Starch bread with a share of non-wheat flours as a source of bioactive compounds in gluten-free diet

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Summary

Maize, buckwheat and amaranth flours were used in formulations for gluten-free bread based on starch and hydrocolloids, replacing 10% of starch. The evaluation of physical parameters of the loaves proved that, at the applied level of addition, their quality was not deteriorated. In the case of buckwheat, some positive influence on texture during storage could be observed. The application of non-wheat flours caused a significant change in the content of nutritionally important constituents of bread. The increase in the content of nutrients and fibre was especially observed in the case of amaranth flour. The bread with buckwheat flour contained the highest amount of polyphenols, including phenolic acids, flavonols, anthocyanins and flavonoids, which resulted in their highest antioxidant activity among all analysed bread samples. Taking into account all analysed gluten-free breads with the share of non-wheat flours, it could be observed that supplementation with buckwheat flour was most effective regarding the increase of nutritional constituents and bioactive compounds with an antioxidant character. Addition of buckwheat flour did not affect physical properties of the crumb and even limited its hardness. This type of addition could be suggested for supplementation of gluten-free bread to assure its pro-health properties.

Keywords

gluten-free bread; amaranth; buckwheat; maize

Celiac disease (enteropathy) affects approximately 1% of European population. It is most often diagnosed in children and young persons, but the group of risk includes also persons between 35 and 50, and over 50 years of age. The disease is more frequent in females. Celiac disease is associated with intolerance to gluten, more specifically cereal prolamins – gliadin (in wheat), secalin (in rye), hordein (in barley) and avenin (in oats). It should be classified as food intolerance because this disease is mainly caused by immaturity of the digestive system, reflecting congenital or acquired disorders of the enzymes in small intestine. Gliadin, as the antigen, forms immune complexes in the intestinal mucosa, favouring the accumulation of "killer" lymphocytes, which is followed by changes in the intestinal mucosa. As a result, partial or complete disappearance of villi of the duodenum and small intestine occurs, which hinders the absorption of nutrients such as proteins, Ca, Fe and vitamins A, E and D. The disease may be accompanied by slower physical and mental development. It can also result in rickets, osteoporosis, anemia and reduce the body's resistance to infections [1, 2].

The only effective method of treatment of celiac disease is a gluten-free diet, which should not contain some basic cereal grains (wheat, rye, barley, oats), and their products. Instead, it should be based on maize, rice, buckwheat, potatoes, millet, peas, beans or amaranth. One of the allowed products is gluten-free bread, typically produced on the basis of starch and hydrocolloids, which are used as structure-forming agents. Neither starch, nor hydrocolloids, provide health benefits usually connected with the consumption of bread, especially in the regarding antioxidants. The increase in antioxidant potential of gluten-free bread could

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only be observed when flours from such grains as maize, buckwheat or amaranth are used as a partial replacement of starch. Amaranth flour is reach in vitamins, maize flour contains ferulic acid, *p*-coumaric acid, carotenoids and tocotrienols, and buckwheat flour provides a large variety of flavonoids such as rutin, quercetin, apigenin or hyperin [3, 4]. So, these flours could be applied in the production of gluten-free bread with a high antioxidant potential. Bioactive constituents present in the above mentioned flours have a protective effect against chronic diseases and cancer [5]. It should be added that gluten-free bread is characterized by relatively low nutritional value in comparison to traditional cereal products, because it is usually deficient in protein, fibre and microelements, which are typical constituents of wheat bread. Non-wheat flours could improve the nutritional value of gluten-free bread [6–8], as well as their pro-health potential. The introduction of new gluten-free products is especially important in the context of growing population with diagnosed gluten intolerance. The research on starch-based bread with the addition of non-wheat flours, which contain high levels of bioactive compounds with pro-health character, seems to be fully justified.

The aim of the study was to analyse the chemical composition of gluten-free bread produced with the addition of buckwheat, maize and amaranth flours. A special attention was given to bioactive components and antioxidant potential of the product. Additionally, the loaves were analysed regarding their colour, volume, as well as crumb texture and structure. Comparison of these results should allow to choose the best ingredients, which should be used in order to obtain best composition of nutritional and bioactive components as well as physical properties of the bread.

MATERIAL AND METHODS

The materials for baking of gluten-free bread were maize starch (Bezgluten, Posądza, Poland), potato starch (Pepees, Łomża, Poland), guar gum (Lotus Gums and Chemicals, Jodhpur, India), pectin (Pektowin, Jasło, Poland), freeze-dried yeast Saf-instant (Lesaffre, Marcq en Baroeul, France) and commercially available products: saccharose, salt and canola oil. Non-wheat flours used for baking included: maize flour (Bezgluten), buckwheat flour (Biofuturo Trade, Kraków, Poland) and amaranth flour (Szarłat, Łomża, Poland).

Bread preparation

Formulation of gluten-free bread contained the following amounts of raw materials: maize starch 400 g, potato starch 100 g, guar gum 8.3 g, pectin 8.3 g, freeze-dried yeast 25 g, saccharose 10 g, salt 8.3 g, oil 15 g, water 517 ml. A part of both starches equal to 10% of their initial amount was replaced with amaranth, buckwheat or maize flour. All the ingredients were mixed for 8 min (Laboratory Spiral Mixer SP 12, Diosna, Osnabrück, Germany). The dough was transferred for 15 min into a fermentation chamber (35 °C, 80% moisture), re-mixed for 1 min and weighed pieces of dough (250 g) were put into greased pans. Final fermentation under the above mentioned conditions continued for 20 min. The loaves were baked in electric oven MIWE Condo type CO 2 0608 (MIWE, Arnstein, Germany) for 30 min at 230 °C. Two batches of 6 loaves were acquired. The loaves were cooled at ambient temperature, packed in polyethylene bags and stored under ambient conditions for further analyses.

Physical parameters of the loaves

Bread volume and height were measured with the use of Volscan Profiler (Stable Micro Systems, Godalming, United Kingdom).

Analysis of crumb colour was performed by instrumental method in CIE $L^*a^*b^*$ system. Determination of reflectance in CIE system [9] was done using Konica Minolta CM-3500d (Konica Minolta Sensing, Osaka, Japan) at an angle of 10°, with a slit width of 30 mm. Samples were put in Petri dishes with a diameter of 55 mm. The measurement allowed determination of the following parameters: L^* – luminance ($L^* = 0$ black, $L^* = 100$ white), a^* – the intensity of green ($a^* < 0$) or red ($a^* > 0$), b^* – the intensity of blue ($b^* < 0$) or yellow ($b^* > 0$). Each sample was analysed in 4 replicates.

Texture profile analysis (TPA) of bread crumb of one loaf from each batch was performed, using texture analyser TA-XT2plus (Stable Micro Systems, Godalming, United Kingdom), according to a standard program, at the compression rate of 5 $mm \cdot s^{-1}$. Loaves to be used for analysis on the following days were stored in plastic bags at a temperature of 22 °C ± 2 °C, humidity 64%. Sample of bread crumb, taken from the centre of the loaf with a height of 2 cm, was pressed to reach 50% deformation by a P/20 aluminum cylinder probe with a diameter of 2 cm, in two cycles with a delay of 5 s. The determined hardness and cohesiveness of the crumb were used as indicators of textural changes during storage. The calculations were performed using the software Texture Exponent (Stable Micro Systems). The analyses were performed after 2 h, 24 h and 48 h after baking.

Chemical evaluation

Prior to chemical analysis, the loaves were dried at 23 °C for 5 days and milled with a grinder Grindomix GM 200 (Retsch, Haan, Germany). The following chemical analyses were performed:

Basic nutritional components

Content of protein was determined by Kjeldahl method AOAC No. 950.36 (using the extraction system Büchi B324, Nx5.7; Büchi Labortechnik, Flawil, Switzerland), fat by Soxhlet method AOAC No. 935.38 (using Büchi B811; Büchi Labortechnik), total (soluble) carbohydrates by AOAC No. 974.06, ash by AOAC No. 923.03 [10].

Non-starch polysaccharides

Content of non-starch polysaccharides, i.e. total, soluble and insoluble dietary fibre, were determined by the method AACC 32-07 [11].

Analysis of phenolic compounds and antioxidant activity in bread extracts

Befor analysis, methanol-acetone and ethanol extracts were prepared:

- Methanol-acetone extracts: 1 g of ground sample was extracted with 40 ml HCl (0.16 $mol \cdot l^{-1}$) dissolved in 80% methanol, at 23 °C for 2 h. The contents were fractionated using MPW-350 centrifuge (MPW, Warsaw, Poland) at 2500 ×*g* for 15 min. The supernatant was stored and the residue was re-extracted with 40 ml of 70% methanol for 2 h (at 23 °C), and centrifuged as before. Both supernatants were combined and stored in a freezer at –20 °C.
- Ethanol extracts: 0.6 g of ground sample was extracted with 30 ml 80% ethanol for 2 h (at 23 °C). The contents were fractionated using MPW-350 centrifuge (MPW) at 2500 ×*g* for 15 min. The extract was stored in a freezer at –20 °C.

Methanol-acetone extracts were used to assess total content of polyphenols and anti-radical activity with the use of synthetic radical cation 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS).

Ethanol extracts were used to assess contents of flavonoids, phenolic acids, flavonols and anthocyanins, as well as tannins, as described further.

Polyphenols

Total content of polyphenols was determined by spectrophotometric method, according to Singleton et al. [12]. A volume of 5 ml of methanol-acetone extract was poured in a 50 ml volumetric flask, and made up with water to the mark. A volume of 5 ml of the solution was transferred to a test tube, treated with 0.25 ml Folin-Ciocalteu's reagent (diluted 1:1 with distilled water) and 0.5 ml 7% Na_2CO_3, mixed and left in dark for 30 min. Absorbance of the solution was measured using Helios Gamma spectrophotometer (Thermo Electron, Cambridge, United Kingdom). The results were expressed as grams of catechin per kilogram of dry mass of the sample.

Flavonoids

Content of flavonoids was determined by spectrophotometric method, according to El Hariri et al. [13]. A volume of 0.5 ml of ethanol extract was mixed with 1.8 ml of distilled water and 0.2 ml of 2-aminoethyldiphenylborate solution. The absorbance of the solution was monitored at 404 nm with Helios Gamma spectrophotometer. The results were expressed as grams of catechin per kilogram of dry mass of the sample.

Phenolic acids, flavonols and anthocyanins

Contents of phenolic acids, flavonols and anthocyanins were determined by spectrophotometric method, according to Mazza et al. [14], with the modification of Oomah et al. [15]. A volume of 0.1 ml of the extract was mixed with 2.4 ml of 2% HCl solution in 75% ethanol. The absorbance of the solution was monitored at 320 nm, 360 nm and 520 nm with Helios Gamma spectrophotometer using ferulic acid, quercetin and cyanidin-3-glucoside as standards for phenolic acid, flavonols and anthocyanins, respectively.

Tannins

Content of tannins was determined by spectrophotometric method, according to Broadhurst and Jones [16].

A volume of 0.05 ml of ethanol extract, 3 ml of 4% methanolic vanillin solution and 1.5 ml of concentrated hydrochloric acid were added. The mixture was allowed to stand for 15 min and the absorbance was measured at 500 nm with Helios Gamma spectrophotometer. Content of tannins was expressed in grams of catechin per kilogram of the dry mass of the sample.

Anti-radical activity

Anti-radical activity was determined by the spectrophotometric method with the use of ABTS, according to Re et al. [17]. ABTS radical was obtained in a reaction of ABTS (7 $mmol \cdot l^{-1}$) with potassium persulphate (2.45 $mmol \cdot l^{-1}$) during 16 h. Appropriately diluted methanol-acetone extracts in phosphate buffer saline (PBS) were mixed with ABTS radical solution and the absorbance at 734 nm was determined using Helios Gamma spectrophotometer after 6 min from the beginning of the reaction. The results were expressed as Trolox equivalent antioxidant capacity (TEAC) in milimoles of Trolox equivalents per kilogram of dry mass of the sample.

Statistical analysis

The experimental data were subjected to analysis of variance (Duncan's test), at the confidence level of 0.05, by the use of software Statistica v. 8.0 (Statsoft, Tulsa, Oklahoma USA). All measurements were done at least in duplicate.

RESULTS AND DISCUSSION

Characteristics of non-wheat flours used in gluten-free bread baking

Tab. 1 shows the contents of nutritionally important compounds in non-wheat flours. It could be observed that amaranth flour was characterized by the highest contents of protein, ash, carbohydrates, fat and dietary fibre, while the lowest contents of these compounds were found in maize flour. The content of these compounds in buckwheat flour was substantial, but 22–57% lower than in the case of amaranth flour. Taking into account the level of total fibre, buckwheat flour contained 33% less of this component than amaranth flour (Tab. 1). The results are comparable with the data reported by other authors [18–20].

Tab. 2 presents the levels of polyphenols, phenolic acids, flavonols and anthocyanins, as well as flavonoids and tannins, in non-wheat flours. Antioxidant potential of the flours was assessed using ABTS radical cation.

It was found that the highest level of total polyphenols was characteristic for buckwheat flour, and both other flours (maize and amaranth) contained significantly less of these bio-active compounds with antioxidant character, by 66% and 74%, respectively. Also the contents of phenolic acids, flavonols and anthocyanins were the highest in the case of buckwheat flour (Tab. 2). The level of flavonoids in buckwheat flour was by 54% higher in comparison to maize and amaranth flours. The presence of tannins was observed only in the case of buckwheat flour, no traces of these compounds were found in maize and amaranth flours (Tab. 2). Antioxidant activity determined by the ABTS method followed the order buckwheat flour > amaranth flour > maize flour. The highest antioxidant activity of buckwheat flour was, in

Tab. 1. Nutritional compounds of non-wheat flours.

Type of flour	Maize	Buckwheat	Amaranth
Protein [$g \cdot kg^{-1}$]	64.0 ± 1.1 ^{a}	116.0 ± 1.5 ^{b}	142.5 ± 1.7 ^{c}
Soluble carbohydrates [$g \cdot kg^{-1}$]	10.0 ± 0.7 ^{a}	16.8 ± 1.0 ^{b}	39.0 ± 1.5 ^{c}
Fat [$g \cdot kg^{-1}$]	24.3 ± 5.4 ^{a}	39.6 ± 1.2 ^{b}	90.9 ± 1.7 ^{c}
Dietary fibre [$g \cdot kg^{-1}$]	27 ± 0.7 ^{a}	27 ± 0.7 ^{a}	85.2 ± 3.4 ^{c}
Ash [$g \cdot kg^{-1}$]	5.0 ± 0.4 ^{a}	21.0 ± 0.3 ^{b}	32.7 ± 0.2 ^{c}

Values are expressed per kilogram of dry matter.
Values signed with the same letters in particular columns are not significantly different at 0.05 level of confidence.

Tab. 2. Phenolic compounds of flours and their antioxidant potential.

Type of flou	Maize	Buckwheat	Amaranth
TPC [$g \cdot kg^{-1}$]	3.22 ± 0.12 ^{b}	9.46 ± 0.15 ^{c}	2.41 ± 0.23 ^{a}
Phenolic acids [$g \cdot kg^{-1}$]	0.056 ± 0.02 ^{b}	0.170 ± 0.017 ^{c}	0 ± 0 ^{a}
Flavonols [$g \cdot kg^{-1}$]	0.020 ± 0.002 ^{b}	0.046 ± 0.001 ^{c}	0 ± 0 ^{a}
Anthocyanins [$g \cdot kg^{-1}$]	0.029 ± 0.007 ^{b}	0.064 ± 0.004 ^{c}	0.011 ± 0.005 ^{a}
Flavonoids [$g \cdot kg^{-1}$]	0.288 ± 0.04 ^{a}	0.412 ± 0.061 ^{b}	0.271 ± 0.011 ^{a}
Tannins [$g \cdot kg^{-1}$]	0 ± 0 ^{a}	1.38 ± 0.07 ^{b}	0 ± 0 ^{a}
TEAC [$mmol \cdot kg^{-1}$]	15.62 ± 0.2 ^{a}	77.1 ± 0.54 ^{c}	26.5 ± 0.72 ^{b}

Values signed with the same letters in particular columns are not significantly different at 0.05 level of confidence
TPC – total phenolic compounds are expressed as grams of catechin per kilogram of dry matter. Phenolic acids are expressed as grams of ferulic acid per kilogram of dry matter. Flavonols are expressed as grams of quercitin per kilogram of dry matter. Anthocyanins are expressed as grams of cyanidin-3-glucoside per kilogram of dry matter. Flavonoids and tannins are expressed as grams of catechin per kilogram of dry matter. TEAC – Trolox equivalent antioxidant capacity is expressed as milimoles of Trolox per kilogram of dry matter.

Tab. 3. Physical parameters of gluten-free bread with addition of different non-wheat flours.

	Control	Gluten-free bread with 10% share of non-wheat flour		
		Maize flour	Buckwheat flour	Amaranth flour
Volume [ml]	594.1 ± 4.6 b	548.6 ± 10.5 a	35.5 ± 6.1 c	563.3 ± 3.7 a
Height [mm]	95.2 ± 0.8 c	80.2 ± 2.1 a	86.3 ± 0.3 b	81.0 ± 0.9 a
Mass [g]	206.8 ± 0.4 a	208.0 ± 0.1 a	204.0 ± 1.2 b	202.7 ± 1.3 b
Specific volume [l·kg^{-1}]	2.87 ± 0.02 c	2.64 ± 0.05 a	3.12 ± 0.01 d	2.78 ± 0.03 b

Values signed with the same letters in particular columns are not significantly different at 0.05 level of confidence.

the first place, caused by the highest contents of polyphenols, including phenolic acids, flavonols, anthocyanins, flavonoids and tannins, in comparison to two remaining flours (Tab. 2). High level of polyphenols, especially flavonoids (rutin, quercetin and hyperin, apigenin) in buckwheat was reported by other authors [3; 4], and the resulting high antioxidant activity of this plant material was earlier determined by SEDEJ et al. [21].

Physical characteristics of gluten-free bread with addition of non-wheat flours

Specific volume of all analysed loaves (Tab. 3) was in the range typical for commercially available gluten-free bread, which was reported to be 1.54–4.79 l·kg^{-1} [22]. The addition of various components had different impact on loaf volume, which decreased after partial replacement of starch with maize and amaranth flours, and increased after the addition of buckwheat flour (Tab. 3). On the other hand, all the applied additives caused a decrease in height of respective loaves, corresponding to more flat surface of their upper part. The loss of water during baking, which was reflected in a final mass of cold loaf, was only slightly affected by the replacement of starch with buckwheat and amaranth flours (Tab. 3).

Improving effects of buckwheat flour on specific volume of gluten-free bread were statistically significant and equalled approx. 8% (Tab. 3). Similar positive effects of buckwheat flour on volume of gluten-free bread was observed by ALVAREZ-JUBETE et al. [8], when it was used instead of potato starch in gluten-free formulation. In the same study, the use of amaranth flour did not cause any significant changes in bread specific volume, although the reported values were generally lower than those reported here (1.29–1.63 l·kg^{-1}).

Hardness of bread crumb was slightly decreased after the introduction of non-wheat flours and, in the case of buckwheat and amaranth flours, this change was statistically significant (Fig. 1). The same pattern was observed throughout the whole storage period and could be explained by the changes in crumb structure caused by the addition of non-wheat flours. The changes in crumb cohesiveness were less pronounced (Fig. 2) and, only in the case of buckwheat flour, a significant retardation of bread crumbling could be observed (higher cohesiveness on the first and second day of

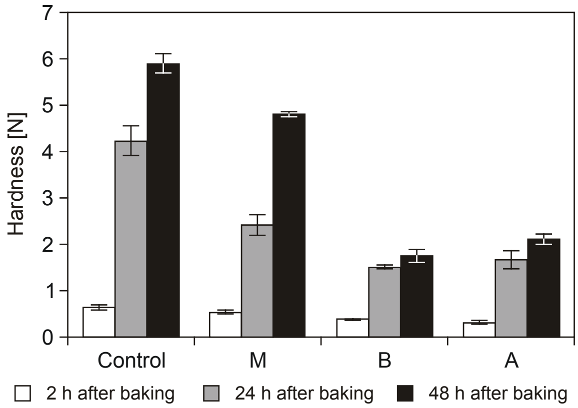

Fig. 1. Hardness of stored bread samples.

M – bread with 10% share of maize flour, B – bread with 10% share of buckwheat flour, A – bread with 10% share of amaranth flour.

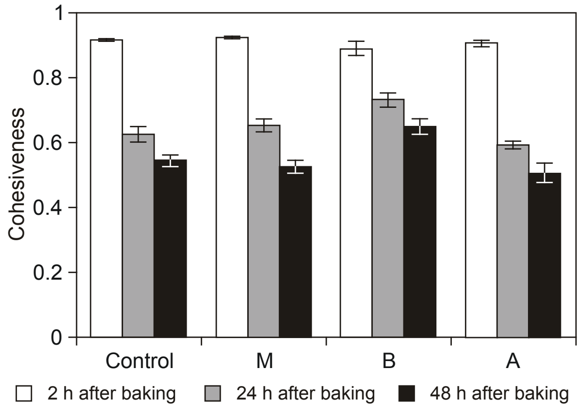

Fig. 2. Cohesiveness of stored bread samples.

M – bread with 10% share of maize flour, B – bread with 10% share of buckwheat flour, A – bread with 10% share of amaranth flour.

storage in comparison to other samples). It should be noticed that the changes in texture parameters did not reflect the alteration in bread volume, as the loaves baked with buckwheat and amaranth flours displayed lower hardness than control, despite that the former were significantly larger and the latter smaller. Reduction of crumb hardness was most probably caused by the decrease in the integrity of the walls of crumb pores, caused by the replacement of starch with cereal fibre [23].

The differences in bread properties could generally be explained by the changes in contents of starch, protein and fibre [23]. The importance of starch and protein, however, seems not to be high in our studies, where the contents of these main components were almost constant at the applied addition level (10%).

More attention should probably be paid to minor constituents of cereal flours, which could play a role as baking improvers, such as lipids and enzymes. The importance of fat content in pseudo-cereals for softening effects on crumb of gluten-free bread was earlier shown in the study of Alvarez-Jubete et al. [8]. The results shown in Tab. 1 demonstrate that the level of lipids in buckwheat and amaranth flours was higher than in maize flour, which could be the reason for lower hardness of the respective loaves in comparison to control (Fig. 1).

In general, it could be observed that, at the applied level, all examined non-wheat flours could be used for bread production without deteriorating its physical characteristics. All applied flours significantly influenced colour of crust, making it lighter in comparison to control bread (Tab. 4). This could be explained by the fact that the level of carbohydrates in flours was lower in comparison to starch, which was replaced by them. The addition resulted also in the increase in yellowness ($b^* > 0$), in comparison to control. The greatest variability was observed for crust redness ($a^* > 0$), which decreased in breads added non-wheat flours in the range from 16% (buckwheat flour) to 53% (maize flour), in comparison to control.

The applied flours caused also a significant change in crumb colour. Gluten-free breads containing amaranth and buckwheat flours had darker colour than control, while the addition of maize flour caused slight but significant increase in lightness (Tab. 4). A substantial variance could be observed for a^* parameter. The addition of non-wheat flours caused a shift from values indicating green colour ($a^* < 0$), which were characteristic for control bread, to the prevalence of red ($a^* > 0$). The greatest change could be observed for bread with addition of buckwheat flour, and the least change after the addition of maize flour. Partial replacement of starch with buckwheat flour did not significantly influence the level of yellowness. The intensity of this colour was, however, significantly increased in crumb with the share of maize flour (by 75% in comparison to control bread). The reported changes in crust and crumb colour, which accompanied the addition of non-wheat flours, were evidently caused by initial appearance of these ingredients, greyish-brown in the case of amaranth and buckwheat flours, and light yellow in the case of maize flour. Similar influence of various flours on the colour of final bread was earlier reported for maize germ flour [24], amaranth flour [20] and buckwheat flour [25, 26]

Influence of non-wheat flours on contents of nutritionally important components and dietary fibre in gluten-free bread

In comparison to traditional cereal products, gluten-free breads are characterized by significantly lower nutritional value, because they contain less protein, fibre and micro-elements. This is

Tab. 4. Colour of crust and crumb of gluten-free bread with addition of non-wheat flours.

	Control	Gluten-free bread with 10% share of non-wheat flour		
		Maize flour	Buckwheat flour	Amaranth flour
Crust				
L^*	42.25 ± 0.42^{a}	57.25 ± 0.38^{d}	55.21 ± 0.21^{c}	54.07 ± 0.33^{b}
a^*	10.42 ± 0.25^{d}	4.90 ± 0.01^{a}	8.77 ± 0.02^{c}	7.65 ± 0.10^{b}
b^*	22.13 ± 0.44^{a}	26.80 ± 0.28^{bc}	27.12 ± 0.20^{c}	26.10 ± 0.39^{b}
Crumb				
L^*	67.48 ± 0.21^{c}	68.14 ± 0.06^{d}	64.96 ± 0.05^{b}	62.19 ± 0.14^{a}
a^*	-0.93 ± 0.01^{a}	0.61 ± 0.00^{b}	1.48 ± 0.06^{d}	1.00 ± 0.03^{c}
b^*	12.49 ± 0.19^{a}	21.91 ± 0.47^{c}	12.88 ± 0.07^{a}	15.15 ± 0.18^{b}

Values signed with the same letters in particular columns are not significantly different at 0.05 level of confidence.

Tab. 5. Nutritional compounds in gluten-free bread with addition of non-wheat flours.

	Control	Gluten-free bread with 10% share of non-wheat flour		
		Maize flour	Buckwheat flour	Amaranth flour
Protein [g·kg^{-1}]	21.2 ± 1.1 ^{a}	31.6 ± 0.1 ^{b}	44.1 ± 1.9 ^{c}	43.3 ± 1.3 ^{c}
Ash [g·kg^{-1}]	19.1 ± 0.3 ^{a}	20.6 ± 0.6 ^{b}	22.6 ± 0.1 ^{c}	23.8 ± 0.1 ^{d}
Soluble carbohydrates [g·kg^{-1}]	4.4 ± 0.3 ^{a}	5.4 ± 0.2 ^{ab}	4.9 ± 0.3 ^{a}	5.2 ± 0.3 ^{ab}
Fat [g·kg^{-1}]	36.6 ± 1.2 ^{a}	34.8 ± 1.7 ^{a}	42.7 ± 1.8 ^{b}	44.8 ± 2.3 ^{b}

Values signed with the same letters in particular columns are not significantly different at 0.05 level of confidence. Values are expressed per kilogram of dry matter.

why gluten-free products should be supplemented with raw-materials, which are naturally free of gluten and contain significant amounts of nutrients e.g. buckwheat, amaranth and maize flours. This is especially important, taking into account high risk of mineral deficiencies and prolonged constipation, caused by diet deprived of dietary fibre, in celiacs [27]. Tab. 5 represents the contents of nutritional compounds in gluten-free bread enriched with non-wheat flours. It was shown that all breads supplemented with non-wheat flours, maize, amaranth and buckwheat, had a higher protein content and a higher ash content compared to standard bread.

The protein content in breads containing amaranth and buckwheat flours increased by 106% compared to control bread and, in gluten-free breads with maize flour, by 50% compared to standard bread (Tab. 5). Ash content was observed to increase with the addition of non-wheat flours, the most pronounced change occurring with the addition of amaranth flour. On the other hand, total carbohydrates level was not affected by bread formulation in a statistically significant way, regardless of the type of non-wheat flour added. Addition of maize flour to gluten-free formulation did not result in a change in the fat content in comparison to the standard bread, as opposed by amaranth and buckwheat flours, which contributed to an increase in fat by approximately 20% compared to control (Tab. 5).

It can be concluded that an increase of protein and ash in gluten-free breads after the addition of non-wheat flours appeared to be of particular value, because such products are considered to be deficient in these two components, which adversely affects the development, growth and functioning of patients with celiac disease, especially children. It should be added that, with the additions of non-wheat flours, gluten-free breads were enriched by proteins of high biological value (high content of lysine: in buckwheat 51 g.kg^{-1}, in maize 28 g·kg^{-1}, in amaranth 62 g·kg^{-1}) and by large quantities of important minerals (especially Ca, Fe, Mg, K), which build bones and other tissues, have hematopoietic and regulatory functions, and stimulate the activity of enzymes in the human body [19, 28].

While healthy people could ensure an adequate supply of minerals by including dark bread or wholemeal products in the diet, this is impossible in the case of people with celiac disease. It is therefore necessary to provide supply of micronutrients usually contained in breads by addition of non-wheat flours. Although the fat content of breads with addition of non-wheat flours increased by 20% (only in the case of flours from amaranth and buckwheat), it should be noted that this fat was rich in polyunsaturated fatty acids [19, 28].

Similar studies on the effect of the addition of pseudo-cereal flour on the nutrient content in gluten-free breads were done by ALVAREZ-JUBETE et al. [7, 8]. The authors found that the level of protein, Ca, Mg, Zn, Fe and vitamin E in gluten-free breads significantly increased by the use of pseudo-cereal flours. Similar conclusions were done by GAMBUŚ et al., who analysed the addition of amaranth flour on gluten-free bread [6]. The authors observed increase in protein, fat and ash after supplementation of gluten-free bread with amaranth flour at 31%; 3% and 9.3%, respectively.

All the applied non-wheat flours enriched bread with nutrients (Tab. 5), but also they provided an increase in dietary fibre as shown in Tab. 6. Fibre is not a bioactive component but has a beneficial effect on human health. It was found that the highest content of total dietary fibre was achieved by the addition of amaranth flour, which should be associated with a high content of this component in this flour (Tab. 1). However, in the loaves prepared with the addition of buckwheat and maize flours, total content of dietary fibre also increased in relation to the control bread, by 5.6% and 12.2%, respectively. In the case of the soluble fraction of dietary fibre, breads prepared with the addition of buckwheat and maize flours contained

Tab. 6. Dietary fibre in gluten-free bread with addition of non-wheat flours.

	Control	Gluten-free bread with 10% share of non-wheat flour		
		Maize flour	Buckwheat flour	Amaranth flour
Insoluble fibre [g·kg^{-1}]	36.6 ± 0.4 [a]	45.3 ± 1.4 [c]	40.0 ± 1.0 [b]	47.6 ± 0.7 [d]
Soluble fibre [g·kg^{-1}]	22.2 ± 0.7 [b]	20.8 ± 0.8 [ab]	22.1 ± 1.5 [b]	26.2 ± 0.6 [c]
Total fibre [g·kg^{-1}]	58.8 ± 0.3 [a]	66.0 ± 0.6 [c]	62.1 ± 2.6 [b]	73.8 ± 0.2 [d]

Values signed with the same letters in particular columns are not significantly different at 0.05 level of confidence.

equal amounts of this component as standard. Bread enriched with amaranth flour contained by 18% more soluble fibre than control. This is especially relevant in the context of hypocholesterolemic, hypoglycemic and anti-tumor activity of this fraction [29].

Insoluble fraction of dietary fibre in all cases raised after the addition of non-wheat flours in the range of 9.3–30% compared to controls (Tab. 6). This is an important result, because insoluble fraction of dietary fibre has anti-cancer and bulking properties [29]. The addition of non-wheat flours to gluten-free bread enriched it in dietary fibre in general and especially in its insoluble fractions. In consequence, the compounds that are an integral part of this fraction, such as polyphenols and other bioactive constituents with an antioxidant activity, could be provided together with the addition of non-wheat flour.

Bioactive components and antioxidant activity of gluten-free bread with a share of non-wheat flours

As it was already mentioned, gluten-free bread is usually low in nutritional value and has no pro-health properties, especially taking into account antioxidant properties. However, such properties may be obtained by partial replacement of starch used in bread formulation with non-wheat flours with high antioxidant potential. It seems that supplementation of gluten-free bread with buckwheat, amaranth and maize flours, which contain high amounts of bioactive components (polyphenols), is fully justified.

Tab. 7 demonstrates data on total polyphenols, phenolic acids, flavonols and anthocyanins in crumb and crust of bread supplemented with non-wheat flours. It could be observed that the highest content of polyphenols was found in crumb and crust of bread with the addition of buckwheat flour, while both other samples contained less polyphenols, but still more than control (Tab. 7).

In the case of bread with maize or amaranth flour, the level of polyphenols in crust was by 12% and 80% higher, respectively, in comparison to control. Their contents in crumb were not so high as at the addition of buckwheat flour, however, still substantially greater than for control crumb, which was virtually deprived of polyphenols (Tab. 7). It could be observed that, although baking causes loss of polyphenols [8, 30], they are not totally destroyed by thermal treatment and enrich the gluten-free bread. In the study of Sakac et al. [18], an increase in polyphenol content with the addition of buckwheat flour was observed, and the level correlated with the share of the flour in the formulation of gluten-free bread. Alvarez-Jubete et al. [8] showed that total polyphenol content increased in the loaves with amaranth, quinoa and buckwheat seeds by 57%, 250% and 630%, respectively, in comparison to control gluten-free bread.

Phenolic acids were more abundant in loaves with the share of buckwheat flour in comparison to control bread, while no traces of them could be detected in crumb and crusts of bread containing other flours, despite that they could be determined in flours before baking (Tab. 2, 7). This fact may be explained by decarboxylation of phenolic acids to 4-vinylguaiacol during baking [31]. Similar tendency could be observed for flavonols, which were detected only in the crumb of breads with maize and buckwheat flours, proportionally to their content in respective flours. The crumb of loaves containing non-wheat flours was enriched in anthocyanins in comparison to standard samples, especially when buckwheat flour was used. No such change could be detected for crust (Tab. 7).

It can be concluded that the highest content of phenolic acids, flavonols and anthocyanins, which was observed for bread supplemented with buckwheat flour, was caused by very high initial content of polyphenols in this raw material in comparison to other non-wheat flours. Despite that thermal processing negatively influences the content of flavonoids [30], they were determined in significant quantities in all breads with the addition of non-wheat flours (Tab. 8). The highest level of flavonoids could be observed for bread with buckwheat flour (crumb and crust), next in the samples with amaranth and maize flours. High content of flavonoids in buckwheat bread was evidently caused by their presence in the flour (Tab. 2, 8). On the other hand, tannins, which are present only in buckwheat flour, were totally destroyed during baking and could not be detected in bread (Tab. 2, 8).

The highest content of polyphenols, including phenolic acids, flavonols, anthocyanins and flavonoids, in bread with buckwheat flour resulted in elevated antioxidant activity (Tab. 7, 8). Its value in bread crust was by 173% higher than in control sample, and an even greater change could be observed for bread crumb, as such activity could not be detected in starch-based bread (Tab. 8). The loaves (crumb, crust) with the addition of amaranth flour contained more total polyphenols and flavonoids than loaves with maize flour, which was also reflected by their relatively high antioxidant activity. Part of antioxidant activity could be attributed to substances that were formed during thermal processing, e.g. in Maillard reaction [32, 33], as well as antioxidant vitamins and polyphenols not determined by methods used in this study. It could be also expected that higher values of antioxidant activity, observed for crust of bread with non-wheat flours, could partly be attributed to the formation of Maillard reaction products, which could mask or compensate the loss of phenolic compounds caused by thermal degradation. SAKAC et al. [18] demonstrated that antioxidant activity of gluten-free bread increased in parallel

Tab. 7. Polyphenolic compounds in gluten-free bread with addition of non-wheat flours.

	Control	Gluten-free bread with 10% share of non-wheat flour		
		Maize flour	Buckwheat flour	Amaranth flour
Crust				
TPC [g·kg^{-1}]	0.25 ± 0.02 c	0.28 ± 0.02 c	1.02 ± 0.07 f	0.45 ± 0.06 d
Phenolic acid [g·kg^{-1}]	0 ± 0 a	0 ± 0 a	0.018 ± 0 b	0 ± 0 a
Flavonols [g kg^{-1}]	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a
Anthocyanins [g kg^{-1}]	0.011 ± 0 b	0.011 ± 0 b	0.011 ± 0 b	0.011 ± 0 b
Crumb				
TPC [g·kg^{-1}]	0 ± 0 a	0.16 ± 0.02 b	0.64 ± 0.05 e	0.31 ± 0.03 c
Phenolic acid [g·kg^{-1}]	0 ± 0 a	0 ± 0 a	0.024 ± 0 c	0 ± 0 a
Flavonols [g kg^{-1}]	0 ± 0 a	0.014 ± 0.001 b	0.049 ± 0.004 c	0 ± 0 a
Anthocyanins [g kg^{-1}]	0 ± 0 a	0.011 ± 0 b	0.03 ± 0 c	0.011 ± 0 b

Values signed with the same letters in particular columns are not significantly different at 0.05 level of confidence
TPC – total phenolic compounds are expressed as grams of catechin per kilogram of dry matter. Phenolic acids are expressed as grams of ferulic acid per kilogram of dry matter. Flavonols are expressed as grams of quercitin per kilogram of dry matter. Anthocyanins are expressed as grams of cyanidin-3-glucoside per kilogram of dry matter.

Tab. 8. Content of flavonoids, tannins and antioxidant activity of gluten-free bread with addition of non-wheat flours.

	Control	Gluten-free bread with 10% share of non-wheat flour		
		Maize flour	Buckwheat flour	Amaranth flour
Crust				
Flavonoids [g·kg^{-1}]	0.181 ± 0.006 e	0.095 ± 0.006 c	0.282 ± 0.013 f	0.133 ± 0.006 d
Tannins [g·kg^{-1}]	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a
TEAC [mmol·kg^{-1}]	6.4 ± 0.23 e	4.31 ± 0.17 c	17.46 ± 0.56 h	7.07 ± 0.27 f
Crumb				
Flavonoids [g·kg^{-1}]	0 ± 0 a	0.067 ± 0.006 b	0.192 ± 0.02 e	0.105 ± 0.007 c
Tannins [g·kg^{-1}]	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a
TEAC [mmol·kg^{-1}]	0 ± 0 a	3.4 ± 0.2 b	13.54 ± 0.47 g	5.26 ± 0.15 d

Values signed with the same letters in particular columns are not significantly different at 0.05 level of confidence.
Flavonoids are expressed as grams of catechin per kilogram of dry matter. Tannins are expressed as grams of catechin per kilogram of dry matter. TEAC – Trolox equivalent antioxidant capacity is expressed as milimoles of Trolox per kilogram of dry matter.

with the share of buckwheat flour (10–30%). Similar results were also published by Lin et al. [25], who observed amelioration of antioxidant activity of wheat bread after the addition of buckwheat flour. Higher antioxidant activity of bread with amaranth, quinoa and buckwheat seeds was also determined in the study of Alvarez-Jubete et al. [8], and the respective increase was 84%, 2-times and 9-times in comparison to control.

In summary, it could be stated that, among all analysed gluten-free breads with the addition of non-wheat flours, the samples with buckwheat flour were distinctive for their nutritional value as well as antioxidant activity. Addition of buckwheat flour seems to be the best way to improve the level of bioactive compounds with antioxidant character, and to minimize the negative effect of baking on antioxidant potential of bakery products.

CONCLUSIONS

It was observed that buckwheat flour was characterized by the highest content of total polyphenols, including phenolic acids, flavonols, anthocyanins, flavonoids and tannins, in comparison to amaranth and maize flours. The level of protein, lipids, carbohydrates and ash was in buckwheat flour high, but lower in comparison to amaranth flour.

All non-wheat flours, when applied as additives for gluten-free bread, did not worsen the quality of the final products and, in the case of bread with buckwheat flour, decreased hardness and increased cohesiveness during storage.

Loaves with the addition of amaranth flour were characterized by the highest nutritional value and most elevated fibre content among the analysed bread samples. The loaves with buckwheat flour had comparable levels of protein, carbohydrates and lipids, and less fibre in comparison to loaves with amaranth flour.

The bread with buckwheat flour contained the highest amount of polyphenols, including phenolic acids, flavonols, anthocyanins and flavonoids, which resulted in the highest antioxidant activity among all analysed bread samples.

Taking into account all analysed gluten-free breads with the share of non-wheat flours, it could be observed that supplementation with buckwheat flour was the most effective approach to increase the levels of nutritional constituents and bioactive compounds with antioxidant character. Addition of buckwheat flour did not affect physical properties of the crumb and even limited its hardness. This type of addition could be recommended for supplementation of gluten-free bread to assure its pro-health properties.

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