

A GC-MS analysis of extracts from *Populus maximowiczii* extracted by supercritical CO₂ with other organic solvents

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Abstract: A GC-MS analysis of extracts from wood biomass *Populus maximowiczii* extracted by supercritical CO₂ with other organic solvents. The aim of this study was to determine chemical compounds extracted from *Populus maximowiczii* wood using supercritical CO₂. Due to the gaseous form of CO₂ at room conditions (pressure 1.013 hPa and temperature 20 °C), this extraction was carried out using other organic solvents with different polar properties (methanol, diethyl ether, cyclohexane). The results indicate that different amounts of extracted compounds are obtained depending on the polar properties of the solvent used. The fundamental solvent, supercritical carbon dioxide, is non-polar due to the molecule's symmetry, so an additive with higher polarity was used. The most compounds were observed in the supercritical CO₂ – diethyl ether extract. The least compounds were observed in the supercritical CO₂ – methanol extract. No detectable quantities of phenolic derivatives such as vanillin or syringaldehyde were detected, while large terpene compounds were detected.

Keywords: extractives, wood, poplar, supercritical CO₂, GC-MS

INTRODUCTION

The world economy depends on several factors such as market, labor force, and the availability of raw materials or energy. Imposing restrictions on greenhouse gas emissions and limiting the extraction of conventional fuels can lead to limitations on the resources required for the global economy. The most important aspect of the global economy, besides access to energy – which is directly affected by the reduction of greenhouse gas production – is the availability of raw materials used in industrial chemical synthesis. These processes make it possible to obtain industrially needed chemical compounds that are difficult to obtain from other sources.

The development of chemical synthesis initiated in 1904-1908 by Haber and Nernst enabled the development of the technology for the chemical synthesis of ammonia in 1913 (Kępiński, 1964, Kostick, 2018). This work contributed to the development of industrial syntheses of compounds such as methanol, the polymerization of ethylene, and the production of synthetic liquid fuels (Kępiński, 1964, Licker, 2003, Skolnik, 1982). Currently, synthesis gas obtained from natural gas is used for chemical synthesis. This raw material, like other fossil fuels, is to be restricted in its use according to the EU RED II directive (Dz. U. UE. L. 328/82). Alternatives to traditional synthesis gas production may include using lignocellulosic biomass to produce synthesis gas or using waste materials generated during biomass pretreatment.

Pretreatment of lignocellulose is one of the intensively developing aspects of its processing to second-generation biofuels (Tutt et al., 2014). As enzymatic hydrolysis is (nowadays) the main way of depolymerizing polysaccharides, pretreatment should make this process easier. One of the more interesting pretreatments that can provide chemicals to other industries is the so-called "supercritical CO₂" (Figure 1) expansion – during which,

it is used as a treatment for LCC materials in biofuel technology. It involves the creation of a supercritical fluid that interacts with biomass under elevated pressure. The additional use of a different solvent enables the extraction of associated substances from the biomass, reducing their impact on subsequent processes. CO₂, due to its size comparable to H₂O molecules, can penetrate the structure of the biomass, leading to a mild hydrolysis process of polysaccharides with a lower degree of polymerization and, thus, improving the availability of the material for enzymatic treatment. When exposed to supercritical CO₂ on wet biomass, it leads to a reduction in lignin content (Alvira et al., 2010, Tomás-Pejó et al., 2011, Sun et al., 2016, Wilk and Krzywonos, 2015).

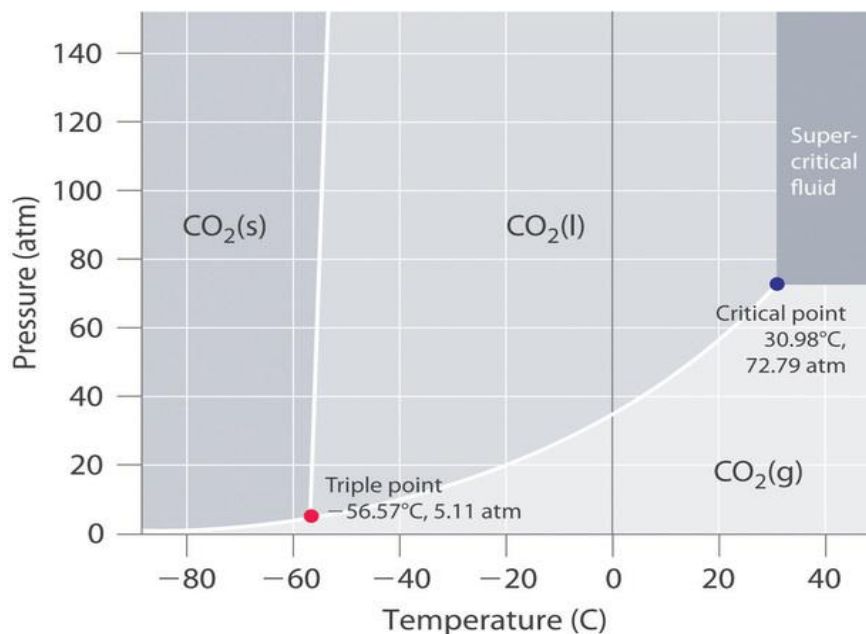


Figure 1. CO₂ phase diagram (temperature, pressure) with the triple point, the critical point, indicated (Brown et al., 2020).

Pretreatment of LCC materials by supercritical CO₂ expansion is much more cost-effective than the steam explosion method and AFEX. In addition, this treatment can use CO₂ from the alcoholic fermentation process or capture it from the atmosphere with special filters to reduce the balance of greenhouse gas emissions and make the process more environmentally friendly.

This is due to the use of much lower temperatures and the possibility of extracting CO₂ generated from the biomass fermentation process. The problem when using this method is the high pressure needed to create a supercritical state for CO₂, which is 7.38 MPa (Alvira et al., 2010, Tomás-Pejó et al., 2011, Sun et al., 2016). The use of supercritical CO₂ is much safer and requires fewer resources than the use of supercritical water, for example (Figure 2).

Obtaining supercritical conditions for this gas is easy – much easier than for water. Supercritical conditions for water: a temperature of 300 °C and a pressure of 22 MPa, while for carbon dioxide, 300 °C and 7 MPa. Figure 1 presents a phase diagram of carbon dioxide – the point of supercritical conditions, and Figure 2 presents a phase diagram of water – the point of supercritical conditions. The extraction abilities of carbon dioxide are the additional advantage of such a process performance (Santos et al., 2016, Feng and Meier, 2016). Carbon dioxide is believed to be able to extract different types

of extractive substances depending on the parameters of extraction and different solvent addition. It may be useful to determine which group of extractives acts like enzymatic hydrolysis inhibitors. Inhibiting the behavior of these compounds is described in the literature (Chandel et al., 2012, Jönsson et al., 2013).

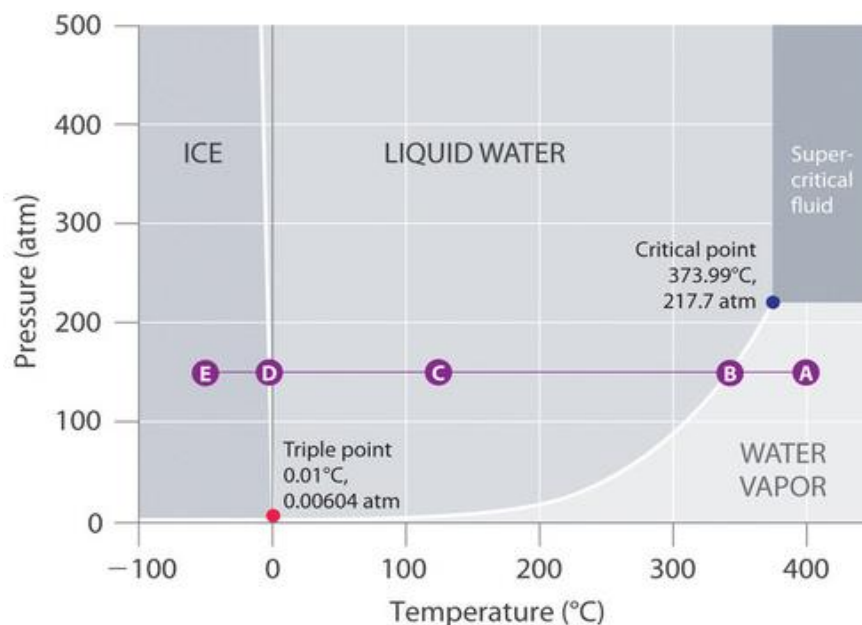


Figure 2. H₂O phase diagram (temperature, pressure) with the triple point, the critical point, indicated (Brown et al., 2020).

The necessity of using additional solvents during extraction with supercritical CO₂ results from the fact that this compound is in the gas phase under normal conditions (1013 hPa and about 20°C). As a result, without adding a solvent normally in liquid form, the compounds dissolved and extracted by supercritical CO₂ will re-deposit on the wood chip structure after expansion. Preventing them from being collected from the biofuel production process. The solvents used should be readily miscible with supercritical CO₂ and, under process conditions, be in a gaseous form that allows better penetration through the material being treated.

Table 1. Extractives in poplar species

Solvent	Extractives						
	Mineral salts	Sugars	Fats, waxes	Steroles	Tanins	Another phenolic substances	Therpens
Water	++	++	-	-	+	+/-	-
Ethyl alcohol	+/-	-	+	+	+	+	+
Cyclohexane	-	-	++	+	-	+/-	++

Extractives in poplar wood, which is the object of interest in this paper, may be divided into the following kinds, as shown in Table 1, with the solvents to be used.

This work aims to develop the method of practical extraction of poplar wood with carbon dioxide in laboratory conditions.

MATERIALS

Poplar species *Populus maximowiczii* was obtained as a result of cooperation within research projects from the experimental plantation of the Department of Plant Genetics, Breeding and Biotechnology of the Faculty of Horticulture, Biotechnology and Landscape Architecture of the Warsaw University of Life Sciences located in Wolica (52 ° 08'42 "N 21 ° 04'07 "E). The wood was obtained from poplars felled at the end of the vegetation period after three years of planting. Before shredding, the raw material was subjected to selection in order to reject the material with defects. The material obtained in this way was dried, debarked and then shredded on a laboratory mill (wood chips fraction between 0.42 -1.00 mm.). These are species of great potential in second-generation biofuel processing. A liquid chromatograph was the device in which the experiment took place. A standard column was used to treat wood dust with supercritical carbon dioxide. The method of sample preparation is presented in Figure 3. A sample of wood dust (1 g) was placed in the column, and dry ice pieces filled the rest of the column volume.

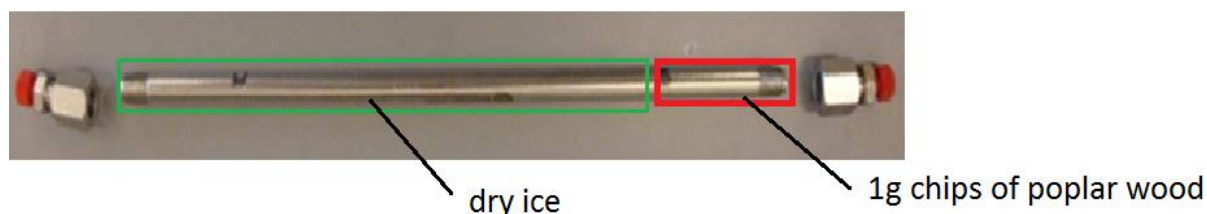


Figure 3. The way of column filling with wood dust and dry ice.

Then, the column was placed in the liquid chromatograph (Shimadzu) to control the temperature, pressure and additional solvent volume – which was methanol, diethyl ether and cyclohexane. The temperature was 40 °C, and pressure varied from 75 to 90 bar – dependent on dry ice mass, which was unable to be the same each time. Additional solvent (1 cm³) was poured inside after obtaining the critical conditions and, then, extraction ran for half an hour. The column was then decompressed and poured out with the solvent. The solvent sample (with extractives) was then analyzed with Shimadzu GC-MS apparatus, starting from 50 °C, running until 230 °C was reached with the heating rate of 10 °C/min and the helium flow of 3.7 cm³/min (column Zebron Z1). NIST11 and NIST11b libraries were used in recognition of chromatographic spectra.

RESULTS

The results obtained with the GC-MS method for *Populus maximowiczii* are presented in Figure 4. Diethyl ether dissolves the highest amount of extractives; some are not present in the case of methanol and cyclohexane.

In extracts extracted with methanol, only three compounds with very high retention times were identified: lup-20(29)-en-3-one (36.846 min), 24 methylenecycloartan-3-one (37.346 min) and stigmast-4-en-3-one (37.467 min), which are terpenoids. In extracts extracted with chloroform, the presence of more compounds was detected, but most of them were hydrocarbons and their derivatives, whose MS spectra are not very characteristic and practically do not allow reliable identification.

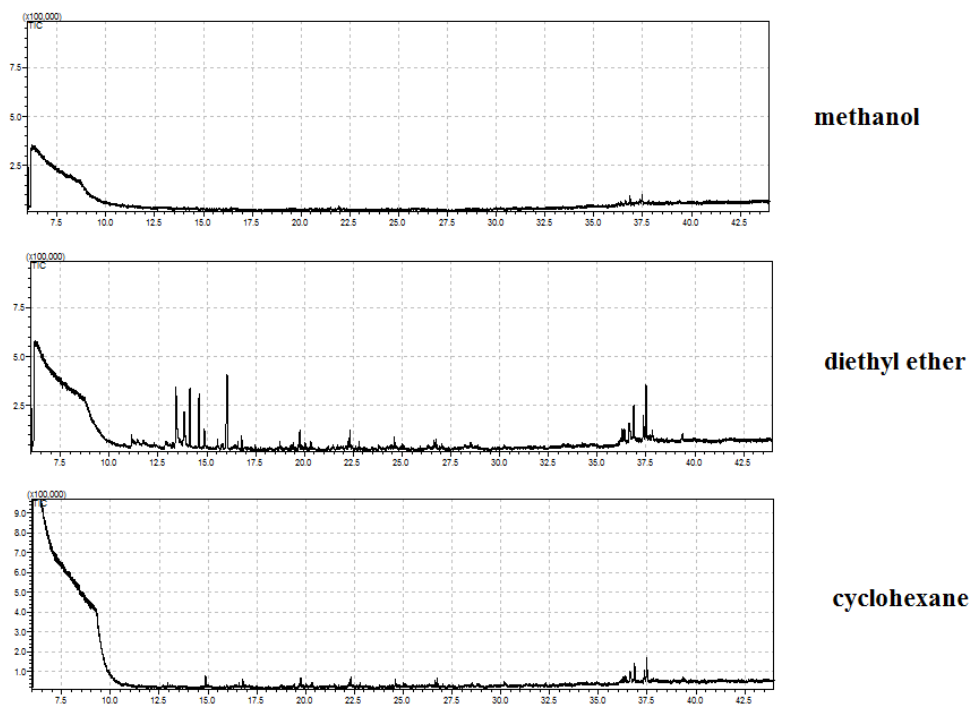


Figure 4. Chromatograms obtained for *Populus maximowiczii* extractives in different solvents after supercritical carbon dioxide application.

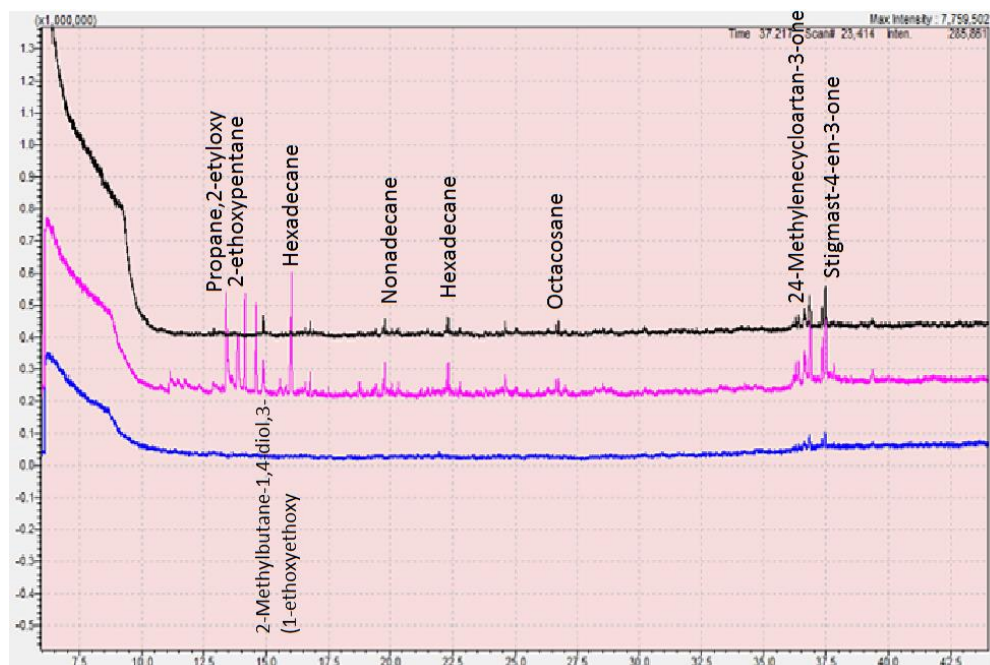


Figure 5. Chromatograms obtained for *Populus maximowiczii* extractives in different solvents after supercritical carbon dioxide application.

Four terpenoids were successfully identified: lup-20(29)-en-3-one (36.617 min), 3-methoxy-stigmasta-5,22-diene (36.856 min), 24-methylenecycloartan-3-one (37.360 min) and stigmast-4-en-3-one (37.485 min). The last two of these compounds were identified from extracts with methanol. When extracted with the least polar additive (i.e., diethyl ether), the highest number of compounds were found, but were mostly difficult to identify hydrocarbon homologues.

Eight terpenoids were identified: lanost-8,24-dien-3-one (36.278 min), two β -amirinone derivatives (36.395 min and 36.871 min), lanost-7-en-3-one (36.627 min), 24-methylenecycloartan-3-one (37.369 min), stigmast-4-en-3-one (37.499 min), stigmast-3,5-dien-7-one (37.827 min) and stigmastane-3,6-dione (39.368 min).

This leads to the conclusion that significantly different extractive substances may be found in *Populus maximowiczii* or different substances are extractable from the wood of this species. Substances identified with the MS detector in the mixture of extractives from *Populus maximowiczii* are shown in Figure 5. These are typical substances present in the extract obtained with the classic method in the Soxhlet apparatus.

The identification is made plausible by a literature search, confirming the presence in the poplar biomass of terpenoids from the β -amirine group (Barnes et al., 2007) and lanostenes (Fernandez, 1999; Fernandez et al., 2011). Abramovitch and Micetich (1963) also report among the extractive substances in *P. tremuloides*, in addition to the two groups mentioned, methylenecycloartanone. The presence of stigmastadiene derivatives is so common and documented that they are proposed as markers of hardwood burning (Goncalves, 2011).

CONCLUSIONS

1. The presented method allows the demonstration of compounds extracted with supercritical CO₂.
2. Supercritical CO₂ allows non-polar compounds to be extracted from lignocellulosic biomass.
3. The presented extraction process with supercritical CO₂ is relatively simple and not complicated.
4. There is a need for further work to verify the compounds extracted with carbon dioxide from other species of lignocellulosic biomass.
5. The results obtained suggest that solvents other than those proposed in the study should be tested.
6. Extraction by supercritical CO₂ with the addition of other solvents allows the selective extraction of groups of compounds from woody biomass.

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Streszczenie: Analiza GC-MS substancji wyekstrahowanych za pomocą nadkrytycznego CO₂ z udziałem innych rozpuszczalników organicznych z biomasy drzewnej *Populus maximowiczii*. Celem pracy było określenie związków chemicznych wyekstrahowanych z drewna *Populus maximowiczii* przy użyciu nadkrytycznego CO₂. Ze względu na gazową postać CO₂ w warunkach pokojowych (ciśnienie 1013 hPa i temperatura 20 °C), ekstrakcję tę prowadzono z wykorzystaniem innych rozpuszczalników organicznych o różnych właściwościach polarnych (metanol, eter dietylowy, cykloheksan). Uzyskane wyniki wskazują, że w zależności od właściwości polarnych użytego rozpuszczalnika uzyskuje się różne ilości ekstrahowanych związków. Podstawowy rozpuszczalnik, CO₂ w stanie nadkrytycznym, jest niepolarny ze względu na symetrię cząsteczki, dlatego zastosowano dodatek o większej polarności. Najwięcej związków zaobserwowano w ekstrakcie z nadkrytycznego CO₂- eter dietylowy. Najmniej związków zaobserwowano w ekstrakcie nadkrytycznym CO₂ - metanolem. Nie znaleziono wykrywalnych ilości pochodnych fenolowych takich jak, wanilina, syringaldehyd, natomiast natrafiono na duże ilości związków terpenowych.

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