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Effects of Milling-process and Pasta Making on ABTS$^{\bullet+}$ Scavenging Activity of Hydrophilic and Lipophilic Extracts of Durum Wheat Varieties

N.A. DI BENEDETTO[1], C. FARES[2], V. MENGA[2], M.N. LAUS[1], D. PASTORE[1] and Z. FLAGELLA[1]*

[1]Department of Agricultural, Food and Environmental Sciences, University of Foggia, Via Napoli, 25 71122 Foggia, Italy
[2]Consiglio per la Ricerca e Sperimentazione in Agricoltura (CRA) – Cereal Research Centre, S.S. 16 Km 675 71122 Foggia, Italy

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Epidemiological studies associated consumption of whole-durum wheat products with reduced incidence of chronic diseases, diabetes and cancer. These health benefits have been mainly attributed to antioxidant activity (AA) due to the unique phytochemical content of wheat. Milling, extrusion and drying process can influence the activity of these beneficial compounds. In order to have a deep insight into the changes of nutritional value from raw material to pasta, the aim of this study was: i) to compare the AA of hydrophilic and lipophilic extracts of five durum wheat genotypes along the pasta chain; ii) to evaluate the changes in antioxidant properties of whole meal after processing in semolina and pasta. To this aim TEAC (Trolox Equivalent Antioxidant Capacity) assay based on ABTS$^{\bullet+}$ [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) scavenging activity was used due to its high reproducibility and simplicity.

Low genotype variability was observed for both hydrophilic and lipophilic extracts. Milling process caused a significant decrease in AA due to the removal of the outside layers of the kernel. This decrease was more marked for lipophilic extracts due to the different distribution of hydrophilic and lipophilic antioxidants along the kernel. Pasta making process while determining a further decrease in AA of lipophilic extracts caused a slight increase in AA of hydrophilic extracts compared to semolina. This might be due to melanoidins formed during Maillard reaction.

Only for lipophilic extracts a predictive evaluation of semolina and pasta ABTS$^{\bullet+}$ scavenging activity was possible by testing raw material.

Keywords: durum wheat, antioxidant activity, TEAC assay (ABTS$^{\bullet+}$), pasta chain

Introduction

Wheat is the main cereal crop used for human consumption in many areas worldwide. Whereas common wheat (*Triticum aestivum* L.) is widely used for bread-making, durum wheat, i.e. *Triticum turgidum* L. subsp. *durum*, is mainly employed in the production of

* Corresponding author; E-mail: z.flagella@unifg.it; Phone +39 0881 589220-336; Fax +39 0881 589502

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other food items, pasta being the most popular. In Italy, the main pasta producer, pasta is a staple food also if its consumption has become widespread in several countries (Cubadda et al. 2009). Health benefits provided by durum wheat products have become a key marketing tool primarily because of increasing consumer awareness of the role of diets in health promotion and disease prevention.

Epidemiological studies have associated consumption of whole-durum wheat products with reduced incidence of chronic diseases (Liu 2007; Tighe et al. 2010), diabetes (He et al. 2010) and cancer (Fung et al. 2003). These health benefits have been mainly attributed to the unique phytochemicals content in wheat.

Durum wheat has a significant level of antioxidants (Fares et al. 2010; Hung and Hatcher 2011) but they are unevenly distributed along the kernel. Liyana-Pathirana and Shahidi (2007) highlighted a different antioxidant capacity of durum wheat fraction (bran, germ and endosperm), grain bran having the highest one.

The most important groups of phytochemicals found in whole durum wheat can be classified as phenolic acids, flavonoids, carotenoids, vitamin E compounds, lignans, β-glucans and inulin, (Liu 2007; Hirawan et al. 2010; Hung and Hatcher 2011). Ferulic acid, vanillic acid, *p*-cumaric acid and sinapic acid are examples of the phenolic acids found in durum wheat (Liyana-Pathirana and Shahidi 2007).

Both genotype (Yu et al. 2002, 2004) and environmental conditions (Yu and Zhou 2004; Menga et al. 2010; Borrelli et al. 2011; Fratianni et al. 2013) may affect phytochemicals profile, oxidative status (Soccio et al. 2010) and AA of raw material. The quality of durum wheat products, in terms of texture, colour, flavour, appearance and antioxidant capacity, is determined by raw material quality and processing methods (Brennan et al. 2011). In literature it is reported that the level of bioactive compounds in extruded products is influenced by extrusion process variables (Brennan et al. 2011). Critical extrusion process variables such as temperature, screw speed, and moisture content may induce desirable modifications, thus improving palatability and technological properties of end products. These conditions have the ability to produce either positive or negative influences on the bioactive compounds of the products.

Several studies have shown that extrusion processing significantly reduces measureable bioactive compounds in food products (Korus et al. 2007; Delgado-Licon et al. 2009). Phenolic compounds during extrusion may undergo decarboxylation due to high barrel temperature and high moisture content, may promote polymerisation of phenols and tannins leading to reduced extractability and AA (Dlamini et al. 2007; Repo-Carrasco-Valencia et al. 2009).

However, in some cases the level of bioactive compounds in extruded products may increase; for example, ferulic acid content was shown to increase three fold in extruded cereal grains (Zielinski et al. 2001). A similar increase in total phenolic compounds was reported after extrusion cooking of durum wheat alone and in combination with vegetables (Stojceska et al. 2008). The increase in the levels of certain phenolic acids in extruded cereal products is generally due to the release from the cell wall matrix (Brennan et al. 2011).

Furthermore, some authors have shown that some antioxidants, formed during processing/extrusion, contribute to the total AA of pasta (Fogliano et al. 1999; Hirawan et al.

2010). Non-enzymatic browning reactions, such as the Maillard reaction, can generate some products that display antioxidant properties (Manzocco et al. 2001; Amarowicz 2009; Hirawan et al. 2010). Pasta has antioxidant or pro-oxidant properties depending on temperature, time and moisture conditions during extrusion and drying processes (Anese et al. 1999; Hirawan et al. 2010).

Some studies report antioxidant properties of non-conventional pasta (Fares et al. 2008, 2010; Hirawan et al. 2010); other ones are focused on phytochemical profile and AA of whole durum wheat, bran and endosperm/germ (Liyana-Pathirana and Shahidi 2007; Hung and Hatcher 2011; Vaher et al. 2011). Instead, to our best knowledge, studies on the changes of AA along pasta chain are missing.

So, in order to have a deep insight into the changes of nutritional value from raw material to pasta, the aim of this work was: i) to compare the AA of hydrophilic and lipophilic extracts of five durum wheat genotypes along the pasta chain; ii) to evaluate the changes in antioxidant properties of whole flour after processing in semolina and pasta. To this aim TEAC assay based on $ABTS^{\bullet+}$ scavenging activity was used due to its high reproducibility and simplicity.

Materials and Methods

Field trial

During the 2009–2010 crop season five durum wheat varieties (Torrebianca, Pietrafitta, Alemanno, Principe, Cannavaro) were cultivated in a field trial carried out in Foggia (Southern Italy, 41°46' N, 15°54'E) on a sandy clay loam soil. N, P and S at a rate of 102, 80 and 30 Kg ha^{-1} were applied. The 2009–2010 temperature trend was regular during the crop cycle (max 16.6°C; min 6.7°C), no temperature extremes were observed, and so the year can be deemed favourable for durum wheat production. On the other hand, the crop season was characterized by scarce rainfall during all the crop cycle (228 mm) and was drier compared with long-term data. Grain samples were harvested on18th June 2010 and mean yield was 2.8 t ha^{-1}.

Sample preparation

The whole grain was ground with a "Cyclotec 1093 Sample Mill" (Foss Italia, Padova, Italy) (1 mm sieve) to obtain the whole meal. For semolina production, the seeds (3 kg), tempered at 16.5% moisture, were milled by a Buhler MLU experimental mill with six breaking and six sizing passages. The semolina yield reached a value of approximately 68%.

Pasta was prepared by mixing semolina (control) of each durum wheat variety with 30% tap water to obtain a total dough water content of 43–44%. The dough was processed into spaghetti (1.70 ± 0.03 mm diameter) with a laboratory press (NAMAD, Rome) with a capacity of 2 kg. Extrusion conditions applied were: temperature 50 ± 5°C, pressure 70 ± 10 atm, and vacuum 700 mm Hg. Extruded spaghetti samples were dried in a laboratory pasta drier (NAMAD, Rome) using a Low Temperature drying procedure for 18 h at about 50°C. Five kinds of spaghetti were produced.

Before analysis spaghetti was crushed and milled through "Cyclotec 1093 Sample Mill" (1 mm sieve). All samples were stored at 4°C up to analysis.

Sample extraction and determination of antioxidant activity

Hydrophilic Extracts. The flour was suspended in deionised water at a (w/v) ratio equal to 1 g/3 mL. The suspension was placed in an ice-water bath for 1 h, stirred at 15 min intervals, and then centrifuged twice at 18700 g × 20 min at 4°C. The final supernatant represents the hydrophilic extract.

Lipophilic Extracts. Lipophilic compounds were extracted according to Fratianni et al. (2013) method with minor modifications. Briefly, whole wheat flour (2 g) was saponified with 60% (w/v) KOH under nitrogen at 70–80°C for 45 min, and then the suspension was extracted twice with 15 mL of n-hexane/ethyl acetate 9:1 (v/v). The organic phases were collected in a flask and evaporated to dryness under vacuum at 40°C using a Buchi rotavapor for AA measurement through TEAC methods, the residue was reconstituted in 1 mL of ethanol.

TEAC (Trolox Equivalent Antioxidant Capacity) Method

TEAC assay according to Re et al. (1999) method was applied. The coloured radical monocation $ABTS^{\bullet+}$ was generated by ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] oxidation with potassium persulfate. The $ABTS^{\bullet+}$ solution was diluted with 5 mM sodium phosphate buffer, pH 7.40 (for AA determination of hydrophilic extracts), or ethanol (for AA determination of lipophilic extract), to obtain an absorbance at 734 nm of 0.70 ± 0.02. The assay mixture contained 1.0 mL of the $ABTS^{\bullet+}$ diluted solution, the extract (or standard antioxidant), and an appropriate volume of sodium phosphate buffer pH 7.40 (or ethanol), to obtain a final volume of the assay mixture equal to 1.1 mL. The absorbance at 25°C and 734 nm was read exactly 5 min and 3 min after hydrophilic and lipophilic extraction (or standard antioxidant). The decrease in A734 (%) measured 5 min after the extract (or standard antioxidant) addition with respect to A734 of the radical cation solution (blank) was used to quantify AA by means of a proper concentration-response curve prepared with Trolox by plotting the decrease of A734 (%) as a function of the standard antioxidant concentration. Trolox was added to obtain a final concentration ranging from 5 to 15 µl and all the analyses were carried out in triplicate.

Statistical analysis

The data were submitted to variance analysis (ANOVA) using MSTAT-C statistical program (version 2.1, 1991; Crop and Soil Sciences Department Michigan State University, East Lansing, MI) and "Statistica 7.1 for Windows" (Stasoft, Tulsa, OK). The significant differences among the mean values were calculated following Duncan test.

The homogeneity of variances for all the studied parameters was evaluated by Barlett's test, performed using SAS JMP statistical analysis software (8). Because the variance of data relative to AA variations of whole meal, semolina and pasta of hydrophilic and

lipophilic extracts was not homogeneously distributed among treatments, natural logarithmic transformed data were used for comparison of these variables.

Results

AA of hydrophilic and lipophilic extracts of whole meal, semolina and pasta of the five durum wheat *cultivars* under study is reported in Figure 1. A significant but slight average variability among genotypes for both hydrophilic and lipophilic extracts (10%) was observed. In whole meal ranges from 6.40 to 5.40 and 0.40 to 0.35 µmol trolox equivalents g^{-1} dry matter (d.m.) were observed, for hydrophilic and lipophilic extracts, respectively. Concerning lipophilic extracts, among the cultivars investigated, Principe showed the highest $ABTS^{\bullet+}$ scavenging activity in whole meal, semolina and pasta (0.41, 0.15 and 0.11 µmol trolox equivalents g^{-1} d.m., respectively) and Alemanno and Pietrafitta the lowest; instead, cultivar Cannavaro showed the highest AA of hydrophilic extracts (6.23 whole meal, 4.0 semolina and 4.7 pasta µmol trolox equivalents g^{-1} d.m) and Alemanno the lowest.

$ABTS^{\bullet+}$ scavenging activity of hydrophilic extracts of the varieties under study always showed values 10 fold higher than lipophilic extracts. Hydrophilic extracts contributed for more than 80% to total AA of durum wheat samples (Fig. 1).

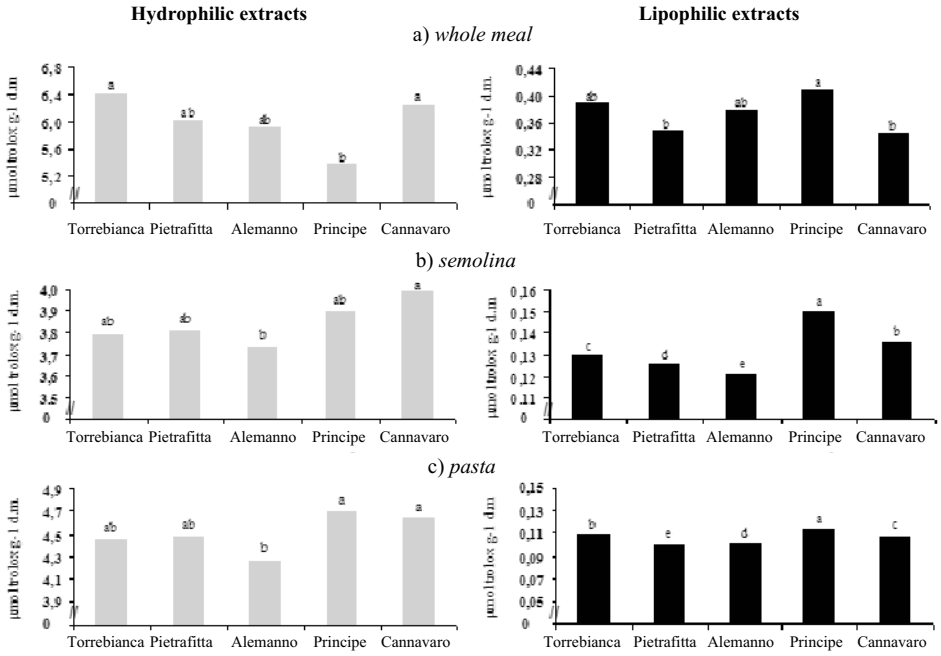

Figure 1. $ABTS^{\bullet+}$ scavenging activity of hydrophilic and lipophilic extracts from whole meal (a), semolina (b) and pasta (c). Different letters indicate significant differences at $P = 0.05$ according to Duncan's test. S.E. = 0.1 and 0.01 for hydrophilic and lipophilic extracts, respectively

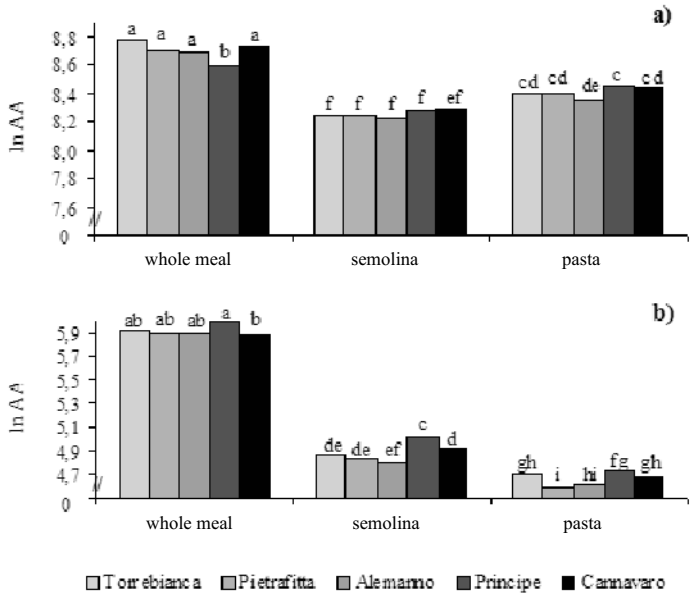

Figure 2. Effect of milling and pasta making on ABTS$^{\bullet+}$ scavenging activity of a) hydrophilic and b) lipophilic extracts. Different letters indicate significant differences at $P = 0.05$ according to Duncan's test. S.E. = 0.05

To evaluate the maintenance of whole meal hydrophilic and lipophilic ABTS$^{\bullet+}$ scavenging activity in the derived products, a combined analysis of transformed natural logarithmic data of whole meal, semolina and pasta was performed (Fig. 2). The antioxidant capacity of the samples was significantly modified by process. In particular, milling process determined a negative effect on ABTS$^{\bullet+}$ scavenging activity of semolina and pasta compared to whole meal, for both hydrophilic and lipophilic extracts.

Table 1. ABTS$^{\bullet+}$ scavenging activity (AA) percent decrease of semolina *vs* whole meal, pasta *vs* semolina and pasta *vs* whole meal on hydrophilic and lipophilic extracts of the five durum wheat *cultivars* under study

Cultivar	AA percent decrease %					
	Semolina *vs* whole meal		Pasta *vs* semolina		Pasta *vs* whole meal	
	Hydrophilic extract	Lipophilic extract	Hydrophilic extract	Lipophilic extract	Hydrophilic extract	Lipophilic extract
Torrebianca	41	66	–17	16	30	72
Pietrafitta	37	64	–18	21	26	71
Alemanno	37	68	–14	16	28	73
Principe	28	63	–21	24	13	72
Cannavaro	35	61	–16	21	25	69
Mean value	35	64	–17	20	24	71

In Table 1 the $ABTS^{\bullet+}$ scavenging activity decreases of hydrophilic and lipophilic extracts of semolina and pasta samples *vs* whole meal of the five genotypes under study are reported; for semolina samples an average decrease of 35 and 64%, respectively, was observed compared to whole meal. In general, the percent decrease of $ABTS^{\bullet+}$ scavenging activity in semolina and pasta lipophilic extracts was higher than that of hydrophilic ones (Table 1).

Moreover the AA of semolina samples was significantly modified by pasta making and a different behaviour for hydrophilic and lipophilic extracts was observed (Fig. 2a and b). For lipophilic extracts a linear diminishing, whole meal>semolina>pasta, of $ABTS^{\bullet+}$ scavenging activity was observed. In particular, lipophilic $ABTS^{\bullet+}$ scavenging activity of pasta showed a decrease of 71%, compared to whole meal (Table 1).

For hydrophilic extracts a percent decrease of 24% of pasta compared to whole meal and a descending order "whole meal>pasta>semolina" were observed (Fig. 2a). Pasta processing significantly modified $ABTS^{\bullet+}$ scavenging activity of samples, which showed an average 15% increase compared to semolina.

Whereas for AA of lipophilic extracts high significant correlations whole meal *vs* semolina ($r = 0.824$), semolina *vs* pasta ($r = 0.597$) and whole meal *vs* pasta ($r = 0.578$) were observed, no correlation was observed for hydrophilic extracts.

Discussion

A low average variability of 10% for both hydrophilic and lipophilic extracts of whole meal, semolina and pasta of the five durum wheat *cultivars* under study was observed.

Data from the literature are in agreement with this result. In particular, by using radical scavenging assays, Moore et al. (2006) and Mpofu et al. (2006) observed an average genotype variability of about 25% and 13% for bran and whole meal wheat, respectively. Furthermore, Moore et al. (2006) also reported a much lower effect of genotype with respect to environment for ABTS cation radical scavenging capacity. In accordance also Menga et al. (2010) by evaluating the effects of genotype and location, on AA of durum wheat by means of TEAC/$ABTS^{\bullet+}$ assay, observed only a 11.5% weight of genotype effect on the total variance.

Antioxidant activity of hydrophilic extracts always showed values 10 fold higher than lipophilic extracts as reported in the literature (Adom et al. 2005; Pastore et al. 2009). This result is mainly due to the different AA of hydrophilic and lipophilic extracts, but also to a lower capability of the method to detect lipophilic antioxidants with respect to other ones (Laus et al. 2012a, 2012b). This is probably dependent both on the chemical basis of the method and on the polarity of the reaction media.

The antioxidant capacity of the samples was significantly modified by process (Fig. 2). In particular, milling process determined a negative effect on $ABTS^{\bullet+}$ scavenging activity of semolina and pasta compared to whole meal, for both hydrophilic and lipophilic extracts. This result may be ascribable to the removal of the outside layers of the kernel, including pericarp, a part of aleurone layer and germ which are more rich in functional com-

ponents (dietary fibre, phytates, minerals, phenolic acids, vitamins, etc.) responsible for AA (Esposito et al. 2005; Borrelli et al. 2008).

Hydrophilic and lipophilic $ABTS^{\bullet+}$ scavenging activity of semolina showed an average decrease of 35 and 64%, respectively, compared to whole meal. In agreement with our results Fares et al. (2010) reported a decrease in $ABTS^{\bullet+}$ scavenging activity of debranned kernels by about 40% of methanol/water extracts. Moreover in crude phenolic extracts of semolina, Liyana-Pathirana and Shaidi (2007) reported a 60% decrease in total antioxidant capacity (TAC) of crude phenolic extracts, compared to whole meal.

In general, after milling process, $ABTS^{\bullet+}$ scavenging activity decrease of semolina and pasta lipophilic extracts was higher than that of hydrophilic ones compared to whole meal (Table 1). Also Adom et al. (2005) reported that milling process may result in a higher decrease in lipophilic AA than in a hydrophilic one. In particular, the authors found the hydrophilic AA of bran/germ samples to be 13–27 fold higher than that of the respective endosperm samples while the lipophilic AA was 28–89 fold higher in the bran/germ fractions than that of the respective endosperm fractions. This is due to the different distribution of hydrophilic and lipophilic antioxidants along the kernel. In fact, as β-carotene and tocopherols are predominant in germ, tocotrienols are prevalent in the aleuron layer and only in trace in endosperm (Borrelli et al. 2008; Fares et al. 2008; Tsao 2008), for lipophilic extracts a higher $ABTS^{\bullet+}$ scavenging activity decrease was expected after milling. Instead hydrophilic compounds, such as phenolic acids, are distributed in all fractions of the kernel (Adom et al. 2005; Fares et al. 2010), therefore, after milling, a lower $ABTS^{\bullet+}$ scavenging activity decrease was expected.

Moreover the antioxidant capacity of the samples was significantly modified by pasta making and a different behaviour for hydrophilic and lipophilic extracts was observed.

Lipophilic $ABTS^{\bullet+}$ scavenging activity showed a further decrease in pasta samples compared to semolina (Table 1). Also Fares et al. (2008) observed a strong reduction in total AA (TAC) of lipophilic extracts of emmer semolina and pasta. Comparing the TAC values of uncooked pasta to the ones of whole meal samples, an average reduction of 95% was observed. This decrease was higher with respect to the decrease of 71% observed in our study. The differences detected in the two studies might be caused by the use of different species (i.e. emmer and durum wheat) as well as by the different conditions adopted during the process; in particular, the high drying temperature adopted by Fares et al. (2008) might have promoted stability and reduced extractability of bioactive compounds causing a decrease in AA (Dlamini, et al. 2007; Repo-Carrasco-Valencia et al. 2009). In fact, pasta as well as other extruded products, has antioxidant properties depending on temperature, time and moisture conditions during the extrusion and drying processes (Anese et al. 1999; Hirawan et al. 2010).

Relative to hydrophilic extracts pasta processing determined an average 15% increase in $ABTS^{\bullet+}$ scavenging activity compared to semolina. This result may be explained on the basis of the formation of new antioxidants during pasta drying process. In the literature it is reported that some antioxidants formed during extrusion and drying processes, might contribute to total AA of pasta (Anese et al. 1999; Fogliano et al. 1999). In particular, a wide number of reactions such as Maillard reaction, caramelisation, and chemical oxida-

tion of phenols take place during pasta making process. The influence of these different reactions on the overall antioxidant capacity of foods has been scarcely investigated (Brennan et al. 2011; Manzocco et al. 2001). Generally AA is associated to the formation of brown melanoidins (White et al. 2010; Brennan et al. 2011). Melanoidins are polymeric and coloured macromolecules originated by the Maillard reaction and formed primarily by interactions between carbohydrates (typically reducing sugars) and compounds characterized by a free amino group, such as amino acids. During recent years, interest in these compounds has increased because of their nutritional antiradical, antimutagenic, chelating properties (Echavarria et al. 2012).

Therefore the increase in $ABTS^{\bullet+}$ scavenging activity of hydrophilic extracts of pasta samples compared to semolina might be due to melanoidins formed during Maillard reaction.

Finally whereas no significant correlation among AA of hydrophilic extracts was observed, for lipophilic extracts it was possible a predictive evaluation of semolina and pasta $ABTS^{\bullet+}$ scavenging activity by testing raw material.

In this study the changes in $ABTS^{\bullet+}$ scavenging activity along pasta chain were investigated. Milling process caused a significant decrease in $ABTS^{\bullet+}$ scavenging activity due to the removal of the outside layers of the kernel. This decrease was more marked for lipophilic extracts, which may be due to the different distribution of hydrophilic and lipophilic antioxidants along the kernel. Pasta making process while determining a further decrease in AA of lipophilic extracts caused a slight increase in AA of hydrophilic extracts compared to semolina. This might be due to melanoidins formed during Maillard reaction. While AA of hydrophilic extracts of whole meal was not significantly correlated to derived products, a high significant correlation was observed for lipophilic extracts. As a consequence breeding for durum wheat genotypes able to provide pasta characterized by high AA appears to be valuable only for lipophilic extracts and not for hydrophilic ones.

Further studies with a higher number of genotypes and different processing conditions have to be carried out in order to optimize the nutritional value of pasta in relation to AA.

Moreover the positive influence of pasta making on AA of hydrophilic extracts highlights that to obtain pasta with high nutritional value, the appropriate combination of suitable raw material and extrusion variables has to be investigated.

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