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# Optimization of hop essential oil extraction by means of supercritical $CO_2$

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Supercritical fluid extraction (SFE) can reduce sample preparation time and the recovery can be equal or better than that of the classical extraction techniques for solid and semi-solid samples. Extraction efficiency and speed can benefit from specific properties of a supercritical fluid.

Essential oils were extracted from "Marynka" variety hop using supercritical carbon dioxide extraction. The highest essential oil recovery was obtained at the optimized conditions of extraction, i. e., trap temperature 5 °C, extraction pressure 85 bar and extraction time 10 min.

Gas chromatography with mass spectrometry was used for determination of quantitative and qualitative composition of the extracted essential oils. The following main compounds were identified in the essential oils from "Marynka" hop calculating retention indices and using mass spectra library: α-pinene, β-myrcene, limonene, humulene, copaene, cubebene, β-caryophyllene and α-caryophyllene.

**Key words:** supercritical fluid extraction, essential oils, hops, gas chromatography – mass spectrometry

## INTRODUCTION

The extraction of essential oils from hops using supercritical fluids has significant advantages over conventional methods such as steam distillation and Soxhlet extraction [1–3]. Higher solubility, mass transfer rates and selectivity make the supercritical fluid extraction more attractive. The selectivity of the compound to be extracted is dependent on the density of the supercritical fluid, which can be altered by varying process conditions. The low latent heat of evaporation and high volatility of the solvent make the extract free from the residual solvent. Moreover, extraction can be conducted at low temperatures, which helps to preserve thermally degradable products [4–6].

Hop (*Humulus lupulus* L.) became a widely grown agricultural plant because of its use to give bitterness and aroma to beer. The beer quality depends partly on the hops used in the brewing process. Since the quality is best maintained by defining raw materials, many, especially traditionally oriented, brewers tend to buy hops of the known variety and origin. As each hop variety has a typical essential oil pattern, hops essential oil analyses can be used to identify them [7]. Dry hops

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contain from 0.5 to 3 % of essential oil, which consists mainly of terpene hydrocarbons and their oxidation products.

As gas chromatography–mass spectrometry (GC–MS) becomes popular, a large number of researchers have turned to the mass spectra for identification of peaks. The most frequent, simple and valid identification method is the comparison of the recorded spectra with the standard MS library. However, when there are peaks of isomers or of similar substances in GC–MS, the identification will be not precise enough and sometimes even wrong, from the chemical structure point of view. As we know, the natural essential oils are usually mixtures of terpenoids (mainly mono-terpenoids and sesquiterpenoids), aromatic compounds and aliphatic compounds. As mass spectra of these compounds are usually very similar, peak identification becomes very difficult and sometimes impossible. In order to address the qualitative determination of compositions of complex samples by GC–MS and to increase the reliability of the analytical results, it seems necessary to utilize the identities of retention indices [8].

In this research work, for the first time the amount of essential oils from hop "Marynka" extracted by supercritical extraction method has been optimized changing extraction conditions, namely, pressure, trap temperature and extraction time.

## EXPERIMENTAL

### Materials

n-heptane for chromatography (purity grade) was purchased from Merck, Germany.

Carbon dioxide (99.9% purity, AGA, Lithuania) was used for the extractions.

### Equipment

For supercritical fluid extraction HP 7680T (Hewlett Packard, USA) apparatus was used. GC–MS analyses were carried out using a model GC-2010 gas chromatograph with a model GCMS-QP2010 mass spectrometer (Shimadzu, Japan), RTX – 5 MS low bleed column (30 m × 0.25 mm × 0.25 μm) coated with non-polar 5% phenyl methylpolysiloxane stationary phase was used for separation of the essential oils. Samples were injected with an autoinjector AOC-20i (Shimadzu, Japan).

### Sample preparation

A sample of the "Marynka" hop variety was obtained from Vytautas Magnus University Botanical Garden in Kaunas (2007). Cones and stalks of hop were collected at the beginning of ripening of seeds (August–September) and dried in an oven at 30 °C. Humidity of cone raw material was less than 10%, and the colour of the sample was greenish-yellow.

Dry raw material was stored in polythene bags in a dry dark place. 4 g sample of dried "Marynka" hop cones was ground for 5 min by means of food processor Braun AG (Frankfurt, Germany). Particle size was from 0.1 to 0.3 cm.

### SFE conditions

Essential oils were extracted from "Marynka" hop using the supercritical carbon dioxide extraction. Following independent variables were examined in this study: extraction pressure (77, 85, 105, 120 and 140 bar), temperature of the trap (–20, –10, –5, 0, 5, 25, 40 and 50 °C) and the extraction time (2, 5, 10, 15 and 20 min). The sample amount of 0.5 g was taken from the well mixed raw material and placed into the 7 ml volume stainless steel extraction cartridge capped with porous frits. Carousel for samples can accommodate up to 8 sample cartridges and process them automatically. The trap volume was 1 ml and it was filled with ocatadecylsilane (ODS) modified silica solid phase extraction particles. After trapping, the essential oils were desorbed in flushing solid phase adsorbent with heptane. The desorbed fractions were collected in the sample collection carousel in 1 ml vials which were later sealed and kept in refrigerator at 4 °C until GC–MS analysis. Volume of the extract fractions was 0.7 ml, $CO_2$ flow rate at the dynamic extraction was 1 ml/min and extraction cartridge temperature was set at 50 °C. Static extraction with supercritical carbon dioxide was set in all experiments for 2 min in order to soak the sample before the dynamic extraction.

### GC–MS conditions

Helium was used as the carrier gas, injector temperature was set at 240 °C, the injected sample volume was 1 μl. Injections were performed in a split mode (split ratio 10 : 1). The column oven temperature was programmed from 60 °C to 150 °C at 2 °C/min speed, held at 150 °C for 5 min, and then programmed to rise at 10 °C/min to 285 °C. Kovats retention indices were determined for each peak from the interpolation of n-alkanes retention times at the same temperature gradient. Electron ionization was performed at 70 eV. To identify and determine the content of the compounds, a mass spectrometer was used in total ion current (TIC) mode.

## RESULTS AND DISCUSSION

Exposure of a raw material of hops to a supercritical fluid during the static extraction leads to soaking and partial dissolving of herbaceous matrix components in compressed supercritical fluid. This may affect matrix of the sample and cause changes of tissue properties and, accordingly, the plant tissue itself might be subjected to swelling. The extent to which changes occur depends on the specific structure of plant material, as well as on the extraction process operation conditions. Since various parameters potentially affect the extraction process, the optimization of experimental conditions represents a critical step in the development of a SFE method. The optimization of the method can be carried out step-by-step or using an experimental design.

**Determination of optimal temperature and pressure**

Selection of the operating conditions depends on the specific compound or compound family to be extracted. Molecular weight and polarity have to be taken into account in every specific case, however some general rules can be applied. Pressure and temperature are two critical variables in $CO_2$ extraction as they both determine the density of $CO_2$ and then its solvating power to the analytes [9]. Hence, the effect of temperature or pressure on the recovery of essential oils should be studied changing one variable while keeping the other one constant.

The increase of temperature reduces density of $CO_2$ (for a fixed pressure), thus reducing the dissolving power of the supercritical solvent. However, it increases the vapour pressure of the compounds to be extracted. Therefore, the tendency of these compounds to pass in the fluid phase is increased. The effect of temperature on the trapping of the essential oils is less investigated, therefore this was one of the tasks of this study. Nonvolatile compounds of the sample can be trapped using a trap filled with inert beads (for instance, stainless steel beads). Nonvolatile compounds precipitate on the trap packing, when supercritical carbon dioxide pressure is released and later washed out to the fraction collector with a small volume of organic solvent. Volatile compounds are trapped using a trap filled with an adsorbent, since these compounds can not be precipitated and trapping should be based on the physical-chemical interaction (adsorption). In this study, the influence of temperature of the trap filled with octadecylated silica, has been investigated in the range from –20 to 50 °C, while keeping extraction pressure at 85 bar and extraction temperature at 50 °C. The experimental results are presented in Fig. 1. Essential oils recovery was calculated as a sum of peak areas in chromatogram relative to that obtained by hydrodistillation method, which was assumed to give 100% recovery.

The temperature effect on trapping of essential oils was dual. Higher temperature was expected to intensify evaporation of the supercritical carbon dioxide as well as volatile analytes. On the other hand, temperature increases nonpolar interaction, therefore adsorption of essential oils on the octadecylsilica favours from it. In Fig. 1, one maximum is at 5 °C, another at 50 °C. It might be, that evaporation of volatile compounds is increased above 5 °C and recovery of essential oils drops, however, further increase of temperature increases hydrophobic interaction with the solid phase adsorbent and the recovery starts to grow.

The optimum temperature for the ODS trap, regarding the recovery of the main compounds, present in the extracted essential oils, is 5 °C (Table 1). This parameter has the greatest effect on operating costs, because lower the temperature of the solid ODS trap causes higher cryogenic $CO_2$ consumption and, hence, increased operating costs. A working temperature was tested. The increase in temperature from 25 to 50 °C inversely affected the recovery of essential oils, both qualitatively (fewer compounds) and quantitatively (lower concentration). So, the highest concentration and recovery of essential oils, such as α-pinene, β-myrcene, α-caryophyllene, limonene, copaene and β-caryophyllene were obtained at 5 °C temperature.

In further experiments, the influence of extraction pressure on the recovery of the essential oils was studied at trap temperature 5 °C under four different pressure regimes, i.e., 77, 85, 105, 120, 140 and 165 bar, respectively. The

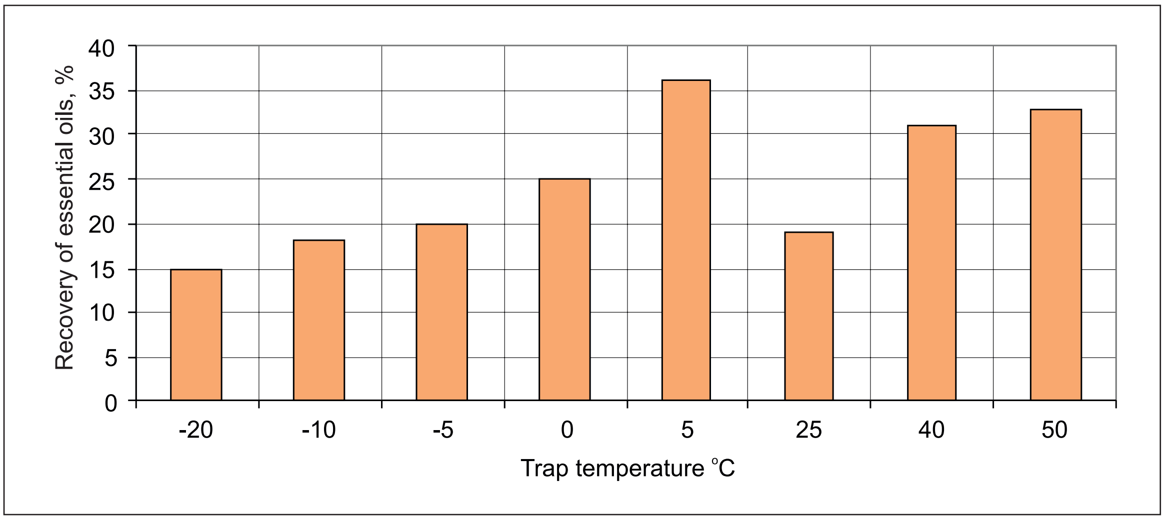

**Fig. 1.** Influence of trap temperature on essential oils recovery

Table 1. **Dependence of relative content of the main components in essential oils from hop "Marynka" on trap temperature**

| Compound | Essential oils (%) at different temperatures | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | –20 °C | –10 °C | –5 °C | 0 °C | 5 °C | 25 °C | 40 °C | 50 °C |
| α-pinene | 0.79 | 0.81 | 0.86 | 0.92 | 1.31 | 1.13 | 0.98 | 0.84 |
| β-myrcene | 1.15 | 0.68 | 1.34 | 1.39 | 1.52 | 1.46 | 1.44 | 1.42 |
| α-caryophyllene | 2.01 | 1.98 | 2.41 | 2.28 | 2.83 | 2.46 | 2.15 | 2.13 |
| limonene | 0.63 | 0.58 | 0.65 | 0.72 | 0.86 | 0.74 | 0.71 | 0.68 |
| copaene | 0.81 | 0.85 | 0.51 | 0.86 | 0.99 | 0.85 | 0.76 | 0.74 |
| β-caryophyllene | 0.82 | 0.83 | 0.98 | 1.38 | 1.67 | 1.42 | 1.39 | 1.37 |

experimental results are given in Fig. 2. The analysis by GC–MS of the recovery of the essential oils showed that less essential oil compounds were extracted when the operating pressure was lower than 77 bar. Therefore, the upper limit for the pressure level was set to 165 bar, since the latter increase of extraction pressure results in extraction of less volatile compounds. Using higher supercritical $CO_2$ densities (pressure 100–200 bar) terpenes and oxygenated terpenes are completely soluble in the supercritical fluid, however other less non-volatile compounds such as fatty acids and waxes can appear in the extract. However, knowledge on solubilities of the pure compounds is encapsulated in the complex biological matrices such as plants and plants cells. In this case, the equilibrium between the solid and fluidic phases should be established.

Table 2 presents the effect of pressure on recovery of essential oils when six various extraction pressures of supercritical $CO_2$ and the constant of extraction trap temperatures (50 and 5 °C, correspondingly) are used. According to the results obtained, at optimal pressure of 85 bar the highest extraction recovery of the main oil components was received.

### Determination of optimal extraction time

Amounts of the active substances are dependent on the static and dynamic extraction time. Static extraction is used to soak and swell the matrix and prepare it for efficient dynamic extraction, when supercritical fluid is flushed via sample at a programmed flow rate (1 ml/min). In the experiments, static extraction was programmed for 2 min. It is estimated that the active substances have a greater diffusion

Table 2. **Dependence of relative content of the main components in essential oils from hop "Marynka" on extraction pressure**

| Compound | Essential oils (%) at different pressure | | | | | |
|---|---|---|---|---|---|---|
| | 77 bar | 85 bar | 105 bar | 120 bar | 140 bar | 165 bar |
| α-pinene | 1.23 | 1.32 | 0.96 | 0.98 | 1.13 | 1.12 |
| β-myrcene | 0.92 | 1.71 | 1.48 | 1.45 | 1.52 | 1.41 |
| α-caryophyllene | 1.98 | 2.49 | 2.13 | 1.65 | 2.48 | 1.53 |
| limonene | 0.41 | 0.63 | 0.54 | 0.56 | 0.61 | 0.53 |
| copaene | 0.52 | 0.81 | 0.71 | 0.61 | 0.68 | 0.64 |
| β-caryophyllene | 0.54 | 0.82 | 0.69 | 0.72 | 0.70 | 0.67 |

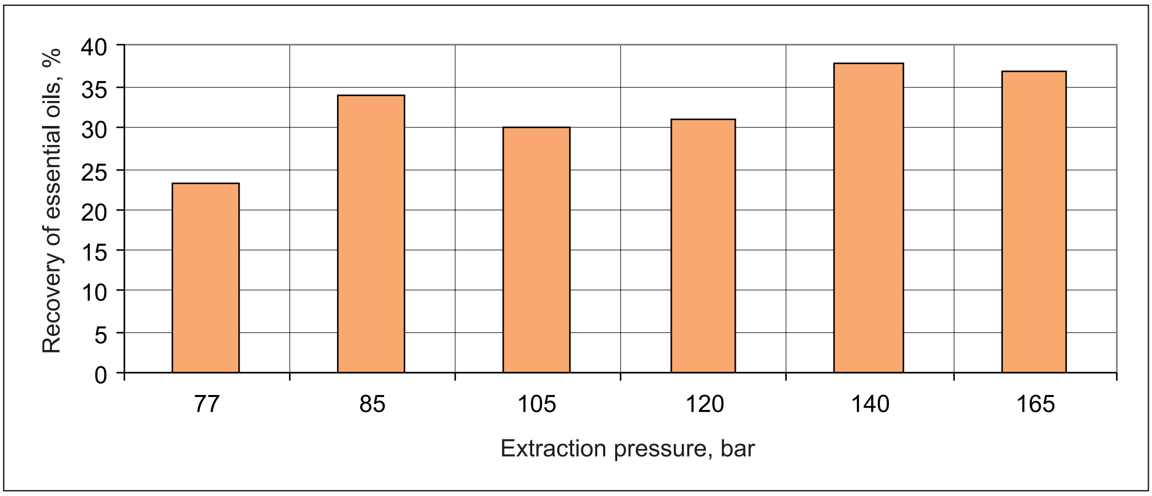

**Fig. 2.** Influence of extraction pressure on essential oils recovery

rate than the ballast materials. The first portion of the extract, obtained during the dynamic extraction, is composed of more active substances. It is important to maximize the contact of the supercritical fluid solvent with the sample material in order to increase the recovery of essential oils and enhance the efficiency of SFE. The influence of the dynamic extraction time on the composition of the essential oils was studied by performing dynamic extraction for 2, 5, 10, 15 and 20 minutes. The extraction time has a nonlinear effect on the oil extraction recovery. While extraction time was longer (15 min), the negative effect also became notable. Further increase in extraction time resulted in a little change in the yield of oil extraction. The optimal time of dynamic extraction was 10 min. There was an appreciable interaction between the temperature and extraction. At low temperature values (from –20 °C to 0 °C), the extraction time was 3.2 times longer when the essential oil extraction recovery reached the highest amount. At higher temperature of trap (5 °C), the extraction time was 10 min when the essential oils recovery reached the highest quantity. The optimal conditions using supercritical carbon dioxide extraction of hops were the following: dynamic extraction time 10 min, trap temperature 5 °C and 85 bar pressure of the supercritical carbon dioxide. The resulted main essential oil compounds are shown in Fig. 3. Using GC–MS method, α-caryophyllene (2.89%), α-pinene (1.61%), β-myrcene (1.81%), limonene (0.95%), copaene (1.15%), β-caryophyllene (1.69%) were determined.

## Identification of essential oil components in "Marynka" hop

The composition of hop essential oil is an important tool for evaluation of hop quality. As each hop variety has a typical essential oil pattern (fingerprint), hop essential oil analyses can be used to distinguish between the hop varieties. Hops essential oil represents a small, volatile fraction of hops (0.5–3.0% v/w), in which ca. 300 compounds have been positively or tentatively identified [7, 10]. The complex mixture can readily be separated into a hydrocarbon fraction (40–80% of the total oil) and an oxygenated fraction [11]. Three or four major compounds are prominent in the hydrocarbon fraction, the monoterpene β-myrcene and the sesquiterpenes β-caryophyllene, α-humulene, and, sometimes, β-farnesene [10]. The most aroma hops contain relatively high levels of α-humulene and low levels of β-myrcene. α-Humulene is being held responsible, at least in part and in combination with a number of other constituents of the essential oil, for the pleasant smell of hops. β-Myrcene and the sesquiterpenes are relatively reactive, and, thus, a great variety of oxidized terpenes can be found in the oxygenated fraction.

It is important to notice that many constituents of the hop essential oil are lost or transformed during the brewing process. It has been shown that the hoppy character of beer is mainly due to oxygenated terpenoids. Sesquiterpene hydrocarbons are very susceptible to auto-oxidation, whereby mainly epoxides are formed, such as β-caryophyllene epoxide and α-humulene-1,2-epoxide [12].

The GC–MS method was used to determine the qualitative and quantitative composition of the obtained hop extracts. Fig. 4 shows a typical chromatogram of hop "Marynka". This chromatogram has been obtained after optimizing the separation conditions. The appearance of the chromatogram demonstrates the complexity of the extract. The compounds of hop "Marynka" were identified by means of retention times, which allow calculating Kovats retention indices and MS spectra compared to that in the mass spectra library of the pure compounds. The reproducibility of

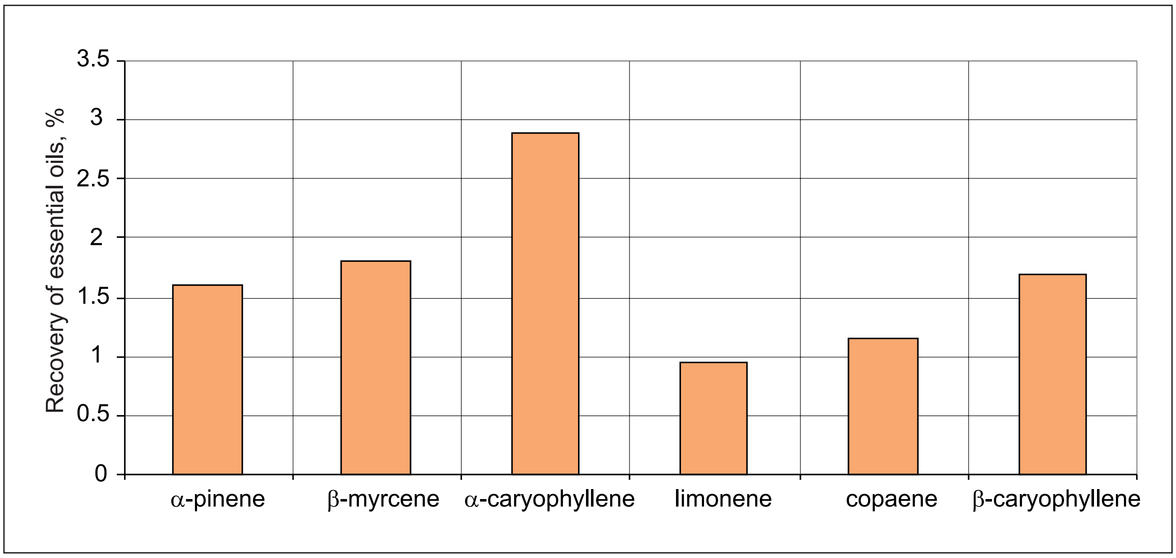

**Fig. 3.** Quantitative composition of the essential oil main compounds of hop "Marynka" at the optimum extraction conditions

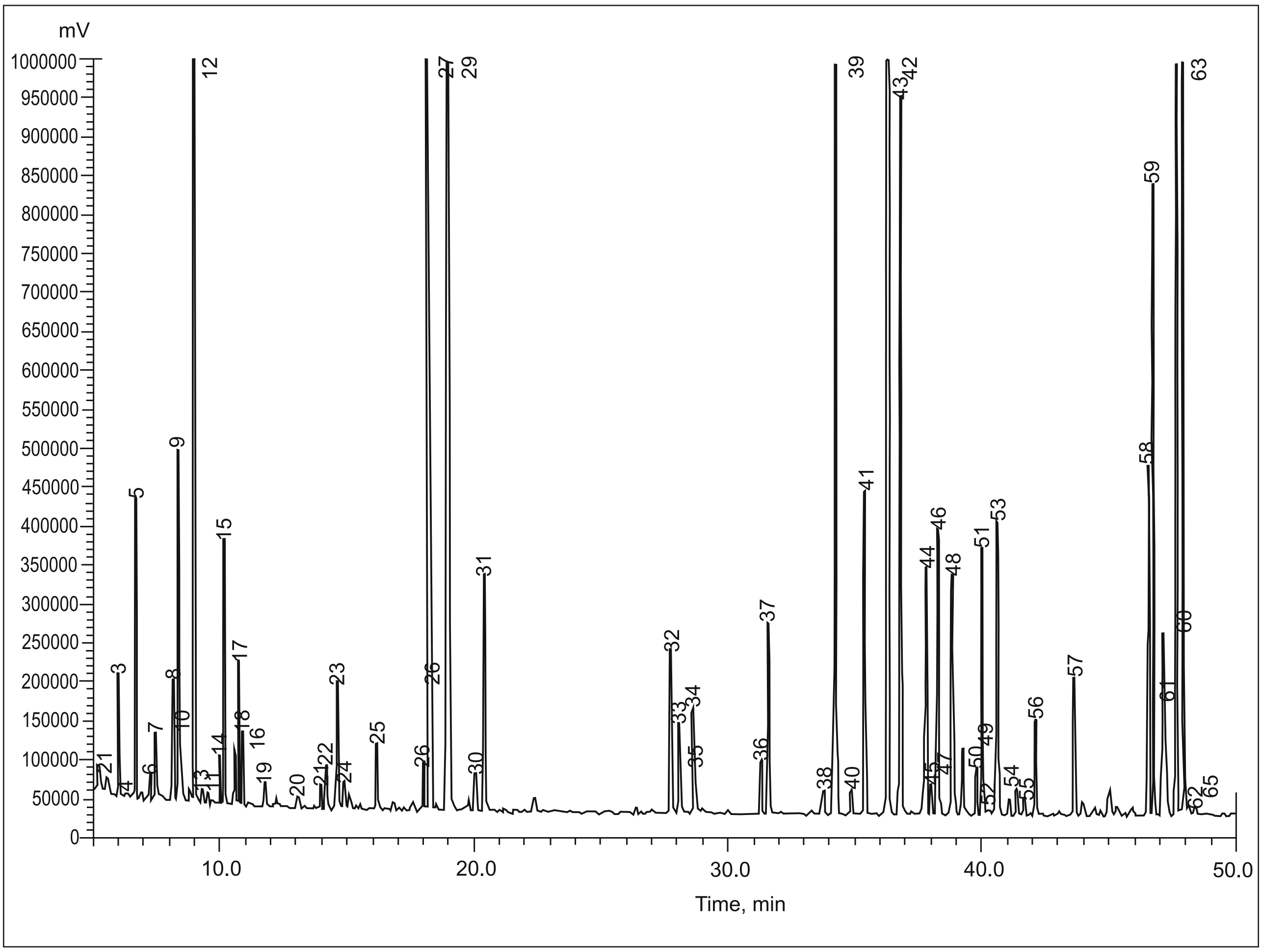

**Fig. 4.** The main essential oil components of hop "Marynka": *1* – α-pinene (921), *2* – β-myrcene (998), *3* – limonene (1028), *4* – humulene epoxide (1310); *5* – α-humulene (1349), *6* – β-ylangen (1370), *7* – copaene (1374), *8* – cubebene (1392), *9* – β-caryophyllene (1410), *10* – α-caryophyllene (1450). Other identified compounds: 2-methylpropyl-2-metilpropionate (1055), 2-nonanone (1470), 2-decanone (1550), 1-pentanone (670), α-cubebene (1590), , α-muurolene (1470), humulene epoxide (1310), β-cadinol (1650), β-farnesene (1450), γ-gurjunene (1160); (in brackets-the calculation of retention index)

the method, performed under optimized conditions, was determined analyzing six samples of the above-mentioned "Marynka" hop variety using a trap temperature of 5 °C, extraction pressure 85 bar, extraction time of 10 min and a sample amount of 0.5 g. The relative standard deviations of relative peak areas exceeding 1% ranged from ±3 to ±5%, and for the peaks in the range between 0.1 and 1%, from ±6 to ±9%.

## CONCLUSIONS

According to our results, the optimum conditions of SFE for the recovery of hop "Marynka" essential oil compounds were: extraction pressure 85 bar, trap temperature at 5 °C and dynamic extraction time 10 min. Based on mean value, it can be shown that the effect of extraction variables on the extraction recoveries decreased in the following order: pressure, trap temperature and extraction time. The extraction pressure played a dominant role in the recovery of the essential oils, while the effect of the dynamic time was less critical. Under the optimum conditions, the highest content of essential oil compound α-caryophyllene was 2.89%. The identification data of the essential oil compounds, obtained by GC–MS in this study, clearly revealed that trap temperature at 5 °C is the most suitable for $CO_2$ extraction, in order to avoid thermal degradation of the sample and maximize the recovery of the essential oils. The essential oil from hop was extracted using the supercritical fluid extraction method in optimized conditions and analyzed using GC–MS. A total of 19 components was identified using similarity searches between the mass spectra obtained and the spectra in the MS database.

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## APYNIŲ ETERINIŲ ALIEJŲ EKSTRAKCIJOS SUPERKRITINIU $CO_2$ OPTIMIZAVIMAS

### *Santrauka*

Analizuojant įvairius junginius, labai svarbus yra bandinio paruošimas, todėl ieškoma efektyvaus bandinio paruošimo metodo, nepavojingo aplinkai, greito, pigaus, kuriam reikia nedidelio ruošiamo kietafazio bandinio kiekio.

Šiame darbe buvo atliktas „Marynka“ apynio veislės eterinių aliejų ekstrakcijos superkritiniu $CO_2$ optimizavimas. Optimizuoti parametrai: slėgis, gaudyklės temperatūra ir ekstrakcijos trukmė. „Marynka“ apynio veislės eterinių aliejų komponentai buvo analizuojami dujų chromatografijos metodu su masių spektrometro detektoriumi.

Tyrimais nustatyti lakiųjų junginių, eterinių aliejų komponentai: α-pinenas, β-mircenas, limonenas, humulenas, α-kubebenas, β-kariofilenas, kopaenas, α-kariofilenas.

Daugiausia „Marynka“ apynio eterinių aliejų gauta ekstrakcijos superkritiniu $CO_2$ metodu, kai gaudyklės temperatūra buvo 5 °C, ekstrakcijos slėgis – 85 bar, ekstrakcijos laikas – 10 minučių.

**Raktažodžiai:** ekstrakcija superkritiniais skysčiais, eteriniai aliejai, apyniai, optimizacija, dujų chromatografija-masių spektrometrija