa seeds in previous studies (Dini and others 2010).

AA of solvent-extractable components of quinoa seeds, as evaluated by the LOX/RNO, ORAC, and TEAC methods, in comparison with durum wheat and emmer

In Figure 1 the sum of AA values of the H, L and FSP components, containing solvent-extractable antioxidant compounds, is reported for quinoa, durum wheat, and emmer flours. As regards the LOX/RNO method (Figure 1A), AA sum for quinoa flour resulted statistically higher than that of durum wheat, but lower with respect to emmer (349 ± 20, 226 ± 20, and 515 ± 53 μmol Trolox eq./g of dry flour [dry weight, d.w.], respectively). In particular, AA sum of quinoa depended on a comparable contribution of water- and fat-soluble antioxidant fractions and to a minor extent on the FSP component; in durum wheat the H and L components showed higher AA values with respect to the FSP one, whereas a clear superiority of H compounds was observed for emmer. The ORAC assay (Figure 1B) measured an AA sum of quinoa flour approximately twice than durum wheat (43 ± 1 and 22 ± 2 μmol Trolox eq./g d.w., respectively), but lower than emmer (60 ± 7 μmol Trolox eq./g d.w.). In all cases, the H component showed an activity much higher than that of the other fractions. As for AA assessment with the TEAC protocol (Figure 1C), a different behavior was observed: quinoa showed a much higher AA than the ones, similar, of durum wheat and emmer (14.8 ± 0.6, 6.3 ± 0.5, and 6.8 ± 0.2 μmol Trolox eq./g d.w., respectively). Also in this case, AA sum depended on a much higher activity of H compounds. The ORAC and TEAC assays resulted unable to point out an important AA of L extracts.

It should be considered that some water-soluble phenolic compounds may be present both in FSP extract and in the H extract. However, this partial overlap in AA should have a limited effect as suggested by the fact that AA of FSP extract is generally low or very low (Laus and others 2012).

AA of IBP fraction of quinoa seeds, as evaluated by the LOX/RNO, ORAC, and TEAC methods, in comparison with durum wheat and emmer

In Figure 2 AA values of the IBP fraction, representing the nonfreely solvent-extractable phenolic component, are reported for quinoa, durum wheat and emmer flours. According to the LOX/RNO method (Figure 2A), the bound phenolic fraction of quinoa showed an AA value approximately equal to a half of that observed for durum wheat and one third of that of emmer (428 ± 4, 800 ± 47, and 1240 ± 21 μmol Trolox eq./g d.w., respectively). Also with the ORAC method, the IBP of quinoa resulted less active than the durum wheat and emmer ones (4.9 ± 0.2, 13.5 ± 1.5, and 26.0 ± 0.4 μmol Trolox eq./g d.w., respectively; Figure 2B). On the contrary, the TEAC assay showed for quinoa an AA value lower than that obtained for durum wheat, but higher with respect to emmer (3.7 ± 0.2, 6.7 ± 0.1, and 1.6 ± 0.1 μmol Trolox eq./g d.w., respectively; Figure 2C), thus showing that the profile of scavenging capacities against ABTS radical cation (TEAC) and peroxyl radical (ORAC;

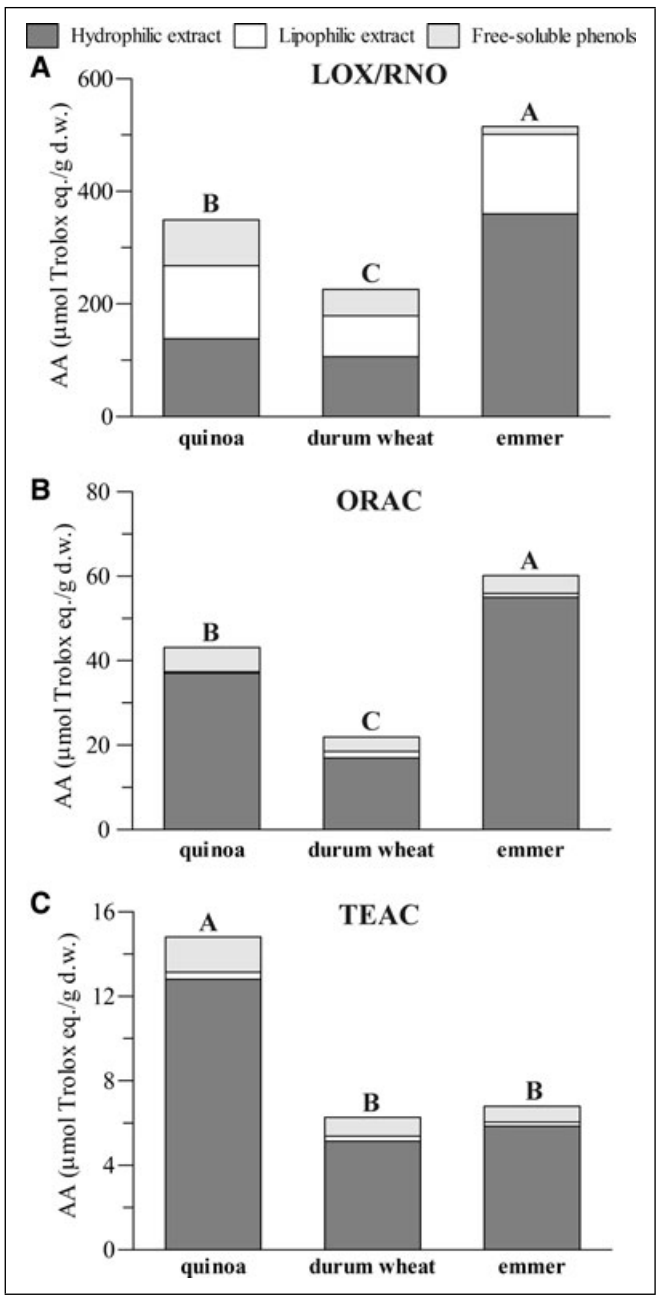

Figure 1–Antioxidant activity (AA), evaluated by means of the (A) LOX/RNO, (B) ORAC, and (C) TEAC methods, of the sum of hydrophilic, lipophilic, and free-soluble phenolic extracts from quinoa, durum wheat, and emmer flours. Data are reported as mean value ($n = 3$). Different capital letters indicate significant differences at 0.01 P level, according to the Duncan's test. As for ORAC measurements, statistical analysis was performed using ln-transformed data.

data from Figure 1 and 2) may deeply differ each other with respect to different extracts and species.

AA of free-soluble antioxidants versus AA of nonextractable bound phenols

In Table 2 the contribution to total AA of solvent-soluble antioxidant components (H, L and FSP) is reported for each species and AA assay under study. Interestingly, in the case of ORAC measurements, AA of the freely soluble antioxidant components of quinoa represented 90% of total AA, much higher than that obtained for durum wheat and emmer; so, the radical scavenging capacity of quinoa seeds is largely attributable to those antioxidant components easily released from flour. Also the ABTS radical cation scavenging capacity of quinoa flour, evaluated by the TEAC assay, resulted largely dependent on the solvent-extractable fractions; the contribution of these components to total AA resulted for quinoa comparable to that of emmer, but higher with respect to durum wheat. The LOX/RNO method showed a contribution of solvent-soluble antioxidant components of quinoa about 1.5- and 2-fold higher than that of emmer and durum wheat, respectively.

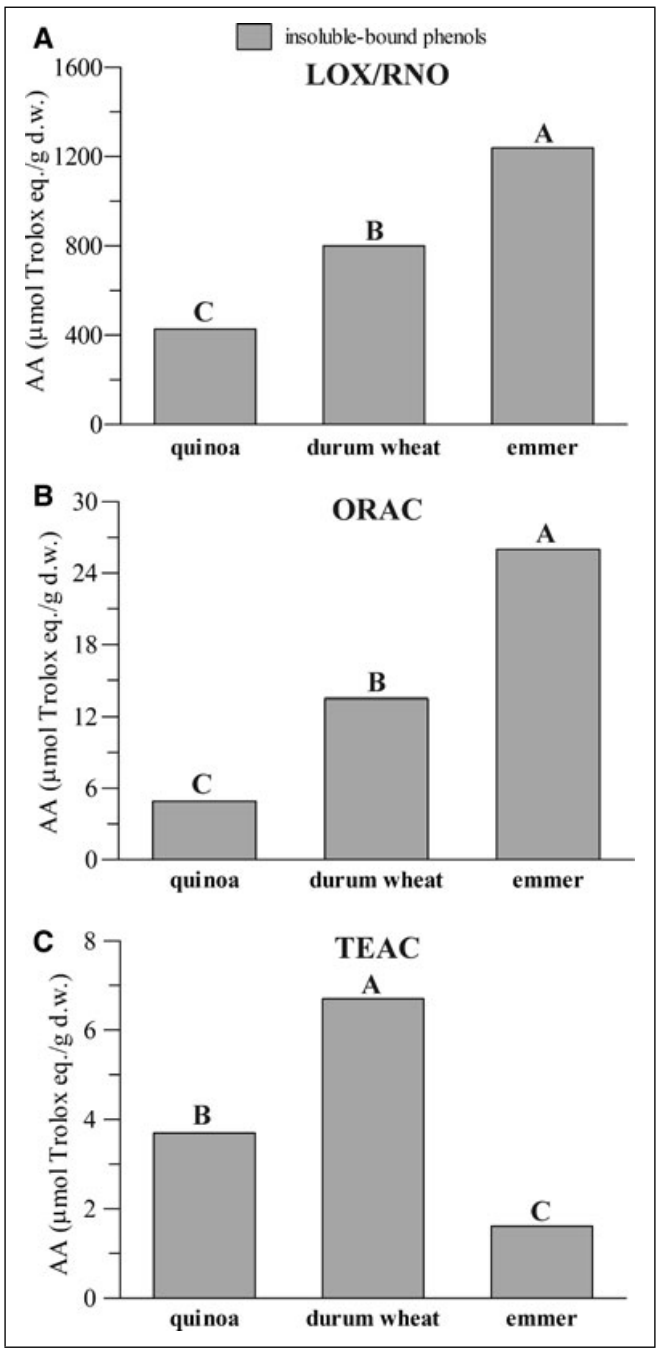

Figure 2–Antioxidant activity (AA), evaluated by means of the (A) LOX/RNO, (B) ORAC, and (C) TEAC methods, of insoluble-bound phenolic extracts from quinoa, durum wheat, and emmer flours. Data are reported as mean value ($n = 3$). Different capital letters indicate significant differences at 0.01 P level, according to the Duncan's test. As for ORAC measurements, statistical analysis was performed using ln-transformed data.

These results are further confirmed by considering the ratio between AA values obtained for the freely soluble antioxidant components and those relative to the IBP fraction (Table 2). The ORAC protocol showed AA values of the freely solvent-extractable antioxidant compounds of quinoa flour about 9-fold higher than that of IBP and this ratio resulted about 4- and 6-fold higher than that of emmer and durum wheat, respectively. In the case of TEAC assay, a value equal to about 4 was found for quinoa, comparable to that of emmer, but 4-fold higher with respect to durum wheat. In the case of the LOX/RNO method, the ratio resulted about 2- and 3-fold higher than that of emmer and durum wheat, respectively.

It is known that phenolic aglycones (free) and some glucosides may be readily absorbed in the small intestine (Manach and others 2004), as well as vitamins C and E (Manach and others 2004); so, they are expected to rapidly exert systemic effects in the body. Contrarily, some glycosides and phenols bound to cell wall polymers are very poorly absorbed or not absorbed at all at upper intestinal segments, so they may be released mostly by colonic microflora digestion (Manach and others 2004). Because absorption occurs in the colon less readily, bound phenols are absorbed less rapidly and less efficiently. So, a major local activity of bound phenols is expected in the terminal intestine, together with a delayed, lower though continuous, systemic activity (Manach and others 2004; Crozier and others 2009; Visioli and others 2011). In the light of this, the ratio $AA_{H+L+FSP}/AA_{H+L+FSP+IBP}$ (or $AA_{H+L+FSP}/AA_{IBP}$) may provide some information about antioxidant accessibility and the high ratio of quinoa may be indicative of a more ready antioxidant accessibility with respect to durum wheat and emmer, which show, on the contrary, high AA of IBP.

Table 2–AA of free antioxidant fractions in relation to both total AA and AA of bound phenols. In the columns different letters, reported as superscript, indicate significant differences at 0.05 (small letters) and 0.01 (capital letters) P levels, according to the Duncan's test. Statistical analysis was performed using sr-transformed data. All data are reported as mean value ($n = 3$).

	$AA_{H+L+FSP}$/total $AA_{H+L+FSP+IBP}$ (%)			$AA_{H+L+FSP}/AA_{IBP}$		
Species	ORAC	TEAC	LOX/RNO	ORAC	TEAC	LOX/RNO
Quinoa	90^{a}	80^{A}	45^{A}	8.82^{A}	3.98^{A}	0.82^{A}
Emmer	70^{b}	81^{A}	29^{B}	2.30^{B}	4.22^{A}	0.42^{B}
Durum wheat	62^{b}	48^{B}	22^{B}	1.64^{B}	0.94^{B}	0.28^{B}

H, hydrophilic extract; L, lipophilic extract; FSP, free-soluble phenolic extract; IBP, insoluble-bound phenolic extract.

Conclusions

In this study antioxidant properties of quinoa was compared with that of the cereals durum wheat and emmer. The comparison showed a new interesting characteristic of this pseudocereal species. Quinoa flour is characterized by a high ratio AAfree antiox./AAtotal antiox., as well as AAfree antiox./AAbound antiox.; these ratios, as evaluated by the LOX/RNO and ORAC assays, are higher than the ones of both durum wheat and emmer, and much higher than durum wheat, according to the TEAC protocol. This may suggest the presence of a fraction of readily accessible antioxidants in quinoa flour which is more favorable for beneficial systemic effects than 2 other cereals traditionally used for human food.

On the whole, the results of this study highlight an excellent antioxidant potential of quinoa seeds. This strongly encourages the use of quinoa for improving nutritive and health-beneficial properties of gluten-free food products, targeted not only for celiac patients, but also for the general population.

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