Evaluation of Antioxidant Activity of Grains From Graminaceae, Pseudocereals and Leguminosae

Damiana Tozzi[1], Anna Gagliardi[1], Donato Pastore[1,2] and Zina Flagella[1,2]

[1] *Dep. Di.S.A.C.D. - University of Foggia - Italy*, z.flagella@unifg.it
[2]*Research Center BIOAGROMED – University of Foggia - Italy*

Regular consumption of fruits, vegetables and whole grains provide a wide range of nutrients and biologically active compounds which may reduce the incidence of various disease (1). Whole grains are rich sources of fibre, vitamins, minerals, and phytochemicals including phenolics, carotenoids, vitamin E, lignans, β-glucan, inulin, resistant starch and sterols (2). Nutritional guidelines put grains and grain products at the base of the food guide pyramid to emphasise grains or grain product consumption as part of normal diet for optimal health (3). While nutritional and technological properties of grain products are widely investigated, few information are available for health implications of grain flour and by-products consumption. Therefore, a great interest is addressed to the determination of antioxidant activity (AA) of grain flour. In this study the AA of seven herbaceous crop species was evaluated. The species under study were the Graminaceae oat (*Avena sativa* L.), teff (*Eragrostis tef* (Zucc.) Trotter) and finger millet (*Eleusine coracana* (L.) Gaertn); the pseudocereals quinoa (*Chenopodium quinoa* Willd; Chenopodiaceae) and amaranth (*Amaranthus* spp., Amarantaceae) and, finally, the Leguminosae faba bean (*Vicia faba* L.) and chickpea (*Cicer arietinum* L.). These crops, belonging to several botanical families are widely spread under different climatic environments. Some evidences are reported in literature in relation to their functional quality and antioxidant activity. The antioxidant capacity of oat is largely due to the presence of tocopherols, tocotrienols, phytic acid, flavonoids, and non-flavonoid phenolic compounds such as avenanthramides (4). Finger millet contains phenolic acids, flavones and is the only millet species containing condensed tannins. Teff is reported to have tannins (5,6). Quinoa and amaranth are rich in several phytochemicals that act as powerful dietary antioxidants (7). Leguminosae provide micronutrients, vitamins, carotenoids (8) and phenols, all of which are considered to be bioactive compounds (9,10). The objective of this investigation was the evaluation of AA of hydrophilic and lipophilic antioxidant components from grain samples. AA of the different species under study was evaluated both on hydrophilic and lipophilic flour extracts.

Methodology

All reagents were purchased from Sigma Chemical Co. (St Louise Mo) or from Fluka Chemie GmbH. Whole grains (5 g) of the investigated species were milled by means of a Cyclotec 1093 Sample Mill (1 mm sieve). To remove external saponin, quinoa grains were previously washed vigorously. Two solvent systems were used to extract hydrophilic and lipophilic antioxidants components from whole grain flours i.e. water and exane/ethyl acetate (9:1 v/v) solution, respectively (11,12). *Extraction with water.* Flour samples were weighted and suspended in distilled water at a w/v ratio equal to 1 g/3 mL. The suspensions were placed in an ice-water bath for 1 h, stirred for 1 min at 15 min intervals; then, they were centrifuged twice at 18700 x g for 20 min at 4°C and the final supernatants (water extracts) were stored in an ice-water bath and daily used. *Extraction with exane/ethyl acetate solution.* 2 g of whole grains flour was saponified under nitrogen by adding ethanolic pyrogallol (60 g/L), ethanol (96%), sodium chloride (1%) and potassium hydroxide (600 g/L). After alkaline digestion at 70°C for 45 min, the samples were cooled in an ice bath and sodium chloride was added. The suspension was then extracted twice with n-hexane/ethyl acetate (9:1 v/v). The organic layer was collected and evaporated to dryness. The dry residue was dissolved in ethanol, stored in an ice-water bath and daily used. To determine the AA of the water and exane/ethyl acetate extracts we used ABTS method (13), with water and ethanol as solvent respectively. ABTS method is based on the ability of some antioxidants to reduce the cationic radical $ABTS^{\bullet+}$. This redox reaction results in a decrease of absorbance of $ABTS^{\bullet+}$ at 734 nm. AA of the hydrophilic and lipophilic components of the flour from

the different species was calculated by means of proper calibration with Trolox. Always, the AA was expressed as μmol Trolox equivalents/g of whole flour.

Results

In Fig. 1 the AA of hydrophilic (A) and lipophilic (B) antioxidant components of the whole flour from the investigated species is reported. Always, the hydrophilic antioxidants were more active than the lipophilic ones. Two different ranking lists were obtained for hydrophilic and lipophilic antioxidants, thus showing a different contribution of these components in determining AA of different grains. In A, ABTS method shows a very high AA value in faba bean, high in chickpea and lower in the other species. In B the highest AA value was observed in chickpea, followed by faba bean, quinoa, teff and amaranth, and the lowest ones in finger millet and oat.

Conclusions

The comparison among the different species under study shows the highest antioxidant activity for leguminosae species and quinoa both in hydrophilic and lipophilic extracts and the lowest activity for oat and finger millet. Further studies will be necessary to evaluate both genetic and environmental influence on AA of the different species, as well as to enlarge evaluation of AA to other methods besides the ABTS one.

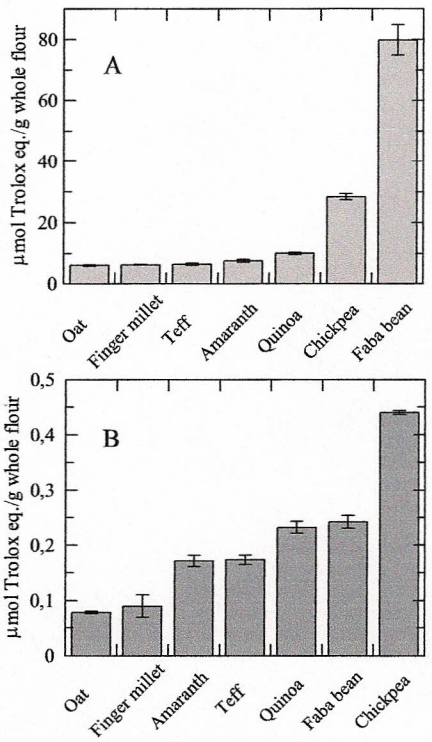

Figure 1. Determination of AA of hydrophilic (A) and lipophilic (B) antioxidant components in the investigated species measured by using ABTS method.

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