SHORT COMMUNICATION

Determination of the content of buckwheat and wheat flours in bread using GC-MS and multivariate analysis

ĐORĐE PSODOROV – MARIJANA AČANSKI – DRAGAN PSODOROV – ĐURA VUJIĆ – KRISTIAN PASTOR

Summary

This paper reports on the analysis of carbohydrate components in bread crusts, made of seven controlled mixtures of buckwheat and wheat flour. These were mono-, disaccharides and some heterocyclic compounds derivatized into their corresponding trimethylsilyl ethers and analysed by a gas chromatography-mass spectrometry system. Selected ion monitoring (SIM) chromatograms with m/z signals of 73, 204 and 217 were selected from total ion current (TIC) chromatograms, as the selected ions are known to be characteristic for silylated carbohydrates. Programmes AMDIS (Version 2.66) and NIST (MS Search Version 2.0), and NIST08 library (National Institute of Standards and Technology, Gaithersburg, Maryland, USA) were used to confirm the presence of carbohydrates. The aim was to evaluate applicability of multivariate analysis of the stated components to determination of the content of buckwheat flour in bread, made of a mixture of buckwheat and wheat flours. It was sufficient to use the principal component analysis and a dendrogram to clearly present the proportion of buckwheat flour in bread.

Keywords

buckwheat; carbohydrate; gas chromatography-mass spectrometry; bread quality control; multivariate analysis

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 saccharides or other sweeteners [1]. Bread is probably the most common and, in terms of quantity, most frequently used type of food of carbohydrate origin in the daily diet of people and, for this reason, bread should have good nutritional properties.

The bakery industry uses mostly wheat flour. The main protein component of wheat flour responsible for the structure, texture and volume of a bakery product is gluten [2]. More and more people are allergic to gluten. Many people live their whole lives suffering from celiac disease not knowing that the struggle they go through is not harmless and it can cause much more serious health disorders [3]. The problem can be solved by substituting wheat flour with buckwheat flour [4].

Buckwheat is a pseudo-cereal that does not contain gluten [5] and therefore, gluten-free buckwheat flour cannot be turned into dough having elastic and plastic properties. There are limitations related to the proportion of buckwheat flour in the formulation for finished bakery products. Enrichment of a bread mixture with white and dark buckwheat flour up to 15% results in the reduction of a specific volume, taste and sensation the product creates in the mouth [6]. The volume of a loaf of bread containing 50% of buckwheat flour is lower compared to the control bread [7].

Buckwheat seed is very rich in nutrients, most of them are carbohydrates (about 73%), proteins (11.7%) and lipids (2.4%). The energy value of 100 g of the edible part of the seed is 1 407 kJ [8]. Buckwheat flour is significantly richer in fibrous carbohydrates in comparison to wheat flour [9]. This is why it probably has a higher content of

Đorđe Psodorov, Đura Vujić, Institute of Food Technology, University of Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia.

Marijana Ačanski, Kristian Pastor, Department of Applied and Engineering Chemistry, Faculty of Technology, University of Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia.

Dragan Psodorov, Department of Management, College of Management and Business Communications, Mitropolita Stratimirovića 110, 21205 Sremski Karlovci, Serbia.

Correspondence author:

Kristian Pastor, tel.: +38163-77-95-821, fax: +38121-450-413, e-mail: pastor@tf.uns.ac.rs

insoluble beta-glucans present in the husk. Considering that beta-glucans are known as immunostimulating polysaccharides [10], consumers who opt for long-term consumption of bread enriched with dark buckwheat flour provide support to their immune system. By studying the content of rutin in buckwheat, raw buckwheat and buckwheat products [11], it was determined that the use of raw buckwheat, due to the high content of rutin, ensures functionality of the final product.

Carbohydrates or their derivatives are usually analysed by liquid chromatography. However, certain carbohydrate samples are analysed by gas chromatography (GC) due to the inherent high efficiency achievable by the technique at short elution times. In addition, gas chromatography–mass spectrometry (GC-MS) is a particularly powerful analytical technique for carbohydrates, especially for their identification. As a prerequisite, appropriate derivatives must be formed to render them sufficiently volatile but still easily recognizable based on their mass spectra [12].

The alternative mode is to use the mass spectrometer as a selective and very sensitive detector by monitoring only a few pre-selected ions from the known fragmentation pattern of compounds to be analysed. This method is called selected ion monitoring (SIM). The principal application of the SIM technique is for quantification of compounds. The combination of specificity and sensitivity makes this method most suitable for residue analysis in the area of food safety. In setting up a SIM experiment, an important decision is the choice of either one or several ions. In some cases, derivatization may provide better chromatographic properties as well as increased molecular weight for better selectivity. The specificity increases with the selection of more ions but, in many cases, two or three may be sufficient [13].

Earlier studies showed that the analysis of derivatized carbohydrates by GC-MS (SIM) in flours facilitated clear differentiation between buckwheat and wheat flours [14]. Considering that buckwheat contains about 70% of carbohydrates, it is reasonable to expect that small quantities of monosaccharides and their derivatives, originating from chemical synthesis of polysaccharides, remain as residues in a dried seed. The purpose of this paper was not to identify and find new compounds, but to compare carbohydrate and heterocyclic components in order to determine the content of buckwheat flour in bread crust samples, made of a mixture of buckwheat and wheat flour. These bread samples were prepared by substituting the content of wheat flour with dark buckwheat flour in the mass amounts of 0%, 20%, 40%, 50%, 60%, 80% and 100%. Results can be applicable in the quality control of buckwheat breads on the market.

MATERIALS AND METHODS

Raw materials and samples

The following raw materials were used for making seven bread samples: dark buckwheat flour (ash content 2.1%, moisture 9.7%; Tehnohemija, Novi Sad, Serbia), wheat flour T-500 (ash content 0.52%, moisture 1.5%; DEM, Kulpin, Serbia), yeast, salt, and a bread dough improver (sodium bicarbonate, disodium dihydrogen pyrophosphate, monocalcium phosphate; Centroproizvod, Beograd, Serbia). The sample breads containing 0%, 20%, 40%, 50%, 60%, 80% and 100% buckwheat flour are shown in Fig. 1.

Sample preparation

Samples were processed in a way that all non-carbohydrate components were removed. The amount of 6 g of bread crusts was defatted in the following manner: 4 ml of hexane were added into a flask with bread crust samples, stirred on a Vortex mixer for 2 min and then centrifuged

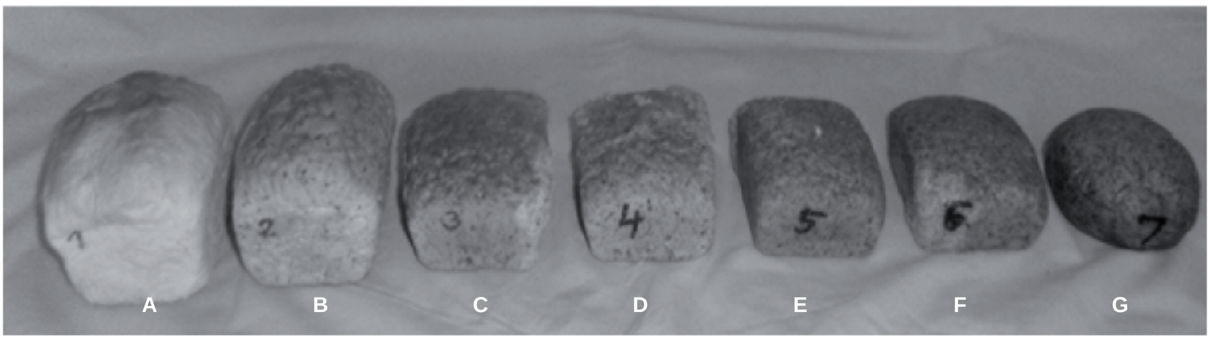

Fig. 1. Bread samples.

Content of buckwheat flour: A – 0%, B – 20%, C – 40%, D – 50%, E – 60%, F – 80%, G – 100%.

5 min at 721.6 ×*g* (radius 160.5 mm), after which the hexane layer was removed. Bread crusts were dried in the air, at room temperature. Then, 10 ml of 96% ethanol were added. The samples were placed on the Vortex mixer for 1 min and then centrifuged again for 5 min at 721.6 ×*g* (radius 160.5 mm). The volumes of 5 ml of 96% ethanol were withdrawn and the mixture was evaporated with a nitrogen flow. Then, 100 µl of pyridine and 100 µl of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA; Macherey-Nagel, Bethlehem, Pennsylvania, USA) were added to the dry residue, by which derivatization of carbohydrates into trimethylsilyl ethers was performed [15].

GC-MS analysis

The GC-MS analyses were performed on the Agilent Technologies 7890 instrument (Agilent Technologies, Palo Alto, California, USA) coupled to MSD 5975 equipment operating in the electron ionization (EI) mode at 70 eV. A DP-5 column (30 m × 0.25 mm × 25 mm; Agilent Technologies) was used. The temperature programme was: 50–130 °C at 30 °C·min^{-1} and 130–300 °C at 10 °C·min^{-1}. The injector temperature was 250 °C. The flow rate of the carrier gas helium was 0.8 ml·min^{-1}. A split ratio of 1 : 50 was used for the injection of 1 μl of the solutions.

Analysis of chromatograms

The chromatograms were analysed in several steps. Total ion current (TIC) chromatograms were first recorded for each sample. Signals for masses, characteristic for carbohydrates, were then separated: m/z =73, m/z = 204 and m/z = 217, although these masses are also contained in MS spectra of some other compounds. Using the programmes AMDIS (Version 2.66) and NIST (MS Search Version 2.0; National Institute of Standards and Technology, Gaithersburg, Maryland, USA), all peaks of m/z =73, m/z = 204 and m/z = 217 were checked. Only those, which were carbohydrates, their alcohols and a few heterocyclic compounds, were taken for the analysis. Traces of trimethylsilyl esters of residual fatty acids were identified and were not taken for data processing. All satisfactory peaks were analysed using AMDIS and NIST programmes, and NIST08 library.

Statistical methods

All carbohydrate peak surfaces were automatically integrated and results introduced into PAST statistical program (Øyvind Hammer, Natural History Museum, University of Oslo, Norway) [16]. Cluster analysis and principal component analysis were used for data processing.

RESULTS AND DISCUSSION

During the baking process, part of starch from the surface of the dough is dextrinated and turned into a bread crust. The major factor in creating dextrin is the oven temperature. In addition to dextrin, the bread crust also contains caramel, which is created during the baking process, and which does not exist in the middle part of the bread. Usually, the caramelization temperature ranges between 140 °C and 150 °C [17]. Due to these reasons, the bread crust has a sweeter taste in comparison to the middle part of the bread. Therefore, we decided to analyse bread crusts.

It was presented that wheat flour can be unambiguously differentiated from buckwheat flour [14]. Therefore, it was interesting to test whether this was also possible with thermally modified flour products, i.e. with a finished food product obtained by mixing wheat and buckwheat flour.

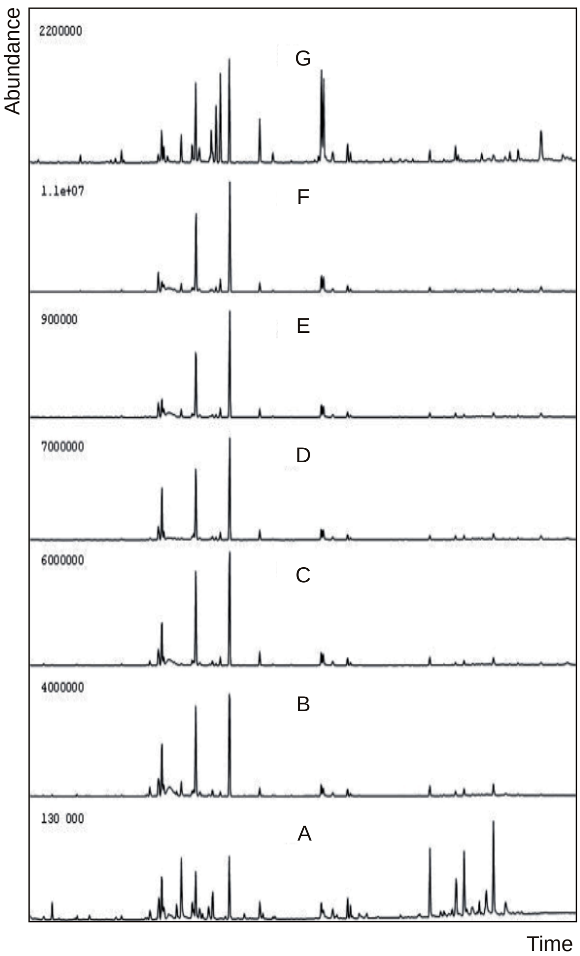

Fig. 2. Total ion current chromatograms of bread crust samples.

Content of buckwheat flour: A – 0%, B – 20%, C – 40%, D – 50%, E – 60%, F – 80%, G – 100%.

Yeast is also used in the preparation of bread, which consumes various types of carbohydrates.

Fig. 2 shows the TIC chromatograms and Fig. 3 the SIM chromatograms of all seven samples of bread crusts. In addition to carbohydrates (pentoses, hexoses, disaccharides, inositols, etc.), several heterocyclic compounds were identified. It is known that they contribute to aromatic properties of finished products that have undergone thermal treatment. Since wheat and buckwheat flours are very different [14], it is expected that a Maillard reaction would create compounds that would make them even more different. Therefore, in addition to carbohydrates and their alcohols, it was decided to also include heterocyclic compounds in a multivariate analysis of samples. It is already known that masses m/z =73, m/z = 204 and m/z = 217 are characteristic for silylated carbohydrate derivatives [14]. Therefore, it was decided to use these masses to compare carbohydrates and their derivatives. These masses were separated and mass spectra analysed only in cases when all three masses had the same apex.

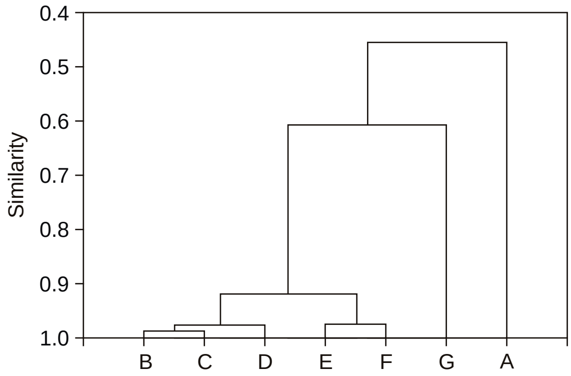

Fig. 4. Dendrogram of silylated components (carbohydrates and heterocyclic compounds) in bread crusts.

Content of buckwheat flour: A – 0%, B – 20%, C – 40%, D – 50%, E – 60%, F – 80%, G – 100%.

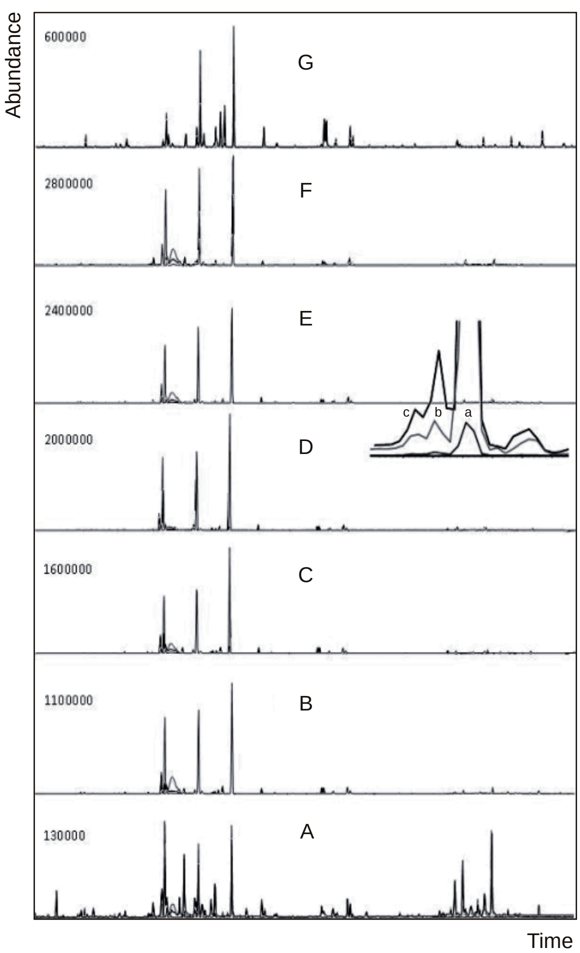

Fig. 3. Selected ion monitoring chromatograms of bread crust samples.

Content of buckwheat flour: A – 0%, B – 20%, C – 40%, D – 50%, E – 60%, F – 80%, G – 100%.
The enlarged picture illustrates the selection of peaks: a – m/z = 73, b – m/z = 217, c – m/z = 204.

In this study, experimental data were subjected to multivariate analysis. The term multivariate not only means many variables, but also means that these variables might be correlated. Each statistical method refers to analysing data, interpreting data, displaying data, and making decisions based on data. Therefore, multivariate statistical methods are collections of methods and procedures that analyse, interpret, display and make decisions based on multivariate data [18].

Fig. 4 shows a Pearson's correlation dendrogram of all crust samples that were analysed. There are two main branches: branch K1 (pure wheat flour) and branch 2 that is divided into K7 (pure buckwheat flour), and the remaining part, which represents combinations of wheat and buckwheat flour. Good differentiation of the studied food products was achieved.

Fig. 5 shows results of principal component analysis (PCA) correlation graphics of the seven tested samples. Samples 1 and 7 are clearly separated, while other samples 2–6 are adequately distributed. This PCA graph is shown with equidistant correlations [16].

It is shown that the content of buckwheat flour in the bread made of a mixture of buckwheat and wheat flour can be determined by applying the multivariate analysis regarding extracted carbohydrates from bread crusts, that were derivatized and analysed by GC-MS. The multivariate analysis of data shows proper classification of samples

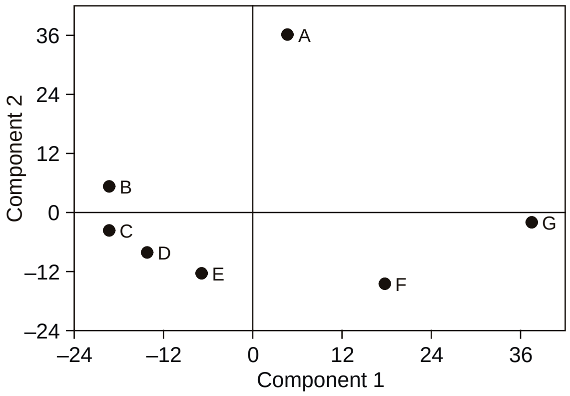

Fig. 5. PCA overview of silylated components (carbohydrates and heterocyclic compounds) in bread crusts.

Content of buckwheat flour: A – 0%, B – 20%, C – 40%, D – 50%, E – 60%, F – 80%, G – 100%.

depending on the mass proportion of buckwheat in its mixture with wheat flour. The method is relatively easy to perform and may be useful for quality control of buckwheat food products.

Acknowledgments

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REFERENCES

1. Story, M. – Stang, J.: Nutrition needs of adolescents. In: Stang, J. – Story, M. (Eds.): Guidelines for adolescent nutrition services. Minneapolis : University of Minnesota, 2005, pp. 21–35. <http://www.epi.umn.edu/let/pubs/adol_book.htm>
2. Edwards, W. P.: The science of bakery products. Cambridge : Bardfield Consultants, Braintree : Royal Society of Chemistry, 2007. ISBN: 978-0-85404-486-3.
3. Catassi, C. – Fasano, A.: Celiac disease. In: Arendt, E. K. – Dal Bello, F. (Eds.): Gluten-free cereal products and beverages. London: Academic Press, 2008, pp. 1–27. ISBN: 978-0-12-373739-7. DOI: 10.1016/B978-012373739-7.50003-4.
4. Gallagher, E.: Gluten-free food science and technology. Chichester: Wiley-Blackwell, 2009. ISBN: 978-1-4051-5915-9.
5. Alvarez-Jubete, L. – Arendt, E. K. – Gallagher, E.: Nutritive value and chemical composition of pseudocereals as gluten-free ingredients. International Journal of Food Science and Nutrition, *60*, 2009, pp. 240–257. DOI: 10.1080/09637480902950597.
6. Lin, L. – Liu, H. – Yu, Y. – Lin, S. – Mau, J.: Quality and antioxidant property of buckwheat enhanced wheat bread. Food Chemistry, *112*, 2009, pp. 987–991. DOI: 10.1016/j.foodchem.2008.07.022.
7. Fessas, D. – Signorelli, M. – Pagani, A. – Mariotti, M. – Iametti, S. – Schiraldi, A.: Calorimetry in food processing: Analysis and design of food systems. In: Kaletunç, G. (Ed.): Use of thermal analysis to design and monitor cereal processing. New York : John Wiley & Sons, 2008, pp. 265–285. ISBN: 9780813814834.
8. Krkošková, B. – Mrázová, Z.: Prophylactic components of buckwheat. Food Research International, *38*, 2005, pp. 561–568. DOI: 10.1016/j.foodres.2004.11.009.
9. Bonafaccia, G. – Marocchini, M. – Kreft, I.: Composition and technological properties of the flour and bran from common and tartary buckwheat. Food Chemistry, *80*, 2003, pp. 9–15. DOI: 10.1016/S0308-8146(02)00228-5.
10. Hozová, B. – Kuniak, L. – Moravčíková, P. – Gajdošová, A.: Determination of water-insoluble β-D-glucan in the whole-grain cereals and pseudocereals. Czech Journal of Food Science, 25, 2007, pp. 316–324. <http://www.agriculturejournals.cz/publicFiles/00507.pdf>
11. Kreft, I. – Fabjan, N. – Yasumoto, K.: Rutin content in buckwheat (*Fagopyrum esculentum* Moench) food materials and products. Food Chemistry, *98*, 2006, pp. 508–512. DOI: 10.1016/j.foodchem.2005.05.081.
12. Scott, R. P. W.: Gas chromatography-mass spectrometry systems. In: Cazes, J.: (Ed.): Encyclopedia of Chromatography. Boca Raton: Marcel Dekker, 2004, pp. 260–270. ISBN: 978-1-4398-7067-9.
13. Gerhardt, K. O.: Gas chromatography-mass spectrometry. In: Gordon, M. H. (Ed.): Principles and applications of gas chromatography in food analysis. Chichester: Ellis Horwood, 1990, pp. 59–85. ISBN: 978-1-4612-8024-8.
14. Ačanski, M. M. – Vujić, Đ. N.: Comparing sugar components of cereal and pseudocereal flour by GC–MS analysis. Food Chemistry, *145*, 2014, pp. 743–748. DOI: 10.1016/j.foodchem.2013.08.138
15. Knapp R. D.: Handbook of analytical derivatization reactions. New York: John Wiley and Sons, 1979, pp. 67–68. ISBN-13: 978-0471034698, ISBN-10: 047103469X.
16. Hammer, Ø. – Harper, D. A. T. – Ryan, P. D.: PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica, *4*, 2001, pp. 4–11. <http://palaeo-electronica.org/2001_1/past/issue1_01.htm>.
17. Schünemann, C. – Treu, G.: Baking: The art and science, A practical handbook for the baking industry. Alberta : Baker Technology, 1988. ISBN: 096937450X.
18. Yang, K. – Trewn, J.: Multivariate statistical methods and quality. New York: The McGraw-Hill, 2004. ISBN: 9780071432085.

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