In vivo and in vitro model studies on noodles prepared with antioxidant-rich pseudocereals

Attila Kiss¹ · Krisztina Takács² · András Nagy² · Magdolna Nagy-Gasztonyi² · Zsuzsanna Cserhalmi² · Zoltán Naár² · Tibor Halasi³ · József Csáki⁴ · Erzsébet Némedi⁵

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Abstract

Pseudocereals are recently positioned at the forefront of interest, as they are eminent sources of several beneficial bioactive components, such as flavonoids. Noodles of specific composition comprising buckwheat and amaranth have been involved in this research. The aim of presented study was to provide authentic tools in order to estimate the actual behaviour of polyphenols of the special noodles throughout the digestion process. In vitro and in vivo digestion models studies were performed with wheat flour noodles fortified with antioxidant rich pseudocereals. Five noodle variants were prepared with supplementation of different flours of amaranth and buckwheat with wheat flour ranging from 5 to 30%. To reveal the effect of human consumption on polyphenols uptake and real biological impacts, in vitro and in vivo experiments were carried out. Different assays were applied to monitor the release of antioxidants in each gastrointestinal phase. Fortification didn't change the cooking quality of noodles, but slightly affected the colour and improved the total polyphenol content (597–920 mg GAE/kg), and the antioxidant capacity (3.47–7.23 mM Fe²⁺/kg) of the noodles. It was established that total polyphenol content and antioxidant capacity exhibited continuous increase in the whole gastrointestinal tracts, due to their gradual release from food matrix. Most pronounced antioxidant capacity was observed in noodles with 30% buckwheat fortification. In vivo model-experiments confirmed the presence of polyphenols in plasma as well, thus their absorption might be accomplished. Therefore, the biological values of these samples were enhanced during digestion. Supplementation prepared pseudocereal resulted in products with improved nutritional properties.

Keywords In vivo · In vitro · Noodle · Polyphenol · Antioxidant capacity · Amaranth · Buckwheat

Introduction

Pseudocereals such as buckwheat and amaranth are proven to be eminent sources of proteins, dietary fibres and lipids, and rich in unsaturated fatty acids. Additionally, these

pseudocereals comprise high amounts of various health-promoting compounds such as flavonoids, phenolic acids, trace elements, minerals and vitamins [1]. It is obvious in recent days that there is a growing demand of healthy food-stuffs, however basic foods health-promoting products are available in limited number. As a consequence efforts to develop and characterize antioxidant pastries meet current needs and nutrition trends of consumers.

The health promoting effects of polyphenols such as catechins with exert positive impact on plasma antioxidant biomarkers and energy metabolism; procyanidins with proven effects on the vascular system; and quercetin which influences the carcinogenesis markers were widely investigated [2]. Hence polyphenols are in the forefront of recent interest, and several studies aim at mapping the polyphenol-profile of food basic materials. The main phenolic compounds in buckwheat seeds are the glycosides of quercetin, followed by the glycosides of apigenin and luteolin [3], while in amaranth

✉ Attila Kiss
kiss.attila@ke.hu

¹ Food Science Innovation Centre, Kaposvár University, Guba Sándor str. 40, Kaposvár 7400, Hungary

² National Agricultural Research and Innovation Centre, Food Science Research Institute, Herman Ottó út 15, Budapest 1022, Hungary

³ Goodmills Magyarország Ltd., Klapka György út 40, Komárom 2900, Hungary

⁴ Exilfood Ltd, Tisza u. 119, Gávavencsellő 4471, Hungary

⁵ Expedit Nodum Ltd, Budapesti út 106, Budapest 1162, Hungary

seeds caffeic acid, *p*-hydroxybenzoic acid and ferulic acid are regarded as major constituents [4].

The versatile beneficial effects of pseudocereals rich in polyphenols make these substances splendid candidates for being utilized in bakery products. In contrast to the comprehensive research data on their composition, much less results are available about their technological properties, as well as behaviour during the digestion process. Our studies provide information in these two niche areas. Much more information should be necessary for laying the foundations of future functional food developments and industrial applications.

There are some previous investigations on revealing the effects of pseudocereals on the nutritional value of certain food products by monitoring the changes in polyphenol content and antioxidant capacity [5]. Fortification with pseudocereals led to an increase in the antioxidant capacity of the breads, as well as a decrease in the sensory perception.

The stability and bioavailability of flavonoids in foods are much conditional on the food matrix and the processing condition [6, 7].

When polyphenols, being attached to sugar moiety, reach the colon, are hydrolysed to aglycones by microflora-generated enzymes in a signifying more absorbable forms [8, 9].

Numerous papers have been published on in vitro models simulating the gastrointestinal digestion processes in distinctive ways; therefore direct comparisons of these results do not lead to unambiguous conclusions [10]. So far large variety of digestive enzymes, as well as different digestion times have been used in previous studies, therefore the COST FA1005 Infogest (improving health properties of food by sharing our knowledge on the digestive process) Action [11] proposed a general standardised and practical static in vitro digestion method [12], which is based on the current state of knowledge on in vivo digestion conditions. This Infogest action is an international network joined by more than 200 scientists from 32 countries working in the field of digestion models. This harmonized; consensus model employs widely available instrumentation and chemicals, as well as physiologically relevant conditions. In general, recent in vitro methods provide limited means for the assessment of polyphenol digestibility and bio-utilization as they do not take the role of the colon into consideration. Thus animal model studies should also be integrated into the research in order to gain more complex and realistic view of the real digestion procedure [13].

Special noodles of unique composition were prepared from different amounts of wheat, amaranth and buckwheat flours with potato flake, egg liquid, oil and salt. Noodle is regarded as a highly popular, traditional type of pastry, thus future consumers' acceptance might be favourable.

The main objective of this research was to study the effect of the partial buckwheat and amaranth flour supplementation of wheat flour on cooking quality, total polyphenol content

and antioxidant capacity of the prepared noodles. As previous literature data on noodles comprising specific components, provide only a few information on the behaviour and absorption of polyphenols, our aim was to estimate their real biological impacts and bioavailability by obtaining relevant proofs. Both digestion model experiments and in vivo (rat) model experiments were implemented. The alteration of total polyphenolic contents of noodles, as well as their antioxidant capacity was monitored. The combined and comparative interpretation of in vivo and in vitro studies may lead to a better understanding of the digestion processes.

Materials and methods

Materials

The ingredients for noodles preparation (egg liquid, potato flake, wheat, buckwheat and amaranth flour, and cooking oil) have been provided by Goodmills Hungary Ltd, Komárom, Hungary and 4 Exilfood Ltd., Gávavencsellő, Hungary. All of the reagents used in our experiments were of analytical grade and were supplied by Sigma Aldrich otherwise stated.

Experimental plan

Different ratios of wheat flour (%W) were replaced with buckwheat flour (%B) and amaranth flour (%A) to increase the nutritional value of the noodles. The composition of different noodles used in our experiment is shown in Table 1. The control noodle (N0) was prepared from 100% wheat flour, N1 noodle contained 5% buckwheat flour and 5% amaranth flour mixed with 90% wheat flour, N2 was composed of 15% buckwheat flour, 15% amaranth flour and 70% wheat flour, N3 comprised 30% buckwheat flour and 70% wheat flour, while N4 contained 30% amaranth flour and 70% wheat flour on the basis of total flour content (100% = 33.5 g).

Preparation of noodles

First of all dry components were mixed together, and then egg liquid, cooking oil and water were slowly added and mixed until the dough had homogenous consistency. The dough was proportioned into a 37 mm × 27 mm × 20 mm silicon form and frozen (−18 °C) until the cooking process (Exilfood Ltd recipe).

Ninety-five g of frozen noodles were cooked for five minutes in 900 mL of boiling distilled water with no added salt. After cooking, noodles were left at room temperature for 2 min to drain the water, weighted, and the investigation of

Table 1 Composition of the noodles

Ingredients	Noodles composition (treatment)				
	N0	N1	N2	N3	N4
Wheat flour (W)	33.5 g (100%)	30.14 g	23.45 g	23.45 g	23.45 g
Amaranth flour (A)	–	1.68 g (5%)	5.03 g (15%)	–	10.06 g (30%)
Buckwheat flour (B)	–	1.68 g (5%)	5.03 g (15%)	10.06 g (30%)	–
Potato flake	9.5 g	9.5 g	9.5 g	9.5 g	9.5 g
Egg liquid	1 mL	1 mL	1 mL	1 mL	1 mL
Cooking oil	1 mL	1 mL	1 mL	1 mL	1 mL
Salt	0.8 g	0.8 g	0.8 g	0.8 g	0.8 g
Water	54.2 mL	54.2 mL	54.2 mL	54.2 mL	54.2 mL

Total flour content of all noodles (N0–N4) was 33.5 g (100%). Wheat flour of the total flour content was supplemented with pseudocereal in 5–5%, 15–15% and 30%

the cooking qualities was immediately conducted (Exilfood Ltd recommendation for consumers).

Firmness measurement

Firmness of prepared noodle samples were measured by texture analyser (Lloyd Instruments LR5K plus, England) equipped with a TG83 probe (3.18 mm probe diameters). We used 50 N load cell and 10 mm/min test speed [14].

All measurements were conducted in triplicates and mean values were calculated.

Colour measurement

Chromate meter CR-400 (Konica Minolta, Japan) was used to measure the colour of the surface of noodles according to the CIE Lab system. The colour was expressed by values as L^* (lightness; 0 = black, 100 = white), a^* [$+a$ = redness (+60), $-a$ = greenness (−60)] and b^* [$+b$ = yellowness (+60), $-b$ = blueness (−60)]. Colour difference (ΔE^*) between two samples were calculated using the following equation: $\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$; where $\Delta a^* = a^*2 - a^*1$, $\Delta b^* = b^*2 - b^*1$, $\Delta L^* = L^*2 - L^*1$.

If the value of the colour difference between the two analysed samples are i, $\Delta E^* < 0.5$ —no difference; ii, $\Delta E^* = 1.5$ –3.0—significant difference, iii, $\Delta E^* > 6.0$ —markedly great difference after visualization [15].

All measurements were conducted in triplicates and mean values were calculated.

In vitro digestion of noodles

In vitro digestion analyses were performed according to the procedure described by Minekus et al. [12], also referred to as a consensus protocol, and developed within the COST Action FA1005 INFOGEST project.

Simulated digestion fluids; Simulated Salivary Fluid (SSF, pH 7.0), Simulated Gastric Fluid (SGF, pH 3.0) and Simulated Intestinal Fluid (SIF, pH 7.0) were prepared and used according to the specification (appropriate electrolyte solutions, enzymes, CaCl_2 and water) by Minekus et al. [12]. The initial amount of the cooked noodle was 5 g, and the ratio of food and simulated fluids was 50:50 (w/v %). In compliance with the harmonised protocol, enzyme activities were adjusted according to the assumable physiological circumstances and used as follows: human salivary alpha-amylase (Sigma A1031-5KU, 75 U/mL) in the final oral bolus, pepsin (Sigma P7012, 2000 U/mL) in the final gastric bolus, pancreatin (Sigma P7545, 100 TAME U/mL, based on trypsin) in the final intestinal bolus. Bile extract (Sigma B8631, 10 mM) was added to the intestinal boluses. The activities of alpha-amylase, pepsin and trypsin were measured according to Bernfeld [16], Anson [17], and Hummel [18], respectively. In practise cooked noodle samples (5 g) were grinded in a mincer, and then 5 mL of SSF was added to conduct a 2-min-long mastication (37 °C, pH 7.0). Oral bolus (2 mL) was taken out for further analytic measurements, while the remained amount (8 mL) was exposed to further gastric digestion. Then the oral bolus was mixed with 8 mL of SGF. The gastric phase digestion was performed at 37 °C for 2 h at pH 3.0. After the gastric digestion step 4 mL of gastric bolus was taken out for further analytical studies, and the residue (12 mL) was forwarded to the further intestinal digestion step. SIF (12 mL) and bile salt extract were added to the retained gastric bolus and shaken at 37 °C for 2 h at pH 7.0. The enzyme reactions were stopped by immediate freezing to −80 °C.

In vivo digestion of noodles

According to the protocol of Pusztai et al. [19] Wistar male rats (Toxi-Coop KKT, Budapest) were weaned at day 21, and from this time they were given free access to commercial

stock diet (Toxi-Coop KKT, Budapest) for 10 days. Tap water was freely available during the experiment. On the first day of the experiment rats (5 animals per group) were fasted for 3 h. Rats in the test groups were given 500 mg of noodles samples by intragastric intubations in 1 mL of saline, while rats in the control group (Blank) were fed only with saline (0.9% NaCl). Rats were killed 90 min subsequent to the intubation. The stomach and the small intestine was removed and washed out, with 1 and 2 mL of phosphate-buffered saline (PBS, pH 7.4) containing 10 mM phenyl-methylsulfonyl fluoride (PMSF), respectively and centrifuged (5000 rpm, 15 min). The blood was collected and centrifuged (3000 rpm, 15 min) in Vacutainer tubes containing EDTA (0.5 M). The supernatants (plasma) were frozen (-80°C) and used for further investigations.

Measurement of total polyphenol content and antioxidant capacity

Suspension of 150 μL of in vitro digested noodles from oral, gastric and intestinal phases was mixed with 850 μL methanol (80%v/v). In case of animal model, plasma (100 μL) and gastric samples (200 μL) were extracted with 200 μL and 400 μL methanol (80%v/v), respectively. The intestinal samples were centrifuged at 12,000 rpm, and then 200 μL supernatant was mixed with 400 μL methanol (80%v/v). Subsequent to storage (24 h, 4°C) all the samples were shaken (120 rpm, 25°C , 30 min), centrifuged (12,000 rpm, 13 min) and the supernatants were used for the analyses.

Total polyphenols were quantified using the Folin–Ciocalteu method [20]. Absorbance of the samples was measured at 750 nm. Total polyphenol content was given in gallic acid equivalent per 1 Litre sample (mg GAE/L).

Antioxidant capacity was measured using ferric reducing-antioxidant power test (FRAP) performed according to Benzie and Strain [21]. Absorbance was examined at 593 nm. The results were expressed in $\text{mmol Fe}^{2+}/\text{L}$ sample.

Statistical analysis

Statistical analyses were performed using SPSS software (Chicago, IL, USA) version 20.0. Kolmogorov–Smirnov test was used to assess the distribution of the data. Our results displayed normal distribution, so the mean $\pm$ standard deviation (SD) values were determined and two-sample t-tests were used for statistical comparison of the experimental data in all examined parameters. The differences were considered to be statistically significant at $p < 0.05$.

Results and discussion

Cooking quality, firmness and colour

Cooking quality and colour of noodles might be regarded as high relevance parameters in respect of food developments and consumers acceptability. The results of these parameters gained from our experimental samples are shown in Table 2.

The weight of frozen noodles (96.16–97.17 g) were found to be similar ($p > 0.05$), while differences were observed in the weight of cooked samples (104.64–121.54 g) compared to the control (106.25 g). The samples supplemented with amaranth (N4), as well as with amaranth and buckwheat (N1, N2) showed higher cooked weight compared to the control (N0), except the sample supplemented with buckwheat (N3) ($p < 0.05$). Firmness referred to the consistency of a product and indicated how much a product could resist an external force (F). The greater the force is, the greater the firmness of the product is. In the respect of firmness, all samples showed similar force values (0.26–0.36 N) as the control (0.36 N) did at $p < 0.05$, which let us concluded that the texture of the noodles were not highly affected by the applied amount of amaranth and buckwheat flours, neither by positive or negative way. The cooking quality of

Table 2 Firmness and colour measurement of noodles

Treatment	Cooked noodle weight (mean $\pm$ SD, g)	Force (mean $\pm$ SD, N)	L* (mean $\pm$ SD)	a* (mean $\pm$ SD)	b* (mean $\pm$ SD)	ΔE^*
N0	106.25 $\pm$ 2.54	0.36 $\pm$ 0.08	68.48 $\pm$ 0.98	− 2.61 $\pm$ 0.05	13.81 $\pm$ 0.42	–
N1	115.37 $\pm$ 4.41*	0.32 $\pm$ 0.06	63.21 $\pm$ 1.13 *	1.00 $\pm$ 0.23 *	14.43 $\pm$ 0.29	6.40
N2	121.54 $\pm$ 1.97*	0.26 $\pm$ 0.06 *	64.14 $\pm$ 1.46 *	2.55 $\pm$ 0.12 *	14.25 $\pm$ 0.52	6.75
N3	104.64 $\pm$ 0.73	0.39 $\pm$ 0.07	58.08 $\pm$ 1.48 *	4.21 $\pm$ 0.54 *	13.60 $\pm$ 1.11	12.43
N4	119.52 $\pm$ 1.67*	0.29 $\pm$ 0.08 *	69.55 $\pm$ 0.53	− 0.51 $\pm$ 0.18 *	17.83 $\pm$ 0.62 *	4.66

Samples: N0 (100% W)-control; N1 (5% B, 5% A, 90% W); N2 (15% B, 15% A, 70% W); N3 (30% B, 70% W); N4 (30% A, 70% W), where % W, B, A means the ratio of Wheat, Buckwheat, Amaranth flour respectively on the basis of the total flour content

ΔE^* : overall differences in colour, L: lightness factor, a*: redness factor, b*: yellowness factor

*Significant difference from N0 sample at $p < 0.05$

all noodles was almost adequate, that of N1 sample was the most prominent, beside the control, while N2, N3, N4 samples were only slightly sticky after cooking.

According to a previous study on dry noodles [22], the increase of ratio of non-gluten protein substituents (5%, 15%, 25%, and 30%) generated by amaranth supplementation for durum wheat flour reduced the gluten strength and weakened the texture of the noodles, resulting decrease in firmness. The decrease in firmness can also be associated with a decrease in starch swelling and gelatinization [23].

Analysing the colour of noodles, it was seen, that a^* values (redness) were increased by both buckwheat and amaranth supplementation. Interestingly, only sample N4 might be characterized by significantly higher b^* value (yellowness) compared to N0, as a consequence of pronounced presence of carotenoid pigments deriving from amaranth flour [24]. Taking the L^* values into consideration, noodles containing buckwheat flour (N1, N2, N3) can be characterized by darker colour compared to the control ($p < 0.05$). The darkest sample was the N3 noodle, because of 30% buckwheat flour, while the L^* of N4 was closely similar to the control (light white butter colour). Darker pasta colour might be associated with an increase in the antioxidant activity. There has been some disagreement about the relationship between total phenolic content and antioxidant activity and colour of food. Colour does not appear to be a factor in the expression of antioxidant-related parameters. For example white wheat or rice varieties showed a higher total phenolic content and antioxidant activities than black wheat or rice varieties [25].

Colour of noodles were of crucial significance in respect of acceptance of consumers, thus the overall differences in colour (ΔE^*) was also investigated. It should be noted, that not only the colour, but also the firmness, the texture and overall elastic traits (cooking quality parameters) play key roles in tailoring the general features and the plausible consumers' acceptance of the noodles. That can be a reason for the finding, that while the smallest colour difference was observed for N4, the consumers' preference of the N1 variant displayed more beneficial overall cooking parameters which are expected to be more pronounced. There was a considerable difference in the colour of the noodle samples. Well-visible colour distinctions were displayed at N1, N2, N4 noodles ($\Delta E^* = 4.66$ – 6.75), and markedly great difference was visualized at N3 noodle ($\Delta E^* = 12.43$), compared to the control.

In summary of the abovementioned arguments it was established that N1 noodle could be suggested for further food industrial applications and human consumption, hence it displayed the most beneficial cooking quality parameters.

Total polyphenol content and antioxidant capacity

The total polyphenol content and antioxidant capacity of the examined noodles are shown in Table 3. Difference in the total polyphenol content from the control noodle was significant only in cases of N2 and N3 samples. Supplementation with 15% buckwheat flour and 15% amaranth flour (N2) led to 62.94% increase in the total polyphenol content, while 30% of buckwheat supplementation (N3) enhanced this parameter by 71.89% ($p < 0.05$). The smallest increase of the polyphenol content (11.51%) was measured in case of the amaranth supplemented noodle (N4), while for N1 (comprising 5% amaranth and 5% buckwheat) 20.78% enhancement was observed. Results of previously performed studies suggested that buckwheat supplementation could be responsible for the increased total polyphenol content. Biney and Beta [26] discovered the same finding, while Alvarez-Jubete et al. [3] obtained significantly lower total phenol content in 50% amaranth containing bread compared to wheat bread. It was also found that 30% of amaranth supplementation in bread did not result in definite decrease of the total polyphenol content, but 50% supplementation had already done.

In the present study a definite increase was observed in the antioxidant capacity for each pseudocereal-supplemented noodle (N1, N2, N3, N4) compared to control sample (N0), and the difference was significant in N2 and N3 samples at $p < 0.05$. In order to improve both the antioxidant capacity and the total phenolic content, amaranth and buckwheat replacements were applied simultaneously in considerable extents, as in case of N1 (5% B, 5% A, 90% W), or N2 (15% B, 15% A, 70% W) samples. Our results are in good correlation with the data obtained by other researchers [3, 5] underpinning that supplementation of wheat flour with

Table 3 Total polyphenol content and antioxidant capacity of noodles

Cooked noodle No	Total polyphenol content (mean $\pm$ SD, mg GAE/kg)	Antioxidant capacity (FRAP) (mean $\pm$ SD, mM Fe ²⁺ /kg)
N0	535 $\pm$ 57	2.52 $\pm$ 0.21
N1	646 $\pm$ 56	3.94 $\pm$ 0.34
N2	872 $\pm$ 77*	6.33 $\pm$ 0.64*
N3	920 $\pm$ 79*	7.23 $\pm$ 0.65*
N4	597 $\pm$ 38	3.47 $\pm$ 0.15

Samples: N0 (100% W)-control; N1 (5% B, 5% A, 90% W); N2 (15% B, 15% A, 70% W); N3 (30% B, 70% W); N4 (30% A, 70% W), where % W, B, A means the ratio of Wheat, Buckwheat, Amaranth flour respectively on the basis of the total flour content

*Significant difference from N0 sample at $p < 0.05$

simultaneously used amaranth or buckwheat flours resulted in a remarkable increase in the total polyphenol content and antioxidant capacity. However, N3 (30% B, 70% W) sample provided the highest antioxidant capacity (7.23 ± 0.65 mM Fe^{2+} /kg) due to the greater extent of buckwheat-supplementation. It is in compliance with the expectation that higher phenolic content contributes to a higher antioxidant capacity. These results were confirmed by previous results of Biney and Beta [26] where significantly higher antioxidant capacity was found for cooked, 30% buckwheat flour containing spaghetti compared to spaghetti from durum semolina used as a control.

Total polyphenol content and antioxidant capacity of in vitro digested cooked noodles

Cooked noodle samples (N0, N1, N2, N3, N4) were exposed to a 3-phased in vitro static digestion process simulating oral, gastric, and intestinal circumstances. Total polyphenol content and antioxidant capacity of in vitro digested cooked noodles were analysed, as presented in Table 4. Several studies indicated that digestion processes largely contribute to the structural modification and antioxidant activity alteration of functional food components such as polyphenols, hence to the change in the antioxidant activity of the consumed foodstuffs. During digestion process, polyphenols, which were entrapped in the food matrix released into digestive

juices and undergoes structural modification with altered bioaccessibility and biological activities [27]. Thus, we focused on studying the behaviour of polyphenols in the prepared noodles whether they stayed available and preserved their antioxidant characteristics or not, during the digestion process. Total polyphenol content exhibited broad variety throughout the digestion process, proceeding from oral to intestinal digestion. After oral phase of the digestion process, N4 (30% A, 70% W) sample (37.40 ± 3.56 GAE/L) showed similar polyphenol content as the NO control did, while all the other samples were characterized by enhanced total polyphenol levels (59.84 – 82.27 GAE/L) compared to the control (32.41 ± 3.51 mg GAE/L) ($p < 0.05$).

In the gastric phase, the released polyphenol concentrations increased comparing to their levels after the oral phase.

If we compared only the gastric phase samples, we established that N2 (15% B, 15% A, 70% W) and N4 (30% A, 70% W) samples exhibited decrease in the total polyphenol content, 154.58 ± 7.01 mg GAE/L and 114.69 ± 7.10 mg GAE/L respectively, while this parameter was highly increased in the case of the N3 (30% B, 70% W) sample (294.19 ± 14.10 mg GAE/L) compared to the control (241.84 ± 3.53 mg GAE/L). In case of N1 sample, no significant alteration was observed (236.85 ± 45.84 mg GAE/L).

The intestinal step of the digestion process could be responsible for the major release of polyphenols of the examined samples. Significantly higher total polyphenol levels were observed in the intestinal phase for N1, N2, N3,

Table 4 Total polyphenol content and antioxidant capacity of noodles after in vitro and in vivo digestion

Cooked noodle No	In vitro digestion			In vivo digestion		
	Oral phase sample	Gastric phase sample	Intestinal phase sample	Gastric phase sample	Intestinal phase sample	Plasma sample
Total polyphenol content (mean $\pm$ SD, mg GAE/L)						
Blank	4.49 ± 1.53	24.93 ± 7.03	870.36 ± 18.53	39.98 ± 12.03	452.81 ± 10.37	291.09 ± 14.95
N0	32.41 ± 3.51	241.84 ± 3.53	924.25 ± 19.90	52.11 ± 13.79	435.74 ± 138.35	347.69 ± 18.34
N1	$64.82 \pm 7.05^*$	236.85 ± 45.84	$1181.76 \pm 21.16^*$	58.99 ± 8.00	489.64 ± 164.59	$533.67 \pm 15.28^*$
N2	$59.84 \pm 7.09^*$	$154.58 \pm 7.01^*$	$1109.46 \pm 10.58^*$	$84.53 \pm 3.78^*$	371.95 ± 114.28	$566.46 \pm 17.45^*$
N3	$82.27 \pm 3.62^*$	$294.19 \pm 14.10^*$	$1214.17 \pm 3.49^*$	$81.31 \pm 17.78^*$	482.46 ± 182.49	$530.08 \pm 19.38^*$
N4	37.40 ± 3.56	$114.69 \pm 7.10^*$	$1266.53 \pm 7.00^*$	59.74 ± 14.08	477.07 ± 120.59	$485.15 \pm 48.87^*$
Antioxidant capacity (FRAP) (mean $\pm$ SD, mM Fe^{2+} /L)						
Blank	0.20 ± 0.01	0.21 ± 0.04	0.71 ± 0.05	0.25 ± 0.03	1.54 ± 0.48	0.28 ± 0.02
N0	0.38 ± 0.02	0.56 ± 0.03	0.85 ± 0.06	0.24 ± 0.03	1.48 ± 0.49	0.33 ± 0.05
N1	$0.56 \pm 0.01^*$	$0.86 \pm 0.08^*$	$1.41 \pm 0.06^*$	$0.36 \pm 0.09^*$	$3.27 \pm 1.38^*$	0.37 ± 0.04
N2	$0.41 \pm 0.01^*$	$0.98 \pm 0.06^*$	$1.63 \pm 0.11^*$	$0.52 \pm 0.18^*$	2.35 ± 1.09	0.35 ± 0.06
N3	$0.59 \pm 0.04^*$	$1.87 \pm 0.13^*$	$2.10 \pm 0.04^*$	$0.35 \pm 0.08^*$	$2.95 \pm 1.11^*$	0.37 ± 0.05
N4	0.36 ± 0.02	$0.43 \pm 0.03^*$	$1.18 \pm 0.02^*$	0.25 ± 0.05	$2.62 \pm 0.87^*$	0.30 ± 0.02

Samples: N0 (100% W)-control; N1 (5% B, 5% A, 90% W); N2 (15% B, 15% A, 70% W); N3 (30% B, 70% W); N4 (30% A, 70% W), where % W, B, A means the ratio of Wheat, Buckwheat, Amaranth flour respectively on the basis of the total flour content

Blank means the background of the measurement

*Significant difference from N0 sample at $p < 0.05$

N4 samples compared to the results obtained in their oral and even gastric phases. N3 and N4 samples gave the highest total polyphenol level (1214.17 ± 3.49 and 1266.53 ± 7.0 mg GAE/L) and N3 resulted the highest antioxidant capacity value (2.1 ± 0.04 mM Fe^{2+} /L). In vitro digestion model showed the same tendency in antioxidant capacity (FRAP) values along the oral, gastric and intestinal phases.

Our results were in agreement with other research findings, as the gastrointestinal tract may acted positively on the accessibility of polyphenols, since they are progressively released from protein and polysaccharide bonds—as the strong interaction between proteins/carbohydrates and polyphenols, an increase in the polyphenol content occurs at gastric and intestinal phases—being available for the absorption and exertion of their biological effects. These findings pointed out the importance of the examined food matrices, the presence/lack of proteins/carbohydrates, as these components apparently contributed to the reduction/increase of polyphenol level [28, 29].

Rodríguez-Roque et al. [29], detected increased phenolic acid and total flavonoid level after gastric digestion of soymilk. In an analogous way, Seiuer et al. [30], observed increased phenol content of olive oil and argan oil after in vitro digestion.

Total polyphenol content and antioxidant capacity of cooked noodles subjected to in vivo digestion

Cooked noodle samples were exposed to in vivo rat digestion model, and both total polyphenol content and antioxidant capacity of 3 biological fluid samples (gastric, small intestinal and blood plasmatic) of each rat were analyzed. Relevant results are summarized in Table 4.

Total polyphenol content in the gastric fluid of rats fed with N2 (15% B, 15% A, 70% W) and N3 (30% B, 70% W) noodles were found to be significantly higher (81.31 ± 17.78 and 81.31 ± 17.78 mg GAE/L, respectively), while in case of N1 (5% B, 5% A, 90% W), N4 (30% A, 70% W) samples no pronounced increase (58.99 ± 8.00 and 59.74 ± 14.08 mg GAE/L, respectively) was observed compared to the gastric fluid of the N0 control noodle fed rats (52.11 ± 13.79 mg GAE/L) ($p < 0.05$). Furthermore, the released and available total polyphenol content significantly increased in intestinal fluids of all rats. In case of surveying the differences in the total polyphenol content among the intestinal fluids, no significant differences could be observed (371.95 – 489.46 mg GAE/L), only the intestinal fluid of N1 (5% B, 5% A, 90% W) sample showed the highest value (489.46 mg GAE/L).

Monitoring with FRAP method the differences in the antioxidant capacity among the gastric fluids of the different noodle digestives, a moderate increase was observed

in cases of N1, N3 samples (0.36 ± 0.09 mM Fe^{2+} /L, N3 0.35 ± 0.08 mM Fe^{2+} /L, respectively) and larger increase was noticed in case of N2 sample (0.52 ± 0.18 mM Fe^{2+} /L) compare to the control (0.24 ± 0.03 mM Fe^{2+} /L). A positive correlation has been observed between the increase in the antioxidant capacity of the intestinal and gastric fluids in all samples corresponding to the increased level of total polyphenol content in the intestinal and gastric fluids. Differences were observed in the antioxidant capacity among all intestinal fluid samples, where a significant increase exhibited in the intestinal biological fluids of N1, N2, N3, N4 noodle fed rats (3.27 ± 1.38 ; 2.35 ± 1.09 , 2.95 ± 1.11 ; 2.62 ± 0.87 mM Fe^{2+} /L, respectively) in comparison with that of the N0 noodle fed rat (1.48 ± 0.49 mM Fe^{2+} /L) ($p < 0.05$). The largest antioxidant capacity appeared at the intestinal fluid of the N1 noodle fed rat (3.27 ± 1.38 mM Fe^{2+} /L).

Results can depend on the solubility of the polyphenols. Soluble polyphenols are mainly low molecular weight structures, soluble in aqueous-organic solvents and they are potentially bioavailable in the small intestine. Non-extractable polyphenols are either polymeric polyphenols or single polyphenols linked to macromolecular food constituents; they are not extracted by common aqueous-organic procedures, they are accessible and bioavailable only in the large intestine [31].

The bioavailability and the absorption of the beneficial compounds such as polyphenols and antioxidants were analysed from blood plasmatic biological fluids. The measurable level of the absorbed polyphenols/antioxidants is presented in Table 4.

Polyphenol content and the antioxidant capacity were detectable, which let us concluded that absorption was performed in case of all samples. Comparing the results of the different plasma samples, we could establish, that the total polyphenol content was significantly increased in all plasma samples (N1, N2, N3, N4) compared to the control ($p < 0.05$). These values in general terms did not differ largely from the results of the intestinal biological fluids of rats. Serra et al. [32], also investigated absorption of phenolic compounds in plasma after ingestion of olive oil using in vivo rat model. After a single ingestion of olive oil phenolic compounds were absorbed, metabolized and distributed through the blood stream to practically all parts of the body, even across the blood–brain barrier.

Antioxidant capacities (by FRAP) of all plasma samples (0.33 – 0.37 mM Fe^{2+} /L) were found to be statistically similar to that of the control plasma sample (0.33 ± 0.05 mM Fe^{2+} /L). Lado et al. [33] evaluated the antioxidant status by means of different methods (FRAP, TBA) and in rat plasma they found similar antioxidant activity values to our results.

Larger values were obtained for the antioxidant capacity of the intestinal than for the plasma samples ($p < 0.05$). It

could be explained by the fact that different types of polyphenols were absorbed into the plasma to distinctive extent [9], thus various antioxidant activity values were observed [34]. Some polyphenols can also be bound to endogenous proteins in the intestinal lumen; therefore, their absorption from the small intestine may be hindered [35]. Also they are metabolized with products detected in plasma that retain at least part of the antioxidant capacity and then excreted, which was supported with different animal experiments [36, 37].

Conclusion

Noodles with higher polyphenol content and antioxidant capacity were made from a mix of wheat and buckwheat and amaranth flours. The texture of the noodles were not highly improved or worsened as an effect of buckwheat and amaranth supplementation, and the results on firmness of all samples did not differ significantly from each other (0.26–0.39 N). According to the consistency and the sensory assessment of N1 noodle exhibited the most acceptable cooking quality, but the other fortified noodles (N2, N3, N4) beside N1 might be favoured by the consumers as functional foods in the future.

In the view of the nutritional value of the noodles, it can be claimed that both buckwheat and amaranth flour supplementation improved the polyphenol content and the antioxidant capacity in all developed noodle products. All noodles showed positive nutritional characteristics. Buckwheat proved to be a better source of polyphenols than amaranth, so N3 (30% B, 70% W) noodle gave the highest polyphenol content (920 ± 79 mg GAE/kg) and antioxidant capacity (7.23 ± 0.65 mM Fe^{2+} /L).

As the behaviour of noodle polyphenols throughout the digestion process has scarcely been revealed so far, so our in vitro and in vivo digestion studies provide novel knowledge on the assessment of their accessibility in the upper part of the gut and their potential availability for the body metabolism. Our results from in vivo model study showed that the extent of accessible polyphenols and antioxidants was unambiguously and gradually enhanced continuously by digestive phases through the digestion process. The accessible polyphenols in the stomach (52.11 – 84.53 mg GAE/L) and in the intestine (371.95 – 489.64 mg GAE/L) and the antioxidant activity (gastric: 0.24 – 0.52 mM Fe^{2+} /L, intestinal: 1.48 – 3.27 mM Fe^{2+} /L) could be monitored. It was concluded that the intestinal digestion phase supposed to largely contribute to the considerable release of the antioxidants from the noodles.

As in vitro digestion model confined to the monitoring the released polyphenols, therefore the absorption of polyphenols was followed by in vivo rat feeding model. It should be

noted that the polyphenols have appeared and were detectable in the plasma that means that they were available for the metabolism.

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Compliance with ethical standards

Conflict of interest The authors declare no potential conflict of interest.

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