Beer ageing: how stable are hop bitter substances?

HOP ADDITION | A number of studies are available on the behaviour of hop bitter substances during beer ageing and potential sensory repercussions; a survey can be found in "Hops – from Cultivation all the way to Beer" [1]. None of these studies tried to address the question whether changes in bitter substances differ between exclusively hot hopped beers and dry hopped beers. To investigate this question, a bottom-fermented test beer was late hopped and, in addition, part of the test beer was dry hopped. The beers were aged systematically and analysed.

IN THE COURSE OF THE TESTS, the two test beers were designated as "late" (only late hopped) and "dry" (late hopped and additionally dry hopped). The original study has been published in BrewingScience volume 73 [2].

The main characteristics of the two beers brewed in the 2 hl research brewery in St. Johann are briefly summarised below:

- 100 % Pilsner malt, infusion mashing process (52/64/72/78 °C); lauter tun; wort clarification in the whirlpool;
- hopping using pellets from the 2016 harvest; 5 g/hl Herkules, pellets type 90 added at the beginning of boiling; 5 g/hl Hallertauer Tradition; pellets type 90 added during mid-time of boiling; 200 g/hl Hallertauer Blanc, pellets type 45 added to the whirlpool (corresponding to 400 g/hl pellets type 90);
- fermentation of the wort (12 % original gravity) with bottom-fermenting yeast (W34/70) at 9 °C;
- separating the green beer after main fermentation into two tanks; one tank conditioned without dry hopping and stored (= late); the other tank was dry hopped with 200 g/hl Hallertauer Blanc type 45 (analogous to whirlpool hopping) (= dry);
- conditioning for 7 days at 15 °C, storage for two weeks at 0 to 1 °C;
- filtration through kieselguhr filter and two membrane filters (pore size 1.2 and 0.45 µm);
- filling into 0.5 l Euro bottles after two pre-evacuations;
- ageing at 4 °C and 22 °C (in line with storage temperature e.g. in a beverage market).

Bitter substances were analysed after 3, 6, 12, 18 and 24 months; bitterness units (IBU) according to EBC 9.8; iso-α-acids and α-acids, humulinones, co-lupulone, xanthohumol and iso-xanthohumol using a modified HPLC method according to EBC 9.47 and a diode array detector measuring at different wave lengths.

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Analysis data

Table 1 lists the absolute measurement values of the beers prior to ageing. When dry hopped, the values for bitterness units, α-acids, humulinones, co-hulupone and xanthohumol go up while iso-α-acids, due to trub separation after dry hopping, drop slightly.

Absolute measurement results after ageing of the beers are converted to % rel. of the starting value as, in this way, components can be compared directly with each other.

ABSOLUTE VALUES OF BITTER SUBSTANCES ...

... in fresh late hopped and additionally dry hopped beer including the standard deviation of analyses in triplicate; values in mg/l (except for bitterness units = IBU)

	late	dry	s
bitterness units	33.2	34.6	0.45
iso-α-acids	31.6	27.6	0.26
cis-Iso-α-acids	21.8	19.0	0.22
trans-Iso-α-acids	9.8	8.6	0.15
α-acids	3.8	5.4	0.18
humulinones	5.0	8.0	0.20
co-hulupone	0.7	0.9	0.08
xanthohumol	0.13	0.25	0.02
iso-xanthohumol	0.54	0.53	0.03

Table 1

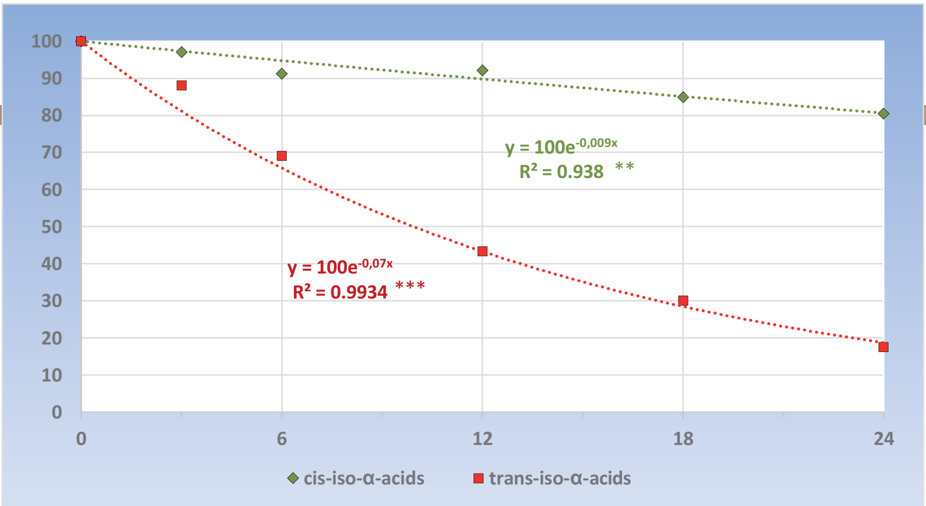

Fig. 1 Slope of decrease in measurement values in % rel. of the value of fresh beer before ageing at 22 °C for two years in the case of cis- and trans-iso-α-acids

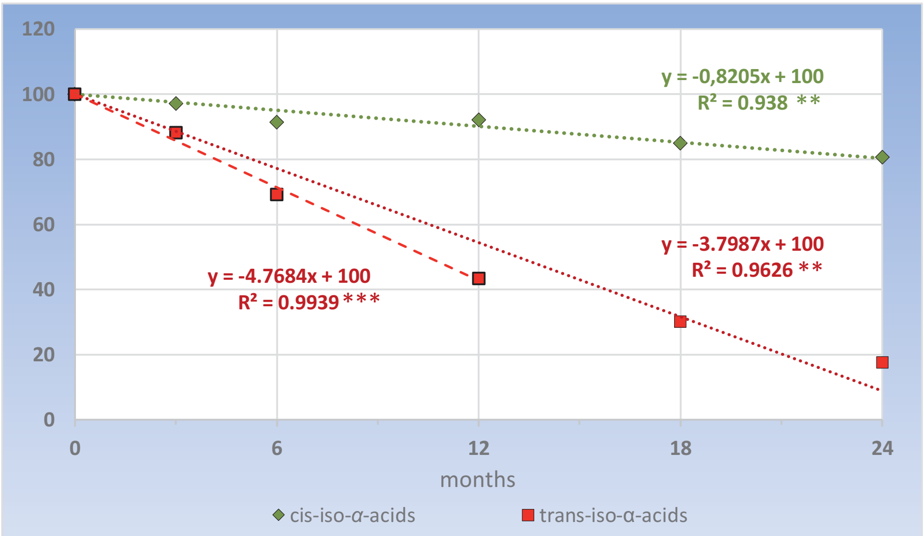

Fig. 2 Assumption of a linear slope for the two iso-α-acids – for the trans-iso-α-acids in the course of one year, for the cis-iso-α-acids in the course of two years – including the associated coefficients of determination and significancies

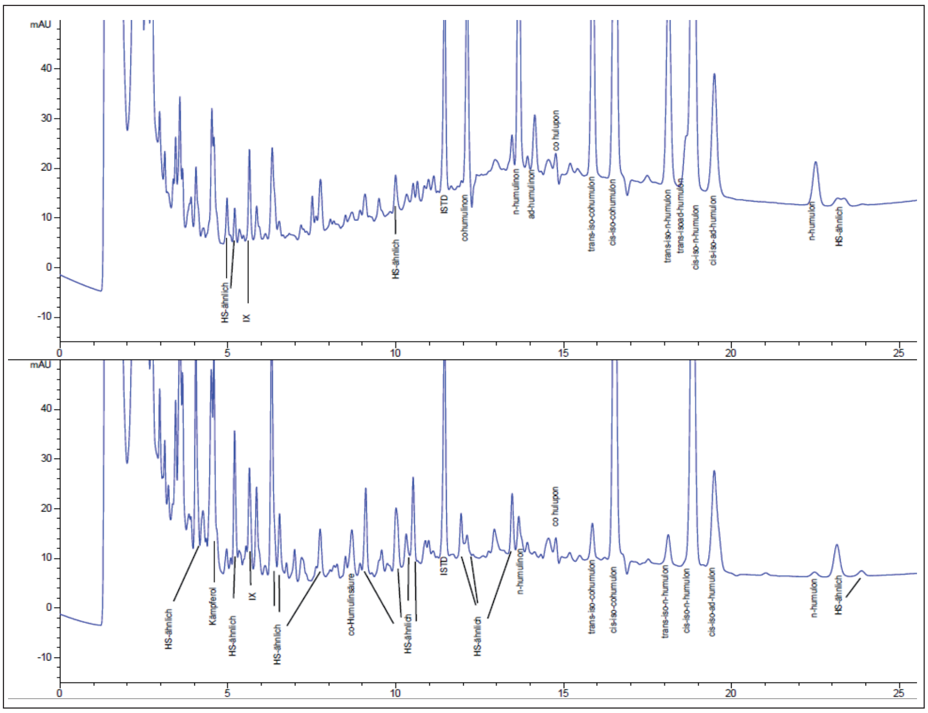

Fig. 3 HPLC chromatogram of a beer hardly aged at 4 °C and a beer stored at 22 °C after two years

Basically, the changes in measurement values follow a function of the first order; this is obvious when looking at the iso-α-acid slope in fig. 1.

Trans-iso-α-acids are significantly less stable than the cis-forms and the exponential character of the curve can be clearly seen. However, all components follow an almost linear slope up until a storage time of twelve months; this is shown in fig. 2. The coefficient of determination R^2 is just marginally inferior in the case of a linear drop compared to an exponential curve.

Table 2 lists the decreases in measurement values in per cent of all substances after storage for one year at 22 °C in order to simplify the comparison. As only slight losses of bitter substances were noted after two years at 4 °C, this data is not shown here.

Conclusions

The following conclusions can be drawn from such data:

- Bitter substances react moderately to ageing. As a result of breakdown of iso-α-acids, substances are formed which also absorb light when measuring the bitterness units at 275 nm, the wavelength used for the IBU measurement. This partly compensates for the loss of iso-α-acids.
- The significantly higher instability of trans-iso-α-acids is due to a trans-specific conversion to cyclic breakdown products and thus confirms results published.
- α-acids are particularly unstable. This test setup was not able to demonstrate whether isomerisation sets in.
- Humulinones are similarly unstable as trans-iso-α-acids.
- Losses in co-hulupones are modest.
- As far as the substances listed here are concerned, losses in all late hopped beers were always higher than in beers that were also dry hopped.
- During storage, xanthohumol decreases significantly but this goes mainly pari passu with an increase in iso-xanthohumol. Isomerisation can be assumed.

Fig. 3 is a comparison of the HPLC chromatograms of a dry hopped beer stored at 4 °C and at 22 °C for two years. Losses are significantly greater at 22 °C. More intensive formation of new components can be noted at the higher temperature. These aged bitter substance components could not be identified by MS but have UV spectrums similar to those of humulinic acids.

Comparison late and dry beers

The phenomenon of greater stability of most measurement values in dry hopped beer has to be investigated in more detail. Fig. 4 shows the more pronounced drop of measurement value in % rel. within a period of 12 months at 22 °C in late hopped beer compared to dry hopped beer.

This is particularly pronounced in cis-iso-α-acids. The authors have not yet arrived at a clear explanation for the phenomenon of higher stability of bitter substances in dry hopped beer. However, two considerations might apply.

During dry hopping, α-acids are again "post-dosed", about 4–10 per cent of these are soluble and thus supply "replenishment" for partial isomerisation. The loss in iso-α-acids thus appears to be lower.

Dry hopping does not just give rise to dosage of aroma and bitter substances. A multitude of other substances are also dissolved e.g. polyphenols. This causes a "more stable" environment with e.g. a higher antioxidative potential. This "more complex" beer can reduce e.g. oxygen that diffuses through the crown cork. Ageing reactions can thus be slowed down.

Summary

A pale lager beer late hopped in the whirlpool (400 g/hl hop equivalent) was compared in terms of ageing to a beer additionally dry hopped with the same quantity. The beers were stored for a period of two years at 4 °C and at 22 °C and the bitter substances were analysed. The beers were almost stable at 4 °C. The following conclusions are deduced from the tests at 22 °C: significant changes in the spectrum of bitter substances are generally noted. The lowest losses are noted for co-hulupone, followed by cis-iso-α-acids, bitterness units, humulinones and trans-iso-α-acids and the highest loss for α-acids. α-acids are e.g. almost 10 times more sensitive than cis-Iso-α-acids. Trans-iso-α-acids show a 6 to 8 times stronger reaction than the cis-forms.

Apart from the losses described, ageing also supports formation of bitter substance components as evidenced by a comparison of the HPLC chromatograms. Most of the bitter components analysed were more stable in the dry hopped beer than in the only late hopped beer. This was particularly evident when looking at the cis-α-acids. It could not be unequivocally determined whether this phenomenon can be attributed to the fact that, during dry hopping, other substances also pass into the beer.

Dosage of reducing polyphenols and other substances could assist in establishing a "more stable environment" with a higher antioxidative potential. Ageing causes a significant decrease in xanthohumol, the major part of which is isomerised to iso-xanthohumol. ■

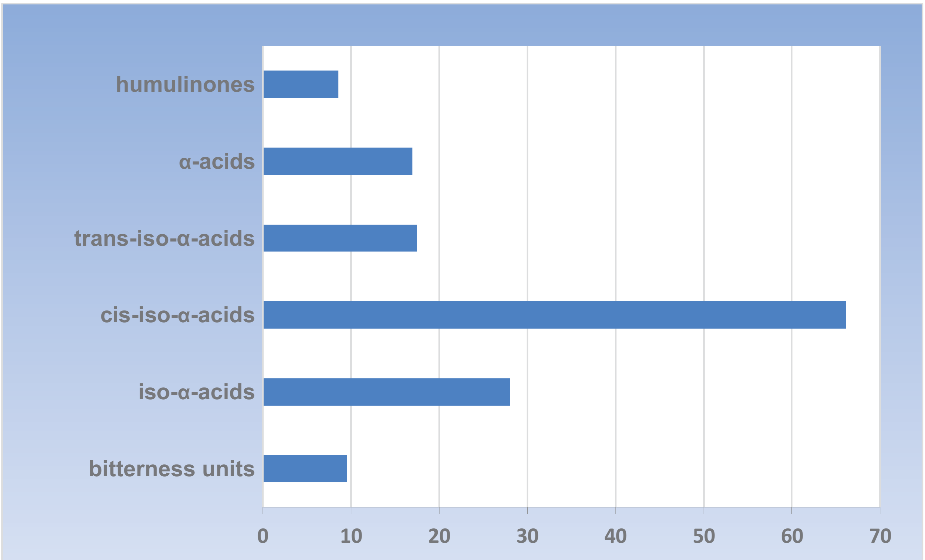

Fig. 4 Difference in per cent between breakdown in late hopped and dry hopped beer

LOSSES AND/OR INCREASES IN BITTER SUBSTANCES …

… in % rel. during storage of beers at 22 °C for a period of 12 months

	late	dry
bitterness units	–14.9	–13.6
iso-α-acids	–21.0	–16.4
cis-iso-α-acids	–9.8	–5.9
trans-iso-α-acids	–57.2	–48.7
α-acids	–77.3	–66.1
humulinones	–49.4	–45.5
co-hulupone	–19.7	–8.9
xanthohumol	–76.9	–80.0
iso-xanthohumol	+18.5	+35.8

Table 2

References

1. Biendl, M.; Engelhard, B.; Forster, A.; Gahr, A.; Lutz, A.; Mitter, W.; Schmidt, R.; Schönberger, C.: "Hopfen – Vom Anbau bis zum Bier"; Fachverlag Hans Carl, Nuremberg, 2012, pp. 61–65 and pp. 253–257.
2. Gahr, A.; Forster, A.; Schüll, F.; Faltermaier, S.; Kellerer, F.: "The Stability of Bitter Substances in Beer During the Ageing Process"; BrewingScience, vol. 73, 2020, pp. 149–157.