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Quality characteristics and *in vitro* digestibility study of barley flour enriched ditalini pasta

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Received: / Accepted: / Published:

Abstract: A ditalini pasta with a mixture of durum wheat and β-glucan enriched barley flour (BF) (60/40%, w/w) was found to have a final content of 5% β-glucan (BF-ditalini). Main quality parameters of BF-ditalini, water uptake and starch-protein texture, were comparable with those of 100% durum wheat ditalini (control). After *in vitro* simulated intestinal digestion, the content of β-glucan in the post intestinal (PI) supernatant of BF-ditalini processed with its cooking water (soup) was six fold higher than that of pasta asciutta. BF-ditalini soup, but not pasta asciutta, strongly delayed the hydrolysis of the starch, without difference of viscosity between PI supernatant and control. PI supernatant from BF-ditalini showed total phenol content and antioxidant capacity significantly higher ($p<0.001$) than control. In conclusion, ditalini pasta fortified with 40% BF may be considered a dietary product with high quality indexes and of functional interest for the abundance of antioxidant phenols, and for the hypoglicemic effect of β-glucan when ingested as a soup.

Keywords: Functional food, (1→3-1→4) β-glucan, barley flour, *in vitro* digestion, antioxidant capacity.

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1. Introduction

(1→3-1→4) β-Glucan, commonly referred to as β-glucan, is a non starch polysaccharide that is present in many cereals such as barley, rye, oat and wheat. Among these, barley and oat have a relatively higher β-glucan content, between 2 and 10% (w/w) (Bamforth, 1982). It has been reported that dietary β-glucan may provide a number of health benefits, including reduced serum cholesterol and

controllable blood glucose levels. (McIntosh et al., 1991; Eastwood, 1992; Bourdon et al., 1999). High viscosity and behavior in solution are considered important for its functional properties (Kahlon et al., 1993; Wood, 1994). The Food and Drug Administration (FDA) has indicated that dietary intake of 3g/ day of barley β-glucan helps to decrease total cholesterol in both the serum and the low-density lipoprotein (FDA, 2006).

Because β-glucan is a functional and bioactive ingredient, cereal-based foods, as well other foods, have been developed and fortified with either grain cell wall fractions or purified β-glucan concentrates. Incorporation of barley flour (BF) in bread, muffins, pasta and noodles, has been accomplished with only moderate success (Izydorczyk et al., 2005) because the incorporation of fibers into the protein-starch texture of wheat flour can alter the sensory and cooking properties of the base food. Spaghetti produced in a pilot plant which replaced semolina with an increasing amount (from 10% to 50%) of BF showed cooking behavior and acceptability similar to 100% semolina pasta, with a maximum β-glucan content around 3% (Lamacchia et al., 2011). A challenge for the food industry is therefore the manufacture of β-glucan fortified products that provide the health benefits of the fiber while simultaneously maintaining satisfactory taste and acceptability. In this study, we evaluated quality parameters of raw and cooked ditalini pasta from durum wheat flour that consisted of 40% BF (w:w). Performing an *in vitro* simulated intestinal digestion of pasta "asciutta" or "soup" (without or with cooking water, respectively), we investigated the effect that cooking and matrix enzyme degradation have on the extractability of β-glucan as well as the influence of barley incorporation on the digestibility of the starch. Finally, soluble fractions of the post-intestinal digesta, isolated by ultracentrifugation, were analyzed for viscosity, molecular characteristics of β-glucan, and antioxidant properties.

2. Materials and Methods

2.1 Materials

2.1.1 Chemicals

2,2'-Azinobis(3-ethyl-benzothiazoline-6-sulphonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX), Folin-Ciocault's phenol reagent, ferulic acid, hydrochloridric acid, sodium hydroxide, sodium phosphate dibasic, sodium phosphate monobasic, porcine pepsin (pepsin crystalline 3,260U/mg protein), bile extract porcine, pancreatin (100,3U/mg amylase activity) were from Sigma-Aldrich. β-Glucan assay kit (Mixed Linkage), β-Glucan MW standard and D-glucose assay kit (GOPOD Format) were from Megazyme International Ireland Ltd. (Wicklow, Ireland). Solvents were reagents grade or HPLC grade.

2.1.2 Pasta samples

Barley flour (Beta Barley Flour 11-BF) containing β-glucan (10.5 to 11.5%, w/w) was provided by Agroalimentare Sud S.p.a. (Melfi, Italy). Ditalini rigati pasta, produced by Pastificio Tomasello (Palermo, Italy), consisted of a mixture of durum wheat flour with barley flour (60:40, w:w) and water. Moisture content of the pasta was adjusted during manufacture to produce visually optimum dough prior to extrusion (43 mL water/100 g flour). Extrusion and dryer procedures were according to the method developed by the factory. The same durum wheat batch flour was used to produce the ditalini pasta that acted as the control. The starch content declared by the manufacturer was 70% and 62% (w:w) for control ditalini and BF fortified-ditalini (BF-pasta), respectively. Dried pasta was stored in sealed bags at room temperature until its use within two months.

2.2 Pasta quality evaluation

2.2.1. Color

The color of raw and cooked ditalini was evaluated according to an established methodology by Majzoobi M. et al. (2014). A Samsung digital camera (ST65) (Samsung Electronics Co, Ltd, China) was installed at a constant distance (30 cm) from the sample surface. The lamp and the camera were placed in a box (50 x 45x 50 cm) with interior white color walls. The angle between the axis of the camera lens and the sample surface was 90°. The angle between the sample surface and the light source was 45°. Illumination was achieved using a 10 Watts fluorescent light lamp.

The digital images of samples were saved in JPEG format and analyzed in the lab using Photoshop (version 8) to obtain color specifications of the samples. The *L* value- ranging from zero (black) to 100 (white)- was measured as an indication of the lightness (brightness). The *a* value is the function of the redness-greenness: positive *a* values indicate redness while negative values indicate greenness. The *b* value indicates blueness-yellowness: positive *b* values are related to yellowness while negative values to blueness.

2.2.2. Size parameters

The ditalini pasta's diameter, thickness and length were measured using a digital micrometer (Mitutoyo Italiana srl, Lainate, Italy) at two different locations in each of twenty specimens.

2.2.3. Cooking conditions and optimal cooking time determination

The ditalini pasta (20 g) was cooked in 125 ml of distilled water. Every 30 s during cooking, five ditalini were withdrawn, cut in half lengthwise, and squeezed between two transparent glass slides, in accordance with the Approved Method 66-50 by the American Association of Cereal Chemistry (AACC, 2000). The point of time at which the starch core completely disappeared, was recorded as the optimal cooking time (OCT).

2.2.4. Cooking loss

The Cooking Loss (CL), the amount of solid substance lost in cooking water, was determined according to Approved Method 66-50 (AACC, 2000). Ditalini pasta was cooked at OCT. The cooking water was collected in an aluminum vessel, placed in an air oven at 105 °C, and evaporated until a constant weight was reached. The residue was weighed and, after correction for tara, reported as a percentage of the starting material.

2.2.5. Dry matter

The moisture of uncooked ditalini pasta and the dry matter of cooked ditalini pasta at OCT were evaluated according to Approved Method 44-15A (AACC, 2000).

2.2.6. Swelling index

The swelling index of cooked pasta (grams of water per gram of dry pasta) was calculated in accordance with the procedure established by Cleary & Brennan (2006). A 20 g sample of ditalini pasta was cooked at OCT, the cooking water was discarded, and the pasta was dried at 105 °C until a constant weight was reached. The swelling index was expressed as

$$[(weight\ of\ cooked\ ditalini) - (weight\ of\ ditalini\ after\ drying)]\text{-}weight\ of\ ditalini\ after\ drying$$

2.2.7 Analysis of β*-glucan*

β-Glucan was determined by the "Mixed Linkage β-Glucan" assay kit from Megazyme International, Ltd., according to the McClearly and Glennie-Holmes method (1985).

2.3. In vitro digestion

Simulated *in vitro* digestion which mimics physic-chemical and biochemical changes that occur in the upper GI was as described in Tesoriere et al., 2008. Briefly, ditalini pasta (20 g) was cooked at OCT as

reported above and separated from cooking water to obtain pasta asciutta. The *oral phase* was carried out by different investigators after an overnight fasting in all simulated digestions (n=8) to take into account inter-individual variations in the saliva composition. After the oral cavity had been rinsed with physiological saline (NaCl 0.9%, pH 7.4, 37 °C, saline), four samples, combined with 2,5 ml each of saline, were chewed 10 times and expelled into a tared becker. The oral cavity was rinsed with 20 ml of saline, and the residual food was expelled into the becker. Cold saline (60 ml) was added, the sample was maintained in ice bath and homogenized in a laboratory blender (Waring, New Hartford, CT) for 2 min. The final volume was adjusted to 120 ml and transferred into a bottle. Aliquot (20 ml) of the post oral digesta (PO) was withdrawn and stored at -20°C until analysis. After acidification at pH=2 with HCl, porcine pepsine (8 mg/ml; 3200-4500 units/mg) was added (*gastric phase*). The bottle was sealed and incubated in a water bath (type M 428-BD, Instruments s.r.l., Bernaggio, Mi, Italy) with shaking (100 rpm), at 37 °C for 2 h. Aliquot (20 ml) of the post gastric digesta (PG) was withdrawn and stored at -20°C until analysis. Then, 200 mM NaH_2PO_4 /Na_2HPO_4 buffer (36 ml) and 5M NaOH were added to a final pH= 7.5. Porcine bile extract (2.4 mg/ml) and pancreatin (0.4 mg/ml) with amylase activity >100 units/mg) were added (*intestinal phase*). The bottle was sealed and incubated in the shaking water bath at 37 °C for 2 h; aliquots of the post-intestinal digest (PI) were stored at -20 °C until analysis. The solution (PI supernatant) was separated from the particulate material (pellet) through centrifugation at 167000 *g* for 35 min at 4 °C. Aliquots of the PI supernatant were stored at -20 °C until analysis.

Alternatively, the oral phase was carried out on cooked pasta in the presence of its cooking water (soup), instead of saline. The final volume was adjusted to 120 ml with saline and processed as described above.

2.3.1 Glucose released during in vitro digestion

Pasta samples were subjected to *in vitro* digestion as reported above, except that, during the intestinal phase, 1 mL aliquots of digesta were removed after 20, 80 and 120 min from the addition of pancreatin and transferred into tubes immersed in boiling water for 15 min to arrest enzymatic digestion. After ultracentrifugation as reported above, the amount of glucose solubilized in the PI supernatant was determined spectrophotometrically at 510 nm utilizing D-glucose assay kit (GOPOD Format) (Megazyme International, Ltd).

2.3.2 Molecular weight determination

Average molecular weight and molecular weight distribution of β-glucan were determined by Size Exclusion Chromatography (HPLC-SEC) using a Yarra SEC 4000 column from Phenomenex kept at 40 °C and connected to a Water 2410 refractive index detector. The mobile phase was 100 mM $NaNO_3$ containing 5 mM NaN_3 at a flow rate of 0.6 ml/min. A calibration curve was obtained by eluting four β-glucan standards (Megazyme International, Ltd.) with a MW in the range 35.600-391.000 Da.

2.3.3 Viscosity measurement

Viscosity of PI supernatants from digesta was measured using a Brookfield mod. DVIII+pro viscosimeter (Brookfield Engineering Laboratories, MA, USA).

2.4. Total phenol (TP) content

The TP content was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton & Rossi, 1965). Raw control ditalini and BF-enriched ditalini (10 g) were homogenated in a blender with 130 ml of saline. Raw pasta homogenate, PO, PG and PI were ultracentrifugated at 167000 g for 35 minutes at 4 °C. Aliquots of the relevant supernatants (0.25-1.0 ml) from both BF– and control pasta, were submitted to the colorimetric assay. The measurements were compared to a

standard curve of ferulic acid (FA) and expressed as milligrams of ferulic acid equivalents (FAE) per gram of raw pasta.

2.5. Total Antioxidant Activity

The total antioxidant activity of raw pasta homogenate, PO, PG and PI supernatants from both BF– and control pasta, was evaluated using the $ABTS^{\cdot+}$ radical cation decolorization assay (Rice-Evans & Miller, 1994). Samples (10, 15 and 20 µl) were analyzed, ~~at three different dilutions~~, within the linearity range of the assay. Purified β-glucan (PM=2.65x10^5 Da) (Megazyme International, Ltd.) dissolved in distilled water was assayed for comparison. The assay was standardized with the synthetic antioxidant Trolox, and results were expressed as Trolox equivalents (TE) per gram of raw pasta or β-glucan.

2.6 Statistical analysis

Results are expressed as mean±SD of *n* independent experiments carried out in triplicate. Statistical comparisons were by Student's *t*- test or by one-way ANOVA test, with Bonferroni's correction for multiple comparisons (Instat-3 statistical software, GraphPad Software Inc., San Diego, CA, USA). In all cases, significance was accepted if the null hypothesis was rejected at the $P<0.05$ level.

3. Results and discussion

3.1 Quality characteristics of ditalini

An industrial procedure was used in this study to obtain ditalini pasta from a mix of durum wheat flour and BF (60/40, w/w). Quality characteristics of the uncooked BF-ditalini are reported in Table 1. The BF-ditalini appeared darker than the control, and color parameters of the samples indicated a reduction

of 27 % in lightness with a net increase of *a* value and a decrease of *b* value, indicating a shift towards redness and blueness. The BF-ditalini incorporated 5% of β-glucan and, at the dryer temperature and relative humidity used in the preparation, showed diameter, thickness and length quite comparable to control ditalini.

Cooking performance is an important factor in a consumer's judgment of pasta quality. When compared to raw pasta, cooked BF-ditalini maintained a similar grade of lightness but showed lower redness-greenness and blueness-yellowness values. In comparison with the control pasta, substitution of wheat flour with 40% BF did not cause any variation of the OCT nor of the swelling index, i.e. the grams of water absorbed per gram of dry pasta (Table 2). The cooking loss is an indicator of the capability of the starch-protein matrix to retain its physical integrity during cooking (Bruneel et al., 2010), and only values lower than 7% are acceptable for a good quality pasta (Sissons et al., 2012). Generally, nonstarch polysaccharide addition increased the cooking loss (Sandhu et al., 2015); this data was confirmed by our study. In fact the cooking loss value of BF-ditalini (4.20 g/100g) was slightly but significantly higher ($p=0.02$) than that of control pasta, indicating that inclusion of BF did not affect remarkably the pasta quality. On the other hand, spaghetti enriched with 4-6% β-glucan, obtained by mixing durum wheat with a concentrated source of barley β-glucan soluble fiber by a laboratory-scale procedure, showed much higher cooking loss values (8 g/100g) (Chillo et al., 2011). Thus, in our BF-ditalini, the use of natural texture of barley flour and/or the adopted industrial procedure appears to preserve the continuity of the flour protein matrix. Furthermore the cooking loss value is also affected by pasta:cooking water ratio (de la Pena & Manthey, 2014). In this study an high pasta:water ratio was used, as it is known that this condition preserves the cooking properties of pasta (de la Pena & Manthey, 2014). Moreover, the dry matter values of BF-ditalini were comparable with those of control pasta ($P>0.05$), indicating a non-significant loss of organic matter during cooking. On the whole, our

BF-fortified product appeared to be characterized by a compact starch-protein network that delays disintegration of the matrix during cooking, thus preserving its quality.

β-Glucan fibers are hydrophilic and their solubilization increases with water temperature (Fincher, 1975; Fleming & Kawakami, 1977). Under the cooking conditions reported, about 50% of β-glucan incorporated in the raw pasta was released (Table 2).

3.2 In vitro digestion

β-Glucan may provide benefits to intestinal function (Shen et al., 2012). To evaluate extractability of β-glucan following ingestion of cooked BF-ditalini, simulated intestinal digestion was performed and PI digesta were submitted to ultracentrifugation in order to measure both the fraction of potentially functional β-glucan in the PI supernatant and the un-extractable fiber (pellet) eventually available to uptake by gut microbiome (Shen et al., 2012). Both the pasta asciutta and the soup were investigated. After digestion of BF-pasta asciutta, about 24% of β-glucan was recovered in the PI supernatant (Table 3). Interestingly, a comparable amount of β-glucan was measured in the same fraction when the digestion procedure was carried out in the absence of hydrolytic enzymes, indicating that release of the fiber from the food matrix does not depend on hydrolysis of the starch-protein network of the pasta, but rather on partition properties of the polysaccharide in the digestive aqueous phase. It is important to emphasize that our *in vitro* digestion procedure, in which 20 g of pasta were processed in a total volume of 160 ml, is consistent with physiological conditions of weight/volume ratio, since a serving of pasta (80 g) is considered dispersed in a total digestive volume of 640 ml (Mahé et al., 1992).

The amount of β-glucan in the PI digesta from BF-ditalini soup was two-fold higher than that measured in PI from BF-pasta asciutta because not only β-glucan found in the food matrix but also the fiber released in the cooking water (Table 3). Accordingly, β-glucan in PI supernatant of the soup were

six-fold more abundant than in that of the pasta asciutta, whereas the unextracted polysaccharide amount in the two relevant pellets was not quantitatively different (Table 3). Overall, these findings indicate that cooking in boiling water is the major extraction step of β-glucan during processing of BF-pasta, and these findings support the idea that a smaller sized BF-pasta, suitable for soup, may be a recommended food for β-glucan fortified diets because it allows the ingestion of the units of fibers solubilized in the cooking water. In addition, ditalini "rigati" pasta, characterized by a high area/ volume ratio, which facilitates the solubilization process, appears to be a dietary product with optimal functional properties.

The glycemic index is a measure of the potential of a carbohydrate-containing food to raise blood glucose concentration after ingestion. *In vitro* digestion has been shown to be a valid proxy to *in vivo* starch digestibility and serves as a predictor for the *in vivo* glycemic response (Monro et al., 2010). Glucose release during *in vitro* pancreatic digestion of either control or BF-pasta, both asciutta and soup, is shown in Fig. 1 and expressed as milligrams glucose normalized for the initial starch amount. With respect to the control, BF-pasta asciutta, including 2.5% β-glucan fiber, caused a decrease of glucose released at 20 min ($P=0.03$). However, no other significant change was observed thereafter ($P>0.05$). On the contrary, release of glucose was significantly lower for BF-pasta soup with respect to control soup, at each considered time-point, although the percentage inhibition decreased with the incubation time (40% at 20 min, 12% at 120 min). These findings indicate that, only BF-soup, containing 5% of β−glucan, significantly decreased the glycemic potency of BF-ditalini.

Hypoglycemic effects by β-glucan-enriched foods have been attributed primarily to the increased luminal viscosity (Wood, et al., 1994; Battilana, et al., 2001; Dikeman & Fahey, 2006). Although the concentration of β-glucan in PI supernatant of digesta from soup was six-fold higher than that from pasta asciutta, no difference in viscosity of the two supernatants was found (data not shown). Overall, our data showed that BF-ditalini, when supplied as a soup, may reduce the glycemic impact of the

pasta without variation in the gut viscosity. Similarly, Chillo et al. (2011) have demonstrated that spaghetti enriched with a 5% β-glucan concentrate, submitted to *in vitro* digestion with its cooking water, significantly reduced the glucose release with respect to the control pasta, without changes in viscosity. Delay in the starch hydrolysis of pasta added with soluble fiber can be attributed to changes in its microstructure and to hydration of the polysaccharide matrix which hinders encapsulation of the protein-starch matrix until the later stages of digestion.

3.3 Total Phenols (TP) and antioxidant activity (TAA)

Phenolic compounds are thought to contribute to the antioxidant activity of cereals (Zieliński & Kozlowska, 2000). Phenolic acid derivatives, flavonoids, and proanthocyanidins are reported in barley (Verardo et al., 2011). TP and TAA of raw control and BF-ditalini used in this study are reported in table 4. TP content of PO, PG and PI supernatants in both BF-ditalini soup and pasta asciutta were determined, in comparison with the relevant control to investigate the impact of digestion on phenol release from the matrix. Data are expressed as milligrams of ferulic acid equivalents (FAE) per gram of raw pasta (table 4), since ferulic acid is the dominant phenolic derivative in cereal grains (Deng et al., 2012). Both in control ditalini pasta and BF-enriched ditalini, a statistically significant increase in total phenol content is evident from the post gastric phase onwards; this is probably due to the phenol release from proteic food matrix and/or to the acid pH. Moreover data repoted reveal that incorporation of barley flour in our pasta formulation increased the content of phenolic compounds by 13% (table 4). Total antioxidant activity of PI supernatant from both the BF-pasta and the control, soup and asciutta, were determined using $ABTS^{\cdot+}$ radical cation decolourization assay and expressed as μmol of Trolox equivalents (TE) per gram of raw pasta. Data demonstrated that the radical scavenging capacity of BF-pasta was about one and a half times higher than that of the not enriched one (Table 4).

β-Glucan is a linear polymer of D-glucopyranosyl residues linked by β-(1→4) linkages forming 3-O-β-cellobiosyl-D-glucose (cellotriose, DP3) and 3-O-β-cellotriosyl-D-glucose (cellotetraose, DP4). Cellotriose and cellotetraose units are linked by β-(1→3) linkages, causing the polymer ramification, and their ratio (DP3:DP4) is different within cereal sources (Lazaridou & Biliaderis, 2007). The antioxidant potential of β-glucan has been reported inversely related to molecular weight (Shah et al., 2015). To investigate the effect of the addition of polysaccharide on the antioxidant activity of BF-ditalini digesta, we first assessed the molar mass of β-glucan in the PI supernatants. HPLC-SEC analysis of PI supernatant from pasta asciutta showed a peak of β-glucan with an apparent molar mass average (Mw) of $2.51x10^5$ Da and low grade of molecular dispersity (Table 5). Molecular weight of β-glucan in the PI supernatant from BF-ditalini soup was not significantly different, indicating that both extraction by boiling water and digestive procedure lead to solubilization of fibers with quite similar high molecular mass (Table 5). The $ABTS^{\cdot+}$ radical scavenging capacity of purified β-glucan with a comparable molecular weight (PM=$2.65 x10^5$ Da) was measured at 15.54 μmol TE/g of β-glucan. Therefore, taking into account the β-glucan amount of the PI supernatant of BF-pasta, both soup and asciutta (Table 3), the contribution of the polysaccharide to the antioxidant capacity of the digesta was calculated 0.38 and 0.08 μmol TE/g of raw pasta. respectively. This indicated that the contribution of β-glucan to the radical scavenging activity of BF-pasta soup and asciutta, was 6.0% and 1.3% respectively. Overall, our data revealed that enrichment of durum wheat with 40% BF improves the antioxidant capacity of pasta digesta and that bioactive phenols, rather than β-glucan, are the main contributors to this effect.

4. Conclusions

An industrially produced 40% BF-fortified ditalini-shaped pasta, with a final 5% content of β-glucan, was compared to 100% durum wheat ditalini pasta (control) to evaluate quality characteristics and bioactive potential. . Quality parameters such as water uptake (OCT and swelling index) and starch-protein texture (cooking loss and dry matter) did not vary substantially from the control, suggesting high potential for consumer acceptance. At the OCT, ditalini retained 2.5% of β-glucan, with the remaining released in the cooking water. A simulated *in vitro* digestion of pasta asciutta (without) and soup (with its cooking water), aimed at investigating the time-course of starch hydrolysis, revealed that soup, but not pasta asciutta, released glucose slower than the control, indicating the role of β-glucan, as far as sufficiently concentrated, as ingredients to reduce the glycemic index of pasta. In addition, the radical-scavenging activity of PI digesta, either asciutta or soup, was higher than the control, apparently due to a higher level of phenol compounds. Thus, the manufacture of a small size 40% BF-enriched pasta to be consumed as a soup is recommended for a reduced glycemic impact, and it is also recommended for its potential protective effect on the gut lumen due to the abundance of reducing components from BF in the final product. Clinical assays are in progress in order to ascertain if 40% BF fortified-ditalini can modulate the glycemic response and improve the plasma antioxidant defense in humans.

Acknowledgments

This work was funded by PON02_00667-PON02_00451_3361785 "Di.Me.Sa-Valorizzazione dei prodotti tipici della Dieta Mediterranea e loro impiego ai fini salutistici e nutraceutici" Ministero dell'Istruzione dell'Università e della Ricerca.

Abbreviations: barley flour (BF), ferulic acid equivalent (FAE), post gastric (PG), post intesinal (PI), post oral (PO), total antioxidant activity (TAA), total phenol content (TP), trolox equivalent (TE).

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