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Rapid Method for Small Grain and Corn Flour Authentication Using GC/EI-MS and Multivariate Analysis

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Abstract

The aim of this study was the application of the GC/EI-MS system and multivariate data analysis to investigate the possibility of chemical differentiation between small grain flour (wheat, barley, oat, triticale, rye) and corn flour samples. All cereal flour samples were first defatted with hexane, after which the extraction with ethanol was performed. Extracted simple sugars (mono-, disaccharides, trisaccharides and sugar alcohols) were analyzed in the form of their corresponding trimethylsilyl-oximes. Peaks of simple sugar derivatives were selected in total ion current (TIC) chromatograms by monitoring exclusively the following characteristic abundant ions: 204m/z, 217m/z and 361m/z. The total surface areas under the selected peaks were subjected to multivariate analysis. Applying principal coordinate analysis and hierarchical cluster analysis to obtained data, samples of corn flour could be very clearly distinguished from all samples of small grain flour, which presented a weaker separation among each other. This method circumvents common analytical procedures by excluding simple sugar identifications, quantitative analysis, the use of analytical standards and calibration curves. Results are applicable in the quality assurance of mixed flour on the market, considering the increased popularity of their consumption in human nutrition.

Key words GC/EI-MS, SIM mode, multivariate analysis, corn, small grains, flour

Introduction

Wheat is of continuing importance worldwide, but non-wheat sources of flour are of increasing interest as food crops. Research involving non-wheat flours has been stimulated tremendously by world food problems resulting primarily from expanding populations. Non-wheat flours have the potential of being economically beneficial to both developing and industrial countries and of making large nutritional contributions (Penfield and Campbell 1990). Consumers are becoming more conscious about the relationship between nutrition and health. Currently, innovations in bread are mainly focused on nutritionally improving bread through enrichment or the use of different flours (Rosell 2011; Doxastakis et al. 2002). The major advantage of incorporating barley, oat and rye into various food products and their consumption stems from their potential health benefits. They represent a rich source of various nutritive and antioxidative compounds, that have been shown to possess numerous beneficial properties, including anti-inflammatory, antiatherogenic, antiproliferative, anticancer, and anti-itch effects (Baik and Ullrich 2008; Kilci and Gocmen 2014; Yang et al. 2014; Zielinski et al. 2007). Triticale is a manmade hybrid of wheat and rye. Triticale is intended to combine the high yield potential and good grain quality of wheat with the disease resistance and environmental tolerance of rye, including winter hardiness. Being a hardy crop with high yield and good nutritional

composition, triticale has been suggested to possess the potential to fight world hunger (Rakha et al. 2011). Corn is the most widely grown crop in the world. It has a wider range of uses than any other cereal. Corn flour and products prepared from it are desirable, mostly due to their taste and nutritional benefits (Žilić et al. 2013). Corn has a higher antioxidant capacity compared to wheat, oat, and rice (Singh et al. 2011).

In our previous research it was proven without doubt that samples of cereal (wheat, spelt) and pseudocereal flour (amaranth, buckwheat) can be clearly distinguished by comparing simple sugar extracts using GC/EI-MS system and multivariate analysis. The process of derivatization was performed using trimethylsilylimidazole (TMSI) reagent (Ačanski and Vujić 2014).

Considering the increasing popularity of mixed flour bakery products, the aim of this paper was to study the possibility of distinguishing flour samples of different small grain cultivars (wheat, barley, oat, rye, triticale) and corn hybrids, both botanically belonging to the group of cereals, comparing their soluble sugar contents using Gas Chromatography-Mass Spectrometry system (GC/EI-MS) and Multivariate Analysis in a simple and rapid way, avoiding any qualitative and quantitative investigations of analyzed samples. To the best of our knowledge, no paper describing the possibility of GC-MS system in flour differentiation between various cereal species was reported in the literature. As TMS-derivatives of simple sugars give many isomers per each sugar compound and, therefore, many peaks on each chromatogram, this time it was decided to include trimethylsilyl-oximes (TMSO) of sugars in the analysis and simplify the chromatogram analysis procedure. Furthermore, the silylation step was performed using other derivatization reagent, bis-(trimethylsilyl)-trifluoroacetamide (BSTFA), which was described as more effective than TMSI according to the literature (Gordon 1990).

Materials and Methods

Sample preparation

All analyzed corn samples were obtained from the Maize Department and all analyzed small grain samples from the Small Grains Department, both at the Institute of Field and Vegetable Crops "NS Seme", Novi Sad, Serbia: wheat-W, barley-B, oats-O, rye-R, triticale-T and corn-C, Table 1. All analyzed cereals were cultivated in the same year and on the same field to avoid influence of environment onto seed composition.

Table 1 Cereal samples used in the analysis

Wheat	Renesansa (W1), Rapsodija (W2), Evropa 90 (W3), Pesma (W4), Milijana (W5), Nataša (W6), Venera (W7), Durumko (W8)
Barley	Novosadski 525 (B1), NS Pinon (B2), NS Zitos (B3), Atlas (B4), Somborac (B5), Rudnik (B6), NS Marko (B7), Golijat (B8), NS Mile (B9)
Oats	Dunav (O1), Jadar (O2), Sedef (O3)
Triticale	NS Karnak (T1), NS Trifun (T2)
Rye	NS Savo (R)
Corn	C1-C17

About 10 g of 23 small grain samples and 17 corn hybrid samples were ground using laboratory mill (Falling Number 3100, Sweden). Each flour sample was homogenized and further treated in the following manner. 0.5 g of flour was poured in a 12 mL cuvette for centrifugation. 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 rpm for 5 min. 3 mL of clear supernatant was poured into a 10 mL glass and left for the analysis of liposoluble (hexane) extracts (Vujić et al. 2012). The procedure was repeated three times, hexane fractions were rejected, and the 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 stirred on Vortex for 2 min and centrifuged at 2000 rpm for 5 min. 2 mL of clear supernatant was separated. 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 which derivatization of oximes into trimethylsilyl-oximes (TMSO) was performed (Macherey–Nagel).

Three sample solutions were prepared from every cereal flour sample.

Instrumentation

Further procedure was conducted on a GC-MS system. The GC–MS analyses were performed on Agilent Technologies 7890 instrument coupled to MSD 5975 equipment (Agilent Technologies, Palo Alto, CA, USA) operating in 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. A split ratio of 1:50 was used for the injection of 1 μL of sample solutions.

Data processing

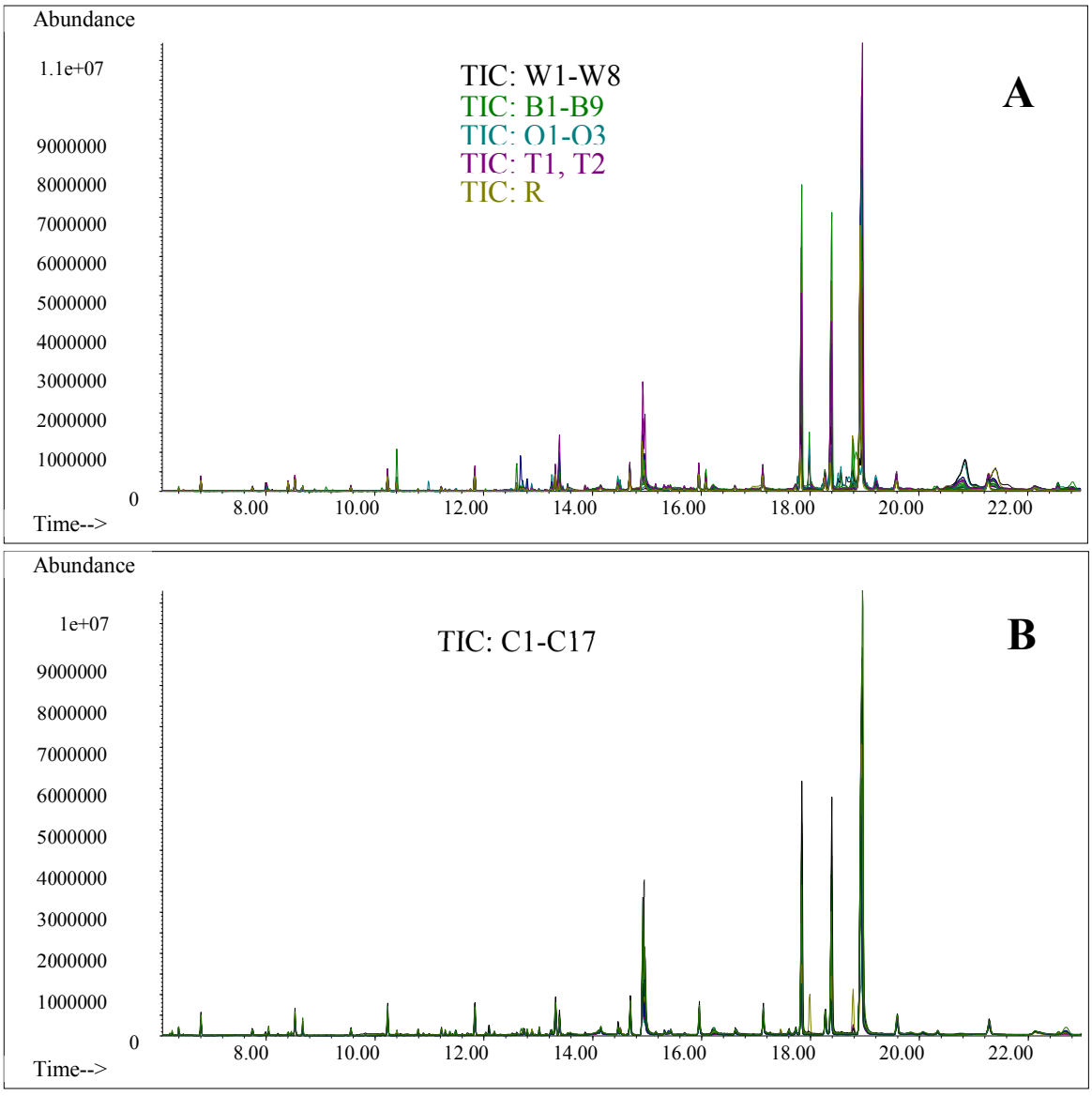
Analysis of obtained chromatograms were performed using the MSD Productivity ChemStation programme.

WILEY 275 library was used for the mass spectrum analysis.

PAST programme was used for the statistical data processing (Hammer et al. 2001).

Results and discussion

By performing GC-MS analysis TIC chromatograms of all cereal flour samples were obtained. Fig. 1 shows the overlapping TIC chromatograms of: all samples of small grains (A), all samples of corn (B), as well as all analyzed samples of flour together (C).



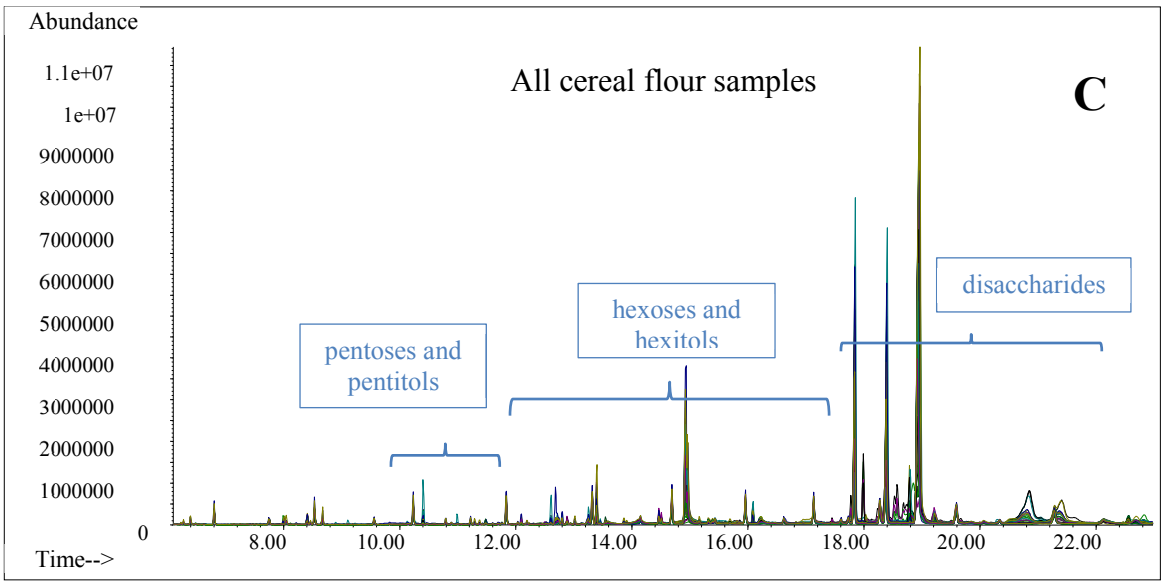


Fig. 1 Overlaid TIC chromatograms: (A) all small grain flour samples, (B) all corn flour samples, and (C) all flour samples together

Trimethylsilyl oximes can be obtained by a two-step derivatization procedure (oximation and silylation) producing two peaks corresponding to the *syn (E)* and *anti (Z)* forms per reducing sugar. A single peak is obtained for any non-reducing carbohydrate present, which do not form oximes. These derivatives are applicable to both aldoses and ketoses and have been widely used for carbohydrate determinations of complex mixtures, as they present good GC properties and provide simple chromatograms (Ruiz-Matute et al. 2011).

An example of a derivatized simple sugar molecule used in the analysis, is presented on Fig. 2. Structural formula of a simple sugar molecule (glucopyranose) is shown on Fig. 2 (A). An oximated simple sugar molecule (B) is obtained in the first derivatization step with hydroxilamine hydrochloride solution, and a trimethylsilylated oxime of the same molecule (C) in the second step of the derivatization procedure, using BSTFA.

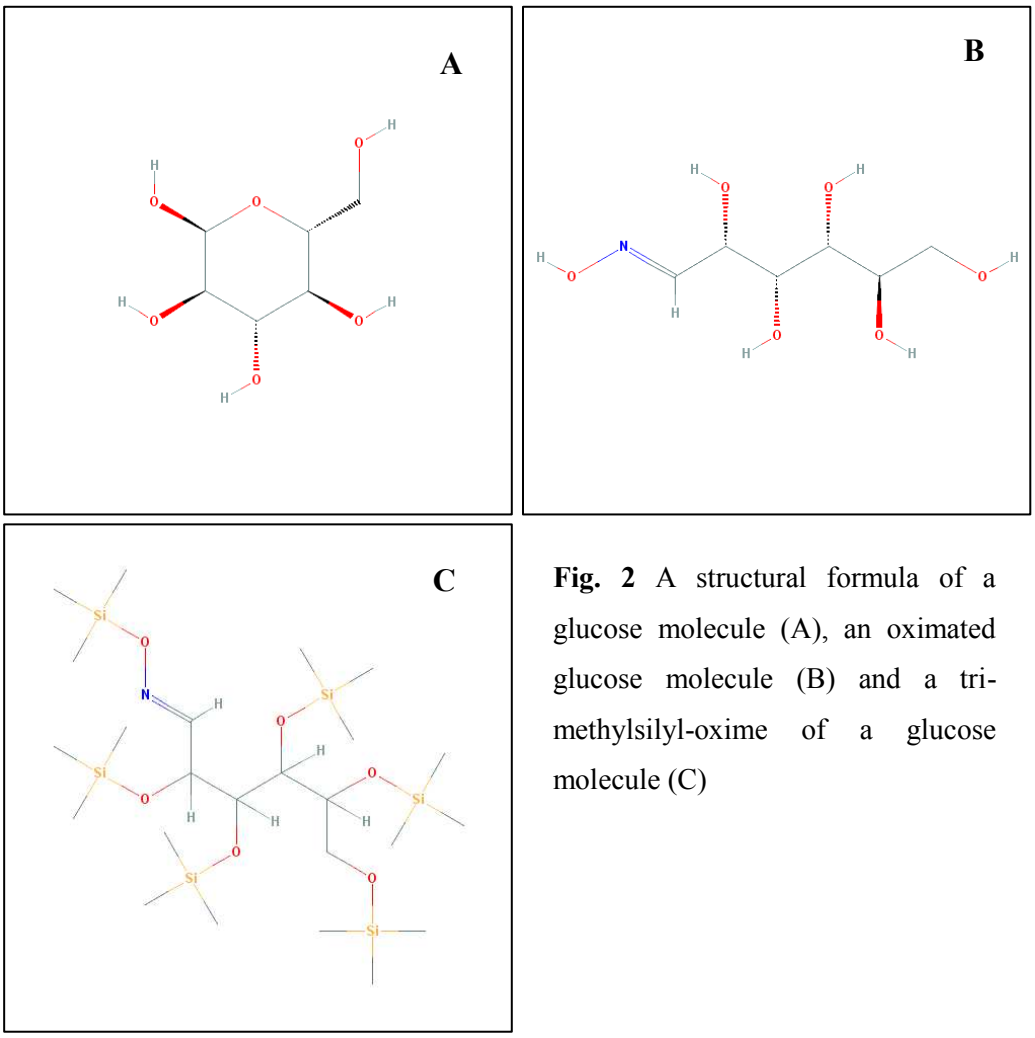


Fig. 2 A structural formula of a glucose molecule (A), an oximated glucose molecule (B) and a tri-methylsilyl-oxime of a glucose molecule (C)

Just by looking at the Fig. 1 it seems that chromatograms show no significant differences. By comparing mass spectra using MSD Productivity ChemStation programme and WILEY 275 library it was determined that pentoses and pentitols elute between 10.00 and 11.38 min, hexoses and hexitols between 12.10 and 17.84 min, and after 17.84 min only various types of disaccharides, Fig. 1. Given that our intention was not to identify individual simple sugar components, the analysis is further done by selecting specific abundant fragment ions of simple sugars. By selecting ions 204 m/z and 217 m/z, characteristics of TMS-derivatives of monosaccharides (pyranose and furanose ring, respectively) (Karady and Pines 1970; Ačanski and Vujic 2014), and 361 m/z, characteristic abundant fragment ion of TMS-derivatives of di- and trisaccharides (Füzfai et al. 2008), the selected ion monitoring (SIM) chromatograms were obtained from complex TIC chromatograms, which simplified the detection procedure of simple sugar derivative peaks in the chromatograms.

Some examples of mass spectra of different simple sugars are given in Fig. 3. The characteristic fragmentation patterns of the peaks representing one hexopyranose (Rt=16.081 min, Fig. 3A), one pentofuranose (Rt=11.381 min, Fig. 3B) and one dissacharide molecule (Rt=18.930 min, Fig. 3C) of completely randomly chosen cereal flour samples (wheat-W2, corn-C9 and barley-B3) are presented.

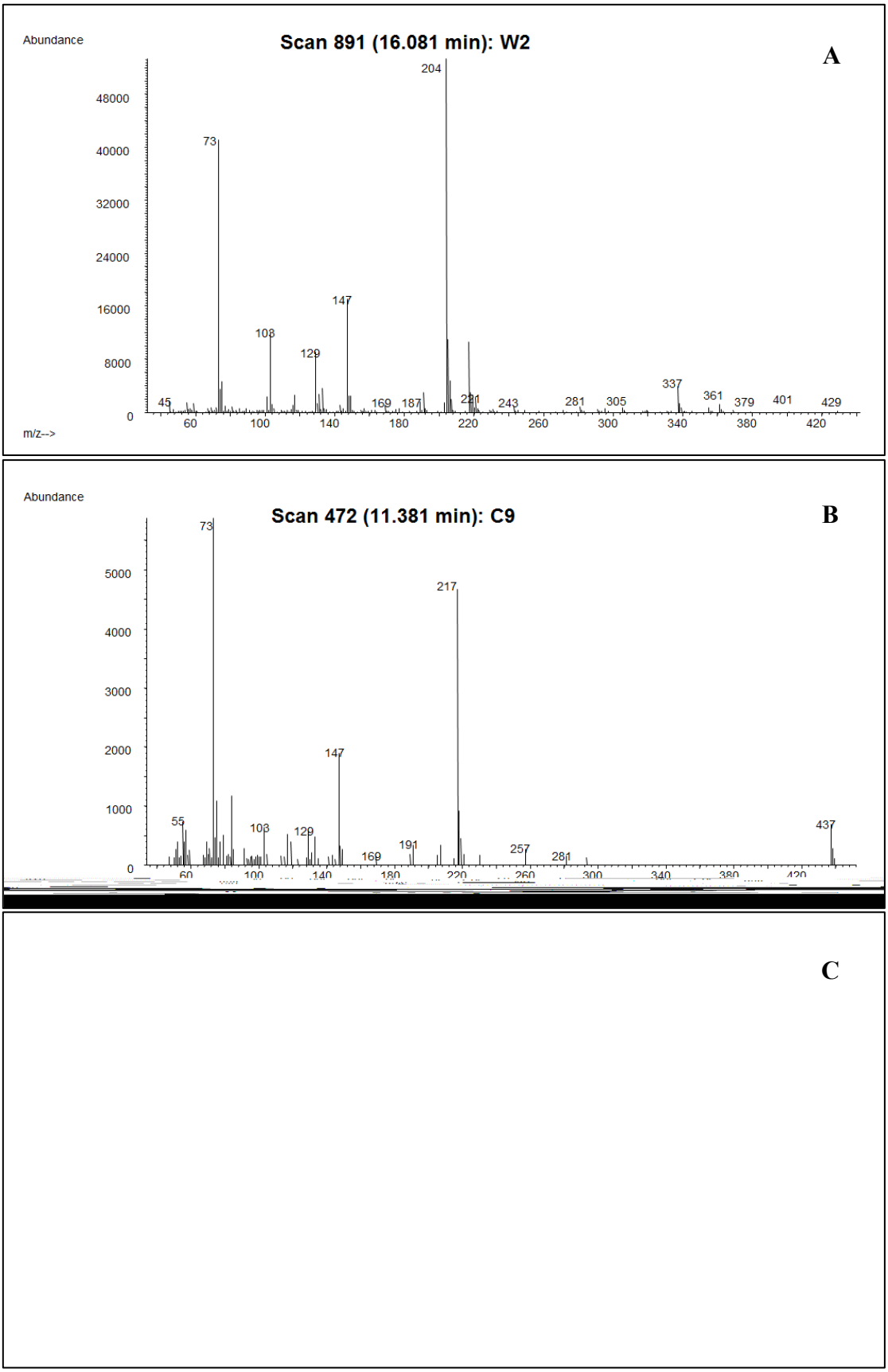


Fig. 3 Mass spectra and characteristic fragment ions of different simple sugar peaks: hexopyranose (A), pentofuranose (B) and a disaccharide molecule (C), of 3 random cereal flour samples

Fig. 4 shows a SIM chromatogram of the wheat sample (W1) with extracted characteristic fragmentation ions for simple sugars (A), and an enlarged example of a simple sugar peak detection in the chromatogram (B). Fig. 4B shows the example of a peak with characteristic abundant fragment ions which was considered for further analysis procedure.

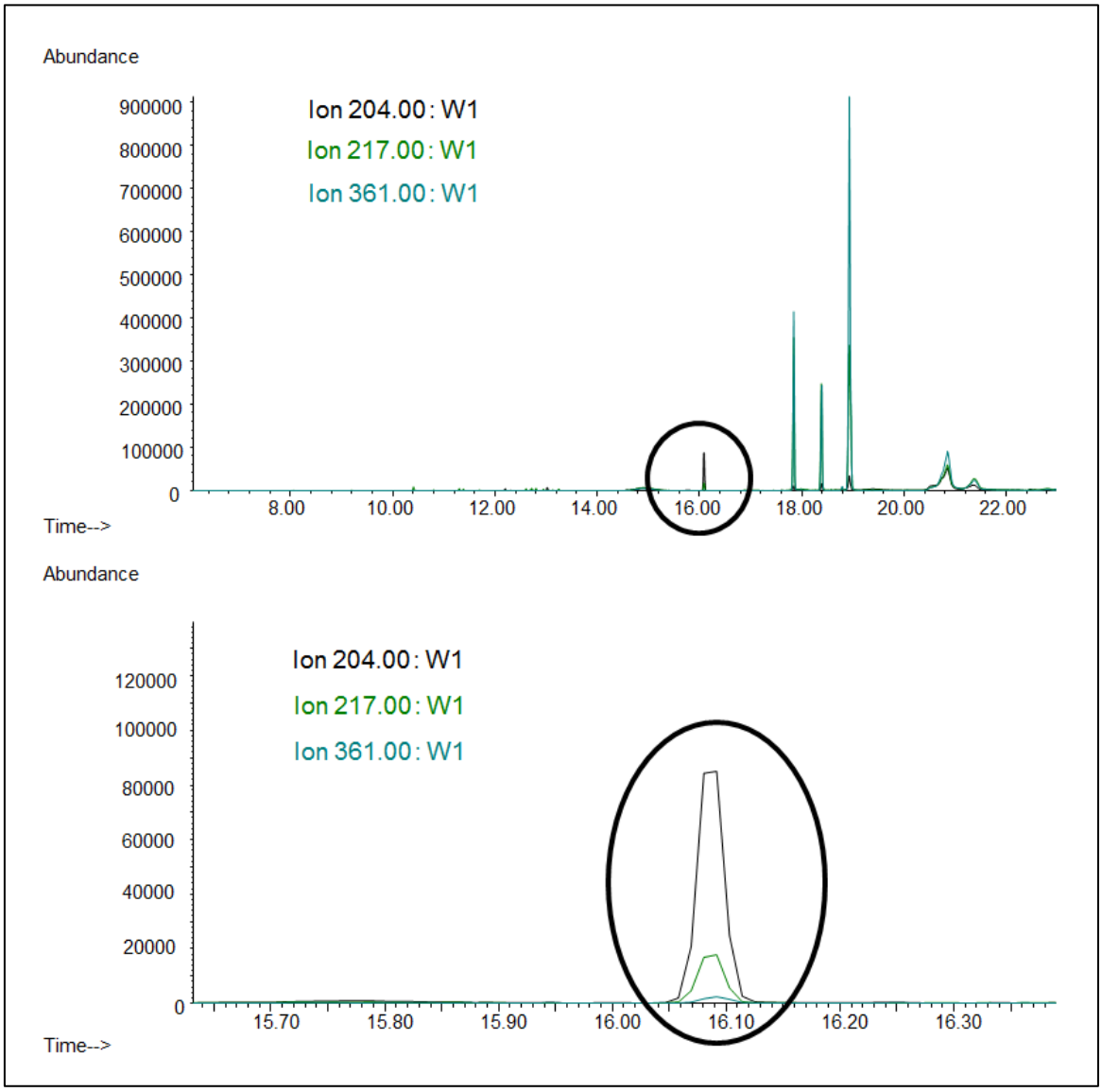


Fig. 4 Example of simple sugar peak detection on SIM chromatogram of a randomly chosen wheat sample (W1)

Only automatic integrated total surface areas of those peaks, which contained at least one of the abovementioned characteristic fragmentation ions (204 m/z, 217 m/z and 361 m/z), were taken into consideration. Peaks which contain an abundant fragment ion 204 m/z should, therefore, represent pyranose ring monosaccharides (Fig. 3A). Peaks with abundant fragment ion 217 m/z represent furanose ring monosaccharides (Fig. 3B), and those with 361 m/z peaks of di- or trisaccharides (Fig. 3C). Every cereal flour sample contained between 10 and 15 of these peaks: corn 13, wheat 11-15, barley 11-15, oats 14-15, triticale 13, rye 10. Since various cereal cultivars were analyzed, some

samples have the particular components (i.e. peaks) that other samples do not contain, so the total number of components that were taken into consideration was 22. This number, therefore, represents the sum of all different components, or all different peaks with specific retention times.

The total surface areas of the corresponding peaks were integrated using the MSD ChemStation software, and the resulting data put into the PAST program in order to perform multivariate data analysis. As the analysis procedure of every cereal flour sample was replicated three times, the mean values of integrated surface areas were used in further data processing. 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).

Mean values of integrated surface areas of the corresponding peaks were first subjected to a Principal coordinate analysis (PCO), which is a computationally fast method. This method is becoming more and more popular in sensory science and product development, and may also be applied in nutritional science (Derndorfer and Baierl 2014). The standard value of transformation exponent (c=2) and Rho similarity index were applied. All data points are plotted in the coordinate system, presented in Fig. 5. The different groups are shown using different symbols and colors, but sample labels are also provided.

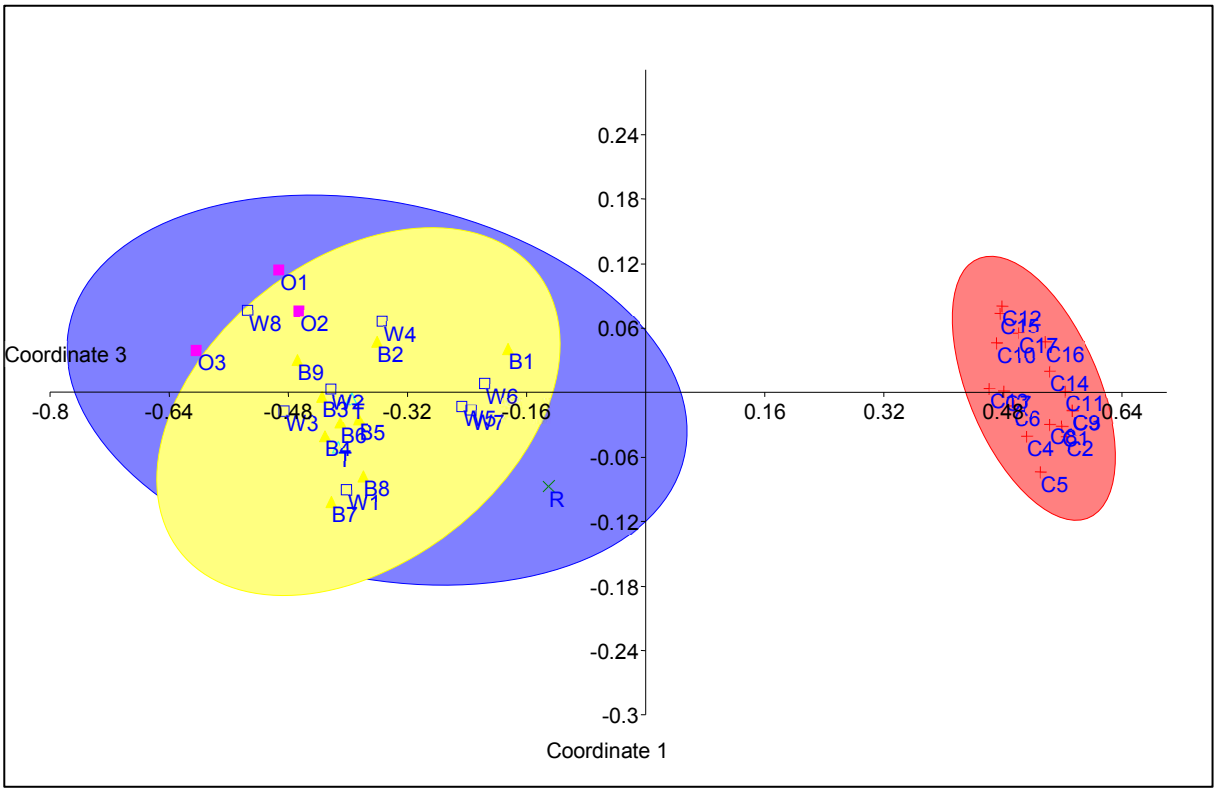


Fig. 5 Diagram of the principal coordinate analysis of flour samples

A very narrow grouping of corn flour samples inside the red ellipse on the right side and wider grouping of small grain flour samples within the blue and yellow ellipses on the left side of the scatter

plot, can be observed. It can be concluded that the PCO analysis clearly separates corn flour samples from small grain flour samples, based on the composition of simple carbohydrates. However, it is insufficiently selective to completely separate small grain flour samples among each other, as they present very strong similarities in a botanical sense.

Integrated surface areas of the corresponding peaks were then subjected to a Cluster analyis. Hierarchical cluster analysis is regarded as a complementary, nonlinear, and widely used method for cluster analysis, with the result represented by a dendrogram. This tree-like diagram shows the objects as ‘‘leaves’’, and branches merge according to the order given by the algorithm. Clusters of similar products can be visually distinguished from dissimilar ones. Paired group algorithm and Rho similarity index were applied. The obtained cophenetic correlation coefficient had a value of 0.9717.

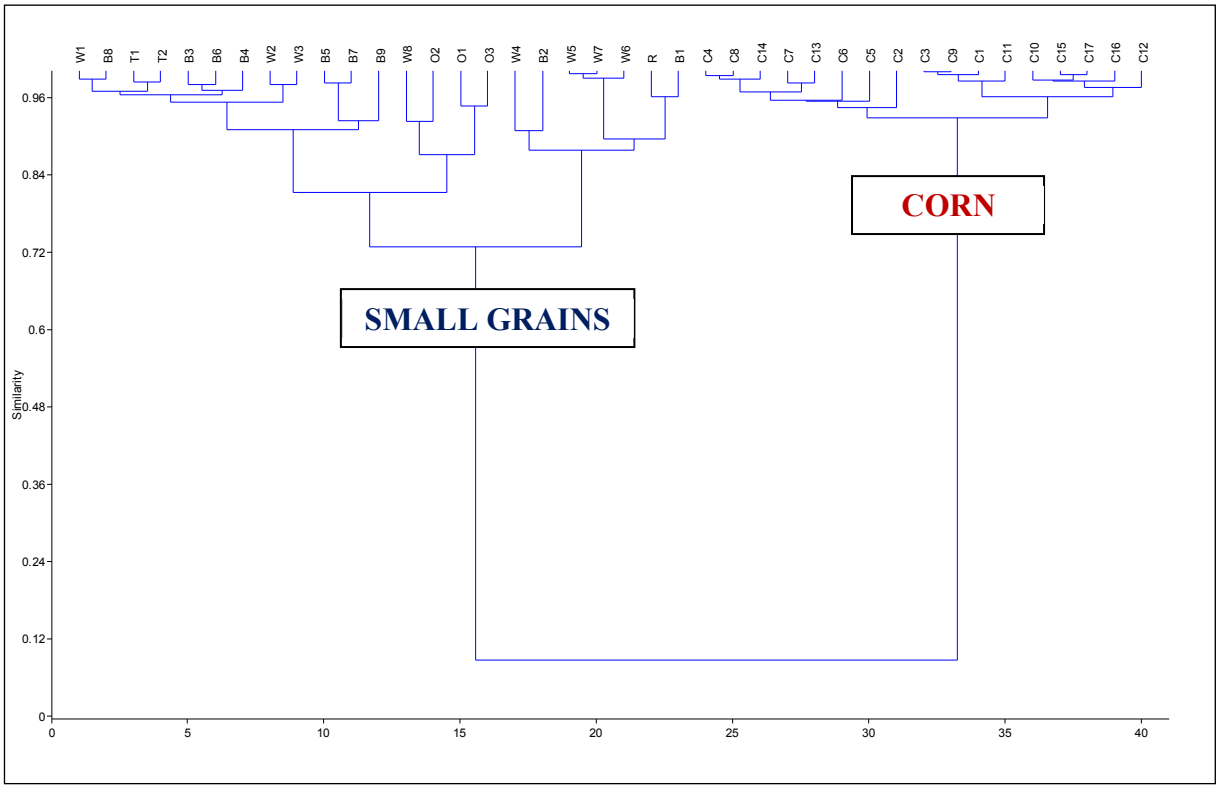


Fig. 6 Dendrogram with clustered data points: corn flour samples (right), samples of small grain flour (left)

Fig. 6 shows that the separation of small grain flour samples from corn flour samples can be very successful using this method, but different cultivars of small grains, once more, do not show a complete separation. A group of triticale samples (T1, T2), two groups of barley samples (B3, B6, B4) (B5, B7, B9), two groups of wheat samples (W2, W3) (W5, W7, W6), and a group of oat samples (O1, O3) are clearly separated from each other, while other samples (W1, B8), (W8, O2), (W4, B2), (R, B1) do not show a complete separation. Generally, the samples of small grain flour demonstrate a weak separation among each other with a relatively high value of a similarity index (more than 0.72),

due to their great botanical similarity. On the other hand, samples of small grain flour and samples of corn flour show a very strong separation between each other with a similarity index less than 0.12.

Conclusions

BSTFA reagent can be successfully applied in derivatization of simple sugars, extracted with ethanol from flour samples of various cereal grains. The analysis procedure of the chromatograms obtained by GC/EI-MS analysis is considerably simplified by forming TMSO derivatives of soluble sugars and by selecting characteristic abundant ions from TIC chromatograms. Using multivariate data processing the corn flour samples can be very succesfully separated from the small grain flour samples, which show weaker separations among each other.

It is interesting to mention that these results are in accordance with the results obtained by comparing cereal flour samples using Synchronous fluorescence spectroscopy, where corn flour samples were also clearly separated from samples of small grain flour, but without complete mutual separation of samples of flour of small grains (Zeković et al. 2012).

This simple method should be applied in quality control, to define the composition of mixed flours of different grain crops, due to the increasing trend of mixed flour application in human consumption. The analysis is rapid and easy to perform, as it does not require identification and quantitation of carbohydrates, and could be of great practical importance.

Acknowledgements

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

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