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SPECTROPHOTOMETRIC AUTHENTICATION IMAGES OF HOPS ESSENTIAL OILS

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Abstract

Hop essential oils are natural, useful substances enriched with biologically active terpene complex. They possess attractive nutritional and energy-stimulating properties and can positively affect the immune-supporting functions. However, they are at risk of falsification, necessitating the development of competent identification methodologies. This research aimed to create methodological, analytical, and informational bases for hop oils to study the possibility of their identification and control against falsification by spectrophotometry. Essential oils of bitter and aromatic hop types were analyzed. The quality and safety parameters of the oils met regulatory requirements. The optical density spectra of hop oil were evaluated and compared in the light absorption wavelength range from 190 nm to 900 nm. It has been established that the identification spectrophotometric images of optical density, when a light beam is transmitted through hop oil samples, discretely, with a wavelength step of 0.05–1 nm, form peaks of total terpene absorption in the ranges of 350–620 nm. These are the main criteria for the formation of spectrophotometric databases of hop oils. Spectrophotometric images and pilot authentication models were developed for high-quality samples of bitter and aromatic hop oils. These findings contribute to the organoleptic authentication of hop oil spectrograms to prevent falsification.

Keywords: essential oil; hops; spectrophotometry; identification; terpenes; quality.

СПЕКТРОФОТОМЕТРИЧНІ АУТЕНТИФІКАЦІЙНІ ОБРАЗИ ЕФІРНИХ ОЛІЙ ХМЕЛЮ

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Анотація

Ефірні олії хмелю – натуральні, корисні речовини, збагаченні біологічно активним терпеновим комплексом, мають привабливі харчові та енергостимулюючі властивості, здатні позитивно впливати на імунопідтримуючі функції організму, але схильні до ризику фальсифікації та потребують створення компетентних методологій для ідентифікації. Метою досліджень було створення методологічної, аналітичної та інформаційної баз олії хмелю та вивчення можливості їх ідентифікації та контролю від фальсифікації методом спектрофотометрії. Для досліджень використовували ефірні олії гіркого і ароматичного типів хмелю. Показники якості та безпеки олій відповідали нормативним вимогам. Проведено оцінку та порівняння спектрів та змін оптичної густини олії хмелю в основних одиницях поглинання світла та у відсотках у діапазоні довжин хвиль від 190 нм до 900 нм. Встановлено, що ідентифікаційні спектрофотометричні образи оптичної густини під час пропускання променю світла через зразки олій хмелю дискретно, з кроком визначення довжини хвилі 0.05–1 нм, формують піки сумарного поглинання терпенів в діапазонах 350–620 нм. Це є основними критеріями для формування спектрофотометричних баз олій хмелю. Встановлені спектрофотометричні образи та створені аутентифікаційні пілотні моделі свідомо якісних зразків олій гіркого та ароматичного типів хмелю. Сформоване методологічне підґрунтя органолептичної аутентифікації спектрограм олій хмелю для запобігання фальсифікації.

Ключові слова: олія ефірна; хміль; спектрофотометрія; ідентифікація; терпени; якість.

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Introduction

Modern trends require manufacturers to focus on the need to reduce the risk counterfeit components, ingredients, additives, etc. in their technologies particularly when using essential oils.

Hop essential oils, enriched with biologically active terpene complex, possess beneficial nutritional and energy-stimulating properties and can positively affect the immune-supporting functions. However, they are prone to falsification. In addition, technological processes to restore oxidized or partially polymerized fractions are essential to maintaining regulatory quality and safety indicators [1]. The development of competent methodologies for essential oil identification improves food quality and ensures consumer safety.

The level of competence in the authentication of essential oils is mainly characterized by the analysis of enantiomers and other forms of stereoisomers related to their components – terpenes (monoterpenes, sesquiterpenes and their derivatives) and the subsequent classification of terpenes by their skeleton structures, oxidation nature, presence (absence) of a double bond, etc. Based on this, developing information libraries supports rapid terpene index calculations, countering sophisticated falsification tactics and allowing reliable judgments about essential oil authenticity [2].

An important stage of quality control is the methodology for identifying biologically active substances in essential oils, which allows determining the concentration of various components in it.

The comparison and classification of essential oils based on their chemical composition, total phenolic content, antioxidant, and antibacterial activity predominantly utilize chemometric methods for analyzing major components [3].

In most cases, gas chromatography, which determines different peak area ratios to accurately detect adulteration [4], voltammetric electronic sensors – voltammetric e-tongue [5] and mass spectroscopy [6] are used as an indicator of adulteration. However, some terpenes in essential oils cannot be identified solely through mass spectra. In such cases, libraries of terpenes with retention times and Kovacs indices are created, enabling reliable identification of more than 95 % of components in common essential oils [7].

The simplest and least expensive method to analyze the composition of essential oils is to use an optical (conventional) spectrophotometer. It allows you to find the appropriate spectral frequencies at which the absorbance is used as an indicator of concentration, while the absorbance and concentration of oil components are in a linear relationship and is the most representative [8].

Furthermore, the identity of essential oils as part of their biostructure is confirmed through infrared absorption spectrophotometry [9]. This method detects structural components characteristic of essential oils based on steric and electronic vibrational bonding effects (neighboring bonds act on the permanent force bond, changing the position of the absorption band). In this case, the identity of essential oils is verified by quantifying structural components and assigning characteristic absorption bands within measured spectrum. Comparing spectra by calculating correlation coefficients is a convenient, fast, and safe method for objectively confirming authenticity of essential oils [10; 11]. In addition, the vibrational infrared spectrum is considered a unique physical property of individual components. Thus, the infrared spectrum can be used for identification by comparing the spectrum from an “unknown” with previously recorded reference spectra [12].

Fourier-transform infrared spectroscopy (FTIR) is also used to determine essential oil compounds. It allows to detect key spectral bands characteristic of essential oils, corresponding to the vibrations of terpene groups (as the most important spectral bands), which can be used as marker bands for distinction and identification [13]. Unlike prism spectroscopes, which are calculated from the signal intensity, these spectral bands are calculated based on time- or space-domain responses [14].

Another effective method for identifying essential oil components is Raman spectroscopy, which significantly alters molecular radiation frequencies using optical radiation. Raman scattering introduces spectral lines absent in primary light spectra, determined by molecular structure. This method effectively supports chemical analysis, composition studies, and structural investigation. Combination scattering spectroscopy for the analysis and classification of the main components of essential oils, in which 96 % of the spectral variation is characterized by the first few components, allows us to identify significant similarities between essential oils and to identify “potential candidates” at risk of falsification of most commercially available substances [15; 16]. Raman spectral correlation methods also enable library formation for widespread surveillance applications, using adaptive spectral region determination for raw material identification and verification [17; 18; 19].

Experimental

For the study, we used serial equipment for obtaining and conducting spectrophotometric studies of hop essential oils.

Materials and equipment. Raw materials for the study included essential oils obtained via steam distillation from different varieties of bitter and aromatic hop types. A SECOMAM spectrophotometer with 10×10 mm quartz cuvettes and appropriate software was used. Additional equipment included a steam generator and a serial installation for the production of essential oil by steam distillation.

Experimental procedure. The essential oils, deliberately obtained as high-quality samples by the method of steam distillation from different varieties of bitter and aromatic hops, were vacuum-filtered. Filtered samples were alternately placed in 10×10 mm cuvettes and analyzed spectrophotometrically. A light beam was transmitted through the samples discretely, with a wavelength step of 0.05–1 nm, altering the wavelength from 350 to 900 nm. Spectrograms of optical density changes of oil samples from the wavelength were recorded, according to these spectrograms, spectrophotometric evaluation and comparison of optical density spectra of hop oils in the main units of light absorption and in percentage at a wavelength range from 190 nm to 900 nm.

At the same time, the obtained spectrogram was examined for the presence of individual links visually close to a straight line and graphical breaks, marking each of the breaks with a separate point and determining the coordinates of each individual point. Authentication involved visual comparisons of obtained spectrograms with reference spectrograms obtained during the study of a deliberately high-quality samples of hop oil (patent application for a 2024 02100, dated 22.04.2024).

Results and discussion

The basic aspects of each technological process require the creation of strong requirements for quality and safety indicators, in addition to cost-effective production. These requirements should focus not only on rational limit ranges, but also form a methodological and rational tool for their determination and control. The functioning of the proper control component to the final product allows to identify quality and separate falsified ones, which accordingly reduces consumer risk.

Modern technologies allow counterfeit oils to be produced using undeclared raw materials or chemically synthesized components, some of which fail to meet quality and safety standards. Authentication techniques counter these issues by verifying both products and individual components.

The aim of the research was to develop spectrometric databases for hop essential oils and investigate identification methods to prevent falsification. The main objective of the research was to form methodological, analytical and information databases of the hop oils for the development of an express method for the presence (identification) of foreign volatile aromatic substances by spectrophotometry. These experiments are based on the study of light absorption intensity spectra of hop oil. Considering that the hop oils optical density is the logarithm of light transmission, which is linearly dependent on terpene concentration, absorption intensity of incident light was determined at different wavelengths in the ultraviolet (200–400 nm), visible (400–760 nm) and infrared (>760 nm) regions of the spectrum. The oils of bitter and aromatic hop varieties were used for the study. Spectrophotometric studies were performed with base oils. According to the experiments, stable maxima are present in the spectra of the studied hop oils. The optical density of these maxima is the result of the total absorption of primary functional groups of essential oils – sesqui- and monoterpenes (acyclic monoterpene myrcene – $C_{10}H_{16}$, sesquiterpenes humulene $C_{15}H_{24}$, beta-caryophyllene $C_{15}H_{24}$, caryophyllene $C_{15}H_{24}$). A SECOMAM spectrophotometer with appropriate software was used for measurements.

Experimental studies were carried out both in the main units of light absorption and in percentage at a wavelength range from 190 nm to 900 nm with bitter and aromatic hop oils (Figs. 1, 2).

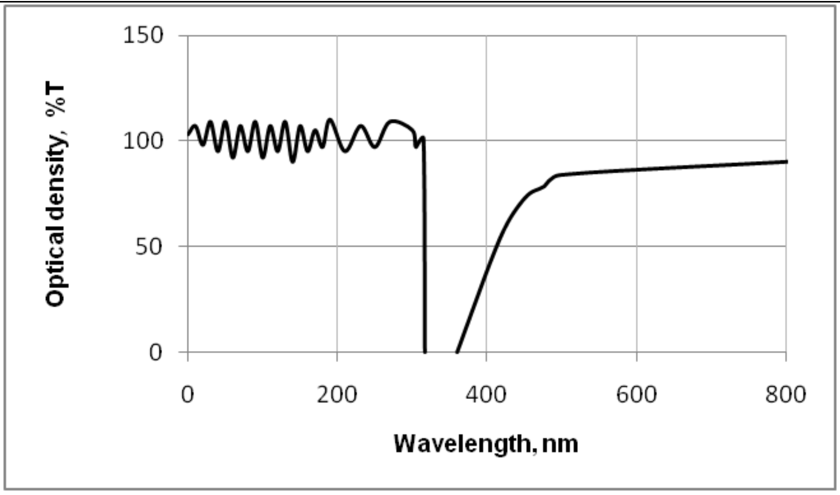

Fig. 1. Intensity of light absorption in bitter hop oil

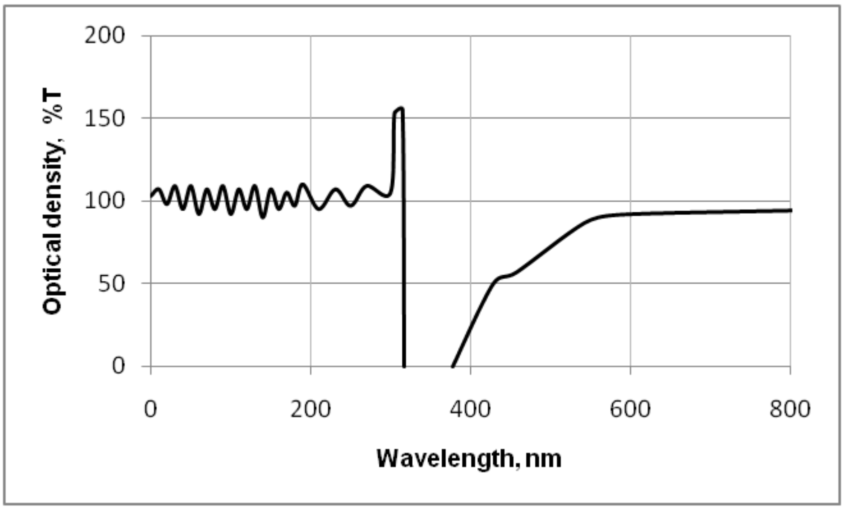

Fig. 2. Intensity of light absorption in the oil of the aromatic hop group

The results of the experiment prove that hop essential oils, regardless of raw material type, form stable and partially similar spectrophotometric image based on the light-absorbing activity of the main terpene compounds of hops (myrcene, humulene, beta-caryophyllene, selenenes, etc.). Minor descrepancies in the spectrograms of the studied samples can be attributed to internal fluctuations in the ratios of optical densities between mono- and sesquiterpenes. Moreover, oils from other hop varieties were similarly studied within narrower spectral ranges (through several repetitions), where spectrophotometric images were formed following the same principle of “similarity”.

The analysis of the optical density in essential oils of a single group (either bitter or aromatic) confirms the relevance of studying these oils in the context of varietal suitability of raw materials. Based on the findings, we have created a database of reference spectrograms for high-quality hop oil samples, categorized by the varietal suitability of Ukrainian-selected hops. These reference images aid in distinguishing oils obtained from blended raw materials within individual groups (bitter and aromatic) and establish stricter authentication criteria for hop oils as a whole.

To enhance the aspects of organoleptic identification, a general spectrophotometric image of a sample from the aromatic hops group was proposed to be segmented into discrete links visually resembling straight lines, with graphical breakpoints (intersections) forming visual markers (Fig. 3). Each breakpoint was marked as a distinct point with specific coordinates. Analyzing the placement of these informational links and points, when comparing the spectrogram of a high-quality reference sample with that of the research sample, allows the identification of organoleptic discrepancies (if present) and the formation of a qualified statement regarding the authentication characteristics of the hop oil sample.

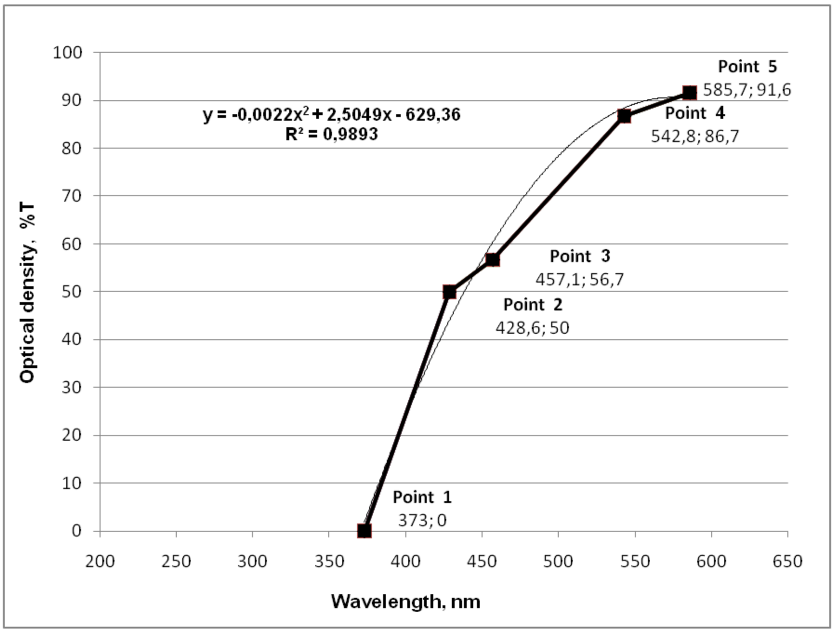

Fig. 3. Discrete links and visual breakpoints of their intersection of the spectrophotometric image of hop essential oil

Additionally, performing value approximation and trend line analysis confirmed that the derived function equation has a significant value of approximation reliability $R^2 = 0.9893$. This value indicates an optimal degree of trend model correspondence to the original data, validating the reliability of the experimental results. The approximation was used to derive the function equation $y = -0.0022X^2 + 2.5049x - 629.36$, whose coefficients also provide an opportunity to indirectly control changes in the light absorption intensity dynamics of hop oils, thereby reducing the risk of counterfeit components.

It is worth noting that all tested oils showed no differences in organoleptic characteristics and met the requirements of the regulatory documentation for hop essential oil FS 42U-2/303-1125-01 in terms of physicochemical requirements and the content of the main terpene compounds. Considering that the experiment was conducted in at least five replicates, the spectrograms presented reflect maximum negative values to provide comprehensive information on the priority of conclusions from spectrophotometric studies.

Conclusions

1. Based on the results of the study, comprehensive image-spectrophotometric databases for essential oils obtained from bitter and aromatic hop varieties were established.
2. Pilot image-models of bitter and aromatic hop oils with defined mathematical equations were developed, with defined mathematical equations whose coefficients improve indirect control of changes in the dynamics of light absorption intensity in the context of hop varieties, thereby reducing the risk of counterfeit components usage.
3. The potential for using image-spectrophotometric studies in authenticating hop essential oils has been substantiated.

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