Use of purple durum wheat to produce naturally functional fresh and dry pasta

Running title. Purple durum wheat: a natural ingredient for functional pasta

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Abstract

In this study, the effects of different milling procedures (roller-milling vs. stone-milling) and pasta processing (fresh vs. dried spaghetti), and cooking on the antioxidant components and sensory properties of purple durum wheat were investigated. Milling and pasta processing were performed using one purple and one conventional non-pigmented durum wheat genotypes, and the end-products were compared with commercial pasta. The results show that the stone milling process preserved more compounds with high health value (total fibre and carotenoids, and in the purple genotype, also anthocyanins) compared to roller-milling. The drying process significantly ($p < 0.05$) reduced the content of anthocyanins ($21.42 \mu\text{g/g}$ vs. $46.32 \mu\text{g/g}$) and carotenoids ($3.77 \mu\text{g/g}$ vs. $4.04 \mu\text{g/g}$) with respect to the pasteurisation process involved in fresh pasta production. The sensory properties of pasta from the purple genotype did not significantly differ from commercial wholemeal pasta, and its *in-vitro* glycemic index was even lower. Thus, it is possible to consider this genetic material as a good ingredient for the production of functional foods from cereals naturally rich in bioactive compounds.

Keywords: Purple durum wheat; anthocyanins; carotenoids; Trolox equivalent antioxidant capacity; milling; pasta processing; sensory/ texture analysis; glycemic index.

Chemical compounds used in this study

Cyanidin 3-*O*-glucoside (PubChem CID: 197081), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (PubChem CID: 40634); 2,2'-azinodi(3-

25 ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) (PubChem CID:
26 6871216); methanol (PubChem CID: 887); 1-butanol (PubChem CID: 263); boric acid
27 (PubChem CID: 7628); sulphuric acid (PubChem CID: 1118); calcium chloride
28 (PubChem CID: 24854) and sodium azide (PubChem CID: 33557).

1. Introduction

Pasta is often consumed daily in the diet, particularly in Mediterranean areas, and it is appreciated for its textural attributes. Conventional pasta is made using the primary ingredient of durum wheat semolina, the yellow colour of which is due to carotenoid pigments (Ficco et al., 2014a). Compared to other starch-based foods, such as bread, pasta has beneficial physiological effects, as seen by low postprandial glycemic and insulinemic responses (Aston, Gambell, Lee, Bryant, & Jebb, 2008).

Over the last few decades, the increased demand for pasta fortified with bioactive compounds has led to the addition of non-durum wheat ingredients to the semolina. This has included legume flours (Fares & Menga, 2012), oregano and carrot leaf (Boroski et al., 2011) and barley flour (Verardo, Gomez-Caravaca, Messina, Marconi, & Caboni, 2011). Also, functional foods made from pigmented cereals (e.g., maize, rice, wheat) that are rich in antioxidant compounds are currently attracting wide interest (Abdel-Aal et al., 2006).

Several recent studies have focussed on the role of whole-grain diets in preventing degenerative diseases (Abdel-Aal & Rabalski, 2008, and refs. therein). These beneficial health properties of whole-grain products have been associated with the presence of variable amounts of antioxidants, as compared to their corresponding refined flours. Many of these are scavengers of free radicals, including carotenoids (Ficco et al., 2014a; De Simone et al., 2010) and anthocyanins (Ficco et al., 2014b; Abdel-Aal, Young, & Rabalski, 2006). The anthocyanins are predominant in pigmented grain and are mainly localised to the pericarp (i.e., the outer layers of the grain) (Zeven, 1991; Abdel-Aal et al., 2006). In contrast, the carotenoids are distributed along the grain

and within the endosperm (i.e., the inner part of the grain) (Borrelli, De Leonardis, Platani, & Troccoli, 2008; Hentschel, Kranl, Hollmann, Lindhauer, Böhm, & Bitsch, 2002).

Borrelli et al. (2008) studied the distribution of the yellow colour components in durum wheat whole-grain and reported that during grain milling, a lower rate of pigment reduction was observed. This arises due to their more favourable distribution along the grain, while the pasta processing affected the residual content, and thus the final colour, to a greater extent (Borrelli et al., 2003; De Simone et al., 2010). Siebenhandl et al. (2007) analysed the phytochemical profile of pigmented cereals (i.e., purple and blue wheats, black barley) using a sieving machine. They observed similar levels of carotenoids in all of the fractions of the purple wheat genotype, with more anthocyanins in the external layers, due to the location of this pigment. In addition they noted 3-fold to 5-fold higher antioxidant activities in the external layers than in the corresponding flours, which decreased further with decreasing particle size from the millstreams.

Conversely, little is known about the loss of anthocyanins and carotenoids during the processing and after the cooking of pigmented wheat products (Pasqualone et al., 2015; Li, Pickard, & Beta, 2007). Li et al. (2007) reported a total loss of anthocyanins in purple wheat-bran muffins blended with other ingredients after heat processing (at 177 °C, for 7-12 min), although other factors might be involved in anthocyanin degradation, such as light and storage conditions (Nayak, Liu, & Juming, 2015).

During processing, as well as pigment losses, many other changes can occur in terms of the appearance, colour, texture, and sensory properties (Padalino,

Mastromatteo, Lecce, Cozzolino, & Del Nobile, 2013). We thus studied a purple durum wheat (PDW) genotype during milling and processing, as semi-wholemeal *versus* semolina, and as fresh pasta *versus* dried pasta. The aim was to investigate: (1) the effects of two milling systems on the anthocyanin and carotenoid levels and the antioxidant capacity of PDW, as compared to a yellow durum wheat (YDW) variety; (2) the effects of the pasta-making and cooking on the antioxidant components; (3) the *in-vitro* estimated glycemic impact; and (4) the sensorial and textural properties of the different PDW pasta, as compared to YDW and commercial durum wheat pasta.

2. Materials and methods

2.1. Raw materials and milling

Two durum wheat genotypes (*Triticum turgidum* subsp. *durum*) were used in the present study: a pigmented durum wheat genotype (accession number CItr14754), with purple grain pericarp (PDW), and the semi-dwarf durum wheat variety PR22D89, released in Italy in 2005, with yellow grain (YDW), and high agronomic and qualitative performance. The raw material used for the processing tests (i.e., the grain) came from a field trial carried out during the growing season 2013-2014 at CREA-CER in Foggia, Italy.

An aliquot (100 g) of grain was milled to wholemeal (Tecator Cyclotec 1093; International PBI, Milan, Italy) and passed through a fine-mesh, 500-micron screen, with almost complete recovery of the sample. For milling into flour (semi-wholemeal and semolina), the grains were conditioned at 16.5% moisture. Using a stone mill (MB250; Partisani, Forlì, Italy), the grains were ground into three fractions, as flour, fine bran and bran. Semi-wholemeal was formed from flour with a particle size below

260 microns plus fine bran with a particle size between 260 microns and 1060 microns, while excluding the 5% to 7% of the bran that corresponded to the outer part of grain. Refined semolina was made using a laboratory mill (MLU 202; Bühler Brothers, Uzwil, Switzerland) with a six-roller system, three breaking and three sizing passages, and an attached semolina purifier, for a fineness range of 200 microns to 315 microns. Commercial durum wheat semolina (Barilla G. & R. Fratelli Spa, Parma, Italy) was purchased in the local market.

2.2. Pasta-making process

Pasta samples were produced using a pilot plant, with an extruder (60VR; Namad, Rome, Italy) for the production of the extruded pasta. Here, 3000 g semi-wholemeal or semolina were mixed with tap water in a rotary-shaft mixer (Namad, Rome, Italy) at 25 °C for 20 min, to obtain dough with 30% moisture content. A screw (length, 30 cm; diameter, 5.5 cm) that ended with a bronze die (hole diameter, 1.70 mm) was used to extrude the dough into a spaghetti shape. The screw speed was 50 rpm. The extrusion pressure was approximately 3.4 bar, and the temperature of the pasta after the extrusion was 27 °C to 28 °C.

An aliquot (40%) of the extruded pasta was pasteurised with steam for 3 min at 90 °C (Camfil Spa, Milan, Italy) and then mechanically transferred into a drying chamber and dried for 1 min with hot air. The final moisture reached in the pasteurised pasta was 25%, which required refrigerated storage. Another aliquot (60%) of the extruded pasta was dried (SG600; Namad, Rome, Italy) according to the following conditions: first step (i.e., external drying): 20 min at 60 °C and 65% moisture; second step (i.e., wrapping): 130 min at 90 °C and 79% moisture; third step (i.e., drying): 150

min at 75 °C and 78% moisture; fourth step (i.e., first cooling phase): 160 min at 45 °C and 63% moisture; fifth step (i.e., second cooling phases): 1040 min at 50 °C and 50% moisture. The final moisture content in the dried pasta was 12.5%. Three independent pasta-making production trials were performed. Commercial dried (Brand 1: Soc. Coop. Consortile Terre e Gusti, Lucera (FG), Italy; Brand 2: Barilla G. & R. Fratelli Spa, Parma, Italy) and fresh (Brand 1) durum wheat pasta made from semolina and wholemeal were purchased at a local market and used as references. Each uncooked and cooked pasta sample was frozen in liquid nitrogen and dried in a laboratory freeze drier. Then, the pasta samples were ground (Tecator Cyclotec 1093), to pass 100% through a 0.5-mm screen. All of the samples were kept at 25 °C until analysis.

2.3. Technological and nutritional assessments

The qualitative analyses were carried out on the flours, as wholemeal, semi-whole meal, and semolina, and on the uncooked and cooked pasta.

The protein content (PC, %N ×5.7, corrected for dried matter) was assayed using the micro-Kjeldahl method, according to AACC approved method 46-13.01 (AACC, 2000).

The gluten index (GluI, %) was determined using an automatic gluten washing apparatus (Glutomatic) followed by centrifugation, as in AACC approved method 38-12.02 (AACC, 2000). This was quantified according to the percentage gluten that remained on an 84 micron polyester sieve, after removing the starch.

Total dietary fibre (TDF, %) was determined following the AACC approved method 32-07.01 (AACC, 2000).

The total anthocyanins (TAC, $\mu\text{g/g}$ dry weight) were quantified as cyanidin-3-*O*-glucoside equivalents and analysed using the pH differential method, as described in Ficco et al. (2014b).

The total carotenoids (YP, $\mu\text{g/g}$ dried matter) were analysed according to AACC approved method 14-50 (AACC, 2000).

The Trolox equivalent antioxidant capacity (TEAC, mmol Trolox equivalent/kg dried matter) was measured by the direct 2,2'-azinodi(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) method, as reported in Serpen, Capuano, Fogliano, & Gökmen, (2007), with slight modifications. Briefly, a sample of 10 mg was added to 6.0 mL ABTS reagent. The mixture was vortexed for 1 min and shaken in an orbital shaker for 5 min in the dark, to facilitate the reaction. Following centrifugation at $9,500\times g$ for 2 min, the absorbance of the optically clear supernatant was measured at 734 nm using a λ 20 UV/vis spectrometer (Perkin-Elmer, Norwalk, Connecticut, USA). The quenching of the absorbance of the sample at 734 nm was interpolated according to that obtained by standard solutions of Trolox.

2.4. In-vitro digestion method and estimation of glycemic index

In-vitro pasta digestion was carried out as described by Padalino et al. (2013). Briefly, 5 g dried spaghetti were cut in 5.0 ± 1.0 cm lengths and weighed. The spaghetti were boiled for the optimal cooking time in a water bath containing 50 mL boiling water. The spaghetti were tipped into a digestion vessel with 50 mL distilled water and 5 mL maleate buffer (0.2 mol/L pH 6.0, containing 0.15 g/L CaCl_2 and 0.1 g/L sodium azide) in a heating block at 37 °C (GFL 1092, Germany) and allowed to equilibrate for 15 min. Digestion was started by adding 0.1 mL amyloglucosidase (EC 3.2.1.3) and 1 mL 2

g/100 g pancreatin (EC 232-468-9) in quick succession, and the vessel was stirred at 130 rpm. At 0, 20, 60 and 120 min, 0.5 mL digested samples were removed for analysis of released glucose. After the 120 min sampling, the digests were homogenised using an Ultraturrax (Ika werke, Germany) to convert them into slurries. The incubation continued for 1 h and 0.5 mL of digested samples was removed for analysis of released glucose. The digestion was carried out in triplicates.

The hydrolysis index (HI) was calculated by dividing the area under the hydrolysis curve (0-180 min) of the sample by the corresponding area obtained for the reference sample (white bread) over the same duration (Goni, Garcia-Alonso, & Saura-Calixto, 1997). The glycemic index (GI) of the sample was estimated according to Granfeldt, Björck, Drews, & Tovar, (1992) as given in Equation (1):

$$GI = 8.198 + 0.862 \times HI \quad (1).$$

2.5. Textural profile

2.5.1. Cooking loss

The cooking loss represents the amount of solid substance lost in the cooking water, and this was determined according to AACC approved method 66-50.01 (AACC, 2000). The pasta (10 g) was cooked in 300 mL boiling distilled water and the optimal cooking time was taken when the white core of the spaghetti completely disappeared after being squeezed between two transparent glass plates. The cooking water was collected in an aluminium vessel, placed in an air oven at 105 °C, and evaporated until a constant weight was reached. The residue was weighed and reported as a percentage of the starting material.

2.5.2. Swelling index and water absorption

The swelling index of cooked pasta (g water/ g dried pasta) was determined according to the procedure reported in Padalino et al. (2013). The spaghetti strands were cut into equal lengths of 40 mm, and 10 g were then weighed accurately, cooked for the optimal cooking time, and dried at 105 °C until a constant weight was reached. The swelling index was measured as given in Equation (2):

$$\frac{(\text{weight of cooked spaghetti}) - (\text{weight of spaghetti after drying})}{(\text{weight of spaghetti after drying})} \quad (2).$$

The water absorption of the drained pasta was determined as in Equation (3):

$$\frac{(\text{weight of cooked spaghetti}) - (\text{weight of raw pasta})}{(\text{weight of raw pasta})} \quad (3).$$

2.5.3. Hardness and adhesiveness

For each hardness and adhesiveness analysis, three spaghetti strands (length, 40 mm) were cooked for the optimal cooking time. After cooking, the spaghetti samples were gently blotted and analysed using a texture analyser (model Z010; Zwick Roell Italia S.r.l., Genova, Italy) equipped with a stainless steel cylinder probe (diameter, 2 cm). The three strands were put side by side on the lower plate, and the upper plate was moved down onto the spaghetti surface. The hardness (mean maximum force, N) and adhesiveness (mean negative area, Nmm) were measured. Six measurements were taken for each sample. The trial specifications were as follows: preload 0.3 N; load cell 1 kN; percentage deformation 25%; crosshead speed constant, 0.25 mm/s.

2.6. Sensory analysis

The fresh and dried pasta samples were tasted by a trained panel of 15 tasters (six men, nine women; 28-45 years old) to evaluate the sensory attributes. The panelists were trained in sensory vocabulary and the identification of particular attributes through evaluation of commercial durum wheat pasta (ISO 11036). They were asked to indicate colour, odour and overall quality of the fresh uncooked pasta, and colour, resistance to breaking and overall quality of the dried uncooked pasta. Elasticity, firmness, fibrous, bulkiness, adhesiveness, colour, odour, taste and overall quality were evaluated for the cooked pasta (Padalino et al., 2013). A nine-point scale, from extremely unpleasant (1), through satisfactory (5), to extremely pleasant (9) was used to quantify each attribute.

2.7. Statistical analysis

The statistical analyses were carried out for triplicates ($n = 3$) and the means $\pm$ standard deviation are reported. The variables were tested for significance using one-way analysis of variance (ANOVA). The differences among the means were assessed using Tukey's tests ($p < 0.05$). Statistica 7.1 for Windows (StatSoft, Inc, Tulsa, OK, USA) was used for these analyses.

3. Results and discussion

3.1. Distribution of qualitative components from different wheat flours

The basic qualitative parameters of the different milling products are reported in Table 1. In the purple and conventional genotypes, the total dietary fibre showed a decreasing trend from wholemeal to semolina, according to Laddomada, Caretto, & Mita (2015),

with higher values in each milling product of PDW. PDW showed significantly lower PC than YDW, although they both showed similar losses in the semolina with respect to the wholemeal (7.0%, on average). The PC of the commercial durum wheat semolina was significantly greater than almost all of the PDW and YDW flour samples. This could be because the commercial durum wheat semolina usually results from a mixture of different durum wheat varieties to always ensure high commercial standards according to market demand. Similarly to PC, GluI is another important selection criterion in genotype breeding. PDW was characterised by weak gluten compared to YDW and commercial durum wheat semolina. This might have arisen because unlike the breeding selection for conventional durum wheats aimed at the improvement of important qualitative factors, such as protein and gluten strength, no breeding programmes have been undertaken for pigmented durum wheats, as these genetic materials have been viewed as unusual (Zeven, 1991) and have been receiving more attention only recently.

3.2. Processing effects on anthocyanin and carotenoid pigments, and antioxidant properties

3.2.1. Milling process

Table 2 showed the levels of bioactive compounds, TAC and YP, and of TEAC, of the different purple and conventional flours and pasta.

In PDW, a significant decrease in TAC was observed going from the wholemeal (73.41 µg/g) to the semi-wholemeal (66.49 µg/g), and finally, to the semolina (24.40 µg/g). These results confirm that the anthocyanins are mainly located in the pericarp of the grain and are gradually lost during the milling process (Zeven, 1991; Abdel-Aal et

al., 2006; Nayaket al., 2015). No TAC were detected in conventional and commercial products, according to Ficco et al. (2014b) and Pasqualone et al. (2015).

YP were generally present at lower concentrations than TAC and were less abundant for PDW with respect to conventional durum wheat. For PDW, the highest YP was in the wholemeal, followed by the semi-wholemeal, with the lowest in the semolina. These data are in agreement with Abdel-Aal, & Hucl (1999).

The same trend was observed in YDW. The minor losses of YP in semolina with respect to wholemeal in both genotypes (24%, on average), unlike the TAC loss in PDW (66.8%), can be explained by the more favourable distribution of YP along the kernel (Adom, Sorrells, & Liu, 2005; Borrelli et al., 2008).

In the commercial durum wheat semolina, the level of YP was comparable to that of the conventional durum wheat. TEAC always showed the highest absolute values and the lowest degree of decay in the different milling products in PDW, due to the presence of anthocyanins. In both genotypes, higher TEAC in the wholemeal and semi-wholemeal with respect to semolina was observed, which is ascribable to the greater proportions of bran and germ, which are richer in many antioxidants, such as phenolics, carotenoids and tocopherols (Chandrika, & Fereidoon, 2007). Conversely, the commercial durum wheat semolina showed the lowest TEAC.

Thus, the milling differently affected the total pigments and the antioxidant properties as a result of their different locations in the kernel. So, a first way to improve the composition of the flours for pasta production would be through the modulation of the milling techniques, to preserve greater amounts of the compounds of interest.

3.2.2. Pasta-making process

The TAC and YP content and the TEAC in extruded pasta and in fresh and dried uncooked pasta are shown in Table 2. The extruded pasta was the common starting point for both the fresh and dried pasta. In addition, Table 2 gives the data related to commercial fresh and dried durum wheat pasta. For these, it needs to be taken in account that while the PDW and YDW pasta were from the same batch of grain that was differently milled and processed, the commercial durum wheat samples were independent of each other. So their use in this study was aimed for the comparison of the pasta products improved in phytochemicals while also meeting the final sensory acceptability of the consumer.

For PDW, the extruded pasta obtained from the semi-wholemeal and the semolina showed similar decreases in TAC (26.0%, on average) with respect to the relative raw materials (Table 2). The fresh pasta derived from the extruded pasta retained most of the TAC (88.5%, on average), while in dried pasta, more consistent decreases were found, and there was total loss in pasta derived from semolina.

Therefore, the critical step during the pasta processing is confirmed to be the drying phase. This might be the consequence of the time/ temperature conditions and water evaporation, with effects on the water solubility of anthocyanins and the exposure of these to polyphenoloxidase and peroxidase activities (Nayak et al., 2015). An in-depth study to optimise the drying conditions to provide better retention of TAC would thus be an appropriate approach in the future. Similarly, decreases in TAC have been observed in other studies during biscuit making from pigmented wheat (Pasqualone et al., 2015) and from dough enriched in grape-marc extract (Pasqualone, Bianco, Paradiso, Summo, Gambacorta, & Caponio, 2014), as well as during the production of muffins with added purple wheat bran (Li et al., 2007). Different decreases in YP in the

extruded pasta obtained from the semi-wholemeal and the semolina were observed in both genotypes (32.1% vs. 16.1%, on average, respectively). This decrease might be explained in terms of the greater activity of enzymes involved in the oxidative degradation of pigments during the mixing phase (Borrelli et al., 2003, and references therein; De Simone et al., 2010). The lower reduction of the YP in the extruded pasta from the semolina with respect to the semi-wholemeal might be due to a more external localisation of these enzymes in the grain, which implies their greater loss in semolina.

In both genotypes, non-significant decreases in YP were observed in all of the fresh pasta, with respect to the related extruded pasta, while dried pasta always showed significant decreases, according to literature (Borrelli et al., 2003; Fratianni, Panfili, & Cubadda, 2015).

Increases in TEAC were observed in the extruded pasta for both PDW and YDW. The rate of increase was similar and not significant in the extruded pasta derived from semolina (1.4%, on average) and more consistent and to a different extent when they were made from semi-wholemeal (7.2% and 26.6%, for PDW and YDW, respectively). During the early stages of processing, the natural phytochemicals present in the flours can undergo different interactions and modifications that are ascribable to the physical conditions to which they are subjected. This can lead to their greater availability in the matrix, contributing to the increase in TEAC. Furthermore, it is known that during extrusion, rather than during drying, different compounds with antioxidant activities can be produced through the Maillard reaction (e.g., Amadori compounds) (Fares, Platani, Baiano, & Menga, 2010). The absolute values of TEAC were higher for PDW with respect to YDW due to presence of anthocyanins, which are mostly responsible for the antioxidant capacity, together with other polyphenols

(Finocchiaro et al., 2007). However, the greater increase observed in extruded YDW pasta made from the semi-wholemeal might be due to the genotype used, PR22D89, which has derived from a genetic selection mainly aimed at increasing the technological and nutritional qualities.

In the PDW and YDW fresh pasta of both the semi-whole meal and the semolina, there were no significant variations in TEAC, while in all dried pasta significant TEAC losses were observed, which were more marked for YDW.

In conclusion, for the compounds that specifically characterise the purple trait of PDW, great attention needs to be paid to optimise the pasta processing conditions, in order to guarantee their greatest preservation.

3.2.3. Pasta cooking

The effects of cooking on the antioxidant components are also reported in Table 2. After cooking, in the semi-wholemeal fresh and dried pasta, the same rates of TAC decrease were observed (20.3%, on average), while no TAC were found in the fresh pasta made from the semolina. This is in agreement with the loss of other phenolic compounds after cooking (Hirawan, Ser, Arntfield, & Beta, 2010; Nayak et al., 2015).

In both genotypes, a similar trend with regard to YP was observed. In the fresh pasta made from semi-wholemeal and semolina a decrease was found, although this was not significant for the semi-wholemeal. Conversely, in the dried pasta, a significant decrease was observed in the pasta from the semolina, while an increase was found in pasta from semi-wholemeal (34.0%, on average).

The pasta samples showed different behaviours after cooking with regard to TEAC. In fresh pasta of both genotypes, a significant TEAC decrease was always

observed, which was greater for the pasta made from the semolina with respect to the semi-wholemeal (18.2% vs. 11.0%, on average, respectively). On the contrary, an increase was always observed in all of the dried pasta, with a similar trend in PDW and YDW.

In the commercial durum wheat pasta, losses of YP and TEAC were always found after cooking, although these were not significant for YP. For TEAC, the losses were significant in all samples except for the dried pasta made from semolina. However, again, it should be noted that these data are not directly comparable with our experimental samples, as they come from independent samples and no information was available on the processing.

Our data regarding the fresh pasta agree with the literature, where decreased antioxidant components after cooking have always been reported (Finocchiaro et al., 2007; Khan, Yousif, Johnson, & Gamlath, 2013; Zaupa, Calani, Del Rio, Brighenti, & Pellegrini, 2015). The degradation and/or leaching of soluble antioxidant compounds during the boiling process could occur more easily in fresh pasta, so promoting their decrease. Instead, the increase in TEAC in dried pasta, due to an enhanced release of bound phenolics from the food matrix and of some Maillard reaction products, is in agreement with Fares et al. (2010). The low decrease in TEAC observed in dried commercial durum wheat pasta has been widely observed also in other cereal foods (Finocchiaro et al., 2007; Hirawan et al., 2010).

3.4. Effects of processing on the estimated glycemic responses for the pasta samples

The different behaviours of the pasta samples in terms of glucose release are reported in Table 3. Overall, compared to both fresh and dried pasta made from semolina, the GI

was lower in the pasta made from the semi-wholemeal for PDW and YDW, and from the wholemeal for the commercial durum wheat pasta. This might be due to the higher content of total dietary fibre in wholemeal and semi-wholemeal pasta, compared to semolina (see Table 1). These data are in agreement with several studies that have investigated the effects of dietary fibre in food and pasta, in particular, on *in-vitro* digestion (Brennan & Tudorica, 2008; Dhingra, Michael, Rajput, & Patil, 2012, and references therein). Increased levels of total dietary fibre in wholemeal and semi-wholemeal might be responsible for the formation of a polysaccharide network that can encapsulate the starch granules during processing. The entrapment of starch reduces accessibility to enzymatic degradation, and therefore, reduces the sugars released in the blood (Foschia, Peressini, Sensidoni, Brennan, & Brennan, 2015). Thus, the lowest GI recorded in the purple pasta compared to YDW and commercial pasta would be attributed to higher fibre levels in the raw PDW material.

Considering the pasta made from semolina, no significant differences were found for all of the fresh pasta samples, while GI was significantly lower in the dried pasta for PDW.

3.5. Cooking quality and pasta texture

The cooking quality and the texture of the pasta are given in Table 4. To investigate consumer acceptance of enriched of TAC products, these parameters were analysed only on pasta made from semi-wholemeal, due to the absence of TAC in pasta made from semolina. The choice of dried pasta was related to a greater availability of dried wholemeal pasta products on the large scale distribution to use as a comparison for consumer response.

The optimum cooking time of Brand 1 commercial durum wheat pasta from wholemeal and semolina was the same (8 min) and was significantly lower with respect to the commercial samples of Brand 2 (10, 11 min) and of PDW and YDW pasta (10 min).

The quantity of the solids released from the pasta into the water during cooking is an important factor for pasta quality, with more textured pasta showing a lower cooking loss. The cooking losses were significantly lower for both of the commercial durum wheat pasta made from semolina, with respect to all of the other pasta. This parameter theoretically reflects the quantity of starch and other biochemical components that are released from the pasta protein matrix, and are subsequently lost in the cooking medium. The differences observed between the semolina and wholemeal/ semi-wholemeal pasta is ascribable to the presence of fibre in the wholemeal/ semi-wholemeal pasta. The interference of fibre with the gluten–protein network of pasta can result in the destruction of the protein–starch matrix and the uneven distribution of water in the pasta structure, which would allow increased loss of solids during cooking (Manthey & Schorno 2002; Tudorica, Kuri, & Brennan, 2002). The significant differences also seen between all of the commercial durum wheat pasta and the semi-wholemeal PDW and YDW pasta might be due to the inherent characteristics of these genotypes as well as to the pilot milling and processing procedures used.

The swelling index is an indicator of the water that is absorbed by the starch and protein during cooking, which is used for gelatinisation of the starch and hydration of the protein. Little or no significant differences in the swelling index were observed among all of the samples studied. Despite these results, significantly different values were found for water absorption, with the highest in both commercial pasta made from

semolina. These results could be due to a bran component that is very rich in fibre in wholemeal and semi-wholemeal flours. During the cooking of pasta, the starch will compete with the fibre for water, so high fibre will mean that the starch will absorb less water, with the consequent lower swelling (Brennan, & Tudorica, 2008). However, this is true only for commercial samples, while in our experimental pasta there was no direct relationship between the two parameters. The lower value for water absorption in PDW respect to YDW, which was ascribable to higher fibre content in its semi-wholemeal flour (Table 1), did not correspond to an evident difference in the swelling index. This might be because the volume change was affected by water absorption, as well as by structure relaxation phenomena and by cooking losses (Ogawa, Hasegawa, & Adachi, 2014), in turn related to gluten strength that was lower in PDW.

The textural characteristics of pasta have essential roles for the final acceptance by consumers (Tudorica, Kuri, & Brennan, 2002). The adhesiveness and hardness are used in this way, showing significantly higher levels in both commercial pasta from semolina than all of the other pasta, which resulted in low cooking quality. Also this aspect might be due to the higher fibre content in pasta from wholemeal and semi-wholemeal, which interferes with the starch network and reduces the amount of pregelatinised starch necessary to structure the dough, giving a better hard pasta (Padalino et al., 2013).

3.6. Sensory analysis

Concurrent with the laboratory evaluations of cooking quality, sensorial analysis was carried out with the aim of comparing the organoleptic features of all of the pasta samples. These data are reported in Table 5.

For the uncooked pasta, there were no significant differences in terms of colour and overall quality among all of the fresh samples, except for odour, for which a significant difference was observed, with YDW showing a higher score. For the dried pasta, only the commercial durum wheat wholemeal pasta of Brand 1 showed lower values, which indicated a perception of inferior quality. Thus, the inherent differences between the genotypes used in this study and the presence of fibre did not interfere in any negative way in the global perception of the panelists.

In general, for the cooked pasta, despite the objective differences among all of the experimental and commercial pasta samples (Figure 1), the sensorial perception showed no substantial differences between the semi-wholemeal PDW and YPW pasta and the commercial wholemeal pasta. However, superior quality was recognized for the commercial pasta made from semolina, both for the fresh pasta and the dried pasta. Interestingly, although made from semi-wholemeal, the colour of the fresh pasta of PDW and YDW promoted positive evaluations, which were similar to that of the commercial durum wheat pasta from semolina.

4. Conclusions

This study has shown that the choice of a suitable milling process is necessary to preserve high antioxidant components, such as TAC, YP and TEAC, to provide good raw materials for pasta processing. In addition, a study aimed to optimise the conditions for pasta processing to consistently reduce the loss of these antioxidant components is necessary.

Moreover, although the sensorial and cooking quality characteristics of these PDW and YDW semi-wholemeal pasta did not reach the quality of the commercial

durum wheat pasta derived from semolina, they were balanced in the purple genotype by the better phytochemical composition and the low estimated GI, making it a good healthy product.

Thus, the production of pasta rich in natural anthocyanins represents an important way to further improve a daily product that is already popular due to its convenience and quality. The residual presence of anthocyanins also in semolina makes this purple genotype particularly interesting also for the preparation of other cereal-based foods derived from refined raw materials.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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Figure caption.

Figure 1. Typical appearance of the different typologies of cooked dried pasta investigated in this study. PDW dried pasta from semi-wholemeal (A) and semolina (A1), YDW dried pasta from semi-wholemeal (B) and semolina (B1), and commercial dried pasta from wholemeal (C) and semolina (C1) of Brand 2.

Table 1. Chemical compositions of the PDW and YDW genotypes according to the different milling techniques, and of the commercial durum wheat semolina. (Means and standard deviations; values expressed on dried matter).

Milling	Durum wheat sample	TDF (%)	PC (%)	GluI (%)
Wholemeal	PDW	9.8 ±0.3 ^a	13.82 ±0.03 ^c	23.25 ±2.03 ^c
	YDW	8.4 ±0.2 ^b	14.24 ±0.08 ^{a,b}	93.72 ±2.20 ^a
Semi-wholemeal	PDW	9.0 ±0.5 ^{a,b}	13.21 ±0.10 ^d	21.90 ±0.78 ^c
	YDW	7.5 ±0.3 ^c	14.11 ±0.02 ^{b,c}	92.55 ±0.51 ^a
Semolina	PDW	3.9 ±0.3 ^d	12.78 ±0.03 ^e	24.08 ±0.60 ^c
	YDW	3.1 ±0.2 ^e	13.28 ±0.08 ^d	94.82 ±0.65 ^a
Commercial semolina	//	3.0 ±0.3 ^e	14.46 ±0.25 ^a	81.83 ±0.76 ^b

PDW, purple durum wheat; YDW, yellow durum wheat.

Wholemeal, milled with a Cyclotec mill 1093; semi-wholemeal, milled with a Partisani stone mill; semolina, milled with a Bühler laboratory mill (see Methods for details).

Data are means ±standard deviations, expressed relative to dried matter.

TDF, total dietary fibre; PC, protein content; GluI, gluten index; n.d., not detected.

Means in the same column followed by different superscript letters differ significantly ($p < 0.05$; Tukey's tests).

Table 2. Total anthocyanins and carotenoids, and antioxidant capacity of pasta from the PDW and YDW genotypes and commercial durum wheat pasta, for uncooked and cooked pasta samples, and from the two different milling systems.

Raw material/ Pasta	Source	PDW			YDW			Commercial durum wheat pasta*		
		TAC (µg/g)	YP (µg/g)	TEAC (mmol Trolox/kg)	TAC (µg/g)	YP (µg/g)	TEAC (mmol Trolox/kg)	TAC (µg/g)	YP (µg/g)	TEAC (mmol Trolox/kg)
Raw material	Wholemeal	73.41 ±1.07 ^a	7.38 ±0.28 ^a	11.63 ±0.04 ^{a,b}	n.d.	7.68 ±0.13 ^a	10.20 ±0.11 ^b			
	Semi-wholemeal	66.49 ±1.33 ^b	6.30 ±0.10 ^b	11.14 ±0.12 ^{b-d}	n.d.	6.76 ±0.54 ^b	8.81 ±0.17 ^c			
	Semolina	24.40 ±0.76 ^f	5.25 ±0.03 ^c	10.81 ±0.44 ^{c,d}	n.d.	6.18 ±0.12 ^c	7.93 ±0.06 ^d	n.d.	6.01 ±0.67 ^a	5.66 ±0.25 ^d
Extruded	Semi-wholemeal	49.36 ±1.36 ^c	4.12 ±0.06 ^{d,e}	11.94 ±0.28 ^a	n.d.	4.76 ±0.20 ^{f,g}	11.15 ±0.07 ^a	n.a.	n.a.	n.a.
	Semolina	18.00 ±1.00 ^h	4.31 ±0.50 ^d	11.00 ±0.10 ^{b-d}	n.d.	5.30 ±0.10 ^{d,e}	8.01 ±0.10 ^d	n.a.	n.a.	n.a.
Uncooked fresh	Semi-wholemeal	46.32 ±0.66 ^d	4.04 ±0.17 ^{d-f}	12.09 ±0.06 ^a	n.d.	4.36 ±0.12 ^{g,h}	10.81 ±0.23 ^a	n.d.	3.84 ±0.10 ^d	10.27 ±0.22 ^a
	Semolina	15.00 ±0.50 ⁱ	4.20 ±0.20 ^{d,e}	10.64 ±0.35 ^{d,e}	n.d.	5.19 ±0.17 ^e	7.70 ±0.20 ^d	n.d.	5.41 ±0.35 ^{a,b}	10.05 ±0.12 ^{a,b}
Uncooked dried	Semi-wholemeal	21.42 ±1.31 ^g	3.77 ±0.03 ^{f,g}	11.48 ±0.13 ^{a-c}	n.d.	4.34 ±0.05 ^h	10.00 ±0.14 ^b	n.d.	5.25 ±0.10 ^{a-c}	10.14 ±0.29 ^a
	Semolina	n.d.	3.50 ±0.24 ^g	10.04 ±0.15 ^e	n.d.	4.97 ±0.08 ^f	6.69 ±0.21 ^e	n.d.	5.22 ±0.05 ^{a-c}	7.89 ±0.07 ^c
Cooked fresh	Semi-wholemeal	37.27 ±0.52 ^e	3.96 ±0.17 ^{e,f}	10.78 ±0.05 ^d	n.d.	4.18 ±0.28 ^h	9.60 ±0.46 ^b	n.d.	3.63 ±0.17 ^d	7.83 ±0.32 ^c
	Semolina	n.d.	3.51 ±0.05 ^g	8.43 ±0.31 ^f	n.d.	4.01 ±0.20 ^h	6.51 ±0.10 ^e	n.d.	5.00 ±0.08 ^{a-c}	7.60 ±0.36 ^c
Cooked dried	Semi-wholemeal	16.89 ±0.85 ^{h,i}	5.20 ±0.08 ^c	11.84 ±0.22 ^a	n.d.	5.64 ±0.16 ^d	10.17 ±0.20 ^b	n.d.	4.88 ±0.61 ^{b,c}	9.44 ±0.21 ^b
	Semolina	n.d.	2.92 ±0.03 ^h	11.53 ±0.25 ^{a,b}	n.d.	4.37 ±0.05 ^{g,h}	7.52 ±0.25 ^d	n.d.	4.38 ±0.12 ^{c,d}	7.77 ±0.15 ^c

PDW, purple durum wheat (Citr 14754); YDW, yellow durum wheat (PR22D89).
*, wholemeal was the source for the commercial durum wheat.
Data are means ±standard deviations, expressed relative to dried matter.
TAC, total anthocyanins; YP, carotenoids (yellow pigment); TEAC, Trolox equivalent antioxidant capacity.
n.a., not applicable; n.d., not detected
Means in the same column followed by different superscript letters differ significantly (p <0.05; Tukey's tests).

Table 3. Estimated glycaemic indices of the different pasta samples.

Source	Fresh pasta		Dried pasta	
	Semolina	Semi-wholemeal	Semolina	Semi-wholemeal
PDW pasta	77 $\pm$ 1.50 ^a	64 $\pm$ 0.40 ^a	66 $\pm$ 1.40 ^a	57 $\pm$ 0.20 ^a
YDW pasta	78 $\pm$ 0.90 ^a	67 $\pm$ 1.00 ^b	74 $\pm$ 1.00 ^b	66 $\pm$ 0.50 ^b
Commercial durum wheat pasta	80 $\pm$ 1.00 ^a	76 $\pm$ 0.10 ^c *	74 $\pm$ 0.80 ^b	70 $\pm$ 1.20 ^c *

PDW: purple durum wheat; YDW: yellow durum wheat.

*, wholemeal was the source for the commercial durum wheat pasta.

Data are means $\pm$ standard deviations, expressed relative to dried matter.

Means in the same row followed by different superscript letters differ significantly ($p < 0.05$; Tukey's tests).

Table 4. Cooking quality of the dried semi-wholemeal pasta of the PDW and YDW genotypes, and of the commercial durum wheat pasta.

Wheat	Source	Optimum cooking time (min)	Cooking loss (%)	Swelling index (g water/g dry pasta)	Water absorption (%)	Adhesiveness (Nmm)	Hardness (N)
PDW	Semi-wholemeal	10 ±0.02 ^b	7.74 ±0.19 ^a	2.02 ±0.08 ^{a,b}	137 ±0.43 ^f	0.75 ±0.03 ^c	7.84 ±0.30 ^c
YDW	Semi-wholemeal	10 ±0.02 ^b	7.66 ±0.06 ^a	1.98 ±0.04 ^{a,b}	156 ±0.50 ^d	0.62 ±0.03 ^c	6.87 ±0.15 ^c
Commercial durum wheat	Wholemeal (Brand 1)	10 ±0.01 ^b	7.08 ±0.20 ^{a,b}	1.72 ±0.01 ^b	150 ±1.96 ^e	0.64 ±0.01 ^c	7.32 ±0.92 ^c
	Semolina (Brand 1)	11 ±0.01 ^a	5.58 ±0.12 ^c	2.24 ±0.06 ^{a,b}	175 ±0.51 ^b	1.04 ±0.08 ^b	10.22 ±0.85 ^b
	Wholemeal (Brand 2)	8 ±0.02 ^c	6.47 ±0.04 ^b	1.93 ±0.01 ^{a,b}	162 ±0.92 ^c	0.74 ±0.15 ^c	7.51 ±0.13 ^c
	Semolina (Brand 2)	8 ±0.01 ^c	4.24 ±0.68 ^d	2.30 ±0.00 ^a	178 ±1.3 ^a	1.25 ±0.05 ^a	13.7 ±1.38 ^a

PDW, purple durum wheat; YDW, yellow durum wheat.
Data are means ±standard deviations.
Means in the same column followed by different superscript letters differ significantly (p <0.05; Tukey's test).

Figure(s)

(A)

(A1)

(B)

(B1)

(C)

(C1)