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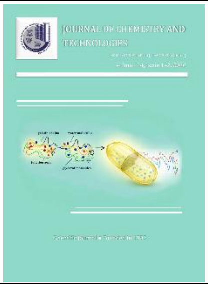

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QUALIFICATION SYSTEM FOR COMPREHENSIVE IDENTIFICATION OF HOP OIL

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Abstract

Modern technological capabilities are causing concern due to the creativity of approaches to the falsification of food products and their components, in particular essential oils. The struggle for quality and safety of end products calls on the global scientific community to improve and create more diverse methodologies with mandatory mathematical comparative analysis of the impact on the same system under changing influencing factors. The aim of the research was to create additional criteria for preventing falsification through spectrophotometric analysis of the intensity of light absorption by ethanol solutions of essential oils of bitter hops. Individual graphical-positional anomalies of wavelengths and spectral intervals were determined, and equations of functions of changes in the concentration of terpene complexes in the studied samples were calculated, coefficients were determined, and the error values were experimentally proven. A rational ratio (oil-ethanol) for dissolution without opalescence was empirically established. The spectra and changes in the optical density of hop oil were evaluated in basic units of light absorption and in percentages in the wavelength range from 190 nm to 900 nm discretely, with a wavelength determination step of 0.05–1 nm. Based on the study of known-quality samples, a wavelength range of 456–780 nm was established, at which the fluctuation in the intensity of the absorbing activity of the main terpenoid compounds facilitates the authentication of other samples of ethanol solutions of hop oils. In addition, fluctuations in the coefficients of mathematical systems for monitoring changes in light absorption intensity in ethanol solutions of hop oil provide an opportunity for comparative control of these changes and, accordingly, create additional criteria for preventing counterfeiting.

Keywords: ethanol extract; essential oil; hops; spectrophotometry; authentication; terpenes; quality.

КВАЛІФІКАЦІЙНА СИСТЕМА ДЛЯ КОМПЛЕКСНОЇ ІДЕНТИФІКАЦІЇ ОЛІЇ ХМЕЛЮ

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Анотація

Технологічні можливості сучасності викликають занепокоєння креативністю підходів до фальсифікації харчових продуктів та їх складових, зокрема ефірних олій. Боротьба за якість та безпеку кінцевих продуктів закликає світову наукову спільноту вдосконалювати та створювати більш різнопланові методології з обов'язковим математичним компаративним аналізом впливу на однакову систему за умови змінення чинників впливу. Метою досліджень було створення додаткових критеріїв запобігання фальсифікації завдяки спектрофотометричному аналізу величини інтенсивності поглинання світла етанольними розчинами ефірних олій гіркого сорту хмелю. Визначені окремі графічно-позиційні аномалії довжин хвиль, спектральні інтервали та розраховані рівняння функцій змін накопичення терпенових комплексів у досліджуваних зразках, визначено коефіцієнти та експериментально доведено значення похибки. Емпірично встановлено раціональне співвідношення (олія-етанол) для розчинення без опалесценції. Оцінку спектрів та змін оптичної густини олії хмелю в основних одиницях світлопоглинання та в відсотках у діапазоні довжин хвиль від 190 нм до 900 нм проводили дискретно, з кроком визначення довжини хвилі 0.05–1 нм. Базуючись на дослідженні свідомо якісних зразків встановлений діапазон довжин хвилі – 456–780 нм, за якого флуктуація інтенсивності поглинаючої активності основних терпенових сполук дозволяє аутентифікувати інші зразки етанольних розчинів олій хмелю. Крім того, коливання коефіцієнтів математичних систем спостереження за динамікою зміни інтенсивності поглинання світла в етанольних розчинах олії хмелю дають нагоду для компаративного контролю їх змін та, відповідно, створює додаткові критерії запобігання фальсифікації.

Ключові слова: етанольний екстракт; олія ефірна; хміль; спектрофотометрія; аутентифікація; терпени; якість.

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Introduction

Technological monitoring of the quality of hop processing products, in particular essential oils, is the basis for their safe use. Modern chemical and technological systems allow them to be synthesized (falsified) from non-natural compounds or by adding cheaper analogues of essential oils, synthetic materials, or diluting essential oils with vegetable oils [1]. This partially legalizes almost any similar component of essential and fatty oils that do not meet regulatory quality and safety standards, and, most unfortunately, this trend is becoming increasingly relevant. Modern counterfeiters have learned to circumvent some “first-pass” chemical tests by creating essential oils that are chemically identical at first glance [2] or by using closely related raw materials for the production of essential oils. Detecting authenticity and counterfeiting is a priority for various stakeholders, such as researchers, consumers, manufacturers, traders, and regulatory authorities [3]. The development of simple, rapid, and effective methods for identifying essential oils comprehensively summarizes the extreme importance of creating quality assessment methods that cover sensory evaluation, assessment of physical and chemical properties, and chemical composition analysis, which are of great importance for assessing the quality and detecting counterfeits of essential oils [4].

A creative approach to developing a comprehensive system for verifying authenticity at the level of using environmentally safe, natural ingredients will raise the bar for quality control of processed products in all consumer sectors. The analysis of existing quality control methodologies in relation to modern technological capabilities for falsification is a matter of critical importance for both consumers and chemical companies, and proves the need to create multi-vector authentication systems for controlling key quality indicators [5]. This proposal is initiated for implementation through the improvement and dissemination of existing methodologies at the level of forming more in-depth research principles and mandatory mathematical analysis of the impact of dependencies between product quality indicators and in the system as a whole. The formation of mathematical patterns, rapid calculation of arithmetic indices [6], the impact of certain chemical systems on the final product, the determination of their coefficients, and experimental proof of possible errors will create additional criteria for quality and safety control and significantly complicate the creation of synthetic analogues.

An example of a methodology for identifying essential oils is the study of a number of indicators: organoleptic (color, smell, transparency, etc.), physicochemical (acid, ester, and iodine numbers, density, solubility, angle of polarization, etc.) [7], content of main components (chromatographic, spectrophotometric study, etc.) and the reaction of the finished product's color to certain chemical systems. This list of control criteria is currently used for regulatory testing of essential oil quality, but it needs to be adjusted to converge with modern technological capabilities. Comparative studies of the properties of essential oils with ethanol extracts are becoming increasingly relevant. In most cases, the antibacterial, immune-protective, energy-stimulating, and other properties of essential oils have similar activity directly in extracts [8]. An important technological aspect is the ability of essential oils to dissolve in certain chemical systems (environments), including those that are permitted and safe for use in the food industry. Considering that the dissolution of essential oils in water leads to phase separation or requires the use of emulsifiers, the scientific community has researched various solvents. The evaluation and selection of the best co-solvents for improving the solubility and bioavailability of essential oils were studied in the presence of propylene glycol (PG), polyethylene glycol 300 (PEG), glycerin, and ethanol. In most of the samples studied, ethanol was determined to be the best solvent in terms of solubility and the total amount of essential oil compounds [9]. The above proves the need to propose, as an additional extension of the control criteria, ethanol systems of the dissolved product with subsequent spectrophotometric research and determination of the coefficients of mathematical equations of quality indicators. The selected ethanol base is justified by the criteria of low cost and safety compared to other solvents. Already at the first stage of the study, in the process of using a system of dissolved hop oil, we obtained spectrophotometric patterns that diverge from traditional findings. In addition, when determining the rational ratio (oil-ethanol), it is necessary to take into account the possibility of opalescence [10]. Some essential oils are completely soluble in one part of 70 % ethanol [11], while other authors reasonably argue that mixing oil requires more than one part of 70 % ethanol [12]. Of course, in order to predict and determine the optimal systems, it is necessary to take into account a number of complex factors: extraction temperature and time, oil-to-ethanol ratio, etc. A number of world scientists studying ethanol extracts suggest a rational ratio of volume parts in the oil-ethanol system of 1:15 [13]. Some authors argue for a wider range of ratios: 1:5; 1 : 10; 1 : 15 [14] or 1 : 10 [15; 16]. However, they all agree on the need for a preliminary experimental study and comparison of factors, as well as the properties of research systems, in order to establish the final optimal conditions for conducting the experiment [17].

A topical issue in establishing conditions and criteria for preventing falsification is the comparison of studies of ethanol compositions with freshly obtained hop oil and oil that has a long but guaranteed shelf life under generally accepted conditions, where the main factors are contact with air, storage time, temperature, and humidity [18]. Numerous studies have been conducted by scientists under conditions of sample storage in a refrigerator (4 °C), freezer (−20 °C), and at room temperature. The results showed that the composition of the oil showed the least changes and retained its initial quality when stored at low temperatures, in particular at −20 °C [19; 20].

Essential oils are often adulterated due to their widespread use and high profit margins, making accurate and precise authentication methods extremely important. These methods include NMR as a universal tool for the quantitative determination of plant oils that are potentially suitable for use as additives or diluents in essential oils. It is possible to determine the concentration of active ingredients, analyze the purity of substances, identify and quantify small molecules, especially in areas where standard materials are not available, and confirm the authenticity and purity of reference materials [21]. In the latter case, it is important to place special emphasis on non-destructive approaches, for which mass spectrometry and chemometric methods have become effective tools for authenticating EO and determining its origin [22]. Another relevant method for detecting and quantifying adulteration in essential oils is attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) combined with chemometric analysis [23]. In this case, it is possible to distinguish between the percentages of EO and impurities within the limits of 97.47 % of the total spectral dispersion, while to strengthen the requirements for counteracting counterfeiting, mathematical processing was performed – the root mean square error of the predicted value obtained from the external validation set and the coefficients of determination were determined. However, verification of the effectiveness of non-targeted approaches based on the synergistic combination of infrared spectroscopy with attenuated total reflection and Fourier transform and discriminant analysis of partial least squares for the classification of species and chemotypes demonstrates the widespread use of mathematical dependencies to create a rational model that provides a fast, intuitive, and practical method for distinguishing components to prevent the adulteration of essential oils [24; 25]. However, it is widely practiced to study a quantitative analysis model, which makes it possible to quickly determine the content of components in essential oil using the near-infrared absorption spectrum method. This involves analyzing spectral absorption peaks in the near-infrared range for the comparative effect of spectral filtering. Based on the analysis of the results of the quantitative model of essential oil components, the root mean square error of prediction (RMSEP) and the orthogonal signal conversion error (OSC) were determined. In addition, by excluding wavelength points (on the spectrogram) that have nothing to do with the composition of the determination or the existing nonlinear correlation, the partial least squares method of the direct interval was used, resulting in 8 spectral intervals, a total of 160 wavelength points, as a data set. By comparing the data sets optimized using OSC-FiPLS with the partial least squares (PLS) method, a rapid quantitative analysis model was created to compare and determine the content of components in lavender essential oil [26]. This opens up new creative opportunities for studying the spectrograms of essential oils for the presence of individual intervals (links) visually close to a straight line, and graphical breaks, marking each break with a separate point and determining the coordinates of each separate point, which will provide priority criteria for organoleptic authentication [27].

Experimental

For the research, mass-production equipment was used to obtain an ethanol solution and conduct spectrophotometric studies of hop essential oils.

Materials and equipment. The raw materials used to obtain the ethanol solution and conduct research were essential oils obtained by steam distillation from freshly harvested bitter hops of the Promin variety, an oil with a long shelf life but with a standard guaranteed shelf life when stored in dark glass, without access to air, at a temperature of –15 °C, whose quality indicators do not meet regulatory requirements. For the experiment, a SECOMAM spectrophotometer with 10×10 mm quartz cuvettes and appropriate software was used.

Experimental procedure. Essential oils obtained by steam distillation from high-quality samples of freshly harvested hops, oils with a shelf life of 1 year, and oils with substandard quality indicators of the bitter hop variety “Promin” were dissolved in ethanol in ratios of oil:ethanol 1 : 15; 1 : 10; 1 : 7; 1 : 5; 1 : 2 and filtered under vacuum; and the optimal ratio was determined to be 1 : 10. After filtration, the samples of ethanol solutions were placed in 10×10 mm cuvettes and examined spectrophotometrically. The light beam was passed through the samples discretely with a wavelength setting step of 0.05–1 nm, changing the wavelength from 190 to 900 nm, and a spectrogram of the ratio of the change in optical density of the oil samples to wavelength was obtained. Based on the specified spectrograms, a spectrophotometric evaluation and comparison of the density spectra of hop oils were performed in basic units of light absorption and in percentages in the wavelength range from 190 nm to 900 nm.

We studied the spectrogram obtained as described above for the presence of separate intervals visually close to a straight line and graphic breaks, marking each break with a separate point and determining the coordinates of each separate point [26]. Authentication was performed visually by comparing three spectrograms constructed during the study of deliberately high-quality hop oil samples obtained from freshly harvested hops, oil with a shelf life of 1 year, and oil with substandard quality indicators. We also determined the equations of functions based on the method of determining individual wavelength points (on the spectrogram) that form spectral intervals. We approximated the values and determined the lines and the reliability of the approximation, which characterizes the optimal degree of correspondence of the trend model to the initial data, i.e., one that has a high probability of experimental results. We analyzed the coefficients of the obtained equations for indirect control of changes in the dynamics of light absorption intensity in three hop oil samples and, accordingly, the reduction of the risks of using counterfeit components [27].

Results and discussion

An important factor in this idea is the possibility of creating closed technological information on quality control for enterprises that manufacture the final product. For example, a factory that manufactures food products containing essential oils conducts research on solutions with known-quality essential oils and determines its own concentration patterns in a closed information field. At the same time, it creates a mathematical system of dependencies, taking into account errors and possible changes in indicators during storage. Subsequently, when any material arrives, it is identified, and high-quality material is separated from low-quality material by comparing spectrophotometric studies and mathematical dependencies.

The aim of the research was to create additional criteria for preventing the falsification of hop essential oils through spectrophotometric analysis of the light absorption intensity values of ethanol solutions of essential oils of bitter hop varieties.

We conducted experimental studies, the results of which determined the spectrograms of ethanol solutions of hop essential oils obtained from freshly harvested hops, oils with a shelf life of 1 year, and oils with non-standard quality indicators.

We identified individual graphical-positional anomalies in wavelength and spectral intervals and calculated the equations of functions for three samples, determined the coefficients, and experimentally proved the magnitude of the error. We experimentally determined the rational ratio (oil-ethanol) for dissolution without opalescence.

Rational concentration ratios were determined organoleptically until opalescence appeared, followed by the calculation of the percentage of losses (sediment). The study was conducted on two samples in five replicates. Prolonged storage in oxidative conditions can lead not only to a decrease in the content of essential oil, in particular, as a result of the more volatile fractions, but also to the degradation and/or conversion of some of the main compounds into their oxidized forms [28]. Given these obstacles, the sample with non-standard quality indicators was not studied, since the goal is to create basic information with known quality indicators, according to which all negatives are falsifications. The first sample is hop essential oil of the Promin variety with a date of receipt of September 2025; the second sample is essential oil of the same hop variety but obtained in September 2024. Storage conditions: dark glassware with a ground-glass stopper (no air access), temperature minus 15 °C. 96 % ethanol was used for the research. The oil-ethanol ratio was 1 : 15, 1 : 10, 1 : 7, 1 : 5, 1 : 2. (Fig. 1).

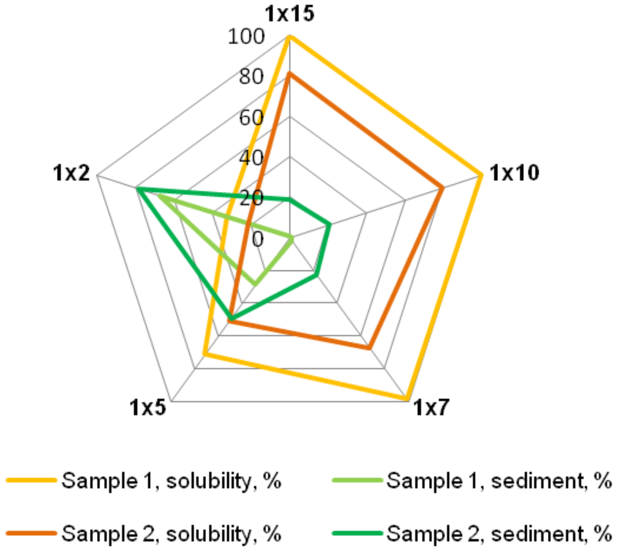
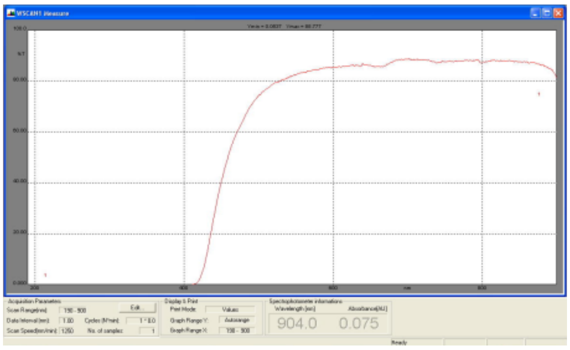


Fig. 1. Rational concentration ratios (hop oil : ethanol) until opalescence appears

In both cases, the greatest opalescence we observed was when the ethanol content was reduced. Considering that in some cases the solubility at a ratio of 1 : 15 and 1 : 10 was low and had insignificant negativity (average fluctuation of only 2 %), the following conditions are proposed for further spectrophotometric studies: for sample 1, use a ratio of 1 : 10.

Based on global research, a methodology has been developed for obtaining a spectrophotometric image of the base solution of hop essential oil. The evaluation of the spectra and changes in the optical density of hop oil in basic units of light absorption and in percentages in the wavelength range from 190 nm to 900 nm was carried out discretely with a wavelength determination step of 0.05–1 nm. This research made it possible to determine individual wavelength points and spectral intervals and calculate function equations, which contributed to the creation of identification spectrophotometric images of optical density during the transmission of light rays through hop oil samples, which form peaks of total terpene absorption.



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Scan Speed(nm/min): 1250
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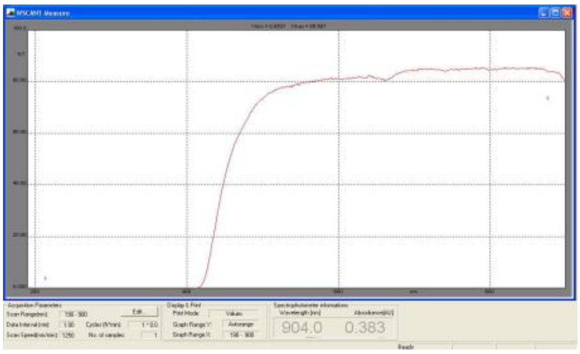
Graph Range X: 190 - 900
Wavelength [nm]: 904.0
Absorbance[AU]: 0.075

Fig. 2. Dynamics of light absorption by an ethanol solution of oil obtained from freshly harvested hops



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Cycles (N*min): 1 * 0.0
Scan Speed(nm/min): 1250
No. of samples: 1
Print Mode: Values
Graph Range Y: Autorange
Graph Range X: 190 - 900
Wavelength [nm]: 904.0
Absorbance[AU]: 0.383

Fig. 3. Dynamics of light absorption by an ethanol solution obtained from hop oil with a storage period of 1 year

The results of spectrophotometric studies show the mutual similarity of spectrophotometric images of light-absorbing activity of the main terpene compounds in samples of ethanol solutions of hop essential oil with standardized quality indicators. In the third sample, the dissolution of oxidized and partially polymerized oil occurs chaotically. It is impossible to determine what exactly dissolves in ethanol and in what proportions, but this shows a significant decrease in light absorption intensity when using low-quality oil and establishes an additional criterion for visual authentication of terpene complexes by spectrogram. At the same time, one of the main criteria for the negativity of spectrograms is the fluctuation in the intensity of light absorption in the wavelength ranges of 456–780 nm. Considering that the research was conducted using deliberately high-quality hop oil samples, it is possible to visually authenticate other samples of ethanol solutions by comparing the above-mentioned spectrograms with the spectrograms of the samples under study.

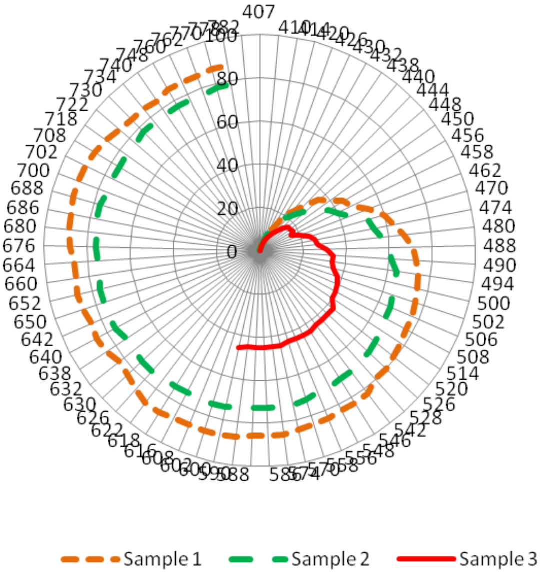

Fig. 4. Intensity of light absorption by ethanol solutions of essential oils of the bitter hop variety "Promin" obtained from freshly harvested hops (sample 1), oil with a shelf life of 1 year (sample 2), and oil with non-standard quality indicators (sample 3)

Systems of linear algebraic equations that include undetermined model parameters serve to describe a wide range of scientific and engineering problems. Considering such systems, where the coefficients of the matrix and the right-hand side are multidimensional polynomials or rational functions of parameters varying within specified intervals, it is possible to calculate a guaranteed and highly accurate interval envelope constraint for the set of solutions, particularly for spectrophotometric dependencies [29].

A higher-order dependency, which leverages the advantages of the general form of estimation functions, defined polynomials with a high degree of approximation accuracy, and a general structure encompassing both common and new higher-order functions, is based on nonlinear optimization methods – specifically coordinate descent and the search for the maximum coefficient of determination (R^2) [30].

The study of spectrophotometric dependencies of the light-absorbing activity of major terpenoid compounds in hop oil ethanol solutions and analytical calculations allowed us to approximate the values, determine the trend line, and derive the functional equation with the optimal approximation confidence (R^2). This coefficient of determination of polynomials, based on a numerical-analytical algorithm for solving a system of differential equations, describes the light absorption dynamics in ethanol solutions of essential oils and illustrates the dependency of the solution error on the polynomial degree (characterizing the optimal fit of the trend model to the initial data and ensuring high validity of experimental results).

The approximation of research results was performed using polynomials with high confidence. The function equations for the samples are as follows:
Sample 1 – y = 1E−11x5 – 6E-08x4 + 0.0001x3 – 0.1101x2 + 44.682x – 6851.6, R^2 = 0.9892;
Sample 2 – y = 8E-11x5 – 3E-07x4 + 0.0004x3 – 0.2775x2 + 94.87x – 12767, R^2 = 0.9923;
Sample 3 – y = 5E–10x5 – 1E-06x4 + 0.0009x3 – 0.3854x2 + 75.119x – 5510.4, R^2 = 0.9954.
Given the accuracy of the coefficients, it is possible to comparatively track changes in the optical property dynamics (light absorption intensity) of the hop oil ethanol extract. Any deviations from the norm indicate adulterated components, allowing for the final elimination of falsified products.

Conclusions

1. A mathematical system for studying graphical-positional anomalies of wavelength

indices, spectral intervals, and calculated coefficients of the equations of the functions of the samples under study creates additional criteria for preventing falsification through spectrophotometric analysis of the light absorption value of ethanol solutions of essential oils of bitter hop varieties.

2. The coefficients of these functions, determined at the error-margin level, provide an opportunity for comparative control of changes in the dynamics of light absorption intensity in ethanol solutions of hop oil and, accordingly, reduce the risks of using counterfeit components.

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