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QUALITATIVE ANALYSIS OF HYDROSOLUBLE FLOUR EXTRACT OF SPELT AND BREAD WHEAT
KVALITATIVNA ANALIZA HIDROSOLUBILNOG EKSTRAKTA SPELTE I HLEBNE PŠENICE

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ABSTRACT

A qualitative analysis of hydrosoluble flour extract of spelt (Triticum aestivum ssp speltae) (three types of spelt: Eko-10, Nirvana and Austrija), and of bread wheat (Triticum aestivum) (seven types of winter bread wheat: Simonida, Dragana, NS-40S, Pobeda, Ljiljana, Zvezdana and Arija), was performed by using gas chromatography with mass spectrometry. TMSI (trimethylsilylimidazole) was used as reagent for derivatization of carbohydrates into trimethylsilylethers. Analysis of the obtained chromatograms helped in identifying trimethylsilyl derivatives of the following sugars in wheat: xylitol, fructose, arabinofuranose, galactopyranose, mannopyranose, mannitol, mannofuranose, glucose and saccharose. However, different composition was determined with the analysis of carbohydrates in spelt. Trimethylsilyl derivatives of the following sugars were identified in spelt: galactofuranose, ribofuranose, arabinofuranose and saccharose. The aim of this study was to verify authenticity of the more expensive spelt flour. Results were analyzed using descriptive statistics (dendrograms and PCA). The results show that this method can be used to make a distinction among different types of flour.

Key words: *spelt, bread wheat, GC-MS, hydrosoluble extract.*

REZIME

Primenom gasne hromatografije sa masenom spektrometrijom urađena je kvalitativna analiza hidrosolubilnog ekstrakta spelte (3 vrste spelte: Eko-10, Nirvana i Austrija) i hlebne pšenice (7 vrsta ozime pšenice: Simonida, Dragana, NS-40S, Pobeda, Ljiljana, Zvezdana i Arija). Reagens za derivatizaciju je bio TMSI (trimetilsililimidazol), čime je izvršena derivatizacija šećera u trimetilsililetre. Analizom dobijenih hromatograma kod pšenice identifikovani su trimetilsilil derivati sledećih šećera: ksilitola fruktoze, arabinofuranoze, galaktopiranoze, manopiranoze, manitola, manofuranoze, glukoze i saharoze. Analizom ugljenih hidrata kod spelte ustanovljen je drugačiji sastav. Naime, kod spelte su identifikovani trimetilsilil derivati sledećih šećera: galaktofuranoze, ribofuranoze, arabinofuranoze i saharoze. Cilj istraživanja je bio da se potvrdi autentičnost skupljeg speltinog brašna. Za obradu rezultata korišćena je metoda deskriptivne statistike (dendrogrami i PCA). Rezultati pokazuju da je ovom metodom moguće napraviti distinkciju između različitih vrsta brašna.

Ključne reči: *spelta, hlebna pšenica, GC-MS, hidrosolublni ekstrakt.*

INTRODUCTION

Spelt (Triticum aestivum ssp. spelta), being an old type of wheat, has the status of minor culture, however, due to its predisposition for growing in organic agriculture, areas under this crop have lately increased throughout Europe: Belgium, Germany, Switzerland, Austria, Poland, Italy, Slovakia, Czech Republic and Hugary (*Trocolli et al 2005; Lacko-Bartošová et al 2007; Zieliński et al 2008*). Comparison of common wheat (Triticum aestivum) and spelt is important as spelt flour is more expensive and, most often, supported by an organic product certificate (*Ruibal-Mendieta, et al., 2005*). Cluster analysis proved to be a very useful tool of descriptive statistics for distinguishing between small grains and grouping them into categories (*Vujić et al., 2012*), and has not been used in previous papers for confirming the authenticity of spelt flour. The objective of this paper is to determine a new method for detecting the authenticity of spelt flour using GC-MS chromatography and cluster analysis of water-soluble extract. 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, it was necessary to determine their content in the sample. The content of carbohydrates in bread wheat flours is commonly within the following limits: starch 64-74%, soluble sugars 2-4%, cellulose 0.1-2% and pentosans 1-5% (*Olson and Frey, 1987; Pomeranz, 1988a; Pomeranz, 1988b; Đaković, 1997*). Sugars also play a role in the process of bread baking since they caramelize at higher temperatures, turn the bread crust to brown color and affect its properties, appearance and flavor.

The sugar content of flour depends on the physiological condition of the processed wheat grain and the presence of enzymes, on the level of grinding, then on the moisture, conditions and duration of flour ripening, etc. Soluble mono- and disaccharides that are present in flour ferment (in the presence of yeast cells) and contribute to the rise of the dough in the first stage of fermentation. In further stages, sugars are continually produced due to the enzyme initiated breaking down of molecules of starch and flour oligosaccharides. Enzyme disintegration of starch produces disaccharide maltosis and its content in the dough for the first 10-15 increases by 10-15 times. Either present in the flour, added to the dough or produced in the dough during fermentation, sugars are used as substrate which the yeast ferments and frees into carbon (IV)-oxide and alcohol. Regardless of the origin, the presence of sugar in the dough affects the porosity, structure and appearance of breadcrumbs. The exception is lactose disaccharide, which the yeast can not ferment. However, hydrolyzed lactose concentrates, or hydrolyzed pure lactose do contribute to fermentation and improve the bread quality.

Purpose of this study is to analyze the flour hydrosoluble extract composed of soluble sugars.

MATERIAL AND METHOD

Sample preparation

About 10 g of the following grains was ground: Simonida (W1), Dragana (W2), NS-40S (W3), Pobeda (W4), Ljiljana (W5), Zvezdana (W6), Arija (W7), Austrija (S1), Eko-10 (S2) and Nirvana (S3). 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 centrifuged at 2000 rotations/min for five minutes. After this 3 mL of clear supernatant was poured into a 10 mL glass and left for the analysis of liposoluable extract (*Vujić et al, 2012*). The procedure was repeated three times, hexane fractions were rejected, so the flour remained defatted. Samples of defatted flour were dried in the air. 5 mL of 96% ethanol (Merck) were 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. 2 mL of clear supernatant was separated and dried by nitrogen flow. Residue was dissolved in 500 μL of pyridine and 50 μL of TMSI (trimethylsilylimidazole, Macherey-Nagel) was added by which derivatization of carbohydrates into trimethylsilylethers was performed (*Knap, 1979*).

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 program 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

RESULTS AND DISCUSSION

Fig. 1 shows the chromatogram of all wheat samples from 10 to 26.00 minutes. Chromatograms of all 7 samples of common wheat are very similar. Table 1 shows retention time of components from the chromatogram presented in Fig. 1. Fig. 2 shows the chromatogram of all spelt samples from 10 to 26.00 minutes. Chromatograms of all 3 spelt samples are also very similar.

Table 1. Retention time (R_t) of common wheat components

Component	R_t
Siloxan	5.27
Siloxan (isomer)	5.54
1,2,3,4,5-pentakis-O-(trimethylsilyl)- Xylitol	10.65
1,3,4,5,6-pentakis-O-(trimethylsilyl)- D-Fructose	11.56
1,2,3,5-tetrakis-O-(trimethylsilyl)- Arabinofuranose	11.64
1,2,3,4,6-pentakis-O-(trimethylsilyl)-β-D-Galactopyranose	11.71
1,2,3,4,6-pentakis-O-(trimethylsilyl)-α-D-Mannopyranose	12.49
Glucose-oxime-hexa-trimethylsilyl	12.86
1,2,3,4,5,6-hexakis-O-(trimethylsilyl)- D-Mannitol	12.93
6-deoxy-1,2,3,5-tetrakis-O-(trimethylsilyl)-β-L-Mannofuranose	13.23
2,3,4,5,6-pentakis-O-(trimethylsilyl)- D-Glucose	13.32
Hexadecanoic acidtrimethylsilyl ester	14.07
9,12-Octadecadienoic acid (Z,Z)-, trimethylsilyl ester	15.59
Oleic acid, trimethylsilyl ester	15.64
unidentified	18.065
Saccharose	18.98

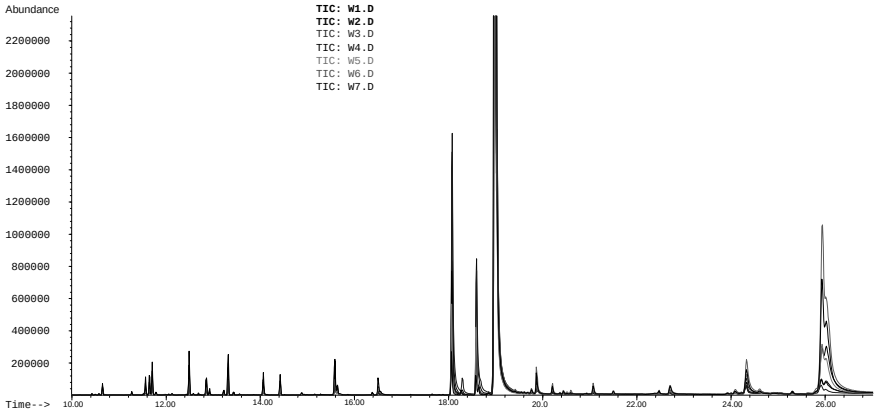

Fig. 1. Chromatograms of all wheat samples

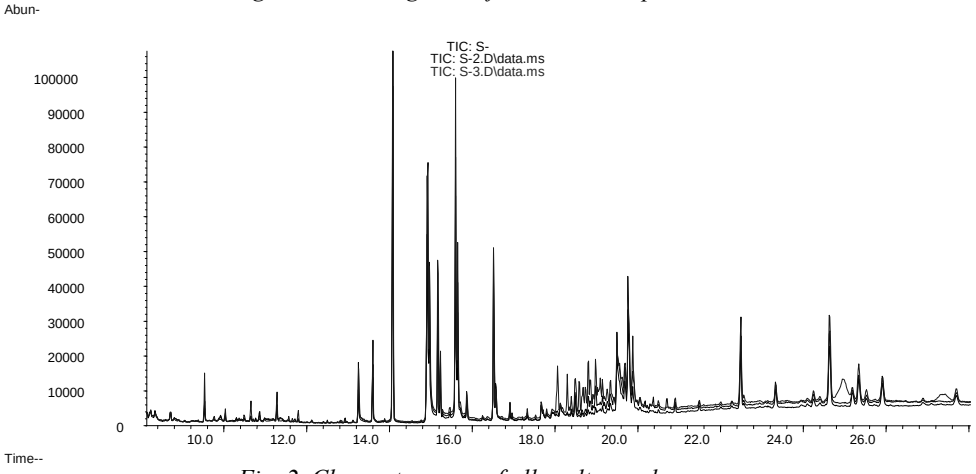

Fig. 2. Chromatograms of all spelt samples

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

Table 2. Retention time (R_t) of spelt components

Component	R_t
alpha.-D-Galactofuranose, 1,2,3,5,6-pentakis-O-(trimethylsilyl)-	9.536
D-Ribofuranose, 1,2,3,5-tetrakis-O-(trimethylsilyl)	11.29
Mannoonic acid, 2,3,5,6-tetrakis-O-(trimethylsilyl)-, lactone	18.064
Arabinofuranose, 1,2,3,5-tetrakis-O-(trimethylsilyl)-	18.797
Saccharose	18.98
alpha.-D-Ribofuranoside, methyl 2,3,5-tris-O-(trimethylsilyl)-	20.70

The analysis of wheat chromatograms identified trimethylsilyl derivatives of the following sugars: xylitol of fructose, arabinofuranose, galactopyranose, mannopyranose, mannitol, mannofuranose, glucose, and sucrose. The analysis of carbohydrates contained in spelt identified a different composition. Namely, spelt was identified to contain trimethylsilyl derivatives of the following sugars: galactofuranose, ribofuranose, arabinofuranose, and sucrose. The purpose of the study was not to identify sugar components, but to compare presence of components in samples of ethanol from wheat and spelt flour. Cluster analysis was used for the comparison of the samples. Single linkage algorithm and similarity measure type of correlation were used (*Hamer et al., 2001*). Fig. 3 shows dendrogram of Pearson's ***r*** correlation of all samples wheat and spelts. A correlation coefficient is shown on the ordinate (Y-axis).

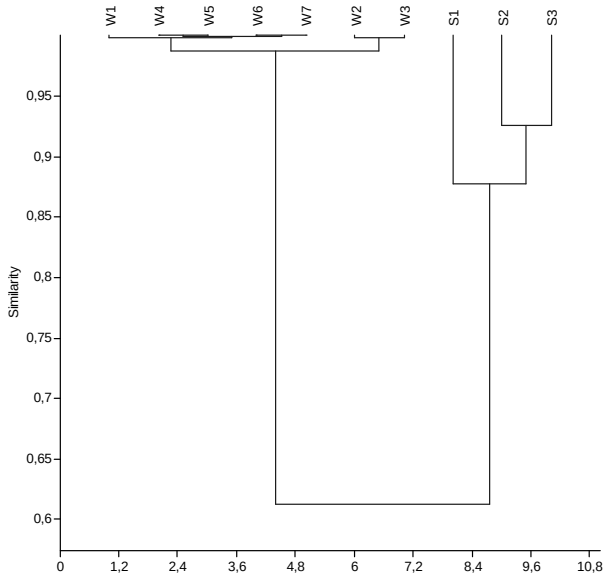

Fig. 3. Dendrogram of components correlations from Table 1 and 2 of 7 samples of common wheat and 3 samples of spelt

Figure 3 shows that all the samples were divided into two clear groups. Wheat and spelt are two separate branches. The dendrogram of Pearson's correlation of all 7 samples of common wheat show that the similarity in the composition of sugars (components listed in Table 1) is significant (r>0.95; Figure 3). Similarity in the composition of sugars of all 3 samples of spelt (components listed in Table 2) is also considerable (r>0.85; Figure 3). The dendrogram of Pearson's ***r*** correlation of carbohydrate components clearly shows that sugars are a very good choice for making a distinction between spelt and wheat flour (r>0.6; Figure 3). A similar result is obtained by using the PCA (*Hammer, O. et al 2001*) analysis of the same samples, Fig. 4.

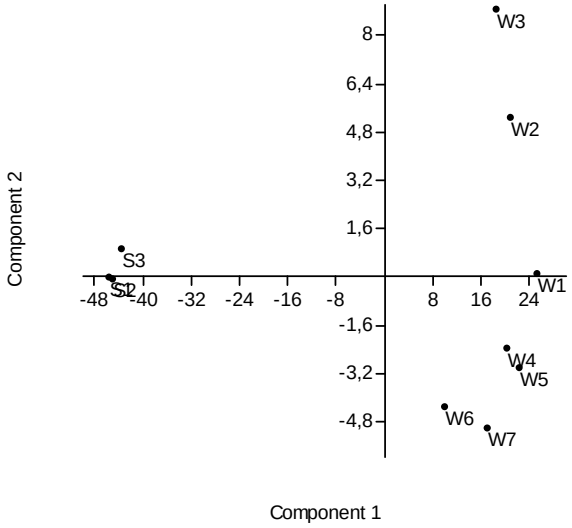

Fig. 4. Principal component analysis of total carbohydrates (monosaccharides, disaccharides) in wheat and spelt flour

Fig. 4 shows that spelt and wheat form two clearly separated groups. This confirms that carbohydrates are a good choice for distinguishing between spelt and wheat flour.

CONCLUSION

Flora is rich in low molecular weight molecules. The most common are: linked fatty acids, carbohydrates, hydrocarbons, sterols, hormones, amino acids and organic acids. Some of them are genetically conditioned and this makes them to suitable for distinguishing between one type from another. This paper has shown that it is possible to compare types of plants through their content of sugars using the GC-MS chromatography and correlation analysis.

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