Microbiologically Important Components in Hoppy Beers (Part 2)

COMMERCIAL-SCALE TESTS | In Part 1 of this contribution (see BRAUWELT International No. 2, 2018, pp. 101-103), microbiological activity of hop components in general was described and identification of a hop-based inhibitory power of beer presented. Part 2 discusses the relevance and significance of this inhibitory power based on commercial-scale tests.

THIS ENTAILED 15 beers being hopped in different ways, tested both microbiologically as well as analytically. They included three test beers and twelve commercial samples. In addition to five Pilsner and three wheat beers, an IPA, a stout and two non-alcoholic beers were included. If known, time of hop addition and quantities added are listed in Table 1. More than half of the beers investigated had been dry hopped.

Hop-Specific Analytical Parameters in Beer

The method Analytica-EBC 9.47 was used for a hop-specific analysis of these beers. Only UV detection was changed and carried out using the diode array detector, not only at 270 nm but also at other wavelengths, depending on analytes.

For external calibration, the HPLC standards ICE-3 (International Calibration Extract no. 3 for alpha and beta acids), ICS-I3 (International Calibration Standard for iso-alpha acids no. 3) and the dicyclohexylamine humulinone complex, pure xanthohumol and pure iso-xanthohumol were used.

When using the HPLC method, all microbiologically relevant hop components could be efficiently separated (Fig. 1). By way of example, the HPLC chromatograph of the stout is shown at a detection wavelength of 270 nm, used for calibration of the cis-/trans-co-/n-/ad-isohumulone (iso-alpha acids) and the co-/n-/ad-humulinone. Depending on the parameter in question, other detections took place at 290 nm for iso-xanthohumol, at 314 nm for co-/n-/ad-humulone and at 370 for xanthohumol (see Materials and methods).

The method EBC-Analytica-EBC 9.8 was used for determining the EBC bitterness units.

The beers investigated had considerable differences in test results (Table 2). Specific attention will be given here to dry hopping. Whereas iso-alpha acid concentrations in dry hopped beers are oftentimes much lower than EBC bitterness units, alpha-acids are remarkably high. After dry hopping, humulinone and, sometimes, xanthohumol levels are also relatively high.

Wheat beer 2, having approximately the same alpha and alpha-acid levels, has been intensely dry hopped. The ratio is even more

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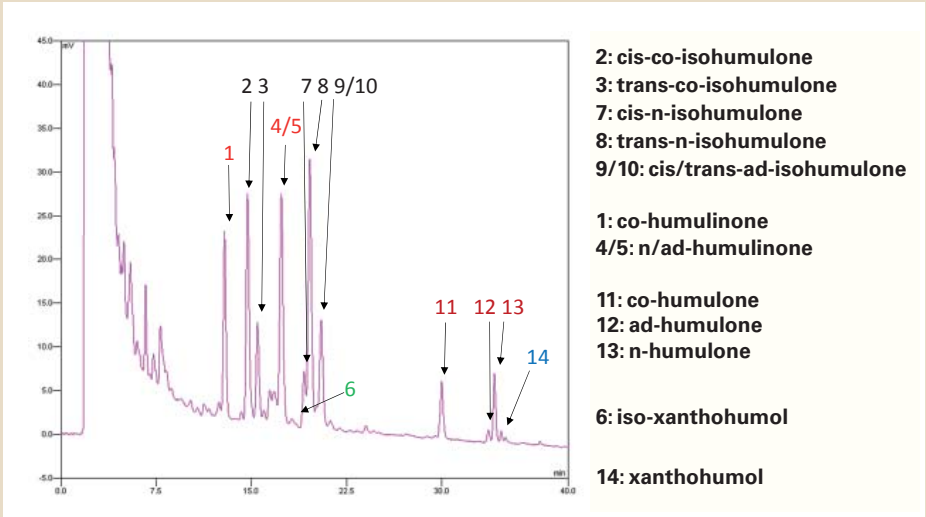

Fig. 1 HPLC chromatograph of beer (stout) in accordance with method Analytica-EBC 9.47 (detection wavelength: 270 nm)

extreme in the stout, with double the quantity of alpha-acids compared to iso-alpha acids and very high levels of humulinones and xanthohumol. Due to the carrier function of certain melanoidins originating from roasted malt, dark beers can contain significantly elevated xanthohumol levels [1]. This prenylated flavonoid has been regarded as having positive effects on health for some time, this has now been confirmed in first human studies [2].

Impact on Microbiological Inhibitory Power

Based on analytical parameters, the microbiological inhibitory power was derived for each beer tested (Table 2), based on the relative inhibitory factors, presented in Part 1, for each component (inhibitory factor 100 % = 1 mg/l of xanthohumol).

Correspondingly lower inhibitory powers of about 450-700 per cent are obtained in more weakly hopped beers with bitterness units below 30. Additional hopping in the whirlpool slightly raises alpha-acid values that may already have a positive effect on inhibitory power (e.g. Pilsner 3 compared to Pilsner 2). Dry hopped beers profit significantly in terms of microbiological inhibitory power in view of raised alpha-acid and humulinone levels. Only dry hopped beers attain inhibitory powers above 1200 per cent due to high alpha-acid, xanthohumol and humulinone concentrations. The highest value of 2780 per cent was found in dry hopped stout. As mentioned above, xanthohumol levels can be significantly higher in dark beers and thus also contribute to the inhibitory power. Dry hopping of non-alcoholic beers also raises alpha acids and humulinones and thus pushes up inhibitory powers. A comparison of test beers 1-3 is interesting. They have been produced with identical amounts of hops dosed at different points in time (Table 1). Dosing hop amounts of 9.1 g alpha per hl results in inhibitory powers between 448 and 684 per cent that have not been significantly influenced by the point in time of hop addition. Better yields achieved with higher levels of iso-alpha acids, with early hop addition in the copper compared to whirlpool or dry hopping, ultimately won the day.

Growth of Obligate Beer Spoilers

Growth tests were based on the method described in Part 1. Representative strains of

HOP ADDITION FOR PREPARING VARIOUS BEERS

Beer type	Point in time of hop addition: x or g/hl, proportion in % (if known)		
	before/after wort boiling	Whirlpool (WP)	Dry hopping (DH)
Test beer 1	9.1 g Alpha		
Test beer 2 WP		9.1 g Alpha	
Test beer 3 DH			9.1 g Alpha
Pilsner 1	x		
Pilsner 2	x		
Pilsner 3 WP	8.5 g Alpha	x	
Pilsner 4 DH	8.5 g Alpha		250 g
Pilsner 5 WP, DH	14 g Alpha	x	250 g
Non-alcoholic 1	x		
Non-alcoholic 2 DH	x		x
Wheat beer 1 WP	50 %	50 %	
Wheat beer 2 DH	18 %	82 %	
Wheat beer 3 DH	30 %	70 %	
IPA WP, DH	32 %	30 %	38 %
Stout WP, DH	36 %	12 %	51 %

Table 1

ANALYTICAL COMPOSITION AND DERIVED MICROBIOLOGICAL INHIBITORY POWER ...

... of various beers produced with different amounts of hops (seeTable 1)

Beer type	EBC BU	iso-alpha mg/l	alpha mg/l	XN mg/l	IX mg/l	HUM mg/l	microbiological inhibition (%)
Test beer 1	25.4	22.1	1.1	< 0.1	0.9	1.3	684
Test beer 2 WP	16.3	10.2	2.1	< 0.1	0.6	1.7	448
Test beer 3 DH	12.2	1.7	5.1	0.1	0.1	2.7	464
Pilsner 1	26.4	23.3	0.2	n.n.	0.3	1.9	627
Pilsner 2	33.3	29.4	0.7	n.n.	0.4	2.5	794
Pilsner 3 WP	34.4	29.3	4.7	0.2	0.9	1.3	1126
Pilsner 4 DH	41.7	25.9	5.6	0.2	0.5	7.6	1222
Pilsner 5 WP, DH	53.3	34.9	5.5	0.3	0.9	10.3	1512
Non-alcoholic 1	27.6	27.7	1.1	< 0.1	0.1	n.n.	772
Non-alcoholic 2 DH	30.7	25.4	3.6	< 0.1	0.2	2.7	980
Wheat beer 1 WP	24.2	15.8	2.3	0.1	0.5	3.0	636
Wheat beer 2 DH	22.8	9.2	8.9	0.6	0.3	2.9	977
Wheat beer 3 DH	36.8	17.2	11.6	0.7	0.8	4.6	1404
IPA WP, DH	60.7	36.3	14.1	0.5	1.1	11.0	2172
Stout WP, DH	45.8	12.5	24.0	6.1	1.1	7.8	2780

Table 2

GROWTH OF OBLIGATE BEER SPOILERS IN BEERS HAVING DIFFERENT MICROBIOLOGICAL INHIBITORY POWERS

Beer type	EBC BU	% microbiological inhibitory power	Growth*			Remark	
			L. brevis 986	*P. damnosus 60*	*L. Lindneri 2*	pH	Alcohol %
Test beer 1	25.4	684	++	+	+		
Test beer 2 WP	16.3	448	+	+	+		
Test beer 3 DH	12.2	464	++	++	+		
Pilsner 1	26.4	627	++	–	s	4.2	5.6
Pilsner 2	33.3	794	s	–	s	4.2	5.6
Pilsner 3 WP	34.4	1126	–	–	–		
Pilsner 4 DH	41.7	1222	–	–	–		
Pilsner 5 WP, DH	53.3	1512	–	–	–		
Non-alcoholic 1	27.6	772	++	s	s	4.1	< 0.5
Non-alcoholic 2 DH	30.7	980	s	–	s	4.1	< 0.5
Wheat beer 1 WP	24.2	636	s	s	s		5.2
Wheat beer 2 DH	22.8	977	+	–	–		4.6
Wheat beer 3 DH	36.8	1404	–	–	–		8.2
IPA WP, DH	60.7	2172	–	–	–		8.2
Stout WP, DH	45.8	2780	–	–	–		6.5

*++ very strong, + strong, s weak/slow, – no growth

Table 3

the three most frequent types of obligate beer-spoilage bacteria, *Lactobacillus brevis* (L 986), *Pediococcus damnosus* (P 60) and *Lactobacillus lindneri* (L 2), were selected. They had been isolated from spoiled beers. In order to maintain beer spoilage properties and yeast tolerance, the strains were kept in a "beer culture" (EBC bitterness units = 23) at all times.

Assessment was based on the criteria "very strong", "strong", weak/slow" or "no growth" of these bacteria within 30 days at 25 °C for the 15 beers selected.

The three obligate beer spoilers exhibited different growths in line with the inhibitory powers determined for the original beers (Table 3). When comparing Pilsner beers 1 and 2 from the same brewery, the higher hopping rate resulted in a noticeable inhibition of *L. brevis*. Microbiological stability was further improved by whirlpool hopping (comparison between Pilsner beers 2 and 3 with similar bitterness units). The two non-alcoholic beers differed in terms of additional dry hopping, resulting in higher inhibitory power and significant growth of *L. brevis* and *P. damnosus*. It should be noted that the low pH certainly also contributed to inhibition.

Compared to wheat beer 1 with 24.2 bitterness units and an inhibitory power of 636 per cent, wheat beer 2, having just 22.8 bitterness units, has a clearly higher inhibitory power of 977 per cent. This goes to explain the complete inhibition of *P. damnosus* and *L. lindneri* and is due to predominantly dry hopping and the resulting considerably elevated alpha-acid levels that have a significantly higher inhibitory power than iso-alpha acids. It also shows that the EBC bitterness units do not necessarily coincide with actual microbiological inhibition. Wheat beer 3, produced with a relatively high amount of hops and combined with dry hopping and an inhibitory power of 1404 per cent, completely inhibited *L. brevis*, *P. damnosus* and *L. lindneri*.

With high bitterness units combined with dry hopping, inhibitory powers were always considerably above 1000 per cent, resulting in complete inhibition of all three test strains in all tests. However, when inhibitory powers were below 700 per cent, vigorous growth of these microorganisms was noted in most instances, levelling off only as of about 30 bitterness units.

Conclusions from Microbiological Tests

In order to have an effect on obligate beer spoilers, a calculated inhibitory power of more than 700 is required. With inhibitory powers between 700 and 1000 per cent, their growth is considerably weakened and the influence of other beer-specific inhibitors (pH, alcohol) plays a particularly important role here. Once inhibitory power is above 1000 per cent, e.g. as a result of additional whirlpool hopping, growth of only particularly hop resistant beer spoilers should be expected. Thus, an inhibitory power of least 1000 per cent and a pH of maximum 4.1 are desirable for non-alcoholic beers. Dry hopping can raise inhibitory powers to significantly more than 1500 per cent that would suppress any growth. In dark beers produced with roasted malt, melanoidins have a carrier function for the weakly soluble xanthohumol. Thus, inhibitory power is highest.

Summary

Specific inhibitory action exhibited by individual hop components acting against obligate beer spoilers was determined and the resulting hop-related inhibitory power defined. It can be calculated and it is thus possible to predict the susceptibility to infections of beers. Based on microbiological tests of various beers with different compositions, the validity of calculated inhibitory power was verified. It was found that microbiological inhibitory power does not always correlate with EBC bitterness units. Hop components have different effects and can be influenced by brewing technology. Dry hopped beers contain higher proportions of alpha acids, humulinones and, in some instances, xanthohumol resulting in significantly improved microbiological stability. ■

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