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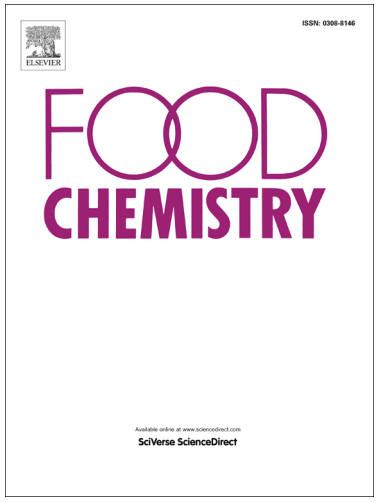

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Different effectiveness of two pastas supplemented with either lipophilic or hydrophilic/phenolic antioxidants in affecting serum as evaluated by the novel Antioxidant/Oxidant Balance approach

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Abbreviations

AAPH, 2,2′-azobis(2-methylpropionamidine) dihydrochloride; ABTS, diammonium-2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate); AC, Antioxidant Capacity; AOB, Antioxidant/Oxidant Balance; AUC, area under curve; BO, bran oleoresin; BW, bran water; DPPD, *N,N*-diethyl-*p*-phenylenediamine; d.w., dry weight; FL, fluorescein, 3',6'-dihydroxy-3H-spiro[2-benzofuran-1,9'-xanthen]-3-one; FSP, Free Soluble Phenolic; f.w., fresh weight; HAT, Hydrogen Atom Transfer; LDLox, oxidized low-density lipoproteins; ln, natural logarithmic; LOX, Lipoxygenase; ORAC, Oxygen Radical Absorbance Capacity; PxL, Peroxide Level; R, reference; RNO, 4-nitroso-*N,N*-dimethylaniline; SC-CO_2, supercritical carbon dioxide; SET, Single Electron Transfer; sr, square root; TEAC, Trolox Equivalent Antioxidant Capacity; Trolox, (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid.

ABSTRACT

Effectiveness in improving serum antioxidant status of two functional pastas was evaluated by the novel Antioxidant/Oxidant Balance (AOB) parameter, calculated as Antioxidant Capacity (AC)/Peroxide Level ratio, assessed here for the first time. In particular, Bran Oleoresin (BO) and Bran Water (BW) pastas, enriched respectively with either lipophilic (tocochromanols, carotenoids) or hydrophilic/phenolic antioxidants extracted from durum wheat bran, were studied. Notably, BO pasta was able to improve significantly (+65%) serum AOB during four hours after intake similarly to Lisosan G, a wheat antioxidant-rich dietary supplement. Contrarily, BW pasta had oxidative effect on serum so as conventional pasta and glucose, thus suggesting greater effectiveness of lipophilic than hydrophilic/phenolic antioxidants under our experimental conditions. Interestingly, no clear differences between the two pastas were observed, when AC measurements of either serum after pasta intake or pasta extracts by *in vitro* assays were considered, thus strengthening effectiveness and reliability of AOB approach.

Keywords: durum wheat antioxidants; functional pasta; human serum; LOX-FL; ORAC; TEAC; Lisosan G.

Chemical compounds studied in this article

AAPH, 2,2'-azobis(2-methylpropionamidine) dihydrochloride (PubChem CID: 76344); ABTS, diammonium-2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (PubChem CID: 9570474); fluorescein, 3',6'-dihydroxy-3H-spiro[2-benzofuran-1,9'-xanthen]-3-one (PubChem CID: 16850); linoleic acid sodium salt (PubChem CID: 23702140); DPPD, *N,N*-diethyl-*p*-phenylenediamine (PubChem CID: 7120); Trolox, (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (PubChem CID: 40634).

1. Introduction

Food industry makes continuous effort to place on the market newer functional products enriched of nutrients and bioactives. Being pasta a widely consumed food product with long shelf life, it has been the object of several supplementation strategies, acting as carrier of vitamins, minerals, polyunsaturated fatty acids, composite plant extracts, and dietary fibers. The most recent functionalizations of pasta include a variety of non-traditional ingredients, such as orange by-product fiber (Crizel, Rios, Cruz, Thys, & Flôres, 2015), mushroom beta-glucans (Kim, Lee, Heo, & Moon, 2016), carob flour (Sęczyk, Świeca, & Gawlik-Dziki, 2016), oregano and carrot leaf (Boroski et al., 2011), not to mention the employment of alternative cereals or pseudo-cereals, such as buckwheat flour and bran (Biney & Beta, 2014), amaranth, teff, quinoa (Kahlon & Chiu, 2015), or the use of legumes, such as soy (Clerici et al., 2011), white bean, yellow pea, and lentil (Wójtowicz & Mościcki, 2014).

Besides reducing the glycemic index, raising protein content and improving the profile of amino acids and fatty acids, one of the main objects of pasta functionalization is improving Antioxidant Capacity (AC) of the end-product. In previous researches, the authors produced durum wheat (*Triticum durum* Desf.) Bran Oleoresin (BO) (Durante, Lenucci, Rescio, Mita, & Caretto, 2012) and durum wheat Bran Water (BW) (Pasqualone et al., 2015) antioxidant extracts, by applying respectively supercritical carbon dioxide (SC-CO_2) or ultrasound-assisted extraction. BO and BW extracts were then used to supplement pasta with lipophilic (tocochromanols, carotenoids) or hydrophilic/phenolic antioxidants, respectively (Pasqualone et al., 2016).

In the present paper, a study was carried out to check both *in vitro* AC of cooked BO and BW pastas and, mostly, their effectiveness in improving the antioxidant status of blood (in particular of serum) by means of *ex vivo* analysis. The latter may take into account bioavailability and metabolism, giving therefore an integrated information of a real effect on blood antioxidant status, which is beyond the original AC of the ingested food. In particular,

the analyses were carried out during digestion, just few hours after food intake, *i.e.* in the window time when glucose released from starch digestion is expected to induce an oxidative effect on blood (Ceriello et al., 2014; Khor et al., 2014). Although the latter may appear as a major aspect to assess functionality of antioxidant-enriched pastas, at our best knowledge, so far only one study reported some information about changes of plasma AC two hours after the intake of pasta containing wholegrain sorghum flour (Khan, Yousif, Johnson, & Gamlath, 2015). On the contrary, many investigations studied *in vitro* AC (Biney & Beta, 2014; Boroski et al., 2011; Sęczyk et al., 2016) or effects of long-term consumption of antioxidant-enriched pastas on serum/plasma AC (Clerici et al., 2011; Durazzo et al., 2014; Whittaker et al., 2016). Unfortunately, in short-term studies (minutes or hours after ingestion), foods often induced limited AC increases, even though very rich in antioxidants (Fernández-Panchón, Villano, Troncoso, & Garcia-Parrilla, 2008; Soccio, Laus, Alfarano, & Pastore, 2016). This finding may depend, at least in part, on some weakness of the analytical approach, which takes into account only changes of AC without considering changes of the oxidative status of serum. This is a central point; in fact, several reports showed that the intake of food antioxidants may induce an increase of AC or a decrease of the oxidation level of serum/plasma or both (Alvarez-Suarez et al., 2014; Khan et al., 2015; Torabian, Haddad, Rajaram, Banta, & Sabaté, 2009). In practice, a fraction of antioxidants is consumed to counteract oxidation and thus cannot be revealed by AC assays. Therefore, a more effective evaluation of antioxidant intake effect should consider simultaneously the effect on AC and that on the oxidation level of serum. With this aim, in this paper, the serum antioxidant status was evaluated by means of a novel approach, presented for the first time in this research. It is based on the determination of "Antioxidant/Oxidant Balance" (AOB), representing the ratio between serum AC and serum oxidant status, evaluated as "Peroxide Level" (PxL). In particular, serum AC was evaluated by three different methods. The first one was the new Lipoxygenase-Fluorescein (LOX-FL) method (Soccio et al., 2016), derived from the LOX/4-

77 nitroso-*N,N*-dimethylaniline (LOX/RNO) one (Pastore, Laus, Tozzi, Fogliano, Soccio, &
78 Flagella, 2009; Pastore, Trono, Padalino, Di Fonzo, & Passarella, 2000), which is able to
79 highlight simultaneously several antioxidant functions and synergy among serum antioxidants
80 (Soccio et al., 2016). The other two methods were the widely used Oxygen Radical
81 Absorbance Capacity (ORAC) and Trolox Equivalent Antioxidant Capacity (TEAC) assays,
82 which mainly give information about antioxidant mechanisms based respectively on
83 Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET) reactions (Huang,
84 Boxin, & Prior, 2005). The combined use of these assays, which have a very different basic
85 rationale, may allow a more complete picture of AC and related AOB.
86 On the whole, the aims of this paper are to assess the reliability of the novel AOB approach in
87 evaluating serum antioxidant status after food intake and to evaluate effects on serum during
88 four hours after BO and BW pasta intake using the new AOB approach. To these purposes,
89 the two antioxidant-supplemented pastas were compared with other foods able to show high
90 AC or to induce pro-oxidant effects (see section 2.2).

2. Materials and methods

2.1. Chemicals

Chemicals and solvents at analytical and HPLC-grade purity were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). In the LOX-FL assay, a dilution in 100 mM Na-borate buffer pH 9.0 of an ammonium sulfate suspension of soybean LOX type V (LOX-1 isoenzyme, E.C. 1.13.11.12) was used, as well as a sodium linoleate solution prepared as described in Pastore et al. (2000). A competitive ELISA kit obtained from Mercodia (Uppsala, Sweden) was used to assay the oxidized low-density lipoprotein (LDLox). Glucose was purchased from Baxter (Rome, Italy). Other chemicals are reported in the sections where their use is described.

2.2. Tested foods

Foods under study included: *i*) pasta enriched with durum wheat BO extract; *ii*) pasta enriched with durum wheat BW extract; *iii)* non-supplemented reference (R) pasta; *iv)* the wheat grain dietary supplement Lisosan G; *v)* glucose; *vi)* R pasta consumed together with Lisosan G.

In particular, Lisosan G is a nutritional supplement certified by Italian Ministry of Health, produced by Agrisan Company (Larciano, PT, Italy) from lysed fine bran and germ of organic wheat (*Triticum aestivum* L.) grains (Pozzo et al., 2015). It was kindly provided by Dr Vincenzo Longo (Institute of Agricultural Biology and Biotechnology-CNR, Pisa, Italy).

As regards extracts, BO was produced by SC-CO_2 extraction accordingly to Durante et al. (2012). Briefly, durum wheat bran, provided by Tomasello milling industry (Casteldaccia, Palermo, Italy), was firstly oven dehydrated at 60 °C to a residual moisture content of 3%. Then, aliquots (3 kg) of dehydrated wheat bran matrix were extracted by SC-CO_2 for 3 h using the following operative parameters: gaseous CO_2 flow rate = 18-20 kg/h; pressure = 35 MPa; temperature = 60 °C. BW extract was obtained by ultrasound-assisted extraction of durum wheat bran (3.5 kg) mixed with tap water (35 L) by means of a pilot plant assembled

by Weal (Milano, Italy). Extraction was performed at 20 °C for 25 min; thirty-second recirculation steps of the suspension into the extraction chamber were carried out every 5 min of ultrasonic treatment. Finally, the suspension was filtered through a metal grid with 1 mm holes to recover the liquid phase (Pasqualone et al., 2015).

As for pastas, the BO-enriched pasta (BO pasta) was obtained by adding 525 g of BO to 9.475 kg of durum wheat (*Triticum durum* Desf., cv. Vertola) semolina; then, the BO-added semolina was mixed with 3 L tap water. As for the BW-supplemented pasta (BW pasta), this was obtained by adding semolina with 3 L of BW extract. The ingredients were processed by using a MAC 60 VR vacuum extruder (Italpast, Fidenza, Italy) at the following conditions: 15 min kneading; 1 bar chamber vacuum; 40 °C die temperature; 25 rpm extruder auger speed. The dough was extruded through a Teflon-coated spaghetti die. Then, pastas were dried in a static dryer (LAB, Namad Impianti, Rome, Italy) according to a high temperature drying program (T max = 78 °C) with linear decrease of relative humidity into the drier from 95% to 40% during the entire drying process (8 h and 50 min). The temperature linearly increased from 40 to 60 °C in 120 min, then from 60 to 68 °C in 120 min, and from 68 to 78 °C until the end of the drying cycle (Pasqualone et al., 2015, 2016). The BO and BW pastas showed color characteristics, cooking performances, viscoelastograph properties and textural parameters resulting not significantly different compared to a conventional pasta in an overall sensory judgment (Pasqualone et al., 2016).

The non-supplemented R pasta was spaghetti produced by TAMMA food industry (Foggia, Italy). This pasta was chosen because in preliminary experiments it showed AC very close to average AC of 20 pastas purchased at local market differing for brand, shape and size (Pastore et al., unpublished results).

Energy/nutrient composition profile of pastas under study is reported in **Table 1**. Analyses were performed by BonassisaLab research and analysis centre (Foggia, Italy) according to international guidelines.

2.3. Extraction of antioxidant compounds and sera collection

2.3.1 Preparation of cooked pasta samples for in vitro AC determination

Pasta cooking was carried out by adding about 100 g of pasta to 1 L of boiling distilled water. All pasta samples were cooked at their optimal time, determined as reported in Pasqualone et al. (2015, 2016), *i.e.* 9, 6 and 5 min for the BO, BW and R pastas, respectively. Cooked pasta samples were then drained with a Buchner funnel. Cooked samples were frozen at -20 °C and then freeze-dried by using the "LIO-5PDGT" lyophilizer (Tecnochimica Moderna, Roma, Italy). Freeze-dried samples were milled using the cryogenic grinder "Cryo Mill" (Retsch, Bergamo, Italy) and stored at -20 °C until needed.

2.3.2 Preparation of hydrophilic, lipophilic and phenolic extracts from cooked pasta samples for in vitro AC determination

Hydrophilic extracts were prepared as described in Laus, Tozzi, Soccio, Fratianni, Panfili, & Pastore (2012b) by extracting samples with deionized water in an ice-water bath for 1 h at a (w/v) ratio equal to 1 g/6 mL.

Lipophilic compounds were extracted according to the procedure described in Laus et al. (2012b), by saponification at 70 °C of samples (2 g) with 2 mL of 60% (w/v) KOH, 5 mL of 6% (w/v) ethanolic pyrogallol, 2 mL of 1% (w/v) NaCl, 2 mL of 96% (v/v) ethanol. After saponification, the suspension was extracted four times with 15 mL of *n*-hexane/ethyl acetate (9:1, v/v). Lipophilic compounds were reconstituted in ethanol.

Free Soluble Phenolic (FSP) compounds were extracted as reported in Laus et al. (2012b), with some modifications. In particular, samples (1.5 g) were extracted twice with 30 mL of 80% (v/v) ethanol for 10 min at room temperature and centrifuged at 14000x*g* for 10 min at 20 °C. The combined supernatants were evaporated under vacuum at 40 °C using a Buchi evaporator and concentrated to approximately 6 mL; then, they were acidified to pH 2-3 using 1 M HCl and centrifuged at 14000x*g* for 10 min at 20 °C. The resultant supernatant was

169 extracted twice with *n*-hexane (at an *n*-hexane/water phase ratio equal to 1:1 by vol.); then,
170 the combined water phases were subjected to three extractions in ethyl acetate (at an ethyl
171 acetate/water phase ratio equal to 1:1 by vol.). The ethyl acetate fractions were combined and
172 evaporated to dryness under vacuum at 40 °C; the dry residue was reconstituted in 1.5 mL of
173 water. All extracts were immediately assayed.
174
175 *2.3.3 Preparation of hydrophilic, lipophilic and phenolic extracts from Lisosan G for in*
176 *vitro AC determination*
177 Extraction of hydrophilic compounds from Lisosan G was performed as reported in Laus et al.
178 (2012b), by adopting a (w/v) ratio equal to 1 g Lisosan G/5 mL of deionized water. Extraction
179 of lipophilic and FSP compounds was carried out as described in Laus et al. (2012b), with
180 final resuspension of dry residues in ethanol and water, respectively.
181 The extracts were straightaway assayed.
182
183 *2.3.4 Collection of sera from volunteers*
184 Seven healthy subjects (3 women and 4 men aged between 24 and 33 years) participated in
185 this study, after providing informed written consent. The study was conducted in according to
186 the guidelines laid down in the Declaration of Helsinki and in accordance with relevant Italian
187 laws and institutional ethical policies. Experiments were approved by both the Board of the
188 SAFE Department of University of Foggia (Italy) and the Scientific Committee of Regional
189 Technological District DARe-Puglia (Italy). The seven volunteers were free of diabetes,
190 cardiovascular, liver, gastrointestinal and kidney diseases. Two days before analysis, the
191 subjects were asked to abstain from alcohol and vigorous physical activity and to follow a diet
192 poor in phenolic antioxidant compounds, avoiding all fresh fruits and vegetables and derived
193 products including fruit juices, tea, chocolate, coffee and wine. Each volunteer attended six
194 sampling sessions at 15 days intervals. At each session each subject consumed 20 g fresh

weight, f.w. (18 g dry weight, d.w.) of Lisosan G, or 50 g of glucose, or 70 g f.w. of BW or BO or R pasta, or 70 g of R pasta consumed together with 20 g (18 d.w.) of Lisosan G. Dose of Lisosan G was determined in preliminary experiments as able to induce an increase of serum AC (Soccio et al., 2016). As for serving of pasta, 70 g, representing a typical meal, was chosen according to Khan et al. (2015) as an amount able to decrease significantly plasma antioxidant status. Dose of glucose represents the amount released from pasta serving size. After 12 h fast, the subjects consumed one of the test foods within 10 min. The subjects assumed glucose as 500 mL of 10% solution or Lisosan G as resuspended in 500 mL of water; pasta was consumed with drinking 500 mL of mineral water. Venous blood samples were collected at baseline (T_0) and exactly 30, 60, 90, 120 and 240 min after food consumption. Blood samples were centrifuged at 3000x*g* for 5 min and the resulting serum samples were stored at -80 °C until analysis.

2.4. AC determination by means of the LOX-FL, ORAC and TEAC methods

2.4.1 LOX-FL method

AC determination by LOX-FL assay was performed as recently described in Soccio et al. (2016). The quenching of FL (3',6'-dihydroxy-3H-spiro[2-benzofuran-1,9'-xanthen]-3-one) was monitored at 37 °C at the excitation and emission wavelengths of 485 and 515 nm, respectively, by means of a LS 55 fluorescence spectrometer (Perkin Elmer, Waltham, MA, USA). The assay mixture (2 mL), consisting of 100 mM Na-borate buffer pH 9.0, 6.3 nM FL, 400 μM Na-linoleate and 1 μL Tween 20/μmol linoleate, was added with 0.5 enzymatic units of soybean LOX-1 to start the reaction. The LOX-FL measurements were performed in both the absence (control) and the presence of sample (extract or serum or the standard antioxidant (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, Trolox). The (%) decrease of the rate of LOX-FL reaction measured in presence of sample was calculated compared to the control. At least three different amounts of sample were analysed in triplicate and the slope of

221 the resulting regression line was obtained. AC was calculated by comparing the slope derived
222 by linear regression analysis of the extract or serum with that of the calibration curve obtained
223 by using Trolox (Di Benedetto et al., 2015; Soccio et al., 2016).
224 Concerning lipophilic antioxidants reconstituted in ethanol (see sections 2.3.2 and 2.3.3),
225 measurements were carried out in presence of a constant ethanol concentration in the reaction
226 mixture to exclude ethanol effect on LOX activity.
227
228 *2.4.2 ORAC method*
229 ORAC measurements were performed as reported by Ou, Hampsch-Woodill, & Prior, (2001),
230 properly modified as in Soccio et al. (2016). A CLARIOstar microplate reader (BMG
231 Labtech, Ortenberg, Germany) and 96-well plates were used for measurements. Assays were
232 conducted in a mixture (final volume of each well: 0.2 mL) consisting of 75 mM Na-
233 phosphate buffer pH 7.4 and 10 nM FL (solubilized in 75 mM Na-phosphate buffer pH 7.4),
234 in tabsence (blank) and presence of sample (extract, serum or Trolox). FL fluorescence
235 decrease was started by adding 40 mM 2,2′-azobis(2-methylpropionamidine) dihydrochloride
236 (AAPH, solubilized in 75 mM Na-phosphate buffer pH 7.4) and monitored by recording
237 fluorescence (λ_{ex}=483 nm, bandwidth 14 nm; λ_{em}=530 nm, bandwidth 30 nm) at 37 °C every
238 30 s. Four different amounts of sample were analysed in triplicate. In order to quantify AC,
239 the difference was calculated between the area under the fluorescence decay kinetic curve
240 (area under curve, AUC) of sample and the AUC of the blank. AC was determined using a
241 dose-response curve obtained by using Trolox. As for measurements of ethanolic extracts, the
242 assay mixture also contained a fixed concentration of ethanol.
243
244 *2.4.3 TEAC method*
245 TEAC assay described in Re, Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans, (1999)
246 was applied with slight modifications as reported in Laus et al. (2015). The aqueous solution

of the diammonium-2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\cdot+}$) was diluted with 5 mM Na-phosphate buffer pH 7.4 (or ethanol in AC measurements of lipophilic extracts). Measurements were carried out in triplicate for three different amounts of sample (extract or serum or Trolox) and AC was quantified using a proper calibration curve obtained with Trolox by plotting the (%) decrease of absorbance at 734 nm as a function of standard antioxidant concentration.

For all three methods, as for *in vitro* measurements of food extracts, AC values were calculated as μmol Trolox eq./g d.w.; then, this value was multiplied for grams of serving size. So, in Table 2 AC values of pasta and Lisosan G extracts were referred to AC per serving. As for *ex vivo* measurements, AC values were calculated as μmol Trolox eq./mL of serum and reported in Table 1 of Laus et al. (submitted). On the other hand, in Figs 1 and 2, in order to facilitate comparison, data were reported as (%) variation with respect to T_0 value.

2.5 Serum PxL and LDLox determination

Serum PxL was spectrophotometrically measured at 37 °C by using a SpectraMax® M5 Multimode Plate Reader (Molecular Devices, Wokingham, UK). The method is based on the capability of hydroperoxide groups to react with Fe^{2+} and generate the corresponding alkoxyl radical, according to the Fenton reaction. This newly formed radical, whose quantity is related to the peroxide content, is chemically trapped with *N,N*-diethyl-*p*-phenylenediamine (DPPD), leading to formation of the corresponding radical cation, which was determined at 512 nm. The method has been preliminarily assessed on the basis of the literature remarks (Erel, 2005) by adding an adequate Fe^{2+} amount in order to promote reaction. Moreover, we adopted as measured parameter the absorbance at the end-point rather than the initial reaction rate, thus significantly improving reproducibility of results. According to Inchingolo et al. (2014) and Simiakakis, Kapsimalis, Chaligiannis, Loukides, Sitaras, & Alchanatis, (2012), we found that

alkylamine reaction strongly enhances response to serum peroxides; although this effect amplifies sensitivity, it also implies calculation of a very high equivalent content of H_2O_2 in comparison with the physiological one (Erel, 2005). For this reason, in this paper we refer the oxidation status of serum to a conventional "Peroxide Level" rather than to a misleading "Peroxide Content".

Briefly, every working well of a 96-well plate contained the assay mixture consisting of 0.2 mL of 100 mM Na-acetate buffer pH 4.8, 6 mM DPPD (solubilized in 100 mM Na-acetate buffer pH 4.8), 30 μM $FeSO_4$ in absence (blank) or in presence of serum. Calculations were based on absorbance at 512 nm evaluated at the end-point (at least after 6 h). Results are expressed as equivalents of H_2O_2 using a proper calibration curve. Three different amounts of sera were evaluated in triplicate for each subject. Serum PxL values after consumption of each food under study are reported in Table 1 of Laus et al. (submitted), while in Fig. 1, to facilitate comparison, data were reported as (%) variation of T_0 value.

LDLox were measured by a commercially available ELISA kit (Mercodia oxidized LDL Competitive ELISA, Uppsala, Sweden).

2.6 Determination of AOB of serum

AOB values of serum after consumption of a tested food were obtained as AC/PxL ratio, *i.e.* by calculating the LOX-FL/PxL, ORAC/PxL and TEAC/PxL ratios. Data were expressed as (%) of T_0 value. It should be outlined that this calculation was carried out individually for each subject, rather than as ratio between the AC and PxL averages of the subjects. So, AOB profiles *vs* time reported in Figs 1 and 2 were obtained by averaging the seven individual AOB (%) profiles obtained by the subjects enrolled in the study.

2.7 Calculation of AC-Area, PxL-Area, AOB-Area, AOB-Index and statistical analysis

298 Areas under the AC, PxL and AOB profiles *vs* time (expressed as % of T_0 *vs* time) were
299 calculated by means of the trapezoidal rule using a Microsoft Excel spreadsheet; then, these
300 areas were expressed as (%) of the basal area, *i.e.* the area below the value at baseline (T_0),
301 and indicated as AC-Area, PxL-Area or AOB-Area. The AOB-Index was calculated, in
302 analogy with the glycemic index, as the ratio between the area under the AOB profile of the
303 tested food and the area under AOB profile of 50 g of glucose, and expressed as (%). Also in
304 this case, the calculation was carried out individually for each subject, rather than as a ratio
305 between the averages of the subjects. In this manner, each subject represented the control of
306 himself.
307 As for statistical analysis, preliminarily data distribution was evaluated using the Shapiro-
308 Wilk and Jarque-Bera tests. Homogeneity of variances was verified by the Bartlett's test.
309 Where necessary, either a natural logarithmic (ln) or square root (sr) transformation was
310 performed (see Table 2). Data were submitted to analysis of variance (ANOVA) and the mean
311 separation was tested by Duncan's test at 0.05 *P* level of significance. ANOVA was
312 performed using Statistica (data analysis software system), version 7.1 (StatSoft, Tulsa,
313 Oklahoma, USA).

3. Results and discussion

In this paper, BO and BW pastas were compared each other by means of *ex vivo* assessment of antioxidant status of serum in terms of the novel AOB approach after pasta intake. Preliminarily, an *in vitro* analysis of AC on pasta extracts was carried out.

3.1. In vitro AC measurements of cooked pastas and Lisosan G

After cooking, BO and BW pastas were compared each other as well as with conventional R pasta and antioxidant-rich dietary supplement Lisosan G in terms of AC determined by *in vitro* assays (**Table 2**). This was made by using LOX-FL, ORAC and TEAC methods on extracts highly enriched of hydrophilic, lipophilic and FSP compounds. Although the insoluble bound phenolic fraction represents the major phenolic component in cereal whole grains (Laus et al., 2012b and refs therein), it was not considered in this study. This fraction, in fact, evades small intestine absorption and might be freed by the gut microbiota with a possible absorption through the colon (Visioli et al., 2011). Therefore, its absorption is delayed and exceeds the four hours chosen to evaluate effectiveness of antioxidants to counteract serum oxidation by glucose.

AC values of extracts were determined per serving of pasta or Lisosan G (see section 2.3.4). Values of Lisosan G were recalculated from Soccio et al. (2016). All data were also reported as (%) of AC of reference (R) pasta (**Table 2**). As expected, because of chemical differences among the assays, LOX-FL, ORAC and TEAC showed different AC values. As regards comparison among different types of extract from the same food, the highest AC values were measured in H component; interestingly, this is observed for every food under study and for each AC assay adopted in this study. As for comparison among different tested foods against the same antioxidant fraction, Lisosan G showed the highest AC value, as evaluated by LOX-FL assay, in both hydrophilic and FSP fractions. Consistently, Lisosan G showed very high protein and phenolic/flavonoid content in hydrophilic and FSP extracts, respectively:

2.37±0.05 g bovine serum albumin eq., 6.21±0.20 mg gallic acid eq. and 0.50±0.03 mg catechin eq. per serving, respectively (Soccio et al., 2016). The most active FSP component following Lisosan G according to LOX-FL method was obtained in BW pasta, resulting 80% compared to Lisosan G and 2.7- and 3.2-fold higher than R and BO pastas, respectively. As for lipophilic component, the highest AC value among all tested foods was obtained by LOX-FL assay in BO pasta, resulting even about 4- and 11-fold higher than R (and BO) pasta and Lisosan G, respectively. This is in general agreement with the different supplementation of BW and BO pastas. In fact, BW extract showed a total phenolic content equal to 1.3 g ferulic ac. eq./L and retained a significant level of ferulic and *p*-coumaric acids (0.31±0.01 and 0.25±0.01 mg/g d.w., respectively), as reported in Pasqualone et al. (2015, 2016). BO extract showed a very high content of lutein, β-carotene and zeaxanthin (4.1±1.5, 1.9±0.4 and 1.6±0.2 µg/g BO, respectively), as well as a high level of tocotrienols and tocopherols (5.2±1.5 and 4.3±0.7 mg/g BO, respectively), as reported in Durante et al. (2012).

Similar to LOX-FL, the ORAC method pointed out a remarkable AC in the hydrophilic and FSP extracts of Lisosan G, as well as a low AC of lipophilic extract. On the contrary, ORAC failed to highlight statistical differences among pastas for FSP fraction. Concerning lipophilic extract, ORAC even showed some superiority of BW and R pastas compared to BO. Interestingly, ORAC pointed out a higher AC value of hydrophilic extract of BO pasta than BW; this is in accordance with results obtained by LOX-FL, also showing a more active hydrophilic fraction of BO in respect of both BW and R pastas. Really, this result is rather unexpected and merits further investigation.

As for TEAC measurements, Lisosan G showed the highest AC in all extracts; unlike LOX-FL and ORAC, TEAC showed also a considerable AC value of lipophilic extract of Lisosan G. Among pastas, TEAC assay highlighted a higher AC value of lipophilic extract of BO pasta compared to R pasta and of FSP extract of BW pasta in respect of both BO and R pastas. Regarding the latter point, it should be reported that using the DPPH assay an AC 20%

366 higher than the control was found in BW pasta (Pasqualone et al. 2015), which is the one
367 obtained by adopting the HT1 drying diagram in that paper.
368 As for the antioxidant compounds present in the type of extracts reported in Table 2, a
369 characterization was already carried out in durum wheat grains. In particular, AC of
370 hydrophilic extract was mainly dependent on flavonoids, as well as on proteins, which are
371 able to show significant *in vitro* antioxidant properties (Laus et al., 2012b); ferulic acid was
372 the most abundant among phenols, while sinapic, vanillic and protocatechuic acids were
373 together about 10% (Pastore et al., 2009); lipophilic antioxidants were essentially β-
374 tocotrienol, α-tocopherol, α-tocotrienol, β-tocopherol and lutein (Laus et al., 2012b).
375 On the whole, all methods showed a very strong AC of hydrophilic and FSP fractions of
376 Lisosan G, resulting from about 2- to 10-fold higher than other wheat species, depending on
377 the cereal species and AC assay (Laus, Gagliardi, Soccio, Flagella, & Pastore, 2012a; Laus et
378 al., 2012b). As for AC of lipophilic extract, only TEAC indicated the highest AC of Lisosan
379 G, while LOX-FL and ORAC gave an opposite result. Some contrasting data were observed
380 also for pastas. Among all tested foods, BO pasta had the highest AC value in lipophilic
381 component only according to LOX-FL, while TEAC pointed out a superiority of lipophilic
382 fraction of BO only with respect to R pasta. As for BW pasta, LOX-FL and TEAC assays, but
383 not ORAC, highlighted a higher AC of FSP extract of BW pasta with respect to both BO and
384 R pastas. These partly incoherent findings may be attributed to the different antioxidant
385 mechanisms highlighted by the three assays. In particular, TEAC mainly assesses a reducing
386 power. Our classical ORAC measurements mainly highlight scavenging activity against
387 peroxyl radicals (Huang et al., 2005), although a recent new version of ORAC may highlight
388 five different free radical species ($ORAC_{MR5}$, Prior et al., 2016). The highly performing LOX-
389 FL method is able to simultaneously detect scavenging capacity against different
390 physiological radicals as well as other antioxidant functions, thus providing a more
391 comprehensive AC evaluation (Soccio et al., 2016).

Although this kind of analysis is useful to characterize a food, in general, the values indicating *in vitro* AC have not been demonstrated to be relevant for the biological effects of specific bioactive compounds (Fraga, Oteiza, & Galleano, 2014). Therefore, the study was enlarged to *ex vivo* analysis of the antioxidant status of serum.

3.2. Effect of intake of Lisosan G, glucose and pastas on the antioxidant status of serum. Assessment of the novel AOB parameter

3.2.1 Lisosan G, glucose and AOB approach

In order to evaluate the effect of functional pasta consumption on serum antioxidant status, the reliability of the novel AOB experimental approach was preliminarily verified by comparing AC and AOB evaluation (**Fig. 1**). In particular, the capability to highlight changes in serum antioxidant status after food antioxidant intake was checked by studying the effect of both an antioxidant-rich food and a pro-oxidant food. In the light of its well-documented high antioxidant properties and bioactivities (Pozzo et al., 2015 and refs therein), the dietary supplement Lisosan G was chosen as a strong source of wheat antioxidants lacking any significant glycemic effect (not shown). On the other hand, glucose was used as pro-oxidant food since its consumption is known to induce serum oxidation (Ceriello et al., 2014; Khor et al., 2014). So, the use of Lisosan G and glucose may impose opposite conditions giving to the serum high antioxidant supply and high oxidation, respectively.

Fig. 1 shows changes of serum AC, evaluated by the three assays, and PxL in comparison with the derived AOBs during four hours after ingestion of either Lisosan G (Fig. 1 A, B and C) or glucose (Fig. 1 A', B' and C'). With the aim of better highlighting changes of the different parameters, data were reported as (%) variation of T_0 value. Lisosan G induced a general increase of AC and decrease of oxidation of serum as measured respectively by LOX-FL and PxL (Fig. 1 A). The data clearly agree with those reported by Torabian et al. (2009) who found an increase of plasma AC and a decrease of plasma lipid peroxidation after

418 consumption of polyphenol-rich walnuts or almonds. These findings indicate that AC and
419 PxL are related each other in such a manner that an antioxidant may both increase AC and/or
420 decrease PxL. Therefore, the novel AOB parameter, considering simultaneously both AC and
421 PxL, may really evaluate in a more comprehensive manner the antioxidant status of serum.
422 Consistently, $AOB_{LOX\text{-}FL/PxL}$ is able to highlight a very strong improvement of antioxidant
423 status of serum up to about 80% at 30-60 min (Fig. 1 A). The trend appears biphasic: a first
424 increase at 30-60 min, compatible with a rapid release and absorption of FSP compounds in
425 the small intestine, was followed by a minimum at 90 min and a subsequent new increase.
426 Although further investigation is required to explain this finding, a possible explanation might
427 be either a secondary release of covalently bound antioxidants or an AC increase following
428 antioxidant metabolism (Visioli et al., 2011).
429 As expected, glucose increased oxidation and decreased AC (Fig. 1 A') in accordance with
430 Khor et al. (2014) who reported an increase of lipid peroxidation and a decrease of AC at 2
431 and 4 h after sugar eating, respectively. In this experiment, the reliability of PxL response was
432 also validated by comparison with the direct determination of serum LDLox. Interestingly, the
433 PxL profile resulted very similar to that of LDLox, with a generally high positive correlation
434 ($r=0.94$, $P<0.01$) of areas under the PxL and LDLox profiles in different experiments. Since
435 PxL determination is easier and inexpensive, it was routinely preferred. As for $AOB_{LOX\text{-}FL/PxL}$,
436 also in the case of glucose it highlighted better than $AC_{LOX\text{-}FL}$ changes of antioxidant status by
437 showing a general decrease up to -30% at 240 min (Fig. 1 A').
438 ORAC and TEAC methods were less performing than the LOX-FL one. ORAC even showed
439 some AC decrease after 90 min following Lisosan G intake (Fig. 1 B), but highlighted a clear
440 AC decrease due to glucose (Fig. 1 B'). TEAC showed no or little changes after Lisosan G or
441 glucose intake, respectively (Fig. 1 C and C'). These data are in accordance with previous
442 results (Lettieri-Barbato, Tomei, Sancini, Morabito, & Serafini, 2013; Soccio et al., 2016),
443 stressing different performances of different AC methods and, in particular, a poor ability of

444 TEAC to assess serum AC changes. These failures may be easily overcome by using AOB; in
445 fact, although results were less performing than those obtained as $AOB_{LOX\text{-}FL/PxL}$, both
446 $AOB_{ORAC/PxL}$ and $AOB_{TEAC/PxL}$ profiles were able to unmask antioxidant (Lisosan G, Fig. 1 B
447 and C) and oxidant (glucose, Fig. 1 B' and C') effects that were hidden when AC alone was
448 measured.
449 As a whole, AOB parameter, taking simultaneously into account both AC and oxidant status
450 of serum, can be considered a much more appropriate and powerful tool than AC
451 measurements for detection of changes in serum antioxidant status due to food intake.
452
453 *3.2.2. Quantification of the effects of Lisosan G, glucose and pastas*
454 In order to quantify changes of serum antioxidant status, areas under profiles of AC, evaluated
455 using the three different methods, and PxL were considered (**Table 3**). In particular, areas
456 were normalized in respect of the area measured in the absence of food intake, *i.e.* the area
457 below the AC or PxL values at baseline (T_0). In comparison with Lisosan G and glucose, the
458 effects of intake of four pastas, containing both antioxidants and starch, were analyzed: BO,
459 BW and R pastas, as well as R pasta consumed with Lisosan G.
460 Unfortunately, the (%) changes were limited and AC-Area of ORAC and TEAC were mostly
461 unable to assess differences between Lisosan G and glucose. Only AC-Area of LOX-FL was
462 able to point out statistical differences between Lisosan G and glucose, showing respectively
463 some increase and decrease of AC. Symmetrically, PxL-Area also showed some decrease and
464 increase of PxL for Lisosan G and glucose, respectively. As a whole, in agreement with data
465 from Fig. 1, data from Table 3 make the ranks obtained by using separately AC and PxL
466 inconclusive.
467 A completely different outline emerges from the use of AOB-derived parameters (**Table 4**).
468 AOB-Area has the same meaning of AC-Area, indicating the (%) change of the area under
469 AOB profile in respect of the basal area, thus showing how serum AOB changes as a result of

food intake. On the other hand, AOB-Index is calculated like glycemic index, *i.e.* as ratio between area under AOB profile of the tested food and area under AOB profile of 50 g of glucose. This last parameter may take into account the effect on AOB of the starch component of pasta (in terms of glucose released from starch digestion). In all cases, a clear highly significant superiority of the very active antioxidant supplement Lisosan G is highlighted in respect of the pro-oxidant glucose (Table 4), thus making reliable the analysis. A remarkable serum antioxidant effect, equal or even higher to that of Lisosan G, is attributed to BO pasta by AOB-Area, evaluated as LOX-FL/PxL or as ORAC/PxL and TEAC/PxL ratios, respectively. As expected, the non-supplemented R pasta showed an effect statistically equal or slightly lower than that of glucose, in terms of AOB-Area determined as LOX-FL/PxL and ORAC/PxL or TEAC/PxL, respectively. Unfortunately, both the BW pasta and the R pasta added with Lisosan G, unlike BO pasta, resulted incapable of exerting a beneficial antioxidant effect on serum, as their AOB-Area values resulted equal or even lower compared to that of glucose (Table 4).

AOB-Index essentially confirmed data of AOB-Area, but had the added dimension of further enhancing the changes, with more than 65% increase of BO pasta in respect of the R one in the case of LOX-FL/PxL assay. AOB-Index also pointed out a significant advantage of R pasta consumed with Lisosan G compared to R pasta alone when analyzed by LOX-FL/PxL and TEAC/PxL. On the contrary, no beneficial effect was observed after BW and R pasta intake, showing an AOB-Index close to 100 or lower; therefore, BW and R pastas are not different from each other and from glucose as for *ex vivo* AC tests.

Interestingly, highly statistically significant positive correlations were obtained between AOB-Area and AOB-Index calculated as LOX-FL/PxL (r=0.988, $P \leq 0.01$), as well as ORAC/PxL (r=0.976, $P \leq 0.01$) and TEAC/PxL (r=0.925, $P \leq 0.05$). Moreover, significant positive correlations were found also among AOB-Area data obtained as different AC/PxL ratios (LOX-FL/PxL *vs* ORAC/PxL r=0.814, $P \leq 0.05$; LOX-FL/PxL *vs* TEAC/PxL r=0.896,

496 $P \leq 0.05$; ORAC/PxL *vs* TEAC/PxL r=0.920, $P \leq 0.05$). This last point strengthens the
497 effectiveness and reliability of AOB approach, able to provide the same results whatever the
498 assay adopted to measure AC.

499 Differences among BO, BW and R pastas, observed in Table 4 in terms of AOB-Area and
500 AOB-Index, are better highlighted in **Fig. 2**, reporting serum AOB profiles of pastas obtained
501 using all three AC methods. The intake of BO pasta caused a very high increase of AOB
502 measured as LOX-FL/PxL up to about 70% after 60 min (Fig. 2 A). Then, AOB gradually
503 decreased, to reach a value of about +20% after 240 min. A significant increase (of about
504 40%) of serum antioxidant status after BO pasta intake was highlighted also by AOB
505 measured as ORAC/PxL and TEAC/PxL. On the contrary, the ingestion of both BW (Fig. 2
506 B) and R (Fig. 2 C) pastas caused a general decrease of AOB values measured as LOX-
507 FL/PxL, up to about -30% and -50% after 90 min, respectively. In both cases, AOB measured
508 by both ORAC/PxL and TEAC/PxL also pointed out a significant decrease. Data from Fig. 2
509 indicate that, whatever the assay used, BO pasta is able to improve AOB of serum, while BW
510 induces a worsening similar to a conventional pasta. In practice, BO pasta is not only able to
511 counteract the detrimental effect of starch/glucose, but even to enhance serum antioxidant
512 status. These results are in accordance with Khor et al. (2014), showing that the ingestion of a
513 phytonutrient-poor food and its individual fat/protein or sugar components rapidly increases
514 plasma oxidative activity, whereas this is not observed after ingestion of a kilojoule-
515 equivalent phytonutrient-rich food. It should be noticed that serum AOB response to the
516 pastas studied in this paper cannot depend on different energy content or different content of
517 macronutrients (Table 1). In particular, only dietary fibre content significantly differed in BO
518 and BW pastas compared to R pasta; nevertheless, AOB responses (Table 4, Fig. 2) appear
519 unrelated to fibre content. These observations strongly suggest that different AOB response
520 after pasta consumption cannot depend on different energy intake in terms of kJ, as well as on
521 different nutrient composition reported in Table 1, but it may be due to the different

522 composition in terms of antioxidant compounds. In particular, BO pasta showed very high
523 total tocochromanol and carotenoid contents (1511±180 and 23±3 μg per serving,
524 respectively), with an enrichment of 3.1 and 3.4 times compared to the non-enriched pasta,
525 respectively (Pasqualone et al., 2016). Specifically, among tocochromanols, α-tocopherol, β-
526 tocopherol and α-tocotrienol even increased about 25, 17 and 10 times, respectively, while,
527 among carotenoids, the highest increase of about 6 times was obtained in both zeaxanthin and
528 β-criptoxanthin contents (Pasqualone et al., 2016).
529 On the contrary, BW pasta enriched in hydrophilic/phenolic compounds failed to induce a
530 positive effect. This may also depend on the low final enrichment in antioxidants in BW
531 pasta, showing a total phenolic content equal to 89±0.7 mg ferulic acid eq., on a serving size
532 basis, with an increase of only 1.3 times compared to the non-enriched pasta (Pasqualone et
533 al., 2016). So, lower amount of antioxidants taken with this pasta in respect of BO pasta may
534 induce lower AOB response. Nevertheless, it should be noticed that also the addition to R
535 pasta of Lisosan G, providing a high content of hydrophilic/phenolic antioxidant compounds
536 (see 3.1 section), cannot elicit increase when evaluated as AOB-Area, while only limited
537 improvement in relation to R pasta was observed when evaluated as AOB-Index.
538 Consequently, addition of pasta with lipophilic rather than hydrophilic/phenolic antioxidants
539 appears more effective in preserving and improving antioxidant status of serum.
540 No statistically significant correlations were found between data of Tables 2 and 4, under our
541 experimental conditions, thus confirming that AC measurements of food extracts by *in vitro*
542 assays may not be predictive of food effects on serum antioxidant status assayed by *ex vivo*
543 approach. However, in the case of Lisosan G, the very high activity measured *in vitro* may
544 explain the high antioxidant effect observed on serum after the dietary supplement intake. As
545 for pastas, only the LOX-FL method was able to highlight a higher AC of lipophilic
546 antioxidants of BO pasta. This result is in accordance with generally good performances of
547 methods based on soybean LOX-1 secondary reactions (Pastore et al., 2000, 2009; Soccio et

548 al., 2016), but the resounding effect on serum AOB remained quantitatively unpredictable on
549 the basis of *in vitro* analysis. This observation is in agreement with several reports stating that
550 assessment of putative beneficial effects of food antioxidants on consumers only by means of
551 widely used *in vitro* AC assays seems to be questionable and rather unrealistic (Fraga et al.,
552 2014; Huang et al., 2005; Pompella et al., 2014).
553 However, accurate *in vitro* dissection of AC of food extracts containing different classes of
554 antioxidants may contribute to characterization of antioxidant-rich foods and may help to
555 point out the more promising ones deserving an in depth investigation by physiological
556 approaches. On the other hand, *ex vivo* analysis of serum AOB after food intake may have a
557 series of advantages. First of all, AOB may implicitly take into account bioavailability, *i.e.* the
558 fraction of an ingested nutrient or compound that reaches the systemic circulation and the
559 specific sites where it can exert its biological action (Visioli et al., 2011). This is an important
560 point, since it has been shown that bioavailability of phytochemicals varies depending on food
561 source and dose. Moreover, AOB will consider antioxidant metabolism, *i.e.* possible
562 transformations by gut microbiota as well as intestinal and hepatic metabolism (*e.g.*
563 glucuronidation, sulfation, and methylation). Gut microbiota, in particular, may deeply
564 transform phenolic compounds (Visioli et al., 2011). Obviously, many transformations can
565 take place beyond the four hours taken into consideration here.
566 By a methodological point of view, the new AOB approach has very high reliability so that,
567 whatever the assay used, the results are the same, thus showing certainty of outcome, while
568 information from serum AC measurements is somehow unclear and also dependent on the
569 assay method adopted.

4. Conclusions

The lipophilic antioxidant-enriched BO pasta, containing high level of tocotrienols, tocopherols and carotenoids, extracted by SC-CO_2 technology from durum wheat bran, allows an improvement of antioxidant status of serum after intake. BO pasta has the capability to compensate serum oxidation due to glucose released from starch and, notably, even increases antioxidant status similarly to the highly active wheat antioxidant-enriched food supplement Lisosan G. This is a prominent effect since blood plays a central role in the homeostasis of cellular redox status by conveying and releasing antioxidants in the body; moreover, the maintenance of blood physiological antioxidant status may preserve endothelial function, which is thought to be an essential determinant of healthy aging (El Assar, Angulo, & Rodríguez-Mañas, 2013). On the contrary, the hydrophilic/phenolic antioxidant-enriched BW pasta induces no different effect on serum antioxidant status than a conventional pasta. Also the conventional reference pasta added with a Lisosan G, which is strongly enriched with hydrophilic/phenolic antioxidants, cannot improve AOB so as BO pasta. These results suggest less effectiveness of hydrophilic/phenolic compounds in respect of the lipophilic ones during the time window when the glucose released from starch digestion induces blood oxidation.

These findings were obtained by using the novel AOB approach. The good performance of this approach depends on the simultaneous evaluation of AC and PxL, the latter being lowered by antioxidants in a manner that cannot be accounted when the AC alone is measured. Therefore, a more comprehensive and effective assessment of antioxidant status of serum after food intake may be carried out. Further studies about serum AOB approach regarding other foods different from pasta are worthwhile.

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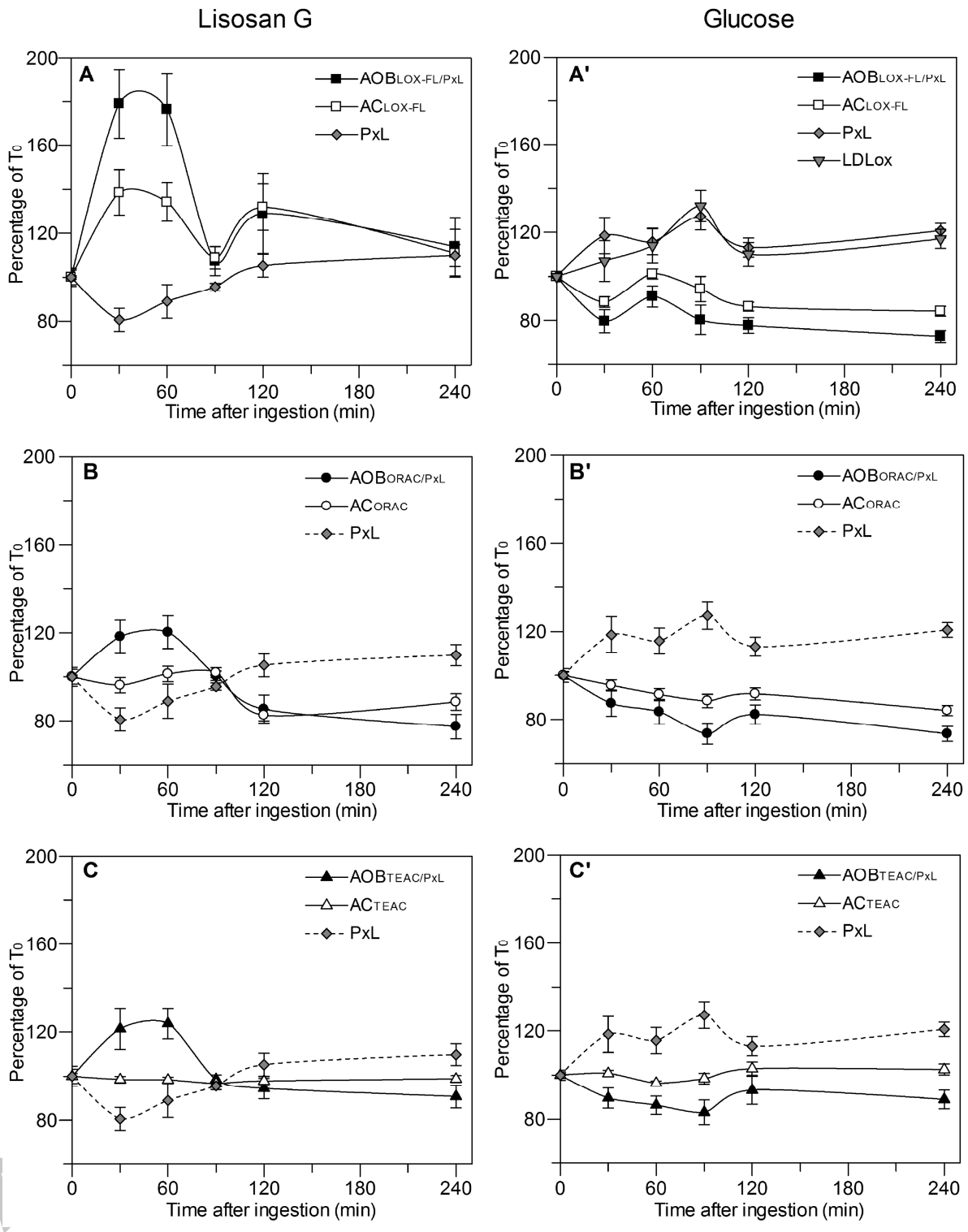
Figure captions

Fig. 1. *Serum Antioxidant Capacity (AC), Peroxide Level (PxL) and Antioxidant/Oxidant Balance (AOB) after consumption of Lisosan G or glucose.* Seven subjects ingested either 20 g (18 g d.w.) of Lisosan G or 50 g of glucose. AC was evaluated by means of LOX-FL, ORAC and TEAC methods. AOB was evaluated as LOX-FL/PxL, ORAC/PxL and TEAC/PxL ratios. PxL values shown in A and A' are reported as dotted lines also in B and C and in B' and C', respectively. The oxidized low-density lipoproteins (LDLox) were also assayed after glucose consumption (A'). AC profiles of Lisosan G shown in A, B, C are from Soccio et al. (2016), here reported to compare them with PxL and AOB profiles. Data are expressed as (%) of T_0 value and are reported as mean value ± SE (n = 7 subjects).

Fig. 2. *Serum Antioxidant/Oxidant Balance (AOB) after consumption of bran oleoresin (BO)-supplemented pasta (A) or bran water extract (BW)-supplemented pasta (B) or reference (R) pasta (C).* Seven subjects ingested 70 g (f.w.) of BO or BW or R pasta. AOB was evaluated as LOX-FL/PxL, ORAC/PxL and TEAC/PxL ratios. Data are expressed as (%) of T_0 values and reported as mean value ± SE (n = 7 subjects).


Lisosan G
Glucose
A
A'
B
B'
C
C'
AOBLOX-FL/PxL
ACLOX-FL
PxL
LDLox
AOBORAC/PxL
ACORAC
AOBTEAC/PxL
ACTEAC
Percentage of T0
Time after ingestion (min)
200
160
120
80
0
60
120
180
240

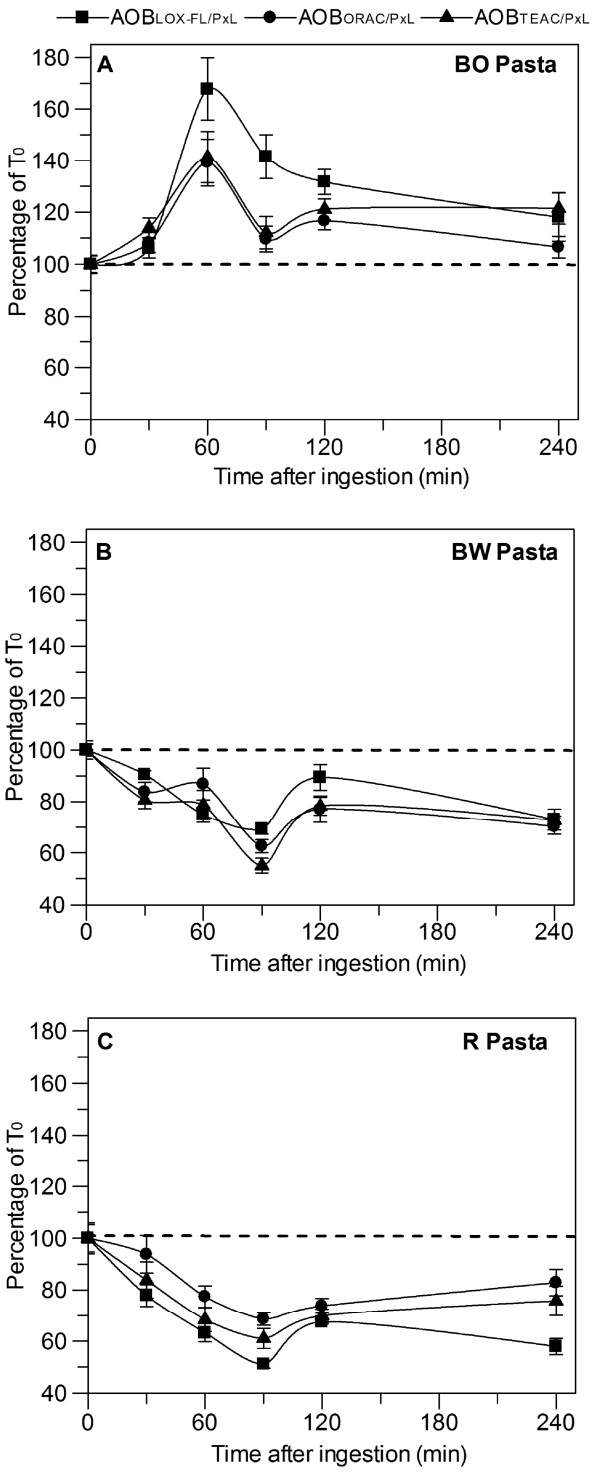


Table 1 - *Energy/nutrient composition of pastas under study.*

	BO pasta[1]	BW pasta[2]	R pasta[3]
Energy (kJ/100 g)	1482	1500	1487
Humidity (%)	10.2	9.5	9.7
Ash (%)	0.83	0.84	0.78
Protein (%)	12.7	12.4	12.5
Total carbohydrate (%)	68.9	68.9	72.7
-soluble sugars (%)	1.03	2.78	1.0
sucrose (%)	*0.18*	*0.28*	*0.22*
maltose (%)	*0.85*	*2.50*	*1.23*
Total fat (%)	1.83	1.73	1.50
-saturated (%)	0.33	0.42	0.30
-saturated (% of total fat)	18.1	24.2	20.0
-monounsaturated (% of total fat)	26.1	18.8	23.9
-polyunsaturated (% of total fat)	55.8	57.0	56.1
Total dietary fibre (%)	5.5	6.6	3.0
Sodium (mg/kg)	12.3	17.4	n.d.

[1] BO pasta: pasta supplemented with bran oleoresin; [2] BW pasta: pasta supplemented with bran water extract; [3] R pasta: reference pasta; n.d.: not determined.

Table 2 - *Antioxidant Capacity (AC), evaluated by LOX-FL, ORAC and TEAC methods, of hydrophilic (H), Free Soluble Phenolic (FSP) and lipophilic (L) extracts obtained from BO, BW, R pastas and Lisosan G.* AC values were reported per serving: 70 g (f.w.) of pasta or 20 g (18 g d.w.) of Lisosan G. Percentages with respect to R pasta values are reported between brackets. In the case of all TEAC measurements and of the LOX-FL measurements of H extracts, statistical analysis was performed using ln-transformed data; as for the LOX-FL measurements of FSP, sr-transformed data were used. Within the same column, different letters indicate significant differences at 0.05 *P* level, according to the Duncan's test. Data are reported as mean value ± SD (n=3 independent experiments).

	AC_{LOX-FL} (μmol Trolox eq. per serving)		
	H	**FSP**	**L**
R pasta[1]	179 ± 6 ^{c} *(100)*	24 ± 1.4 ^{c} *(100)*	29 ± 2.8 ^{b} *(100)*
BW pasta[2]	167 ± 6 ^{c} *(94)*	64 ± 2 ^{b} *(271)*	25 ± 1.4 ^{b} *(85)*
BO pasta[3]	205 ± 8 ^{b} *(115)*	20 ± 0.7 ^{d} *(85)*	111 ± 7 ^{a} *(385)*
Lisosan G	1471 ± 85 ^{a} *(824)*	77 ± 7 ^{a} *(325)*	10 ± 1.1 ^{c} *(34)*
	AC_{ORAC} (μmol Trolox eq. per serving)		
	H	**FSP**	**L**
R pasta[1]	361 ± 39 ^{bc} *(100)*	102 ± 11 ^{b} *(100)*	78 ± 8 ^{a} *(100)*
BW pasta[2]	322 ± 14 ^{c} *(89)*	105 ± 2 ^{b} *(103)*	77 ± 7 ^{a} *(99)*
BO pasta[3]	422 ± 34 ^{b} *(117)*	118± 13 ^{b} *(116)*	60 ± 8 ^{b} *(77)*
Lisosan G	2214 ± 108 ^{a} *(614)*	461 ± 13 ^{a} *(454)*	23 ± 0.4 ^{c} *(30)*
	AC_{TEAC} (μmol Trolox eq. per serving)		
	H	**FSP**	**L**
R pasta[1]	160 ± 14 ^{b} *(100)*	14 ± 2.1 ^{c} *(100)*	10 ± 1.4 ^{c} *(100)*
BW pasta[2]	164 ± 8 ^{b} *(102)*	20 ± 0.7 ^{b} *(145)*	11 ± 0.7 ^{bc} *(107)*
BO pasta[3]	149 ± 7 ^{b} *(93)*	13 ± 0.7 ^{c} *(95)*	13 ± 0.2 ^{b} *(130)*
Lisosan G	864 ± 54 ^{a} *(539)*	128 ± 22 ^{a} *(913)*	67 ± 14 ^{a} *(680)*

[1] R pasta: reference pasta; [2] BW pasta: pasta supplemented with bran water extract; [3] BO pasta: pasta supplemented with bran oleoresin.

Table 3 - *"Antioxidant Capacity"-Area (AC-Area), evaluated by LOX-FL, ORAC and TEAC methods, and "Peroxide Level"-Area (PxL-Area) of serum after consumption of different foods in seven subjects.* For each tested food, AC-Area and PxL-Area values are reported, representing the area under profiles of AC or PxL *vs* time (from 0 to 240 min) of the tested food respectively, expressed as (%) of basal area. Data are reported as mean value (n = 7 subjects). Within the same column, different letters indicate significant differences at 0.05 *P* level, according to the Duncan's test.

Food	Serving size (g f.w.)	AC-Area (% of basal area)			PxL-Area (% of basal area)
		LOX-FL	ORAC	TEAC	
BO Pasta[1]	70	107 ^{b}	96 ^{b}	101 ^{ab}	87 ^{d}
Lisosan G	20	123 ^{a}	92 ^{bc}	98 ^{abc}	100 ^{c}
R Pasta[2]+Lisosan G	70+20	98 ^{bc}	85^{c}	102 ^{a}	113 ^{b}
BW Pasta[3]	70	103 ^{b}	96 ^{b}	94 ^{c}	128 ^{a}
R Pasta[2]	70	86 ^{d}	105 ^{a}	96 ^{bc}	96 ^{a}
Glucose	50	90 ^{cd}	91 ^{bc}	101 ^{ab}	116 ^{b}

[1] BO pasta: pasta supplemented with bran oleoresin; [2] R pasta: reference pasta; [3] BW pasta: pasta supplemented with bran water extract.

Table 4 - *"Antioxidant/Oxidant Balance"-Area (AOB-Area) and "Antioxidant/Oxidant Balance"-Index (AOB-Index) of serum, evaluated as LOX-FL/PxL, ORAC/PxL and TEAC/PxL ratios, after consumption of different foods in seven subjects.* For each tested food, AOB-Area and AOB-Index values are reported. The AOB-Area values represent the area under the profile of AOB *vs* time (from 0 to 240 min) of the tested food, expressed as (%) of basal area. As for the AOB-Index, values represent area of each tested food expressed as (%) of area relative to consumption of glucose. Data are reported as mean value (n=7 subjects). Within the same column, different letters indicate significant differences at 0.05 *P* level, according to the Duncan's test.

Food	**Serving size (g f.w.)**	**AOB-Area (% of basal area)**		
		LOX-FL/PxL	**ORAC/PxL**	**TEAC/PxL**
BO Pasta[1]	70	127 [a]	113 [a]	119 [a]

Lisosan G	20	133 [a]	95 [b]	101 [b]
R Pasta[2]+Lisosan G	70+20	89 [b]	76 [c]	89 [c]
BW Pasta[3]	70	82 [b]	78 [c]	76 [d]
R Pasta[2]	70	66 [c]	80 [c]	74 [d]
Glucose	50	79 [bc]	80 [c]	89 [c]

Food	**Serving size (g f.w.)**	**AOB-Index (% of glucose area)**		
		LOX-FL/PxL	**ORAC/PxL**	**TEAC/PxL**
BO Pasta[1]	70	165 [a]	145 [a]	137 [a]
Lisosan G	20	173 [a]	123 [b]	119 [a]
R Pasta[2]+Lisosan G	70+20	132 [b]	106 [bc]	120 [a]
BW Pasta[3]	70	110 [bc]	99 [c]	87 [b]
R Pasta[2]	70	98 [c]	111 [bc]	98 [b]

[1] BO pasta: pasta supplemented with bran oleoresin; [2] R pasta: reference pasta; [3] BW pasta: pasta supplemented with bran water extract.

Highlights

1. The new Antioxidant/Oxidant Balance (AOB) is presented for the first time
2. AOB simultaneously considers serum Antioxidant Capacity (AC) and oxidant level
3. AOB assesses very well serum antioxidant status while AC cannot do it
4. A lipophilic antioxidant-enriched pasta strongly improves serum AOB
5. Contrarily, a hydrophilic/phenolic antioxidant-enriched pasta is ineffective