HOMOGENITY OF OIL AND SUGAR COMPONENTS OF FLOUR AMARANTH INVESTIGATED BY GC-MS

Article Highlights
• GC-MS and correlation analysis have been used
• GC-MS showed that the lipid composition of different varieties of amaranth are very similar
• GC-MS showed that the sugar composition of different varieties of amaranth are less similar

Abstract
Gas chromatography with mass spectrometry (GC-MS) was used for performing a qualitative analysis of liposoluble and hydrosoluble flour extracts of three genotypes of Amaranthus sp. All three samples were first defatted with hexane. Hexane extracts were used for the analysis of fatty acids of lipid components. TMSH (trimethylsulfonium hydroxide, 0.2 M in methanol) was used as the transesterification reagent. With transesterification reaction, fatty acids were esterified from acilglycerol to methyl-esters. Defatted flour samples were dried in the air and then extracted with ethanol. Ethanol extracts were used for the analysis of soluble carbohydrates. TMSI (trimethylsilylimidazole) was used as a reagent for the derivatisation of carbohydrates into trimethylsilylethers. The results show that the dominant methyl-esters of fatty acids are very similar in all the three samples. Such a similarity was not detected in the analysis of soluble sugars. The following test cluster analysis was used for the comparison of liposoluble and hydrosoluble flour extracts of three genotypes of Amaranthus sp.

Keywords: Amaranthus sp., GC-MS, liposoluble and hydrosoluble composition, correlation.

In ancient times, amaranth was cultivated by the Aztecs and Incas. Today, it is grown in many tropical, subtropical, and temperate countries [1,2]. Amaranthus includes over 75 wild and weedy species native to tropical and temperate regions of the whole world but is most diverse in the Americas [3]. Presently, cultivated grain is grown in many areas of the world, including Central and South America, Africa, India, China, and the United States [4].

Amaranth oil contains about 18.6-23.4% palmitic (C16:0), 22.7-31.5% oleic (C18:1n-9), and 39.4-49.8% linoleic (C18:2n-6) acids [5-7]. The lipid content in amaranth is between two and three times higher than in common cereals such as wheat [8,9]. Amaranth is a robust crop and can withstand a wide range of climatic conditions. Amaranth is rich in proteins, which have a balanced amino acid profile and are rich in lysine [10,11]. Several studies have reported significant amounts of important unsaponifiable components in amaranth oil [3-8] such as tocopherols, phytosterols, and squalene. Tocopherols are well-known for their cardiovascular benefits and antioxidant capacity, whereas phytosterols and squalene have the ability to decrease serum total cholesterol [5]. Squalene has also been studied for its anticarcinogenic and antioxidant activities [4,10,12].

Since carbohydrates, as the most common group of compounds found in nature, are the main elements of foods of plant origin, as well as of industrially processed products thereof, and since they
constitute series of compounds among which sugars, sugar derivatives and sugar polymers are the most important ones [13]. The content of starch in amaranth flours is about 61.4% [8]. Considering that the starch was investigated in many studies [14-16], but there are not many studies about soluble carbohydrates, it was necessary to determine the content of the samples.

The purpose of this study is to determine liposoluble and hydrosoluble components in three genotypes of Amaranthus sp. and obtain preliminary information on their variability, and then to determine the possibility of differentiation of three genotypes of Amaranthus sp. flour by creating the dendrograms of liposoluble and hydrosoluble extracts. Such studies have been conducted on wheat [17,18], spelt [19,20] and buckwheat [submitted for publication]. It was detected that each plant species has a characteristic composition of liposoluble and hydrosoluble extracts. The final objective of the study is to easily and quickly determine flour authenticity on the basis of liposoluble [submitted for publication] and hydrosoluble composition of obtained chromatograms without an analysis of individual components [21]. Results should also be applicable in the quality control of finished products [submitted for publication].

EXPERIMENTAL

Preparation of samples for analysis of oil components

About 10 g of the three genotypes of Amaranthus sp. were grinded: 2 (A1),16 (A2) and 31 (A3) [22].

Each sample was homogenized and then treated in the following manner: 0.5 g of flour was poured in 12 mL cuvette for centrifugation with the precision of 0.01 g. The cuvette was additionally filled with 5 mL of n-hexane (Merck) and stirred on Vortex for 2 min, after which the mixture was centrifuged at 2000 rpm for 5 min. 2 mL of clear supernatant were separated and dried on a nitrogen flow. The residue was dissolved in 500 µL of pyridine and 50 µL of TMSI (trimethylsilylimidazole, Macherey-Nagel) were added, by which derivatisation of carbohydrates into trimethylsilyl ethers was performed.

Preparation of samples for analysis of sugar components

Samples of defatted flour were dried in the air. Five mL of 96% ethanol (Merck), were added to the dried samples and stirred on Vortex for 2 min, after which the mixture was centrifuged at 2000 rpm for 5 min. 2 mL of clear supernatant were separated and dried on a nitrogen flow. The residue was dissolved in 500 µL of pyridine and 50 µL of TMSI (trimethylsilylimidazole, Macherey-Nagel) were added, by which derivatisation of carbohydrates into trimethylsilyl ethers was performed.

GC–MS Analyses

All the tests were performed on a gas-chromatography system.

The GC–MS analyses were performed on an Agilent Technologies 7890 instrument paired with MSD 5975 equipment (Agilent Technologies, Palo Alto, CA, USA) operating in El mode at 70 eV. A 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. 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 the solutions.

WILEY 275 library was used for the mass spectrum analysis.

PAST programme was used for the statistical data processing [23].

RESULTS AND DISCUSSION

Figure 1 shows the chromatogram of hexane extracts of all three Amaranthus sp. genotypes, from 10 to 21 min. Chromatograms of all three genotypes are very similar. The peak integration shows a ratio of components areas 1:150.

Table 1 shows the retention time of components from the chromatogram presented in Figure 1.

The profile of fatty acids of analysed samples was the same in all three genotypes.

The following fatty acids have been identified as dominant in the form of methyl esters: palmitic acid, oleic acid and linoleic acid. These results are in accordance with our earlier investigations of liposoluble extracts of small grains [18]. The high intensity peak in Figure 1 with the retention time 20.40 min is squalene [4].

Figure 2 shows the chromatogram of all three Amaranthus sp. genotypes, from 10 to 21 min in ethanol extract.

The analysis of amaranth chromatogram in Figure 2 identified trimethylsilyl derivatives of the sugars presented in Table 2. Chromatograms of all three genotypes are also similar, but less than in Figure 1.

Figure 2 shows two areas with silyl derivatives of carbohydrates. The results of the chromatogram
revealed the presence of following compounds: from 10 to 12 minutes there is a presence of tetrose TMSI ethers, and from 18 to 21 min there is a presence of pentoso and hexsoso TMSI ethers. GC-MS allows identification of the presence of TMSI esthers of fatty acids, from 12 to 18 min, which remain after incomplete defattening. However, this is not the focus of the research at the moment.

The carbohydrate content was much lower than the unbound triglyceride content [17], and therefore the remains of triglycerides are higher than the content of carbohydrates.

The purpose of the study was to identify liposoluble and hydrosoluble components and to compare presence of components in samples of hexane and ethanol extracts from amaranth flour. Cluster analysis was used for comparing the samples. A single linkage algorithm and similarity measure type of correlation has been used [22]. Figures 3 and 4 show dendrograms of Pearson's $r$ correlation of liposoluble, i.e., hydrosoluble samples.

Table 1. Retention time (Rt) of components of three genotypes Amaranthus sp. in the liposoluble (hexane) extracts

<table>
<thead>
<tr>
<th>Rt / min</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.808</td>
<td>Tetradecanoic acid, methyl ester</td>
</tr>
<tr>
<td>11.881</td>
<td>Pentadecanoic acid, methyl ester</td>
</tr>
<tr>
<td>12.953</td>
<td>Hexadecenoic acid, methyl ester</td>
</tr>
<tr>
<td>13.642</td>
<td>Hexadecanoic acid, methyl ester</td>
</tr>
<tr>
<td>13.907</td>
<td>Heptadecanoic acid, methyl ester</td>
</tr>
<tr>
<td>14.569</td>
<td>9,12-Octadecadienoic acid (Z,Z)-, methyl ester, methyl linoleate</td>
</tr>
<tr>
<td>14.675</td>
<td>9-Octadecenoic acid, methyl ester</td>
</tr>
<tr>
<td>14.887</td>
<td>Octadecanoic acid, methyl ester</td>
</tr>
<tr>
<td>15.522</td>
<td>Nonadecanoic acid, methyl ester</td>
</tr>
<tr>
<td>16.635</td>
<td>Eicosanoic acid, methyl ester</td>
</tr>
<tr>
<td>18.264</td>
<td>Docosanoic acid, methyl ester</td>
</tr>
<tr>
<td>19.019</td>
<td>Tricosanoic acid, methyl ester</td>
</tr>
<tr>
<td>19.50</td>
<td>Tetracosanoic acid, methyl ester</td>
</tr>
<tr>
<td>19.79</td>
<td>Ergost-5-en-3-ol</td>
</tr>
<tr>
<td>20.30</td>
<td>Pentacosanoic acid, methyl ester</td>
</tr>
<tr>
<td>20.40</td>
<td>Squalene</td>
</tr>
<tr>
<td>20.93</td>
<td>Hexacosanoic acid, methyl ester</td>
</tr>
<tr>
<td>21.14</td>
<td>$\gamma$-Sitosterol</td>
</tr>
<tr>
<td>21.32</td>
<td>Ethylcholestanol</td>
</tr>
</tbody>
</table>
Figure 2. Chromatograms of three genotypes of Amaranthus sp. (A1, A2 and A3) in the hydrosoluble (ethanol) extract.

Table 2. Retention time (Rt) of components of three genotypes Amaranthus sp. in the hydrosoluble (ethanol) extract

<table>
<thead>
<tr>
<th>Rt / min</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.401</td>
<td>1,2,3,5-Tetrakis-O-(trimethylsilyl)-tetrose isomer</td>
</tr>
<tr>
<td>12.63</td>
<td>1,2,3,5-Tetrakis-O-(trimethylsilyl)-tetrose isomer</td>
</tr>
<tr>
<td>18.01</td>
<td>1,2,3,5-Tetrakis-O-(trimethylsilyl)-pentose isomer</td>
</tr>
<tr>
<td>18.514</td>
<td>D-Ribofuranose, 1,2,3,5-tetrakis-O-(trimethylsilyl)- (CAS)</td>
</tr>
<tr>
<td>18.98</td>
<td>Gluconic acid, 2,3,5,6-tetrakis-O-(trimethylsilyl)-, lactone</td>
</tr>
<tr>
<td>19.43</td>
<td>Glucofuranoside, methyl-tetrakis-α-(trimethylsilyl)-</td>
</tr>
<tr>
<td>19.547</td>
<td>2,3,5-tris-O-(trimethylsilyl)-hexose isomer</td>
</tr>
<tr>
<td>19.70</td>
<td>2,3,5-tris-O-(trimethylsilyl)-hexose isomer</td>
</tr>
<tr>
<td>20.046</td>
<td>PER-TMS D-Hexose isomer</td>
</tr>
</tbody>
</table>

Figure 3. Dendrogram of component correlations from Table 1 of three genotypes of Amaranthus sp. (A1, A2 and A3).

Figure 4. Dendrogram of component correlations from Table 2 of three genotypes of Amaranthus sp. (A1, A2 and A3).
A correlation coefficient is shown on the ordinate (Y-axis). X-axis is labelled distance and refers to a distance measure between clusters.

The dendrograms of Pearson’s r correlation show that the similarity in the composition of lipophilic substances of amaranth (components listed in Table 1) is significant (r > 0.9942, Figure 3).

Similarity in the composition of carbohydrates of three types of amaranth components listed in Table 2 is much smaller (r > 0.64, Figure 4).

CONCLUSION

Gas chromatography-mass spectrometry was used to show that the lipid compositions of all three genotypes of *Amaranthus sp.* are, from the nutritional aspect, very similar.

Gas chromatography-mass spectrometry also showed that the sugar compositions of all three genotypes of *Amaranthus sp.* are, from the nutritional aspect, less similar.

Three genotypes of *Amaranthus sp.* have a characteristic composition of liposoluble and hydrosoluble extracts. This was also detected in other plant species (wheat, spelt and buckwheat).

Therefore, the final objective of this study is to determine the authenticity of the flour and finished products without an analysis of individual components of the extracts in a relatively simple and quick manner.

Acknowledgements

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HOMOGENOST UZORAKA ULJNIH I ŠEČERNIH KOMPONENTI BRAŠNA AMARANTUSA ISPITANIH GC-MS HROMATOGRAFIJOM


Ključne reči: Amaranthus sp., GC-MS, liposolubilne i hidrosolubilne komponente, korelaciju.