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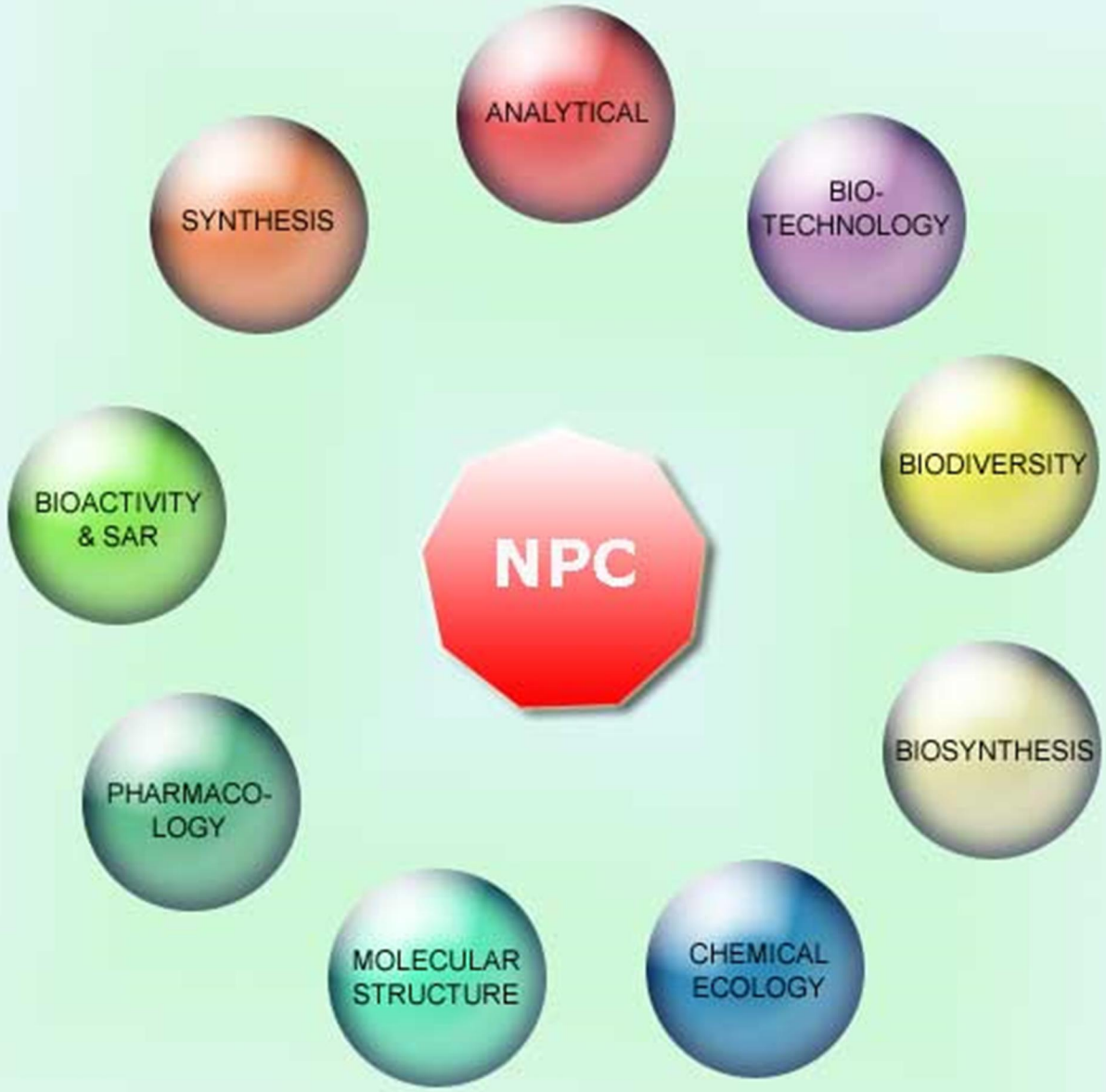

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Effect of Supplementation with Wheat Bran Aqueous Extracts Obtained by Ultrasound-Assisted Technologies on the Sensory Properties and the Antioxidant Activity of Dry Pasta

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Phenolic compounds have antioxidant properties and activate endogenous detoxification defense systems able to scavenge the reactive species of oxygen. The external layers of wheat caryopsis, largely constituting by-products of the milling industry such as bran and various middlings, contain relevant amounts of phenolic compounds. The aim of the research has been to evaluate the effect of supplementation with wheat bran aqueous extracts, obtained by ultrasound-assisted technologies, on the sensory properties and antioxidant activity of dry pasta. The HPLC-DAD characterization of the extract evidenced the presence of ferulic and *p*-coumaric acids. The supplemented pasta showed significantly higher antioxidant activity and phenolic content than the control, coupled to good overall sensory judgment. In addition, two different pasta drying diagrams were adopted, and the comparison of the corresponding end-products allowed it to be pointed out that the processing technology has to be carefully set up to prevent possible detrimental effects on the antioxidant activity. The proposed utilization of bran might add value to a milling by-product that, otherwise, is mostly employed in animal feeding.

Keywords: Phenolic extracts, Ultrasound, Pasta, Bran, Functional foods.

In recent years the role played by reactive oxygen species in many diseases related to aging has been extensively reported [1]. Functional foods, providing health benefits beyond basic nutrition [2], are the object of increasing interest by both producers and health-conscious consumers. In particular, redox and antioxidant systems are among the most addressed targets in functional food science.

Phenolic compounds have been demonstrated to have clear antioxidant properties *in vitro* and *in vivo* [3]. The consumption of phenolics may hence contribute in preventing diseases related to aging [4,5]. The ascertained toxicity of synthetic phenolic antioxidants, such as butylated hydroxy anisole and butylated hydroxy toluene [6,7], focused attention towards the phenolic compounds commonly found in natural sources such as fruits, vegetables, and cereals [8]. In particular, the external layers of wheat caryopsis, largely constituting by-products of the milling industry such as bran and various middlings, contain relevant amounts of phenolic compounds [9-11]. These by-products are commonly destined for animal feeding. Alternatively, they are used in whole wheat bread- and pasta-making to an increasing, but still marginal, extent. However, the textural features of the end-product, such as pasta firmness and elasticity, are negatively affected by fiber [12,13], and an increase of cooking loss, swelling index, and water absorption has been reported [14].

The enrichment of fresh pasta with bran phenolic extracts, after preliminary KOH-induced hydrolysis, affects dough consistency and pasta taste [15]. Ultrasound-assisted technology, an environmentally-friendly system for extracting bioactive compounds from natural sources [16], could be exploited to recover phenolic compounds from bran. The aim of this research has been to evaluate the effect of pasta supplementation with wheat bran aqueous extract, obtained by ultrasound-assisted technologies, on the sensory properties and antioxidant activity of the end-product.

The bran aqueous extract was submitted to quali-quantitative characterization by HPLC-DAD (Table 1). The most represented compound was ferulic acid, as already observed by other authors [17,18], followed by *p*-coumaric acid. None of the other phenolic acids usually found in wheat was detected. Higher levels of phenolics have been reported in the literature for extractions carried out by acid or basic hydrolysis of bran followed, after neutralization, by methanol or ethyl acetate extraction [17,19], but these methods are not suitable for food supplementation purposes.

Table 1: HPLC-DAD quali-quantitative characterization of aqueous extract obtained from wheat bran by means of ultrasound-assisted technologies.

Phenolic acid	Content(mg g^{-1}d.m.)
p-Hydroxy benzoic acid	n.d.
Vanillic acid	n.d.
Syringic acid	n.d.
Sinapic acid	n.d.
Ferulic acid	0.31±0.01
p-Coumaric acid	0.25±0.01
o-Coumaric acid	n.d.

n.d. = not detected.

The semolina used in the trials showed good pasta-making features (Table 2). The gluten index was in the highest strength category (>80) [20], ensuring good pasta consistency and resistance to overcooking [21]. The yellow index was above the ranges previously observed in quality surveys of Southern Italian semolina [22-24]. The bright yellow color, due to carotenoid pigments, is typical of semolina and is partly transferred to the end-product. The ash content fell within the limits of the current rules [25].

Table 2: Main quality characteristics of semolina used in the pasta-making trials.

Parameter	Value
Ash (% d.m.)	0.83±0.01
Dry gluten (% d.m.)	12.7±0.2
Gluten index	91±3
Yellow index	26.3±1.0

Semolina was processed with either aqueous bran extract (supplemented pasta) or water (control) into 'mezzi rigatoni'-shaped pasta, and dried according to two different drying diagrams (Fig. 1). The content of phenolic substances of the supplemented pasta was in the range reported for whole meal pasta [26] and was significantly higher in both the supplemented types than in the corresponding controls. The antioxidant activity of supplemented pasta was similar to the levels reported in soft wheat whole meal or in pigmented wheat [27,28], and was higher than the control only when the HT1 drying diagram was adopted. This result was probably due to the more severe thermal effect of the HT2 than HT1 process.

Table 3: Content of phenolic compounds and antioxidant activity of pasta supplemented with aqueous bran extract and of control pasta. Both pasta types were dried according to two different drying programs, HT1 and HT2, reported in Fig. 1.

Pasta type	Antioxidant activity		Total soluble phenolic compounds
	µmol Trolox g-1 (d.m.)	DPPH SC%	mg FAE g-1 (d.m.)
Control-HT1	0.42±0.003^{b}	10.62±0.42^{b}	1.03±0.01^{b}
Supplemented-HT1	0.49±0.002^{a}	12.77±0.13^{a}	1.16±0.01^{a}
Control-HT2	0.41±0.004^{b}	10.17±0.64^{b}	1.01±0.05^{b}
Supplemented-HT2	0.45±0.002^{ab}	11.56±0.23^{ab}	1.11±0.03^{a}

Different letters in the same column indicate significant differences at $p< 0.05$;DPPH SC% = 2,2-diphenyl-1-picrylhydrazyl scavenging capacity percentage; Trolox = 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

Good cooking quality performances of pasta include low stickiness, and high firmness and elasticity. These features can be evaluated either by a texture analyzer or by sensory evaluation, the latter approach being more similar to the final consumer evaluation than the former. The sensory analysis of supplemented and control pasta was performed to evaluate 15 descriptors, defined in Table 4, so as to express an overall judgment. The addition of bran extract did not cause significant detrimental effects on textural characteristics, such as stickiness, elasticity, chewiness, and resistance to cutting (Table 5). Moreover, the supplemented pasta interestingly showed higher scores for the firmness sensory descriptor than control pasta. In addition, pasta color did not show significant differences between supplemented and control trials.

As far as taste was concerned, sweet and salty descriptors did not show differences by comparing supplemented pasta with the corresponding controls, whereas a slightly greater astringent note was perceived in pasta to which the bran extracts had been added, but without significant differences with the control. Regarding odor and flavor descriptors, no significant differences were observed. As a consequence, the overall evaluation was similar for supplemented pasta and control.

These findings demonstrated the effectiveness of the strategy of transferring phenolic compounds from bran to pasta by means of an aqueous extract, without encountering the typical drawbacks of whole meal pasta-making due to fiber interference with the gluten network [14]. The supplementation of pasta with aqueous phenolic extracts obtained from durum wheat bran by ultrasound-assisted technologies allowed us to obtain pasta with higher antioxidant activity than the control, coupled to good sensory properties. In addition, the proposed utilization of bran might valorize a milling by-product that otherwise is essentially employed in animal feeding.

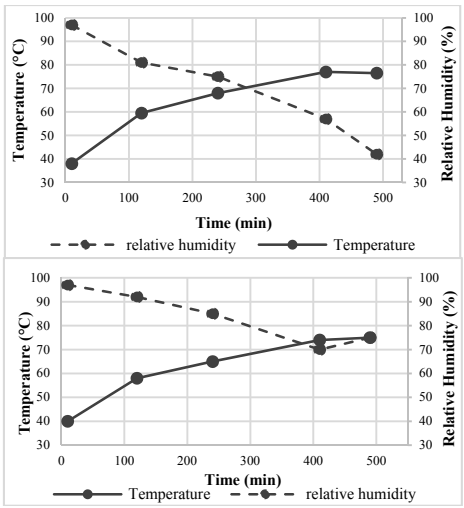

Figure 1: Variations of temperature and relative humidity in the drier during HT1 (top) and HT2 (bottom) pasta drying programs.

Table 4: Descriptors selected to describe the sensory properties of pasta.

Descriptor	Definition
Color	Intensity of amber-yellow color
Stickiness	Degree of adherence of pasta to the teeth (while chewing) and to the palate (by pressing a pasta piece onto palate, then removing it by the tongue)
Elasticity	Degree of recovery evaluated by stretching (spaghetti) or bending (other types) a pasta piece
Resistance to cutting	Resistance of pasta to being cut by a fork
Firmness	Degree of resistance while compressing a pasta piece by fingers
Odor of semolina	Aroma characteristic of durum wheat semolina perceived by orthonasal olfaction
Odor of cooked pasta	Aroma characteristic of cooked pasta perceived by orthonasal olfaction
Off-odor	Anomalous odor perceived by orthonasal olfaction
Flavor of semolina	Retronasal aroma characteristic of semolina perceived when pasta is in the mouth
Flavor of cooked pasta	Retronasal aroma characteristic of cooked pasta perceived when pasta is inthe mouth
Off-flavor	Retronasal anomalous aroma perceived when pasta is in the mouth
Sweet taste	Taste of sucrose perceived in the mouth while chewing
Salty taste	Taste of sodium chloride perceived in the mouth while chewing
Astringency	Sensation of dryness and coarseness perceived in the mouth while chewing
Chewiness	Duration and number of chews needed before swallowing pasta
Overall judgment	Overall evaluation of pasta acceptability

It has to be remarked that the differences in the levels of antioxidant activity and phenolic compounds between supplemented pasta and control, although being statistically significant, were modest. However, these results can be considered satisfactory because the sensory properties of the end-product were not altered. Moreover, the typically high frequency of pasta consumption in the Italian diet could ensure beneficial health effects even for slight augmentations of pasta antioxidant activity. Finally, the comparison of two different drying diagrams allowed it to be pointed out that the processing technology has to be carefully set up to prevent possible detrimental effects on the antioxidant activity of pasta.

Experimental

Chemicals and reagents: Authentic standards of phenolic acids (*p*-hydroxybenzoic acid, vanillic acid, syringic acid, *p*-coumaric acid,

o-coumaric acid, sinapic acid, and ferulic acid), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), and Folin-Ciocalteu reagent, acetonitrile, and phosphoric acid from Carlo Erba Reagent (Milan, Italy).

Ultrasound-assisted production of bran aqueous extract: Durum wheat bran (3.5 kg) was mixed with tap water (35 L) and the suspension was submitted to ultrasound-assisted extraction at 20°C for 25 min in a pilot plant assembled by Weal (Milano, Italy) whose working principle and details are reported in Punzi *et al.* [29], then the suspension was filtered through a metal grid with 1 mm holes to recover the liquid phase. About 25 L of aqueous extract was obtained that was used immediately after its production.

Table 5: Sensory properties of pasta supplemented with aqueous bran extract and of control pasta. Both pasta types were dried according to two different drying programs, HT1 and HT2, as reported in Fig. 1.

Sensory descriptor	Pasta type			
	Control-HT1	Supplemented-HT1	Control-HT2	Supplemented-HT2
Color	4.75±1.00^{a}	4.66±1.29^{a}	4.50±1.27^{a}	3.83±1.92^{a}
Stickiness	5.00±2.33^{a}	5.33±1.21^{a}	4.83±1.60^{a}	4.75±2.23^{a}
Elasticity	6.25±1.73^{a}	6.83±1.48^{a}	5.91±1.50^{a}	6.33±1.03^{a}
Resistance to cutting	6.00±1.34^{a}	6.66±1.95^{a}	6.83±1.29^{a}	6.75±1.78^{a}
Firmness	4.50±1.75^{a}	6.08±1.78^{b}	3.83±1.95^{a}	6.00±2.11^{b}
Semolina odor	6.58±1.50^{a}	6.66±1.44^{a}	6.33±1.31^{a}	6.41±1.50^{a}
Cooked pasta odor	5.75±1.88^{a}	6.00±2.22^{a}	5.08±1.29^{a}	6.00±2.00^{a}
Off-odor	2.08±1.19^{a}	2.00±1.98^{a}	1.83±1.30^{a}	1.66±1.95^{a}
Sweet	4.50±1.24^{a}	4.33±2.28^{a}	4.91±1.56^{a}	4.91±1.97^{a}
Salty	2.08±1.40^{a}	2.75±1.73^{a}	2.16±1.22^{a}	2.25±2.30^{a}
Astringency	3.41±1.82^{a}	4.25±2.02^{a}	3.25±2.11^{a}	3.91±2.90^{a}
Chewiness	5.91±2.11^{a}	5.25±1.66^{a}	5.33±2.07^{a}	5.25±2.00^{a}
Semolina flavor	6.25±1.56^{a}	5.33±1.76^{a}	6.41±1.44^{a}	5.66±1.97^{a}
Cooked pasta flavor	4.50±1.71^{a}	4.33±1.23^{a}	5.00±1.68^{a}	5.08±1.50^{a}
Off-flavor	2.16±1.86^{ab}	3.41±1.53^{b}	1.75±2.21^{a}	2.16±2.39^{ab}
Overall judgment	5.50±1.09^{a}	4.58±1.17^{a}	5.50±1.51^{a}	5.41±1.44^{a}

Different letters in the same row indicate significant differences at $p< 0.05$.

Pasta production: Durum wheat (*Triticum durum* Desf.) cv 'Vertola' was used to perform the experiments. Grains were milled to semolina by means of a MLU 202 mill (Buhler, Uzwil, Switzerland), after conditioning at 17.5% moisture. Semolina was added to either the aqueous bran extract at 30% level (on semolina weight basis) to produce supplemented pasta, or 30% water, for control pasta. Specifically, about 5.5 kg semolina was kneaded with either water or aqueous bran extract for 15 min by using a MAC 60 VR 1-bar vacuum extruder (Italpast, Fidenza, Italy). The dough, extruded through a Teflon die, was shaped into 'mezzi rigatoni' having a length of 18.60±1.20 mm, diameter 6.66±0.16 mm, and thickness 1.00±0.08 mm. Then, each pasta type was divided into 2 batches and dried in a static dryer (LAB, Namad Impianti, Rome, Italy) according to 2diverse high temperature (HT) drying programs (coded HT1 and HT2; reported in Fig. 1), both reaching the maximum temperature of 78°C but involving different variations of relative humidity (RH).

HPLC quali-quantitative analysis of bran aqueous extract: Quantitative measurements of individual phenolic acids were achieved using an Agilent 1100 (Santa Clara, CA, USA) liquid chromatograph equipped with a photodiode array detector (DAD). Wavelengths of 280, 295 and 320 nm were used for quantification of the phenolic acids. Phenolic acid separation was achieved by using a Phenomenex-luna (Macclesfield, UK) 5 µm C18 100 Å column (250 × 4.6 mm) and a column temperature of 30°C. The flow rate of the mobile phase was 1.0 mL/min, and the injection volume was 20 µL. A gradient-elution program was utilized with a mobile phase consisting of acetonitrile (solution A) and 1%, v/v, phosphoric acid in water (solution B) as follows: isocratic elution, 100% B, 0-30 min; linear gradient from 100% B to 85% B, 30-55 min; linear gradient from 85% B to 50% B, 55-80 min; linear gradient from 50% B to 30% B, 80-82 min; post time, 10 min before the next injection. The flow rate of the mobile phase was 1.0 mL/min, and the injection volume was 20 µL. Phenolic acids were quantified using calibration curves of phenolic acid standards (previously prepared as stock solutions at 2 mg mL^{-1} in 80:20, v/v, ethanol/water and stored in the dark at -18°C, where it remained stable for over 3 months). The analyses were carried out in triplicate.

Basic chemical and physical determinations of semolina and pasta: Semolina was submitted to the determination of ash content according to the AACC method 08-12 [30]. Dry gluten and gluten index were determined as in the AACC method 38-12.02 [30]. Yellow index (corresponding to *b**) was determined by means of the reflectance colorimeter Chroma Meter CR-300 (Konica Minolta Sensing, Osaka, Japan).

Quantification of total phenolic compounds and antioxidant activity of pasta: The total phenolic compounds (specifically the fraction composed of soluble free phenolic acids and soluble phenolics bound to low molecular mass components) and the antioxidant activity, assessed by the DPPH radical scavenging capacity assay, were determined as in Pasqualone *et al*. [31]. All tests were carried out in triplicate.

Sensory analysis: Pasta was cooked in boiling distilled water at 1:10, w/v, pasta to water ratio, without the addition of salt. Optimum cooking time was determined according to the AACC Method 16-50 [30]. After cooking and draining, the samples were allowed to rest for 5 min. Subsequent analysis was carried out according to the UNI method 10957 [32], by a trained panel of 12 assessors, previously selected over a group of 16 people for their reliability, consistency, and discriminating ability. After a cycle of lectures on the basic notions of sensory analysis, the selection involved a series of tests to monitor the performance in recognizing the basic tastes, odors, flavors, and colors. Prior to actual sample evaluation, the selected panelists were trained via six pre-test sessions held to (i) familiarize them with the vocabulary regarding the descriptors of pasta, (ii) define the list of sensory terms to be evaluated in the samples studied, (iii) establish the order of their testing, (iv) define their intensity range, and (v) fix their scale anchors. Samples similar to those objects of the study were used in this phase. The list of sensory terms included descriptors of visual appearance (color), texture (stickiness, elasticity, resistance to cutting, firmness, chewiness), tactile in mouth (astringency), odor and flavor (semolina, cooked pasta, off-odor and off-flavor), taste (salty, sweet) and an overall judgment, defined in Table 4. They were rated on an anchored line scale that provided a 1-9 score range. The samples, presented in white dishes coded using a three-digit number, were distributed simultaneously and anonymously to all panel members. Evaluations were led in single boxes and two sensory testing sessions were carried out. The order of presentation was randomized among judges and sessions. All data were acquired by a direct computerized registration system (FIZZ Biosystemes. ver. 2.00 M, Couternon, France).

Statistical analysis: Data were submitted to one-way analysis of variance (ANOVA) by XLStat software (Addinsoft SARL, New York, NY, USA).

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