PAPER

CHANGES IN THE ANTIOXIDANT CAPACITY AND POLYPHENOLS CONTENT OF RYE-BUCKWHEAT CAKES FORTIFIED WITH SPICES DURING THEIR LONG-TERM STORAGE

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ABSTRACT

The long-term storage of cakes may affect their functional and physicochemical properties. In this study, we investigated changes in the antioxidant capacity and polyphenols content of rye-buckwheat cakes with selected spices, e.g. cloves, nutmeg, cinnamon, vanilla, allspice and a commercial blend of spices, during their 18-month storage at 23°C, 60% humidity. Measurements were performed after each 6 months. During storage, chemical markers decreased considerably. The observed decrease in the antioxidant capacity was highly correlated with contents of total phenols, flavonoids and rutin. The use of cloves, cinnamon and a spice mix in rye-buckwheat recipe offered a higher functionality of cakes.

Keywords: antioxidant capacity, flavonoids, rye-buckwheat cakes, storage, total phenolics, spices

1. INTRODUCTION

According to the report of the Polish Agriculture Market Agency, the consumption of cookies and cakes had been increasing till 2008 and became stable for many years (AGRICULTURE MARKET AGENCY, 2013). Producers develop new recipes to attract consumers with new flavors and enrich confectionery products with healthy ingredients. Spices are well-known sources of natural antioxidants, which might be used as preservatives or flavor enhancers in cookies and biscuits (EMBUSCADO, 2015). A wide range of compounds with different molecular structures has been suggested to be responsible for the high antioxidant status of spices (YASHIN *et al.*, 2017). Therefore, they might significantly influence the quality and sensory parameters of confectionery products, but also increase their functional properties like e.g. their antioxidant activity (NANDITHA and PRABHASANKAR, 2009). PASQUALONE *et al*. (2014) described the positive influence of grape marc (by-product with high antioxidant potential) on biscuits' flavor and sensorial parameters, e.g. by more intense color and fruity odor formation. Powders are the main form in which spices are incorporated into confectionery products. Therefore, BAJEJ *et al.* (2006) demonstrated that better bakery parameters as well as consumer acceptability were achieved for biscuits incorporating mint powder than for biscuits with either mint extract or menthol. In some cases, however, essential oils of spices – which are generally acceptable by consumers – are added to provide the antioxidant capacity of confectionary goods (BASUNY *et al.*, 2012). Cinnamon, mint, nutmeg and cloves were indicated as the most commonly used spices and herbs in confectionary (NANDITHA and PRABHASANKAR, 2009). Garlic, ginger, basil, turmeric or coriander powders were also used in some studies with wheat bread (DZIKI *et al*., 2014). The addition of spices, as the main sources of antioxidants, is expected to prolong storage stability of confectionery products, ensure consumer acceptability and provide healthy properties.

According to many reports, consumer acceptability may be affected by the formation of oxidative compounds, which might create an unpleasant aroma and/or rancid and bitter taste in stored products. Oxidation may be effectively inhibited by antioxidants, therefore recipes of confectionery products were modified to increase antioxidants content in many ways. For example, WATANABE *et al.* (2014) reported that the addition of quinoa flour to wheat cookies increased their antioxidant capacity and then improved their oxidative stability during 50-day storage period (lower peroxide value of cookies incorporating 7.5% of quinoa flour). ZIELIŃSKI *et al.* (2012) demonstrated a greater increase in the antioxidant capacity of ginger cakes made of rye flour, instead of wheat flour, after long-time storage. They speculated this might also be due to the formation of some of the Maillard reaction products, e.g. melanoidins, which was indicated by the higher values of the browning index. In turn, JENSEN *et al.* (2011) reported no significant changes in the oxidative status during storage of wheat bread incorporated with a rosemary extract or an α-tocopherol solution. However, enhanced hydroperoxide formation was determined in the samples prepared with α-tocopherol, which means that the rosemary extract protected food from oxidation. In studies of NING *et al.* (2017) the addition of green tea powder to wheat bread significantly increased its overall antioxidant capacity and inhibited the formation of peroxides during 8-day storage (which was described as lower peroxide values, even at only 1% of green tea addition) as compared to wheat bread. Also interesting results were obtained by DAR *et al.* (2016), who developed rice snacks with a combination of cereal brans. According to their results, the antioxidant capacity of these snacks decreased significantly during 6 months of their storage, i.e. about 25 up to 28%. They also noticed the negative effect of storage on total phenolic contents, which might be due to low-stability of molecules in the matrix and advanced degradation process of phenolic

compounds. The reason of these changes was explained as "dilution of antioxidant components by increased moisture" or oxidation of antioxidant molecules during long-term storage, but these are only speculations as causes these changes are still unknown. Little literature focused on changes in the antioxidant capacity and phenolic content in cereal products is available today.
Therefore, the aim of this study was to determine changes in total phenolic and flavonoid contents during 18-month storage of rye-buckwheat cakes fortified with selected spices. The spices used in rye-buckwheat cakes formula were: cloves, nutmeg, cinnamon, vanilla, allspice and a commercial blend of spices for cakes. The content of rutin, as the main polyphenol occurring in buckwheat flour and honey, was determined to control its changes during storage period. Furthermore, antioxidant capacity was evaluated by measurements of the scavenging ability against $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ radicals. This study focused also on the antioxidant stability of rye-buckwheat cakes during storage. Moreover, spices with the best impact on storage stability of the analyzed cakes were selected. The measurements were performed every 6 month till the 18^{th} month of storage period and compared with results obtained immediately after baking.

2. MATERIAL AND METHODS

2.1. Chemicals

Standards of rutin (quercetin-3-rutinoside), gallic acid and catechin as well as chemicals used for antioxidant capacity determination: 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), sodium nitrite, aluminum chloride, and sodium carbonate were purchased from Sigma (USA). Ethanol, acetonitrile and formic acid were obtained from Merck (Germany). Folin-Ciocalteu reagent was provided by POCh (Poland). For HPLC analysis, water was purified with the Mili-Q-system (Milipore, USA).
The spices used in rye-buckwheat cakes formula as: cloves (*Syzygium aromaticum* L.), nutmeg (*Myristica fragrans* H.), cinnamon (*Cinnamomum verum* J.), vanilla (*Vanilla* Mill.), allspice (*Pimenta dioica* L.) and a commercial blend of spices for cakes, were provided from local market (Kotányi, Poland). According to the producer's declaration, commercial spice mix contains cinnamon, pepper, clove, anise, coriander, fennel and nutmeg. TPC values of each spice were previously calculated in our studies (PRZYGODZKA *et al.*, 2014): clove 156.67±3.52 (mg GAE g^{-1}), nutmeg 11.80±0.55, cinnamon 180.59±14.23, vanilla 8.08±0.49, allspice 183.92±1.26, and spice mix 101.33±5.66. Also TF of selected spices was measured (mg rutin g^{-1}): clove 19.78±0.54, nutmeg 10.21±0.17, cinnamon 96.81±1.95, vanilla 3.97±0.37, allspice 16.82±0.07, and spice mix 64.21±1.82.

2.2. Preparation of rye-buckwheat cakes and their storage conditions

Preparation of rye-buckwheat cakes with selected spices was described in detail in our previous work (PRZYGODZKA *et al.*, 2016).The composition of ingredients used is listed in Table 1. The rye-buckwheat cakes fortified with cloves, nutmeg, cinnamon, vanilla, allspice, and a spice mix were prepared while rye-buckwheat cakes without spices served as a control sample. After baking, the cakes were cooled down and part of them were freeze-dried, ground into powder and then used directly for analyses ("0"), whereas the others were packed into plastics bags with clips, stored at room temperature, without light

for 6, 12 and 18 months, and afterwards defrosted and ground into powder to prepare cake extracts.

Table 1. Composition of rye-buckwheat cakes with selected spices.

Ingredients (g)	C	Cakes with spices
rye flour	70	70
light buckwheat flour	30	30
buckwheat honey	50	50
sugar	20	20
baking powder	3	3
butter	25	25
cloves/ nutmeg/ cinnamon/ vanilla/ allspice or spice mix	0	2

2.3. Rye-buckwheat cake extracts preparation

About 100 mg of powdered rye-buckwheat cakes were placed in 2-mL Eppendorf flasks, to which 1 mL of an extraction solvent (ethanol/water 1:1, v/v, the solvent was selected according to our previous study (PRZYGODZKA *et al*., 2014) was added. The mixture was vortexed for 1 min and then extracted with ultrasonic waves for 30 sec. Then, it was centrifuged at the maximum speed and a temperature of 4ºC for 15 min. The supernatant was then transferred to a 5-mL volumetric flask and the residues were re-suspended in another 1-mL portion of the ethanol/water solution. This step was repeated until 5 mL of the extract had been obtained. These extracts were used for further analyses of rutin, total phenols and flavonoids contents, and for antioxidant capacity determination with ABTS and DPPH assays.

2.4. Determination of rutin, total phenolics and flavonoids contents

The content of rutin in rye-buckwheat cakes was determined with the HPLC system (Shimadzu, Japan) with a UV detector (SPD-10A) set up at 330 nm according to the procedure presented by ZIELIŃSKA *et al*. (2010). Five concentrations of a rutin standard were prepared in triplicate in the range of 1.0-40 μM, then solutions were filtered through nylon filter membranes, with pore size of 0.45 μm, before injection. The results were expressed in μg g^{-1} of dry weight. The TPC and TF contents were determined as previously described by PRZYGODZKA *et al.* (2014). TPC content was expressed in terms of mg gallic acid equivalents (GAE) g^{-1} of dry weight, whereas TF content was expressed as mg of catechin (CA) g^{-1} of dry weight.

2.5. Determination of antioxidant capacity (AC) by ABTS and DPPH assays

The antioxidant capacity of rye-buckwheat cakes enriched with spices was determined by ABTS assay as described by PRZYGODZKA *et al.* (2014). Also scavenging ability against DPPH radicals was measured according to PRZYGODZKA *et al.* (2015). The results provided by both ABTS and DPPH assays were expressed as μmol of Trolox equivalents (TE) $g^{-1}$of dry weight.

2.6. Statistical analysis

The results of the analyses are given as the means and the standard deviation of three independent extractions (mean±SD). Statistical one-way analysis of variance (ANOVA) using Fischer test was performed. The significance level was set at $p< 0.01$. Pearson correlation coefficients were calculated to enable correlations analysis of the results of rutin, total phenolic and flavonoids contents, and results of ABTS and DPPH assays. The correlations were calculated for each type of rye-buckwheat cakes fortified with selected spices over 18-month storage period.

3. RESULTS AND DISCUSSION

3.1. Changes of rutin, total phenols and flavonoids contents of rye-buckwheat cakes during storage

Changes in rutin content in cakes made of rye and buckwheat flour mixtures with selected spices during 18-month storage are illustrated in Fig. 1. Generally, a progressive reduction of rutin content was observed in all samples. It the control samples, it decreased significantly after 6 months of storage (almost 45%), to reach finally 86% decrease after 18 months of storage. In the case of cakes with spices addition, after 6 months the lowest decrease was noticed in cakes with allspice and cloves, from 173 to 89 $\mu g\ g^{-1}$ and 320 to 190 $\mu g\ g^{-1}$, respectively.

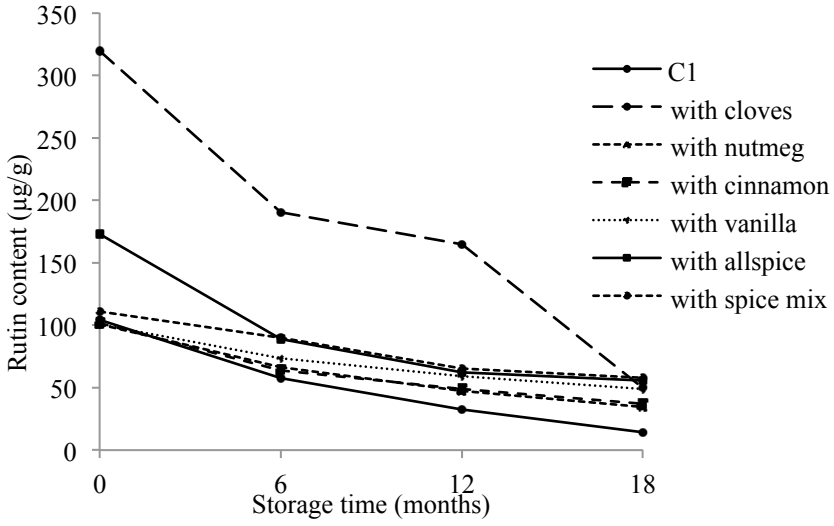

Figure 1. The influence of storage time on rutin content in rye-buckwheat cakes fortified with selected spices. SD were not higher than 10%.

A further decrease in rutin content was observed after 12 months, and after 18 months the lowest rutin content was determined in the cakes with cinnamon and the highest one - in the cakes with the spice mix and allspice. The final rutin content was the highest in the cakes fortified with the spice mix, cloves and vanilla. Our observations are in an agreement with findings reported by SAKAČ *et al.* (2016), who also observed a decrease of rutin content in rice-buckwheat cookies (80:20, v/v) during 16-month storage. They noticed the difference between rutin content in unpacked and packed cookies after the

first 6 months of storage, while afterwards no difference was demonstrated. In this case, it might be hypothesized that the lower rutin content was associated with the formation of new molecules with another compound like lysine, as it was described by ZHANG *et al.* (2016), or that rutin might be degraded to quercetin during the baking process (LUŠKIČ *et al*, 2016). Moreover, DADÁKOVÁ *et al.* (2011) noticed that 12- month storage of black elderflowers at 22ºC impacted a decrease of rutin amount, even much more than of chlorogenic acid. However, storage of asparagus at 4ºC or as freeze-dried flour had no significant influence on rutin content (STOFFYN *et al*., 2012). Moreover, rutin content decrease could be ascribed to the oxidation processes. In model studies of BUCHNER *et al.*(2006), a decrease in rutin concentration was observed in solutions with rutin kept under air perfusion. This effect might be reduced with vacuum packaging of cakes. Not many studies have been focused on changes in contents of individual polyphenols in bakery and/or confectionery products during storage, whereas a high number of research works is available on the monitoring of changes of total phenolic compounds.

Therefore, the total phenolic compounds (TPC) content was monitored every 6 months in rye-buckwheat cakes fortified with selected spices (Table 2). Its decrease was observed in all cakes over the entire period of storage. After 6-month storage it decreased by 6% in the control cakes and by 2% and 24% in the samples with spice mix and nutmeg, respectively. After another 6 months of storage, a significant decrease of TPC content was noticed in almost all cakes samples except for control and with allspice. Then, after 18 months the greatest loss of TPC was determined in the cakes with nutmeg (33%) and the lowest one in the cakes with the spice mix (9%). A significant decrease in TPC content, by 73 and 58%, was also observed in the cakes with cloves and spice mix. Despite the fact that the TPC content decreased by more than a half, still a very high TPC content was noted in the cakes with cloves. Moreover, the highest TPC content was reached in the cakes made of light buckwheat flour and fortified with spice mix, cloves, cinnamon and then allspice. The changes in TPC contents were higher compared to these presented previously by ZIELIŃSKI *et al.* (2012), which might be a consequence of rapid rutin degradation in cakes made of buckwheat flours. Therefore, the higher TPC content in rye-buckwheat cakes before the storage might be due to higher amounts of honey and ginger in their recipe. Our results are in accordance with findings reported by SAKAČ *et al.* (2016), who observed that TPC decreased along the prolonged storage period. After 6-month storage, the TPC content in rice-buckwheat cakes decreased by about 25%, then after 12 months by up to 55%. Moreover, these authors found no difference between TPC contents in the samples stored at 23 and 40°C, whereas slightly higher TPC values were observed in unpacked than in packed cakes.

Table 2. Content of total phenolic compounds (TPC) and flavonoids (TF) in rye-buckwheat cakes fortified with selected spices: cloves, nutmeg, cinnamon, vanilla, allspice and spice mix.

	TPC [mg GAE g^{-1}]						
	Storage time						
spices	**0 m**	**6 m**	**0-6 m**	**12 m**	**0-12 m**	**18 m**	**0-18 m**
C (without spices)	1.12 ± 0.03^{gA}	1.05 ± 0.07^{eAB}	↓ 6%	1.04 ± 0.01^{fB}	↓ 7%	0.85 ± 0.03^{eC}	↓ 24%
cloves	2.11 ± 0.04^{cA}	2.04 ± 0.06^{bA}	↓ 3%	1.73 ± 0.06^{bcB}	↓ 18%	1.68 ± 0.03^{bB}	↓ 20%
nutmeg	1.56 ± 0.10^{eA}	1.18 ± 0.04^{dB}	↓ 24%	1.09 ± 0.01^{eC}	↓ 30%	1.04 ± 0.01^{dD}	↓ 33%
cinnamon	2.28 ± 0.05^{bA}	2.01 ± 0.06^{bB}	↓ 12%	1.80 ± 0.06^{bC}	↓ 21%	1.66 ± 0.02^{bD}	↓ 27%
vanilla	1.32 ± 0.08^{fA}	1.25 ± 0.07^{dB}	↓ 5%	1.14 ± 0.01^{dC}	↓ 14%	1.01 ± 0.04^{dD}	↓ 23%
allspice	1.84 ± 0.07^{dA}	1.63 ± 0.05^{cB}	↓ 11%	1.62 ± 0.05^{cB}	↓ 12%	1.57 ± 0.02^{cB}	↓ 15%
spice mix	2.70 ± 0.09^{aA}	2.64 ± 0.03^{aA}	↓ 2%	2.53 ± 0.05^{aB}	↓ 6%	2.46 ± 0.03^{aB}	↓ 9%

	TF [mg CA g^{-1}]						
	Storage time						
spices	0 m	6 m	0-6 m	12 m	0-12 m	18 m	0-18 m
C (without spices)	2.02 ± 0.01^{fA}	1.59 ± 0.02^{eB}	↓ 21%	1.37 ± 0.07^{gC}	↓ 32%	1.24 ± 0.01^{eD}	↓ 38%
cloves	3.45 ± 0.01^{aA}	3.11 ± 0.06^{aB}	↓ 10%	3.01 ± 0.07^{aB}	↓ 13%	2.86 ± 0.05^{aC}	↓ 17%
nutmeg	2.20 ± 0.01^{eA}	2.13 ± 0.02^{dB}	↓ 3%	1.98 ± 0.01^{fC}	↓ 10%	1.63 ± 0.02^{dC}	↓ 26%
cinnamon	2.95 ± 0.05^{bA}	2.81 ± 0.05^{bB}	↓ 5%	2.46 ± 0.05^{bC}	↓ 16%	2.00 ± 0.02^{bD}	↓ 32%
vanilla	2.47 ± 0.02^{dcA}	2.35 ± 0.02^{cB}	↓ 5%	2.18 ± 0.01^{dC}	↓ 12%	1.65 ± 0.03^{dD}	↓ 33%
allspice	2.21 ± 0.02^{eA}	2.15 ± 0.05^{dAB}	↓ 3%	2.10 ± 0.02^{eB}	↓ 5%	1.83 ± 0.04^{cC}	↓ 17%
spice mix	2.96 ± 0.03^{bA}	2.79 ± 0.08^{bA}	↓ 6%	2.54 ± 0.04^{cB}	↓ 14%	1.94 ± 0.05^{bC}	↓ 34%

a, b- means in the same column with different letters as superscripts are significantly different ($p < 0.001$).
A, B- means in the same row with different letters as superscripts are significantly different ($p < 0.001$).

In our case, however, the changes in TPC contents were smaller. DAR, SHARMA and NAYIK (2016) also found a smaller decrease of TPC content (at about 17%) in snacks prepared from rice flour and a blend of three brans: wheat, oat and rice (2:1.5:1.5), during storage at room temperature. Also LIANG and WERE (2018) reported on the loss of phenols in butter cookies with different addition of sweeteners. Furthermore, the greatest decrease in TPC contents after 24-hour storage was observed in the cookies with honey and the lowest one in these with xylitol. This might be due to interactions between sugars (glucose, fructose etc.) and formation of Maillard reaction compounds. The loss of TPC was also explained by the formation of favorable polyphenol-sugar adducts which were further rearranged to brown pigments (melanoidins) (ZHANG, CHEN and WANG, 2013). The Pearson coefficient of correlation between TPC and rutin content (Table 4) was very high for almost all samples of cakes fortified with cinnamon (0.987), allspice (0.985), spice mix (0.982), nutmeg (0.974), and vanilla (0.937).

The main flavonoids of common buckwheat kernels are flavonol glycosides: rutin, quercetin, and kaempferol-3-rutinoside (TIAN, LI and PATIL, 2002). Total flavonoid (TF) content was also monitored during rye-buckwheat cakes storage and its significant changes were determined. In the control samples, TF values decreased from 2.02 for "0" time, through 1.59 after 6 months and 1.37 after 12 months and finally to 1.24 mg g^{-1} after 18 months. Furthermore, after 6 months no significant decrease was observed in the cakes with addition of nutmeg, and allspice, whereas 5% decrease was noticed in cinnamon and vanilla, and 6% decrease of TF was noticed in spice mix. Then, 12-month storage caused a further decrease in TF content in the range from 5 to 16% in the cakes with allspice and cinnamon, respectively. After 18 months, the least changes of TF contents were observed in the cakes fortified with cloves and allspice. Therefore, the highest TF content was determined in the cakes with cloves (2.86 mg g^{-1}), cinnamon (2.00 mg g^{-1}), and spice mix (1.94 mg g^{-1}). The decrease in TPC and TF contents in all samples of cakes was observed, however their values in the samples fortified with spices were still higher than in the control sample. According to results of TPC and TF content determination, after long-time storage of the spices-fortified cakes, the cloves, spice mix, and cinnamon might be recommended as good additives for confectionery goods. According to values of correlation coefficients (Table 4), it can be concluded that in the control samples and in the cakes with cloves and spice mix, rutin might have the highest contribution to TF levels in these samples. Whereas, a correlation between TPC and TF contents was the highest in the samples with vanilla (0.970), spice mix (0.950), and cinnamon (0.936).

3.2. Changes of the antioxidant capacity of rye-buckwheat cakes during storage

In the next step of our study, antioxidant capacity was monitored in rye-buckwheat cakes for 18 months of storage based on the scavenging ability of ABTS and DPPH radicals. Results of these determinations are presented in Table 3.
In control cakes, the ABTS values decreased only by 1 and 4% after 6 and 12 months of storage, respectively. Then, a high decrease (27%) was noticed after 18 months. In rye-buckwheat cakes fortified with spices and stored for 6, 12 and 18 months, the least changes in the antioxidant capacity were observed in the cakes with the spice mix (from 1 to 12%) and vanilla (from 4 to 14%). In turn, the greatest decrease in the antioxidant capacity was noticed in the cakes with cinnamon (17%) and allspice (14%) after 6 months, and in the cakes fortified with cinnamon (25- 27%) after 12 and 18 months. In the samples with nutmeg no change was observed after 6 months, then after 12 months the antioxidant capacity dropped drastically to 21% and finally after 18 months to 25%. A decrease of the antioxidant capacity of snacks enriched with bran was also described by DAR, SHARMA and NAYIK (2016). They reported that after 6 months of storage, ABTS values decreased by about 17-18% (conventional and microwave-assisted extraction, respectively). In turn, SAKAČ *et al.* (2016) demonstrated that in packed samples of cookies formulated from 80% of rice and 20% of buckwheat, the antioxidant capacity increased in the first months of storage but after 10 months started to decrease. Whereas from the beginning of storage period, a decreasing tendency was noticed in the packed samples, which were stored at higher than room temperature.

Table 3. Antioxidant capacity determined with ABTS and DPPH assays in rye-buckwheat cakes fortified with selected spices: cloves, nutmeg, cinnamon, vanilla, allspice and spice mix.

				ABTS [µmol TE g^{-1}]			
				Storage time			
spices	**0 m***	**6 m**	**0-6 m**	**12 m**	**0-12 m**	**18 m**	**0-18 m**
C (without spices)	21.13 ± 0.88^{fA}	20.94 ± 0.32^{fAB}	<1%	20.36 ± 0.94^{fB}	↓ 4%	15.36 ± 0.15^{gC}	↓ 27%
cloves	55.52 ± 2.73^{bA}	47.91 ± 0.40^{bB}	↓ 14%	45.62 ± 1.16^{bB}	↓ 18%	43.18 ± 0.40^{bC}	↓ 22%
nutmeg	30.49 ± 0.84^{eA}	30.47 ± 0.28^{eA}	<1%	24.16 ± 0.17^{eB}	↓ 21%	22.98 ± 0.36^{eC}	↓ 25%
cinnamon	49.38 ± 0.19^{cA}	41.10 ± 0.14^{cB}	↓ 17%	37.24 ± 0.41^{cC}	↓ 25%	36.04 ± 0.29^{cD}	↓ 27%
vanilla	21.87 ± 1.21^{fA}	20.91 ± 0.78^{fAB}	↓ 4%	19.83 ± 0.76^{gB}	↓ 9%	17.12 ± 0.25^{fD}	↓ 14%
allspice	40.86 ± 2.28^{dA}	35.98 ± 0.75^{dB}	↓ 12%	34.09 ± 0.63^{dB}	↓ 17%	32.55 ± 0.87^{dC}	↓ 20%
spice mix	63.24 ± 1.31^{aA}	62.61 ± 0.57^{aA}	<1%	57.03 ± 0.13^{aB}	↓ 10%	55.23 ± 0.72^{aC}	↓ 12%
				DPPH [µmol TE g^{-1}]			
				Storage time			
spices	**0 m***	**6 m**	**0-6 m**	**12 m**	**0-12 m**	**18 m**	**0-18 m**
C (without spices)	17.48 ± 0.57^{cA}	16.94 ± 0.67^{cAB}	↓ 3%	16.71 ± 0.10^{bB}	↓ 4%	16.30 ± 0.80^{bB}	↓ 7%
cloves	22.35 ± 0.70^{aA}	21.96 ± 0.88^{aA}	↓ 2%	21.99 ± 0.68^{aA}	↓ 2%	21.56 ± 0.04^{aA}	↓ 4%
nutmeg	9.99 ± 0.16^{gA}	9.96 ± 0.49^{gA}	↓ <1%	9.57 ± 0.35^{eA}	↓ 4%	9.08 ± 0.06^{fB}	↓ 10%
cinnamon	21.39 ± 0.61^{bA}	19.62 ± 0.16^{bB}	↓ 8%	16.38 ± 0.27^{bC}	↓ 23%	16.76 ± 0.65^{bC}	↓ 22%
vanilla	12.26 ± 0.72^{fA}	12.29 ± 0.53^{fA}	↑ <1%	12.11 ± 0.09^{dA}	↓ 2%	11.08 ± 0.02^{eB}	↓ 10%
allspice	15.96 ± 0.60^{dA}	13.78 ± 0.26^{eB}	↓ 12%	13.88 ± 0.08^{cB}	↓ 13%	12.07 ± 0.26^{dC}	↓ 24%
spice mix	15.12 ± 0.19^{eA}	15.23 ± 0.54^{dA}	↑ <1%	13.83 ± 0.25^{cC}	↓ 9%	14.71 ± 0.04^{cB}	↓ 3%

a, b- means in the same column with different letters as superscripts are significantly different ($p < 0.001$).
A, B- means in the same row with different letters as superscripts are significantly different ($p < 0.001$).

Table 4. Coefficients of correlations between rutin, TP and TF contents and antioxidant capacity provided by ABTS and DPPH assays for 18-month stored of rye-buckwheat cakes fortified with selected spices.

Type of cakes	rutin *vs* TPC	rutin *vs* TF	TPC *vs* TF	rutin *vs* ABTS	TPC *vs* ABTS	TF *vs* ABTS	rutin *vs* DPPH	TPC *vs* DPPH	TF *vs* DPPH	ABTS *vs* DPPH
C (without spices)	0.842	**0.999**	0.821	0.734	**0.983**	0.707	**0.987**	0.713	0.833	0.831
cloves	0.863	**0.981**	0.881	**0.966**	0.875	**0.998**	**0.989**	0.751	0.742	0.926
nutmeg	0.974	0.859	0.735	0.872	0.754	0.881	0.855	0.914	0.975	0.932
cinnamon	**0.987**	0.877	**0.936**	**0.995**	0.978	0.842	**0.931**	**0.958**	0.964	**0.948**
vanilla	0.937	0.848	**0.970**	0.890	**0.986**	**0.996**	0.703	**0.971**	**0.997**	**0.950**
allspice	**0.985**	0.695	0.717	**0.988**	0.977	0.798	0.908	0.946	**1.000**	**0.950**
spice mix	**0.982**	**0.885**	**0.950**	0.955	**0.982**	**0.916**	0.666	**0.947**	**0.987**	0.698

The antioxidant capacity was also measured using the DPPH method (Table 3) and a decreasing tendency was observed in DPPH values of the rye-buckwheat cakes. This result is in agreement with study of RINALDI *et al.* (2014), who observed DPPH decrease in chestnut- wheat bread even after 3 days of storage. The ability of control cake to scavenge DPPH· radicals mildly decreased from 3 to 7% during 18-month storage period. The 6-month storage had no significant influence on DPPH value decrease of ginger cakes with nutmeg, spice mix (both around 1%) and cloves (2%), whereas in the cakes with vanilla addition a slightly increase of DPPH value was observed (less than 1%). With time of storage, successive reduction was observed in the scavenging ability against DPPH radicals. In the cakes fortified with cinnamon, the DPPH value decreased even by 23%, whereas in the samples with cloves addition no significant change was noted. After 18 months, the highest reduction in DPPH values was determined in the cakes with cinnamon (22%) and allspice (24%) addition. In contrast, the least changes in DPPH values were observed in the cakes with spice mix (3%) and cloves (4%). Our observations are in accordance with findings reported by SAKAČ *et al.* (2016), and by DAR, SHARMA and NAYIK (2016), who observed that DPPH values increased by about 20% and 17% during storage of cereal products, respectively. It may be suggested that not only storage conditions but also the type and amount of flours used in formulae of cookies can influence the antioxidant capacity during storage. However more studies are needed in this respect because in other studies a decrease of the antioxidant capacity was also observed after 1 and 2 days of storage of barley bread, but it was not recognized as a significant change (HOLTEKJØLEN *et al*., 2008). According to ABTS and DPPH methods, the highest values of antioxidant activity were found in the buckwheat cakes with cloves, cinnamon and spice mix. Moreover, other methods could be used to evaluate the antioxidant activity to ensure better verification of tendencies in antioxidant activity changes. The highest coefficients of correlation (Table 4) between rutin content and ABTS values were calculated for the samples with cinnamon (0.995), allspice (0.988), cloves (0.966), and spice mix (0.955). Furthermore, high values of TPC *vs*. ABTS correlation were noted for the samples with vanilla (0.986), spice mix (0.982), cinnamon (0.978), and allspice (0.977). The high coefficients of correlation between TF and ABTS were calculated for the samples with cloves (0.998), vanilla (0.996), and spice mix (0.916). Whereas the DPPH radical method demonstrated a high correlation between rutin content and DPPH value in the buckwheat cakes with cloves (0.989), cinnamon (0.931), and allspice (0.908); as well as between TPC and DPPH value in the samples with addition of vanilla (0.971), cinnamon (0.958), spice mix (0.947), allspice (0.946), and nutmeg (0.914). Similar Pearson coefficients were calculated for the correlation between TF content DPPH value. It can be concluded

that the antioxidant capacity of rye-buckwheat cakes fortified with spices was highly related to bioactive compounds composition. The neo-formed antioxidants during baking or storage period have no significant contribution to the final antioxidant potential.

4. CONCLUSIONS

In this study, polyphenols, flavonoids and rutin contents, and antioxidant capacity of rye-buckwheat cakes enriched with cloves, nutmeg, cinnamon, spice mix, allspice or vanilla were monitored during 18-month storage. The loss of rutin, flavonoids and polyphenols, and antioxidant capacity was observed in both non-enriched and spices-fortified cakes. However, in the rye-buckwheat cakes fortified with selected spices all parameters were still higher as compared to the non-fortified ones. It was shown that the rye-buckwheat cakes fortified with cloves, cinnamon and spice mix maintained the highest functional parameters as TPC, TF, and rutin contents and antioxidant capacity after 18-month storage. Moreover, the loss of antioxidant capacity of the cakes fortified with spices was highly correlated with phenolics content. Therefore, the cloves, cinnamon and spice mix may be recommended to improve the quality of rye-buckwheat cakes. In future studies, it would also be advisable to develop cake formulas with other types of flour originating from tartary buckwheat or tartary buckwheat sprouts (being reach sources of antioxidants), whereas amounts of these ingredients need to be carefully studied to achieve also high consumer acceptance and mask their bitter taste and potential a stringent after taste in the finished product. Therefore, except for polyphenol-rich ingredients, a vacuum packaging or more appropriate bags should be used to prevent rapid degradation of polyphenols and/or formation of oxidation products.

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Paper Received July 30, 2018 Accepted October 8, 2018