

Розкрито основні сучасні методи виділення терпенів, вказано переваги та причини цього технологічного перероблення ефірних олій, зокрема лимонної. Якісний склад і кількісні співвідношення компонентів лимонної ефірної олії визначено газохроматографічним методом на насадковій колонці (нерухома фаза – динонілфталат). З даних хроматографічного профілю з'ясовано, що ключовим компонентом олії є монотерпен d-лімонен (70,60±4,61%), що маскує гармонійний прояв лимонного аромату.

Представлено результати теоретичних розрахунків вибіркового фракціонування під вакуумом, що відпрацьовані на лабораторній установці фракційної перегонки ефірних олій, яка моделює повний цикл фракціонування. Основні вузли установки узгоджені із схемами універсальних установок, поширених в промисловості.

Наведено відпрацьовані на установці режими відбору терпенової та терпеноїдної фракцій, а саме: значення граничних та робочого флегмового числа, тиску та діапазонів температур кипіння.

Фракція терпенів виділена при температурі куба від 67 °C до 70 °C при тиску 2,64 кПа та флегмовому числі 1:3 становить 30,0 % мас. Фракція терпеноїдів виділена при температурі куба від 84 °C до 96 °C при залишковому тиску, що зменшується від 0,66 до 0,33 кПа та флегмовому числі 1:14 становить 60,0 % мас. У результаті органолептичного дослідження стало зрозуміло, що терпенова фракція має лимонний запах, за рахунок високого вмісту (80,0 %) лімонену з нотами бергамоту (β-мірцен – 5,12 %). Наявний серед терпеноїдів цитраль вносить в аромат мускатний тон, який разом з гераніолом і ліналоолом набуває шляхетного аромату цитрусу з тонкою квітковотою нотою.

Всі фракції ефірної олії доцільно використовувати у харчовій та парфумерно-косметичній технологіях, оскільки фракція терпенів може стати цінним компонентом у створенні оригінальних композиційних ароматів

Ключові слова: лимонна ефірна олія, фракціонування, детерпенізація, газова хроматографія, насадкова колонка, лімонен

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SEPARATION OF TERPENES FROM LEMON ESSENTIAL OIL BY SELECTIVE FRACTIONATION UNDER A VACUUM

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1. Introduction

Essential oils (EO) are almost not used in the raw form by modern industry, they are exposed to various technological treatments. One of the reasons is considered to be the oxidation and polymerization of their components, namely terpene hydrocarbons, under the influence of oxygen in the air, moisture, heat, and light. This, in turn, contributes to an increase in the viscosity and the plane of polarization, to a change in color, to deterioration of aroma and taste of EO.

The requirements by consumers contribute to the implementation of new principles for prolonging the shelf life, to the refinement and elaboration of new methods and procedures for studying the factors that influence the quality of a product. Such a technological tool is the selection of the fraction of terpene hydrocarbons – deterpenation.

Selective fractionation is one of the few techniques to implement the deterpenation of EO that makes it possible to quickly and accurately separate a multi-component mixture into fractions at its minimal losses. However, to this end, it is necessary to conduct theoretical calculations, and then specify the limits of technological modes of operation, which

in turn requires skilled workers and leads to the unjustified losses of EO.

2. Literature review and problem statement

The deterpenation of EO positively affects their application in the perfume and cosmetic industry. Paper [1] specifies the advantages of terpene-free EO, namely: an intense aroma, the absence of bitterness characteristic of a natural oil, prolonged storage life.

Terpene-free EO require much smaller consumption of alcohol in their further processing. For example, the citrus EO, as reported in work [2], contain up to 90 % of monocyclic terpene – limonene, which is practically insoluble in aqueous medium and requires a significant amount of alcohol.

Worth noting additionally is the benefit of deterpenation in the production of soft drinks. The citrus EO with a significant content of terpene hydrocarbons leave a sediment while dissolving in water or in a water-alcohol solution, which is hard to separate from the product even by modern systems of filtration. In addition, the use of terpene-free fractions of EO makes it

possible to avoid the difficulties associated with the instability of the colloidal system of thick drinks. According to data from experts [3], the deterpenized lemon EO is 25...30 times more aromatic than the natural oil. A separated fraction of terpenes has its own flavor and is used in the perfumery-cosmetic industry and to flavor medicinal remedies.

The pine and citrus EO are mostly exposed to deterpenation. In addition, terpene-free EO are obtained from bergamot, lime, orange oils. A study into a terpene-free bergamot EO, reported in paper [4], confirms its special value by a wide use in the production of elite perfume products, beverages, confectionery, meat products.

According to modern knowledge about their chemical composition, which is described in study [5], terpene hydrocarbons are represented by aliphatic compounds, which are characterized by the presence of three double bonds and cyclic compounds that can contain one, two, or three cycles in a molecule. These are myrcene, ocimene, d-limonene, α , β -pinenes, α , β -phellandrenes, sabinene, etc., as well as the representatives of sesquiterpenes – bisabolene, caryophyllene, which is contained by the EO of black currant buds.

Known deterpenation techniques can be combined into the following groups:

- fractional distillation by atmospheric pressure or under a vacuum;
- fractional extraction, including by liquefied gases (CO₂, chladones);
- adsorption of non-volatile/volatile components on a neutral carrier;
- chemical treatment with the selection of terpenes in the form of solid sediment.

Of growing popularity are the techniques of deterpenation by extracting a terpene fraction with supercritical carbonic acid (CO₂-extraction). The advantage of using supercritical liquids is the fractionation at low temperatures. Control over a change in temperature or pressure enables a selective fractionation [6]. The result is achieved through the adsorption of terpenes on a solid polar adsorbent, for example on silicon acid at a temperature of 18 °C, followed by the selection of monoterpenes and terpenes [7].

Another deterpenation technique, whose results are described in paper [8], is the fractional extraction by liquid extragents. In this case, the authors use a pairwise extraction, that is, employing different solvents that are not mixed together. One solvent easily extracts terpenes (petroleum ether, pentane) and the other one – oxygenated components (methanol, ethanol). When these solvents mix with EO, two layers form: one is composed mainly of terpenes, the other one – terpenoids. The terpenoids that are in methyl alcohol are removed with water, followed by drying.

The selection of terpenes by fractional distillation under atmospheric pressure was described in detail in work [9]. However, this technique does not always yield the expected results because a series of oxygen-containing components of EO with close boiling temperatures also penetrate the terpene fraction. In addition, under atmospheric pressure, there occurs a partial osmolation or even decomposition of highly-boiling terpenes (α -pinene, α -phellandrene, caryophyllene and β -myrcene). Therefore, the fractional deterpenation is most often carried out under a vacuum.

Studies into EO, reported in papers [9, 10], established that at a temperature of distillation in the range of 105...250 °C there is a possibility for the decomposition of terpenes with the formation of volatile sesquiterpene lactones, which significantly

deteriorates the flavor of EO. The reason for this could be the improperly chosen pressure and the reflux number, which is associated with the large costs for the execution of residual modes at an industrial installation.

The solution to this problem is proposed in paper [11] by using a laboratory installation for selective fractionation that makes it possible to reduce the consumption of raw materials, time and money, in order to establish the optimum modes of deterpenation. However, determining technological regimes for the selection of a terpene fraction of lemon EO at the laboratory installation also requires preliminary theoretical calculations and their further verification. Therefore, the present research deals with the procedure for establishing the technological modes of selective fractionation of lemon EO.

3. The aim and objectives of the study

The aim of this study is to establish technological modes for the selective fractionation of lemon EO in order to separate the terpene fraction.

To accomplish the aim, the following tasks have been set:

- to identify the components of lemon EO using a gas chromatography method;
- to calculate and set the limits of technological modes for the deterpenation of lemon EO at a laboratory installation;
- to analyze the obtained fractions of lemon EO.

4. Materials and research methods

The quantitative and qualitative composition of lemon EO was investigated using a procedure of gas chromatography analysis of raw materials and products in the technology of fragrances at the packed column with stationary phase 20 % dinonylphthalate [11]. The advantage of using a packed column in our research is explained by the possibility of introducing a sample to the chromatograph in the amount of 5...10 μ l. In addition, the packed column, compared to capillary, has a wider choice of the stationary phase [12].

We have tested the theoretically calculated modes for the deterpenation of lemon EO at a laboratory installation for fractional distillation (IFD), which was described in detail in earlier papers [13]. A laboratory IFD simulates a full cycle of EO separation into fractions and includes the following sections – a rectification node, a pump area, a system to supply raw materials and to collect fractions, as well as an industrial automatic system to control the process. The basic nodes of the laboratory installation are aligned with the circuits of universal installations for the fractional distillation IFD, common in the industry. That ruled out the need for a special design of the installation.

5. Results from the deterpenation of lemon EO through selective fractionation

5.1. Identification of the component composition of lemon EO using a gas chromatography method

Lemon EO is obtained by pressing fresh peel of lemons in the amount of 0.4...0.6 %. It is a transparent or slightly greenish liquid with a smell of lemon and a pleasant bitter aftertaste. Given the high content of terpenes, EO quickly oxidizes that gives rise to the smell of turpentine, which sig-

nificantly hinders the expression of a pleasant citrus scent. In addition, the oil becomes more viscous, darkens, its physical and chemical characteristics change.

The negative effects of EO storage, specifically lemon oil, can be eliminated either during its production or after the term of storage through selective fractionation. However, in order to establish the technological regimes of this process, one needs to know the chemical composition of EO, which is a multicomponent mixture. The optimal method of analysis in this case is a high-performance gas-liquid chromatography that employs capillary columns, which provide for the maximum separation of the sample and the identification of its components. However, the sample volume can vary from 0.01 to 0.05 μl , which complicates studying the quantitative composition of aromatic substances in food products.

The highly efficient packed columns make it possible to obtain the clearly separated peaks with an optimum width of foot, in order to reliably compute the quantitative ratios between sample's substances. That contributes to the clear registration of small chromatographic peaks by the detector. We identified the component composition based on the results from a gas chromatography analysis (Fig. 1) and verified it by the mass-spectra, optical isometry.

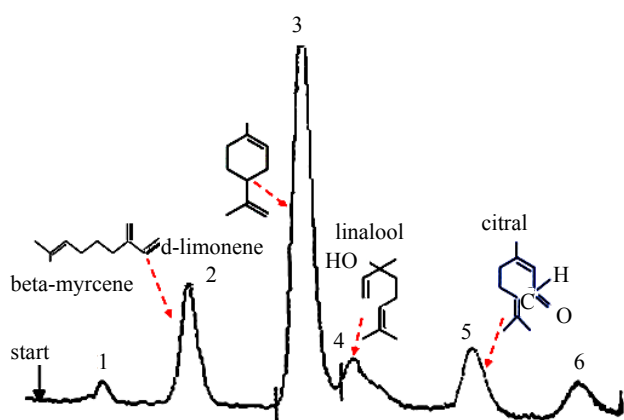


Fig. 1. Chromatogram of lemon EO

Table 1 gives the qualitative composition and quantitative ratios between the ingredients of lemon EO. We identified the component composition of oil based on the relative time of aging. The results were obtained from a series of parallel distillation and refined based on Kovach indexes.

Table 1

Quantitative ratios of ingredients in lemon EO

No. of entry	Component name	Mass fraction, %
1	α -pinene	1.40
2	β -myrcene	3.80
3	d-limonene	80.40
4	linalool	4.04
5	citral	7.12
6	geraniol	3.24

Note: the average values are given at probability level $p=0.95$

It was established based on data from the chromatographic profile that the key component of lemon EO is monoterpene d-limonene, as its amount is the largest – 80.40 ± 4.61 %. It is the high content of terpenes that disguises the harmonious manifestation of a lemon flavor.

5.2. Calculation and refinement of deterpenation modes of lemon EO at the laboratory IFD

As noted above, the selective fractionation, implying the selection from the component composition of EO of fraction of terpenes, would ensure the stability in its quality indicators. To implement a given technique, one should theoretically calculate the deterpenation modes of lemon EO.

Terpenes demonstrate different volatility that depends on pressure of the saturated vapor and the boiling temperatures. These indicators relate the structural construction of terpenes and their physical and chemical properties to the thermodynamic parameters for fractional deterpenation.

A difference of boiling temperatures for terpenes in a vacuum can be significantly greater than that at atmospheric pressure. The anchor points of equilibrium pressure at the stages of lemon EO fractionation are found from the dependence of boiling temperature of the components on the respective pressure of saturated vapor. Based on experimental data, a significant number of substances that boil under atmospheric pressure at a temperature of 250°C and above with decomposition are distilled without changing the structure under pressure of 1.33 kPa and a temperature of $160\text{...}210^\circ\text{C}$, or within the temperatures from 100 to 130°C under pressure 0.00133 kPa, or at temperatures from 40 to 60°C in a vacuum.

Data on the dependence of saturated vapor pressure of terpenes on boiling temperature are limited in the information field and, in a particular case, should be refined based on a Miller correlation, an Antoine equation [14]. One can use the diagram by Capson and Frolich, built in the $\lg P-1/T$ coordinates for the lower hydrocarbons [15], etc.

The minimum number of theoretical plates during separation of lemon EO (n_{\min}) was established based on the equation by Fenske-Underwood [16] for all possible equivalent EO subsystems; the maximum value out of the derived values was accepted as n_{\min} for the entire system.

We implemented a given technique at the laboratory installation during trial fractionation. A working reflux number (V) was determined from practical tests between the values of the boundary conditions for distillation V_{\min} and V_{∞} . In this case, we established the optimal values for residual pressure and temperature intervals of fraction selection. The resulting modes for the selective fractionation of lemon EO are given in Table 2.

Table 2

Technological modes for the deterpenation of lemon EO

Stages at deterpenation	Temperature, $^\circ\text{C}$		Pressure, kPa	Reflux number	Content, % by weight
	cube	head			
Heating a column	50...65	14...15	2.64	∞	–
Terpene fraction	67...70	17...19	2.64	1 : 3	30.0...32.0
Terpenoid fraction	84...96	30...36	0.66...0.33	1 : 14	60.0...62.0
Cube balance	115...127	–	0.33	–	4.55 ± 0.5

Note: losses amount to $2.0\text{...}6.0$ % by weight.

The result is the two obtained fractions – terpenes and terpenoids, as well as cube balance with a total content of $94\text{...}96$ % by weight. Collected fractions are drained from the receiver into a sealed container made of dark glass and stored.

5. 3. Analysis of the obtained fractions of lemon EO

The qualitative and quantitative composition of EO fractions was determined using a gas chromatography method at the packed column (20 % dinonylphthalate) under conditions that have been devised for EO. The results obtained from a series of parallel distillations are given in Table 3.

Table 3

Qualitative composition and quantitative ratios between components of lemon EO fractions

Terpenes – 30...32 % by weight		Terpenoids – 60...62 % by weight	
Component	Content, %	Component	Content, %
α -pinene	2.30	citral	20.28
β -myrcene	5.12	l-linalool	17.36
d-limonene	80.0	geraniol	14.75
Losses	4.50	d-limonene	42.00
Cube balance	4.55	Losses	5.67

The qualitative composition and quantitative ratios between components of fractions confirm the effectiveness of deterpenation with the selection of a terpene fraction (up to limonene inclusive) and a terpenoid fraction, which accounts for more than 60 % of the total composition of lemon EO. The data from a gas chromatography are useful when predicting the aroma of the fraction, when designing new fragrances [17].

The industrial application of reliable data on the aroma of EO components is the key to create a variety of aromatic compositions with stable characteristics. That also relates to the flavor of food products, as well as the creation of perfumes. Table 4 gives the results from an organoleptic analysis of the obtained fractions.

Table 4

Organoleptic indicators for the products of lemon EO deterpenation

Indicator	Lemon EO	Fraction	
		Terpenes	Terpenoids
Production technique	Distillation	Fractional distillation	
Physical appearance	Movable liquid, light yellow to yellow color		
Aroma	lemon	lemon	lemon-muscat
Taste	From bitter to sweet-bitter		

The fraction of terpenes has a lemon scent, and the content of β -myrcene adds a note of bergamot. Citral that exists among terpenoids introduces a muscat tone to the aroma, which, together with geraniol and linalool, acquires a noble aroma of citrus with a fine floral note.

All EO fractions are expedient to use in the technology of fragrances since the fraction of terpenes could be a valuable component in creating original composition fragrances. This has a positive aspect as the separated fraction of terpene is successfully utilized in the chemical, cosmetic, food, and

other industries. For example, to obtain carvacrol – an active natural bactericidal substance of phenolic nature.

6. Discussion of results of the deterpenation of lemon EO through selective fractionation

The expediency of the performed deterpenation of lemon EO relates to the separation of a fraction of terpenes from its component composition, which are easily oxidized thereby acquiring a foreign smell, which significantly hinders the expression of a pleasant citrus scent and worsens the physical-chemical characteristics. In contrast to common techniques, the proposed technique for selective fractionation makes it possible to separate the fraction of terpenes.

The study conducted proves the manageability of EO deterpenation modes taking into consideration different volatility of the components. Preliminary calculation and practical implementation of EO deterpenation at a laboratory setup makes it possible to: establish clear limits for the technological regimes of fraction separation, to determine the quantitative ratios between their components, and to predict the aroma. The obtained fractions of lemon EO are natural concentrated flavorings of stable quality for use in various industries. It should be noted that limonene, as a key component of citric EO, is included in both fractions, however, it dominates the first. To completely separate limonene, it is necessary to repeat the fractionation of the second fraction (if necessary).

However, even a partial separation of d-limonene and the complete removal of unstable α -pinene and β -myrcene from the fraction of terpenoids increases the shelf life of EO. Determining the term of EO storage, as well as products of its processing, requires further research and additional experimental data about quality loss by a finished product over time.

7. Conclusions

1. We have identified the qualitative composition and quantitative ratios between components of lemon EO. The key component is the monoterpene d-limonene in the amount of 70.60 % \pm 4.61.

2. Using a laboratory installation for the fractional distillation, we have defined the technological modes for selective fractionation of the fraction of terpenes in the amount of 30.0 % by weight, namely: a cube temperature is 67...70 °C, pressure is 2.64 kPa, the reflux ratio is 1:3. The fraction of terpenoids in the amount of 60.0 % by weight is separated at a cube temperature of 84...96 °C, under residual pressure that decreases from 0.33 to 0.66 kPa and at a reflux ratio of 1:14.

3. The results of an organoleptic study have shown that the terpene fraction has a lemon scent, due to the high content of limonene (80.0 %) with notes of bergamot (β -myrcene, 5.12 %) Citral that exists among terpenoids introduces a muscat tone to the fraction's aroma, which, together with geraniol and linalool, acquires a noble aroma of citrus with a fine floral note.

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