

DETERMINATION OF VOLATILE COMPOUNDS IN FRUITS OF *DIOSPYROS VIRGINIANA* L.

Objective — to investigate the qualitative and quantitative content of volatile organic compounds in American persimmons (*Diospyros virginiana* L.) fruits of the collection of M.M. Gryshko National Botanical Garden of the NAS of Ukraine.

Material and methods. The objects of study were fruits of 10 years old plants of 7 genotypes (DV-01 — DV-07) of *Diospyros virginiana* which were collected in October 2015. The investigation of the volatiles was conducted by the method of Chernohorod and Vinogradov (2006) using chromatography-mass spectrometry. Basic statistical analyses were performed using PAST 2.17; hierarchical cluster analyses of similarity between genotypes were computed on the basis of the Bray—Curtis similarity index.

Results. During the analysis of qualitative composition and quantitative content of volatiles of fruits from the 7 genotypes 129 compounds were detected, and 118 compounds among them were identified. Total content of volatile compounds was from 121.60 to 676.50 mg/kg. Identified compounds belong to hydrocarbons, aldehydes, carboxylic acids and their ethers, monoterpenes, sesquiterpenes, triterpenes. In the fruits of investigated genotypes prevailed fatty acids (mg/kg): myristic acid (from 18.5 to 234.6), palmitic acid (from 14.9 to 125.7), lauric acid (from 29.8 to 50.5), 7,10,13-hexadecatrienic acid (from 18.6 to 33.4), 11-hexadecenoic acid (from 12.3 to 22.1). For all investigated genotypes 14 components were typical: furfural, linalool, phenylacetaldehyde, α -terpineol, geraniol, lauric acid, ethylmyristate, myristic acid, pentadecanoic acid, ethyl palmitate, ethyl palmitoleate, palmitic acid, palmitoleic acid, and squalene.

Conclusions. Detected volatile compounds in the fruits of *Diospyros virginiana* belong to alcohols, saturated and unsaturated aldehydes, ketones, fatty acids, esters, and terpenoids. Shares of keeping them in total identified fruits volatile substances vary and depend on the genotype of plants. The fruits are rich in fatty acids. They are considered as precursors of many specific aroma compounds. Found aldehydes are thought to be responsible for the decreasing of astringency by persimmon fruits. Study of the quantitative and qualitative content of volatile compounds of *Diospyros virginiana* fruits suggest about making sense of conducting further pharmacognostic investigations.

Key words: *Diospyros virginiana*, Forest-Steppe of Ukraine, fruits, volatile compounds.

Introduction

The deterioration of ecological situation in the world caused the need for finding of new plant species which is valuable source of biologically active compounds. It is source of prevention and treatment for human health by natural products of organic origin, the so-called non-traditional plant species: *Aronia Mitschurinii* A.K. Skvortsov & Maitul., *Cornus mas* L., *Cydonia oblonga* Mill., *Diospyros* spp., *Elaeagnus multiflora* Thunb., *Morus nigra* L., *Pseudocydonia sinensis* Schneid., *Ziziphus jujuba* Mill. [6; 12; 15; 16; 23; 25; 26; 28; 33; 44].

American persimmons (*Diospyros virginiana* L.) are known as a widespread culture in a traditional use by Native Americans as food product [5; 10; 18; 31; 39].

The natural range of *Diospyros virginiana* includes the eastern part of North America from Connecticut to Iowa and from Kansas to Florida [9; 41]. Today more than 200 cultivars of *Diospyros virginiana* exist and their fruits have differences in fruits shape, size, color and ripening [11; 13; 14; 17; 42; 46].

The *Diospyros virginiana* is of great practical interest for fruit growing. The fruits of American persimmon are an excellent dietary product, they are used in fresh condition and from them are prepared pastes, jams, syrups, marinades. The fruits were also used to make wine, brandy, white wine vinegar and beer [3; 5]. In addition, the American persimmon is a valuable decorative and medicinal plant. *Diospyros virginiana* since ancient times is used in the folk medicine [5; 10; 18; 31]. The fruit has been used medicinally as antiseptic and for the treatment of burns, diphtheria, dropsy, diarrhoea,

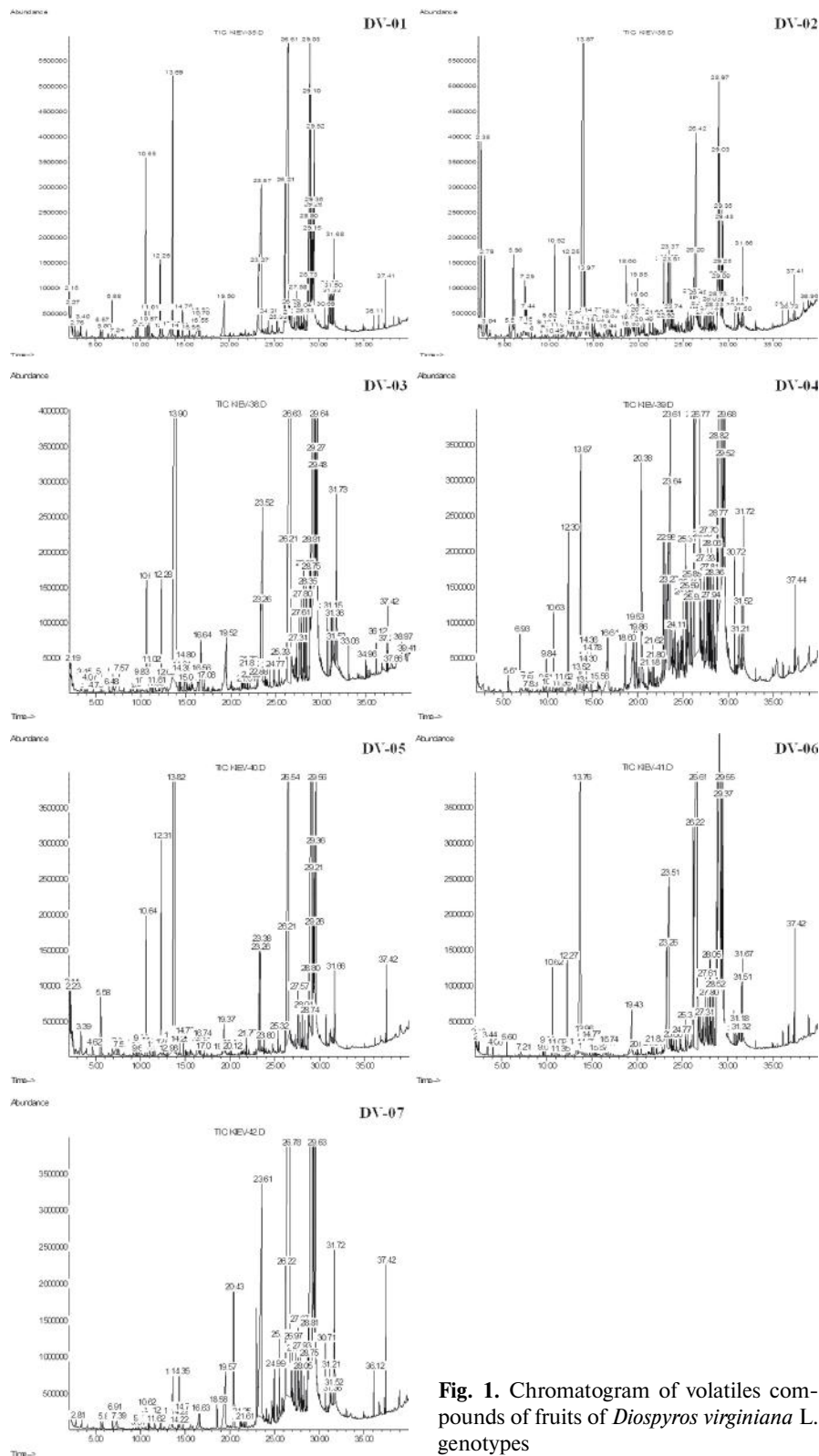


Fig. 1. Chromatogram of volatiles compounds of fruits of *Diospyros virginiana* L. genotypes

gonorrhoea, candidiasis, dysentery, fevers, thrush, fungal and bacterial infections, gastrointestinal bleeding, sore throats [5]. Fruits exhibit the antimicrobial, antifungal [38] and antioxidant activities [16]. Fruits and leaves have an antitumor [40], antimicrobial [20; 22], and antifungal effects [45]. The bark has an antiseptic [5], hepatoprotective and antipyretic [36; 37] action.

Objective — to investigate the qualitative and quantitative content of volatile organic compounds in American persimmons (*Diospyros virginiana* L.) fruits of the collection of M.M. Gryshko National Botanical Garden of the NAS of Ukraine.

Material and methods

Locating trees and data collection

The fruits of 7 genotypes of *Diospyros virginiana* (DV-01—DV-07) collected in the M.M. Gryshko National Botanical Garden of NAS of Ukraine (NBG) were the objects of these investigations. The raw material was collected in the period (October) of full ripeness.

Volatile compounds analysis

The investigation of the volatiles was conducted at the National Institute of Viticulture and Wine “Maga-rach” by the method of Chernogorod and Vinogradov (2006) [21]. Volatiles were investigated by the method of chromatography-mass spectrometry using the chromatograph Agilent Technologies 6890 N with the mass spectrometric detector 5973 N (USA) and a capillary column DB-5 length is 30 mm and an internal diameter is 0.25 mm. The carrier gas velocity (Helium) was 1.2 ml/min. The injector heater temperature was 250 °C. The temperature of thermostat was programmed from 50 °C to 320 °C at the speed 4 °C. The mass spectra library NIST 05 WILEY 2007 with 470 000 spectra and AMDIS, NIST programs were used to identify the investigated compounds. The identification was conducted by comparing obtained mass spectra to mass spectra of standards. The method of internal standard used to determine the quantitative content of compounds.

Statistical analyses

Basic statistical analyses were performed using PAST 2.17; hierarchical cluster analyses of similarity between phenotypes were computed on the basis of the Bray-Curtis similarity index.

Results and discussion

Plants emit a great diversity of volatile compounds from leaves, bark, roots, flowers, and fruits [7; 8; 24; 27; 29; 30; 35].

The volatile compounds have remained an overlooked trait in plant phenotyping [30] that enables the identification of specific chemotypic profiles among and within species [34].

In this study, 129 volatiles compounds in the fruits of *Diospyros virginiana* were detected. Among them, 118 compounds were identified.

The chromatogram of volatiles compounds of fruits of selected genotypes of *Diospyros virginiana* is represented in Fig. 1.

The identified components belong to different chemical classes, including hydrocarbons, alcohols, aldehydes and phenylaldehydes, terpenes, esters and fatty acids.

The widest spectrum of volatiles compounds was characterized by the fruits of genotype DV-02 (74 compounds), among which were identified 64 substances (Fig. 2).

Non-identified compounds have peak with square no more 0.4 % and respectively extremely low intensity of mass-spectrums, which not allows achieving the required reliability of results in the librarian searching.

Fruits of DV-04 genotype had the higher content of total volatile compounds (676.5 mg/kg), and fruits of DV-02 genotype — the least one (121.6 mg/kg) (Fig. 3).

Obtained results of a content of total volatile compounds were confirmed by data of cluster

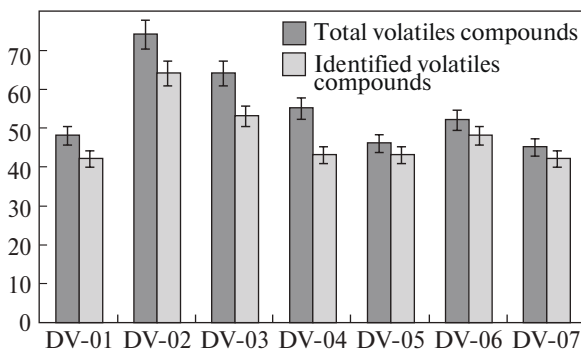


Fig. 2. The number of volatiles compounds (total and identified) of *Diospyros virginiana* L. genotypes

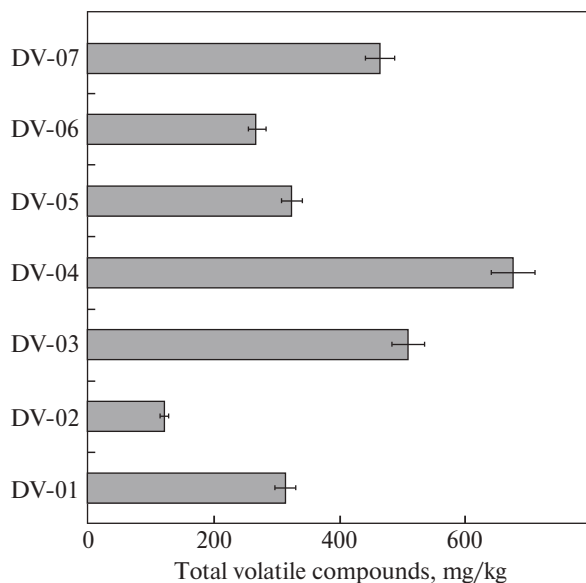


Fig. 3. The total volatile compounds of fruits of *Diospyros virginiana* L. genotypes

analysis (Fig. 4). The cluster analysis (CA) was performed according to the hierarchical cluster analysis (HCA) method using the mean value to distinguish similar groups among the various volatiles compounds. In this study, seven genotypes were grouped into the two main clusters based on highest similarities. In Group 1 concluded genotypes (DV-03, DV-04 and DV-07) with the biggest content of volatile compounds. Rest genotypes concluded in the Group 2.

Common for all investigated genotypes was the presence of 14 components: furfural, linalool, phenylacetaldehyde, α -terpineol, geraniol, lauric acid, ethylmyristate, myristic acid, pentadecanoic acid, ethyl palmitate, ethyl palmitoleate, palmitic acid, palmitoleic acid, and squalene.

Among the fatty acids of genotypes fruits were found myristic acid from 15.21 (DV-02) to 43.45 (DV-06) %, palmitic acid from 6.34 (DV-06) to 29.09 (DV-05) %, lauric acid from 3.45 (DV-02) to 12.27 (DV-01) %, palmitoleic acid from 3.28 (DV-06) to 11.03 (DV-0) %, and 7,10,13-hexadecatrienic acid from 3.29 (DV-02) to 6.57 (DV-03) % (Fig. 5). The last component wasn't identified in the genotypes of DV-05 and DV-06, but presence 11-hexadecenoic acid in the range from 6.85

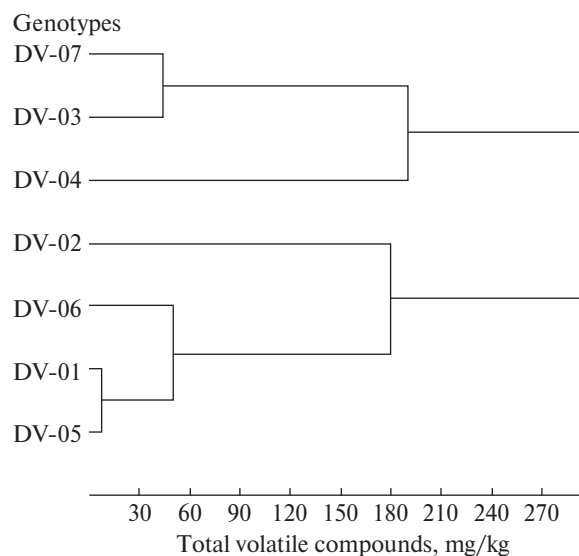


Fig. 4. Cluster diagram of volatile compounds of fruits of *Diospyros virginiana* L. genotypes

(DV-05) to 7.94 (DV-06) %. The share of other components varied from 14.69 (DV-07) to 65.38 (DV-02) %.

Palmitic acid was identified as minor constituents by Horvat et al. (1991) [19]. Among other saturated fatty acids were identified butyric, caproic (hexanoic), caprylic (octanoic), nonanoic, capric (decanoic), lauric (dodecanoic), tridecanoic, pentadecanoic ones. Also, we identified that some genotypes contain stearic acid $C_{17}H_{35}COOH$ and it's unsaturated derivatives: oleic acid $C_{17}H_{33}COOH$ (one double bond), linoleic acid $C_{17}H_{31}COOH$ (two double bonds) and linolenic acid $C_{17}H_{29}COOH$ (three double bonds). Later they were identified in all genotypes.

In the present study, isoamyl alcohol and heptanol-4 (DV-01), octanol (DV-01, DV-02, DV-03), benzyl alcohol (DV-03, DV-04, DV-05, DV-07) were identified.

According to Besada et al. (2013) [4], the high accumulation of phenylacetaldehyde and lipid-derived aldehydes are related to loss of astringency of fruits. Regarding to the previously described volatile compounds of the *Diospyros kaki*, Besada et al. (2013) [4] benzyl alcohol and some related compounds such as acetaldehyde, hexanol-1, 3-me-

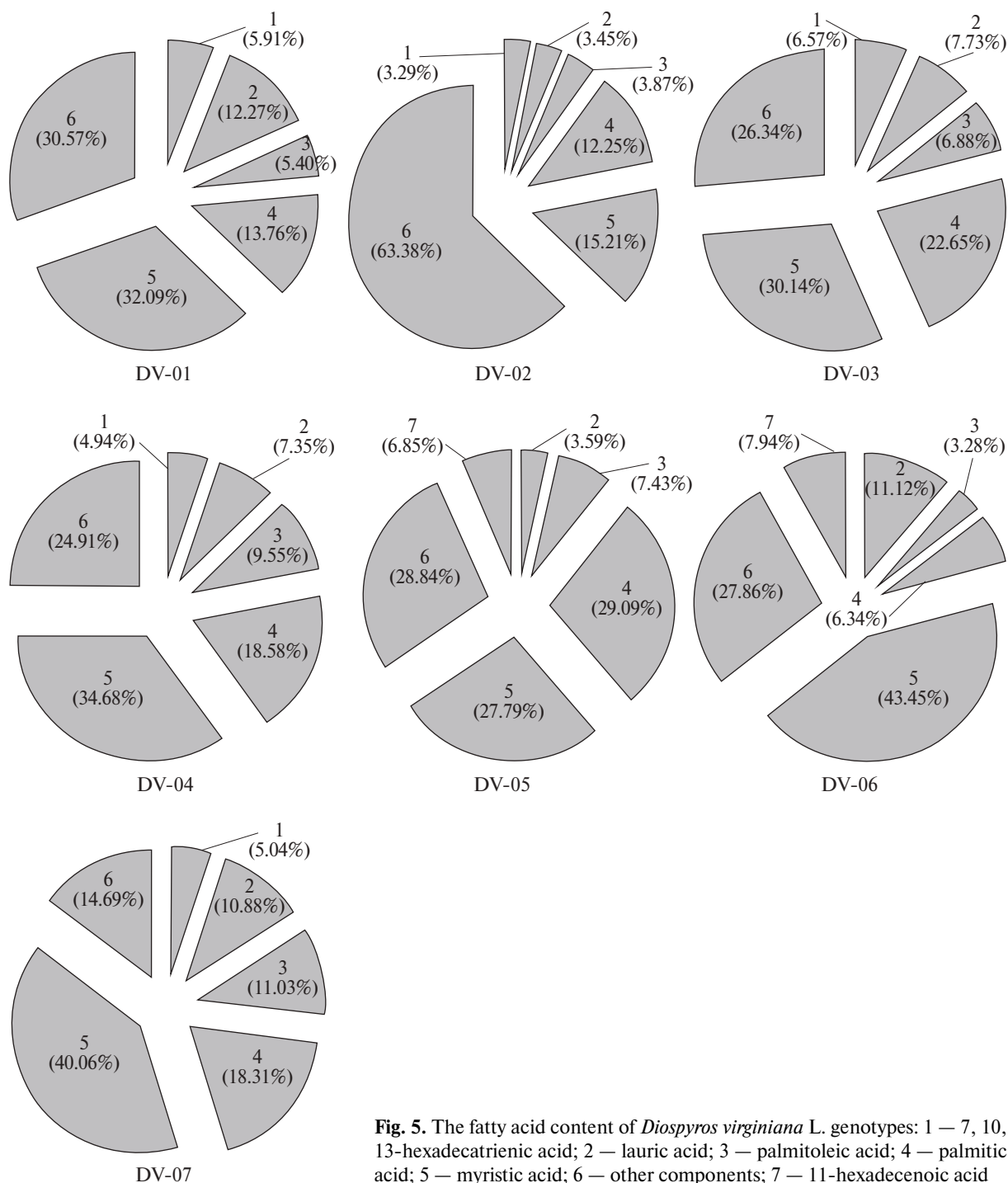


Fig. 5. The fatty acid content of *Diospyros virginiana* L. genotypes: 1 – 7, 10, 13-hexadecatrienic acid; 2 – lauric acid; 3 – palmitoleic acid; 4 – palmitic acid; 5 – myristic acid; 6 – other components; 7 – 11-hexadecenoic acid

thyl-1-heptanol, 1-undecanol, and aliphatic saturated and unsaturated aldehydes such as hexanal, heptanal, octanal, decanal, (E)-2-octenal, (Z)-2-

nonenal, (E)-2-decenal, (E,E)-2,4-heptadienal were identified. Taira et al. (1995) identified such volatile compounds of astringent *Diospyros kaki*

fruits as n-butanol, hehanol-1, (Z)-3-hexen-1-ol, 2-methyl hexanol, acetoin and acetic acid [43].

Flavour and aroma are important quality features in American persimmon fruits. Flavour is formed by the combination of sweetness and sourness from carbohydrates, organic acids and aroma volatile compounds [4]. In general, fruit volatile compounds refer to aliphatic esters, alcohols, aldehydes, ketones, lactones, terpenoids (monoterpenes, sesquiterpenes) and apocarotenoids. Fatty acids are the major primary precursor substrates of many character-impact aroma compounds in most fruits. Aliphatic alcohols, aldehydes, ketones, organic acids, esters and lactones, ranging from C₁ to C₂₀, are all derived from fatty acid precursors through three key biosynthetic processes: α-oxidation, β-oxidation and the lipoxygenase pathway. Sensor analysis is used for the estimation of the ripening stage and storage life of *Diospyros kaki* fruits [2].

Among the identified volatiles responsible for flavour in this study were linalool, α-terpineol and geraniol in all the genotypes and nerol in DV-01 and DV-06, terpinene-4-ol in DV-04 and DV-06 genotypes. They all belong to terpenoids. Geraniol and nerol have a rose odour, nerol has a weaker odour [1]. As was stated by Martineli et al. (2013) [32] the volatiles from *Diospyros kaki* were mainly represented by terpen hydrocarbons, followed by straight-chain esters.

The fact that less part of identified volatile compounds in American persimmon flesh in this study were reported by other scientists could be explained, first of all by the absence of available studies for *Diospyros virginiana*. Therefore, our results were compared with studies performed for *Diospyros kaki*.

Conclusions

Identified volatile compounds in the fruits of *Diospyros virginiana* belong to alcohols, saturated and unsaturated aldehydes, ketones, fatty acids, esters, and terpenoids. Shares of keeping them in identified fruits volatile substances vary and depend on the genotype of plants. The fruits are rich in fatty acids. They are considered as precursors of many specific aroma compounds. Aldehydes are thought to be responsible for the loss of astringency by persimmon fruits.

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ВИЗНАЧЕННЯ ЛЕТКИХ РЕЧОВИН В ПЛОДАХ *DIOSPYROS VIRGINIANA* L.

Мета — дослідити якісний склад і кількісний вміст летких речовин у плодах хурми віргінської (*Diospyros*

virginiana L.) з колекції Національного ботанічного саду імені М.М. Гришка НАН України.

Матеріал та методи. Об'єктом дослідження були плоди 7 генотипів (DV-01—DV-07) 10-річних рослин *Diospyros virginiana*, заготовлені у жовтні 2015 р. Дослідження летких речовин проводили за методикою Черногорода та Виноградова (2006) з використанням хромато-мас-спектрометрії. Основні статистичні аналізи виконували за допомогою PAST 2.17. Ієрархічний кластерний аналіз подібності між генотипами обчислено за індексом подібності Брей—Кертиса.

Результати. У плодах 7 генотипів визначено 129 компонентів, з них ідентифіковано 118. Сумарний кількісний вміст летких компонентів становив від 121,60 до 676,50 мг/кг. Визначені сполуки належать до вуглеводнів, альдегідів, карбонових кислот та їх ефірів, монотерпенів, сесквитерпенів, тритерпенів. В плодах досліджуваних генотипів переважають жирні кислоти (мг/кг): міристинова (від 18,5 до 234,6), пальмітинова (від 14,9 до 125,7), лауринова (від 29,8 до 50,5), 7,10,13-гексадекатринова (від 18,6 до 33,4), 11-гексадеценінова (від 12,3 до 22,1). Для всіх досліджуваних генотипів були характерні 14 компонентів: фурфурол, ліналоол, феніл-ацетальдегід, α -терпінеол, гераніол, лауринова кислота, етилмеристат, міристинова кислота, пентадеканінова кислота, етилпальмітат, етилпальмітолеат, пальмітинова кислота, пальмітолеїнова кислота та сквален.

Висновки. Визначені леткі сполуки в плодах *Diospyros virginiana* належать до спиртів, насичених та ненасичених альдегідів, кетонів, жирних кислот, ефірів та терпеноїдів. Їх частка в ідентифікованих летких речовинах плодів варіює та залежить від генотипу. Плоди багаті на жирні кислоти. Їх вважають прекурсорами багатьох специфічних ароматичних сполук. Виявлені альдегіди сприяють зменшенню терпкості в плодах хурми. Дослідження якісного складу та кількісного вмісту летких компонентів плодів *Diospyros virginiana* свідчить про доцільність проведення подальших фармакогностичних досліджень.

Ключові слова: *Diospyros virginiana*, Лісостеп України, плоди, леткі речовини.

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ОПРЕДЕЛЕНИЕ ЛЕТУЧИХ ВЕЩЕСТВ В ПЛОДАХ *DIOSPYROS VIRGINIANA* L.

Цель — исследовать качественный состав и количественное содержание летучих веществ в плодах хурмы виргинской (*Diospyros virginiana* L.) из коллекции Национального ботанического сада имени Н.Н. Гришко НАН Украины.

Материал и методы. Объектом исследования были плоды 7 генотипов (DV-01—DV-07) 10-летних растений *Diospyros virginiana*, заготовленные в октябре 2015 г. Исследование летучих веществ проводили по методике Черногорода и Виноградова (2006) с использованием хромато-масс-спектрометрии. Основные статистические анализы выполняли с помощью PAST 2.17. Иерархический кластерный анализ сходства между генотипами рассчитан на основе индекса сходства Брей—Кертиса.

Результаты. В плодах 7 генотипов определены 129 компонентов, из них идентифицированы 118. Суммарное количественное содержание летучих компонентов составляло от 121,60 до 676,50 мг/кг. Идентифицированные соединения относятся к углеводородам, альдегидам, карбоновым кислотам и их эфирам, монотерпенам, сесквитерпенам, тритерпенам. В плодах исследуемых генотипов преобладают жирные кислоты (мг/кг): миристиновая (от 18,5 до 234,6), пальмитиновая (от 14,9 до 125,7), лауриновая (от 29,8 до 50,5), 7,10,13-гексадекатриновая (от 18,6 до 33,4), 11-гексадеценовая (от 12,3 до 22,1). Для всех исследуемых генотипов были характерны 14 компонентов: фурфурол,

линалоол, фенилацетальдегид, α -терпинеол, гераниол, лауриновая кислота, этилмеристат, миристиновая кислота, пентадеканоиновая кислота, этилпальмитат, этилпальмитолеат, пальмитиновая кислота, пальмитолеиновая кислота и сквален.

Выводы. Идентифицированные летучие соединения в плодах *Diospyros virginiana* принадлежат к спиртам, насыщенным и ненасыщенным альдегидам, кетонам, жирным кислотам, эфирам и терпеноидам. Их доля в идентифицированных летучих веществах плодов варьирует и зависит от генотипа. Плоды богаты жирными кислотами. Их считают прекурсорами многих специфических ароматических соединений. Идентифицированные альдегиды способствуют уменьшению терпкости в плодах хурмы. Исследование качественного состава и количественного содержания летучих компонентов плодов *Diospyros virginiana* свидетельствует о целесообразности проведения дальнейших фармакогностических исследований.

Ключевые слова: *Diospyros virginiana*, Лесостепь Украины, плоды, летучие вещества.