UDC 663.423: 663:41:633.791

INVESTIGATION OF PROPERTIES OF BIOLOGICALLY ACTIVE SUBSTANCES AND THEIR CONTENT IN CONES OF UKRAINIAN HOP VARIETIES

L. V. Protsenko [1], M. I. Liashenko [2], A. S. Vlasenko [3], T. P. Hryniuk [4], O. O. Dobrovolny [5]

[1, 2, 3, 4] Institute of Agriculture of Polissia NAAS, 131, Kyivske shosse, Zhytomyr, Ukraine 10007
[5] PJSC SIC "Borshchahivskiy CPP" 17, Myru Str. Kyiv, Ukraine, 03134

e-mail: Lidiya.procenko@ukr.net [1], Lyashenko@ ukr.net [2], alenavlasenko27@gmail.com [3], tatjana.ch74@gmail.com [4], aad@bhfz.com.ua [5]

Received on August 15, 2018

Introduction. According to the data of foreign studies, prenyl flavonoids and bitter substances of hop have significant antioxidant, antiviral, antimicrobial, anti-inflammatory and anti-carcinogenic properties. **Methods**. A complex of the following methods was used: a monographic method, analysis, synthesis and comparison, mathematical-statistical and modern physical-chemical methods of determining qualitative indices of hop, special and common in hop-growing industry, which allowed obtaining scientifically valid results. **Results.** The modern level of knowledge about biologically active compounds of hop and their properties was analyzed, in particular, bitter substances and xanthohumol. The quantitative and qualitative content of bitter substances, essential oil and xanthohumol in hop varieties of Ukrainian, European and American breeding was studied. Among the Ukrainian varieties, the highest amount of xanthohumol was found in Ruslan and Xanthus varieties – 1.16 % and 1.06 % against dry substances respectively, and its minimal amount was detected in the cones of the bitter Alta variety. Among the European varieties, the highest amount of this substance is contained in the German variety HallertauerTaurus – 0.9–1.0 %, up to 1 % in the Czech variety Agnus and the English variety Admiral, with the content of 0.95 %–1.09 %. There is a strong correlation between the accumulation of xanthohumol and alpha acids in the formation and ripening of the cones. The maximal amount of xanthohumol is formed in the hop cones in the phase of complete technical ripeness. The content of this substance in hop cones depends on the breeding variety and is a varietal trait, genetically fixed for each variety. The quantitative amount of xanthohumol may be one of biochemical criteria of identifying the variety. **Conclusions.** By their characteristics, the Ukrainian hop varieties correspond to the world's analogues, namely, according to their biochemical and technological indices, hop varieties Klon-18 and Zlato Polissia correspond to the characteristics of the hop of the Czech Saaz variety, the biochemical indices of the bitter Alta variety correspond to the German variety Magnum, and such varieties as Slovianka and Zahrava exceed the world's analogues considerably in the composition and quality of bitter substances and essential oils and are unique.

Keywords: prenyl flavonoids, xanthohumol, alpha acids, hop varieties.

DOI: 10.15407/agrisp5.02.052

INTRODUCTION

Hop is a unique plant, whose cones contain over 100 bitter substances, not found in other plants, about 325 components of essential oil and over 70 polyphenol compounds [1–3]. The use of hop in beer brewing is conditioned by a number of numerous plants therein, which belong to biologically active compounds that ensure biological resistance of beer, promote its conservation, create foam and unique bouquet of taste and aroma properties. Different varieties of hop create a unique remarkable aroma and taste in beer [1–3]. In

addition to beer brewing, hop is used in baking bread, in pharmacology, scientifically grounded and folk medicine. As for the latter, hop cones have been used for over 2,500 years. Currently the world's pharmaceutical industry produces over 100 medicinal preparations on the basis of hop components. The main substances, conditioning the biological activity of hop cones, are bitter substances, phenol compounds and essential oil [4, 5].

Current level of knowledge about biologically active compounds of hop and their properties on the basis of recent studies and publications analysis. Bitter substances, contained in hop cones, were not revealed in other plants by chemical structure, physical-chemical indices and organoleptic properties [2, 6]. Alpha-, and especially, beta-acids, which are components of bitter substances of hop, inhibit the development of gram-positive, gram-negative bacteria and acid-forming microorganisms, but they do not impact the development and activity of yeasts [1, 4, 5]. This is of great relevance for beer technology, as the optimal content of bitter substances therein increases its microbiological resistance. Specific qualities of beer are mostly influenced by isomers of initial bitter substances of hop, which are in insignificant quantities in the cones, and formed in the process of boiling wort with hop. In addition to antibiotic properties regarding bacterial microflora, humulone, one of alpha-acid homologues, inhibits the growth of some harmful fungi.

Japanese scientists have proven that beta-acids of hop inhibit the growth of *Helicobacter pylori* bacteria which have infected almost a half of the world's population and conditions the development of gastritis and peptic ulcer. The interrelation between the presence of these bacteria and the occurrence of stomach cancer has been established [7].

It is known that one of beta-lupulinic acids – colupulone – prevents the development of many microorganisms, including such pathogens as *Staphylococcus aureus*, *Mycobacterium tuberculosis* and *Mycobacterium plhei*. It was studied as far as in 1949 that α- and β-acids were efficient in suspending the growth of tuberculosis bacteria [8]. They are notable for both antiseptic and sedative effect [7, 9].

Different content of polyphenol compounds in different hop varieties impacts the taste and aroma of beer made of them. Hop polyphenols are antioxidants, increasing the restoring property of beer and affecting taste stability. They promote protein precipitation and the formation of complex protein-polyphenol complexes during boiling, which promotes wort clearing and protection of bitter substances of hop from oxidation and losses. However, during long-term boiling high molecular polyphenols of hop create an unpleasant astringent flavor of beer. Thus, polyphenol substances of hop affect the taste and quality of beer not independently, but in the complex with bitter substances of hop, protein, and aminoacids of wort.

Salach and other researchers analyzed the quality of hop depending on the content of polyphenols therein and noted that in Czech varieties the latter are much more numerous compared to hop varieties of other countries. For instance, the content of polyphenols in hop of Czech variety, Zhatetsky, which is remarkable for the highest quality, is 5.2–5.9 %, whereas the same compound in American varieties does not exceed 2.6 %. The scientists deemed high content of polyphenols in Zhatetsky hop to be its advantage over other varieties. Modern researchers also believe that beer, made of hop, containing about 5 % of polyphenols, has the highest quality [10, 11].

Among hop phenols there are also such unique compounds as prenyl flavonoids of chalcone and flavanone types. Previously prenyl flavonoids were paid sufficient attention neither from the standpoint of hop-breeding industry, nor from the standpoint of using them in the process of beer production. Only at the end of the last century [12, 13] scientists started studying these substances actively due to detecting their high biological activity. Currently [14, 15] over two dozen of compounds have been revealed in hop, which belong to the group of prenyl flavonoids. They have an extremely wide spectrum of biological activity. In particular, xanthohumol is currently studied as a potential anti-cancer means. Prenyl flavonoids of hop manifest anti-carcinogenic, phytoestrogen, antioxidant and antiviral properties. Scientists presented rather a wide spectrum of antiviral effect of prenyl flavonoids of hop [5, 8, 16–22]. In the cell culture, hop extracts, enriched with xanthohumol, moderately inhibit the reproduction of bovine viral diarrhea virus which serves as a surrogate model of hepatitis C virus (HCV), herpes simplex virus type 2 (HSV-2) and rhinovirus [23].

The antioxidant activity of prenyl flavonoids was also determined, their effect was manifested in neutralizing active radicals of oxygen and inhibiting the processes of free radical oxidation, which lie in the foundation of cardio-vascular diseases [24, 25]. The most relevant chalcone of hop is xanthohumol, the content of which fluctuates in the range of 0.2–1.1 %

from the mass directly after harvesting of different varieties [7, 9, 24, 25]. During the process of boiling wort and hop about 70 % of xanthohumol is isomerised into isoxanthohumol, which also has a high anti-carcinogenic potential [26].

It is noteworthy that according to the data of experimental studies xanthohumol manifested considerable chemoprophylactic activity regarding the processes of oncogenesis, including specific stages of mechanisms of inhibiting proliferation and metastasis [27]. It was established that it was an active modulator of enzyme activity, in particular, quinone reductase and enzyme CYP450 as well as the ones, taking direct or indirect part in metabolism and detoxication of carcinogens (inhibiting the formation and absorption of reactive oxygen species, including the formation of superoxide-anion and nitrogen oxide) [28].

There are current works, dedicated to the significance of phytoestrogens in human nutrition [29]. It is believed that the nutrition of the population of Western and Central Europe lacks phytoestrogens which may have a negative effect on health condition. For instance, the concentration of phytoestrogens in the nutrition of Europeans is about 100 times smaller compared to the Asian countries. Therefore, an urgent current issue is studying the sources and possibilities of increasing their daily consumption. One of these sources may be found in prenyl naringenins of hop which manifest phytoestrogen activity. Phytoestrogens manifest anti-carcinogenic effect and decrease the risk of hormone-dependent formations considerably, for instance, breast cancer, uterine and prostate cancer [8].

Taking into consideration substantiated practical interest in prenyl flavonoids of hop and, in particular, in 8-prenyl naringenin, noteworthy is the main nutritional source of these components – beer and food products, in which hop is used. Depending on the technological process of beer brewing and used variety of hop, the concentration of these substances in the product may reach 4 mg/l. It is known that the dominating prenyl flavonoid in raw material is chalcone xanthohumol (up to 1 %), but thermal isomerization occurs in the process of wort boiling, due to which a larger part of xanthohumol is transformed into isoxanthohumol. As a result, the main prenyl flavonoid of beer is isoxanthohumol, whose concentrations fluctuate from 500 mcg/l (lager/pilsner) to 4 mg/l (strong ale). As for another chalcone, desmethylxanthohumol, after thermal isomeration, it is transformed to 8-prenyl naringenin and 6-prenyl naringenin in variable ratios between them. Although the maximal concentration of 8-prenyl naringenin in beer may reach 100 mcl/l, total estrogen activity of the beverage is 500–1000 times lower compared to the hazardous concentration, determined in rats *in vivo* (≈100 mg/l) [28]. In addition, modern mass production of beer uses hop extracts, but not its solid raw material, which decreases or excludes the content of 8-prenyl naringenin in the final product considerably [28].

Xanthohumol and isoxanthohumol are supposed to be the main compounds, determining the positive effect of beer on human health on condition of its moderate consumption [5, 17, 30]. Due to this fact, an urgent task of current practice of beer production is increasing the content of prenyl chalcones in beer, elaborating the technologies of producing beer and hop extracts, enriched with xanthohumol, designed for beer brewing, food, pharmaceutical, and other branches of economy [17, 28, 30]. To solve this problem and to obtain high quality hop raw materials with high content of prenyl flavonoids, one should study the content of xanthohumol and other biologically active compounds in hop cones of Ukrainian varieties and determine, at which stage of cone ripeness the maximal amount of this compound is accumulated.

The aim of the study was to investigate the properties of biologically active hop compounds, to study their content in the hop cones of Ukrainian varieties, the dynamics of accumulating alpha-acids and xanthohumol and to determine the interrelations between them.

MATERIAL AND METHODS

The studies were conducted in 2011–2017 in the accredited laboratory of the Department of biochemistry of hop and beer of the Institute of Agriculture of Polissia, NAAS of Ukraine.

Solving the set tasks envisaged the application of the following methods: monographic method – to study positive experience on these issues, analysis, synthesis, and comparison, studying the results of investigations and forming the conclusions, mathematical-statistical and modern physical and chemical methods of determining qualitative indices of hop, special and common for hop-breeding industry. The study investigated the samples of hop cones of aromatic and bitter varieties of Ukrainian breeding and foreign hop products. Hop samples of each variety were selected in the phase of complete technical maturity from at least 10 bushes from the medium layer of plants. The mass of the average sample for identification and biochemical stu-dies was at least 1 kg of dry hop. Hop samples were dried to

standard humidity of 9–12 %. The number of alpha-acids – a conductometric index of bitterness in hop cones – was determined by the methods of European Brewery Convention (EBC 7.4) [2, 31, 32]. The method is based on conductometric titration of hexane extract of bitter substances using the solution of lead acetate and further estimation of the mass content of alpha-acids. Bitter substances, including alpha-acids, beta-acids and xanthohumol were extracted with organic solvent – methanol. The ratio between the mass of hop cones and extraction agent was 1 : 10. The amount of xanthohumol, alpha- and beta-acids and their homologues were detected by the method of highly efficient liquid chromatography. The detection was conducted by EBC method 7.7 [32]. Chromatography was conducted with liquid chromatograph Ultimate 3000 with UV detector at 35 °C. A column of 100 × 2.1 mm, filled with a sorbent Pinacle DB C18 3 mc, was used. The solution of methanol, water, and acetonitrile in the ratio 38 : 24 : 38 was used as a mobile phase. The quantitative determination of xanthohumol involved the use of standard-reference of xanthohumol with the content of this compound of 99.8 %, and the determination of bitter substances – the international reference ICE 3.

RESULTS OF INVESTIGATIONS

The main index of brewing quality of hop variety and a price-forming factor of hop raw material is the presence of cones of bitter substances, in particular, the content of alpha-acids therein. The content of alpha-acids in hop cones depends both on the variety, agrotechnical measures, cultivation technologies and on weather conditions of the year, especially in the period of formation and maturity of cones. It should be noted that abnormally high temperatures of July-August 2015 and insufficient precipitation during the period of synthesis of bitter and other biologically active substances led to uncharacteristically low accumulation of these substances in this year, thus, the values of indices of alpha-acids and xanthohumol for 2015 were not considered in further analysis and characterization of varieties.

The average values of the content of alpha-acids in hop varieties of Ukrainian breeding are presented in Table 1. The analysis of the data demonstrated that the indices of the amount of alpha-acids were considerably different both by specific varieties and by the years of studies. The amount of alpha-acids in the varieties changed from 2.9 % in the cones of fine aroma variety Klon-18 to 13.0 % in bitter variety Alta. Among the varieties of fine aroma type, the highest content of alpha-acids was determined in cones of Natsionalny variety, the average value – 6.1 %, of aroma type – in Zahrava variety, the average value – 6.5 %, and bitter group – in cones of Alta variety, the average value – 10.7 %.

The studies allowed determining a complex biochemical estimation of Ukrainian and foreign varieties of hop, the products of processing of which are of considerable interest for Ukrainian brewers. The characteristics of hop of these varieties are presented in Table 2. Quality indices of foreign hop varieties, determined by

Table 1. The content of alpha-acids in Ukrainian hop varieties, % to dry matter (average for 2011–2017)

Hop variety	Years of studies						
	2011	2012	2013	2014	2016	2017	Average, %
Fina aroma type of hop							
Klon-18	3.8	4.8	4.3	2.9	3.7	4.5	4.0
Zlato Polissia	5.1	3.1	4.8	3.8	3.9	4.2	4.2
Slovianka	5.3	5.9	5.6	4.5	4.8	5.8	5.3
Natsionalny	7.6	6.2	7.2	7.3	4.2	4.2	6.1
Aroma type of hop							
Zahrava	6.8	7.3	6.1	7.5	5.3	5.7	6.5
Haidamatsky	4.5	6.0	5.0	3.2	4.7	4.3	4.6
Bitter type of hop							
Alta	10.6	9.8	13.0	9.7	9.6	11.7	10.7
Promin	8.7	7.1	6.7	6.5	6.1	7.2	7.1

us, coincide with passport data of the countries-producers of these varieties.

The study of biochemical composition of hop cones of the best aroma varieties in the world: Klon-18 (Ukraine), Saaz (Czech Republic), Lublin (Poland), Tettnanger (Germany) and hop of other varieties which are used at brewing plants of Ukraine, demonstrated that the characteristic specificity of fine aromatic and aroma varieties was a considerable advantage in resins of the shares of beta-acids over the share of alpha-acids. Thus, they preserved a positive coefficient of aromaticity between the content of beta- and alpha-acids which is over 1. This is one of decisive features while estimating the brewing quality of hop. Also, the main criteria of referring the variety to a certain type are the amount and qualitative composition of bitter substances, essential oil and xanthohumol, i.e. the classification is composed by varietal features. In the opinion of scientists and brewery specialists of Germany, the Czech Republic, the United States of America, and Slovenia, the mass share of cohumulone in the composition of alpha-acids for fine aroma varieties should not exceed 30. The lowest amount of this homologue of alpha-acids among European and American varieties is attributed to German variety Saphir – from 11 to 17 %. As for Ukrainian varieties, the lowest amount of this substance was detected in Natsionalny hop variety.

In aroma hop of Ukrainian varieties – Klon-18, Zlato Polissia, Slovianka, high quality composition of bitter substances is combined with fine aroma of farnesyl type, remarkable for the best European varieties, such as Saaz, Lublin, Tettnanger.

There is a noted high brewing estimation of Klon-18 hop variety which carries a historic tradition of Ukrainian hop-breeding and brewing. Last century it was one of the best fine aroma varieties in the world which is a part of a large family of Zatec hops. The variety is not high-yielding, the content of alpha-acids is at the low level, but due to aroma properties, it is one of hop varieties, popular among brewers. Klon-18 has a very fine aroma with grass, fruit and flower notes. Due to a low content of bitter substances, the advantage of this hop variety is its aroma, though Klon-8 is also used to add some bitterness to beer. The comparison of char-

Table 2. The biochemical indices of hop varieties of Ukrainian and foreign breeding (average for 2011–2017)

Variety of hop	Content of α-acids, %, EBC method 7.4	Content of β-acids, %, EBC method 7.7	β/α, EBC method 7.7	Content of essential oil, ml/100 g of dry hop	Cohumulone in the content of α-acids, %, method EBC 7.7
Fina aroma type of hop					
Klon-18 (Ukraine)	2.5–5.3	3.5–6.5	0.9–1.3	0.4–0.7	23.5–28.8
Saaz (Czech Republic)	2.5–6.0	4.5–7.0	1.2–1.4	0.4–0.8	24.7–28.3
Lublin (Poland)	3.8–4.8	2.7–3.5	0.7–1.0	0.5–0.7	25.3–29.7
Tettnanger (Germany)	2.5–5.4	3.0–5.1	0.9–1.1	0.3–0.8	22.3–28.1
Saphir (Germany)	1.8–3.9	3.2–6.8	1.7–2.3	0.6–1.0	11.5–16.5
Slovianka (Ukraine)	4.2–6.9	5.3–8.7	1.3–1.8	1.3–2.0	21.7–25.8
Natsionalny (Ukraine)	5.0–7.9	4.6–8.1	0.9–1.2	0.8–1.2	20.4–24.6
Aroma type of hop					
Zahrava (Ukraine)	5.1–7.5	4.8–6.4	0.9–1.3	1.8–2.5	21.1–26.7
Hallertauer Tradition (Germany)	4.6–6.5	3.8–5.5	0.8–0.9	0.4–0.8	24.1–28.5
Haidamatsky(Ukraine)	3.2–6.0	4.0–6.2	1.2–1.5	0.5–1.0	26.5–29.7
Bitter type of hop					
Alta (Ukraine)	9.6–13.0	6.3–7.0	0.5–0.6	1.0–2.0	23.2–25.6
Magnum (Germany)	11.3–15.7	3.4–7.2	0.5–0.6	1.1–1.8	22.8–29.0
Columbus (USA)	14.0–16.0	4.5–5.8	0.28–0.35	1.5–3.0	29.0–39.0
Tomahawk (USA)	15.0–17.8	4.9–5.7	0.27–0.33	1.6–4.0	26.0–30.0

acteristics of hop varieties Klon-18, Zlato Polissia and Saaz demonstrated that these varieties were remarkable for almost the same content of bitter substances. The amount of beta-acids in the varieties exceeds the content of alpha-acids, i.e. the positive coefficient of aromaticity is preserved. The content of essential oil in these varieties is about 1 %. It is presented by cariofilen, humulene and farnesene.

Especially high brewing properties are noted for fine aroma and high-resinous hop variety Slovianka, which presents the elite group of fine aroma varieties. It is valued for the unique composition of bitter substances and essential oil of farnesene type. Among the investigated varieties of Ukrainian and foreign breeding, Slovianka has the highest content of beta-acids which is one and a half times higher compared to alpha-acids. This regularity is preserved for many years and is a varietal specificity. The index of the ratio between beta- and alpha-acids, the amount and unique composition of bitter substances and essential oil in combination with other components characterize hop of this variety as an especially valuable fine aroma form of hop for brewing. The finest hop aroma and tender bitterness of this hop add unique exquisite bouquet of taste and noble aroma with grass and flower-fruity flavor with nectar notes to different kinds of beer.

The same properties are remarkable for Natsionalny hop variety. This variety is remarkable for the highest content of alpha-acids in the fine aroma group and rather stable index of the ratio between beta- and alpha-acids. The essential oil is presented with a considerable amount of farnesene. This combination of aroma and bitter substances in hop cones defined excellent brewing capacities of this variety.

Zahrava is a highly resinous aroma variety. The unique character of bitterness and taste is due to low content of cohumulone in the composition of alpha-acids which refers it to noble hop. This is one of aroma varieties of the world which has the highest amount of essential oil – up to 2.5 ml per 100 g of dry hop. Among foreign varieties, this amount of essential oil is usually present in bitter varieties such as Columbus, Tomahawk, *etc.* The balanced composition of aroma oil with a sufficient amount of farnesene promotes the formation of spicy flower aroma with pronounced grassy character and fruit notes. Zahrava is a universal variety in beer production which is widely used while producing beer of all the traditional types. It is deemed to be double-purpose hop and is used both for bitterness and aroma.

The abovementioned demonstrates that Ukraine is famous for its fine aroma and aroma varieties. Unfortunately, Ukrainian varieties lack the ones like a German variety Magnum, American varieties Columbus and Tomahawk, the content of alpha-acids in which exceeds 13 %. However, Alta variety has its criteria, similar to the famous German variety Magnum, the products of which are popular among beer producers but it has fewer bitter substances.

Table 3. The content of xanthohumol in hop varieties of Ukrainian breeding, (average for 2011–2017), % to dry matter

Hop variety	Years of studies						
	2011	2012	2013	2014	2016	2017	Average, %
Fina aroma type of hop							
Klon-18	0.27	0.23	0.22	0.27	0.26	0.22	0.25
Zlato Polissia	0.31	0.24	0.26	0.26	0.27	0.24	0.26
Slovianka	0.46	0.38	0.40	0.47	0.34	0.36	0.40
Natsionalny	0.59	0.52	0.55	0.57	0.52	0.50	0.54
Aroma type of hop							
Zahrava	0.49	0.50	0.44	0.46	0.42	0.46	0.46
Haidamatsky	0.34	0.27	0.29	0.32	0.33	0.30	0.31
Bitter type of hop							
Alta	0.21	0.17	0.19	0.20	0.22	0.21	0.20
Promin	0.39	0.29	0.31	0.30	0.30	0.33	0.32

We have also investigated the amount of xanthohumol in hop cones of Ukrainian varieties. The content of this compound during the years of studies is presented in Table 3.

As seen from the data in Table 3, the amount of xanthohumol in different varieties fluctuates from 0.17 % in Alta cones to 0.59 % in Natsionalny variety. Within the years of studies, the highest amount of xanthohumol was found in hop cones of the harvest for 2011, which was remarkable for favorable natural and climatic conditions during the period of vegetation and maturity of this crop.

It should be noted that the highest amount of alpha-acids was present in the cones of Alta variety (Table 1), however the content of xanthohumol was the lowest – 0.17–0.22 % to dry matter, i.e. the accumulation of this substance in hop cones is a varietal property, genetically fixed for each variety. The analysis of the data of Table 1 and Table 3 established that the quantitative content of xanthohumol in the cones of the investigated hop varieties did not depend on the type of hop, was a varietal feature and may be one of biochemical criteria of variety identification.

Ukraine's breeders bred and registered new hop varieties with the increased content of xanthohumol – Ruslan and Xanthus, the characteristics of which are presented in Table 4, in the State Register of Plant Varieties, Suitable for Dissemination in Ukraine.

The data, obtained within 2011–2017, demonstrated that the average content of xanthohumol in hop cones of Xanthus variety fluctuated from 0.72 % to 1.06 % to the mass of dry matter at the content of alpha-acids in the range from 7.1 % to 10.6 %. Low values of the content of alpha-acids and xanthohumol in hop cones of late-season variety Xanthus, the harvest of 2014, were explained by the fact that these samples of hop were taken according to the experiment scheme and analyzed at the end of the first decade of September 2014. Further on, early night frost, observed in the middle of the second decade of September, led to brown discoloring of the cones, the loss of alpha-acids and xanthohumol, *i.e.* in general, to the deterioration in the quality of hop cones. As in the second decade of September the cones of these varieties were already unsuitable for further studies, the investigations with this variety were stopped and the data for 2014 were not taken into consideration in the estimation. The average value of xanthohumol in Xanthus variety is 0.96 % to the mass of dry matter at the content of alpha-acids of 9.84 %. Among the Ukrainian hop varieties, the maximal amount of xanthohumol was found in Ruslan variety – 1.0 % on average to the mass of dry matter at the values of experimental data in the range from 0.88 to 1.16 % and the content of alpha-acids in the range of 8.3–10.8 %. In this respect the latter variety and Xanthus variety may be compared to the best world's varieties which have a high amount of prenyl flavonoids, for instance, to the German variety Hallertauer Taurus, the content of xanthohumol in the cones of which was 0.9–1.0 %, the Czech variety Agnus, which contains about 1 % of xanthohumol or the English variety Admiral with the content of this compound of 0.95–1.09 % [7]. Thus, during six years of studies hop varie-

Table 4. The content of alpha-acids and xanthohumol in promising hop varieties of Ukrainian breeding (2011–2017), % to dry matter

Years of studies	Xanthus hop variety		Ruslan hop variety	
	Quality indices, % to dry matter			
	Content of alpha-acids	Content of xanthohumol	Content of alpha-acids	Content of xanthohumol
2011	10.4	1.06	10.8	1.16
2012	9.2	0.90	9.2	0.92
2013	9.1	0.92	10.2	1.10
2014	7.1	0.72	10.0	1.02
2016	10.6	0.96	8.3	0.88
2017	9.9	0.96	9.2	0.91
Average	9.84	0.96	9.61	1.00
HIP_{05}	0.37	0.03	0.33	0.04

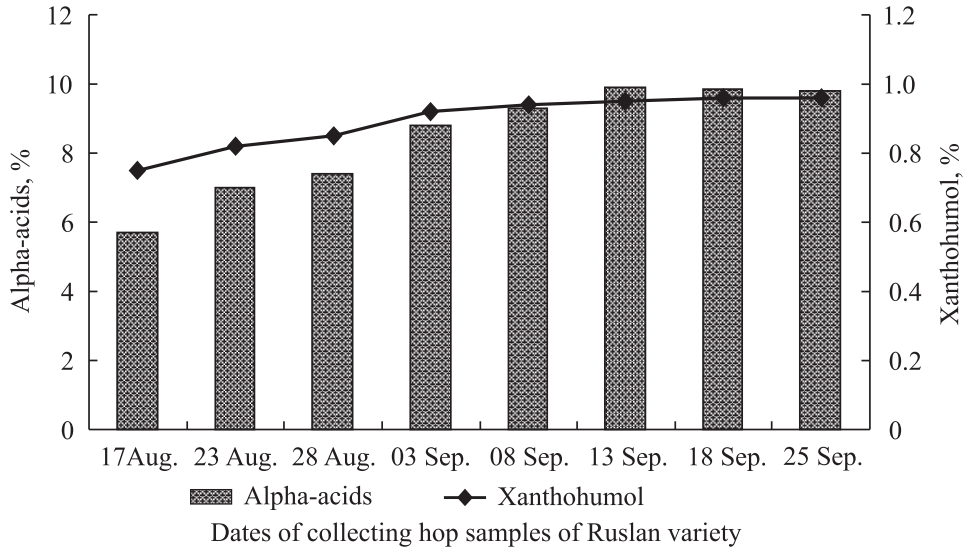

Fig. 1. The dynamics of accumulation of xanthohumol and alpha-acids in hop cones of Ruslan variety

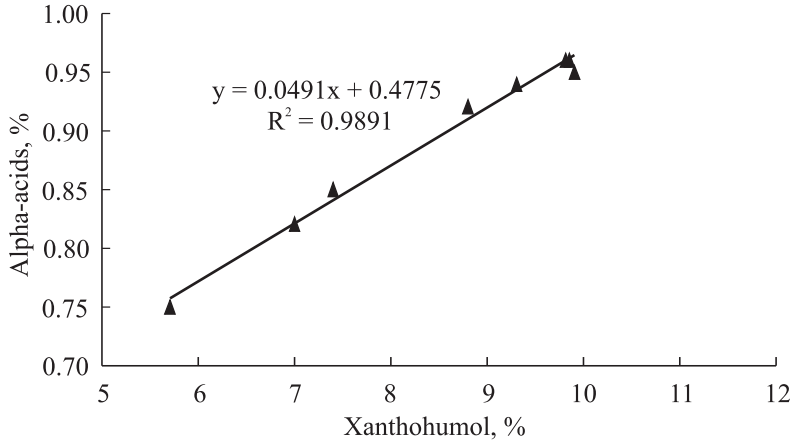

Fig. 2. The correlation dependence between the accumulation of xanthohumol and alpha-acids in hop cones of Ruslan variety

ties Xanthus and Ruslan had a high and stable content of xanthohumol, the amount of which fluctuated in the range of 0.86–1.16 %. Unfortunately, these varieties were not widely disseminated in Ukrainian farms.

In 2012–2017 we also investigated the interrelation between the accumulation of xanthohumol and alpha-acids in these hop varieties in the process of cone formation and ripening. The dynamics of accumulation for these substances in hop cones of Ruslan variety is presented in Fig. 1.

Depending on natural-climatic conditions of the year, the recommended terms of harvesting hop cones, when xanthohumol and alpha-acids are maximally accumulated in Ruslan variety, are the end of the first and the start of the second decade of September.

Fig. 2 demonstrates the correlation dependence field of xanthohumol accumulation on the content of alpha-acids in the samples of Ruslan hop variety, regression equations and determination coefficient R^2, with the building of the trend line.

The dependence of xanthohumol accumulation on the amount of alpha-acids in hop cones of Ruslan variety is expressed in the correlation equation: $\tilde{y}_x = 0.0491x + 0.4775$. There is a direct correlation between the properties. The correlation coefficient ($r = 0.99 \pm 0.1$) demonstrates a strong relation between the accumulation of xanthohumol and alpha-acids. The determination coefficient $R^2 = 0.9891$ indicated that 98.91 % of fluctuations in accumulation indices for hop xanthohumol was related to the accumulation of alpha-acids, and the remaining 1.09 % – to other impact factors, which were not considered in this case, namely, temperature regime, precipitation during vegetation, damage by pests and diseases, *etc.*

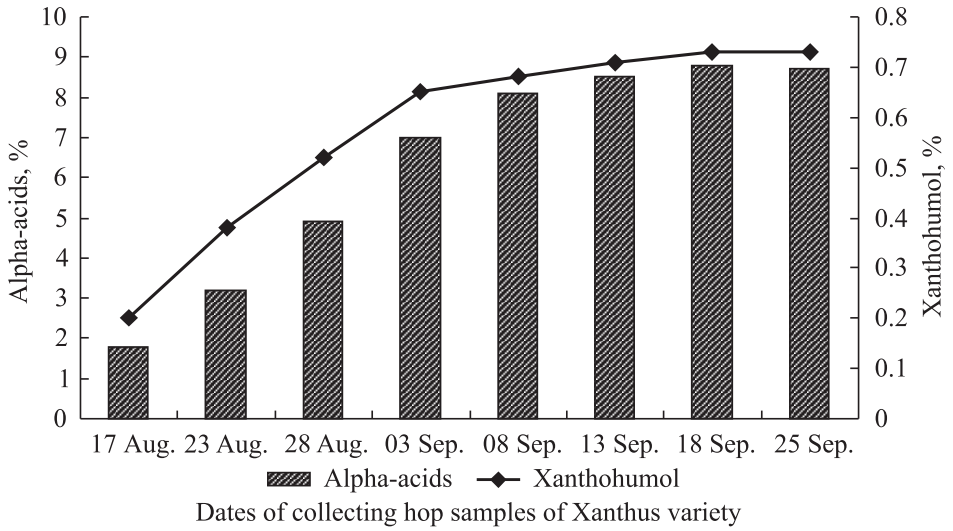

Fig. 3. The dynamics of accumulation of xanthohumol and alpha-acids in hop cones of Xanthus variety

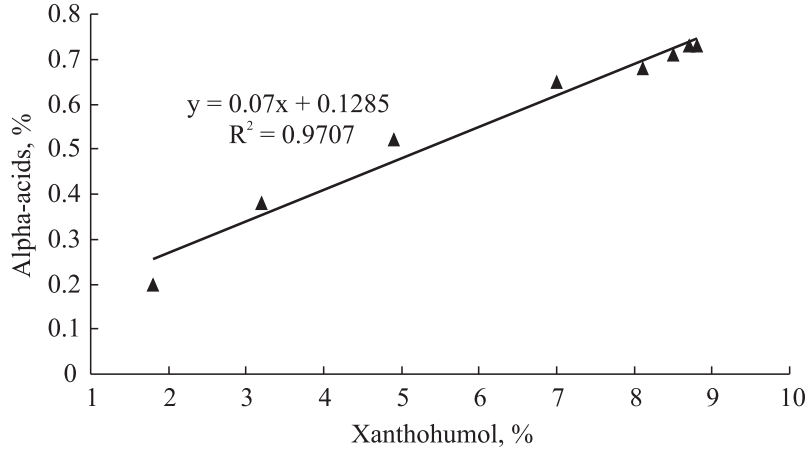

Fig. 4. The correlation dependence between the accumulation of xanthohumol and alpha-acids in hop cones of Xanthus variety

The dynamics of accumulation of alpha-acids and xanthohumol in hop cones of Xanthus variety during the period of their formation is presented in Fig. 3.

The recommended terms of harvesting hop cones of Xanthus variety, when xanthohumol and alpha-acids are maximally accumulated, are the end of the second and the beginning of the third decade of September.

Fig. 4 demonstrates the correlation field of the dependence of xanthohumol accumulation on the content of alpha-acids in the samples of Xanthus hop variety.

As for Xanthus variety, this dependence is expressed via a correlation equation: $\tilde{y}_x = 0.07x + 0.1285$. There is a direct correlation between the properties. There is also a strong correlation between two factors – the accumulation of xanthohumol and alpha-acids which is proven by the correlation coefficient ($r = 0.98 \pm 0.2$). We have noticed that 97.07 % of total variation in the content of xanthohumol was conditioned by changes in the content of alpha-acids, and the remaining 2.93 % – by other impact factors, which were not considered in this case.

The analysis of the study results demonstrated that the process of accumulating xanthohumol and bitter substances in the investigated hop varieties during the formation and ripening of cones was the same.

The studies, conducted in cooperation with the Public joint-stock company "Scientific-industrial center Borshchahivskiy Chemical-Pharmaceutical Plant", based on biologically active substances of cones of Xanthus variety, allowed elaborating the method of obtaining hop extract, enriched with prenyl flavonoids with

estrogen effect and the patent for this method was received [28].

Testing positive properties of xanthohumol and other substances, analogous to it, is constantly going on *in vitro* and in natural conditions. Taking this fact into consideration, one may expect that hop of the investigated varieties with a high content of biologically active substances will be widely applied in other branches of economy as well, and the production of hop raw material of these varieties will increase in future.

CONCLUSIONS

The main substances, conditioning the biological activity of hop cones, are bitter substances, phenol compounds and essential oil. According to the data of Ukrainian and foreign studies, prenyl flavonoids and bitter substances of hop have significant antioxidant, antiviral, antimicrobial, anti-inflammatory and anti-carcinogenic properties.

The maximal amount of xanthohumol is in hop varieties of Ukrainian breeding – Ruslan and Xanthus – 1.16 % and 1.06 % respectively, and its minimal amount was detected in the cones of bitter variety, Alta. Among the European varieties, the highest amount of xanthohumol is contained in the German variety HallertauerTaurus – 0.9–1.0 %, up to 1 % in the Czech variety Agnus and the English variety Admiral, with the content of 0.95–1.09 of this substance. The content of xanthohumol in hop cones depends on the breeding variety and is a varietal trait, genetically fixed for each variety. The quantitative amount of xanthohumol may be one of biochemical criteria of identifying the variety.

The maximal amount of xanthohumol is formed in the hop cones in the phase of complete technical ripeness. There is a strong correlation between the accumulation of xanthohumol and alpha acids in the formation and maturing of the cones.

The studies established that the quantitative content and qualitative composition of bitter substances, essential oil, polyphenol compounds and xanthohumol in hop cones of Ukrainian and foreign production was stable and corresponded to passport data of the variety. It was proven that by their characteristics they correspond to the world's analogues, namely, according to their biochemical and technological indices, hop varieties Klon-18 and Zlato Polissia correspond to the characteristics of the hop of the Czech Saaz variety, the biochemical indices of the bitter Alta variety correspond to the German variety Magnum, and such varieties, as Slovianka and Zagrava exceed the world's analogues considerably in the composition and quality of bitter substances and essential oils and are unique.

The comparative biochemical characterization of Ukrainian hop varieties and the hop, produced in the European countries and the USA, established the correspondence of their quality to the world's level.

Уміст біологічно активних речовин в шишках українських сортів хмелю та дослідження їх властивостей

Л. В. Проценко [1], М. І. Ляшенко [2], А. С. Власенко [3], Т. П. Гринюк [4], О. О. Добровольний [5]

e-mail: Lidiya.procenko@ukr.net, Lyashenko@ ukr.net, alenavlasenko27@gmail.com, tatjana.ch74@gmail.com, aad@bhfz.com.ua

[1, 2, 3, 4] Інститут сільського господарства Полісся НААН, шосе Київське, 131, м. Житомир, Україна,10007,
[5] ПАТ НВЦ «Борщагівський ХФЗ», вул. Миру, 17, м. Київ, Україна, 03134

Мета. Пренілфлавоноїди і гіркі речовини хмелю, за даними зарубіжних досліджень, мають значні антиоксидантні, антивірусні, антимікробні, протизапальні та антиканцерогенні властивості. **Методи.** Використано комплекс методів: монографічний, аналіз, синтез та порівняння, математично-статистичні та сучасні фізико-хімічні методи визначення якісних показників хмелю, спеціальні та загальноприйняті в хмелярській галузі, що дозволило отримати науково обґрунтовані результати. **Результати.** Проаналізовано сучасний рівень знань про біологічно активні сполуки хмелю та їх властивості, зокрема: гіркі речовини та ксантогумол. Досліджено кількісний вміст та якісний склад гірких речовин, ефірної олії та ксантогумолу в сортах хмелю української, європейської та американської селекції. Серед українських сортів найбільше ксантогумолу міститься в сортах Руслан і Ксанта – 1,16 і 1,06 % до сухих речовин відповідно, а мінімальна його кількість визначена в шишках гіркого сорту Альта. Серед європейських сортів найбільше даної сполуки міститься в німецькому сорті Hallertauer Taurus – 0,9–1,0 %, до 1 % в чеському сорті Agnus та англійському сорті Admiral, з вмістом 0,95–1,09 %. Між накопиченням ксантогумолу та альфа-кислот при формуванні та дозріванні шишок існує сильний зв'язок. Максимальна кількість ксантогумолу формується в шишках хмелю у фазі повної технічної стиглості. Вміст даної сполуки в шишках хмелю залежить від селекційного сорту і є сортовою ознакою, генетично закріпленою для кожного сорту. Кількісний вміст ксантогумолу може бути одним з біохімічних критеріїв ідентифікації сорту. **Висновки.** Українські сорти хмелю за своїми характеристиками відповідають світовим аналогам, а саме: хміль сортів Клон 18 та Злато Полісся за біохімічними та технологічними

показниками відповідають характеристиці хмелю чеського сорту Saaz, біохімічні показники гіркого сорту Альта відповідають німецькому сорту Magnum, а такі сорти як Слов'янка та Заграва за складом і якістю гірких речовин та ефірної олії значно перевищують світові аналоги та є унікальними.

Ключові слова: пренілфлавоноїди, ксантогумол, альфакислоти, сорти хмелю.

Исследование свойств биологически активных веществ и их содержание в шишках украинских сортов хмеля

Л. В. Проценко [1], М. И. Ляшенко [2], А. С. Власенко [3], Т. П. Гринюк [4], О. О. Добровольный[5]

e-mail: Lidiya.procenko@ukr.net, Lyashenko@ ukr.net, alenavlasenko27@gmail.com, tatjana.ch74@gmail.com, aad@bhfz.com.ua

[1, 2, 3, 4] Институт сельского хозяйства Полесья НААН, шоссе Киевское, 131, г. Житомир, Украина, 10007,
[5] ПАО НПЦ «Борщаговский ХФЗ», ул. Мира, 17, г. Киев, Украина,03134

Цель. В пренилфлавоноидах и горьких веществах хмеля, по данным зарубежных исследований, выявлены значительные антиоксидантные, антивирусные, антимикробные, противовоспалительные и антиканцерогенные свойства. **Методы.** Использован комплекс методов: монографический, анализа, синтеза и сравнения, математико-статистические и современные физико-химические методы определения качественных показателей хмеля, специальные и общепринятые в хмелеводческой отрасли, что позволило получить научно обоснованные результаты. **Результаты.** Проанализирован современный уровень знаний о биологически активных соединениях хмеля и их свойствах, в частности: горьких веществах и ксантогумоле. Исследовано количественное содержание и качественный состав горьких веществ, эфирного масла и ксантогумола в сортах хмеля украинской, европейской и американской селекции. Среди украинских сортов максимальное количество ксантогумола содержится в сортах Руслан и Ксанта – 1,16 и 1,06% к сухому веществу соответственно, а минимальное его количество определено в шишках горького сорта Альта. Среди европейских сортов наибольшее количество ксантогумола содержится в немецком сорте Hallertauer Taurus – 0,9–1,0 %; до 1% в чешском сорте Agnus и английском сорте Admiral, содержащий 0,95–1,09 % данного соединения. Меж-ду накоплением ксантогумола и альфа-кислот при формировании и созревании шишек существует сильная связь. Максимальное количество ксантогумола формируется в шишках хмеля в фазе полной технической спелости. Содержание данного соединения в шишках хмеля зависит от селекционного сорта и является сортовым признаком, генетически закрепленным для каждого сорта. Количественное содержание ксантогумола может быть одним из биохимических критериев идентификации сорта. **Выводы.** Украинские сорта хмеля по своим характеристикам соответствуют мировым аналогам, а именно: хмель сортов Клон-18 и Злато Полесья по биохимическим и технологическим показателям соответствуют характеристике хмеля чешского сорта Saaz, биохимические показатели горького сорта Альта соответствуют немецкому сорту Magnum, а такие сорта как Славянка и Заграва по составу и качеству горьких веществ и эфирного масла значительно превышают мировые аналоги и являются уникальными.

Ключевые слова: пренилфлавоноиды, ксантогумол, альфа-кислоты, сорта хмеля.

REFERENCES

1. *Liashenko NI.* Biochemistry of hop and hop products. Zhytomyr: Polissia. 2002:388 p.
2. *von Narziß L, Back W. AbrissderBierbrauerei.* Wiley-VCH: Weinheim, 2005:163–70.
3. Biendl M., Pinzl C. Arzneipflanze Hopfen: *Anwendungen, Wirkungen, Geschichte,* Deutsches Hopfenmuseum Wolnzach: Wolnzach, 2007:127 p.
4. *Danila AO, Gatea F, Radu GL.* Polyphenol composition and antioxidant activity of selected medicinal herbs. Chem. Nat. Compounds. 2011;**47**(1):22–26.
5. Żołnierczyk *AK, Mączka WK, Grabarczyk M, Wińska K, Woźniak E, Anioł M.* Isoxanthohumol – biologically active hop flavonoid. Fitoterapia. 2015;**103**:71–82. doi:10.1016/j.fitote.2015.03.007.
6. *Hanke S.* Untersuchungen zum Einfluss der Hopfungstechnologie auf die Geschmacksstabilität und Harmonie untergäriger Biere. Technische Universität München: Freising. 2010:307 p.
7. *Protsenko L, Rudyk R, Hryniuk T, Vlasenko A, Protsenko A, Litvynchuk S, Ovadenko O.* Beer enrichment with biologically active hop compounds. Ukrain. Food J. 2018;**7**(1):65–78. doi: 10.24263/2304-974X-2018-7-1-7.
8. *Stevens JF, Page JE.* Xanthohumol and related prenylflavonoids from hops and beer: to your good health! Phytochemistry. 2004;**65**(10):1317–30. doi:10.1016/j.phytochem.2004.04.025
9. *Fukao T, Sawada H, Ohta Y.* Combined effect of hop resins and sodium hexametaphosphate against certain strains of *Escherichia coli*. J. Food Prot. 2000;**63**(6): 735–40.
10. *Pavlovič V, Pavlovič M, Čerenak A, Košir IJ, Čeh B, Rozman Č, Turk J, Pazek K, Krofta K, Gregorič G.* Environment and weather influence on quality and market value of hops. Plant Soil Environ. 2012;**58**(4):155–60.
11. *Srečec S, Zechner-Krpan V, Marag S,* Špoljarić *I, Mršić G.* Morphogenesis, volume and number of hop (*Humulus lupulus* L.) glandular trichomes, and their influence on

alpha-acids accumulation in fresh bracts of hop cones. Acta Bot. Croat. 2011;**70**(1):1–8. doi.org/10.2478/v10184-010-0017-2.

12. *Stevens JF, Miranda CL, Buhler DR, Deinzer ML.* Chemistry and biology of hop flavonoids. J. Am. Soc. Brew. Chem. 1998;**56**(4:136–45.
13. *Miranda CL, Stevens JF, Ivanov V, McCall M, Frei B, Deinzer ML, Buhler DR.* Antioxidant and prooxidant action of prenylated and nonprenylated chalcones and flavanones *in vitro*. J. Agric. Food Chem. 2000; **48**(9):3876–84.
14. *Magalhães PJ, Carvalho DO, Cruz JM, Guido LF, Barros AA*. Fundamentals and health benefits of xanthohumol, a natural product derived from hops and beer. Nat. Prod. Commun. 2009;**4**(5):591–610.
15. *Protsenko L, Litvynchuk S.* Features of using hops and CO_2-extract in brewing, Ukrain. Food J. 2017;**6**(1):77–84.doi:10.24263/2304-974x-2017-6-1-9.
16. *Strathmann J*, *Klimo K, Sauer SW, Okun JG, Prehn JHM, Gerhäuser C.* Xanthohumol-induced transient superoxide anion radical formation triggers cancer cells into apoptosis via a mitochondria-mediated mechanism. FASEB J. 2010;**24**(8):2938–50. doi: 10.1096/fj.10-155846.
17. *Ferk F, Huber WW, Filipic M, Bichler J, Haslinger E, Misík M, Nersesyan A, Grasl-Kraupp B, Zegura B, Knasmüller S.* Xanthohumol, a prenylated flavonoid contained in beer, prevents the induction of preneoplastic lesions and DNA damage in liver and colon induced by the heterocyclic aromatic amine amino-3-methyl-imidazo[4,5-f]quinoline (IQ). Mutat. Res. 2010;**691**(1–2):17–22. doi: 10.1016/j.mrfmmm.2010.06.006.
18. *Monteiro R, Calhau C, Silva AO, Pinheiro-Silva S, Guerreiro S, Gärtner F, Azevedo I, Soares R.* Xanthohumol inhibits inflammatory factor production and angiogenesis in breast cancer xenografts. J. Cell. Biochem. 2008;**104**(5):1699–707. doi: 10.1002/jcb.21738.
19. Xiu-Li Zhang, Yong-Dong Zhang, Tao Wang, Hong-Yun Guo, Qi-Ming Liu, and Hai-Xiang Su. Evaluation on Antioxidant Effect of Xanthohumol by Different Antioxidant Capacity Analytical Methods J. Chem.2014, doi: 10.1155/2014/249485.
20. *Zamzow DR, Elias V, Legette LC, Choi J, Stevens JF, Magnusson KR.* Xanthohumol improved cognitive flexibility in young mice. Behav. Brain. Res. 2014;**275**:1–10. doi:10.1016/j.bbr.2014.08.045.
21. *Warnakulasuriya SN, Ziaullah, Vasantha Rupasinghe HP.* Long chain fatty acid acylated derivatives of quercetin-3-o-glukoside as antioxidants to prevent li-pid oxidation. Biomolecules. 2014;**4**(4):980–93.doi: 10.3390/biom4040980.
22. *Żołnierczyk AK, Baczyńska D, Potaniec B, Kozłowska J, Grabarczyk M, Woźniak* E, *Anioł M.* Antiproliferative and antioxidant activity of xanthohumolacyl derivatives. Med. Chem. Res. 2017;**26**(8):1764–71.doi: 10.1007/s00044-017-1887-9.
23. *Yang M, Li N, Li F, Zhu Q, Liu X, Han Q, Wang Y, Chen Y, Zeng X, Yi Lv, Zhang P, Yang C, Liu Z.* Xanthohumol, a main prenylated chalcone from hops, reduces liver damage and modulates oxidative reaction and apoptosis in hepatitis C virus infected *Tupaia belangeri*. Int Immunopharmacol. 2013;**16**(4):466–74. doi:10.1016/j.intimp.2013.04.029.
24. *Stevens JF, Page JE.* Xanthohumol and related prenylflavonoids from hops and beer: to your good health! Phytochemistry. 2004;**65**(10):1317–30. doi:10.1016/j.phytochem.2004.04.025.
25. *Protsenko LW, Svirchevska OW, Wlasenlo AS.* Research of biologically active substance Xanthohumol in the cones of the Ukrainian hop varieties. Agropromyslove vyrobnytstvo Polissya. 2015;(8):68–73.
26. *Protsenko L, Grynuk T, Litvynchuk S*. Influence of the constituent alpha acids of Ukrainian varieties of hops and hop preparations on quality indicators of mash and beer, Ukrain. Food J., 2014;**3**(3):389–96.
27. *Dobrovolny OO, Shalamai AS, Shmatenko OP, Protsenko LV.* The study of hop varieties as initial raw material of the extract with increased content of prenylated flavonoids. Farmatsevtychny chasopys. 2014;**2**(30):11–7.
28. *Patent of* Ukraine No. 103469, MPK A61K 35/00. A method of obtaining the hop extract, enriched with prenylated flavonoids with estrogenic effect, utility model patent / Dobrovolny OO, Bezpalko LV, Slobodianiuk you, Shmatenko OP, Fesenko SO, Protsenko LV, Rudyk RI; appl. 04.04.2014, publish. 25.12.2015, Bul. #24.
29. *Stevens, JF, Page, JE.* Xanthohumol and related prenylflavonoids from hops and beer: to your good health? Phytochemistry 65, 2004. 1317–1330.
30. *McCullough MP, Volpe R.* Can Beer Consumption be Part of a Healthy Diet? J. Agricultural and Applied Economics Association. 2017;**32**(3):1–7.
31. *Yermolaeva GA.* Reference book of a laboratory specialist of beer brewing plant. StPb: Publishing House "Profession". 2004. 535 https://elibrary.ru/item.asp?id=19614429
32. *Analytica* – EBC (1987), European Brewery Convention, fourth edition.