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VARIABILITY IN THE CONTENTS OF IMPORTANT COMPOUNDS FOR PHARMACEUTICAL AND BREWING INDUSTRIES WITHIN HOP GENE POOL

VARIABILITA OBSAHU FARMACEUTICKY A PIVOVARSKY VÝZNAMNÝCH LÁTEK U GENOFONDU CHMELE (*Humulus lupulus L.*)

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Totally 1546 hop samples have been determined for statistical assessment of hop database in 2006–2007. The average content of alpha acids is 6.07% with the variability of 64.6%. The range is from 0.37 to 17.82% w/w. The average content of beta acids is 4.5% w/w with the variability of 33.4%. The range is from 0.54 to 10.89% w/w. The average content of cohumulone is 27.3% relative (rel.) and 48.1% rel. in the case of colupulone. This compound shows lower variability (CV = 27%, resp. 18%). Important ingredients for pharmacy are xanthohumol and desmethylxanthohumol (DMX). The average content of xanthohumol amounts to 0.36% w/w under variability CV = 48%. Minimal content is 0.02% and maximal content is 0.94%. The average content of DMX amounts to 0.09% under variability CV = 56%. Minimal content of DMX is 0.01% and maximal one is 0.2%. Positive correlations of alpha acid content (r = 0.78) as well as beta acid content (r = 0.52) with the contents of xanthohumol have been determined as well. Nearly the same positive correlation has been determined for the influence of alpha and beta acids on the content of DMX (r = 0.61, resp. 0.60). Although positive correlations have been determined for xanthohumol and DMX (r = 0.58), we can conclude that it is impossible to combine breeding for high contents of these both compounds together.

Key words: hop, *Humulus lupulus* L., genetic resource, alpha and beta acids, xanthohumol, desmethylxanthohumol

Economic value of the hop plant is derived from its worldwide utilisation as an essential flavoring ingredient for the beer brewing. The impact of hops on beer quality is manifold, but by far, most important are specific features attributed to beer bitter taste and hoppy aroma. With this respect the most important compounds of hops are so-called alpha acids and beta acids, also known as humulones and lupulones. Alpha and beta acids are precursors of beer bittering agents; iso-alpha acids are the most relevant. Each series represents a mixture of several analogs, from which cohumulone, humulone and adhumulone and colupulone, lupulone and adlupulone, respectively are the most abundant (de Keukeleire et al. 2003).

Over the past few years, hop research has been largely dedicated to prenylflavonoids in view of their extremely interesting bioactivities. Prenylflavonoids are secreted into lupulin glands that are abundantly present in cones of the female hop plant, together with alpha acids, beta acids and hop oils. This is very important property because it facilitates their analytical determination by liquid chromatography. Prenylflavonoids form a sort of transition between hop resins and polyphenols. Initially formed prenylflavonoids in hops are xanthohumol (X) and

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desmethyl-xanthohumol (DMX). Both prenylflavonoids coexist in various ratios with predominance of xanthohumol (up to 1.5% in dry hop cones) (de Keukeleire et al. 2007). Contents of DMX are usually up to 0.20% in dry hop cones. DMX is considered to be a proestrogen, because by its cyclization it generates 8-prenylnaringenin (8-PN) which is one of the most potent phytoestrogen currently known (Milligan et al. 1999).

Xanthohumol and other prenylated flavonoids are now attracting big attention in the medical field. Thus xanthohumol showed antiproliferative activity in cancer cell lines derived from human breast cancer, colon cancer and ovarian cancer *in vitro* (Miranda et al. 2000a, b). In addition to its anti-cancer activity, xanthohumol showed antioxidant activity in inhibiting low-density lipoproteins oxidation (Rodriguez et al. 2001). Tobe et al. (1997) have revealed that bone resorption is strongly inhibited with the help of some ingredients contained in hops, at first by xanthohumol and humulone, which are supposed to be perspective therapeutic substances in the struggle against osteoporosis.

Ratios between alpha and beta acids as well as their contents are decisive for the division of hop cultivars on aroma, bitter and superbitter (alpha). High content of beta acids is very important aspect for aroma hops as well (Nesvadba et al. 2003). Cohumulone contents also influence character of beer bitterness (Nesvadba and Krofta 2007). Among the hop cultivars the contents of xanthohumol, DMX, alpha acids and beta acids vary in the large scale. Also ratios of hop prenylflavonoids and alpha acids or beta acids are widely different (Krofta 2003).

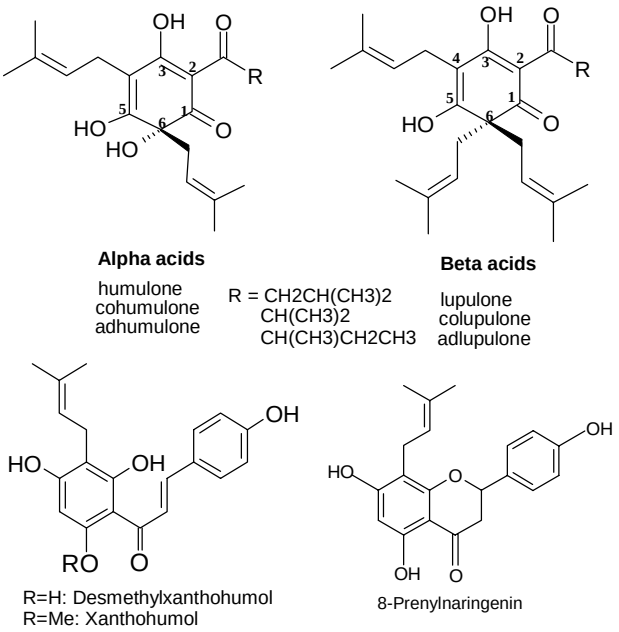

Fig. 1. Structures of alpha acids, beta acids, xanthohumol, DMX and 8-prenylnaringenin

MATERIAL AND METHODS

The contents of the alpha acids, beta acids, xanthohumol and DMX were obtained from the collection of wild hops; genetic resources of hop and breeding material. Totally 154 samples were assessed wild hops having their origin in Europe (Czech Republic, Austria, France, Belgium). The collection of world hop varieties include hop cultivated varieties obtained since the beginning of the 20th century till present, original regional (country) varieties as well as some important breeding genotypes (822 samples). In the collection of breeding material perspective genotypes of aroma as well as bitter types, obtained in the last 15 years, are included (570 samples). Most of all investigated hops were cultivated and harvested at the experimental farm of Hop Research Institute in Stekník. Locality is situated at the altitude 200 m above see level nearby Ohře river. Totally 1546 hop samples were analyzed in the period 2006–2007. Each

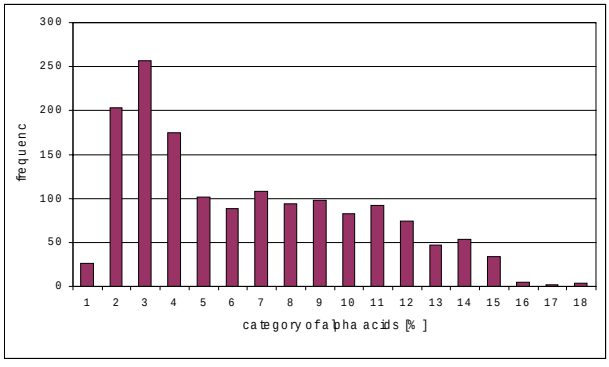

Fig. 2. Frequency of alpha acid contents

sample was harvested at experimental hop-picking machine "Volf" and in this way we got an average sample within each genotype. Hop samples were kept in an air-conditioned room under the temperature of 3°C. Before each analysis hops were ground with the help of centrifugal mill "Retch ZM 1".

Analyses of alpha and beta acids, xanthohumol and DMX were carried out by liquid chromatography on SHIMADZU LC 10A and LC 20A appliances. The methodology issues from EBC 7.7. method (Analytica EBC 1997). Hops were extracted in the mixture ethylether – methanol. Aliquot part of ether phase was after diluting injected on the column of liquid chromatograph (10 ml). The instruments were equipped with diode array detectors (DAD). Analytical signal was recorded simultaneously under wavelength of 314 nm (alpha and beta acids) and 370 nm (xanthohumol, DMX). Separation of analytes was carried out on the column Nucleosil RP C_{18}, 5 mm, 250 × 4 mm (Macherey-Nagel). Mobile phase was made up of the mixture methanol/water/phosphoric acid (c = 85%) in the ratio 850:190:5 vol:vol:vol. Flow of mobile phase was 0.8 ml/min^{-1}. Quantification of alpha and beta acids was carried out by external calibration on the external standard ICE 2. Standard for xanthohumol with the purity of 99% was bought from Phytochem GmbH (Germany), standard for DMX determination given us from University of Gent (Belgium). All analytical data were calculated to dry substance.

Statistical evaluation of experimental data was carried out with the help of the QC.Expert 2.5 program developed by TriloByte, Co., Ltd. (Pardubice, CR).

RESULTS AND DISCUSSION

The average content of alpha acids in the studied hops reached the value of 6.07%. Variability of alpha acid content expressed by a hundredfold of the coefficient of variability amounts to 64.6%. The lowest contents of alpha acids under the level of 1.0% were found out in some wild hops. On the contrary, the highest contents of alpha acids above the level of 17.0% were revealed in some genotypes within new breeding lines. It is apparent from Figure 2 that the highest frequency of genotypes is in the group with the contents of 2.0% to 3.0%. The range is given by the origin of the genotypes. Average content of alpha acids in wild hops is 2.5%, whereas in the collection of genetic resources it amounts to 4.0% w/w. High average content of alpha acids was revealed in new breeding lines (10.0%) under low variability (28.5%). It is due to the main objective of hop breeding in the nineties of the 20th century. Previously hop breeding was rather aimed at aroma hops with the contents of alpha acids on the level between 3.0 and 5.0%.

The average content of beta acids in hops was determined at the level of 4.5%. Variability of beta acid content amounts to 33.4%. The lowest contents under the level of 1.0% w/w

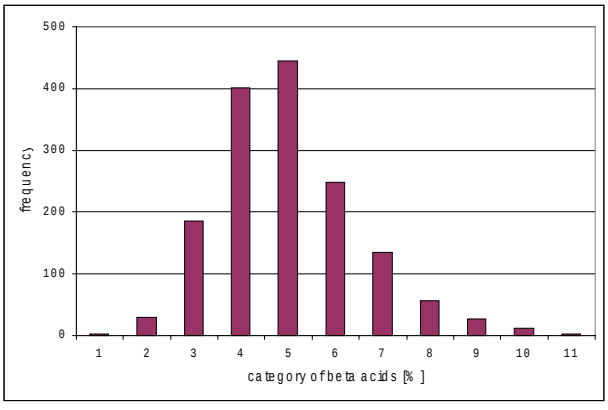

Fig. 3. Frequency of beta acid contents

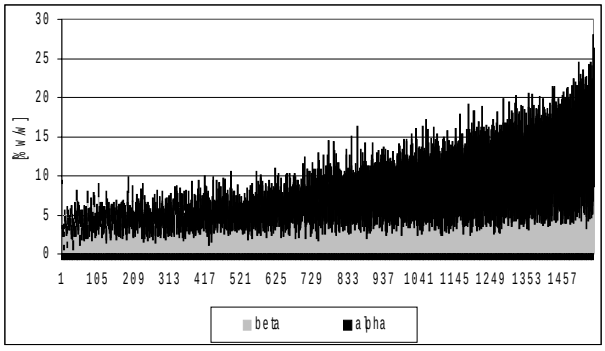

Fig. 4. Influence of alpha acids content on beta acids content

were in wild hops. The highest contents of beta acids (> 10.0%) were determined in the genotypes belonging to hop breeding material. It is evident that the genotypes are the most frequent in the group with the content of beta acids between 4.0 and 5.0% (Fig. 3).

It is obvious from the results that beta acid content does not correlate with the content of alpha acids (r = 0.48). We can suppose that the content of beta acids is influenced by the content of alpha acids only from 23%. This phenomenon is shown in Figure 4, where we can see the list of all the tested genotypes sorted out according to alpha acid contents. It is evident that with the increasing contents of alpha acids the content of beta acids is nearly constant. This knowledge is very important for breeding aimed at high contents of beta acids in aroma hops.

The average content of cohumulone in hops was determined at the level of 27.3% (rel.). Variability in alpha acid content amounts to 26.9%. The lowest cohumulone ratio shows the clone K26 from Poland (11.0% rel.) as well as a wild hop from Caucasus (13.0%). The highest ratio of cohumulone was found out in wild hops from North America (45.6–69.0% rel.). These hops are characterized by a high content of cohumulone. American varieties Aquila and Talisman show the highest ratio of cohumulone (>49% rel.) within the collection of genetic resources. The most numerous are genotypes in the groups with the ratio of cohumulone in the range between 20 and 30% (Fig. 5).

Ratio of colupulone has a similar trend. Average ratio of colupulone was determined at the level 48.1% rel. Variability in the contents of this substance amount to 17.7%. The lowest ratio of colupulone was revealed in a German variety Hallertauer (29.7% rel.) and a Japanese variety Sapporo 5 (30.0% rel.). The high content of colupulone was determined again in wild hops from North America (66.4–84.4% rel.). American variety Talisman has a colupulone ratio higher than 90% rel. The studied genotypes are most numerous in the groups with the colupu-lone ratio between 40 and 50% rel. (Fig. 6). Co-humulone and colupulone ratio are not independent parameters. Positive correlation between both variables was found by Likens et al. (1978).

The average content of xanthohumol was determined at the level of 0.36%. Variability in the contents of this ingredient amounts to 47.9%. The lowest content of xanthohumol was revealed in Clone 10 (Czech clone from Saaz genetic group), which belongs to the collection of genetic resources. On the contrary, the highest content was found out within the genotypes belonging to the breeding material group (1.02%) and commercial varieties Admiral, Taurus, Agnus and Target. It is obvious from Figure 7 that the most numerous are genotypes in the group with xanthohumol content in the range 0.20–0.30%. The basic statistical data show that normality of data was rejected whereas homogeneity was accepted. Disruption of data homogeneity is caused by goal-directed breeding process aimed at a high content of xanthohumol.

The average content of desmethylxanthohumol (DMX) was determined at the level of 0.09%. Variability of this substance amounted to

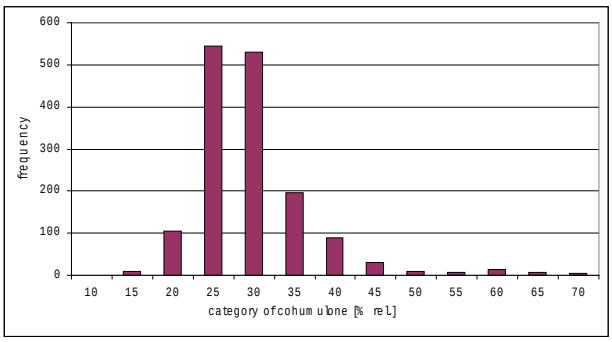

Fig. 5. Frequency of cohumulone contents

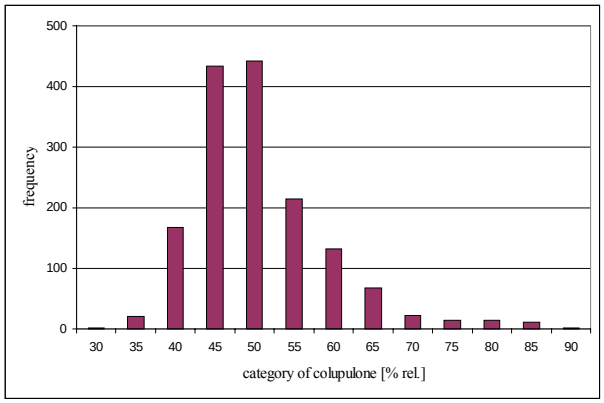

Fig. 6. Frequency of colupulone contents

56.2%. The lowest content of DMX was 0.01% (wild hops from Caucasus and hop varieties Wyoming, Target and Pride of Ringwood). On the contrary, the highest content was revealed in the genotypes within the hop breeding material group (0.46%). High variability issues from obtaining genotypes with extremely high content of DMX. From Figure 8 it is evident that the most numerous are the genotypes in the group with DMX content in the range 0.08-0.12%. The basic statistical data show that both normality as well as homogeneity were rejected.

As hops in the breweries are generally used on the basis of their alpha acid contents, beside the absolute concentration the ratio of xant-hohumol to the alpha acid is also taken into consideration (Biendl 2002a). Consequently the amount of xanthohumol in alpha dosage can be determined. Typical contents of xanthohumol, alpha acids and their ratio in Czech hop cultivars are summarized in the Table 1. The data show that aroma hops Saaz and preferably Sládek are notable by a high xanhohumol/alpha acid ratio which is much higher than in bitter hops.

Contents of xanthohumol and alpha acids significantly correlate. Data of xanthohumol and alpha acid contents in Premiant variety (crop harvest 2006) are shown on Figure 9. Individual data points represent samples of the variety taken in various localities within all the growing regions (Saaz, Auscha, Tršice) indicating the effect of growing site on secondary metabolites content. Such rich data concerning xanthohumol contents in hops are not usually available. In the papers the data taken from a single sample are mostly published (Biendl 2002b).

T a b l e 1

Typical contents of xanthohumol, alpha acids and their ratio in Czech hop cultivars

Variety	Xanthohumol [% w/w]	Alpha acids [% w/w]	Ratio X/ Alpha.10^2
Saaz	0.25–0.45	3.0–4.0	9.0–13.0
Bor	0.40–0.60	7.0–10.0	4.0–7.0
Sládek	0.45–0.80	4.0–8.0	9.0–15.0
Premiant	0.30–0.50	7.0–11.0	3.5–4.5
Agnus	0.70–1.10	9.0–14.0	6.0–8.0

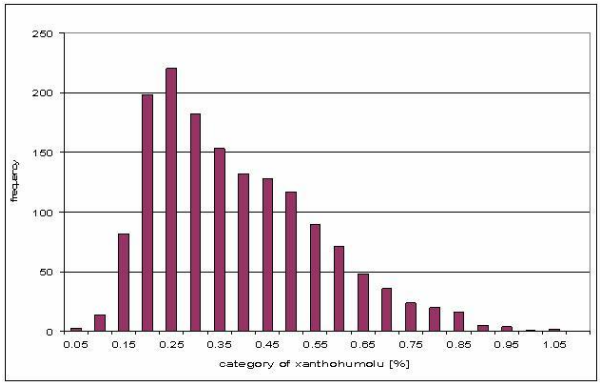

Fig. 7. Frequency of xanthohumol contents

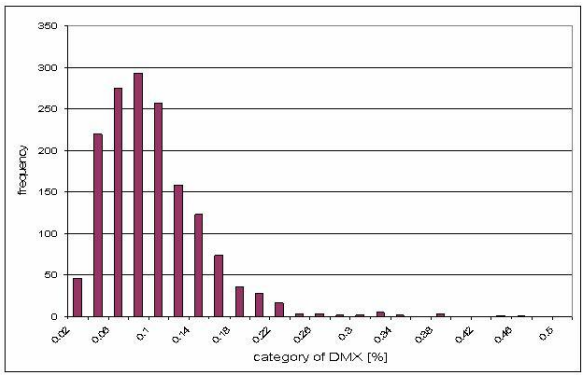

Fig. 8. Frequency of desmethylxanthohumol (DMX) contents

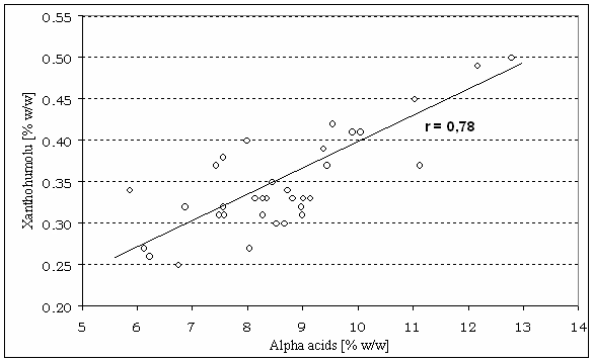

Fig. 9. Correlation between alpha acid and xanthohumol contents in Premiant variety (crop harvest 2006 in various localities)

CONCLUSION

Alpha bitter contents show non-normal distribution within the assessed collection. Breeding of hops aimed at high contents of this ingredient has caused it. On the contrary, beta acid content is typical by normal distribution. We did not determine the influence of alpha acids on the content of beta acids. Ratio between cohumulone and colupulone is based on genetic origin. American hops show higher contents of these components. Positive correlation between these two ingredients has been determined. Contents of xanthohumol and DMX have the character of non-normal distribution. Content of these compounds is influenced by breeding process. Plant breeding programmes have led to the development of hop varieties that combine unusually high contents of alpha acids with greatly improved resistance against the most relevant diseases (powdery and downy mildew). Hop genetics began to be explored in hop breeding finding markers of important traits and quality parameters. With respect to amazing properties of hop prenylflavonoids targeted breeding to their high contents in hop cones has been started in several countries (Germany, Czech Republic).

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SOUHRN

Statistické hodnocení databáze chmele z pohledu obsahu a složení vybraných sekundárních metabolitů bylo v letech 2006 a 2007 provedeno na souboru 1546 vzorků chmele. Průměrný obsah alfa kyselin byl 6,1 % hmotnostních (hm.) s variabilitou 64,6 % a rozmezím individuálních hodnot v intervalu 0,4 % hm. až 17,8 % hm. Průměrný obsah beta hořkých kyselin byl zjištěn 4,50 % hm. a variabilitou 33,4 % a rozmezím 0,5 % hm. až 10,9 % hm. Průměrný podíl kohumulonu byl 27,3 % relativních (rel.) a kolupulonu 48,1 % rel. Z farmakologického hlediska jsou důležitými látkami chmele prenylflavonoidy xanthohumol a desmethyxanthohumol (DMX). Průměrný obsah xanthohumolu ve zkoumaném souboru vzorků byl 0,36 % hm. při variabilitě V = 48 %, při minimálním obsahu 0,02 % a při maximálním obsahu 0,94 %. Průměrný obsah DMX byl 0,09 % hm. s intervalem hodnot od 0,01 % do 0,20 % hm. Mezi obsahem alfa kyselin i beta kyselin a obsahem xanthohumolu byla stanovena pozitivní korelace na hladině významnosti a = 95 %. Cíleným šlechtěním byly získány odrůdy, které mají vysoký obsah některého ze sekundárních metabolitů. V České republice i Německu byly rozpracovány šlechtitelské programy s cílem získat odrůdy s vysokým obsahem xanthohumolu či DMX. Přesto, že byla stanovena vzájemná pozitivní korelace obsahu xanthohumolu a DMX (r = 0,58), ukazuje se, že šlechtění na vysoký obsah obou prenylflavonoidů současně nelze spojovat.

Klíčová slova: chmel obyčejný, genetické zdroje, alfa a beta-kyseliny, desmethylxanthohumol, xanthohumol