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A study of dynamics of bitter acids and xanthohumol in hop pellets during storage

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Abstract. Eight varieties of hop pellets were analyzed for the contents of α-acids, ß-acids and xanthohumol according to the EBC 7.7 analytical method. The pellets were extracted with acidified mixture methanol - diethylether and analyzed using HPLC with a diode-array detector and a Nova-Pak column C_{18}. Four series of analyses were performed: immediately after the unpacking of the pellets and then after five, seven and nine months of storage at 4 °C. According to the first series of analyses, the contents were assayed as following (α-acids, ß-acids, xanthohumol resp., all in weight % in pellets): Galaxy (13.4, 8.0, 0.74), Citra (11.1, 3.0, 0.48), Tradition (8.2, 8.0, 0.58), Cascade (4.5, 5.2, 0.25), Northern Brewer (4.0, 2.9, 0.37), Sládek (3.5, 4.0, 0.48), Saaz (2.0, 3.4, 0.24), and Triskel (1.7, 3.6, 0.18). According to these results, variety Galaxy was found as the richest in all three parameters. After nine months of storage at 4 °C, the weight loss of α-acids ranged from 4.1% (Citra and Triskel) to 66.4% (Galaxy). The losses of β-acids and xanthohumol were less distinctive (from zero to 31.3% and 25.7%, resp.) and indicated good long storage possibilities of these compounds at convenient conditions (darkness, low temperature, elimination of direct influence of oxygen).

Key words: hop pellets, hop varieties, storage, α-acids, ß-acids, xanthohumol, HPLC.

INTRODUCTION

Hop growing at the territory of the present-day Czech Republic has a long tradition; the first news concerning hop growing come already from the 8th and 9th centuries. Thanks to the specific soil and climatic conditions, the best results in the quality of hops have been achieved in Žatec (Saaz) region. Several important hop varieties exported to many foreign countries originate from this region, e.g. Saaz semi-early red-bine hop, Saaz Late or Sládek (Nesvadba, 2013).

From the chemical point of view, the most important criteria of hop quality are the contents of hop resins (especially α-acids and β-acids), hop polyphenols (xanthohumol and related compounds), and the total contents and composition of essential oils (European Brewery Convention, 2013). The main constituents of α-acids are humulone,

cohumulone and adhumulone, the main constituents of β-acids are lupulone, colupulone and adlupulone (Fig. 1).

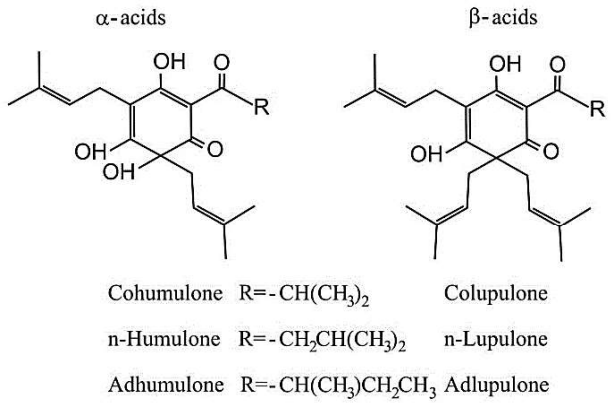

Figure 1. Chemical formulas of the main constituents of α- and β-acids (Saugspier et al., 2012).

During the brewing process, humulones are thermally transformed into iso-humulones (iso-α-acids), which are responsible for the specific bitter taste and the stability of beer foam (Česlová et al., 2009). Due to structural differences, many properties of β-acids are different from the properties of α-acids. The presence of another isoprenyl side chain causes the molecule as a whole to have a more hydrophobic character. Therefore, β-acids are much less soluble in water and cannot isomerize during the brewing process, but their oxidation occurs in the course of processing and storage of hops and to a small extent also during beer production. The unique chemical structure of β-acids is a source of their antimicrobial properties and other physiological effects on the human organism (Krofta & Mikyška, 2014). Detailed analytical studies of α- and β-acids using high-performance liquid chromatography (Hermans-Lokkerbol & Verpoorte, 1994) and HPLC-mass spectrometry (Česlová et al., 2009) were published.

The hops contain many polyphenolic compounds in addition to bitter acids. The important hop flavonoids are xanthohumol and related prenylflavonoids as isoxanthohumol, desmethylxanthohumol, 6-prenylnaringenin and many others. They have a positive effect on the human health due to their antioxidant, anticancer, antimicrobial, and anti-inflammatory properties (Stevens & Page, 2004). Xanthohumol is the most frequently investigated polyphenol in hops and hop products (Fig. 2).

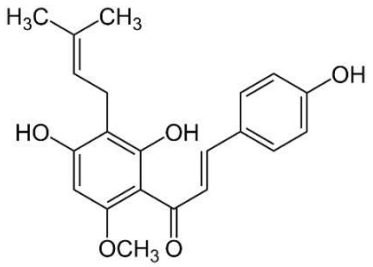

Figure 2. Chemical formula of xanthohumol (https://en.wikipedia.org/wiki/Xanthohumol#/media/File:Xanthohumol.svg).

Hop pellets are a hop product added to the kettle to provide bitterness and a hop character that is not distinguishable from that achieved using raw hops. Pellets provide improved homogeneity, better storage stability and reduced storage/transport costs

compared to raw hops. Their chemical composition and the demands for their quality assessment are similar as for the raw hops. Hop pellets are usually packed in laminated foils with an aluminium layer as a barrier against diffusion of oxygen and they can be shipped for long distances (Barth & Schönberger, 2014). Thus, the using of hop pellets made of miscellaneous hop varieties is typical for contemporary brewery practice including breweries in the Czech Republic, where the use of traditional Czech varieties predominated in past years (Nesvadba, 2013).

Because the quality of input raw materials is very important, we decided to arrange an analytical study including the determination of α-acids, β-acids and xanthohumol in eight varieties of hop pellets being frequently used in the Czech Republic. The main goal of this study was to evaluate the influence of storage on the quality of hop pellets and to compare the properties of analyzed hop varieties with data from literature.

MATERIALS AND METHODS

Hop pellets

Hop pellets manufactured from eight miscellaneous hop varieties were studied (country of origin is given in brackets): Cascade (USA), Citra (USA), Galaxy (Australia), Northern Brewer (Germany), Saaz (Czech Republic), Sládek (Czech Republic), Tradition (Germany), and Triskel (France). All pellets were type 90, cylindrical shape with diameter about 6 mm, packed in laminated aluminium foils per 5 kg, year of harvest 2015 (only Cascade 2014, weight of package 10 kg).

All samples were obtained in cooperation with a medium-size brewery located in East Bohemia (beer production ca. 130 000 hl per year). Most of analyzed hop pellets were supplied by Joh. Barth & Sohn Gmbh & Co. KG (Nuremberg, Germany), only the Saaz variety was supplied by a Czech company Chmel Polepská blata, Ltd., Polepy. All other identification data (batch numbers, delivery sheets etc.) are available at the authors.

Sampling and storage

About 100 g of hop pellets was taken from each batch delivered into the brewery immediately after packing foil opening and placed into a zip reclosable plastic bag. Before closing the bag, the access of air was removed carefully and tightly closed bags were stored in a refrigerator at 4 °C. The first series of analyses was performed up to 48 hours from sampling, the next series of analyses were performed after five, seven, and nine months of storage in the refrigerator at the same conditions.

Extraction of pellets

Extraction of pellets was performed according to the EBC 7.7 analytical method (Krofta, 2008; European Brewery Convention, 2013). 10 g of hop pellets were blended and poured into a 250 mL glass jar with a screw cap. 20 mL of methanol, 100 mL of diethyl ether, and 40 mL of hydrochloric acid ($c = 0.1$ mol L^{-1}) were added. Tightly closed jars were shaken for 40 minutes in a laboratory shaker and then left to stand for 10 minutes at least (to let liquid phases to separate). 5 mL of the clear upper layer were pipetted into a 50 mL volumetric flask, filled up with methanol and homogenized carefully. About 2 mL of this solution were taken into a plastic syringe and transferred through a syringe filter into a glass vial for the HPLC analysis. For each sample of pellets, two parallel extractions were made.

High-performance liquid chromatography

HPLC instrument Waters equipped with a diode-array detector and a Nova-Pak column C_{18} (150 mm x 3.9 mm i.d., particle size 4 μm) was used for the analysis of samples. The composition of the mobile phase was slightly modified to improve the separation (isocratic elution with methanol/water/orthophosphoric acid = 764 : 227 : 9, v/v/v, flow rate 0.8 mL min^{-1}, column temperature 40 °C). The new International Calibration Standard ICE–3 (Labor Veritas, Zürich) was used for the calibration of the HPLC instrument according to the EBC 7.7 method. Two repeated injections (10 μl) of the calibration solution were made before and after the ending of each series of analyses for the quantification of α- and ß-acids at 314 nm. The solutions of a pure analytical standard (Sigma-Aldrich, Prague) in the concentration range 0.012–0.24 mg mLl^{-1} were used for the quantification of xanthohumol at 370 nm. Two repeated injections (10 μl) were made for each extract of hop pellets. Example of a chromatogram at 314 nm (variety Triskel) is given in Fig. 3

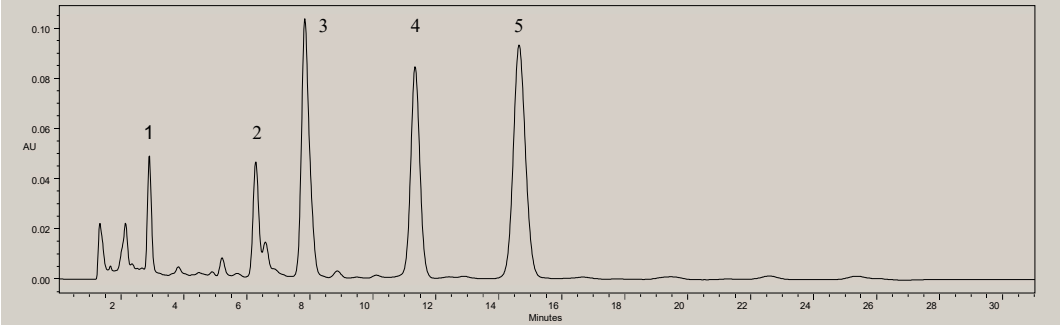

Figure 3. Chromatogram of hop pellets at 314 nm, variety Triskel (1 = xanthohumol, 2 = cohumulone, 3 = humulone + adhumulone, 4 = colupulone, 5 = lupulone + adlupulone).

RESULTS AND DISCUSSION

The contents of α-acids, β-acids, and xanthohumol were determined in pellets manufactured of eight hop varieties. The first series of analyses included pellets which were analyzed immediately after packing foil opening (up to 48 hours), the next series of analyses were done after five, seven and nine months storage of pellets in tightly closed plastic bags at 4 °C. The percentual loss of weight after nine months storage for each analyzed component and the α/β ratio were calculated. All results are given in Table 1 (including data from literature).

According to the contents of α-acids measured in the first series of analyses, the richest varieties were Galaxy (13.4%) and Citra (11.1%), the lowest contents were in Saaz (2.0%) and Triskel (1.7%). In most cases, the measured values of α-acids were almost in the same range (Cascade, Citra, Galaxy) or about 20% lower (Saaz and Sládek) than data from literature (see notices under Table 1). Substantial differences between measured values and literature data were found for three varieties: Tradition (8.2% vs. 4–7% in lit.), Northern Brewer (4.0% vs. 8–10%), and especially Triskel (1.7% vs. 8–9%).

Table 1. Contents of α-acids, β-acids and xanthohumol in hop pellets

Variety (country of origin)	Component	Contents (data from literature)	Measured contents* (after package opening)	Measured contents* (after 5 months)	Measured contents* (after 7 months)	Measured contents* (after 9 months)	Loss of mass
		% (w/w)	% (w/w)	% (w/w)	% (w/w)	% (w/w)	% (rel.)
Cascade	α-acids	4.5–7.0[2)]	4.45 ± 0.15	4.21 ± 0.16	4.20 ± 0.06	4.22 ± 0.06	5.2
(USA)	β-acids	4.8–7.0[2)]	5.24 ± 0.26	5.12 ± 0.25	5.12 ± 0.11	5.19 ± 0.11	1.0
	xanthohumol	0.30–0.32[3)]	0.25 ± 0.01	0.26 ± 0,01	0.25 ± 0,01	0.26 ± 0,01	0.0
	α/β ratio	0.9–1.0[2)]	0.85	0.82	0.82	0.81	
Citra	α-acids	11.0–13.0[2)]	11.10 ± 0.14	10.92 ± 0.10	10.81 ± 0.04	10.65 ± 0.03	4.1
(USA)	β-acids	3.5–4.5[2)]	3.01 ± 0.04	3.01 ± 0.03	2.98 ± 0.01	2.93 ± 0.06	2.7
	xanthohumol	0.44 [2)]	0.48 ± 0.01	0.50 ± 0.00	0.49 ± 0.00	0.49 ± 0.00	0.0
	α/β ratio	2.4–3.7 [2)]	3.69	3.62	3.63	3.63	
Galaxy	α-acids	13.5–14.8[2)]	13.38 ± 0.09	7.74 ± 0.02	5.84 ± 0.03	4.49 ± 0.02	66.4
(Australia)	β-acids	5.0–6.5[2)]	8.03 ± 0.06	6.46 ± 0.0.1	5.86 ± 0.02	5.52 ± 0.02	31.3
	xanthohumol	0.5–0.9[2)]	0.74 ± 0.01	0.65 ± 0.00	0.59 ± 0.00	0.55 ± 0.00	25.7
	α/β ratio	2.1–2.9[2)]	1.67	1.19	0.99	0.81	
Northern Brewer	α-acids	8.0– 0.0[2)]	3.98 ± 0.11	3.90 ± 0.04	3.78 ± 0.05	3.81 ± 0.06	4.3
(Germany)	β-acids	3.0–5.0[2)]	2.87 ± 0.09	2.92 ± 0.03	2.87 ± 0.05	2.96 ± 0.06	0.0
	xanthohumol	0.6[2)]	0.37 ± 0.01	0.38 ± 0.00	0.37 ± 0.01	0.38 ± 0.01	0.0
	α/β ratio	no data found	1.39	1.33	1.32	1.29	
Saaz	α-acids	2.5–4.5[1)]	1.95 ± 0.04	1.81 ± 0.06	1.80 ± 0.05	1.80 ± 0.02	7.7
(Czech R.)	β-acids	4.0–6.0[1)]	3.41 ± 0.07	3.32 ± 0.12	3.27 ± 0.09	3.35 ± 0.04	1.8
	xanthohumol	0.3–0.5[1)]	0.24 ± 0.01	0.24 ± 0.01	0.23 ± 0.01	0.24 ± 0.00	0.0
	α/β ratio	0.6–1.0[1)]	0.57	0.55	0.55	0.54	
Sládek	α-acids	4.5–8.0[1)]	3.47 ± 0.04	3.08 ± 0.03	2.83 ± 0.08	2.76 ± 0.01	20.5
(Czech R.)	β-acids	4.0–7.0[1)]	3.95 ± 0.06	3.81 ± 0.06	3.50 ± 0.13	3.67 ± 0.02	7.1
	xanthohumol	0.50–0.75[1)]	0.48 ± 0.01	0.48 ± 0.01	0.47 ± 0.01	0.47 ± 0.00	2.1
	α/β ratio	0.7–1.3[1)]	0.88	0.81	0.81	0.75	

Table 1 (continued)

Tradition (Germany)	α-acids	4.0–7.0[2)]	8.24 ± 0.11	6.97 ± 0.24	6.42 ± 0.05	6.05 ± 0.02	26.6
	β-acids	3.0–6.0[2)]	7.98 ± 0.07	7.58 ± 0.21	7.24 ± 0.09	7.28 ± 0.05	8.8
	xanthohumol	0.3–0.5[2)]	0.58 ± 0.00	0.57 ± 0.02	0.55 ± 0.01	0.54 ± 0.01	6.9
	α/β ratio	no data found	1.03	0.92	0.89	0.83	
Triskel (France)	α-acids	8.0–9.0[2)]	1.71 ± 0.01	1.62 ± 0.01	1.66 ± 0.02	1.64 ± 0.03	4.1
	β-acids	4.0–4.7[2)]	3.60 ± 0.05	3.55 ± 0.03	3.61 ± 0.06	3.65 ± 0.08	0.0
	xanthohumol	0.2–0.6[2)]	0.18 ± 0.00	0.18 ± 0.00	0.17 ± 0.00	0.18 ± 0.00	0.0
	α/β ratio	no data found	0.48	0.46	0.46	0.45	

[1)] Nesvadba (2013); [2)] Barth & Schönberger (2014); [3)] Forster & Gahr (2014)

[*]calculated as the arithmetic mean of n = 4 (two repeated injections of two extracts).

The highest contents of β-acids were in Galaxy (8.0%) and Tradition (8.0%), the lowest contents were in Citra (3.0%) and Northern Brewer (2.9%). The greatest differences compared to literature data were found for Galaxy (8.0% vs. 5.0–6.5% in lit.) and Tradition (8.0% vs. 3.0–6.0%). Extremely high value of α/β ratio was found in Citra (3.69), the lowest value in Triskel (0.48). The trend of α/β ratio in analyzed hop varieties was similar as the trend of α-acids contents.

It is generally known that contents and ratios of α- and β-acids can be very variable depending on the year of harvest. For example, in a long-term study of Czech hops (1994–2013) the contents of α-acids in dried hop cones of Saaz variety ranged from 1.85% to 5.41%, the contents of β-acids ranged from 1.56% to 5.36%, and the α/β ratios ranged from 0.60 to 1.40 (Mikyška & Jurková, 2014). These data collected in a very long time period are in a good agreement with reference values given in other literature sources (Nesvadba, 2013) and also with data given in our study.

The differences depending on the growing area were also recorded. For example, the average values of α-acids contents in Saaz hop cones from three different growing areas in the Czech Republic (Žatec, Úštěk and Tršice) harvested in 2013 were resp. 3.5%, 3.2% and 2.9% (Mikyška & Jurková, 2014). The declared contents of β-acids in variety Cascade from Oregon (USA) were 4.8–7.0%, in the same variety from Hallertau (Germany) only 3.4% (Barth & Schönberger, 2014). Further literature data about varieties Northern Brewer and Triskel, which showed the most remarkable differences in our study, were not available according to our best knowledge and analyses of these varieties should be the subject of additional investigations.

After nine months of storage at 4 °C, the contents of α- and β-acids decreased in most of analyzed varieties. The relative decline of α-acids was more distinctive than the decline of β-acids; the α/β ratio decreased in all cases consequently. The loss of α-acids ranged from 4.1% (Citra and Triskel) to 66.4% (Galaxy), the loss of β-acids ranged from zero (Northern Brewer and Triskel) to 31.3% (Galaxy). Generally, the highest losses of both groups of acids occurred in Galaxy, where the value of α/β ratio changed remarkably from 1.67 to 0.81% during storage.

Relatively low losses of β-acids measured in this study are in a certain disagreement with a generally accepted opinion about their low stability. For example, the loss of pure β-acids after their 96 h deposition on an inert carrier (micronized cellulose) under open air was 94% at 4 °C and 25.7% at -18 °C (Krofta & Mikyška, 2014); the same authors also refer to low stability of β-acids in dried hop cones stored under open air. On the base of all these results it is possible to assume that principal factors causing low stability of β-acids are influence of oxygen and storage temperature. On the contrary, relatively simple adjustment of storage conditions can improve the stability of these compounds.

CONCLUSIONS

The measured contents of α- and β-acids in eight analyzed hop varieties were in most cases in a good agreement with literature data excluding varieties Tradition (much higher measured contents), Northern Brewer and Triskel (much lower measured contents). The measured contents of xanthohumol responded very well to literature data with the exception of Northern Brewer. According to the results acquired in this study, variety Galaxy was found as the richest in all three analyzed parameters (α-acids, β-acids, xanthohumol).

After nine months of storage at 4 °C, considerable decline of α-acids was observed, but substantial differences among analyzed varieties were recorded. The losses of β-acids and xanthohumol were less distinctive and indicated good long storage possibilities of these compounds at convenient conditions (low temperature, elimination of direct oxygen influence).

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