SIMILARITY OF SUGAR COMPONENTS IN WASTE BUCKWHEAT FLOUR DETERMINED USING GC-MS

R.Razmovski[1], M. Ačanski[1], V.Puškaš[1], V. Vučurović[1], U. Miljić[1], K. Pastor[1]

Abstract: Gas chromatography with mass spectrometry (GC-MS) was used for performing a qualitative analysis of the hydrosoluble flour extracts of 9 buckwheat samples, taken as leftovers from restaurants. All 9 samples were first defatted with hexane. Samples of defatted flour were dried in the air and then extracted with ethanol. Ethanol extracts were used for the analysis of soluble carbohydrates. TMSi (trimethylsilylimidazole) was used as a reagent for the derivatization of carbohydrates into trimethylsilyl-ethers. The results show that the dominant trimethylsilyl-ethers of sugars are actually very similar in all buckwheat samples. The following cluster analysis was used for the comparison of the hydrosoluble extracts of 9 waste buckwheat flour samples.

Key words: waste buckwheat flour, gas chromatography-mass spectrometry, correlations of hydrosoluble composition

Introduction

Common buckwheat was domesticated and first cultivated in Asia, in the Yunnan region of China and Tibet, around 6000 BC, and from there spread to Central Asia and Europe.[1] Although the cultivation of buckwheat is the largest in China, Russia, and North America, it is now grown in Europe, India, Tibet, Tasmania, Australia, Argentina, Bhutan, South Africa, Brazil and many other countries.[2,3]

Buckwheat belongs to *Polygonaceae* family, unlike major cereals such as wheat, rice, and corn.[4,5] Cultivation of buckwheat was in decline in the past century. More recently, cultivation has increased due to the growing interest in organic farming, alternative culture, old and traditional diet, outstanding nutritive properties, and not to mention numerous studies which support the claim that buckwheat has had a positive effect on human health.[6-8] The most commonly cultivated buckwheat species are: common buckwheat (*Fagopyrum esculentum* Moench) and tartary buckwheat (*Fagopyrum tataricum* Gaertner).[9,10] Buckwheat is now mainly grown for grain, which is often processed into flour. Buckwheat seed is peeled, and then peeled grain is milled and sieved.[11]

The purpose of this study is to quickly and relatively easy determine similarity between 9 types of waste buckwheat flour, taken from different restaurants, by creating the dendrograms of hydrosoluble extracts.

[1]University of Novi Sad, Faculty of Technology, Boulevard Cara Lazara 1, 21000 Novi Sad, Serbia (corresponding author: macanski@tf.uns.ac.rs)

Material and methods

About 10 g of each of the following samples were grinded: Godijeva (H1), Bambi (H2), Darja (H3), Francuska (H4), Prekumurska (H5), Češka (H6), Čebelica (H7), Novosadska (H8), and Spacinska (H9). Each sample was homogenized and then treated in the following manner: 0.5 g of flour was poured in 12 mL cuvette for centrifugation with the precision of 0.01 g. The cuvette was additionally filled with 5 mL of n-hexane and stirred on vortex for 2 min, after which the mixture was centrifuged at 2000 rotations/min for 5 min. After this, the clear supernatant was poured into a 10 mL glass. Samples of defatted flour were dried in the air. 5 mL of 96% ethanol (Merck) were added to the dried samples and stirred on vortex for 2 min, after which the mixture was centrifuged at 2000 rotations/min for 5 min. 2 mL of clear supernatant were separated and dried on a nitrogen flow. The residue was dissolved in 500 μL of pyridine and 50 μL of TMSi (trimethylsilylimidazole, Marchery-Nagel) were added, by which derivatization of carbohydrates into trimethylsilyl-ethers was performed.

All the tests were performed on a gas chromatography system. The GC-MS analyses were performed on Agilent Technologies 7890 instrument paired with MSD 5979 equipment (Agilent Technologies, Palo Alto, CA, USA) operating at EI mode at 70 eV. The 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. The 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.[12]

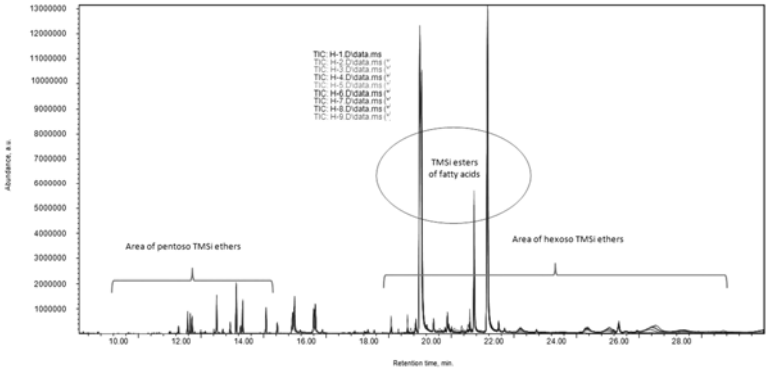
Results and discussion

Sucrose and raffinose were presented as the major free sugars in buckwheat seeds, while only small quantities of rhamnose, fructose, glucose, maltose were detected.[13]

Soluble carbohydrates (1-6%), including sucrose and fagopyritol, are located predominantly in the germ (71.4% of the total soluble carbohydrates). The content of fagopyritol in tartary buckwheat is two times lower than in common buckwheat, but tartary buckwheat contains other soluble carbohydrates, not present in common buckwheat, and this is assumed to be rhamnosyl-glucoside, which is present in the quantity of max. 31%. Fagopyritols can have a positive effect on human health.[14,15] Horbowicz et al. (1998) claim that the germ of buckwheat is unique because it accumulates sugar alcohols instead of raffinose series oligosaccharides.

Figure 1 shows the chromatogram of ethanol extracts of waste buckwheat flour samples from 13 to 21.60 min. On Figure 1 two areas containing silyl-derivatives of carbohydrates can be seen. The results of the chromatogram revealed the presence of the following compounds: the presence of pentose TMSi-ethers and pentose alcohols, from 10 to 12 min, and the presence of hexitols, hexose, several disaccharide TMSi-ethers, from 18 to 21 min. GC-MS allows us to identify the presence of TMSi-esters of fatty acids, from 14 to 18 min, which remained after incomplete defattening. However, this is not the focus of our research at the moment.

The chromatograms of all nine samples (Figure 1) are very similar. The peak integration shows the ration of components areas 1:150.



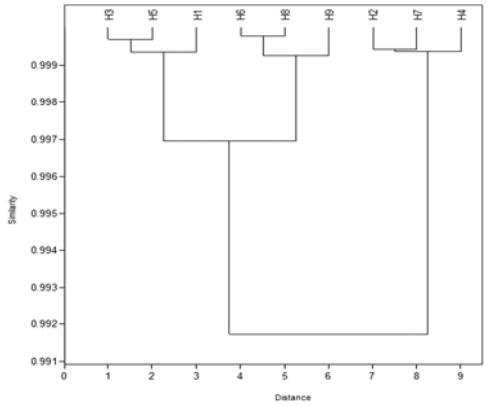
Slika 1. Hromatogrami komponenata u hidrosolubilnim (etanolnim) ekstraktima svih uzoraka heljde

Figure 1. Chromatograms of all buckwheat sample components in the hydrosoluble (ethanol) extracts

Generally, the similarity in the composition of the hydrophilic components was detected in all nine buckwheat species, which is in line with previous studies.[16]

Figure 2 shows Pearson's r correlation dendrograms of nine hydrosoluble samples. The ordinate (Y-axis) presents the correlation coefficient.

The Pearson's r correlation dendrograms show that the similarity in the composition of hydrophilic substances in buckwheat (components listed in Table 1) is significant (r > 0.991), Figure 2.



Slika 2. Dendrogram korelacije komponenata 9 uzoraka heljde iz Tabele 1

Figure 2. Dendrogram of component correlations from Table 1 of 9 buckwheat species

Table 1 shows the retention time of components in the chromatogram presented in Figure 1.

Tabela 1. Retenciona vremena (R_t) komponenanta heljde u hidrosolubilnom (etanolnom) ekstraktu

Table 1. Retention time (R_t) of buckwheat components in the hydrosoluble (ethanol) extracts

Rt/min	Jedinjenje *Compound*
8.23	ERYTHRITOL PER-TMS
9.32	D-Arabino-Hexitol, 2-deoxy-1,3,4,5,6-pentakis-O-(trimethylsilyl)-
10.51	XYLITOL 5TMS
10.72	Ribitol, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-
11.16	1,2,3,4,5-pentakis-O-(trimethylsilyl)-pentose
11.57	Arabinofuranose, 1,2,3,5-tetrakis-O-(trimethylsilyl)-
11.72	Sorbopyranose, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-
11.80	D-Galactose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-
12.00	beta.-D-Galactofuranose, 1,2,3,5,6-pentakis-O-(trimethylsilyl)-
12.07	2-Deoxy-galactose, tetrakis(trimethylsilyl)
12.14	Mannopyranose pentakis(trimethylsilyl)
12.41	Talose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-
12.50	D-Mannopyranose, 1,2,3,4,6-pentakis-O (trimethylsilyl)-
12.59	alpha.-D-Galactopyranose, 1,2,3,4,6-pentakis-O-(trimethylsilyl)-
12.93	D-Glucitol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-
13.13	Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-, allo-
13.33	Glucopyranose pentaTMS $$ Glucopyranose, 1,2,3,4,6-pentakis-O-(trimethylsilyl)-, D-
13.83	Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-, scyllo-
14.21	ALLOSE PER-TMS
14.44	Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-isomer
18.09	D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-
19.00	alpha.-D-Glucopyranoside,1,3,4,6-tetrakis-O-(trimethylsilyl)-.beta.-D-fructofuranosyl2,3,4,6-tetrakis-O-(trimethylsilyl)-
20.72	PER-TMS D Hexose isomer
21.15	MELIBIO PER-TMS SE 8TMS
21.52	PER-TMS D Hexose isomer
25.93	Maltose, octakis(trimethylsilyl)-
28.70	alpha.-D-Galactoside, methyl tetrakis-O-(trimethylsilyl)-

Conclusion

This analysis proves that it is possible to compare types of plants through their content of sugar using gas chromatography-mass spectrometry method and correlation analysis. This technique was used for showing that the sugar composition of different buckwheat varieties is very similar, regarding nutritional aspect. These results can be of great significance for further usage of waste flour in production of bioethanol.

Acknowledgment

The authors gratefully acknowledge the financial support Science and Technological Development of Autonomous Province of Vojvodina, 114-451-3830/2013-02).

References

Ohnishi O. (1998). Economic Botany. 52: 123-133.
Bonafaccia G., Marocchini M., Kreft I. (2003). Food Chemistry. 80: 9-15.
Im J., Huff H. E., Hsieh F. (2003). Journal of Agricultural and Food Chemistry. 51: 659-666.
Sauerbeck G., Greef J. M. (2002). Journal of Agronomy and Crop Science. 189: 198-198.
Mitsuru W. (1998). Journal of Agricultural and Food Chemistry. 46: 839-845.
Wieslander G., Norback D. (2001). In: Symposium of Buckwheat. The proceeding of the 8th: 608-612.
Alvarez-Jubete L., Arendt E. K., Gallagher E. (2010). Trends in Food Science and Technology. 21: 106-113.
Li S., Zhang Q. H. (2001). Food Science and Nutrition. 41 (6): 451-464.
Mazza G., Oomah B. D. (2003). In: Encyclopedia of Food Sciences and Nutrition, B. Caballero (Ed.), Oxford, United Kingdom: Academic Press.
Krkoškova B., Mrazova Z. (2005). Food Research International. 38: 561-568.
Ikeda K. (2002). Advances in Food and Nutrition Research. 44: 395-434.
Hammer O., Harper D. A. T., Ryan P. D. (2001). Paleontological Statistics Software Package for Education and Data Analysis. Paleontologia Electronica. 1: 4-11.
Kim S. L., Kim S. K., Park C. H. (2004). Food Research International. 37: 319-327.
Wijngaard H. H., Arendt E. K. (2006). Cereal Chemistry. 83: 391-401.
Kim S. L., Son Y. K., Hwang J. J., Kim S. K., Hur H. S., Park C. H. (2001). Fagopyrum. 18: 49-54.
Vujić Đ. N., Ačanski M. M., Bodroža-Solarov M. I., Hristov N. S., Krunić M. N. (2012). CI&CEQ. 18(4): 555-561.

SLIČNOST UGLJENOHIDRATNOG SASTAVA OTPADNOG BRAŠNA HELJDE DOKAZANA PRIMENOM GASNE HROMATOGRAFIJE SA MASENOM SPEKTROMETRIJOM

R.Razmovski[1], M. Ačanski[1], V.Puškaš[1], V. Vučurović[1], U. Miljić[1], K. Pastor[1]

Izvod

Kvalitativna analiza hidrosolubilnih komponenti 9 uzoraka otpadnog brašna heljde izvedena je pomoću gasne hromatografije sa masenom spektrometrijom (GC-MS). Svi uzorci prethodno su obezmašćeni heksanom. Uzorci obezmašćenog brašna osušeni su na vazduhu, nakon čega je izvršena ekstrakcija etanolom. Etanolni ekstrakti upotrebljeni su za analizu rastvorljivih ugljenih hidrata. Kao reagens za derivatizaciju ugljenih hidrata u metilsilil-etre korišćen je TMSi (trimetilsililimidazol). Rezultati pokazuju da je sastav najzastupljenijih ugljenih hidrata, odnosno njihovih trimetilsilil-etara, veoma sličan u svih 9 uzoraka heljde. U cilju poređenja hidrosolubilnog sastava etanolnih ekstrakata uzoraka otpadnog brašna heljde primenjena je klaster analiza.

Ključne reči: otpadno brašno heljde, gasna hromatografija-masena spektrometrija, korelacije hidrosolubilnog sastava

[1]Univerzitet u Novom Sadu, Tehnološki Fakultet, Bulevar Cara Lazara 1, 21000 Novi Sad, Srbija (autor za kontakt: macanski@tf.uns.ac.rs)