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EFFECTS OF BUCKWHEAT FLOUR (*FAGOPYRUM ESCULENTUM* MOENCH) ON THE QUALITY OF GLUTEN-FREE BREAD

Małgorzata Wronkowska[1], Agnieszka Troszyńska[2], Maria Soral-Śmietana[1], Agnieszka Wołejszo[2]

[1]Department of Functional Properties of Food; [2]Department of Sensory Analysis of Food, Division of Food Science, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn

Key words: buckwheat flour, gluten-free bread, resistant starch, elements, sensory evaluation

Buckwheat flour was studied as an ingredient of commercial gluten-free formulation NISKOBIAŁKOWA. The effect of exchange of formulation mass by buckwheat flour (BF) in 10, 20, 30, 40 and 50% was analysed and experimental products were subjected to sensory evaluation. Higher contents of nutrients, proteins and elements were determined in gluten-free bread with buckwheat flour. Also, the increase of resistant starch content was noticed.

The sensory evaluation was carried out with the method of sensory profiling (QDA) and in hedonic tests. In QDA a panel (n=8) rated the breads for colour, odour, taste and texture. In the affective tests the consumers (n=30) evaluated the samples for overall quality. The results proved that the overall quality of bread with BF was significantly higher than that of pure formula (control). The average overall quality of scores for BF bread ranged from 4.9 units (50% BF) to 5.8 units (30% BF), whereas 3.4 units for the control. The QDA demonstrated significant differences ($p<0.05$) between the breads for the following attributes: colour, rancid odour, "rancid" taste, buckwheat odour and "buckwheat" taste, bitter taste, aftertaste and moistness. The principal component analysis (PCA) indicated that the first (PC1) and the second (PC2) component together explained 97.15% of the variation of sensory quality of the experimental gluten-free bread.

INTRODUCTION

Recent epidemiologic studies have shown that the prevalence of celiac disease or non-typical celiac disease, or allergic reaction/intolerances to gluten has been significantly underestimated [Gallagher *et al*., 2004]. Those diseases have been connected with life-long intolerance to a gliadin fraction of wheat and other prolamines: rye (secalin), barley (hordein) and possibly oats (avenin). The reaction to gluten ingestion, among patients suffering from celiac diseases, is the inflammation of the small intestine which leads to the malabsorption of several important nutrients. The only effective treatment for celiac disease is keeping a strict gluten-free diet throughout the patient's lifetime. Many of gluten-free products available on the market are of low quality, exhibiting a dry crumbling crumb that results in poor mouthfeel and flavour [Arendt *et al.,* 2002]. The acceptability of gluten-free bread is connected with its quality characteristics which need to be similar to those of wheat flour or mixed wheat-ray flour bread. This is why in recent study an increasing interest was observed in the improvement of structure and sensory parameters of gluten--free breads by incorporation of starches, dairy proteins and hydrocolloids into a gluten-free formula bases that could mimic the viscoelastic properties of gluten [Gallagher *et al*., 2003; Gujral *et al*., 2003, Ahlborn *et al*., 2005]. However, the diet based on gluten-free products is often characterised by a low content of some nutritional components such as proteins and mineral components, as well as non-nutritional but physiologically important components, like dietary fibre.

Buckwheat grains are a rich source of a special type of starch [Soral-Śmietana *et al*., 1984a; Acquistucci & Fornal, 1997] with dietary lipids [Soral-Śmietana *et al*., 1984b] and contain many valuable compounds, such as proteins with a low content of α-gliadin fraction [Kreft *et al.*, 1996], antioxidative substances [Stempińska *et al.,* 2007; Michalska *et al*., 2007], trace elements and dietary fibre [Steadman *et al*., 2001; Stempińska & Soral-Śmietana, 2006]. Proteins content in buckwheat flour has been reported to range from 8.5 to 18.9%, depending on the variety [Krkošková & Mrázová, 2005; Stempińska & Soral-Śmietana, 2006]. Buckwheat proteins have a high biological value due to well-balanced amino-acids composition, although their digestibility is relatively low [Kato *et al*., 2001; Tomotake *et al*., 2006].

Despite the growing interest in the nutritional and health aspects of buckwheat grains their consumption in many Western countries is low. One of the most substantial reasons for its limited use, as well as for search of the new applications for buckwheat grains, arises from its specific flavour. However, the information about the effect of buckwheat flour on the sensory properties of bread is scant. Thus, the aim of the present research was to investigate effects of buckwheat flour, incorporated into the commercially-available gluten free formula, on the technological and sensory properties of gluten--free formulation.

Author's address for correspondence: dr Małgorzata Wronkowska, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, ul. Tuwima 10,10-747 Olsztyn, Poland; tel: (48 89) 523 46 18; fax: (48 89) 524 01 24; e-mail: m.wronkowska@pan.olsztyn.pl

MATERIAL AND METHODS

Materials and bread-making

The gluten-free formulation named: NISKOBIAŁKOWA and buckwheat flour were purchased from a local market in Olsztyn, Poland. The gluten-free formulation contained: wheat and corn starches, guar gum, pectin, glucose, dietary fibre, emulsifiers and sugar (according to producer declaration). Buckwheat flour substitutes 10, 20, 30, 40 or 50% w/w of gluten--free formulas basis. The amount of added water was 88 g per 100 g of gluten-free formulation. Sunflower oil (6%), fresh yeast (2%), salt (1%) and sugar (3%) were also added. The mixture was blended with a planetary rotation of mixing within 5-speed mixer (Kitchen Aid, USA) for 12 min. The dough was proofed at 35-40°C for 40 min and baked at 215°C for 25-35 min. Baking tests were carried out in an electric oven with an incorporated proofing chamber (ZBPP, Bydgoszcz, Poland). The breads baked were subjected to physicochemical analysis and sensory evaluation abbreviated as: control (100% formula); 10% BF (10% buckwheat flour and 90% formula); 20% BF (20% buckwheat flour and 80% formula); 30% BF (30% buckwheat flour and 70% formula); 40% BF (40% buckwheat flour and 60% formula); and 50% BF (50% buckwheat flour and 50% formula).

Physicochemical analysis

The volume of breads was determined following the national standard methods of Poland [PN-A-74123, 1996]. Measurements of the loaves were carried out after cooling to room temperature (20°C). The content of protein (Nx6.25) was determined with the Kjeldahl method [AOAC, 1990]. Resistant starch (RS) was estimated according to the method described by Champ *et al*. [1999]. The measurement of elements content in the crumb was carried out using the atomic absorption spectroscopy (AAS) method by a Unicam 939 spectrometer equipped with data base ADAX, background correction and cathode lamps [Soral-Śmietana *et al*., 2001]. Before elements determination all samples were wet mineralised with a mixture of acids: nitric and perchloric (3:1). Potassium was assayed with the photometric flame method and phosphorus was investigated with the colorimetric method by molybdate with hydroquinonate and sodium sulfate (IV). For the validation of calcium measurement, the solution of lanthanum chloride was added to all samples in the amounts assuring 0.5% concentration of La3+.

TABLE 1. Definitions of sensory attributes used for sensory evaluation of the gluten-free bread crumb by QDA analysis.

Attribute	Definition
	Appearance
Colour	Visual impression of the bread colour (from light to dark)
Porosity	Visual impression of the bread crumb porosity (poreless – porous)
	Odour
Rancid	Odour typical of rancid nut oil (none – very intensive)
Sweet	Odour characteristic to bun produced from wheat flour (none – very intensive)
Yeast	Odour characteristic to yeast-raised bread produced from wheat flour (none – very intensive)
Buckwheat	Odour typical of boiled buckwheat (none – very intensive)
Acidulous	The intensity of the acidulous odour (none – very intensive)
	Taste
Sweet	Basic taste illustrated by sucrose diluted in water 1.5% (none – very intensive)
Rancid	Taste typical of rancid nut oil (none – very intensive)
Bitter	Basic taste illustrated by caffeine diluted in water 0.5% (none – very intensive)
Buckwheat	Taste characteristic to boiled buckwheat (none – very intensive)
Acidulous	The intensity of the acidulous taste (none – very intensive)
Yeast	Taste characteristic to yeast-raised bread produced from wheat flour (none – very intensive)
Aftertaste	Aftertaste which continued after the removal of sample (none – very intensive)
	Texture (by finger)
Springiness	Degree of springiness in bread crumb by pressing with finger (not springy – springy)
Elasticity	Response to stretching (not elastic – elastic)
	Texture (mouth feel)
Mastication	Degree of perceived resistance while chewing the sample 10 times (not mastic – mastic)
Adhesiveness	Degree of adhesiveness perceived while chewing the sample 10 times (low – high)
Gumminess	Degree of gumminess perceived while chewing the sample 10 times (not gummy – gummy)
Moistness	Degree of moistness perceived while chewing the sample 10 times (dry – moist)

Sensory evaluation

Sensory methods and evaluation conditions

Quantitative descriptive analysis (QDA) was used to determine differences in the sensory characteristics of the breads [Stone & Sidel, 1993; Lawless & Heymann, 1999]. Prior to the analysis, vocabularies of the sensory attributes were developed by the panel in a round-table session, using a standardised procedure [ISO/DIS 13299:1998]. Twenty attributes related to the appearance, odour, taste and texture of breads were selected and thoroughly defined for profiling. Definitions and description of these sensory attributes are summarised in Table 1. The panelists evaluated the intensity perceived for each sensory attribute on unstructured graphical scales. The scales were 10 cm long and verbally anchored at each end and the results were converted to numerical values (from 0 to 10 units) by a computer. Loaves were sliced (15 mm thickness) and served to the assessors in transparent plastic boxes. The samples were coded with a three-digit number and presented to the panelists in random order. Mineral water was offered between the samples. The assessments were carried out at a sensory laboratory room, which fulfils the requirements of the ISO standards [ISO 8589:1998]. The results were collected using a computerised system ANALSENS (IAR&FR PAS, Olsztyn, Poland). Each sample was tested in two replications.

Sensory panel

Sensory assessments of the samples (QDA) were carried out by a panel consisting of 8 members (7 females and 1 male, ranging in age between 26-39 years) previously selected and trained according to ISO guidelines [ISO 8586-1:1993]. All assessors have passed the basic taste test, the odour test and the colour vision test. Prior to their participation in the experiments, the subjects were trained on sensory descriptors for breads purchased from a local supermarket.

Consumer test

A semi-consumer panel of 30 members (including the Institute staff, graduate, and undergraduate students of the Isntitute) has made hedonic evaluation of the samples. In the test, each panelist was asked to assess the breads for overall quality, based on the overall colour, odour, taste and texture. An unstructured graphical scale was 10 cm long and anchored on both ends: disliked (0) – extremely liked (10).

Statistical analysis

The sensory attributes were analysed by ANOVA using Fisher's Least Significant Difference test (LSD). Principal component analysis (PCA) was performed in order to describe the variance among all sensory data obtained. Statistical analyses were performed using software package (StatSoft Inc., v. 7.1, Tulsa, OK, USA).

RESULTS AND DISCUSSION

Physicochemical characteristics

The loaf volume of the control gluten-free bread was the highest in comparison with all breads supplemented with buckwheat flour (BF) (Table 2). In general, the incorporation of BF into the gluten-free dough influenced the reduction of bread loaf volume by about 9.8-20.5%, however, they still complied the requirements of the Polish Standard for gluten-free bread [Polish Standard PN-A-74123, 1997]. Literature data indicated that the volume of gluten-free bread supplemented with different kinds of hydrocolloids ranged from 205 to 268 cm^3/100 g of bread [Lazaridou *et al*., 2007]. According to the Polish Standard [PN-A-74108, 1996], the volume of wheat flour bread is about 300 cm^3/100 g of bread. Volumes of gluten-free breads obtained in this experiment were not much different than those of wheat flour bread. The experimental bread volume was probably affected by the presence of buckwheat flour proteins of low molecular mass [Soral-Śmietana, 1984]. More than 50% of proteins of buckwheat flour have the molecular mass of 11,400 and 19,100 Da, and about 20% of proteins are of 10,700 Da [Soral-Śmietana, 1984; Fornal *et al.,* 1985]. Buckwheat starch granules have a small size in the range of 1-9 μm, with the most frequent size of 3-5 μm [Soral-Śmietana *et al*., 1984a; Acquistucci & Fornal, 1997] and indicate high viscosity at small range of gelatinisation temperature on Brabender viscogram curves, between 68 to above 80°C. The same tendency was also detected in a mixture of corn grits and buckwheat flour, at the ratio of 80:20, [Soral-Śmietana, 1992]. As a result of rising BF share in dough, small porosity crumb structure was obtained.

The gluten-free formula NISKOBIAŁKOWA used in this study was characterised by a low content of proteius, near 1.5% d.m. (Table 3). The presence of buckwheat flour, with protein content up to 12.6% d.m., within the experimental formulas caused an increase in the total proteins content of crumb, from 1.5% (in the control) up to 9.3% (in bread with 50% w/w buckwheat flour on formula base). Gluten-free diet has relatively low nutritional value [Bardella *et al*., 2000], therefore the increased content of protein obtained in the study could be considered as a valuable achievement. The content of starch fraction resistant to hydrolysis with α-amylase in NISKOBIAŁKOWA formula was 2.5% d.m., and after the baking process some increase in its value was detected. Comparing the resistant starch contents in all experimental crumbs, the increase in RS content was no-

TABLE 2. Effect of buckwheat flour on volume of gluten-free breads.

NISKOBIAŁKOWA gluten-free formula	Volume (cm^3/100 g of bread)
Control	268 ± 3
10% BF	242 ± 5
20% BF	230 ± 5
30% BF	223 ± 4
40% BF	230 ± 3
50% BF	213 ± 5

TABLE 3. Content of proteins and resistant starch in the investigated materials and gluten-free crumb of breads.

Samples	Proteins (% d.m.)	Resistant starch (% d.m.)
NISKOBIAŁKOWA formula	1.53 ± 0.24	2.52 ± 0.13
Buckwheat flour	12.61 ± 0.18	6.56 ± 0.20
Crumb of bread:		
control	1.52 ± 0.05	4.18 ± 0.19
10% BF	2.39 ± 0.07	4.82 ± 0.29
20% BF	4.47 ± 0.14	5.51 ± 0.12
30% BF	4.55 ± 0.18	6.12 ± 0.21
40% BF	7.18 ± 0.21	6.67 ± 0.33
50% BF	9.26 ± 0.14	6.84 ± 0.15

TABLE 4. Micro– and macroelements in crumb of gluten-free control and experimental breads.

Elements (mg/100 g)	Crumb of bread					
	control	10% BF	20% BF	30% BF	40% BF	50% BF
Cu	0.04 ± 0.01	0.08 ± 0.01	0.15 ± 0.02	0.18 ± 0.01	0.23 ± 0.01	0.29 ±0.03
Zn	0.54 ± 0.01	0.82 ± 0.01	0.95 ± 0.01	1.18 ± 0.02	1.47 ± 0.01	1.87 ± 0.04
Mn	0.04 ± 0.01	0.22 ± 0.01	0.39 ± 0.01	0.54 ± 0.01	0.69 ± 0.01	0.83 ± 0.02
Fe	0.52 ± 0.02	0.69 ± 0.01	0.95 ± 0.02	1.04 ± 0.08	1.31 ± 0.09	1.48 ± 0.02
Ca	12.18 ± 0.12	13.23 ± 0.10	14.21 ± 0.05	15.67 ± 0.12	16.39 ± 0.05	19.31 ± 0.22
Mg	5.22 ± 0.01	18.64 ± 0.08	31.25 ± 0.23	44.28 ± 0.38	55.81 ± 0.87	68.76 ± 0.06
K	24.26 ± 0.10	61.19 ± 0.09	93.90 ± 0.95	133.00 ± 1.23	163.8 ± 1.04	207.00 ± 2.10
P	30.21 ± 0.05	62.62 ± 0.07	91.60 ± 0.96	114.9 ± 1.02	147.00 ± 1.22	192.00 ± 1.85
Na	370.00 ± 2.28	390.00 ± 1.80	400.00 ± 2.88	410.00 ± 3.55	380.00 ± 1.98	390.00 ± 2.01

ticed, especially significant in the crumb with the highest BF share. The obtained enrichment in that non-nutritional component is very significant as it plays very important physiological functions [Soral-Śmietana & Wronkowska, 2004]. Celiac patients often suffer from malnutrition as their intestinal absorption is impaired. Celiac disease is manifested by inflammation of the small-intestinal mucosa, causing deficiencies of the fat-soluble vitamins, iron, folic acid, and calcium and other minerals [Murray, 1999]. Many authors have published on the bone mineral content in people with celiac disease [Barera *et al*., 2004; Mora *et al*., 1993], or zinc and calcium metabolism in celiac disease [Crofton *et al*., 1990, Barera *et al*., 2004]. In this study, the content of micro- and macroelements in the crumb of gluten-free breads was determined as well (Table 4). The content of individual elements in the control gluten-free bread was very low in comparison with that reported in wheat flour breads, as presented by Soral-Śmietana *et al*. [2004] or El-Adawy [1997]. The substitution of the experimental bread with buckwheat flour caused a significant increase of all investigated elements content, except for sodium, which remained at the same very high level. It was connected with the composition of commercial gluten-free formula. In comparison with control gluten-free bread crumb, in crumb of bread with 10% of BF the content of manganese was observed to increase 5 times and that of potassium to increase 3 times. The increase of the content of phosphorous and copper (2-times) was also connected with the lowest supplementation of buckwheat flour. The content of calcium, zinc and iron increased together with the increasing amount of buckwheat flour.

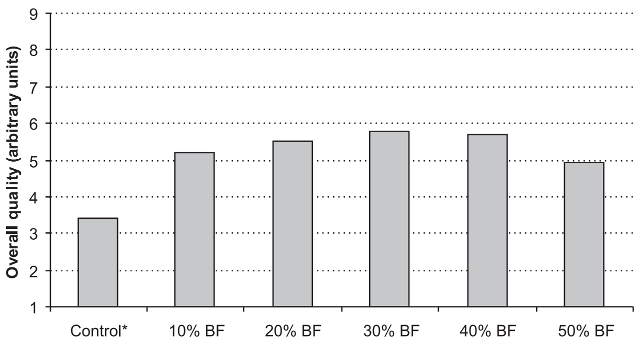

FIGURE 1. Overall quality of gluten-free breads (*see abbreviations in Table 2).

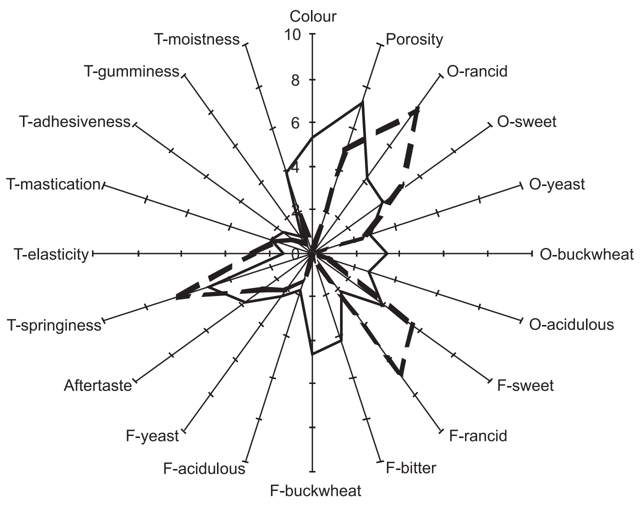

FIGURE 2. The sensory profiles of gluten-free breads: – – – Control (the lowest score of overall quality); —— 30% BF (the highest score of overall quality).

Sensory evaluation

The effects of the incorporation of buckwheat flour (BF) into gluten-free formula on the overall quality of breads are shown in Figure 1. The results indicated that palatability of the breads with BF (10% BF, 20% BF, 30% BF, 40% BF and 50% BF) was significant ($p<0.05$) higher than those of the control (the bread made from 100% gluten-free formula). The average overall quality of scores for BF breads ranged from 4.9 units (50% BF) to 5.8 units (30% BF) whereas the control obtained 3.4 units (in the scale of 10 units). It suggests that BF might contribute to the improvement of the sensory properties of gluten-free bread. To find attributes which influenced the sensory quality of breads, quantitative descriptive analysis (QDA) was used in the study. Descriptive analysis is the most sophisticated tool of sensory evaluation and involves the discrimination and description of both the qualitative and quantitative sensory attributes of a product by trained panels [Lawless & Heymann 1999; Murray *et al*., 2001]. The mean sensory ratings for the samples and the analysis of variance are presented in Table 5. The results of ANOVA showed that there were significant differences ($p<0.001$) in the intensity of attributes such as: colour, rancid odour "rancid" taste, buck-

TABLE 5. Mean descriptive analysis ratings of gluten-free breads.

Attributes[1]	Bread[2]					
	Control	10% BF	20% BF	30% BF	40% BF	50% BF
Colour	0.3^{a}	2.0^{b}	3.6^{c}	5.3^{d}	8.2^{e}	8.8^{e}
Porosity	4.9^{a}	7.1^{b}	6.2^{ab}	7.3^{b}	4.8^{a}	6.4^{ab}
O-rancid	8.1^{c}	5.6^{b}	4.9^{b}	4.2^{ab}	3.1^{a}	2.5^{a}
O-sweet	5.1^{b}	4.1^{ab}	4.7^{ab}	4.0^{ab}	3.2^{a}	2.8^{a}
O-yeast	2.4^{ab}	1.3^{a}	2.3^{ab}	2.8^{ab}	3.1^{b}	3.1^{b}
O-buckwheat	0.1^{a}	0.4^{a}	1.7^{b}	3.4^{c}	6.7^{d}	6.8^{d}
O-acidulous	0.9^{a}	1.6^{a}	2.0^{ab}	2.7^{abc}	4.3^{c}	3.8^{bc}
F-sweet	5.6^{a}	4.6^{a}	3.8^{a}	4.0^{a}	3.9^{a}	3.6^{a}
F-rancid	6.8^{c}	4.1^{b}	2.9^{ab}	2.2^{a}	2.2^{a}	1.8^{a}
F-bitter	0.4^{a}	1.0^{ab}	1.8^{b}	4.2^{c}	5.5^{c}	6.9^{d}
F-buckwheat	0.1^{a}	0.8^{ab}	2.0^{b}	4.6^{c}	6.9^{d}	8.2^{e}
F-acidulous	1.3^{ab}	0.8^{a}	1.3^{ab}	1.8^{abc}	2.5^{bc}	2.9^{c}
F-yeast	2.1^{a}	2.1^{a}	1.7^{a}	2.4^{a}	3.0^{a}	2.4^{a}
Aftertaste	2.7^{a}	3.0^{a}	3.3^{a}	3.8^{a}	5.7^{b}	6.1^{b}
T-springiness	6.4^{a}	5.2^{a}	6.6^{a}	5.0^{a}	5.0^{a}	4.9^{a}
T-elasticity	2.7^{a}	2.0^{a}	1.8^{a}	1.3^{a}	1.5^{a}	1.9^{a}
T-mastication	1.8^{a}	2.1^{a}	2.1^{a}	2.0^{a}	2.3^{a}	2.4^{a}
T-adhesiveness	1.1^{a}	1.8^{a}	1.1^{a}	1.6^{a}	1.7^{a}	2.2^{a}
T-gumminess	0.6^{a}	0.6^{a}	1.0^{ab}	0.9^{ab}	1.1^{ab}	1.7^{b}
T-moistness	1.8^{a}	2.2^{a}	2.9^{ab}	3.9^{bc}	4.2^{bc}	4.8^{c}

[1]O=odour, T=texture, F=taste. [2]Values followed by the same letter in the same row are not significantly different ($p<0.05$).

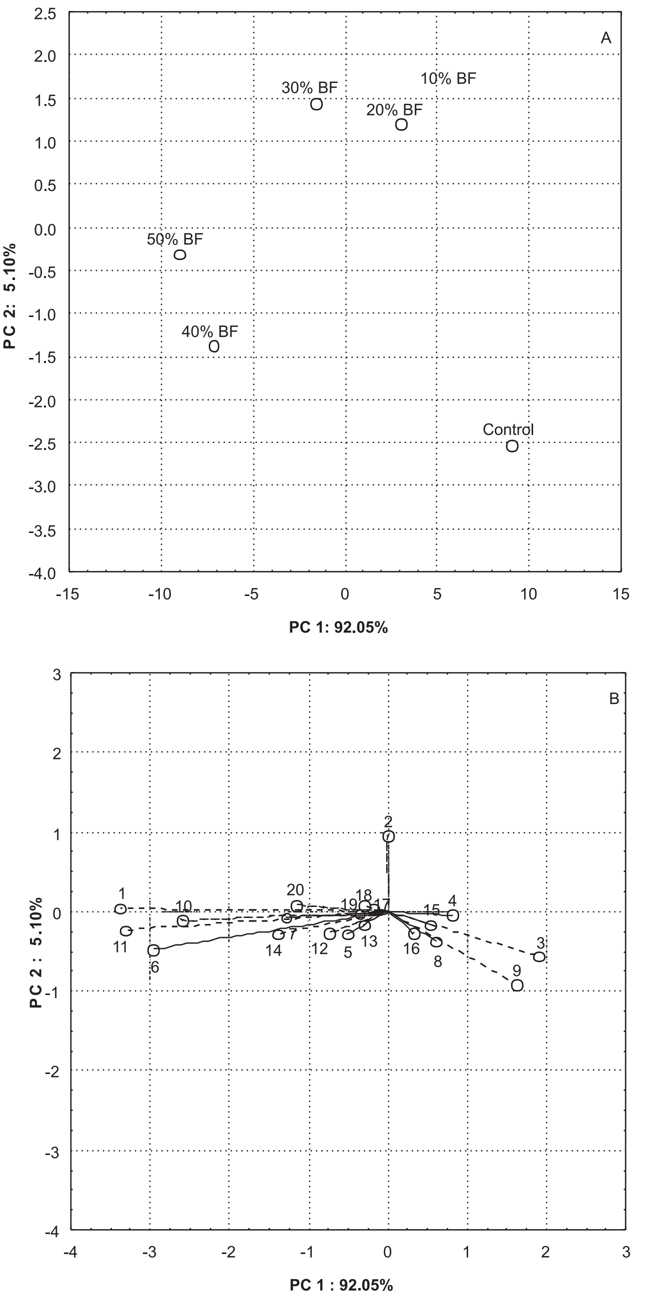

FIGURE 3. Principal component analysis (PCA) scores (a) and loadings (b) of sensory data for gluten-free bread.
Vectors: 1 – colour, 2 – porosity, 3 – rancid odour, 4 – sweet odour, 5 – yeast odour, 6 – buckwheat odour, 7 – acidulous odour, 8 – sweet taste, 9 – rancid taste, 10 – bitter taste, 11 – buckwheat taste, 12 – acidulous taste, 13 – yeast taste, 14 – aftertaste, 15 – springiness, 16 – elasticity, 17 – mastication, 18 – adhesiveness, 19 – gumminess, 20 – moistness.

wheat odour and "buckwheat" taste, bitter taste, aftertaste and moistness caused by the kind bread. In order to observe the above differences in the analysed samples more clearly, the sensory profiles of 30% BF bread (with the highest scores of overall quality) and control bread (with the lowest scores of overall quality) were displayed as spider diagrams in Figure 2. It can be seen that the sensory profiles of these samples were significantly different in the intensity of attributes for appearance, odour and taste. In the profile of control there dominated the sensory attributes desirable for the consumers such as rancid odour and "rancid" taste. It should be emphasised that the control demonstrated approximately three times as high "rancid" taste as the bread with 30% BF. This notes probably did affect the sensory overall quality. In contrast, in the profile of 30% BF bread the "buckwheat" attribute dominated, being accompanied by bitterness. It should be stressed that in the sensory profiles of the samples there were no distinct differences in the attributes of texture except moistness. The results proved that the moistness of breads increased with the addition of BF. Samples with a greater addition of BF were characterised by a higher intensity of these notes. It suggests that BF might kept the softness of gluten-free bread during storage.

Data obtained by means of the QDA method were subjected to principal component analysis (PCA) which was performed on the covariance matrix of the samples with no rotation. Five principal components (PC1–PC5) were extracted among which the first two principal components (PC1 and PC2) had eigen value greater than one and accounted for 97.15% of the total variance. The first two principal components were plotted in Figures 3a and 3b. It can be seen that the PCA technique differentiated the samples by the kind of breads (Figure 3a). The samples formed distinctly separate cluster found along the first principal component, which indicated their different sensory characteristics. Figure 3b shows the loadings of the PC1 and PC2. It can be seen that the PC1 was differentiated by all attributes except porosity (attribute 2). It is a common knowledge that the PC1 contains the most important information and includes more important characteristics. The attributes having a decisive effect on the variation in the sensory quality of the samples were features connected with bread appearance, odour and taste such as: colour (attribute 1), rancid odour (attribute 3), buckwheat odour (attribute 6), "rancid" taste (attribute 9), bitter taste (attribute 10), and "buckwheat" taste (attribute 11). This was indicated by the length of the plotted vectors describing the sensory attributes.

CONCLUSIONS

Based on the results obtained in this study, it could be concluded that the partial substitution of commercial gluten-free formula with buckwheat flour resulted in (i) enrichment in nutrients, especially in proteins and elements as well as resistant starch, and (ii) improvement of the overall sensory quality of bread with BF.

Experimental gluten-free bread could be offered to the consumers, especially recommendable is bread with the supplementation of 30% of buckwheat flour.

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