APTEFF, 46, 1-269 (2015) UDC: 633.16:547.915:664.1:543.544.3
DOI: 10.2298/APT1546065P BIBLID: 1450-7188 (2015) 46, 65-75
Original scientific paper

LIPID AND SUGAR PROFILES OF VARIOUS BARLEY CULTIVARS *(Hordeum vulgare)*

Kristian A. Pastor[1], Marijana M. Ačanski[1], Djura N. Vujić[1], Ankica Đ. Kondić-Špika[2] and Nikola S. Hristov[2]*

[1] University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[2] Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia

The lipid components and soluble sugars in flour samples of different cultivars of barley (Hordeum vulgare), involving winter malting barley, winter forage barley, spring barley, and hulless barley, were identified). Fatty acids were extracted from flour samples with n-hexane, and derivatized into volatile methyl esters, using TMSH (trimethylsulfonium hydroxide) in methanol. Soluble sugars were extracted from defatted and dried samples of barley flour with 96% ethanol, and further derivatized into the corresponding trimethylsilyl (TMS) oximes, using hydroxylamine hydrochloride solution and BSTFA (N,O-bis-(trimethylsilyl)-trifluoroacetamide). The hexane and alcoholic extracts of barley cultivars were analyzed by GC-MS system. Lipid and sugar compositions were very similar in all barley cultivars. Therefore, multivariate analysis was applied to numerical values of automatically integrated areas of the identified fatty acid methyl esters and TMS oximes of soluble sugars. The application of hierarchical cluster analysis showed a great similarity between the investigated flour samples of barley cultivars, according to their fatty acid content (0.96). Also, significant, but somewhat less similarity was observed regarding the content of soluble sugars (0.70). These preliminary results indicate the possibility of distinguishing flour made of barley, regardless of the variety, from flours made of other cereal species, just by the analysis of the contents of fatty acids and soluble sugars.

KEY WORDS: barley cultivars, lipid composition, soluble sugar composition, GC-MS, cluster analysis

INTRODUCTION

Research involving non-wheat flours has been stimulated tremendously by world food problems resulting primarily from expanding populations. Developing countries are much too dependent on costly imported grain; insufficient food and inadequate nutrition are widespread problems. Non-wheat flours have the potential of being economically benefi-

* Corresponding author: Pastor Kristian, University of Novi Sad, Faculty of Technology, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia, e-mail: pastor@tf.uns.ac.rs

cial to both developing and industrial countries and of making large nutritional contributions, particularly in developing countries (1). Small grains of high quality have been dominant on the world market in the last decade. Special importance is given to the improvement of yield and quality potential of alternative small grains due to their constant demand and shortage on the world market. Improvement of small grains processing has great potential primarily in increasing quality of raw materials, improvement of processing equipment and quality of final products, which would significantly affect the commercial sector (new products, new processing methods, expanding the range of small grain products etc.) (2). Barley *(Hordeum vulgare)* is used almost entirely as feed and as a grain for brewing and for ethanol production (1). Fortification of bread with barley significantly improves the nutritional quality of bread due to increased levels of dietary fiber and β-glucans, and qualifies the product (in some countries) to carry a health claim, relating the presence of β-glucans with reducing the risk of cardiovascular diseases, heart diseases, constipation (3) and type II diabetes (4).

Barley is a rich source of tocols, including tocopherols and tocotrienols, which are known to reduce serum LDL cholesterol through their antioxidant action (5). Soong et al. (2014) investigated total phenolic content, which is found to be positively correlated with total antioxidative capacity in muffins made of different cereal species. They proved that muffins made with barley flour had the highest phenolic content compared to corn, oat, wheat and rise (6). Wholegrain barley foods also appear to be associated with increased satiety and weight loss.

There is great potential to utilize barley in a large number of cereal-based food products as a substitute partially or wholly for currently used cereal grains such as wheat *(Triticum aestivum)*, oat *(Avena sativa)*, rice *(Oryza sativa)*, and maize *(Zea mays)*.

Barley is arguably the most widely adapted cereal grain species with production at higher latitudes and altitudes and farther into deserts than any other cereal crop. It is in extreme climates that barley remains a principal food source today, e.g., Himalayan nations, Ethiopia, and Morocco.

The aim of this study consists of two different parts: (i) the application of gas chromatography-mass spectrometry system (GC-MS) to determine lipid and soluble sugar composition in hexane and ethanol extracts of various barley cultivars; and (ii) the application of multivariate data analysis to investigate the similarity of analyzed cultivars and the possibility to determine the authenticity of barley flour based upon mutual similarities among the samples, considering the importance of barley flour addition in non-wheat and mixed flour bakery products. These results could be integrated in the procedures for flour quality assurance.

EXPERIMENTAL

Sample preparation

All analyzed barley samples were obtained from the Small Grains Department at the Institute of Field and Vegetable Crops "NS Seme", Novi Sad, Serbia: three cultivars of winter malting barley (Novosadski 525 - B1, NS Pinon - B2, NS Zitos - B3), three culti-

vars of winter forage barley (Atlas - B4, Somborac - B5, Rudnik - B6), one spring barley cultivar (NS Marko - B7), and one hulless barley cultivar (Golijat - B8). All barley samples were grown in the same year and at the same location, thus enabling a comparison independent from the differences in the environmental conditions.

About 10 g of each barley cultivar were ground using a laboratory mill (Falling number 3100, Sweden). An amount of 0.5 g of barley flour sample was poured in a 12 mL cuvette for centrifugation. The cuvette was afterwards filled with 5 mL of n-hexane and stirred on Vortex for 2 min, after which the mixture was centrifuged at 2000 rpm for 5 min. A volume of 3.00 mL of clear supernatant of each sample was separated into a 10 mL glass beaker and dried under nitrogen flow. The residue was first dissolved in 400 μL of methylene chloride, and then 100 μL of 0.2 M *trimethylsulfonium hydroxide* in methanol (TMSH, Macherey–Nagel) was added, to achieve the derivatization into volatile methyl-esters (7).

After removing the hexane fractions, the barley flour samples remained defatted. Samples of defatted flour were dried in the air. 5 mL of 96% ethanol (Merck) was added to each dried sample. The mixture was vortexed for 2 min and centrifuged at 2000 rpm for 5 min. A volume of 2.00 mL of clear supernatant was separated and 50 μL of 10% sodium hydroxide in ethanol and 50 μL of 10% hydroxilamine hydrochloride solution were then added, through which oximes of sugars were obtained in ethanol solution. The mixture was dried under nitrogen flow. The residue was first dissolved in 400 μL of methylene chloride and 50 μL of BSTFA (N,O-bis-(trimethylsilyl)-trifluoroacetamide, Macherey-Nagel) was added, by oximes were derivatized into trimethylsilyl-oximes (TMSO) (8). By creating TMSO, two peaks corresponding to the syn (E) and anti (Z) forms per reducing sugar are obtained and a single peak for any non-reducing carbohydrate which does not form oximes. These derivatives are applicable to both aldoses and ketoses, and have been widely used for the determination of carbohydrate complex mixtures, such as flour of cereals, as they present good GC properties and provide simple chromatograms (9).

All samples were prepared and analyzed in triplicates.

GC-MS analysis

The GC-MS analyses were performed on an Agilent Technologies 7890 gas chromatograph coupled to 5975 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA), operating in EI mode at 70 eV. A DB-5 MS column (30m × 0.25mm × 25μm) was used. The same experimental conditions were used to determine both lipid and simple sugar profiles. The temperature program was: 50-130^{o}C at 30^{o}C/min and 130-300^{o}C at 10^{o}C/min. The injector temperature was 250^{o}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 sample solutions.

Data processing

The analysis of the obtained chromatograms was performed using the MSD Productivity ChemStation program. WILEY 275 library was used for the mass spectra analysis.

UDC: 633.16:547.915:664.1:543.544.3
BIBLID: 1450-7188 (2015) 46, 65-75
Original scientific paper

PAST program was used for statistical data processing (10). Hierarchical cluster analysis of integrated surface areas of derivatized lipid and simple sugar compounds were performed. Data points were clustered using paired group algorithm and correlation similarity measure.

RESULTS AND DISCUSSION

Figure 1 shows the overlaid chromatograms of 8 samples of barley flour extracts: (A) overlaid chromatograms of lipid components; (B) overlaid chromatograms of soluble sugar components.

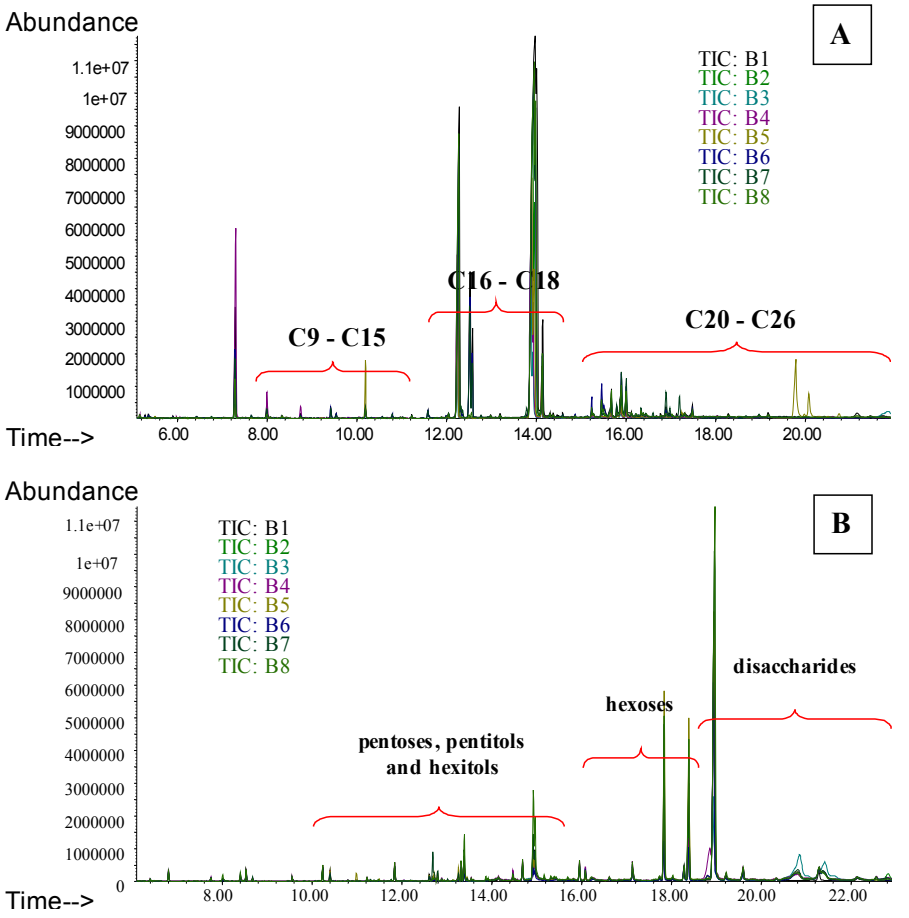

Figure 1. Overlaid chromatograms of 8 samples of barley flour: (A) lipid components; (B) soluble sugar components

The Wiley 275 mass spectra library was used for the identification of detected components in both hexane and ethanol extracts. The components of each barley flour extract were identified according to the characteristic fragmentations for each group of compounds – lipid and soluble sugar compounds.

Using the MSD Productivity ChemStation program the peaks of compounds of interest were integrated automatically from the chromatograms of both hexane and ethanol extracts of barley flour samples. The numerical values of integrated surface areas were subjected to the multivariate analysis using PAST program. A fundamental idea in multivariate data analysis is to regard the distance between objects in the variable space as a measure of the similarity of the objects. Hierarchical cluster analysis is a complementary, nonlinear, and widely used method for cluster analysis, with the result represented by a dendrogram (11). Paired group algorithm and correlation similarity measure were applied in all cases, because it provided the highest values of cophenetic correlation coefficient.

Hexane extracts analysis

Table 1 lists the identified lipid components in hexane extracts.

Table 1. Compounds detected in hexane extracts of analyzed barley samples

Lipid compound	Rt
Dodecanoic acid, methyl ester	8.08
Nonanedioic acid, dimethyl ester	8.34
Tetradecanoic acid, methyl ester	10.20
9-Dodecenoic acid, methyl ester, (E)-	10.98
Pentadecanoic acid, methyl ester	11.23
9-Hexadecenoic acid, methyl ester	12.04
Hexadecanoic acid, methyl ester	12.25
Heptadecanoic acid, methyl ester	13.19
9,12-Octadecadienoic acid (Z, Z), methyl ester	13.92
9-Octadecenoic acid (Z)-, methyl ester	13.96
Octadecanoic acid, methyl ester	14.12
11-Eicosenoic acid, methyl ester	15.67
Eicosanoic acid, methyl ester	15.90
Heneicosanoic acid, methyl ester	16.69
Ricinoleic acid methyl ester	16.90
Octadecanoic acid, 9,10-dihydroxy-methyl ester	17.23
13-Docosenoic acid, methyl ester	17.31
Docosanoic acid, methyl ester	17.48
15-Tetracosenoic acid, methyl ester	18.98
Tetracosanoic acid, methyl ester	19.17
Hexacosanoic acid, methyl ester	21.45

*The components written in italic are present in all barley flour samples

By looking at the overlaid chromatograms in Figure 1 (A) and Table 1 it can be said that the chromatograms can be divided into three different parts. First part includes methyl esters of minor fatty acids with less than 16 carbon atoms in the molecule (Rt ≤ 11.23 min). The second part includes methyl esters of the most abundant fatty acids, i.e. saturated and unsaturated fatty acid methyl esters with 16 and 18 carbon atoms in the molecule (Rt=12.04÷14.12 min): hexadecanoic (palmitic), 9,12-octadecadienoic (linoleic), 9-octadecenoic (oleic), and octadecanoic (stearic) acid. 9-hexadecenoic (palmitoleic) and heptadecanoic (margaric) acid methyl esters were also detected in the second part, but in much smaller quantities. The third part of the chromatograms is composed of methyl esters of saturated and unsaturated fatty acids with more than 18 carbon atoms in the molecule, which also appear in smaller amounts in all barley samples (Rt ≥ 15.25 min).

The dendrogram of 8 barley flour samples constructed based on the lipid components detected in the hexane extracts is presented in Figure 2.

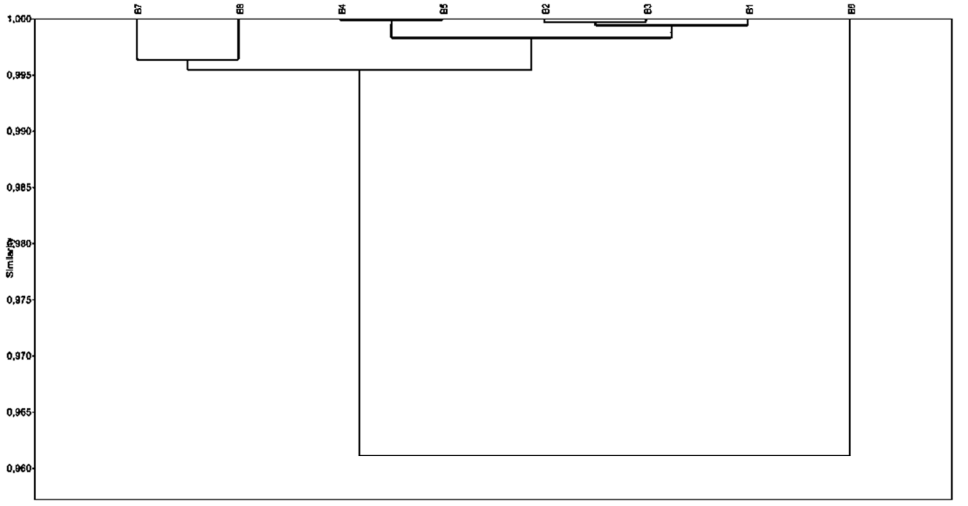

Figure 2. Dendrogram of lipid component correlations from Table 1, present in 8 samples of barley flour

The correlation similarity measure among barley samples is very high (r≥0.96). The flour samples of barley hybrids are divided in three groups (B1, B2, B3), (B4, B5), (B7, B8), but they still represent great similarities between the groups. The properties of barley flour sample B6 slightly differ compared to the other barley samples, regarding its lipid composition, but its similarity with the other samples is still very high (r~0.96).

The obtained value of cophenetic correlation coefficient was very high (0.9951), which means that the dendrogram preserves very faithfully the pairwise distances between the original unmodeled data points.

APTEFF, 46, 1-269 (2015) UDC: 633.16:547.915:664.1:543.544.3
DOI: 10.2298/APT1546065P BIBLID: 1450-7188 (2015) 46, 65-75
Original scientific paper

Analysis of ethanol extracts

Table 2 lists the identified soluble sugars in ethanol extracts of 8 barley flour samples.

Table 2. Compounds detected in ethanol extracts of the analyzed barley samples

Simple sugar compound	Rt
Xylitol, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-	10.40
Arabinopyranose, tetrakis-O-(trimethylsilyl)-α-D-	11.30
D-Mannitol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-	12.61
D-Glucitol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-	12.69
Xylitol, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-	12.80
D-Glucitol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-	13.25
D-Glucopyranose, 1,2,3,4,6-pentakis-O-(trimethylsilyl)-	16.09
α-D-Glucopyranoside, 1,3,4,6-tetrakis-O-(trimethylsilyl)-β-D-fructofuranosyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-	17.84
α-D-Glucopyranoside, 1,3,4,6-tetrakis-O-(trimethylsilyl)-β-D-fructofuranosyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-	18.38
D-Turanose, heptakis(trimethylsilyl)-	18.57
Melibiose, octakis(trimethylsilyl)-	18.80
α-D-Glucopyranoside, 1,3,4,6-tetrakis-O-(trimethylsilyl)-β-D-fructofuranosyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-	18.92
α-D-Glucopyranoside, 1,3,4,6-tetrakis-O-(trimethylsilyl)-β-D-fructofuranosyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-	20.85
α-D-Glucopyranoside, 1,3,4,6-tetrakis-O-(trimethylsilyl)-β-D-fructofuranosyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-	21.39

* The components written in italic are present in all barley flour samples

By observing the overlaid chromatograms in Figure 1 (B) and the detected components in Table 2 it can be concluded that soluble sugar components, present in all the barley flour samples include monosaccharides and sugar alcohols: pentose and pentitol (arabinose and xylitol), hexose and hexitols (glucose, glucitol and mannitol), but also some reducing disaccharides (turanose and melibiose). The most abundant component detected in the chromatograms of ethanol extracts is definitely a disaccharide, identified as the non-reducing sugar - sucrose, which represents the highest peaks on the chromatograms, eluting at 17.84 min, 18.38 min and 21.39 min.

The similarity of the barley flour samples obtained by applying hierarchical cluster analysis of compounds selected in the chromatograms of the ethanol extracts, by using all sugar components that are present in the obtained chromatograms (monosaccharides, disaccharides and sugar alcohols), is shown in Figure 3. The figure depicts the dendrogram of the analyzed barley samples obtained according to soluble sugars.

UDC: 633.16:547.915:664.1:543.544.3
BIBLID: 1450-7188 (2015) 46, 65-75
Original scientific paper

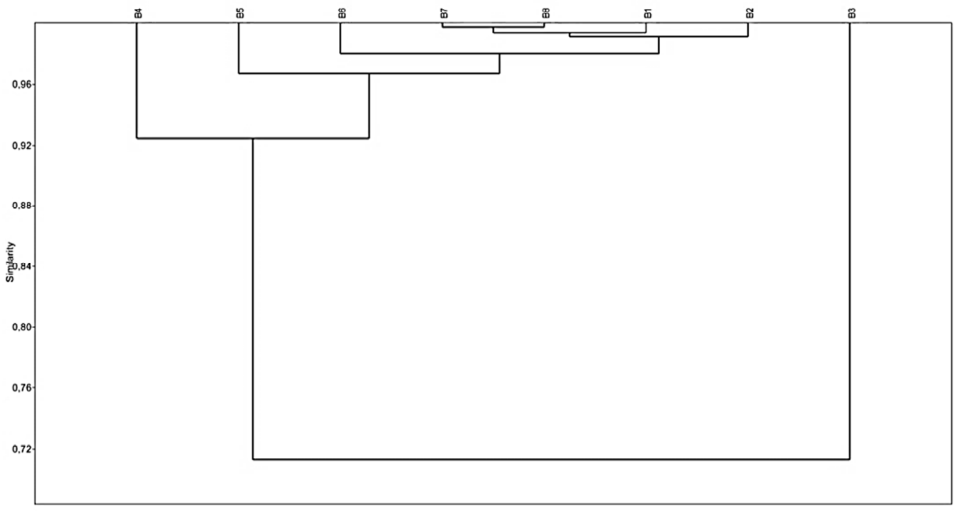

Figure 3. Dendrogram of simple sugar component correlations from Table 2, present in 8 samples of barley flour

In this case, based on the soluble sugar content, the branch with the sample B3 is separated from the other samples, which are much more similar among each other. The correlation similarity measure for all the samples was, however, very high, above $r \geq 0.995$. According to the soluble sugar content, samples B7 and B8 are grouped together in one branch because of the highest similarity, while samples B1, B2, B6, B5 and B4 manifest slight differences and their branches join individually in the order mentioned.

It can be said that the similarity among the barley samples, when comparing their ethanol extracts, was above 0.70, which is significantly lower compared to the similarity between the hexane extracts of the same samples, but still represents a relatively high similarity.

The obtained value of cophenetic correlation coefficient was again very high (0.9837).

CONCLUSIONS

All samples of flour produced of different barley cultivars give similar chromatograms of hexane and ethanol extracts, lipid and soluble sugar compositions and, therefore, high values of similarity measures. With the application of multivariate analyses to integrated surface areas of identified lipid and soluble sugar compounds, the samples of barley flour of different cultivars can be grouped together into clusters on the dendrograms based on the mutual similarities of their hexane and ethanol extracts. Studies regarding various cultivars of other cereal (corn, wheat) and pseudocereal species (amaranth, buckwheat), as well, has already proven high similarities of the lipid and simple sugar profiles of samples with the same botanical origin. In this way, our future research will be direc-

ted towards the identification of barley flour during procedures concerning the flour quality control on the world market, apart from the analyzed samples of barley cultivars.

Acknowledgement

The authors gratefully acknowledge the financial support of the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project TR 31066) and of the Provincial Secretariat for Science and Technological Development of Vojvodina (Project No. 114-451-1361/2014-03).

REFERENCES

1. Penfield, M.P.; Campbell, A.M. Flour. In *Experimental Food Science*; Penfield, M.P., Campbell, A.M., Eds.; Academic press: London, UK, 1990; pp 384-405.
2. Vujić, Dj.N.; Ačanski, M.M.; Bodroža-Solarov, M.I.; Hristov, N.S.; Krunić, M.N. Performance of GC-MS analysis for differentiation of various types of flour by creating dendrogram of liposoluble extract. *Chem. Ind. Chem. Eng. Q. (CI&CEQ)* **2012**, *18* (4), 555-561.
3. Izydorczyk, M.S.; McMillan, T. Chapter 31: Barley β-Glucans and Fiber-Rich Fractions as Functional Ingredients in Flat and Pan Bread. In *Flour and Breads and their Fortification in Health and Disease Prevention*; Preedy, V.R., Watson, R.R., Patel, V.B. Eds.; Elsevier: USA, 2011; pp 337-353.
4. King, R.A.; Noakes, M.; Bird, A.R.; Morell, M.K.; Topping, D.L. An extruded breakfast cereal made from a high amylose barley cultivar has a low glycemic index and lower plasma insulin response than one made from a standard barley. *J. Cereal Sci.* **2008**, *48*, 526-530.
5. Baik, B.-K.; Ullrich, S.E. Barley for food: Characteristics, improvement, and renewed interest. *J. Cereal Sci.* **2008**, *48*, 233-242.
6. Soong, Y.Y.; Tan, S.P.; Leong, L.P.; Henry, J.K. Total antioxidant capacity and starch digestibility of muffins baked with rice, wheat, oat, corn and barley flour. *Food Chem.* **2014**, *164*, 462-469.
7. Ačanski, M.M.; Vujić, Dj.N.; Psodorov Dj.B. Pratical method for the confirmation of authentic flours of different types of cereals and pseudocereals. *Food Chem.* **2015**, *172*, 314-317.
8. Ačanski, M.M.; Vujić, Dj.N. Comparing sugar components of cereal and pseudocereal flour by GC-MS analysis. *Food Chem.* **2014**, *145C*, 743-748.
9. Ruiz-Matute, A.I.; Hernández-Hernández, O.; Rodríguez-Sánchez, S.; Sanz, M.L.; Martínez-Castro, I. Derivatization of carbohydrates for GC and GC–MS analyses. *J. Chromatogr. B,* **2011**, *879*, 1226-1240.
10. Hammer, O.; Harper, D.A.T.; Ryan, P.D. *PAST-Paleontological statistics software package for education and data analysis*; Palaeontologia Electronica 4(1): Oslo, Norway, 2001; pp 4-11.
11. Varmuza, K.; Filzmoser, P. *Introduction to Multivariate Statistical Analysis in Chemometrics;* CRC Press Taylor & Francis Group: Boca Raton, FL, 2008; 265-270.

12. Pastor, K.A.; Ačanski, M.M.; Hristov, N.S.; Vujić, Dj.N.; Kondić-Špika, A.Dj. Application of multivariate analysis in wheat and corn flour differentiation, 4th International Congress Engineering, Environment and Materials in Processing Industry, Jahorina, Pale, Bosnia and Herzegovina, 4-6 March 2015, Book of Abstracts, pp 69-70.
13. Pastor, K.A.; Ačanski, M.M.; Bekavac, G.F.; Vujić, Dj.N. Qualitative analysis of liposoluble flour extracts of different corn hybrids using GC-MS system, 4th International Congress Engineering, Environment and Materials in Processing Industry, Jahorina, Pale, Bosnia and Herzegovina, 4-6 March 2015, Book of Abstracts, pp 349-350.
14. Psodorov, Dj.B.; Ačanski, M.M.; Vujić, Dj.N.; Brkljača, J.; Psodorov, D.Dj. Homogenity of oil and sugar components of amaranth flour investigated by GC-MS. *Chem. Ind. Chem. Eng. Q. (CI&CEQ)* **2015**, *21* (1), 71-76.
15. Ačanski, M.M.; Pastor, K.A.; Razmovski, R.N.; Kravić, S.Ž.; Psodorov, Đ.B.; Vujić, Đ.N. Homogenity of oil and sugar components of buckwheat flour investigated by GC-MS system and multivariate analysis, International Congress on Natural Science and Engineering, Kyoto, Japan, 7-9 May 2015, Conference Proceedings, Part 2, pp 403-410.

ЛИПИДНИ И ШЕЋЕРНИ ПРОФИЛИ РАЗЛИЧИТИХ СОРТИ ЈЕЧМА (*Hordeum vulgare*)

Кристиан А. Пастор[1], Маријана М. Ачански[1], Ђура Н. Вујић[1], Анкица Ђ. Кондић-Шпика[2], Никола С. Христов[2]

[1] Универзитет у Новом Саду, Технолошки факултет, Булевар цара Лазара 1, 21000 Нови Сад, Србија
[2] Институт за ратарство и повртарство, Максима Горког 30, 21000 Нови Сад, Србија

У овом раду идентификоване су липидне компоненте и растворљиви шећери у узорцима брашна различитих сорти јечма (*Hordeum vulgare*). Испитане су следеће сорте јечма: 3 узорка сорте озимог пивског јечма, 3 узорка сорте озимог сточног јечма, и по један узорак сорти јарог и голозимог јечма. Из узорка брашна јечма прво су хексаном екстраховане масне киселине, које су затим дериватизоване у одговарајуће, испарљиве метил-естре, применом реагенса за дериватизацију TMSH (триметилсулфонијум-хидроксид у метанолу). Након тога, обезмашћени и осушени узорци јечменог брашна подвргнути су екстракцији 96%-тним етанолом, чиме су издвојени растворљиви шећери, који су даље дериватизовани у одговарајуће TMS-оксиме, применом раствора хидроксиламин-хидрохлорида и BSTFA (N, O-бис-(триметилсилил)-трифлуороацетамид). Хексански и алкохолни екстракти сорти јечма анализирани су применом гасне хроматографије - масене спектрометрије (GC-MS). Састав липидних, као и шећерних компонената је био врло сличан код свих сорти. Стога је за даљу обраду података примењена мултиваријантна анализа нумеричких вредности интегрисаних површина идентификованих метил-естара масних киселина и триметилсилил-оксима растворљивих шећера. Примена хијерархијске кластер анализе показује веома велике сличности између испитаних узорака брашна сорти јечма, по садржају масних киселина (0.96). Такође значајна, али нешто ма-

ња сличност установљена је код садржаја растворљивих шећера (0,70). Ови прелиминарни резултати указују на могућност разликовања јечменог брашна, без обзира на сорту, од брашна других житарица анализом садржаја масних киселина и растворљивих шећера.

Кључне речи: сорте јечма, липидни састав, састав простих шећера, GC-MS, кластер анализа

Received: 9 April 2015.
Accepted: 18 June 2015.