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A New “Functional” Pasta Containing Tartary Buckwheat Sprouts as an Ingredient Improves the Oxidative Status and Normalizes Some Blood Pressure Parameters in Spontaneously Hypertensive Rats.

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ABSTRACT

Epidemiological studies have reported that some foods, particularly those rich in (poly)phenols, may reduce cardiovascular risk and metabolic disorders such as hypertension. Buckwheat sprouts have been suggested as a new raw material for the production of functional foods due to their high content of healthy compounds as rutin and quercetin. The aim of this paper is to evaluate the biological hypotensive and antioxidant responses of pasta containing tartary buckwheat sprouts (TBSP) on spontaneously hypertensive rats (SHR). In this study, dry tartary buckwheat sprouts were milled to obtain a powder that was used in the production of pasta containing 30% dry buckwheat sprouts and 70% durum wheat semolina. Afterwards, we analyzed the *in vitro* TBSP features compared with the control (durum wheat flour pasta, DWFP), and the *in vivo* effects of TBSP on SHR and their normotensive counterpart, Wistar Kyoto rats (WKY). The total phenolic content and antioxidant activity were higher in the TBSP compared to DWFP. The results showed that SHR fed TBSP exhibited higher plasma levels of the endogenous vasodilators bradykinin (BK) and nitric oxide (NO), a lower level of the vasoconstrictor endothelin-1 (ET-1), and an improved antioxidant capacity. These data suggest that TBSP may help reduce hypertension and oxidative stress *in vivo*.

INTRODUCTION

Epidemiological studies have reported that some foods, particularly those rich in phenolic compounds, may reduce cardiovascular risk¹ and metabolic disorders², such as hypertension.^{3,4} Hypertension affects more than 30% of adults and is the leading cause of morbidity and mortality worldwide.⁵ Numerous factors, such as the upregulation of the renin-angiotensin system (RAS)⁶, decreases in NO levels⁷, increases in ET-1 release⁸, reduction in the antioxidant capacity⁹, and increases in the inflammatory status¹⁰, are implicated in its pathophysiology and etiology. Thus, nutraceuticals and functional foods with high polyphenol contents might represent a potential alternative therapy for the treatment of hypertension, especially for borderline patients with marginally high blood pressure that does not warrant the prescription of blood pressure-lowering medications.¹¹

Buckwheat and, in particular, buckwheat sprouts have received increased interest in recent years due to the growing awareness of their potential as healthy foods.¹² Indeed, the content of polyphenols, such as rutin and quercetin, and of vitamins B1, B6, and C in buckwheat sprouts is higher than that found in buckwheat seeds and flour.¹³ Furthermore, *in vivo* studies have shown that buckwheat sprouts exhibit beneficial effects in the prevention of cardiovascular diseases¹⁴, the promotion of hypocholesterolemic and hypotriglyceridemic activities in a hamster model¹⁵, and the enhancement of anti-inflammatory effects. A recent study reported that buckwheat flour displays *in vitro* inhibitory activity against the angiotensin-I converting enzyme (ACE)¹⁶ most likely due to its high content of rutin¹⁷ and to 2"-hydroxynicotianamine.¹⁸ However, to the best of our knowledge, the antihypertensive effect of buckwheat sprouts as food has not yet been demonstrated *in vivo*.

In this study, we first compared the germinability and sprout yield of three different types of buckwheat seeds and sprouts (two common buckwheat cultivars and one tartary buckwheat landrace). The total polyphenol content and antioxidant capacities in seed flour and in dried sprouts were measured. Subsequently, we made pasta using the dry tartary buckwheat sprouts that showed the best germinability and *in vitro* antioxidant capacity as an ingredient and verified whether the healthy characteristics of the buckwheat sprouts were maintained throughout all of the production phases. To evaluate the biological hypotensive and antioxidant responses of pasta containing tartary buckwheat sprouts, we studied the biochemical effects *in vivo* by analyzing the levels of some blood pressure parameters, such as BK, NO, and ET-1, and the antioxidant status in WKY rats and SHR.

MATERIALS AND METHODS

Materials

Common buckwheat and tartary buckwheat were obtained from the Rangus mill (Vrhpolje pri Šentjerneju, Slovenia). All of the chemical reagents (methanol, ethanol, chloridric acid, Folin Ciocalteu reagent, sodium carbonate, gallic acid, iron (III) chloride hexahydrate, 2,4,6-(2-tripyridyl)-s-triazine, acetic acid, sodium acetate trihydrate, ferrous sulfate heptahydrate, 2,2-diphenyl-1-picrylhydrazyl, dihydrogen ammonium phosphate, orthophosphoric acid, acetonitrile, rutin hydrate and quercetin hydrate, Tween 20) were purchased from Sigma-Aldrich (St. Louis, USA). The bioassay kits (nitric oxide detection kit, bradykinin ELISA kit, endothelin-1 ELISA kit, and protein carbonyl ELISA kit) were manufactured by Enzo Life Science (Farmingdale, NY, USA). The hydroxyl radical antioxidant capacity (HORAC) assays were manufactured by Oxford Biomedical Research (Oxford, MI, USA), and the lipid peroxidation (MDA) assay kit was obtained from Biovision Research Products (Mountain View, CA, USA).

Seeding and sprout productions

Initially, two species of *Fagopyrum*, *F. esculentum* Moench (common buckwheat) and *F. tataricum* Gaertn (tartary buckwheat) were used. The former was represented by two commercially available cultivars, namely Lileja (Russian origin) and Darja (Slovenian cultivar), whereas the latter was represented by the Slovenian landrace Ljse.

For each seed sample, the weight of 1000 seeds was estimated using three replicates, each of which contained 100 seeds. The seed water content was estimated by measuring the weight loss after 3 days at 130°C.

The germination test was carried out in Petri dishes using sterile filter paper. The paper was water-saturated at the beginning and kept moist during the test. The germinability and germination power were estimated by recording the germination rates of four replicates ($n = 25$) for each seed sample. For each replicate, the number of seeds germinated was counted daily for 10 days. Germinability was reported as the mean percentage of germinated seeds at the tenth day. The percentage of germination calculated for each day was used to estimate the germination power of each accession.

For buckwheat sprout production, 200 g of seeds was surface-sterilized with UV rays for 3 h and pre-soaked in sterile water (with 3-4 drops of Tween 20) for 4 h. The germination trays were also UV-sterilized for 8 h. Two seed germination systems were compared. In the first (referred to as the “free-water” system), the seeds were placed into 30×45 cm plastic nets that were stretched over trays containing free water (about 10 L) agitated with an air pump. In the second system (referred to as the “no-free-water” system), the seeds were directly placed into 30×45 cm plastic trays with two foils of filter paper saturated with water. The germinating systems were incubated in a growth chamber at 22±2°C under dark conditions. In the “no-free-water” system, the filter paper was maintained moist (without free water) throughout the experiment. The seedlings in both systems were also sprayed daily to facilitate pericarp shedding. Ten days after the start of the experiments, sprouts were cut 2 mm above the crown. The length of the aerial was measured in 12 representative seedlings per accession. The plant material was weighted and dried at 55±2°C for 24 h, and the drying time was estimated to achieve a water content of approximately 7% in the biomass produced. To remove the remnants of the pericarp, the dried sprouts were crumbled by hand and sieved. The final weight of the dried sprout powder was recorded.

Pasta-making process

For our *in vitro* and *in vivo* studies, we used two different types of pasta (spaghetti), namely DWFP and TBSP. The DWFP was commercial pasta purchased from the local market. The TBSP was obtained by modifying basic pasta recipe: 30% of tartary sprout powder (milled with particle size similar to that of semolina) was mixed with durum wheat semolina. The pasta-making process consisted of kneading (pre-mixing for 15 min at room temperature and kneading for 10 min in vacuo at 40°C), extrusion (pressure range of 9.1-12.1 MPa and vacuum of 70 mmHg), and drying at low temperature (30°C for pre-drying and 4 h at 50-58°C for drying). The total moisture content of TBSP dough was 9,6%

To assess the cooking quality of the pasta, each type of spaghetti (100 g) was cooked in 1 L of boiling tap water, and the optimal cooking time was recorded as the time to when the white cores of the strands disappeared after squeezing them between two glass plates, according to Approved Method 66-50 of AACC International.¹⁹

The total organic matter (TOM) was determined by the standard method described by D'Egidio²⁰, and this amount represents the amount of surface material released from the cooked pasta

into the washing water after rinsing. TOM values greater than 2.1 g/100 g corresponded to low quality, whereas TOM values between 2.1 and 1.4 g/100 g corresponded to good quality, and TOM values less than 1.4 g/100 g indicated very good quality. The centesimal analysis of the pasta samples was performed using the method reported in the document “Decreto Ministeriale” 1994.²¹

Extract preparations

To measure the antioxidant activity, total polyphenol contents (free and bound) and rutin and quercetin concentrations, all samples were commingled and frozen prior to being freeze-dried (Thermo Electron Corporation, Waltham, MA, USA). For the preparation of soluble extracts, 1 g of the freeze-dried (seed flour and sprouts) was extracted with 6 mL of 80% methanol, under agitation for 2 h at room temperature. After centrifugation at 1000 g for 10 min, the supernatant was collected. The extraction residues were further used to extract the bound phenolic compounds according to the method described by Pellegrini.²² Finally the antioxidant activity and total polyphenol contents were expressed as the sum of the results obtained by the two extracts.

We also measured the antioxidant activity, total polyphenol content and free rutin and quercetin concentration in both the uncooked and cooked pasta products. The cooked samples were prepared as follows: 1 L of water was brought to a boil, and 100 g of pasta was then added. The pasta was boiled until it was optimally prepared (approximately 10 min). The cooked pasta samples were frozen prior to being freeze-dried. Subsequently, the freeze-dried samples (1 g) were extracted twice by shaking at room temperature in 4 mL of a methanol:water solution (80:20 v/v) and acidified with 1% HCl for 2 h.²³

To analyze the polyphenols released into the water during boiling, 35 mL of acetone was added to 15 mL of cooking water.²⁴ The supernatant was separated from the precipitated starch by centrifugation at 1000 g for 10 min, and the polyphenol content of the supernatant was then determined.

Total phenolic content determination

Total phenols (TP) were determined using a modification of the Folin-Ciocalteu standard method.²⁵ Briefly, the assay was conducted by mixing 4 mL of deionized water, 0.25 mL of extracts (see ‘Extract preparations’ in this section), 0.25 mL of Folin-Ciocalteu reagent, and 0.5 mL of Na₂CO₃. After 30 min at room temperature, the absorbance of the mixture at 725 nm was measured. A

standard curve was prepared with gallic acid. The final results were expressed as mg of gallic acid equivalents (GAE)/g of dry weight (DW). All of the analyses were conducted in triplicate and the results report the sum of bound and soluble phenolic compounds.

Determination of rutin and quercetin concentration

Sample extracts were filtered through a syringe filter unit (0.22 μm , Millex Durapore PVDF, Millipore, Billerica, USA). The filtrate was injected, using a 20 μL loop, into a HPLC system (DIONEX – Thermo Scientific, Chicago, IL, USA) that consisted of a quaternary gradient pump (P680), a diode array UV-visible detector (PDA-100), and a Chromeleon software as data processing system. An Acclaim® 120 C18 column (5 μm 120Å, 4.6 \times 250 mm), kept at 30°C, was used as stationary phase. Separation, according to Ritchey and Waterhouse²⁶, was performed by a multistep gradient of ternary mobile phase: solvent A was 50 mM dihydrogen ammonium phosphate adjusted to pH 2.6 with orthophosphoric acid, solvent B was 20% A with 80% acetonitrile, and solvent C was 0.2 M orthophosphoric acid adjusted with NaOH to pH 1.5. The flow rate was 0.5 mL/min, and the effluent was monitored at 365 nm. Rutin and quercetin were quantified on the basis of calibration curve of external standards prepared in 80% methanol. Results were expressed as mg/g of dry weight.

DPPH radical scavenging activity assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was measured according to the method described by Beta and co-workers.²⁷ In detail, 6×10^{-5} mol/L of DPPH radical was prepared in methanol. The DPPH solution (3.9 mL) was then added to 0.1 mL of the extracts, and the reaction was allowed to run for 30 min. The absorbance (A) of the solution was measured at 515 nm against a blank of methanol at $t = 30$ min. The DPPH radical scavenging activity (%) of the samples was calculated using the following formula: $(1 - [A_{\text{sample}}_{t=30}/A_{\text{control}}_{t=0}] \times 100)$. All of the analyses were conducted in triplicate.

ORAC assay

The oxygen radical absorbance capacity (ORAC) was determined using the hydroxyl radical antioxidant capacity (HORAC) assay kit according to the manufacturer's instructions. The final ORAC values were expressed as gallic acid equivalents and determined according to the standard curve. All of the analyses were conducted in triplicate.

Determination of FRAP activity

A ferric reducing antioxidant power (FRAP) assay was performed according to the method described by Benzie and Strain²⁸, which was adapted for 96-well plates and an automatic reader (Infinite 2000, Tecan, Salzburg, Austria). The method is based on the reduction of the Fe³⁺-2,4,6-tripyridyl-s-triazine (TPTZ) complex to its ferrous form at a low pH. Briefly, 160 µL of FRAP assay solution (consisting of 20 mM ferric chloride solution, 10 mM TPTZ solution, and 0.3 M acetate buffer at pH 3.6) was prepared daily, mixed with 10 µL of the sample, standard, or blank, and dispensed into each well of a 96-well plate. The absorbance was measured at 595 nm at 37°C after 30 min of incubation. All of the analyses were conducted in triplicate. The final results are expressed as µmol Fe²⁺ equivalents/L of plasma or mmol Fe²⁺ equivalents/g of the DW of the samples, and the results were obtained using a standard curve with different concentrations of ferrous sulfate heptahydrate (FeSO₄·7H₂O).

Experimental animals and diets

Twelve-week-old male SHR and normotensive WKY rats were obtained from the Charles River Laboratory (Lecco, Italy). The rats were housed under conventional conditions of adequate temperature (23°C) and humidity (60%) control with a 12-h light/12-h dark cycle and were acclimatized to these conditions for 7 days with free access to food (AIN93-M diet, purchased from Laboratories Dottori Piccioni, Milano) and water.

All of the experiments with rats were performed in accordance with the Italian Laws (D.L. 116/92 and following additions, which enforce the EU86/609 directives: Council Directive 86/609/EEC of 24 November 1986 on the approximation of law, regulations, and administrative provisions of the member states regarding the protection of animals used for experimental and other scientific purposes) and with the approval of Health Ministry (authorization number DGSAF 0009805-A-23/05/2012) and of animal care committee of Tuscia University.

After the acclimatization period, the rats were randomly divided into two groups (n = 5), namely DWFP rats and TBSP rats. Every day for six weeks, the rats were fed approximately 5 g of the respective type of pasta. The calories of the different types of pasta are shown in Table 1.

The body weight and food intake were recorded weekly over the six-week experimental period. The treatment was stopped 1 day before the end of the experiment to study the long-term effects of the pasta without acute administration effects. At the end of the experimental period, the

animals were fasted overnight, anesthetized with isoflurane, and sacrificed. Blood was drawn from the heart into tubes containing EDTA, sodium citrate, or heparin (Venosafe Terumo, Leuven Belgium). The plasma was separated by centrifugation at 3000 g and 4°C for 20 min, and the supernatant was utilized for analysis.

Plasma biochemical analysis

The antioxidant capacity was analyzed using the FRAP and ORAC methods as described above. The concentration of carbonyl proteins was analyzed by a protein carbonyl ELISA kit according to the manufacturer's instructions. The protein carbonyl content was expressed as nmol/mg protein.

The concentration of malondialdehyde (MDA) was analyzed using a lipid peroxidation assay kit according to the manufacturer's instructions. The MDA content was expressed as nmol/mL plasma. The endothelin-1 and bradykinin levels were analyzed by immunoassay kits (endothelin-1 ELISA kit and bradykinin ELISA kit, respectively). The plasma NO metabolite (NO₂ and NO₃) levels were analyzed through a colorimetric assay kit (nitric oxide detection kit). NO₂ and NO₃ are stable metabolites of NO after deproteinization of the plasma through concentrator-based filtration (Corning Spin-X concentrators, Corning Incorporated, MA, USA).

Statistical Analysis

According to the comparisons tested, the data were subjected to one- or two-way analyses of variance (ANOVA), and the means were separated by Duncan's multiple range test. The percentages of germinability recorded at day 10 were subjected to ANOVA after angular transformation. All of the other results are expressed as the mean \pm S.E (Standard Error).

RESULTS

Cultivation of buckwheat sprouts

The two *F. esculentum* cultivars showed a 1000-seed weight greater than 20 g, although Lileja had significantly larger seeds than Darja (Table 2). The *F. tataricum* species had much smaller seeds, which weighed approximately one-half of that of the cultivated buckwheat (Table 2). In the germination test, all three seed samples (Lileja, Darja, and Ljse) showed a high germination power,

ranging from 85 to 90% of the total germinable seeds on the third day (data not shown). The final germinability was significantly higher for Ljse and Lileja compared with the Darja cultivar and reached a maximum of 90% in the *F. tataricum* landrace (Table 2).

The sprout length was significantly longer for the Darja and Ljse cultivars compared with Lileja (Table 2). The sprout yield, which is expressed as fresh weight, was higher in *F. tataricum* compared with *F. esculentum* (Table 2). The sprout yield of the Darja cultivar was the lowest, which is expected due to its lower germinability. The Ljse sprout production was higher than that of Lileja in the “free-water” system, although the difference in the dried weight was not significant (Table 2). The mean Ljse sprout production obtained in the “no-free-water” system was approximately two-thirds of that obtained in the “free-water” system (data not shown). Because the second system is easier and allows the management of more trays, we adopted this system for the production of the main stock of dried tartary sprouts.

Total polyphenol content and antioxidant capacity of flour and dried sprouts

Total polyphenols and antioxidant capacities of buckwheat seed flour and dried sprouts were examined. The results showed that, once dried, all the studied types of sprouts had higher polyphenol contents and increased antioxidant capacity compared with the respective seed flour (Fig. 1). However, the dried sprouts of tartary buckwheat (Ljse) exhibited higher total polyphenol content (Fig. 1; A) and antioxidant capacity (Fig. 1; C, D, and E) compared with the dried sprouts of common buckwheat (Lileja and Darja).

Characteristics of the pasta

The spaghetti containing buckwheat sprout powder had a slightly rough irregular surface with a light brown appearance, which was related to the innate color of dried buckwheat sprouts. The centesimal analysis, which is reported in Table 3, showed that TBSP had a good level of proteins and was characterized by a lower level of carbohydrates compared to DWFP. The sensorial evaluation of the cooked pasta samples revealed that the sprout-enriched sample exhibited a lower quality compared with commercial pasta (Table 3), as shown by the higher values of TOM. This result indicates that it is necessary to improve the quality of this product.

Total polyphenol content, rutin and quercetin concentration, and antioxidant capacity in the flour and pasta

The main effect highlighted from the pasta-making process was a reduction in the total polyphenol concentration and in rutin in the experimental pasta (Fig. 1 panel B, and Table 4) compared with the original ingredients (Fig. 1 panel A, and Table 4). In addition, our results showed that during the pasta-making process there was an increase in quercetin (Table 4). Subsequently, the cooking of the pasta resulted in a further reduction in the total polyphenol content compared with the original content (Fig. 1 panel B) due to their release in cooking water (about 40,6 % of loss). Nevertheless the reduction of rutin and quercetin observed in the cooking pasta was not significant (Table 4).

The measurement of the antioxidant capacity of cooked and uncooked pasta was performed through three different techniques (FRAP, DPPH, and ORAC). As shown in Fig. 1 (panels F, G, and H) the pasta production process induced an increase in antioxidant capacity compared with the original content (Fig. 1; C, D, and E).

In vivo studies

To study the biological responses of pasta containing tartary buckwheat sprouts *in vivo*, we used SHR as the animal model and their normotensive counterpart, WKY rats, as the controls. There were no significant differences in the weight and the consumption of food or water (Table 1) between the two experimental groups considering that the two types of pasta had a similar caloric amount.

The main effect observed after six weeks of feeding was a general improvement in the plasma antioxidant capacity of the rats that received TBSP with respect to those fed DWFP (Fig. 2; A and B); note that this effect was observed in both WKY rats and SHR. Furthermore, the plasmatic oxidative response (protein carbonyl concentration and MDA) was greatly improved in the rats fed the spaghetti made with buckwheat sprouts (Fig. 2; C and D).

Moreover, our results showed that the SHR fed pasta enriched with dried buckwheat sprouts for six weeks exhibited higher plasma levels of the endogenous blood pressure-lowering parameters BK and NO and a reduction in the vasoconstrictor ET-1 level compared with SHR fed with DWFP (Fig. 2; E, F, and G).

DISCUSSION

In this report, we demonstrate the excellent effect of a new pasta, which was developed in our laboratory and contains 30% dry tartary buckwheat sprouts, for the improvement of the oxidative status and some blood pressure parameters in SHR.

This research was performed in three different phases. In the first step, to ensure that our starting raw material was the best both from a commercial point of view (productivity) and from a healthy aspect, we compared the germinability, sprout yield, total phenol content, and antioxidant capacities of three different types of buckwheat seeds flour and sprouts (two common buckwheat cultivars and one tartary buckwheat landrace). After this comparative study, we analyzed the effects of food processing and cooking on the availability of these beneficial components and then studied the biological responses *in vivo*.

As a first result, we found that tartary buckwheat sprouts had the best germinability, *in vitro* antioxidant capacity and rutin content (the major flavonoid found in buckwheat) both with respect to the buckwheat seed flour that within the three varieties of sprouts evaluated. This result was in line with the research study conducted by Liu²⁹ who found that the polyphenol and rutin content was 5-fold higher in tartary than in common buckwheat sprouts. Based on these results, the dried Ljse tartary buckwheat sprouts were chosen as the ingredient for the formulation and production of functional pasta.

To achieve the maximum benefits of healthy food compounds, it is critical to understand the effects of food formulation, food processing, and cooking on the availability of the beneficial components. Therefore, this research investigated the effect of processing and cooking on the polyphenol contents and antioxidant properties of the different types of pasta in comparison with the raw materials before the pasta-making process.

Our results showed that the main effect of the pasta-making process was a general reduction in the polyphenol concentration compared with the original ingredients. The observed reduction in the polyphenol content during pasta processing is in agreement with previous studies, which found a combined impact from the addition of water, oxygen, and high temperature during kneading on the oxidative degradation of polyphenols.^{30,31} Therefore, despite of the observed polyphenol reductions, the spaghetti made with buckwheat sprout powder maintained high level of total phenolic compounds. Moreover, when the pasta was cooked, there was a further reduction in the total polyphenol content

compared with the original content, but the polyphenol amount after cooking was still higher in the spaghetti made with buckwheat sprouts compared with commercial pasta. The loss of polyphenols during pasta making is always charged to the free fraction of antioxidants and is for this reason that we have determined both the free and bound fraction of polyphenols. It is also important to emphasize that the reduction of rutin and quercetin observed when the pasta was cooked was not significant.

The analysis of the antioxidant capacity before and after the pasta-making process and after cooking showed that the pasta production process induced an increase in the antioxidant capacity. This increase is consistent with the fact that different food preparation methods can influence the antioxidant activity.³² For example, it is possible that the cooking process releases, or at least exposes, some Maillard reaction products that contribute to the overall antioxidant activity.^{33, 34} Moreover we observed that during the pasta making process the rutin was in part transformed in quercetin and is known that quercetin has higher antioxidant activity than rutin.³⁵

To study the *in vivo* biological responses to a diet containing TBSP, we used hypertensive rats as an animal model and their normotensive counterpart, WKY rats, as the controls. The main effect observed after six weeks of feeding was a general improvement in the plasma antioxidant capacity of the rats that received TBSP compared with those fed DWFP; this effect was observed in both WKY rats and SHR. Furthermore, the plasmatic oxidative response (protein carbonyl concentration and MDA) was strongly improved in rats fed the spaghetti made with buckwheat sprouts.

The past decade has exhibited a growing interest in the use of buckwheat for the treatment of hypertension. Indeed, this pseudocereal is rich in blood pressure-lowering compounds, such as rutin and γ -aminobutyric acid (GABA).^{36,37} Buckwheat also contains a very potent ACE-inhibitor noted as 2''-hydroxynicotianamine.³⁸ These pressure-lowering buckwheat compounds appear to be more plentiful in buckwheat sprouts than buckwheat seeds, and it has been reported that germinated buckwheat grains exert antihypertensive effects in SHR.¹⁴ Moreover, Koyama³⁹ recently identified and purified some antihypertensive peptides from fermented buckwheat sprouts.

Despite all of these potential activities, the actual antihypertensive effect of buckwheat sprouts as food has not yet been demonstrated. Thus, the other biochemical parameter analyzed *in vivo* was the levels of important molecules involved in blood pressure control, such as BK, NO, and ET-1. Our results showed that SHR fed TBSP for six weeks exhibited higher plasma levels of the blood pressure-lowering parameters BK and NO and a reduction in the level of the vasoconstrictor ET-1 compared with SHR fed DWFP. Our data indicate that the TBSP diet is able to restore the levels of

BK, NO and ET-1 in SHR to levels similar to those observed in WKY rats. Because we highlighted that the pasta made with buckwheat sprouts exhibited a higher level of rutin but in particular of its aglycone quercetin, the effect observed *in vivo* may be due either to the higher amount of total phenolic content in TBSP or to this specific flavonoid compounds. However, other compounds, which were not investigated in this research but are present in buckwheat sprouts, could have exerted a combined effect with flavonoids that resulted in the observed results.

It is important to note that the levels of blood pressure-related biochemical parameters in the normotensive control rats did not change significantly, which indicates that TBSP does not have any contraindications for a healthy group of animals.

In conclusion, we suggest that pasta enriched with dried tartary buckwheat sprouts may help reduce hypertension and oxidative stress *in vivo*. We are currently conducting studies to identify other TBSP compounds responsible for the observed hypotensive effects.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1 Growth performance and energy intake of rats fed the experimental diets

Experimental pasta	WKY		SHR	
	DWFP	TBSP	DWFP	TBSP
Initial body weight (g) [†]	244.8 ± 9.2	254.2 ± 12.8	254.1 ± 11.8	254.6 ± 14.7
Final body weight (g) [‡]	459.2 ± 21.3	476.2 ± 17.5	435.8 ± 16.5	448.3 ± 19.4
Pellet intake (g/rat/die)	28.2 ± 2.8	31.9 ± 2.1	29.8 ± 1.4	30.3 ± 2.6
Pellet energy intake (kcal/rat/die)	101.5 ± 10.1	114.8 ± 7.5	107.3 ± 5.1	109.1 ± 9.4
Cooked pasta intake (g/rat/die)	5	5	5	5
Cooked pasta energy intake (kcal/rat/die)	7.5	7.5	7.5	7.5

[†]twelve-week-old male rats.
[‡]body weight after six weeks of experimental diets.
The values are expressed as means ± S.E.

Table 2 Seed and seedling shoot traits in the two *Fagopyrum esculentum* cultivars and in the *F. tataricum* landrace studied

Species	Cultivar/ landrace	Seed traits				Seedling shoot traits			
		1000-seed weight (g)	No. of seeds in 200 g	Seed water content (%)	Germinated seeds at 10 DAS (%)	Hypocotyl length (mm)	FW yield (g) [†]	DW yield (g) [†]	
<i>F. esculentum</i>	Lileja	24.0 ^a	8409 ^b	13.9 ^a	73.5 ^b	82.4 ^b	202.4 ^b	13.3 ^{ab}	
	Darja	22.3 ^b	8740 ^b	13.6 ^a	57.0 ^c	124.9 ^a	165.0 ^b	6.1 ^b	
<i>F. tataricum</i>	Ljse	12.4 ^c	16166 ^a	11.5 ^b	90.0 ^a	117.8 ^a	397.6 ^a	16.9 ^a	

Means in a column with different superscript letters (a, b, c) are significantly different for $P \leq 0.05$

DAS, days after sowing

[†] Data for sprout yield refer only to the “free-water system” (see text) and are expressed as fresh weight (FW) or dry weight (DW) of the biomass obtained after the germination of 200 g of seed

Table 3 Centesimal analysis (expressed as g/100 g) and cooking behavior of pasta samples

Samples	DWFP	TBSP
Humidity	10.8	9.6
Ashes	0.9	2.3
Protein	9.6	15.2
Lipids	1.2	1.2
Carbohydrates	77.5	71.7
kcal	340	347
OCT [†]	10	10
TOM [‡]	0.9	3.5

[†]OCT optimal cooking time, min
[‡]TOM total organic matter

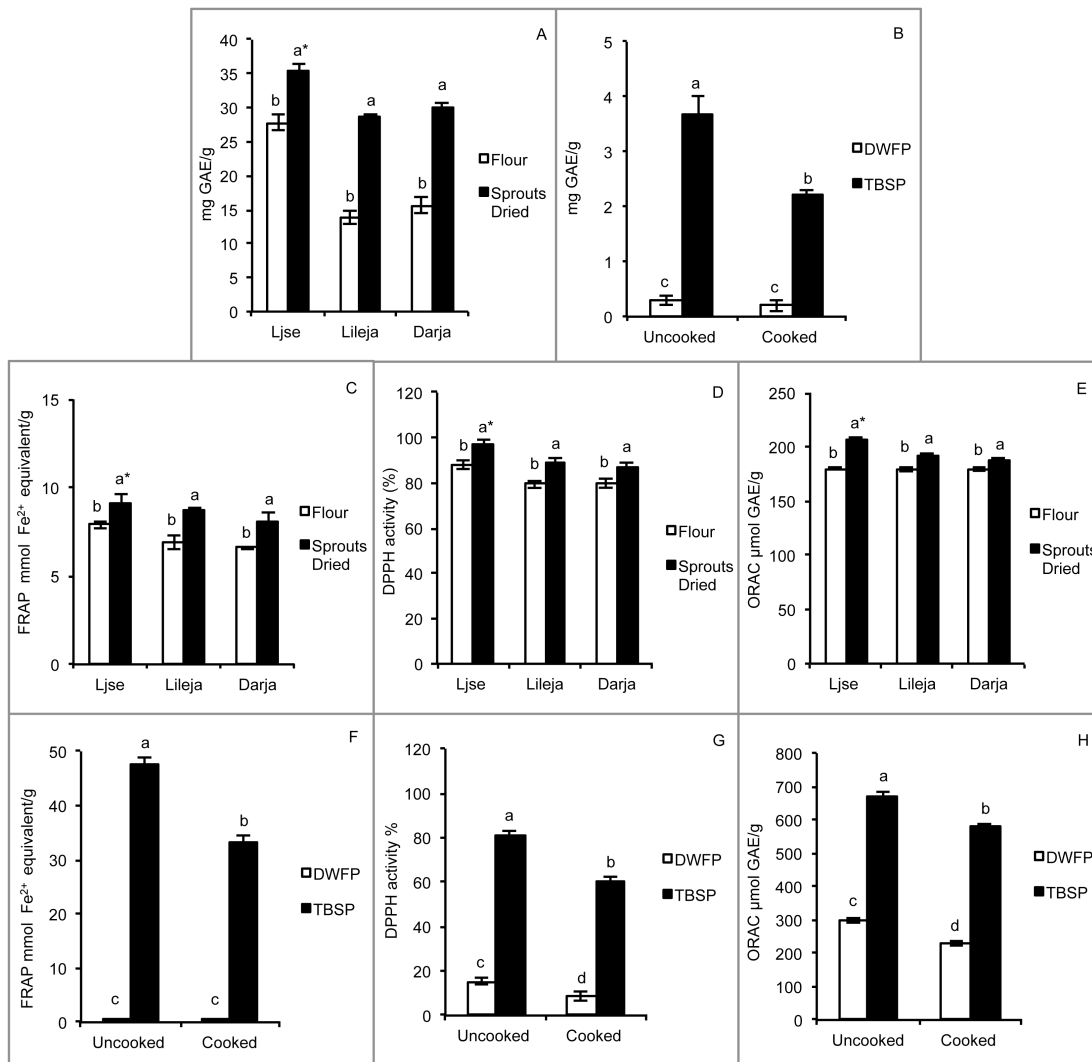
Table 4 HPLC rutin and quercetin determination on raw material and pasta samples

	Rutin (mg/g) [†]	Quercetin (mg/g) [†]
<i>Raw material samples</i>		
Semolina	n.d. [‡]	n.d. [‡]
TB Seed flour	7.6 ± 1.1	0.4 ± 0.1
TB Sprout powder	24.6 ± 1.2 ^{a*}	0.8 ± 0.2 ^{b*}
<i>Uncooked samples</i>		
DWFP	n.d. [‡]	n.d. [‡]
TBSP	0.8 ± 0.02 ^b	3.0 ± 1.2 ^a
<i>Cooked samples</i>		
DWFP	n.d. [‡]	n.d. [‡]
TBSP	0.6 ± 0.4 ^b	2.7 ± 0.2 ^a

[†]of DW samples[‡] non detectable

The values are expressed as means ± S.E. Means in a column with different superscript letters (a, b) are significantly different for P≤0.05

^{*} Indicates that the value is significantly different (P≤0.05) only in the raw material samples



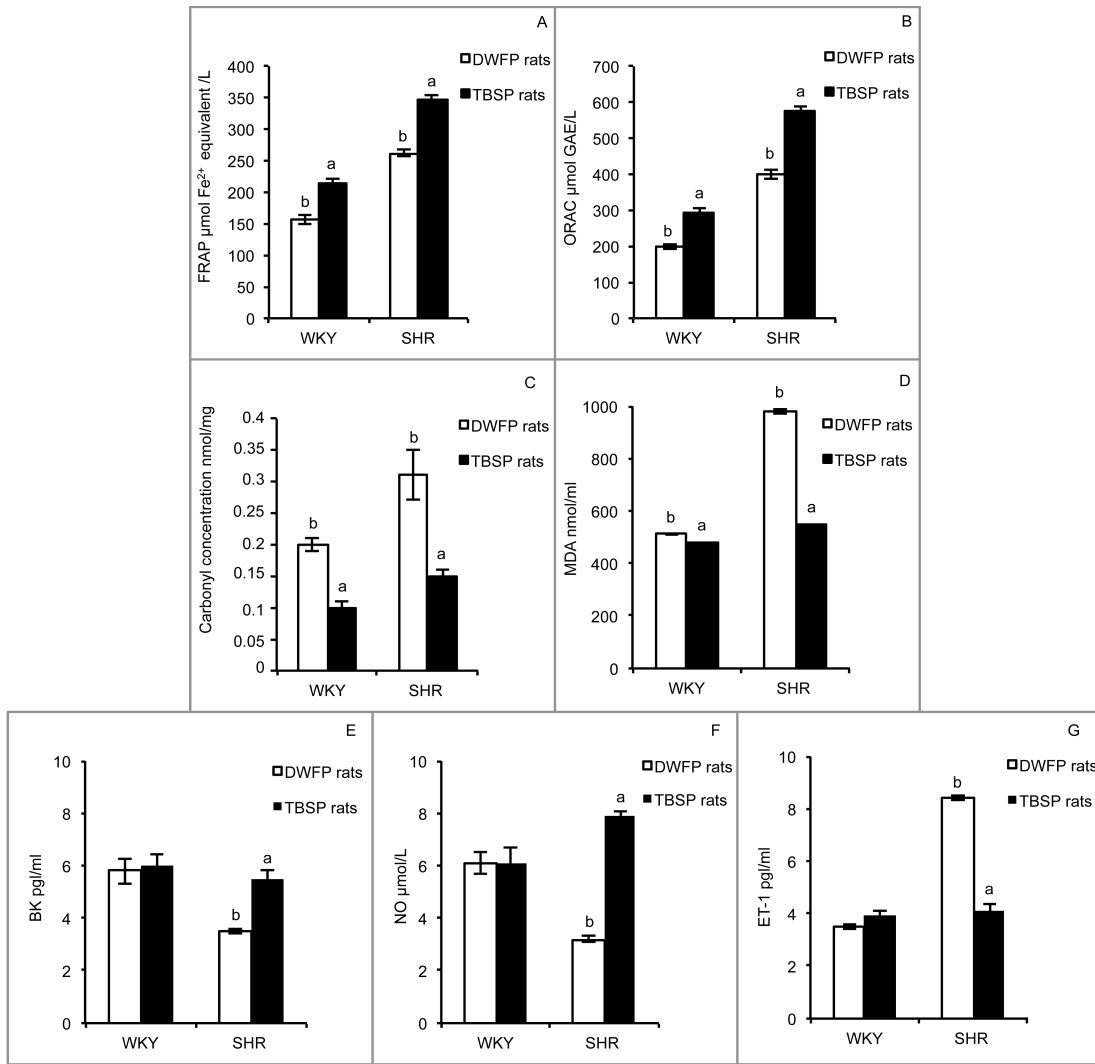


FIGURE CAPTIONS

Fig. 1 *In vitro* analysis of total polyphenol content and antioxidant capacity of raw material samples (flour and sprouts), and cooked/uncooked pasta samples.

Total polyphenol content (panels A and B) and antioxidant capacity (FRAP, panels C and F; DPPH, panels D and G; ORAC, panels E and H) of flour, sprouts of the different cultivars, and cooked/uncooked pasta samples. Each value is expressed as the mean \pm S.E. (n = 3).

Different letters (a, b, c, d) indicate that the value exhibit a significant difference ($P \leq 0.05$) for each comparison (only within the same group in the raw material samples: Ljse, Lileja, and Darja).

* Indicates that the value is significantly different ($P \leq 0.05$) from all other data within each panel only in the raw material samples.

Fig. 2 *In vivo* effects of experimental pasta on animal models.

The plasma total antioxidant capacity determined by FRAP (panel A) and ORAC (panel B), plasma oxidative status (protein carbonyl concentrations, panel C; MDA concentrations, panel D), and plasma blood pressure parameters (BK, panel E; NO, panel F; ET-1, panel G) of WKY and SHR fed the experimental pasta.

Each value is expressed as the mean \pm S.E. (n = 5).

Different letters (a, b) denote significant differences ($P \leq 0.05$) within each panel (only within the same group: WKY and SHR).