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ORIGINAL ARTICLE

Nutritional and dietary value of gluten-free rolls enriched in amaranth flour

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Abstract: The subject of the study was to evaluate the influence of the enrichment of gluten-free rolls with amaranth flour on the uptake of selected nutritional and dietary compounds by laboratory rats, as compared to control rolls (not enriched). Chemical composition of rolls obtained by laboratory baking was assessed by AOAC methods, amino acid composition was measured with amino acid analyzer (AAA-400, INGOS). After the agreement of Bioethical Committee nutritional experiments were performed on Wistar laboratory rats. Animals were fed for 28 days with standard and enriched rolls (2 groups), after then plasma levels of Cu, P, Mg and Fe were measured, and lipid profile was evaluated. Selected micro and macro elements were also checked in liver and bones. It was found, that the addition of amaranth flour caused a significant increase in all nutritional components of the rolls, as compared to control. No influence of amaranth enrichment was detected in plasma lipid profile of the rats. Significant increase in minerals deposited in plasma (Ca and Mg), liver (K, P, Mg, Zn, Cu and Mn) and bones (P, Mg and Cu) of animals was observed.

Key words: gluten-free rolls, amaranth flour, rats, nutritional components, minerals

INTRODUCTION

More than 4000 years ago baking technology was invented, and since then bread became basic staple food despite the changes of eating patterns. Cereal products have been considered the main sources of plant proteins, dietary fibre, many vitamins and minerals [1-3]. In contrast to normal wheat-based products, these nutrients are lacking in gluten-free bread, which is often produced from starch, rice flour and mixtures of different hydrocolloids, replacing gluten [4, 5]. The results of several surveys show that people following a gluten-free diet are endangered by the lack of proteins, minerals (calcium, iron), vitamins (folic acid, vitamin B_{12}, and lipid soluble vitamins) and dietary fibre [6, 7]. At the present time various methods of enrichment may be used to increase the nutritional value of gluten-free bakery products. Gluten-free diet should be enriched with products that are naturally free of gluten, and gluten-free products should contain such plant raw materials as buckwheat, millet, soy, amaranth, sunflower, sesame and nuts [5, 7, 8]. It is also necessary to establish new formulations and technologies for gluten-free products, which could lead to the increase of their nutritional value [7].

Because of the chemical compositional and nutritional value, amaranth seeds are regarded as a valuable component for gluten-free products. They are distinguished by high level (up to 19%) of gluten-free protein. Amaranth protein is rich in exogenous amino acids, and has high biological value [9, 10]. The seeds are also abundant in dietary fiber, which is in a large part soluble [7, 11]. Fat is present in amaranth seeds at the levels higher than in cereals, and contains more squalene than popular plant oils [12-14].

Amaranth flour contains much more minerals, especially iron, potassium and magnesium, as compared to cereals [7, 11]. It has a considerable importance, because many studies suggest, that patients with newly diagnosed disease, as well as those which do not strictly follow dietary recommendations, are lacking minerals, which could negatively influence mineral density of bones, and destabilize equilibrium between among selected microelements [5, 15, 16].

The subject of the study was to check the influence of amaranth flour, at the level of 10 % as calculated on starch basis, on the uptake of selected nutritional and dietary compounds by laboratory rats, as compared to control rolls (not enriched).

MATERIALS AND METHOD

Materials. The material consisted of gluten-free control rolls (C) and gluten-free rolls enriched with amaranth flour (A). Gluten free dough consist of corn starch, corn meal, potato starch, gluconic acid lactone and a mixture of hydrocolloids (hydroxypropylmethylcellulose (HPMC), guar gum, highly methylated pectin, locust bean gum). Corn starch was partially replaced with amaranth flour at the level 10% of the whole mass of starch used in the formulation. Milk powder, rapeseed oil, sugar and salt were used as technological additives. Water was added in amounts that allowed obtaining a consistency of 350 units, as measured by a farinograph. Fermentation was conducted for 35 minutes, with the use of freeze-dried yeast (Lesaffre). Rolls weighing 70 g were baked for 20 minutes at 230°C. After cooling they were dried and milled. The obtained material was chemically analyzed and used as a diet for laboratory rats.full cream milk powder, yeast, salt, sugar, oil and water.

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Chemical composition. Chemical composition in gluten-free rolls was assessed according to the AOAC methods (2006): total protein (Met. 950.36), dietary fibre TDF and IDF (Met. 991.43), raw fat (Met. 935.38) and ash (Met. 930.05) [17].

Composition of amino acids was assessed by ion-exchange chromatography, by means of amino acid analyzer AAA 400 (INGOS), according to the standard protocol [18]. Basing on the amino acid composition, Chemical Score and Exogenic Amino Acid Index were calculated, app FAO/WHO [19].

The preparation of samples for the evaluation of ash components conducted according to EN 13804 [20]. Mineralization performed using the dry-ashing method, through the modified method AOAC 985.01 [17]. The modification concerned a lowered temperature and prolonged time of ashing, in order to reduce the risk of loss of assessed minerals resulting from the formation of volatile compounds. The risk increases with rising ashing temperature. The applied temperature was lowered from 500 to 460ºC and ashing time in both steps was three times longer than in the original method. The contents of Ca, Mg, P, K, Na, Fe, Mn, Cu, Zn in the solutions obtained after mineralization were measured by the inductively coupled plasma atomic emission spectrometer JY 238 Ultratrace (Jobin-Yvon, France) following the rules presented in EN-14084 [21].

Animals, diets and experimental design. All experimental procedures complied with the Polish Ethical Standards, and were approved by the 1-st Local Animal Ethics Commission in Krakow (nr 30/2008). Twelve, 5-week old, growing male rats of Wistar strain, weighing initially 90-120 g, were obtained from the Institute of Animal Production in Warsaw. They were randomly assigned to two experimental groups, 6 animals each, and housed at 3 rats steel cages, in an isolated room with controlled temperature (25°C) and ambient humidity, with 12-h light-dark cycle. Prior to experiments all rats were for 4 days fed with gluten-free control rolls, until their weight was stabilized. The rats were fed of experimental diets (40 g per day per cage) and had free access to distilled water. Group I, which was control group, obtain non-enriched rolls (C), while the second group (Group II) receive rolls supplemented with amaranth flour (A). Each of the two group were fed for 4 weeks on gluten-free crumbs. To simulate the nutritional deficiencies, that may be present in gluten-free diets, bread crumbs were the only source of nutrients and no additional sources of protein, vitamins, and minerals were used in experimental diets. Food intake was measured daily and animal body mass was recorded weekly.

On the last day of the experiment the rats were anaesthetized with thiopental (Biochemie GmbH, Austria; 25 mg/100 g body mass), blood, liver and thighbones were used for further analyses. Blood was rapidly collected by cardiac puncture, transferred to a centrifuge tube, and serum was separated by low-speed centrifugation (1500 g, 15 min). The serum samples were stored at -20ºC until analysis. Livers and thighbones were removed, washed in a cold solution of 0.9% sodium chloride, dried and weighted. The tissue samples were stored at -20ºC until the analysis.

Serum total cholesterol (TC) and HDL fraction were analyzed enzymatically with standard kits (BioVendor cat.-no 10851 and BioVendor cat.-no 10855 respectively). The LDL+VLDL fraction of the cholesterol was calculated as the difference between TC and HDL-C. Triacylglycerol content was estimated enzymatically with standard kits (BioVendor cat.-no 12805). The serum levels of Ca (Roche cat.-no 20763128322), Mg (Roche cat.-no 20737593), Fe (Roche cat.-no 03183696122), and P (Roche cat.-no 0318379322) were measured with using commercial kits.

Measurement of minerals in liver and thigh bones. The amounts of selected minerals in the liver and bones of the rats were measured after their mineralization by wet-ashing in high-pressure, microwave system (Milestone 1200) according to EN 13805 [22]. The contents of Ca, Mg, P, K,Na, Fe, Mn, Cu, Zn in solutions obtained after mineralization were measured using the inductively coupled plasma atomic emission spectrometer JY 238 Ultratrace (Jobin-Yvon, France)

Statistical analysis. The effect of bread treatments was analyzed by one-way ANOVA generated by the STATISTICA version 8.0 package (StatSoft, Tulsa, OK.). Where appropriate, treatment means were compared by the LSD Fisher test and p values <0.05 were considered as showing a significant difference between treatment means. All the data was expressed as a mean ±SD.

RESULTS AND DISCUSSION

Existing data clearly shows that gluten-free bakery and other food products have significantly lower nutritional value in comparison to corresponding products, which contain gluten [5, 7, 23]. Comparing the content of basic chemical components (Table 1) in standard (C) and amaranth enriched rolls (A), a significant increase in protein, fat, ash and dietary fibre especially insoluble dietary fibre, which significantly improves nutritional value of the rolls. The effect is caused by a 10% replacement of corn starch with amaranth flour. The increase in dietary fiber seems to be especially important, because it is often reported that celiac people, who follow gluten free diet consume lower amounts of fiber, than it is advised [5, 23, 24]. Vegetables, fruit and whole-meal products, such as buckwheat, flaxseed, millet, quinoa, brown rice and amaranth seem to be a good source of fiber in gluten-free diet [5, 7]. Appropriate supply of fiber in human diet is especially required for proper functioning of dietary tract, regulation of cholesterol level, and development of bacterial micro flora in colon. Dietary fiber reduces absorption of carbohydrates and toxic substances from food, but on the other hand causes also a decrease in bioavailability of macro and microelements [25-27].

As a result of the studies a significant increase in fat content could be observed, which seems to be beneficial, taking into accounts many reports on valuable fatty acid profile in amaranth seeds, and especially large amounts of squalene, which could positively impact cholesterol metabolism in humans [9, 12, 14].

The increase in ash found for rolls with 10% of amaranth flour (Table 1) is accompanied by the rise in the levels of selected minerals. Significant change was found for almost all studied macro- and microelements. Only the content of K was the same for standard and amaranth enriched rolls (Table 1).

The application of amaranth caused a significant rise of protein content in rolls with added amaranth flour (Table 1), however the resulting protein proved to be slightly,

Table 1 Chemical composition of control rolls(C) and rolls enriched with amaranth flour (A).

		C		A	
Total protein N × 6.25	[% d.m.]	4.42±0.09	a	6.37±0.08	b
Fat		4.92±0.16	a	5.74±0.03	b
Dietary fibre Insoluble		3.32±0.01	a	3.90±0.06	b
Soluble		2.90±0.08	a	3.08±0.01	a
Total		6.22±0,09	a	6.98±0.05	b
Ash		2.83±0.01	a	2.94±0.03	b
P	[mg/kg d.m.]	1,131±6	a	1,757±26	b
K		2,294±51	a	2,222±7	a
Ca		569±11	a	768±13	b
Mg		206±3	a	476±9	b
Fe		13.81±0.30	a	19.85±0.15	b
Zn		13.52±0.12	a	16.50±0.24	b
Mn		0.80±0.00	a	6.68±0.06	b
Cu		0.67±0.03	a	1.29±0.01	b

Values are means ± SD
Means followed by different letters in rows are significantly different at p<0.05

but significantly less valuable as compared to control rolls. Because of the replacement of plant proteins by milk proteins (addition of milk powder), the resulting control bread (C) contained protein of full value; CS = 100%, EEAI = 100% (Table 2). It should be noticed that this rolls contain much more protein, therefore more exogenic amino acids. Due to the presence of milk powder, the lack of lysine, characteristic for cereal products, was not observed. In spite of using milk protein in rolls enriched with amaranth was distinguished by lower protein quality. Biological value of amaranth protein was a subject of many scientific studies. Despite the lack of consensus about the limiting amino acid, some authors believe that this should be attributed to leucin. Its content might be regulated by amaranth species and applied processing [10, 11, 28, 29].

Table 2 Chemical Score (CS) and Essential Amino Acid Index (EAAI) of protein present in control rolls (C) and rolls enriched with amaranth flour (A).

		C			A	
CS	Lysine	100.0 ± 1.7	b	Lysine	95.8 ± 1.0	a
EAAI		100.0 ± 0.1	b		99.5 ± 0.1	a

Values are means ± SD
Means followed by different letters in rows are significantly different at p<0.05

Standard and amaranth enriched rolls, were used as solitary components of the diet for laboratory rats. They induced varying effect on the body mass of rats during 4 weeks of experiment (Table 3). In general, all animals fed with rolls enriched with amaranth flour gained weight faster in comparison to standard group (I). The highest average body mass, after 5-days adaptation period, as well as at the end of experiment was observed for group II. The animals in this group were characterised by significantly larger livers.

Table 3 Body gain and liver weight of experimental rats.

	Group I:	C	Group II:	A
Body gain [g]	6.0±1.9	a	36.2±6.8	b
Liver weight [g]	5.14±0.72	a	7.37±0.59	b

Values are means ± SD
Means followed by different letters are significantly different at p<0.05

There was no significant decrease of total cholesterol, and its HDL and LDL fractions, in serum of rats fed with amaranth enriched rolls, as compared to standard (Table 4). The level of triglycerides in blood of animals fed with supplemented rolls was unchanged in comparison to standard group (Table 4), it should be underlined that despite the changes in plasma cholesterol between animals fed by amaranth and control rolls were not significant, lower cholesterol content was measured in group II, which might indicate a tendency of amaranth enriched rolls to decrease cholesterol level. Most studies on the influence of amaranth on cholesterol level, shows its hypocholesterolemic activity, and it is commonly believed that squalen and tocoferols are the compounds responsible for this effect. It was however reported, that even defatted amaranth seeds may positively impact cholesterol level [7, 13, 14, 30, 31]. Significant influence of amaranth enrichment on lipid metabolism could probably be proved in a separate experiment, with the use of hypercholesterolemic diet, but it was out of the scope of the present study.

Table 4 Lipid profile and concentration of some minerals in serum of experimental rats.

	Group I C		Group II A	
TC [mmol/l]	1.61±0.33	a	1.47±0.20	a
HDL [mmol/l]	1.34±0.23	a	1.24±0.12	a
TG [mmol/l]	0.49±0.15	a	0.44±0.14	a
LDL+VLDL [mmol/l]	0.28±0.11	a	0.23±0.08	a
Fe [µmol/l]	32.1±2.3	b	25.5±2.1	a
Ca [mmol/l]	2.38±0.08	a	2.51±0.07	b
Mg [mmol/l]	0.88±0.09	a	1.09±0.11	b
P [mmol/l]	2.36±0.28	a	2.46±0.23	a

Values are means ± SD
Means followed by different letters in rows are significantly different at p<0.05

Slight, but significant increase of Ca and Mg in serum of rats fed with amaranth enriched rolls in comparison to standard group. No influence of the diet on P serum level was observed.

The largest differences between groups were found for Fe in serum, because in groups II (fed with rolls with Amaranth), its level was significantly lower as compared to standard group (I).

In livers of rats fed with amaranth enriched rolls, significant increase of K, P, Mg, Zn, Cu and Mn, as compared to control group, was observed (Table 5). The level of Ca was significantly lower, while Fe level in live was unchanged in of animals fed

Table 5 Concentration of minerals in liver of experimental rats.

mg in liver/ 100 g body mass rat	GROUP I C		GROUP II A	
K	10.73±0.47	a	11.88±0.96	b
P	10.89±0.69	a	12.56±1.01	b
Mg	0.81±0.05	a	0.90±0.15	b
Fe	0.60±0.05	a	0.57±0.06	a
Ca	0.19±0.02	b	0.16±0.02	a
Zn	0.073±0.008	a	0.087±0.007	b
Cu	0.009±0.001	a	0.010±0.001	b
Mn	0.0079±0.0005	a	0.0090±0.0014	b

Values are means ± SD
Means followed by different letters in rows are significantly different at p<0.05

with amaranth enriched rolls, in comparison to standard rolls.

The largest differences in content of minerals were noted for bones of rats fed with standard and amaranth enriched rolls (Table 6). Enrichment with amaranth flour resulted not only in higher body mass, but also caused the increase in concentration of P, Mg and Cu in bones. Taking into account the levels of Ca, K, Fe, Zn and Mn, no differences between bones of control rats (I) and group II were found.

Table 6 Concentration of minerals in bones of experimental rats.

mg/kg bone	GROUP I C		GROUP II A	
Ca	138138±4377	a	139495±9080	a
P	58565±1863	a	61044±2112	b
K	1914±46	a	1990±80	b
Mg	1907±98	a	1982±102	b
Zn	103.9±2.0	a	108.2±1.8	a
Fe	68.6±4.5	a	69.5±3.4	a
Cu	0.91±0.08	a	0.99±0.04	b
Mn	0.42±0.06	a	0.44±0.07	a

Values are means ± SD
Means followed by different letters in rows are significantly different at $p<0.05$

The results obtained in an experiment on animals show a beneficial impact of amaranth addition on accumulation of macro and microelements in animal tissues, especially phosphorus, magnesium and copper. The increase of minerals in bones seems to be of the greatest importance, because both phosphorus and magnesium levels are higher, while the content of calcium is unchanged. It should be noted, that although animals fed with amaranth enriched flour gained more weight, the percentage of minerals in bones was not negatively affected. It is an important information for celiac people, because the adherence to gluten-free diet, which is generally low in minerals, may lead to osteoporosis [7, 32]. Gluten free diet which has to be obeyed by those people contains less nutritional compounds, so its enrichment in highly nutritive products is very important [23, 33, 34]. Strict adherence to gluten free diet and care to balance its nutritional value, leads to a significant health improvement of persons with newly diagnosed disease, including the progress in mineral density of bones [5, 35, 36].

The above results proved, that the application of amaranth flour as additives for gluten free rolls significantly improved nutritional and dietary value of the rolls, as compared to control. The products were characterized by high bioavailability of micro- and macro elements. Better absorption of minerals was found despite of the increase in fiber level, caused by amaranth addition, although it is commonly known, that such an increase may be accompanied by limited bioavailability of nutrients, especially minerals [25]. Unfortunately the application of amaranth did not cause an increase in deposition of iron in the studied animal tissues, so it seems necessary to find other ways of iron supplementation in gluten free products, such as application of iron salts [5, 8].

CONCLUSION

The results allow to conclude, that:

1. Rolls enriched with amaranth flour were considerably richer in protein, fat, dietary fibre, and ash, in comparison to standard products.
2. Standard rolls were characterized by high protein quality, therefore rolls enriched with amaranth were distinguished by lower protein quality.
3. All animals fed with the rolls enriched with amaranth were characterised by significantly higher gain of body mass as compared to control group.
4. The level of total cholesterol, its LDL and HDL fractions, and triglycerides in serum was the same for rats fed with standard and amaranth enriched rolls.
5. Significant increase of Ca and Mg in serum of rats fed with amaranth enriched rolls, as compared to control group was observed.
6. In livers of rats fed with amaranth enriched rolls a significant increase in the levels of K, P, Mg, Zn, Cu and Mn, as compared to control group, was observed.
7. Enrichment of rolls with amaranth flour positively influenced the concentration of P, Mg, and Cu in bones of rats fed with these rolls, irrespective of the baking method.

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