Polish Journal of Food and Nutrition Sciences

DE GRUYTER OPEN

Pol. J. Food Nutr. Sci., 2015, Vol. 65, No. 3, pp. 191–198
DOI: 10.1515/pjfns-2015-0038
http://journal.pan.olsztyn.pl

Original article
Section: Food Quality and Functionality

Evaluation of *In Vitro* Inhibitory Activity of Rye-Buckwheat Ginger Cakes with Rutin on the Formation of Advanced Glycation End-Products (AGEs)

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Key words: rye-buckwheat ginger cakes enhanced with rutin, inhibitory effect, AGEs formation, phenolic compounds, rutin, D-*chiro*-inositol

In this study, the relationship between the inhibitory effects of extracts from rye-buckwheat ginger cakes supplemented with low and high rutin dosage baked without or with dough fermentation step on the formation of fluorescent advanced glycation end-products (AGEs), and phenolic compounds, rutin, D-*chiro*-inositol and antioxidant capacity were addressed. The cakes were based on rye flour substituted by light buckwheat flour or flour from roasted buckwheat groats at 30% level, and were produced with or without dough fermentation step. The inhibitory effect against AGEs formation was studied in bovine serum albumin (BSA)-glucose and BSA-methylglyoxal (MGO) systems. The antioxidant capacity was measured by 2,2-diphenyl--1-picrylhydrazyl (DPPH) and cyclic voltammetry (CV), rutin and D-*chiro*-inositol contents by HPLC and total phenolics (TPC) by spectrophotometric assays. The study showed the inhibitory effects of extracts from rye-buckwheat ginger cakes supplemented with low and high rutin dosage. The results of the inhibitory activity were highly correlated in two applied model systems. Enrichment of rye-buckwheat ginger cakes with rutin improved their antioxidant properties. The correlation studies showed that the inhibitory effects of rye-buckwheat ginger cakes produced with dough fermentation step and enhanced with rutin against formation of AGEs were highly correlated with TPC, rutin and D-*chiro*-inositol contents, and antioxidant capacity. Moreover, the effect of rutin enrichment was clearly seen in cakes obtained with dough fermentation step, even the inhibitory activity was slightly lower as compared to the cakes produced without dough fermentation.

INTRODUCTION

Dietary advanced glycation end products (dAGEs) are formed in thermally-treated food *via* Maillard reaction [Markowicz Bastos & Gugliucci, 2015]. dAGEs are an important contributor to the total pool of AGEs formed in the living organism and can induce an oxidant stress and inflammation resulting in an increasing risk of diabetic and cardiovascular diseases [Uribarri *et al*., 2005; Yamagishi *et al*., 2007; Ames, 2009]. Furthermore, the list of dAGEs obtained from food products with special guidelines for diabetic patients was elaborated by Uribarii *et al.* [2010]. In the past decade, the extensive attention was focused on searching and developing drugs against AGEs-related diseases. The novel insights into the mechanism and drugs inhibiting AGEs formation was briefly reported by Zuwało-Jagiełło [2009] and Alam *et al.* [2013]. It is noteworthy that two non-pharmacologic prevention ways were proposed instead of the pharmaceutical treatment. The AGEs accumulation in the body can be regulated by low dAGEs diet recommended by Kellow & Savige [2013] and Vlassara & Uribarri [2014]. On the other hand, consumption of foods with natural AGEs inhibitors was proposed [Peng *et al.,* 2011]. According to the latest research conducted by Harris *et al.* [2011] and Peng *et al.* [2011], plant extracts, which are good sources of antioxidant polyphenols, could contribute to the reduction of AGEs formation by preventing oxidative damage of proteins. Green tea and tea infused with selected herbs [Ho *et al.,* 2010], tomato paste [Kiho *et al*., 2004] or spices [Tosun & Khan, 2015] was highlighted as a potential inhibitor of AGEs formation. Therefore, the positive correlation between anti-glycation potential and antioxidant properties in herbs was observed [Ramkissoon *et al.,* 2013]. Searching for inhibitors of AGEs formation, focus is put on polyphenols. Recently, the potential medical properties of quercetin-3-*O*--rutinoside (rutin) have been discussed [Zhang *et al.,* 2012] in the light of its impact on diabetes and its complications [Hao *et al.,* 2012; Kamalakkannan & Prince, 2006; Annapurna *et al.,* 2009]. Rutin extracted from *Piper betle* Linn. leaf [Bhattacherjee & Chakraborti, 2013] as well as obtained from *Teucrium polium* [Esmaeili, 2014] and rutin metabolites [Cervantes--Laurean *et al.,* 2006; Pashikanti *et al.,* 2010] were proved to have the ability to inhibit AGEs formation. In comparison to other plant species, common buckwheat (*Fagopyrum esculentum*) is a good source of rutin [Jiang *et al.,* 2007]. Thereby, the confirmation of high anti-glycation potential of extracts from wheat bread enhanced with buckwheat has been recently reported by Szawara-Nowak *et al.* [2014].

Besides rutin, common buckwheat is a good source of another bioactive compound – D-*chiro*-inositol (DCI) [Arendt

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& Zannini, 2013]. Results of animal studies showed a decreasing effect of DCI on glucose concentration in blood of streptozocin-induced rats [Kawa *et al.,* 2003] and blood pressure, plasma triglyceride and glucose concentrations in type 2 diabetic mice [Yao *et al.,* 2008]. Moreover, the important role of DCI glycans as a second messenger in insulin abnormal response was explained by Larner *et al.* [2010]. Medical studies focused on oral DCI administration showed a positive correlation with a decreasing effect on insulin resistance in women with the polycystic ovary syndrome [Cheang *et al.,* 2008; Takahama, *et al*. 2011]. This finding highlighted the importance of food formulated with partial contribution of buckwheat flour that may be useful in diabetes and polycystic ovary syndrome intervention. Up-to-date results of DCI phosphoglycans accumulation in human placentas of women diagnosed with preeclampsia were observed, thus their involvement in metabolic alternations in the insulin signalling activation in placenta was presented by Scioscia *et al.* [2012].

The objective of this study was to find the relationship between bioactive compounds and the inhibitory activity of extracts from rye-buckwheat ginger cakes supplemented with low and high rutin dosage on the formation of fluorescent advanced glycation end-products (AGEs). The importance of this study to the industry was related to the elaboration of a new product containing natural AGEs inhibitors which can be further recommended as a part of wider prophylactic strategies against diabetes and its complications.

MATERIAL AND METHODS

Chemicals and reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), D-*chiro*-inositol, rutin, D-glucose, sodium azide, bovine serum albumin (BSA) and methylglyoxal (MGO) were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA). Folin-Ciocalteu reagent, sodium carbonate and methanol (HPLC purity) were provided by POCh (Gliwice, Poland). Water was purified with Mili-Q-system (Milipore, Bedford, USA).

Ginger cakes formula preparation

Rye flour (type 720), light buckwheat flour (from *Fagopyrum esculentum*) and buckwheat honey were provided from an organic food store located in Olsztyn (Poland). Flour from roasted buckwheat groats (*F. esculentum* var. Kora) was provided by a local producer from Poland. A commercial spice mix for ginger cakes (according to producer's declaration including cinnamon, anise, fennel, allspice, cloves, coriander, nutmeg) was obtained from Mäspoma (Zvolen, Slovakia).

The cookie making process involved dough preparation by mixing rye flour (70% w/w) and light buckwheat flour or flour from buckwheat groats (30% w/w). The recipe of rye-buckwheat ginger cakes was modified by adding low and high amounts of rutin to the mixture of flours. The addition of rutin to rye-buckwheat ginger cake formula corresponded to the rutin content in one tablet of pharmaceutical drug available in drugstore (25–50 mg of rutin per tablet). After that step, buckwheat honey and other bakery ingredients were added and well mixed, as it was shown in Figure 1. Next, one half of dough was set aside and the second one was directly cut into 0.5-cm thick discs of 5.5 cm diameter and baked at 180°C for 18 min in an electric oven (DC-32E, Sveba-Dahlen, Fristad, Sweden). The first half of dough was held for 72 h at 21°C and then ginger cakes were baked in the same conditions. In the final step, the cakes were freeze-dried and ground. The powdered samples were sieved through a 60-mesh screen and then stored at –20°C until analyzed.

Preparation of ginger cake extracts for the measurement of phenolics and rutin contents, and antioxidant capacity

Powdered ginger cakes samples (100 mg) were extracted with 1 mL of 80% (v/v) methanol. Next, the mixture was treated by ultrasounds for 30 sec, then vortexed for 30 sec, again treated by ultrasounds and vortexed, and centrifuged for 5 min (5000 × *g* at 4°C). That step was repeated 5 times

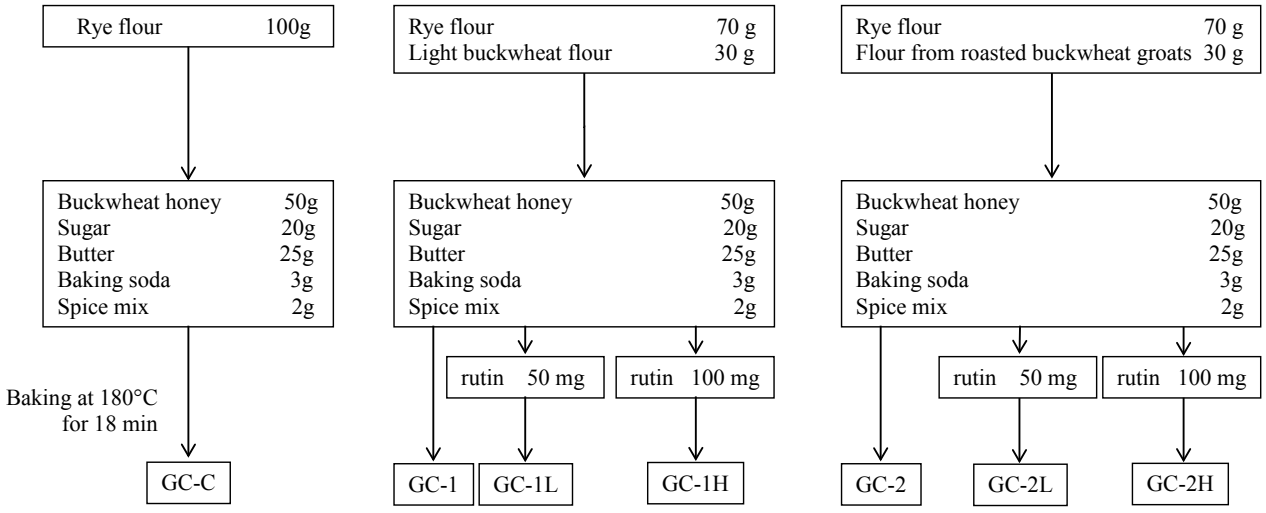

FIGURE 1. Flow diagram of rye-buckwheat cakes making process.

and the supernatants were collected into 5-mL flask. Final extract concentration was 20 mg/mL. Extraction of each sample was performed in triplicate. The fresh extracts were used to determine rutin and total phenolics content and the antioxidant capacity by DPPH and CV methods.

Total phenolic content (TPC) determination

The total phenolic content was determined with Folin-Ciocalteu reagent in 80% methanol cakes extracts according to Singleton & Rossi [1965] with modifications [Przygodzka *et al.,* 2014]. Exactly 0.25 mL of the extract was mixed with 0.25 mL of the Folin-Ciocalteu reagent previously diluted with distilled water (1:1 v/v), 0.5 mL of saturated sodium carbonate (Na_2CO_3) and 4 mL of water. The mixture was incubated at room temperature for 25 min and centrifuged at 2000 × *g* for 10 min. Supernatant absorbance was measured at 725 nm using a spectrophotometer (UV-160 1PC, Shimadzu, Japan). The total phenolic content was standardized against rutin and expressed as mg rutin equivalents per g of dry matter (DM). The measurements were done in triplicate.

Rutin analysis by HPLC

Rutin analysis in rye-buckwheat cakes was determined with HPLC (Shimadzu, Japan) with UV detector (SPD-10A) set up 330 nm as it was recently described by Zielińska *et al.* [2010]. Chromatographic determinations were performed in triplicate. The results were calculated as μg per g DM.

D-*chiro*-inositol analysis

The quantitative method with slight modifications for determining the total D-*chiro*-inositol present in rye-buckwheat ginger cakes samples was conducted according to Yang & Ren [2008]. To 0.25 grams of lyophilized rye-buckwheat ginger cakes 1 mL of 50% ethanol aqueous solution was added and the solutions were mixed for 1 h at room temperature with 1400 rpm shaking using termomixer (Comfort R, Eppendorf, Germany). The samples were centrifuged (5000 × *g*, 20 min, 4°C) and 0.5 mL of supernatants were collected and concentrated up to a half of the volume (1400 rpm, 1 h, 30°C). Then, 0.5 mL of 2 mol/L trifluoroacetic acid was added to hydrolase for 4 h at 70°C. After hydrolysis, the samples were dried under nitrogen stream. Before the analysis, the samples were dissolved in 0.2 mL of methanol.

The HPLH system (Shimadzu, Japan) equipped with a refractometric detector (RID-6A, Shimadzu, Japan) was used. The separation was conducted on a Unison UK-Amino column (3 μm, 250x4.6mm, Imtakt, Japan) with oven temperature set up at 35°C, in an isocratic system with 90% acetonitrile with eluent flow rate of 0.8 mL/min. Identification and quantification was based on the retention time and calibration curve of D-*chiro*-inositol standard. Results were expressed as μg per g DM. Analysis was performed in triplicate.

Antioxidant capacity determination

DPPH assay

The DPPH• radicals scavenging activity assay was based on a modified method of Brand-Williams *et al.* [1995]. In this assay antioxidants present in the sample reduce the DPPH• radicals which have an absorption maximum at 515 nm. The DPPH• solution was prepared by dissolving 10 mg of DPPH in 25 mL of methanol. The decrease in absorbance of the resulting solution was monitored at 517 nm using a spectrophotometer (UV-160 1PC, Shimadzu, Kyoto, Japan). The Trolox standard solution (concentration 0.1––2.5 mmol/L) in methanol was prepared and assayed under the same conditions. The DPPH• scavenging activity was expressed as Trolox equivalents, on the basis of percentage inhibition of absorbance at 515 nm of standards and samples.

Cyclic voltammetry

A potentiostat/galvanostat G 750 (Gamry Ins., USA) was used for voltammetric experiments. Measurements were performed as it was recently reported in details by Zielińska *et al.* [2010]. Cyclic voltammetric experiments were performed on 80% methanol ginger cakes extracts (50 mg/mL) mixed with 0.2 mol/L sodium acetate-acetic buffer (pH 4.5) at a ratio of 1:1 (v/v) in 80% methanol [Cosio *et al.,* 2006]. The measurements were performed at room temperature using apparatus cell (volume 200 μL). The cyclic voltammograms were acquired in the range of -100 to +1200 mV at a scanning rate of 100 mV/s at 2 mV intervals. The 80% methanol solutions of Trolox within the concentration range of 0.05–2.50 mmol/L were used to prepare calibration curve. The results were expressed as μmol Trolox/g DM. Triplicate samples were run for each set.

Anti-glycation assays: BSA-glucose and BSA-MGO systems

Two model systems were used for determination of the anti-glycation activity of rye-buckwheat ginger cakes: BSA-MGO system which was identified with middle stage formation of oxidative cleavage products and BSA-glucose model describing the final stage of AGEs formation [Bhattacherjee & Chakraborti, 2013]. About 0.5 gram of powdered cakes were extracted with 5 mL of 80% methanol aqueous solution (40 min, 25°C) followed by centrifugation (10 min, 4°C, 16000 × *g*) to the final concentration of 50 mg/mL. After centrifugation, the extracts were dried under vacuum at 40°C using a rotary evaporator. After vacuum drying, the samples were dissolved in 5 mL of 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 330 nm and emission wave 410 nm) was measured using luminescent spectrophotometer (LS 50B, Perkin Elmer, USA). Triplicate samples were run for each set and the percent inhibition of AGEs formation by a cake extract or aminoguanidine solution (1 mmol/L) used as a positive control, was calculated.

Statistical analysis

The results are given as the means and the standard deviation of three independent measurements. Statistical one-way analysis of variance (ANOVA) using Fischer LSD test ($p \leq 0.05$) was performed. The correlation tests between anti-glycation activity and rutin, total phenolics and D-*chiro*-inositol content were performed and the Pearson correlation coefficients were calculated. Statistical analyses were performed using software package (StatSoft Inc., v. 7.1, Tulsa, OK, USA).

TABLE 1. Total phenolic compounds (TPC), rutin and *D-chiro*-inositol contents and antioxidant capacity measured against radical (DPPH•) and directly by cyclic voltammetry (CV) method of rye-buckwheat ginger cakes enriched with rutin obtained without dough fermentation step.

Ginger cake type	TPC (mg of rutin /g DM)	Rutin (μg/g DM)	D-*chiro*-inositol (mg/g DM)	Antioxidant capacity (μmol TE/g DM)	
				DPPH	CV
GC-C	2.52±0.10^{e}	26.55±1.78	3.51±0.37^{c}	5.15±0.65^{f}	9.87±0.73^{d}
GC-1	3.85±0.27^{d}	48.10±3.84	4.53±0.18^{a}	7.53±1.14^{e}	17.36±0.16^{b}
GC-1L	3.79±0.16^{d}	346.21±36.59^{c}	3.63±0.09^{c}	9.16±0.10^{d}	17.13±0.29^{b}
GC-1H	4.97±0.16^{c}	723.31±55.74^{a}	4.15±0.01^{b}	9.37±0.24^{d}	19.72±0.77^{a}
GC-2	4.66±0.32^{c}	32.41±1.08	4.60±0.17^{a}	8.74±0.77^{e}	16.76±0.52^{b}
GC-2L	5.52±0.36^{b}	381.91±12.87^{c}	2.71±0.07^{e}	12.15±0.36^{b}	14.99±0.87^{c}
GC-2H	7.20±0.46^{a}	476.58±87.79^{b}	3.04±0.11^{d}	17.74±0.29^{a}	14.58±0.44^{c}

Sample description: GC-C: control ginger cake formulated on rye flour; GC-1: rye-buckwheat ginger cake formulated on rye and light buckwheat flours (70:30; w/w); GC-1L: rye-buckwheat ginger enriched with low rutin content; GC-1H: rye-buckwheat ginger enriched with high rutin content; GC-2: rye-buckwheat ginger cake formulated on rye flour and flour from roasted buckwheat groats(70:30; w/w); GC-2L: rye-buckwheat ginger enriched with low rutin content; GC-2H: rye-buckwheat ginger enriched with high rutin content.
Values are means ± standard deviation (n=3). Values in each column with different small superscript letters are significantly different (p≤0.05).

TABLE 2. Total phenolic compounds (TPC), rutin and *D-chiro*-inositol contents and antioxidant capacity measured against radical (DPPH•) and directly by cyclic voltammetry (CV) method of rye-buckwheat ginger cakes enriched with rutin obtained with dough fermentation step.

Ginger cake type	TPC (mg of rutin /g DM)	Rutin (μg/g DM)	*D-chiro*-inositol (mg/g DM)	Antioxidant capacity (μmol TE/g DM)	
				DPPH	CV
GC-C	4.80± 0.14^{d}	17.62± 0.16^{e}	3.12±0.09^{e}	6.87±0.05^{f}	11.55±1.99^{d}
GC-1	4.91±0.23^{cd}	32.41±1.08^{de}	4.88±0.39^{b}	7.20±0.21^{ef}	13.41±0.54^{bc}
GC-1L	5.71±0.49^{b}	467.45±9.81^{c}	4.63±0.49^{bc}	7.78±0.28^{de}	12.58±1.04^{cd}
GC-1H	5.80±0.42^{b}	829.79±29.87^{a}	6.55±0.16^{a}	8.18±0.08^{d}	14.42±0.41^{b}
GC-2	4.26±0.14^{e}	44.64±0.42^{d}	4.11±0.48^{cd}	9.40±0.35^{c}	12.64±0.25^{cd}
GC-2L	4.63±0.36^{de}	470.13±9.98^{c}	3.72±0.01^{d}	11.02±0.45^{b}	13.81±0.85^{bc}
GC-2H	8.43±0.37^{a}	787.23±2.68^{b}	6.63±0.09^{a}	14.55±0.68^{a}	16.93±0.33^{a}

Sample description as under Table 1. Values are means ± standard deviation (n=3). Values in each column with different small superscript letters are significantly different (p≤0.05).

RESULTS AND DISCUSSION

Rutin and total phenolics contents

Currently, it is generally accepted that rutin is the main bioactive compound in buckwheat-based products [Zhang *et al*., 2012]. The content of rutin and total phenolic compounds in rye-buckwheat ginger cakes enhanced with rutin obtained without and with dough fermentation step is presented in Table 1and Table 2, respectively.

Rutin content in control ginger cakes formulated on rye flour (GC-C) obtained with and without dough fermentation step was related to the buckwheat honey usage in the recipe. Apart from buckwheat honey, the next important source of this flavonoid was light buckwheat flour and flour from roasted buckwheat groats. In this study, rutin content increased almost twice in GC-1 and GC-2 cakes due to the substitution of rye flour by buckwheat flours at 30% level (Table 1 and Table 2). Generally, the higher rutin content was noted in ginger cakes made of light buckwheat flour (GC-1) than in ginger cakes made of flour from roasted buckwheat groats (GC-2) without dough fermentation step (Table 1), however a contrast finding was found for the cakes produced with dough fermentation step (Table 2). When GC-1 and GC-2 cakes without fermentation step were enhanced with low and high rutin doses, its content increased 7 times in GC-1L and 15 times in GC-1H. The same tendency was observed in GC-2L and in GC-2H, 12 times and 15 times, respectively (Table 1). A similar trend was noted in rye-buckwheat ginger cakes enhanced with rutin obtained with dough fermentation (Table 2). The dough fermentation caused some changes in cake matrices resulting in better extractability and hence higher rutin content was noted in cakes formulated on flour from roasted buckwheat groats rather than on light buckwheat flour (Table 2) as compared to the results in Table 1. Generally, rutin content was in the following order: GC-1H> GC-1L> GC-1> GC-C and GC-2H> GC-2L> GC-2> GC-C. It can be concluded that high temperature during baking process did not have an impact on rutin stability. Rutin content in rye-buckwheat ginger cakes (GC-1 and GC-2 with dough fermentation step) was higher than it was noted in ginger nut biscuits (wheat-buckwheat flour, 70:30) elaborated by Filipčev *et al.* [2011].

Table 1 and 2 show TPC contents in rye-buckwheat ginger cakes prepared with and without dough fermentation step, respectively. The substitution of rye flour by buckwheat flours at the level of 30% w/w on total flour basis resulted in higher TPC content in rye-buckwheat ginger cake GC-1 and GC-2 obtained without dough fermentation step as compared to control rye ginger cake GC-C. In contrast, this effect was not seen in GC-1 and GC-2 cakes obtained after dough fermentation. As expected, the addition of low and high rutin doses increased the TPC values. The highest TPC was noted in GC-2H cake obtained with dough fermentation step, the result was almost 2-times higher in comparison to GC-2. The higher TPC was noticed in ginger cakes evaluated after longer dough storage, which is likely to be related to the positive influence of temperature during dough storage on increased production of antioxidants [Hur *et al.,* 2014]. All types of rye-buckwheat ginger cakes reached higher TPC values in comparison to ginger nut biscuits [Filipčev *et al*., 2011], which means that this innovative buckwheat-based product is a good source of phenolic compounds.

D-*chiro*-inositol content

The content of D-*chiro*-inositol in rye-buckwheat ginger cakes enriched with rutin produced without dough fermentation is resumed in Table 1. The presence of D-*chiro*-inositol in control rye ginger cake (GC-C) was related to the addition of buckwheat honey to ginger cakes formula. The substitution of rye flour by buckwheat flours at the level of 30% w/w on total flour basis resulted in increased D-*chiro*-inositol content by 30% in GC- 1 and Gc-2 as compared to control rye ginger cake GC-C. In contrast, the amount of D-*chiro*-inositol in ginger cakes with low and high doses of rutin (GC-1L, GC-1H and GC-2L, GC-2H) was between the range noted for GC-C and GC-1 and GC-2. The rye-buckwheat ginger cakes obtained with dough fermentation showed also higher content of D-*chiro*-inositol up to 56% as compared to the content in the control sample (GC-C) (Table 2). The use of low rutin dosage in cake recipe showed no effect on D-*chiro*-inositol content, however the amount of D-*chiro*-inositol after high rutin enrichment was by 34 and 61% higher in GC-1H and GC--2H cakes as compared GC-1 and GC-2 samples. The results of current studies indicate the impact of technology and possible phenolics and D-*chiro*-inositol interaction, which hindered clear explanation of the provided findings. Therefore, more research is required in this area. On the other hand, the data shown in our study confirm that rye-buckwheat ginger cakes enhanced with high rutin dosage obtained after dough fermentation may be recognized as DCI-rich product similarly to crackers made of tartary buckwheat flour as described by Yang & Ren [2008]. It should also be pointed out that a much higher dosage of DCI oral supplementation to observe the positive influence on decreasing insulin sensitivity was required to rich a fast-observed effect [Cheang *et al.*, 2008].

Antioxidant capacity provided by DPPH assay and CV method

The 80% methanol extracts of ginger cakes obtained without dough fermentation were examined for their free radical scavenging activity against DPPH$^{\bullet}$ radicals. The results are shown in Table 1. The positive effect of rutin addition on the antioxidant capacity was observed in all samples. The rank of antioxidant capacity in ginger cakes was as follows: GC-1H= GC-1L>GC-1> GC-C and GC-2H> GC--2L> GC-2> GC-C. The higher antioxidant capacity was noted for ginger cakes made of flour from roasted buckwheat groats. The 16% increase of antioxidant activity values was noted in GC-2 in comparison to GC-1, 32% in GC-2M to GC-1M, 89% in GC-2H to GC-1H. The highest scavenging of DPPH$^{\bullet}$ radicals was noted for GC-2H samples. A similar increasing trend of antioxidant capacity *vs.* rutin addition was found for rye-buckwheat ginger cakes with dough fermentation step (Table 2). The antioxidant capacity determined by DPPH test for rye-buckwheat ginger cakes was higher compared to ginger cakes from dark rye or brown rye flours [Zieliński *et al.,* 2012].

The antioxidant capacity of rye-buckwheat ginger cakes enhanced with rutin obtained with and without dough fermentation step provided by CV method confirmed data obtained by DPPH test with one exception made to ginger cakes formulated from rye flour and flour from roasted buckwheat groats where no increasing effect on the antioxidant capacity was found after rutin enrichment (Table1). It can be suggested that the application of buckwheat flours in ginger cake formula was more effective in antioxidant activity increase in comparison to ginger cakes from dark rye or brown rye flours [Zieliński *et al.,* 2012].

Anti-glycation ability determined in BSA-glucose and BSA-MGO model systems

In this research, the inhibitory activity of extracts from rye-buckwheat ginger cakes were evaluated in two model BSA--glucose and BSA-MGO systems. Aminoguanidine (AG) solution (1 mmol/L) was employed in both systems as a reference inhibitor of glycation process [Zuwało-Jagiełło, 2009]. AG (a hydrazine compound) is a representative drug, which prevents AGEs formation by trapping intermediates at the initial glycation stages [Thornalley, 2003].

The inhibitory effects of the extracts from rye-buckwheat ginger cakes rutin obtained without and with dough fermentation evaluated by BSA-glucose and BSA-MGO model systems are displayed in Figure 2 and Figure 3, respectively. It was found that in BSA-glucose model the inhibitory activity of cake extracts formulated on light buckwheat flour and produced without dough fermentation (GC-1) showed a threefold higher value as compared to the activity of control rye ginger cake extract (GC-C) (Figure 2) while the inhibitory effect of AG was higher reaching 80%. The enrichment of ginger cakes with rutin increased the inhibitory activity and the highest value was noted in cakes with high dose of rutin (GC-1H). Moreover, the application of flour from roasted buckwheat groats in ginger cakes formula offered also higher values of inhibitory activity against AGEs formation as compared to control cake (GC-2). In this study all cakes produced with dough fermentation step showed a lower inhibitory activity compared to those with fermentation step however the values were still higher than that found in control GC-C cake (Figure 2). An exception was made to cakes GC-2L and GC-2H for which a higher inhibitory activity was noted as compared

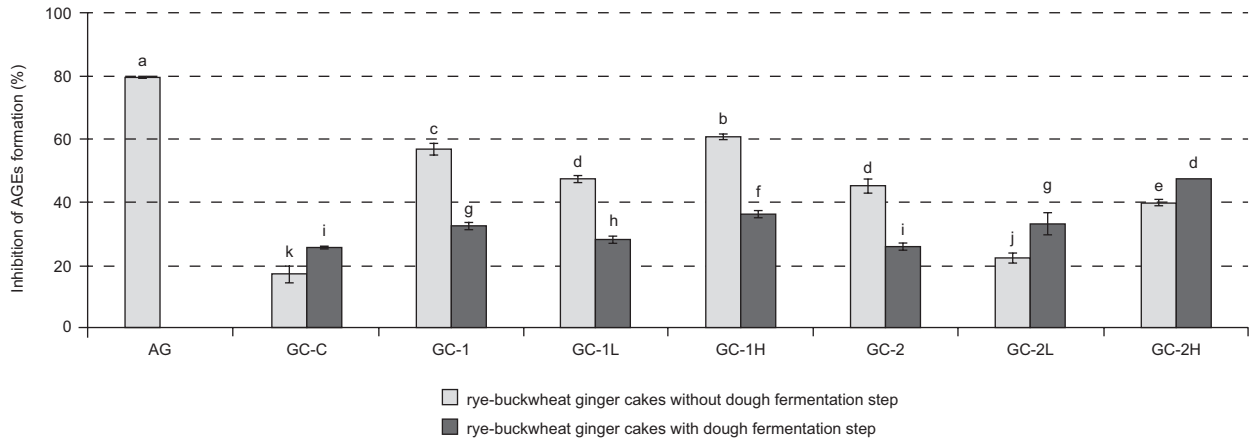

FIGURE 2. The inhibitory effects of rye-buckwheat ginger cakes enriched with rutin and produced with or without dough fermentation step against AGEs formation in BSA/glucose model system. (Sample description as under Table 1).

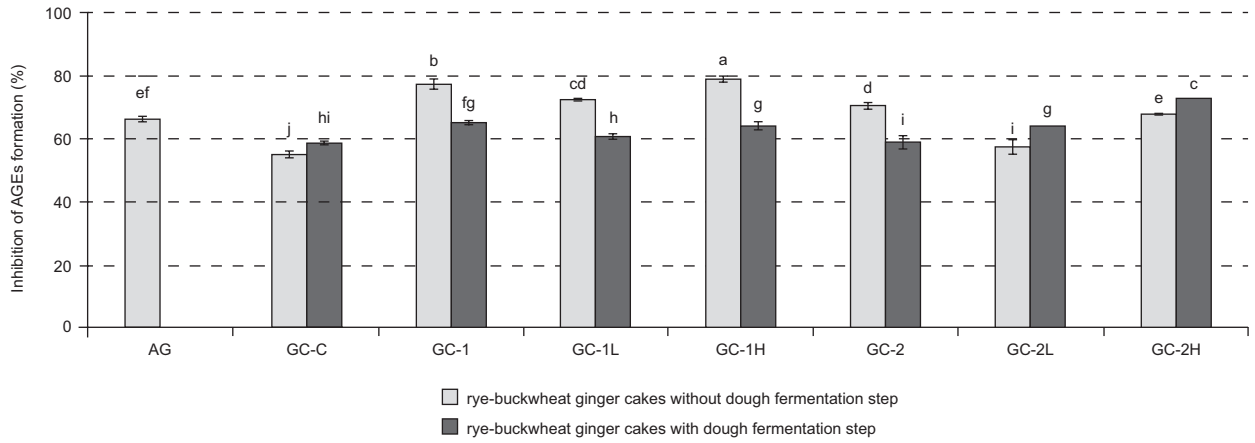

FIGURE 3. The inhibitory effects of rye-buckwheat ginger cakes enriched with rutin and produced with or without dough fermentation step against AGEs formation in BSA/MGO model system. (Sample description as under Table 1).

to respective cakes without dough fermentation. However, this finding was negligible due to the generally lower activity as compared to GC-2 cake.

The values of the inhibitory activity of ginger cakes evaluated in the second model system BSA-MGO clearly confirmed those results provided by BSA-glucose model system and the highest inhibitory activity was also noted for GC-1H obtained without the dough fermentation step (Figure 3). The inhibitory effects of extracts from rye-buckwheat ginger cakes enriched with rutin determined by BSA-glucose and BSA-MGO assays were highly correlated (r=1.00 for cakes without dough fermentation and r=0.98 for cakes with dough fermentation).

Correlation studies

Correlations between the inhibition of AGEs formation and the contents of TPC, rutin, D-*chiro*-inositol and antioxidant capacity in rye-buckwheat ginger cakes enhanced with rutin are illustrated in Table 3. The correlation studies showed that the inhibitory effects of rye-buckwheat ginger cakes enhanced with rutin against formation of AGEs were highly correlated with bioactive compounds content and antioxidant capacity determined in the cakes obtained with dough fermentation step. It seems that the fermentation step changes the cake's matrix, which resulted in liberating more compounds with scavenging activity against DPPH radicals. This effect was not seen in the cakes obtained without the fermentation step since no correlation was noted between inhibitory activity and antioxidant capacity. It should be pointed out that the effect of rutin enrichment was clearly seen in the cakes obtained with the dough fermentation step even the inhibitory activity was slightly lower as compared to the cakes produced without dough fermentation.

These findings confirmed the theory that the antiglycation activity is strongly correlated with the scavenging ability of polyphenolic compounds in reducing oxidation of Amadori compounds [Harris *et al*., 2011]. The high positive correlation between antiglycation and antioxidative activities of spices in BSA-glucose model system was confirmed (TPC/BSA-glucose, r=0.9; DPPH/BSA-glucose, r=0.6) by Ramkissoon *et al.* [2013]. Our observations on the antiglycation effect related to buckwheat are in agreement with our earlier research in which the inhibitory effect of buckwheat-enhanced wheat breads against AGEs formation in BSA-glucose and BSA-MGO systems was dependent on the level of the substitution of dark or white wheat flours with buckwheat flour in wheat bread formulas [Szawara-

TABLE 3. Correlation between the inhibition of AGEs formation and the contents of total phenolic compounds (TPC), rutin (Ru), D-*chiro*-inositol (DCI) and antioxidant capacity (AC) measured by CV and DPPH assays in rye-buckwheat ginger cakes enhanced with rutin.

Type of rye-buckwheat ginger cakes enhanced with rutin	Model systems		Bioactive compounds/ antioxidant capacity
	BSA/glucose	BSA/MGO	
Obtained without dough fermentation step	r=0.69	r=0.12	TPC
	r=0.31	r=0.31	Ru
	r=0.69	r=0.69	DCI
	r=0.89	r=0.89	AC by CV
	r=0.00	r=0.00	AC by DPPH
Obtained with dough fermentation step	r=0.88	r=0.84	TPC
	r=0.75	r=0.63	Ru
	r=0.81	r=0.74	DCI
	r=0.79	r=0.96	AC by CV
	r=0.98	r=0.77	AC by DPPH

The Pearson correlation coefficient (r) was calculated for each model system.

-Nowak *et al.,* 2014]. However, the high AGEs inhibitory potential of coriander (one ingredient of spice mix) was noted [Ramkissoon *et al*., 2013]. It means that also each spice from the spice mix for gingerbreads may contribute to the total antiglycation ability of rye-buckwheat ginger cakes. In accordance to researches conducted by Bhattacherjee & Chakraborti [2013] and Esmaeili [2014], in this study it was confirmed that phenolic compounds as well as D-*chiro*-inositol are the active antiglycating agents. However, the mechanism of their antyglycating action needs further research.

CONCLUSIONS

Data on total phenolics, rutin and DCI contents, antioxidative capacity measured by DPPH and CV methods, and inhibitory activity against protein glycation *in vitro* in BSA-glucose and BSA-MGO systems of rye-buckwheat ginger cakes enriched with rutin were provided. Enrichment of rye-buckwheat ginger cakes with rutin improved their antioxidant properties. High inhibitory anti-glycation potential of rye-buckwheat ginger cakes enriched with rutin was confirmed. The relationship between antiglycation effect with rutin content and antioxidant capacity was found. The addition of buckwheat flours as well as rutin supplementation in ginger cakes caused an increase of AGEs inhibitory potential. Moreover, rye-buckwheat ginger cakes enriched with high dose of rutin were shown to express a similar antiglycation ability as aminoguanidine. Besides antiglycation findings, this study confirmed that the developed cakes are a good source of DCI. It can be concluded that rye-buckwheat ginger cakes produced with dough fermentation step can be recommended for diabetes for two various reasons: firstly – ginger cakes can be a source of natural therapeutic agent against AGEs formation, and secondly – they may increase DCI intake from natural sources.

ACKNOWLEDGEMENTS

This research was supported by grant PRELUDIUM No.2012/07/N/NZ9/02250 from the National Science Centre. The article is a part of the Ph.D. thesis of Małgorzata Przygodzka.

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Submitted: 17 April 2015. Revised 21 May 2015. Accepted: 22 May 2015. Published on-line: 7 July 2015