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Determination of Free and Bound Phenolic Compounds in Buckwheat Spaghetti by RP-HPLC-ESI-TOF-MS: Effect of Thermal Processing from Farm to Fork

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ABSTRACT: Nowadays there is considerable interest in the consumption of alternative crops as potential recipes for gluten-free products production. Therefore, the use of buckwheat for the production of gluten-free pasta has been investigated in the present study. RP-HPLC-ESI-TOF-MS has been applied for the separation and characterization of free and bound phenolic compounds in buckwheat flour and buckwheat spaghetti. Thus, 32 free and 24 bound phenolic compounds in buckwheat flour and spaghetti have been characterized and quantified. To the authors' knowledge, protochatechuic-4-*O*-glucoside acid and procyanidin A have been detected in buckwheat for the first time. The results have demonstrated a decrease of total free phenolic compounds from farm to fork (from flour to cooked spaghetti) of about 74.5%, with a range between 55.3 and 100%, for individual compounds. The decrease in bound phenols was 80.9%, with a range between 46.2 and 100%. The spaghetti-making process and the cooking caused losses of 46.1 and 49.4% of total phenolic compounds, respectively. Of the total phenolic compounds present in dried spaghetti, 11.6% were dissolved in water after cooking.

KEYWORDS: buckwheat, spaghetti, noodles, phenolic compounds, RP-HPLC-ESI-TOF-MS, cooking loss

INTRODUCTION

Numerous foods are already associated with health promotion and disease prevention. Inappropriate nutrition is a primary factor in unattained genetic potential, reduced physical and cognitive performance, and increased risk of some diseases. [1] Celiac disease is one of the most common lifelong disorders worldwide with an estimated mean prevalence of 1% of the general population. [2] It is an autoimmune enteropathy triggered by the ingestion of gluten-containing grains (wheat, barley, rye, and possibly oats) in genetically susceptible individuals. The only treatment for celiac disease is lifelong adherence to a gluten-free diet. [3,4] For instance, in many areas of the world staple foods such as bread, bakery products, and pasta contain gluten and, therefore, should be avoided in the diet. At present there is considerable interest in the consumption of alternative crops as potential recipes for gluten-free products production.[5] Several of them are considered minor cereals, that is, underutilized cereals (for example, sorghum, rye, and millet); others are pseudocereals, crops evolutionarily distant from cereals, which produce grains (for example, quinoa, amaranth, and buckwheat). Gluten-free cereal foods are frequently made using refined gluten-free flour or starch and are generally not enriched or fortified. Sorghum is often recommended as a safe food for celiac patients, because it is only distantly related to the Triticeae tribe cereals wheat, rye, and barley,[6] being a member of the Panicoideae subfamily, which also includes maize and most millets.[7] Traditional flatbreads from sorghum and millets were produced in India and Ethiopia. Many alternatives to common gluten-containing grains exist, such as the pseudocereals amaranth, quinoa, and buckwheat. [8−10]

Buckwheat has been used in different bread formulations[11−15] and in the production of other gluten-free bakery products.[8,16,17] The results have suggested that processing conditions, including tempering moisture, heating temperature, and heating time, significantly influence the physical and chemical qualities of buckwheat products such as specific volume, hardness, integrity, color, internal structure, and rutin content. In addition, Bojňanská and co-workers[18] have demonstrated an increase of the total antioxidant status in vivo when buckwheat-enriched bread is consumed.

Traditional foods made from buckwheat flour are consumed in China, Japan, Korea and Bhutan, with Japanese buckwheat consumption primarily in the form of noodles. Buckwheat, or soba, noodles are normally made from a blend of common wheat flour and buckwheat flour. The Japanese Food Agency stipulates that a minimum of 35% buckwheat must be present in noodles to be

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called soba. Most of the soba noodles contain at least 60% buckwheat. Some handmade soba noodles, available only in selected restaurants, are made with 100% buckwheat flour.[19]

In recent years, different researchers have studied the effect of hydration level on processing properties and the effects of hydration level, concentration of buckwheat bran flour, and drying temperature on the physical and cooking qualities of spaghetti.[20−23]

To our knowledge, the single free and bound phenolic compounds have not been previously identified in buckwheat spaghetti or in cooking water. For this reason, the aim of this study was to analyze the content of phenolic compounds in flour, dried spaghetti, cooked dried spaghetti, and cooking water. This matrix is particularly interesting because buckwheat is considered to be very promising for the development of gluten-free and functional products. Thus, the free and bound phenolic compounds were characterized by HPLC-ESI-TOF-MS and were determined in the raw material and end product to establish the stability of these phytochemicals during pasta processing and after cooking.

MATERIALS AND METHODS

Reagents and Chemicals. HPLC grade acetonitrile, ethanol, and methanol were purchased from Labscan (Dublin, Ireland). Acetic acid analytical grade (assay >99.5%) was purchased from Fluka (Buchs, Switzerland). Water was purified by using a Milli-Q system (Millipore, Bedford, MA). Other reagents not listed were of an analytical grade. Syringic acid, (+)-catechin, rutin, and myricetin (Sigma-Aldrich, St. Louis, MO) were used for the calibration curves.

Samples. Organic whole buckwheat flour (*Fagopyrum esculentum* Moench) was used.

Pasta was manufactured in a pilot pasta-making plant (Namad, Rome, Italy). Whole buckwheat flour was mixed with tap water (30 °C) for 15 min to obtain a dough suitable for extrusion (dough moisture ≈ 30%). The press (capacity = 5 kg) was equipped with a vacuum mixing and extruding system as well as with a water-cooling jacket of the barrel and extrusion head to reduce heat and to maintain a constant extrusion temperature of <50 °C. The dough was processed into spaghetti (1.8 mm diameter). Spaghetti was dried using a high-temperature program as described by Cubadda et al.[24] with a maximum of temperature of 85 °C in a static dryer (Pavan, Padova, Italy). The sampling was the result of four different production batches of spaghetti.

Extraction of Free Phenolic Compounds from Flour and Dried Spaghetti. To isolate the free phenolic fraction from flour and dried spaghetti, the protocol of Van Hung and Morita[25] with some modifications was applied. Dried spaghetti samples were milled using a refrigerated laboratory mill (model IKA A10, IKAWERKE GmbH & Co. KG, Staufen, Germany).

Briefly, 2 g of buckwheat flour (4 g of dried spaghetti) was extracted twice in an ultrasonic bath at 40 °C with 40 mL of ethanol/water (4:1 v/v) for 10 min. The supernatants were collected, evaporated at 40 °C in a rotary evaporator, and finally reconstituted with 2 mL of methanol/water (1:1 v/v). The extracts were stored at −18 °C until use.

Extraction of Free Phenolic Compounds from Cooked Dried Spaghetti. To isolate the free phenolic fraction from cooked dried spaghetti, 10 g of dried spaghetti was cooked in 400 mL of water for 13 min. Optimum cooking time for each sample was determined using AACC method 66-50.[26] Cooked spaghetti were freeze-dried before milling. Afterward, the cooked pasta was extracted twice in an ultrasonic bath with a solution of ethanol/water (4:1 v/v). The supernatants were collected, evaporated at 40 °C in a rotary evaporator, and reconstituted in 3 mL of methanol/water (1:1 v/v). The extracts were stored at −18 °C until use.

Extraction of Bound Phenolic Compounds from Flour and Uncooked and Cooked Spaghetti. Residues of free phenolic extraction were digested with 300 mL of 2 M NaOH at room temperature for 4 h by shaking under nitrogen gas as reported by Van Hung and Morita.[25] The mixture was acidified (pH 2−3) with hydrochloric acid in a cooling ice bath, to remove the lipids, and extracted with 500 mL of hexane. The final solution was extracted five times with 100 mL of 1:1 diethyl ether/ethyl acetate (v/v). The organic fractions were pooled and evaporated to dryness at 40 °C in a rotary evaporator. The phenolic compounds were finally reconstituted in 2 mL of methanol/water (1:1 v/v).

Extraction of Phenolic Compounds from Cooking Water. Water residue after cooking was acidified to pH 2−3 by adding 10 M hydrochloric acid in a cooling ice bath and extracted three times with 100 mL of 1:1 diethyl ether/ethyl acetate (v/v). The organic fractions were pooled and evaporated to dryness at 40 °C in a rotary evaporator. The phenolic compounds were reconstituted in 2 mL of methanol/water (1:1 v/v).

RP-HPLC-ESI-TOF-MS Analysis. RP-HPLC analysis was performed by an Agilent 1200 series rapid resolution LC system (Agilent Technologies, Palo Alto, CA) consisting of a vacuum degasser, an autosampler, and a binary pump equipped with a reversed-phase C_{18} analytical column (4.6 × 250 mm, 1.8 μm particle size, Agilent ZORBAX Eclipse plus). The mobile phase and gradient program were used as previously described by Verardo et al.[27] All solvents were filtered with a 0.45 μm filter disk. A gradient elution was carried out using the following solvent system: mobile phase A, water/acetic acid (99:1, v/v); mobile phase B, mobile phase A/acetonitrile (60:40, v/v). The gradient program was as follows: from 2 to 6% B in 16 min, from 6 to 10% B in 4 min, from 10 to 17% B in 4 min, from 17 to 36% B in 14 min, from 36 to 38.5% B in 2 min, from 38.5 to 60% B in 13 min, from 60 to 100% B in 5 min, and from 100 to 2% B in 2 min. A 10 min re-equilibration time was used after each analysis. The flow rate used was set at 0.50 mL/min throughout the gradient. The effluent from the RP-HPLC column was split using a T-type phase separator before being introduced into the mass spectrometer (split ratio = 1:3). Thus, the flow that arrived in the ESI-TOF-MS detector was 0.2 mL/min. The column temperature was maintained at 25 °C, and the injection volume was 10 μL. The RP-HPLC system was coupled to a microTOF (Bruker Daltonics, Bremen, Germany), an orthogonal−accelerated time-of-flight mass spectrometer (oaTOF-MS) equipped with an ESI interface. Parameters for analysis were set using negative ion mode with spectra acquired over a mass range from m/z 50 to 1300. The optimum values of the ESI-MS parameters were capillary voltage, + 4.5 kV; drying gas temperature, 190 °C; drying gas flow, 7.0 L/min; and nebulizing gas pressure, 21.7 psi.

The accurate mass data of the molecular ions were processed through the newest software Data Analysis 4.0 (Bruker Daltonics), which provided a list of possible elemental formulas by using the Smart Formula Editor. The Editor uses a CHNO algorithm, which provides standard functionalities such as minimum/maximum elemental range, electron configuration, and ring-plus double bond equivalents, as well as a sophisticated comparison of the theoretical with the measured isotope pattern (sigma value) for increased confidence in the suggested molecular formula. The widely accepted accuracy threshold for confirmation of elemental compositions has been established at 5 ppm. During the development of the HPLC method, external instrument calibration was performed using a Cole Palmer syringe pump (Vernon Hills, IL) directly connected to the interface, passing a solution of sodium formate cluster containing 5 mM sodium hydroxide in the sheath liquid of 0.2% formic acid in water/isopropanol 1:1 (v/v). Using this method, an exact calibration curve based on numerous cluster masses each differing by 68 Da (NaCOOH) was obtained. Due to the compensation of temperature drift in the microTOF, this external calibration provided accurate mass values (better than 5 ppm) for a complete run without the need for a dual sprayer setup for internal mass calibration.

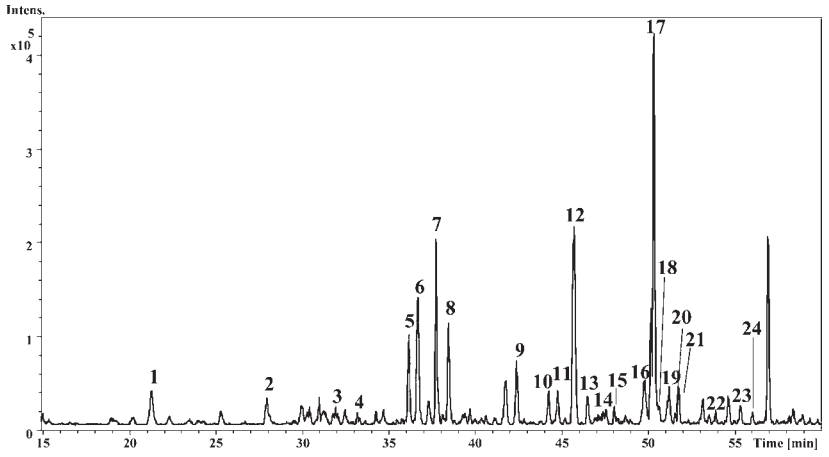

Figure 1. Base peak chromatogram of a bound phenolic extract of buckwheat flour. The numbers of the peaks in this figure coincide with the compound numbers in Table 1.

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 comparisons (one-way ANOVA), $p < 0.05$ level, were evaluated using Statistica 6.0 software (2001, StatSoft, Tulsa, OK).

RESULTS AND DISCUSSION

Identification of Bound Phenolic Compounds in Flour Hydrolyzed Extract. The identification of free phenolic compounds has already been performed in our previous work;[27] therefore, the first step of this work was the identification of bound phenolic compounds.

The analysis of the hydrolyzed extracts by RP-HPLC-ESI-TOF-MS revealed that flavonoids were the major class of bound phenolic compounds in buckwheat flours. Twenty-four phenolic compounds, 17 flavonoids and 7 derivative phenolic acids, were characterized.

The base peak chromatogram (BPC) of a hydrolyzed extract is illustrated in Figure 1. Under the used RP-HPLC-ESI-TOF-MS method, a large number of phenolic compounds present in buckwheat flours were detected. These are summarized in Table 1, with their formula, selected ion, experimental and calculated m/z, MS fragments, error (ppm), sigma (σ) value, tolerance, and retention time.

All detected compounds observed in Table 1 exhibited good σ values smaller than 0.05 and mass accuracy (ppm) as indicated by the error values even when a low tolerance was chosen (5 ppm).

To identify compounds for which no commercial standards were available, the generated molecular formula, obtained by the Smart Formula Editor, and the obtained fragments of each were used.

All of the compounds, except compounds **2**, **10**, **12**, **14**, and **16**, have been previously identified,[27] and their presence in free and bound form has been confirmed. [28]

The ion at m/z 315.0695 (peak 2) at 27.9 min reported a molecular formula $C_{13}H_{15}O_9$ $[M - H]^-$ and a fragment ion at m/z 153.0126 (molecular formula $C_7H_5O_4$). The molecular formula of the fragment suggested the presence of protocatechuic acid, and the loss of 162 u showed the presence of glucose; for this reason, compound **2** was identified as protocatechuic-4-*O*-glucoside acid. As far as we know, this is the first time that this compound has been determined in buckwheat, although the presence of protocatechuic acid in buckwheat has been already reported by Watanabe and co-workers.[29]

The ion in negative mode at m/z 317.0291 (compound **10**) was identified as myricetin according to the generated molecular formula ($C_{15}H_9O_8$) and its retention time at 44.2 min. The identity of this compound was sustained by coelution with the respective standard. The presence of myricetin in buckwheat in its free form was reported by Kalinova and Vrchotova,[30] but, as far as we know, this is the first time that it has been identified as a bound phenolic compound in buckwheat flour.

At a retention time of 45.7 min, a signal with m/z 197.0451 (molecular formula $C_9H_9O_5$) was detected. In this case, the coelution with standard confirmed the presence of syringic acid.

The *p*-coumaric acid at m/z 163.0398 (molecular formula $C_9H_7O_3$) was detected at a retention time of 46.8 min (peak 14). The presence of this phenolic acid in buckwheat has been reported by other authors[28] and was confirmed by coelution with a commercial standard.

Another signal at m/z 575.1157 was revealed at 49.6 min (peak 16), which showed two fragments at m/z 289.0625 and 285.1020. The same fragmentation pattern was reported for procyanidin A dimer ((epi)catechin−(epi)catechin) by Monagas et al.[31]

The limit of quantitation (LOQ) of the method was assessed for syringic acid, (+)-catechin, rutin, and myricetin. Solutions at 0.87, 0.29, 0.93, and 1.04 μg/mL gave signal-to-noise ratios of approximately 9 (S/N $\approx$ 9) corresponding to the LOQs of syringic acid, (+)-catechin, rutin, and myricetin, respectively.

Impact of Spaghetti Making and Cooking Process on Phenolic Content. The free phenolic contents of the raw ingredient and end products investigated in this study are shown in Table 2. Rutin and swertiamacroside resulted in the first and second component of buckwheat flour (331.38 $\pm$ 1.13 mg rutin/kg and 235.93 $\pm$ 0.80 mg syringic acid/kg, respectively). Besides rutin and swertiamacroside, 2-hydroxy-3-*O*-β-D-glucopyranosyl-benzoic acid, protocatechuic-4-*O*-glucoside acid, and caffeic acid hexose were also found in significant quantities (54.81, 65.61, and 41.93 mg/kg, respectively) in buckwheat free fraction as phenolic acid derivatives. Also, some flavan-3-ol derivatives were quantified in buckwheat flour; (epi)afzelchin−(epi)catechin, (epi)afzelchin−(epi)catechin-*O*-dimethyl gallate, and (−)-epicatechin were the most representative compounds of this class. Important quantities of catechin-glucoside, (epi)afzelchin−

Table 1. Bound Phenolic Compounds Determined by RP-HPLC-ESI-TOF-MS in an Alkaline Extract of Buckwheat Flour

				m/z						
no.	compound	mol formula	selected ion	exptl	calcd	MS fragment	error (ppm)	σ value	tolerance (ppm) in generated mol formula	retention time (min)
1	2-hydroxy-3-*O*-β-D-glucopyranosylbenzoic acid	$C_{13}H_{15}O_9$	$[M-H]^-$	315.0704	315.0722		3.7	0.0051	5	21.2
2	protocatechuic-4-*O*-glucoside acid	$C_{13}H_{15}O_9$	$[M-H]^-$	315.0695	315.0722	153.0126	4.4	0.0055	5	27.9
3	caffeic acid hexose	$C_{15}H_{17}O_9$	$[M-H]^-$	341.0859	341.0878	179.0329	4.6	0.0223	5	32.1
4	catechin-glucoside	$C_{21}H_{23}O_{11}$	$[M-H]^-$	451.1209	451.1246	289.0678	4.2	0.0116	5	33.3
5	caffeic acid hexose	$C_{15}H_{17}O_9$	$[M-H]^-$	341.0860	341.0878	179.0342	3.3	0.0116	5	36.1
6	catechin	$C_{15}H_{13}O_6$	$[M-H]^-$	289.0698	289.0718		3.7	0.0395	5	36.6
7	swertiamacroside	$C_{21}H_{27}O_{13}$	$[M-H]^-$	487.1457	487.1463	179.0322	5.0	0.0011	5	37.7
8	(epi)afzelchin-(epi) catechin isomer A	$C_{30}H_{25}O_{11}$	$[M-H]^-$	561.1364	561.1402	543.1264, 435.1099, 425.0850, 289.0686, 271.0516	3.8	0.0155	5	38.4
9	epicatechin	$C_{15}H_{13}O_6$	$[M-H]^-$	289.0688	289.0718		3.6	0.0373	5	42.3
10	myricetin	$C_{15}H_9O_8$	$[M-H]^-$	317.0291	317.0303		3.8	0.0281	5	44.2
11	(epi)afzelchin-(epi) catechin isomer B	$C_{30}H_{25}O_{11}$	$[M-H]^-$	561.1361	561.1402	543.1264, 425.0831, 289.0701, 271.0516	4.4	0.0086	5	44.7
12	syringic acid	$C_9H_9O_5$	$[M-H]^-$	197.0451	197.0455		2.1	0.0002	5	45.7
13	orientin	$C_{21}H_{19}O_{11}$	$[M-H]^-$	447.0911	447.0933		4.9	0.0261	5	46.5
14	*p*-coumaric acid	$C_9H_7O_3$	$[M-H]^-$	163.0398	163.0401		1.7	0.0304	5	46.8
15	isorientin	$C_{21}H_{19}O_{11}$	$[M-H]^-$	447.0902	447.0933	357.0614, 327.0480	3.9	0.0184	5	47.5
16	procyanidin A	$C_{30}H_{23}O_{12}$	$[M-H]^-$	575.1157	575.1195	289.0625, 285.1020	3.6	0.0236	5	49.6
17	rutin	$C_{27}H_{29}O_{16}$	$[M-H]^-$	609.1434	609.1461		4.5	0.0057	5	50.3
18	vitexin	$C_{21}H_{19}O_{10}$	$[M-H]^-$	431.0843	431.0882		4.8	0.0130	5	50.3
19	hyperin	$C_{21}H_{19}O_{12}$	$[M-H]^-$	463.0882	463.0882		4.0	0.0155	5	51.1
20	epicatechin-gallate	$C_{22}H_{17}O_{10}$	$[M-H]^-$	441.0796	441.0827	289.0694, 169.0103	3.0	0.0087	5	51.2
21	isoquercitrin	$C_{21}H_{19}O_{12}$	$[M-H]^-$	463.0882	463.0849		3.3	0.0155	5	51.8
22	epiafzelchin-epicatechin-*O*-methyl gallate	$C_{38}H_{31}O_{15}$	$[M-H]^-$	727.1635	727.1668	455.1003, 289.0842, 271.0516	4.6	0.0155	5	53.4
23	quercitrin	$C_{21}H_{19}O_{11}$	$[M-H]^-$	447.0906	447.0933		4.9	0.0143	5	55.2
24	(−)-epicatechin-3-(3″-*O*-methyl) gallate	$C_{23}H_{19}O_{10}$	$[M-H]^-$	455.0966	455.0984	289.0696, 183.0348	3.8	0.0295	5	56.0

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Table 2. Free Phenolic Compounds Determined by RP-HPLC-ESI-TOF-MS in Whole Buckwheat Flour and Uncooked and Cooked Spaghetti[a]

compound	whole buckwheat flour	uncooked spaghetti	cooked spaghetti
2-hydroxy-3-*O*-β-D glucopyranosylbenzoic acid[b]	54.81 ± 0.03 (a)	34.28 ± 0.12 (b)	12.30 ± 1.17 (c)
caffeic acid hexose[b]	65.61 ± 0.22 (a)	36.98 ± 1.41 (b)	10.63 ± 1.86 (c)
protocatechuic-4-*O*-glucoside acid[b]	41.93 ± 0.87 (a)	21.81 ± 0.07 (b)	8.07 ± 1.14 (c)
catechin-glucoside[c]	4.52 ± 0.15 (a)	3.37 ± 0.01 (b)	1.70 ± 0.04 (c)
catechin-glucoside[c]	22.10 ± 0.08 (a)	10.11 ± 0.15 (b)	1.30 ± 0.02 (c)
(+)-catechin[c]	10.81 ± 0.04 (a)	8.96 ± 0.08 (b)	3.75 ± 0.18 (c)
catechin-glucoside[c]	12.67 ± 0.04 (a)	4.35 ± 0.16 (b)	2.43 ± 0.07 (c)
swertiamacroside[b]	235.93 ± 0.80 (a)	75.14 ± 1.12 (b)	23.58 ± 1.15 (c)
procyanidin B_2 [c]	7.39 ± 0.03 (a)	3.90 ± 0.10 (b)	1.40 ± 0.08 (c)
(−)-epicatechin[c]	31.17 ± 0.11 (a)	19.19 ± 0.36 (b)	10.25 ± 0.40 (c)
(epi)afzelchin-(epi)catechin[c]	11.34 ± 1.96 (a)	5.70 ± 0.37 (b)	1.12 ± 0.04 (c)
procyanidin B_2-3-*O*-gallate[c]	3.93 ± 0.01 (a)	1.04 ± 0.02 (b)	0.38 ± 0.02 (c)
orientin[d]	1.38 ± 0.10 (a)	0.47 ± 0.00 (b)	nd[f]
isorientin[d]	1.80 ± 0.10 (a)	0.48 ± 0.01 (b)	0.19 ± 0.00 (c)
rutin[d]	331.38 ± 1.13 (a)	243.20 ± 0.02 (b)	148.18 ± 0.70 (c)
vitexin[d]	3.22 ± 0.01 (a)	1.25 ± 0.17 (b)	0.73 ± 0.02 (c)
(epi)afzelchin−(epi) afzelchin−(epi)catechin[c]	9.46 ± 0.03 (a)	3.79 ± 0.26 (b)	1.35 ± 0.30 (c)
hyperin[d]	1.26 ± 0.01 (a)	0.91 ± 0.00 (b)	0.33 ± 0.03 (c)
(epi)catechin-gallate[c]	4.82 ± 0.02 (a)	2.81 ± 0.02 (b)	0.71 ± 0.14 (c)
isoquercitrin[d]	2.66 ± 0.01 (a)	1.31 ± 0.10 (b)	0.33 ± 0.03 (c)
(epi)afzelchin−(epi)catechin[c]	27.83 ± 0.09 (a)	11.59 ± 0.00 (b)	4.60 ± 0.49 (c)
(epi)afzelchin−(epi) catechin-*O*-methyl gallate[c]	14.48 ± 0.05 (a)	6.41 ± 0.05 (b)	2.86 ± 0.12 (c)
luteolin-glycoside[d]	<LOQ	nd	nd
(epi)afzelchin−(epi)catechin[c]	<LOQ	nd	nd
(epi)afzelchin−(epi)catechin[c]	7.23 ± 0.02 (a)	1.30 ± 0.03 (b)	0.65 ± 0.01 (c)
quercitrin[d]	1.18 ± 0.00 (a)	0.47 ± 0.07 (b)	nd
(−)-epicatechin-3-(3″-*O*-methyl) gallate[c]	5.70 ± 0.02 (a)	3.45 ± 0.24 (b)	1.08 ± 0.10 (c)
procyanidin B_2 dimethyl gallate[c]	11.42 ± 0.04 (a)	3.54 ± 0.07 (b)	1.54 ± 0.07 (c)
(epi)afzelchin−(epi) catechin-*O*-dimethyl gallate[c]	46.90 ± 0.16 (a)	23.11 ± 1.65 (b)	10.02 ± 1.21 (c)
(epi)catechin-*O*-3,4-dimethyl gallate[c]	23.04 ± 0.08 (a)	7.54 ± 0.56 (b)	4.11 ± 0.00 (c)
quercetin[e]	1.50 ± 0.04 (a)	0.37 ± 0.01 (b)	nd
dihydroxytrimethoxyisoflavan[c]	11.44 ± 0.04 (a)	8.06 ± 0.67 (b)	3.59 ± 0.48 (c)
sum	1008.91 ± 2.50 (a)	545.50 ± 4.97 (b)	275.15 ± 1.65 (c)

[a] Different letters in the same row indicate significantly different values ($p < 0.05$). [b] mg syringic acid/kg dw. [c] mg (+)-catechin/kg dw. [d] mg rutin/kg dw. [e] mg myricetin/kg dw. [f] nd = not detected.

(epi)catechin-*O*-methyl gallate, (epi)catechin-*O*-3,4-dimethyl-gallate, (+)-catechin, procyanidin B_2, and their derivatives were also detected. Moreover, the typical buckwheat glycosylflavone isomer compounds, such as orientin/isoorientin, quercitrin/isoquercitrin, hyperin, and vitexin were quantified. As reported in our recent work,[32] dihydroxytrimethoxyisoflavan was characterized. Small quantities of quercetin were detected in whole buckwheat flours. The flavonoids constituted 60% of the free phenolic fraction.

The pasta-making process (mixing, extrusion, and drying) caused a loss of 45.9% of the total phenolic compounds. This can be explained by the temperature rise during the extrusion and the high temperature (about 95 °C) reached during the drying. The single phenolic compounds showed a different rate of degradation; swertiamacroside, procyanidin B_2-3-*O*-gallate, orientin, isorientin, epiafzelchin−epiafzelchin−epicatechin, (epi)afzelchin−(epi)catechin, epiafzelchin−epicatechin-*O*-methyl gallate, quercitrin, procyanidin B_2 dimethyl gallate, epicatechin-*O*-3,4-dimethylgallate, and quercetin were reduced by 50.6−82.0%. The degradation of the other phenolic compounds was in the range of 17.1−49.8%.

Further degradation of phenolic compounds was obtained after cooking of spaghetti. The boiling process induced a degradation of 52.9% of total phenolic compounds.

For all of the compounds, except epicatechin, rutin, and epicatechin-*O*-3,4-dimethylgallate, were reported losses of >50%.

Table 3. Bound Phenolic Compounds Determined by RP-HPLC-ESI-TOF-MS in Whole Buckwheat Flour and Uncooked and Cooked Spaghetti[a]

compound	whole buckwheat flour	uncooked spaghetti	cooked spaghetti
2-hydroxy-3-*O*-β-D-glucopyranosylbenzoic acid[b]	15.45 ± 0.64 (a)	2.29 ± 0.02 (b)	0.16 ± 0.00 (c)
protocatechuic-4-*O*-glucoside acid[b]	8.45 ± 0.42 (a)	1.19 ± 0.05 (b)	0.20 ± 0.00 (c)
caffeic acid hexose[b]	1.42 ± 0.03 (a)	1.04 ± 0.06 (b)	0.35 ± 0.03 (c)
catechin-glucoside[c]	0.16 ± 0.02	nd[f]	nd
caffeic acid hexose[b]	22.04 ± 1.65 (a)	11.38 ± 1.66 (b)	3.94 ± 0.02 (c)
(+)-catechin[c]	125.39 ± 2.66 (a)	44.85 ± 0.11 (b)	19.47 ± 2.13 (c)
swertiamacroside[b]	42.27 ± 0.44 (a)	18.15 ± 0.17 (b)	7.65 ± 0.72 (c)
(epi)afzelchin−(epi)catechin[c]	64.09 ± 1.36 (a)	27.13 ± 3.87 (b)	12.47 ± 0.04 (c)
(−)-epicatechin[c]	80.75 ± 1.72 (a)	18.68 ± 0.29 (b)	7.94 ± 0.38 (c)
myricetin[e]	3.72 ± 0.01 (a)	1.56 ± 0.10 (b)	nd
(epi)afzelchin−(epi)catechin[c]	34.71 ± 0.74 (a)	11.88 ± 0.53 (b)	3.69 ± 0.01 (c)
syringic acid[b]	76.08 ± 0.26 (a)	43.26 ± 1.37 (b)	30.57 ± 0.86 (c)
orientin[d]	0.56 ± 0.01 (a)	0.30 ± 0.01 (b)	0.10 ± 0.01 (c)
p-coumaric acid[b]	6.00 ± 0.13 (a)	2.59 ± 0.07 (b)	1.45 ± 0.00 (c)
isorientin[d]	1.95 ± 0.04 (a)	1.09 ± 0.00 (b)	0.69 ± 0.05 (c)
procyanidin A[c]	7.25 ± 0.02 (a)	5.30 ± 0.13 (b)	3.90 ± 0.00 (c)
rutin[d]	43.00 ± 0.15 (a)	20.01 ± 0.72 (b)	10.01 ± 0.70 (c)
vitexin[d]	9.28 ± 0.20 (a)	5.43 ± 0.02 (b)	1.87 ± 0.46 (c)
hyperin[d]	4.91 ± 0.10 (a)	2.83 ± 0.01 (b)	1.78 ± 0.17 (c)
epicatechin-gallate[c]	21.82 ± 0.46 (a)	10.62 ± 0.32 (b)	2.53 ± 0.00 (c)
isoquercitrin[d]	7.52 ± 0.16 (a)	3.62 ± 0.01 (b)	1.51 ± 0.12 (c)
epiafzelchin−epicatechin-*O*-methyl gallate[c]	4.35 ± 0.08 (a)	3.34 ± 0.07 (b)	1.54 ± 0.00 (c)
quercitrin[d]	4.08 ± 0.09 (a)	1.67 ± 0.01 (b)	0.81 ± 0.15 (c)
(−)-epicatechin-3-(3″-*O*-methyl) gallate[c]	27.08 ± 0.58 (a)	10.53 ± 1.18 (b)	4.83 ± 0.00 (c)
sum	612.33 ± 8.93 (a)	248.74 ± 10.03 (b)	116.93 ± 3.48 (c)

[a] Different letters in the same line indicate significantly different values ($p < 0.05$). [b] mg syringic acid/kg dw. [c] mg (+)-catechin/kg dw. [d] mg rutin/kg dw. [e] mg myricetin/kg dw. [f] nd = not detected.

The phenolic degradation after boiling was significantly different ($p < 0.05$) with respect to the degradation obtained after the technological process. This effect can be laid to the solubility of phenolic compounds in cooking water.

Table 3 reports the bound phenolic content in buckwheat flour and spaghetti. The phenolic composition was similar to the free phenolic fraction; additionally, some phenolic acids such as syringic and *p*-coumaric acid, procyanidin A, and myricetin were determined.

Bound phenols content in buckwheat flour was 612.33 mg/kg and, according to Inglett et al.,[28] represents 36% of the total phenolic content. The principal components were represented from (+)-catechin and (−)-epicatechin for which were reported contents of 125.39 and 80.75 mg/kg, respectively. Syringic acid was the third bound phenolic compound at 76.08 mg/kg. Interesting quantities of swertiamacroside, (epi)afzelchin−(epi)catechin, rutin, epicatechin-gallate, and (−)-epicatechin-3-(3″-*O*-methyl) gallate were also quantified. Seventy-two percent of the bound phenolic fraction was represented by flavonoids.

Likewise for the free phenolic fraction, the technological process affected the bound phenolic fraction; indeed, the total bound phenolic content of buckwheat spaghetti was 248.74 mg/kg. That represented a decrease of 59.4% of phenolic compounds. This value was higher than the value obtained for the free fraction. These data confirmed the results of Dietrych-Szostak and Oleszek,[33] who demonstrated that the buckwheat flavonoids experienced a drastic reduction when buckwheat seeds were thermally treated.

The cooking process determined a decrease of total bound phenolic content of 53%; indeed, the total phenolic content was 116.93 mg/kg. The loss of the single phenolic compound ranged from 26.4 to 100%.

Table 4 shows the content of total phenolic compounds (free + bound) in uncooked and cooked spaghetti and in cooking water.

The results showed that only 46% of total phenolic compounds tolerated the cooking process; 11.6% of the total phenolic fraction was dissolved in cooking water and 42.4% was degraded. Rutin, procyanidin A, and syringic acid demonstrated the highest tolerance to the cooking process with cooking losses of <40%. Cooking water showed interesting quantities of catechin and epicatechin and (epi)afzelchin−(epi)catechin. Nevertheless, quercetin and myricetin showed a total degradation during the cooking process. This confirmed the data of Buchner and coworkers,[34] who reported the degradation of quercetin in an aqueous model system.

In this work, a powerful RP-HPLC-ESI-TOF-MS analytical method has been used to characterize bound phenolic compounds present in buckwheat. This method was used to determine free and bound phenolic fractions in raw material and uncooked and cooked buckwheat spaghetti. The results showed that technological processing and cooking negatively affected the phenolic content detected in whole meal, and this result was in

Table 4. Total (Free + Bound) Phenolic Compounds Determined by RP-HPLC-ESI-TOF-MS in Uncooked and Cooked Spaghetti and in Cooking Water[a]

compound	uncooked spaghetti	cooked spaghetti	% of loss	cooking water
2-hydroxy-3-*O*-β-D-glucopyranosylbenzoic acid[b]	36.57 ± 0.85	12.45 ± 0.23	65.9	0.09 ± 0.00
protocatechuic-4-*O*-glucoside acid[b]	22.99 ± 0.19	8.27 ± 0.31	64.0	0.01 ± 0.00
caffeic acid hexose[b]	49.40 ± 0.33	14.92 ± 0.19	69.8	0.45 ± 0.01
catechin-glucoside[c]	17.83 ± 0.10	5.43 ± 0.09	69.5	0.48 ± 0.01
(+)-catechin[c]	53.81 ± 0.24	23.23 ± 0.37	56.8	25.98 ± 0.45
swertiamacroside[b]	93.30 ± 0.47	31.22 ± 1.02	66.5	1.66 ± 0.03
procyanidin B_2[c]	3.90 ± 0.13	1.40 ± 0.02	64.2	2.22 ± 0.04
(−)-epicatechin	37.87 ± 0.29	18.19 ± 0.26	52.0	15.93 ± 0.28
(epi)afzelchin−(epi)catechin[c]	57.60 ± 0.36	17.28 ± 0.51	70.0	17.94 ± 0.13
procyanidin B_2-3-*O*-gallate[c]	1.04 ± 0.06	0.38 ± 0.00	63.6	0.17 ± 0.00
orientin[d]	0.77 ± 0.02	0.10 ± 0.00	87.6	0.22 ± 0.00
isorientin[d]	1.57 ± 0.04	0.88 ± 0.06	44.2	0.48 ± 0.02
rutin[d]	263.21 ± 2.01	158.19 ± 1.87	39.9	3.54 ± 0.06
vitexin[d]	7.28 ± 0.16	2.60 ± 0.10	64.3	4.60 ± 0.08
(epi)afzelchin−(epi)afzelchin−(epi)catechin[c]	3.79 ± 0.08	1.35 ± 0.05	64.5	0.14 ± 0.00
hyperin[d]	3.75 ± 0.04	2.11 ± 0.09	43.7	1.10 ± 0.04
epicatechin-gallate[c]	13.43 ± 0.25	3.24 ± 0.47	75.9	1.91 ± 0.03
isoquercitrin[d]	4.93 ± 0.32	1.84 ± 0.02	62.6	2.96 ± 0.05
epiafzelchin−epicatechin-*O*-methyl gallate[c]	9.74 ± 0.61	4.41 ± 0.08	54.8	3.52 ± 0.06
quercitrin[d]	2.14 ± 0.09	0.81 ± 0.03	62.3	1.24 ± 0.02
(−)-epicatechin-3-(3″-*O*-methyl) gallate[c]	13.98 ± 0.13	5.91 ± 0.10	57.7	2.77 ± 0.05
procyanidin B_2 dimethyl gallate[c]	3.54 ± 0.05	1.54 ± 0.07	56.6	0.17 ± 0.00
(epi)afzelchin−(epi)catechin-*O*-dimethyl gallate[c]	23.11 ± 0.35	10.02 ± 0.18	56.7	nd[f]
(epi)catechin-*O*-3,4-dimethylgallate[c]	7.54 ± 0.99	4.11 ± 0.33	45.6	2.85 ± 0.05
quercetin[e]	0.37 ± 0.01	nd	100	nd
dihydroxytrimethoxyisoflavan[c]	8.06 ± 0.60	3.59 ± 0.14	55.5	nd
myricetin[e]	1.56 ± 0.03	nd	100	nd
syringic acid[b]	43.26 ± 2.22	30.57 ± 1.01	29.3	1.76 ± 0.03
p-coumaric acid[b]	2.59 ± 0.38	1.45 ± 0.24	44.1	nd
procyanidin A[c]	5.30 ± 0.98	3.90 ± 0.47	26.4	nd
sum	794.25 ± 7.89	369.36 ± 3.61	53.5	92.17 ± 0.63

[a] Phenolics in the cooking water expressed as mg/kg dw of the uncooked spaghetti. [b] mg syringic acid/kg dw. [c] mg (+)-catechin/kg dw. [d] mg rutin/kg dw. [e] mg myricetin/kg dw. [f] nd = not detected.

agreement with the data present in the literature.[35,36] The results emphasize that the high drying temperature affected the phenolic components, but this temperature was necessary to obtain spaghetti with good structural and textural properties during the making and cooking processes.[37] Indeed, a high drying temperature was applied when nonconventional raw materials were used as ingredients.[38,39] For this, further studies will be carried out to investigate the possibilities of producing gluten-free buckwheat spaghetti with a mixture of other raw materials and using a lower drying temperature.

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