

## Quality of gluten-free supplemented cakes and biscuits

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### Abstract

Gluten-free confectionery products were used as controls for comparison with the products, which included different supplements such as linseed meal, amaranth and/or buckwheat. The latter were expected to increase nutritional values of confectionery products. Cookies were analyzed in terms of volume, selected textural parameters (hardness, cohesiveness), organoleptic quality, shelf-life, and different chemical components. All supplemented gluten-free products received high consumer scores, exceeding in some cases those of control samples. Supplementation of gluten-free confectionery products with linseed meal, amaranth and/or buckwheat flours enhanced their final nutritional quality. A significant rise was observed in the protein content and dietary fiber, and in the case of linseed meal also  $\alpha$ -linolenic acid. All of the supplemented gluten-free confectionery products contained more macro-elements and micro-elements (i.e. potassium, phosphorus, magnesium, calcium, iron, manganese, zinc and copper), as compared with the controls. Taking into account the amino-acid composition, amaranth proved a more beneficial supplement of gluten-free products than linseed.

**Keywords:** *Celiac disease, gluten-free cakes, linseed, amaranth, buckwheat*

### Introduction

Celiac disease is common among the white population of Europe, North America and Australia, where cereals containing gluten are traditionally everyday food. This malfunction can appear at any age, but the classical symptoms are most frequently observed during infancy and early childhood, when foods containing gluten are introduced into the diet. Sometimes the disease develops during adolescence or adulthood (usually at age 30–40 years). Preliminary results based on seroepidemiologic studies suggest that each recognized case of celiac disease is accompanied by three to seven undetected cases (Rewers 2005).

Cereal products have for thousands of years been one of the basic food products and thus represent one of the main sources of proteins in the human diet. Gluten makes them expand during baking, and helps to retain moisture for some time (He and Hosney 1990; Rasmussen and Hansen 2001). Lack of gluten results in changes of the

shape, texture, smell and taste in bakery, as well as in quick drying of the crumb (Gambus et al. 2001; Gallagher et al. 2003a; Lazaridou et al. 2007).

The preparation of gluten-free bakery products requires application of different flours in exchange for wheat flour, so the resulting taste very often does not resemble that of classical, gluten products. The role of food technologists is to design such recipes for gluten-free products, which would improve their expansion, structure and taste (Gambus et al. 2001; Gallagher et al. 2003b, 2004), and would help the people with celiac disease to fulfill the nutritional directions, which imply everyday consumption of dietary fiber, minerals and other food constituents (Thompson 2000; Case 2005). The above-mentioned also applies to confectionery products, which often better suit children's taste than ordinary bread. Gluten-free products are usually protein-free products. Removal of proteins deprives the raw material of minerals and vitamins, which negatively impacts its nutritional value. This is the reason why gluten-free products should be supplemented by raw materials naturally free of gluten, and rich in additional nutrients (Korus et al. 2006, Kiskini et al. 2007).

Baking of gluten-free product allows introducing various additions in the amounts of several percent, depending on their type and properties. Supplementation of gluten-free bakery products may be done by the addition of synthetic or natural nutrients. The first group includes such substances as salts of calcium and iron, thiamine, riboflavin, and sometimes pyridoxine (Thompson et al. 2005). In the second group the most common substances are milk powder, whey, dried yeast, soy preparations and various oilseeds, legumes and pseudo-cereals or non-bread cereals, such as buckwheat. Supplementation with natural products is more reasonable, as they include many important constituents. The impact of additives on technological properties of the dough is usually more or less negative. The dough made of gluten-free products is difficult to shape, which is the main technological problem in the production of confectionary products (Gambus et al. 2001; Gallagher et al. 2004; Lazaridou et al. 2007). The final recipe must take into account both nutritional and technological issues.

Amaranth and linseed are the raw materials that still seem to be underestimated in the technology of traditional and gluten-free bread. The content and quality of bioactive compounds present in those plants is higher than in popular cereals (Lehmann 1996; Gambus et al. 2004).

Amaranth seeds contain up to 19% protein, which has better biological value than milk proteins and is very rich in essential amino acids. Amaranth flour contains also more minerals in comparison with common cereals, especially iron, potassium, calcium and magnesium. It is also abundant in dietary fiber, which is in large part soluble. Fat is present in amaranth seeds at a higher level than in cereals, and contains more squalene than popular plant oils (Bressani et al. 1992; Williams and Brenner 1995; Písaríková et al. 2005).

Proteins of amaranth are characterized by high biological value (>80% of biological value of egg protein) and beneficial amino acid composition (Paredes-Lopez 1994).

Linseed may be a very good supplement for gluten-free products, due to its unique chemical composition (>20% protein of high digestibility; >30% dietary fiber, mostly water soluble; >40% fat rich in polyunsaturated fatty acids [PUFA], especially deficient in the diet alpha-linolenic acids (ALA)) and the lack of gluten proteins (Oomah 2001; Gambus et al. 2004, 2007; Tarpila et al. 2005). It should be

mentioned that the linseed protein is easily available, and digestible in 85–90%, which makes it one of the most nutritive plant proteins with a high influence of immunological response of the human organism (Thompson 1995; Oomah 2001).

Another interesting raw material is buckwheat, which contains easily available protein, minerals, organic acids and vitamins. The popularity of buckwheat has increased in the past decades. Products based on buckwheat were used for dietary purposes, and also in the case of celiac disease. Buckwheat groats contain 10–15% protein with a biological value equivalent to 95% defatted milk and 81.5% egg protein. Easily available and valuable protein, a high content of minerals, organic acids and vitamins caused buckwheat flour and groats to be extensively used in child nutrition and for people with disorders of the dietary tract (Steadman et al. 2001; Krkošková and Mrázová 2005; Wijngaard and Arendt 2006).

The aim of the present study was the application of naturally gluten-free raw materials rich in nutrients deficient in the average gluten-free diet (i.e. amaranth flour, linseed meal, and buckwheat flour) for baking cakes and biscuits with improved chemical composition, and with retained attractive appearance, texture and organoleptic, as well as storage properties. Apart from a demonstration of the influence of these supplements on the quality of the final products, another purpose of the study was to check out the nutritional benefits resulting from their presence in the modified recipes.

## Materials and methods

### *Materials*

Sucrose (crystalline) was from Krajowa Spółka Cukrowa S.A. (Torun, Poland), sucrose (powder) from Cukrownia i Rafineria Chybie S.A. (Chybie, Poland), vanilin from Delecta (Włocławek, Poland), cinnamon and ginger ground from Ducros (Monteux, France), vegetable oil from ZT 'Kruszwica' S.A. (Kruszwica, Poland), margarine from Unilever Polska S.A. (Warszawa, Poland), coconut chips from Uno Fresco Tradex Sp. Zo.o. (Janów Podlaski, Poland), raisins and walnuts from Bakal Center Sp. Zo.o. (Dabrowa Górnicza, Poland), salt from Janikosoda S.A. (Janikowo, Poland), milk (UHT, 2% fat) from Mlepol (Grajewo, Poland) and gluten-free baking powder from Celiko S.A. (Poznan, Poland). Carrots and eggs were purchased at the local market.

Materials for the further study were cakes and cookies produced according to the modified recipes published by Fenster (2004). They included two types of cakes with short shelf-life (sponge cake and carrot cake), as well as biscuits and coconut cakes of longer durability. Products basing on the original recipes were used as controls for comparison with gluten-free products, which included different supplements. Each gluten-free product was supplemented with raw materials, which were expected to increase its nutritional value.

Sponge cake (SC) contained eggs (150 g), sucrose (120 g), gluten-free baking powder (5 g), and 'gluten-free flour' (120 g).

Carrot cake (CR) consisted of 'gluten-free flour' (170 g), xanthan gum (7 g), gluten-free baking powder (9 g), cinnamon (7 g), ginger (1 g), salt (6 g), eggs (100 g), sucrose (250 g), milk (150 g), oil (60 g), vanilin (5 g), finely shredded carrot (220 g), coconut chips (50 g), raisins (80 g) and finely ground walnuts (45 g).

Biscuits (B) were prepared from 'gluten-free flour' (260 g), pectin (10 g), margarine (150 g), gluten-free baking powder (13 g), eggs (45 g), sucrose (120 g) and vanilin (2 g).

Coconut cakes (CN) contained 'gluten-free flour' (250 g), margarine (250 g), sucrose powder (185 g), gluten-free baking powder (10 g), shredded coconut (100 g) and vaniline (5 g). Additionally, 14 g cocoa was added in the formulation CN 2.

The differences in the formulations of 'gluten-free flour' used for cakes and cookies under study are presented in Tables I and II, respectively.

### Methods

**Baking.** Confectionery cookies were baked in an electric oven (Meteor MD 08/065; Victus s.r.l., Parma, Italy). Sponge cake was baked at 160°C for 40 min and carrot cake at 170°C for 50 min in appropriate baking pans. Biscuits were baked for 15–20 min at 210°C, and coconut cakes for 20–25 min at 190°C. A detailed preparation of the products is described by Fenster (2004).

**Sensory analysis.** Sensory evaluation of the products was performed according to PN-ISO-6658:1998 (1998). The tested and experienced organoleptic panel consisted of 14 persons. A five-point hedonic scale was established separately for each product. The following quality attributes (adequate weighting coefficients) were taken into account: shape (0.1), color (0.1), surface (0.15), consistency (0.15), fracture (0.1), smell (0.15), and taste (0.25). Basing on the total points, the overall consumer acceptance was rated: <2.90 = unacceptable, 3.0–3.50 = acceptable, 3.51–4.50 = good, and 4.51–5.0 = very good.

**Volume of cakes.** After cooling for 1 h at ambient temperature, the volume of sponge cake was measured by the rapeseed displacement method. Other products were only analyzed visually, because of either too big a volume (CR 1 and CR 2) or because the volume of biscuits and coconut cakes do not play a role either as a technological attribute or as a sensory attribute.

Table I. Composition of 'gluten-free flour' used for preparation of sponge cake (SC) and carrot cake (CR).

Raw material (g)	Sponge cake (395 g dough)			Carrot cake (1,160 g dough)	
	SC 1 standard	SC 2	SC 3	CR 1 standard	CR 2
Corn flour (250–50; BOLY ZRT, Boly, Hungary)	60	20	–	170	60
Potato starch (Superior; P. P. Z. Bronislaw Spółka Zo.o., Strzelno, Poland)	60	40	60	–	–
Amaranth flour (Szarlat s.c., Lomza, Poland)	–	60	60	–	–
Linseed meal (Sztabinski Andrzej, Nowy Dwór Gdanski, Poland)	–	–	–	–	110

SC 1 and CR 1, standard; SC 2, sponge cake with amaranth flour; SC 3, sponge cake with amaranth flour without corn flour; CR 2, carrot cake with linseed meal.

Table II. Composition of 'gluten-free flour' used for preparation of biscuits and coconut cakes.

Raw material (g)	Biscuits (600 g dough)			Coconut cakes (1,000 g dough)	
	B 1 standard	B 2	B 3	CN 1 standard	CN 2
Corn flour (250–50; BOLY ZRT, Boly, Hungary)	130	60	–	–	–
Potato starch (Superior; P. P. Z. Bronislaw Spółka Zo.o., Strzelno, Poland)	130	70	78	–	–
Rice paste (Biofuturo, Kraków, Poland)	–	–	–	250	125
Buckwheat flour (BIO Aleksandra i Mieczyslaw Babalscy, Pokrzydowo, Poland)	–	130	104	–	–
Amaranth flour (Szarlat s.c., Lomza, Poland)	–	–	78	–	–
Linseed meal (Sztabinski Andrzej, Nowy Dwór Gdanski, Poland)	–	–	–	–	125

B 1 and CN 1, standard; B 2, biscuits with buckwheat flour; B 3, biscuits with buckwheat and amaranth flour; CN 2, coconut cakes with linseed meal.

*Texture profile analysis.* The bakery ageing process was monitored using texture profile analysis and crumb moisture. The measurements were performed by means of texture analyzer TAXT 2, equipped with software XTR1 (Stable Micro Systems Ltd, Surrey, UK). Hardness was evaluated for all samples, while cohesiveness was only calculated for sponge and for carrot cakes. The analyses were done on the day of baking and then after 1, 2, and 3 days of storage. Additional measurements of texture were performed for sponge cake after 4 days of storage, and for biscuits and coconut cookies after 4, 5 and 30 days of storage. Sponge cakes and carrot cakes were stored in bread storage containers, under ambient conditions, while biscuits and coconut cakes were stored in glass jars, at ambient temperature.

*Chemical analyses.* Samples were air dried and their chemical composition was assessed according to the AOAC (2006) methods: total protein, Method 950.36; dietary fiber (total dietary fiber and insoluble dietary fiber), Method 991.43; raw fat, Method 935.38; and ash, Method 930.05.

Preparation of samples for evaluation of ash components was conducted according to EN 13804 (2002). Mineralization was performed by the dry-ashing method, a modified version of AOAC Method 985.01. The modification concerned lowered temperature and prolonged time of ashing, in order to reduce the risk of loss of assessed minerals resulting from formation of volatile compounds. The risk increases with rising ashing temperature. The applied temperature was lowered from 500°C to 460°C and the ashing time in both steps was three times longer than in the original method. The contents of calcium, magnesium, phosphorus, potassium, sodium, iron, manganese, copper, and zinc in the solutions obtained after mineralization were measured by inductively coupled plasma atomic emission spectrometer (JY 238 Ultratrace; Jobin-Yvon, Longjumeau, France) following the rules presented in EN-14084 (2003).

Te composition of amino acids was assessed by ion-exchange chromatography, by means of an amino acid analyzer (AAA 400; INGOS, Prague, Czech Republic), according to the control protocol (Smith 2003).

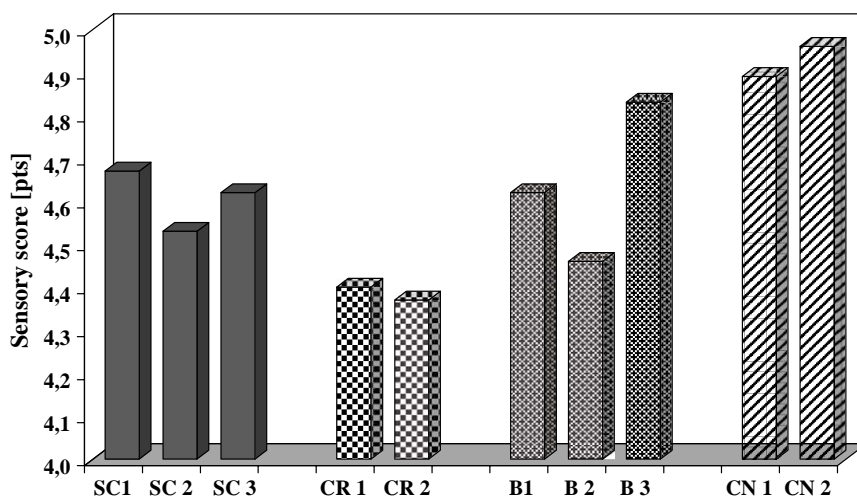


Figure 1. Sensory assessment of gluten-free confectionery products. For description of products SC 1, SC 2, SC 3, CR 1 and CR 2 see Table I; for description of products B 1, B 2, B 3, CN 1 and CN 2 see Table II.

The fatty acid composition was measured after preparation of fatty acid esters. To this end, sodium hydroxide in methanol was added to about 5 mg samples of oils (2 ml of 0.5 M) and incubated for 10 min at 60°C. Next 2 ml of 14% boron trifluoride in methanol was added and incubated for 10 min. After cooling the mixture, 2 cm<sup>3</sup> saturated NaCl was added and the required methyl esters were extracted with hexane (2 ml). The organic layer was separated using Pasteur pipettes and dried over anhydrous sodium sulfate.

Samples of extracted fat were analyzed by means of gas chromatography coupled with a mass spectrometer (Shimadzu QP 5050A; Shimadzu Europa GmbH, Duisburg, Germany): helium; SP<sup>TM</sup>-2560 columns, 100 m; film thickness, 0.25 µm; film diameter, 0.25 mm; column and detector temperatures, 60–220°C and 245°C, respectively. Identification of individual components was achieved using library search software (NIST 1.7) and a reference mixture of fatty acid methyl esters (FAME Mixture Me 100; Larodan Fine Chemicals, Malmoe, Sweden).

Biscuits and cookies used in the study were stored for 3 months, and every month the peroxide number was measured using PN-EN-ISO 3960 (2004). Basing on these values, the shelf-life of cakes and cookies was evaluated.

### *Statistical analysis*

All of the analyses were performed in at least two replications. The significance of differences between average values was evaluated using Duncan's test ( $\alpha = 0.05$ ).

## **Results and discussion**

The amounts of linseed meal and non-bread flours were adjusted in such a manner as to obtain significant improvement in chemical composition of the confectionery products.

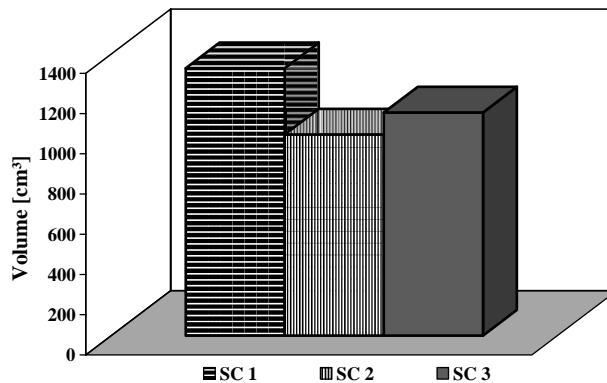


Figure 2. Volume of sponge cake. For description of products SC 1, SC 2 and SC 3 see Table I.

It is obvious that any nutritional benefits resulting from the exchange of potato starch or corn flour with the above-mentioned raw materials may be considered only if the obtained products are tasty and accepted by the consumers.

The organoleptic assessment is shown in Figure 1. All of the sponge cakes were highly accepted by the consumers (scores over 4.51), and can be described as very good. The best score was given to the control, and comparable results were obtained by SC 3 cake, where corn flour was completely exchanged with amaranth flour. Slightly lower scores were given to the SC 2 recipe, which contained 50% amaranth flour and 27% corn flour. The latter product revealed also the lowest volume (Figure 2).

Comparable scores were obtained from the two types of carrot cake, control CR 1 (score of 4.40) and CR 2 with 64% of milled linseed instead of corn flour. These scores were denoted as good (Figure 1). Similar results were obtained by Hussain et al. (2006), who used flaxseed flour for supplementation of wheat flour in cookies. The authors noticed, however, that increasing the flaxseed content in cookies caused lower acceptance by the consumers.

Among biscuits, the highest scores were received by B 3 products (score of 4.83) supplemented with 40% buckwheat flour and 30% amaranth flour in exchange for corn flour (Figure 1). Similarly to the B 1 control they were estimated as very good. The B 2 biscuits, which contained 50% buckwheat flour instead of potato starch and corn flour, were slightly less attractive (score of 4.46), because of the intensive smell and taste of buckwheat.

The use of a 50% share of linseed meal in coconut cakes CN 2 positively influenced sensory parameters (total score of 4.96), so that they were regarded as very good, just like the control CN 1 (score of 4.89; Figure 1). In order to improve the color, 1.5% coconut was added together with the supplement of linseed.

Because hardness and cohesiveness are the most important texture parameters of gluten-free products, they were closely monitored in the analyzed products.

Both in case of sponge cake as well as carrot cake the classical increase of hardness over the storage period was observed (Figure 3a and Figure 4a). It was similar to the changes in crumbs of gluten-free breads (Gambus et al. 2004). From the day of baking the lowest hardness was measured for sponge cake without supplements. The difference between control and other samples was growing during the storage period. The hardness of both amaranth-supplemented cakes was comparable. The statistically

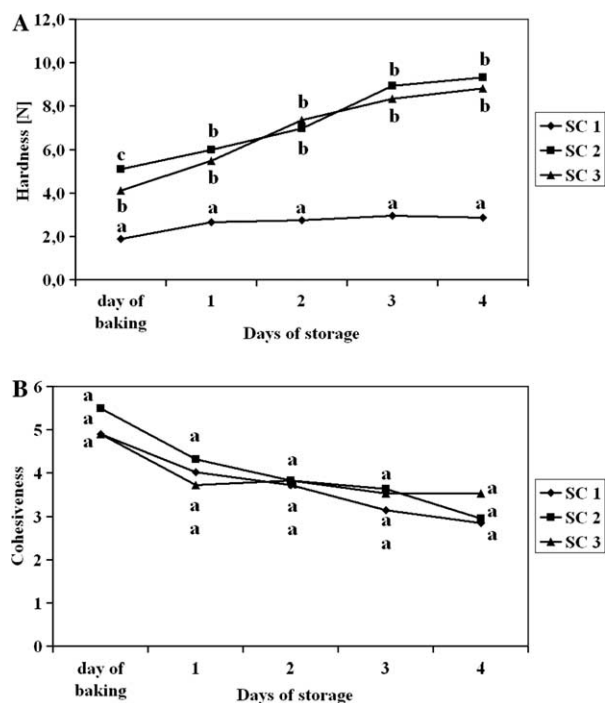


Figure 3. Changes in crumb (a) hardness and (b) cohesiveness of sponge cake during storage. Values denoted by different letters for selected days differ significantly at the level  $\alpha=0.05$ . For description of products SC 1, SC 2 and SC 3 see Table I.

insignificant difference between samples may be attributed to the content of dietary fiber, which was highest when both amaranth and corn flours were applied (Table I). The negative impact of the amount of dietary fiber on the quality of sponge cake was even more pronounced concerning volume (Figure 2) and consumer acceptance (Figures 1 and 2).

The addition of new components did not significantly influence cohesiveness of the sponge and/or carrot cakes (Figure 3b and Figure 4b), which should be noted because natural raw materials usually deteriorate this property. On the other hand, significant changes in hardness were observed after supplementation (Figure 3a and Figure 4a).

Despite the lower expansion, carrot cake with the addition of linseed meal received as good quality scores as a control CR 1 (Figure 1). Its hardness on the day of baking and during storage was significantly reduced (Figure 4a).

Hardness of biscuits with the share of buckwheat and corn flour B 2 was insignificantly higher than in case of the products prepared with amaranth flour B 3. Significantly lower values were measured for control biscuits B 1 (Figure 5). During 4 days of storage the parameter was almost unchanged; only in case of B 3 was some hardening observed after the first day. After 30 days, the hardness of biscuits B 1 and B 2 was slightly lower than initially, but the changes were statistically insignificant (Figure 5). A similar pattern was also found for B 3 biscuits, apart from the above-mentioned hardening process on the day of baking, which was probably caused by the specific properties of amaranth starch. This starch contains mostly amylopectin, which has a smaller tendency for retrogradation than the starches of the other



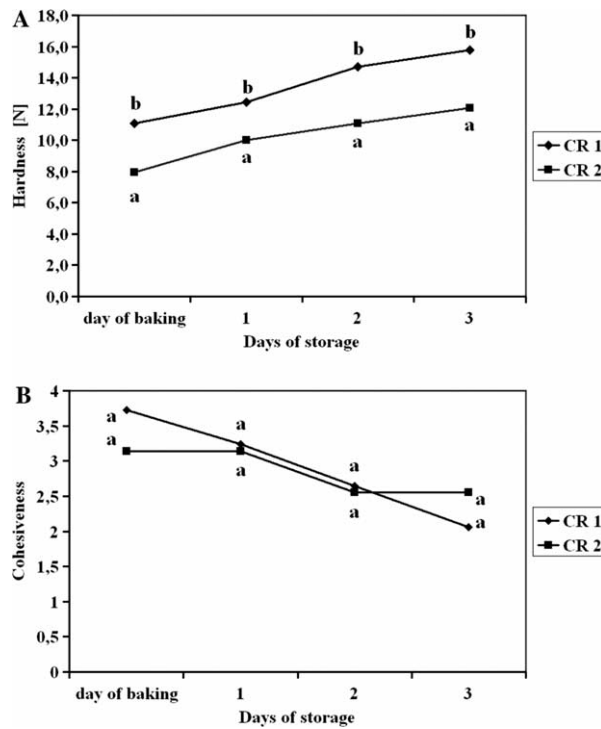


Figure 4. Changes in crumb (a) hardness and (b) cohesiveness of carrot cake during storage. Values denoted by different letters for selected days differ significantly at the level  $\alpha=0.05$ . For description of products CR 1 and CR 2 see Table I.

botanical origin. Slower retrogradation caused lower values of hardness for biscuits on the day of baking.

Coconut cakes displayed a slightly different pattern of texture changes. The share of linseed meal in CN 2 cookies resulted in their lower hardness in comparison with CN 1 control on the day of baking, which may be attributed to high content of lipids in this product. The hardness rose for 3 days and then began to fall, reaching a minimum value 30 days after baking (Figure 6).

According to the standard method PN-EN-A-86902:1997/Az1 (2001), the peroxide number should be smaller than 3 mg active oxide/1 g fat. After 3 months of storage, the peroxide numbers in fat extracted from the products were in the ranges acceptable by the standards, so they were suitable for consumption (Table III).

Evaluation of the nutritive value of the products included the measurement of basic chemical components: total protein, raw fat, dietary fiber (soluble and insoluble fractions), total ash and selected macro-elements and micro-elements. Additionally, the amino acid composition of protein present in the products and the fatty acid composition of fat were evaluated.

Taking into account gluten-free cakes (Table IV) the highest protein content was measured for sponge cake SC 2 with 50% amaranth and 27% corn flours (38% more than in control cake SC 1). This was caused by the considerable addition of amaranth flour, because this pseudo-cereal contains approximately 19% protein of beneficial amino acid composition (Písáriková et al. 2005). A little protein was also added with

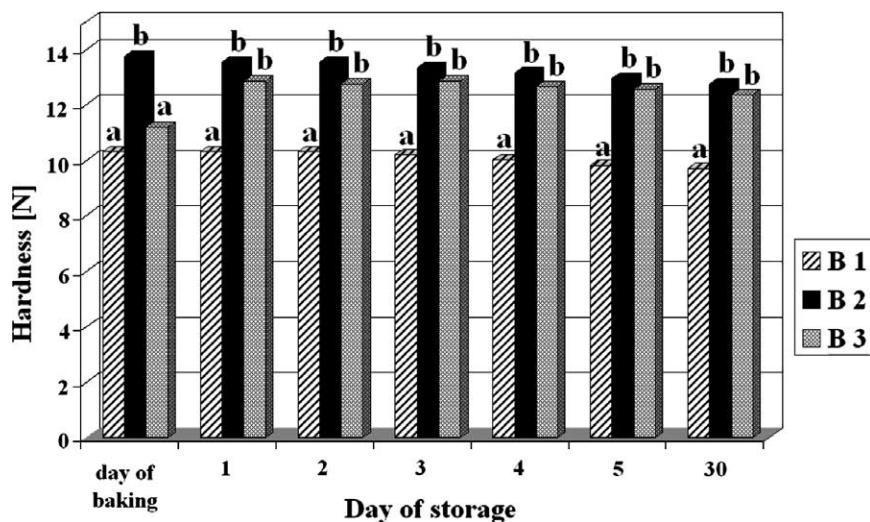


Figure 5. Changes in hardness of biscuits during storage. Values denoted by different letters for selected days differ significantly at the level  $\alpha = 0.05$ . For description of products B 1, B 2 and B 3 see Table II.

corn flour, as in the cake SC 3; without its addition, the protein content was by 0.29% in comparison with the cake SC 2. The SC 2 cake contained also the largest amounts of dietary fiber, especially its soluble fraction, because amaranth and corn flours are a rich source of this fraction of non-starch polysaccharides (Bressani et al. 1992; Williams and Brenner 1995).

In carrot cake, which should be called carrot-linseed because of the 64% share of linseed meal, the amount of protein in dry substance was increased by 2.18 percentage points in comparison with control—which means a change by more than one-third.

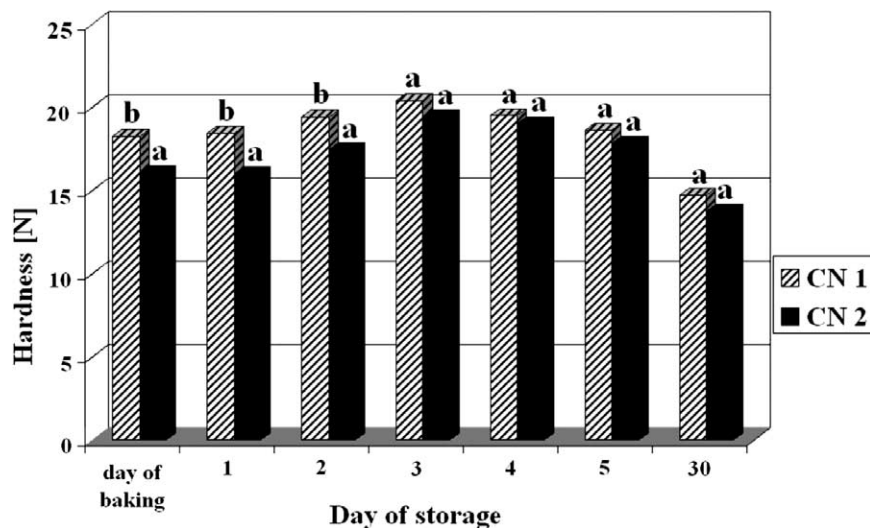


Figure 6. Changes in hardness of coconut cakes during storage. Values denoted by different letters for selected days, differ significantly at the level  $\alpha = 0.05$ . For description of products CN 1 and CN 2 see Table II.

Table III. Peroxide number of fat in biscuits and coconut cakes during storage.

Type of product		Peroxide number of fat ( $\mu\text{g O}_2/\text{g fat}$ )			
		Month of baking	1 month of storage	2 months of storage	3 months of storage
Biscuits	B 1 control	$0.71 \pm 0.02^A$	$1.07 \pm 0.01^A$	$1.25 \pm 0.02^A$	$1.36 \pm 0.04^A$
	B 2	$0.81 \pm 0.03^B$	$1.17 \pm 0.05^B$	$1.35 \pm 0.04^B$	$1.40 \pm 0.05^A$
	B 3	$0.94 \pm 0.04^C$	$1.23 \pm 0.04^C$	$1.57 \pm 0.05^C$	$1.63 \pm 0.06^B$
Coconut cakes	CN 1 control	$0.95 \pm 0.04^A$	$1.25 \pm 0.02^A$	$1.38 \pm 0.04^A$	$1.45 \pm 0.03^A$
	CN 2	$1.05 \pm 0.03^A$	$1.30 \pm 0.03^A$	$1.47 \pm 0.06^A$	$1.52 \pm 0.02^A$

Values denoted by different uppercase superscript letters for selected days differ significantly at the level  $\alpha = 0.05$ . For description of products see Tables I and II.

This is due to the application of linseed, which contains approximately 20% protein (Gambus et al. 2004).

In cake CR 2 the level of dietary fiber more than doubled in comparison with control CR 1, and its soluble fraction was increased almost 10 times. Fiber is especially valuable for people with celiac disease, because they often suffer prolonged constipation due to adherence to a gluten-free diet, which is low in dietary fiber (Green and Jabri 2003; Kupper 2005).

Taking into account gluten-free cookies (Table IV), the highest amount of protein was measured for biscuits B 3 with 40% buckwheat and 30% amaranth flours. The observed protein level was three times higher in comparison with control B 1.

However, buckwheat has a special taste, which is not always acceptable by the consumers, so B 2 biscuits with the share of buckwheat and without amaranth flour received the lowest consumer scores of all analyzed biscuits (Figure 1). Supplementation of biscuits with buckwheat and amaranth flours at the same time, caused a double benefit—limitation of the specific smell and taste of buckwheat, and multiplication of the protein level in the final products. In the case of these biscuits a visible, significant

Table IV. Protein content, raw fat and dietary fiber in analyzed gluten-free confectionary products.

Sample		Protein content (% dry matter)	Raw fat (% dry matter)	Dietary fiber (% dry matter)		
				Total	Unsoluble	Soluble
Sponge cakes	SC 1	$6.90 \pm 0.03^A$	$3.68 \pm 1.11^A$	$1.49 \pm 0.01^A$	$0.04 \pm 0.01^A$	$1.45 \pm 0.01^A$
	SC 2	$9.51 \pm 0.03^B$	$4.58 \pm 0.95^B$	$3.61 \pm 0.03^C$	$1.60 \pm 0.01^C$	$2.01 \pm 0.01^C$
	SC 3	$9.22 \pm 0.01^B$	$5.21 \pm 0.80^C$	$2.65 \pm 0.01^B$	$0.86 \pm 0.01^B$	$1.83 \pm 0.01^B$
Carrot cakes	CR 1	$6.14 \pm 0.03^A$	$17.90 \pm 0.56^A$	$6.98 \pm 0.01^A$	$5.49 \pm 0.01^B$	$1.49 \pm 0.01^A$
	CR 2	$8.32 \pm 0.03^B$	$22.33 \pm 1.13^B$	$16.14 \pm 0.03^B$	$1.86 \pm 0.01^A$	$14.28 \pm 0.01^B$
Biscuits	B 1	$1.81 \pm 0.01^A$	$21.84 \pm 0.69^B$	$2.60 \pm 0.03^A$	$1.26 \pm 0.04^A$	$1.34 \pm 0.01^A$
	B 2	$3.51 \pm 0.01^B$	$22.61 \pm 0.56^C$	$3.00 \pm 0.01^B$	$1.45 \pm 0.01^B$	$1.55 \pm 0.01^B$
	B 3	$5.25 \pm 0.01^C$	$20.79 \pm 0.86^A$	$3.50 \pm 0.01^C$	$1.85 \pm 0.03^C$	$1.64 \pm 0.01^C$
Coconut cakes	CN 1	$5.39 \pm 0.01^A$	$28.54 \pm 1.5^A$	$1.73 \pm 0.01^A$	$1.37 \pm 0.01^B$	$0.00 \pm 0.00$
	CN 2	$8.53 \pm 0.01^B$	$36.28 \pm 1.20^B$	$11.67 \pm 0.03^B$	$1.23 \pm 0.03^A$	$10.44 \pm 0.01^B$

Values denoted by different uppercase superscript letters for selected days differ significantly at the level  $\alpha = 0.05$ . For description of products see Tables I and II.

increase of dietary fiber was also observed (Table IV), although its degree was lower than in the case of cakes.

In coconut cakes CN 2, the addition of 50% linseed meal resulted in the increase of protein by 3.14 percentage points; that is, more than one-half its initial content in control cakes (Table IV). The same level of linseed supplementation caused a sevenfold growth of dietary fiber, especially its soluble fraction, which was 10 times higher than in the control (Table IV).

In all supplemented products, the content of amino acids was higher than in control recipes (Table V). There was also a rise in essential amino acids, especially in the case of biscuits B 3—where their level was 2.5 times higher than in control B 1 and almost 30% increased in comparison with B 2, which were given poor consumer scores. Such a large increase of essential amino acids was not observed for carrot cake and coconut cakes supplemented with linseed flour (Figure 7), which suggests that amaranth is more beneficial for supplementation of gluten-free cakes in protein than linseed. In the evaluated sponge cakes there was evidence of a positive impact of corn flour on the quality and quantity of the protein, because SC 2 products contained more essential amino acids than SC 3 without addition of corn flour; however, the change was not statistically significant (Figure 7).

With the exception of biscuits B 3, which received high consumer scores, all of the supplemented products contained significantly higher amounts of raw fat (Table IV). As was expected, the increase was higher in products supplemented with linseed in comparison with those containing amaranth (Gambus et al. 2004, 2007; Písaríková et al. 2005). The effect of supplementation depends more on the proportion between saturated and monounsaturated fatty acids than on their amounts. Saturated and monounsaturated fatty acids may be synthesized in human or animal organisms, contrary to the PUFA such as linoleic acid (18:2, n-6) or  $\alpha$ -linolenic acid (18:3, n-3) that have to be imported with food (Vaisey-Genser and Morris 1997; Goyens et al. 2006). As implied by the data presented in Table VI, the supplemented products had an improved proportion of PUFA compared with other fatty acids, with the exception of B 3 biscuits where this ratio was the same as in the control. Comparing the amounts of saturated and unsaturated fatty acids in dry substances, the products supplemented with linseed (Figure 8), such as carrot cake CR 2, revealed an increase of unsaturated fatty acids by 50% of its initial content in control recipe CR 1. A similar change was observed for coconut cakes CN 2, where the level of unsaturated acids was by 30% higher than in control CN 1. It is worth mentioning that the content of  $\alpha$ -linolenic acid in these products (Figure 9) was 2.5 times higher in carrot cake after linseed supplementation, and 30 times higher in coconut cakes. This is why linseed is considered a good source of PUFA in food products, an alternative to fish oil (Oomah 2001; Curran et al. 2002).

Sponge cake with amaranth flour, carrot cake with linseed meal, biscuits with buckwheat and amaranth flour, as well as coconut cakes with milled linseed were positively affected by the supplementation, taking into account the content of ash, macro-elements (except sodium) and micro-nutrients (Table VII).

Sponge cakes SC 2 and SC 3 contained three times more magnesium, twice as much iron and zinc, and 10 times more manganese than the control (SC 1). In carrot cake CR 2, the increase in magnesium, manganese, zinc, and copper after supplementation was 85%, 53%, 55%, and 66%, respectively, in comparison with control cake (Table VII).

Table V. Amino acid composition of protein present in gluten-free confectionery products.

Amino acid	mg amino acid/100 g dry mass of the product									
	SC 1	SC 2	SC 3	CR 1	CR 2	B 1	B 2	B 3	CN 1	CN 2
Asp	737±5 <sup>A</sup>	890±13 <sup>B</sup>	900±13 <sup>B</sup>	631±15 <sup>A</sup>	785±9 <sup>B</sup>	267±10 <sup>A</sup>	474±6 <sup>B</sup>	628±16 <sup>C</sup>	1,315±150 <sup>A</sup>	1,308±57 <sup>A</sup>
Thr	346±5 <sup>A</sup>	417±7 <sup>B</sup>	418±6 <sup>B</sup>	268±5 <sup>A</sup>	340±2 <sup>B</sup>	132±5 <sup>A</sup>	214±4 <sup>B</sup>	278±8 <sup>C</sup>	525±60 <sup>A</sup>	561±19 <sup>B</sup>
Ser	499±3 <sup>A</sup>	623±9 <sup>B</sup>	630±12 <sup>C</sup>	368±10 <sup>A</sup>	501±5 <sup>B</sup>	183±8 <sup>A</sup>	282±5 <sup>B</sup>	391±11 <sup>C</sup>	707±81 <sup>A</sup>	780±29 <sup>B</sup>
Glu	998±21 <sup>A</sup>	1,321±27 <sup>C</sup>	1,276±23 <sup>B</sup>	1,182±32 <sup>B</sup>	1140±9 <sup>A</sup>	479±21 <sup>A</sup>	780±13 <sup>B</sup>	1,056±23 <sup>C</sup>	2487±276 <sup>B</sup>	2,123±87 <sup>A</sup>
Pro	351±5 <sup>A</sup>	396±10 <sup>C</sup>	379±6 <sup>B</sup>	397±3 <sup>B</sup>	334±3 <sup>A</sup>	200±0 <sup>A</sup>	252±2 <sup>B</sup>	273±2 <sup>C</sup>	614±72 <sup>B</sup>	537±6 <sup>A</sup>
Gly	267±3 <sup>A</sup>	444±6 <sup>B</sup>	443±7 <sup>B</sup>	250±3 <sup>A</sup>	312±3 <sup>B</sup>	121±5 <sup>A</sup>	254±6 <sup>B</sup>	425±15 <sup>C</sup>	627±66 <sup>B</sup>	620±24 <sup>A</sup>
Ala	450±5 <sup>A</sup>	489±10 <sup>C</sup>	483±7 <sup>B</sup>	370±9 <sup>A</sup>	457±3 <sup>B</sup>	205±10 <sup>A</sup>	282±7 <sup>B</sup>	314±10 <sup>C</sup>	666±73 <sup>A</sup>	696±23 <sup>B</sup>
Val	491±4 <sup>A</sup>	560±8 <sup>B</sup>	560±8 <sup>B</sup>	380±9 <sup>A</sup>	517±5 <sup>B</sup>	189±8 <sup>A</sup>	291±10 <sup>B</sup>	355±8 <sup>C</sup>	730±83 <sup>A</sup>	795±24 <sup>B</sup>
Ile	378±7 <sup>A</sup>	447±6 <sup>B</sup>	450±8 <sup>B</sup>	289±4 <sup>A</sup>	382±6 <sup>B</sup>	138±6 <sup>A</sup>	218±9 <sup>B</sup>	275±5 <sup>C</sup>	577±66 <sup>A</sup>	619±19 <sup>B</sup>
Leu	686±7 <sup>A</sup>	740±10 <sup>C</sup>	730±14 <sup>B</sup>	574±14 <sup>A</sup>	693±7 <sup>B</sup>	326±15 <sup>A</sup>	420±15 <sup>B</sup>	458±9 <sup>C</sup>	935±107 <sup>A</sup>	1,015±30 <sup>B</sup>
Tyr	292±4 <sup>A</sup>	350±9 <sup>B</sup>	360±7 <sup>C</sup>	238±4 <sup>A</sup>	345±2 <sup>B</sup>	132±6 <sup>A</sup>	191±8 <sup>B</sup>	251±14 <sup>C</sup>	422±46 <sup>A</sup>	482±6 <sup>B</sup>
Phe	418±11 <sup>A</sup>	486±7 <sup>C</sup>	482±12 <sup>B</sup>	397±6 <sup>A</sup>	452±2 <sup>B</sup>	171±9 <sup>A</sup>	271±9 <sup>B</sup>	337±7 <sup>C</sup>	734±86 <sup>B</sup>	704±26 <sup>A</sup>
His	222±1 <sup>A</sup>	267±5 <sup>B</sup>	268±6 <sup>B</sup>	188±2 <sup>A</sup>	221±22 <sup>B</sup>	099±2 <sup>A</sup>	146±13 <sup>B</sup>	202±11 <sup>C</sup>	341±40 <sup>A</sup>	341±13 <sup>A</sup>
Lys	439±3 <sup>A</sup>	575±9 <sup>B</sup>	575±8 <sup>C</sup>	274±6 <sup>A</sup>	407±2 <sup>B</sup>	107±3 <sup>A</sup>	247±12 <sup>B</sup>	361±8 <sup>C</sup>	518±58 <sup>A</sup>	603±18 <sup>B</sup>
Arg	493±11 <sup>A</sup>	779±12 <sup>B</sup>	779±11 <sup>B</sup>	622±6 <sup>A</sup>	678±6 <sup>B</sup>	169±4 <sup>A</sup>	409±32 <sup>B</sup>	662±29 <sup>C</sup>	1,470±103 <sup>B</sup>	1,288±54 <sup>A</sup>
Cys	246±5 <sup>A</sup>	358±7 <sup>C</sup>	296±32 <sup>B</sup>	215±2 <sup>A</sup>	310±6 <sup>B</sup>	102±4 <sup>A</sup>	170±3 <sup>B</sup>	220±5 <sup>C</sup>	251±16 <sup>A</sup>	393±7 <sup>B</sup>
Met	303±6 <sup>A</sup>	372±7 <sup>C</sup>	359±13 <sup>B</sup>	171±2 <sup>A</sup>	337±4 <sup>B</sup>	093±4 <sup>A</sup>	146±3 <sup>B</sup>	194±5 <sup>C</sup>	227±22 <sup>A</sup>	397±7 <sup>B</sup>

Values denoted by different uppercase superscript letters for selected days differ significantly at the level  $\alpha=0.05$ . For description of products see Tables I and II.

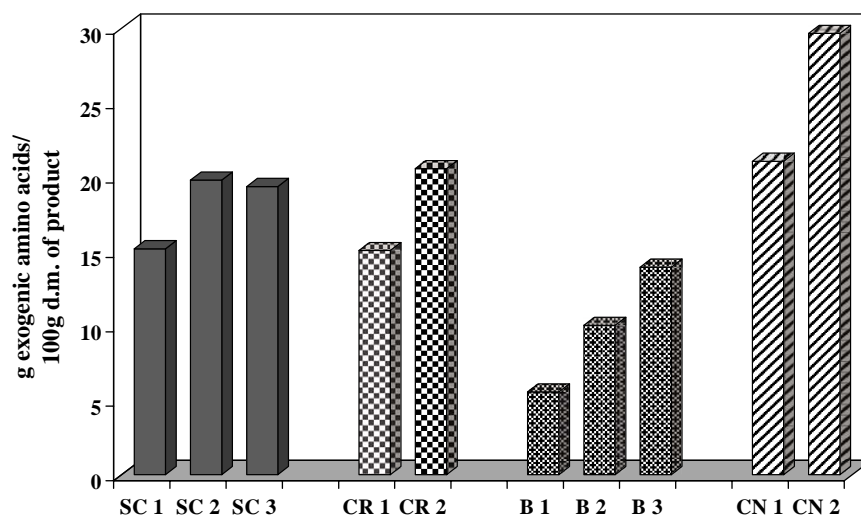


Figure 7. Content of essential amino acids in gluten-free confectionery products. For description of products SC 1, SC 2, SC 3, CR 1 and CR 2 see Table I; for description of products B 1, B 2, B 3, CN 1 and CN 2 see Table II.

Because of the higher consumer acceptance, the application of both flours seems to be especially beneficial for biscuits B 3, where due to their presence multiplied contents of potassium (three times), magnesium (five times), calcium (two times), zinc (2.5 times), and copper (two times) were observed (Table VII).

In the case of coconut cakes, the most pronounced multiplications of beneficial nutrients were found for potassium (four times), magnesium (three times), calcium (two times), iron (four times), manganese (1.5 times), zinc (two times) and copper (two times) (Table VII).

The introduction of significant amounts of macro-elements and micro-elements in the assessed confectionery products, due to the supplementation with the applied gluten-free raw materials, should be considered an important result of this study. The results of clinical studies have demonstrated that there was a risk of nutritional deficiencies in the group of people with celiac disease that were following gluten-free diet. The risk was applied mainly to insufficient intake of calcium, iron, zinc and selenium (Kupper 2005).

Zinc is the component of more than 80 enzymes, and its deficiencies lead to retarded development of children, skin affections, acne and weakening of the taste. Deficiency of iron in children may reduce the ability to learn, and in adults negatively affects their working efficiency (da Silva et al. 2003). The presence of easily available iron ions makes amaranth flour especially valuable additive for gluten-free products (Paredes-Lopez 1994).

Supplementation of food with magnesium is rarely necessary for healthy people. However the deficit of this mineral may accompany excessive alcohol consumption, disorders of absorption, inadequate availability of proteins and diarrhea. The last symptoms are typical for people (especially children) with celiac disease. The best way to overcome such deficit in healthy persons is to include cereal products, which are abundant in this mineral, such as wholemeal bread and groats (Raymond et al. 2006). This addition, however, is impossible to apply in the case of people with celiac disease.

Table VI. Fatty acid composition of the fat in gluten-free confectionery products.

Sample		Share of fatty acid (%)						Ratio of saturated/monounsaturated acids to PUFAs
		Palmitic acid (16:0)	Stearic acid (18:0)	Oleic acid (18:1)	Linoleic acid (18:2, n-6)	$\alpha$ -Linolenic acid (18:3, n-3)	Gadoleic acid (20:1)	
Sponge cakes	SC 1	21.7 $\pm$ 0.11 <sup>A</sup>	6.4 $\pm$ 0.07 <sup>A</sup>	42.7 $\pm$ 0.23 <sup>C</sup>	21.0 $\pm$ 0.12 <sup>A</sup>	1.6 $\pm$ 0.02 <sup>B</sup>	–	3.1
	SC 2	23.6 $\pm$ 0.12 <sup>B</sup>	6.6 $\pm$ 0.07 <sup>A</sup>	39.3 $\pm$ 0.21 <sup>B</sup>	24.0 $\pm$ 0.13 <sup>C</sup>	1.6 $\pm$ 0.02 <sup>B</sup>	–	2.7
	SC 3	24.0 $\pm$ 0.12 <sup>C</sup>	6.9 $\pm$ 0.07 <sup>A</sup>	38.3 $\pm$ 0.20 <sup>A</sup>	23.3 $\pm$ 0.13 <sup>B</sup>	1.1 $\pm$ 0.01 <sup>A</sup>	–	2.8
Carrot cakes	CR 1	9.4 $\pm$ 0.07 <sup>B</sup>	3.5 $\pm$ 0.03 <sup>A</sup>	34.4 $\pm$ 0.16 <sup>B</sup>	24.5 $\pm$ 0.13 <sup>B</sup>	9.4 $\pm$ 0.05 <sup>A</sup>	1.2 $\pm$ 0.03 <sup>A</sup>	1.3
	CR 2	8.7 $\pm$ 0.07 <sup>A</sup>	4.0 $\pm$ 0.04 <sup>B</sup>	31.9 $\pm$ 0.15 <sup>A</sup>	22.6 $\pm$ 0.12 <sup>A</sup>	18.6 $\pm$ 0.11 <sup>B</sup>	1.0 $\pm$ 0.02 <sup>A</sup>	1
Biscuits	B 1	23.7 $\pm$ 0.12 <sup>A</sup>	6.4 $\pm$ 0.07 <sup>A</sup>	27.2 $\pm$ 0.14 <sup>B</sup>	23.7 $\pm$ 0.13 <sup>B</sup>	1.0 $\pm$ 0.01 <sup>A</sup>	–	2.4
	B 2	23.7 $\pm$ 0.12 <sup>A</sup>	6.0 $\pm$ 0.06 <sup>A</sup>	27.5 $\pm$ 0.14 <sup>B</sup>	24.6 $\pm$ 0.14 <sup>C</sup>	1.0 $\pm$ 0.01 <sup>A</sup>	–	2.2
	B 3	23.3 $\pm$ 0.12 <sup>A</sup>	7.1 $\pm$ 0.09 <sup>B</sup>	26.8 $\pm$ 0.13 <sup>A</sup>	22.9 $\pm$ 0.13 <sup>A</sup>	1.2 $\pm$ 0.02 <sup>B</sup>	–	2.4
Coconut cakes	CN 1	23.2 $\pm$ 0.12 <sup>B</sup>	6.4 $\pm$ 0.07 <sup>B</sup>	27.0 $\pm$ 0.14 <sup>A</sup>	23.5 $\pm$ 0.13 <sup>B</sup>	1.2 $\pm$ 0.02 <sup>A</sup>	–	2.1
	CN 2	19.8 $\pm$ 0.11 <sup>A</sup>	5.4 $\pm$ 0.06 <sup>A</sup>	27.5 $\pm$ 0.14 <sup>B</sup>	18.6 $\pm$ 0.10 <sup>A</sup>	14.3 $\pm$ 0.10 <sup>B</sup>	–	1.7

Values denoted by different uppercase superscript letters for selected days differ significantly at the level  $\alpha = 0.05$ . For description of products see Tables I and II.

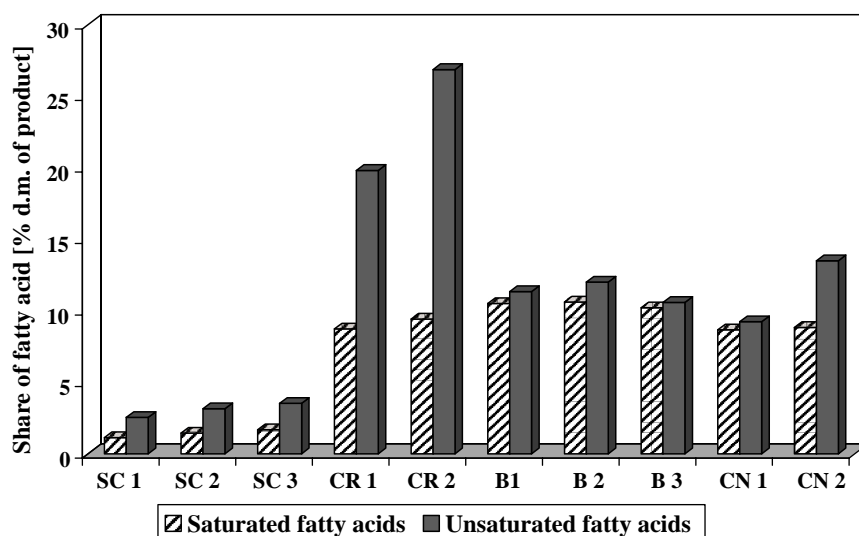


Figure 8. Saturated and unsaturated fatty acids in the examined products. For description of products SC 1, SC 2, SC 3, CR 1 and CR 2 see Table I; for description of products B 1, B 2, B 3, CN 1 and CN 2 see Table II.

The introduction of magnesium in gluten-free products with added amaranth flour or linseed meal therefore seems advisable.

Cereal products, which are absent in the gluten-free diet, are the rich source of manganese (60% of total intake) in the everyday diet (Green and Jabri 2003; Kupper 2005). An increase of its level in gluten-free products after supplementation should also be considered an important outcome of the study. Manganese is a component of metal-enzyme complexes and acts as an activator in reactions catalyzed by glycosyl

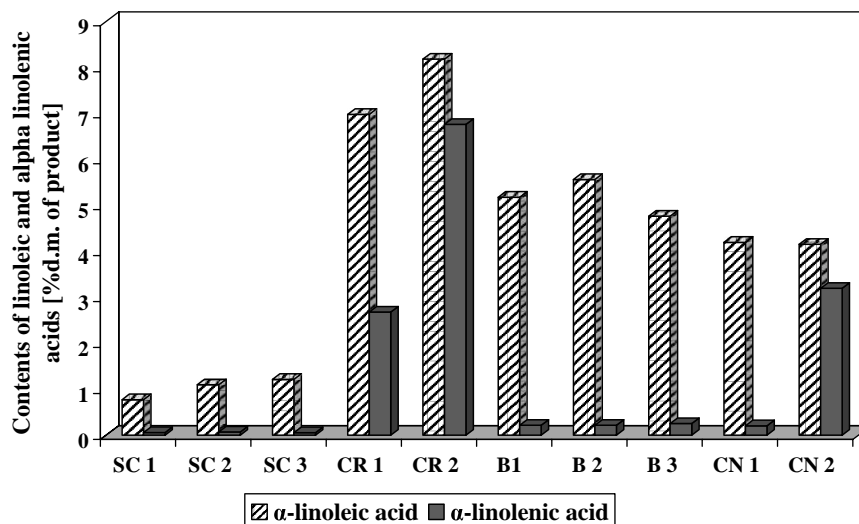


Figure 9. Contents of linoleic and  $\alpha$ -linolenic acids in analyzed gluten-free confectionery products. For description of products SC 1, SC 2, SC 3, CR 1 and CR 2 see Table I; for description of products B 1, B 2, B 3, CN 1 and CN 2 see Table II.



Table VII. Ash content and micro-elements and macro-elements in gluten free confectionery products.

Sample		Ash content (% dry matter)	mg/kg dry matter of product								
			Potassium	Phosphorus	Sodium	Magnesium	Calcium	Iron	Manganese	Zinc	Copper
Sponge cakes	SC 1	1.41 ± 0.22 <sup>A</sup>	1110 ± 345 <sup>A</sup>	3045 ± 283 <sup>A</sup>	3342 ± 202 <sup>A</sup>	69 ± 12 <sup>A</sup>	121 ± 11 <sup>A</sup>	14.1 ± 0.2 <sup>A</sup>	1.1 ± 0.1 <sup>A</sup>	7.2 ± 0.1 <sup>A</sup>	1.5 ± 0.4 <sup>A</sup>
	SC 2	1.83 ± 0.18 <sup>B</sup>	2057 ± 563 <sup>B</sup>	4134 ± 378 <sup>B</sup>	3177 ± 485 <sup>A</sup>	223 ± 23 <sup>B</sup>	173 ± 14 <sup>B</sup>	28.0 ± 0.4 <sup>B</sup>	11.1 ± 0.2 <sup>C</sup>	15.6 ± 0.3 <sup>C</sup>	2.7 ± 0.6 <sup>B</sup>
	SC 3	2.1 ± 0.32 <sup>C</sup>	2032 ± 456 <sup>B</sup>	4773 ± 273 <sup>C</sup>	3808 ± 171 <sup>B</sup>	232 ± 41 <sup>B</sup>	188 ± 21 <sup>B</sup>	30.4 ± 0.1 <sup>C</sup>	9.1 ± 0.3 <sup>B</sup>	14.8 ± 0.2 <sup>B</sup>	2.7 ± 0.6 <sup>B</sup>
Carrot cakes	CR 1	2.72 ± 0.15 <sup>A</sup>	3464 ± 345 <sup>A</sup>	2446 ± 344 <sup>A</sup>	5913 ± 278 <sup>A</sup>	149 ± 37 <sup>A</sup>	264 ± 54 <sup>A</sup>	26.0 ± 0.7 <sup>A</sup>	7.2 ± 0.6 <sup>A</sup>	9.5 ± 0.3 <sup>A</sup>	3.0 ± 0.9 <sup>A</sup>
	CR 2	3.12 ± 0.26 <sup>B</sup>	4445 ± 212 <sup>B</sup>	3251 ± 414 <sup>B</sup>	6034 ± 414 <sup>A</sup>	275 ± 21 <sup>B</sup>	292 ± 34 <sup>A</sup>	32.0 ± 0.3 <sup>B</sup>	11 ± 1.1 <sup>B</sup>	14.7 ± 0.6 <sup>B</sup>	5.0 ± 0.8 <sup>B</sup>
Biscuits	B 1	1.86 ± 0.24 <sup>A</sup>	619 ± 34 <sup>A</sup>	4530 ± 331 <sup>A</sup>	5932 ± 212 <sup>B</sup>	55 ± 12 <sup>A</sup>	87 ± 9 <sup>B</sup>	12.8 ± 0.2 <sup>A</sup>	1.3 ± 0.3 <sup>A</sup>	4.5 ± 0.2 <sup>A</sup>	1.5 ± 0.9 <sup>A</sup>
	B 2	2.30 ± 0.18 <sup>B</sup>	1319 ± 121 <sup>B</sup>	5211 ± 414 <sup>B</sup>	5743 ± 636 <sup>B</sup>	120 ± 31 <sup>B</sup>	75 ± 7 <sup>A</sup>	24.2 ± 0.1 <sup>B</sup>	4.5 ± 0.8 <sup>B</sup>	8.8 ± 0.6 <sup>B</sup>	2.4 ± 0.8 <sup>B</sup>
	B 3	2.08 ± 0.13 <sup>C</sup>	1847 ± 99 <sup>C</sup>	4347 ± 271 <sup>A</sup>	4064 ± 849 <sup>A</sup>	249 ± 24 <sup>C</sup>	158 ± 11 <sup>C</sup>	30.8 ± 0.3 <sup>C</sup>	8.9 ± 0.4 <sup>C</sup>	12.3 ± 0.7 <sup>C</sup>	3.2 ± 0.7 <sup>C</sup>
Coconut cakes	CN 1	1.03 ± 0.09 <sup>A</sup>	886 ± 141 <sup>A</sup>	2377 ± 107 <sup>A</sup>	2732 ± 478 <sup>A</sup>	77 ± 14 <sup>A</sup>	89 ± 14 <sup>A</sup>	8.3 ± 0.4 <sup>A</sup>	4.4 ± 0.6 <sup>A</sup>	9.3 ± 1.1 <sup>A</sup>	1.8 ± 0.6 <sup>A</sup>
	CN 2	2.02 ± 0.11 <sup>B</sup>	3045 ± 407 <sup>B</sup>	3525 ± 98 <sup>B</sup>	2773 ± 307 <sup>A</sup>	257 ± 28 <sup>B</sup>	168 ± 20 <sup>B</sup>	34.3 ± 0.2 <sup>B</sup>	7.3 ± 0.2 <sup>B</sup>	20.9 ± 0.8 <sup>B</sup>	4.1 ± 0.7 <sup>B</sup>

Values denoted by different uppercase superscript letters for selected days differ significantly at the level  $\alpha = 0.05$ . For description of products see Tables I and II.

transferase, glutamate synthase and other enzymes. It is also necessary for the formation of connective tissue of bones, and functioning of the brain and pancreas (Takeda 2003).

## Conclusions

- All of the examined gluten-free products supplemented with buckwheat flour, amaranth flour and linseed meal received good or very good consumer scores.
- The analyzed biscuits and coconut cakes, irrespective of the applied nutritional supplements, had a shelf-life of at least 3 months.
- The replacement of 50% corn starch by amaranth flour in the sponge cake resulted in an increase of protein content by 40%, and triplication of the dietary fiber content in comparison with the control sponge cake.
- The replacement of 65% corn flour by linseed meal in carrot cake increased the protein content by 30% and doubled the amount of dietary fiber content in comparison with the control carrot cake.
- The replacement of corn flour and potato starch with 50% buckwheat flour in biscuits was accompanied by a twofold rise of the protein content, while the use of both buckwheat flour (40%) and amaranth (30%) caused the additional increase of protein and dietary fiber contents.
- Coconut cakes with a share of linseed meal instead of 50% rice paste had more protein (by 60%), total dietary fiber (sevenfold), and its soluble fraction (10-fold).
- Supplementation of confectionery products with linseed meal allowed one to introduce  $\alpha$ -linolenic acid (18:3, n-3), which belongs to the important PUFAs.
- All of the supplemented gluten-free confectionery products contained a few times more macro-elements and micro-elements (i.e. potassium, phosphorus, magnesium, calcium, iron, manganese, zinc and copper) compared with the controls.

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