THE CONTENT OF BUCKWHEAT FLOUR IN WHEAT BREAD

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Seven samples of bread were prepared in a special way. Samples were made of a mixture of wheat and buckwheat flour in defined proportions. Carbohydrates from crumbs of bread were derivatized with BSTFA and analyzed by GC-MS. Multivariate analysis was applied. The purpose of this study was not the identification and quantification of carbohydrate composition. The results demonstrated a clear relation between the carbohydrate composition of the bread samples and the type of flour used for their production.

KEY WORDS: bread, wheat, buckwheat, GC-MS, cluster analysis

INTRODUCTION

Dietary guidelines suggest that 50% or more of all consumed calories during a day should be of carbohydrate origin, where a maximum of 10-25% originates from sugars or other sweeteners (1). Bread is probably the most common and, in terms of the quantity, most frequently used type of food of carbohydrate origin in a daily diet of people, and, for this reason, bread should have good nutritional properties.

The bakery industry uses mostly wheat flour (2). Production of existing baked “functional” foods usually requires mixing of at least two types of flour. The substitution of white or whole grain wheat flour with spelt, buckwheat or amaranth flour may increase nutritional and/or functional value of the product, which could have a positive impact on human health if consumed regularly (3, 4).

By studying the content of routine in buckwheat, raw buckwheat and buckwheat products (5), it was determined that the use of raw buckwheat, due to a high content of routine, ensures functionality of the final product (6).

Earlier studies (7, 8) showed that it is possible to quickly and clearly differentiate between types of flour, using the GC-MS analysis of derivatized small molecules of flour (lipids and carbohydrates) and multivariate analysis, without compound identifications.

The objective of this work was to investigate physical properties of bread samples that contain different proportions of wheat and buckwheat flour and also to differentiate carbohydrate content in bread crumbs. Bread samples were prepared by substituting the con-
tent of wheat flour with buckwheat flour in the mass amounts of 0, 20, 40, 50, 60, 80 and 100%. The tested bread samples, shown in Figure 1, are labeled as S1, S2, S3, S4, S5, S6 and S7, respectively.

![Bread samples](image)

**Figure 1. Bread samples**

### EXPERIMENTAL

#### Physical properties

The basic characteristics of the whole grain buckwheat flour and wheat flour T-500 are shown in Table 1.

**Table 1. Indicators of the quality of whole grain buckwheat flour and wheat flour T-500 used for bread-making**

<table>
<thead>
<tr>
<th>Quality indicator</th>
<th>Buckwheat flour Value</th>
<th>Wheat flour T-500 Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>12.80</td>
<td>12.20</td>
</tr>
<tr>
<td>Mineral substances (% in dry matter)</td>
<td>2.44</td>
<td>0.49</td>
</tr>
<tr>
<td>Raw protein (% in dry matter)</td>
<td>13.70</td>
<td>10.70</td>
</tr>
<tr>
<td>Fat (% in dry matter)</td>
<td>2.60</td>
<td>0.95</td>
</tr>
<tr>
<td>Starch (% in dry matter)</td>
<td>63.0</td>
<td>73.20</td>
</tr>
<tr>
<td>Total dietary fiber (% in dry matter)</td>
<td>9.06</td>
<td>0.50</td>
</tr>
<tr>
<td>Degree of acidity</td>
<td>3.20</td>
<td>2.50</td>
</tr>
</tbody>
</table>

Results are shown as a mean value ± standard deviation (n = 3). Analysis were performed using standard procedures.

### A technological procedure for making bread with whole grain buckwheat flour

The following raw materials were used for making bread with whole grain buckwheat flour: whole grain buckwheat flour, wheat flour T-500, yeast, salt, and a bread dough improver. The technological procedure for making bread that contains buckwheat has four phases:
Phase I. Homogenization and soaking of the whole grain buckwheat flour in hot water. Processing with warm water is done to enhance hydration of particles of the whole grain buckwheat flour that contains different particles ranging, e.g. from 35 µm to 450 µm. Preparation of the whole grain buckwheat flour is carried out under the following conditions: hot water (70-85°C) was poured over a prepared quantity of whole grain buckwheat flour and the mass was cooled off to 35-40°C. The ratio between hot water and buckwheat flour was 1:1. This phase is very important as it allows overcoming deficiencies in textural properties of the bread containing whole grain buckwheat flour, which, if the flour is not soaked in hot water, irritates the throat while swallowing the bread (damages or “scratches” the lining of the throat).

Phase II. Selection of dough consistency (preparing “soft” dough) and shaping of dough pieces in special silicone molds is done according to the special raw material composition. A measured quantity of T-500 flour and wet whole grain buckwheat flour mass (the water added for soaking the flour is included) were poured into a container used for mixing, 2% of table salt and 3% of compressed yeast and 0.3% of a bread improver (commercial powder-like bread additive) were added. Mixing, during which dough is made, is done in a two-speed spiral mixer in the duration of 12 (4+8) min. The dough temperature after mixing should range between 28 and 32°C, and dough fermentation in the mass takes about 40 min. The shaping of dough pieces is done manually by dividing the dough into desired mass of up to 100 g/piece and by shaping it into the prepared silicone molds.

Phase III. The final fermentation process is conducted in a fermentation chamber at the optimum temperature (32-36°C) and optimum humidity of over 85%. The duration of the final fermentation process depends on dough growing biochemical process as well as on the proportion of buckwheat flour in the mass, and, on average, lasts about 35 min.

Phase IV. Baking of the fermented dough pieces in a deck oven at a temperature ranging from 215 to 230°C, with duration between 14 to 18 min. The bread was taken out of a mold and cooled off at room temperature until the temperature of about 40°C in the middle part of the bread was reached.

Preparation of the Samples

The amounts of 6 g of the bread crumb samples were defatted with hexane in the following manner: each sample and 40 mL of n-hexane were added into a flask, stirred on a Vortex mixer for 1 min, after which the mixture was centrifuged at 2000 rpm for 5 min. The hexane layer was then removed. The procedure was repeated three times, the hexane fractions were rejected, so the flour remained defatted. Samples of defatted bread crumbs were dried in the air, at room temperature. 10 mL of 96% ethanol (Merck) were added and stirred on a Vortex mixer for 1 min, after which the mixture was centrifuged at 2000 rpm for 5 min. 5 mL of clear supernatant were separated and dried by nitrogen flow. Dry residues were dissolved in 100 µL of pyridine and 100 µL of BSTFA (N,O-Bis(trimethylsilyl)trifluoroacetamide) (Macherey-Nagel), to derivatize carbohydrates into their trimethylsilyl ethers (8).
**GC-MS Analysis**

The GC–MS analyses were performed on an Agilent Technologies 7890 instrument coupled with MSD 5975 equipment (Agilent Technologies, Palo Alto, CA, USA) operating in the EI mode at 70 eV. A DP-5 MS column (30 m; 0.25 mm; 25 μm) was used. The temperature program was: 50-130°C at 30°C/ min and 130-300°C at 10°C/ min. The injector temperature was 250°C. The flow rate of the carrier gas (helium) was 0.8 mL/min. A split ratio of 1:50 was used for the injection of 1 μL of the solutions.

WILEY 275 library was used for the mass spectrum analysis.
PAST program was used for the statistical data processing (9).

**RESULTS AND DISCUSSION**

**Results for the physical and organoleptic properties**

The addition of whole grain buckwheat flour into white wheat flour affected the physical and organoleptic characteristics of the bread, Figure 2. An increase in the amount of buckwheat resulted in the reduction of the volume of the bread. The bread crust turned brown-green. This complies with previous studies (10, 11, 12).

- Substituting white wheat flour with buckwheat flour in the bread formulation did not significantly affect the specific volume of the bread. Considering that the specific volume of standard bread should be 6 cm³/g, and should not go below 3.5 cm³/g (13), it appears that all seven breads had satisfactory specific volume.
- In general, adding from 20% to 50% of buckwheat flour into the bread formulation was rated as acceptable. Although breads S2, S3 and S4 were smaller in their volume, their taste was aromatic and pleasant. Their elasticity was also satisfactory. Unfortunately, adding more than 50% of whole grain buckwheat flour was rated as unacceptable. Breads S5, S6 and S7 had an unacceptable color, they were floppy, rubbery, soggy and slightly bitter.

**Results of GC-MS analysis**

Figure 3 shows TIC (Total Ion Current) chromatograms of seven crumb samples of bread. In this study, as in previous research (7), the intention was not to search for new ingredients or their quantification. Only peaks that had the same apex of masses 73m/z and 217m/z in SIM chromatogram (Selected Ion Chromatogram) were separated from the chromatograms presented in Figure 3, and their mass spectra were analyzed as in the previous studies (7), where the attention was focused on flour (7). Therefore, it was interesting to investigate if the same can be concluded for thermally modified flour, i.e. with a finished food product obtained by mixing wheat and buckwheat flour and by analyzing carbohydrate content of bread crumbs. Yeast is also used in the preparation of bread, which consumes various types of carbohydrates.
Figure 2. Volume (a), height (b) and width (c) of tested breads (S1-S7)
Figure 3. TIC chromatograms of bread crumb samples (S1-S7)

Judging from the chromatograms in Figure 3, it is difficult to determine the difference between the bread samples (care should be taken that the abundances of chromatograms in Figure 3 are different). That is why the signals 73m/z and 217m/z are extracted from the TIC chromatograms. For the purposes of determining similarity or differences among
these 7 samples, experimental data were subjected to a multivariate analysis, using 18 carbohydrate variables (derivatised mono- and disaccharides and their alcohols).

Figure 4 shows the dendrogram of carbohydrate contents in the bread crumb samples. There are three main branches: branch S1 (pure wheat flour), branch S7 (pure buckwheat flour), and the remaining part which represents combinations of wheat and buckwheat flour. The values on the y axis show that this method is very suitable for differentiating among the stated food products.

**Figure 4.** Dendrogram of carbohydrate contents in bread crumb samples (S1-S7)

**CONCLUSION**

By analyzing the physical and organoleptic properties of the final product it was concluded that the addition of 20% to 50% of whole grain buckwheat flour into the bread formulation was rated as acceptable, but bread samples containing more than 50% of buckwheat flour were rated as unacceptable. This paper shows that the content of buckwheat flour in the bread made from a mixture of buckwheat and wheat flour can be determined by applying the multivariate analysis. This was achieved by extracting carbohydrate-
tes (mono- and disaccharides) from bread crumbs. The extracted carbohydrates were derivatized and analyzed in the GC-MS system. The multivariate analysis of data clearly shows proper classification of the samples depending on the mass proportion of buckwheat in its mixture with wheat flour. This shows that the method is relatively easy to perform and apply in the quality control of buckwheat food products.

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REFERENCES


**SADRŽAJ HEЉDINOG BРАШНА У ХЛЕБУ ОД ПШЕНИЦЕ**

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Седам узорака хлеба припремљено је на посебан начин. Узорци су направљени од мешавине пшеничног и хељдиног брашна у дефинисаним пропорцијама. Угљени хидрати из средине хлеба су дериватизовани са BSTFA и анализиране помоћу GC-MS. Примењена је и мултиваријантна анализа. Сврха ове студије није била идентификација и квантификација угљено-хидратног састава. Резултати су показали јасну везу између угљено-хидратног састава узорака хлеба и врсте брашна коришћене за њихову производњу.

Кључне речи: хлеб, пшеница, хељда, GC-MS, кластер анализа

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