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COMPARATIVE QUANTITATIVE STUDY ON PHYTOCHEMICAL CONTENTS OF HOPS (*Humulus lupulus* L.)

Summary

Hop cones (Humulus lupulus L.*) are mainly used as the main flavoring ingredient in beer production. Cones contain active compounds that determine not only the aromatic characteristic, but also functional properties. The aim of this study was to determine the basic anatomical characteristics of hops of the following varieties: Marynka, Lubelski, Magnum, and the assessment of the content of selected phytochemicals in water extracts. The different level of the content of chlorophyll depending on the cultivar studied was shown. The cones of the Marynka variety (0.49 mg/g) had the highest content of total chlorophylls. All tested hop cone extracts showed a similar content of carotenoid pigments. The highest amount of polyphenolic acids was found in extracts prepared from the Lubelski variety cones (5.22 mg chlorogenic acid/g), and the lowest in the cones of Marynka variety (3.36 mg chlorogenic acid/g). Based on the research, it was shown that hop cones can be a good raw material for the production of water extracts due to the high content of biologically active compounds. Hop cones can be a raw material for the production of functional food.*
Keywords: *hops, hop cones, bioactive compounds, polyphenolic acids, chlorophyll, carotenoids, proanthocyanidis*

CHMIEL (*Humulus lupulus L.*) JAKO SUROWIEC O WŁAŚCIWOŚCIACH PROZDROWOTNYCH – ZAWARTOŚĆ FITOZWIĄZKÓW W WYBRANYCH ODMIANACH CHMIELU

Streszczenie

Szyszki chmielowe (Humulus lupulus L.*) wykorzystywane są głównie jako podstawowy składnik aromatyzujący w produkcji piwa. Zawierają one bowiem związki aktywne, które decydują nie tylko o walorach organoleptycznych, ale także o właściwościach prozdrowotnych. Celem niniejszej pracy była podstawowa charakterystyka towaroznawcza szyszek chmielu odmian Marynka, Lubelski i Magnum, a także ocena zawartości wybranych fitozwiązków w wodnych ekstraktach z szyszek. Wykazano zróżnicowanie zawartości chlorofilu w zależności od badanej odmiany. Najwyższą zawartością sumy chlorofili charakteryzowały się ekstrakty z szyszek odmiany Marynka (0,49 mg/g). Wszystkie badane ekstrakty z szyszek chmielowych wykazały podobną zawartość barwników karotenoidowych. Polifenolokwasy w największej ilości znajdowały się w ekstraktach sporządzonych z szyszek odmiany Lubelski (5,22 mg kwasu chlorogenowego/g), a w najmniejszej w szyszkach odmiany Marynka (3,36 mg kwasu chlorogenowego/g). Na podstawie wykonanych badań wykazano, że szyszki chmielu mogą stanowić dobry surowiec do wytwarzania ekstraktów wodnych z uwagi na wysoką zawartość związków biologicznie aktywnych. Szyszki chmielu mogą stanowić również surowiec do produkcji żywności funkcjonalnej.*
Słowa kluczowe: *chmiel, szyszki chmielowe, związki bioaktywne, polifenolokwasy, chlorofil, karotenoidy, proantocyjanidyny*

1. Introduction

Hops (*Humulus lupulus* L.) is a plant known primarily as a raw material used in brewing. The part of the plant used is hop cones, rich in compounds that determine the characteristic aroma and taste of beer. In Poland, hops are grown mainly in the Lublin region, in the Poznań region (near Nowy Tomyśl), and in the Opolskie region (near Nysa). Both aromatic and bitter varieties are grown there. In Poland, 12 varieties of hops are grown, and in significant quantities, these are the varieties Lubelski (aromatic), Marynka, and Hallertau Magnum (bitter). To obtain a more intense bitterness effect, bitter hops are added to the wort at the beginning of cooking. Aromatic hops, on the other hand, will not work if cooked for too long, so they are added later in the process. Alpha acids in hops are responsible for the taste, and essential oils for the smell, so the content of these substances determines the properties of individual varieties of this plant. Hop cones are harvested in late summer or early autumn when their water content is 75-80%, and the content of metabolites in lupulin is the highest (essential oils, containing humulene, β-myrcene, farnezen, β-caryophyllene, etc., tannins, alcohols: myrcene and its esters with isovaleric, pelargonic, butyric and acetic acids, resin compounds: humulone (α-bitternessmielic acid), lupulin (i.e. β-bitternessmielic acid), colupulone, adlupulone, xanthohumol, chalcones, flavonoids, and others) [1].

After harvesting, the cones are dried at a temperature of 60-75°C to obtain a humidity of about 10%. They are then kneaded and cooled, then packaged and refrigerated until they are used, sold, or processed into hop products. Thus prepared hops can be stored for several months, preferably at a temperature close to 0°C, which significantly slows down the oxidation processes and ensures the stability of compounds in cones [1].

Hops have been used as a medicine for centuries. It was used primarily as a mild sedative in the treatment of insomnia

and relieving anxiety, it also prevented headaches, and also improved appetite, and relieved tooth and ear pain. Currently, the cones of female hops are used almost exclusively as an ingredient for the brewing industry [2]. One hop cone can be used to brew 0.5 liters of beer [3].

The aim of the study was to assess the content of selected active compounds in various varieties of hop cones and their basic commodity assessment.

2. Material

The research material was the cones of common hop (*Humulus lupulus* L.) of Magnum, Lubelski, and Marynka varieties. The material was obtained from a common hops plantation in Malice (Kujawsko-Pomorskie Voivodeship, Kcynia Commune: 52°59'52.6"N 17°31'20.6"E. The cones were collected, depending on the variety, at the turn of August and September 2019. Then they were dried and frozen to a temperature of -35ºC, and then they were subjected to the freeze-drying process in a CHRIST 1-4 LSC freeze dryer with constant parameters: condensation temperature: -48ºC, the temperature on the shelf of the freeze dryer: + 20ºC, product temperature: -4ºC, under reduced pressure, during 48 hours. The material obtained was ground in a Retsch Grindomix 664-14872 for 30 seconds at a speed of 3000 rpm. Water extracts were prepared from the amount of 4 g of crushed cones and 100 ml of solvent at 85°C. The whole was shaken in a water bad for 60 minutes and then vacuum filtered on a Büchner funnel. The prepared extracts were stored tightly closed until further analyzes under refrigeration at 4°C.

3. Methods

The total content of polyphenolic acid compounds was determined in the aqueous extracts of hop cones [4]. The principle of the method was based on the colorimetric measurement of the reaction mixture at a wavelength of 490 nm (Meterec SP-830) using the Arnov reagent described in the Polish Pharmacopoeia [4]. The obtained data on the content of polyphenolic acids were converted into chlorogenic acid based on the drawn standard curve and expressed in mg/g of dry mass extract. The content of proanthocyanidins in the extracts was determined by the method of reducing iron Fe (NH_4SO_4) 3 in 2 M HCl. It was determined spectrophotometrically at a wavelength of 550 nm. The obtained results were presented in the values of A550 nm absorbance per 1 mg of the extract [5].

The content of chlorophyll and carotenoid was determined by the spectrophotometric method. The absorbance of the solution was measured at the wavelengths: 662 nm, 644 nm, and 440 nm [6]. The assessment of the significance of mean differences for the content of bioactive ingredients was performed using ANOVA and Tukey's test at the significance level of $\alpha = 0.05$ in the Statistica 13.1 program.

4. Results

4.1. Characteristics of hop cones (*Humulus lupulus* L.)

After drying, the hops of the Marynka, Magnum, and Lubelski varieties were measured (height and width) and weighed. Then the color and aroma of the cones were assessed using the organoleptic method. The results are presented in Table 1. The cones of the Lubelski variety were yellow-green, about 3 cm long, and characterized by a delicate aroma. On the other hand, the cones of the Marynka variety were light green, had the smallest size, and had an intense aroma. The Magnum variety was characterized by a dark green color, a very strong aroma, and larger sizes.

Similar characteristics of both the cones and other morphological parts of hops were also made when comparing Polish and foreign hop varieties [7].

4.2. Polyphenols content

Polyphenolic compounds are a large group of compounds in which there are flavonoids, stilbenes, lignans, and phenolic acids. An important group of polyphenols in plant raw materials are phenolic acids, which can be divided into hydroxybenzoic acids (e.g. gallic acid) and hydroxycinnamic acids (e.g. caffeic, chlorogenic, or p-coumaric acids). Significant amounts of polyphenolic acids, are found in fruits, grains, and seeds, flowers, roots, and leaves of plants. Phenolic acids of all polyphenols constitute up to 30% of the average diet. In food products of plant origin, they are often in the form of glycosides or esters [8, 9].

The results were expressed as chlorogenic acid and are presented in Fig. 1.

Hop cones of the Lubelski variety (5.22 mg/g) were characterized by the highest content of polyphenolics among all the tested preparations, and the smallest content of the Marynka variety (3.36 mg/g). Polyphenolic acids accounted for approximately 12-14% of the total content of phenolic compounds in hop extracts.

Inui et al. [10] analyzed the content of 23 polyphenolic compounds in hop cones. Among the phenolic acids, they determined a significant content of *p*-coumaric, salicylic, chlorogenic, and caffeic acids. However, they mention xanthohumol, C1 procyanidin, epigallocatechin, quercetin and, kaempferol as the main polyphenolic compounds of hops. Similarly to this study, they found that polyphenolic acids constitute approx. 10% of the total content of phenolic compounds in hops. They also indicated that this content significantly depends on the harvest date of the plant [10]. Analyzing the commercial hop extract Hopsphenon®, it was shown that polyphenolic acids constitute only 3% of the total content of phenolic compounds in this preparation [11].

Table 1. Commodity characteristics of freeze-dried hop cones
Tab. 1. Charakterystyka towaroznawcza liofilizowanych szyszek chmielu

Variety	Height [mm]	Width [mm]	Mass [g]	Color	Aroma
Marynka	$30.02^{a} \pm 2.31$	$19.01^{a} \pm 1.04$	$2.01^{a} \pm 0.03$	light green	intense hoppy, slightly floral
Magnum	$37.89^{b} \pm 5.22$	$20.21^{a} \pm 2.32$	$2.02^{a} \pm 0.04$	dark green	intense hop
Lubelski	$35.17^{b} \pm 3$	$19.15^{a} \pm 1.54$	$2.02^{a} \pm 0.02$	yellow to green	delicate hoppy, pleasant

Data show mean values of triplicates and standard deviation. Mean values marked with different lowercase letters in the same column indicate the significance of the differences ($p \leq 0.05$)

Source: own work / Źródło: opracowanie własne

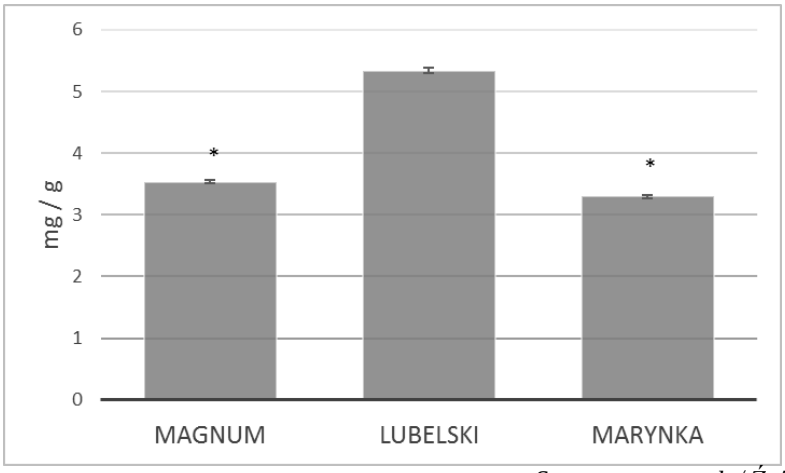

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Fig. 1. The content of polyphenolic acids in extracts from hop cones (data show mean values from three repetitions and standard deviation) * indicate the significance of differences for $p \leq 0.05$

*Rys. 1. Zawartość polifenolokwasów w ekstraktach z szyszek chmielowych (dane przedstawiają wartości średnie z trzech powtórzeń oraz odchylenie standardowe) * świadczy o istotności różnic (p≤0,05)*

4.3. The content of proanthocyanidins

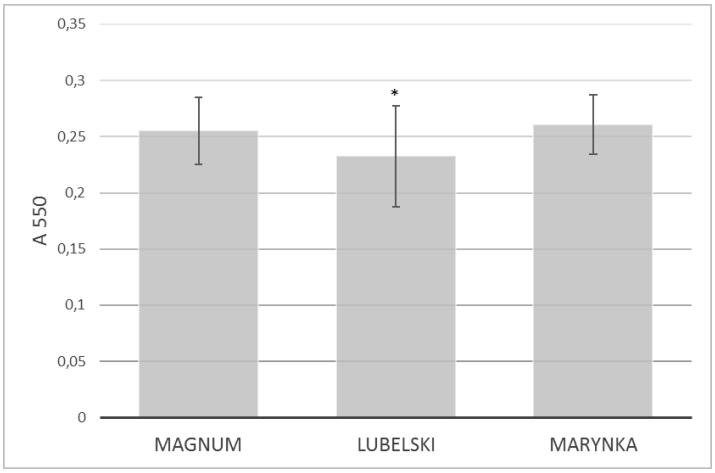
An important group of compounds belonging to polyphenols is proanthocyanidins, better known as condensed tannins. They are mixtures of oligomers and polymers consisting of flavanone-3-ol, contained in fruits, vegetables, cereals, seeds, nuts, spices and cocoa. Proanthocyanidins are of interest due to their potential health benefits and, chemopreventive properties [5]. The content of proanthocyanidins in hop is shown in Fig. 2.

The highest content of proanthocyanidins was determined in the extract of cones of cultivar Marynka (A550 nm/mg of extract = 0.260), and the lowest in the extract of cultivar Lubelski (A550 nm /mg of extract = 0.200). Research shows that the content of bioactive compounds in hops is high. Proanthocyanidins constitute a significant part of this. Proanthocyanidin B3 was identified as the dominant proanthocyanidins in hop extracts, followed by B1, B4 and, C2 [12]. The proportions of various proanthocyanidins differ significantly depending on the variety and climatic conditions, which indicates differences in their content in the cones of the same variety from different geographic regions [13]. In addition, research has shown that these compounds stabilize the organoleptic properties and color of beer, and give it the desired effects of astringency.

Recent studies have also shown that these compounds can also have significant beneficial effects on health. Proanthocyanidins have been shown to inhibit the growth of various human neoplastic cells [14]. Nagasako-Akazome et al. [11] examined the content of proanthocyanidins in the commercial extract of Hopsphenon® hops and showed that their share in the total content of phenolic compounds was as high as 38%.

4.4. The content of chlorophylls and carotenoids

Chlorophyll dyes and carotenoids are plant compounds that occur in complexes with protein-chloroplasts. There are naturally two forms of chlorophyll: chlorophyll a ($C_{55}H_{72}O_5N_4Mg$), a blue-green color, and chlorophyll b ($C_{55}H_{70}O_6N_4Mg$), a yellow-green color. Plants are dominated by chlorophyll a, which is responsible for the green color.



Source: own work / Źródło: opracowanie własne

Fig. 2. The content of proanthocyanidins in hop cone extracts (data contain mean values from three replications and standard deviation) * indicate the significance of differences for $p \leq 0.05$

*Rys. 2. Zawartość proantocyjanidyn w ekstraktach z szyszek chmielu (dane zawierają wartości średnie z trzech powtórzeń i odchylenie standardowe) * świadczy o istotności różnic dla p ≤ 0,05*

Chlorophylls are classified as porphyrin pigments. The difference between chlorophyll a and b is that in the molecule of chlorophyll a there is a methyl group (-CH_3), and that of chlorophyll b there is an aldehyde group (CHO) [15].

The content of chlorophylls a and b in hop cones and the sum of chlorophylls were converted into mg/g of product (dried material) and presented in Fig. 3.

It was shown that the variety content of chlorophyll in extracts from hop cones depended on the variety. The cones of the Marynka cultivar (0.49 mg/g) had the highest content of these compounds, followed by the Lubelski cones (0.47 mg/g), and the lowest chlorophylls were those of the Magnum cultivar (0.42 mg/g). Chlorophyll a was the dominant form of chlorophyll in all samples, and its content was the highest in the Marynka variety (0.38 mg/g). Chlorophyll b was present in much lower amounts in all analyzed samples, from 0.1 mg/g to 0.11 mg/g for the plant cone extracts.

Croft et al. [16] showed that the content of chlorophyll in leaves depends on seasonality and the physiological processes taking place in them. At the same time, as shown by Polak et al. [18] and Śledź and Witrowa Rajchert [17], the content of chlorophyll dyes is also influenced by the method of raw material preparation, especially the parameters of the drying process and storage conditions.

The literature knows the antioxidant and pro-oxidative properties of chlorophyll, which, depending on the conditions of the reaction system, either accelerates or slows down the oxidation reactions [19]. It concerns the ability to easily exchange Mg2 + ions of a chlorophyll molecule with ions of other divalent metals. As a result of these reactions, the color changes to gray-brown when magnesium is replaced with Fe2 + ions and to green after Cu2 + or Zn2 + ions are introduced into the chlorophyll molecule. Chlorophyll dyes are also easily broken down by acids and bases. In an acidic environment, the magnesium ion is exchanged into two hydrogen ions, and depending on the acid concentration, fat-soluble pheophytin (pH <7) or water-soluble pheophorbide (pH <7) is formed [20]. On the other hand, in the presence of bases, the hydrolysis of both ester bonds takes place, without removing magnesium, resulting in the formation of chlorophyllins, and as a result of the action of chlorophyllase, water-soluble phytol-free chlorophyllides are formed, which still contain a methoxy group [19].

Carotenoids, like chlorophylls, are natural pigments located in chloroplasts of plant cells, permanently attached to proteins. They give plants a color from yellow, through orange to red, and are also important in the photosynthesis process. Some of the carotenoids are responsible for the synthesis of pigments responsible for the vision process in humans and animals. Carotenoid pigments are precursors of vitamin A and have antioxidant properties, which makes them important in the prevention of diseases caused by oxidative stress [21].

The results of the total content of carotenoid pigments in extracts from cones were expressed in mg/g and are presented in Fig. 4.

All samples contained a similar amount of carotenoid dyes. The cones of the Lubelski variety (0.46 mg/g) and Marynka (0.46 mg/g) were characterized by the highest content, while the Magnum variety cones showed only a slightly lower content of the tested compounds, namely 0.45 mg/g. Comparing the results of the carotenoid content determination to the previously discussed results of chlorophyll content, it was found that the content of carotenoid and chlorophyll pigments in hop cones was at a similar level.

Sivanesan et al. [22] analyzed the content of carotenoids in black chokeberry shoots. They showed that their content was approx. 191.4 μg/g of fresh material. This value is lower than that obtained in the present research for hop cones. As in the case of chlorophylls, the content of carotenoids in plants is influenced by irrigation and fertilization of plants as well as exposure to sunlight [23, 24]. The carotenoid content is also influenced by the method of preparation of the raw material, especially the conditions of the drying process, which was confirmed in this study, and the product storage conditions [18, 19].

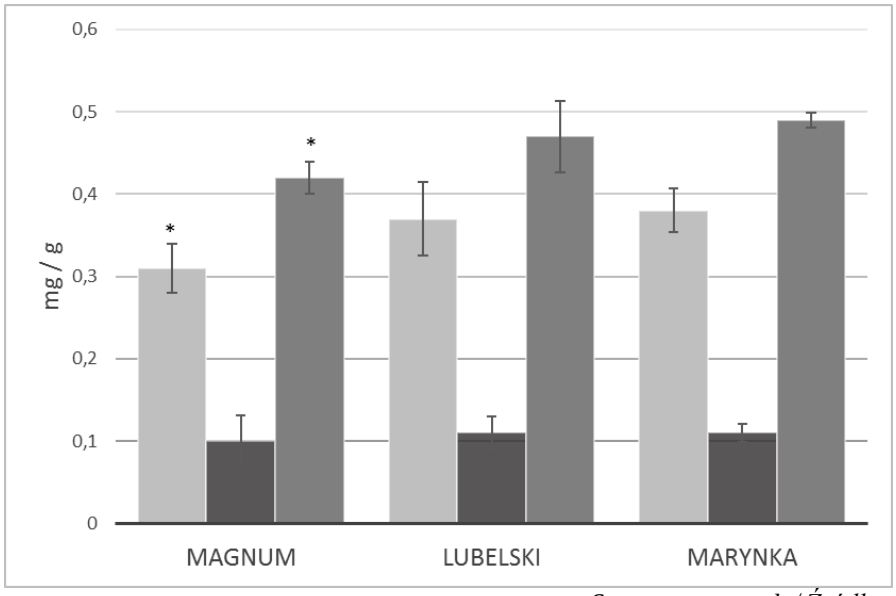


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Fig. 3. Chlorophyll content in hop cones extracts (data show mean values from three repetitions and standard deviation) * show the significance of differences for $p \leq 0.05$

*Rys. 3. Zawartość chlorofili w szyszkach chmielu (dane przedstawiają wartości średnie z trzech powtórzeń oraz odchylenie standardowe) * świadczy o istotności różnic dla $p \leq 0,05$*

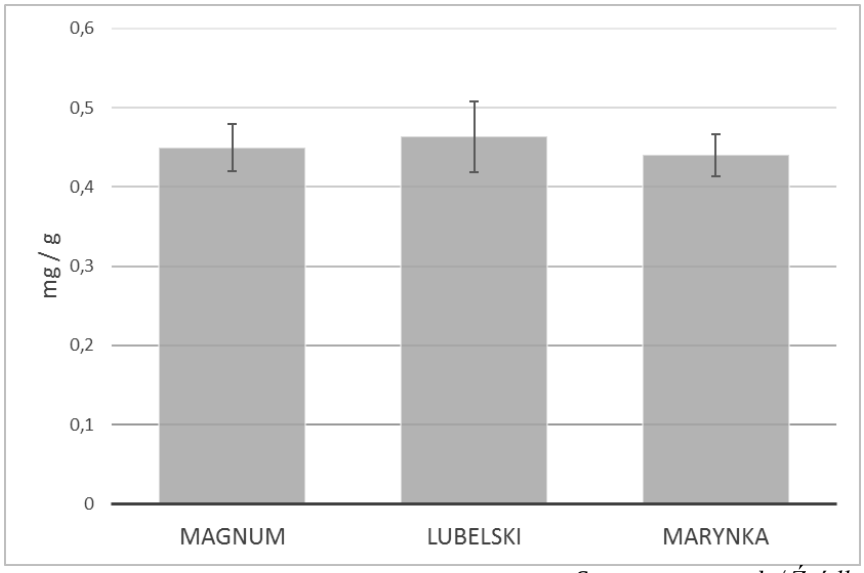

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Fig. 4. Carotenoids content in hop cones (data show mean values from three repetitions and standard deviation) * show the significance of differences for $p \leq 0.05$

*Rys. 4. Zawartość karotenoidów w szyszkach chmielu (dane przedstawiają wartości średnie z trzech powtórzeń oraz odchylenie standardowe) * świadczy o istotności różnic dla $p \leq 0,05$*

5. Conclusion

The extracts of hops (*Humulus lupulus* L.) have a wide range of properties resulting from the high content of bioactive compounds.

Humulus lupulus L. hop cones vary in size (height, width and weight), color (from light green to dark green) and aroma (from delicate to intense hop) depending on the variety.

Water extracts from common hops contain bioactive compounds such as polyphenols, including proanthocyanidins, phenolic acids, carotenoids and, chlorophyll.

The best known properties of phenolic acids are antioxidant properties. It has been shown that they are related to the chemical structure of polyphenolic acids. The antioxidant properties of these compounds directly depend on the number of hydroxyl groups in the compound molecule. In addition, numerous studies prove the health-promoting properties of polyphenolic acids. Chlorogenic, caffeic, ellagic and gallic acids have chemopreventive properties.

In this study the highest content of total polyphenolic acids was found in the extract of hop cones of the Lubelski variety (5.22 mg of chlorogenic acid/1 g). On the other hand, the highest number of proanthocyanidins was determined in the extract of cones of cultivar Marynka (A550 nm / mg extract = 0.260). It was shown that the content of chlorophyll in extracts from hop cones depended on the variety. The cones of the Marynka variety (0.49 mg/g) had the highest content of these compounds, while chlorophyll "a" was the dominant form of chlorophyll in all the samples. Moreover, all samples contained a similar amount of carotenoid dyes. The cones of the Lubelski variety (0.46 mg/1 g) and Marynka (0.46 mg/1 g) were characterized by the highest content, while the Magnum variety cones showed only a slightly lower content of the tested compounds, namely 0.45 mg/g.

Hop cones can be a valuable raw material used in the food or pharmaceutical industry as a source of bioactive compounds with multidirectional action.

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