

# Authenticity of Lipid and Sugar Profiles of Various Buckwheat Cultivars Investigated by GC-MS System and Multivariate Analysis

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**Abstract** Gas chromatography with mass spectrometry (GC-MS) was used to perform a qualitative analysis of the liposoluble and hydrosoluble flour extracts of nine buckwheat samples. All samples were first defatted with hexane. Hexane extracts were used for the analysis of the fatty acids of lipid components. Transesterification reagent was TMSH (Trimethylsulfonium hydroxide, 0.2M in methanol). With transesterification reaction, fatty acids esterified from acilglycerol to methyl esters. Samples of defatted flour 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 derivatization of carbohydrates into trimethylsilylethers. The results show that the dominant fatty acid methyl esters and sugar composition are very similar in all investigated samples. The cluster analysis was used to compare the liposoluble and hydrosoluble flour extracts of nine buckwheat cultivars to establish homogeneity among them and the possibility of their authentication.

**Keywords** Buckwheat, GC-MS, Correlations of Liposoluble and Hydrosoluble Composition

## 1. Introduction

Common buckwheat was domesticated and first cultivated in Asia, in the Yunnan region of China and Tibet, around 6000 BC, and from there spread to Central Asia and Europe [1].

Although the cultivation of buckwheat is the largest in China, Russia and North America, it is now grown in Europe, Japan, India, Tibet, Tasmania, Australia, Argentina, Bhutan, South Africa, Brazil and many other countries [2].

Buckwheat belongs to the Polygonaceae family, unlike major cereals such as wheat, rice, and corn [3]. Cultivation of buckwheat was in decline in the past century. More recently, cultivation has increased due to the growing interest in organic farming, alternative culture, old and traditional diet,

outstanding nutritive properties, and not to mention numerous studies which support the claim that buckwheat has had a positive effect on human health [4-6].

The most commonly cultivated buckwheat species are: common buckwheat (*Fagopyrum esculentum* Moench) and tartary buckwheat (*Fagopyrum tataricum* Gaertner) [7,8]. Buckwheat is now mainly grown for grain, which is often processed into flour. Buckwheat seed is peeled, and then peeled grain is milled and sieved [9]. In China, India, and Nepal, the leaves are also used as a dried or pickled vegetable. In Japan, buckwheat inflorescences are utilized as a functional food, due to their high rutin content. A green flour obtained by milling the dried flowering buckwheat plants is added as a natural food colorant to pasta, ice cream, and other products in Japan and South Korea [10].

Whole buckwheat groats contain 55% starch, 12% protein, 4% lipid, 2% soluble carbohydrates, 7% total dietary fibre, 2% ash and 18% other components (organic acids, phenolic compounds, tannins, phosphorylated sugars, nucleotides and nucleic acids, and unknown compounds) [2,8,11]. The content of every component depends on buckwheat species.

The purpose of this study was to, quickly and relatively easy, determine the similarity between 9 samples of flour produced of various buckwheat cultivars by creating the dendrograms of liposoluble and hydrosoluble extracts. Liposoluble and hydrosoluble components were analysed and identified using gas chromatography with mass spectrometry. The aim was also to show if it is possible to authenticate buckwheat flour based on lipid and simple sugar profiles, simply by introducing automatically integrated peak surface areas into multivariate analysis, avoiding any quantitative determinations and applications of analytical standards.

## 2. Experimental

About 10 g of the following samples was grounded: Godijeva (H1), Bambi (H2), Darja (H3), Francuska (H4), Prekumurska (H5), Češka (H6), Čebelica (H7), Novosadska

(H8) and Spacinska (H9). Each sample was homogenized 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 an oily residue the amount of 10  $\mu$ L was taken, reconstituted to 400  $\mu$ L of methanol, and additionally 100  $\mu$ L of the transesterification reagent - TMSH (Trimethylsulfonium hydroxide, 0.2M in methanol, Macherey-Nagel) was added. With such a transesterification reaction, the fatty acids esterified from acylglycerol to methyl esters.

Samples of defatted flour were dried in the air. A 5 mL of 96% ethanol (Merck) was added to the dried samples and stirred on Vortex for 2 minutes, after which the mixture was centrifuged at 2000 rotations/min for five minutes. The amount of 2 mL of clear supernatant was separated and dried under nitrogen flow. The residue was dissolved in 500  $\mu$ L of pyridine and 50  $\mu$ L of TMSI (*Trimethylsilylimidazole, Macherey-Nagel*) was added by which derivatization of carbohydrates into trimethylsilyl ethers was performed.

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 DB-5 MS column (30 m, 0.25 mm, 25  $\mu$ m) 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. The split ratio of 1:50 was used for the injection of 1  $\mu$ L of the solutions. WILEY 275 library was used for mass spectra analysis. PAST programme was used for statistical data processing [12].

### 3. Results and Discussion

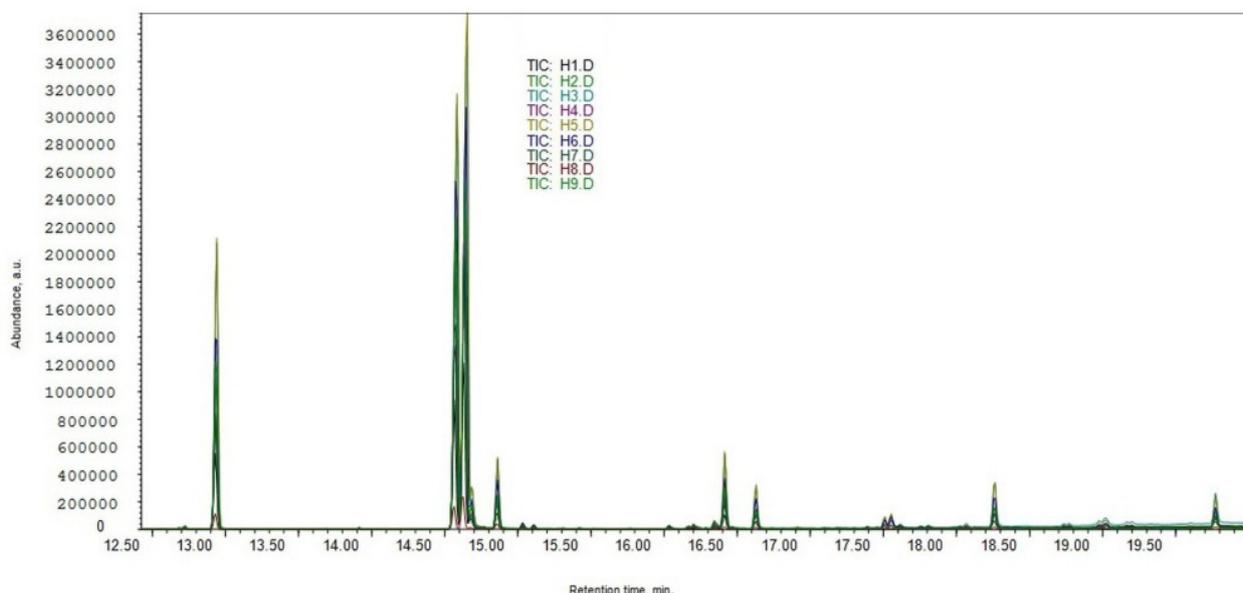
**Analysis of liposoluble extracts.** The lipid content of buckwheat seeds varies, depending on the variety of buckwheat, region in which it is grown, and differences can occur due to the different time of harvest [13]. The dominant fatty acids of buckwheat seeds are unsaturated fatty acids (18:1 and 18:2). Linoleic (18:2), oleic (18:1) and palmitic acids (16:0) constitute 88% of the total fatty acids of buckwheat seeds [14, 15].

With typically 80% unsaturated fatty acids and more than 40% of fatty acids, such as the polyunsaturated essential fatty acid - linoleic acid [14], buckwheat is nutritionally superior to cereal grains in the fatty acid composition [16]. Fig. 1 shows the chromatogram of the hexane extracts of buckwheat samples from 13 to 21.60 min. Chromatograms of all nine flour samples are very similar. The peak integration shows the ratio of components areas 1:150.

Table 1 shows the retention times of the components from the chromatogram presented in Fig. 1.

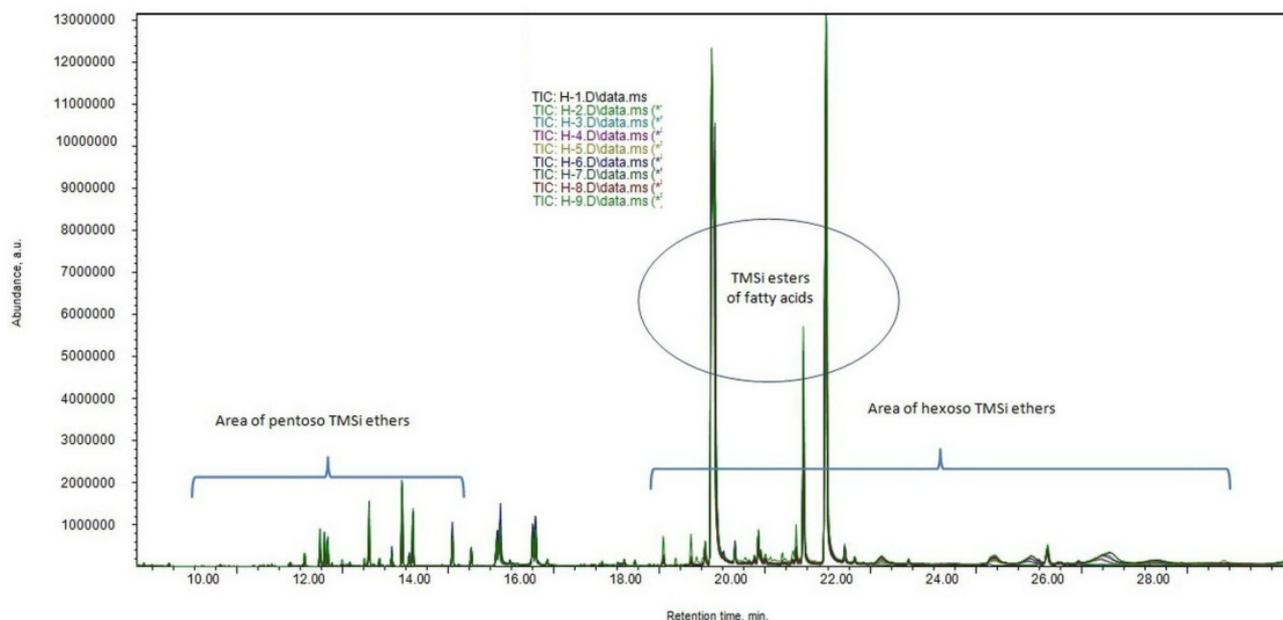
**Table 1.** Retention times ( $R_t$ ) of buckwheat components in the liposoluble (hexane) extracts

$R_t$ [min]	Compound
12.940	Hexadecanoic acid, methyl ester
14.581	9,12-Octadecenoic acid (Z,Z)-,methyl ester
14.647	9-Octadecenoic acid, methyl ester
14.864	Octadecanoic acid, methyl ester
16.419	Eicosenoic acid, methyl ester
16.632	Eicosanoic acid, methyl ester
17.51	Unknown
17.56	Unknown
18.264	Docosanoic acid, methyl ester
19.776	Tetracosanoic acid, methyl ester



**Figure 1.** Overlaid TIC chromatograms of the liposoluble (hexane) extracts of all buckwheat cultivars





**Figure 3.** Overlaid TIC chromatograms of the hydrosoluble (ethanol) extracts of all buckwheat cultivars

The chromatograms of all investigated buckwheat cultivars (Fig. 3) are very similar. The peak integration shows the ratio of components areas 1:150.

Table 2 shows the retention times of components from the chromatogram presented in Fig. 3.

**Table 2.** Retention times ( $R_t$ ) of buckwheat components in the hydrosoluble (ethanol) extracts

$R_t$ [min]	Compound
8.23	ERYTHRITOL PER-TMS
9.32	D-Arabino-Hexitol, 2-deoxy-1,3,4,5,6-pentakis-O-(trimethylsilyl)-
10.51	XYLITOL 5TMS
10.72	Ribitol, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-
11.16	1,2,3,4,5-pentakis-O-(trimethylsilyl)-pentose
11.57	Arabinofuranose, 1,2,3,5-tetrakis-O-(trimethylsilyl)-
11.72	Sorbopyranose, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-
11.80	D-Galactose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-
12.00	beta.-D-Galactofuranose, 1,2,3,5,6-pentakis-O-(trimethylsilyl)-
12.07	2-Deoxy-galactose, tetrakis(trimethylsilyl)
12.14	Mannopyranose pentakis(trimethylsilyl)
12.41	Talose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-
12.50	D-Mannopyranose, 1,2,3,4,6-pentakis-O (trimethylsilyl)-
12.59	alpha.-D-Galactopyranose, 1,2,3,4,6-pentakis-O-(trimethylsilyl)-
12.93	D-Glucitol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-
13.13	Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-, allo-
13.33	Glucopyranose-pentaTMS-Glucopyranose 1,2,3,4,6-pentakis-O-(trimethylsilyl)-, D-
13.83	Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-, scyllo-
14.21	ALLOSE PER-TMS
14.44	Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-isomer
18.09	D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-
19.00	alpha.-D-Glucopyranoside,1,3,4,6-tetrakis-O-(trimethylsilyl)-.beta.-D-fructofuranosyl-2,3,4,6-tetrakis-O-(trimethylsilyl)-
20.72	PER-TMS D Hexose isomer
21.15	MELIBIO PER-TMS SE 8TMS
21.52	PER-TMS D Hexose isomer
25.93	Maltose, octakis(trimethylsilyl)-
28.70	alpha.-D-Galactoside, methyl tetrakis-O-(trimethylsilyl)-

Generally, the similarity in the composition of the hydrophilic components was detected in all nine flour samples of analyzed buckwheat cultivars, which is consistent with previous studies [17].

Fig. 4 shows dendrograms of Pearson's  $r$  correlation of nine hydrosoluble extracts. The dendrograms of Pearson's  $r$  correlation show that the similarity in the composition of simple sugar substances of buckwheat (components listed in Table 2) is significant ( $r > 0.991$ ), Fig. 4.

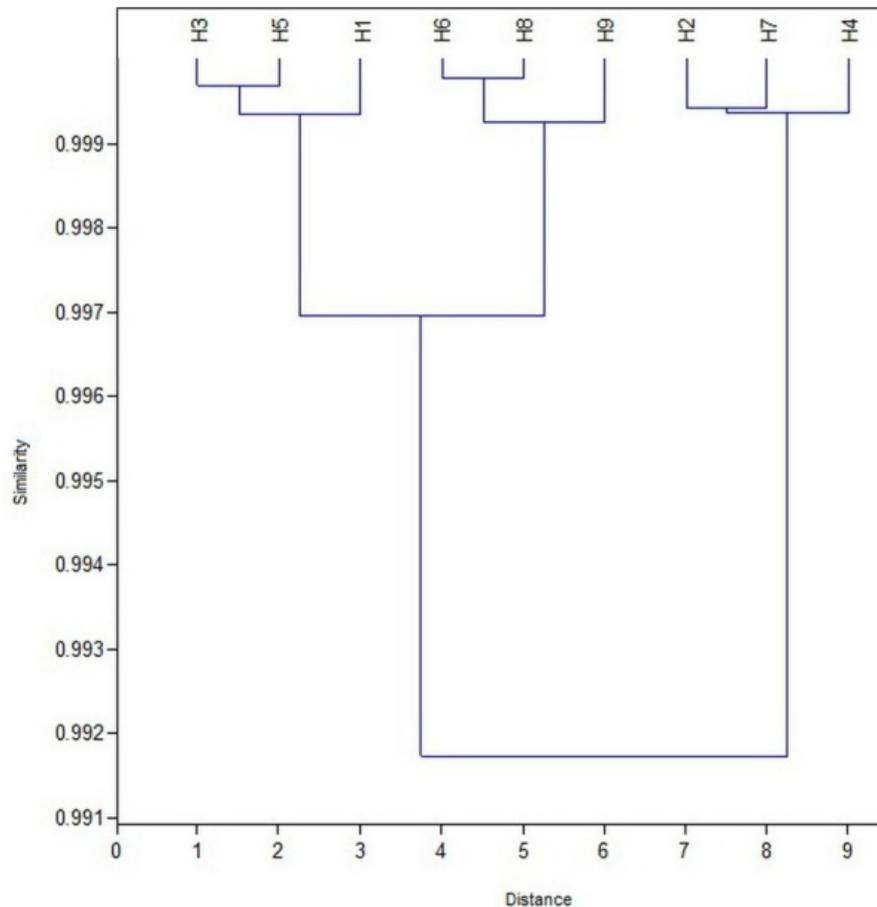


Figure 4. Dendrogram of components correlations from Table 2 of the nine types of buckwheat

## 4. Summary

The results in this paper show that the lipid and sugar composition of different cultivars of buckwheat are, from the nutritional aspect, very similar. The obtained results are fully in accordance with our previous research, where it has been proved that homogeneity of various cultivars of amaranthus can be determined using the same method [20].

In general, this paper shows that it is possible to compare types of plants through their oil and sugars content using GC-MS system and correlation analysis, avoiding quantitative determinations of identified compounds.

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