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ENRICHMENT OF GLUTEN-FREE ROLLS WITH AMARANTH AND FLAXSEED INCREASES THE CONCENTRATION OF CALCIUM AND PHOSPHORUS IN THE BONES OF RATS

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The utilization of nutrients present in amaranth flour and flaxseed, used as ingredients in gluten-free rolls, was examined in rats. The chemical composition of amaranth flour, flaxseed and enriched rolls was compared. In the nutritional study Wistar rats were fed diets consisting solely of air-dried gluten-free rolls and water, to check the hypothesis that the enrichment of gluten-free rolls with flaxseed or amaranth can improve biochemical indices in rats. The animals were divided into 3 groups of 6 rats. Group I was fed control (not enriched) rolls, group II and group III were fed amaranth and flaxseed, respectively, enriched rolls. The experiment was conducted over 30 days. At the end of the experiment, the blood and organs were removed from the anesthetized rats. The lipid profile and content of some elements were measured in blood serum, while other tissues were checked for Ca, P, Mg, Fe, Na, K, Cu, Zn and Mn content. Weight gain was observed only in treatment groups of animals. Both applied ingredients decreased the TC and HDL-cholesterol content of serum in both treatment groups of animals. They also increased Ca content in the serum of the rodents. The concentration of calcium and phosphorus was significantly higher in the bones of rats fed with diets with amaranth or flaxseed enriched rolls.

INTRODUCTION

Celiac disease is one clinical absorptive disorder, and its only treatment is strict adherence to a gluten-free diet (often throughout one's whole life) [Mora *et al.*, 1993]. This is why an important role in the treatment is played by gluten-free products, which should provide all the necessary nutrients. The results of several surveys show that people following a gluten-free diet are at risk of a lack of proteins, minerals (calcium, iron), vitamins (folic acid, vitamin B_{12}, and lipid-soluble vitamins) and dietary fiber [Presutti *et al.*, 2007]. Bread has been, for the many thousands of years one of the basic food products, and thus it is considered the main source of plant proteins and many minerals in the everyday diet. This, however, is not valid for gluten-free bread, which is often produced from starch (wheat or corn), rice flour and mixtures of different hydrocolloids, replacing gluten [Gambuś *et al.*, 2007]. Apart from the technological difficulties in the shaping and structure formation of such bread, a significantly lower nutritional value could be observed in comparison with traditional, gluten-containing products. Gluten-free bread contains lower amounts of minerals, vitamins and dietary fiber [Thompson, 2000]. Various methods of enrichment have been used to increase the nutritional value of gluten-free bakery products. Among the best is an addition of raw materials, which are naturally free of gluten, such as amaranth or flaxseed, which enrich the products with nutrients. This is especially important for people who suffer from other food allergies together with celiac, and do not tolerate, for example, milk products, which deprives their diet of proteins, calcium and other minerals [Kupper, 2005]. Amaranth is a gluten-free plant rich in many nutrients. Its proteins have a high biological value [Písaříková *et al.*, 2005]. The content of minerals and dietary fiber is also higher in comparison to wheat, rye and barley. Functional properties of flaxseed are mainly connected with α-linolenic acid (18:3 n-3, ALA) and lignans, because flax is the richest source of those components in nature [Oomah, 2001]. A positive physiological action is exerted by gums, which are part of the soluble dietary fiber present in these seeds [Tarpila *et al.*, 2005]. Flaxseed protein is characterised by a high level of branched-chain amino acids (valine, leucine, isoleucine), low amounts of aromatic amino acids and high, comparable to soy, Fischer ratio. Such proteins are required by the producers of physiological and functional food, intended for special purposes, *e.g.* for undernourished patients with cancer, burns, liver diseases, and as nutritional supplementation for children with chronic or severe celiac sprue or allergy to milk proteins [Oomah, 2001]. Although dietary fiber beneficially impacts human physiology, it may negatively affect the bioavailability of selected nutrients, mainly minerals. The total content of minerals in food products is not the veritable measure of their nutritional value, because bioavailability depends on many factors, such as digestive conditions and absorption capability in the small intestine [Harland, 1989].

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The aim of the study was to check the hypothesis that the enrichment of gluten-free rolls with flaxseed or amaranth can improve biochemical indices and mineral status of rats. This is important to the field of nutrition, to show some paths to balance nutritional deficiencies connected with the gluten-free diet.

MATERIALS AND METHODS

MATERIALS

Material consisted of amaranth flour, flaxseed meal and gluten-free rolls, baked from gluten-free flour, consisting of corn starch, potato starch, corn flour and a mixture of hydrocolloids (guar gum, pectin, locust bean gum, hydroxypropylmethylcellulose). Corn starch was partially replaced with amaranth flour or flaxseed meal at the level of 10% of the whole mass of starch used in the formulation. Milk powder and rapeseed oil 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 min, with the use of freeze-dried yeast (Lesaffre). Rolls weighing 70 g were baked for 20 min at 230°C. After cooling, they were dried and milled. The material obtained was analysed chemically and used as a diet for laboratory rats.

METHODS

Assessment of the chemical composition of amaranth flour, flaxseed meal and rolls

AOAC methods were used for the determination of total protein (950.36), total dietary fiber (TDF) and insoluble dietary fiber (IDF) (991.43), raw fat (935.38) and ash (930.05) in amaranth flour, flaxseed meal and rolls [AOAC, 2006].

Amino acid composition

The composition of amino acids was assessed by ion-exchange chromatography, by means of an amino acid analyzer AAA 400 (INGOS), according to the standard protocol [Davidson, 2003]. Based on the amino acid composition, the Chemical Score (CS) and Essential Amino Acid Index (EAAI) were calculated [FAO-WHO, 1991].

Fatty acid profile measurement

The lipid profile of extracted fat was established by means of a gas chromatograph coupled with a mass spectrometer (Shimadzu QP 5050A) (helium; SPTM-2560 columns – 100 m, film thickness of 0.25 μm and diameter of 0.25 mm, column and detector temperatures were 60–220°C and 245°C, respectively).

Measurements of minerals in amaranth, flaxseed and control and enriched rolls

The preparation of samples for the evaluation of ash components was conducted according to EN 13804 Standard [EN-13804, 2002]. Mineralization was performed using the dry-ashing method, modified acc. to the AOAC 985.01 method [AOAC, 2006]. The modification concerned a lowered temperature and prolonged time of ashing, in order to reduce the risk of loss of the assessed minerals resulting from the formation of volatile compounds. The risk increases with a rise in ashing temperature. The applied temperature was lowered from 500 to 460°C 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 (ash was dissolved in HNO_3) were measured with the inductively coupled plasma atomic emission spectrometer JY 238 Ultratrace (Jobin-Yvon, France) following procedures presented in EN-14084 Standard [EN-14084, 2003].

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. They were performed according to the ethical rules recommended by Close *et al.* [1997]. Eighteen 5-week old growing male rats of the Wistar strain, initially weighing 90-120 g, were obtained from the Laboratory of Animals Husbandry in Warsaw, Poland. They were randomly assigned to three experimental groups (n=6), and housed individually in screen-bottomed stainless steel cages, in an isolated room with controlled temperature (25°C) and ambient humidity, with a 12-h light-dark cycle. These rats were fed 10 g of air-dried crumbs per day and had free access to distilled deionized water. According to Brylińska & Kwiatkowska [1996], the intake of dry feed by growing rats varies in the range of 8–20 g, and the amounts that were used in the experiment were not fully consumed by animals. Each of the three experimental groups were fed for 4 weeks with gluten-free crumbs. Group I (C), which was the control group, obtained non-enriched rolls, while treatment groups received rolls enriched with amaranth – group II (A) and flaxseed – group III (F). The animals' body weight was recorded on the first day of the experiment and after 5, 10 and 30 days. On the last day of the experiment, the animals were anaesthetized with thiopental (Biochemie GmbH, Austria; 25 mg/100 g body weight). 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 -20°C until analysis. Livers, kidneys and thighbones were removed, washed in a cold solution of 0.9% sodium chloride, dried and weighed. The tissue samples were stored at -20°C until the analysis.

The serum's total cholesterol (TC) and high density lipoproteins (HDL) fraction were analysed enzymatically with standard kits (BioVendor cat. no 10851 and BioVendor cat. no 10855 respectively). The low-density lipoproteins and very low-density lipoproteins (LDL+VLDL) fractions of the cholesterol were calculated as the difference between TC and HDL-C. The 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 the use of commercial kits.

Measurement of minerals in livers, kidneys, and thighbones

The contents of selected minerals in the kidneys, liver and bones of the rats were measured after their decomposition by wet-mineralization performed in teflon vessels under approx. 100 Ba in a high-pressure, microwave system (Milestone 1200) according to EN 13805 [EN-13805, 2003]. The sample containing approx. 0.5 g d.m. was treated with 5 cm^3 HNO_3 and 1 cm^3 H_20_2 (30%) and heated, increasing temperature in 5 steps. Suprapur Merck reagents were used for analysis. The contents of Ca, Mg, P, K, Na, Fe, Mn, Cu, Zn in the solutions obtained after mineralization were measured using the inductively coupled plasma atomic emission spectrometer JY 238 Ultratrace (Jobin-Yvon, France). Precision of the method was established based on the relative standard deviation (RSD) calculated from triple measurement of the same sample. The analysis was accepted if RSD was less than 5%.

Accuracy of the method was evaluated by analysis of certified material obtained from the International Atomic Energy Agency (Certified Reference Material IAEA/V-10 HAY; Powder).

Statistical analysis

All the data were analysed with a single factor analysis of variance using Microsoft Excel (Redmond, WA, USA) spreadsheet. Where appropriate, treatment means were compared with the Student's t-test and p values <0.05 were considered as showing a significant difference between treatment means.

RESULTS

The chemical composition of amaranth flour and flaxseed is shown in Table 1. Comparing the ingredients used for gluten-free rolls, it could be observed that linseed contained more nutritionally important constituents, such as protein, fiber, and minerals.

Replacing part of corn starch with amaranth flour or flaxseed meal in experimental rolls affected the content of some nutrients (Table 1). Ground flaxseed had a higher content of total protein in comparison to amaranth flour. The enrichment of rolls with ground amaranth and flaxseed increased the level of total protein, non-soluble dietary fiber, and ash as compared to the control rolls. Higher concentrations of copper, iron, calcium, magnesium, potassium and manganese were found in the enriched rolls. The level of fat, soluble dietary fiber fraction and total dietary fiber were significantly higher ($p<0.05$) in rolls enriched with flaxseed as compared to rolls enriched by amaranth flour (Table 1).

On average flaxseed contained ten times more unsaturated fatty acids as compared to amaranth flour (Table 2). As a result, a significantly higher level of unsaturated fatty acids was determined in rolls enriched with flaxseed compared to the amaranth and control rolls. The most pronounced change was observed in the content of α-linolenic acid, which was almost absent in amaranth flour, and present in large amounts in flaxseed. In bakery products enriched with flaxseed the level of this acid was significantly higher than in rolls with amaranth flour (Table 2). Flaxseed also contained three times more linoleic acid (C18:2, n-6) as compared to amaranth flour (Table 2). Similarly, the level of this acid was higher in rolls with flaxseed than those with amaranth, however the difference observed was not statistically significant.

Flaxseed had a higher level of all measured amino acids than amaranth flour (Table 3). Similarly, the content of all measured amino acids in rolls enriched with flaxseed was found to be higher than in those enriched with amaranth. However, the level of amino acids in amaranth rolls was significantly higher than in the control rolls (Table 3).

TABLE 1. Chemical composition of amaranth flour, flaxseed, control rolls (C), and rolls supplemented with amaranth flour (A), or flaxseed meal (F); mean ± standard deviation.

Component		Amaranth flour	Flax seeds	C	A	F
Total protein (% d..m.) N x 6.25		13.9±0.7	20.8±0.2	4.33±0.03^a	5.37±0.11^b	5.75±0.09^b
Fat (%d.m.)		5.83±0.31	40.79±0.47	3.79±0.05^a	3.92±0.03^a	6.89±0.41^b
Dietary fiber (%d.m.)	Soluble fiber	2.97±0.09	5.80±0.01	2.61±0.01^a	2.38±0.15^a	3.94±0.33^b
	Insoluble fiber	6.72±0.26	20.52±0.28	3.37±0.08^a	3.80±0.05^b	4.30±0.24^c
	Total dietary fiber	9.69±0.17	26.33±0.28	5.98±0.09^a	6.18±0.11^a	8.23±0.50^b
Ash (% d.m.)		3.42±0.02	4.78±0.01	2.56±0.02^a	2.81±0.02^b	2.92±0.01^c
Minerals (mg / kg d.m.)	Cu	7.59±0.03	16.3±0.1	0.73±0.01^a	1.39±0.01^b	2.18±0.01^c
	Fe	67.0±0.5	66.6±1.7	16.1±0.9^a	29.6±0.4^c	22.8±0.1^b
	Mn	29.5±0.2	40.0±0.2	0.95±0.11^a	3.78±0.10^b	4.88±0.01^c
	P	6430±10	7310±120	1060±10^a	1600±20^b	1620±10^b
	Zn	54.0±2.4	92.2±0.6	24.1±1.8^a	23.6±1.1^a	28.5±1.4^a
	Ca	1050±10	1360±10	615±2^a	646±2^b	714±9^c
	Mg	3080±50	3160±20	253±3^a	543±6^b	568±8^b
	Na	255±4	275±7	7080±20^a	7110±30^a	7090±30^a
	K	5760±30	11160±40	1970±30^a	2540±10^b	3020±10^c

Values in rows denoted with different letter are significantly different as checked by multiple Student's t-test at $p\leq0.05$.

TABLE 2. Content of fatty acids in amaranth flour, flaxseed, control rolls (C) and rolls supplemented with amaranth flour (A) or flaxseed meal (F), (% of dry matter); mean ± standard deviation.

Fatty acids	Amaranth flour	Flaxseed	C	A	F
Palmitic 16:0	1.23±0.11	2.73±0.31	0.47±0.06[a]	0.54±0.05[b]	0.65±0.08[c]
Stearic 18:0	0.28±0.04	1.71±0.02	0.18±0.02[a]	0.22±0.04[a]	0.30±0.02[b]
Oleic 18:1	1.66±0.99	9.46±1.23	1.90±0.31[a]	1.70±0.29[a]	2.51±0.46[b]
Linoleic 18:2 n-6	2.19±0.16	6.32±0.21	0.78±0.11[a]	0.87±0.20[a]	1.21±0.14[a]
α-Linolenic 18:3 n-3	0.06±0.01	20.6±4.3	0.21±0.09[a]	0.23±0.06[a]	1.99±0.11[b]

Values in column with different letter are significantly different at p≤0.05.

TABLE 3. Amino acid composition of amaranth flour, flaxseed, control rolls (C), and rolls supplemented with amaranth flour (A) or flaxseed meal (F); mean ± standard deviation.

Amino acid	Amino acid composition (g amino acid / 100 g d.m. of the examined sample)				
	Amaranth flour	Flaxseed	C	A	F
Asp	1.22±0.04[a]	5.70±0.35[b]	0.33±0.01[a]	0.41±0.01[b]	0.51±0.02[c]
Thr	0.54±0.02[a]	2.32±0.14[b]	0.17±0.01[a]	0.21±0.01[b]	0.25±0.01[c]
Ser	0.86±0.03[a]	2.81±0.17[b]	0.19±0.01[a]	0.25±0.01[b]	0.29±0.01[c]
Glu	2.27±0.07[a]	11.42±0.76[b]	0.61±0.03[a]	0.78±0.02[b]	1.01±0.04[c]
Pro	0.59±0.02[a]	2.22±0.15[b]	0.271±0.019[a]	0.321±0.003[b]	0.357±0.004[c]
Gly	1.14±0.03[a]	3.66±0.22[b]	0.15±0.01[a]	0.23±0.01[b]	0.27±0.01[c]
Ala	0.57±0.02[a]	2.76±0.18[b]	0.22±0.01[a]	0.25±0.01[b]	0.31±0.01[c]
Val	0.64±0.02[a]	3.12±0.20[b]	0.23±0.01[a]	0.27±0.01[b]	0.33±0.01[c]
Ile	0.53±0.03[a]	2.50±0.15[b]	0.18±0.01[a]	0.21±0.01[b]	0.26±0.01[c]
Leu	0.83±0.03[a]	3.57±0.23[b]	0.35±0.02[a]	0.41±0.02[b]	0.47±0.01[c]
Tyr	0.58±0.02[a]	1.68±0.09[b]	0.17±0.01[a]	0.20±0.01[b]	0.22±0.01[c]
Phe	0.64±0.02[a]	2.87±0.19[b]	0.19±0.01[a]	0.23±0.01[b]	0.29±0.01[c]
His	0.42±0.02[a]	1.37±0.07[b]	0.117±0.006[a]	0.134±0.004[b]	0.161±0.005[c]
Lys	0.89±0.03[a]	2.42±0.16[b]	0.25±0.01[a]	0.30±0.01[b]	0.32±0.01[c]
Arg	1.60±0.05[a]	6.91±0.49[b]	0.21±0.01[a]	0.31±0.02[b]	0.42±0.02[c]
Cys	0.50±0.02[a]	1.55±0.04[b]	0.085±0.02[a]	0.123±0.02[b]	0.133±0.01[c]
Met	0.44±0.02[a]	1.37±0.05[b]	0.088±0.002[a]	0.117±0.001[b]	0.127±0.001[c]
Limiting amino acid	Leucine	Lysine	Lysine	Lysine	Lysine
CS	75.5±3.0 [b]	59.2±3.8[a]	100.0±5.3[b]	95.4±2.7[b]	81.5±3.0[a]
EAAI	95.2±1.3 [b]	89.8±2.4[a]	99.7±0.3[b]	99.4±0.4[b]	97.5±0.4[a]

Values in column with different letter are significantly different at p≤0.05.

The Chemical Score (CS) and Essential Amino Acid Index (EAAI) were higher in amaranth flour than in ground flaxseed. Because milk powder was the only source of protein in the control rolls, these indices were maximum in this case. The admixture of plant protein in flaxseed-enriched rolls reduced these values, while there was no difference between the control rolls and the amaranth rolls, due to a high biological value of amaranth proteins. Because of the presence of milk powder, the lack of lysine, characteristic for cereal products, was not observed (Table 3).

The weight gain of animals was significantly different in the experimental groups. Rodents fed with control rolls did not gain body weight over the experimental period. In contrast, body weight gain was observed in animals fed rolls with an addition of amaranth flour and flaxseed (Table 4). Liver weight was significantly higher in the group of rodents fed rolls with amaranth. In turn, no statistically significant differences were noticed in the weight of kidneys between the groups.

The level of total cholesterol (TC) and HDL was significantly higher ($p<0.05$) in the serum of rats of both treatment groups: II (A) and III (F), as compared to the control (Table 5). The concentration of LDL+VLDL cholesterol and TG were the lowest ($p<0.05$) in the group fed rolls with amaranth flour. There was no difference in this parameter levels in the serum of rats fed rolls containing flaxseed and the control group.

There was no difference in the concentration of Fe and Mg in the serum of treatment rats compared to the control group, while the content of Ca was found to be slightly higher (Table 5). The level of Fe in the serum of both treatment groups

TABLE 4. Body gain, liver, and kidneys weight of experimental rats; mean (n=6) ± standard deviation.

Parameter	Group I (C)	Group II (A)	Group III (F)
Body weight gain	-5.6±6.4[a]	6.5±3.4[b]	9.8±4.1[b]
Liver weight	4.12±0.59[a]	5.94±0.61[b]	4.60±0.59[a]
Kidneys weight	0.77±0.23[a]	0.87±0.17[a]	0.93±0.23[a]

Values in rows denoted with different letters are significantly different compare to group I at p≤0.05.

TABLE 5. Lipid profile and concentration of some minerals in serum of experimental rats; mean (n=6) ± standard deviation

	Unit	Group I (C)	Group II (A)	Group III (F)
TC	(mmol/L)	1.62±0.27[b]	1.13±0.26[a]	1.30±0.18[a]
HDL-cholesterol	(mmol/L)	1.17±0.18[b]	0.90±0.22[a]	0.87±0.08[a]
LDL+VLDL-cholesterol	(mmol/L)	0.41±0.09[b]	0.23±0.09[a]	0.42±0.13[b]
TG	(mmol/L)	0.70±0.27[b]	0.38±0.09[a]	0.50±0.15[ab]
Fe	[μmol/l]	24.6±8.4[a]	25.9±5.5[a]	27.5±6.9[a]
Ca	(mmol/L)	2.35±0.07[a]	2.48±0.07[b]	2.51±0.08[b]
Mg	(mmol/L)	1.19±0.14[a]	1.27±0.19[a]	1.28±0.09[a]
P	(mmol/L)	2.62±0.22[a]	2.77±0.21[ab]	3.05±0.25[b]

Values in rows denoted with different letters are significantly different at p≤0.05.

tended to be higher than in the control group. On the other hand, the level of iron in the livers of rats fed flaxseed rolls tended to be lower than in the control group (Table 6).

Significant differences were observed in the concentration of zinc in the liver, which was the lowest in the control group, and the highest in the group of rats fed amaranth enriched rolls. Enrichment with amaranth also resulted in the higher concentration of phosphorus, manganese, magnesium, and the sodium level in this organ, as compared to the other groups of animals. The concentration of some minerals in the bones of animals was significantly different between the control and treatment groups. Rats fed rolls enriched with amaranth flour and ground flaxseed had a higher concentration of calcium and phosphorus in their bones as compared to the control group. A difference was also found between both treatment groups (Table 7). Animals fed rolls containing amaranth had a higher concentration of calcium and phosphorus in their bones as compared to the bones of rodents fed the flaxseed diet The content of copper was higher in the bones of rats fed flaxseed rolls.

There was no difference in the concentration of minerals in the kidneys of rats (Table 6).

DISCUSSION

Amaranth seeds contain a considerably higher concentration of carbohydrates than flaxseed [Bressani *et al.*, 1992], and this feature results in a much lower content of non-starch components in amaranth flour. In consequence, the products obtained with ground flaxseed were richer in nutritionally important compounds, especially polyunsaturated fatty acids.

It is well established that ALA is required for the synthesis of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. They are factors in many processes, such as lipid metabolism (cholesterol, triacylglycerols), brain development, blood pressure, clot formation, and they help prevent diseases. These acids are also known to inhibit cholesterol synthesis by

TABLE 7. Concentration of minerals in bones of rats per 100 g of body mass of experimental rats; mean (n=6) ± standard deviation

Minerals	Group I (C)	Group II (A)	Group III (F)
P (g)	40.2±3.3[a]	50.9±3.6[c]	43.1±0.9[b]
Ca (g)	83.4±3.7[a]	91.9±6.3[c]	87.2±3.7[b]
Mg (g)	1.13±0.07[a]	1.15±0.07[a]	1.15±0.07[a]
K (g)	3.17±0.22[a]	3.03±0.18[a]	3.15±0.15[a]
Na (g)	3.02±0.28[a]	3.17±0.27[a]	2.970±0.26[a]
Fe (mg)	54.6±6.9[a]	53.4±6.5[a]	49.8±6.0[a]
Mn (mg)	0.55±0.08[a]	0.54±0.09[a]	0.49±0.08[a]
Zn (mg)	88.1±6.1[a]	87.1±8.5[a]	86.4±6.6[a]
Cu (mg)	1.16±0.10[a]	1.11±0.16[a]	1.35±0.17[b]

Values in rows denoted with different letters for each tissue are significantly different at p≤0.05.

TABLE 6. Concentration of minerals in liver and kidneys per 100 g body mass of experimental rats; mean (n=6) ± standard deviation.

Minerals	Liver			Kidneys		
	Group I (C)	Group II (A)	Group III (F)	Group I (C)	Group II (A)	Group III (F)
P (mg)	10.8±0.4[a]	14.3±1.3[b]	11.2±0.9[a]	2.1±0.3[a]	2.1±0.6[a]	2.3±0.4[a]
Ca (mg)	0.38±0.04[ab]	0.43±0.04[b]	0.35±0.04[a]	0.12±0.02[a]	0.11±0.03[a]	0.12±0.03[a]
Mg (mg)	0.78±0.04[b]	0.96±0.08[c]	0.70±0.04[a]	0.15±0.03[a]	0.15±0.04[a]	0.16±0.02[a]
K (mg)	5.47±0.54[ab]	6.83±0.73[b]	5.14±0.30[a]	0.93±0.20[a]	0.91±0.22[a]	1.06±0.21[a]
Na (mg)	1.93±0.03[a]	2.49±0.19[c]	2.12±0.02[b]	0.60±0.12[a]	0.61±0.11[a]	0.69±0.13[a]
Fe (mg)	0.56±0.09[b]	0.60±0.07[b]	0.44±0.07[a]	0.043±0.008[a]	0.045±0.014[a]	0.050±0.005[a]
Mn (μg)	6.0±1.0[a]	9.0±1.0[b]	7.0±1.0[a]	0.7±0.1[a]	0.7±0.2[a]	0.8±0.1[a]
Zn (μg)	81.0±9.0[a]	132.0±21.0[c]	98.0±16.0[b]	14.0±3.0[a]	16.0±4.0[a]	20±3.0[a]
Cu (μg)	10.0±1.0[a]	11.0±1.0[a]	12.0±1.0[b]	2.7±0.5[a]	2.9±0.6[a]	3.4±0.7[a]

Values in rows denoted with different letters for each tissue are significantly different at p≤0.05.

the inactivation of the 3-hydroxyl-3-methylglutaryl coenzyme A reductase (HMG CoA).

ALA is the substrate for synthesis of arachidonic (C 20:4, n-6) and dihomogamma-linolenic acid (C20:3, n-6). Polyunsaturated fatty acids, especially those with 20 and more carbon atoms, are components of cell membranes, and play an important role in signaling pathways. Taking into account these physiological effects, the increased level of polyunsaturated fatty acids (PUFA) in flaxseed-enriched rolls is very promising, and could have a beneficial effect on health, especially in the group of people affected by celiac disease.

It should be noticed that the chemical methods, based on the amino acid composition of protein, have a limited value for the evaluation of protein quality, as they do not take into account the digestibility and absorption of selected amino acids, which are modified by the applied technological (mostly thermal) processes and the presence of non-nutritive substances in food products. This is the reason for discrepancies between the results of chemical analyses and the real nutritional value of proteins. This is also the reason for performing nutritional studies on animals.

The lack of body weight gain in the control group was caused by the applied diet, unbalanced in protein, fat, carbohydrates, minerals and vitamins, and not by the lack of feed, because at the applied level it was not fully consumed by animals. Such a diet was aimed to simulate the deficiency of nutrients in the diet of people having celiac disease. Taking into account the requirements of the AIN'93 diet [Reeves *et al.*, 1993], the use of rolls enriched with amaranth and flaxseed decreased the deficiency of magnesium, phosphorus and potassium in diets as compared to the control.

The total cholesterol in serum of rodents fed with a diet containing flaxseed was lower as compared to the control group. As was already mentioned, flaxseed is a rich source of unsaturated fatty acids, mainly α-linolenic acid, which affect the level of TC.

To date there has been no comparison of the effects of amaranth and flaxseed on the lipid metabolism in laboratory animals. However experiments were performed separately on both these raw materials, which showed that the level of cholesterol fractions in animals or people with hypercholesterolemia was decreased following the consumption of amaranth oil [Martirosyan *et al.*, 2007] and flaxseed oil [Czerwinski *et al.*, 2004]. Similar dietary effects were found for milled flaxseed [Gambuś *et al.*, 2004] or amaranth flour [Czerwinski *et al.*, 2004].

On the other hand, flaxseed oil did not significantly influence the lipid profile of rats with a normal cholesterol level [Czerwinski *et al.*, 2004], and amaranth had no effect on the lipid profile of healthy pigs [Zraly *et al.*, 2004].

The present results show a more pronounced hypocholesterolemic effect of amaranth as compared to flaxseed in rodents with a normal cholesterol level. Amaranth is a good source of squalene compounds and tocotrienols which are inhibitors of cholesterol synthesis in the liver [Martirosyan *et al.*, 2007]. It has been reported that α-linolenic acid has no direct effect on cholesterol synthesis and acts only after conversion to eicosapentaenoic or docosahexaenoic acid, which is limited in mammals [Czerwinski *et al.*, 1993]. This may be the reason why the levels of TC and LDL+VLDL were lower in the serum of rats fed rolls containing amaranth [Czerwinski *et al.*, 1993].

Qureshi *et al.* [1996] in the experiment with chickens fed a diet containing amaranth seeds showed similar results to our data. Chickens fed amaranth seeds had a lower level of total and LDL cholesterol in their serum as compared to the control group. An addition of amaranth oil to the diet inhibited the HMG CoA.

The concentration of iron in rolls enriched with flaxseed was significantly lower as compared to those enriched with amaranth flour. It caused a lower intake of this mineral from the diet, and may be a reason for the lower content of Fe in the liver. It might be suggested that rats fed flaxseed rolls had a limited intake of iron, which resulted in the release of this microelement from the liver, and kept the normal level of this mineral in the serum. Flaxseed is a good source of dietary fiber, which decreases the bioavailability of mineral components from the diet. It may be suggested that this was a reason for the lower level of iron (20%) in the liver as compared to the control group. It seems that the bioavailability of iron from rolls enriched with amaranth flour was better as compared to bakery products based on flaxseed. These results confirm previously published reports, showing that amaranth flour is a good source of bioavailable iron [Whittaker & Ologunde, 1990].

Calcium and phosphorus are the main minerals of bones and the presented results, showing their increasing content after enrichment, suggest that amaranth flour and flaxseed are good sources of these elements.

The level of magnesium in experimental diets was significantly different compared to the control group, however it did not affect the level of magnesium in the bones of animals. These results are similar to the data published by Coudray *et al.* [2002], who did not show any significant difference in the balance of magnesium and its concentration in the bones of animals fed diets with an addition of 300 mg or 600 mg of this element. A lower concentration of magnesium in the bones and fluids of rodents was measured when the experimental diet contained 150 mg of this mineral. Magnesium deficiency in the diet caused its lower excretion by animals, which seems probable also in our study.

The above results are especially valuable taking into account that persons with celiac diseases usually exhibit the deficiency of mineral components [Mora *et al.*, 1993]. This study showed that amaranth flour and flaxseed may be good ingredients for gluten-free bakery products, resulting in an increase of bioavailability in some minerals and polyunsaturated fatty acids. Additionally, at the applied enrichment levels, they have no negative influence on sensory parameters.

It may be suggested that the rolls containing amaranth flour had a better bioavailability of mineral components compared to rolls enriched with ground flaxseed. The main factor which decreased the intake of nutrients (minerals) from the flaxseed enriched rolls was most probably soluble dietary fiber [Harland, 1989].

CONCLUSIONS

The applied enrichment of gluten-free rolls with amaranth flour and ground flaxseed increased the content of total proteins, non-soluble dietary fiber fraction, and some minerals,

e.g.: Cu, Fe, Mn, P, Ca, Mg, and K as compared to the control rolls. Ground flaxseed added to rolls significantly increased the level of fat, polyunsaturated fatty acids and soluble dietary fiber fraction as compared to the control rolls.

The beneficial effects of enrichment were shown in the nutritional experiments, where gluten-free rolls were used as a diet for rats. Weight gain was observed only in treatment groups of animals. Both applied ingredients decreased the TC and HDL content of serum in both treatment groups of animals. They also increased Ca content in the serum of the rodents. The concentration of calcium and phosphorus was significantly higher in the bones of rats fed diets with amaranth or flaxseed enriched rolls.

The results suggest that the applied ingredients: amaranth flour and flaxseed, should be recommended for the production of bakery goods for persons following the gluten-free diet.

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