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Effect of altitude on the composition of suberin monomers in the outer bark of Scots pine (*Pinus sylvestris* L.)

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ABSTRACT

The changes in the amount and composition of suberin monomers of Scots pine outer bark, which is grown natively in Turkey, was investigated as a function of growing altitude. Samples were taken from every 100 m to 1300 m which was the highest point of the sampling area. While the total amount of suberin monomers is varied within the range 17.56–47.20 mg/g, there was no correlation between total amount and altitude. Total amount of alkanols of suberin monomers decreased when the altitude increased. Alcohol 24:0 was seen as the dominant constituent and its amount decreased from 100 m to 1300 m. There was not a clear change in the total amount of alkanoic, dioic and hydroxy acids. Acid 1,18-hydroxy-18:1 and acid 1,18-dioic-18:1 were determined as the main compounds in all samples.

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1. Introduction

Suberin is a nonextractable, complex and natural lipidic biopolymer found in the cell walls of normal and wounded external tissues of aerial and subterranean organs of plants. In higher plants, it is one of the main components with an important role of thermal and hydric insulation in the outer bark cells (Holloway, 1983; Sakai, 2001; Gandini et al., 2006).

This renewable resource is gaining dramatically interest in the last few years as the implementation of biorefineries in agroforest-activities. Scientists pointed suberin as a valuable precursor to novel macromolecular material. Its carboxylic and hydroxy groups and of side hydroxy and epoxy moieties on long chain monomers make them particularly suited as building blocks for polymers and surfactant (Gandini et al., 2006). Also, it is a potential carbon source for cultivation of microorganisms or microbiological transformation in food technology (Norin, 1971).

Chemical composition of suberin varies not only within the species but also in the same species due to the geographical origin and the status of the trees (García-Vallejo et al., 1997). Owing to these factors, the effect of altitude on the composition of suberin monomers was studied in the present paper. As known, temperature and air-pressure decreases with the increase of altitude. Thus, one can expect some changes in the chemical composition of the tree bark.

2. Materials and methods

2.1. Materials

Scots pine is one of the rare species able to grow both at sea-level and at higher altitudes so it has been chosen as a study material. The samples were taken from Ayancik, Sinop, Turkey. Scots pine (*Pinus sylvestris* L.) can grow up at 1300 m altitude in this region. Sampling was done between healthy trees, having approximately same diameter and height, at the northern side of the mountain where growing was better. As there was no Scots pine at sea level (0 m altitude), samples were obtained from every 100 m between 100 m and 1300 m altitudes. Data of the collected samples can be seen in Table 1.

After trees were fallen, cross-sections were removed first from the bottom (0.50 m), second from the middle of the tree and third from upper part of the stem, and bark was separated from the samples. The bark was also separated into inner and outer bark. All of the samples were stored at −24 °C until analyses. After breaking into small pieces, outer barks were freeze-dried and grounded by Willey mill to 1 mm (Ekman, 1983; García-Vallejo et al., 1997). A second drying procedure was treated to the grounded outer bark prior to extraction to remove all the volatile compounds.

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Table 1
Data of the collected samples.

Altitude (m)	Height (m)	Diameter ($d_{0.50}$ cm)	Annual ring
118	21.5	22.0	24
205	20.5	25.5	27
319	21.0	25.0	26
427	22.5	21.0	23
526	19.5	21.5	25
632	20.5	19.0	23
701	20.0	21.0	24
822	19.0	18.0	20
914	19.5	16.0	20
1031	21.0	23.0	26
1095	21.5	24.5	25
1190	23.0	22.0	21
1296	22.0	18.5	20

2.2. Extraction, hydrolysis and identification

Approximately 4 g of grounded outer bark sample from each altitude was extracted in an ASE apparatus (Accelerated Solvent Extractor, ASE 200, Dionex Inc.) first with *n*-hexane and then acetone:water (95:5, v:v) mixture to remove extractives (Willför et al., 2003; Kilic et al., 2011).

A certain amount of cholesterol, used as an internal standard, was dissolved in acetone and 1 ml of the solution was put in a 50-ml test tube. The solvent was evaporated in a water-bath under nitrogen. Extractive-free, grounded 100 mg outer bark and 10 ml 0.5 M KOH in 90% ethanol were added to the same tube. Hydrolysis was performed at 70 °C for 1.5 h with continuous stirring. After hydrolysis was completed 1 ml was taken from liquid phase and put into a 15-ml test tube. Then 1 ml distilled water and 2–3 drops of bromocresol green were added. To get pH 3.5, 2–3 drops of 0.25 M H_2SO_4 were added. Then 2 ml of MTBE (methyl *tert* buthyl ether) was added into the tubes and shaken tightly thereafter MTBE phase was moved to another test tube. This phase was evaporated in a water bath under nitrogen prior to silylation (Ekman, 1983).

Quantitative analyses of suberin monomers from the outer bark of Scots pine were performed with a Pelkin Elmer Autosystem XL gas chromatograph equipped with HP-1 (J&W), 25 m × 0.2 mm (0.11 μm film thickness) column and flame ionization detector (FID), carrier gas was H_2 at 0.8 ml/min, temperature program was 120 °C raised by steps of 6 °C/min to 320 °C. Injector temperature was 260 °C and FID temperature was 320 °C. 1 μl of the aliquot was injected to the GC (split ratio: 1:24). To identify individual compounds HP 6890-5973 gas chromatography/mass spectrometry instrument equipped with HP-1 column was used. The temperature program was the same as above.

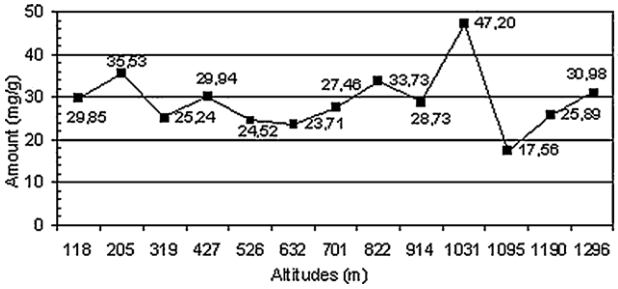

Fig. 1. Total amount of suberin monomers (mg/g).

3. Results and discussion

Effect of altitude on the total amount and the composition of suberin monomers from the outer bark of Scots pine were studied. As seen in Fig. 1, in various altitudes, total amount of suberin monomers was changed with in the range of 17.56–47.2 mg/g. The amount of suberin monomers for different coniferous species was previously reported as 16.4 mg/g in Scots pine, 18.0 mg/g in Norway spruce and 25–30 mg/g in cedar (Ekman and Reunanen, 1983; Hafızoğlu and Reunanen, 1987).

The composition of suberin monomers is given in Table 2. Identified dioic and hydroxy acids were the main group of the components followed by alkanoic acids. Sitosterol, catechin and ferulic acid were found to minor compounds in the outer bark of Scots pine. Mass chromatogram of the suberin monomers is given in Fig. 2.

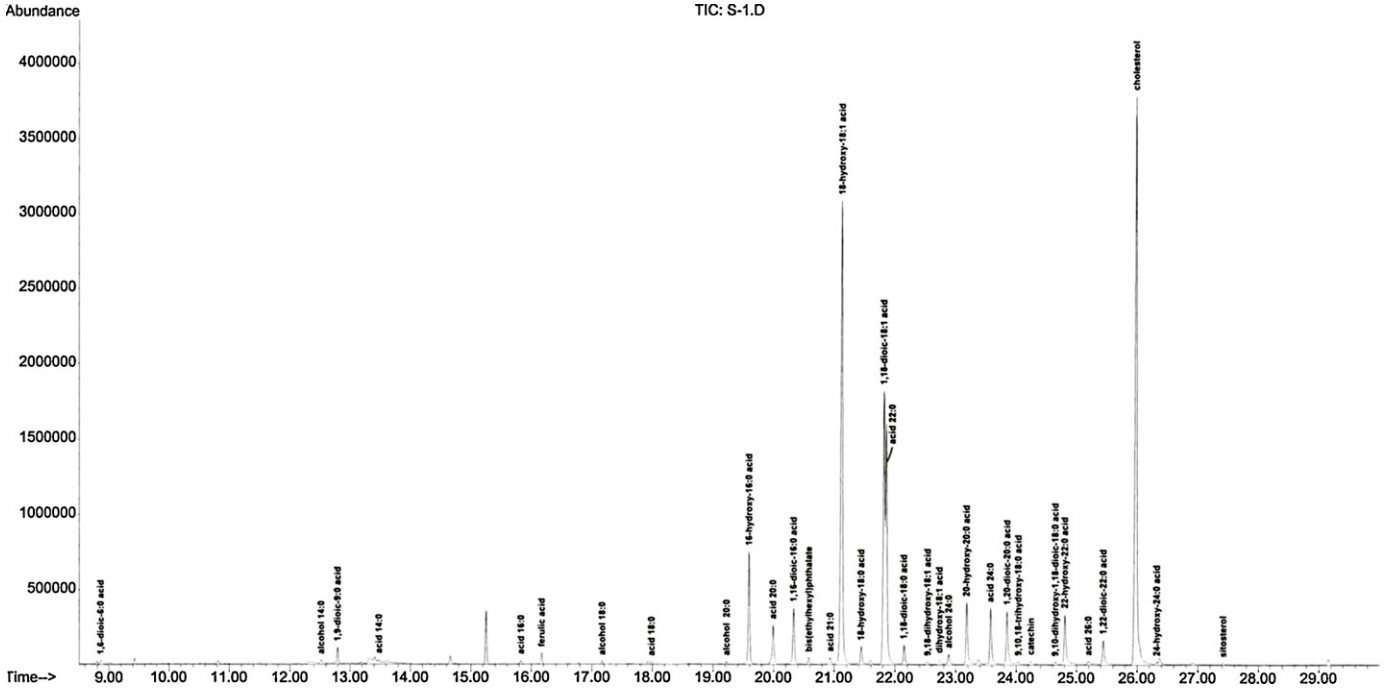

Fig. 2. Mass chromatogram of the suberin monomers of Scots pine outer bark.

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