

BIOACTIVE SUBSTANCES IN HOPS AND BEER



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Abstract

The contents of prenylflavonoids (xanthohumol, desmethylxanthohumol, isoxanthohumol) were investigated in Czech and world hop cultivars during the period 2000-2004. The concentrations of xanthohumol varied between 0.2 and 1.2% (w/w) with the highest amounts found in the high-alpha hop cultivars Taurus, Admiral and Agnus. The highest ratio of xanthohumol/alpha acids, greater than 10^1 , was determined for Sladek, Czech hybrid aroma cultivar. A positive correlation was observed for the xanthohumol and alpha acids. Fresh hops contain also small amount of isoxanthohumol up to $2 \cdot 10^{-2}$ % (w/w). Desmethylxanthohumol was present in hop cultivars in the range of 0.05 to 0.30% (w/w). The contents of prenylflavonoids, predominantly isoxanthohumol, in Czech beers were usually between 0.3 and 1.9 mgL^{-1} . Low isoxanthohumol levels were found if hop CO_2 extracts were used. Prenylflavonoids were very minor constituents in draught or diet beers. Losses of isoxanthohumol on beer production were highest during wort cooling and trub removal.

Bioactive substances in hops and beer

Hops have a long history in herbal history. They have been used to treat a variety of complaints. Hops have been used for the relief of insomnia, as herbal antibiotics and anti-inflammatory agents. Hops also have a reputation for ability to treat menstrual disturbances. Recent research in bioactive effects revealed many remarkable properties of many compounds contained in hops. Pharmaceutical companies have taken increasing interest in herbal remedies and hops have been considered as a potential source of new-plant derived medicines. Practically all hops secondary metabolites exhibit more or less pronounced bioactive effects (Kondo, 2003).

Antioxidative activity of hop bitter acids and their analogues has a cancer preventive effect since they can quench free radicals which cause oxidation of the DNA in the body and thus cause genetic defects (Tagashira, 1995). Humulon has been known for its preventive effect against osteoporosis (Tobe, 1997). Humulon hinders the growth of certain leukemia cells especially with the combination of vitamin D. Humulones (α -acids) were found to be present in beer at concentration levels of 150-200 μgL^{-1} , while β -acids were absent (Hofte, 1998). The results of one recent study confirmed that beer components are protective against the genotoxic effects of heterocyclic amines on target organs associated with tumorigenesis „in vivo“ (Arimoto-Kobayashi, 2005). Isohumulones prevent the developments of non-insulin dependent diabetes and hyperlipidemia. Oral administration of isohumulones may improve insulin sensitivity in patients with type 2 diabetes (Kondo, 2003). Antibiotic activities were detected not only in hop resins but in the essential oils as well. Although less performant than traditional antiseptics, hop oils components are able to eliminate specifically some inauspicious microorganism of the skin, pathogens or saprophytes with high levels of odour (Chaumont, 1997).

Recently the high attention has been devoted to hop prenylflavonoids, plant secondary metabolites that are prominently present in hops (*Humulus lupulus* L.), (Stevens, 1997). The most important hop prenylflavonoids are xanthohumol (X), desmethylxanthohumol (DMX), isoxanthohumol (IX), 6-prenylnaringenin (6-PN) and 8-prenylnaringenin (8-PN). Importance of prenylflavonoids consists in wide range of biological effects (Stevens, 1999a; Miranda, 1999). Xanthohumol enters biochemical pathways of many xenobiotics and promote to eliminate them out of organism. For example X at micromolar levels stimulates the activity of detoxifying enzyme quinone reductase. Stimulating effect of some enzymes contributes to cancer prevention (Miranda, 2000). Hops are also source of phytoestrogens.

8-Prenylnaringenin is the most phytoestrogen currently known (Milligan, 1999). Phytoestrogens are plant-derived substances with oestrogenic activity. They are thought to protect against several chronic diseases or breast and prostate cancer (Walker, 2000). The composition of prenylflavonoids largely depends on the variety. The ripening stage and storage conditions constitutes up to 90 % of the mixture of prenylflavonoids (Biendl, 2002). During beer brewing X is isomerized to IX, hence this is the most prevalent prenylflavonoid in beer (Fig. 1). Bioactivity of isoxanthohumol is usually lower than of xanthohumol. It is compensated by higher concentrations of IX and easy availability in beer. Similarly DMX is converted to 6-PN and 8-PN. Beer is the most important source of prenylflavonoids in the human diet (Stevens et. al, 1999b).

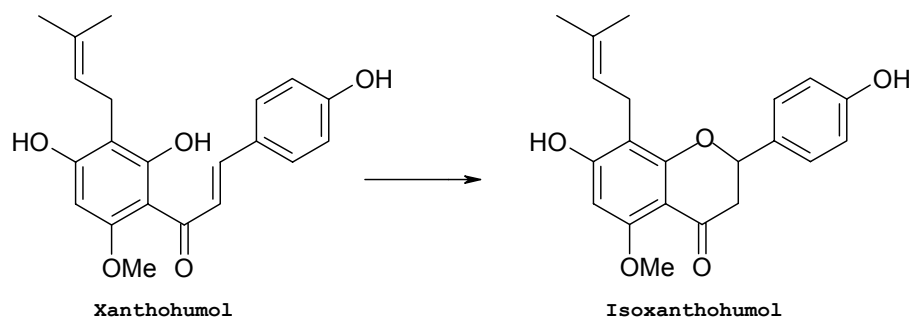


Fig.1: Isomerization of xanthohumol to isoxanthohumol during beer brewing

In the recent years contents of prenylflavonoids in many hop cultivars and beer brands have been investigated in Hop Research Institute in Zatec, CZ. Obtained results are presented further.

MATERIAL AND METHODS

Hop cultivars from the harvest seasons 2000-2004 were collected for quantitative analyses of prenylflavonoids by liquid chromatography and with UV (LC/UV) and mass detection (LC/MS). Various types of Czech beers (lagers, draught beers, diet and special beers) were investigated during 2003-2004. Beers were bought at local store. X and DMX (detection at 370 nm) were determined according to the EBC 7.7 method (Analytica-EBC) simultaneously with alpha and beta acids. Mobile phase consisted of 900 ml of methanol with 190 ml of water containing 5 g of 85% phosphoric acid. It was mixed and filtered through 0,45- μ m nylon filter. The analytical column was Nucleosil EC C18 Hop (4 x 250 mm). The column temperature was 40°C and the flow rate was 0,8 ml/min. Beers preconcentration was effected by solid-phase extraction (Supelclean LC-18, 500 mg/6 ml cartridges). Cartridges were conditioned with methanol (2 x 10 ml) and water (2 x 5 ml). Beer (50ml) with addition of 0,1 ml phosphoric acid were soaked through the cartridge. Fraction of interest was eluted with 2,5 ml of methanol. Eluate (10 μ l) was injected in the HPLC instrument (detection at 290 nm). MS detection was performed in the positive chemical ionization mode at atmospheric pressure (LC-API-MS) using an ion trap FINNIGAN LCQ Deca mass detector. The HPLC eluent was introduced into mass spectrometer via a heated nebulizer interface 450 °C. Ionisation of the analyte vapor mixture was initiated by a corona discharge needle at 6 kV and discharge current 5 μ A. Prenylflavonoids were detected in single ion monitoring mode. Typical chromatograms of the analysis of prenylflavonoids in hops and beer performed by LC/UV are shown on Fig. 2, 3.

X was purchased from Phytochem, Germany, DMX was obtained from Dr. A. Heyerick (Ghent University, Belgium), standards of 6-PN and 8-PN were given by Dr. F. Stevens (Oregon State University, Corvallis, USA).

RESULTS AND DISCUSSION

The concentration ranges of X and alpha acids in Czech and world hop cultivars are summarized (Table 1, Table 2). X contents are in the interval of 0.2 to 1.2 % w/w. The highest amount of xanthohumol was found in Taurus, Admiral and Agnus cultivars. Sládek showed remarkably high xanthohumol/alpha acids ratio. The ratio is an important parameter from brewer's perspective. Positive correlation between X and alpha acid contents was demonstrated (Fig. 4). The relationships between X and alpha acid contents in German, American, Czech, English and Slovenian hop cultivars are shown (Fig. 5). As the content of

alpha acids increases, levels of X also increase. Scattering of X levels in high-alpha hops suggests that X is influenced by environment and genetic factors too. DMX contents in hop cultivars varied between 0,05 and 0,30 (w/w) (Table 2, Table 3) in agreement with published data (J. de Keukeleire, 2003). Also small amounts of IX were observed in raw hops, up to 0.02 % w/w. A direct correlation with the concentrations of X is evident (Table 4).

The summarised contents of prenylflavonoids for various brands of Czech beers are shown (Table 5). IX is the most abundant prenylflavonoid in beers. Widely varying contents reflect different hopping regimens. When hop extracts (carbon dioxide) or advanced hop products are used low or negligible amounts of prenylflavonoids are found in the final beers. The highest concentration of prenylflavonoids, appr. 2 mg/L, was determined in Pilsner Urquell 12 % lager. Czech lager beers contained significantly more prenylflavonoids than did draught beers. If xanthohumol enriched products are used for hopping concentration of isoxanthumol in final beer can increase up to 6 mgL⁻¹ (Biendl, 2000). Relatively high concentrations of xanthohumol were found in some stout and porters style beers, up to 1,2 mgL⁻¹. This phenomenon is explained by inhibition effect of roasted malt to xanthohumol isomerization (Walker, 2004).

Significant losses of prenylflavonoids occur during brewing (Stevens, 1999b). The contents of IX were monitored at several stages in the course of lager production (Fig 6). IX was lost particularly during wort cooling and trub removal. IX was drastically removed while further losses downstream were less pronounced. The utilisation of isoxanthohumol from hot wort to final beer was about 50 %. In spite of high losses in the course of production beer remains the most important source of prenylflavonoids and other hops bioactive compounds in human diet. Beer also provides a number of important nutrients including carbohydrates, aminoacids, minerals (Mg, K, P, Si) and vitamins. Beer reduces the general level of mortality if drunk moderately and regularly (Keil, 1997). All these aspects improve image of beer in consumers. It is not without a reason that beer is called „liquid bread“.

CONCLUSIONS

The concentrations of X and DMX in hop cultivars were found in the ranges of 0.2 to 1.2 % (w/w) and 0,05 to 0,30 % (w/w) respectively. Taurus, Admiral and Agnus are the richest cultivars in xanthohumol, Magnum, Galena and Newport are richest in desmethylxanthohumol. Total contents of prenylflavonoids in Czech beers may be as high as 2 mg/L. Czech lager beers contain more prenylflavonoids than draught or diet beers. The most important losses of IX in the course of brewing occur during wort cooling and trub removal. In spite of high losses in the course of production beer remains the most important source of prenylflavonoids and other hops bioactive compounds in human diet.

ACKNOWLEDGEMENTS

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Table 1. Concentration ranges of xanthohumol (X) and alpha acid in Czech hop cultivars (2000-2004).

Cultivar	<u>X</u> (% w/w)	Alpha acids (% w/w)	Ratio <u>X</u> /Alpha.10 ²
Saaz	0.25 – 0.45	3.0 - 4.0	9 – 13
Bor	0.40 – 0.60	7.0 – 10.0	4 – 7
Sládek	0.45 – 0.80	4.0 – 8,0	9 - 15
Premiant	0.30 – 0.50	7.0 – 11.0	3.5 – 4.5
Agnus	0.70 – 1.10	10.0 – 15.0	6 – 8

Table 3. Concentrations (mean values and ranges) of desmethylxanthohumol (DMX), xanthohumol (X) and alpha acids in Czech hops (2003-2004).

Cultivar	Year	<u>DMX</u> (% w/w)	<u>X</u> (% w/w)	Alpha acids (% w/w)
Saaz	2003	0.073 (0.053 – 0.120)*	0.35 (0.28 – 0.42)*	3.3 (2.3 – 4.4)*
	2004	0.111 (0.080 – 0.167)	0.40 (0.33 – 0.46)	3.8 (2.6 – 5.0)
Sládek	2003	0.120 (0.050 – 0.202)	0.48 (0.29 – 0.62)	4.1 (1.6 – 5.7)
	2004	0.176 (0.122 – 0.300)	0.75 (0.60 – 1.04)	6.8 (4.4 – 9.5)
Premiant	2003	0.081 (0.051 – 0.110)	0.29 (0.22 – 0.41)	6.7 (4.8 – 8.7)
	2004	0.116 (0.064 – 0.191)	0.43 (0.35 – 0.51)	10.6 (7.5 – 12.7)
Agnus	2003	0.111 (0.090 – 0.160)	0.77 (0.74 – 1.00)	11.1 (7.9 – 13.1)
	2004	0.150 (0.119 – 0.190)	1.00 (0.86 – 1.18)	13.0 (12.4 – 15.4)

*mean value/interval of experimental results

Table 2: Contents of xanthohumol, desmethylxanthohumol and alpha acids in world hop cultivars (2002-2004)

Cultivar	Alpha acids (w/w)			Xanthohumol (% w/w)			DMX (% w/w)	
	2002	2003	2004	2002	2003	2004	2003	2004
GERMANY								
Hallertauer	-	2,4	4,8	-	0,25	0,35	0,031	0,075
Hall. Tradition	7,8	3,5	7,5	0,41	0,32	0,52	0,051	0,135
Hersbrucker	3,7	1,5	3,9	0,23	0,15	0,28	0,065	0,111
Magnum	15,1	12,8	18,8	0,54	0,41	0,63	0,163	0,228
Northern Brewer	9,4	6,4	11,7	0,65	0,51	0,89	0,077	0,175
Nugget	12,1	9,4	13,4	0,73	0,60	0,95	0,118	0,196
Perle	6,8	3,9	5,4	0,46	0,32	0,51	0,067	0,115
Saphir	4,3	-	5,4	0,33	-	0,53	-	0,095
Spalter Select	5,8	2,3	6,4	0,48	0,28	0,58	0,027	0,116
Taurus	17,5	12,4	20,4	1,08	0,77	1,27	0,041	0,115
USA								
Cascade	7,7	5,6	6,5	0,36	0,27	0,38	0,156	0,169
Cluster	5,8	7,6	5,9	0,36	0,38	0,37	0,073	0,080
Columbus	13,7	15,9	15,4	0,74	0,73	0,91	0,134	0,138
Fuggle	6,2	4,4	3,1	0,38	0,28	0,19	0,049	0,039
Galena	12,0	12,2	12,4	0,39	0,50	0,59	0,197	0,260
Chinook	14,1	13,2	13,4	0,47	0,47	0,51	0,043	0,064
Liberty	5,0	4,4	4,2	0,27	0,26	0,24	0,087	0,104
Mount Hood	5,7	6,1	5,0	0,30	0,30	0,30	0,100	0,106
Newport	-	10,3	10,0	-	0,61	0,63	0,197	0,258
Nugget	10,8	13,3	13,6	0,58	0,69	0,77	0,153	0,181
Perle	6,4	8,2	5,1	0,40	0,42	0,35	0,100	0,083
Sterling	6,4	4,2	6,0	0,26	0,21	0,29	0,111	0,127
Tettnanger	4,0	3,4	4,6	0,24	0,22	0,31	0,052	0,063
Willamette	5,2	4,0	4,2	0,46	0,36	0,36	0,065	0,064
SLOVENIA								
Aurora	9,9	6,4	10,9	0,53	0,40	0,56	0,045	0,106
Bobek	5,8	3,5	5,5	0,53	0,36	0,54	0,086	0,084
Celeia	4,5	1,9	3,7	0,24	0,16	0,25	0,035	0,057
Savinjski Golding	3,4	2,5	3,8	0,29	0,24	0,35	0,019	0,030
ENGLAND								
Admiral	15,0	15,3	-	1,09	1,00	-	0,132	-
Fuggle	4,9	5,1	-	0,20	0,24	-	0,051	-
Target	11,4	10,5	-	0,80	0,72	-	0,070	-
First Gold	9,3	8,7	-	0,46	0,48	-	0,109	-
Goldings	6,4	5,6	-	0,32	0,27	-	0,065	-
Pilgrim	10,8	11,1	-	0,65	0,75	-	0,092	-

Table 4. Concentrations (%w/w) of xanthohumol (X) and isoxanthohumol (IX) in Czech hops (2002)

Odstraněno:

Cultivar	Locality	X (% w/w)	IX (% w/w)
Saaz	Drahonice	0.20	0.004
Saaz	Ročov	0.30	0.006
Sládek	Žatec	0.51	0.015
Premiant	Staňkovice	0.22	0.005
Premiant	Brozany	0.20	0.004
Bor	Nesuchyně	0.29	0.006
Agnus	Staňkovice	1.03	0.018

Table 5. Concentrations of prenylflavonoids in Czech beers (X = xanthohumol; IX = isoxanthohumol; 6-PN = 6-Prenylnaringenin; 8-PN = 8-Prenylnaringenin).

Brewery	Beer brand	Prenylflavonoids ($\mu\text{g}\cdot\text{L}^{-1}$)			
		X	IX	6-PN	8-PN
A	draught	9	206	5	< 2,0
B	lager 12°P	-	620	-	-
B	draught	34	450	21	9
C	draught	< 0,2	< 20	< 2,0	< 2,0
D	diet beer	1,9	250	7	5
E	lager 12°P	28	1350	31	15
F	lager 11°P	28	1180	30	12
G	lager 12°P	26	1910	29	22
H	lager 12°P	16	860	27	25
H	draught	-	320	-	-
I	lager 12°P	-	338	-	-
K	lager 12°P	-	270	-	-
K	draught	-	74	-	-
L	lager 12°P	-	730	-	-
L	draught	-	350	-	-
M	lager 12°P	-	725	-	-
M	draught	-	570	-	-

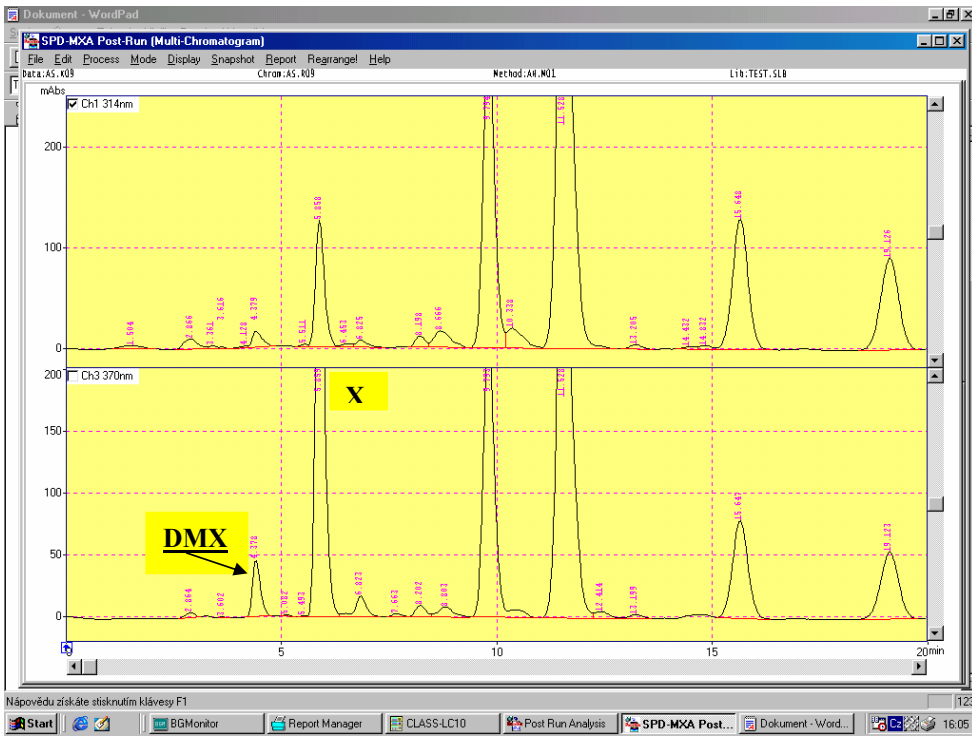


Fig. 2: The chromatogram of simultaneous analysis of bitter acids and prenylflavonoids in raw hops by liquid chromatography with UV detection (Agnus cultivar, 2004).

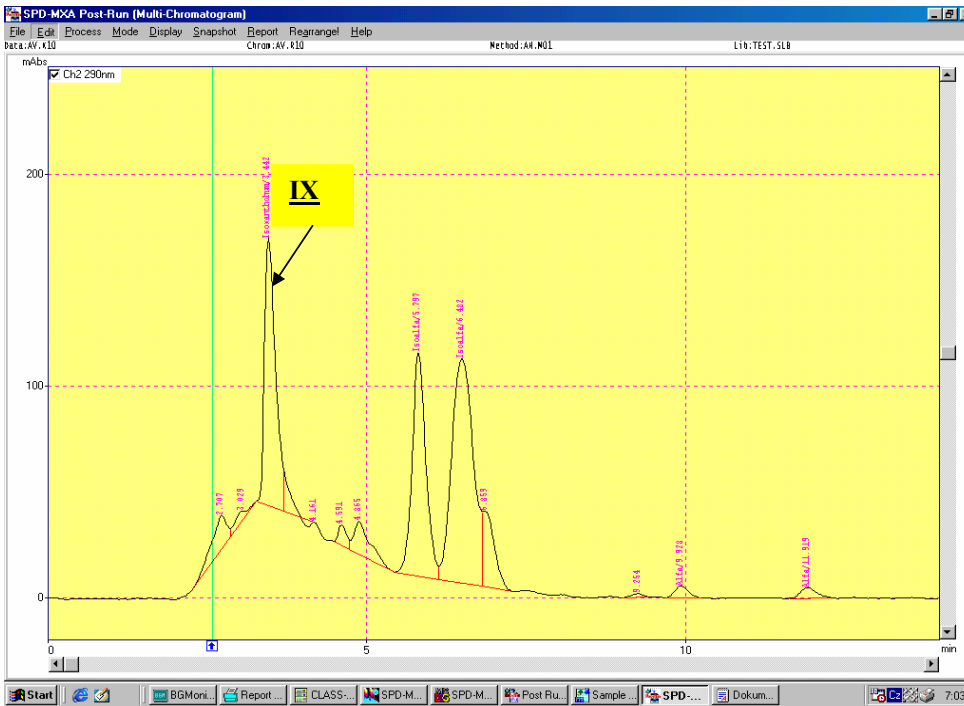


Fig. 3: The chromatogram of isoxanthohumul analysis in beer lager after SPE pre-concentration

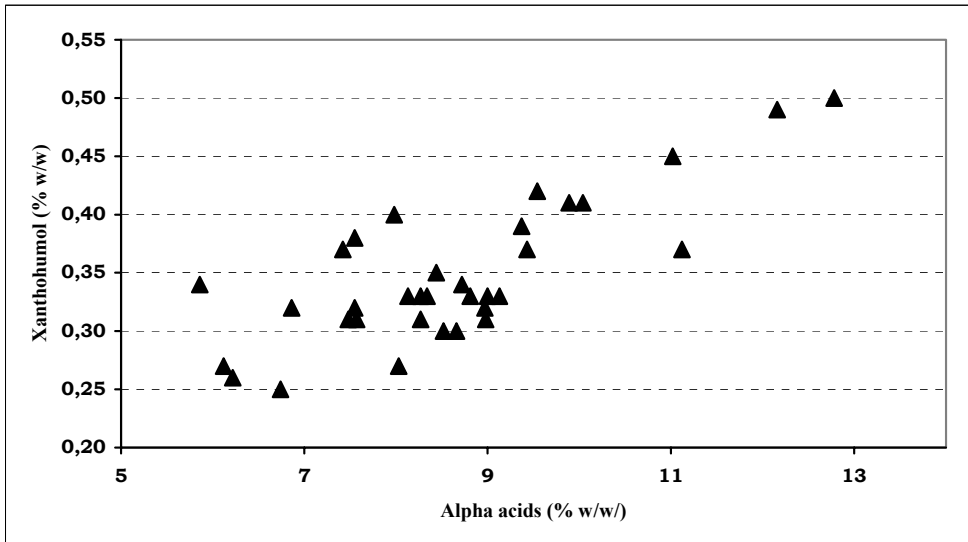


Fig. 4: Relationship between the concentrations of xanthohumul and alpha acids contents in Czech cultivar Premiant, harvest season 2002. Triangles represent various localities in growing areas, $r = 0,780$.

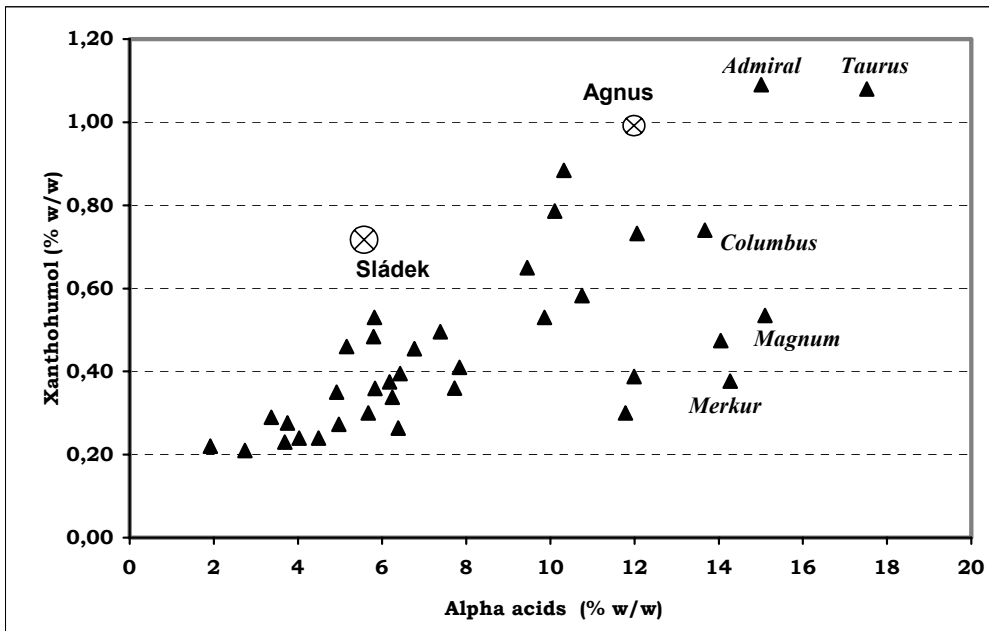


Fig. 5: Relationship between the concentrations of xanthohumul and alpha acids contents in various hop cultivars, harvest season 2002. Triangles represent hop cultivars from USA, Germany, England, Slovenia and Czech Republic.

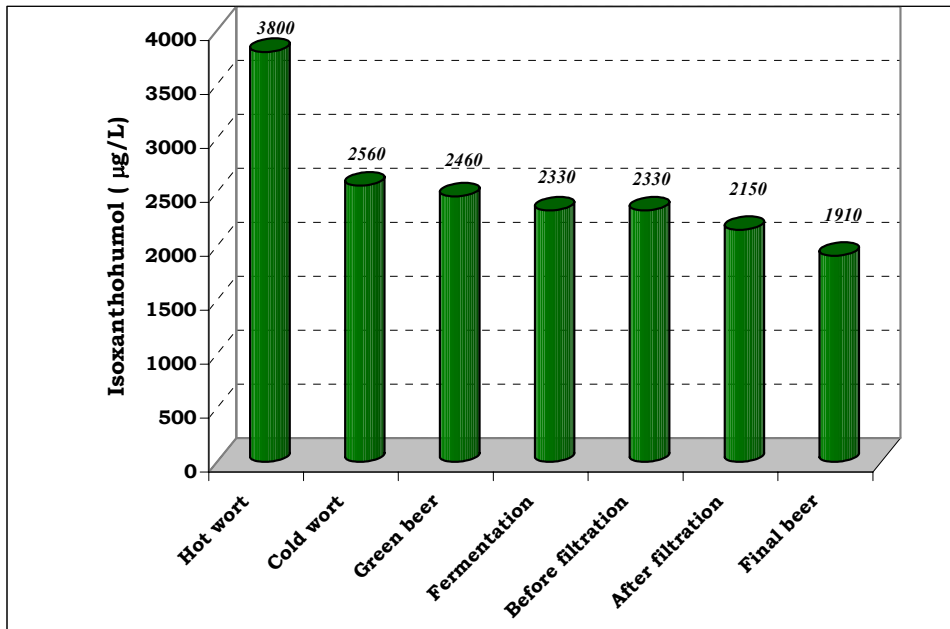


Fig. 6: Contents of isoxanthohumul in semi-products and final beer in the course of 12°P lager production

