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Determination of optimum conditions for the analysis of volatile components in pine needles by double-shot pyrolysis–gas chromatography–mass spectrometry

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Abstract

The optimum conditions for the analysis of the volatile organic components of pine needles from *Pinus densiflora* using double-shot pyrolysis–gas chromatography–mass spectrometry (DSP–GC–MS) were investigated with respect to thermal desorption temperature and duration of heating. A total of 41 compounds were identified using thermal desorption temperatures of 150 °C, 200 °C, 250 °C and 300 °C. Thermal decomposition products, which include acetol, acetic acid, furfurals and phenols, were observed only at thermal desorption temperatures exceeding 250 °C: they were not observed in the extract from a simultaneous distillation extraction (SDE) method. Heating times of 1 s, 6 s, 30 s, 150 s and 300 s were investigated at the thermal desorption temperature of 200 °C, whence it was found that thermal decomposition products were produced only at heating times over 30 s. The optimum pyrolyzer conditions for the analysis of pine needles using DSP–GC–MS is 200 °C for 6 s. Under these conditions, this method gives comparable results to the SDE method.

Keywords: Pine needle; Thermal desorption; Double-shot pyrolyzer; Volatile components

1. Introduction

Pine trees represent the most widely used species of tree in Korea. *Pinus densiflora* S. is common throughout Korea: it is known in the west as Japanese red pine [1]. Pine needles have long been valued for their medical effects and have been used in popular medicines for the treatment of hepatosis, various neurological disorders, and arteriosclerosis [2]. They are also valued for their flavouring properties: the essential oil of pine needles has found wide commercial use and is a constituent of certain beverages, cookies, detergents, cosmetics, amongst others [3,4]. In recent years, there has been an increase in the need for natural flavourings, following increases in the demand for natural products, as opposed to nature-identical or synthetic products. It is likely that this trend will continue for the time being. In response to this situation, there have been some studies on the volatile ingredients of pine needles. Hong et al. [5] have analyzed the volatile organic components of *Pinus rigida* needles using steam distillation and solvent extraction. Woo et al. [6] reported a difference in the composition of volatile ingredients of pine twigs from *P. densiflora* S. using of supercritical fluid extraction and steam distillation. In addition, Roussis et al. [7] analyzed the volatile components of five Greek varieties of pine needles and found large differences between the varieties. More recently, Yu et al. [8] have analyzed the volatile organic compounds in the pine needles of *P. densiflora* S. using SDE and headspace solid-phase microextraction (HS-SPME) and Stevanovic et al. [9] have analyzed the essential oil of the needles and twigs of the dwarf pine *Pinus mugo* Turra.

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Previously, steam distillation methods have been widely used to extract volatile ingredients from plant material. However, solid-phase microextraction (SPME) [10] and headspace [11] methods are more commonly used at present. Steam distillation has certain advantages, such as the use of small amounts of solvent, but it also has certain drawbacks, such as major changes in composition resulting from thermal decomposition due to the necessary maintenance of high temperatures during the extraction process [12]. SPME has been preferred to headspace methods since it is simple and can be used on a small sample without any organic solvents. However, the efficiency of this method has been found to be much lower than that of headspace methods [13,14].

Thermal desorption methods, which use a directly connected gas chromatograph–mass spectrometer (GC–MS), can analyze volatile compounds using small samples (lower than 0.01 g) and is economical, due to the ability to treat many samples in a short time. For example, Sanz et al. [15] analyzed volatile compounds by injecting them into the GC–MS after collecting them in a Tenax TA and desorbing *Lavandula luisier* L. at 320 °C. However, it has been shown that the composition of volatile components of a given sample varies with both the thermal desorption temperature and the heating time, and hence requires careful selection of optimum conditions. Moreover, thermal decompositions of certain components occur at higher temperatures or over prolonged heating times [16,17,18]. González-Vila et al. [18] reported that there were large differences in the volatile compound composition with regard to heating time, when Rye grass (*Lolium rigidum*) was heated at 350 °C using a curie-point pyrolyzer and GC–MS.

This study presents the results of an investigation to find the optimum conditions for the analysis of the volatile organic components of pine needles, using a double-shot pyrolyzer GC–MS set up, by varying the thermal desorption temperature and heating time. The double-shot pyrolyzer is a type of furnace where the range of temperature setting is wide compared with the more usual curie-point pyrolyzer.

2. Experimental

2.1. Plant material and reagents

P. densiflora pine needles were collected from mountains near Daejeon, South Korea in August 2004, stored in solvent-cleaned glass jars with aluminium foil-lined lids and were refrigerated at 3 °C in the laboratory until required for analysis. Pine needles were cut to 2 mm lengths immediately before use. All organic solvents were of analytical grade and were purchased from Sigma.

2.2. Thermal desorption using a double-shot pyrolyzer

Volatile fractionation was carried out by using a double-shot pyrolyzer 2020iD (Frontier Lab, Japan), which was connected directly to the injector of the GC. The Pyrolyzer was composed of a plunger for the sample, the sample cup, a deactivated needle (into the GC injector) and a furnace. Helium (high purity, 99.99%) was used both as the GC carrier gas and as the inert atmosphere for thermal desorption.

Pine needles (10 mg) and internal standard *n*-decanol (0.3 μl of a 0.45 mg ml^{-1} ethanol solution) were introduced into the sample cup, which was then placed in the furnace. In order to evaporate the solvent (ethanol) before commencing the thermal desorption, the system was purged for a short time (30 s) with the carrier gas. After purging, the sample cup was heated, whence the volatile organic components were transferred from the furnace to GC–MS without significant loss.

Experiments were carried out for 6 s (heating time) on separate samples at temperatures of 150 °C, 200 °C, 250 °C and 300 °C, respectively), in triplicate.

Also, to investigate the best heating time at the thermal desorption temperature of 200 °C, thermal desorptions were carried out for 1 s, 6 s, 30 s, 150 s, 300 s, respectively.

2.3. SDE

Pine needles (60 g), distilled water (500 ml), and *n*-decanol internal standard (1 g of a 0.45 mg ml^{-1} ethanol solution) were placed in a 2 l round-bottom flask. Diethyl ether (30 ml) and pentane (30 ml) were placed in a 100 ml round-bottom flask, and the two flasks were connected to the modified Likens-Nickerson micro SDE apparatus [19]. The extraction was performed for 2 h, during which time chilled water was circulated through the cold finger condenser. The fractions in the solvent flask were dried with anhydrous sodium sulfate, filtered and then concentrated by blowing with nitrogen. Three replications of the extraction and analysis procedure were performed for each of the samples.

2.4. GC–MS analysis

The GC–MS equipment consisted of an Agilent 6890 gas chromatograph equipped with an Innowax capillary column (50 m, 0.25 mm i.d., 0.25 μm film; polyethylene glycol as stationary phase). The double-shot pyrolyzer was directly connected to the GC injector, which was maintained at 230 °C, with a 1:100 split ratio at the initial time. The detector consisted of an Agilent 5973 mass selective detector operating in the scan modus. Mass spectra were recorded in the electron impact (EI) mode at 70 eV, scanning the *m*/*z* 30–500 range. Interface and source temperature were 250 °C and 230 °C, respectively. The carrier gas used was helium with a controlled flow of 1.0 ml min^{-1}. The GC oven temperature was programmed from 50 °C (3 min) to 220 °C (20 min) by increasing the temperature at the rate of 2 °C min^{-1}.

2.5. Qualitative and quantitative analysis

The identification of the separated volatile organic compounds was achieved through retention times (retention indices; RI) and mass spectrometry by the comparing mass