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Bitter Substances in the Hop Lupulin

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Aim. To investigate the quantity and structure of α-, β-acids and xanthohumol in lupulin grains of different hop varieties that essentially differ in these biochemical indexes, and also the presence of the substances both in staminate racemes and in the leaves. **Methods**. High performance liquid chromatography (HPLC), up-to-date physical-chemical methods of hop quality indicators' definition, special and standard in the hop-growing branch, were applied. **Results**. It was stated that lupulin of aroma and bitter varieties contains various quantity of α- and β-acids. Therefore, the ratio of α- to β-acids in aroma hop varieties is above one (1), whereas in varieties of bitter type this ratio is much lower than one (1). No correlation between the quantity of lupulin and the contents of α- and β-acids was found. It was noted that the color of lupulin depends upon the quantity of xanthohumol. **Conclusions**. The performed tests give evidences on lupulin glands are located on anthers of staminate racemes and on the leaves as well, though in much less quantity and less educed. It was found that the quantity and structure of bitter substances in lupulin grains from selection varieties does not depend upon lupulin content in hop cones, but it is a grading factor. Lupulin from the staminate racemes received from various plants essentially differs in quantity of α- and β-acids. This fact is of key importance for pair selection. In petal glands on the leaves of a hop plant bitter substances are represented only by β-acids, mainly lupulone and adlupulone.

Keywords: bitter substances, lupulin grains, xanthohumol, α-acids, β-acids, staminate racemes.

INTRODUCTION

Because of the presence of approximately 100 bitter substances, also more than 320 components of essential oils and 70 substances of polyphenolic compounds and almost 20 prenylated flavonoids in the hop cones, this culture plant is an irreplaceable raw material for the beer production. Furthermore, the hop cones are widely used in perfume, baking industries and medicine. At the moment more than 100 complex medicines include the hop or its components. But the main consumer of hop (more than 90 per cent) is the brewery industry. The unique bitter substances never found in any other plant, essential oil and polyphenols of hop provide to beer the typical bitter taste and aroma, cause purification and foam formation, also increase biological and colloidal storability of this beverage [1].

It is known that the bitter substances, essential oil and prenylflavonoids of hop are synthesized and accumulated in the lupulin glands (peltate trichomes) located on the inner side of the hop cones' perulas, and also at the ovary and cone's spindle. Lupulin glands are multicellular hairsprings formed from the cells of epidermis. Peltate trichomes synthesize the biologically active materials exuded under the cuticle. As long as the peltate glands are greatly filled with bitter substances, essential oil and xanthohumol, lupulin begins to accumulate. Depending on the ripeness of cones and weather conditions they have various forms, are sticky by touch and become of gold yellow color.

The lupulin glands are also located on the anthers of staminate racemes,while on leaves they are considerably less in number and are also less developed. Therefore, quantity and state of lupulin is one of the important organoleptic indices of the hop cones' quality [2].

The brewing value of hop and its products are determined by the quality properties of the hop variety: the biochemical composition of bitter substances and essential oil, harvesting periods, processing technology and conditions, also standard rates and ways of application to wort. It is known, the varieties of hop are distinguished not only by the α-acids content, but also β-acids, essential oil and polyphenols concentration at the rate of 1 g of α-acids.

Since 1990 many scientists in various countries pay considerable attention to the exploration of hop's prenylated flavonoids, which are extremely active biologically, in particular, anticarcinogenic, phytoestrogen, antioxidant, antimicrobial and antiviral properties [3, 4]. It is known that hop's prenylflavonoids include

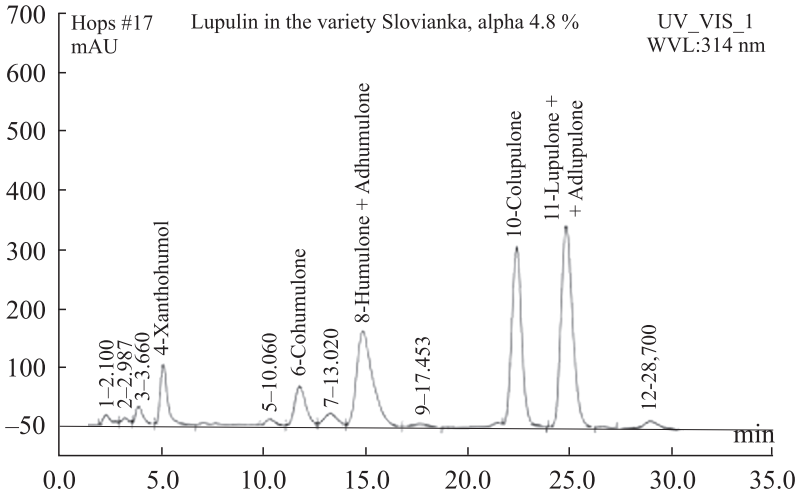

Fig. 1. Lupulin bitter substances chromatogram for the fine-aroma variety Slovianka: X-axis: bitter substances components yield time (min), Y-axis: signal scope (mAU)

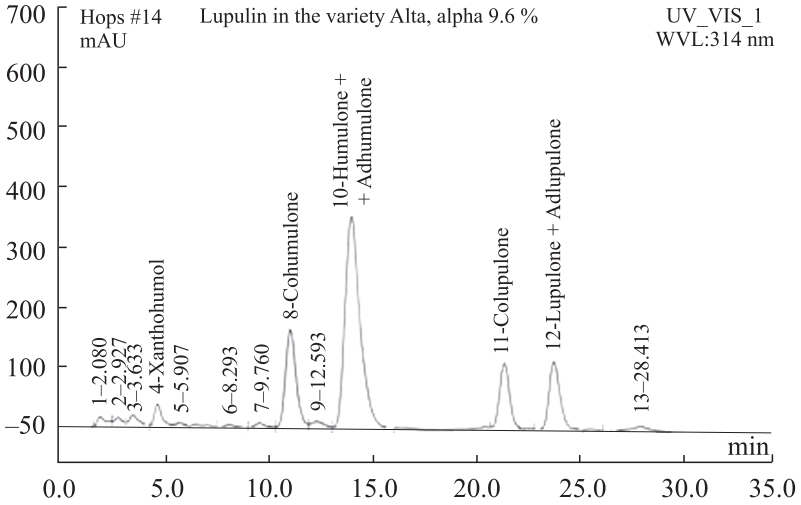

Fig. 2. Lupulin bitter substances chromatogram for the bitter variety Alta: X-axis: bitter substances components yield time (min), Y-axis: signal scope (mAU)

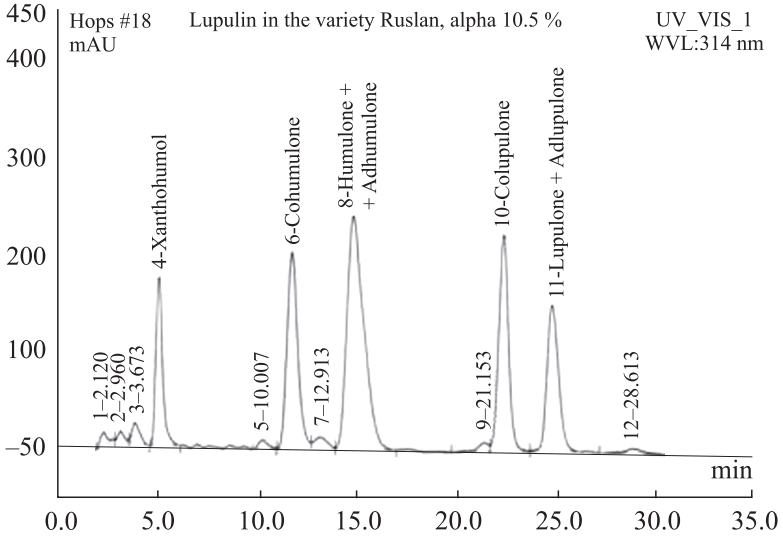

Fig. 3. Lupulin bitter substances chromatogram for the variety Ruslan: X-axis: bitter substances components yield time (min), Y-axis: signal scope (mAU)

more than 20 compounds: xanthohumol, 6-prenylnaringenin, 8-prenylnaringenin, 6-geranilnaringenin, dysmethilxanthohumol, dehydrocycloxanthohumol and others. As Stevens *et al*. [4] state, xanthohumol is of the greatest value, its content composes 80-90 per cent of the prenylchalcones' overall mass in the hop. The presence of xanthohumol in the hop cones is established in 1967. However, as this connection was

in the fraction of hard resins considered undesirable among the bitter substances in the beer production, it was not given an adequate consideration and practically not investigated.

The anticarcinogenic properties of xanthohumol were reported for the first time in 1998 at the conference of the American Toxicology Society in Seattle by researchers from the Oregon University. It is very efficient during the treatment of the diseases caused by fungi, staphylococci, streptococci, also the viruses of herpes and hepatitis. Xanthohumol is also studied as potential anticarcinogenic agent [5−7]. During the biological tests it proved to be the most active connection among prenylflavonoids, especially as for the influence on the cancerous cells in the patients with cancer of the large intestine, mammary gland, ovaries, prostate and blood, at the same time no influence on healthy cells was stated. Stevens and Miranda investigated that xanthohumol suppresses the cancerous cells of mammary gland 200 times more active in comparison to resveratrol contained in red wine [4, 6, 7]. The anticarcinogenic action of xanthohumol is explained with its antioxidant properties. This very agent activates the ferments impeding a tumor growth, also destroys cancerous cells and stunts control metastases increase [7, 8].

Co-author of this article, M. I. Liashenko, investigated for the first time a quantity of xanthohumol in various domestic varieties of hop, and also its accumulation in the process of cones' forming and ripening in the beginning of 1980s. The xanthohumol concentration in the hop cones depends on its variety and normally is from 0.2 per cent to 1.6 per cent. For the domestic hop varieties Ruslan, Xanta, and Chaklun the quantity of the agent is within 1.0−1.6 per cent, whereas the foreign varieties – German Taurus and Czech Agnus – demonstrate maximally 1.0 per cent.

During wort pitching xanthohumol is converted into isoxanthohumol. Therefore, the latter considerably exceeds xanthohumol in the beer. The use in the brewing of the hop varieties with α-acids carbonic acid extracts high content, where there is practically no xanthohumol, leads to a decrease of this valuable for the human organism agent in the beer.

The correlation between the quantity of lupulin glands on the bracteal perulas of the hop cones and total number of bitter substances was established earlier [9].

The aim of this research consisted in the study of the quantity and qualitative composition of α-, β-acids and xanthohumol into the lupulin of the hop cones for particular varieties essentially differing by these biochemical qualitative indexes and by their presence in the staminate racemes and leaves.

MATERIALS AND METHODS

The research was conducted in the certified laboratory of the Hop and Beer Biochemistry Department in the Institute of Agriculture of Polissia, NAAS of Ukraine (hereinafter referred to as the Institute).

The contemporary physical-chemical methods of the hop qualitative estimation, both specially designed and common for the hop-growing according to DSTU 4099: 2009 Hop.; the sampling rules and the testing techniques [1, 10], and also mathematical-statistical methods involving the dispersion and correlation-regression analyses for the authenticity evaluation of the obtained results were applied in the current research.

The hop samples of the aromatic and bitter varieties grown in the test field of the Institute were analyzed. The hop samples of each variety were selected in the industrial dead-ripe stage, not less than from 10 bushes within the middle canopy of plants, according to the ef-

Table 1. Lupulin Bitter Substances Content in Hop Cones of Both Aroma and Bitter Varieties

Parameter	Hop Variety							
	Klop 18	Slovianka	Serebrianka	Zahrava	Promin	Ruslan	Alta	Mahnum
Cohumulone, %	3.3	3.7	4.9	6.1	9.4	11.2	8.5	11.0
Humulone + adhumulone, %	14.3	12.3	18.5	20.2	27.5	19.6	24.8	27.4
α-acids, %	**17.6**	**16.0**	**23.4**	**26.3**	**36.9**	**30.8**	**33.3**	**38.4**
Cohumulone as part of α-acids, %	23.3	23.1	20.9	23.2	25.5	36.2	25.6	28.6
Colupulone, %	8.9	17.2	10.6	14.1	10.4	13.0	6.1	7.4
Lupulone + adlupulone, %	11.2	20.8	15.4	17.9	9.9	9.4	6.3	8.2
β-acids, %	**20.1**	**38.0**	**26.0**	**32.0**	**20.3**	**22.4**	**12.4**	**15.6**
Colupulone as part of β-acids, %	44.3	45.3	40.8	43.9	51.2	58.0	48.8	47.4
β-acids:α-acids	**1.14**	**2.38**	**1.11**	**1.21**	**0.55**	**0.73**	**0.37**	**0.41**
α- i β-acids in total, %	**37.7**	**54.0**	**49.4**	**58.4**	**57.2**	**53.2**	**45.7**	**54.0**
α-acids ratio to α- and β-acids amount, %	46.7	29.6	47.4	45.2	64.5	57.9	72.9	71.1
Xanthohumol, %	**1.13**	**1.88**	**1.41**	**1.57**	**1.72**	**3.0**	**0.65**	**1.16**

fective regulation [10]. The average sample mass for identification and biochemical studies is equal to at least 1 kg of dry hop. The hop samples were dried to standard humidity of 9−12 per cent.

Pure hop lupulin was obtained by the screen separation of the industrially ripe cones' petal mass through the 0.25 mm-meshes sieve; while the mixture of pollen and lupulin was obtained from the staminate racemes. The content and composition of α -, β-acids and xanthohumol were determined by the method of high performance liquid chromatography [1, 10].

RESULTS AND DISCUSSION

The content and qualitative composition of bitter substances and xanthohumol in lupulin for both the aromatic and bitter hop varieties are determined. Lupulin bitter substances and xanthohumol chromatograms for fine-aroma variety Slovianka, bitter one – Alta and high xanthohumol bitter-aroma variety Ruslan are represented in Fig. 1−3.

The basic components of bitter substances are α- and β-acids. On the chromatograms α-acid are represented with the summation of cohumulone, humulone and adhumulone; whereas β-acid – with colupulone, lupulone and adlupulone. The given chromatograms prove the fact that the lupulin in the aroma variety Slovianka demonstrates considerably higher peaks of β-acids components than the same α-acids compounds, whereas the lupulin in the bitter variety Alta has more α-acids, on the contrary. Furthermore, on the chromatogram of the lupulin in the variety Ruslan the xanthohumol highest peak is observed among the given chromatograms, together with increased content of cohumulone in the α-acids compounds and colupulone in β-acids.

A quantity and composition of the lupulin bitter substances in the hop cones of both aroma and bitter varieties are given in Table 1.

It has been previously established that a α-acids quantity varies from 1 per cent to 18 percent, and β-acids – from 2 per cent to 12 per cent [1, 2] depending on the selective variety of hop. This gives evidences on the fact that the α-acids quantity in the lupulin of the aroma varieties of hop is considerably less (14.3−26.3 per cent) in comparison with the bitter varieties (30.8−38.4 per cent), whereas β-acids quantity is more in the lupulin of aroma varieties: 20.1−38.0 per cent, while 12.4−22.4 per cent in the bitter varieties, on the contrary. For this very reason the ratio of β-acids quantity to α-acids in the aroma varieties is more than one, whereas in bitter varieties it is considerably less. The α-acids portion in the total amount of α- and β-acids varies within 29.6−72.9 per cent depending on selective variety in the investigated lupulin. Thus, it is impossible to forecast the α- and β-acids content on the lupulin quantity. For example, in the variety Aromat Polissia the cones contained a significant quantity of lupulin in some years, however, the α-acids content could be not more than 2 per cent.

The study of α- and β-acids composition showed that the portion of cohumulone in α-acids and colupulone in β-acids considerably varies and serves as a grading factor. Thus, α- and β-acids definite content and qualitative composition are one of the basic biochemical indices of both brewing value and hop selective varieties identification.

For the first time the xanthohumol quantitative content in the lupulin of different varieties was analyzed by the method of highly effective liquid chromatography. It was established that depending on variety its quantity varies from 0.65 per cent in Alta to 3.0 per cent in Ruslan. Since xanthohumol is rich yellow, its portion in lupulin varies lupulin color from light-yellow to gold yellow.

The bitter substances in the pollen and lupulin mixture of the hop staminate racemes were also investigated. As a result, it was stated that α- and β-acids quantity and their content in various plants differ essentially (Table 2).

Thus, an α-acids portion in the studied plants varied from 0.22 per cent to 5.14 per cent, β-acids – from 0.34 per cent to 5.63 per cent, cohumulone portion as a part of α-acids increased almost twice (22−44 per cent),

Table 2. α- and β-acids Contents in Hop Staminate Racemes (Pollen and Lupulin Mixture), %

Plant Number	Content, %			
	α-acids	β-acids	Cohumulone as part of α-acids	Colupulone as part of β-acids
22-52	0.22	0.34	41.0	68.0
A-224	1.18	0.52	34.0	54.0
4-25	2.14	1.28	22.0	32.0
4-26	3.27	2.32	31.0	50.0
A-225	5.14	5.63	34.0	41.0

Table 3. β-acids Content in Lateral Branches Leaves of Hop

Hop Variety	β-acids, 1 mg per 100g of Dry Substance	β-acids, Content, %	
		Colupulone	Lupulone + Adlupulone
Klon 18	9.8	21.4	78.6
Slovianka	32.1	28.0	72.0
Zhytran	140.7	26.7	73.3
Zmina	57.3	28.0	72.0
Haydamatskyi	55.0	32.9	67.1
Poliskyi	9.8	21.4	78.6

and colupulone as part of β-acids was 32 per cent to 68 per cent. Thus, it is necessary to consider the results of these explorations during the pair selection for the breeding.

In leaf glands of hop only β-acids were reported; however, their quantity considerably varied depending on the selective variety (Table 3).

It results from Table 3 that β-acids quantity greatly differs in lateral branches leaves of the different hop varieties. The highest content of β-acids was reported in the leaves of the aroma variety Zhytran, while the least – in the varieties Klon 18 and Poliskyi. It should be noted that the β-acids content is relatively constant in the leaves with the tangible advantage of lupulone and adlupulone over colupulone.

CONCLUSIONS

The quantitative content and composition of α-, β-acids and cohumulone in the lupulin from particular varieties does not depend on its content in the hop cones, though it is a grading factor at the same time. α- and β-acids account for almost half of the lupulin mass.

The lupulin from the Ruslan variety contains the highest rate of xanthohumol among all tested samples.

The lupulin in the staminate racemes obtained from various plants essentially differs in α- and β-acids content. The rate of cohumulone in the composition of α-acids and colupulone in β-acids essentially changes in the separate plants, just as into the hop cones lupulin.

The bitter substances in the hop leaves glands are represented only with β-acids, moreover lupulone and adlupulone in essence.

Гіркі речовини лупуліну хмелю

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Мета. Вивчити кількісний та якісний склад α-, β-кислот і ксантогумолу у лупуліні шишок сортів хмелю, який суттєво різниться за цими біохімічними показниками якості та їхньою присутністю в тичинкових суцвіттях і листках. **Методи**. Використано високоефективну рідинну хроматографію, сучасні фізико-хімічні методи визначення якісних показників хмелю, спеціальні та загальноприйняті в хмелярській галузі. **Результати**. Визначено, що лупулін ароматичних і гірких сортів містить різну кількість α- і β-кислот. Так, співвідношення β- до α-кислот в ароматичних сортах становить більше одиниці, тоді як у сортах гіркого типу воно значно менше. Залежності між кількістю лупуліну та вмістом α- і β-кислот не виявлено. Відмічено, що забарвлення лупуліну визначається кількостю ксантогумолу. **Висновки**. З проведених досліджень випливає, що лупулінові залозки присутні також на пиляках тичинкових суцвіть, на листках їх значно менше і вони менш розвинені. Встановлено, що вміст і склад гірких речовин у лупуліні селекційних сортів не залежить від його кількості у шишках хмелю, а є сортовою ознакою. Лупулін у тичинкових суцвіттях, одержаний з різних рослин, суттєво різниться за кількістю та якісним складом α- і β-кислот, що має важливе значення у селекційному процесі при підборі пар для схрещування. У залозках на листках хмелю гіркі речовини представлені лише β-кислотами, в основному, лупулоном і адлупулоном.

Ключові слова: гіркі речовини, лупулін, ксантогумол, α-кислоти, β-кислоти, тичинкові суцвіття.

Горькие вещества хмеля

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Цель. Изучить количественный и качественный состав α-, β-кислот и ксантогумола в лупулине шишек сортов хмеля, который существенно отличается по этим биохимическим показателям качества и их присутствию в тычиночных соцветиях и листьях. **Методы**. Использованы высокоэффективная жидкостная хроматография, современные физико-химические методы определения качественных показателей хмеля, специальные и общепринятые в хмелеводческой отрасли. **Результаты.** Определено, что лупулин ароматических и горьких сортов содержит различное количество α- и β-кислот. Так, соотношение β- и α-кислот в ароматических сортах составляет больше единицы, тогда как в сортах горького типа оно значительно меньше. Зависимости между количеством лупулина и содержанием α- и β-кислот не обнаружено. Отмечено, что цвет лупулина определяется количеством ксантогумола. **Выводы**. Из проведенных исследований следует, что лупулиновые железки присутствуют также на пыльниках тычиночных соцветий, на листьях их значительно меньше, и они менее развиты. Установлено, что содержание и состав горьких веществ в лупулине селекционных сортов не зависит от его количества в шишках хмеля, а является сортовым признаком. Лупулин в тычиночных соцветиях, полученный из разных растений, существенно различается по количеству и качественному составу α- и β-кислот, что имеет важное значение в селекционном процессе при подборе пар для скрещивания. В железках на листьях хмеля горькие вещества представлены только β-кислотами, в основном, лупулоном и адлупулоном.

Ключевые слова: горькие вещества, лупулин, ксантогумол, α-кислоты, β-кислоты, тычиночные соцветия.

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