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ORIGINAL ARTICLE

Effect of fermented and unfermented buckwheat flour on functional properties of gluten-free muffins

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Abstract Selected functional properties of four types of gluten-free muffins made of unfermented and fermented (by *Lactobacillus plantarum*) buckwheat flour in comparison with control muffins made using commercial gluten-free corn flour were evaluated in this study. The proximate chemical composition, antioxidant capacity analysed by ABTS, photochemiluminescence and cyclic voltammetry assays, and inhibitory activity against protein glycation in vitro in BSA/Glu systems were investigated. The content of the total phenolic compounds, available lysine, furosine, free and total FIC, browning index and antioxidant capacity of buckwheat-enhanced gluten-free muffins were higher compared to the control samples. Gluten-free muffins made of the fermented buckwheat flour showed a significantly higher antioxidant capacity, an increased activity against AGEs formation and an increased available lysine content, as well as a higher FAST index and browning index as compared to the muffins obtained with unfermented buckwheat flour. The study showed that buckwheat flour fermented by *L. plantarum* could be used as an ingredient for improving the functional properties of gluten-free muffins.

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Introduction

Muffins are one of the popular sweet snacks known all around the world. People suffering for celiac disease are unable to consume them because they are traditionally prepared from wheat flour with added eggs, sugar, oil or fat and milk, yeasts or baking powder. Nowadays, scientific data is available regarding gluten-free muffins prepared from different types of flours, like chickpea (Herranz et al. 2016), rice (Nozawa et al. 2016; Singh et al. 2015, 2016), corn (Marcet et al. 2015) or buckwheat (Ciesarová et al. 2016). Also protease treatment is used to produce wheat flour with partially hydrolysed gluten that may be used for preparing hypoimmunogenic muffins as presented by Umashankar et al. (2016).

In their review article, Zannini et al. (2012) presented the feasibility of applying microbial fermentation to produce gluten-free products with improved quality. They emphasised how important it is to look for the optimal microbial starter cultures to reengineer gluten-free products and processes. Cereals are good sources of nutrients for a number of species of the *Lactobacillus* genus (Charalampopoulos et al. 2009; Müller et al. 2001). Fermentation of cereals with lactic acid bacteria, including lactobacilli, may result in many types of metabolites with putative bioactivity like, e.g., cobalamin, reuterin, riboflavin, and γ-aminobutyrate (Russo et al. 2014; Stromeck et al. 2011). Ciesarová et al. (2016) demonstrated that muffins made of unfermented and fermented buckwheat flour suspension showed higher contents of potassium, magnesium, zinc and

manganese compared to the control muffins made of gluten-free corn flour.

Buckwheat (*Fagopyrum esculentum* Moench) has been used as a component of gluten-free products and reported to improve the technological and overall sensory quality of bread (Dapčević Hadnađev et al. 2013; Wronkowska et al. 2013). This pseudocereal is a significant source of rutin, catechins and polyphenols, with their potential antioxidant activity having a significant effect on a nutritional value (Wronkowska et al. 2010). Giménez-Bastida and Zieliński (2015) presented an overview of recent in vitro and in vivo studies concerning health benefits resulting from buckwheat consumption. They pointed to the need for designing future in vitro studies which will allow indicating compounds responsible for the observed beneficial effects, trying to identify the mechanisms which underlie the positive impact on health.

The aim of this study was to evaluate the selected functional properties of four types of gluten-free muffins made of unfermented and fermented, by *Lactobacillus plantarum,* common buckwheat flour in comparison with muffins made of commercial gluten-free corn flour.

Materials and methods

Ingredients for muffin formulation

The following ingredients were used: commercial granulated sugar (Korunný cukor, Slovenské cukrovary s.r.o., Sered', Slovakia), sunflower oil (Prommiena, produced for Lidl, Nemšová, Slovakia), eggs (medium size 53–63 g, produced for Lidl, Nemšová, Slovakia), salt (Castello, produced for Lidl, Nemšová, Slovakia), and sodium bicarbonate p.a. ($NaHCO_3$, Slavus, Bratislava, Slovakia). Gluten-free corn flour (containing: corn starch, corn flour, guar gum and dextrose, according to producer's declaration) was provided by Dr. Schär AG/SPA (Italy). Flour from common buckwheat was provided by the local industry from North–East Poland.

Muffin-making process

The procedure for the preparation of buckwheat or gluten-free corn flour suspensions and for the preparation of a fermented suspension of flour was described in detail by Ciesarová et al. (2016). Briefly, 25 g of buckwheat flour or gluten-free corn flour was mixed with 100 mL of water, boiled (up to thick consistency of the flour suspension) and then sterilised in an autoclave (121 °C for 15 min, at 200 kPa). The fermented buckwheat flour suspension was prepared by mixing the suspension with 1 mL of inoculum of *L. plantarum* S-lak 1 (collection from Stuvital, Ltd., Slovakia) and then incubation for 24 h at 25 °C, without mixing. The basic formulas of control and experimental muffins are shown in Table 1. The mixture of all ingredients was blended (planetary rotation of mixing) using a 5-speed KitchenAid mixer Model 5KSM150PS (Artisan, USA). The dough (50-g portions) was baked in paper cups at 180 °C for 25 min. Baking tests were carried out in an electric oven (Miwe Condo, Germany). The mass of fresh muffins, after cooling, was circa 40 g. The dry matter (DM) of muffins was determined after 48-h pre-drying at ambient temperature using a moisture analyser IR-120 (Denver Instrument, Germany). Sample abbreviations were presented under Table 1.

Chemicals and reagents

2,2′-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), rutin (quercetin-3-rutinoside), D-glucose, bovine serum albumin (BSA), and lysine (N^{α}-acetyl-L-lysine) were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA). The kit for the photochemiluminescence (PCL) assay was from Analytik Jena AG (Jena, Germany). o-phtaldialdehyde for fluorescence (OPA) and sodium dodecylsulfonate (SDS) were supplied by Fluka (Buchs, Switzerland). Furosine (2-furoylmethyl-lysine) was purchased from PolPeptide (Strasbourg, France). Methanol (HPLC purity) was provided by POCh (Gliwice, Poland). Water was purified with the Mili-Q-system (Milipore, Bedford, USA).

Methods

The protein, ash and fat content of the gluten-free muffins was determined using AOAC (2005) method while total carbohydrate was determined by difference.

Assays of available lysine, furosine, free fluorescence intermediary compounds (FIC), FAST index and browning index were conducted as described by Michalska et al. (2008). Materials for these analyses were prepared as follows: dry samples were mixed with 6% of aqueous SDS, incubated for 30 min with stirring every 10 min for 30 s and filtrated, and then the filtrates were used for the analyses.

The OPA assay was used to determine the content of available lysine (fluorescence at $\lambda_{Ex} = 340$ and $\lambda_{Em} = 455$ nm). For the quantitative analysis of available lysine, the external standard method was used and values obtained were expressed as FI/mg of dry matter (DM).

Furosine, 2-furoylmethyl-lysine, content was determined with the chromatographic method. The samples were hydrolysed with 8 mL of 8 M HCl at 110 °C for 23 h under anaerobic conditions, and then the hydrolysates were

Table 1 Recipe of experimental muffins

Ingredients (g)	Control	Gluten-free buckwheat		Gluten-free buckwheat/corn	
		Unfermented UB	Fermented FB	Unfermented UG	Fermented FG
Buckwheat flour	–	54	54	–	–
Gluten-free corn flour	54	–	–	54	54
Gluten-free corn flour in suspension	36	–	–	–	–
Unfermented buckwheat flour in suspension	–	36	–	36	–
Fermented buckwheat flour in suspension	–	–	36	–	36
Eggs	116 ± 10	116 ± 10	116 ± 10	116 ± 10	116 ± 10
Sugar	50	50	50	50	50
Sunflower oil	10	10	10	10	10
Salt	0.3	0.3	0.3	0.3	0.3
$NaHCO_3$	2	2	2	2	2

Control, control muffins from unfermented gluten-free corn flour suspension with gluten-free corn flour; UB, muffins from unfermented buckwheat flour suspension with buckwheat flour; FB, muffins from fermented buckwheat flour suspension with buckwheat flour; UG, muffins from unfermented buckwheat flour suspension with gluten-free corn flour; FG, muffins from fermented buckwheat flour suspension with gluten-free corn flour

filtrated and used for further analysis. The external standard method was applied for the quantitative analysis of furosine by using of a commercial standard of pure furosine, and data were expressed as milligrams per one grams of DM sample.

The free fluorescence intermediary compounds (FIC) were measured at $\lambda_{Ex} = 353$ and $\lambda_{Em} = 438$ nm. FIC data were expressed as arbitrary fluorescence intensity per one milligram of DM sample (FI/mg).

The FAST index was calculated according to Birlouez-Aragon et al. (2001), based on the analysis of fluorescence due to advanced MRPs measured at $\lambda_{Ex} = 353$ and $\lambda_{Em} = 438$ nm and tryptophan fluorescence at $\lambda_{Ex} = 290$ and $\lambda_{Em} = 340$ nm. Data of FAST index were expressed in percent (w/w).

The formation of brown pigments in the examined muffin samples was estimated according del Castillo et al. (2002). The assay was performed in a Coulter DU 800 spectrophotometer (Beckman Instruments Inc., Fullerton, CA), at the absorbance 420 nm. Results were expressed as arbitrary absorbance units.

For determination of rutin and total phenolic compounds (TPC) content, and for ABTS and PCL assays of antioxidant capacity, extracts from muffins were prepared as follows: 100 mg of muffin powder were extracted with 1 mL of 80% (v/v) methanol, after ultrasonic vibration for 30 s, the solution was mixed and centrifuged at 5000x*g* at 4 °C for 5 min. That step was repeated 5 times and the supernatants were collected into a 5-mL flask. The final extract concentration was 20 mg/mL.

Rutin content was analysed in an HPLC system (Shimadzu, Kyoto, Japan) consisting of two pumps (LC-10 AD), a UV detector (SPD-10A) set at 330 nm, an autosampler set for 5 µL injection (SIL-10 ADVP), a column oven (CTO-10 ASVP), and a system controller (SIL-10 ADVP) according to the method described by Zielińska et al. (2010).

Total phenolic compounds (TPC) was determined according to Shahidi and Naczk (1995). Muffin extracts (0.25 mL) were mixed with 0.25 mL of the Folin-Ciocalteu reagent, 0.5 mL of Na_2CO_3 solution and 4 mL of water, the mixture was left for 25 min at room temperature, and then was centrifuged at 2000x*g* for 10 min. A UV-160 1PC spectrophotometer (Shimadzu, Japan) was used to measure the absorbance at 725 nm. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of DM.

Antioxidant capacities of 80% aqueous methanol extracts from muffin samples were determined against $ABTS^{+\bullet}$ radical cation using a spectrophotometric assay by Re et al. (1999) with a minor modification. For the photometric assay, 1.48 mL of the $ABTS^{+\bullet}$ solution and 20 µL of muffin extracts or Trolox standards were mixed and measured immediately, and again after 6 min at 30 °C and 734 nm using a UV-160 1PC spectrophotometer (Shimadzu, Kyoto, Japan). Appropriate solvent blanks were run in each assay. The antioxidant capacity was calculated on the basis of percentage inhibition of absorbance at 734 nm using a Trolox standard curve and was expressed as µmol Trolox equivalents (Trolox Eq) per one g of sample DM.

Antioxidant capacities were analysed using the photochemiluminescence (PCL) method described by Popov and Lewin (1999) with PHOTOCHEM® apparatus (Analytik Jena, Leipzig, Germany). The muffin extracts were analysed according to Analytic Jena protocols. The total antioxidant capacity (PCL) was calculated as the sum of the values obtained for lipophilic (PCL-ACL) and hydrophilic (PCL-ACW) extracts of muffins and was expressed as μmol Trolox equivalents (Trolox Eq) per one g of sample DM.

Cyclic voltammetry experiments (CV) were performed in 80% methanol extracts of gluten-free muffins (200 mg/mL) mixed with 0.2 M sodium acetate–acetic buffer (pH 4.5) at the ratio of 1:1 (v/v) according to Zieliński et al. (2012). The sodium acetate–acetic buffer acted also as a supporting electrolyte for cyclic voltammetry measurements. A micro-electrochemical cell (with the total volume of 200 μL), made of Teflon, was used in the experiment. The cell comprised three electrodes: a glassy carbon (GC) working electrode (BAS MF-2012, 3 mm diameter), an Ag/AgCl (3.5 M KCl) reference and a Pt (0.5 mm diameter coiled Pt wire) counter electrode. The cyclic voltammetry experiment were performed in the range of −100 to 1200 mV at a potential sweep-rate of 100 mV s^{-1} at room temperature using a G 750 potentiostat/galvanostat (Gamry Ins., USA). The higher total charge under anodic current wave indicates a higher reducing capacity of the investigated muffin extracts. The reducing capacity of gluten-free muffins was expressed in terms of μmol Trolox per one g of sample DM.

To analyse the inhibitory activity against protein glycation (AGEs) in vitro in a bovine serum albumin/glucose (BSA/Glu) assay, muffin extracts were prepared as follows: 0.5 g of powdered muffins were extracted with 5 mL of the 80% methanol aqueous solution (40 min, 25 °C) to the final concentration of 50 mg/mL, and then dried. The dried samples were dissolved in 5 mL of a phosphate buffer (0.1 mol/L, pH 7.4) and used directly for the anti-glycation tests as it was described in details by Szawara-Nowak et al. (2014). Fluorescence intensity (excitation wave of 330 nm and emission wave of 410 nm) was measured using an LS 50B luminescent spectrophotometer (Perkin Elmer, USA). Triplicate samples were run for each set and the percent inhibition of AGEs formation by a muffin extract or aminoguanidine solution (1 mmol/L) used as a positive control, was calculated.

Statistical analysis

The measurements were performed in three replications for each type of muffins obtained from two separate baking processes for every formulation. The reported data are the mean results for each formulation with the standard deviation. The results obtained were analysed by one-way ANOVA. Fisher's Least Significant Difference Test was performed at a significance level of $p < 0.05$ for post hoc comparison.

Results and discussion

The proximate composition of gluten-free muffins made of fermented and unfermented buckwheat flour is presented in Table 2. The highest protein content was found in the muffins prepared from unfermented and fermented buckwheat flour (UB and FB, respectively). The highest fat content was found in control muffins, whereas the lowest in UB muffins. The gluten-free muffins obtained from unfermented or fermented, by *L. plantarum*, common buckwheat flour suspension mixed with buckwheat flour showed the highest content of ash compared to the other analysed samples. As presented Ciesarová et al. (2016), buckwheat flour is a better source on macro- and microelements compared to gluten-free corn flour (7.7 and 1.1 g/kg of ash, respectively). The total carbohydrate content was similar in the analysed muffins, with the lowest value determined in FB muffins. The muffins prepared from gluten-free corn flour with unfermented or fermented buckwheat flour (UG and FG, respectively) showed higher protein content as compared to the control samples. It is important from the nutritional point of view, because the

Table 2 Proximate chemical composition of gluten-free muffins

Type of muffin	Dry matter (%)	Proteins content (g/100 g DM)	Fats content (g/100 g DM)	Ash content (g/100 g DM)	Carbohydrates content (g/100 g DM)
Control	92.8 ± 0.1b	7.6 ± 0.7c	11.3 ± 0.1a	1.2 ± 0.3c	72.7 ± 0.1b
UB	92.3 ± 0.2b	10.4 ± 0.9a	9.8 ± 0.2c	1.8 ± 0.3a	70.3 ± 0.6c
FB	95.5 ± 0.1a	10.7 ± 0.8a	10.9 ± 0.1b	1.8 ± 0.3a	72.1 ± 0.3b
UG	90.7 ± 0.3c	8.3 ± 0.6c	10.2 ± 0.1b	1.6 ± 0.3b	70.6 ± 0.1c
FG	95.3 ± 0.2a	9.2 ± 0.3b	10.4 ± 0.1b	1.6 ± 0.3b	74.1 ± 0.2a

Values are mean ± standard deviation ($n = 3$). Values in each column with different letters are significantly different ($p < 0.05$). *DM* dry matter; *UB, FB, UG, FG* abbreviation as in Table 1

Table 3 Content of rutin and total phenolic compounds (TPC), and antioxidant capacity of gluten-free muffins analysed with PCL, ABTS and CV methods

Type of muffin	Rutin (μg/g DM)	TPC (mg GAE/g DM)	Antioxidant capacity (μmol of Trolox Eq/g DM)		
			PCL	ABTS	CV
Control	10.49 ± 0.74	21.06 ± 0.54d	0.40 ± 0.01d	18.00 ± 1.79c	0.62 ± 0.22d
UB	11.60 ± 1.02	26.28 ± 0.26a	0.75 ± 0.00b	22.46 ± 2.60b	1.45 ± 0.12c
FB	11.81 ± 2.87	25.44 ± 0.55ab	0.97 ± 0.01a	28.99 ± 0.59a	1.80 ± 0.28b
UG	11.07 ± 0.97	24.78 ± 1.05c	0.51 ± 0.01c	20.49 ± 2.05c	2.29 ± 0.18a
FG	11.11 ± 3.09	24.48 ± 0.33c	0.90 ± 0.00a	24.20 ± 0.33b	2.24 ± 0.26a

Values are mean ± standard deviation ($n = 3$). Values in each column with different letters are significantly different ($p < 0.05$). *TPC* total phenolic acids; *GAE* gallic acid equivalents; *DM* dry matter; *UB, FB, UG, FG* abbreviation as in Table 1

Table 4 Tryptophan, available lysine and Maillard reaction products (MRPs) in gluten-free muffins

Type of muffin	Tryptophan (FI/mg DM)	Available lysine (mg/g DM)	Early MRPs	Advanced MRPs			Final MRPs
			Furosine (mg/g DM)	Free FIC (FI/mg DM)	Total FIC (FI/mg DM)	FAST index (%)	Browning index (AU)
Control	7.3 ± 0.38a	3.07 ± 0.14d	0.37 ± 0.08d	4.5 ± 0.27d	32.11 ± 0.14c	61.64d	0.096 ± 0.009b
UB	5.8 ± 0.61c	4.32 ± 0.22b	1.32 ± 0.09a	12.6 ± 1.08b	84.52 ± 7.05b	217.24b	0.098 ± 0.008b
FB	6.1 ± 0.15bc	5.52 ± 0.38a	0.99 ± 0.04b	15.4 ± 1.04a	90.22 ± 6.72b	252.46a	0.168 ± 0.016a
UG	6.9 ± 1.00ab	3.38 ± 0.33 cd	0.81 ± 0.04c	11.1 ± 0.96bc	118.44 ± 11.49a	160.87c	0.167 ± 0.013a
FG	5.3 ± 0.25c	3.80 ± 0.53bc	0.70 ± 0.03c	10.8 ± 0.66c	118.71 ± 2.58a	203.77b	0.198 ± 0.026a

Values are mean ± standard deviation ($n = 3$). Values in each column with different small superscript letters are significantly different ($p \leq 0.05$). *FI* fluorescence intensity; *AU* arbitrary units; *UB, FB, UG, FG* abbreviation as in Table 1

amino acid composition of buckwheat proteins is well balanced, they are rich in arginine and lysine being the primary amino acids limiting the content of proteins in cereals (Wronkowska et al. 2010). Also their digestibility was relatively low (Kato et al. 2001), and they exhibited some functional properties such as hypercholesterolaemic activity in rats fed a high-cholesterol diet as reported by Tomotake et al. (2002).

Buckwheat groats are sources of quercetin and rutin (Kreft et al. 2006). However, muffins produced exclusively from buckwheat flour did not show statistically significant differences in the content of rutin compared to the other muffins (Table 3). The content of total phenolic compounds (TPC) was higher in the muffins prepared from unfermented or fermented buckwheat flour suspension (UB and FB, respectively) compared to the other samples. The fermentation by *L. plantarum* had no significant effect on TPC contents compared to the muffins made of the unfermented buckwheat suspension. Soong et al. (2014) found that the total phenolics content in the muffins baked with corn was higher compared to the muffins made of oat, wheat, and rice flour, but determined the highest total phenolics content in the muffins from barley flour. As presented by Dordević et al. (2010), fermentation of buckwheat by *S. cerevisiae* and *L. rhamnosus* caused TPC increase compared to the non-fermented samples. A significant increase of phenolic acids content in soybean fermented with different microorganisms (*Aspergillus oryzae, Rhizopus oryzae* and *Bacillus subtilis*) was also found by Dueñas et al. (2012).

The antioxidant capacity of gluten-free muffins was analysed with PCL, ABTS and CV methods (Table 3, Fig. 1). The muffins made of fermented and unfermented buckwheat flour had the highest antioxidant capacity compared to the control muffins prepared from the unfermented gluten-free corn flour suspension with gluten-free corn flour. It should be noted that the fermented buckwheat flour suspension used in the recipes was significantly increasing the antioxidant capacity of the muffins (FB and FG). As reported by Soong et al. (2014), the antioxidant capacity of corn, wheat and oat muffins was comparable, while barley muffin demonstrated the highest ability to scavenge ABTS and DPPH free radicals. The fermentation process can enhance the level of many bioactive compounds in cereals, but of course the type of fermentation affects the potentially bioactive constituents obtained. Fermentation of buckwheat, wheat germ, barley and rye by lactic acid bacteria or yeast showed that this process led to

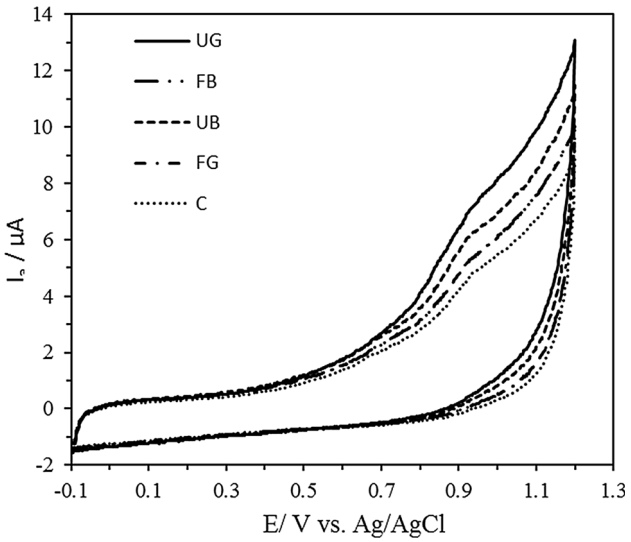

Fig. 1 Cyclic voltammograms of gluten-free muffins. Measurements were performed with 80% methanol extracts (200 mg/mL) mixed with 0.2 M sodium acetate-acetic buffer (pH 4.5) at the ratio of 1:1 (v/v); scan rate 100 mV s^{-1}. Sample description as under Table 1

an increase of antioxidant activities, as presented by Dordević et al. (2010).

Table 4 summarises tryptophan content and results obtained in the OPA assay. In the control samples, prepared from the unfermented gluten-free corn flour suspension with gluten-free corn flour, the content of tryptophan was the highest (7.3 FI/mg DM). Available lysine content in the control samples was lower (3.1 mg/g DM) compared to other analysed muffins. The fermentation procedure used to prepare the fermented buckwheat flour suspension had a beneficial effect since the available lysine content was higher compared to the muffins with the unfermented buckwheat flour suspension. Cereal grains, like corn, have low lysine and tryptophan content as compared to buckwheat (Arendt and Zannini 2013). Bilgiçli (2009) showed that 40% addition of buckwheat flour to prepare of tarhana (traditional Turkish fermented cereal food) significantly increased lysine content.

Contents of the early, advanced and final Maillard reaction products in gluten-free muffins are shown in Table 4. Furosine is one of the markers of the early stage of the Maillard reaction. In our study, the production of furosine was observed in all types of muffins. Its lowest content was found in control muffins (0.4 mg/g DM). Muffins prepared exclusively from buckwheat flour, UB and FB, had the highest content of furosine (1.32 and 0.99 mg/g DM, respectively). For both muffins with the fermented buckwheat flour suspension (FB and FG), a decrease of the furosine content (not significant for FG) was observed compared to the muffins with the unfermented buckwheat flour suspension.

Advanced MRPs analyses in this study included: free and total fluorescence of the intermediary compounds (FIC), also the FAST index was calculated. The content of Free FIC content of muffins prepared exclusively from buckwheat flour (UB and FB) was about 2.5-times higher compared to the control sample, and it was higher compared to UG and FG muffins. The opposite situation was found during the determination of the total FIC, the highest content was found for UG and FG as compared to UB and FB and it was about three times higher compared to the control muffins. These findings indicate that UG and FG muffins contained higher content of linked-to-protein fluorescent compounds as compared to muffins prepared from buckwheat flour (UB and FB). The lowest level of linked-to-protein fluorescent compounds was found in control sample.

The FAST index, calculated as a ratio of FIC to tryptophan fluorescence, is important from the nutritional point of view for the food produced by heat treatment. The significant increase of FAST index was observed for all investigated muffins compared to the control sample. The UG and FG muffins, prepared from unfermented or fermented buckwheat flour suspension with gluten-free corn flour, showed lower values of the FAST index compared to the UB and FB muffins, which could indicate better nutritional quality of the latter. That index was used by Damjanovic Desic and Birlouez-Aragon (2011) as a sensitive indicator which provides reliable information on the nutritional damage induced by heat treatment in, e.g. infant formulas. Thermal conditions used for rye bread baking caused the increase of FIC and FAST index, for bread crust, as demonstrated by Michalska et al. (2008).

Browning index is used as an indicator of the formation of melanoidin during the final stage of Maillard reaction (Table 4). Muffins prepared from unfermented and fermented buckwheat flour suspension with gluten-free corn flour (UG and FG, respectively) showed higher browning index compared to the muffins prepared exclusively from buckwheat flour (UB and FB). The fermentation process used caused browning index increase in both types of muffins (FB and FG), but not statistically significant for the FG sample. Proteins and reducing sugar could be released from the matrix during the fermentation process. Then after baking process used to produce gluten-free muffins, higher values of the browning index were noticed for muffins FB and FG compared to UB and UG muffins due to the final stage of Maillard reaction. Przygodzka et al. (2015) demonstrated an increase of the browning index in rye-buckwheat cake with the selected spices compared to cakes without spices. Slight formation of brown polymer MRP took place during long-term storage (5 years) of ginger cakes, as presented by Zieliński et al. (2012).

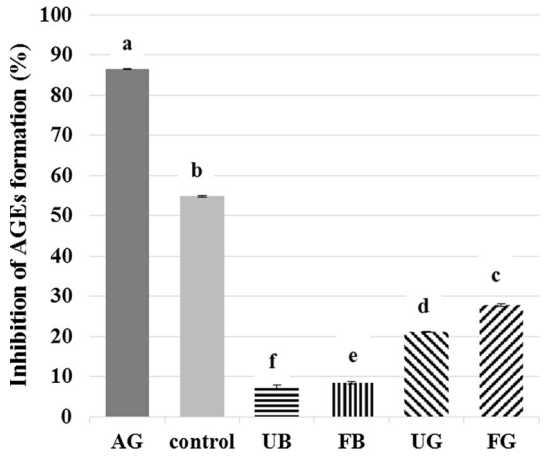

Fig. 2 The inhibitory effects of gluten-free muffins against AGEs formation in the BSA/glucose model system (sample description as under Table 1; *AG* aminoguanidine)

The inhibitory effects of the extracts from buckwheat gluten-free muffins evaluated using the BSA-glucose model are depicted in Fig. 2. The aminoguanidine (AG) solution (1 mmol/L) was used as a reference inhibitor of the glycation process (Zuwało-Jagiełło 2009). AG (a hydrazine compound) prevents AGEs formation by trapping intermediates at the initial glycation stages (Thornalley 2003). It was found that in the BSA-glucose model the inhibitory activity of extracts from muffins prepared from unfermented and fermented buckwheat flour suspension with buckwheat flour (UB and FB, respectively) showed a six-fold lower value as compared to the activity of control muffins from unfermented gluten-free corn flour suspension with gluten-free corn flour. Whereas for extracts from muffins made of the unfermented or fermented buckwheat flour suspension with gluten-free corn flour (UG and FG, respectively) this difference was only about two-fold compared to the control muffin. The inhibitory effect of AG was high and reached 87%. It is noteworthy that the use of the buckwheat flour suspension in the muffins prepared from gluten-free corn flour (UG and FG) affected the improvement of the inhibitory effect of these muffins against AGEs formation in the BSA/glucose model system. Another interesting observation is that the fermentation process applied affected the increase in the degree of inhibition of FB and FG muffins against AGEs formation compared to the muffins prepared from unfermented buckwheat flour suspension (UB and UG). Szawara-Nowak et al. (2014) showed for the buckwheat-enhanced wheat bread that the inhibitory effect against AGEs formation in BSA-glucose system depended on the level of buckwheat substitution and type of wheat flour used. Przygodzka and Zieliński (2015) made a similar observation for rye-buckwheat ginger cakes enriched with rutin. They found that the addition of buckwheat flours as well as rutin supplementation in ginger cakes caused an increase of AGEs inhibitory potential. Also these authors showed that the cakes produced with dough fermentation step had a lower inhibitory activity compared to those without any fermentation step.

Conclusion

The proximate chemical composition, antioxidant capacity of gluten-free muffin extracts against $ABTS^{\bullet+}$ and $O_2^{\bullet-}$ radicals (by ABTS and PCL assays) and their inhibitory activity against protein glycation in vitro in BSA/Glu systems were investigated. The increase of protein content in the muffins with the use of buckwheat flour (UG and FG) compared to the control samples is important from the nutritional point of view because the amino acid composition of buckwheat proteins is well balanced. No statistically significant differences were observed in the content of rutin in various muffins. The content of total phenolic compounds (TPC), available lysine, furosine, free and total FIC, browning index and antioxidant capacity of the muffins were higher compared to the control samples. The fermentation process used for buckwheat flour suspension significantly increased the antioxidant capacity, available lysine content, FAST index, browning index and the degree of inhibition against AGEs formation compared to the samples with unfermented buckwheat flour suspension. The fermentation of buckwheat flour suspension by *L. plantarum* represents a novel technological solution, which could lead to the production of innovative functional products.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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