BRAUWELT
INTERNATIONAL
TECHNICAL FEATURE

Use of a xanthohumol-rich hop product in beer production

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In on-going in vitro investigations, xanthohumol prenylflavonoid exhibits a surprisingly great diversity of positive attributes. A test series was run to establish the behaviour of a xanthohumol-rich hop product in beer production (compared to α-acids) and to evaluate the resulting bitter impression.

First results about the biological effect of xanthohumol were summarised in a review article published in 1998 (1).

Brief survey on status of research into xanthohumol

Biological effects

Growth inhibition of certain tumour cells, antioxidative and other anti-carcinogenic properties (activation of the "Quinon-reductase" enzyme or inhibition of human enzymes of the "P450 CYP1A2" class) has been reported. Prophylactic protection against osteoporosis and arteriosclerosis has also been mentioned.

Moreover, antimutagenicity (2) as well as a high activity vis-à-vis oxidation of low density lipoproteins has been determined recently (3). This protective effect may have prophylactic value in preventing coronary diseases, myocardial infarction, arteriosclerosis and strokes.

The overall favourable pharmacological potential of xanthohumol can be classed as being very high. But to-date, its action was proven only in tests using isolated cells (in vitro). Confirmation by in vivo tests (animal experiments, clinical studies) are not yet available.

Behaviour during beer production

Depending on variety, xanthohumol content of hops fluctuates between 0.1 and 1.1% (4). Its behaviour during beer production has been investigated in great detail (5). During wort boiling, xanthohumol is converted to isoxanthohumol (Fig. 1).

After addition of whole hops 15 min after the beginning of wort boiling and a further boiling period of 75 min, a yield of 69% was noted after a 15-minute whirlpool rest. Only very low quantities of non-isomerised xanthohumol could be detected. 13% of xanthohumol added with hops was measured in the spent hops and 12% in the hot break. Further losses arose during cold break removal (6%) and fermentation (11%). 30% of xanthohumol originating from the hops was eventually found in the final beer after storage. 98% of this had been converted to isoxanthohumol.

Other authors describe even larger losses. With divided hop addition in the form of pellets, only 15% of xanthohumol originally added was detected in beer after filtration (6).

Isoxanthohumol also exhibits positive biological effects. Compared to xanthohumol, the activity of the former is, however, slightly reduced, though it is higher particularly in inhibition of enzymes of the "P450 CYP1A2" class (7). These enzymes can catalyse development of carcinogenic substances. Inhibition by isoxanthohumol was e.g. found in metabolisation of B1 aflatoxin, whereby conversion to the carcinogenic aflatoxin M1 compound can be prevented.

Arimoto-Kobayashi and co-workers report that beer inhibits the mutagenic action of so-called heterocyclic amines present in cigarette smoke or charred meat (8). This could even be confirmed in vivo in an experiment with mice. The corresponding active beer constituents are still unknown. It has been ascertained recently that xanthohumol and isoxanthohumol can inhibit the carcinogenic effect of certain heterocyclic amines (2,7). Xanthohumol,

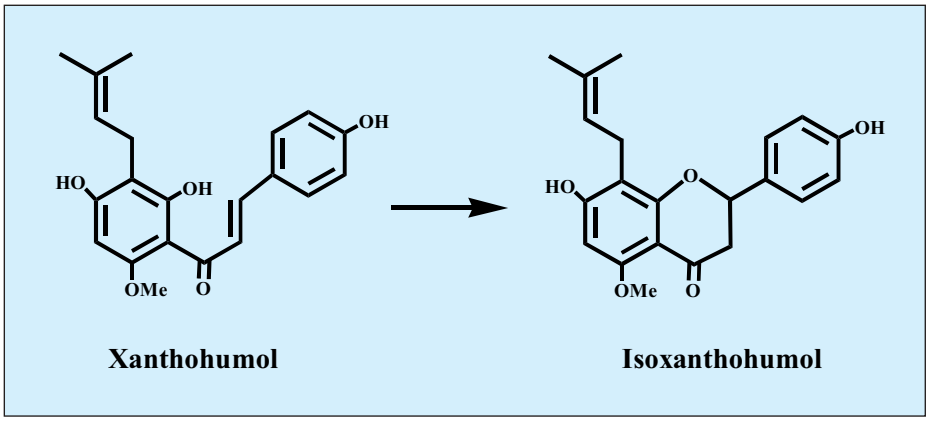

Fig. 1 Conversion of xanthohumol to isoxanthohumol

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Table 1 Hop products used

	Ethanol extract	Pellets	"Xanthohumol product"
Iso- and α-acids (HPLC) %	42.8	9.7	2.9
β-acids (HPLC) %	22.3	5.1	0.4
Total resins %	82.8	n.b.	19.3
Xanthohumol %	1.9	0.5	2.1
n.b.: not determined			

Table 2 Hop addition

	Ethanol extract	Pellets	"Xanthohumol product"
α-acids mg/l	102	96	38
Xanthohumol mg/l	4.1	4.4	30.5

and especially isoxanthohumol available in very much higher concentrations due to conversion during wort boiling, may possibly make a contribution to the anti-carcinogenic property of beer ascertained by Arimoto-Kobayashi and co-workers.

Isoxanthohumol content in beer

The isoxanthohumol content in beer varies widely. *Stevens* and co-workers report concentrations ranging from 0 to 3.4 ppm (9). Studies of a larger international range of polyphenol-rich and strongly hopped beers showed isoxanthohumol contents of up to 2.7 ppm (10).

The isoxanthohumol content of beer can be influenced by selection of hop variety and hop product. *Kammhuber* and co-workers report xanthohumol contents of different hop varieties and different hop products (4). The "Hallertauer Taurus" bitter hops has e.g. a xanthohumol content of 1.1%, the corresponding figure in "Hallertauer Magnum" is only 0.6%. Processing also gives rise to clear differences. Hop xanthohumol is retained almost quantitatively in pellets and ethanol extracts, whereas it is not extracted by carbon dioxide due to its polarity and remains in the spent hops.

Being a prenylated flavonoid, isoxanthohumol counts as a polyphenol. Measures aimed at reducing this substance class (e.g. stabilisation by PVPP) consequently also lead to a decrease in isoxanthohumol. Forster and Köberlein state that stabilisation with PVPP reduces isoxanthohumol yield by about 30% (6).

Table 3 Wort analyses

	Unit	Ethanol extract Beginning of boiling[3]	Pellets Beginning of boiling[1]	"Xanthumol product" Beginning of boiling[3]	"Xanthhumol product" End of boiling[2]	50% "Xanthohumol product 50% Ethanol extract[1]
		Reference E	Reference P	Test KB	Test KE	Test M
Extract, apparent	% w/w	11.3	11.4	11.3	11.3	11.4
pH value	–	5.56	5.50	5.55	5.57	5.55
Colour	EBC	6.5	6.7	7.7	7.4	7.1
Bitterness units	EBC	46	46	43	37	48
Attenuation, apparent	%	81.1	80.9	80.6	80.6	81.1
Tannins	mg/l	193	215	216	214	202
Anthocyanogens	mg/l	112	110	125	118	123
Iso-α-acids	mg/l	40.0	32.7	19.5	9.6	33.6
α-acids	mg/l	14.9	16.5	5.1	10.8	8.8
Total humulones	mg/l	55	49	24	21	42
Degree of isomerisation	%	73	66	79	46	79
Total humulones/ BE	mg/l/ EBC	1.18	1.07	0.56	0.55	0.88
[1]) 1 test; [2]) 2 tests; [3]) 3 tests						

Test arrangement

Hop products used

A- and β-acids can be extracted from hops with ethanol as well as with carbon dioxide. But xanthohumol is dissolved only in ethanol and not in carbon dioxide. Thus, a combination of the two processes achieves a (partial) separation of xanthohumol and α-/β-acids. The xanthohumol-rich hop product was prepared from the Taurus variety. Ethanol extract from Brewers Gold, Northern Brewer as well as Magnum and pellets type 90 from Northern Brewer as well as from Magnum were used as a reference.

Table 1 shows composition of the xanthohumol product used, compared with other hop products used in the brewing tests. The xanthohumol content of pellets and ethanol extract is lower by a factor of about 20 than the concentration of alpha-/iso-alpha acids, the corresponding ratio in the xanthohumol product is 0.72.

With a total resin content of 19.3%, the xanthohumol product contains only "neutral" hop matrix (cellulose, high molecular weight carbohydrates and proteins) in addition to hop resins. Water-soluble low molecular weight components have been separated off. The specifically identified constituents add up to 5.4%. More than two-thirds of the total resin fraction are unspecific soft and mainly hard resins. The hard resin fraction of the hops is almost completely contained in the xanthohumol product.

Hop addition

As the xanthohumol product has a very low alpha-/iso-alpha acid content, hop addition was based on total resins (about 300 mg/l). When using xanthohumol product, xanthohumol addition was higher by a factor of 7.5 compared to the reference brews (Table 2).

Hop products were added at the beginning of boiling. In addition, in a further test, the xanthohumol product was added at the end of boiling.

Brewing method

The tests were carried out one to three times in the pilot brewery of Bitburger Brauerei on a 20-hl scale. Boiling time was 75 minutes in each instance, 55 minutes of this under pressure at 102 – 103 °C.

Analysis of isoxanthohumol

Isoxanthohumol in wort and beer was analysed by means of solid-phase extraction and subsequent HPLC/UV measurement (10). Pure xanthohumol and pure isoxanthohumol were used as an external calibration standard (purity each >95% using DAD-HPLC, supplier: Phytochem Referenzsubstanzen GbR mbH, 89335 Ichenhausen, Germany).

Tasting

Tasting was carried out in five different operations and institutes based on different tasting systems.

Results

Wort analyses

As a result of the xanthohumol product, wort colour rose by up to 1.2 EBC (Table 3). Despite a low total humulone content, these worts had high bitterness unit values similar to those of the reference worts. Only in test KE were bitterness units lower, this had been expected due to the shorter isomerisation time. Tannin and anthocyanogen levels in tests KB and KE were slightly above those of the ethanol extract brew and comparable to the contents when using pellets. The degree of isomerisation of humulones was higher in the test brews than in reference E, this might be attributed to lower concentrations. Due to the spent hop matrix, the pellets led to a further 7% reduction of the degree of isomerisation. Test KE yielded the lowest isomerisation due to the limited time.

When using a mixture of xanthohumol product and ethanol extract, the analytical values were about half-way between the values when using 100% of either material.

Total xanthohumol (xanthohumol + isoxanthohumol) yield in the wort of 61 and 73% was higher in reference E compared to the tests (Table 4). The significantly lower yield from pellets compared to ethanol extract was striking. The pellets yielded just 50%, this was almost also reached in test KB despite significantly increased xanthohumol addition. The spent hops obviously lead to delayed leaching and isomerisation. Higher losses due to the increased trub fraction in the whirlpool might also be a reason. Accordingly, the spent hop matrix reduces yield to a much more pronounced degree than the raised concentration when using xanthohumol product. In the case of late hop addition, yield drops considerably, whereas an over-proportionally good yield was achieved in test M.

The degree of isomerisation of xanthohumol was over 90%. Only test KE, with 42%, had a substantially lower degree of isomerisation. No significant secondary isomerisation was noted in the whirlpool although isoxanthohumol contents of the pitched worts were clearly higher than those of cast wort (the values for cast and pitched worts originate from different brews).

Table 4 Xanthohumol in cast / pitched wort

	Unit	Reference E	Reference P	Test KB	Test KE	Test M
Xanthohumol	mg/l	0.2[1]/0.3[2]	0.2[1]	1.1[1]/1.1[2]	2.6[1]	0.9[2]
Isoxanthohumol	mg/l	2.3[1]/2.7[2]	2.0[1]	12.7[1]/13.4[2]	1.9[1]	10[2]
Sum	mg/l	2.5[1]/3.0[2]	2.2[1]	13.8[1]/14.5[2]	4.5[1]	10.9[2]
Degree of isomerisation	%	92[1]/90[2]	91[1]	92[1]/92[2]	42[1]	92[2]
Yield*	%	61[1]/73[2]	50[1]	45[1]/48[2]	15[1]	63[2]

*[1]) cast wort, test 2; [2]) pitched wort, test 3; *) based on xanthohumol addition*

Table 5 Beer analyses

	Unit	Reference E [3]	Reference P [1]	Test KB [3]	Test KE [2]	Test M [1]
Extract, apparent	% w/w	2.5	2.5	2.5	2.5	2.5
Alkohol	Vol.-%	4.8	4.8	4.7	4.7	4.7
Stammwürze	Gew.-%	11.3	11.4	11.3	11.3	11.3
Attenuation, apparent	%	78.3	78.2	77.5	77.7	77.7
Colour	EBC	5.5	5.7	6.2	6.3	5.8
pH		4.41	4.39	4.41	4.43	4.42
Bitterness units	EBC	33	33	33	28	33
Tannins	mg/l	188	208	198	194	191
Anthocyanogens	mg/l	96	107	104	94	107
Foam stability (NIBEM)	s	293	272	272	260	268
Iso-α-acids	mg/l	34.2	29.8	16.2	12.6	24.6
α-acids	mg/l	1.3	1.1	0.9	1.0	1.2
CO_2 content	%	0.50	0.50	0.50	0.50	0,49

[1]) 1 test; [2]) 2 tests; [3]) 3 tests

Beer analyses

In final beer (Table 5), colour of test beers was again slightly higher compared to reference beers. Tannin and anthocyanogen contents tended to be higher whereas foam stability, due to the lower iso-α-acid content, was adversely affected. Despite a low iso-α-acid and α-acid content, bitterness units were approximately as high as in the reference beers, corresponding to results from the wort. Accordingly, the xanthohumol product led to a pronounced bitter impression not attributable to iso-α-acids. As had been expected, the KE test contained the least bitterness units.

The isoxanthohumol content in the final beer (Table 6) could be increased by 200% to up to 6.0 mg/l, compared to hopping with pellets or ethanol extract. But total xanthohumol yield was reduced from 36 to 47% to 15 – 20%.

Table 6 Xanthohumol in beer

	Unit	Reference E [3]	Reference P [1]	Test KB [3]	Test KE [2]	Test M [1]
Xanthohumol	mg/l	0,1	0,1	0,1	0,1	0,1
Isoxanthohumol	mg/l	1,9	1,5	6,0	4,6	4,5
Total yield*	%	47	36	20	15	27

*[1]) 1 test; [2]) 2 tests; [3]) 3 tests; *) based on xanthohumol addition*

Absolute assessment: 0 = very poor/low; 9 = very good/high				
	Reference E	Reference P	Test KB	Test KE
Smell	5.8	6.0	5.8	5.7
Taste	5.9	5.5	5.1	4.7
Intensity of bitterness	5.4	5.8	5.5	4.5
Body	5.5	5.5	5.4	4.9
Liveliness	5.7	5.7	6.0	5.5
Aftertaste	4.6	5.4	5.0	4.3
Quality of beer	5.5	5.8	5.2	4.8
Rank	1	2	3	4

Table 7 Tasting (panel I) test series II

absolute assessment: 0 = very poor/low; 9 = very good/high			
	Reference E	Test KB	Test M
Smell	5.7	6.4	6.4
Taste	6.0	6.2	6.4
Intensity of bitterness	6.4	5.9	5.5
Body	5.4	5.4	5.1
Liveliness	5.8	5.4	5.9
Aftertaste	4.7	4.7	4.3
Quality of beer	6.0	6.2	6.4
Rank	2	2	1

Table 8 Tasting (panel I) test series III

Absolute assessment: 0 = very old; 9 = very fresh			
	Reference E	Test KB	Test M
Aging	7.1	.6	6.4

Table 9 Tasting after forced aging (panel I) test series III

Tasting

All five taster panels were able to differentiate the beers. Differences occurred mainly in taste, bitterness and quality of the beer. Whereas panel I (Tables 7 and 8) found a correlation largely between the sensory intensity of bitterness and analytically determined bitterness units, other panels did not establish any relationship for this property. The bitterness units (26.6 – 30.2) estimated by some panels were below those analytically measurable. The quality of bitterness was also assessed very differently so that no clear-cut conclusion can be drawn. As the various taster panels each preferred a different beer (individual data not presented), no beer was found to have a clear advantage or disadvantage. The beers normally tasted by a panel might possibly have an influence on the subjective preference for a certain beer type, due to the effect of habituation.

Use of xanthohumol product resulted in a slightly inferior taste stability compared to ethanol extract (Table 9). This was largely confirmed in the other test series (individual data not presented).

Summary

By a combination of ethanol and CO_2 extraction of hops, a xanthohumol-rich hop product was obtained, this was compared with ethanol extract and pellets in brewing tests on a 20-hl scale. This "xanthohumol product" is characterised by its very high xanthohumol/a-acid ratio of 0.72 (ethanol extract: 0.04). Total resin content proved to be a good measure of hop addition. Xanthohumol is isomerised during wort boiling, yield fluctuates between 15 and 70% as a function of time of hop addition, concentration of xanthohumol added and spent hop portion. Only 15 – 50% of the xanthohumol added remains in the beer. Yields determined in wort and beer are in some instances above those described by other authors (5,6).

Despite a 7.5 times increase in the xanthohumol used, it was possible only to triple total xanthohumol content. The content in the ethanol extract beer of 2 mg/l can be regarded as high (compare also 10).

Despite highly fluctuating iso-a-acid contents, bitterness units of worts and beers were largely comparable, this could also be confirmed by sensory analysis. In sensory terms, the beers could be differentiated without any one of the hop products used being clearly preferred or found wanting.

References

1. Stevens, J. F., Miranda, C. L., Buhler, D. L., Deinzer, M. L.: "Chemistry and biology of hop flavonoids", Journal American Chemical Society Brewing Chemists 56, 136 – 145, 1998.
2. Miranda, C. L., Yang, Y.-H., Henderson, M. C., Stevens, J. F., Santana-Rios, G., Deinzer, M. L., Buhler, D. R.: "Antimutagenic activity of prenylated flavonoids towards 2-Amino-3-Methylimidazo[4,5-F]quinolin (IQ), a carcinogenic heterocyclic amine from cooked food", Proceedings of the American Association for Cancer Research, Volume 41, 847, 2000.
3. Miranda, C. L., Stevens J. F., Ivanov, F., McCall, M., Frei, B., Deinzer, M. L., Buhler, D. R.: "Antioxidant and prooxidant actions of prenylated and nonprenylated chalcones and flavanones in vitro", J. Agricultural Food Chemistry, 48, 3876 – 3884, 2000.
4. Kammhuber, K., Zeidler, C., Seigner, E., Engelhard, B.: "Stand der Erkenntnisse zum Hopfeninhaltsstoff Xanthohumol", Brauwelt **138**, 1633 – 1636, 1998.
5. Stevens, J. F., Taylor, A. W., Clawson, J. E., Deinzer, M. L.: "Fate of xanthohumol and related prenylflavonoids from hops to beer", Journal Agricultural Food Chemistry 47, 2421 – 2428, 1999.
6. Forster, A., Köberlein, A.: "Der Verbleib von Xanthohumol aus Hopfen während der Bierbereitung", Brauwelt **138**, 1677 – 1679, 1998.
7. Henderson, M. C., Miranda C. L., Stevens, J. F., Deinzer, M. L., Buhler, D. R.: "In vitro inhibition of human P450 enzymes by prenylated flavonoids from hops, *Humulus Lupulus*", Xenobiotica 30, 235 – 251, 2000.
8. Arimoto-Kobayashi, S., Sugiyama, C., Harada, N., Takeuchi, M., Takemura, M., Hayatsu, H.: "Inhibitory effects of beer and other alcoholic beverages on mutagenesis and DNA adduct formation induced by several carcinogens", J. Agric. Food Chem. 47, 221 – 230, 1999.
9. Stevens, J. F., Taylor, A. W., Deinzer, M. L.: "Quantitative analysis of xanthohumol and related prenylflavonoides in hops and beer by liquid chromatography-tandem mass spectrometry", Journal Chromatography A 832, 97 – 107, (1999).
10. Piendl, A., Biendl, M.: "Über die physiologische Bedeutung der Polyphenole und Hopfenbitterstoffe des Bieres", Brauwelt **140**, 526/539 – 543, 2000. ■