Quantitative and Qualitative Seasonal Changes of Scots Pine and Norway Spruce Foliage Essential Oils in Latvia, and the Extraction Dynamics Thereof

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Abstract

The tree foliage (needles and non-lignified shoots) of the main Latvian tree species – the Scots pine *(Pinus sylvestris* L.) and the Norway spruce *(Picea abies* (L.) Karst.) – contains essential oils, a product with useful properties. Prior to this, there has been no research done in Latvia about the yields and quality indices of essential oils during the different seasons. This study examines oil content and quality during the autumn, winter, spring and summer periods. Essential oil distillation dynamics were investigated to determine the optimum distillation length for the recovery of essential oil content in tree foliage over the course of a year. Samples of tree foliage were collected twice per month during a one year investigation period, and the content of essential oils was determined. The obtained essential oils were analyzed, determining the density, refractive index and the amount of the main terpenes (α-pinene, camphene, β-pinene, 3-carene, limonene and bornyl acetate). The pycnometer, refractometer and gas chromatography-mass spectrometry (GC-MS) methods were used to determine the density, refractive index and terpene content, respectively. The results show that oil amount and physicochemical properties are variable during the course of a year. This study demonstrates that it is possible to produce pine and spruce essential oils throughout the whole year, and that the product corresponds to the Latvian standards for an industrial product – pine and spruce essential oils.

Key words: Scots pine, Norway spruce, tree foliage, essential oils, seasonal dynamics, terpenes, distillation dynamics.

Introduction

An important product of plant origin is volatile substances, which fall into various chemical compound classes and are used in aromatherapy, perfumery, etc. These substances are called essential oils, and are contained in various plants, including trees, from which they can be extracted in industrial quantities.

Latvian coniferous trees (pine, spruce, juniper, and others) also contain these essential oils. They are mainly contained in the needles and non-lignified shoots. The essential oil yield of the main coniferous trees growing in Latvia is as follows: in pine needles – up to 0.87 % (hereinafter – per dry matter) (Daugavietis et al. 2002) and in spruce needles – up to 0.68 % (Polis et al. 2009). The essential oils of the main coniferous tree species of Latvia – the pine and the spruce – have bactericidal and also insecticidal properties, and they have found various practical applications (Produkti 2012, Беспалов и Некрасова 2007, Polis 1995). Pine and spruce essential oils, their extraction and properties have not been extensively studied in Latvia (Polis et al. 2009, Daugavietis et al. 2005, Daugavietis et al. 2002, Галванс и Лиепиньш 1973). Investigations conducted in other countries shows that the essential oil content of the Scots Pine (*Pinus sylvestris*) needles is on average 0.4 – 1.0% (Сотникова и Степень 2001, Репях и Степень 2000, Колесникова 1998,). Their chemical composition is also studied (Kupcinskiene et al. 2008, Maciag et al. 2007, Сотникова и Степень 2001), The essential oil content of the Norway Spruce (*Picea abies*) needles is average 0.2-0.4% (Колесникова 1998, Orav et al. 1996). Information can be found in literature on the changes of the amount of essential oils in different seasons, as well as on their chemical composition (Radulescu et al. 2011, Orav et al. 1996).

To obtain pine and spruce essential oils in industrial amounts, they are extracted through steam distillation of the raw material – tree foliage (Лобанов 2006,

Черняева и Голиков 1977**)**. Steam distillation of essential oils is affected by several factors – distillation intensity, duration, steam parameters (in the case of the so-called blowing method) etc. (Лобанов 2006, Бараков 1977, Черняева 1977, Никифоров и Калинин 1977**).** A method of processing tree foliage extractives to obtaining essential oils is also known (Левин и Репях 1984, Ягодин 1981, Продниекс и Дрожжин 1974).

Therefore, due to the lack of research on the extraction and properties of essential oils of the main Latvian coniferous tree species (pine and spruce), this research work was performed determining the essential oil yield of these tree species over the course of the year and the chemical composition and physical properties of the extracted oils. We also have examined the dynamics of essential oil distillation aiming to improve the methodology for determining the amount of essential oils.

We assume that obtained results will allow specialists in forestry of other countries to expand their knowledge about Latvian coniferous essential oils.

Materials and Methods

Plant material

The raw material for analysis (pine and spruce foliage) was gathered twice per month (at the beginning and in the middle of each month) from September 2010 to September 2011. The tree foliage was gathered from State forests in Iecava municipality, Vidusdaugava forestry, within a 1 km radius around the polar coordinates 56°44.100 N and 024°15.403 E. Pine foliage was gathered in stands of the 1st age class (trees up to 20 years old) while spruce foliage was obtained from 20-40-year-old trees in stands of the 2nd – 3rd age class, both of the II site index, according to Orlov (Словарь 1984). The average sample was taken from all sides of 20 tree crown in height up to 2 – 2.5 m of the total mass of ~ 20 kg. The samples were kept in cold storage at +4 °C. The analyses were conducted within 3 days after obtaining the samples (taking them from the tree).

For the purposes of analysis, along with the analytic sample, a second sample of tree foliage was taken from average sample to determine dry matter content as a percent of total mass when sampled. Dry matter was determined by drying a previously weighed foliage sample until it reached a constant (invariable) mass in a drying oven at a temperature of 105°C.

The weighing was conducted to the accuracy of ± 0.0001 g. The dry matter was determined by repeating the procedure 3 times and taking the arithmetic mean of the three results.

Determinig essential oil distillation dynamics

The dynamics of the hydrodistillation process were studied in order to determine the shortest length of time needed to extract the maximum quantity of essential oils. The dynamics were determined for both spruce and pine foliage, in two ways for each: for non-shredded foliage (10–15 cm long, needle-covered branches) and shredded foliage. Shredding was conducted with an extruder-type shredder M2. Samples were taken from the shredded and non-shredded foliage in order to determine the dry matter, so that the yield could be calculated for completely dry foliage. Dry matter was calculated in accordance with above described method.

The hydro-distillation method consists of placing non-shredded foliage in a 4-L round-bottom flask, weighed to the accuracy of ± 0.1 g, and water is poured in to immerse the foliage; an oil trap (Clevenger apparatus) and a reflux condenser are connected, followed by boiling on an electric laboratory hot plate. The beginning point of oil distillation is assumed to be the moment when the first drops of condensate appear in the reflux condenser. From this moment, the amount of distilled oil in the trap is read every 15 minutes (the oil receiver of the Clevenger apparatus is gradated to an accuracy of ± 0.1 cm^{-3}). The hydro-distillation is continued until the amount of oil in the Clevenger apparatus has not increased for 3 readings in a row. The dynamics of oil extraction from shredded foliage was examined in the same way. Thus, the maximum yield of essential oils obtainable through the tree foliage hydro-distillation process is the amount recorded in the trap at the end of the distillation, when the amount of distilled essential oils is no longer increasing. Each version (both with shredded and non-shredded foliage) is repeated 5 times. In each experiment, the distilled/recorded essential oil yield is calculated every 15 minutes (as a percentage of the maximum yield). The result of the experiment is assumed to be the average essential oil yield across the five repeats.

Determining the amount of essential oils

The amount of essential oils in tree foliage was determined with the hydro-distillation method. The tree foliage to be analyzed was shredded in extruder-type shredder M2. During the shredding process, the tree foliage is inside the shredder for approximately 5 seconds and does not heat up enough for the essential oils to evaporate in a considerable amount. An exact weighed amount (approx. 700 g, weighed to accuracy of ± 0.1 g) was taken from the shredded foliage and placed in a 4-L round-bottom flask made of glass. A sufficient amount of water was poured into the flask

to immerse the entire foliage, and a Clevenger apparatus for determination of volatile oil lighter than water and a reflux condenser were connected. The content of the flask was heated on an electric laboratory hot plate. With the liquid boiling, the azeotropic mixture of essential oils and steam enters the reflux condenser, condenses and runs off to the Clevenger apparatus. Non-water-soluble essential oils remain in the Clevenger apparatus, but the water runs back to the flask. The process is continued until the amount of essential oils in the Clevenger apparatus is no longer increasing (visual inspection). The distillation was continued for 3 hours, as the prior research of hydrodistillation dynamics showed that after 3 hours the essential oils have been quantitatively extracted from both pine and spruce foliage. The process was repeated 3 times. The extracted essential oils were combined, and their volume was measured. The essential oils were dried with anhydrous sodium sulfate and stored in a refrigerator until the conduction of analyses. The amount of essential oils in tree foliage was calculated in accordance with the following formula (1):

$$A = \frac{V \times d_{oils} \times 10000}{M \times S}, \quad (1)$$

where A is essential oil content in tree foliage, % of dry matter; V is volume of essential oils distilled off the analytic sample, cm^{-3}; d_{oils} is density of essential oils, g cm^{-3}; M is mass of tree foliage sample, g; S is dry matter in tree foliage, %.

Determining essential oil density

The density was determined with a pycnometer (Gallova 2011). The method is based on comparing the mass of the same volume of the analytic material and a substance with precisely known density. We used distilled water, which has certain changes in density depending on the temperature (data are available in corresponding handbooks). First, a previously weighed pycnometer is filled to the specific mark with distilled water, the temperature of which has been measured. The filled pycnometer is weighed. The distilled water is then poured out; the pycnometer is dried and again filled to the mark with the analytic liquid – pine or spruce essential oils – and weighed. All weighing is conducted to the accuracy of ± 0.0001 g.

The density of the essential oils is calculated in accordance with the formula (2):

$$d_{oils} = \frac{m_{oils}}{m_{H2O}} \times d_{H2O}, \quad (2)$$

where d_{oils} is density of essential oils, g cm^{-3}, m_{oils} is mass of essential oils in pycnometer (excl. mass of pycnometer), g, m_{H2O} is mass of water in pycnometer (excl. mass of pycnometer), g, d_{H2O} is density of water (at specific temperature in accordance with handbooks data), g cm^{-3}.

The density measurement was repeated 5 times for each essential oil sample, and the arithmetic mean of these five measurements was taken as the result.

Determining the refractive index of essential oils

The refractive index of the essential oils was determined with Abbe bench refractometer 2WAJ ('OPTIKA Microscopes', Italy). The refractive index was determined in room temperature for each sample by nearing the boundary line to the center of cross-lines five times "from the top" and five times "from the bottom", in accordance with refractometer manual. The arithmetic mean of these ten measurements was taken as the result.

Determining essential oil content

Extracted essential oils were evaluated for the qualitative and quantitative presence of the following terpene compounds: α-pinene , camphene, (-)-β-pinene, (+)-3-carene, (R)-(+)-limonene and bornyl acetate; they were selected as the most characteristic monoterpene components of pine and spruce essential oils.

The gas chromatography-mass spectrometry (GC-MS) method was used to determine the quantitative content of terpenes in pine and spruce tree foliage essential oils. Analysis was carried out with a mass spectrometer (Hewlett-Packard 5973) connected with a Hewlett-Packard gas chromatograph (HP 6890). Column: Zebron ZB-5MS, 30 m × 0.25 mm, film thickness 0.25µm. Carrier gas: helium, flow rate 1 ml min^{-1}. Tem-

	% area						
Essential oil	0,1%	1%	3%	5%	10%	R^2	Linear equation
□-pinene	10518	1E+05	3E+05	5E+05	1E+06	1.000	y=10161069.893x+1495.410
camphene	6644	65258	2E+05	3E+05	691731	1.000	y=6583840.643x+591.625
(-)-□-pinene	8615	90395	3E+05	4E+05	985254	1.000	y=8606184.344x+2223.556
(+)-3-carene	5374	58710	2E+05	3E+05	785370	1.000	y=6048170.889x-1327.388
(R)-(+)-limonene	1986	25628	97780	2E+05	609513	0.993	y=3773434.562x-8076.136
bornyl acetate	1943	25373	71662	1E+05	383147	0.989	y=2835284.291x-3952.718

Table 1. Terpene standard linear equations for quantitative determination with GC-MS

perature ranges from 50 to 280 °C at a rate of 4°C min^{-1}. Total time of analysis: 62.5 min.

α-Pinene (from Aldrich), camphene (from Supelco), (-)-β-pinene (from Fluka), (+)-3-carene (from Fluka), (R)-(+)-limonene (from Fluka) and bornyl acetate (from Fluka) standards were used. The reference substances were used to make reference solutions at several concentrations, corresponding to the expected content in the essential oils to be analyzed. A chart of each reference substance was prepared depending on the concentration, and a linear equation was derived from it and used to calculate the content of the respective component in the analyzed essential oils. The results have been summarized in Table 2 and Table 3.

Results

The essential oil distillation dynamics investigation results (both on non-shredded and shredded pine and spruce foliage) have been summarized in Figures 1 and 2. The experiments show that essential oils are completely extracted from non-shredded pine foliage with the hydro-distillation method in nearly four hours (225 min), while extraction from shredded pine foliage takes only two and a half hours (135 min.). Complete extraction from non-shredded spruce foliage takes approximately three hours (195 min); extraction from shredded spruce foliage also takes nearly three hours (165 min). In line with these results, in all subsequent

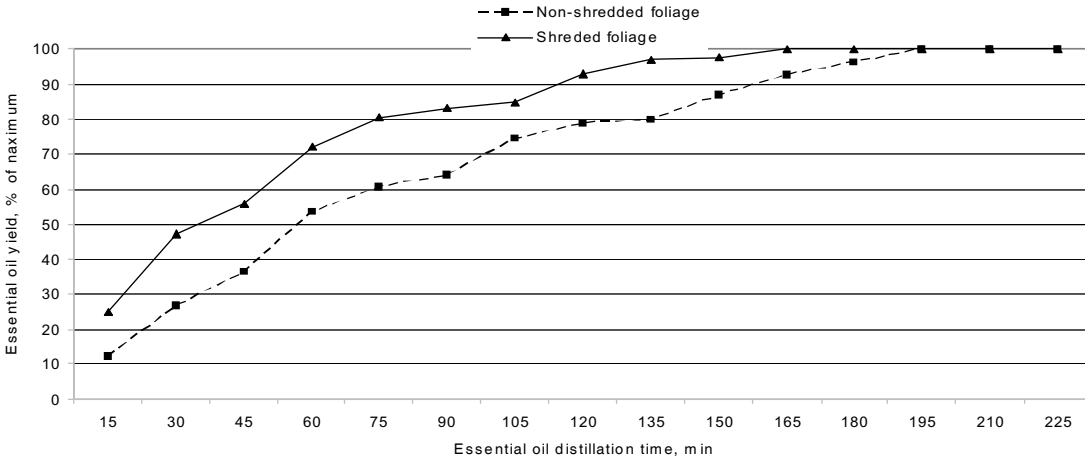

Figure 2. Essential oil distillation dynamics of Norway spruce foliage collected from trees till height of 2-2.5 m

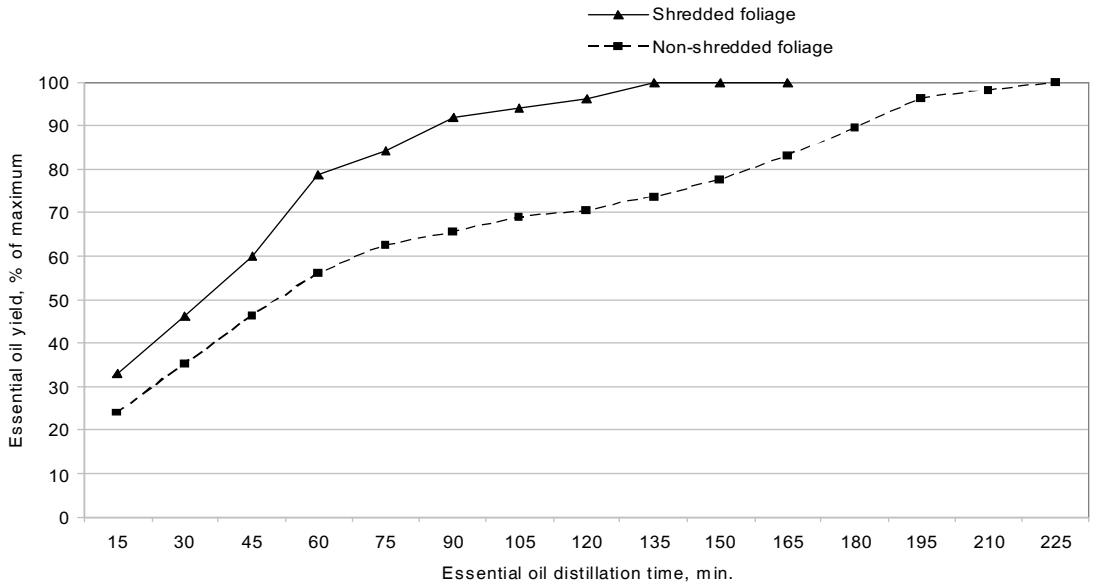

Figure 1. Essential oil distillation dynamics of Scots pine foliage collected from trees till height of 2-2.5 m

parts of this study, the distillation was conducted for three hours, and the foliage was shredded before the distillation.

In accordance with the methodologies described above, the yield of pine and spruce essential oils was determined twice per month over the course of the entire year, and the density and the refractive index of the obtained essential oils were recorded (Korica et al. 2011). The results of the research are presented in Tables 2 and Table 3.

Essential oil content in tree foliage over the course of one year was variable. In pine foliage, it ranged from 0.53% to 0.88% (percentage of the dry matter of tree foliage). The average annual yield was 0.56%. Although the peak amount of oil was observed in the winter (December), variations were observed throughout the year. The obtained results do not indicate a specific pattern of oil content changes over the course of the year ($p < 0.5$). In spruce foliage, the oil content yield ranged from 0.26% to 0.61%.

The density of pine foliage essential oils ranged during the year from 0.864 to 0.888 g cm^{-3}, annual average – 0.877 g cm^{-3}, and pronounced patterns of change have not been found. The same applies to changes in the density of spruce foliage essential oils over the year – it ranged from 0.892 g cm^{-3} to 0.910 g cm^{-3} with average annual density 0.900 g cm^{-3}.

The refractive index of pine essential oils ranged over the course of the year from 1.4760 to 1.4840, annual average – 1.4808. The refractive index of spruce essential oils ranged from 1.4695 to 1.4790, annual average – 1.4740.

The content of the main terpenes of essential oils (α-pinene, camphene, (-)-β-pinene, (+)-3-carene, (R)-(+)-limonene and bornyl acetate) was determined in accordance with the presented methodology once per month throughout the year. The obtained results are presented in Tables 2 and 3.

In pine essential oils, the content of α-pinene (on average – 2.01% of the oil content) and 3-carene (1.19%) is the highest among the analyzed terpenes; in spruce essential oils, the highest content was found by bornyl acetate (1.94%), camphene (0.88%) and limonene (0.74%).

In pine essential oils, the maximum content of α-pinene was recorded in February (2.77%), the minimum – in July (1.36%). The respective maximum and minimum content of other analyzed terpenes in spruce foliage essential oils was as follows: camphene – 0.29% in April and 0.17% in January, June and July; β-pinene – 0.53% in February and 0.08% in June; 3-carene – 2.66% in January and 0.42% in December; limonene – 0.42% in May and October and 0.26% in January; bornyl acetate – 0.43% in April and 0.18% in January.

Table 2. Analysis of Scots Pine essential oils

Date of analysis	Content, % of dry matter	Density, g cm	Refractive index
15.09.2010	0.75 ± 0.02	0.877 ± 0.008	1.4805 ± 0.001
03.10.2010	0.72 ± 0.02	0.878 ± 0.007	1.4822 ± 0.0008
17.10.2010	0.71 ± 0.01	0.878 ± 0.007	1.4803 ± 0.001
01.11.2010	0.58 ± 0.01	0.871 ± 0.008	1.4811 ± 0.001
15.11.2010	0.57 ± 0.01	0.877 ± 0.006	1.4822 ± 0.0009
01.12.2010	0.73 ± 0.02	0.876 ± 0.007	1.4828 ± 0.0008
16.12.2010	0.60 ± 0.02	0.881 ± 0.006	1.4824 ± 0.001
03.01.2011	0.53 ± 0.01	0.869 ± 0.008	1.4800 ± 0.001
17.01.2011	0.88 ± 0.02	0.870 ± 0.006	1.4780 ± 0.001
02.02.2011	0.70 ± 0.01	0.864 ± 0.08	1.4775 ± 0.001
14.02.2011	0.63 ± 0.02	0.871 ± 0.007	1.4760 ± 0.001
01.03.2011	0.54 ± 0.01	0.873 ± 0.009	1.4805 ± 0.0009
15.03.2011	0.61 ± 0.01	0.874 ± 0.008	1.4805 ± 0.001
30.03.2011	0.71 ± 0.01	0.877 ± 0.008	1.4815 ± 0.0009
15.04.2011	0.71 ± 0.01	0.878 ± 0.010	1.4815 ± 0.001
29.04.2011	0.64 ± 0.01	0.875 ± 0.008	1.4805 ± 0.0008
16.05.2011	0.53 ± 0.02	0.882 ± 0.009	1.4785 ± 0.0008
02.06.2011	0.66 ± 0.01	0.888 ± 0.008	1.4820 ± 0.0009
16.06.2011	0.63 ± 0.02	0.888 ± 0.007	1.4840 ± 0.001
04.07.2011	0.70 ± 0.02	0.876 ± 0.008	1.4805 ± 0.001
18.07.2011	0.61 ± 0.01	0.871 ± 0.008	1.4805 ± 0.009
01.08.2011	0.58 ± 0.02	0.881 ± 0.008	1.4830 ± 0.0007
15.08.2011	0.57 ± 0.01	0.881 ± 0.007	1.4810 ± 0.0007
02.09.2011	0.59 ± 0.01	0.882 ± 0.007	1.4810 ± 0.001
15.09.2011	0.62 ± 0.01	0.881 ± 0.009	1.4815 ± 0.0007

Table 3. Analysis of Norway spruce essential oils

Date of analysis	Content, % of dry matter ± SD	Density, g m^{-3} ± SD	Refractive index ± SD
15.09.2010	0.42 ± 0.02	0.905 ± 0.008	1.4740 ± 0.001
03.10.2010	0.47 ± 0.001	0.903 ± 0.009	1.4740 ± 0.0008
17.10.2010	0.36 ±0.001	0.899 ± 0.009	1.4729 ± 0.0008
01.11.2010	0.48 ± 0.001	0.899 ± 0.009	1.4728 ± 0.0008
15.11.2010	0.57 ± 0.002	0.904 ± 0.008	1.4725 ± 0.0009
01.12.2010	0.61 ± 0.001	0.900 ± 0.007	1.4755 ± 0.008
16.12.2010	0.33 ± 0.001	0.909 ± 0.008	1.4770 ± 0.001
03.01.2011	0.42 ± 0.002	0.896 ± 0.008	1.4740 ± 0.0008
17.01.2011	0.43 ± 0.002	0.892 ± 0.008	1.4745 ± 0.0008
02.02.2011	0.30 ± 0.010	0.893 ± 0.008	1.4755 ± 0.0008
14.02.2011	0.30 ±0.01	0.902 ± 0.009	1.4760 ± 0.0009
01.03.2011	0.44 ± 0.01	0.897 ± 0.008	1.4740 ± 0.0008
15.03.2011	0.38 ± 0.01	0.906 ± 0.008	1.4735 ± 0.0008
30.03.2011	0.32 ± 0.01	0.899 ± 0.009	1.4790 ± 0.001
15.04.2011	0.36 ± 0.01	0.896 ± 0.009	1.4750 ± 0.009
29.04.2011	0.36 ± 0.01	0.897 ± 0.007	1.4745 ± 0.0009
16.05.2011	0.36 ± 0.01	0.910 ± 0.008	1.4720 ± 0.0008
02.06.2011	0.45 ± 0.01	0.899 ± 0.008	1.4730 ± 0.0009
16.06.2011	0.26 ± 0.01	0.901 ± 0.009	1.4755 ± 0.0008
04.07.2011	0.43 ± 0.01	0.907 ± 0.009	1.4725 ± 0.0008
18.07.2011	0.46 ± 0.01	0.898 ± 0.007	1.4695 ± 0.0009
01.08.2011	0.53 ± 0.02	0.897 ± 0.008	1.4730 ± 0.001
15.08.2011	0.38 ± 0.01	0.902 ± 0.008	1.4725 ± 0.008
02.09.2011	0.38 ± 0.01	0.902 ± 0.009	1.4755 ± 0.008
15.09.2011	0.39 ± 0.01	0.899 ± 0.009	1.4710 ± 0.008

The respective maximum and minimum content of the individual terpenes in spruce foliage essential oils over the course of the year was as follows: bornyl ace-

tate – 2.42% in April and 1.05% in January; α-pinene – 0.76% in October and 0.34% in February; camphene – 1.16% in September and 0.63% in February and April; β-pinene – 0.45% in October and 0.15% in July; 3-carene – 0.38% in July and 0.06% in January; limonene – 0.97% in September and 0.53% in February.

Table 4. Analysis of terpenes in Scots pine essential oils

Date of analysis	α-pinene, % of oils	camphene, % of oils	(-)-β-pinene, % of oils	(+)-3-carene, % of oils	(R)-(+)-limonene, % of oils	bornyl acetate, % of oils
15.09.2010	1.72	0.21	0.16	0.90	0.34	0.24
17.10.2010	1.81	0.26	0.16	0.68	0.35	0.37
15.11.2010	1.97	0.25	0.26	1.36	0.36	0.37
16.12.2010	1.85	0.18	0.16	0.42	0.34	0.24
17.01.2011	2.53	0.17	0.18	2.66	0.26	0.18
14.02.2011	2.77	0.20	0.53	0.82	0.38	0.22
15.03.2011	1.81	0.25	0.18	1.25	0.38	0.29
15.04.2011	2.37	0.29	0.25	1.03	0.39	0.43
16.05.2011	2.23	0.26	0.16	1.01	0.42	0.30
16.06.2011	1.79	0.17	0.08	1.88	0.30	0.32
18.07.2011	1.36	0.17	0.13	1.27	0.38	0.41
15.08.2011	1.87	0.24	0.15	1.01	0.42	0.35

Table 5. Analysis of terpenes in Norway spruce essential oils

Date of analysis	α-pinene, % of oils	camphene, % of oils	(-)-β-pinene, % of oils	(+)-3-carene, % of oils	(R)-(+)-limonene, % of oils	bornyl acetate, % of oils
15.09.2010	0.66	1.16	0.29	0.15	0.97	2.39
17.10.2010	0.76	1.11	0.45	0.22	0.82	2.04
15.11.2010	0.48	1.06	0.18	0.09	0.58	2.15
16.12.2010	0.40	0.71	0.33	0.11	0.59	1.99
17.01.2011	0.57	0.98	0.41	0.06	0.76	1.05
14.02.2011	0.34	0.63	0.24	0.07	0.53	1.77
15.03.2011	0.50	0.87	0.26	0.19	0,79	2.09
15.04.2011	0.40	0.63	0.30	0.11	0.80	2.42
16.05.2011	0.67	0.99	0.47	0.25	0.73	1.54
16.06.2011	0.40	0.67	0.26	0.13	0.78	1.98
18.07.2011	0.39	0.76	0.15	0.38	0.83	2.28
15.08.2011	0.63	0.98	0.25	0.35	0.73	1.61

Discussion and Conclusions

The difference in the time needed for complete extraction of essential oils from pine and spruce foliage as determined by the current research on hydrodistillation dynamics, is explained by the differing structure of pine and spruce needles. This gives rise to different dynamics in the mixing of essential oils and steam (steam penetration to the essential oil depositories in the needles). In addition, quantitative distillation of essential oils is faster with shredded foliage than with non-shredded foliage, as the structure of the needles and twigs is partially broken down, thereby increasing the contact surface of water and essential oils (Satish Kumar 2010). Estimation of minimum necessary time for complete essential oils distilling allows reducing time for essential oils content analysis.

Previous research has found that for several conifers the minimum essential oil content in tree foliage is found in spring and summer, and the maximum – in autumn and winter (Цюпко 2002, Степень и др. 1981, Томчук и Томчук 1973). The results of our analysis show that the content of essential oils in pine and spruce tree foliage is variable across the 12-month collection period. The content of spruce essential oils in the foliage was more changeable over the course of the year than that of pine oils.

The refractive indices of the tree foliage essential oils were recorded throughout the entire year. The indices of both pine and spruce essential oils changed throughout the year. It indicates that the chemical composition and the proportions of the components of foliage essential oils change little over the course of the year, as the refractive index depends on both the chemical composition of the individual essential oil components and on their proportions in the product.

The densities of essential oils in both pine and spruce foliage also varied throughout the year. As with the refractive index, the density of essential oils also depends on the chemical composition of the individual components and on the quantitative proportions thereof.

As the refractive index and also the density of essential oils is determined by their chemical composition and the quantitative proportions of the components, these two values are the ones that determine the identity of each specific essential oil, because it is virtually impossible to fake plant essential oils by making them as mixtures of other substances in such a way as to ensure that both their organoleptic properties and the refractive index and density correspond to the original essential oils. That is why Latvian technical regulations on industrially extracted pine and spruce foliage essential oils specify density and refractive index among the indicators (US 2006).

Prior to this investigation of the content of the main components of essential oils obtained from the main Latvian conifer species – pine and spruce – and changes thereof over the course of the year has not been previously studied. The only known research is conducted by Галванс (1973). More extensive studies on the content of conifer essential oils, their amount and the variations of individual components across seasons have been conducted in Europe and Russia (Kupcinskiene et al. 2008, Maciag et al. 2007, Цюпко 2002). According to these studies, the minimum content of essential oils in various fir species (*Abies nephrolepis*, *Abies sachalinensis*, *Abies mayriana*, *Abies holophylla*) has been observed in needles in summer, and the maximum content – in winter. The

content of 3-carene is relatively invariable, with a small tendency of increasing in summer. The same can be said about the changes in α-pinene content. For bornyl acetate, the minimum content is observed in summer, and the amount is elevated in winter months. No seasonal correlations among the individual components of essential oils were found in literature. Explicit patterns of change in the components over the course of the year were also not discovered during our study.

In summary, it can be concluded that the essential oil content in the foliage of the main Latvian tree species, Scots pine and Norway spruce, is variable throughout the year. The same conclusion can be made about the characteristics of the essential oils – their density, refractive index and content of individual components – they vary throughout the year, but show no explicit patterns.

The amount of essential oils in pine tree foliage is greater than in spruce tree foliage throughout the entire year. The amount of spruce essential oils is more variable over the course of the year than that of pine oils (the annual peak content is twice as large as the minimum content).

The essential oil content in both pine and spruce tree foliage in Latvia is sufficient for the oils to be obtained throughout the year, regardless of the season. It is an advantage compared to the extraction of essential oils from agricultural plants, which has a seasonal character.

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