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Gas chromatography–mass spectrometry system applied to determine botanical origin of various types of edible vegetable oils

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Abstract: This study represents a new strategy for discrimination of 59 samples of various cold-pressed, virgin and refined edible vegetable oils according to the corresponding botanical origin. Samples were produced from 17 plant species: olive, sunflower, safflower, flax, pumpkin, sesame, hemp, walnut, hazelnut, almond, grape, black cumin, apricot, plum, soybean, wheat and rapeseed. A GC/MS device performing in a ion current (IC) mode, combined with multivariate clustering, was employed in the analysis. Derivatization reaction occurred in the injector of a gas chromatograph. The discriminations between species were based on marker-peaks of 9 molecular ions of dominant fatty acid methyl esters (FAMEs), which were chosen as descriptors: *m*/*z* 268, 270, 292, 294, 296, 298, 324, 326 and 354. Dendrogram obtained after performing cluster analysis shows clear discriminations of the analyzed samples, based on the belonging botanical origin. These results demonstrate that IC-GC/MS approach with cluster analysis could be a useful tool in rapid screening for botanical origin of commercial samples of various edible vegetable oils.

Keywords: authenticity of oils; botanical origin; GC/MS; multivariate cluster analysis.

INTRODUCTION

Vegetable oils of different types and quality are important from nutritional and economical point of view. They are widely used in homemade cooking and food industry.[1,2] Virgin olive oil is a big success on the market, but over the last years other vegetable oils that are produced by mechanical extraction without the

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use of any solvent emerged and are now available for the consumer, resulting in increased competition for market share.[3,4] These oils are a natural source of bioactive compunds, which are proposed to have a wide spectrum of biological and health beneficial effects including anti-inflammatory, anti-oxidative, anti-carcinogenic and cholesterol-lowering.[5] A report of a joint WHO/FAO expert consultation demonstrates a decreased risk of cardiovascular disease for unsaturated fatty acids, such as linoleic acid, α-linolenic acid, oleic acid, but also plant sterols and stanols.[6]

Higher quality products demand higher market prices, therefore unscrupulous traders may attempt to increase profits by deliberately mislabeling foods, or by increasing the volume of a good quality batch through adulteration with low value ingredients. The substitution or adulteration of food products with a cheap ingredient is not only an economic fraud, but may also have severe health implications to consumers.[7–9]

Edible vegetable oils can be also purposely modified using different methods to enhance their commercial applications, to improve their nutritional quality and to create new specific products at affordable prices. These products are characterized by desired textural, oxidative and nutritional properties, such as changed fatty acid composition and increased bioactive components and natural antioxidants. Therefore, in the future, there are many economical and health reasons for the production of new oil blends using new and conventional oil sources to be introduced to the market.[10] Verifying the description of food in terms of its composition, processing or origin is challenging, but important in protecting consumers and enforcing food law. Consumers need clear and accurate information so that they can make informed choices about their diet and the foods they buy.[11] The assessment of quality needs to be based on the exploitation of high-tech analytical methodologies. At the present, chromatographic methods are the most popular ones used in routine measurements in monitoring authenticity, adulteration, and in traceability studies of fats and oils. The analysis of fatty acid methyl esters by GC-FID or GC/MS is the simplest and most commonly used technique.[7,12]

The analysis performed by the new and sophisticated analytical instruments provide a vast amount of data, which is difficult to process. However, developments in computer science have allowed the extensive use of multivariate procedures, in order to efficiently extract the maximum useful information from obtained data.[13,14] Therefore, the information content of the total fatty acid profile, obtained after GC/MS analysis, can be more efficiently exploited by multivariate data analysis in order to classify oils or determine their authenticity.[15]

Various analytical methods utilizing GC, HPLC, MS, NMR, FTIR, Raman spectroscopy etc., associated with different multivariate techniques, have been extensively used for the characterization and authentication of different types of edible vegetable oils.[16–25] The aim of this paper was to demonstrate the potential

of a new and rapid analytical approach, utilizing a GC/MS device in IC mode combined with a multivariate clustering tool, that will discriminate 59 samples of various edible oils according to belonging botanical origin. The aim was not to discover new or identify known eluting components, but to establish a rapid approach for differentiation based on comparing the influences of selected factors present in and specific for an edible oil sample of each botanical origin.

EXPERIMENTAL

Analyzed samples

The number of 29 samples of certified cold-pressed (21), virgin (7) and refined (1) edible vegetable oils were purchased from the Department of Food Preservation Engineering, Faculty of Technology Novi Sad, Republic of Serbia. The other 30 samples of non-certified cold-pressed oils were obtained from the Oil Crops Department at the Institute of Field and Vegetable Crops, Novi Sad, Republic of Serbia. These samples were extracted mechanically, by pressing the oilseeds under 200 bar and kept in the nitrogen atmosphere until the analysis. Analyzed certified and non-certified edible oil samples, their origin, source and labeling are given in Table I.

TABLE I. Certified and non-certified samples of cold-pressed, virgin and refined edible vegetable oils used in the analysis, their origin, source and labels

Botanical origin and source	Oil sample labels	
	Certified	Non-certified
Olive	O1-O5 (virgin)	–
Sunflower (seed)	Su1 (refined), Su2, Su3	–
Safflower (seed)	–	Sf1-Sf8
Flax (seed)	Fx1	Fx2 - Fx7
Pumpkin (seed)	Pu1, Pu2	Pu3, Pu4
Sesame (seed)	Se1 (virgin), Se2	Se3
Hemp (seed)	He1, He2	–
Walnut	Wn1, Wn2	–
Hazelnut	Hn1 (virgin), Hn2	–
Almond	Al	–
Grape (seed)	Gs1, Gs2	–
Black cumin	Cu1, Cu2	–
Apricot (seed)	Ap1, Ap2	–
Plum (seed)	Pl	–
Soybean	So	–
Wheat germ	Wh	–
Rape (seed)	–	Rs1 - Rs13

Sample preparation

The volume of 10 µl of each sample was pipetted into a glass vial with a micropipette, and further dissolved by the addition of 1 ml of methylene chloride. The volume of 50 µl of a 0.2 M TMSH derivatization solution (trimethylsulfonium hydroxide, Macherey-Nagel) in methanol, was added, and each sample was transferred to a GC/MS device. Transesterification derivatization procedure occurred in the injection port of a GC/MS device itself, thus con-

verting the components of oil saponifiable fraction into corresponding volatile fatty acid methyl esters (FAMEs).

GC/MS analysis

A gas chromatography device (Agilent Technologies 7890) with mass spectrometric detection (Agilent Technologies 5975 MSD), was employed in the analysis. Electron ionization with the energy of 70 eV was applied for the purpose of fragmentation. A DB-5 MS column (30 m×0.25 mm×25 μm) was used for separation. The temperature of the injection port was 250 °C, the flow of the carrier gas (helium) 0.8 ml min^{-1}, and the applied temperature program was: 50–130 °C, 30 °C min^{-1} and 130–300 °C, 10 °C min^{-1}. The volume of 1 μl of each sample was injected with a split ratio of 1:50. Samples were analyzed in duplicate.

Data matrix construction

Chromatograms were processed using a ChemStation program (Agilent Technologies, Palo Alto, USA). The retention times/the time position of peaks of the eluting FAMEs were established in total ion current (TIC) mode using both NIST14 and WILEY7 mass spectra libraries, giving the match quality of over 90 %. Molecular ions of methyl esters of 9 dominant fatty acids were extracted in IC mode from TIC chromatograms of all investigated oil samples. Peaks of the selected molecular ions on IC chromatograms with the following *m*/*z* ratios were integrated: 268 (*cis*-9-hexadecenoic acid, methyl ester), 270 (hexadecanoic acid, methyl ester), 292 (*cis*,*cis*,*cis*-9,12,15-octadecatrienoic acid, methyl ester), 294 (*cis*,*cis*-9,12--octadecadienoic acid, methyl ester), 296 (*cis*-9-octadecenoic acid, methyl ester), 298 (octadecanoic acid, methyl ester), 324 (*cis*-11-eicosenoic acid, methyl ester), 326 (eicosanoic acid, methyl ester) and 354 (docosanoic acid, methyl ester). Mean numerical values of ion peak surface areas were used in further data processing.

Data processing

Collected raw numerical data matrix of the IC-GC/MS analysis was firstly variance--scaled, and then subjected to multivariate data analysis. Cluster analysis (CA) comprises classification algorithms designed to understand the information of data matrices, to describe similarities and dissimilarities among objects and to single out categories grouping similar objects.[26,27] The performances of CA with Correlation algorithm were employed in order to classify investigated edible oil samples according to the corresponding botanical origin of plant species used in the oils production. Multivariate statistical procedures were carried out using a freely available PAST 3.21 program (Palaeontological Association).[28]

RESULTS AND DISCUSSION

Marker ions of all lipid compounds eluting on chromatograms of every analyzed oil sample were taken into consideration for discrimination purposes. Marker ions present only in oil samples of certain plant species represent a good discriminating factor of these samples. Overlaid chromatograms of 9 marker ions of edible oil samples, each thereby representing one specific vegetable oil group, are given in Supplementary material to this paper. One oil sample of each plant species was chosen for visualization purposes. The abundances of selected ions are shown on vertical axes and their specific retention times on horizontal axes. Chromatograms of plum oils were not presented because they belong to the same

group like apricot oil, and are therefore grouped together in a dendrogram shown on Fig. 1.

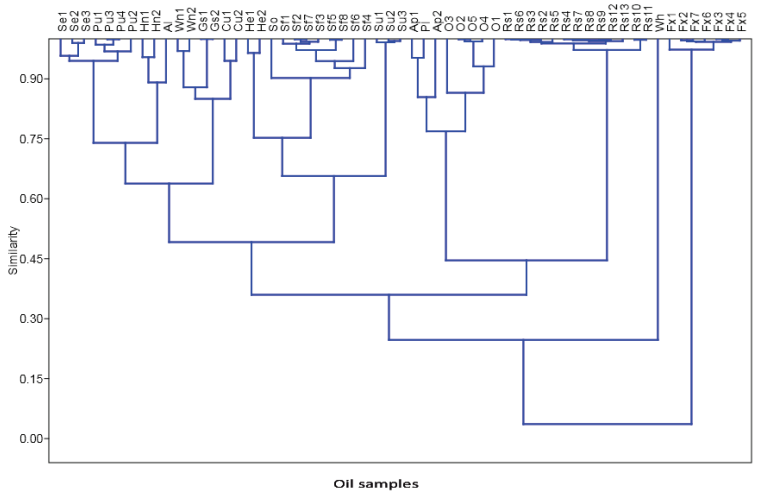

Fig. 1. Multivariate clustering of investigated edible vegetable oil samples, discriminated according to botanical origin. Labels of cold-pressed, virgin and refined oil samples are indicated in Table I.

Selected marker ions show the similar qualitative pattern, based on the observed retention times, but different amounts of eluting FAMEs in oil samples belonging to different botanical group. However, differences in ionic functions are scarcely visually observed. Therefore, a multivariate statistical tool has to be applied in order to visualize the differences between analyzed edible oil samples, taking into account 9 FAMEs, as discriminating variables.

The obtained dendrogram of the ratios of 9 discriminating marker ions which are present in 59 investigated edible vegetable oil samples is presented in Fig. 1.

According to the literature, the number of discriminating variables, i.e. chemical compounds, should be lower than objects, *i.e.*, samples.[26] Obtained dendrogram suggested that 9 observed FAMEs contributed sufficiently to the separation of 59 edible oil samples into 16 groups: 1) cold-pressed flax seed oils Fx1––Fx7; 2) cold-pressed wheat germ oil; 3) cold-pressed rapeseed oils Rs1–Rs13; 4) virgin olive oils O1–O5; 5) cold-pressed apricot and plum seed oils; 6) refined Su1 and cold-pressed sunflower seed oils Su2 and Su3; 7) cold-pressed safflower seed oils Sf1–Sf8; 8) cold-pressed soybean oil; 9) cold-pressed hemp seed oils He1 and He2; 10) cold-pressed black cumin oils Cu1, Cu2; 11) cold-pressed grape seed oils Gs1 and Gs2; 12) cold-pressed walnut oils Wn1 and Wn2; 13) cold-pressed almond oil; 14) virgin Hn1 and cold-pressed Hn2 hazelnut oils; 15) cold-pressed pumpkin seed oils Pu1–Pu4; 16) virgin Se1 and cold-pressed Se2

and Se3 sesame seed oils. The overlap between some edible oil samples, specifically samples of cold-pressed apricot and plum seed oils, might be related to either location, growing season or, even more probable, due to a high level of genetic similarity between varieties of these plants used for cold-pressing in oil extraction. They express a high degree of mutual similarities of FAMEs ratios. The group of oil samples of flaxseed (group 1) is mostly differentiated from all the other samples. The samples of olive oil, apricot seed oil and plum seed oil (groups 4 and 5) express high levels of similarities, as well as the samples of safflower and soybean oil (groups 7 and 8), samples of cumine, grapeseed and walnut oil (groups 10, 11 and 12), samples of almond and hazelnut oil (groups 13 and 14), and samples of pumpkin seed and sesame seed oils (groups 15 and 16). Rapeseed oils (group 3) and sunflower seed oils (group 6) show strong mutual groupings and inter-group separations in the obtained dendrogram.

There was an established opinion that the ability of an analytical method to characterize a vegetable oil is based on the identification and quantification of minor constituents, such as fatty alcohols, waxes, hydrocarbons, tocopherols and tocotrienols, phenolic compounds, volatiles, pigments and triterpenic acids, that are expected to be in connection with the oil's origin. This is, however, a difficult task because these groups contain numerous compounds with a wide range of polarities, concentrations and chemical structures. Therefore, such approach would require the isolation and analysis of minor constituents by means of several procedures of separation, identification and quantification.[30] Furthermore, it is possible to "de-sterolize" oils and remove sterols and related minor compounds characteristic to a certain botanical oilseed species. Also, levels of tocopherols and tocotrienols can be affected by oil age and refining process.[30] This study delivers a rapid, qualitative approach without the analysis of minor constituents, which are present in higher quantities in samples of cold-pressed oils. In contrast, this approach is focused on the characteristic ratios of fatty acid profiles between the analyzed edible vegetable oil samples, as major and mandatory constituents of such products.

CONCLUSIONS

An approach which combines the utilization of a IC-GC/MS device with multivariate clustering has been employed to discriminate a wide spectrum of edible vegetable oils, based on relative proportions of 9 dominant fatty acids, as discrimination factors. Oil samples were clustered into 16 groups based on corresponding botanical origin, independently of the production procedure. It is important to emphasize that the proposed qualitative approach does not require exact qualitative nor quantitative determinations of eluting FAMEs. In addition, a simple sample processing is required and derivatization occurs directly in an injector of a gas chromatography device. Also, using this approach determin-

ations of various minor constituents using other instrumental techniques or/and methodologies is thus avoided. Therefore, the proposed approach displays a high potential to rapidly confirm the correctness of the label on expensive and valuable vegetable oil products, and determine possible adulterations in their production process and sale on the market.

SUPPLEMENTARY MATERIAL

Additional data are available electronically from the journal web site: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

ПРИМЕНА СИСТЕМА ГАСНЕ ХРОМАТОГРАФИЈЕ–МАСЕНЕ СПЕКТРОМЕТРИЈЕ У ОДРЕЂИВАЊУ БОТАНИЧКОГ ПОРЕКЛА РАЗЛИЧИТИХ ЈЕСТИВИХ БИЉНИХ УЉА

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Ова студија представља нову стратегију разликовања 59 узорака различитих хладно пресованих, девичанских и рафинисаних јестивих биљних уља према одговарајућем ботаничком пореклу. Узорци су произведени од 17 биљних врста: маслине, сунцокрета, шафрана, лана, бундеве, сусама, конопље, ораха, лешника, бадема, коштице грожђа, црног кима, коштице кајсије, коштице шљиве, соје, пшеничних клица и уљане репице. У анализи је коришћен GC/MS уређај у режиму јонске струје (IC), уз мултиваријантну кластер анализу добијених података. Реакција дериватизације се одиграва директно у инјектору гасног хроматографа. Разлике између биљних врста базирају се на маркер-пиковима молекулских јона метил-естара 9 доминантних масних киселина (FAMEs), који су изабрани као дескриптори. Ови резултати показују да би IC-GC/MS приступ у комбинацији са кластер анализом могао бити корисно средство у брзом разликовању комерцијалних узорака различитих врста јестивих биљних уља према ботаничком пореклу.

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