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Authenticity of Lipid and Soluble Sugar Profiles of Various Oat Cultivars *(Avena sativa)*

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***Abstract*—**The identification of lipid and soluble sugar components in flour samples of different cultivars belonging to common oat species (*Avena sativa* L.) was performed: spring oat, winter oat and hulless oat. 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 then extracted from defatted and dried samples of oat flour with 96% ethanol, and further derivatized into corresponding TMS-oximes, using hydroxylamine hydrochloride solution and BSTFA (N,O-bis-(trimethylsilyl)-trifluoroacetamide). The hexane and ethanol extracts of each oat cultivar were analyzed using GC-MS system. Lipid and simple sugar compositions are very similar in all samples of investigated cultivars. Chemometric tool was applied to numeric values of automatically integrated surface areas of detected lipid and simple sugar components in their corresponding derivatized forms. Hierarchical cluster analysis shows a very high similarity between the investigated flour samples of oat cultivars, according to the fatty acid content (0.9955). Moderate similarity was observed according to the content of soluble sugars (0.50). These preliminary results support the idea of establishing methods for oat flour authentication, and provide the means for distinguishing oat flour samples, regardless of the variety, from flour samples made of other cereal species, just by lipid and simple sugar profile analysis.

***Keywords*—**Authentication, chemometrics, GC-MS, lipid and soluble sugar composition, oat cultivars.

I. Introduction

OAT, like all other grain varieties, belongs to the *Poaceae* family. Common oat (*Avena sativa* L) is the most important among the cultivated oats [1].

World oat production peaked at an average 52 million tons on 1970/74 but fell progressively to 39 million tons in 1990/91. Traditionally, most of the oat crop has been consumed as animal feed, but nonfeed use (as food or industrial raw materials) has risen in recent years. Therefore, the focus has shifted to food usage, as consumers have become aware of the potential benefits of oats [2].

Oat is distinct among the cereals due to its multifunctional characteristics and nutritional profile. It is a good source of dietary fiber, especially β-glucan, minerals, fat, protein, B complex vitamins [1], [3]. Soluble β-glucan and other bioactive compound from cereals such as oats and their fractions have been shown to have potential nutritional benefits within the food industry (for example, bread baking industry). Many clinical studies associate the relationship between the consumption of oat β-glucan and reduction of plasma total and low-density lipoprotein (LDL) cholesterol [4], [5], and glucose levels [3], [5], [6], as well as the enhancement of postprandial satiety [7]. Oats contain much higher levels of lipid than any other cereal grain, which makes them an excellent source of energy and unsaturated fatty acids [2]. Oat is also a source of many compounds that exhibit antioxidant activity, such as vitamin E (tocols), phytic acid, phenolic compounds (vanillic, ferulic and gallic acids) [3], more than 25 avenanthramide compounds [8], flavonoids and sterols. A few examples show that an oat-containing diet boosted the antioxidant capacity of serum or meat in animals [9].

Considering the health benefits, many researchers have recently incorporated different levels of oat flour or oat bran in food bakery products [1], [5].

Authenticity testing of cereal-based products is necessary to comply with labeling rules, avoid unfair economic competition, and mainly to ensure consumer protection against fraudulent practices commonly observed in unscrupulous trade [10]. Chromatographic techniques are among the most important methods used in food authentication and adulteration [10]-[13].

It can be said that the aims of this paper are: (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 oat cultivars; and (ii) the application of chemometric tools to investigate the similarity of analyzed cultivars and a possibility to determine the authenticity of oat flour in general, considering the importance of oat flour addition in non-wheat and mixed flour bakery products. These preliminary results could be integrated in the procedures for flour quality assurance.

II. Experimental

A. Sample Preparation

The investigated samples of oats were obtained from the cultivated, living collection of Small Grains Department at the Institute of Field and Vegetable Crops "NS Seme", Novi Sad, Republic of Serbia: Dunav (spring oat, O1), Jadar (winter oat, O2), and Sedef (hulless oat, O3). The 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 3 oat cultivars were ground using a laboratory mill (Falling number 3100, Sweden). 0.5 g of each oat flour sample was poured in a 12 mL cuvette for

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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 *trimethylsulfonium hydroxide* in methanol (TMSH, Macherey–Nagel) was added, by which derivatization into volatile methyl-esters was performed (Macherey–Nagel) [14].

After removing the hexane fractions, the oat 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 N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA, Macherey–Nagel) was added, by which derivatization of oximes into trimethylsilyl-oximes (TMSO) was performed (Macherey–Nagel) [15].

By creating trimethylsilyl-oximes 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 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, such as flour of cereals, as they present good GC properties and provide simple chromatograms [16].

Three hexane and three ethanol sample solutions were prepared from each oat flour sample.

B. GC-MS Conditions

Analytical procedure was conducted on a GC-MS system. The analyses were performed on Agilent Technologies 7890 instrument coupled with MSD 5975 equipment (Agilent Technologies, Palo Alto, CA, USA) operating in electron ionization mode at the energy of 70 eV. A DB-5 MS column (30 m length, 0.25 mm i.d., 25 µm film thickness, Phenyl Arylene polymer, Agilent Technologies) 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. Helium was used as the carrier gas with a constant flow rate of 0.8 mL/min. A split ratio of 1:50 was used for the injection of 1 µL of sample solutions. Triplicate injections were made for each sample.

C. Data Processing

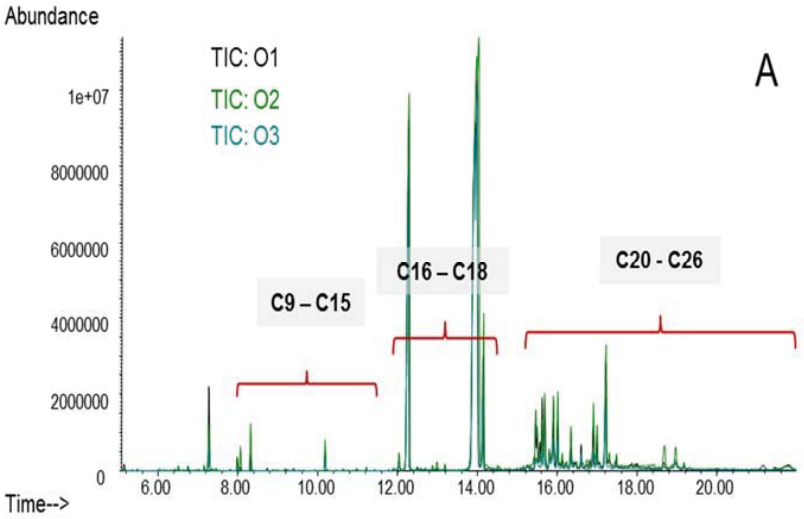
The GC-MS data in the form of full-scan chromatograms were acquired by Agilent MSD Productivity ChemStation software. Compound identifications involved comparison of the mass spectra with the WILEY 275 MS database using a probability-based matching algorithm (a match quality of 95% minimum was used as a criterion).

D. Chemometric Analysis

PAST programme was used for statistical data processing [17]. Hierarchical cluster analysis of integrated surface areas of derivatized lipid and simple sugar compounds were performed. Hierarchical cluster analysis is a complementary, nonlinear, and widely used method for cluster analysis, with the result represented by a dendrogram [18].

III. Results and Discussion

Fig. 1 presents the overlaid total ion current (TIC) chromatograms of liposoluble (A) and hydrosoluble (B) extracts of investigated oat flour samples.



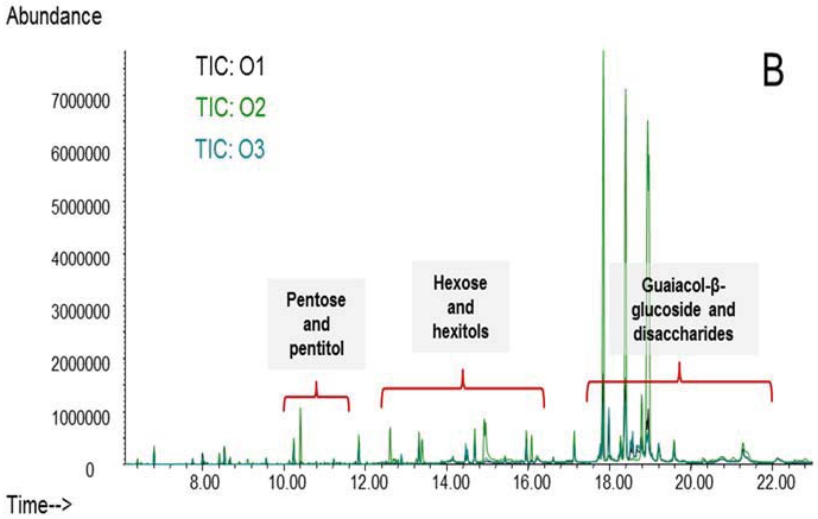


Fig. 1 Overlaid TIC chromatograms of lipid components (A) and soluble sugar components (B) of investigated samples of oat flour

The components in liposoluble and hydrosoluble extracts were identified by comparing their mass spectra with Wiley mass spectra library. Components identifications were performed according to their characteristic fragmentations using a probability-based matching algorithm. The performances of the system of gas chromatography coupled to mass spectrometry were completely satisfactory for that purpose.

MSD Productivity ChemStation programme was used to automatically integrate the peaks of detected fatty acid methyl esters and TMS-oximes of simples sugars in hexane and ethanol oat flour extracts, respectively. The chemometric tool was applied to classify the investigated samples of oat cultivars using numerical values of integrated surface areas from obtained chromatograms. The results are represented by a dendrogram.

A. Analysis of Lipid Components

Table I shows the detected fatty acid methyl esters in hexane extracts of investigated oat flour samples.

The overlaid TIC chromatograms on Fig. 1 (A) can be separated in three parts according to the eluting components and their abundances. First part includes methyl esters of minor fatty acids with up to 15 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, 17 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 of the chromatograms, but in much lower quantities. Third part of the chromatograms consists of methyl esters of saturated and unsaturated fatty acids with more than 18 carbon atoms in molecule, which also appear in smaller amounts in each investigated oat cultivar (Rt ≥ 15.67 min).

TABLE I
DETECTED COMPOUNDS IN LIPOSOLUBL EXACTS OF ANALYZED OAT CULTIVARS AND THEIR CORRESPONDING ELUTION TIMES

Lipid compounds	Rt
Dodecanoic acid, methyl ester	8.08
Nonanedioic acid, dimethyl ester	8.34
Tetradecanoic acid, methyl ester	10.20
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
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

*All detected lipid components are present in every oat cultivar analyzed.

Fig. 2 presents the dendrogram of fatty acid methyl esters detected in hexane extracts of oat flour.

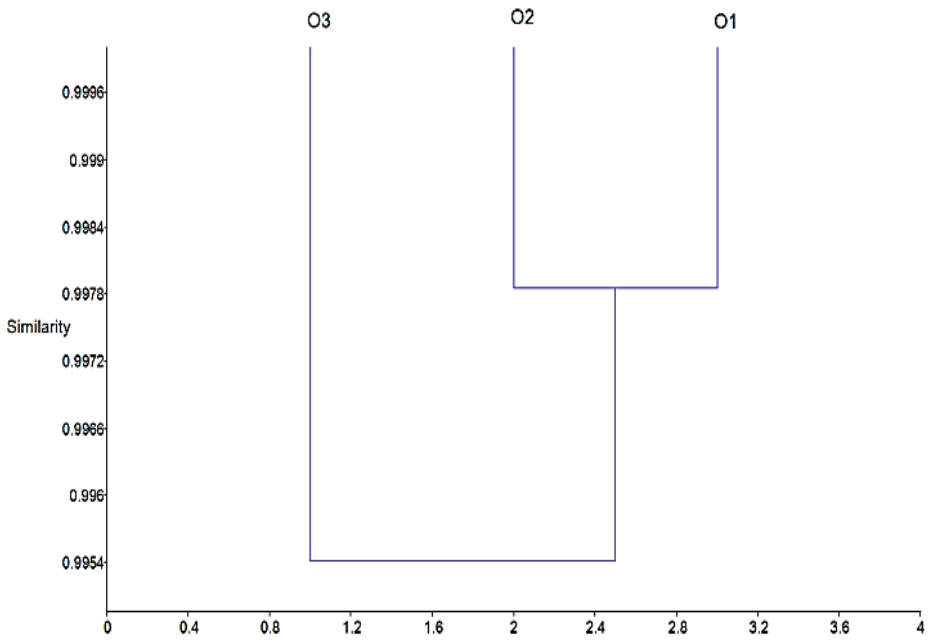


Fig. 2 Correlation dendrogram of lipid components listed in Table I

Paired group algorithm and Cosine similarity measure were applied, presenting the highest values of Cophenetic correlation coefficient. The obtained value of cophenetic correlation coefficient was 0.9738, which means that the dendrogram very faithfully preserves the pairwise distances between the original unmodeled data points.

Even the lowest value of the correlation similarity measure among investigated oat cultivars was very high (r≥0.9955). The flour samples of oat cultivars O1 and O2 represent higher similarities among each other (r≥0.9979). It can be said that the properties of oat flour sample O3 slightly differ regarding its lipid composition.

B. Analysis of Simple Sugar Components

Table I shows the detected TMS-oximes of simple sugars in ethanol extracts of investigated oat flour samples.

Simple sugar components present in flour samples of investigated oat cultivars include TMS-oximes of monosaccharides and sugar alcohols: pentose and pentitol (Arabinose and Xylitol), hexose and hexitols (Glucose, Glucitol and Mannitol), non-reducing and reducing disaccharides (Sucrose, Turanose and Melibiose), and Guaiacol-β-glucoside. Among compounds detected in ethanol extracts, the most abundant are peaks of disaccharide, Sucrose, that elutes at 18.92 min, 20.85 min, and 21.39 min.

Fig. 3 presents the dendrogram of TMS-oximes of simple sugars detected in ethanol extracts of oat flour. In this case, Paired group algorithm and Raup-Crick similarity measure were applied, presenting the highest values of Cophenetic correlation coefficient. The obtained value of cophenetic correlation coefficient was the highest possible, 1.

TABLE II
DETECTED COMPOUNDS IN HYDROSOLUBLE EXACTS OF ANALYZED OAT CULTIVS AND THEIR CORRESPONDING ELUTION TIMES

Simple sugar compounds	Rt
Xylitol, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-,	10.40
Arabinopyranose, tetrakis-O-(trimethylsilyl)-, .alpha.-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
.alpha.-D-Glucopyranoside, 1,3,4,6-tetrakis-O-(trimethylsilyl)-.beta.-D-fructofuranosyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-	17.84
.alpha.-D-Glucopyranoside, 1,3,4,6-tetrakis-O-(trimethylsilyl)-.beta.-D-fructofuranosyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-	18.38
Guaiacol-.beta.-D-glucopyranoside-TMS	18.52
D-Turanose, heptakis(trimethylsilyl)-	18.57
Melibiose, octakis(trimethylsilyl)-	18.80
.alpha.-D-Glucopyranoside, 1,3,4,6-tetrakis-O-(trimethylsilyl)-.beta.-D-fructofuranosyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-	18.92
.alpha.-D-Glucopyranoside, 1,3,4,6-tetrakis-O-(trimethylsilyl)-.beta.-D-fructofuranosyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-	20.85
.alpha.-D-Glucopyranoside, 1,3,4,6-tetrakis-O-(trimethylsilyl)-.beta.-D-fructofuranosyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-	21.39

*The component written in italic is present only in the oat sample (O2). The rest of detected simple sugar components are present in every oat cultivar analyzed.

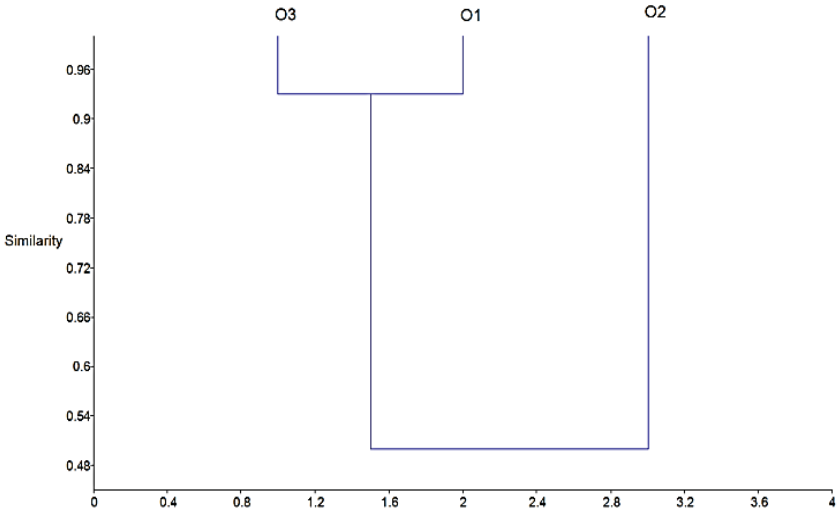


Fig. 3 Correlation dendrogram of simple sugar components listed in Table II

The lowest value of the correlation similarity measure among investigated oat cultivars was moderate (r≥0.50), indicating that the flour sample of winter oat cultivar, O2, differs according to the simple sugar content. The flour samples of spring and hulless oat cultivars, O1 and O3, represent much higher similarities among each other (r≥0.93).

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

IV. Conclusion

Lipid and simple sugar profiles of hexane and ethanol extracts of oat flour samples, respectively, can be successfully established with the application of the GC-MS technique.

Using the proposed GC-MS method combined with a chemometric tool, the obtained similarity among flour samples of spring, winter and hulless oat cultivars was, in general, very high. However, the investigated oat cultivars presented higher general similarity regarding lipid components, considering diversity of the winter oat cultivar from the others according to the simple sugar profile.

The aim of the preliminary results presented is directed towards the identification and authenticaton of an oat flour during procedures concerning the mixed flour quality assessment on the world market.

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