The chromatographic analysis of extracts from poplar (*Populus* sp.) - Laying program GC-MS

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Abstract: *The chromatographic analysis of extracts from poplar (Populus sp.) - Laying program GC-MS.* The aim of the study was to develop the method of analysis by gas chromatography of the liquid obtained after extraction with cyclohexane of wood of different poplar varieties (Populus sp.). After applying an appropriate method, the application of gas chromatography with mass detector facilitates the analysis of the chemical composition of extracts from different types of lignocellulosic biomass. It is also possible to verify included compounds as well as to compare the content of individual compounds contained in the analysed sample. Moreover, this sample will make it possible to determine the significance of the influence of given substances on biofuel production processes based on lignocellulosic materials. One of the key chemical substances influencing the process of enzymatic hydrolysis and fermentation are extraction substances contained in lignocellulose materials used in 2nd and 3rd generation biofuels. These compounds can inhibit the whole process of producing biofuels from lignocellulosic biomass.

Keywords: GC-MS, poplar wood, extractives, biofuels

INTRODUCTION

The industrial use of wood and raw materials is associated with solving a wide range of problems related to the physical and chemical properties of this material (Król et al. 2017, Kučerová and Výbohová 2018). The chemical composition of wood and lignocellulosic raw materials affects the technological process of weighing and processing of lignocellulosic materials and determines their subsequent parameters such as strength. The substances constituting the chemical composition of the biomass influence the weaving of glue joints in products made of e.g., solid or shredded wood. They also determine the amount of hardeners added to resins used in the production of chipboards and hardeners in the case of surface refinement with paints or varnishes (Król et al. 2017, Stachowiak-Wencek et al.2019).

One of the main group of substances influencing these parameters are extraction substances. These substances have different structures and properties that are depending on the compounds in which they are included. However, the composition of extraction substances obtained from lignocellulose biomass is not the subject of the chemical analysis due to their content in lignocellulose (Stachowiak-Wencek et al.2019). Nevertheless, extraction substances as chemical compounds are directly responsible for the resistance of lignocellulose biomass to biological corrosion, i.e., the action of fungi that cause white, brown and grey decomposition and resistance to the action of feeding insects. At the same time, such a buffer capacity for the sealing of particleboard carpets (Król et al. 2017) or chemical and biochemical processes during the production of biofuels from lignocellulosic materials (Kučerová et al. 2019, Szadkowski et al. 2017) directly affect the chemical processes in the processing of lignocellulosic biomass. Additionally, the extraction substances have an influence on the strength influence the strength and corrosion of equipment used in lignocellulosic biomass processing.

MATERIALS

A number of analyses of the sample obtained by extraction with cyclohexane of white poplar (Populus alba L.) (Lewandowska 2013) were performed in order to determine the

appropriate programme on the gas chromatography. The analyses were performed for a carrier gas (helium) flow of 0.8 cm^3/min, a pressure of 36.9 kPa and an injector (injection) temperature of 250°C. The remaining parameters of the analysis, i.e., the division of the analyte stream (split), the temperature program of the column and the detector voltage were changed according to Table 1.

Table 1. GCMS chromatographic analysis programme

Name of programme	Split	Temperature programme	Detector voltage	Injection temperature
G4	1:2	50°C maintained for 7 minutes, an increase of 10°C/min to 300°C, maintained for 15 minutes	0,3 kV	250°C
G5	1:2	50°C maintained for 7 minutes, an increase of 10°C/min to 320°C, maintained for 10 minutes	0,8 kV	250°C
G6	splitless	50°C maintained for 7 minutes, an increase of 10°C/min to 320°C, maintained for 10 minutes	1,1 kV	250°C
G9	direct	50°C maintained for 7 minutes, an increase of 10°C/min to 320°C, maintained for 10 minutes	1,3 kV	250°C

RESULTS

The distinct programs differed in the detector voltage and the way the sample was dosed per column. In program G4 the detector voltage was determined according to the tuning performed on the gas chromatograph.
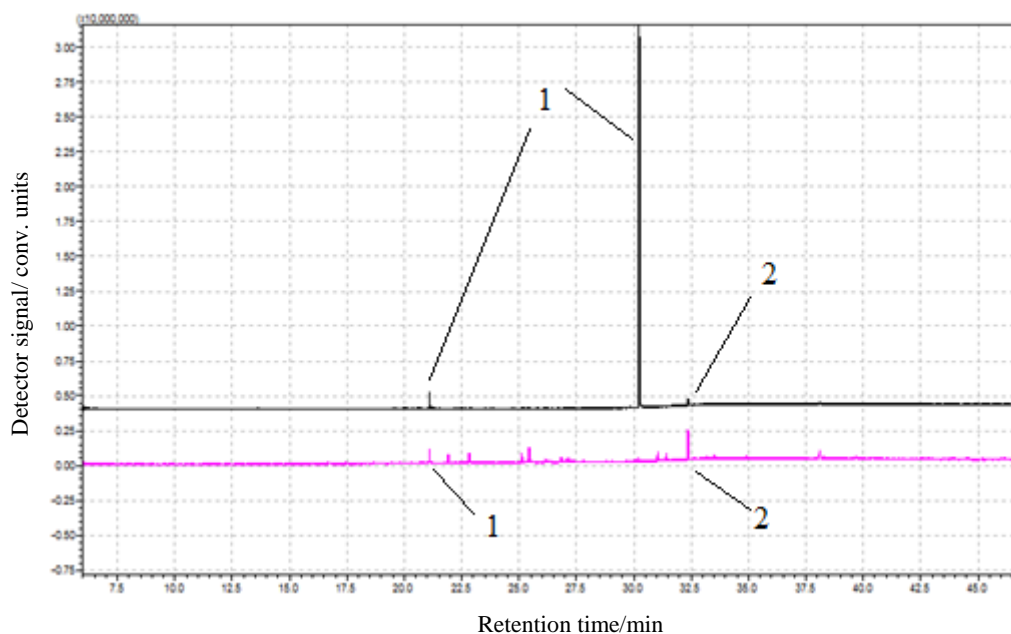

Figure. 1 Chromatograms in two different programs black spectrum - program G5, pink spectrum G4 (1- phthalate, 2- squalene)

The voltage (as shown in figure 3) is insufficient to obtain a readable chromatogram; therefore, it was decided to increase the detector voltage (Fig. 1, Fig. 2) and at the same time the way of dosing the samples by means of the injector per column was analysed (Fig. 3).

After the analysis of the chromatograms produced in different programs, it was decided to dose the sample directly to the column. The identified compounds presented in the figure are phthalates in both spectra identified as diisooctyl phthalate, diethyl phthalate. These are salts and esters of phthalic acid which are also organic compounds used primarily in the manufacture of plastics to improve their performance. Plastics containing phthalates are more flexible; therefore, they are used in the manufacture of many everyday items. They are found in septa in vial caps containing samples. Squalene is also found in the G4 spectrum - it is one of the components of wood extraction, tri-terpene.
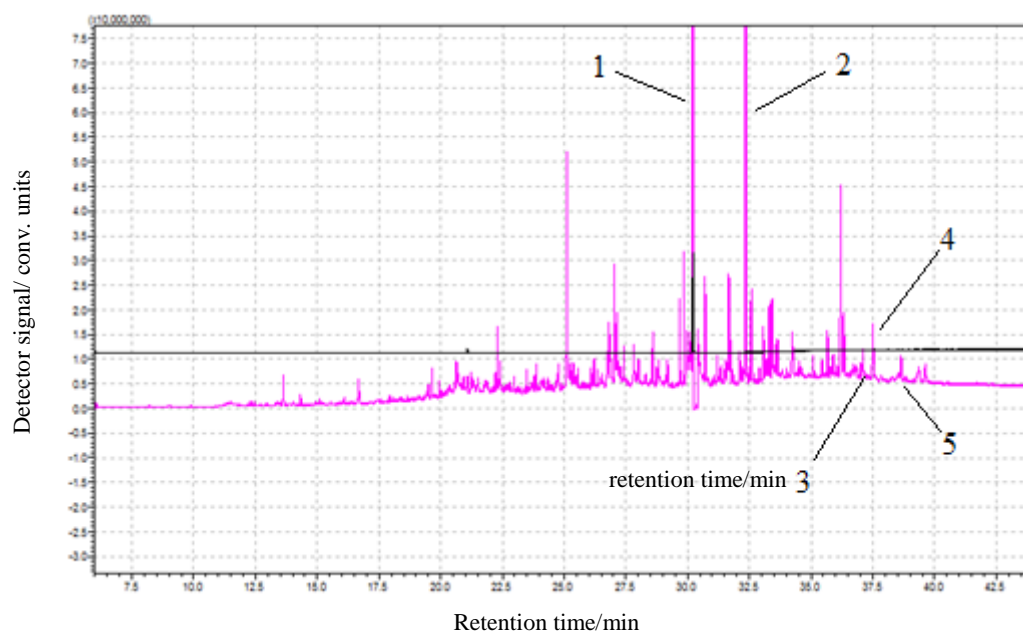

Figure. 2 Chromatograms in two different programs black spectrum - program G5, pink spectrum G6 (1- phthalate; 2- squalene; 3- sigmasterol; 4- stigmastanol; 5- sigmasterol)
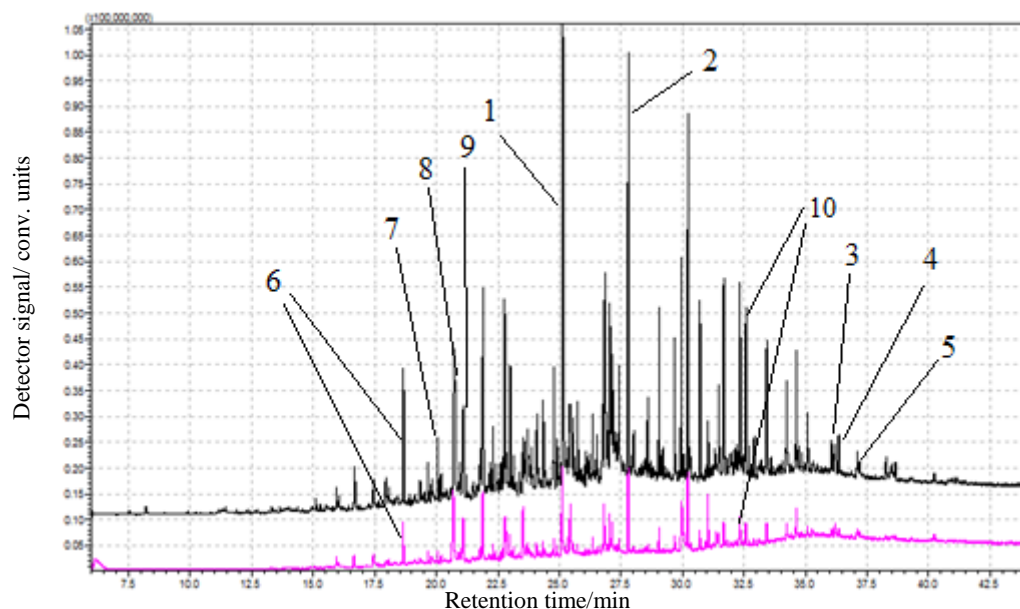

Figure. 3 Chromatograms in two different programs black spectrum - program G6, pink pectrum G9 (1- phthalate; 2- squalene; 3- sigmasterol; 4- stigmastanol; 5- sigmasterol; 6- vanillin, 7- isovanillin; 8- 4-hydroxy-3,5-dimethoxy-benzaldehyde; 9- oleic acid; 10-squalene)

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The key to obtaining a readable chromatograph was the detector voltage. When it is set at an adequate level to the tuning of the apparatus (program G4), the chromatogram is a straight line in which the only peaks are phthalates. When we increase the voltage first to 0.8 kV (program G5), we notice an increase in the number of peaks. The best results of the number of peaks on the chromatogram were obtained at the detector voltage of 1.1 kV (program G6) and 1.3 kV (program G9) this is shown at figure 2 and 3.

Another important issue in achieving a satisfactory chromatogram is the way of dosing the sample onto the chromatograph column. We have applied three different dosing methods - direct to the column, with 1:2 split and splitless. The most effective methods are splitless and direct. Unfortunately, split 1:2 did not give a satisfactory spectrum (G4, G6) shown in Figures 1 and 2. The comparison of the application of splitless and split 1-2 is in Figure 2 - you can see that changing the dosage gives us more peaks. The comparison of splitless and direct dosing is present in Figure 3 - here you can see that the individual elements of the program should be selected in relation to each other because of the fact that too high accuracy can affect the readability of the chromatogram (G6 spectrum shown in Figure 3).

In programmes G6 and G9 it has already been possible to identify individual extraction compounds derived from poplar wood. Using the automatic identification of peaks in the G6 spectrum we obtain, among others, sterols (gamma-sitosterol, stigmastanol, stigmasterol), higher fatty acids (Oleic acid), terpenes (squalene). In the G9 spectrum, the automatic identification of peaks also identified lignin derivatives – (mainly vanillin and isovanillin, as well as 4-hydroxy-3,5-dimethoxy-benzaldehyde). Obviously, the use of manual identification of the peaks brings better results - you can reject compounds that are not the part of the wood extraction substances and identify the correct identification of the library. They assign the individual peaks to compounds that are in the first place of spectrum compatibility in the library. Unfortunately, the compounds derived from wood are often on the search list further on.

CONCLUSION

The result of chromatographic analysis of extracts from different poplar varieties. The analysis of extracts obtained during the determination of the amount of extraction substances of selected poplar varieties listed in point. Chromatograms allow of the identification of substances present in particular extracts as well as substances present in all poplar varieties were obtained. The best condition for the analysis of the extracts is to increase the temperature program to 50°C maintained for 7 minutes, increase 10°C/min to 320°C maintained for 10 minutes. The carrier gas used in the chromatography is helium 6.0, the flow was set at 0.8 ml/min. The sample was introduced directly into the column, the injection temperature was 250°C, and the detector voltage was set at 1.2 kV. The use of automatic peak identification in wood extracts is not recommended.

REFERENCES
1. BOGOLISTYN K.G. GUSAKOVA M.A., SLOBODA A.A., POKRYSHKIN S.A., 2014: *Chemical composition of extractives of normal and rotten aspen (Populus tremula) wood*, Russian Chemical Bulletin, 63, 9, 2169-2174
2. CHAUDHRY A.S., 2000, Rumen degradation in sacco in sacco in sheep of wheat straw treated with calcium oxide, sodium hydroxide and sodium hydroxide plus hydrogen peroxide, Animal Feed Science and Technology 83(3-4), 313-323
3. KRÓL P., TOŁOCZKO-MAMIŃSKA R., MAMIŃSKI M., 2017: A critical role for the presence of lignocellulosic material in the determination of wood buffering capacity, Journal of Wood Chemistry and Technology 37(6), 478-484

4. KUČEROVÁ V., VÝBOHOVÁ E., 2018, Release of saccharides during hot-water pretreatment of willow wood (*Salix Alba* L.), Cellulose Chemistry and Technology 52 (5-6), 381-386
5. KUČEROVÁ V., LAGAŇA R., HÝROŠOVÁ T., 2019, Changes in chemical and optical properties of silver fir (*Abies alba* L.) wood due to thermal treatment, Journal of Wood Science 65:21, 1-10
6. PAUL S., DUTTA A., 2018, Challenges and opportunities of lignocellulosic biomass for anaerobic digestion, Resources, Conservation and Recycling 130, 164-174
7. STACHOWIAK-WENCEK A., ZBOROWSKA M., WALISZEWSKA H., WALISZEWSKA B., 2019: Chemical changes in lignocellulosic biomass (Corncob) influenced by pretreatment and anaerobic digestion (AD), BioResources 14 (4), 8082-8099
8. SZADKOWSKA D., RADOMSKI A., DEREWIAKA D., LEWANDOWSKA A., DROŻDŻEK M., ZAWADZKI J., PIETRYKOWSKI M., ZIELENKIEWICZ T., 2016: Chromatographic analysis of extracts isolated from different poplar species as potential inhibitors of enzymatic hydrolysis, Przemysł Chemiczny, 95, 11, 2179-2182
9. SZADKOWSKI J., RADOMSKI A., ANTCZAK A., SZADKOWSKA D., LEWANDOWSKA A., MARCHWICKA M., KUPCZYK A., 2017, The yield of model hydrolysis and fermentation in the technology of bioethanol production from poplar wood (*Populus* sp.), Przemysł Chemiczny 96,3, 518-520

Streszczenie *Analiza chromatograficzna ekstraktów z drewna topoli (Populus sp.)- układanie programu GC-MS.* Celem pracy było opracowanie metodyki przeprowadzania analizy przy pomocy chromatografii gazowej cieczy pozyskanej po ekstrakcji za pomocą cykloheksanu drewna różnych odmian topoli (*Populus* sp.). Zastosowanie chromatografii gazowej z detektorem mas (GC-MS) umożliwia po zastosowaniu odpowiedniej metody na analizę składu chemicznego ekstraktów z różnych rodzajów biomasy lignocelulozowej oraz weryfikacje związków w niej występującej, a także porównanie zawartości poszczególnych związków w analizowanej próbce, co umożliwi określenie istotności wpływu danych substancji na procesy wytwarzania biopaliw w oparciu o materiały lignocelulozowe. Jednymi z kluczowych substancji chemicznych wpływających na proces hydrolizy enzymatycznej oraz fermentacji są substancje ekstrakcyjne zawarte w materiałach lignocelulozowych wykorzystywanych w biopaliwach 2 i 3 generacji. Związki te mogą inhibitować cały proces wytwarzania biopaliw z biomasy lignocelulozowej

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