Bulletin of the *Transilvania* University of Brasov
Series II: Forestry • Wood Industry • Agricultural Food Engineering • Vol. 15(64) No. 2 – 2022
https://doi.org/10.31926/but.fwiafe.2022.15.64.2.12

THE EFFECT OF WHOLE BUCKWHEAT FLOUR ADDITION ON PHYSICO-CHEMICAL CHARACTERISTICS, BIOLOGICAL ACTIVE COMPOUNDS AND FATTY ACIDS PROFILE OF BREADS

Gjore NAKOV[1] Mishela TEMKOV[2]
Stanka DAMYANOVA[3] Silviya IVANOVA[1]

Abstract: To evaluate the impact of fortification of the wheat bread with whole buckwheat flour on chemical characteristics, bioactive components and fatty acids, six types of breads with different whole-grain buckwheat flour concentration (0, 10, 20, 30, 40 and 50) were prepared. The breads made with whole buckwheat flour had inferior physical characteristics in comparison to the bread made solemnly with wheat flour that was taken as a reference sample. The quantities of ash (1.66 g/100g), fats (3.42 g/100g), proteins (16.23 g/100g), total fibers (soluble and insoluble) (80.0318.95 g/100g dry matter), total polyphenols (13.24 g GAE/100g DM) and antioxidant activity increased with the increment of the added content of whole buckwheat flour up to 50%WBF, whereas the quantity of carbohydrates was decreased. The amount of total monounsaturated fatty acids also increased with subsequent higher concentrations of whole buckwheat flour in bread (39.01 g/100 g fats against 20.90 g/100 g fats for the control bread), the most prominent of which was oleic acid. The results lead to conclusion that the whole buckwheat fortified breads have better chemical and nutritional characteristics than the wheat bread. Sensory evaluation test ranked the one with 30% whole buckwheat flour as the most acceptable. The flour blend (wheat flour – buckwheat flour) can be included in development of new innovative and high in nutrition products.

Key words: buckwheat, breads, cereals, composite flours, functional food.

[1] Technical University of Sofia, College of Sliven, 59 Bourgasko Shose Blvd., 8800, Sliven, Bulgaria;
[2] Department of Food Technology and Biotechnology, Faculty of Technology and Metallurgy, “St. Cyril and Methodius” University in Skopje, Ruger Boskovic 16, Skopje, R.N. Macedonia;
[3] University of Ruse “Angel Kanchev”, branch Razgrad, blv. Aprilsko vastanie, 47 Razgrad, Bulgaria;
Correspondence: Gjore Nakov; email: gore_nakov@hotmail.com.

1. Introduction

Pseudo-cereals were part of humans' daily diet in the past. Today, these crops are totally neglected, underestimated and underused. This group encompasses buckwheat culture (*Fagopyrum* Mill. Polygonaceae) among others, which has 30 different species, but only two (Tartary and common buckwheat) are being used for nutritional purposes [20]. Production and consumption of the buckwheat is traditional for Central and Eastern Europe (Croatia, Russia, Slovenia, Poland) and the grain is often used as rice substitution [22]. On the other hand, the countries from Southeastern Europe (Bulgaria, North Macedonia, Albania, and Greece) do not have common practice in inserting the grain in their daily diet. The buckwheat grain, as well as the buckwheat flour, are rich in minerals (Fe, Zn, Cu, K, Mn, Na), vitamins (B, E, PP), proteins, dietary fibers and bioactive compounds (phenolic acids and flavonoids). In addition, they possess high antioxidant capacity, positively influencing consumers' health [23]. Bakery products, especially bread are the most commonly consumed manufactured goods made of wheat flour [30]. In modern days, there is a growing interest in producing bread and bakery products from other grains such as: rye [32], barley [33], buckwheat [8], oats [5], and corn [15]. There are several reasons for this growing trend: implementing healthy life style, use of whole grain flour in human's diet for greater health benefits and welfare [35]. However, the use of buckwheat flour as a bakery raw material is limited due to the lack of glutenins and gliadins, the proteins that form the gluten network in wheat. The recent advances in processing and development of buckwheat flour were fruitful in developing a variety of bakery and non-bakery products [13]. Although the buckwheat is a historic cereal, there is a gap in the research literature report regarding physical, rheological and nutritional characteristics of bread enriched with buckwheat flour (BF). Among them rice bread fortified with BF, Torbica et al. [36] technologies are reported. Moreover, there are published results for different flour blends composed of buckwheat flour and some other type of flour [8, 28]. Lin et al. [22] showed that wheat flour substituted with 15% of either husked or unhusked buckwheat exhibited higher content of carbohydrates, free amino acids, higher flavor 5'-nucleotides, and 2 – to 3-fold higher total volatile content in comparison to wheat bread. This research indicated that buckwheat flour could be incorporated into bread recipe, providing buckwheat-enriched wheat bread with more carbohydrates, a stronger umami taste and more distinctive aroma. Bojňanská et al. [4] reported that a substitution of 30% wheat flour with buckwheat flour yielded a buckwheat enhanced wheat bread acceptable from the technological, sensory and healthy point of view. The antioxidant capacity increased in the plasma after four weeks of consumption of 30% buckwheat enriched wheat bread. The research also provided information that the addition of buckwheat increased the content of proteins, minerals, fibers as well as rutin in bread. Vogrinčič et al. [37] showed an increase in the antioxidant activity and rutin content in dough and breads containing growing percentage of tartary buckwheat flour. Chlopicka et al. [6]

reported that buckwheat-enriched wheat bread exhibited the highest phenolic content and antioxidant activity in comparison with amaranth and quinoa enriched wheat bread.

The aim of this research is to study the influence of whole buckwheat flour (WBF) replacing part of the WF on the nutritional and sensory characteristics of newly produced breads. The first step involves blend WF and WBF five different blends of WF and WBF (90:10, 80:20, 70:30, 60:40, 50:50). Later, these blends and 100% WF was used to make dough that eventually were transformed into breads which were characterized in terms of their physico-chemical, nutritional and sensory properties. Special attention is laid on the fatty acid composition of the produced breads.

2. Materials and Methods

2.1. Materials

Bread loaves were made using two types of flour namely wheat flour (type 500) and whole buckwheat flour ground with an IKA MF10 grinder (IKA®-Werke GmbH & Co. KG, Staufen, Germany). From these two types, six different mixtures were prepared: bread with 100% wheat flour (control); bread with 90% wheat flour and 10% whole tartary buckwheat flour (10% WBF); bread with 80% wheat flour and 20% whole buckwheat flour (20% WBF); bread with 70% wheat flour and 30% whole buckwheat flour (30% WBF); bread with 60% wheat flour and 40% whole buckwheat flour (40% WBF); bread with 50% wheat flour and 50% whole buckwheat flour (50% WBF). The other ingredients (salt and fresh yeast) where bought form a local market in Razgrad (Bulgaria).

2.2. Methods

2.2.1 Bread Preparation

Six bread formulation (control, 10, 20, 30, 40 and 50% WBF) were produced according to the method presented by Dimov and Georgieva [10]. After the baking process, breads were cooled to room temperature (25°C) and analyzed within 24 h. Two sets of each sample bread were prepared.

2.2.2. Physical Characteristics

The parameters: width (mm), height (mm), volume (cm^3), and specific volume (cm^3/g) of prepared breads with different amounts of WBF were determined using a Volscan Profiler (Stable Micro Systems Ltd. Godalming, Surrey, UK), on three samples. The color expressed in the CIE L* a* b* system, was recorded with a Chroma Meter CR-400 colorimeter (Konica Minolta, Tokyo, Japan) on two sets of five random points.

2.2.3. Textural Characteristics

The textural properties of the breads (hardness, springiness, cohesiveness, chewiness and resilience) were measured using a TA-XT2 Plus texture analyzer (Stable Micro System Ltd. Godalming, Surrey, UK). TPA test included double compression of slices to 40% of their thickness with a 25 mm aluminum cylindrical probe The initial force employed (10 g) was applied twice, with a 5 s delay between the two applications; the probe speed was 5 mm/s until a 40% deformation of the sample center.

2.2.4. Bread Crumbs Image J Analysis

High-resolution images (600 dpi) of three slices from each batch were acquired using digital camera (Canon EOS 1100 D) in brightness, contrast as default software values, and saved in TIFF format. Before shooting, the camera was calibrated with a Datacolor Spyder CHECKR™ calibration plate. A single 40 mm x 40 mm square field of view (with a spatial resolution of 1 cm = 237 pixels) was analyzed in each image to obtain the morphogeometry and crumb cell characteristics by an image analysis program (Image J, NIH, USA). After cropping, the images were pre-processed and converted to 8-bit greyscale. Segmentation (thresholding) was performed manually, by binarization of greyscale images into black-and-white images using the Otsu algorithm according to Aleixandre et al. [1]. The samples were characterized by several parameters that were extracted and calculated: cells count (objects); mean and total cell area (cm^2); mean cell density (cells/cm^2) calculated by dividing the number of objects by the mean cell area, circularity (cell shape), and surface porosity (%) calculated as the coefficient between total cell area and total crumb studied area [25]. The results are expressed as mean values from six different measurements randomly cropped in the bread slices.

2.2.5. Chemical Characteristics

The chemical properties were evaluated for all flours and newly formulated breads. Moisture and ash content were performed according to the methods ISO 712:2009 and ISO 2171:2007 [18, 19], respectively. The lipid concentration was measured according to Soxhlet method 136 ICC [17]. The protein content was determined following the modified Lowry method, as described by Mæhre et al. [24]. The content of total, soluble and insoluble fiber was assessed as in methods AACC 32–07.01 [2]. Carbohydrate content was calculated by difference. The total polyphenols and antioxidant activity capacity using the DPPH method were investigated according to the method presented by Nakov et al. [26]. The results for total polyphenols were expressed as g galic acid equivalent (GAE)/100 g DM. The antioxidant activity was recorded as percentage of inhibition of the DPPH radical. For each test, the measurements were performed in triplicate.

2.2.6. Fatty Acid Profile

Fatty acid profile of flours (wheat flour and buckwheat flour) as well as of different breads was determined as described by Bligh and Dyer [3]. Methyl esters of fatty acids were obtained using chloroform and methanol as solvent. A Shimadzu GC-2010 chromatograph (Kyoto, Japan) equipped with a capillary column CP7420 (100 m x 0.25 mm i.d., 0.2 m, Varian Inc., Palo Alto, CA) and a flame ionization detector (FID) were employed. The results were expressed as g of fatty acid per 100 g total fatty acids.

2.2.7. Sensory Analysis

Twenty untrained panelists who habitually consume bread carried out the sensory evaluation of newly formulated breads. The different formulations were evaluated by overall acceptability using a nine-point hedonic scale (9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much; 1 = dislike extremely) [22].

2.2.8. Statistical Analysis

One-way analysis of variance (ANOVA) and, when significant, Fisher's Least Significant Difference test (LSD) at $p < 0.05$ were performed with the software XL STAT 2019 (Addinsoft Inc. Long Island City, NY, USA) and Office Excel 2016 (Microsoft, Redmond, WA, USA).

3. Results and Discussion

3.1. Physical Characteristics

Physical characteristics (width, thickness, volume, specific volume, hardness, springiness, cohesiveness and resilience) are presented in Table 1. These characteristics in bread are formed during the process of baking and are influenced by the dough processing, baking process and used ingredients [26]. Although the dough was leavened in molds before the baking process giving the breads' width fixed values, the addition of WBF actually showed an influence on this parameter decreasing it significantly ($p<0.05$) from 104.78 to 100.50 mm. Significant reduction ($p<0.05$) in thickness (from 103.95 to 62.13 mm), volume (from 1018.50 to 755.45 cm^3) and specific volume (from 2.43 to 1.82 cm^3/g) in the samples were determined.

The changes in these parameters are induced even at the lowest added concentration (10%) of WBF. The dough is a very complex system, where several phases have specific roles. The substitution of wheat flour implies changes in gluten network and starch concentration. However, reduced gluten concentration will influence the dough pore size, which additionally will affect other phenomena like heat transfer, mass transfer, biochemical reactions, starch gelatinization etc.[15]. Reduced specific volume of breads containing increasing amount of WBF is also confirmed by Fessas et al. [11].

Regarding textural characteristics, the control sample (100% wheat flour) is softer (1537.15 N) in comparison to the samples in which the wheat flour was substituted with 10, 20, 30, 40 and 50% WBF (1809.91; 1881.89; 2191.58; 2246.18 and 2825.21 N, respectively). Springiness for the analyzed samples was not significantly different ($p<0.05$). Cohesiveness of the bread with 50% substituted WF with WBF was statistically the lowest (0.40) in comparison to other samples. Chewiness of the analyzed samples was measured as 663.79 N for control bread and rose up to 958.67 N for the bread made with composite flour of 50% WF and 50% WBF. Even minimal substitution of WF with WBF causes significant reduction ($p<0.05$) of the resilience parameter.

Baking is the phase in which the dough transforms into bread with specific characteristics (texture, volume, color). The color formation of the crust is linked with Maillard reaction (reaction between amino groups and carboxyl groups) [26]. In Table 1 color data are shown for the crust and crumb of different types of bread, determined with CIE L* a* b* system. ANOVA (not shown) demonstrated significant differences ($p<0.05$) between crust and crumb color for the bread that contain WBF and the control one. L* values of crust and crumb are decreasing i.e. the samples are turning darker with the augmentation of WBF in breads, whereas the control bread has the lightest color of crust and crumb (53.31 and 53.10, respectively). The values for a* (green-red) and b* (blue-yellow) measured for the crust and crumbs are the highest for the bread with the highest amount of WBF (a* crust 2.62, crumb 12.50; b* crust 17.16, crumb 27.92). This result implies that the WBF incorporation intensifies yellow and red tones due to the composition of the grain that contains minerals and biologically active components having darker color [14]. The same results in color when using WBF were demonstrated by Nasir et al. [27]. Bread with greater specific volume has higher porosity, better appearance and is softer. The link between bread pores and their structure is considered very important. However, the degree of their expressiveness is not fully understood [38]. Thin slices of various types of buckwheat substituted bread were scanned to be examined for the alveolar homogeneity and crumb structure as predominant quality indicators in texture properties by Image J analysis. For high quality bread, small and uniform pores are prerequisite. The number of pores shall be high as small number indicates gas leakage during fermentation resulting gap in the gluten network [31]. The cross-sectional images of bread loaves where the wheat flour was replaced with WBF in different quantity (0, 10, 20, 30, 40, and 50%) are presented in Figure 1, whereas the crumb features are presented in Table 2.

The partial replacement of wheat flour with whole buckwheat flour increased the total count of the pores from 1648 for the control sample to 2284 for the sample containing 50% WBF. In addition, as the pore count increased so did the pore area from 30.7% to 43.6% for the highest amount of added WBF with the respect to the control sample. This result can be interpreted as dilution of gluten proteins and weakening the gluten matrix caused by buckwheat flour which is high in fiber, consequently changing the appearance of the baked product [33]. However, this did not influence the circularity of the alveoli, which had 0.78-0.79 of a perfect circular shape and did not differ one from another. What is normally expected to be seen is the irregular shape of the alveoli, due to the soluble non-starch polysaccharides present in the buckwheat that affect the gluten matrix which is anyway in a lower content. However, the globular proteins found in the buckwheat flour might act as gas/dough interface surfactant, counterbalancing the previous effect. The highest total area was observed for the breads enriched with the highest concentration of buckwheat flour (7.11 mm), rather than the control bread, gradually decreasing up to (5.01 mm). The increased surface area is in accordance with the increased value of pore count. Likewise, the average size of the alveoli was observed to gradually decrease from 0.00328 mm to 0.00217 mm in the breads prepared with WBF in the range of 0 to 50%, respectively. The size of the alveoli is linked with the gluten elasticity that expands while incorporating air throughout fermentation, while the buckwheat protein do not have the same elasticity and cannot greatly expand under the gas pressure, resulting in more dense and compact fortified breads [9].

Table 1

Mean values (±standard deviation) of the physical characteristics of the wheat flour (WF) and whole buckwheat (WBF) enriched breads

		WF	10%WBF	20% WBF	30% WBF	40% WBF	50% WBF
Width [mm]		104.78^{a}±0.31	104.11^{a,b}±0.30	103.33^{b,c}±0.17	102.72^{c}±0.25	104.66^{d}±0.33	100.50^{e}±0.71
Thickness [mm]		103.95^{a}±0.07	80.53^{b}±0.39	70.63^{c}±0.10	67.84^{d}±0.23	63.35^{e}±0.49	62.13^{f}±0.18
Volume [cm^3]		1018.50^{a}±2.12	922.69^{b}±0.01	916.72^{b}±0.00	866.18^{c}±12.69	837.10^{d}±0.14	755.45^{e}±0.64
Specific volume [cm^3/g]		2.43^{a}±0.04	2.20^{b}±0.00	2.18^{b}±0.01	2.08^{c}±0.01	2.00^{d}±0.01	1.82^{e}±0.01
Hardness [N]		1537.15^{c}±7.80	1809.91^{b,c}±6.87	1881.89^{b,c}±7.63	2191.58^{b}±8.74	2246.18^{b}±6.86	2825.21^{a}±4.15
Springiness		0.91^{a}±0.00	0.86^{a}±0.07	0.86^{a}±0.07	0.84^{a}±0.02	0.83^{a}±0.01	0.82^{a}±0.01
Cohesiveness		0.57^{a}±0.02	0.48^{b}±0.02	0.45^{c}±0.01	0.45^{c}±0.00	0.42^{c,d}±0.01	0.40^{d}±0.01
Chewiness [N]		663.79^{b}±23.63	663.79^{b}±20.45	759.36^{a,b}±21.89	791.95^{a,b}±21.89	842.56^{a,b}±3.20	958.67^{a}±6.60
Resilience		0.25^{a}±0.01	0.19^{b}±0.01	0.17^{b,c}±0.01	0.17^{b,c}±0.01	0.16^{c}±0.01	0.15^{c}±0.00
Crust	*L**	53.31^{a}±3.26	47.64^{b}±1.51	46.36^{b,c}±1.48	45.54^{b,c}±2.36	45.16^{b,c}±3.08	43.50^{c}±4.38
	*a**	6.72^{b}±1.75	7.58^{b}±1.15	11.90^{a}±1.11	12.18^{a}±0.87	12.58a±0.89	12.50^{a}±0.75
	*b**	23.90^{b}±1.00	24.26^{a,b}±3.43	25.34^{a,b}±2.68	25.96^{a,b}±0.62	26.04^{a,b}±3.42	27.92^{a}±3.67
Crumb	*L**	53.10^{c}±1.55	56.80$^{b,c±}$1.88	6.38^{a,b}±5.68	61.04^{a}±1.37	62.22^{a}±3.51	64.34^{a}±2.25
	*a**	-1.18^{f}±0.11	-0.26^{e}±0.18	0.58^{d}±0.11	1.46^{c}±0.21	2.12^{b}±0.32	16.54^{a}±0.77
	*b**	10.80^{e}±0.73	12.52^{d}±0.59	13.74^{c}±0.97	15.54^{b}±0.26	16.54^{a}±0.77	17.16^{a}±0.65

The means are computed from three repetitions. Values in the same row with different exponents are significantly different ($p<0.05$) following Fisher\`s LSD test

Table 2

Mean values (±standard deviation) of crumb features of white flour (WF) and whole buckwheat (WBF) enhanced breads

Slice	Count	Total area [mm]	Average size	Area [%]	Circ.
0% WBF	1648.6^{b}±155	5.01^{d}±0.45	0.0033^{a}±0.26	30.70^{d}±2.7	0.786^{a,b,c}±0.00
10% WBW	1762.2^{b}±136	5.29^{d}±0.20	0.0030^{a}±0.00	32.50^{d}±1.2	0.791^{a,b}±0.01
20% WBW	1764.8^{b}±194	5.46^{c,d}±0.33	0.0029^{a}±0.25	33.55^{c,d}±2.0	0.791^{a,b}±0.01
30% WBW	1817.4^{b}±51	5.98^{b,c}±0.08	0.0025^{a}±0.28	36.68^{b,c}±0.5	0.784^{b,c}±0.01
40% WBW	2280.4^{b}±52	6.21^{b}±0.33	0.0023^{a}±0.21	38.09^{b} ±2.1	0.780^{c}±0.01
50% WBW	2284.4^{a}±99	7.11^{a}±0.25	0.0022^{a}±0.18	43.58^{a}±1.6	0.794^{a}±0.00

The means are computed from three repetitions. Values in the same column with different exponents are significantly different ($p<0.05$) following Fisher`s LSD test.

Table 3

Fatty acids composition of lipid form wheat flour (WF), whole buckwheat flour (WBF) and bread with various portion of WBF obtained by GC analysis

Component [g/100 g oil]	WF	WBF	0% WBF	10% WBF	20% WBF	30% WBF	40% WBF	50% WBF
Palmitic acid	30.53±0.73	15.05±0.73	22.22^{a}±0.08	20.28^{b}±0.55	19.69^{b}±0.81	16.28^{c}±0.28	14.15^{d}±0.21	13.50^{d}±0.47
Stearic acid	13.38±0.13	4.86±0.20	13.42^{a}±0.22	13.31^{a}±0.54	12.81^{a}±0.16	9.76^{b}±0.17	8.90^{c}±0.15	8.39^{c}±0.12
Linoleic acid	31.72±0.23	20.76±0.16	31.51^{a}±0.03	29.59^{b}±0.57	27.57^{c}±0.53	25.54^{d}±0.49	23.51^{e}±0.45	21.48^{f}±0.41
γ linolenic acid	0.33±0.01	0.34±0.01	0.33^{a}±0.01	0.32^{a}±0.02	0.32^{a}±0.01	0.33^{a}±0.01	0.33^{a}±0.01	0.33^{a}±0.02
α linolenic acid	0.19±0.10	0.29±0.01	0.17^{d}±0.03	0.22^{c}±0.01	0.24^{b,c}±0.01	0.26^{a,b}±0.00	0.28^{a}±0.02	0.29^{a}±0.01
Oleic acid	0.05±0.01	47.02±0.02	0.09^{f}±0.04	4.76^{e}±0.00	9.44^{d}±0.03	14.12^{c}±0.06	18.80^{b}±0.09	23.48^{a}±0.12
TS	44.29±0.11	21.39±0.59	41.92^{a}±2.10	36.49^{b}±0.08	35.94^{b,c}±0.03	34.44^{b,c}±0.68	33.28^{c,d}±0.86	31.04^{d}±0.86
TMUS	19.78±0.01	47.59±0.59	20.90^{d}±0.47	32.74^{c}±0.18	34.32^{b,c}±2.69	36.42^{a,b}±0.62	37.33^{a}±0.02	39.01^{a}±0.78
TPUS	35.41±0.74	30.17±0.67	35.00^{a}±0.78	34.02^{a}±0.33	28.72^{b}±0.42	26.86^{c}±0.47	25.09^{d}±0.89	20.71^{e}±0.61

TS: total saturated fatty acids content; TMUS: total monounsaturated fatty acids content; TPUS: total polyunsaturated fatty acids content; Breads values in the same row with different letters are significantly different ($p<0.05$) following Fisher`s LSD test.

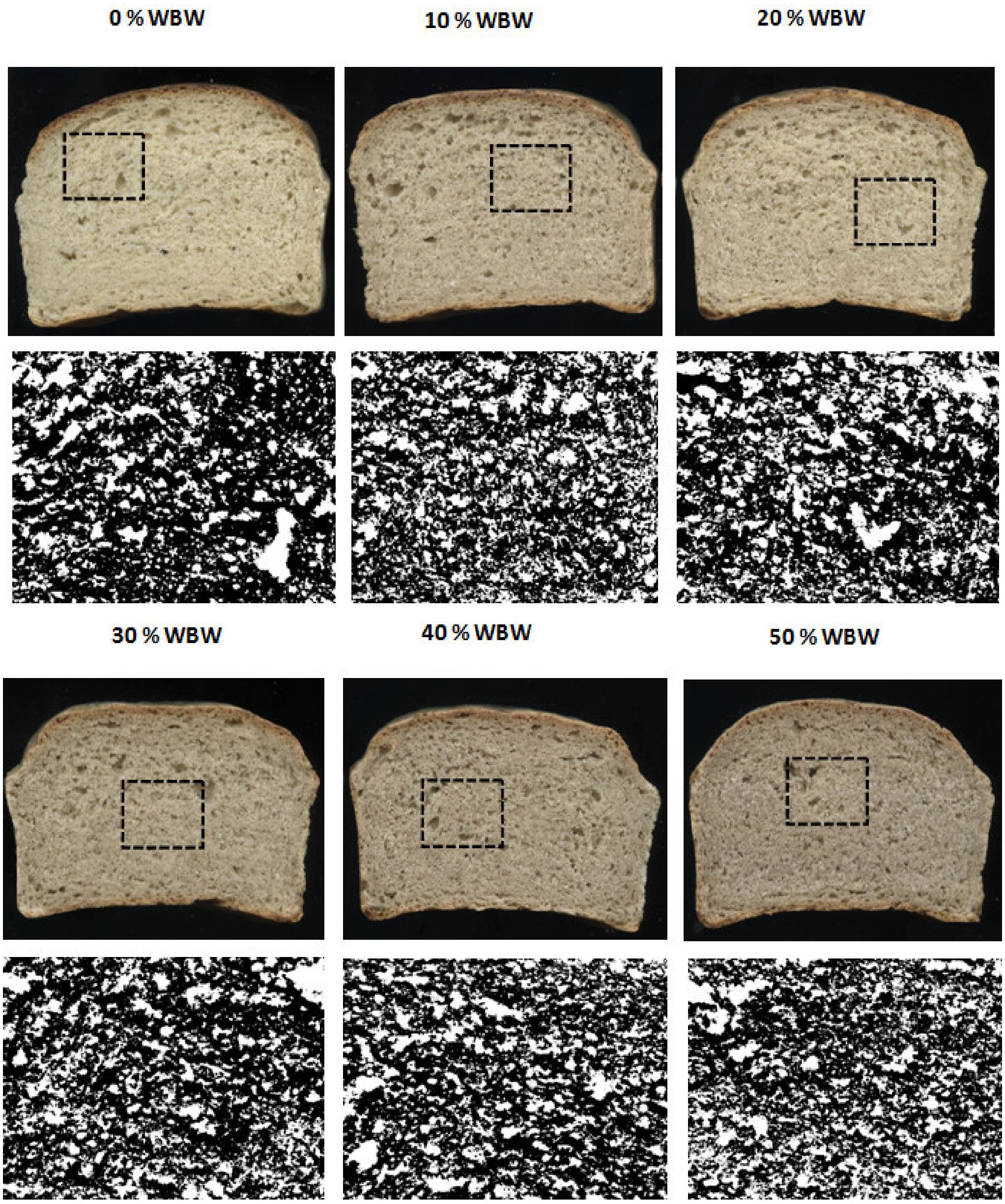

Fig. 1. *Appearance of bread made of buckwheat flour (as a substitution of wheat flour in concentration 0, 10, 20, 30, 40, 50%) and details of their porous structure obtained by Image J analysis software*

3.2. Chemical Characteristics

The ANOVA for moisture, ash, lipid, protein dietary fiber (total dietary fiber - TDF, soluble dietary fiber - SDF and insoluble dietary fiber - IDF), total carbohydrates, total phenols and antioxidant activity highlighted significant differences among the six breads types. All chemical components and their part in breads with different quantity of WBF, as well as the flours, are presented in Table 3.

The control bread (0% WBF) has the highest moisture (34.39 g/100g) that progressively decreases as the WBF fraction was increased in breads, reaching 31.13g/100 g in the bread with 50% WBF. Mineral content is higher in WBF in comparison to WF (1.66 g/100g and 0.46 g/100g, respectively), while the breads containing higher content of WBF also have high contents of minerals. This result was expected knowing the fact that in WBF grain bran is included bearing high quantity of micro and macro elements [25]. Lipid content in WBF is higher (3.42 g/100g) in comparison to WF (2.11 g/100g). From the results presented in Table 3 can be noticed higher lipid content in breads with higher WBF incorporation. According to Christa and Soral-Śmietana [7], lipid content in buckwheat flour is not greater than 3%. Our results are in accordance with the results presented by Nikolić et al. [28] which examined the lipid quantity in WF and BF in blends in different ratios (3, 5, 10, 20 and 30 g/100g). They determined the lipid content in BF as 2.2 g/100g, and by increasing the part of this flour in the mixture, the lipid content increased. Protein content was found to be lower in WF (13.21 g/100g) in comparison to WBF (16.23 g/100g) (Table 3). Different researches published different values for protein content in BF in ranges from 10.55 to 16.4g/100g [5, 23]. The difference comes from different fractions obtained during milling that are present in BF in various proportions, besides the type and species of the buckwheat grain. Protein content follows the same trend i.e. it increases as the WBF substitution amount gradually increases. From the limited published results on protein content in bread fortified with 50% BF Stokić et al. [34] found 14.00 g/100 g dry matter. Polyphenols and dietary fibers (soluble and insoluble) present in WBF contribute to functional properties of breads enriched with WBF. From the results presented in Table 3 it can be noticed that the augmentation of TDF, IDF and SDF is almost linear as the quantity of WBF gets higher as part of the bread. SDF (oligosaccharides, gums, pectins, beta glucans) are digested in humans' colon. These fibers reduce the cholesterol in blood and control sugar level in blood among other benefits. IDF (resistant starch, cellulose, hemicellulose, and lignin) are not digestible in the gut and improve peristalsis; additionally, they are partially fermented in the colon and thus support the growth of intestinal microflora (including probiotic bacteria) [12]. The quantity of total carbohydrates decreases with increment of the WBF part in the bread from 83.91 to 80.03 g/100g dry matter (Table 3). There were significant differences in total polyphenolic content (TPC) and antioxidant activity (AOA) in samples with different WBF quantity (ANOVA not shown). The quantity of TPC and AOA in the control sample were at the lowest level and grow linearly with gradual increasing of the WBF amount in the bread samples (Table 3) reaching the highest level in the bread with 50% WBF (2.40 g/100g DM and 13.24% DPPH, respectively). The most common polyphenols in BF are rutin and quercetin which have the ability to reduce the digestibility of proteins [23]. Higher level of TPC and AOA in bread samples with more WBF come from their initial higher content in the flour [8]. However, TPC and

AOA content depend on type, species, and agro-technical conditions of cultivation of the plant [7]. Molecular complexes between bioactive compounds and starch or proteins from flour can be formed in food systems, which are influenced by the nutritional content, pH, temperature and consequently to change bioactive profile in the samples [29]. The quantity of fatty acids contained in WF, WBF, additionally in breads prepared with substitution of WF with WBF are presented in Table 3. The ANOVA for all fatty acid (TS: total saturated fatty acids content, TMUS: total monounsaturated fatty acids content, TPUS: total polyunsaturated fatty acids content) highlighted significant differences ($p<0.05$) among the six analysed bread types. Saturated fatty acids in WF were in quantity of 44.29 g/100 g fats; whereas the content of the same in WBF was two times lower (21.39 g/100 g fats). In addition, WF has higher content of polyunsaturated fatty acids (35.41 g/100 g fats) with the linoleic acid at the highest concentration 30.17 g/100 g fats. On the other hand, the fatty acids present in lower quantity in WBF have an important role in determining the quality of food produced from this type of flour [21]. Generally, fatty acids in buckwheat grain are mono- and polyunsaturated [16]. The quantity of monounsaturated fatty acids in WBF was found to be 47.59 g/100 g fats. This value is two times higher than the one found in WF (19.78 g/100 g fats). The main representative of this group of fatty acids is oleic acid, which in WF is 0.05 g/100 g fats, while in WBF its content was 47.02 g/100 g fats. The use of WBF in bread production leads to creation a product with lower content of the unwanted saturated fatty acids. The reduction of the saturated acids in our samples with various amounts of WBF is due to the lower content of palmitic and stearic fatty acids in WBF (15.05 and 4.86 g/100 g fats, respectively). The use of WBF in bread production also leads to reduction of linoleic acid as the percentage of WBF in bread increases (from 31.51 g/100 g fats in control bread to 21.48 g/100 g fats in bread with 50% WBF).

3.3. Sensory Analysis

The choice of the consumers for food products is influenced by visual and sensory impression. Therefore, in the process of development of new products, sensory test is mandatory that will determine the acceptance of the product. This analysis presented on Figure 2a shows that among all bread samples that contain WBF in different quantities, the one with 30% is highly liked. The overall acceptance of newly formulated breads is presented on Figure 2b. In this case, the same sample is the most accepted for the consumers. Literature data related to the influence of WBF on the sensory profile of bread and other bakery products is scarce and very often refers to blends of buckwheat flour and other types of flour for production of “gluten-free” products, which is different from our goal in this paper. Therefore, further studies are needed regarding the sensory acceptability of bread made of blend of wheat and buckwheat flour [8].

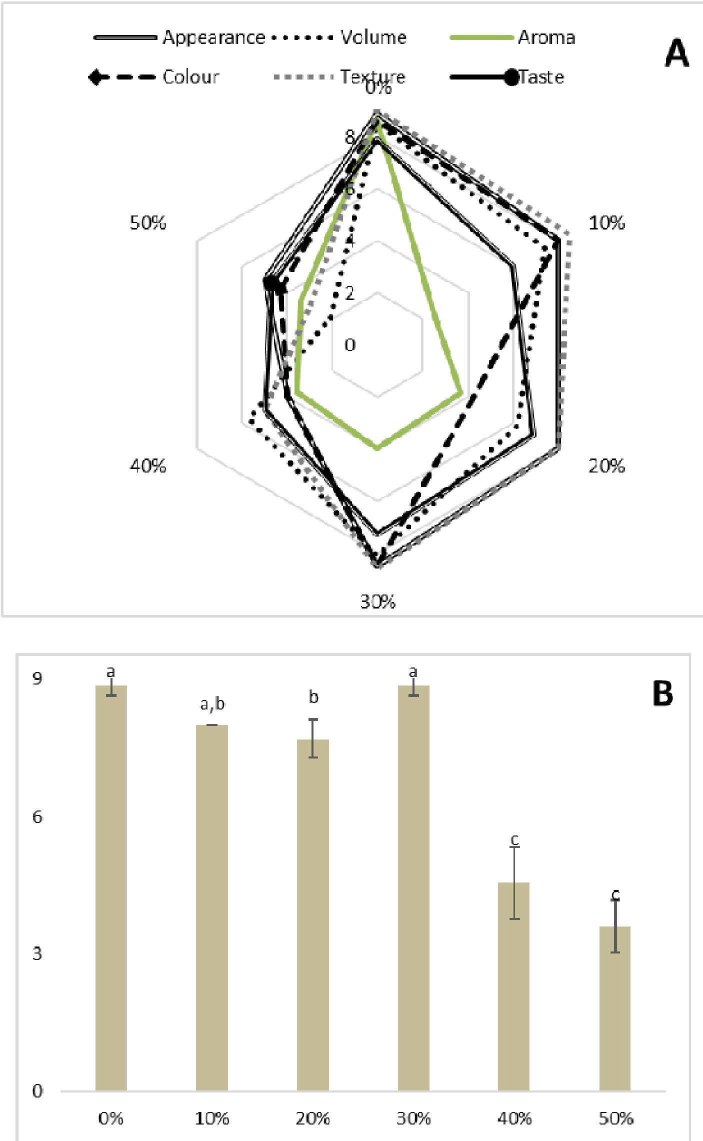

Fig. 2. *Sensory characteristics (A) and overall sensory quality (B) of six bread with increasing quantities of whole buckwheat flour (0, 10, 20, 30, 40 and 50). Scale from 1 (dislike extremely) to 9 (like extremely). Columns with different letters are significantly different (p<0.05) following Fisher`s LSD test*

4. Conclusions

Results in this study highlight the advantages of use of whole buckwheat flour in combination with white wheat flour in bread production with ameliorated nutritional properties and reduced content of gluten. Bread fortified with whole buckwheat flour has inferior physical characteristics (width, thickness, volume and specific volume); however, it contains higher content of ash, fats, dietary fiber (total, soluble and insoluble) as well as total polyphenols and antioxidant activity. The quantity of oleic acid is increasing with the content of WBF in breads. The sensory analyses showed that the bread containing 30% whole buckwheat flour is highly accepted and appreciated by consumers. Therefore, the blend of white wheat flour and whole buckwheat flour can offer new functional cereal product with health benefits, reduced gluten content and high content of biologically active compounds.

Acknowledgements

The financial support of this work by the Bulgarian Ministry of Education and Science, National Research Fund under the contract number КП-06-М56/3-2021 is gratefully acknowledged.

References

1. Aleixandre A., Benavent-Gil Y., Velickova E. et al., 2021. Mastication of crisp bread: Role of bread texture and structure on texture perception. In: Food Research International, vol. 147, ID article 110477. DOI: 10.1016/j.foodres.2021.110477.
2. American Association of Cereal Chemists (AACC) International, 2000. Approved methods of analysis. 10th Edition. Method 32-37. St. Paul, MN, USA.
3. Bligh E.G., Dyer W.J., 1959. A rapid method of total lipid extraction and purification. In: Canadian Journal of Biochemestry and Physiology, vol. 37(8), pp. 911-917.
4. Bojňanská T., Frančáková H., Chlebo P. et al., 2009. Rutin content in buckwheat enriched bread and influence of its consumption on plasma total antioxidant status. In: Czech Journal of Food Science, vol. 27(1), pp. 5236-5240. DOI: 10.17221/967-CJFS.
5. Burešová I., Tokár M., Mareček J. et al., 2017. The comparison of the effect of added amaranth, buckwheat, chickpea, corn, millet and quinoa flour on rice dough rheological characteristics, textural and sensory quality of bread. In: Journal of Cereal Science, vol. 75, pp. 158-164. DOI: 10.1016/j.jcs.2017.04.004.
6. Chlopicka J., Pasko P., Gorinstein S. et al., 2012. Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereal breads.In: LWT – Food Science and Technology, vol. 46(2), pp. 548-555. DOI: 10.1016/j.lwt.2011.11.009.
7. Christa K., Soral-Śmietana M., 2008. Buckwheat grains and buckwheat products - Nutritional and prophylactic value of their components - A review. In: Czech Journal of Food Science, vol. 26(3), pp. 153-162. DOI: 10.17221/1602-cjfs.
8. Coronel E.B., Guiotto E.N., Aspiroz M.C. et al., 2021. Development of gluten-free premixes with buckwheat and chia flours: Application in a bread product. In: LWT – Food Science and Technology, vol. 141, ID article 110916. DOI: 10.1016/j.lwt.2021.110916.
9. Dhen N., Ben Rejeb I., Boukhris H. et al., 2018. Physicochemical and sensory properties of wheat- Apricot kernels composite bread. In: LWT – Food Science and Technology, vol. 95, pp. 262-267. DOI: 10.1016/j.lwt.2018.04.068.
10. Dimov I., Georgieva A., 2013. Quality indicators of wheat bread enriched with buckwheat flour. In: Mag. Food Indicators, vol. 3, pp. 44-45.

11. Fessas D., Signorelli M., Pagani A. et al., 2008. Guidelines for buckwheat enriched bread: Thermal analysis approach. In: Journal of Thermal Analysis and Calorimetry, vol. 91(1), pp. 9-16. DOI: 10.1007/s10973-007-8594-6.
12. Foschia M., Peressini D., Sensidoni A. et al., 2013. The effects of dietary fibre addition on the quality of common cereal products. In: Journal of Cereal Science, vol. 58(2), pp. 216-227. DOI: 10.1016/j.jcs.2013.05.010.
13. Giménez-Bastida J.A., Piskula M.K., Zieliñski H., 2015. Recent advances in processing and development of Buckwheat derived bakery and non-bakery products - A review. In: Polish Journal of Food and Nutrition Sciences, vol. 65(1), pp. 9-20. DOI: 10.1515/pjfns-2015-0005.
14. Gómez M., Gutkoski L.C., Bravo-Núñez Á., 2020. Understanding whole-wheat flour and its effect in breads: A review. In: Comprehensive Reviews in Food Science and Food Safety, vol. 19(2), pp. 3241-3265. DOI: 10.1111/1541-4337.12625.
15. Grassi de Alcântara R., Aparecida de Carvalho R., Maria Vanin F., 2020. Evaluation of wheat flour substitution type (corn, green banana and rice flour) and concentration on local dough properties during bread baking. In: Food Chemestry, vol. 326, ID article 126972. DOI: 10.1016/j.foodchem.2020.126972.
16. Huda M.N., Lu S., Jahan T. et al., 2021. Treasure from garden: Bioactive compounds of buckwheat. In: Food Chemestry, vol. 335, ID article 127653. DOI: 10.1016/j.foodchem.2020.127653.
17. International Association for Cereal Chemistry (ICC), 2014. Standard method: 14026. In: ICC Standard Methods. Vienna, Austria.
18. International Standard Organization (ISO), 2007. ISO 2171:2007: Cereals, pulses and by-products — Determination of ash yield by incineration, Vienna, Austria.
19. International Standard Organization (ISO), 2009. ISO 712:2009 - Determination of moisture content. Vienna, Austria.
20. Janovská D., Jágr M., Svoboda P. et al., 2021. Breeding buckwheat for nutritional quality in the czech republic. In: Plants, vol. 10(7), ID article 1262. DOI: 10.3390/plants10071262.
21. Krumina-Zemture G., Betaine I., 2017. Fatty acid composition in buckwheat (*Fagopyrum esculentum* M.) flours and their extrudet products. In: Proceedings of the 8th International Scientific Conference, 23-24 November, 2017, Aleksandras Stulginskis University. DOI: 10.15544/RD.2017.017.
22. Lim J., 2011. Hedonic scaling: A review of methods and theory. In: Food Quality and Preference, vol. 22(8), pp. 733-747. DOI: 10.1016/j.foodqual.2011.05.008.
23. Luthar Z., Zhou M., Golob A. et al., 2021. Breeding buckwheat for increased levels and improved quality of protein. In: Plants, vol. 10(1), ID article 14. DOI: 10.3390/plants10010014.
24. Mæhre H.K., Dalheim L., Edvinsen G.K. et al., 2018. Protein determination—method matters. In: Foods, vol. 7(1), ID article 5. DOI: 10.3390/foods7010005.
25. Morreale F., Garzón R., Rosell C.M., 2018. Understanding the role of hydrocolloids viscosity and hydration in

developing gluten-free bread. A study with hydroxypropylmethylcellulose. In: Food Hydrocolloids, vol. 77, pp. 629-635. DOI: 10.1016/j.foodhyd.2017.11.004.

26. Nakov G., Brandolini A., Ivanova N. et al., 2018. The effect of einkorn (*Triticum monococcum* L.) whole meal flour addition on physico-chemical characteristics, biological active compounds and in vitro starch digestion of cookies. In: Journal of Cereal Science, vol. 83, pp. 116-122. DOI: 10.1016/j.jcs.2018.08.004.
27. Nasir S., Allai F.M., Gani M. et al., 2020. Physical, textural, rheological, and sensory characteristics of amaranth-based wheat flour bread. In: International Journal of Food Science, vol. 2020, ID article 8874872. DOI: 10.1155/2020/8874872.
28. Nikolić N., Sakač M., Mastilović J., 2011. Effect of buckwheat flour addition to wheat flour on acylglycerols and fatty acids composition and rheology properties. In: LWT – Food Science and Technology, vol. 44(3), pp. 650-655. DOI: 10.1016/j.lwt.2010.08.017.
29. Păcularu-Burada B., Turturică M., Rocha J.M. et al., 2021. Statistical approach to potentially enhance the postbiotication of gluten-free sourdough. In: Applied Sciecne, vol. 11(11), ID article 5306. DOI: 10.3390/app11115306.
30. Pentikäinen S., Sozer N., Närväinen J. et al., 2014. Effects of wheat and rye bread structure on mastication process and bolus properties. In: Food Research International, vol. 66, pp. 356-364. DOI: 10.1016/j.foodres.2014.09.034.
31. Protonotariou S., Stergiou P., Christaki M. et al., 2020. Physical properties and sensory evaluation of bread containing micronized whole wheat flour. In: Food Chemestry, vol. 318, ID article 126497. DOI: 10.1016/j.foodchem.2020.126497.
32. Przygodzka M., Piskula M.K., Kukurová K. et al., 2015. Factors influencing acrylamide formation in rye, wheat and spelt breads. In: Journal of Cereal Science, vol. 65, pp. 96-102. DOI: 10.1016/j.jcs.2015.06.011.
33. Robles-Ramírez M., del C. Ortega-Robles E., Monterrubio-López R. et al., 2020. Barley bread with improved sensory and antioxidant properties. In: International Journal of Gastronomy and Food Science, vol. 22, ID article 100279. DOI: 10.1016/j.ijgfs.2020.100279.
34. Stokić E., Mandić A., Sakač M. et al., 2015. Quality of buckwheat-enriched wheat bread and its antihyperlipidemic effect in statin treated patients. In: LWT – Food Sceince and Technology, vol. 63(1), pp. 556-561. DOI: 10.1016/j.lwt.2015.03.023.
35. Świeca M., Regula J., Suliburska J. et al., 2020. Safeness of diets based on gluten-free buckwheat bread enriched with seeds and nuts—effect on oxidative and biochemical parameters in rat serum. In: Nutrients, vol. 12(1), ID article 41. DOI: 10.3390/nu12010041.
36. Torbica A., Hadnadev M., Dapčević T., 2010. Rheological, textural and sensory properties of gluten-free bread formulations based on rice and buckwheat flour. In: Food Hydrocolloids, vol. 24(6-7), pp. 626-632. DOI: 10.1016/j.foodhyd.2010.03.004.
37. Vogrinčič M., Timoracka M.,

Melichacova S. et al., 2010. Degradation of rutin and polyphenols during the preparation of tartary buckwheat bread. In: Journal of Agricultural and Food Chemestry, vol. 58(8), pp. 4883-4887. DOI: 10.1021/jf9045733.

38. Wang S., Karrech A., Regenauer-Lieb K. et al., 2013. Digital bread crumb: Creation and application. In: Journal of Food Engineering, vol. 116(4), pp. 852-861. DOI: 10.1016/j.jfoodeng.2013.01.037.