Microbiologically Important Components in Hoppy Beers (Part 1)

HOP-BASED INHIBITORY POWER | Hops as raw material contribute significantly to microbiological stability of beer. In the present contribution, determination of a so-called hop-based inhibitory power is described in order to assess the antimicrobial potential of beer. This will be verified in Part 2 based on commercial-scale tests.

HOPS WERE USED on various occasions already in the 8th century AD e.g. in must, wine or sourdough. In 715, St. Emmeram recognised the preservative effect of hops. It is reported that there was a hop garden in Geisenheim in 736 and hops were cultivated in the Hochstift Freising in 768. However, in the Chronicle of Spalt, use of hops in beer is mentioned – verifiably – only in 1030, with the addendum that this was supposed to have been a very good and agreeable drop.

Biological Stability

Prior to Linde having invented the refrigeration machine (1876), hopping of beers played a significant role in storing them for a longer period, especially in summer months. The different biological stabilities can be observed right up to the present day as a function of hop content and/or bitter values.

But hops are not the only beer protectant. The low pH (about 4.5), the anaerobic atmosphere (CO_2 content 4.5-6 g/l and simultaneous lack of oxygen of 0.05-0.5 g/l), alcohol content (about 5 %) and lack of utilisable nutrient and growth substances (e.g. monosaccharides, amino acids) resulting from prior yeast fermentation are responsible for the fact that only a few, very specialised microorganisms can grow in the selective beer medium [1, 2].

It is worthwhile pointing out that neither pathogens nor heat-resistant organisms (endosperm-forming organisms) can propagate in beer. The latter criterion is of major technological and qualitative importance as beer can thus be pasteurised at moderate temperatures (65-75 °C) that do not damage the product. In contrast, wort is a so-called collective medium in which many microorganisms find suitable conditions for nutrition and growth [3, 4].

Previous studies suggested that the five selective criteria in beer account for approximately the percentages of inhibitors of potential beer spoilers as shown in Table 1. Accordingly, hops contribute significantly to biological stability of beer. However, the question is very interesting to what extent the high level of hopping, currently often common also in view of hopping in the whirlpool or in the cold section (e.g. for dry hopped craft beers), influences biological stability of resulting beers.

Appropriate test strains have to be selected so as to provide informative results. Therefore, obligate beer-spoiling bacteria that often cause biological problems are particularly suitable [4, 5]. These are the ten most frequent species that can multiply in common beers in accordance with Table 2.

So-called potential beer spoilers (e.g. *L. plantarum*) have limited tolerance to the five selective features of beer (and also to hops in most instances) and are unsuitable for hop resistance tests.

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SELECTIVE CRITERIA FOR ANTIMICROBIAL ACTIVITY IN BEER

Component	Percentage selectivity
Hops	30
Alcohol	10
Anaerobic atmosphere	20
Low pH	25
Lack of nutrient and growth substances	15

Table 1

Independent of growth of obligate and, in some cases, also potential beer spoilers in more or less strongly hopped beers, it is also worth noting that a very varied microflora exists on hops harvested and on various hop products (e.g. pellets). These are ubiquitous microorganisms that may be found everywhere, also e.g. on raw materials for brewing. Conidiospores of mould fungi, various yeast types, enterobacteriaceae as well as bacillus and clostridium types forming endospores, micrococci, enterococci and others are frequently encountered. These microorganisms are of no consequence unless moisture goes up. Leaving hopped wort or premixed hop solution stand for an extended period (>6h) at temperatures below 55 °C, as sometimes was done during casting wort on coolships in the past, is e.g. problematic. These bacteria that are mostly gram-negative are often very tolerant to hops, they can propagate very fast in these substrates and form off-flavours and toxins. Such problems can, however, be eliminated by proper drying or further processing.

OBLIGATE BEER SPOILERS

Genus	*Lactobacillus*	*Pediococcus*	*Pectinatus*	*Megasphaera*
Type	*brevis* *backi* *lindneri* *(para-)collinoides* *paucivorans* *(brevisimilis)*	*damnosus*	*cerevisiiphilus* *frisingensis* *haikarae*	*cerevisiae*

Table 2

MICROBIOLOGICALLY RELEVANT COMPONENTS IN HOPS AND BEER*

*(incl. dry hopped beers)

	Bitter hops (%)	Aroma hops (%)	Beer (mg/l)**
Alpha acids (alpha)	10 - 20	2 - 10	0,2 - 20
Beta acids (beta)	4 - 8	4 - 7	n.n. - 1
Iso-alpha acids (iso-alpha)	< 0,1	< 0,1	15 - 50
Humulinone (HUM)	0,3 - 0,5	0,1 - 0,3	n.n. - 20
Polyphenols (POLY)	3 - 4	4 - 6	150 - 300
Xanthohumol (XN)	0,5 - 1,0	0,3 - 0,5	n.n. - 6
Isoxanthohumol (IX)	< 0,1	< 0,1	n.n. - 2
Essential hop oil (EHO)	1,5 - 3	0,6 - 1,5	n.n. - 2

Table 3 ***n.n.: untraceable*

Microbiologically Relevant Components in Hops and Beer

Previous investigations have reported that alpha and beta-acids, in particular, as well as xanthohumol are microbiologically relevant components of hops. Polyphenols, humulinones and hop oils have also some antimicrobial effects. According to Table 3, particularly significant differences between bitter and aroma hops exist in terms of alpha acids. As far as xanthohumol and the humulinones are concerned, values in bitter hops are, on average, also slightly higher whereas polyphenols are present to a lesser extent.

Hardly any iso-alpha acids are present in cone hops. Important isomerisation of alpha acids takes place in the brewhouse during boiling. Iso-alpha acids make a dominant contribution to the bitter value (=100 %) of beer. In comparison, humulinones, with a bitter value of 66 per cent, also have some effect on beer taste [6] whereas the bitter value of alpha acids amounts to only about ten per cent. Humulinones are almost quantitatively transferred during brewing on account of their high polarity and, therefore, concentration increases most when intensive dry hopping is carried out.

Microbiologically relevant components in beer are quite different compared to hops (Table 3). In beer, iso-alpha acids amounting to about 15-50 mg/l are dominant while only low concentrations of alpha acids are found in most beers. This also applies likewise to xanthohumol because both alpha acids and xanthohumol are not just isomerised during wort boiling but are excreted to a large extent during brewing on account of their poor solubility. This loss even affects nonpolar beta acids to a still greater degree. Elevated values of alpha acids and xanthohumol are obtained during hop addition in the whirlpool and, in particular, during dry hopping. Even humulinones (usually below 2 mg/l) can have higher concentrations (clearly above 10 mg/l). However, nonpolar beta acids are mostly untraceable in beer, even after dry hopping.

Larger quantities of polyphenols are present, 150-300 mg/l, but stabilisation procedures during beer filtration have a strong influence on levels. Moreover, a major proportion of total polyphenols in beer originates from malt. Levels of isoxanthohumol, formed from hop xanthohumol during wort boiling, are relatively low, below two mg/l, compared to the level of total polyphenols. Concentrations of hop oil components in beer are even significantly lower though their aroma can express itself when present only in trace concentrations.

Determining Relative Microbiological Inhibitory Powers

As mentioned, the inhibitory power of iso-alpha acids, alpha acids and xanthohumol in beer was investigated in previous studies. Original isolates from beer were used as test organisms for these microbiological investigations. Their strain maintenance took place in pale standard vollbier (15 EBC bitterness units, pH 4.6, alcohol 4.5 % v/v, CO_2 4.5 g/l, apparent attenuation 78 %) (inoculum one drop to about 20 ml test medium using a Pasteur pipette in reaction tubes filled to the brim.

Three strains of *Lactobacillus brevis, L. lindneri, Pediococcus damnosus* each, one strain of *L. casei, Pediococcus inopinatus, Lactococcus lactis, Mircococcus kristinae, Pantoea agglomerans* each, one strain of *Pectinatus cerevisiiphilus, Pect. frisingensis, Megasphaera cerevisiae* each were tested).

Tests were carried out with xanthohumol additions of 1, 2.5 and 5 mg/l to standard

RELATIVE INHIBITORY POWERS OF MICROBIOLOGICALLY RELEVANT COMPONENTS IN BEER

	% Microbiological inhibitory power (XN=100%)	Average pale vollbier (mg/l)	% Inhibitory power per litre of beer (1 mg/l XN=100%)
Alpha	70	1	70
Beta	ca. 70	< 0,1	< 10
Iso-Alpha	25	20	500
HUM	ca. 20	1	20
POLY	ca. 0,5	150	75
XN	100	0,2	20
IX	20	1	20
EHO	20	< 0,05	< 1

Table 4

vollbier (EBC BU=15). Moreover, three different concentrations of alpha acids (2, 4, 10 mg/l) and iso-alpha acids (10, 20, 30 mg/l) were tested. The respective levels of alpha and iso-alpha acids were adjusted by mixing appropriate base beers and, if required, unhopped beer. A differentiation was made in the assessments between positive and no growth within 30 days at 25 °C. Following evaluation of the growth test, the average percentage inhibitory power was based on 1 mg of xanthohumol. Accordingly, the inhibitory power of 1 mg of xanthohumol was, on average, just as strong as that of about 1.5 mg of alpha acids or that of about 4 mg of iso-alpha acids (test results, cf. Back, Doctoral Thesis 1974, Postdoctoral Thesis 1980; Back: Screening Test in the context of the development of NBB detection media 1976, deposited at Döhler, Darmstadt, Germany).

When looking at comparable concentrations, xanthohumol has the highest inhibitory power, classified at 100 per cent as a reference value (Table 4). Compared to xanthohumol, alpha acids are about 70 per cent and, accordingly, also have a major inhibitory power whereas iso-alpha acids have an inhibitory power of just 25 per cent. But as significantly higher concentrations of these are found in beer (15-50 mg/l), they still contribute to microbiological stability. Beta acids should behave similarly to alpha acids.

Neither are systematic test results available for humulinones, polyphenols, iso-xanthohumol and hop oils. They are empirical values or estimates as, resulting from combination effects (enhancer effects), it is not possible to reach clear conclusions.

Hop-Based Microbiological Inhibitory Power of Beer

The proportionate inhibitory powers for an average pale vollbier are derived in Table 4 by way of example. The main effect comes as a result of dominance of 20 mg/l of iso-alpha acids with an inhibitory power of 500 per cent. 1 mg/l of alpha acids and 170 mg/l of polyphenols account together for an additional inhibitory power of 145 per cent. Low levels of xanthohumol, isoxanthohumol and humuliones are present, each contributing an inhibitory power of just 20 per cent. The overall resulting inhibitory power amounts to about 700 per cent, this is not sufficient for satisfactory inhibition of obligate beer spoilers from hops. In order to verify the relevance and significance of this microbiological inhibitory power derived from analytical values, 15 beers produced with different hop additions were examined microbiologically as well as using HPLC. Beta-acids and hop oils were not analysed as only very low concentrations of these hop components are present in beer. Polyphenols were also ignored as they can be introduced by hops and also by malt. Part 2 of this publication (BRAUWELT International No. 3, 2018) will report on results from the microbiological and analytical examination of these 15 beers. ■

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