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THE IMPACT OF PLANT PROTECTION AND FERTILIZATION ON CONTENT OF BIOACTIVE SUBSTANCES IN ORGANIC HOPS

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Abstract. Hop (*Humulus lupulus*) is one of the basic raw materials employed in brewing. All hops secondary metabolites exhibit pronounced bioactive effects. Therefore beer is the most important source of hop bioactive compounds in the human diet. Xanthohumol is the major prenylated flavonoid occurred only in the hops plant. The aim of this study was to determine the content of selected biologically active compounds in organic hops in comparison with conventional depending on plant protection and fertilization regimes. Organic hops had higher contents of the most important biologically active substances as xanthohumol, flavanols and alpha-acids, especially affected by probiotic microorganisms with plant extracts as plant protection agent. Significant differences in the content of xanthohumol were found between all types of tested organic fertilizers, but the highest content of this compound was recorded in result of plant fertilization with basalt meal. Both the probiotic microorganisms enhanced plant extracts as plant protection agents and basalt meal and basalt meal with manure and probiotic microorganisms as organic fertilizers the most positive influenced the biochemical parameters of hops.

Key words: plant extracts, probiotic microorganisms, basalt meal, manure, xanthohumol

INTRODUCTION

The hop (*Humulus lupulus* L.) is one of the basic raw materials employed in brewing. Practically all hops secondary metabolites exibit more or less pronounced bioactive effects. Therefore beer is the most important source of hop bioactive compounds in the human diet. Flavonoids are a group of constituents of food and drinks commonly consumed by humans, including compounds of different chemical classes such as flavones (7,8-benzoflavone), flavonols (quercetin), flavan-3-ols (cathechin), flavanones (naringenin), isoflavones (genistein), and chalcones (xanthohumol). Xanthohumol (XN) is the

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chalcone occurred only in the hops and represents 82–89% of the total prenylated flavonoid identified in various European hop varieties [Ruefer et al. 2005, Česlová et al. 2009]. Hops have long been used in folk medicine and are commonly employed in the brewing industry to flavor beer. Hops in beer is a major dietary source of prenylated flavonoids, which has been found at concentrations up to 0.96 $mg \cdot l^{-1}$ (1.95 μM). Flavonoids, including chalcones, have been shown to inhibit tumor cell proliferation and growth [Gerhauser et al. 2002]. For example, XN inhibits human breast (MCF-7), colon (HT-29), and ovarian cancer (A-2780) cell proliferation in vitro [Miranda et al. 1999]. Small animals clinical studies support that consumption of organically produced food is beneficial to human health. This related to an increase content of mineral nutrient, especially trace elements, vitamins and some secondary metabolites in organically grown foods [Hallmann and Rembiałkowska 2012]. There is a growing concern that the levels of some phenolics may be lower than optimal for human health in conventional foods [Lairon 2009]. This concern arises because conventional agricultural practices utilize levels of pesticides and fertilizers that can result in a disruption of the natural production of phenolic metabolites in the plant [Solarska et al. 2010]. Factors such as resource availability and plant protection compounds are known to affect levels of nutrients in plants [Grzyb et al. 2012]. The organic management system is important to use organic fertilizers which are a source of humus and micronutrients contributes to the maintenance of soil biological efficiency. These include manure, compost, slurry and basalt meal [Grabowska-Orządała et al. 2007]. Biological products for protecting plants against pests and diseases are not a threat to the human, animals and beneficial insects. For the plant protection in organic production system are used plant extracts, pheromone, natural enemies and probiotic microorganisms [Mayer et al. 2010]. Content of biologically active compounds, such as polyphenols which are a very strong antioxidants, increases in organic crops [Young et al. 2005, Hallmann and Rembiałkowska 2012].

The aim of this study was to determine the content of selected biologically active compounds in organic hops in comparison with conventional depending on the types of plant protection and fertilization.

MATERIALS AND METHODS

Hop protection and fertilization. The experiment was carried out in 2010–2012 on hop cv. Marynka growing in organic and conventional hop-gardens in the same locality. The biologically active compounds were assessed in cones of the hops. The plant protection products and ways of fertilization are shown in Table 1. The probiotic microorganisms (PM) with fermented extracts of nettle, horsetail, dandelion and field sow thistle were prepared as follows: one thousand liters container was filled with shredded mass of the whole plants and then poured 20 dm^3 of PM, topping up with water, and it was covered tightly and the content was allowed to fermentation for 2 to 3 weeks. Before spraying the liquid was strained and 20 dm^3 PM added to it. In the season 5 treatments using PM with fermented plant extracts and 5 treatments using alone PM were applied.

Table 1. Characteristics of plant protection and fertilization regime used for organic and conventional cultivation of hop cv Marynka in 2010–2011

Growing system	Plant protection products	Fertilizers
Organic	probiotic microorganisms with plant extracts in dose 40 l PM in1000 l water·ha	horse manure composed (35 t·ha^{-1}) with probiotic microorganisms (1 l·m^{-3} manure)
Organic	probiotic microorganisms in dose 40 l PM in 1000 l water·ha^{-1}	
Conventional	Aliette 80 WP (Fosetyl AL) 4 kg a.i.·ha^{-1} Zato 50 WG (trifloxystrobin) 250 g a.i.·ha^{-1} Confidor 200 SL (imidachlopryd) 166 g a.i.·ha^{-1} Siarkol 80 WG (sulphur) 6 kg a.i.·ha^{-1}	manure (30 t·ha^{-1}) mineral fertilizations: nitrogen 180 kg·ha^{-1} phosphorus 45 kg·ha^{-1} potassium 160 kg·ha^{-1}

In 2012 the effect of different types of organic fertilizers on the content of biologically active compounds in two varieties of hops – Marynka and Magnum was examined. Types of used fertilizers are shown in Table 2. To protect hop plants against diseases and pests PM with the plant extracts were applied.

Table 2. Fertilizers used in growing of hops cvs Marynka and Magnum in 2012

Term of fertilization	Type of fertilization		
April	composed horse manure (15 t·ha^{-1}) after spraying with probiotic microorganisms in amount of 1 l·m^{-3} of manure		
May	basalt meal (1 t·ha^{-1}) (BM)	horse manure composed (20 t·ha^{-1}) with probiotic microorganisms (1 l·m^{-3} manure) and basalt meal (2 t·ha^{-1}) (BM+M+PM)	horse manure composed (20 t·ha^{-1}) with probiotic microorganisms (1 l·m^{-3} manure) (M+PM)

The hop cones of tested cultivars were sampled in the same state of ripeness from two experimental farms. From each trial object 5 kg of cones of each cultivar was sampled.

Preparation of plant material. The hop cones for analysis were shredded in the device Thermomix TM 31 (Vorwerk).

Concentrations of alpha- and beta-acids. Alpha- and beta-acids were analysed by spectrophotometric method, following the procedure proposed by Canbaş et al. [2001].

Total phenolic content. The concentrations of polyphenols were analysed by a modified Folin-Ciocalteau method descibed by Magalhães et al. [2010] and were expressed as mg of gallic acid equivalent per gram of dry weight (mg GAE·g^{-1}).

Total flavan-3-ols and proanthocyanidins contents. The content of flavan-3-ols and proanthocyanidins was analysed with vanillin reagent according to the method described by Magalhães et al. [2010] and was expressed as mg of catechin equivalent per gram of dry weight (mg CE·g^{-1}).

Concentrations of xanthohumol. The content of xanthohumol in the methanolic extracts of hop cones was analysed by the high-performance liquid chromatography with diode array detection, according to the method described by Magalhães et al. [2007]. The HPLC system consisted of two Gilson 306 Separation Module piston pumps, Gilson PhotoDiode Array Detector 170, Gilson loop (0.02 cm^3), manometric module Gilson 805, dynamic mixer 811C and Gilson Autoinjector. Separation was achieved on Water Symmetry C18 column (USA, 250 mm, 4.6 mm, 5 µm), and Waters Symmetry® C_{18} pre-column (5 µm, 8 × 20 mm). The results were expressed as g of xanthohumol per kg of dry matter.

Concentrations of tannins. Tannins was analysed by spectrophotometric method using Folin-Denis reagent described by Canbaş et al. [2001]. Tannins were determined as g tannic acid·kg^{-1} of dry matter.

Dry matter. The dry matter of hop cones was determined using the method given in the relevant Polish Norm [PN-90/A-75101/03] and calculated in g·kg^{-1} and received values were basis for calculations of all above parameters.

Statistical analysis. All experiments were performed in triplicate and the results were expressed as the mean value ± the standard deviation. The differences between the averages were evaluated post hoc Tukey's test. Data were verified statistically with STATISTICA 8.0 for Windows (StatSoft, Poland). The results were analysed using ANOVA analysis of variance. Significant difference was defined as $P < 0.05$.

RESULTS AND DISCUSSION

Effects of plant protection on the bioactive substances content in the hop cones. The fundamental differences in organic and conventional agricultural systems, regarding fertilization methods, soil fertility management and plant protection protocols, affect the nutrient composition in plants [Mitchell et al. 2007, Grzyb et al. 2012]. The own study have shown that there are significant differences in the quality of hops of the two cultivation systems depending on the plant protection. The organic hop cones protected by PM with plant extracts were characterized by a significantly higher content of the most important biologically active compounds as xanthohumol, proanthocyanidins and flavan-3-ols compared with conventional hops (tab. 4).

Jadesha et al. [2012] also found positive effect of plant extracts used as protection agent on composition of active biological compounds in banana fruits. The obtained results indicate that plant protection had significant effect on alpha-acids content (tab. 3). The content of the substances was highest in organic hop cones in object at which PM with plant extracts were used. However, in combination with using only PM the content of alpha-acids was lowest i.e. lower than in conventional hop cones. Plant protection had significant effect on content of beta-acids (tab. 3). The significantly higher content of beta-acids was found in cones from object with chemical control in comparison with hop protection by PM and PM with plant extracts. There was also significant higher content of beta-acids in hop protected using PM with plant extracts compared with alone PM (tab. 3). The plant protection, growing season and interaction between them had significant impact on total polyphenols production in hop cones

Table 3. Content of alpa and beta acids in hop cones cv Marynka from organic and conventional systems in two growing seasons (2010–2011)

	Plant protection, average and *P*-value	Year	Alpha acids ($g \cdot kg^{-1}$)	Beta acids ($g \cdot kg^{-1}$)
Plant protection	PM	2010	90.2 ±1.7	75.1 ±2.3
		2011	92.0 ±2.3	76.7 ±2.5
	PM with plant extracts	2010	106.0 ±2.5	82.9 ±5.0
		2011	106.8 ±4.8	84.7 ±3.7
	chemical protection	2010	102.6 ±4.8	95.5 ±3.3
		2011	99.6 ±4.4	94.7 ±3.7
Average	for PM	–	92.0 ±1.3a	75.9 ±1.6a
	for PM with plant extracts	–	106.4 ±2.4b	83.8 ±2.8b
	for chemical protection	–	101.1 ±3.0b	95.6 ±2.2c
P-value	plant protection	–	0.006	0.000
	year	–	NS	NS
	interaction	–	NS	NS

(tab. 4). Although chemically protected hop had significantly higher content of total polyphenols compared to biologically protected ones, the content of the most important polyphenol – xanthohumol was the largest in the object in which PM with plant extracts were used. Biologically protected hops were characterized by lower content of tannins in comparison with chemically protected ones but there were no significant differences (tab. 4).

Table 4. Content of total polyphenols, flavan-3-ols, xanthohumol and tannins in hop cones cv Marynka from organic and conventional systems in two growing seasons (2010–2011)

	Plant protection, average and *P*-value	Year	Total polyphenols ($mg\ GAE \cdot g^{-1}$)	Flavan-3-ols and proantho-cyanidins ($mg\ CE \cdot g^{-1}$)	Xanthohumol ($g \cdot kg^{-1}$)	Tannins ($g \cdot kg^{-1}$)
Plant protection	PM	2010	29.16 ±0.14	4.64 ±0.10	1.49 ±0.03	184.35 ±1.63
		2011	29.16 ±0.18	4.66 ±0.15	1.96 ±0.07	182.70 ±0.91
	PM with plant extracts	2010	30.58 ±0.33	5.54 ±0.40	3.49 ±0.24	179.73 ±2.49
		2011	31.59 ±0.27	6.75 ±0.23	4.27 ±0.17	180.90 ±1.37
	chemical protection	2010	36.62 ±0.23	5.48 ±0.04	1.80 ±0.03	185.18 ±0.68
		2011	33.77 ±0.34	5.48 ±0.16	1.82 ±0.02	184.07 ±2.87
Average	for PM	–	29.16 ±0.11a	4.65 ±0.07a	1.72 ±1.1a	183.53 ±1.02a
	for PM with plant extracts	–	30.92 ±0.26b	5.95 ±0.31c	3.75 ±2.1b	180.12 ±1.64a
	for chemical protection	–	35.19 ±0.51c	5.48 ±0.08b	1.81 ±0.2a	184.63 ±1.34a
P-value	plant protection	–	0.000	0.000	0.000	NS
	year	–	0.024	NS	0.019	NS
	interaction	–	0.000	NS	NS	NS

The use of plant natural compounds against fungal diseases and pests in organic agriculture is an important activity, because it would permit to obtain crops of highest quality.

Effects of organic fertilization on the bioactive substances content in the hop cones. The use of the different types of organic fertilization on hops had an effect on the bioactive compounds compositions. With manure fertilization, increase soil organic matter contents. Higher soil organic matter contents have been found to have positive effects on yield components of plants [Mitchell et al. 2007]. Greater availability of nitrogen in conventional systems reduces the plant's ability to defense against pathogens. If plants grow relatively slowly, they build up their chemical defences to a level that prevents most diseases and pests. However, if a plant is allowed to grow unusually fast by providing it with an abundance of nitrogen, the accumulation of defence compounds i.e. plant secondary metabolites is reduced [Brandt and Mølgaard 2006]. There are many indications that secondary plant metabolites are responsible for beneficial effects of some crops. Hop is rich source of flavonoids such as flavan-3-ols and xanthohumol which have positive health effects [Gerhauser et al. 2002, Magalhães et al. 2010]. Highest content of xanthohumol and flavanols in cones of hops fertilized with basalt meal and manure with basalt meal and probiotics microorganisms proves that a good supply of plants in trace elements derived from basalt meal significantly affects the increase in the plants most important biologically active substances. Phenolic compounds may act as antioxidants and exert other protective effects in the body. Concentrations of particular phenolic compounds vary with climate and plant variety, but have also been reported to be higher in many organically grown crops as compared to their conventionally grown counterparts [Woese et al. 1997, Worthington 2001, Mitchell et al. 2007, Lima and Vianello 2011, Hallmann and Rembiałkowska 2012]. Obtained results of own study indicate that agricultural practices such as protection and fertilization of organic crops are an important tool in the creation of high-quality food.

Table 5. Content of alpa and beta acids in hop cvs Marynka and Magnum from organic system with different types of fertilization in 2012

	Fertilizer, average and P-value	Variety	Alpha acids ($g \cdot kg^{-1}$)	Beta acids ($g \cdot kg^{-1}$)
Fertilizer	BM	Marynka	77.9 ±5.55	36.1 ±2.78
		Magnum	88.3 ±3.19	78.3 ±1.72
	M + BM + PM	Marynka	73.3 ±2.36	37.3 ±0.27
		Magnum	87.6 ±2.22	73.8 ±2.10
	M + PM	Marynka	72.0 ±1.76	41.1 ±1.87
		Magnum	99.6 ±5.24	34.2 ±1.54
Average	for BM	–	83.1 ±3.7a	57.2 ±9.6b
	for BM + M + PM	–	80.5 ±3.5a	55.5 ±8.2b
	for M + PM	–	85.8 ±6.7a	37.7 ±1.9a
P-value	fertilizer	–	NS	0.000
	variety	–	0.000	0.000
	interaction	–	NS	0.000

Table 6. Content of total polyphenols, flavan-3-ols, xanthohumol and tannins in hop cvs Marynka and Magnum from organic system with different types of fertilization in 2012

	Fertilizer, average and P-value	Variety	Total polyphenols (mg GAE·g^{-1})	Flavan-3-ols and proantho-cyanidins (mg CE·g^{-1})	Xanthohumol (g·kg^{-1})	Tannins (g·kg^{-1})
Fertilizer	BM	Marynka	28.91 ±0.14	4.74 ±0.04	8.80 ±8.08	180.92 ±2.80
		Magnum	23.0 ±0.41	3.43 ±0.11	7.12 ±0.06	121.79 ±1.87
	M + BM + PM	Marynka	28.0 ±0.26	5.07 ±0.17	7.51 ±0.05	178.12 ±3.22
		Magnum	23.47 ±0.36	3.28 ±0.25	7.99 ±0.02	109.79 ±3.06
	M + PM	Marynka	29.03 ±0.92	4.50 ±0.13	8.04 ±0.05	177.19 ±2.90
		Magnum	24.62 ±0.25	2.77 ±0.10	7.22 ±0.04	99.72 ±0.20
Average	for BM	–	25.95 ±1.34a	4.09 ±0.30ab	7.96 ±0.38c	151.35 ±13.31c
	for BM + M + PM	–	25.73 ±1.03a	4.17 ±0.42b	7.75 ±0.11b	143.95 ±15.41b
	for M + PM	–	26.83 ±1.08a	3.64 ±0.39a	7.63 ±0.19a	138.45 ±17.37a
P-value	fertilizer	–	NS	0.007	0.000	0.001
	variety	–	0.000	0.000	0.000	0.000
	interaction	–	NS	NS	0.000	0.013

The use of the different types of organic fertilization on hops had an effect on the bioactive compound compositions. Highest content of xanthohumol and flavanols found in cones of hops fertilized with basalt meal and manure with basalt meal and probiotics microorganisms.

The significant impact on content of all tested biologically active compounds in the hop fertilized with different organic fertilizers had cultivar (tabs 5–6). There was no difference in the content of alpha-acids in cones of hop fertilized with different organic fertilizers. The different types of organic fertilization, growing season and interaction between them had significant impact on content of beta-acids in hop cones (tab. 5). It was observed that hop fertilized using alone basalt meal (BM) and manure with basalt meal and probiotic microorganisms (M + BM + PM) contained more beta-acids compared to hop fertilized using manure with probiotic microorganisms (M + PM). The studied types of organic fertilization did not differ each other concerning the content of total polyphenol content in hop cones in the 2 years of the experiment (tab. 6). Besides cultivar also type of fertilization had significant impact on flavan-3-ols and proanthocyanidins content in hop cones (tab. 6). Highest contents of the compounds were found in the cones of hop fertilized using M + BM + PM and BM compared to hop fertilized using M + PM. The different organic fertilization had modified xanthohumol content in hop cones (tab. 6). The highest content of this compound was recorded at object with BM. In case of tannins also all examined types of organic fertilization had significant effect on their content (tab. 6). The highest content of this compounds was recorded at object with BM.

CONCLUSIONS

1. Probiotic microorganisms with plant extracts for plant protection of organic hop increased significantly content of xantohumol, flavan-3-ols and proanthocyanidins in cones compared with conventional hops.

2. Basalt meal and manure with basalt meal and probiotic microorganisms used for fertilization of hop significantly affected the increase content of important biologically active substances.

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WPŁYW SPOSOBU OCHRONY I NAWOŻENIA ROŚLIN NA ZAWARTOŚĆ SKŁADNIKÓW BIOLOGICZNIE CZYNNYCH W CHMIELU EKOLOGICZNYM

Streszczenie. Chmiel jest jednym z podstawowych surowców wykorzystywanych w browarnictwie. Wszystkie wtórne metabolity chmielu wykazują znaczące działanie bioaktywne. W związku z tym piwo jest najważniejszym źródłem bioaktywnych składników chmielu w diecie człowieka. Podstawowym prenyloflawonoidem występującym tylko w roślinach chmielu jest ksantohumol. Celem badań było określenie zawartości wybranych biologicznie czynnych składników chmielu ekologicznego w porównaniu z konwencjonalnym w zależności od sposobów ochrony i nawożenia. Chmiel ekologiczny zawierał więcej związków biologicznie aktywnych, tj. ksantohumolu, flawanoli i alfa-kwasów, szczególnie w przypadku zastosowania jako czynników biologicznej ochrony mikroorganizmów probiotycznych z ekstraktami roślinnymi. Rodzaj nawożenia miał istotny wpływ na zawartość ksantohumolu, przy czym największą zawartość tej substancji zanotowano w wyniku nawożenia roślin mączką bazaltową. Zarówno probiotyczne mikroorganizmy z ekstraktami roślinnymi jako czynnik ochrony roślin, jak i mączka bazaltowa lub mączka bazaltowa z obornikiem jako nawożenie organiczne w największym stopniu wpłynęły korzystnie na skład biochemiczny chmielu.

Słowa kluczowe: ekstrakty roślinne, mikroorganizmy probiotyczne, mączka bazaltowa, obornik, ksantohumol

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