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ANALYSIS OF HEXANE EXTRACTS OF CORN HYBRIDS

ANALIZA HEKSANSKIH EKSTRAKATA BRAŠNA HIBRIDA KUKURUZA

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ABSTRACT

Groups of corn hybrids with common genetic constitutions were analyzed. The lipid compounds from 13 samples of corn flour were extracted with n-hexane. Trimethylsulfonium hydroxide (TMSH) was applied for derivatization of fatty acids into volatile methyl esters. Derivatized extracts were analyzed by GC-MS.Simplified SIM chromatograms were extracted from TIC chromatograms by selecting the ion 74 m/z, which is characteristic only for lipid components. By using cluster analysisof the peak areas of lipid substances a dendrogram was obtained. It was observed that the best separation among corn hybrids can be achieved by introducing automatic integrated areas of peaks from third part of the chromatograms (lipid components that elute after 14.25 min) in multivariate analysis. However, it was also observed that samples of corn hybrids are grouped into clusters independently from their corresponding genetic constitution, no matter which part of the chromatogram is to be applied in cluster analysis.

Keywords: *corn hybrids, extraction and derivatization, GC-MS, lipid composition, cluster analysis.*

REZIME

Sistemom gasne hromatografije sa masenom spektrometrijom (GC-MS) analizirane su grupe hibrida kukuruza različitih genetskih konstitucija. Uzorci su najpre samleveni na laboratorijskom mlinu i homogenizovani. Lipidna jedinjenja iz 13 uzoraka brašnahibrida kukuruza ekstrahovana su prvobitno n-heksanom. Trimetilsulfonijum hidroksid (TMSH) je primenjen za derivatizaciju masnih kiselina u njihove odgovarajuće, isparljive metil estre. Derivatizovani ekstrakti brašna hibrida kukuruza potom suanalizirani primenom GC-MS sistema, pri čemu se dobijaju relativno kompleksni TIC hromatogrami. Pojednostavljeni SIM hromatogrami izdvojeni su sa TIC hromatograma odabirom fragmentnog jona 74 m/z (tzv. jon McLafferty-jevog premeštanja), koji je karakterističan samo za lipidne komponente. Na ovaj način se iz dalje analize isključuju druga nepolarna jedinjenja, koja se iz analiziranih uzoraka takođe ekstrahuju heksanom (npr. ugljovodonici). Pikovi lipidnih jedinjenja identifikovani su primenom masenog spektrometra povezanog sa Wiley bibliotekom masenih spektara. Primenom klaster analize automatski integrisanih površina pikova svih identifikovanih lipidnih komponenti dobija se dendrogram. Primećeno je da se najbolje razdvajanje među uzorcima hibrida kukuruza može postići unošenjem automatski integrisanih površina pikova lipidnih komponenti isključivo iz trećeg dela hromatograma, odnosno komponenti koje eluiraju posle 14,25 min, u multivarijantnu klaster analizu. Međutim, takođe je uočeno da se uzorci hibrida kukuruza na dendrogramina grupišu u klastere potpuno nezavisno od njihove odgovarajuće genetske konstitucije, bez obzira na to koji deo hromatograma je primenjen u klaster analizi.

Ključne reči: *hibridi kukuruza, ekstrakcija i derivatizacija, GC-MS, lipidni sastav, klaster analiza.*

INTRODUCTION

Cereals and cereal-based products have constituted the major component of the human diet throughout the world since the earliest times (*Rosell, 2011*). Problems with a lack of food resources in the world, which are caused by growing populations, stimulate research on non-wheat types of flour. Developing countries depend on the import of expensive grains,thereforeinsufficient food and inadequate nutrition are widespread problems. The non-wheat types of flour have the potential of economic benefits, both for developing countries and industrial countries (*Penfield and Campbell, 1990*).

Corn is the most widely grown cereal in the world, due to the high productivity and high adaptability. It has a wider scope of application than any other cereal (*Milenković et al., 2014*; *Žilić et al., 2013*).

In the era of the greatest technological development of human civilization, there is a growing need for the production of safe food (*Milenković et al., 2014*).

Corn flour and its products are preferred because of their taste, but also because of good nutritional properties (*Žilić et al., 2013*). Different bioactive components of fruits and vegetables, such as carotenoids, anthocyanins and phenolic compounds, whose biological activities are associated with health promotion and disease prevention, are also present in corn (*Singh et al., 2011*). In addition, corn is most frequently used as feed in animal nutrition, but because it is rich in starch, it is also one of the major raw materials for the energy production *(Ačanski et al., 2014; Semenčenko et al., 2014)*. Corn flour is used in dry mixtures for obtaining various products, such as bread, cereals and extruded products (*Penfield and Campbell, 1990*).

The lipid content in corn seeds is between 2 and 6 %. The lipids can be subdivided into nonpolar, polar and non-saponifiable. By much the most abundant type is the nonpolar fraction consisting of fatty acids, triglycerides rich in linoleic, followed by oleic acid. Linoleic acid is considered to be the only essential fatty acid in human nutrition, and corn oil contains more than 50 % of this acid. In previous studies it was found by using various methods that about 99 % of the corn oil composition consists of fatty acids such as palmitic (16: 0), stearic (18: 0), oleic (18: 1), linoleic (18: 2) and linolenic (18: 3), and that in some cases myristic (14: 0), eicosanoic (20: 0) and eicosenoic acid (20: 1) are also present *(Jimenez et al., 2009; Preciado-Ortiz et al., 2013)*. Among flour lipids, the so-called ‘free fraction’ that can

be extracted by non-polar solvents is of great interest. Also, the content of this fraction is much more susceptible to changes in relation to the content of bound lipids (*Konopka et al., 2006*).

In our previous investigations it was already proven that it is possible to differentiate samples of cereal flour (wheat and spelt) from samples of pseudocereal flour (buckwheat and amaranth) using gas chromatography-mass spectrometry (GC-MS) with multivariate analysisof their liposoluble extracts *(Ačanski et al., 2015)*. The aim of this work is the application of GC-MS system to identify lipid components in the samples of flour made from 13 types of corn hybrids with different genetic constitutions, and determining the ability to distinguish them on the basis of their liposoluble extracts by using multivariate analysis. Furthermore, it was interesting to verify the potential correlation between the similarity of the samples of corn hybrids based on their genetic constitutions and composition of liposoluble extracts.

MATERIAL AND METHOD

All analyzed samples of corn hybridswere obtained from the Corn Department at the Institute of Field and Vegetable Crops "NS Seme", Novi Sad, Serbia. The 13 analyzed corn hybrid samples are grouped according to similar genetic constitutions: (C1, C2), (C3, C4), (C5, C6), (C7, C8), (C9, C10, C11, C12, C13). All corn hybrid samples were grown in the same year and at the same location, thus enabling a comparison independent from differences in environmental conditions.

About 10 g of 13 corn hybrid samples were ground using a laboratory mill (Falling number 3100, Sweden). 0.5 g of each sample of corn flour 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. 3 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 t*rimethylsulfonium hydroxide* in methanol (TMSH, Macherey–Nagel) was added, by which derivatization into volatile methyl-esters was performed (Macherey–Nagel) *(Ačanski et al., 2015)*.

Further procedure was conducted on a GC-MS system. The GC–MS analyses was performed on Agilent Technologies 7890 instrument coupled to MSD 5975 equipment (Agilent Technologies, Palo Alto, CA, USA) operating in EI mode at 70 eV. An 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 sample solutions. Analysis of obtained chromatograms were performed using the MSD Productivity ChemStation programme.WILEY 275 library was used for the mass spectrum analysis. PAST programme was used for statistical data processing (*Hammer et al., 2001*). Hierarchical cluster analysis of integrated surface areas of lipid compounds was performed. Data points were clustered using paired group algorithm and correlation similarity measure.

RESULTS AND DISCUSSION

After GC-MS analysis total ion chromatograms (TIC) were obtained. Overlaid TIC chromatograms of all corn hybrid flour samples are shown in Figure 1 (A).

TIC chromatograms are rather complicated for the analysis since they include the peaks of all non-polar components extracted with hexane.using the MSD Productivity ChemStation programme for processing data that were obtained by GC-MS analysis, simplified selected ion monitoring chromatograms (SIM) were extracted from the TIC chromatograms by selecting the appropriate 74 m/z ion (the so-called McLafferty rearrangement ion), which is characteristic only for the lipid components.In this waypeaks of other compounds extracted with hexane, such as hydrocarbon peaks,were excluded from further analysis. The overlaid SIM chromatograms of all corn hybrid flour samples are shown in Figure 1 (B). The difference in complexity between overlaid TIC and SIM chromatograms is obvious. The peaks identification of lipid components of corn hybrids was performed by mass spectral analysis, by comparing mass spectra of peaks representing lipid compounds on SIM chromatograms with Wiley library of mass spectra. Identified compounds in liposoluble flour extracts made from all corn hybrid samples are listed in Table 1.

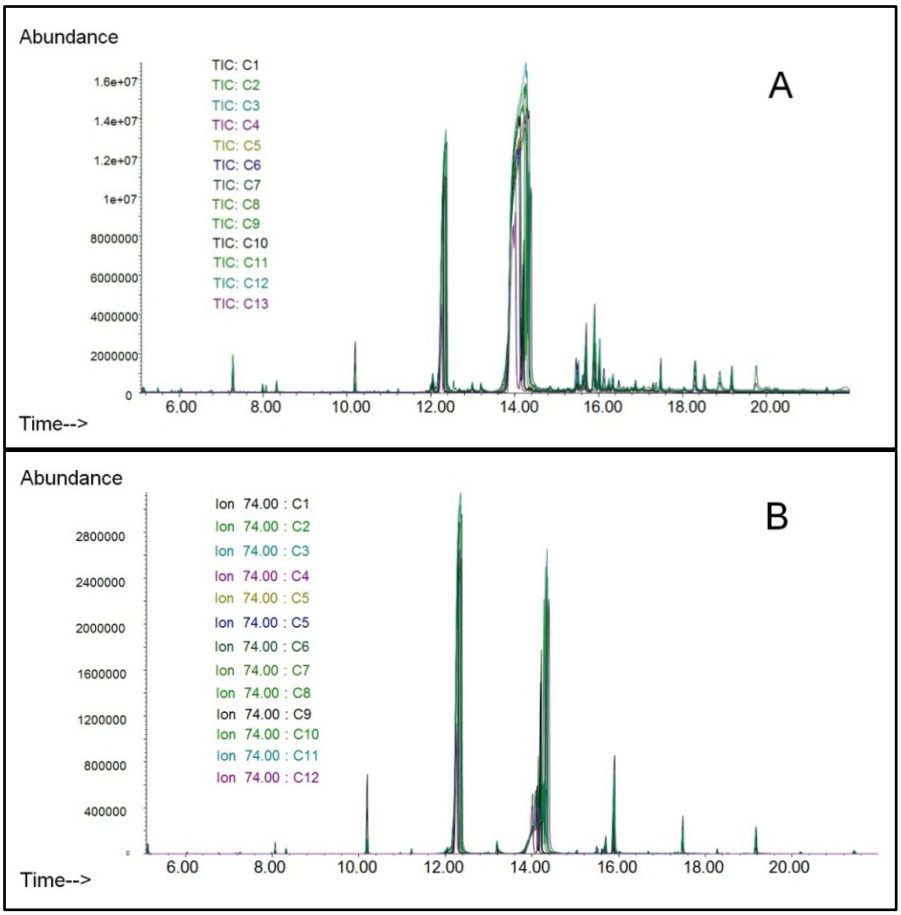

Fig. 1. Overlaid TIC chromatograms (A), and SIM chromatograms (B) of all corn hybrid flour samples

Table 1. Identified lipid components in corn hybrid flour samples

Compound	Rt
Nonanoic acid, methyl ester	**5.15**
Nonanoic acid, 4-oxo-, methyl ester	**6.52**
Nonanoic acid, 9-oxo-, methyl ester	**7.17**
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**
*10-Nonadecenoic acid, methyl ester**	**14.86**
Nonadecanoic acid, methyl ester	**15.04**
11-Eicosenoic acid, methyl ester	**15.67**
Eicosanoic acid, methyl ester	**15.87**
Heneicosanoic acid, methyl ester	**16.69**
13-Docosenoic acid, methyl ester	**17.31**
Docosanoic acid, methyl ester	**17.48**
Tetracosanoic acid, methyl ester	**19.17**
Campesterol	**20.01**
Pentacosanoic acid, methyl ester	**20.21**
Squalene	**20.27**
Stigmasterol	**20.88**
Hexacosanoic acid, methyl ester	**21.45**

**Lipid components that elute in the third part of the chromatograms are writen in italic*

Mass spectrometer was able to successfully identify lipid compounds without the use of any sort of analytical standard. Obtained match values for all identified lipid compounds were higher than 90 %. Figure 1 clearly indicates that each of these chromatogramscould be separated into three different parts. The first part elutes untill 11.23 min and consists of fatty acid methyl esters with a lower number of carbon atoms in the molecule, up to 16, such as Dodecanoic, Tetradecanoic acid, Pentadecanoic acid etc. The second part consists of methyl estersof saturated and unsaturated fatty acid with 16 to 18 carbon atoms in the molecule (9-Hexadecenoic acid, Hexadecanoic acid, Heptadecanoic acid, 9,12-Octadecadienoic acid (Z, Z), 9-Octadecenoic acid (Z)-, and Octadecanoic acid), which elute between 12.04 and 14.12 min.

These two parts of chromatograms are typical for each sample of cornhybrids, and therefore are not a good basis for distinguishing them. The third part consists of fatty acid-methyl esters with more than 18 carbon atoms (such as Nonadecanoic, Eicosanoic, Heneicosanoic, Docosanoic, Tetracosanoic, Pentacosanoic Hexacosanoic acid) and unsaponifiable compounds (Campesterol, Squalene andStigmasterol). This part of the chromatograms includes all lipid compounds that elute after 14.12 min.

Thesurface areas of the peaks of all identified lipid compounds listed in Table 1, were automatically integrated using the MSDChem Station Software, and the resulting data put into thePASTprogram in order to perform multivariate data analysis.

In general, 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 (*Varmuza and Filzmoser, 2009*). By the application of hierarchical cluster analysis, a dendrogram was obtained, Figure 2.

The lowest value of similarity measure between corn hybrid flour samples obtained in this way was still very high, about 0.9966. This means that the samples manifest strong similarities in liposoluble extract compositions. Some of the samples are divided in 3 groups (C2, C8, C12, C13), (C4, C6, C9), (C3, C10), while the branches with samples C1, C5, C7, and C11 join individually, expressing different properties of liposoluble extracts.

In our previous research it was concludes that better separation between samples of cereal and pseudocereal flour can be obtained by introducing only the automatically integrated surfaces of lipid compounds that elute in third part of the chromatograms into multivariate analysis (*Vujić et al., 2012*). This time it was decided to apply this method on differentiation between flour samples made of corn samples exclusively, i.e. of the same cereal species, considering their significantly higher botanical similarity. A dendrogram obtained using this method is presented on Figure 3.

It includes all lipid compounds from liposoluble extracts that elute after 14.12 min, Table 1. By observing similarity measure presented on y-axes it can be concluded that this time a better separation among corn hybrid flour samples is achieved.The value of similarity measure between corn hybrid flour samples is lower this time, about 0.81. This time the most of the samples are clearly divided in 4 groups (C1, C4), (C6, C8), (C3, C7), and (C2, C9, C11, C12, C13) while only the samples C5 and C10 stand separated in dendrogram presenting individual characteristics.

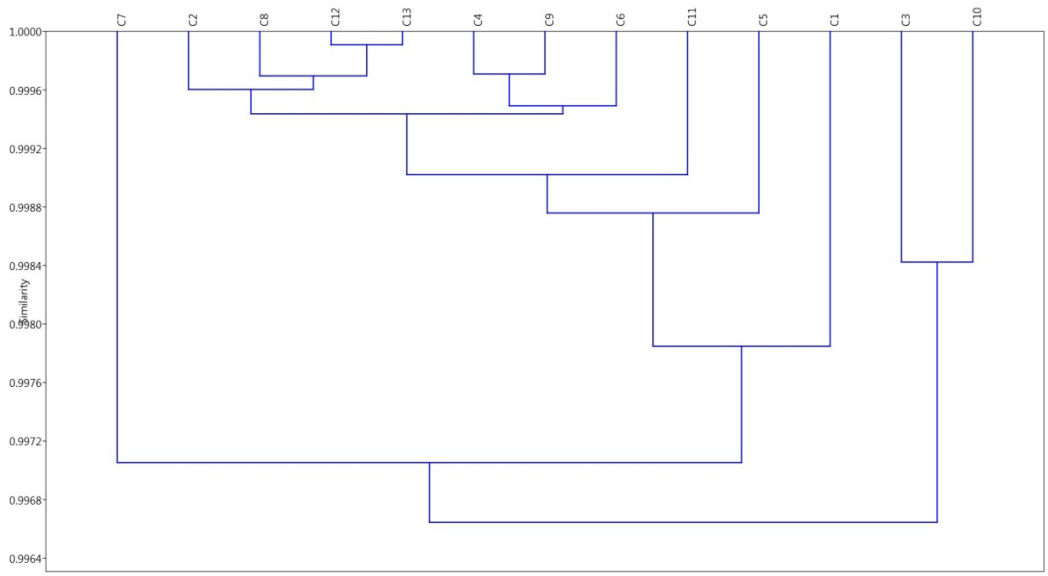

Fig. 2. Dendrogram of all lipid compounds of corn hybrid flour samples

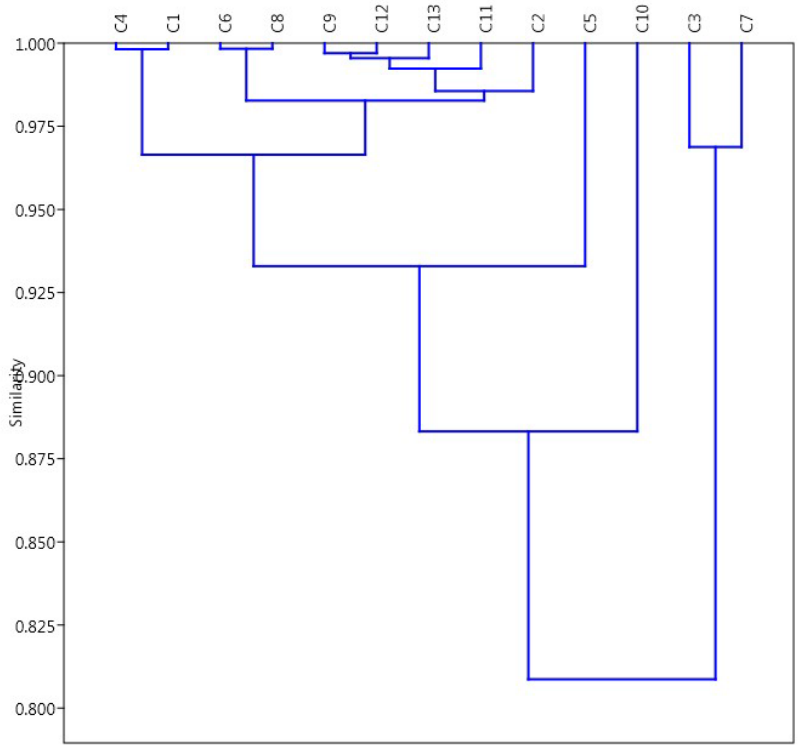

Fig. 3. Dendrogram of lipid compounds after 14.12 min of corn hybrid flour samples

CONCLUSION

After GC-MS analysis of all groups of corn hybrids with common genetic constitutions, similar TIC chromatograms were obtained. Using characteristic abundant fragment ion 74 m/z, simplified SIM chromatograms can be extracted from TIC chromatograms, thus excluding non-lipid components from further analysis procedure (such as liposoluble hydrocarbons). The performances of mass spectrometer used in lipid compound identifications were completely satisfying, giving strong match values, above 90 %, for every lipid component. Obtained chromatograms could be clearly divided in 3 parts. Applying hierarchical cluster analysis of automatically integrated surface areas of lipid compounds detected it can be stated that better separations among corn hybrid flour samples can be achieved using only those lipid components that elute in third part of the chromatograms, after 14.12 min (methyl esters of fatty acids with more than 18 C atoms in molecules, and non-saponifiable compounds), rather than all lipid compounds identified in this analysis procedure. However, no matter which part of the chromatogram is to be applied in cluster analysis, it can be concluded that samples of corn hybrids are grouped into clusters independently from their corresponding genetic constitution.

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