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Development of Functional Spaghetti Enriched in Bioactive Compounds Using Barley Coarse Fraction Obtained by Air Classification

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ABSTRACT: Barley byproducts obtained by air classification have been used to produce a different barley functional spaghetti, which were compared to different commercial whole semolina samples. Total, insoluble, and soluble fiber and β-glucan contents of the barley spaghetti were found to be greater than those of commercial samples. Furthermore, it was proved that barley spaghetti reached the FDA requirements, which could allow these pastas to deserve the health claims "good source of dietary fiber" and "may reduce the risk of heart disease". When the barley coarse fraction was used, a flavan-3-ols enrichment and an increase of antioxidant activity were reported, while commercial samples showed the absence of flavan-3-ols and a higher presence of phenolic acids and tannins. Whole semolina commercial spaghetti had a significantly higher content of phenolic acids than semolina spaghetti samples. Besides, it was observed that when vital gluten was added to the spaghetti formulation, phenolic compounds were blocked in the gluten network and were partially released during the cooking process.

KEYWORDS: Barley byproduct, spaghetti, phenolic compounds, capillary electrophoresis, dietary fiber, β-glucans

INTRODUCTION

Whole grains are rich in a wide range of compounds with known health benefits. Whole-grain products have a high content of dietary fiber, certain vitamins, minerals, and phytochemicals, and there is much published research[1−3] that supports a protective role for these compounds. Nevertheless, it is important to take into account that in the grain-refining process, the bran is removed, resulting in a loss of dietary fiber, vitamins, minerals, lignans, phyto-estrogens, and phenolic compounds. Despite that, cereal fiber and whole-grain intakes have been consistently associated in the epidemiological literature with reduced mortality and risk of chronic disease including obesity, cardiovascular disease (CVD), and type 2 diabetes.[4]

The excellent nutritional value of minor cereals is quite well-known, but the presence of minor components with functional properties has been poorly investigated. For this reason, the research on the functional properties of minor cereals focused on the development of new products with functional effects becomes of great interest for the food industry.[5]

Barley grain is an excellent source of healthy dietary fiber[6] and other bioactive constituents, such as vitamin E, including tocotrienols,[7] B complex vitamins, minerals, and phenolic compounds,[8] and consequently, it is gaining renewed interest as an ingredient for the production of functional foods.

In barley, as in most cereals, phenolic compounds are concentrated mainly in the bran. Normally, the bran is removed, but in recent years, significant changes to milling technology have occurred. To this end, in the last years, food researchers and the food industry have been investigating the possibility of producing barley flours naturally enriched in bioactive compounds. Some authors[7,9] have studied the separation of different fractions of whole barley flour by pearling and have demonstrated that the outermost fractions yielded the highest phenolic content. Pearled barley fractions are suitable for use in many food preparations, including different types of bread[10] and pasta.[11]

Recently, air classification has been used as an effective way to separate materials into fractions with different sizes, properties, and different contents in chemical components.[12] Different studies[13,14] have been developed about the possibility of producing barley flours enriched in β-glucans, and the results indicated that the coarse fractions (corresponding to the external layer of caryopsides) were enriched on β-glucans. Also, Verardo et al.[15] have demonstrated that the same fractions are naturally enriched in phenolic compounds, particularly flavan-3-ols, and they have already been used as ingredients in bakery products.[16,17] This represents an excellent opportunity to add value to this fraction that represents a byproduct of the milling process.

Because of the high content of β-glucans and flavan-3-ols of the barley coarse fraction (cv. Scarlett), the objective of this work was to use it as an ingredient for the formulation of functional spaghetti. The influence of the addition of barley coarse fraction, used in several percentages, on the quality of spaghetti was checked. Finally, the

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contents of β-glucans and phenolic compounds were evaluated, and the results were compared to those of commercial samples.

MATERIALS AND METHODS

Reagents and Chemicals. Chloroform, methanol, ethanol, acetone, *n*-hexane, isopropanol, sulfuric acid, barium chloride dehydrate, acetonitrile, water, isopropanol for high-performance liquid chromatography (HPLC), glacial acetic acid, ethanol, formic acid (96%), and sodium hydroxide were purchased from Merck KGaA (Darmstadt, Germany). Anhydrous sodium sulfate, potassium chloride (99%), ferric chloride hexahydrate, 2,2-diphenyl-1-picrylhydrazyl (DPPH) (95% free radicals), and (+)-catechin (purity = 98%) were provided by Sigma-Aldrich (St. Louis, MO); ferrous KH_2PO_4 was provided by Carlo Erba Reagents (Milan, Italy). Sodium tetraborate decahydrate, capillary electrophoresis (HPCE)-water, and sodium dodecyl sulfate (SDS) were purchased from Fluka Biochemika (Buchs, Switzerland). Amyloglucosidase, protease, and α-amylase were purchased from Sigma-Aldrich. Technical nitrogen was supplied by Sapio Industries (Monza, Italy), and double-distilled water was produced in situ by Elix system of 10 Millipore (Billerica, MA).

Samples. *Barley Flour Air Classification.* Hulled grain of barley (cv. Scarlett) was dehulled and pin-milled (whole meal). Air classification (pilot plant of Scientific and Technological Park Moliseinnovazione, Campobasso, Italy) was developed to separate flours into various particle sizes using air currents. The classification of flour was achieved under the influence of two opposing forces, air traction and centrifugal force. Selected particle size ranges were obtained by adjusting baffles that rotate forming a barrier that allowed specific particle-size ranges to pass. Whole meal was air-classified into a coarse fraction (40%, w/w) and a fine fraction (60%, w/w). Particle-size ranges were 120−477 and 45−120 μm for coarse and fine fractions, respectively. The soluble and insoluble dietary fiber and the β-glucan content in coarse fraction were 15.6, 7.8, and 10.6% dm, respectively.

Gluten Extraction. Durum wheat semolina (2 kg) was mixed in a dough mixer (prototype built by NAMAD, Roma, Italy) with 1.2 L of water at room temperature for 4 min at 95 rpm and 6 min at 140 rpm. The obtained dough was allowed to rest for 15 min at room temperature. The dough was then poured over the sieve (pore size 420 μm) of the gluten washing machine (prototype as above). After 30 min of washing, the gluten fraction was recovered from the sieve and dried under vacuum at <50 °C in a special drier (NAMAD). The dried gluten, 80−82% protein (dm) and 5% moisture, was then milled in a laboratory mill to pass a 150−200 μm mesh size, sealed in plastic bags, and kept at room temperature before use.

Functional Spaghetti. Three different functional spaghetti were produced: BS50 was produced by replacing 50% durum wheat semolina with coarse fraction air classified barley flour (cv. Scarlett); BS45 was produced by replacing 45% durum wheat semolina with coarse fraction air classified barley flour and adding 5% vital wheat gluten; BS95 was produced with 95% coarse fraction air classified barley flour and adding 5% vital wheat gluten. The composite flours were used to manufacture spaghetti (long pasta) under conditions previously reported (85 °C drying temperature, 7 h drying cycle).[11] An experimental pasta-making apparatus composed by a press and a dryer (Pavan, Padova, Italy) was used. The diameter of the dried spaghetti was 1.65−1.70 mm.

Furthermore, three semolina spaghetti (commercial semolina spaghetti, CSS), three organic semolina spaghetti (commercial organic semolina spaghetti, COSS), three whole semolina spaghetti (commercial whole semolina spaghetti, CWSS), and three organic whole semolina spaghetti (commercial organic whole semolina spaghetti, COWSS) samples were purchased in different local markets.

Moisture Determination. The moisture content of samples was determined according to ICC methods 110/1.[18] Seven grams of grounded spaghetti were stored in a drying oven for 90 min at 130 °C until constant weight, and the moisture was calculated.

Ashes Content. The ashes content was determined according to ICC method 104/1.[18] One gram of grounded spaghetti was collected in a porcelain crucible, previously conditioned in muffle furnace at 525 °C for 1 h, and then cooled. Afterward, the sample, out of muffle, was burnt with ethyl alcohol and then put in muffle at 525 °C. Ashing was completed when the cooled residue was white or nearly white. After cooling, porcelain crucibles were weighed, and the ash content was calculated.

Lipid Extraction. The lipid content was determined by the method 136 of ICC.[18] Briefly, the spaghetti samples were manually ground in a mortar. Afterward, 6 g of sample were extracted in a Soxtec apparatus (1045 Soxtec Extraction Unit, Foss Tecator, Foss Italia S.p.A., Padova, Italy).

Protein Determination. To determine the protein content of spaghetti, the ICC method 105/2 was carried out;[18] 1 g of sample was subjected to mineralization of organic matter with 10 mL of sulfuric acid, in the presence of copper sulfate. Therefore, nitrogen was converted in ammonium sulfate and treated with NaOH. The ammonia released was collected in a solution of 4% boric acid and titrated with 0.05 M sulfuric acid. The factor used to convert nitrogen into protein was 6.25.

Evaluation of Fiber Content. To evaluate insoluble, soluble, and total dietary fiber content in analyzed spaghetti, duplicate test portions of defatted sample were gelatinized with heat-stable α-amylase and then enzymatically digested with protease and amyloglucosidase to remove protein and starch in accordance to Prosky et al.[19]

Insoluble dietary fiber (IDF) was removed by filtering and washing residue with water. Soluble dietary fiber (SDF) in the filtrate was precipitated by adding 95% ethanol to it. The precipitate was filtered and washed with 78% ethanol, 95% ethanol, and acetone; dried; and weighed. One duplicate was used to analyze proteins, and the other one was incinerated at 525 °C to determine ash. SDF was expressed as the weight of residue minus the weight of protein and ash.

Determination of β-Glucans. As reported in AACC method 32-23.01,[20] (1-3)(1-4)-β-D-glucan was determined using lichenase to generate soluble mixed-linkage β-oligosaccharides, which were separated from insoluble polysaccharides by centrifugation. The oligosaccharides were hydrolyzed to glucose with β-glucosidase. Subsequent colorimetric measurement of the derived glucose was performed using glucose oxidase/peroxidase reagent.

Total Organic Matter (TOM). TOM, the amount of surface material released in the washing water after thoroughly rinsing the cooked pasta, was determined using the standard chemical method 153, ICC 1995.[18]

Sensory Analysis. Sensory assessment was made by a trained panel of five experts of Scientific and Technological Park "Moliseinnovazione" and University of Molise, Campobasso (Italy) (two women and three men aged between 24 and 40 years). The stickiness, bulkiness, and firmness of the cooked spaghetti were determined according to the sensory assessment procedure reported by Cubadda et al.[21]

The degree of stickiness, which is the amount of material that adheres to the surface of the cooked pasta, was assessed visually and by handling the samples. Bulkiness, which was assessed in the same way, is the degree to which the strands of spaghetti adhere to each other. Firmness is the degree of resistance of the cooked spaghetti when either pressed between the fingers or chewed. The scale for both stickiness and bulkiness was as follows: 20 = very high, 40 = high, 60 = rare, 80 = almost absent, and 100 = absent. The firmness scale was as follows: 20 = rare, 40 = insufficient, 60 = sufficient, 80 = good, and 100 = very good. The score of each organoleptic parameter was the arithmetic mean of the values given by the assessors. The total score was obtained by summing the scores of the parameters, multiplying the total by 33.3, and dividing it by 100.

Extraction of Phenolic Compounds from Spaghetti. To isolate the phenolic fraction from spaghetti, the protocol of Hirawan et al.[22] was used. Spaghetti samples were milled using a refrigerated laboratory mill (model IKA A10-Ikawerke GmbH & Co. KG, Staufen, Germany). Briefly, 4 g of spaghetti was extracted twice in an ultrasonic bath with a solution of ethanol (95%) and 1 M HCl/95% ethanol (w/v, 15/85). The supernatants

Table 1. Chemical Composition (g/100 g Dry Matter) of Commercial and Barley Spaghetti[a]

				fiber			
samples	ash	proteins	fats	total	soluble	insoluble	β-glucan
				CSS			
CSS1	0.6 d	12.1 b,c	1.6 a,b	3.0 c	1.3 c	1.7 d	0.2 c
CSS2	0.5 d	13.9 b	1.7 a,b	2.8 c	1.1 c	1.7 d	0.2 c
CSS3	1.1 c	13.7 b	1.9 a,b	4.0 b	1.7 c	2.3 d	0.2 c
				COSS			
COSS1	0.8 c,d	13.5 b	1.3 b	4.4 c	1.4 c	3.0 d	0.3 c
COSS2	1.1 c	13.9 b	1.7 a,b	4.2 c	1.5 c	2.6 d	0.2 c
COSS3	1.0 c	13.3 b	1.9 a	3.7 c	1.0 c,d	2.7 d	0.2 c
				CWSS			
CWSS1	1.1 c	14.3 b	1.7 a,b	7.0 b	1.5 c	5.5 b	0.4 c
CWSS2	1.2 c	14.2 b	2.0 a	9.5 b	2.6 c	6.9 b	0.5 c
CWSS3	1.1 c	13.9 b	1.4 b	7.2 b	1.6 c	5.6 b	0.4 c
				COWSS			
COWSS1	1.0 c	13.8 b	2.2 a	6.7 b	2.3 c	4.4 b,c	0.4 c
COWSS2	0.9 c	13.3 b	1.7 a,b	6.3 b	1.9 c	4.4 b,c	0.4 c
COWSS3	1.0 c	13.5 b	1.6 a,b	7.0 b	2.5 c	4.5 b,c	0.5 c
				developed barley spaghetti			
BS50	1.6 b	11.4 b,c	2.5 a	19.3 a	7.8 b	11.5 a	5.2 b
BS45	1.6 b	15.6 a	2.6 a	21.0 a	8.6 b	12.4 a	4.8 b
BS95	2.3 a	13.7 b	2.6 a	24.5 a	14.7 a	9.8 a	10.2 a

[a] Different letters in the same column mean significantly different values ($p < 0.05$).

were collected, evaporated, and reconstituted with H_2O/formic acid (99.7:0.3 v/v). The extracts were stored at −18 °C until use.

Determination of Phenolic Compounds. The electrophoretic conditions used in this work were optimized in a previous publication.[17] Calibration curves of (+)-catechin, ferulic acid, and tannic acid were carried out from 1 to 1000, 1 to 2000, and 10 to 5000 μg/mL, respectively. Detection was performed at 214 nm. The linearity range was assessed for each analyte using 8, 10, and 10 concentration levels, respectively. The capillary electrophoretic analysis was replicated three times for each extract and for each calibration point ($n = 3$).

Antioxidant Activity of Phenolic Extracts. To determine the antioxidant activity of the ethanol extracts, the DPPH radical scavenging and the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging method were performed as described by Dudonné et al.[23]

Statistical Analysis. Unless otherwise stated, the results reported in this study are the averages of three repetitions ($n = 3$). Tukey's honest significant difference multiple comparison (one-way analysis of variance), Pearson's linear correlations, both at the $p < 0.05$ level, and principal component analysis were evaluated using Statistica 6.0 software (2001, StatSoft, Tulsa, OK).

RESULTS AND DISCUSSION

Chemical Composition of Commercial and Developed Spaghetti. The proximate composition of commercial and developed spaghetti is shown in Table 1. Barley spaghetti reported the higher ash content (1.6−2.3%) that increased with the addition of the barley coarse fraction. The protein content was in a range within 12.1 and 15.6% dm. As expected, there is an increase on the protein content in BS45 sample; the explanation about this behavior has been associated to the vital gluten added during the semolina mixing. In fact, BS45 was formulated with 50% of semolina (that contains endogenous proteins) plus 5% gluten.

The total dietary fiber content was significantly ($p < 0.05$) lower in CSS and COSS samples with a range between 2.8 and 4.4% dm. All whole semolina spaghetti samples (CWSS and COWSS) reported a total fiber content higher than CSS and COSS samples with values in the range of 6.3−9.5% dm. However, barley spaghetti showed the highest content of total dietary fiber, and as expected, BS95 presented the major content, which was correlated with the barley coarse fraction content.

Barley spaghetti contained from 5.1 to 7.7 times more dietary fiber than CCS and COSS samples and from 2.3 and 3.4 times more dietary fiber than CWSS and COWSS samples. The observed high total dietary fiber contents of barley spaghetti may be mainly attributed to the high inherent fiber of barley coarse fraction as compared to semolina or whole semolina that were used to prepare the commercial samples.

On the basis of the normative of the European Union,[24] commercial samples with whole semolina (CWSS and COWSS) and barley spaghetti can benefit from the claim "high fiber". According to this normative, a food can be consider "high fiber" if the product contains at least 6 g of fiber per 100 g.

The IDF content in spaghetti samples ranged from 1.7 to 12.4% dm, the mean value content was 1.9, 2.8, 6.0, 4.4, and 11.2 g/100 g dm for CSS, COSS, CWSS, COWSS, and BS, respectively. The ratio of IDF/SDF varied from 0.7 to 3.6, and the lowest and highest values correspond to BS95 and CWSS1 samples, respectively. Generally, BS95, BS45, BS50, CSS1, and CSS3 reported the lowest IDF/SDF ratio values.

The SDF content of different spaghetti ranged from 1.0 to 14.7% dm; the lowest and the highest values were found for COSS3 and BS95, respectively. Commercial samples were between 1.0 and 1.7% dm. The developed barley spaghetti showed a content of SDF ranging between 7.8 and 14.7% dm. Izydorczyk and Dexter[25] correlated the content of SDF with β-glucans content. In fact, the β-glucans content was enormously higher in barley spaghetti than in commercial samples. Commercial samples reported a content of β-glucans lower than 0.6% dm, while barley spaghetti showed a content of 5.2, 4.9, and 10.2% dm of β-glucans in BS50, BS45, and BS95 spaghetti, respectively. These contents meet the FDA requirements[26] of 0.75 g of β-glucans per serving, which could allow these pastas to deserve the health claims "good source of dietary fiber" and "may reduce the risk of heart disease". A recent work[27] demonstrated that high quantities of β-glucans significantly decrease the glycemic response and the glycemic index of spaghetti.

Cooking Quality of Barley Spaghetti. The spaghetti (100 g) were cooked in 1 L of unsalted boiling tap water. The optimum cooking time was taken at the moment that the white core of the pasta disappeared when squeezed between two test glasses. The cooking time for spaghetti is shown in Table 2.

BS45 and BS95 spaghetti had the same firmness, bulkiness, and stickiness values (Table 2) that can be compared with data obtained by Marconi et al.[11] for durum wheat pasta. BS50 spaghetti reported the absence of bulkiness and stickiness and a good firmness. Overall, this sample showed a good quality but lower than BS45 and BS95 samples. According to literature,[28,29] the addition of vital gluten in BS45 and BS95 samples decreased the stickiness and increased the firmness.

Effectively, TOM values of barley spaghetti were lower than 1.4 g/100 g, precisely 0.9, 0.8, and 0.5 g/100 g for BS50, BS45, and BS95, respectively. Spaghetti supplemented with vital gluten showed the lowest cooking loss according to literature.[30]

Phenolic Content and Antioxidant Activity in Uncooked and Cooked Spaghetti. Table 3 reports the phenolic content in uncooked and cooked pasta. The results evidenced the different phenolic composition between semolina and barley spaghetti. Indeed, the electropherograms shown in Figure 1 demonstrated the different phenolic profile between semolina (A), a mixture of semolina and barley coarse fraction (B), and barley coarse fraction (C) flours used in this experimentation. As reported in our previous work,[31] when aqueous ethanol was used, flavan-3-ols, phenolic acids, and hydrolyzable tannins were extracted. In fact, electropherogram obtained by injection of the ethanolic extracts presented two separate peak zones confirmed by spiking trials. The former one, from 1.4 to 2.7 min, was represented by flavan-3-ols, their oligomers, and by phenolic acids, while the latter one, from 2.8 to 4.7 min, was a big group of unresolved peaks that well matched with the tannic acid standard and that represents the complex fraction of tannins.

Incorporation of barley coarse fraction as ingredient in spaghetti formulation improved the content of simple phenolic compounds; particularly, the barley coarse fraction supplied the flavan-3-ols. In the case of commercial pasta, the only peak identified was *trans*-ferulic by spiking with the commercial standard and spectral analysis by UV-DAD; moreover, several compounds that reported the typical spectra data of phenolic acids were determined. The presence of (+)-catechin and prodelphinidin B3 was verified in barley spaghetti samples by comigration of commercial standards; also, other peaks with

Table 2. Cooking Quality of Developed Barley Spaghetti[a]

	BS45	BS50	BS95
cooking time (min)	12.35	13.00	12.58
stickiness	98 (absent)	81 (almost absent)	98 (absent)
firmness	94 (good/excellent)	91 (good)	96 (good/excellent)
bulkiness	96 (absent)	92 (absent)	96 (absent)
TOM[b]	0.83	0.93	0.55

[a] As general reference, ICC Method 153 proposed the following classification: spaghetti with a TOM value equal or lower than 1.4 = good quality, from 1.5 to 2.1 = fair quality, and equal or higher than 2.2 = poor quality. [b] TOM is expressed as g/100 g of dry spaghetti.

Table 3. Comparison of Phenolic Content in Uncooked and Cooked Commercial and Barley Spaghetti[a]

		phenolic acids[b]			flavan-3-ols[c]					% inhibition	
samples		ferulic acid	others	sum	(+)-CAT	PD B3	others	sum	complex phenols[d]	DPPH	ABTS
						CSS					
CSS1	uncooked	3.75 e	46.97 e	50.72 d	ND	ND	ND	ND	1755.53 a	5.79 d,e	2.11 h,i
	cooked	1.94 f	34.21 f	36.15 e,f	ND	ND	ND	ND	1370.35 b	4.41 e	1.42 l
CSS2	uncooked	2.95 f	36.89 f	39.84 e	ND	ND	ND	ND	2037.65 a	4.75 e	2.70 g
	cooked	1.61 g	24.22 g	25.83 g	ND	ND	ND	ND	1558.43 b	3.38 f	1.78 i
CSS3	uncooked	3.76 e	31.64 f,g	35.40 f	ND	ND	ND	ND	1852.68 a	4.77 e	1.94 i
	cooked	2.04 f,g	17.08 h	19.12 h	ND	ND	ND	ND	1435.12 b	3.69 f	1.33 l
						COSS					
COSS1	uncooked	3.47 e	63.69 d	67.16 d	ND	ND	ND	ND	1162.83 b	4.21 e	2.21 h
	cooked	1.89 f,g	47.23 e	49.12 d,e	ND	ND	ND	ND	997.15 c	2.55 g	1.40 i
COSS2	uncooked	2.98 f	63.12 d	66.10 d	ND	ND	ND	ND	1249.87 b	4.53 e	2.52 g,h
	cooked	1.74 g	45.08 e	46.82 e	ND	ND	ND	ND	1089.78 c	2.67 g	1.38 i
COSS3	uncooked	4.02 e	73.39 d	77.41 c	ND	ND	ND	ND	1442.28 b	4.65 e	2.65 g,h
	cooked	2.16 f,g	46.17 e	48.33 d,e	ND	ND	ND	ND	1102.56 c	2.81 f,g	1.72 i
						CWSS					
CWSS1	uncooked	20.35 c	88.48 b	108.83 b	ND	ND	ND	ND	1931.93 a	6.70 d	5.64 e
	cooked	13.47 d	45.96 e	59.43 d	ND	ND	ND	ND	1459.57 b	3.80 e	2.99 g
CWSS2	uncooked	29.11 a,b	90.73 b	119.84 b	ND	ND	ND	ND	2016.05 a	7.14 d	5.98 e
	cooked	16.88 c	48.10 e	64.98 d	ND	ND	ND	ND	1538.34 b	3.85 e	4.00 f
CWSS3	uncooked	31.98 a	80.57 c	112.55 b	ND	ND	ND	ND	1879.43 a	6.57 d	4.39 f
	cooked	20.02 c	40.39 e	60.41 d	ND	ND	ND	ND	1422.91 b	4.01 e	3.31 g
						COWSS					
COWSS1	uncooked	37.60 a	118.67 a	156.27 a	ND	ND	ND	ND	1288.71 b	7.61 d	6.91 e
	cooked	21.69 c	66.65 d	88.34 c	ND	ND	ND	ND	987.12 c	4.21 e	5.28 f
COWSS2	uncooked	32.41 a	81.37 c	113.78 b	ND	ND	ND	ND	1417.95 b	7.41 d	6.49 e
	cooked	20.84 c	48.29 e	69.13 c,d	ND	ND	ND	ND	1077.29 c	3.99 e	5.67 f
COWSS3	uncooked	29.90 a,b	74.22 d	104.12 b	ND	ND	ND	ND	1334.79 b	6.78 d	5.91 e
	cooked	18.77 c	40.32 e,f	59.09 d	ND	ND	ND	ND	1040.26 c	3.75 e	4.34 f
						developed barley spaghetti					
BS50	uncooked	2.06 g	18.69 h	20.75 g	100.22 a	80.96 a	374.27 a	555.45 a	48.14 a	34.93 a	48.14 a
	cooked	0.93 i	12.58 i	13.51 h	63.91 b	42.60 b	192.94 c	299.45 c	36.10 b	24.17 b	36.10 b
BS45	uncooked	1.20 h	6.11 j	7.31 i	63.38 b	37.61 c	102.65 d	203.64 d	25.85 c	19.26 c	25.85 c
	cooked	0.71 j	5.62 j	6.10 i	38.29 c	26.46 c,d	103.32 d	168.07 e	23.74 c	16.81 d	23.74 c
BS95	uncooked	ND	2.15 k	2.15 j	102.15 a	86.48 a	217.85 c	406.48 b	33.50 b	26.12 b	33.50 b
	cooked	ND	1.89 k	1.89 j,k	61.99 b	52.09 b	265.59 b	379.67 b,c	31.09 b,c	21.03 c	31.09 b,c

[a] Different letters in the same column mean significantly different values ($p < 0.05$). (+)-CAT, (+)-catechin; PD B3, prodelphinidin B3; ND, not detected. [b] μg ferulic acid/g dw. [c] μg (+)-catechin/g dw. [d] μg tannic acid/g dw.

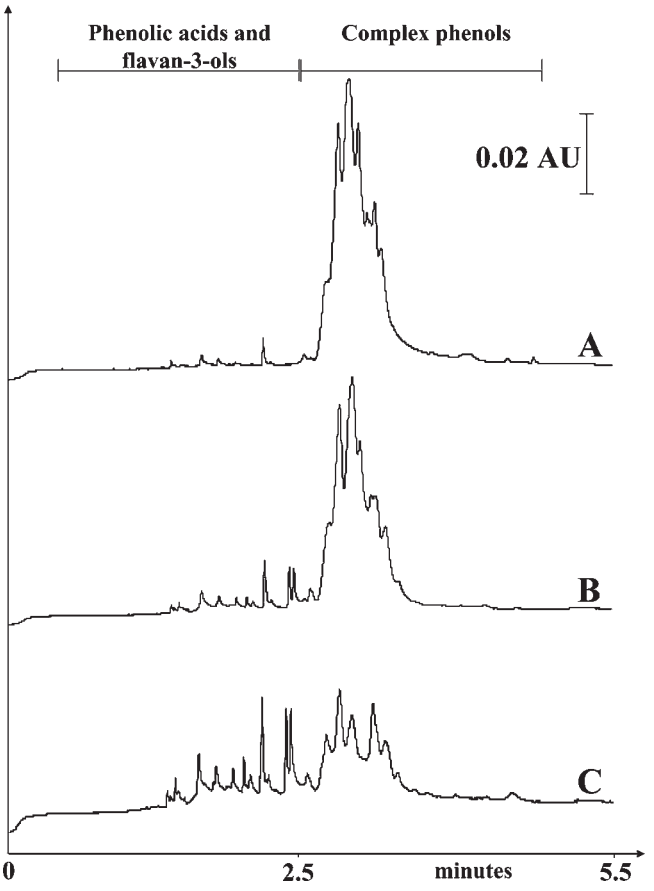

Figure 1. Electropherograms of semolina (A), a mixture of 50% semolina + 50% barley coarse fraction (B), and a mixture of 95% barley coarse fraction + 5% vital gluten (C).

specific UV absorption of flavan-3-ols were detected. The correlation of MEKC analysis data with data obtained by other analytical methods has been demonstrated in our previous work.[15]

The presence of these compounds in barley was confirmed in the literature,[9] and their enrichment in barley coarse fraction was clarified in a precedent work.[15] As reported in literature,[22] the semolina pasta contained phenolic acids that were sourced from semolina. Effectively, the commercial spaghetti reported the presence of only phenolic acids, as simple phenolic fraction, and a higher quantity of tannins. Differences between commercial semolina and CWSS were reported.

Uncooked Spaghetti. Regarding the uncooked spaghetti, it can be said that the spaghetti formulated with whole semolina showed an increase of phenolic acids content. The uncooked semolina (CSS) and organic semolina (COSS) spaghetti (Table 3) exhibited a phenolic acid content, with an average of 56.1 μg ferulic acid/g of dry weight and a range from 35.4 to 77.4 μg ferulic acid/g of dry weight.

The content of ferulic acid in CSS and COSS samples was in the range of 4.5−5.2 and 7.4−10.6% of total phenolic acid content, respectively. However, no statistical differences in total phenolic acids content were found between uncooked CSS and COSS samples (with the exception of sample COSS3). Statistical differences ($p < 0.05$) were reported between CSS and COSS regarding the tannin fraction. In fact, the COSS samples showed the lowest content of tannins. The ranges were 1755.5−2037.6 and 1162.8−1442.3 μg tannic acid/g for CSS and COSS samples, respectively.

The phenolic acids content in uncooked commercial spaghetti formulated with whole semolina ranged from 104.1 to 156.3 μg of ferulic acid/g of dry weight, and no statistical differences in phenolic acids content were found among whole semolina spaghetti samples. The ferulic acid content in whole spaghetti samples ranged from 20.3 to 37.6 μg of ferulic acid/g. Statistical differences were not found between CWSS and COWSS samples, except for sample CWSS1.

As revealed for the CSS and COSS samples, the whole semolina spaghetti showed statistical differences as compared to COWSS. Indeed, CWSS samples reported a higher content (from 1879.4 to 2016.0 μg of tannic acid/g of dry weight) of tannins than COWSS samples (1288.7−1417.9 μg of tannic acid/g of dry weight).

Table 3 evidently reports that the highest amount of catechins and proanthocyanidins were found in only in barley spaghetti. Surprisingly, uncooked BS50 reported the highest content in all of the phenolic classes (phenolic acid, flavan-3-ols, and tannins) with respect to uncooked BS45 and BS95 samples. In particular, the content of phenolics compounds in uncooked BS50 spaghetti was about twice higher than in BS45 and about the same quantity respect to uncooked BS95 sample.

The BS50 sample showed a content of ferulic acid 2-fold as compared to BS45; the same compound was not identified in the BS95 sample. The total phenolic acid content is 3-fold higher in BS50 spaghetti than in the BS45 sample. The lowest content was determined in the BS95 sample. Significant differences ($p < 0.05$) were found between the content of flavan-3-ols in BS50 and BS45 samples. In fact, the contents of (+)-catechin and prodelphinidin B3 (PD B3) were 100.2 and 81.0 μg (+)-catechin/g and 63.4 and 37.6 μg (+)-catechin/g, respectively. The sample BS95 reported a content of (+)-catechin and PD B3 similar to BS50 sample. These data underlined an influence of gluten added in the BS45 and BS95 formulation on the extractability/bioavailability of the phenolic compounds.

To evaluate the antioxidant activity of the phenolic compounds quantified in the samples, the results obtained by capillary electrophoresis were compared with the spectrophotometric determinations by DPPH and ABTS assays. There were no significant differences in DPPH and ABTS radical scavenging activity for CSS and COSS and for CWSS and COWSS. Significant differences were noticed between semolina and whole semolina samples. The whole semolina spaghetti (CWSS and COWSS) reported a higher antioxidant activity justified by the major content of phenolic acids. The low contribution of tannins to the antioxidant activity was also previously illustrated by Bonoli and co-workers.[31] The DPPH and ABTS data confirmed the high antioxidant activity of flavan-3-ols; in fact, the spaghetti formulated with barley coarse fraction reported the highest antioxidant powers. In fact, barley contained substantial amounts of phenolic antioxidants that effectively scavenge free radicals.[32] Furthermore, BS50 sample displayed the highest values of DPPH and ABTS as compared to BS45 and BS95 samples. This confirmed the data obtained by capillary electrophoresis.

The relationship between the antioxidant activity of the extracts and their content of catechins and proanthocyanidins was confirmed by the existing positive Pearson's correlation between flavan-3-ols content and DPPH ($r^2 = 0.8440$, $p < 0.0001$) and flavan-3-ols content and ABTS ($r^2 = 0.8201$, $p < 0.0001$), while the tannin fraction was not correlated with ABTS and DPPH, which confirmed that hydrolyzable tannins did not contribute to the antioxidant capacity of the extracts, while the catechins and proanthocyanidins gave the highest antioxidant power to the extracts. A good positive Pearson's correlation was found between DPPH and ABTS ($r^2 = 0.7371$, $p < 0.05$).

Cooked Spaghetti. The effect of cooking on phenolic content in commercial spaghetti and barley spaghetti was also evaluated. All of the samples showed significant differences before and after cooking (Table 3).

Commercial pasta showed a phenolic acid loss that varies from 26.9 to 46.3% according to Hirawan et al.[22] The ferulic acid content reported a loss in the range of 33.8 and 48.3%. The tannin fraction reported a loss in the range of 12.8−24.4%.

Furthermore, barley spaghetti reported a phenolic degradation after cooking. BS50 spaghetti showed a cooking loss of flavan-3-ols and phenolic acids of 46.1 and 34.9%, respectively, and a degradation of tannin fraction equal to 15.2%. The BS45 and BS95 reported a lower cooking loss; as a matter of fact, the phenolic acid degradation was 16.5 and 12.2%, and the flavan-3-ols loss was 17.5 and 6.6% for BS45 and BS95, respectively.

The loss of ferulic acid and other derivatives was statistically different between BS50 and BS45 samples; indeed, BS45 reported a loss of ferulic acid and other derivatives of 40.8 and 8.0%, respectively, as compared to 54.9 and 32.7% obtained in BS50 sample.

Interesting data were shown by the catechin and PD B3 contents in barley spaghetti. The barley spaghetti reported the same loss ($p < 0.05$) of (+)-catechin for BS50, BS45, and BS95 samples (36.2, 39.6, and 39.3%, respectively). The loss of PD B3 in gluten-added samples was statistically lower than the BS50 sample. Moreover, the content of other flavan-3-ols in BS95 increased after cooking, no statistical difference was found in BS45, but they decreased in the percentage of 48.4% in BS50 sample.

The antioxidant activity by DPPH and ABTS confirmed these data. Also, the tannin fraction reported the same trend of the simple phenolic fraction; tannin degradations were 15.2, 9.7, and 9.7% in BS50, BS45, and BS95, respectively.

It can be supposed that the phenolic compounds in BS45 and BS95 can be physically blocked in a gluten network (due to the replace with vital gluten) and can be partially released during the cooking process. These hypotheses were supported by the studies of several authors[33,34] that demonstrated the interaction between the proteins and the phenolic compounds in foods.

According to Hirawan and co-workers,[22] the phenolic compounds content was affected by the cooking process; the cooking temperature encourages both the dissolution of these compounds in the cooking water and their degradation. Anyway, after cooking, the barley spaghetti reported an interesting content of catechins and proantocyanidins; their consumption has been implicated in improved antioxidant status and decreased DNA damage in humans and reduced development of aortic atherosclerosis and delayed tumor production in animal test systems.[35,36]

Principal component analysis contributed to a further profiling of the samples considered, based on the overall polyphenolic composition and the antioxidant activity. PC1, explaining 79.2% of total variation, is clearly linked to phenolic composition. As illustrated (Figure 2), this component was the only one that differs between commercial and barley spaghetti. From Figure 2, it is therefore clear that PC1 discriminates the commercial samples from the barley spaghetti because commercial samples are characterized by the presence of a higher content of phenolic acid derivatives and tannins (at the right side of PC1 axis), whereas barley spaghetti are characterized by the presence of flavan-3-ols with high antioxidant activity (collocated at the left side of PC1).

PC2 (Figure 2) explains 11.0% of variability and separates, on the one hand, spaghetti with semolina from the spaghetti made with whole semolina and, on the other hand, the barley spaghetti

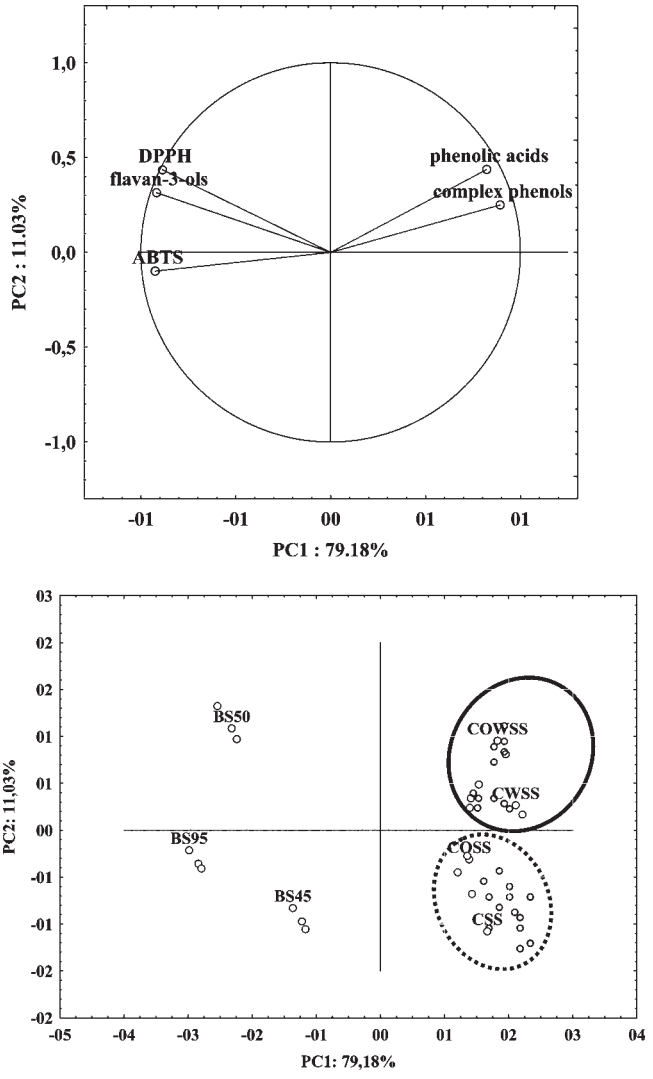

Figure 2. Principal component score of the investigated barley and commercial spaghetti according to PC1 and PC2 obtained by contents of phenolic compounds and their antioxidant activity. Percentages represent the variance of each component. The continued circle indicates the whole spaghetti, and the dotted circle indicates the semolina spaghetti.

with vital gluten (BS45 and BS95) from BS50. In fact, this component was positively correlated with phenolic acids and tannin fraction and flavan-3-ols. This component showed the separation of the CWSS, COWSS, and BS50 samples (above the PC2 axis) and the CSS, COSS, BS45, and BS95 (below than PC2 axis).

These results confirmed the data obtained by capillary electrophoresis; whole semolina spaghetti, according to the literature,[22] were characterized to have a higher content of phenolic acids than semolina samples. In addition, vital gluten added in BS45 and BS95 recipes influences the flavan-3-ols bioavailability and degradation.

This study deals with the relevance of barley coarse fraction as a good source of dietary fiber and flavan-3-ols. To develop these studies, different formulations of barley spaghetti were developed. The barley spaghetti showed an increase of β-glucans in the range of 4.3−9.0%, proportionally at the barley coarse fraction replacement. This content meets the FDA requirements, which could allow these pastas to deserve the health claims "good source of dietary fiber" and "may reduce the risk of heart disease".

The results highlight the separation of the commercial spaghetti and barley spaghetti (formulated with barley coarse fraction) mostly on the basis of the phenolic profile. The phenolic acids content was higher in the commercial samples, whereas flavan-3-ols (that were not detected in commercial samples) were the phenolic compounds present in a higher concentration in barley spaghetti samples. Because of that, the barley coarse fraction has demonstrated to be an useful ingredient to enrich spaghetti in flavan-3-ols. Moreover, the enrichment with vital gluten improved the cooking quality of spaghetti and influenced the extraction and protection of phenolic compounds during cooking process. Further studies will be needed to elucidate the in vivo antioxidant power of pasta when vital gluten will be added in the recipe.

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ABBREVIATIONS USED

$ABTS^{+\bullet}$, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation; BS45, barley spaghetti with 50% semolina, 5% vital gluten, and 45% barley coarse fraction; BS50, barley spaghetti with 50% semolina and 50% barley coarse fraction; BS95, barley spaghetti with 5% vital gluten and 95% barley coarse fraction; CSS, commercial semolina spaghetti; COSS, commercial organic semolina spaghetti; COWSS, commercial organic whole semolina spaghetti; CVD, cardiovascular diseases; CWSS, commercial whole semolina spaghetti; $DPPH^{\bullet}$, 2,2-diphenyl-1-picrylhydrazyl radical; HPCE, capillary electrophoresis; IDF, insoluble dietary fiber; PD B3, prodelphinidin B3; SDF, soluble dietary fiber; TOM, total organic matter

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