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Research Paper

Preconditions for industrial use of foliage as felling by-product of Scots pine for essential oil production

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A R T I C L E I N F O

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A B S T R A C T

The aim of the study was to analyse some preconditions for the industrial production of Scots pine (*Pinus sylvestris* L.) essential oils including effects of storage temperatures of harvested foliage on the percentages of essential oils, α- and β-pinenes, and an estimate of potential resources of the foliage obtained from by-products of forest felling. The hypothesis was tested whether uncommon winter temperatures could provide favourable storage conditions of pine foliage to produce essential oils without a loss of yield. The study revealed that neither of the tested storage conditions of pine foliage (one month at +4 °C, and one month at −24 °C) had adverse effects on the total yield of essential oils if compared to the freshly collected foliage. The essential oil yield varied within 0.43–0.64%, showing the highest amount after storage at +4 °C. Percentages of α and β isomers of pinene, the main compound of the essential oils, did not change significantly after the storage at both temperatures. Independently of the storage conditions, the (1R)-(+)-α-pinene was more prevalent than (1S)-(−)-α-pinene: the chiral analysis showed that (+) enantiomer was 3.1–5.8 times more abundant than (−) enantiomer. The tested storage temperatures span over the common temperatures of winter time in the region, when forest felling is being performed, and occur more notably with the climate change. Nevertheless, this provide favourable preconditions to produce essential oils without a loss from a pine foliage which could be obtained in large amounts from by-products of forest felling. The equations developed by different authors for the estimation of pine foliage biomass in the study area were used, and it was estimated that a mature 1-m^3-stem-volume pine tree produces at least 10 kg of fresh foliage biomass. Considering that the rate of the final felling of mature pinewoods in Lithuania remains at the current level of 700,000 m^3 of stem wood a year, the total estimated harvest of foliage is 7000 t. This amount of foliage contains at least 35 t of essential oils or 17.4 kg of essential oils per hectare of mature pinewood on average. The resources of such magnitude are available on a yearly basis.

1. Introduction

In recent years, the use of biomass as a renewable source of energy has been significantly encouraged in the European Union. The Renewable Energy Directive (Directive 2009/28/EC) has played a decisive role in this field and gave an impetus for the biomass-targeted activities at the national level. In the result, forest felling by-products, such as branches and tops of trees, are more thoroughly collected, although utilized predominantly for fuel. Meanwhile, vast amounts of felling by-products collected from different tree species allow for the diversification of biomass usage. The use of foliage of Scots pine (*Pinus sylvestris* L.) for the essential oil production is among the most potential ones. Within its natural distribution range, *P. sylvestris* often forms pure stands, meaning that its resources are spatially concentrated, which is very important for the industrial use of the biomass. Also, the latest developments of machinery used in forest felling facilitate collecting, packaging and transportation of nearly all felling by-products.

A global demand of natural essential oils as a source of natural perfumes and cosmetics is constantly growing. The total world fragrance and flavour market was estimated for 2016 to be US $ 25.25 billion, and showed an increase of 10.26% if compared to 2012 (Leffingwell & Associates, 2017). In Lithuania, the import of essential oils, resinoids, perfume and related materials in 2016 amounted to € 416.15 million, and showed an increase of 83.76% if compared to 2012 (Statistics Lithuania, 2017). Meanwhile, the country has a plethora of untapped natural resources for the conifer essential oil production as its forests are dominated by two conifer species, Scots pine and Norway spruce, occupying 56% of the total forest area (State Forest Service, 2015). Moreover, an increasing potential for forest growth has been reported as one of the key impacts of projected climate change for the

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whole boreal region in Europe (European Environment Agency, 2017). Among the main bioactive compounds of pine essential oils are monoterpenes α- and β-pinenes, widely recognized as antimicrobial (antifungal, antibacterial, antiviral) substances (Bakkali et al., 2008; Kozioł et al., 2014; Krauze-Baranowska et al., 2002; da Silva et al., 2012), as well as possessing antioxidative, anticancer, and genotoxic properties (Aydin et al., 2013); both monoterpenes naturally exist in two enantiomeric forms: dextrorotatory (1R)-(+)-form and/or as levorotatory (1S)-(−)-form. The pinenes are also used as industrial solvents (Schmidt, 2010).

This report aims at the analysis of some preconditions for the industrial production of Scots pine (*Pinus sylvestris* L.) essential oils, such as effects of storage temperatures of winter-harvested foliage on the contents of essential oils, as forest felling is being performed predominantly in winter, and an estimate of potential resources of foliage obtained from by-products of forest felling. Climate change projections for the boreal region in Europe suggest that there will be a larger than average temperature increase, in particular in winter (European Environment Agency, 2017). Therefore, and because of a lack of the research data on the effects of storage temperatures, a hypothesis is tested in this study whether uncommon winter temperatures (+4 °C and −24 °C), likely conditioned by the ongoing climate change, are in favour with the yield of essential oils in pine foliage stored for a reasonable time (one month).

2. Materials and methods

2.1. Plant material sampling and preparation

To avoid influence of needle age on essential oil contents, the annual shoots of *P. sylvestris* were sampled. Five individual trees were used for the material sampling. The trees were located 20–30 m apart from each other in a pure pine stand, Vilnius Forestry Enterprise, East Lithuania; WGS coordinates 54.766847 N and 25.311983 E. Average stand characteristics: stem diameter at the breast height (1.3 m from ground level) 33 cm, height 27.5 m and age 106 years-old (State Forest Service, 2017). Sampling was made from the lower part of the crown at a height of 2–4 m, exposed to the forest opening, in midwinter, January 11, 2017. The local temperature during collection was −8 °C, while the average low temperature recorded in Vilnius in winter 2016–2017 was −5.3 °C and the average high −0.6 °C (AccuWeather.com, 2017). The collected annual shoots of each tree were divided into three, approximately equal parts: the first part was taken for the laboratory analysis the next workday after sampling (fresh), while, the second and third parts were put to storage for one month (30 days) in open plastic bags at +4 °C in refrigerator and at −24 °C in freezer (refrigerator-freezer model Whirlpool WSC5511A + NX), respectively, before the analysis. To aid interpretation and repeatability of the experiment, the biomass of each of two botanical fractions, needles and defoliated twigs, was estimated in each part of the plant material by weighting it just before the analysis.

2.2. Evaluation of water content in needles

For measuring of water content in fresh needles and needles stored at different temperatures for one month, 2 g of needles were dried for 5 h at +104 °C in drying oven. Percent of water content was calculated by the formula $W = (m_o - m_d/m_o) \cdot 100$, where W is water content of a sample (%), m_o − mass of original sample (g), and m_d − mass of dried sample (g), in 3 replicates.

2.3. Isolation of essential oils

Essential oils were isolated from needles by hydrodistillation in the Clevenger apparatus during two hours (European Pharmacopoeia, 2008). The isolation of essential oils was carried out from 70 g of fresh (i.e., not dried) needles, separated from twigs of each individual tree in 3 replicates in each of the three variants (freshly collected and after storage at two different temperatures); essential oils were stored in dark phials at −24 °C until further analysis.

2.4. Analysis of pinene isomers by GC-FID and their enantiomers by chiral-phase capillary GC

Essential oils solutions of 1% were prepared in mixture of diethyl ether and n-pentane (1:1) for further investigations. The analysis of monoterpene pinene isomers was carried out using a FOCUS GC (Thermo Scientific) gas chromatograph with a flame ionisation detector (FID). Data were processed with the CHROM-CARD S/W. The silica capillary column TR-5 (30 m, i. d. 0.25 mm, film thickness 0.25 μm) was used for the analysis of the α- and β-pinene with the following GC parameters: carrier gas helium flow rate 1.6 mL/min; temperature programme from 40 °C to 250 °C increasing at 4 °C/min; detector temperature 260 °C; splitless mode injection was used, split injector was heated at 250 °C, injection volume 1 μL. The identification of α- and β-pinene was carried out by the comparison of the retention time (RT) of their GC peaks in the FID chromatograms with the RT of α- and β-pinene analytical standards (Sigma-Aldrich). Retention times of α- and β-pinene analytical standards were 9.31 min and 10.82 min, respectively; RT of α- and β-pinene in investigated essential oils were 9.27–9.30 min and 10.79–10.82 min, respectively. The percentages of pinene isomers were recalculated according to the areas of the FID chromatographic peaks assuming that all constituents of the essential oil comprise 100%.

α-Pinene enantiomers were separated on HP − Chiral − 20 B column (30 m length, 0.249 mm id, 0.25 μm film thickness) with helium as carrier gas. The following GC parameters were used for the analysis of the α-pinene enantiomers: helium flow rate of 1.6 mL/min; temperature programme from 85 to 160 °C increasing at 5 °C/min; detector temperature 260 °C; splitless mode injection was used, split injector was heated at 250 °C, injection volume 1 μL. The identification of (1R)-(+)-α-pinene and (1S)-(−)-α-pinene was carried out by the comparison of the retention time (RT) of its GC peak in FID chromatograms with the RT of (1R)-(+)-α-pinene and (1S)-(−)-α-pinene analytical terpene standard (Sigma-Aldrich; purity (GC area%) ≥98.5% and ≥99.0%, respectively) under the same GC parameters and column. Retention times of (1S)-(−)-α-pinene and (1R)-(+)-α-pinene analytical standards were 8.09 min and 8.34 min, respectively; RT of (−) and (+) enantiomers of α-pinene were 8.09–8.19 min and 8.31–8.42 min, respectively, in investigated essential oils. The percentage amounts of α-pinene enantiomers were recalculated according to the areas of the FID chromatographic peaks assuming that monoterpene α-pinene fraction is 100%.

2.5. Data analysis

Statistical data processing, including calculation of means, standard errors, Spearman's rank correlation coefficients (R), the Kruskal–Wallis test and probabilities (p), was carried out with the STATISTICA® 7 and MS Excel.

2.6. Assessment of foliage biomass resources

Lithuanian Statistical Yearbook of Forestry data as for January 1, 2015 (State Forest Service, 2015) was used to assess mature pine stand resources in Lithuania. The empirical formulas describing relations between the conventional sylvicultural measurements and foliage biomass were used as reported in the research papers from the region. Out of more than 20 equations for the assessment of foliage biomass of a Scotch pine tree in Europe (Jelonek et al., 2012; Lehtonneen, 2005; Muukkonen and Mäkipää, 2006; Socha and Wezyk, 2007; Turski et al., 2008; Zianis et al., 2005) six were chosen based on the geographic