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Functional, textural and sensory properties of dry pasta supplemented with lyophilized tomato matrix or with durum wheat bran extracts produced by supercritical carbon dioxide or ultrasound

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Abstract
A study was carried out to produce functional pasta by adding bran aqueous extract (BW) and bran oleoresin (BO) obtained using ultrasound and supercritical CO_2, respectively, or a powdery lyophilized tomato matrix (LT). The bioactive compounds, hydrophilic and lipophilic antioxidant activity (HAA and LAA) *in vitro*, were evaluated. BW supplementation did not improve antioxidant activity, whilst LT pasta showed unconventional taste and odor. BO pasta had good levels of tocochromanols (2551 μg/100 g pasta f.w.) and carotenoids (40.2 μg/100 g pasta f.w.), and the highest HAA and LAA. The oleoresin altered starch swelling and gluten network, as evidenced by scanning electron microscopy, therefore BO pasta had structural characteristics poor compared with the control (4.8% vs. 3.2% cooking loss), although this difference did not affect significantly overall sensory judgment (74 vs. 79 for BO and control, respectively). BO supplementation was most effective for increasing antioxidant activity without jeopardizing pasta quality.

Keywords
Functional pasta, Green extraction technologies, Carotenoids, Phenolics, Tocochromanols, *Solanum lycopersicum*, *Triticum durum*

1. Introduction
The increasing demand for healthy foods has encouraged food companies to direct new research and development activities towards products providing, beyond basic nutritional functions, beneficial effects for health and/or reducing the risk of chronic diseases, i.e. functional foods (Roberfroid, 2002). Potentially, pasta is a useful carrier for substances acting as nutrition enhancers or providing specific physiological functions and has, thus, been the object of many functionalization strategies (Li, Zhu, Guo, Brijs, & Zhou, 2014). To improve protein content and essential amino acid profile, pasta has been supplemented with flour made from split pea and faba bean (Petitot, Boyer, Minier, & Micard, 2010), common bean (Gallegos-Infante et al., 2010), lupin (Doxastakis et al., 2007), and chickpea and lentil (Zhao, Manthey, Chang, Hou, & Yuan, 2005). Other supplements aimed to increase omega-3 polyunsaturated fatty acid content to prevent/ reduce cardiovascular diseases. For this purpose, functional ingredients of marine origin, such as seaweeds, have been exploited (Prabhasankar, Ganesan, & Bhaskar, 2009). Microencapsulated fish oil, rich in long chain omega-3 polyunsaturated

fatty acids, was proposed by Iafelice et al. (2008). Other pasta formulations, supplemented with carrot and oregano leaf powders (Boroski et al., 2011), black carrot concentrate (Day, Seymour, Pitts, Konczak, & Lundin, 2009), carob flour (Sęczyk, Świeca, & Gawlik-Dziki, 2016) or germinated pigeon pea seeds (Torres, Frias, Granito, & Vidal-Valverde, 2007) showed higher antioxidant activity *in vitro* as well as increased levels of phenolic compounds than unsupplemented controls.

Tomato and durum wheat are among the major food crops in the Mediterranean area and there is huge economic interest in evaluating alternative uses for these crops, including products, by-products and waste derived from their industrial processing. Food industry by-products and waste, as well as cultivars specifically selected for the high content of a specific bioactive compound, are potentially valuable sources of functional ingredients suitable for incorporation into pasta.

The outermost layers of wheat caryopsis constitute bran, an abundant by-product of the milling industry. Destined mainly for animal feed, bran contains antioxidants including phenolics (Yu, 2008), carotenoids, and tocochromanols (Durante, Lenucci, Rescio, Mita, & Caretto, 2012). Wheat bran extracts, obtained by preliminary KOH-induced hydrolysis, have been used in fresh pasta in a previous study (Delvecchio & Pasqualone, 2013). However, addition of the extract reduced dough machinability and affected the sensory properties of the end product, due to salts derived from KOH neutralization. Ultrasound-assisted and supercritical carbon dioxide (SC-CO_2) technologies represent effective and non-toxic systems for extracting nutraceuticals (Wang & Weller, 2006), which can be exploited to recover bioactive compounds from bran without the need for chemical pretreatments.

Rich in antioxidant molecules, such as lycopene, ascorbic acid, vitamin E, carotenoids, flavonoids, and phenolic compounds (Raffo et al., 2002), tomato has undergone intense breeding, especially in regard to lycopene content. As a result, a number of high lycopene hybrids, with good agronomic and biochemical traits, have been introduced to the global market (Ilahy et al., 2011, Ilahy et al., 2016) and, recently, used to produce antioxidant enriched lyophilized powders suitable for addition to innovative functional foods (Lenucci et al., 2010, Lenucci et al., 2015).

Until now, there have been no reports about either the addition to pasta of functional extracts from bran, obtained by ultrasound-assisted and SC-CO_2 technologies, or lyophilized matrices from high lycopene tomatoes. Further, there are no comparative studies on the properties of pasta made with aqueous, oleaginous or powdery supplements. These materials, although similar in terms of antioxidant activity, are very different in composition, and could have different effects on the pasta-making process. The aim of this study was to explore the feasibility of producing functional pasta, with quality characteristics similar to conventional pasta, by adding antioxidant extracts or powdery matrices derived from food industry by-products or specifically selected cultivars. In particular, supplementation was realized by addition to the semolina: (i) a bran aqueous extract obtained using ultrasound-assisted technology; (ii) a bran oleaginous extract (oleoresin) prepared using SC-CO_2; (iii) a powdery lyophilized tomato matrix with high lycopene content. Functional, textural, and sensory properties of the supplemented pasta were evaluated.

2. Materials and methods

2.1. Wheat bran oleoresin production

Durum wheat (*Triticum durum*, Desf.) bran was provided by Tomasello s.p.a. milling industry (Casteldaccia, Palermo, Italy) and processed into a dehydrated matrix accordingly to Durante, Lenucci, Laddomada, Mita, and Caretto (2012). Briefly, wheat bran was oven dehydrated at 60 °C to a residual moisture content of 3%. The dehydrated material (with an average granulometry of ~600 μm) was used directly for SC-CO_2 oleoresin extraction. The matrix was vacuum-packaged in food grade oxygen impermeable plastic bags and stored in a freezer at −20 °C until SC-CO_2 extraction. SC-CO_2 extractions were performed in the pilot plant described by Vasapollo, Longo, Rescio, and Ciurlia (2004). Aliquots (3 kg) of the wheat bran matrix were packed into a 5 L stainless-steel extraction vessel and extracted dynamically for 3 h using CO_2 at a rate of 18–20 kg/h.

The other operative parameters were pressure = 35 MPa and temperature = 60 °C. The obtained oleoresin was stored at −20 °C in a food-grade polyethylene terephthalate (PET) bottle until use.

2.2. Wheat bran aqueous extract production

Durum wheat bran (3.5 kg) was mixed with tap water (35 L) (pH 7.6, Maximum Contaminant Levels 315 mg/L, hardness 20 °fH, conductibility 451 μS/cm at 20 °C). The suspension was subjected to ultrasound-assisted extraction at 20 °C for 25 min by means of a pilot plant assembled by Weal (Milano, Italy), consisting of a stainless steel cylindrical extraction chamber (32-cm diameter, 102-cm height) equipped with a Sonic Digital LC 1000 SD 25-P Premium ultrasound generator (Weber Ultrasonics, Karlsbad-Ittersbach, Germany), and a Sonopush Mono titanium alloy transducer bar (Weber Ultrasonics, Karlsbad-Ittersbach). A centrifugal electrical-pump allowed recirculation of the suspension of water and bran into the extraction chamber, with the aim of ensuring homogeneous cavitation in the mass to be extracted. Thirty-second recirculation steps were carried out every 5 min during ultrasonic treatment. Finally, the suspension was filtered through a metal grid with 1 mm holes to recover the liquid phase. The extract obtained, with a total phenolic concentration of 1.30 g/L expressed as ferulic acid equivalents (FAE), was stored at −20 °C until use.

2.3. Lyophilized tomato matrix production

Open field grown red-ripe tomatoes (*Solanum lycopersicum* L.), high lycopene HLY 18 cultivar, were processed in a dehydrated matrix as described by Lenucci et al. (2010). Briefly, tomatoes were blanched in water at 70 °C for 5 min, crushed and sieved using a Reber 9004 N tomato squeezer (Reber, Luzzara, Italy) to obtain a tomato purée free from skins, seeds and vascular tissues. The purée was centrifuged at 27000×*g* for 10 min to remove water-soluble substances. The pellet was dehydrated to a constant weight using a Christ ALPHA 2-4 LSC freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). The lyophilized tomato pellet was ground in a laboratory ultracentrifugal mill (ZM200, Retsch GmbH, Haan, Germany) through a 35-mesh (500 μm) sieve. The powdered matrix obtained was vacuum-packaged in food grade oxygen impermeable plastic bags and stored in a freezer at −20 °C until use.

2.4. Pasta production

Durum wheat (*Triticum durum* Desf.) cv. 'Vertola' was used to perform all the experimental work. This cultivar, released in 2003, was developed by the Agricultural and Forest Sciences Department of the University of Palermo (Italy). It is characterized by good agronomic features (short plant stature, early heading and maturity, high yield potential). Sowing was carried out during the first week of December 2013 and production of the grain was conducted under rain-fed conditions at the Pietranera farm (Santo Stefano Quisquina, Italy; 37° 30′ N, 13° 31′ E), located in a hilly area of Sicily. It has a semiarid Mediterranean climate with a mean annual rainfall of 552 mm, most of which falls in the autumn/winter (74%) and spring (18%). There is a dry period from May to September. The mean air temperature is 15.9 °C in autumn, 9.8 °C in winter, and 16.5 °C in spring. The average minimum and maximum annual temperatures are 10.0 °C and 23.3 °C, respectively. The weather data were collected from a weather station located within 500 m of the experimental site. Grains were harvested at full ripening stage during the first week of July 2014. Grains were milled to semolina by means of MLU 202 mill (Buhler, Uzwil, Switzerland), after conditioning at 17.5% moisture, the semolina was used to produce five types of pasta: (i) control pasta; (ii) pasta containing bran oleoresin (BO); (iii) pasta containing bran aqueous extract (BW); (iv) pasta containing the lyophilized tomato matrix at 1.5% level (1.5LT) and (v) at 2.5% level (2.5LT). Specifically, BO pasta was obtained by adding 525 g bran oleoresin to 9.475 kg semolina; 1.5LT and 2.5LT pasta were prepared by adding 150 g and 250 g of lyophilized tomato matrix to 9.850 kg and 9.750 kg semolina, respectively, while BW pasta was obtained mixing 3 L bran aqueous extract to 10 kg of semolina (i.e. by completely substituting processing water with the aqueous extract). Tap water (3 L) (pH 7.6, Maximum Contaminant Levels 315 mg/L, hardness 20 °fH, conductibility 451 μS/cm at 20 °C) was, instead,

used to prepare BO and LT dough. The amount of BW to be added was determined by means of preliminary trials, which aimed to achieve a significant increase in phenolics with respect to control (data not shown). BO and LT amounts were determined with the purpose of significantly contributing to the recommended daily allowance (RDA) of the prevalent antioxidant molecule or class of molecules (lycopene, isoprenoids). The ingredients were processed using a MAC 60 VR vacuum extruder (Italpast, Fidenza, Italy) under 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 and the pasta dried in a static dryer (LAB, Namad Impianti, Rome, Italy) according to a high-temperature drying program (T max = 78 °C) with linear decrease in the relative humidity from 95% to 40% over the entire process (8 h and 50 min). The temperature increased linearly from 40 to 60 °C in 120 min, then from 60 to 68 °C in 120 min, and from 68 to 78 °C to the end of the drying cycle.

2.5. Chemical, biochemical, and physical determinations in semolina and pasta

Semolina was subjected to determination of dry gluten and gluten index, according to the AACC method 38-12.02 (AACC, 2000), and ashes as described in the AACC method 08-12 (AACC, 2000). Color indices of semolina and pasta (yellow index, corresponding to b^*; red index, corresponding to a^*; brown index, corresponding to 100-L) were determined by means of the reflectance colorimeter Chroma Meter CR-300 (Konica Minolta Sensing, Osaka, Japan). The linoleate:oxygen oxidoreductase (LOX, EC1.13.11.12) activity of semolina was determined spectrophotometrically according to Pastore et al. (2000) following the conversion of linoleate into the corresponding hydroperoxide. Briefly, durum wheat semolina (5 g) was suspended in 10 mL of sodium phosphate buffer (pH 7.0) and stirred at 4 °C for 1 h. The mixture was centrifuged at 35,000×g for 15 min at 4 °C. Aliquots (2 μL) of the supernatant (crude enzyme extract) were added to 2 mL of 400 μM linoleate in mL 50 mM sodium phosphate buffer (pH 6.6) containing 0.4 μL/mL Tween 20 (Sigma-Aldrich Chemical Co., St. Louis, MO, USA). The absorbance increase was monitored at 234 nm. One enzyme unit (EU) of linoleate hydroperoxidation activity corresponded to the formation of 1 μmol of conjugated diene per min at 25 °C.

2.6. Pasta cooking test

Pasta was cooked in boiling distilled water at 1:10 (w/v) pasta to water ratio, without the addition of salt. Optimum cooking time (OCT) was determined preliminary by removing spaghetti strands from the boiling water at 30 s intervals and squeezing it between two transparent glass slides until the white, opaque center core of the strand disappeared, according to the AACC method 16-50 (AACC, 2000). After cooking and draining, samples were rinsed with distilled water and allowed to rest for 5 min. Cooking losses were evaluated by combining the cooking and rinse waters, measuring total volume, putting 20 mL in a tarred Petri dish, and evaporating to dryness in an air-oven at 105 °C until a constant weight was reached (Alireza Sadeghi & Bhagya, 2008). The residue, scaled up to total volume, was expressed as a percentage of the original pasta sample weight. The physical swelling index (PSI) of pasta was determined as:PSI=E-E0E0where E and E_0 were the thickness of cooked and raw pasta, respectively, both measured by Z1.0 TN texture profile analyzer (Zwick Roell, Ulm, Germany). By moving down a stainless steel probe at the speed of 10 mm/min, the analyzer registered first contact with spaghetti, measuring the difference in height between the spaghetti upper surface and the lower plate where spaghetti were positioned. Data were acquired by means of the TestXPertII v. 3.41 software (Zwick Roell, Ulm, Germany) at 400 Hz. The water absorption (A) of pasta was determined as:A=W-W0W0100where W and W_0 were the weight of cooked and raw pasta, respectively. Cooking tests and subsequent determinations were performed in duplicate.

2.7. Pasta sensory evaluation

A trained panel of eight assessors (4 males and 4 females, aged 31–49 y) carried out the sensory evaluation. After cooking at OCT and draining, without any rinsing, the pasta samples were allowed

to rest for 5 min in white dishes, randomly coded by three-digit numbers, and submitted for evaluation of: (i) stickiness, related to the organic matter released during cooking and still adhering to the surface of pasta, which was evaluated by pressing a single spaghetti strand against the palate and determining the force required to remove it with the tongue; (ii) bulkiness, expressing adhesion of spaghetti strands to each other, evaluated both visually and manually by pressing two spaghetti strands together and determining the force required for detachment; (iii) firmness, which is the resistance of cooked pasta to chewing measured while cutting the spaghetti strand using the front teeth; (iv) smell, perceived by olfaction, and (v) taste, perceived during mastication, which was intended to be the typical odor and smell of durum wheat pasta without anomalies, such as sour, rancid, or foreign notes. Each descriptor was scored from 10 to 100. The scores for firmness were: 10 = very low, 100 = very high; for stickiness and bulkiness: 10 = very high, 100 = absent (as in D'Egidio, Mariani, Nardi, & Novaro, 1993); for smell and taste: 10 = strong presence of anomalous notes, 100 = high intensity of typical smell/taste without anomalous notes. The overall sensory judgment (SJ) was calculated for each sample as the mean of scores attributed to all the descriptors.

2.8. Pasta texture profile analysis and viscoelastograph analysis

Both the texture profile analysis (TPA) and viscoelastograph analysis were performed on pasta, cooked at OCT, by means of a Z1.0 TN texture analyzer (Zwick Roell, Ulm, Germany) equipped with a stainless steel square probe (4 cm side) and a 1 kN load cell. Data were acquired by means of the TestXPertII v. 3.41 software (Zwick Roell, Ulm, Germany) at the frequency of 400 Hz. For each test, 20 spaghetti strands were cut 6-mm long pieces, put side-by-side on the lower plate to obtain a homogeneous surface, and anchored to it using the blocking tool of the analyzer. The probe was then moved down on to the spaghetti surface. Five replicates for each sample were performed. The TPA conditions in the cyclic compression test were: 1 mm/s probe compression rate; 25% sample deformation in both the compressions; and a 10 s pause before second compression. The following parameters were calculated: (i) hardness (N), defined as the peak force attained during the first compression; (ii) springiness or elasticity, i.e. the adimensional ratio between the time samples needed to recover maximum height during the second compression cycle and the time needed during the first compression cycle; (iii) cohesiveness, i.e. the adimensional ratio of the positive force area during the second compression cycle to the positive force area recorded during the first compression cycle, or Area 2/Area 1; (iv) chewiness (N), i.e. the product of hardness, cohesiveness, and springiness. The viscoelastograph analysis, which is a peculiar type of TPA specific to pasta, was set up as in D'Egidio et al. (1993): load, 500 g; time of loading, 40 s; time of recovery after loading off, 20 s. The following parameters were taken from the viscoelastograph strain-time curve: initial spaghetti thickness (E, mm); thickness before loading off (e_1, mm); final thickness (e_2, mm). Then, firmness (F), elastic recovery (ER), and overall viscoelasticity index (VI) were calculated as follows:

$$F = (e_1/E)*100$$
$$ER = [(e_2-e_1)/(E-e_1)]*100$$
$$VI = ER \times E$$

2.9. Scanning electron microscopy (SEM) imaging of pasta

Raw and cooked pasta were cut transversally with a sharp blade without damaging the structure. Samples were critical-point-dried (K850 Critical Point Drier, Quorum Technologied LTD, Ashford, UK) using liquid CO_2, mounted on carbon adhesive stubs and gold coated with a Balzers SCD 040 sputter coater (BAL-TEC AG, Balzers, Lichtenstein; thickness of gold layer: 40 nm). Microstructure observation of transversal cross sections were carried out using SEM with a ZEISS EVO HD 15 (Carl Zeiss Microscopy GmbH, Oberkochen, Germany) operating under high-vacuum at an accelerating voltage of 20 keV, at a magnification of 5000×.

2.10. Determination of isoprenoid compounds of pasta

Control, BO, 1.5LT and 2.5LT pasta were ground in a laboratory ultracentrifugal mill (ZM200, Retsch GmbH, Haan, Germany) through 35 mesh (500 μm) sieve. For tocols and carotenoids, triplicate aliquots (1.0 g) from control and BO samples were extracted according to the method of Sadler, Davis, and Dezman (1990), modified by Perkins-Veazie, Collins, Pair, and Roberts (2001). The solvent was vacuum evaporated, and the oleaginous extract dissolved in 1 mL of ethyl acetate before being passed through a 0.45 μm syringe filter (Millipore Corporation, Billerica, MA, USA) and immediately analyzed by HPLC. Quali-quantitative analyses of tocols and carotenoids were carried out using the method of Fraser, Pinto, Holloway, and Bramley (2000), slightly modified for an Agilent 1100 Series HPLC-DAD system (Agilent Technologies, Santa Clara, CA, USA) equipped with a reverse-phase C30 column (5 μm, 250 × 4.6 mm) (YMC Inc., Wilmington, NC, USA). The mobile phases were: methanol (A), 0.2% ammonium acetate aqueous solution/methanol (20/80, v/v) (B) and *tert*-methyl butyl ether (C). The isocratic elution was as follows: 0 min, 95% A and 5% B; 0–12 min, 80% A, 5% B and 15% C; 12–42 min, 30% A, 5% B and 65% C; 42–60 min, 30% A, 5% B and 65% C; 60–62 min, 95% A, and 5% B. The column was re-equilibrated for 10 min between runs. The flow rate was 1 mL/min and the column temperature was maintained at 25 °C. The injection volume was 10 μL. Absorbance was registered at 475 nm for carotenoids and 290 nm for tocols. Peaks were identified by comparing their retention times and UV–Vis spectra to those of authentic isoprenoid standards.

Lycopene extraction and determination was conducted as described by Fish, Perkins-Veazie, and Collins (2002) on triplicate independent aliquots (0.3 g) of control, 1.5LT and 2.5LT milled pasta. The method uses a mixture of hexane/ethanol/acetone (2:1:1 by vol.) containing 0.05% butylated hydroxytoluene (BHT). The absorbance of the hexane extract was read at 503 nm using a Cecil BioQuest CE 2501 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). Hexane was used as a blank. During the extraction process and analysis, some precautions were taken, such as working with reduced lighting in the room and wrapping glass materials in aluminium foil to minimize lycopene loss through photo-oxidation. Lycopene molar extinction $\varepsilon = 17.2\ 10^4\ M^{-1}cm^{-1}$ in *n*-hexane was used for lycopene content determination and results are expressed as mg/100 g pasta fresh weight. The same procedures were used for cooked pasta, which was freeze-dried previously in a Christ ALPHA 2-4 LSC freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). In this case, the results are expressed as per 100 g f.w. of raw pasta.

2.11. Determination of total phenolic compounds of pasta

Total phenolic compounds were extracted from BW pasta with methanol and were, subsequently, subjected to the Folin-Ciocalteu reaction and absorption measure at 765 nm under the conditions reported in Pasqualone, Delvecchio, Mangini, Taranto, and Blanco (2014). The bran aqueous extract (BW) was subjected to Folin-Ciocalteu reaction and analyzed using the same conditions. A calibration curve was built with ferulic acid (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) at concentrations between 0.1 and 2 g L^{-1} ($y = 0.0007 \times +0.0089$; $R^2 = 0.9985$). The results are expressed as mg/g FAE fresh weight.

2.12. Determination of the hydrophilic and lipophilic antioxidant activity of raw and cooked pasta

Hydrophilic and lipophilic antioxidant activities of raw and cooked pasta were evaluated using the 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalent antioxidant capacity (TEAC) assay, as described by Re et al. (1999), and the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical discoloration method. Cooked pasta was freeze-dried to constant weight using a Christ ALPHA 2-4 LSC freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). Raw and cooked pasta were ground in a laboratory ultracentrifugal mill (ZM200, Retsch GmbH, Haan, Germany) through 35 mesh (500 μm) sieve. Hydrophilic and

lipophilic antioxidants were extracted from 0.5 g of each milled sample (three independent replicates) with 100% methanol or 100% acetone, respectively, at 4 °C with constant shaking (300 rpm) for 20 h. Samples were centrifuged at 8800×*g* for 7 min. Supernatants were recovered and used for antioxidant activity measurements at 734 nm in a Beckman DU650 spectrophotometer (Beckman Coulter Ltd, High Wycombe, UK). Two different calibration curves were constructed using freshly prepared Trolox for hydrophilic and lipophilic antioxidant activity determinations. The linear reading of the standard curves was from 0 to 16 μM Trolox for both hydrophilic and lipophilic antioxidant activities. Values are expressed as μM of Trolox Equivalents (T.E.)/100 g dry weight.

2.13. Statistical analysis

Results are presented as the mean value ± standard deviation of three independent replicate experiments ($n = 3$). Statistical analysis was based on a one-way ANOVA test. Tukey's post hoc method was applied to establish significant differences between means ($p < 0.05$). All statistical comparisons were performed using SigmaStat version 11.0 software (Systat Software Inc., Chicago, IL).

3. Results and discussion

3.1. Main characteristics of the starting materials

The semolina used for pasta preparation showed good quality characteristics (Table 1). Yellow index and gluten quantity were particularly high, and above the average for Italian semolina quality in many surveys (Raffo et al., 2003, Pasqualone et al., 2004, Brescia et al., 2007). Gluten index was also very high and within the "best quality" category according to Cubadda, Carcea, and Pasqui (1992). Good values for gluten quantity and gluten index are expected to lead to excellent pasta in terms of consistency and cooking performances (D'Egidio et al., 1993). The ash content was within the limits of current regulations (Italian Republic, 2001).

Table 1. Main quality characteristics of semolina cv. Vertola used in the pasta-making trials. Data are given on dry weight (d.w.).

Parameter	Value
Ash (% d.w.)	0.83 ± 0.01
Dry gluten (% d.w.)	12.7 ± 0.2
Gluten index	91 ± 3
Yellow index	26 ± 1
LOX activity (EU g^{-1})[a]	0.062 ± 0.002

[a] *LOX = Linoleate:oxygen oxidoreductase (EC1.13.11.12); EU = enzyme unit.*

The very low LOX activity (0.062 ± 0.002 EU/g) indicated that the semolina was suitable for the addition of bioactive antioxidant compounds, without being affected excessively by endogenous enzymes.

The chemical composition of wheat bran oleoresin extracted by SC-CO_2 was as described previously in Durante, Lenucci, Rescio et al. (2012). Lutein was the most abundant carotenoid (4.1 ± 1.5 μg/g oleoresin), followed by β-carotene (1.9 ± 0.4 μg/g oleoresin) and zeaxanthin (1.6 ± 0.2 μg/g oleoresin), while tocotrienols were slightly more abundant (5.2 ± 1.5 mg/g oleoresin) than tocopherols (4.3 ± 0.7 mg/g oleoresin).

The wheat bran aqueous phenolic extracts contained 1.30 g/L total phenolic compounds, expressed as FAE, whereas the lyophilized tomato matrix was characterized by high lycopene content (12.4 ± 0.8 mg/g dw) accordingly with Lenucci et al. (2015). The latter was also rich in cell wall polysaccharides, mainly cellulose, pectins (homogalacturonan and rhamno-galacturonan)

and hemicelluloses (xyloglucans, xylans and mannans), which have a role as soluble and insoluble dietary fibers (Lenucci, Durante, Montefusco, Dalessandro, & Piro, 2013).

3.2. Physical properties of pasta

Table 2 reports the color characteristics, cooking performance, texture-related parameters, and sensory evaluation of control and supplemented pasta. Pasta color is an essential attribute that influences strongly consumer choice at purchase, bright yellow pasta being more appealing than discolored products. Marked shifts in color indices were observed among the samples, with the exception of BW pasta where no significant differences were observed with respect to control. The yellow index (b^*) was significantly higher in control and BW pasta than in the other samples. The red (a^*) and brown ($100 - L^*$) indices were significantly higher in 1.5LT and 2.5LT pasta than in all other samples, due to the reddish color of the lyophilized tomato matrix used for supplementation. However, no significant differences in red and brown indices were observed between 1.5LT and 2.5LT despite the different amounts of tomato added. BO pasta had significantly higher brown and red indices than control pasta, and a lower yellow index, reflecting the supplementation.

*Table 2. Color characteristics, cooking performances, viscoelastograph parameters, textural parameters and sensory evaluation of control and supplemented pasta (BO = pasta added of bran oleoresin; BW = pasta added of bran aqueous extract; 1.5LT = pasta added of 1.5% lyophilized tomato matrix; 2.5LT = pasta added of 2.5% lyophilized tomato matrix). Data are the mean ± standard deviation of three independent replicates (*n = 3*). Different letters in row indicate significant differences at* $p < 0.05$.

	Control	**BO**	**BW**	**1.5LT**	**2.5LT**
Color characteristics					
Yellow index (b^*)	$40.38^{a} \pm 1.33$	$35.56^{b} \pm 1.50$	$38.91^{a} \pm 1.16$	$18.08^{d} \pm 1.28$	$24.46^{c} \pm 2.90$
Red index (a^*)	$5.31^{c} \pm 0.18$	$6.69^{b} \pm 0.36$	$5.96^{c} \pm 0.51$	$23.86^{a} \pm 2.08$	$27.32^{a} \pm 3.37$
Brown index ($100 - L^*$)	$29.93^{c} \pm 1.33$	$36.01^{b} \pm 2.08$	$31.12^{c} \pm 1.30$	$54.59^{a} \pm 3.03$	$57.54^{a} \pm 2.45$
Cooking performances					
Cooking loss (%)	$3.2^{b} \pm 0.1$	$4.8^{a} \pm 0.3$	$3.3^{b} \pm 0.1$	$3.3^{b} \pm 0.3$	$3.2^{b} \pm 0.2$
Water absorption (%)	$109^{a} \pm 12$	$101^{a} \pm 11$	$113^{a} \pm 9$	$113^{a} \pm 10$	$110^{a} \pm 11$
Swelling index	$0.65^{a} \pm 0.01$	$0.62^{a} \pm 0.02$	$0.66^{a} \pm 0.01$	$0.67^{a} \pm 0.02$	$0.65^{a} \pm 0.01$
Viscoelastograph parameters					
Consistency (%)	$72.96^{a} \pm 1.01$	$70.96^{b} \pm 0.58$	$73.23^{a} \pm 1.17$	$73.63^{a} \pm 1.33$	$73.43^{a} \pm 1.28$
Elastic recovery (%)	$73.97^{ab} \pm 2.15$	$69.62^{c} \pm 1.78$	$72.22^{bc} \pm 1.95$	$74.61^{ab} \pm 1.17$	$77.78^{a} \pm 2.23$
Viscoelasticity index	$273.60^{a} \pm 10.44$	$239.71^{b} \pm 12.20$	$269.83^{a} \pm 13.10$	$279.11^{a} \pm 14.04$	$292.75^{a} \pm 13.13$
Textural parameters					
Hardness (N)	$20.9^{ab} \pm 0.4$	$20.1^{b} \pm 0.3$	$20.5^{ab} \pm 0.4$	$20.6^{ab} \pm 0.3$	$21.3^{a} \pm 0.5$
Springiness (-)	$0.67^{ab} \pm 0.11$	$0.60^{b} \pm 0.08$	$0.70^{ab} \pm 0.05$	$0.71^{ab} \pm 0.05$	$0.78^{a} \pm 0.08$
Cohesiveness (-)	$0.60^{a} \pm 0.02$	$0.51^{b} \pm 0.02$	$0.61^{a} \pm 0.02$	$0.59^{a} \pm 0.03$	$0.60^{a} \pm 0.02$
Chewiness (N)	$8.4^{b} \pm 0.3$	$6.2^{c} \pm 0.1$	$8.7^{b} \pm 0.2$	$8.6^{b} \pm 0.2$	$10.1^{a} \pm 0.3$

	Control	BO	BW	1.5LT	2.5LT
Sensory evaluation					
Bulkiness	$78^{a} \pm 5$	$72^{a} \pm 6$	$77^{a} \pm 5$	$74^{a} \pm 5$	$75^{a} \pm 5$
Stickiness	$78^{a} \pm 5$	$73^{a} \pm 6$	$76^{a} \pm 4$	$75^{a} \pm 5$	$76^{a} \pm 7$
Firmness	$79^{a} \pm 4$	$71^{a} \pm 5$	$77^{a} \pm 4$	$76^{a} \pm 7$	$76^{a} \pm 6$
Typical odor	$79^{a} \pm 4$	$78^{a} \pm 4$	$79^{a} \pm 4$	$43^{b} \pm 5$	$21^{c} \pm 4$
Typical taste	$78^{a} \pm 5$	$76^{a} \pm 4$	$77^{a} \pm 5$	$41^{b} \pm 4$	$23^{c} \pm 5$
Overall judgment	$79^{a} \pm 2$	$74^{a} \pm 3$	$78^{a} \pm 3$	$62^{b} \pm 3$	$54^{c} \pm 3$

As far as cooking performances are concerned, good quality pasta is characterized by minimal cooking losses and stickiness, and high firmness and springiness (Sozer, Dalgıç, & Kaya, 2007). These characteristics are important factors affecting consumer acceptance and product quality. The cooking loss, due to gelatinized starch leaching into the cooking water, was significantly higher in BO pasta than in the other samples, including control pasta, indicating a reduced ability of the BO pasta gluten network to retain gelatinized starch. The non-polar nature of bran oleoresin added to the dough probably interferes with semolina hydration, which is essential for proper formation of the gluten network, resulting in a less compact protein matrix than control. All other supplemented pasta has cooking losses similar to the control. Water absorption and swelling index, related to the hydration degree and tolerance of pasta to cooking, did not show significant differences among samples.

As far as viscoelastograph parameters were concerned, the BW, 1.5LT and 2.5LT supplemented pasta showed consistency values similar to those of the control while BO pasta was significantly less consistent and elastic, which are also related to interference by oleoresin with formation of the gluten network. The elastic recovery of 2.5LT pasta was higher than in the other supplemented samples. The viscoelasticity index was significantly lower in BO pasta than in control, in agreement with cooking loss data. Overall, the observed viscoelastograph parameters were in agreement with those reported for high-temperature dried pasta (D'Egidio et al., 1993).

Textural profile analysis confirmed the viscoelastograph data and added other information. In particular, hardness and springiness, the elastic recovery that occurs when a compressive force is removed, were significantly higher in 2.5LT than in BO pasta, but the differences observed were not statistically significant with respect to the control. Cohesiveness, which is related to the force of internal bonds holding the pasta structure and indicates how the sample holds together upon cooking (Sissons, Egan, Alexander, & Batey, 2006), was lower in BO-supplemented pasta than in all other samples. Chewiness, defined as the effort required to masticate spaghetti to the point of swallowing (Sissons et al., 2006), best distinguished differences among pasta types. The 2.5LT pasta has the highest chewiness followed by BW and 1.5LT pasta, both similar to control. BO pasta exhibited the lowest chewiness. The different chewing behavior of LT- and BO-supplemented pasta is probably due to the polarity of the two components, with the non-polar bran oleoresin interfering with gluten formation and the polar, fiber-rich, lyophilized tomato matrix having a positive impact on chewiness.

The sensory descriptors related to pasta structure and texture, such as bulkiness, stickiness, and firmness received high scores (Table 2) due to a high gluten content and quality of the semolina, coupled with the adoption of high-temperature pasta drying technology. Although these parameters scored less in BO than in other pasta, the differences were not statistically significant. According to Sissons et al. (2006) instrumental tests are more discriminating than sensory analysis in evaluating textural features, thus the moderate differences observed in TPA parameters were not expected to affect the sensory evaluation. Odor and taste received markedly lower scores in 1.5LT and 2.5LT pasta than in control, BO, and BW pasta. In particular, these parameters were worst in 2.5LT pasta compared with 1.5LT pasta. Hence, increasing lyophilized tomato progressively altered both odor

and taste of the end product, adding a sharp tomato note without any other defect (such as sour or rancid notes). The low scores for taste and smell negatively influenced the overall sensory judgment of 1.5LT and 2.5LT pasta, the latter being lowest in sensory judgment.

3.3. SEM observations of raw and cooked pasta

SEM observations of cross sections of raw control (C), BW and 2.5LT pasta (Fig. 1i) revealed a well developed protein matrix with starch granules trapped and embedded in the gluten network. Few imprints of missing starch granules (starch shadows) were also evident especially in C, BW and 2.5LT samples. Small cracks were present in the protein-starch matrix of the same samples. They might have been due to shrinkage during sample preparation or tension within the pasta dough during drying. A more homogeneous and compact structure was observed in raw BO pasta (Fig. 1i), where starch granules were deeply embedded in a protein and oleoresin matrix and difficult to distinguish. The presence of cracks was reduced, probably due to the lubricant action of oleoresin during extrusion and pasta drying processes. The structural differences observed in raw pasta were more evident after cooking (Fig. 1ii). C and BW pasta showed similar swollen starch granules embedded in a complex filamentous and porous protein network. In 2.5LT pasta, swollen starch granules were completely surrounded by and covered with an amorphous matrix, indicating that the presence of the lyophilized tomato powder did not interfere with gluten organization. Irregular particles of different diameter, likely tomato matrix particles, were also embedded in the protein network and might be the cause of increased chewiness observed in 2.5LT pasta. A less structured protein network with less swollen starch granules characterized BO pasta. Swelling of the starch appeared reduced, probably due to the hydrophobic nature of the bran oleoresin, which might interfere with pasta hydration during cooking. Oleoresin components (e.g. charged lipids, mono- and diglycerides) might also act as emulsifiers forming complexes with amylose during cooking, leading to reduced starch swelling according to Bustos, Perez, and Leon (2015). Thus, increased cooking losses observed in BO pasta, as well as lower cohesiveness and chewiness, might result from a balance between the reduced starch swelling and the leaching of gelatinized starch in the cooking water due to altered formation of the gluten network.

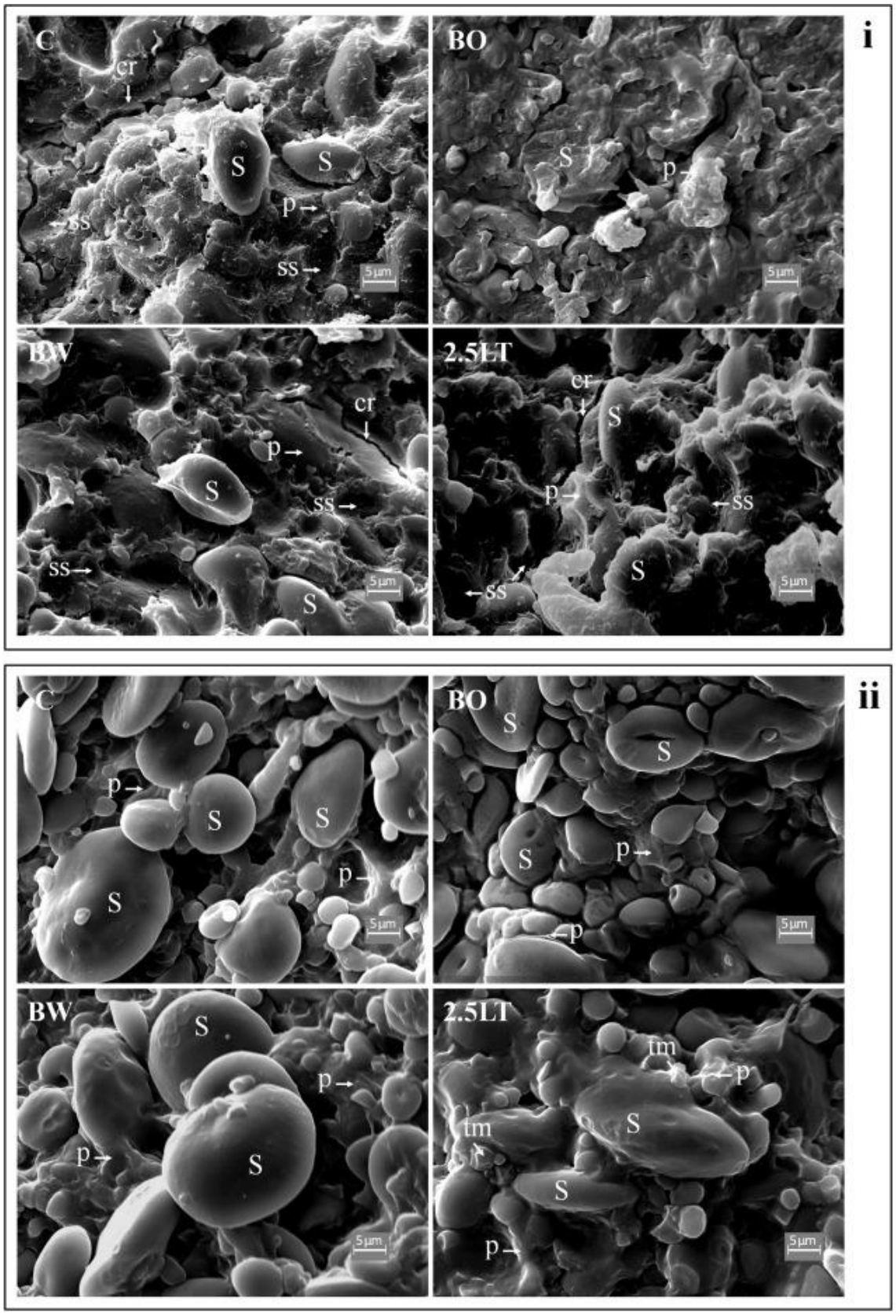

Fig. 1. SEM micrographs of cross sections of raw (i) and cooked (ii) pasta. C, control pasta; BO, pasta supplemented of bran oleoresin; BW, pasta supplemented of bran aqueous extract; 2.5LT, pasta supplemented of 2.5% lyophilized tomato matrix. S, starch granules; ss, starch shadows; p, protein network; cr, craks; tm, tomato matrix granules. Magnification 5000×.

3.4. Bioactive compounds and antioxidant activity of pasta

The analysis of bioactive compounds confirmed the effectiveness of supplementations. Isoprenoid profiles of raw BO and control pasta, analyzed by HPLC, were markedly different (Table 3). An increase of about 2.4-, 21-, and 3.3-times in the content of tocotrienols, tocopherols, and total carotenoids, respectively, was observed in BO pasta compared to control. Among the carotenoids, β-carotene (15.3 ± 1.2 μg/100 g pasta f.w.) and zeaxanthin (14.5 ± 0.8 μg/100 g pasta f.w.) were the most abundant followed by lutein (7.2 ± 1.7 μg/100 g pasta f.w.). The modest increase in BO pasta lutein content, in spite of its abundance in the extract, confirmed the higher susceptibility of lutein to degradation than other carotenoids during pasta preparation, as previously reported by Hidalgo, Brandolini, and Pompei (2010). A 15–20% reduction in the amounts of isoprenoids was registered after cooking (Table 3). The amounts of carotenoids in control pasta agreed with the ranges reported in literature (Hidalgo et al., 2010).

*Table 3. Tocochromanol and carotenoid profiles of control and bran oleoresin (BO) supplemented pasta. Data of both raw and cooked pasta are referred to 100 g raw pasta fresh weight (f.w.) and are the mean ± standard deviation of three independent replicates (*n = *3). Different letters in row indicate significant differences at* $p < 0.05$.

	Control		**BO**	
	Raw	**Cooked**	**Raw**	**Cooked**
Tocochromanols[a] (μg/100 g pasta f.w.)				
δ-T3	$726^{a} \pm 68$	$596^{a} \pm 88$	$1308^{b} \pm 111$	$1099^{b} \pm 124$
γ-T3	$44^{a} \pm 6$	$36^{a} \pm 9$	$188^{b} \pm 18$	$160^{b} \pm 22$
α-T3	$47^{a} \pm 9$	$40^{a} \pm 9$	$486^{b} \pm 65$	$418^{b} \pm 71$
β-T	$14^{a} \pm 7$	$12^{a} \pm 8$	$240^{b} \pm 8$	$201^{c} \pm 11$
α-T	$13^{a} \pm 3$	$11^{a} \pm 5$	$329^{b} \pm 24$	$280^{c} \pm 29$
Total	*$844^{a} \pm 93$*	*$694^{a} \pm 119$*	*$2551^{b} \pm 226$*	*$2158^{b} \pm 257$*
Carotenoids (μg/100 g pasta f.w.)				
Lutein	$5.2^{a} \pm 0.3$	$3.9^{a} \pm 0.5$	$7.2^{b} \pm 0.7$	$5.4^{a} \pm 0.9$
Zeaxanthin	$2.5^{a} \pm 0.3$	$2.0^{a} \pm 0.3$	$14.5^{b} \pm 0.8$	$12.2^{c} \pm 1.4$
β-criptoxanthin	$0.3^{a} \pm 0.0$	$0.2^{a} \pm 0.1$	$1.4^{b} \pm 0.1$	$1.2^{b} \pm 0.3$
α-carotene	$0.5^{a} \pm 0.0$	$0.4^{a} \pm 0.2$	$1.8^{b} \pm 0.2$	$1.4^{c} \pm 0.3$
β-carotene	$3.7^{a} \pm 0.2$	$3.2^{a} \pm 0.5$	$15.3^{b} \pm 1.2$	$12.3^{c} \pm 1.9$
Total	*$12.2^{a} \pm 0.8$*	*$9.7^{a} \pm 1.6$*	*$40.2^{b} \pm 2.1$*	*$32.5^{c} \pm 4.8$*

[a]Tocochromanol's form abbreviations are as follows: α-T3 = α-tocotrienol; γ-T3 = γ-tocotrienol; δ-T3 = δ-tocotrienol; α-T = α -tocopherol; β-T = β-tocopherol.

The content of lycopene and total phenolic compounds in control BW and LT pasta is shown in Table 4. Both LT raw pasta were rich in lycopene (11.9 ± 0.6 mg/100 g f.w. in 1.5LT pasta and 20.0 ± 0.6 mg/100 g f.w. in 2.5LT pasta). A 23–27% loss of lycopene was registered during pasta cooking (data not shown), but 70 g of 1.5LT and 2.5LT cooked pasta provided about 84% and 156%, respectively, of the daily dosage for lycopene for adults suggested by scientific literature (5–10 mg/day, mean value 7.5) (Rao & Shen, 2002). The level of total phenolic compounds of BW pasta accounted for 127 ± 1 mg/100 g pasta f.w. as FAE, whereas in control pasta f.w. 97 ± 1 mg/100 g

was detected. Phenolic compounds in control and BO pasta were within ranges reported in literature for whole-wheat and conventional pasta (Hirawan, Ser, Arntfield, & Beta, 2010).

*Table 4. Lycopene and total phenolic compounds of pasta supplemented with bran aqueous extract (BW), or lyophilized tomato matrix at 1.5% (1.5LT) and 2.5% (2.5LT) level, compared to control pasta. Data are given on fresh weight (f.w.) and are the mean ± standard deviation of three independent replicates (*n *= 3). Different letters in row indicate significant differences at* $p < 0.05$.

	Control	BW	1.5LT	2.5LT
Lycopene (mg/100 g pasta f.w.)	–	n.d.	$11.9^{a} \pm 0.6$	$20.0^{b} \pm 0.6$
Total phenolic compounds (mg FAE/100 g pasta f.w.)[a]	$97^{a} \pm 1$	$127^{b} \pm 1$	n.d.	n.d.

[a]*FAE = Ferulic Acid Equivalents; n.d. = not determined.*

An increase of antioxidant activity is one of the main aims of pasta supplementation (Boroski et al., 2011, Day et al., 2009, Sęczyk et al., 2016, Torres et al., 2007). The hydrophilic and lipophilic antioxidant activities (HAA and LAA) of control, BO, BW, and both LT pasta types were evaluated on raw and cooked samples (Table 5). BO pasta showed the highest HAA and LAA values, 3.3- and 10-times those of control, respectively. In contrast, no significant differences were found between BW raw pasta and control with respect to HAA and LAA, evidencing the need for achieving further enrichment of the BW extract, possibly by including a concentration step. The HAA values of 1.5LT and 2.5LT pasta were both higher than control, without significant differences between the two levels of enrichment, while only LAA in 2.5LT pasta was more higher than the control. Similar increases have been reported by other authors, using various enrichment strategies: the antioxidant activity of pasta was doubled with the addition of 0.5% black carrot concentrate (Day et al., 2009), whilst increases of 18- and 3-fold in antiradical activity and reducing power, respectively, were observed for pasta fortified with 5% of carob flour (Sęczyk et al., 2016).

*Table 5. Hydrophilic antioxidant activity (HAA) and lipophilic antioxidant activity (LAA) of control and supplemented pasta (BO = pasta added of bran oleoresin; BW = pasta added of bran aqueous extract; 1.5LT = pasta added of 1.5% lyophilized tomato matrix; 2.5LT = pasta added of 2.5% lyophilized tomato matrix). Data are expressed as µmol 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) Equivalents (T.E.)/g pasta on dry weight (d.w.) and represent the mean ± standard deviation of three independent replicates (*n *= 3). Different letters in row indicate significant differences at* $p < 0.05$.

	Control		BO		BW		1.5LT		2.5 LT	
	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked
HAA (µmol T.E./g pasta d.w.)	$1.2^{fg} \pm 0.1$	$1.1^{fg} \pm 0.2$	$4.0^{a} \pm 0.2$	$2.0^{cd} \pm 0.1$	$1.5^{ef} \pm 0.2$	$0.9^{g} \pm 0.3$	$2.2^{bc} \pm 0.1$	$1.8^{de} \pm 0.1$	$2.5^{b} \pm 0.1$	$2.3^{bc} \pm 0.1$
LAA (µmol T.E./g pasta d.w.)	$0.3^{de} \pm 0.1$	$0.3^{de} \pm 0.1$	$3.0^{a} \pm 0.2$	$2.9^{a} \pm 0.2$	$0.2^{e} \pm 0.1$	$0.2^{e} \pm 0.1$	$0.6^{cd} \pm 0.1$	$0.9^{c} \pm 0.1$	$0.9^{c} \pm 0.1$	$1.6^{b} \pm 0.1$

Cooking was associated with a negligible decrease in HAA in most samples, with the exception of BO and BW pasta were it was approximately halved. LAA was not affected by cooking in control, BO and BW pasta, while a significant increase was observed in both LT pasta. It is likely that this is related to partial dissolution of lycopene crystals during cooking, which increased solubility in the solvents used for LAA determination, or to formation of lipophilic degradation products with higher antioxidant activity than lycopene.

4. Conclusions

Overall, the technological features of the proposed supplemented pasta types were good, due to high gluten content of the starting semolina and adoption of high-temperature pasta drying technology. BO pasta, characterized by the highest HAA and LAA values, had different physical characteristics to the control when evaluated instrumentally, although within the normal quality variation of commercial pasta products on the market (Sissons et al., 2006). SEM observed few ultrastructural differences in control and supplemented pasta. In BO pasta, reduced swelling of starch granules was observed, probably due to the lipophilic nature of the extract. Moreover, the extent of these differences in physical and ultrastructural features was moderate, and did not affect the sensory evaluation. Based on the sensory and antioxidant activity data, BO supplementation was best at increasing antioxidant activity without jeopardizing pasta quality. Even the non-conventional taste and odor of 1.5LT and 2.5LT pasta, with good HAA and LAA values, might be accepted by consumers, if communicated appropriately. The BW supplementation would need further research to increase the concentration of phenolic compounds and improve significantly the *in vitro* antioxidant activity of the end product.

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