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Antioxidant Capacity of Durum Wheat Large Flour Particles May Be Evaluated by QUENCHER$_{ABTS}$ Assay by Adopting a Proper Calculation Mode

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Assessment of Antioxidant Capacity (AC) of foods is useful to consider cumulative/ synergistic action of all dietary antioxidants, thus providing a more integrated information than the simple sum of measurable antioxidants. Among the different AC assays, the QUENCHER$_{ABTS}$ (QUick, Easy, New, CHEap and Reproducible) procedure is based on the direct reaction of ABTS$^{\bullet+}$ reagent with fine solid food particles without extraction of antioxidants. This assay is able to measure both soluble and insoluble antioxidants, that simultaneously come into contact with ABTS$^{\bullet+}$ molecules by either liquid–liquid or solid–liquid interactions, respectively. These interactions may change depending on the particle diameter. Usually, particles having 0.1–0.3 mm size are used. Here, AC was evaluated on whole flour (WF), derived from a mix of grains of ten durum wheat varieties, characterized by three different particle sizes: a smaller one, ≤0.2 mm (control, WF$_{0.2}$), and two larger ones, ≤0.5 mm and ≤1 mm (WF$_{0.5}$ and WF$_1$, respectively). Moreover, a novel AC calculation procedure based on the slope value of the regression line of ABTS$^{\bullet+}$ response *vs* flour amount is presented in detail. The classical QUENCHER$_{ABTS}$ procedure provided for WF$_{0.2}$ an AC value of 42.0±2.7 µmol eq. Trolox/g d.w. A similar result was obtained for WF$_{0.5}$ (38.3±0.9 µmol eq. Trolox/g d.w.), thus indicating that these large particles may be analyzed by the QUENCHER$_{ABTS}$ assay provided that the "slope" calculation procedure is used. On the contrary, WF$_1$ showed about half AC (20.3±0.2 µmol eq. Trolox/g d.w.), thus showing that very large particles cannot be used even adopting the "slope" calculation.

Keywords: antioxidant capacity, QUENCHER$_{ABTS}$, durum wheat grains, particle size

Abbreviations: ABTS, 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid); AC, antioxidant capacity; d.w., dry weight; f.w., fresh weight; QUENCHER, QUick, Easy, New, CHEap and Reproducible; Trolox, ±-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid; WF, whole flour

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Introduction

To date, a large diversity of analytical methods for *in vitro* measurement of Antioxidant Capacity (AC) of foods is available. These assays differ from each other mainly in terms of chemical mechanism/s involved, and oxidant species/probe(s) and techniques used to monitor the reaction (Magalhaes et al. 2008; Carocho et al. 2013). Moreover, some assays provide results of questionable physiological relevance and often not related to individual dietary antioxidants or to different phytochemicals synergically acting. These aspects are discussed in Pastore et al. (2009) and Laus et al. (2012, 2013). From a methodological point of view, most of the AC assays used up to now require the preliminary extraction of antioxidants from food matrices before AC measurement. The use of different extraction procedures, showing different efficiency and recovery of antioxidants, is an important source of variation in the reported AC values. This limits a proper inter-laboratory comparison of data and it can explain many discrepancies among independent studies (Serpen et al. 2007, 2008; Gökmen et al. 2009).

During recent years, a new simple and direct procedure for AC assessment of food matrices avoiding any extraction and hydrolysis step has been proposed (Serpen et al. 2008). It involves a direct reaction of food solid particles with the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation, followed by centrifugation to obtain an optically clear supernatant for absorbance measurement at 734 nm. The new approach, abbreviated as "QUENCHER$_{ABTS}$" (QUick, Easy, New, CHEap and Reproducible), takes advantage from liquid-liquid interactions occurring in the solvent between soluble antioxidants and $ABTS^{\bullet+}$ molecules, and it may also assess AC of antioxidants bound to insoluble matter by surface solid-liquid interactions (Serpen et al. 2007, 2008; Gökmen et al. 2009). This direct procedure, avoiding any pretreatment of food samples, may give AC values generally higher than that obtained by different extraction and hydrolysis procedures used for comparison (Serpen et al. 2008). Moreover, these values are potentially related to a true antioxidant action in food or in human gastrointestinal trait (Serpen et al. 2008; Gökmen et al. 2009), thus showing possible physiological relevance of results. At this regard, only complex *in vitro* enzymatic digestion designed to mimic digestion in the gastrointestinal tract may be more related to physiological effects (Gong et al., 2013). The QUENCHER procedure has been successfully applied to measure AC of very different foods (Serpen et al. 2008, 2012 a, b, c; Acar et al. 2009; Ciesarova et al. 2009; Rufian-Henares et al. 2009; Serpen and Gökmen 2009; Amigo-Benavent et al. 2010; Delgado-Andrade et al. 2010; Žilić et al. 2012, 2013). Moreover, the QUENCHER assay has been successfully performed also replacing $ABTS^{\bullet+}$ as radical probe with DPPH (Gökmen et al. 2009; Serpen et al. 2012 a, b, c), as well as using the colour generation of FRAP (Serpen et al. 2012 a, b) and CUPRAC reagents (Tufan et al. 2013) or fluorescence as in ORAC protocol (Amigo-Benavent et al. 2010; Kraujalis et al. 2013).

Obviously, in this approach, total surface area and solid food particle sizes may play a crucial role in determining the reaction rate and, in turn, the measured AC (Gökmen et al. 2009). To date, the QUENCHER procedure has been generally applied to finely ground

food solid particles having generally a diameter not exceeding 0.2–0.3 mm. Nevertheless, some food matrices of interest may be produced and used as larger size particles. At this purpose, industrial milling process of wheat grains generates by-products showing different particle size distribution mainly dependent on milling process and grain hardness (Devaux et al. 1998). Large particle by-products may display special properties. As for wheat bran, the addition of bran having a larger particle size (≥ 0.5 mm) has been reported to positively influence technological performance, as well as quality and sensory characteristics of some integrated fiber-rich foods (Noort et al. 2010; Chen et al. 2011). Moreover, large particle-bran has been also shown to induce a greater acetate production in an *in vitro* fermentation system (Stewart et al. 2009) and to influence the extent of starch retrogradation and in turn starch digestibility in bran-enriched bread during storage (Cai et al. 2014), so suggesting that large particles may exert physiological effects. In the light of this, AC determination of large particles may have a physiological interest.

So, the goal of this study was to test the applicability of the QUENCHER$_{ABTS}$ protocol to solid particles having large size. This was performed by analyzing whole flour (WF) derived from a mix of grains of ten durum wheat (*Triticum turgidum* L. subsp. *durum*) varieties, characterized by three different particle sizes: a smaller one ($\leq$0.2 mm, control, $WF_{0.2}$) and two higher ones ($\leq$0.5 mm and $\leq$1 mm, $WF_{0.5}$ and WF_1, respectively), larger than that so far reported in literature. To do this, a novel AC calculation procedure based on the slope value of the regression line of $ABTS^{\bullet+}$ response *vs* flour amount was used for $WF_{0.5}$ and WF_1 and results were compared with that obtained for $WF_{0.2}$ using classical procedure.

Materials and Methods

Chemicals

ABTS, ethanol, potassium persulfate, ±-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich Corp. (St. Louis, Mo., USA).

Plant material

Grain samples from ten durum wheat varieties (Quadrato, Torrebianca, Pietrafitta, Vendetta, Alemanno, Principe, Cannavaro, Gattuso, Simeto and Duilio) were stored under vacuum at 4 °C for no longer than 2 months. Before use, a balanced mix of whole grains were daily milled by means of a Cyclotec 1093 Sample Mill (using 1 mm or 0.5 mm sieves). Ground samples were passed through 1 mm, 0.5 mm and 0.2 mm certified test sieves (Giuliani Tecnologie, Turin, Italy) to obtain WF_1 (as not passing through 0.5 mm sieve), $WF_{0.5}$ (as not passing through 0.2 mm sieve) and $WF_{0.2}$ (as passing through 0.2 mm sieve), respectively.

Determination of AC by the direct QUENCHER$_{ABTS}$ procedure

The $ABTS^{\bullet+}$ radical cation was generated by chemical oxidation with potassium persulfate as described by Re et al. (1999) and then diluted in a mixture of ethanol:water (50:50, v/v) to obtain an absorbance value at 734 nm (A_{734}) of 0.70±0.02. Measurements were carried out in triplicate by adding the $ABTS^{\bullet+}$ diluted solution with flour sample and vigorously stirring the suspension to facilitate a surface reaction between the solid particles and the $ABTS^{\bullet+}$ reagent. Then, optically clear supernatant obtained after centrifugation at 9200×*g* for 2 min was used to measure A_{734}. The (%) decrease of A_{734} measured after sample incubation (A_f) with respect to A_{734} of $ABTS^{\bullet+}$ solution (A_0) was calculated by the following equation: (%) decrease of $A_{734} = [1 - (A_f/A_0)] \times 100$. To develop a properly adapted protocol for $WF_{0.5}$ and WF_1, different reaction times (ranging from 5 to 300 min for WF_1 and from 5 to 270 min for $WF_{0.5}$) and whole flour amount (ranging from 0.40 to 1.66 mg fresh weight, f.w./mL of $ABTS^{\bullet+}$ solution for WF_1 and from 0.10 to 1.60 mg f.w./mL for $WF_{0.5}$) were analyzed. Note that, depending on the particle size, the interactions between $ABTS^{\bullet+}$ molecules and insoluble-bound antioxidants are different. As a consequence, different flour amounts were necessary to assure AC values giving linearity of response. This linear dependence of the (%) decrease of A_{734} on sample amount was verified for each incubation time by linear regression analysis of data. AC was obtained by comparing the slope derived by linear regression analysis with that of the Trolox-derived calibration curve. As for $WF_{0.2}$, measurements were carried out for 60 min and using flour amount ranging from 0.10 to 0.35 mg f.w./mL of $ABTS^{\bullet+}$. In this case, AC was classically calculated by linear interpolation of data.

Results

Firstly, the QUENCHER$_{ABTS}$ procedure was applied to $WF_{0.2}$ particles, having a diameter ≤0.2 mm, that is included in the 0.1–0.3 mm range generally used in literature. In Figure 1 the linear dependence of (%) decrease of A_{734} on the ratio between $WF_{0.2}$ amount and volume of $ABTS^{\bullet+}$ solution is shown. The straight line obtained by linear regression analysis of $WF_{0.2}$ data, as well as the calibration curve obtained with Trolox, showed *y*-axis intercepts very close to zero. Linear interpolation of $WF_{0.2}$ data into Trolox calibration curve provided an AC value for $WF_{0.2}$ of 42.0±2.7 μmol eq. Trolox/g of dry weight, d.w.

In order to test the applicability of the QUENCHER$_{ABTS}$ procedure to solid particles having diameter higher than 0.2 mm, possible methodological adaptation of classical QUENCHER$_{ABTS}$ assay was evaluated, with particular attention to both reaction time and flour amount. So, both $WF_{0.5}$ and WF_1 particles were analyzed by carrying out the reactions for increasing incubation times and using increasing amounts of flour sample (see also Methods). Results relative to both $WF_{0.5}$ and WF_1 are shown in Figure 2. In particular, in Figures 2a and d, the profile of (%) decrease of A_{734} *vs* the incubation time is reported for each tested amount of $WF_{0.5}$ and WF_1, respectively. The profile relative to the $ABTS^{\bullet+}$ diluted solution in the absence of sample is also shown. An undesired complete bleaching of the $ABTS^{\bullet+}$ reagent (100% decrease of A_{734nm}) was observed in the presence

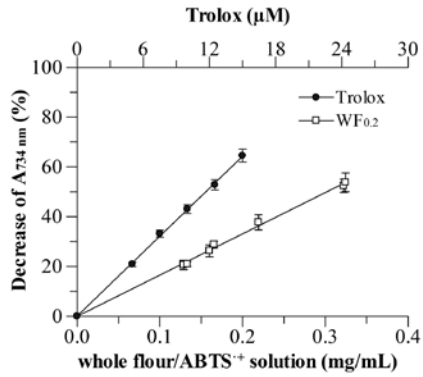

Figure 1. Dependence of the absorbance decrease (%), measured at 734 nm by using the QUENCHER$_{ABTS}$ assay, on the amount of durum wheat whole flour (≤0.2 mm particle size, $WF_{0.2}$). Measurements were carried out as described in Methods, after 60 min incubation of $ABTS^{\bullet+}$ diluted solution with different amounts (f.w.) of $WF_{0.2}$ mix. The straight line obtained by linear regression analysis of data relative to $WF_{0.2}$ mix is reported. Calibration curve obtained with Trolox is also shown. Data are reported as mean value ± SD (n = 3 different experiments)

of both 1.60 mg $WF_{0.5}$/mL and 1.66 mg WF_1/mL, thus indicating that $ABTS^{\bullet+}$ molecules are limiting with respect to the sample. This saturation response is a condition that prevents AC determination. However, using lower flour amounts, $ABTS^{\bullet+}$ quenching curves were obtained tending to a different plateau below saturation, so allowing AC quantification. To define the most appropriate measurement condition, data of Figs. 2a and d were plotted as (%) decrease of A_{734} *vs* the flour amount/$ABTS^{\bullet+}$ volume ratio (Figs. 2b and e). In both cases, two distinct flour amount ranges were found, in which a different relationship between WF amount and (%) decrease of A_{734} was observed (ranges I and II). A possible explanation of this phenomenon is that the increasing of the "insoluble antioxidants/$ABTS^{\bullet+}$" ratio over a given value might result in a lowering of the reaction rate constant. This effect is more pronounced as the reaction time increases, since the unreacted available $ABTS^{\bullet+}$ radical decreases. As expected, in the light of a lower diameter and higher total surface area of $WF_{0.5}$ with respect to WF_1, the ranges I and II are different between the two particle sizes. For both $WF_{0.5}$ and WF_1, linear regression analyses of data in the range I (0.10–0.30 mg $WF_{0.5}$/mL and 0.40–0.66 mg WF_1/mL) generated straight lines generally almost parallel whatever the reaction time (Figs 2c and f). However, none of these straight lines passed through the origin of axes, but the lines of range I showed intercepts to the *y*-axis much closer to zero with respect to the regression curves obtained in the range II. Since the theoretical intercept is zero (see $WF_{0.2}$ and Trolox in Figure 1), the range I has to be used to calculate AC values. Moreover, the values of *y*-axis intercepts have to be adequately subtracted in AC calculation. To do this, the equation of the linear regression curve is calculated; then, the slope value is divided by that of the Trolox calibration curve (slope value = 4.28) of Fig. 1. The final AC value is expressed as μmol eq. Trolox and referred to grams of d.w. All calculations are reported in Table 1. In particular, equations of the linear regression curves are reported, obtained in the linearity range I for

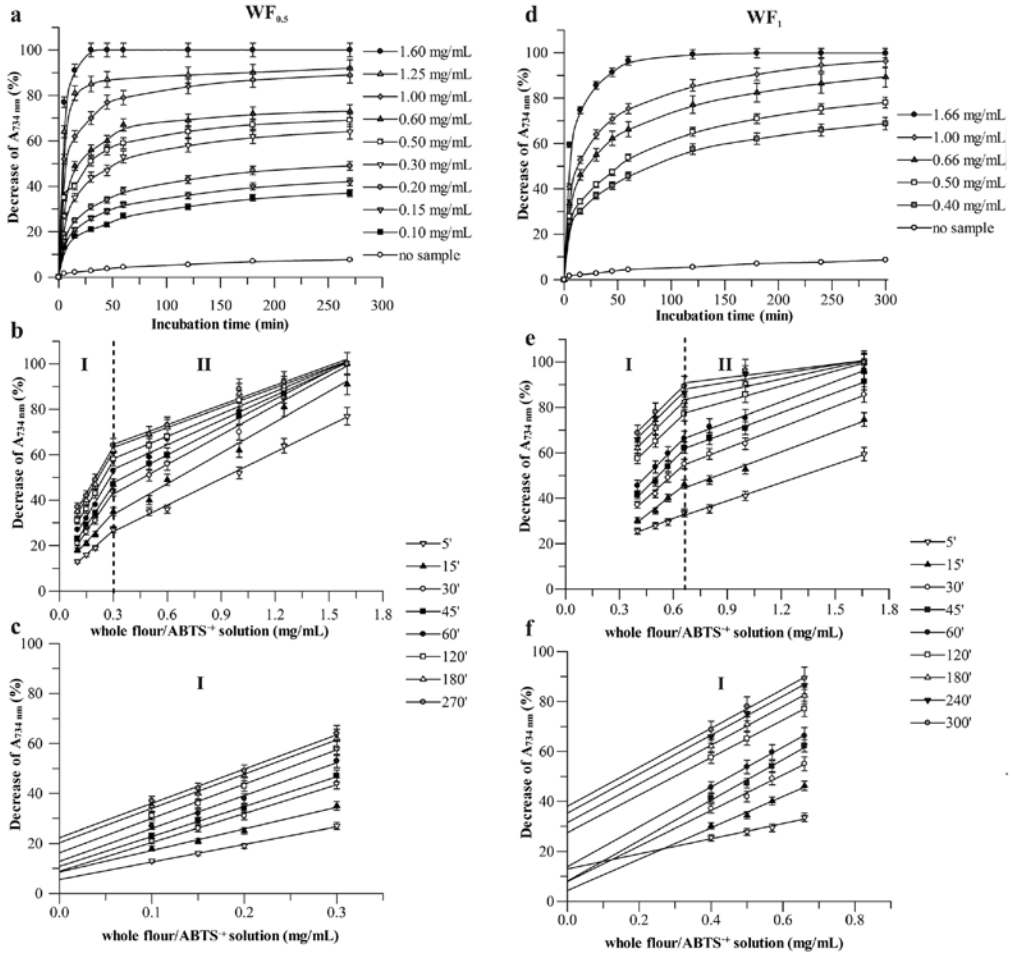

Figure 2. Dependence of the absorbance decrease (%), measured at 734 nm by using the QUENCHER$_{ABTS}$ assay, on the reaction time of the ABTS$^{\bullet+}$ radical cation with durum wheat whole flour (a, d) and on the amount of whole flour (b, c, e, f) ABTS$^{\bullet+}$ diluted solution was incubated for different times with different amounts (f.w.) of either $WF_{0.5}$ or WF_1. In (a) and (d) the profiles *vs* reaction time are reported for $WF_{0.5}$ and WF_1, respectively. In (b) and (e) the straight lines obtained by two separate linear regression analyses of data in the ranges I and II (0.1–0.30 and 0.30–1.60 mg (f.w.) $WF_{0.5}$/mL and 0.40–0.66 and 0.66–1.66 mg (f.w.) WF_1/mL, respectively) are shown. In (c) and (f) the extrapolation to *y*-axis of the curves obtained by linear regression analyses of data in the range I of Fig. 2b and Fig. 2e is shown in detail. Data are reported as mean value ± SD (n = 3 different experiments)

Table 1. Antioxidant Capacity (AC) evaluated by the QUENCHER$_{ABTS}$ assay of durum wheat whole flour having ≤0.5 mm ($WF_{0.5}$) and ≤1 mm (WF_1) particle sizes

$WF_{0.5}$		
Time of incubation (min)	Equation of straight line	AC* QUENCHER$_{ABTS}$
5	$y = 72.86x + 4.04$	20.2±0.4
15	$y = 90.82x + 6.23$	25.2±0.6
30	$y = 116.7x + 6.26$	32.4±0.8
45	$y = 123.30x + 8.10$	34.3±0.7
60	$y = 137.95x + 9.07$	38.3±0.9
120	$y = 138.57x + 13.45$	38.5±1.2
180	$y = 140.30x + 17.41$	39.0±1.5
270	$y = 138.28x + 19.16$	38.4±1.3
WF_1		
Time of incubation (min)	Equation of straight line	AC* QUENCHER$_{ABTS}$
5	$y = 29.60x + 13.25$	7.7±0.3
15	$y = 61.31x + 4.96$	16.0±0.5
30	$y = 69.51x + 8.63$	18.1±0.5
45	$y = 79.24x + 8.90$	20.7±0.5
60	$y = 77.86x + 14.72$	20.3±0.2
120	$y = 73.20x + 28.36$	19.1±0.1
180	$y = 75.54x + 32.36$	19.7±0.4
240	$y = 76.04x + 36.21$	19.8±0.5
300	$y = 76.09x + 38.94$	19.8±0.5

Equations of straight lines obtained by the linear regression analysis of data relative to $WF_{0.5}$ (Fig. 2c) and WF_1 (Fig. 2f). In the equations, x represents the (%) decrease of A_{734} and y represents whole flour amount expressed as mg f.w./mL of $ABTS^{\bullet+}$ solution. AC values were calculated by comparing the slope of each straight line with that of the Trolox calibration curve of Fig. 1, having a slope value of 4.28.

*Data are expressed as μmol eq. Trolox/g d.w. and reported as mean value ± SD (n = 3 different experiments). Moisture content was 15% and 11% in the $WF_{0.5}$ and WF_1 experiments, respectively.

each incubation time using either $WF_{0.5}$ (Fig. 2c) or WF_1 (Fig. 2f). The corresponding AC values obtained by the "slope" calculation mode are also reported. The linear regression analysis of data relative to $WF_{0.5}$ generated curves with higher slopes, up to about a doubling, with respect to WF_1. Consistently, AC values obtained for $WF_{0.5}$ by the slope calculation mode resulted up to about 2-fold higher than that calculated for WF_1. For both $WF_{0.5}$ and WF_1, AC values were found to increase with increasing incubation time up to 45 or 60 min, respectively. On the contrary, AC values remained statistically equal over from 45–60 min, with the ones obtained at 60 min (38.3±0.9 for $WF_{0.5}$ and 20.3±0.2 μmol eq. Trolox/g d.w. for WF_1) affected by the lowest experimental error. On the basis of these results, the reaction time of 60 min appears to be the most suitable. In the whole, AC value obtained for $WF_{0.5}$ using 60 min reaction time and by exploring the range 0.10–0.30 mg of flour/mL, resulted similar to the one measured for $WF_{0.2}$ (42.0±2.7 μmol eq.

Trolox/g d.w.) by the classical interpolation mode, so indicating as appropriate this experimental condition for $WF_{0.5}$ measurements. On the contrary, no experimental condition useful for WF_1 analysis was identified.

Discussion

The QUENCHER$_{ABTS}$ method allows for AC evaluation of compounds without preliminary extraction when they are still bound to the insoluble food matrix. Recently, we have shown that in whole flour of durum wheat the QUENCHER$_{ABTS}$ assay highlights AC mainly due to bound phenols (Laus et al. 2015). So QUENCHER$_{ABTS}$ assay appears to be a useful approach to prevent some misjudgements; for example, acid hydrolysis of bound phenolic compounds may produce 5-hydroxymethyl-2-furfural and derivatives, able to display AC, that may induce an incorrect AC determination when extracts are analyzed (Chen et al. 2014).

As for AC measurements of cereal grains by using this direct procedure, it has been reported that an accurate grinding is required to obtain particles having a diameter ranging between 0.1 and 0.3 mm (Gökmen et al. 2009). Nevertheless, since the use of larger particles of some cereal milling products has been reported to exert positive effect on technological performance and quality of some derived foods (Noort et al. 2010; Chen et al. 2011), as well as to induce some physiological effects (Stewart et al. 2009; Cai et al. 2014), the study of AC of larger particles may be of interest. Here, we show that adopting little changes with respect to the original QUENCHER$_{ABTS}$ method, it is possible to analyze large particles up to 0.5 mm, without mistakes in measured AC values. This result extends the findings of Serpen et al. (2008), who found no relevant changes (within 20%) in AC value in the 0.105–0.177 mm particle size range. In particular, the observed feature of large particles to generate straight lines that do not pass through origin appears to be a characteristic of the QUENCHER$_{ABTS}$ procedure. Under these conditions, the calculation procedure based on comparison between slopes is strongly advisable to avoid AC overestimation, while classical AC measurement through interpolation would lead to incorrect values. So, our QUENCHER$_{ABTS}$ approach extends the potentiality of the QUENCHER procedure and improves its range of applicability. On the contrary, as expected in the light of the strong total surface area/volume ratio decrease due to the doubling of particle size, the AC measured was much lower when particles up to 1 mm were analyzed. So, to avoid AC underestimation, analysis of 1 mm-particles by the QUENCHER procedure should be avoided. On the whole, when comparing literature AC data obtained by means of the QUENCHER procedure, it is very important to know the adopted experimental conditions with particular attention to particle size.

In conclusion, by adopting the novel mode of calculation based on the slope value of the regression line of $ABTS^{\bullet+}$ response *vs* flour amount, it is possible to use the QUENCHER$_{ABTS}$ method to measure, without alteration of results, AC of particles having larger size (up to 0.5 mm) than that believed so far. This finding extends the applicability of the method. However, whatever the calculation mode, very large particles having 1 mm size cannot be analyzed by the QUENCHER$_{ABTS}$ method.

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References

Acar, O.C., Gökmen, V., Pellegrini N., Fogliano, V. 2009. Direct evaluation of the total antioxidant capacity of raw and roasted pulses, nuts and seeds. Eur. Food Res. Technol. **229**:961–969.

Amigo-Benavent, M., del Castillo M.D., Fogliano, V. 2010. Are the major antioxidants derived from soy protein and fructo-oligosaccharides model systems colored aqueous soluble or insoluble compounds? Eur. Food Res. Technol. **231**:545–553.

Cai, L., Choi, I., Hyun, J.N., Jeong, Y.K., Baik, B.K. 2014. Influence of bran particle size on bread-baking quality of whole grain wheat flour and starch retrogradation. Cereal Chem. **91**:65–71.

Carocho, M., Ferreira, I.C.F.R. 2013. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem. Toxicol. **51**:15–25.

Chen, J.S., Fei, M.J., Shi, C.L., Tian, J.C., Sun, C.L., Zhang, H., Ma, Z., Dong, H.X. 2011. Effect of particle size and addition level of wheat bran on quality of dry white Chinese noodles. J. Cereal Sci. **53**:217–224.

Chen, P.X., Tang, Y., Zhang, B., Liu, R., Marcone, M.F., Xihong, L., Tsao, R. 2014. 5-hydroxymethyl-2-furfural and derivatives formed during acid hydrolysis of conjugated and bound phenolics in plant foods and the effects on phenolic content and antioxidant capacity. J. Agric. Food Chem. **62**:4754–4761.

Ciesarova, Z., Kukurova, K., Bednarikova, A., Morales, F.J. 2009. Effect of heat treatment and dough formulation on the formation of Maillard reaction products in fine bakery products-benefits and weak points. J. Food Nutr. Res. **48**:20–30.

Delgado-Andrade C., Conde-Aguilera J.A., Haro A., Pastoriza de la Cueva S., Rufian-Henares J.A. 2010. A combined procedure to evaluate the global antioxidant response of bread. J. Cereal Sci. **52**:239–246.

Devaux, M.F., Le Deschault de Monredon, F., Guibert, D., Novales, B., Abecassis, J. 1998. Particle size distribution of break, sizing and middling wheat flours by laser diffraction. J. Sci. Food Agric. **78**:237–244.

Gökmen, V., Serpen, A., Fogliano, V. 2009. Direct measurement of the total antioxidant capacity of foods: the 'QUENCHER' approach. Trends Food Sci. Tech. **20**:278–288.

Gong, L., Jin, C., Wu, X., Zhang, Y. 2013. Relationship between total antioxidant capacities of cereals measured before and after *in vitro* digestion. Int. J. Food Sci. Nutr. **64**:850–856.

Kraujalis, P., Venskutonis, P.R., Kraujalienė, V., Pukalskas, A. 2013. Antioxidant properties and preliminary evaluation of phytochemical composition of different anatomical parts of amaranth. Plant Foods Hum. Nutr. **68**:322–328.

Laus, M.N., Di Benedetto, N.A., Caporizzi, R., Tozzi, D., Soccio, M., Giuzio, L., De Vita, P., Flagella, Z., Pastore, D. 2015. Evaluation of phenolic antioxidant capacity in grains of modern and old durum wheat genotypes by the novel QUENCHER$_{ABTS}$ approach. Plant Foods Hum. Nutr. DOI: 10.1007/s11130-015-0483-8, *in press.*

Laus, M.N., Soccio, M., Pastore, D. 2013. Evaluation of synergistic interactions of antioxidants from plant foods by a new method using soybean lipoxygenase. J. Food Nutr. Res. **52**:256–260.

Laus, M.N., Tozzi, D., Soccio, M., Fratianni, A., Panfili, G., Pastore, D. 2012. Dissection of antioxidant activity of durum wheat (*Triticum durum* Desf.) grains as evaluated by the new LOX/RNO method. J. Cereal Sci. **56**:214–222.

Magalhaes, L.M., Segundo, M.A., Reis, S., Lima, J.L.F.C. 2008. Methodological aspects about *in vitro* evaluation of antioxidant properties. Anal. Chim. Acta **613**:1–19.

Noort, M.W.J., van Haaster, D., Hemery, Y., Schols, H.A., Hamer, R.J. 2010. The effect of particle size of wheat bran fractions on bread quality - Evidence for fibre-protein interactions. J. Cereal Sci. **52**:59–64.

Pastore, D., Laus, M.N., Tozzi, D., Fogliano, V., Soccio, M., Flagella, Z. 2009. New tool to evaluate a comprehensive antioxidant activity in food extracts: bleaching of 4-nitroso-*N,N*-dimethylaniline catalyzed by soybean lipoxygenase-1. J. Agric. Food Chem. **57**:9682–9692.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. **26**:1231–1237.

Rufian-Henares, J.A., Delgado-Andrade, C. 2009. Effect of digestive process on Maillard reaction indexes and antioxidant properties of breakfast cereals. Food Res. Int. **42**:394–400.

Serpen, A., Capuano, E., Fogliano, V., Gökmen, V. 2007. A new procedure to measure the antioxidant activity of insoluble food components. J. Agric. Food Chem. **55**:7676–7681.

Serpen, A., Gökmen, V. 2009. Evaluation of the Maillard reaction in potato crisps by acrylamide, antioxidant capacity and color. J. Food Compos. Anal. **22**:589–595.

Serpen, A., Gökmen, V., Fogliano, V. 2012a. Solvent effects on total antioxidant capacity of foods measured by direct QUENCHER procedure. J. Food Compos. Anal. **26**:52–57.

Serpen, A., Gökmen, V., Fogliano, V. 2012b. Total antioxidant capacities of raw and cooked meats. Meat Sci. **90**:60–65.

Serpen, A., Gökmen, V., Mogol, B.A. 2012c. Effects of different grain mixtures on Maillard reaction products and total antioxidant capacities of breads. J. Food Compos. Anal. **26**:160–168.

Serpen, A., Gökmen, V., Pellegrini, N., Fogliano, V. 2008. Direct measurement of the total antioxidant capacity of cereal products. J. Cereal Sci. **48**:816–820.

Stewart, M.L., Slavin, J.L. 2009. Particle size and fraction of wheat bran influence short-chain fatty acid production *in vitro*. Brit. J. Nutr. **102**:1404–1407.

Tufan, A.N., Celik, S.E., Ozyurek, M., Guclu, K., Apak, R. 2013. Direct measurement of total antioxidant capacity of cereals: QUENCHER-CUPRAC method. Talanta **108**:136–142.

Žilić, S., Akillioglu, H.G., Serpen, A., Peric, V., Gökmen, V. 2013. Comparison of phenolic compounds, isoflavones, antioxidant capacity and oxidative enzymes in yellow and black soybeans seed coat and dehulled bean. Eur. Food Res. Technol. **237**:409–418.

Žilić, S., Serpen, A., Akıllıoglu, G., Jankovic, M., Gökmen, V. 2012. Distributions of phenolic compounds, yellow pigments and oxidative enzymes in wheat grains and their relation to antioxidant capacity of bran and debranned flour. J. Cereal Sci. **56**:652–658.