UDC 57.08 + 631

ECONOMICALLY PROFITABLE NOVEL QUALITY EVALUATION METHOD FOR RAW HOP *(HUMULUS LUPULUS L.)*

A. L. BOJKO, *Doctor of Biological Science, Professor, Academician of NAAS, Leading Researcher*
E-mail: agroecology_naan@ukr.net
YU. A. NYKYTYUK, *Doctor of Economic Sciences, Senior Research Officer*
E-mail: andreyniks2@gmail.com
Institute of Agroecology and Environmental management of NAAS

M. YA. SPIVAK, *Doctor of Biological Science, Professor, Corresponding Member of the NAS of Ukraine, Head of the Department of Problems of interferon and immunomodulators*
E-mail: spivak.spivakn@gmail.com
Danylo Zabolotny
Institute of Microbiology and Virology of NAS Ukraine
O. A. BOJKO, *Candidate of Biological Science (Ph. D.), Associate Professor, Head of the Department of Physiology, Plant Biochemistry and Bioenergetics*
E-mail: olga_bojko@ukr.net
National University of Life and Environmental Science of Ukraine

R. I. RUDYK, *Candidate of Agricultural science (Ph. D.), Director*
E-mail: isgpo_zt@ukr.net
Institute of Agriculture of the Polissya of NAAS

YA. V. CHABANYUK, *Doctor of Agricultural Science, Senior Research Officer, Head of the Department of Agroecology and Biosafety*
E-mail: nauka25@ukr.net
N. P. SUS, *Postgraduate Student*
E-mail: nazar.sus@gmail.com
Y. O. SOLOHUB, *Postgraduate Student*
E-mail: happ1ness8@ukr.net
V. O. TSVIHUN, *Candidate of Biological Sciences (Ph. D.)*
E-mail: vika-natcevich@ukr.net
A. V. ORLOVSKYY, *Postgraduate Student*
E-mail: mumuk6500@gmail.com
Institute of Agroecology and Environmental management of NAAS

https://doi.org/10.31548/bio2018.03.001

Based on years of research the technology of determining the raw hops quality on the results of morphological and structural indices of lupulin glands of hop cones study has been proposed. Several variants of the methodology have been developed based on scanning electron, luminescent and light microscopy. All fragments of the technique have

a significantly lower cost and a simpler algorithm compared to commonly accepted European methods for determining the quality of raw hops, namely the content of the bitter substances and the main component - -acid. The hop`s Carlavirus, which has a considerable spread on hop plantations, caused a significant reduction in the quality of raw materials of this valuable crop, was used in the work as a model system. The proposed technique can be applied (with simple microscopy) in field conditions on different hop types; for express evaluation of raw materials at customs; for evaluation of lupulin at brewery and pharmaceutical, food, cosmetic, special laboratories; for analysis of lupulin after treatment of hops with preparations of various composition.

Introduction. Common hop (*Humulus lupulus L.*) – culture of multipurpose use. The raw material of this culture is used for various industries: baking, pharmaceutical, food, fodder, brewing, alcoholic beverage, textile, official and traditional medicine, cosmetology, paintwork, ornamental horticulture, etc. *Humulus lupulus* L. female plants propagated by cuttings are cultivated for industrial purposes. *Humulus lupulus* L. female inflorescence form a cone which is the most valuable part of this plant due to the complex of specific resins, polyphenolic compounds, essential oils and biologically active substances that have not only taste and aromatics, but also antibiotic, antioxidant and therapeutic properties. From the vegetative mass of plants the silage and feed meal are prepared or it is used for the manufacture of durable rough fiber and in fresh form - for feeding animals.

The bitter substances (the most valuable in brewing is -acid humulene) are most important for production among all the compounds present in the cones (fig.1). They give the beer a pleasant bitter taste and a specific (bear) aroma.

The valuable part of the hop cones are lupus glands (lupulin grains, lupulin) – multicellular thyroid hairs, which are formed from the the epidermis cells and are on the inside of the scales of hop cones, as well as ovary and spindle bumps, in which the synthesis of biologically active substances takes place. Lupular glands are also on male anthers inflorescences, they are less on leaves and they are small (only -acids are synthesized) [1-3].

So quality control of raw hops, in particular the determination of the bitter substances content and α-acids are extremely important. High-performance liquid, fine-layer, paper-based chromatography, conductomet-

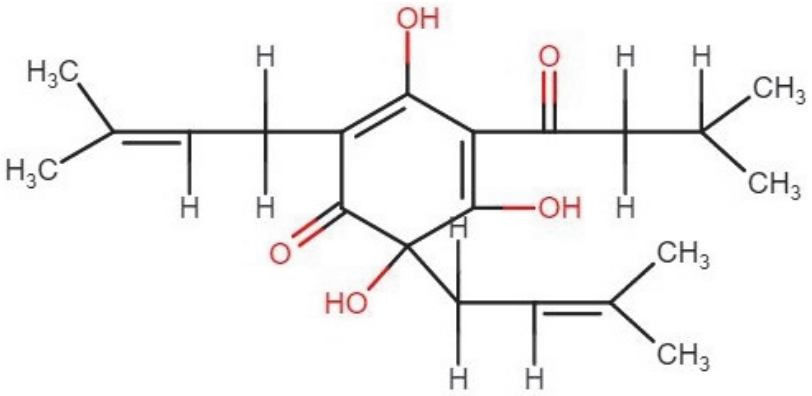

Fig.1. The humulene structural formula [2–4]

ric, gravimetric, spectrophotometric and other methods are used to determine the content of certain components of bitter substances. It should be noted that the important criteria of our proposed method for controlling the quality of raw hops are widely available in practice. It is important that in conditions of agrocenoses hops are affected by pathogens of different taxonomic groups and raw materials from such plants require a dynamic analysis of its quality.

Particular danger to hops is caused by pathogens of viral nature. Among them *Carlavirus* has a significant spread. It affects up to 32 or more plants. The virus has a length of 632 nm, diameter – 10-12 nm, genome (+)RNA-containing. It is well observed in the cytoplasm in ultrathin cuts. Pathogen causing significant degradation of cell organelles, causes destruction of the leaves and the whole plant. The *Carlavirus* also infects hops along with the *Ilarvirus* and the *Tobamovirus*.

Purpose. The purpose of our research was to develop economically profitable express method for determining quality of raw hop, which propagate under different conditions of cultivation technological processes: cuttings, in vitro.

Methods. Our work is based on the results of many years of research on raw hops under the conditions of a virus infection [4-7].

The samples were studied using raster electron, luminescent and light microscopy.

Previous studies of structural differences between lupulin glands of plants, that were virus-infected and not infected plants carried out using a scanning electron microscope JSM-U3 by scanning electron microscopy method. Fixing electroconductive glue coated grid were used before applying the samples, which then were uniformly sprayed with technical gold in a vacuum [4-6]. To study the samples by the method of luminescent microscopy unlike the light one, lupulin glands were fixed in 1.5% acetic acid solution for 2 minutes and washed with distilled water for 3 minutes. the preparations were stained with a fluorescent dye - acridine orange for 5 minutes and visualized in a luminescent microscope ML-2 with a set of photocells: VL-2, WL-8, YL-18, YGL-19 and with lenses 20,0 x 0,40; 40 x 0,65; 90,0 x 1,25 [4-6]. As a model system of influence on the indices of lupulin glands the infectivity of the *Carlavirus* genus virus, which infected hop plants, was used. Non-viral plants were as controls in experiments. Infected plants were selected by plant-indicator, electron microscopic and ELISA. For possible stimulation of growth and development of hop plants, which were sprayed with biological Bioecofunge-1 composition, which was based on the biochemical fractions of mushrooms (Basidiomycetes) (developed by the National University of Life and environmental science of Ukraine) [8].

Results. Our researches show that there is a causal relationship between structural features of the form of lupulin glands and the content of bitter substances and their -acids component Based on this dependence and based on our developed methods for determining the quality of raw hops, which, unlike commonly used technologies, have lower cost and are much simplified [2]. This is especially true of technological processes using light and luminescent microscopy.

The cause-effect relationships are detected between size, color, shape, quantitative composition of lupulin glands, the varietal affiliation of hops donor plants and eco-factors (climatic, data processing of plants with pesticides, biopreparations, hop damage by viruses of various taxa, etc.), which has an extraordinary negative impact on these plants. For example, for the favorable influence of the listed factors on hop plants their lupine grains always have amber-yellow, golden-green color and rounded-ribbed form when visualization with SEM and other methods) [5].

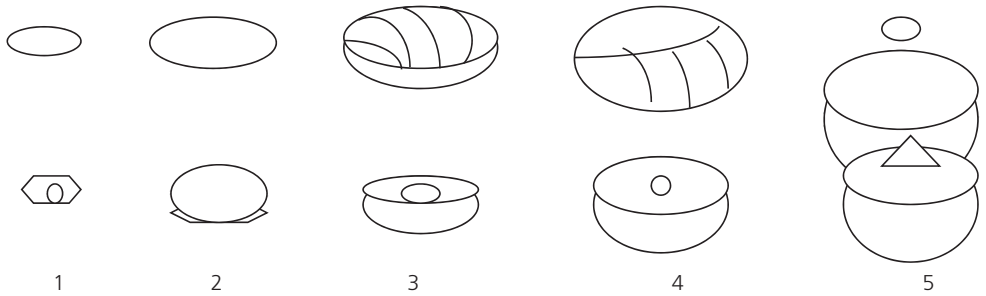

Fig. 2. A schematic representation of themorphology of lupulin grains that supplement the graph (fig. 2)
1 - strong destruction lupulin glands, 2 - average destruction lupulin glands, 3 - weak destruction lupulin glands
4 - almost healthy plants lupulin glands, 5 - plants (0,5%) treated with Bioecofunge-1»

The methods for determining the quality of raw hops were developed based on the detected changes in the structural features of shape, color, the size of lupulin and their casual dependence on the content of bitter substances.

The essence of the proposed techniques is to determine the degree of destruction of lupulin grains, by their visualizing with methods of light and luminescent microscopy and comparing the observed image with those in the schematic determinant (fig. 2), and after determining the degree of destruction, the total content of the bitter substances is estimated according to the calibration schedule (fig .3.).

Thus, taking into account the advantages of the proposed methodological processes, they can be applied (with simple microscopy) in field conditions on different hop types; for express evaluation of raw materials at customs; for evaluation of lupulin at brewery and pharmaceutical, food, cosmetic, special laboratories; for analysis of lupulin after treatment of hops with preparations of various composition.

Discussion. The analysis of morphological and structural indices of lupulin seeds of hops cones provides an opportunity, based on calibration indicators (graphs), to evaluate the quality of raw materials on the pro-

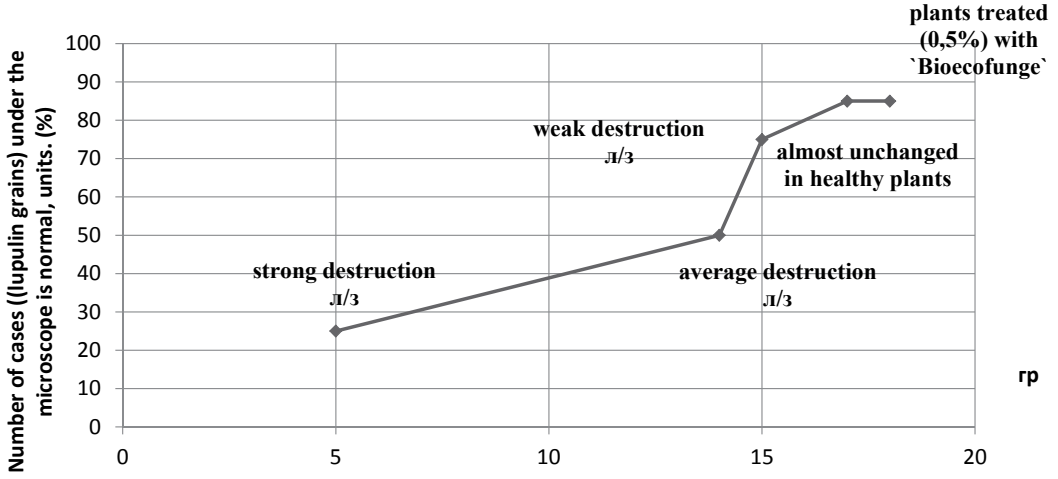

Fig. 3. Adaptive total content of bitter substances (%dry matter) corresponding degradation lupulin grains hops

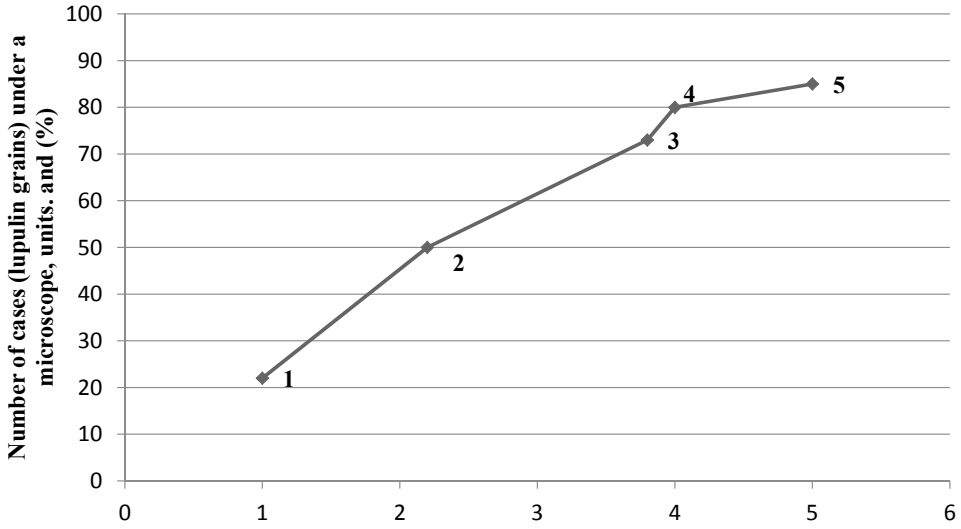

Fig. 4. The content of α-acids according to the morphological and structural analysis of hops lupulin grains 1 – 22%grains of normal; 2 – 50%grains of normal; 3 – 73% grains of normal; 4 – 80% grains of normal; 5 – 85% grains of normal (processing of sample plants 0,5% with `Bioekofunge-1` solution)

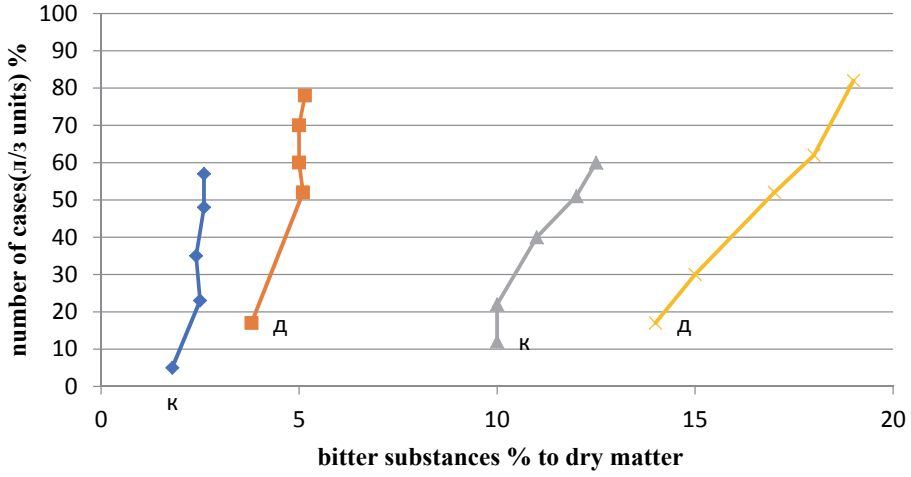

Fig 5. The content of α-acid and bitter substances in hops plant affected with Carlavirus and treated «Bioekofunge – 1» (0,5 %) C – control (untreated plants); R – research (double spray); lupulin glands – 10–30% - strong destruction; lupulin glands – 40–50% - average destruction; lupulin glands – 60–85% - weak destruction

posed economically profitable method of express analysis, which is more profitable in 2,3 - 3,0 times in comparison with known cost prototypes. Based on calibration graphs the developed technology provides an opportunity to evaluate the formation of lupulin grains under different conditions of hop growing: viral infection, growth stimulation and the development of plants when using biocompounds and other factors.

References

1. Boyko, A.L, Nikityuk, Yu.A. (2015) Ekonomichna otsinka syrovyny khmelyu za umov urazhennya virusnoyu infektsiyeyu [Economic evaluation of the hop raw materials under conditions of the lesions with viral infection]. Balanced Nature Using, 4, 38–44.
2. Lyashenko N.I. (2002) Biohimiya hmelya i hmeleproduktov [Biochemistry of hops and hop products]. Zhitomir: Polissya, 388.
3. Lyashenko, M. I., Protsenko, L. V., Rudyk, R. I., Bober, A.V., Hryniuk,T. P., Vlasenko, A.S. (2017). Doslidzhennya hirkykh rechovyn lupulinu riznykh sortiv khmelyu [Research of bitter substances of lupulin in different types of hops]. Naukovyy visnyk NUBiP Ukrayiny, 269, 224-233.
4. Mamedova, S.A., Boyko, A.L., Vasiliev, A.D. (1974). Isledovaniye metodom skaniruyushchey elektronnoy mikroskopii lupulinovykh zeren khmelya, porazhennogo virusnymi zabolevaniyami [Research with the scanning electron microscopy of lupulin grains of the hops infected with viral diseases]. Nauchnyye doklady vysshey shkoly. Biologicheskiye nauki, 8, 130-132.
5. Boyko, A.L. (1976). Virusy i virusnyye zabolevaniya khmelya i rozy efiromaslichnoy [Viruses and viral diseases of hops and oil-bearing roses]. Naukova dumka, 111.
6. Boyko, A.L. (1990). Ekologiya virusov rasteniy [Ecology of plant viruses]. Vyshcha shkola, 165.
7. Boyko, A.L., Zaritsky, M.M., Tovkach, F.I. (2012). Virusolohiia v elektronnohrafiiakh: albom [Virology in electronography: album]. Kyiv: DIA, 54.
8. Boyko, O.A., Veselskiy, S.P., Grygoryuk, I.P., Melnychuk, M.D. (2014). Stvorennia biopreparativ na osnovi biokhimichnykh komponentiv riznykh vydiv bazydiomitsetiv ta vyshchykh roslyn [The creation of biopreparation based on biochemical components of different basidiomycetes species and higher plants]. Naukovyy visnyk NUBiP Ukrayiny, 204, 120-126.

АНОТАЦІЯ

А. Л. Бойко, Ю. А. Никитюк, М. Я. Співак, О. А. Бойко, Р. І. Рудик, Я. В. Чабанюк, Н. П. Сус, Ю. О. Сологуб, В. О. Цвігун, А. В. Орловський. *Економічно-вигідний оригінальний спосіб оцінки якості сировини хмелю (humulus lupulus l.) // Біоресурси і природокористування.– 2018. – **10**, №3–4. – С.5–10. https://doi.org/10.31548/bio2018.03.001*

На основі багаторічних досліджень запропонована технологія визначення якості сировини хмелю на результатах вивчення морфолого-структурних показників лупулінових залоз шишок хмелю. Розроблено декілька варіантів методики, що ґрунтуються на скануючій електронній, люмінесцентній та світловій мікроскопії. Всі фрагменти методики мають значно нижчу собівартість та простіший алгоритм у порівнянні з загальноприйнятими європейськими методами визначення якості сировини хмелю, а саме вмісту гірких речовин та головного компонента α-кислоти. Як модельну систему в роботі використано Carlavirus хмелю, який має значне розширення на хмелеплантаціях, де він викликає значне зниження якості сировини цієї цінної культури. Запропонована методика може бути застосована (при звичайному мікроскопіюванні) у польових умовах на різних сортах хмелю; для експрес-оцінки сировини на митницях; для оцінки лупуліну на пивзаводах та фармацевтичних, харчових, косметичних, спец-лабораторіях; для аналізу лупуліну після обробки рослин хмелю препаратами різного складу.

Ключові слова: *сировина хмелю, метод, Carlavirus, BTM, лупулін*

АННОТАЦИЯ

А. Л. Бойко, Ю. А. Никитюк, Н. Я. Спивак, О. А. Бойко, Р. И. Рудик, Я. В. Чабанюк, Н. П. Сус, Ю. А. Сологуб, В. А. Цвигун, А. В. Орловский. *Экономически выгодный оригинальный способ оценки качества сырья хмеля (humulus lupulus l.)// Биоресурсы и природопользование. – 2018. – **10**, №3–4. – С.5–10. https://doi.org/10.31548/bio2018.03.001*

На основе многолетних исследований предложена технология определения качества сырья хмеля по результатам изучения морфолого-структурных показателей лупулиновых желез шишек хмеля. Разработано несколько вариантов методики, основанных на сканирующей электронной, люминесцентной и световой микроскопии. Все фрагменты методики имеют значительно более низкую себестоимость и простой алгоритм по сравнению с общепринятыми европейскими методами определения качества сырья хмеля, а именно содержания горьких веществ и главного компонента α-кислоты. Как модельная система в работе использован Carlavirus хмеля, который имеет широкое распространение на хмелеплантациях, где он вызывает значительное снижение качества сырья этой ценной культуры. Предложенная методика может быть применена (при обычном микроскопировании) в полевых условиях на различных сортах хмеля; для экспресс-оценки сырья на таможнях; для оценки лупулина на пивзаводах и фармацевтических, пищевых, косметических, спец-лабораториях; для анализа лупулина после обработки растений хмеля препаратами различного состава.

Ключевые слова: *сырье хмеля, метод, Carlavirus, BTM, лупулин*