TITLE: Practical method for the confirmation of authentic flours of different types of cereals and pseudocereals

AUTHORS: Marijana M. Ačanski, Đura N. Vujić, Đorđe B. Psodorov

PRACTICAL METHOD FOR THE CONFIRMATION OF AUTHENTIC FLOURS OF DIFFERENT TYPES OF CEREALS AND PSEUDOCEREALS

Marijana M. Ačanski[1] Djura N. Vujić[2], Djordje B. Psodorov[2]

[1]*Faculty of Technology, University of Novi Sad, Bulevar Cara Lazara 1, Novi Sad, Serbia*

[2]*FINS, University of Novi Sad, Bulevar Cara Lazara 1, Novi Sad, Serbia*

[2]

Corresponding author

E–mail: macanski@tf.uns.ac.rs (Marijana Ačanski)

Department of Applied and Engineering Chemistry

Faculty of Technology

University of Novi Sad

Bulevar Cara Lazara 1

21000 Novi Sad

Serbia

Phone: + 381 21 485 3692

Fax: +381 21 450 413

Abstract

Gas chromatography with mass spectrometry was used for performing a qualitative analysis of the liposoluble flour extract of different types of cereals (bread wheat and spelt) and pseudocereals (amaranth and buckwheat). In addition to dominant fatty acids the liposoluble extract also contains minor amounts of fatty acids with more than 20 carbon atoms, higher hydrocarbons and phytosterols. TMSH (Trimethylsulfonium hydroxide, 0.2M in methanol) was used as a transesterification reagent. In a transesterification reaction, triglicerides esterifyed from acilglycerol to methyl-esters. SIM (Selected ion monitoring) technique was used for the extraction of methyl-esters of fatty acids using the fragment 74 Da which originates from McLafferty movement, typical for methyl-esters. GC-MS system is used for the transesterification of triglycerides to methyl-esters of fatty acids in the gas chromatographic injector. This eliminated laboratory preparation of methyl-esters of fatty acids starting from triglycerides.

The tests cluster analysis was used for comparing the liposoluble flour extract of different types of cereals and pseudocereals. Statistical data show that the analysis of the liposoluble extract enables determination of flour origin. Obtained results are unambiguous and may be used for the quality control.

Key words: cereals and pseudocereals, GC-MS, correlations of liposoluble composition

1. Introduction

Global production has been increasingly introducing grain crops, which botanically do not fall into cereals (buckwheat, amaranth), as well as archaic forms of wheat (spelt). They are used as raw materials in the bakery business or for the production of special products (Bognar & Kellermann, 1993; Bojnanska & Francakova, 2002). The substitution of white or whole-wheat flour with spelt, buckwheat or amaranth flour increases nutritional and functional value of products, which, if consumed regularly, have a positive impact on human health.

The analysis of triglycerides in a certain number of samples of small grains has already been carried out (Vujić, Ačanski, Bodroža, Solarov, Hristov & Krunić, 2012) for the purposes of determining variability within the group. A similar procedure was applied in a more comprehensive analysis of triglycerides of different plant species. In this case, those were wheat, spelt, amaranth and buckwheat. This paper includes analysis of methyl-esters of fatty acids of triglycerides. It was carried out using the GC-MS system and an appropriate library to correctly identify methyl-esters and present hydrocarbons and phytosterols. The GC-MS system also allows us to select interesting compounds and to eliminate unimportant and secondary compounds, mostly contaminants. Secondly, SIM technique may be used for extracting methyl-esters with the mass, i.e. fragment 74 Da that originates from McLafferty movement, typical for methyl-esters. There is another, very important advantage of use of the GC-MS system and that is that transesterification of triglycerides to methyl-esters of fatty acids occurs in the gas chromatographic injector. This eliminates laboratory preparation of methyl-esters of fatty acids starting from triglycerides.

Production of functional food requires mixing of two types of flour. The procedure presented in this paper enables a definite distinction of raw materials, i.e. determining the exact origin of each component.

An important feature of the composition of cereals and pseudocereals is their fat content. Lipid content in amaranth is between 2 and 3 times higher than in buckwheat and common cereals such as wheat (Alvarez-Jubete, Arendt & Gallagher, 2009), and spelt has a higher lipid content than winter wheat (Ruibal-Mendieta, Delacroix & Meurens, 2002).

The following spices have been analysed: 7 types of winter wheat (Simonida, Dragana, NS-40S, Pobeda, Ljiljana, Zvezdana and Arija), 3 types of spelt (Austrija, Eko-10 and Nirvana), 3 types of amaranth (2A, 16A and 31A), and 9 types of buckwheat (Godijeva, Bambi, Darja, Francuska, Prekumurska, Češka, Čebelica, Novoadska and Spacinska).

2. Experimental

2.1. Sample preparation

About 10 g of the following samples was grounded: Simonida (W1), Dragana (W2), NS-40S (W3), Pobeda (W4), Ljiljana (W5), Zvezdana (W6), Arija (W7), Austrija (S1), Eko-10 (S2), Nirvana (S3), 2A (A1), 16A (A2), 31A (A3), Godijeva (H1), Bambi (H2), Darja (H3), Francuska (H4), Prekumurska (H5), Češka (H6), Čebelica (H7), Novoadska (H8) and Spacinska (H9). Each sample was homogenised and further treated in the following manner. A 12 mL cuvette for centrifugation was used for pouring 0.5 g of flour with the precision of 0.01 g. The cuvette was additionally filled with 5 mL of n-hexane and

stirred on Vortex for 2 minutes, after which the mixture was centrifugated at 2000 rotations/min for five minutes. After this 3 mL of clear supernatant was poured into a 10 mL glass and left to steam up on the ambient temperature. From the oily residue was taken an amount of 10 μL, reconstituted to 400 μL of methanol and additionally added 100 μL of the transesterification reagent: TMSH (Trimethylsulfonium hydroxide, 0.2M in methanol, Macherey-Nagel). With such a transesterification reaction, fatty acids from acilglycerol esterify to methyl-esters.

All the testing was conducted on a gas chromatography system.

The GC–MS analyses were performed on Agilent Technologies 7890 instrument coupled with 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 programme was: 50-130°C at 30°C/ min and 130–300°C at 10°C/ min. 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 the solutions.

For the mass spectrum analysis was used WILEY 275 library.

For the statistical data processing was used PAST programme (Hammer, Harper & Ryan, 2001).

3. Results and discussion

More than two decades ago, many authors investigated the lipid content of cereals (Rozenberg et al., 2003; Ruibal-Mendieta et al., 2004; Ruibal-Mendieta et al., 2005; Caboni, Iafelice, Pellilo & Marconi, 2005; Iafelice, Verardo, Marconi & Caboni, 2009; Dinelli et al. 2009; Pelillo, M., Ferioli, F., Iafelice, G., Marconi, E. & Caboni, M. F., 2010;

118 Dinelli et al. 2011) and pseudocereals (Kim, Kim & Park, 2004; Bonafaccia, Marocchini &
119 Kreft, 2003; Alvarez-Jubete, Arendt & Gallagher, 2010; Pina-Rodriguez.& Akoh, 2009).

120 Our intention in this paper is not to investigate new ingredients or to determine
121 them quantitatively, but to search for a possibility to definitely distinguish cereals and
122 pseudocereals both in flour and in finished products.

123 Chromatograms of methyl-esters of fatty acids were obtained in TIC (Total ion
124 chromatogram) mode. Mass spectra were recorded in the range 30-500 Da. This enabled
125 not only a comprehensive analysis of methyl-esters but also of other existing ingredients
126 such as present hydrocarbons and steroid structures. For the extraction of methyl-esters of
127 fatty acids alone, the following procedure should be carried out:

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130
131 Fig. 1.
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133 Blue colour marks the total chromatogram. Red colour marks only methyl-esters of
134 fatty acids. They show McLafferty fragmentation of methyl-esters. The fragmentation is
135 characterised by ion 74 Da which represents methyl-esters of acetic acid. The intensity of
136 this peak is the strongest with saturated fatty acids and it decreases with the increase in the
137 number of double bonds in the molecule (e.g. ione 74 is smaller in oleic in comparison to
138 palmitic acid, whereas in case of linoleic acid, the intensity is even smaller.) When the peak
139 with the mass 74 coincides with the peak on TIC chromatogram, control is done in the data
140 base whether this is really the methyl-ester of the fatty acid. If this is confirmed, the peak of
141 this retention time is taken for data processing.

142 Multivariate analysis is suitable for rapid identification of essential differences
143 among the analysed samples. The Figure 2 shows the Pearson correlation dendrogram.

Many forms of correlations were examined. In all of them amaranth, wheat and buckwheat were grouped equally, and three spelt samples mutually slightly differed. For example, in one case S2 is in the group with wheat (Fig. 2), and in another case the group contains sample S3. This shows that amaranth, wheat and buckwheat certainly definitely and totally differ from one another in all cases. On the other hand, spelt or two out of three spelt samples are also extracted. This probably concerns hybrids.

This procedure shows that the analysis of methyl-esters of fatty acids, hydrocarbons and phytosterols may be used to reliably distinguish among the stated four types of flour.

Fig. 2

The same data obtained from the chromatogram were subjected to PCA analysis in the overview with PC1 and PC3 coordinates, Fig. 3. This shows that the four types of flour are completely distinguished.

Fig. 3

4. Conclusion

This paper has shown that it is possible to compare types of plants against the content of lipids with the GC-MS chromatography and correlation analysis. The GC-MC analysis allows elimination of compounds (peaks) which do not have a biological origin or origin specific for analysed samples.

The obtained results create great opportunities for carrying out similar testing of the authenticity of items in the food industry. Other small molecules suitable for GC-MS

analysis may also be used together with triglycerides for the confirmation of authentic items.

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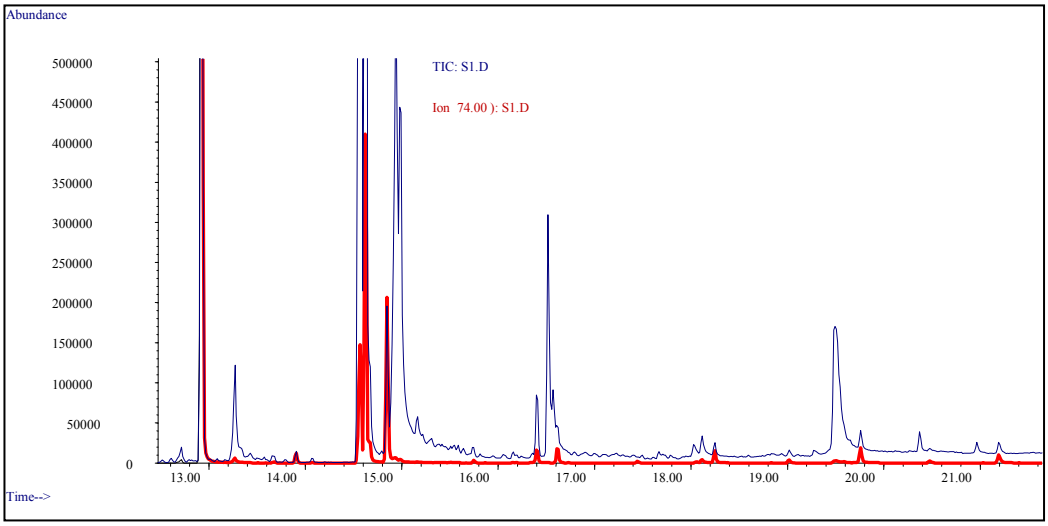
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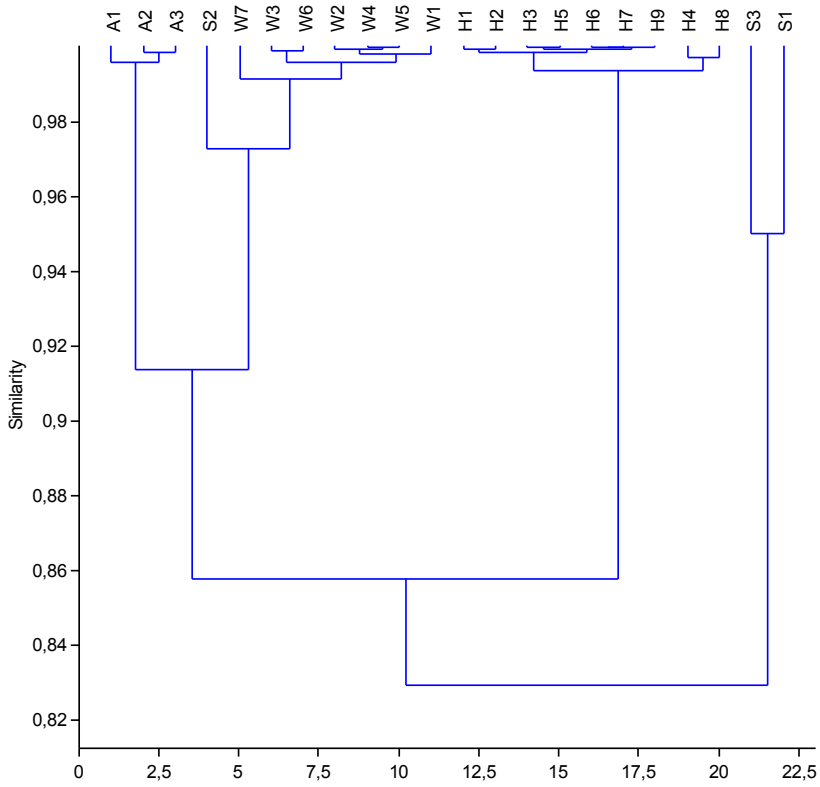
Fig. 1



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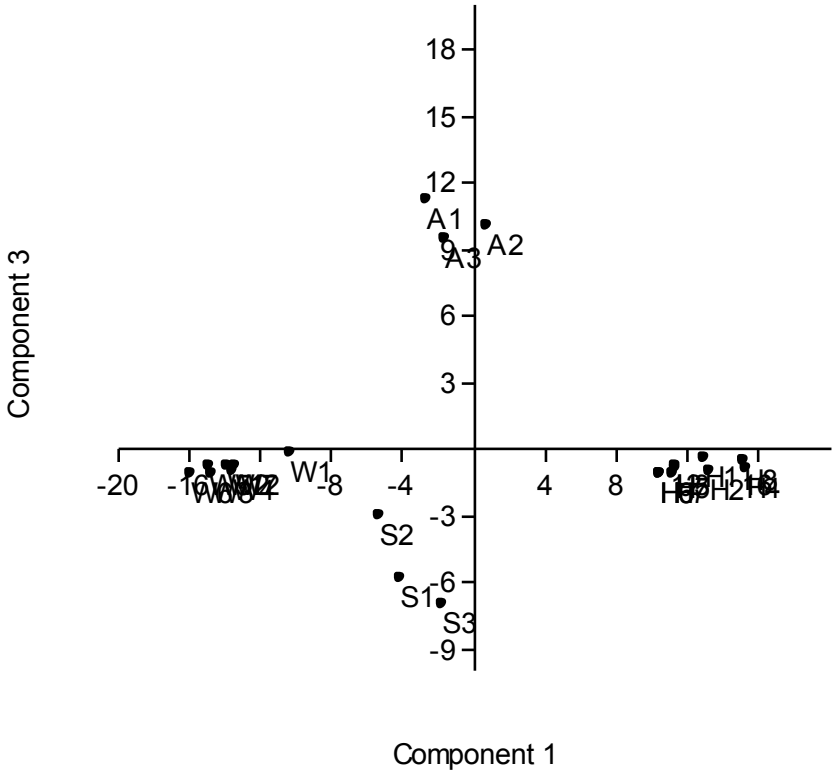
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Fig. 3.

Figure captions:

Fig. 1. Chromatogram of the sample spelt, showed as TIC and SIM of 74Da

Fig. 2. Dendrogram of Pearson´s correlations of investigated cereals and pseudocereals

Fig. 3. Principal component analysis of investigated cereals and pseudocereals