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DEVELOPMENT OF ORGANIC BUCKWHEAT GLUTEN-FREE BREAD, CHARACTERIZED BY A HIGH LEVEL OF BIOACTIVE COMPOUNDS

Summary

In the present work the formulation of gluten - free bread made of wholegrain buckwheat flour or mixtures of this flour with rice and maize flour (each on substitution level 20%) was evaluated. The buckwheat flour was selected from organic flours available on market after evaluation of their chemical characteristics, mainly on the basis of phenolic compounds content and antioxidant activity. The breads were prepared with the use of buckwheat sourdough obtained with starter culture composed of autochthonous lactic acid bacteria strains. The obtained breads were characterized in terms of quality and biologically active phenolic compounds content and antioxidant activity (AOA). The deprivation of total phenols and AOA in bread in relation to their content in the flour reached 48%. In conclusion, the results demonstrated that organic buckwheat flour could be used as a sole raw material for baking GF bread, acceptable in terms of taste and flavor and characterized by high amounts of phenolic compounds (on the level 387 mg GAE/100g) and antioxidant activity).
***Key words**: gluten free bread, buckwheat flour, phenolic compounds, sourdough*

OPRACOWANIE EKOLOGICZNEGO, GRYCZANEGO PIECZYWA BEZGLUTENOWEGO, CHARAKTERYZUJĄCEGO SIĘ WYSOKIM POZIOMEM ZWIĄZKÓW BIOAKTYWNYCH

Streszczenie

W niniejszej pracy oceniono możliwość otrzymywania pieczywa bezglutenowego, wykonanego z mąki gryczanej pełnoziarnistej lub mieszaniny tej mąki z mąką ryżową i kukurydzianą (każda zastępująca mąkę gryczaną na poziomie 20%. Mąkę gryczaną wybrano z dostępnych na rynku, ekologicznych mąk po przeprowadzeniu ich oceny pod względem składu chemicznego, zwłaszcza na podstawie zawartości związków fenolowych i aktywności antyoksydacyjnej. Pieczywo zostało przygotowane z wykorzystaniem zakwasów gryczanych otrzymanych z kulturą starterową, składającą się z autochtonicznych szczepów bakterii fermentacji mlekowej. Uzyskane chleby zostały scharakteryzowane pod względem jakości i zawartości biologicznie aktywnych fenolowych i aktywności antyoksydacyjnej. Zmniejszenie zawartości związków fenolowych i AOA w chlebie w stosunku do ich zawartości w mące sięgało 48%. Podsumowując, otrzymane wyniki wskazują, że ekologiczna mąka gryczana może być stosowana jako jedyny surowiec do wypieku chleba GF, akceptowalnego pod względem smaku i zapachu i charakteryzującego się dużą zawartością związków fenolowych (na poziomie 387 mgGAE/100g) i aktywnością przeciwutleniającą.
***Słowa kluczowe**: pieczywo bezglutenowe, mąka gryczana, związki fenolowe, zakwasy piekarskie*

1. Introduction

It is estimated that currently about 1% of the human population suffers from the celiac disease and is forced to remain on a strictly gluten-free diet (GF- gluten free) [7, 8]. Besides the growing number of people remaining on the gluten-free diet for therapeutic reasons, an additional aspect of the increased interest in gluten-free food is the media spread opinion about the negative effects of gluten on the health of healthy people, so the number of people who follow the "fashion" of such a diet grows.

The effect of the long-term diet with elimination of gluten products, i.e. the most of the cereal products, results in animbalanced daily intake of nutrients. The level of B vitamins, dietary fiber and such trace elements as Mg, Fe, Zn in patients on a gluten-free diet is significantly lower than in the traditional nutrition. The gluten-free foods are often low in the fiber and such valuable biologically active ingredients of plant origin as polyphenols [8, 9, 32].

The bread production with the use of such ingredients or additives as starches, dairy products, egg proteins causes crumbling texture of bread and accelerates bread staling [8, 30]. Likewise, the lack of gluten in cereal flours affects their baking properties, having negative impact on the quality of produced bread (also pasta, cookies). The deterioration in volume and texture of GF bread is a consequence of lack of gluten network in GF dough. To imitate the unique rheological properties of wheat dough in GF dough enzymes are often used or hydrocolloids (hydroxypropyl methylcellulose - HPMC, guar –gum, xanthan-gum) [1, 9, 15, 18, 29].

Increasing the nutritional value of bread and improving the balance of a gluten-free diet can be achieved through the use, for bread production, of pseudo-cereals grains flour (quinoa, buckwheat and amaranth), considered as raw materials with high "nutrient density".

The proteins of buckwheat, quinoa and amaranth are comprised mainly of the soluble proteins - globulin and albumin, and a very low amount of prolamin [4, 5, 30]. The amino acid composition in the buckwheat is characterized by a high lysine content, amounting to about 6 mg/100 g of proteins, containing more of other amino acids such as arginine and less glutamic acid and proline than the prolamines [5]. The first limited amino acid in buckwheat is leucine [28]. The pseudocereals are a good source of fiber. In the grain of buckwheat a total dietary fiber content reaches value 27,38 [5].

Buckwheat contains a resistant starch and consist of a part of the suggested components of the proposed food with a low GI [2, 9, 14, 29]. Buckwheat seed are characterized by high antioxidant activity, the main antioxidants in buckwheat are rutin and quercetin [17, 25]. Among the compounds of pro-health importance, it contains phytosterols, fagopyritols, D-chiro-inositol, myo-inositol as well as components with healing benefits: flavonoids and flavones, therefore can be considered as a good source of phenolic compounds [2, 13, 25, 29]. The lipid composition in buckwheat is nutritionally advantageous/beneficial, in fatty acids prevail linoleic acid (C18:2) and oleic acid (C18:1) [5].

By dint of the high nutritional quality and easy accessibility, the use of buckwheat flour in bread making is growing. The addition of buckwheat flour gives the bread an intense flavor. Bread prepared with buckwheat flour easily grows, its structure is soft, but it is characterized by poor crumb and crust properties, presenting short shelf-life mainly because of bread staling [8]. Therefore, the most common buckwheat flour is used as an additive increasing the nutritional value of bread and the attempts of use of buckwheat flour as the sole raw material for bread are infrequent. An investigation by Mariotti et al. [2013] demonstrated that the inclusion of 40% dehulled and puffed buckwheat flour in substitution levels 40% does not reduce, but actually improves the baking performances of the commercial GF mixtures, based on starch. Moreover, the presence of a small amount of those flours turned out to prevent too fast loss of water from the bread crumb and reduced staling kinetics during storage. According to Torbica et al. [2010] mixtures of rice flour and husked or unhusked buckwheat flour (up to 30% of buckwheat) expressed rheological properties similar to wheat flour. Currently, the need for improving the nutritional value of GF bread should be associated with the attempts of increasing sensory quality of baked goods.

The ecologically friendly method of improving bread quality is sourdough (SD) technology, which is a well-established practice in the production of wheat and rye bread [3, 10, 11, 31]. Effects of microbial fermentation, induced by the lactic acid bacteria (LAB) and yeast, are associated with the decrease in pH in sourdough due to organic acids synthesized by LAB, resulting in, inter alia, activation of endogenous dough/flour proteases and release of metal ions of the phytate complex by endogenous phytases [14, 31]. In order to prevent malfermentation, currently starter cultures containing selected LAB or LAB and yeast are often used. Several studies have been performed on the microorganisms of sourdough prepared from cereals (wheat, rye, barley), GF cereals (oat, rice, maize, millet), pseudocereals (amaranth, quinoa, buckwheat) [19, 20, 32, 33]. It was found that during fermentation of different row materials specific microbiota established themselves, composed of the bacteria and yeast competitive in each ecological niche and well adapted to the substrates. It indicated, that in the case of sourdough fermentation of different substrates than common cereals, it is more effective to initiate it with suitable starter cultures composed of autochthonous microorganisms than to use commercial starter cultures. The use of SD technology appears to be the right tool to improve the bread making performance of GF grains in terms of the need for high-quality GF buckwheat bread with increased concentration of health beneficial compounds.

The aim of the work was to characterize organic buckwheat flour available on the Polish market and to develop gluten-free bread with high phenolic compounds and of dietary fiber content, characterized by antioxidant activity.

Therefore, this paper attempts to optimize the preparation of gluten-free buckwheat bread using starter cultures containing selected indigenous lactic acid bacteria (LAB).

2. Materials and methods

The experimental material consist of five batches of flour made from organic buckwheat, available in local stores. These flours were labeled as MG 1, MG 2, MG 3, MG 4, MG 5 and were produced by Polish manufactures. The samples MG3 and MG5 were different batches of flour produced by the same manufacturer. Two of the tested flours were labeled as whole-meal flour; in the case of the other samples the producers did not give any additional information. The authors do not wish to reveal the names of the companies producing the organic buckwheat flour, so as not to influence the choices of consumers and prevent exploitation of presented results in marketing. Rice and maize were used in baking test flour (protein content 7,9%; 6,9 %, respectively) as substitution of buckwheat flour. The control bread was made with the of organic wheat flour type 550 characterized by 29% gluten content. Chemical analyses (protein and ash content) were performed according to standard methods: protein content by Kjeldahl method, using a nitrogen to protein conversion factor of 5.75 [3] - PN EN ISO 20483: 2007, total ash and moisture content according to PN-EN ISO 2171: 2010 and PN-EN ISO 712:2012.

The dietary fiber content was evaluated using the Megazyme enzyme assay kit as described by the manufacturer.

Determination of antioxidant properties and phenolic compounds content was performed in samples extracted with a mixture of acetone and water in a ratio of 7: 3 (v: v) and with a mixture of methanol and water in a ratio of 7: 3 (v: v). The content of phenolic compounds was determined with the Folin-Ciocalteu reagent (with the use of spectrophotometric readings) and expressed in gallic acid as standard equivalents (GAE) [13,24]. The antioxidant activity was evaluated as total free radical scavenging capacity of flour extracts, using the ABTS + (2,2-azynobis- (3-ethylbenzthiazoline-6-sulfonate) [22] and free radical scavenging reagent DPPH (1,1-diphenyl-2-pikrylohydrazyl) [6, 21] and expressed in equivalents Trolox (TE) (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), used as a reference antioxidant. The alpha-tocopherol quantification was performed by HPLC according to standard method (PN-EN 12822: 2002).

Assessment of microbiological quality of flour was made according to standards PN-EN ISO 4833:2004, ISO 21528-2:2005, ISO 21527-2:2009, ISO 15214: 2002; BS EN ISO 7932: 2005; BS EN ISO 6887-1: 2000. The following was assessed: the number of yeast, lactic acid bacteria, bacteria of the species *Bacillus cereus* and *Bacillus subtilis,* spore-forming bacteria and the number of yeast and mold.

The results of the analyses of at least two independent experiments are reported by arithmetic means with standard deviation.

The baking experiments were conducted with the use of the buckwheat flour MG5 characterized by the highest dietary fiber content and phenolic compounds, as well as high antioxidant activity. In order to improve sensory characteristics of bread, 15% or 25% of the flour was introduced as sourdough (SD). The fermentation of sourdough was initiated with the starter culture containing two strains of *Pediococcus pentosaceus* ZFG2, *Pediococcus pentosaceus* ZFG4, in an amount of 0.5% relative to the flour. These strains were previously isolated from buckwheat sourdough. Buckwheat sourdough fermentation was conducted for 24 hours at 30°C. Sourdough was obtained with yield 300, what means proportion of ingredients 100 g flour to 200 g of water. An experiment was also made with buckwheat flour substitute for rice (20%) and maize (20%) flour and adding 45% buckwheat flour as SD. Here are the basic bread variants tested and the dough ingredients are given in table 1.

1 - 100% buckwheat bread with the use of buckwheat SD 15%,
2 - 100% buckwheat bread with the use of buckwheat SD 25%,
3 - Bread made with buckwheat flour (60%), maize flour (20%) and rice flour (20%) with use of buckwheat SD 45%,
4 -100 % wheat bread (control).

Table 1. The dough ingredients
Tab. 1. Skład ciasta

Ingredients, g	1 (15% SD)	2 (25% SD)	3 (45% SD)	4 (wheat bread)
Buckwheat flour	850	750	150	-
Buckwheat SD*	450	750	1350	-
Wheat flour	-	-	-	1000
Rice flour	-	-	200	-
Maize flour	-	-	200	-
Yeast	40	40	40	20
Salt	20	20	20	20
Guar gum	15	15	15	-
Oil	30	30	30	-
Sugar	30	30	30	30
Water	650	450	50	620

SD – sourdough. *Source*: own work / *Źródło*: praca własna

A baking test was conducted on three loaves from each bread type (including the control bread). In assessing the overall physical and chemical characteristics of sourdoughs and bread doughs according to PN-A-74100: 1992, the following tests were performed: determination of pH and total acidity by titration, expressed as titratable acidity in degrees (ml 0.1 mol NaOH/10g).

Performance and dough fermentation time were established. Bread quality was assessed according to Polish standard (PN-A-74108: 1996). Hardness of the bread crumb, one of the texture parameters, was measured using analyser Instron 1140, according to the manufacturer's instruction, at the compression rate 50 mm·min^{-1} and load 50 N. A Sample of bread crumb taken from the central part of the loaf with a height of 30 mm was pressed by 50%, by aluminium sampler with a diameter of 35 mm.

Hardness, expressed as the force (in N) required to achieve a predetermined deformation of the breads, was determined on the basis of the graph (the maximum peak height).

Statistical analysis

The data were statistically analysed using Statistica 8, StatSoft INC. An analysis of variance and post hoc test (Tukey) were performed.

3. Results and discussion

The chemical composition of buckwheat flours is given in Tables 2 and 3.

The analysis (Table 2) showed that the organic buckwheat flour on the Polish market differed in terms of chemical compounds irrespectively of declared type. The highest ash content was found in sample of flour MG3 (labeled as made from whole grain), samples MG1 and MG5 characterized similar level of ash, higher than in MG4 and MG2. Similarly the highest protein content (13.4 g/100g dw) was in flour MG5 and the lowest (9.1 g/100g dw) in MG2. The results of these analayses are similar to the characteristics of three varieties of buckwheat grain given by Stempińska and Soral-Śmietana [28]. The relatively high content of dietary fiber in buckwheat flour MG5 indicates their potential for preventative nutrition in GF diet.

This flour has the highest content of polyphenols and antioxidant activity (Table 3).

It is believed that the phenolic compounds have a role in protecting the body from oxidative stress, diets rich in these compounds lowers the risk of diseases such as cancer and cardiovascular disease. Buckwheat has a total phenolic content, according to data collected by Taylor at al. [29], ranging from 29 to 1371 mg/100 g depending on extraction method and standard used.

Table 2. Chemical composition of buckwheat flour
Tab. 2. Skład chemiczny mąki gryczanej

Quality factors Content	Sample of buckwheat flour				
	MG1	MG2	MG3	MG4	MG5
moisture, %	12.3 ± 0.3	13.0± 0.1	10.9 ± 0.2	11.7 ± 0.1	11.4 ± 0.2
ash g/100 g dw	1.79± 0.12 ab	1.21± 0.22 a	2.01± 0.13 b	1.45± 0.21 a	1.81± 0.02 ab
total protein, g/100 g dw	12.0 ± 0.4ac	9.1± 0.4b	12.8 ± 0.5cd	11.9 ± 0.1a	13.4 ± 0.2d
dietary fiber g/100 g dw	6.1± 0.2a	3.9 ± 0.1b	9.2 ± 0.2c	3.9 ± 0.2b	12.6 ± 0.1d

± sd. – standard deviation for three independent determination, *Source*: own work / *Źródło*: praca własna

dw – dry weight.
Means with different letters within a row are significantly different (P<0.05).

Table 3. Characteristics of buckwheat flour in terms of sum of phenolic compounds, tocopherol content and antioxidant activity (AOA)
Tab. 3. Charakterystyka mąki gryczanej pod względem zawartości sumy związków fenolowych, tokoferolu i aktywności antyoksydacyjnej

Sample of flour	sum of phenolic compounds [mg GAE/100g]	AOA with DPPH [mg TE/100g]	AOA with ABTS [mg TE/100g]	tocopherol (vitamin) mg/100g
MG1	578.0 ± 5.7 a	775.5 ± 10.8 a	1603.8 ± 17.0 a	0.31±0.02a
MG2	375.2± 10.8 b	501.0± 10.3 b	1001.5± 0.6 b	0.22±0.02b
MG3	657.8 ± 5.4 c	881.9 ± 6.6 c	1885.7± 44.0 c	0.25±0.01c
MG4	447.5 ± 2.5 d	567.0 ± 4.3 d	1157.3±24.7 d	0.24±0.01c
MG5	748.9±5.1 e	1202±17.1 e	2215.5±50.9 e	0.27±0.10d

GAE - gallic acid equivalent, TE - trolox equivalent, *Source*: own work / *Źródło*: praca własna
dw – dry weight, ± sd. – standard deviation for tree independent determination.
Means with different letters within a row are significantly different (P<0.05).

Table 4. Microbiological analysis of buckwheat flour
Tab.4. Analiza mikrobiologiczna mąki gryczanej

Micro-organisms	sample of flour, presence of microorganisms, cfu/g				
	MG 1	MG 2	MG 3	MG 4	MG5
yeast	n.d. a	n.d. a	1.0x10 b	1.3x10 b	n.d. a
LAB	$4.0x10^3$ $\pm 1.4x10^2$ a	$2.4x10^2$ $\pm 2.9x\ 10^1$ b	$5.0x10^2$ $\pm 1.4x\ 10^2$ c	$6.0x10^2$ $\pm 1.4x\ 10^2$ c	$6.0x10^3$ $\pm 2.5x10^2$ c
mould	$6.0x10^3$ ± 7.1x10 a	$3.0x10^2 \pm 0$ b	$6.0x10^2$ $\pm 1.4x10^2$ h	$2.0x10^2$ ± 7.1x10 c	$2.0x10^2$ ± c
Bacillus cereus	$8.0x10^2 \pm 0$	0.5x10± 7.1x10 a	$1.0x10^2$ ± 2.1x10 a	$1.0x10^4$ $\pm 2.5x10^3$ b	$1.0x10^4$ $\pm 7.1x10^2$ b
Bacillus subtilis	$1.0x10^3$ $\pm 1.4x10^2$ a	$1.0x10^2$ ± 2.1x10 a	$3.0x10^2$ ± 7 a	$6.0x10^4$ $\pm 2.8x10^3$ b	$6.0x10^4$ $\pm 1.4x10^3$ b
spore-forming bacteria	$4.0x10^3$ $\pm 2.1x10^2$ a	$5.0x10^2 \pm 7$ b	$5.4x10^2$ ± 2.1x10 b	$9.0x10^4$ $\pm 7.1x10^2$ c	$9.0x10^4$ $\pm 1.4x10^3$ c

LAB - lactic acid bacteria, n. d. - not detected. *Source*: own work / *Źródło*: praca własna
Means with different letters within a row are significantly different (P<0.05).

Table 5. Technological parameters of bread dough
Tab. 5. Parametry technologiczne ciasta chlebowego

Parameters		Type of dough			
		B 100% 15% SD*	B 100% 25% SD	B 60%,M*20% R 20% 45% SD	Control W100%
sourdough 0 h	titratable acidity, degrees	2.8±0.1			-
	pH	7.71±0.1			-
sourdough 24h	titratabre acidity, degrees	12.3±0.2			-
	pH	4.1±0.1			-
baking dough	titratable acidity, degrees	5.2±0.05	6.0±0.04	8.8±0.03	3.1±0.06
	pH	5.67±0.02	5.33±0.05	4.39±0.02	6.1±0.02
yield of dough (flour +water)		195	195	195	162
proof time, min.		25	25	30	55

B - buckwheat flour content in recipe, M - maize flour content in recipe, R - rice flour content in recipe,
W -wheat flour content in recipe, SD – sourdough, the exact composition of each dough are shown in table 1.
± sd. –standard deviation for tree independent determination. *Source*: own work / *Źródło*: praca własna

Table 6. Quality parameters of sourdough buckwheat bread as compared to wheat bread
Tab. 6. Parametry jakościowe zakwasowego pieczywa gryczanego w porównaniu do chleba pszennego

Bread characteristic	B 100% 15% SD*	B 100% 25% SD	B60% M20% R20% 45% SD	Control W100%
Yield of bread made with100g flour	186.8	185.9	186.7	137.3
Volume of bread, cm^3/100g	208±2.5	208.4±2.6	186.8±2.1	380±3.0
Volume of bread, cm^3/100 g flour	388.5±2.7	387.4±3.8	348.8±1.9	522±2.1
Crumb moisture content, %	51.0±0.2	51.3±0.4	49.5±0.5	43.5±1.0
Titratable acidity , degrees	4.4±0.2	4.8±0.1	5.3±0.1	1.2±0.1
Crumb hardness (Instron), [N]	35.8±1.3	35.3±2.0	41.7±0.8	14.8±1.2

Organoleptic evaluation, according to PN, points	29.1±4.1	30.5±2.8	33.3±3.2	36.0±1.2

B - buckwheat flour content in recipe, M - maize flour content in recipe, R - rice flour content in recipe,
W -wheat flour content in recipe, SD – sourdough, the exact composition of each bread are shown in table 1.
± sd. –standard deviation for three independent determinations. *Source*: own work / *Źródło*: praca własna

Table 7. Characteristics of buckwheat bread, in terms of antioxidant activity and phenolic compounds
Tab.7 Charakterystyka pieczywa gryczanego pod względem zawartości związków fenolowych i aktywności antyoksydacyjnej

Designation breads samples	Moisture [%] dry weight	Sum of phenolic compounds [mgGAE/100g]	Antioxidant activity (AOA) with	
			DPPH [mg TE/100g]	ABTS [mg TE/100g]
1. B* 100% 15% SD*	90.6±0.4 a	387.1±3.8 a	501.9±7.3 a	1168.0±5.8 a
2. B 100% 25% SD	91.2±0.3 a	382.7±6.6 a	487.3±4.0 ab	1133.9±11.3 b
3. B 60%M*20% R*20%, 45% SD	90.9±0.2 a	369.2±3.3 b	478.7±9.6 b	1008.5±18.7 b
4. Control bread 100% wheat	90.1±0.1	36.0±0.5	13.5±1.9	154.0±6.4

B*- buckwheat flour, M* - maize flour, R*- rise flour, SD*- sourdough *Source*: own work / *Źródło*: praca własna
Means with different letters within a column are significantly different (P<0.01)
± sd. –standard deviation for three independent determinations

The results of evaluation of antioxidant activity (AOA) were higher than those obtained by Chlopicka et al. [7] i.e. in this study AOA with DPPH were of 20.0 mmol Trolox/kg dw to 48 mmol TE/kg dw (after conversion) while according to Chlopicka et al. the antioxidant activity of the buckwheat flour with DPPH was 8.8 mmol TE/kg dw. However the results of the analyses of total phenolic compounds were at similar levels 375.2 to 748.9±5.1 mg GAE/100g as obtained by Chlopicka et al. [7] - 725 mg/g dw. Additionally both the results of determinations of total phenols and antioxidant activity (as DPPH scavenging capacity) were at similar levels in polish organic flours as those observed by Alvarez-Jubete et al. [2] respectively - 323.3 mg GAE/100g and 620 mg TE/100g dw. These values were lower than demonstrated in analyzed organic flours. As in the case of phenolics content, range of value of antioxidant activity, obtained by different authors, varied reaching a maximum value on the level of 5740 µmol Trolox/100g [29].

The microbial analysis of organic buckwheat flour, showed in Table 4, revealed relatively low number of lactic acid bacteria. Only in two samples the number of LAB reached level of 10^3 cfu/g. Regarding the high presence of mould (number on the level 10^2 to 10^3 cfu/g) and spore forming bacteria in organic flour, use of competitive starter culture is purposeful.

The dough fermented for 25 to 55 minutes and then was divided into pieces (950 g, 590 g – control dough), which were placed into tins. The proofing of dough pieces was performed at 75% of relative humidity atmosphere, and the temperature of 35°C. Baking was carried out in a baking oven (Winkler-Wachtel), at temperature 230°C, with steam injection. The evaluation of bread characteristics is presented in table 6.

The breads obtained with buckwheat sourdough was characterized by relatively low volume (cm^3/100 g), high acidity and hardness. Use of sourdough influenced such sensory attributes as specific "buckwheat" aroma with nutty, acidic note, sour taste/flavor. Use for bread making of whole-meal flour caused a pungent sense of taste. The addition of maize and rice flour improved the mouthfeel of the crumb. This bread was better assessed than 100% buckwheat bread. Taking into account/it was observed that phenols content and antioxidant activity of bread made of three flours did not differ significantly from100% buckwheat bread (Table 7) so it could be concluded that the flour quality determined the bioactive compounds in bread more than bread-making. The characteristics of the buckwheat breads in terms of phenolic compounds and antioxidant activity are shown in Table 7.

The resulting buckwheat bread was characterized by a high content of polyphenols and antioxidant activity. In relation to the flour (748.9 mg GAE/100g) content of phenolic compounds in the tested bread was reduced by approximately 50%, which is in accordance with the data presented by other authors [2, 7, 26]. These results are broadly in line with expectations, since the thermal treatment has a negative effect on the flavonoid and total phenolic content in food products. Baking process lowers content of compounds with antioxidant activity in bread, including polyphenols and flavonoids [2, 12, 31]. However, during baking the compounds may be formed of an antioxidant activity as certain Maillard reaction products (melanoidins) in the crust of the bread [2, 16].

Alvarez - Jubete et al. [2] determined the polyphenol content of the bread (containing 50% of buckwheat flour) in an amount of 64.5 mg GAE /100 g dw and antioxidant activity (with DPPH) at 58.8 mg TE/100g dw, thus the reduction of the polyphenol content in the bread relative to the raw material by approximately 80% was observed. Reduction of about 50 % of phenolic compounds in relation to flour (748.9±5.1) was reported by other authors, according to Chlopicka et al. [7] and Zhang at al. [34] the buckwheat breads showed a higher loss of flavonoids after thermal processing than for total phenolic compounds. The breads obtained in this work showed relatively high levels of functional important phenolic compounds ranging from 369.2 to 387.1 mg GAE/100g.

4. Summary

Many studies have been reported that buckwheat is generally rich in phenolic phytochemicals, which may have important health-promoting properties [2, 7, 17, 29]. However, there have been large differences between the values observed by different authors in the tested flours (e.g. made from dehulled grain, whole grain, white buckwheat flour). Taking into account the diversity of the obtained results of analyses the organic buckwheat flours available on the Polish market, the manufacturer shall provide a uniform material for bread making what ensure high concentration of bioactive compounds. Use of the sourdough, obtained with starter culture, in bread-making allows to produce gluten-free bread with good sensory characteristics. It was determined that the starter culture containing strains of *Pediococcus pentosaceus* G4 and *P. pentosaceus* G2 is good starter for buckwheat sourdough fermentation.

The use of starter cultures allowed to develop gluten-free bread with functional features, prepared using buckwheat flour as the sole raw material.

This conclusion is in line with the observation of some researches [8, 20, 32] that using the sourdough technology improves sensory characteristics of bread, produced from gluten-free flours which refers also to pseudocereals flour. The good quality bread made with organic buckwheat flour is good, healthy food for both remaining on the gluten-free diet as well as other consumers because it is rich source of biologically active compounds including compounds, dietary fiber, and is characterized by antioxidant properties. The obtained breads had significantly higher levels of these nutritionally important compounds then white wheat bread. Contrary to many gluten-free products buckwheat bread consumption does not carry the risk of unbalancing the diet in terms of nutrients.

5. References

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