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Measurement of biological antioxidant activity of seven food-grade sorghum hybrids grown in a Mediterranean environment

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Abstract

Sorghum is source of antioxidant compounds including phenolic acids, flavonoids and condensed tannins. In this study, we measured the antioxidant capacity of seven white food-grade sorghum hybrids grown in Southern Italy using the Ty1antiROS cell-based test and the chemical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis-(3-ethylbenzothiazolin-6-sulfonic acid) (ABTS) assays. Samples were also analyzed for phenolic contents. Ty1antiROS test showed that all hybrids possessed antioxidant activity and were effective in scavenging the $ABTS^{\bullet+}$ target species but they exerted a weak $DPPH^{\bullet}$ antiradical effect. Furthermore, all samples had similar values for total phenolic content. Statistical analyses showed a positive correlation between the ABTS and DPPH data (R = 0.61) and a weakly positive correlation between the ABTS and Ty1antiROS data (R= 0.38). The utilization of both biological and chemical tests for the antioxidant capacity evaluation make us able to select varieties of sorghum with high antioxidant potential useful to promote human health.

Keywords: sorghum hybrid, Ty1antiROS, DPPH, ABTS.
Abbreviations: ABTS_2,2'-azinobis-(3-ethylbenzothiazolin-6-sulfonic acid); DPPH_2,2-diphenyl-1-picrylhydrazyl; GAE_gallic acid equivalent; RACI_relative antioxidant capacity index; ROS_reactive oxygen species; %RSC_percentage of radical scavenging capacity; TEAC_Trolox equivalent antioxidant capacity; TPC_total phenol content.

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop in the world after wheat, rice, corn and barley. Sorghum outperforms other cereals under various environmental stresses and is, thus, generally more economical to produce (ICRISAT, 1996). On a global scale, more than 35% of sorghum is grown directly for human consumption. The rest is used primarily for animal feed, alcohol and industrial products. The United States is the largest producer and exporter of sorghum, accounting for 17% of world production in 2013-2014 (WSP, 2015) and almost 76% of world sorghum exports in 2012-2013 (USDA, 2015). In many developing countries, sorghum has traditionally been used in food products and various food items (Pontieri et al., 2011; Pontieri et al., 2014). The white food sorghums are processed into flour and other products, including expanded snacks, cookies and ethnic foods, and gaining more popularity in areas like Japan (USGC, 2001). Sorghum is considered as a safe food for celiac patients suffering from symptoms associated with an immune reaction to gluten proteins, being found in all *Triticum* species and closely related cereals such as barley and rye (Ciacci et al., 2007). White sorghum can be used to substitute for wheat in products for people allergic to wheat gluten (Fenster, 2003). Recently, molecular evidence demonstrating the absence of toxic gliadin-like peptides in sorghum was reported, confirming that sorghum can be considered safe for consumption by people with celiac disease (Pontieri et al., 2013). Sorghum contains various phytochemicals, including polyflavanols (procyanidins) (Gu et al., 2002), anthocyanins (Awika, 2003), phenolic acids (Waniska et al., 1989), and other antioxidant compounds. Phenols help in the natural defense of plants against pests and diseases, while the plant sterols and policosanols are mostly components of wax and plant oils (Hwang et al., 2002). Phytochemicals have increasingly attracted interest due to their antioxidant activity, cholesterol lowering properties and other potential health benefits. Sorghums could; thus, be an important source of ingredients for use in functional foods and other

applications (Awika et al., 2003). However, data are hard to find on antioxidant activities of the specialty sorghums and/or their products. Such information is critical, if sorghum is to become a competitive source of the phytonutrients.

A quick, reliable, and cost effective method is necessary for screening sorghum samples for antioxidant activity. Numerous methods are used to evaluate antioxidant activities of natural compounds in foods or biological systems with varying results. Two free radicals that are commonly used to assess antioxidant activity *in vitro* are DPPH and ABTS. However, both of these radicals are foreign to biological systems. The DPPH method is widely used to determine antiradical/antioxidant activity of purified phenolic compounds as well as natural plant extracts (Fukumoto and Mazza, 2000). The method, however, has not been correlated with biological effects; hence, its actual relevance to *in vivo* antioxidant efficacy is unknown. The ABTS method is rapid and can be used over a wide range of pH values (Lemanska et al., 2000), in both aqueous and organic solvent systems. It also has good repeatability and is simple to perform; hence, it is widely reported. However, like DPPH, it has limited, if any, relevance to biological systems. An alternative is given by the recently reported Ty1antiROS cell-based test, in which an *in vitro* reaction between oxidative radicals and antioxidants is carried out in living cells (Dimitrov et al., 2013).

The present study was conducted to determine the antioxidant activity of sorghum hybrids grown in a temperate Italian environment utilizing both the Ty1antiROS cell-based test and the chemical DPPH and ABTS tests.

Results

In vivo antioxidant activity of sorghum hybrids

Sorghum flours and their extracts have a complex nature rich in sugars, vitamins, proteins and other bioactive molecules that might interfere with biology of yeast cells and compromise results in the Ty1antiROS test. Therefore, in preliminary experiments the influence of sorghum extracts on viability and growth rate was studied. In these experiments, the tester cells were treated with concentrations of sorghum extracts, exceeding twice the highest concentration used in the Ty1antiROS test. Statistically meaningful differences with untreated controls were not detected (data not shown), indicating that the Ty1antiROS test can be used for determination of the antioxidant activity of sorghum flour extracts.

The results of Ty1antiROS test (Fig. 1) show that all seven studied sorghum varieties (Table 1) possess antioxidant activity. The ID_{50} values of the studied samples range between 28-250 µg/mL, indicating an almost tenfold difference in antioxidant activities between the sorghum samples. Results obtained by other authors in the study of biological products such as honey, have shown the existence of variations in their antioxidant properties, which depend on the geographic origin, soil characteristics and seasonal changes. The antioxidant activity variations found in these studies were small and did not reach a two folds difference between the lowest and the highest value measured (Kumazawa et al., 2004). The existence of 10-fold variations in the samples of sorghum varieties studied here strongly suggests that the differences in the antioxidant activities can be genetically determined. It confirms the results of previous studies, evidencing the existence of genetic control mechanisms of antioxidant properties in sorghum (Dykes et al., 2014). Considering the biological effects of sorghum, the values obtained in the Ty1antiROS assay show antioxidant activities that are significantly lower compared to those of the referent antioxidant vitamin C (Fig 1, Controls). The ID_{50} values of sorghum samples are comparable to the ID_{50} values found for royal jelly and honey (Dimitrov et al., 2013), suggesting a moderate *in vivo* antioxidant activity for sorghum. However, given the usage of sorghum as human food and food additive, it might be concluded that its effect as an antioxidant is expected to be comparable to that of honey.

TPC and radical scavenging activity

Sorghum contains large quantities of phenolics and other compounds of use in human foods to prevent health deterioration (Dykes and Rooney, 2006). Since phenolic phytochemicals appear to be mainly responsible for the antioxidant efficacy of plant matrices, the Folin-Ciocalteau reagent method was applied to estimate the TPC of the investigated sorghum hybrids. Results reported in Table 2 clearly show that all samples had similar levels of TPC, with the hybrid Hy F-X715 Ch being slightly higher than the others (44.82 mg GAE/100 g). The results of this study confirm the characteristic of sorghum as a rich source of phenolic compounds (Dykes et al., 2014; Dykes and Rooney, 2006).

The antiradical effectiveness of sorghum hybrids was also evaluated by $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ methods. All the samples were effective in scavenging the $ABTS^{\bullet+}$ target species (Fig. 2A), whereas they exert only a weak $DPPH^{\bullet}$ antiradical effect (Fig. 2B). The scavenging efficiency was strongly dependent on the dose tested. The data show that Hy ArchX-02 hybrid was the most effective at inhibiting $ABTS^{\bullet+}$with an ID_{50} of 88.6 µg/mL. In general, these data confirm previous findings reporting that the ABTS method was more suitable for determining radical scavenging activity of sorghums than the DPPH method (Awika et al., 2003).

RACI test for antioxidant activity in seven food-grade sorghum hybrids

RACI correlated with each chemical assay and might be used as a reference for measuring food antioxidant capacity (Sun and Tanumihardjo, 2007). Although it is a relative index and may not represent a specific antioxidant property of different food items, RACI provides a reasonably accurate rank of antioxidant capacity among foods. Therefore, it can be used as an integrated approach to evaluate food antioxidant capacity. Thus, data obtained from TPC, ABTS, DPPH and Ty1antiROS tests were used to calculate RACI value for each hybrid sorghum extract. The correlation coefficients of antioxidant activity among the various methods were calculated and the results are shown in Table 3. The analysis showed a positive correlation between ABTS and DPPH ($R = 0.61$). The ABTS data weakly but positively correlated to those from Ty1antiROS data (0.38). RACI calculation represents the average of the standard scores obtained from the raw data for the various methods (Fig 3). The results emphasized that the Hy ArchX-02 and Hy F-X715 Ch (RACI 1.08 and 0.45, respectively), were stronger antioxidants than Hy X10341 (RACI 0.19), whereas other sorghum hybrids did not present any antioxidant effect ($RACI > 0$).

Discussion

Specialty sorghum hybrids contain high levels of diverse antioxidant compounds that may provide health benefits.

Table 1. List of sorghum hybrids.

Hybrid name (Hy)	Source	Kind of hybrid
Hy X10341	Richardson Seeds, Ltd (Vega, TX)	F_1
Hy X10315	Richardson Seeds, Ltd (Vega, TX)	F_1
Hy X87341	Richardson Seeds, Ltd (Vega, TX)	F_1
Hy F-X525	Richardson Seeds, Ltd (Vega, TX)	F_1
Hy ArchX-02	Richardson Seeds, Ltd (Vega, TX)	F_1
Hy SP-X303	Richardson Seeds, Ltd (Vega, TX)	F_1
Hy F-X715 Ch	Richardson Seeds, Ltd (Vega, TX)	F_1

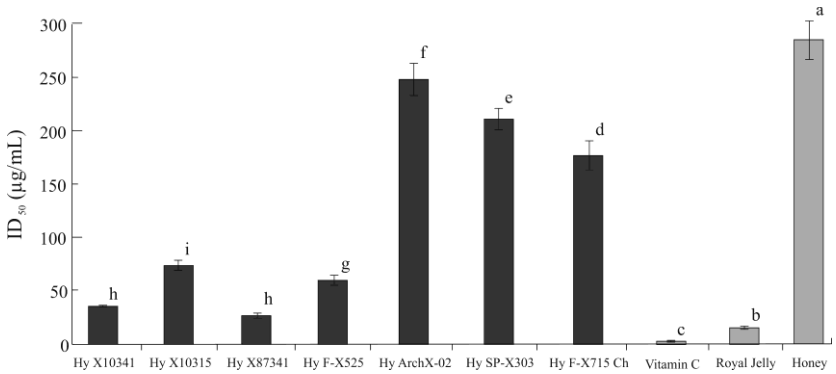

Fig 1. Variation in antioxidant activity of sorghum extracts in the TyantiROS test. Data are mean ± SE (n = 5) per sorghum hybrids. Different letters above the columns indicate significant differences ($p \leq 0.05$) as determined by the least significant difference (LSD) test.

Table 2. TPC of investigated flour sorghum hybrids. Values are reported as mg GAE/100 g of flour. Values are means (±SD) of triplicate analyses (n = 3) and are expressed on weight basis.

Hybrid	mg GAE/100 g
Hy X10341	39.97±0.88 [a]
Hy X10315	36.87±1.28 [b]
Hy X87341	39.49±0.86 [a]
Hy F-X525	41.96±1.51 [c]
Hy ArchX-02	31.14±0.85 [d]
Hy SP-X303	37.07±0.05 [b]
Hy F-X715 Ch	44.82±4.65 [e]

Means followed by same letter are not significantly different ($p \leq 0.05$) according to LSD test.

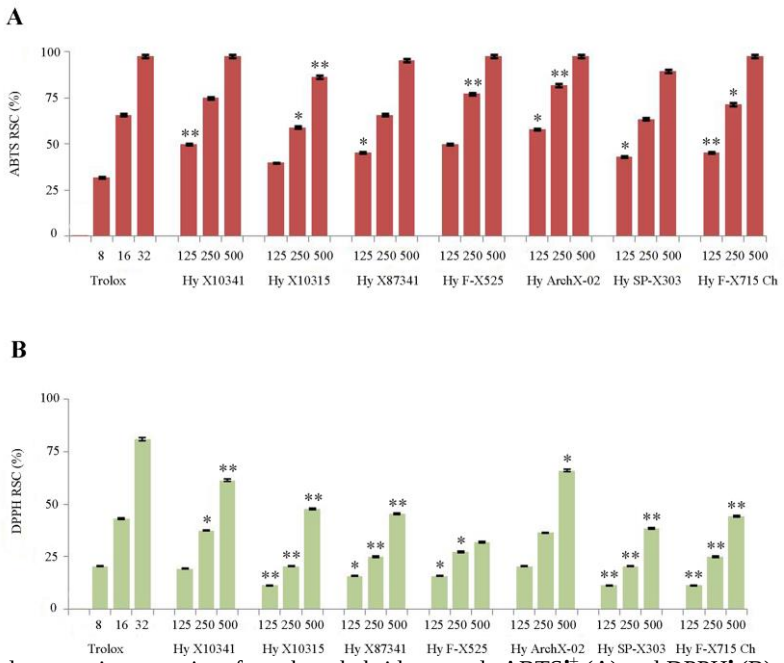

Fig 2. Percentage of radical scavenging capacity of sorghum hybrids towards $ABTS^{\bullet+}$ (A) and $DPPH^{\bullet}$ (B). Data presented are means ± SD from five (n=5) independent experiments and are expressed on weight basis. Least significant difference (LSD) test significance of sorghum hybrids compared to the positive control (Trolox; * for $p \leq 0.05$ or ** for $p \leq 0.01$).

Table 3. Correlation coefficients of antioxidant activity among the different antioxidant methods applied.

	ABTS	Ty1antiROS	TPC	DPPH
ABTS	1	0.38	-0.41	0.61
Ty1antiROS		1	-0.46	0.29
TPC			1	-0.43
DPPH				1

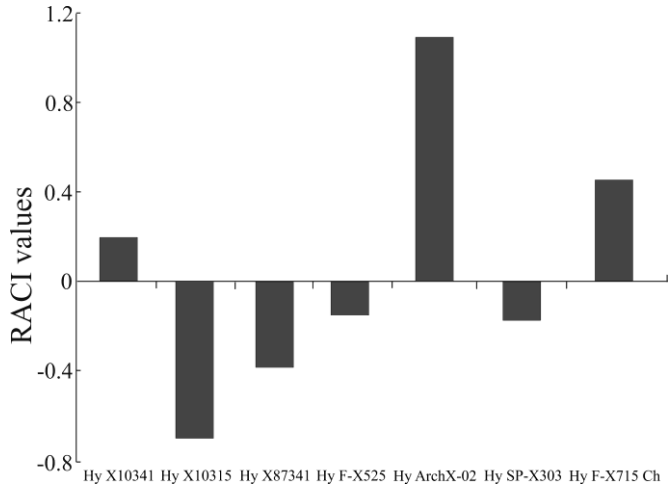

Fig 3. Relative antioxidant capacity index (RACI) of sorghum hybrids. RACI values were developed from data obtained by antioxidant chemical and biological methods applied.

High levels of polyflavanols (procyanidins), anthocyanins, phenolic acids, and other antioxidant compounds have been reported in sorghums (Awika et al., 2003). Sorghums could; thus, be an important source of ingredients for use in functional foods and other applications to prevent deterioration of human health. Numerous methods are used to evaluate the antioxidant activities of natural compounds in foods or biological systems with varying results (Prior et al., 2005; Magaehales et al., 2008). The increasing interest in the role of free radicals in the pathogenesis of human diseases and the benefits of consumption of foods with antioxidant properties has led to an increased necessity to develop new techniques and testing systems to measure antioxidants *in vitro* and *in vivo*. There is a general agreement (Honzel et al., 2008) that *in vivo* cell-based antioxidant methods are a relevant way to study the biological effect of antioxidants. Considering the biological effect of antioxidants, these methods may have to take into account as the main characteristics and complex nature of antioxidant action. Consequently, antioxidant activity needs to be assayed by methods considering the ability of antioxidants to penetrate cells and target sub-cellular structures in active form, which is necessary to neutralize oxidative radicals shortly after their generation. Few of the existing tests for determination of antioxidant activity reach these criteria. Analytical *in vitro* assays for measuring antioxidant activity are based on chemical reactions resulting from direct interactions between antioxidants and oxidative radicals (Prior et al., 2005; Magaehales et al., 2008). Although these chemical *in vitro* antioxidant assays are easy, fast, cheap, and suitable for measuring antioxidant properties of food and dietary supplements, they are conducted under non-physiological conditions and the obtained results cannot be extrapolated to the *in vivo* situation. An alternative is given by *in vivo* tests such as the recently published Ty1antiRos cell-based test (Dimitrov et al., 2013). The main advantage of cell-based assays is that they directly measure the penetration of the studied antioxidant into cells, and the ability of the original compound or its metabolites to neutralize oxidative radicals inside the living cells. In our study, the results obtained in determination of the antioxidant activity of seven sorghum hybrids measured with an *in vivo* cell-based and two chemical *in vitro* tests were compared. The Ty1antiROS test measures the antioxidant activity by changes in a cellular process – the Ty1 transposition. This feature implies that the measurement of antioxidant activity with the Ty1antiROS test reflects the antioxidant effects only on oxidative radicals that are physiologically active and able to trigger cellular processes. In most of the other cell-based assays, the antioxidant activity is determined by chemical reactions with all available oxidative species in tester cells irrespective to their physiological state (Dimitrov et al., 2013).

Methanol extracts of sorghum flour possess antioxidant activity as evidenced by the positive results obtained in the Ty1antiROS test, as shown in Fig. 1. Therefore, the evidence that sorghum extracts have antioxidant properties *in vivo* demonstrates that sorghum is a biological antioxidant able to penetrate cells and to modulate the redox state of live cells. The differences found between the sorghum samples studied in Ty1antiROS test reflect the different biological effect that these samples have when used as food or food additives. Considering the biological effects of sorghum, the values obtained in the Ty1antiROS assay show antioxidant activities that are significantly lower compared to those of the reference antioxidant vitamin C (Fig. 1, Controls in grey). The ID_{50} values of sorghum samples are comparable to the ID_{50} values found for royal jelly and honey (Dimitrov et al., 2013), suggesting a moderate *in vivo* antioxidant activity for the white-food grade sorghum lines tested here. However, given the usage of sorghum as human food and food additive it might be concluded that white food-grade sorghum is effective as an antioxidant and is expected to be comparable to that of honey.

The antioxidant capacity of the sorghum samples analyzed was also evidenced by chemical tests. Results of Table 2 clearly show that all samples had similar values for TPC with

the Hy F-X715 Ch hybrid exhibiting slightly higher levels than the other hybrids (44.82 mg GAE per 100 g of flour). The results of this study confirm the characteristic of sorghum to be a rich source of phenolic compounds (Dykes et al., 2014; Dykes and Rooney, 2006). The antiradical effectiveness of sorghum hybrids was evaluated by performing $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ methods. As shown in Fig. 2A, all the samples were effective in scavenging the $ABTS^{\bullet+}$ target species. The ABTS values for sorghum extracts were relatively similar but, unexpectedly, these values negatively correlated with the TPC found for the studied sorghum varieties (-0.41) (Table 3). Negative correlation was also found between the antioxidant activity measured in the Ty1antiROS test and the content of total polyphenols (-0.46) (Table 3). Though well correlated to $ABTS^{\bullet+}$, all sorghum extracts exerted a weaker (two-fold) $DPPH^{\bullet}$ antiradical effect (Fig. 2B). DPPH is stable nitrogen radical that bears no steric accessibility to the highly reactive and transient peroxyl radicals involved in ABTS assay. Many antioxidants that react quickly with peroxide radicals may react slowly or may be even inert to DPPH due to steric inaccessibility. Thus, antioxidant capacity is not fairly rated by the ability of antioxidants to react with DPPH (Prior et al., 2005).

Interestingly, ABTS data weak but positively correlated to those from Ty1antiROS data (0.38) (Table 3). Consistently with ABTS and Ty1antiROS results, the sorghum Hy ArchX-02 hybrid flour exhibited the highest RACI (Fig. 3**)**.

Materials and Methods

Plant cultivars

The seven plant cultivars and their sources employed in this study are indicated in Table 1. The cultivars were chosen because they were both acclimated to Mediterranean environment and carefully characterized at both genetic and biochemical level (Pontieri et al., 2011, 2013, 2014). Furthermore, since all seven cultivars are hybrid lines, the F_1 generation is the one that presents the strongest genetic characters. The F_1 hybrids are excellent food quality grain producers. Grain threshes very free of the glumes in combine harvesting while the caryopses are very round and process well in the food industry. In particular, the hybrids exhibit: (a) white translucent grain color; (b) strongly vitreous grain, easy and clean threshing; and (c) high test weight.

Experimental site

Field trials were conducted at San Bartolomeo in Galdo (BN) south of Italy on a clay-loam soil during summer 2012. San Bartolomeo in Galdo is an inland area at the east of the Campania Region, about 530 m above the sea level. Seven hybrids of sorghum (Table 1) were sown on May 6, 2012 in row plots (2 m × 5 m) replicated 3 times in a randomized block design. Before sowing, a complex fertilizer (NPK [Mg, S] 12-12-17 [2, 14]) was applied during the growing crop cycle. Besides, urea (N46%) was distributed at stem elongation stage. An herbicide treatment of glyphosate (4 l/ha) was applied to the field to eliminate weeds before planting. After planting, weeds were controlled by hand hoeing as necessary. Plants were grown without supplemental irrigation. The hybrids were harvested starting from the end of August to mid-September.

Other materials and chemicals

Commercially available honey, propolis, and royal jelly were obtained from the Bulgarian Association of Honey Producers. Honey and royal jelly were dissolved in sterile water, whereas propolis was first treated with dimethylsulfoxide for 10 min and then diluted with 9 parts of the initial volume of sterile water. Stock solutions were filter sterilized and kept at +4 °C until used. Folin-Ciocalteau reagent, gallic acid, DPPH, ABTS, solvents and salts were obtained from Sigma-Aldrich S.r.l. (Milan, Italy).

Flour sample preparation

Sorghum samples were milled into flour using a two-roll mill (Chopin mod. Moulin CD1). Then, milled samples were sieved with a planetary sieve (Buhler), through a 120 μm^2 sieve opening. The sample so obtained is the edible part of the seed.

Preparation of methanol extracts from sorghum flour for Ty1antiROS test

One gram of sorghum flour was extracted with 1 mL methanol for 4 h, centrifuged and the supernatant evaporated in vacuum at ambient temperature. The dried residue from supernatant was weighed, dissolved in sterile water at a concentration of 1 mg/mL and centrifuged. Cleared supernatants were used immediately for determination of antioxidant activity.

Ty1antiROS test for antioxidant activity

This test is based on the inhibition by antioxidants of the Ty1 transposition process induced proportionally to the level of ROS generated by treatment of tester cells with carcinogens. Ty1antiROS test was performed essentially as described (Dimitrov et al., 2013) with strain *Saccharomyces cerevisiae* 551 as tester organism. Briefly culture aliquots of *S. cerevisiae* 551 cells growing exponentially were treated with increasing amounts (25-150 µL) of sorghum extracts, washed and suspended in fresh medium containing hexavalent chromium (Cr[VI]) as inducer of ROS. After 30 min, cultivation cells were collected by centrifugation and Ty1 transposition rates were determined as previously described (Pesheva et al., 2005). Ty1 transposition rates were plotted against the amounts of sorghum extracts and the ID_{50} values determined. The ID_{50} value is the amount of sorghum extract inhibiting 50% of the Ty1 transposition rate in the control sample processed in the same way, but without addition of sorghum extract. In this study the results obtained for the ID_{50} values are presented as micrograms of evaporated extracts in 1 mL of assay mixture (µg/mL) and; therefore, the data obtained from the different sorghum samples are directly comparable.

Extracts preparation for antioxidant capability evaluation of food-grade sorghum hybrids

In order to assess the antioxidant capability, crude extracts were prepared from investigated hybrids (~ 500.0 mg) by sonication (model UP 200S, Hielscher Ultrasonics GmbH, Teltow, Germany) for 2 h using methanol as extracting solvent. Samples were centrifuged at 3500 rpm for 10 min in a Beckman GS-15R centrifuge with rotor S4180 (Beckman Coulter, Milan, Italy), and supernatants were dried under vacuum obtaining crude extracts. Tests were carried out performing three replicate measurements for three samples of each examined extract.

Determination of TPC

The total phenols amount of both the crude extracts was measured according to the Folin-Ciocalteau procedure (Di

Maro et al., 2013). Sample extract (1.0 mg/mL) was mixed with 0.5 mL of Folin-Ciocalteau reagent and 4.0 mL of Na_2CO_3 (7.5% w/v). The reaction mixture was stirred at room temperature for 3 h. The absorbance was read at 765 nm. The content of total phenols of the samples is expressed as mg GAE per 100 g of flour.

Determination of DPPH• scavenging capacity

In order to estimate the DPPH• scavenging capability (Pacifico et al., 2012), investigated samples (10, 25, 50, 75, 100 and 200 μg/mL; final concentration) were dissolved in a DPPH• methanol solution (9.4×10^{-5} M; 1.0 mL) at ambient temperature. After 30 min of incubation the absorption at 515 nm was measured by a Shimadzu UV-1700 spectrophotometer in reference to a blank. The assay evaluates the percentage decrease of the initial DPPH• absorption by the test samples, and results are expressed in terms of % RSC. The DPPH ID_{50} value defined as the extract amount causing 50 % inhibition of absorbance was determined from the plotted curves. Furthermore, using Trolox® regression curve, the DPPH• scavenging activity in terms of TEAC (μg/mL) values was calculated.

Determination of ABTS•+ scavenging capacity

Determination of $ABTS^{\bullet+}$ solution scavenging capacity was estimated according to previously work (Re et al., 1999). $ABTS^{\bullet+}$ was generated by reacting ABTS (7.0 mM) and potassium persulfate (2.45 mM) and allowing the mixture to stand in the dark at room temperature for 12–16 h. Thus, the $ABTS^{\bullet+}$ solution was diluted with PBS (pH 7.4) in order to have an absorbance of 0.70 at 734 nm. The samples (10, 25, 50, 75, 100 and 200 μg/mL; final concentration) were dissolved in 1.0 mL of diluted $ABTS^{\bullet+}$ solution. After 6 min of incubation, the absorption at 734 nm was measured by a Shimadzu UV-1700 spectrophotometer in reference to a blank. The assay evaluates the percentage decrease of the initial $ABTS^{\bullet+}$ absorption by the test samples, and results are expressed in terms of %RSC ABTS ID_{50} and TEAC values as described above for DPPH test.

Statistical analysis and calculation of RACI

All analyses were performed in quintupled (n=5), and the results are presented as the mean ± SD. The Least significant difference test (LSD) at $p\leq 0.05$ was applied using the GraphPad Prism 6.0 (GraphPad Software Inc., La Jolla, CA 92037 USA).

The RACI was determined as described in previous research (Sun and Tanumihardjo, 2007; Gorjanovic et al., 2013). The samples were ranked by the mean value and standard deviation of the assays, and the dimensionless scores were calculated by subtracting the mean from the raw data divided by the standard deviation. The standard scores of a sample for different antioxidant assays were the specific combination of data from different methods with no unit limitation and no variance among methods. All applied assays were considered, i.e., TPC, ABTS, DPPH and Ty1antiROS.

Conclusions

In this study, a combination of TyantiROS, ABTS and DPPH tests were used to evaluate the antioxidant properties of seven white food-grade sorghum hybrids grown in a Mediterranean environment. Our results demonstrated that all analyzed sorghum hybrids possessed a moderate antioxidant activity comparable to those of royal jelly and honey. Furthermore, the relative antioxidant capacity of the studied sorghum hybrids could be experimentally determined. The utilization of both biological and chemical tests for the determination of antioxidant capacity will make us able to select varieties of sorghum in the future breeding programs with high antioxidant potential with the aim to promote human health.

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