BISCUITS FROM FERMENTED ROASTED BUCKWHEAT FLOUR - PHENOLICS PROFILE AND BIOACCESSIBLE ANGIOTENSIN CONVERTING ENZYME INHIBITORY ACTIVITY

– Research paper –

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Abstract: The bioaccessible angiotensin converting enzyme (ACE) inhibitory activity of biscuits formulated from roasted common buckwheat flour after fermentation by select bacteria was studied. The same content of total phenolic compounds was found in fermented flour and in biscuits obtained from them. Generally, fermentation of flour did not changes the ACE inhibitory activity, whereas baking process significantly increased the ACE inhibitory activity of examined products. The potential bioaccessible ACE inhibitory activity from biscuits was very high. Phenolic acids such as protocatechuic, vanillic and syringic acids as well as flavonoids: kaempferol and epicatechin in the digested buckwheat biscuits have the highest impact on ACE inhibitory activity. A high significant correlations were found between IC₅₀ and total phenolic compounds of fermented flours, biscuits before and after digestion. The data obtained in this study closely associates phenolic compounds with ACE inhibitory activity.

Keywords: buckwheat; roasting; fermentation; baking; *in vitro* digestion; ACE inhibition

INTRODUCTION

Food bioactive compounds, such as peptides with potent angiotensin-I converting enzyme (ACE) inhibitory activity can fulfill other roles than energetic or nutritional. This type of peptide have been separated and characterized from enzymatic hydrolysates of many foodstuffs, such as casein (Miguel et al., 2009), tuna bone protein (Je et al., 2007), soybean (Kuba et al., 2005), wheat germ (Matsui et al., 1999), lentil (Torino et al. 2013), apricot almond meal (Wang et al., 2011), and many other (Ren et al., 2014). Iwaniak et al. (2014) have been reviewed in details the food-originating ACE inhibitors and antihypertensive peptides which could be used for prevention in blood pressure reduction. Lacaille-Dubois et al. (2001) presented ACE inhibitory activity of flavonoids isolated from plants. It should be noted that this activity in food of plant origin results not only from flavonoids but also from other unidentified components present in the extracts. Cardiovascular function could be modulated by flavonoids through regulation of blood pressure

(Galleano et al., 2010), oxidative stress (Cordova et al., 2012) and inflammation (Pan et al., 2009) in the cardiovascular system. ACE plays a key physiological role in the control of blood pressure in the rennin-angiotensin system (Fujita et al., 2000). The synthetic drugs that inhibit ACE, such as captopril and enalapril (Kim et al., 2001) are commonly used for hypertension treatment. But as presented by Kumar et al. (2010) synthetic ACE inhibitors can have adverse side effects such as cough, taste disturbances, skin rashes or angioneurotic edema.

Giménez-Bastida and Zieliński (2015) showed that consumption of buckwheat is associated with differentiated activities such as: antioxidant, hypotensive, antispasmodic, hypocholesterolemic, hypoglycemic, anticancer, and anti-inflammatory anti-glycation activities. Higasa et al. (2011) showed that buckwheat have high levels of angiotensin I-converting enzyme (ACE)-inhibitory activity. The greatest ACE inhibitory activity (IC₅₀ = 30 µg/mL) was found for the extracts of buckwheat hulls (Tsai et al., 2012). These finding suggest the impact of polyphenols on ACE inhibitory activity, because hull is a rich source of

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flavonoids and phenolics acids (Zielińska et al., 2013). Recently it was found that lentil polyphenol extract prevents angiotensin II-induced hypertension, vascular remodeling and perivascular fibrosis (Yao et al. 2012). As presented Jakobek (2015) health-promoting features of the polyphenols is due to the fact that it could be solubilized in digestive fluids, then absorbed and metabolized in gastrointestinal tract. It has been reported that thermal processing of cereals may positively or negatively affect the content of polyphenols in cereal and pseudo-cereals bakery products (Giménez-Bastida et al., 2015). Spínola et al. (2018) showed for Madeira sorrel, a wild leafy-vegetable, that *in vitro* digestion model could be used to predict the bioavailability of phenols, and these types of analysis is simpler and easier to perform than the animal model.

The fermentation of cereals or pseudo-cereals is related to lactic acid bacteria (Charalampopoulos et al., 2009; Muller et al., 2001). The development

of flavour, enhancement of the nutritional value and shelf life, and removing toxic or antinutritional factors of obtained products could be indicated as those properties associated with cereal fermentation (Coda et al., 2014). As presented by Baráth et al. (2004) that effects are connected with the production of organic acids (lactic and acetic) and pH lowering. Zieliński et al. (2017) showed that fermentation of buckwheat flour by *L. plantarum* could be used for the production of functional products.

The aim of this study was to investigate the potential bioaccessible ACE inhibitory activity under the influence of the applied process: fermentation, baking and *in vitro* digestion of products obtained from roasted buckwheat flour fermented by selected bacteria. The contribution of total phenolic compounds (TPC) to the ACE inhibitory activity of roasted buckwheat fermented flours and obtained biscuits before and after *in vitro* digestion were also investigated.

MATERIALS AND METHODS

Chemicals

Sigma-Aldrich (St. Louis, MO, USA) was supplier of captopril (No C4042, St. Louis, MO, USA), angiotensin-converting enzymes (ACE) from porcine kidneys (EC 3.4.15.1), the Folin and Ciocalteu's phenol reagent, standards of phenolic acids and flavonoids, acetonitrile, methanol, water and formic acid. Extrasynthese used for their IC₅₀ calculation was obtained from Genay (France). A three Tris-base buffers were as follows: buffer A: 300 mM Tris-base buffer (pH 8,3) with 2 mM ZnCl₂; buffer B: 150 mM Tris-base buffer (pH 8,3) and buffer C: 150 mM Tris-base buffer (pH 8,3) with 1,125 M NaCl. From BACHEM (Bubendorf, Switzerland) was obtained *o*-aminobenzoylglycyl-*p*-nitrophenylalanylproline (Abz-Gly-Phe(NO₂)-Pro). Avantor Performance Materials Poland S.A. (Gliwice, Poland) was supplier of the diethyl ether (Et₂O), hydrochloric acid (HCl) and sodium hydroxide (NaOH). The Mili-Q-system (Milipore, Bedford, USA) was used for water purification.

Roasted buckwheat flours – fermentation process

The roasted common buckwheat groats (*Fagopyrum esculentum* Moench) (Melvit S.A., Kruki, Poland) were milled into flour and then were called roasted buckwheat flour. Zieliński et

al. (2009) described the roasting process used in the industry in previous publication. Wronkowska et al. (2018) and Zieliński et al. (2019) presented the processes used for flour pre-treatment, the origin of selected bacteria used, and the fermentation process. Freeze-drying was used for dehydration process

Biscuits from roasted buckwheat fermented flours – baking process

The AACC 10–52 method (2000) with the modification proposed by Hidalgo and Brandolini (2011) was used for biscuit dough preparation. In the recipe used in our study sugar, shortening and non-fat dry milk have not been applied. All ingredients were mixed for 3 minutes (Kitchen Aid, St. Joseph, MI, USA), then the dough was cut with a square cookie cutter (60 mm). The biscuits were baked in electric oven (Sveba Dahlen AB, Fristad, Sweden) at temperature 220°C for 30 min. The obtained biscuits were freeze-dried, milled and stored in a refrigerator until analysis.

Biscuits from roasted buckwheat fermented flours – *in vitro* digestion

The biscuits were subjected to successive *in vitro* digestion according Delgado-Andrade et al. (2010). In first step: samples were incubated with α -amylase solution (37°C/30 min., pH 7.0). In second step: after the reduction of the pH to 2.0

samples were incubated with pepsin solution (37°C/120 min.). In the last step: the pH was adjusted to 6.0, and a mixture of pancreatin and bile salts extract was added. Then the samples were incubated 120 min. at 37°C after the pH adjusted to 7.5. For inactivating of the enzymes the heating at 100°C for 4 minutes was used, then samples were centrifuged. Obtained supernatant were stored at -20°C for the evaluation of TPC and bioaccessible ACE inhibitory activity.

The bioaccessibility index (BI) were determined for illustration of the bioaccessible ACE inhibitory activity *in vitro*:

$$BI = (1/IC_{50})_{GD} / (1/IC_{50})_{BB};$$

where $(IC_{50})_{GD}$ is the ACE inhibitory activity after *in vitro* digestion (GD) and $(IC_{50})_{BB}$ is the ACE inhibitory activity of buckwheat biscuits (BB). BI value higher than one indicates high bioaccessibility and BI value lower than one indicates low bioaccessibility.

Roasted buckwheat fermented flours and biscuits before and after *in vitro* digestion - determination of TPC

Analysed samples (100 mg) were extracted with 80% MeOH, then were vortexed/sonicated/vortexed, and centrifuged. This procedure was repeated 5 times, the final extract concentration was 20 mg/mL. Then extracts as well as supernatants obtained after *in vitro* digestion were mixed with the Folin-Ciocalteu reagent, left for 25 min at room temperature, and centrifuged. Spectrophotometer (model A UV-1601PC, Shimadzu, Japan) was used for the absorbance determination at 725 nm, results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry matter (d.m.) sample (Zieliński et al., 2017).

Extraction and isolation of the main phenolic compounds from roasted buckwheat fermented flour and biscuits before and after *in vitro* digestion

Freeze-dried samples were extracted with 80% methanol according Wiczowski et al. (2016) assay. Free forms of polyphenols were isolated by diethyl ether, but conjugated forms (esters and glycosides) samples were initially hydrolysed with NaOH and HCl. After each hydrolysis, the extraction process was conducted in triplicates, the collected ether extracts were evaporated to dryness. For the analysis of profile and content of

phenolic acids and flavonoids the system HPLC-MS/MS involving an HALO column was applied (Wiczowski et al., 2016).

Angiotensin-I-converting enzyme (ACE) inhibitory assay

Freeze-dried samples (300 mg) were 5-times extracted with 70% aqueous methanol. Before assay evaporated extract were dissolved in deionized water, but the supernatants obtained after *in vitro* digestion of biscuits were used directly. Each sample was diluted to various concentrations and then used for the evaluation of the ACE inhibitory activities (expressed as IC_{50}).

Method proposed by Sentandreu and Toldra (2006) was used for determination of ACE inhibitory activities. Exactly 50 μ L of ACE working solution (7.5 mg/mL in 150 mM Tris-base buffer, pH 8.3) was added to each microliter-plate well, then adjusted to 100 μ L by adding either deionized water (control) or samples in the inhibition studies. The enzyme reaction was initiated by the addition of 200 μ L of substrate working solution of Abz-Gly-Phe(NO₂)-pro (in tris-base buffer, pH 8.3), immediately mixed and incubated at 37°C. The generated fluorescence was measured immediately after mixing (0 min) and then after 30 min of reaction using multiscan microplate fluorometer at an excitation of 365 nm and an emission of 405 nm. To calculate the % of inhibition of ACE, the following equation was used:

$$\text{Relative ACE activity \%} = 100 -$$

$$- (\Delta RFU_{\text{sample}} * 100 / \Delta RFU_{\text{negative control}})$$

where $\Delta RFU = RFU_{\text{at time 30}} - RFU_{\text{at time 0}}$.

Statistical analysis

The mean values and the standard deviation of three independent measurements were presented as the illustration of the obtained results. Student's t-test for less numerous groups ($P < 0.05$) was used for determination of the differences in the IC_{50} or TPC in control sample compared to other analysed samples. All data were analysed using one-way analysis of variance (ANOVA) with Fisher's Least Significant Difference test ($P < 0.05$). The Pearson correlation coefficient was used for the correlation analysis ($P < 0.05$). All analysis were performed in STATISTICA for Windows (StatSoft Inc., Tulsa, USA, 2001).

RESULTS AND DISCUSSION

Effect of fermentation on ACE inhibitory activity of roasted buckwheat flours

The IC₅₀ of control, non fermented roasted buckwheat flours, was 60.86 mg mL⁻¹ (Table 1). According to definition proposed by Mojica et al. (2015) the lower IC₅₀, the higher ACE inhibitory activity of analysed product. Compared to control sample, non fermented roasted buckwheat flour, the significantly higher ACE inhibitory activity was noticed for roasted flours fermented by two *Lactobacillus* strains: *L. delbrueckii* subsp. *bulgaricus* 151 and *L. plantarum* W42 (Table 1). For eight bacteria strains, the ACE inhibitory activity were at a similar level as was provided for control sample, whereas for four bacteria a decrease in ACE inhibitory activity was noticed ($P < 0.05$). Summing up the results, it should be stated that ACE inhibitory activity did not change generally under the influence of fermentation by selected bacteria.

Currently it is noted increased interest in natural sources of ACE inhibitory peptides due to the fact that they are considered to come from safe sources (Rui et al., 2015). The ACE inhibitory effect may depend on the plant material used and also microorganisms used as it was showed by: Pyo and Lee (2007), Torino et al. (2013), and Juan et al. (2010). These authors demonstrated the ACE inhibitory activity of *Monascus* fermented soybean, *Lactobacillus plantarum* fermented lentil, and *Bacillus* spp. fermented black soybeans, with IC₅₀ of 0.29; 0.20 and 1.81–2.35 mg/mL, respectively. Navy bean and chickpea do not

showed the ACE inhibitory activity, but fermented products obtained from them exhibit this property (Rui et al., 2015; Xiao et al., 2015).

Effect of baking on ACE inhibitory activity of biscuits obtained from roasted buckwheat fermented flour

Table 1 showed the results of ACE inhibition after the baking process (220°C/30 min), control biscuits from not fermented roasted buckwheat flour get IC₅₀ values of 81.31 mg mL⁻¹. Generally, significantly increase of the ACE inhibitory activity under the influence of baking process was observed. For eleven biscuits significant decrease of IC₅₀ values compared to control biscuit was found (Table 1). While Mojica et al. (2015) did not found differences of IC₅₀ values of raw and precooked bean, and those authors suggested that ACE inhibitory potential of common bean was not affected by processing.

Effect of *in vitro* digestion on ACE inhibitory activity of biscuits obtained from roasted buckwheat fermented flour

In Table 1 were presented the results of ACE inhibitory activity of supernatant obtained after *in vitro* digestion of buckwheat biscuits. All analysed supernatants showed significantly higher ACE inhibitory activity (e.g. lower IC₅₀ values) compared to flours and biscuits. The IC₅₀ values of supernatant obtained after digestion of control biscuits was 3.07 mg mL⁻¹. Only for sample fermented by *L. plantarum* IB and *L. acidophilus* V significant decrease of IC₅₀ compared to digested control sample was observed.

Table 1. The IC₅₀ of samples obtained from roasted buckwheat (mg mL⁻¹)

No	Strain/sample	Flour	Biscuits	Digested biscuits
1	Control	60.86 ± 1.46b	81.31 ± 4.76a	3.07 ± 0.04c
2	<i>L. plantarum</i> IB	57.15 ± 2.76b	70.86 ± 3.45*a	2.71 ± 0.07*c
3	<i>L. plantarum</i> W42	54.97 ± 0.76*b	75.91 ± 7.38a	3.25 ± 0.05*c
4	<i>L. delbrueckii</i> subsp. <i>bulgaricus</i> 151	43.63 ± 2.11*b	60.24 ± 4.20*a	3.16 ± 0.32c
5	<i>L. casei</i> Ley	81.30 ± 2.58*a	36.92 ± 6.43*b	3.59 ± 0.20*c
6	<i>Streptococcus thermophilus</i> MK-10	68.76 ± 2.64b	109.50 ± 7.93*a	3.21 ± 0.29c
7	<i>L. acidophilus</i> La5	79.60 ± 1.34*a	67.50 ± 5.12*b	3.35 ± 0.13*c
8	<i>L. acidophilus</i> V	77.31 ± 0.49*a	61.16 ± 6.47*b	2.80 ± 0.08*c
9	<i>L. acidophilus</i> 145	58.48 ± 2.31b	110.98 ± 1.15*a	4.14 ± 0.40*c
10	<i>L. casei</i> 2K	67.04 ± 3.09a	21.77 ± 3.54*b	3.79 ± 0.53c
11	<i>L. delbrueckii</i> subsp. <i>bulgaricus</i> K	66.99 ± 3.65a	38.16 ± 4.61*b	3.75 ± 0.33*c
12	<i>L. rhamnosus</i> GG	68.18 ± 0.52*b	73.41 ± 0.77*a	3.48 ± 0.32c
13	<i>L. rhamnosus</i> 8/4	63.41 ± 3.52a	66.71 ± 3.52*a	3.60 ± 0.03*b
14	<i>L. rhamnosus</i> K	61.74 ± 3.84a	56.99 ± 1.82*a	3.30 ± 0.18b
15	<i>L. salivarius</i> AWH	69.41 ± 3.10a	54.32 ± 3.53*b	2.96 ± 0.24c
Average (from 2 to 15)		65.57 ± 10.20	64.60 ± 24.68	3.36 ± 0.40

Data presented as mean ± standard deviation (n = 3). *means in each column followed by asterisk are significantly different at $P < 0.05$ based on the Student's t-test for less numerous groups. Different letters (a-c) within the same row indicate statistically significantly different at $P < 0.05$ in LSD Fisher test.

The bioaccessible ACE inhibitory activity of biscuits obtained from roasted buckwheat fermented flour

The ACE inhibitory activity of the digested biscuits were significantly higher compared to no digested biscuits (Table 1). In our study, for the digested biscuits the BI values from 5.7 to 34.1 (Figure 1) were noted, and the highest value was found for *Streptococcus thermophilus* MK-10 (34.1), followed by *L. acidophilus* 145 and *L. rhamnosus* GG, whereas BI value for digested biscuits made of non-fermented roasted buckwheat flour was 26.5 (Figure 1). Generally, high potential bioaccessibility of ACE inhibitory activity from experimental biscuits was observed in this study. Gawlik-Dziki et al. (2016) showed that BI could be used for coffee and coconut as useful parameter to study the phenolics bioaccessibility. For tartary buckwheat Li et al. (2002) showed that pepsin hydrolysis has not effect on increased the ACE inhibitory action. But these authors showed that when combined action of pepsin and trypsin/chymotrypsin was applied the significant increase in the inhibitory activity was noted. Kawakami et al. (1995) found that enzymatic hydrolysis of buckwheat proteins positively effects on the production of ACE inhibitors, and those could suggest that gastrointestinal digestion support the ability to inhibit ACE.

Effect of fermentation, baking and in vitro digestion on the content of TPC of analysed samples

Generally, significant increase in TPC in roasted buckwheat flour fermented by selected bacteria strains was observed as compared to the non-

fermented roasted flour (Table 2). Moreover, baking process did not change significantly the TPC content and similarly like for flours a slight increase of TPC in biscuits was noticed. Significantly higher TPC content was found in samples obtained after digestion of examined biscuits compared to the fermented flours or biscuits before digestion. But compared to digested control biscuits there were not significantly different in TPC, except for three type of the biscuits obtained from flour fermented by: *L. delbrueckii subsp. bulgaricus* 151, *S. thermophilus* MK-10 and *L. rhamnosus* GG (Table 2).

As it was showed by other authors the increase of TPC content was observed during the solid-state fermentation of oat by fungus *Monascus anka* GIM 3.592 (Bei et al., 2020) and lentils by *Aspergillus oryzae* and *A. niger* (Alves Magro et al., 2019).

The average levels of main phenolic compounds from analysed samples – profile and content

In all analysed samples ten phenolic acids and eight flavonoids were found (Table 3 and 4). Phenolic acids included derivatives of hydroxycinnamic and hydroxybenzoic acids: chlorogenic, p-coumaric, sinapic, ferulic, t-cinnamic, syringic, vanillic, isovanillic, protocatechuic and caffeic acids. The average (calculated for all analysed samples) level of detected phenolic acids is shown in Table 3. Isovanillic acid was identified only in biscuits before and after digestion.

Figure 1. The bioaccessibility index (BI) of biscuits formulated from roasted buckwheat fermented flours

Table 2. The content of total phenolic compounds (TPC) in samples obtained from roasted buckwheat (mg GAE/g d.m.).

No	Strain/sample	Flour	Biscuits	Digested biscuits
1	Control	0.89 ± 0.07b	0.80 ± 0.05b	5.22 ± 0.14a
2	<i>L. plantarum</i> IB	1.16 ± 0.07*b	1.23 ± 0.07*b	4.83 ± 0.18*a
3	<i>L. plantarum</i> W42	1.14 ± 0.06*b	0.96 ± 0.03*b	4.94 ± 0.29a
4	<i>L. delbrucki subsp. bulgaricus</i> 151	1.00 ± 0.16b	0.90 ± 0.01*b	5.67 ± 0.24*a
5	<i>L. casei</i> Lcy	1.02 ± 0.15b	1.03 ± 0.04*b	5.24 ± 0.24a
6	<i>Streptococcus thermophilus</i> MK-10	1.11 ± 0.05*b	0.83 ± 0.06c	6.02 ± 0.14*a
7	<i>L. acidophilus</i> La5	1.25 ± 0.16*b	1.18 ± 0.03*b	5.42 ± 0.38a
8	<i>L. acidophilus</i> V	1.25 ± 0.09*b	1.07 ± 0.06*b	5.63 ± 0.27a
9	<i>L. acidophilus</i> 145	1.21 ± 0.06*b	0.82 ± 0.01c	4.94 ± 0.09*a
10	<i>L. casei</i> 2K	0.93 ± 0.31b	0.74 ± 0.03b	4.80 ± 0.09*a
11	<i>L. delbrucki subsp. bulgaricus</i> K	0.96 ± 0.10b	0.83 ± 0.04b	4.99 ± 0.13a
12	<i>L. rhamnosus</i> GG	1.16 ± 0.14*b	0.91 ± 0.01*b	5.72 ± 0.24*a
13	<i>L. rhamnosus</i> 8/4	0.95 ± 0.04b	0.69 ± 0.02*c	5.09 ± 0.24a
14	<i>L. rhamnosus</i> K	0.88 ± 0.16b	0.78 ± 0.01b	4.47 ± 0.19*a
15	<i>L. salivarius</i> AWH	0.87 ± 0.14b	0.84 ± 0.01b	4.37 ± 0.12*a
Average (from 2 to 15)		1.06 ± 0.13	0.92 ± 0.16	5.15 ± 0.49

Data presented as mean ± standard deviation (n = 3). *means in each column followed by asterisk are significantly different at P<0.05 based on the Student's t-test for less numerous groups. Different letters (a-c) within the same row indicate statistically significantly different at P<0.05 in LSD Fisher test.

In non-fermented and fermented roasted buckwheat flours: caffeic, vanillic and ferulic acids were in the highest concentration among average content of other identified phenolic acids (Table 3). Concentration of individual phenolic acids in fermented roasted buckwheat flours was differentiated. The average content of t-cinnamic acid was almost 50% lower in fermented flour compared to non-fermented ones. Baking process caused a reduction of content of phenolic acids with exception of syringic and protocatechuic acids (Table 3). Abdel-Aal and Rabalski (2013) observed for wholegrain bakery products that thermal process influenced on the content and profile of phenolic acids. Generally, after digestion the total average concentration of phenolic acids increased as compared to non-digested biscuits.

The highest increase was noticed for caffeic and sinapic acids, 8 and 5-times compared to their content in biscuits before digestion, respectively. Only for syringic acid the decrease of its level was observed after digestion.

In this study flavonoids in analysed samples included: luteolin, kaempferol, quercetin, rutin, vitexin, apigenin, orientin and epicatechin and their average levels is shown in Table 4. In non-fermented and fermented roasted buckwheat flours the highest average concentration was observed for rutin and epicatechin. Generally, fermentation process did not change the content of flavonoids compared to unfermented roasted buckwheat flour. Only concentration of rutin and epicatechin decrease was observed.

Table 3. Average levels of phenolic acids (µg/mL) in samples obtained from roasted buckwheat

Sample	Non-fermented flour	Fermented flour	Biscuits	Digested biscuits
Chlorogenic	0.07±0.01	0.05±0.02	0.08±0.06	0.10±0.01
p-Coumaric	13.5±0.1	19.3±8.5	13.6±8.3	13.1±3.4
Sinapic	8.7±0.6	6.1±2.2	2.1±0.7	10.5±1.5
Ferulic	70.5±2.4	59.3±7.9	2.6±0.4	4.8±0.9
t-Cinnamic	10.0±0.2	5.8±3.6	5.4±2.1	11.7±3.2
Syringic	24.7±0.7	30.4±5.1	114.8±23.5	68.2±8.3
Vanillic	68.6±1.5	65.7±10.4	50.9±16.6	86.6±15.1
Isovanillic	nd	nd	4.2±3.1	13.2±3.4
Protocatechuic	25.2±0.3	25.2±6.5	38.3±10.1	128.2±31.1
Caffeic	89.3±3.8	61.5±36.6	2.0±1.0	17.7±6.9
Total	310.6	273.4	234.0	354.1

Table 4. Average levels of flavonoids ($\mu\text{g/mL}$) in samples obtained from roasted buckwheat.

Sample	Non-fermented flour	Fermented flour	Biscuits	Digested biscuits
Luteolin	0.24 $\pm$ 0.01	0.30 $\pm$ 0.08	0.18 $\pm$ 0.04	0.35 $\pm$ 0.05
Kaempferol	0.78 $\pm$ 0.01	1.00 $\pm$ 0.30	4.6 $\pm$ 1.4	18.3 $\pm$ 2.1
Quercetin	3.8 $\pm$ 0.1	3.9 $\pm$ 1.3	3.3 $\pm$ 1.8	5.8 $\pm$ 1.8
Rutin	172.5 $\pm$ 0.3	139.9 $\pm$ 25.1	65.0 $\pm$ 8.6	2.1 $\pm$ 0.5
Vitexin	22.4 $\pm$ 1.2	16.4 $\pm$ 2.1	12.3 $\pm$ 2.7	6.1 $\pm$ 0.8
Apigenin	0.70 $\pm$ 0.05	0.5 $\pm$ 0.1	1.3 $\pm$ 0.7	1.0 $\pm$ 0.1
Orientin	14.2 $\pm$ 0.5	9.7 $\pm$ 2.3	6.2 $\pm$ 1.9	2.2 $\pm$ 0.4
Epicatechin	138.5 $\pm$ 1.6	96.6 $\pm$ 33.0	11.9 $\pm$ 11.3	17.7 $\pm$ 3.9
Total	353.1	268.3	104.8	53.5

Baking process used (220°C, 30 min) causes a decrease in the content of investigated flavonoids, but most noticeable again in the case of rutin and epicatechin (Table 4). After *in vitro* digestion the average level of rutin, apigenin and orientin were further reduced, whereas the average concentration of kaempferol, quercetin, luteolin and epicatechin was increased. Generally, after digestion the total average concentration of flavonoids was reduced twice as compared to non-digested biscuits (Table 4). The highest decrease was found for rutin, the main buckwheat flavonoid.

Park et al. (2007) observed increments of phenol and flavonoids after the fermentation of soybean meal with different microorganisms. Broccoli co-products, which was used as a crackers ingredient, increase of TPC content and also after simulated gastrointestinal digestion the amount of phenolic and antioxidant compounds was higher as found by Lafarga et al. (2019).

Correlation study focused on the contribution of TPC, phenolic acids and flavonoids to the ACE inhibitory activity of analysed samples

High significant correlation ($r=0.79$) was found between IC_{50} and TPC of non fermented and fermented flours, biscuits before and after digestion (Table 5). Almost for all analysed phenolic acids the correlation coefficient were ranged from $r=0.14$ (syringic acid) to $r=0.99$ (protocatechuic acid). These evidences clearly indicate for the contribution of phenolic compounds in ACE inhibitory activity of the samples obtained from roasted buckwheat fermented by selected bacteria strains.

The opposite situation was noticed for flavonoids, the correlation coefficients $r= -0.67$ were found

between IC_{50} and TPC, total flavonoids of non-fermented and fermented flour, biscuits before and after digestion (Table 5). But it should be noted that correlation coefficients found between IC_{50} and selected flavonoids, such as luteolin, kaempferol and quercetin were 0.75; 0.98 and 0.97, respectively.

Table 5. The values of the Pearson correlation coefficient between $1/\text{IC}_{50}$ and TPC, phenolic acids and flavonoids of non-fermented and fermented roasted buckwheat flours, biscuit before and after *in vitro* digestion ($P<0.05$)

Bioactive compounds/samples	Samples based on roasted buckwheat flour*
Total phenolic acids	0.79
Chlorogenic	0.80
<i>p</i> -Coumaric	-0.40
Sinapic	0.67
Ferulic	-0.55
<i>t</i> -Cinnamic	0.75
Syringic	0.14
Vanillic	0.85
Isovanillic	0.95
Protocatechuic	0.99
Caffeic	-0.41
Total flavonoids	-0.67
Luteolin	0.75
Kaempferol	0.98
Quercetin	0.97
Rutin	-0.81
Vitexin	-0.79
Apigenin	0.24
Orientin	-0.77
Epicatechin	-0.52

*non-fermented flour; fermented flours; biscuit and sample after digestion

CONCLUSIONS

Generally, the fermentation by selected bacteria of roasted buckwheat flour did not change of ACE inhibitory activity, but was strains dependent. Whereas, baking and digestion significantly increase those properties of biscuits prepared from fermented roasted buckwheat flour. A high

significant correlations were found between IC₅₀ and TPC of fermented flour, biscuit before and after digestion, what should indicate contribution of phenolic compounds in ACE inhibitory activity.

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