Behaviour of linalool during beer staling

HOP AROMA | Hop aromatic beers have been around forever and a day. Linalool is frequently referred to as an indicator substance for sensory impression [1-4]. The rediscovery of dry hopping resulted in many hoppy, palateful beers coming on the market. But what about stability of hop aroma? Opinions voiced in the literature differ. This contribution analyses the behaviour of linalool in different beers during ageing at 20 and 30 °C based on many sample withdrawals. A sensory assessment was not part of the analyses to start with.

THE FAST-PACED DEVELOPMENT of craft brewing, with the rediscovery of dry hopping, resulted in a multiplicity of hop aromatic beers coming on the market, in particular pale ales and India pale ales. Taste stability of these palateful, often high-alcohol and hoppy beers is usually better than that of more weakly hopped pale lager beers. In the course of beer staling, sensory impressions originating from hops change, leading to questions about the stability of hop aroma in beer.

Investigations carried out were limited to the behaviour of linalool as the key aroma substance in staling of beer. Depending of hop type, linalool levels usually range from 4 to 12 mg/100 g [1]. As a function of the boiling system, transfer rates of linalool are between about 20 and 60 per cent relative to late hop addition into the wort copper and/or the whirlpool [1-4]. Dry hopping achieves transfer rates of 40 to almost 100 per cent, depending on technology [5, 6]. With late hopping, about 20 to 100 µg/l of linalool would be expected in beer. Examples indicate that even more than 500 µg/l were transferred to dry hopped beers [7].

Linalool is comprised of two enantiomers, an R and an S form. Sensory intensity of R linalool having an approximate threshold value of 10 to 20 µg/l of beer is considerably higher than that of S linalool at about 200 µg/l. *Fritsch* et al. mention a aroma threshold value of 2.2 for R linalool and of 170 µg/l for S linalool [8]. The share of R linalool in fresh hops is about 90 per cent, a ration wghich is the same in beers brewed with fresh hops. Aged hops has increasing S and decreasing R shares, resulting in lower R linalool values and a lower sensory intensity in beers [1, 2].

Contradictory data about the behaviour of linalool during beer staling is found in the literature. *Tressl* et al. [9] reported of a total loss (96 %) for moderate beer staling at 4 °C over a period of eight months. However, *Peacock* et al. [10] found a decrease in linalool of 11 per cent after beer storage at 20 °C for 57 days. *Kaltner* et al. [11] did not detect any linalool losses after beer storage for one year at 20 °C. Ageing simply resulted in a moderate shift from R to S linalool: the R share dropped from 72 to 64 per cent.

Qian [12] recently reportet about conversions of linalool during beer ageing. In

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TEST SERIES AND NUMBER OF RESPECTIVE BEERS, STORAGE TEMPERATURES AND DAYS ...

... and number of measurements

Serie	Beers	Ageing Temperatures [°C]	Storage time [days]	Number of measurements
1	5	20	700	5
2	16	20	420	16
3	6	20	79, 140	12
4	4	20	28, 56, 84, 112	16
		30	21, 42, 63, 84, 105	20
5	3	20	28, 105, 112	9
		30	21, 42, 63, 84, 105	15
6	4	20	56, 84	8
Total	38			101

Table 1

addition to racemisation from R to S linalool, he observed a conversion to S and R alpha terpineol as well as to nerol.

The investigations described here show the staling behaviour of linalool in different beers and in many samples withdrawn during ageing at 20 and 30 °C. R and S linalool were separated using gas chromatography. At this time, sensory evaluation was not carried out, the focus was on analysis. Results have been published in detail in [13].

38 test beers were exactly defined

The hop varieties listed below were used for the test beers:

- HA = Hallertauer mittelfrueh;
- SR = Saphir;
- HT = Hallertauer Tradition;
- TU = Taurus;
- SP = Spalter from Spalt;
- HE = Hersbrucker;
- MB = Mandarina Bavaria;
- HC = Hallertau Blanc;
- HN = Huell Melon;
- PA = Polaris;
- CA = Cascade (Hallertau and Yakima).

Hops came from the Hallertau unless otherwise indicated.

All beers investigated were produced in the 2 hl research brewery of Hopfenveredlung St. Johann. With the exception of three, all beers were bottom-fermented lagers hopped with different varieties and quantities. All had the same characteristics:

- 90 per cent Pilsner malt, 10 per cent Carahell®;
- infusion method:
- internal boiler, boiling time 70 min;
- hopping at the beginning of boiling, mid-time of boiling; late hopping divided into 50 per cent at the end of boiling (EB) and 50 percent into the whirlpool (WP);
- main fermentation with W 34/70 at 8 °C, about one week;
- conditioning at 14 °C, 1 week;
- storage at 0 °C, 2 weeks;
- kieselguhr and membrane filtration;
- filling in bottles with double pre-evacuation using scavenger crown corks.

For dry hopping, pellets went into the storage tank during change of tank after main fermentation. This results in a contact time of one week at 14 °C and two weeks at 0 °C. A number of test series were run:

- Series 1 in accordance with [6]: late hopped reference beer (HT, HA) and, additionally, dry hopped with MB, HN, HC and PA (5 beers);
- series 2: comparison of late (EB and WP) and additionally dry hopped beers with HC, MB, HN, PA, HA, SR and CA from the US and the Hallertau (8 late and 8 additionally dry hopped beers = 16 beers);
- series 3: beer without hopping at the beginning of boiling; hopping at mid-time + and end of boiling with HT and MB, only at end of boiling with HT and SR and dry hopped only with TU and HC (six beers);
- series 4: comparison of two types of late hopped and, additionally, dry hopped: PA and Hüll breeding line 89/002/025 (4 beers);
- series 5: beer with oil additions from TU; addition only at end of boiling as well as end of boiling + dry hopped (3 beers);
- series 6: exceptional beers according to [7], all late and dry hopped:
  – light ale: late hopped with SR and HA; dry hopped with CA, PA, HC;
  – IPA: late hopped with SR; dry hopped with HC, PA;
  – light wheat beer: late hopped with SR, MB; dry hopped with MB;
  – bottom-fermented festival beer, late hopped with HA, HE, SR and SP; dry hopped with HC, PA, MB, HN.

Table 1 shows the temperatures and the number of storage days for the particular series up until sample withdrawal. Stored at two temperatures and investigating 38 beers, 101 samplings were analysed with measured values for R and S linalool in accordance with the particular storage times.

Analysis of R and S linalool

Aroma substances from wort and beer are

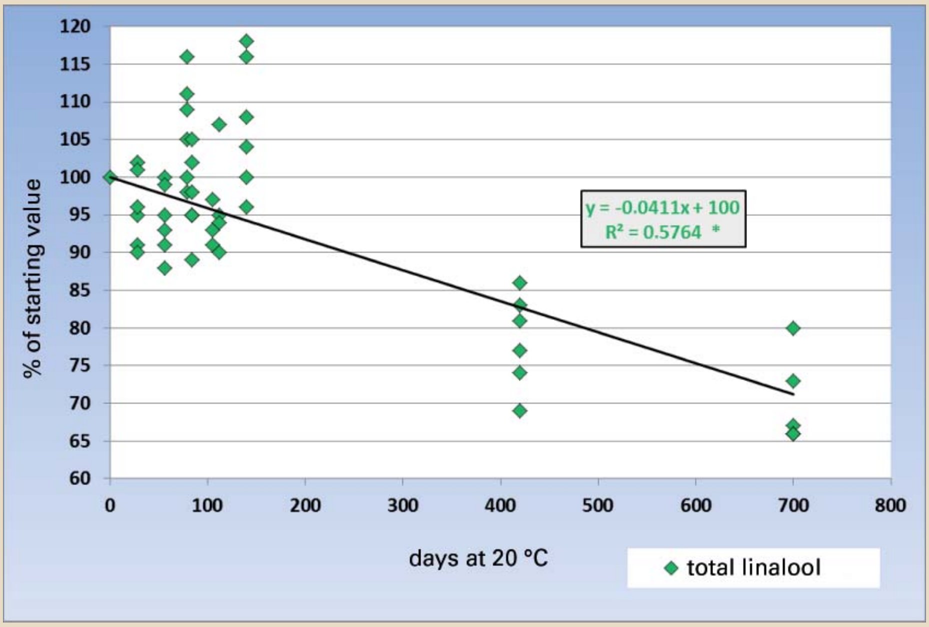

Fig. 1 Curve of averaged changes in measured values after 6 series after storage for 700 days at 20 °C; values in % rel. from starting value

R AND S LINALOOL VALUES IN FRESH BEERS OF THE VARIOUS SERIES ...

... and their ranges of variation (values in µg/l); R share in % rel.

Series	R linalool	S linalool	R share
1	45-123	4-9	92-94
2	44-112	4-13	90-92
3	50-666	5-35	91-95
4	36-342	6-20	86-94
5	66-254	5-16	93-94
6	119-264	10-21	92-93

Table 2

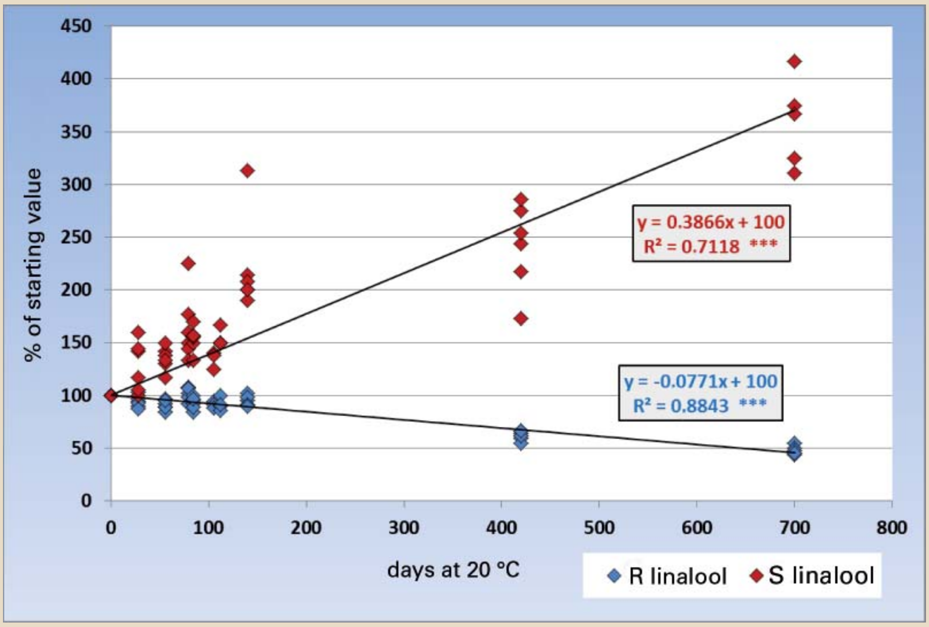

Fig. 2 Curves of changes in measured values of R and S linalool, averaged over 6 series after storage at 20 °C and up to 700 days; values in % rel. from starting values

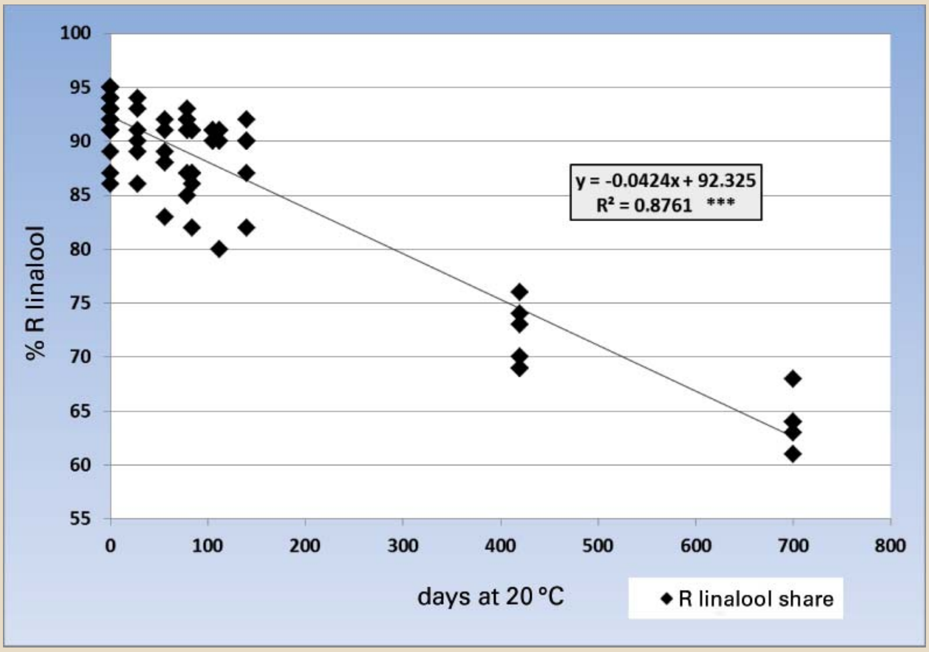

Fig. 3 Curve of R share as part of total linalool/values in % rel.

concentrated using solid phase extraction and then separated by means of GC. This will result in chiral separation of linalool. Using FID, R and S enantiomers of linalool are detected and quantified; stability of calibration is ensured also over longer time periods [13].

As all beers were strongly hopped, absolute levels fell within a comparatively high range, thus leading to reliable data. Standard deviations for the triple determination were ±2 µg/l for R linalool and ±1 µg/l for S linalool.

Data evaluation is based on the calculation given below:

$$\text{Change in \% rel.} = \frac{\text{measured value after storage x 100\%}}{\text{measured value before storage}}$$

This can mean both increases and decreases. Coefficients of variation of the relative changes in measured values range from ±3 to ±5 per cent for R linalool and from ±4 to ±8 per cent for S linalool in view of the lower absolute values. In addition to changes in measured values, the share of R linalool can be calculated as follows:

$$\text{R share in \% rel.} = \frac{\text{R linalool x 100\%}}{\text{total linalool}}$$

Here the standard deviation is ±3 per cent.

Absolute linalool levels of beers

In view of the abundance of data, individual measured values for R and S linalool as well as total linalool calculated are not indicated here as only changes in measured values caused by beer staling are of interest. Table 2 lists only the range of variation of absolute linalool levels in freshly filled beers in the various test series (values in µg/l). Taste threshold for R linalool is always exceeded. The literature quotes 2 to 80 µg [1]. In our St. Johann panel, it ranges from 5 to 20 µg/l, depending on beer type. On average, the relative R share of all beers is more than 90 per cent, fluctuating between 86 and 95 per cent. This high ratio points to use of fresh or appropriately stored hops and pellets produced thereof.

Change in measured values/ storage at 20 °C

Changes in measured values of the various test series were averaged and plotted against storage time. Figure 1 shows the relative decreases in measured values of total linalool over a storage period of 700 days. Relative error deviations of ±5 per cent were determined. No clear picture emerged over the first 140 days. Some losses of up to twelve per cent were compensated for by increases of up to 18 per cent.

The trend became unambiguous after 420 and 700 days. On average, 15 per cent of total linalool per year was lost. Linalool is thus not a stable substance in beer. However, the trend will make itself clearly felt only after an extended time period.

Figure 2 shows the separation of R and S linalool. This data is much more informative. Rise in S linalool is highly significant. An average increase per annum of about 1,5 times is calculated. The drop in R linalool per annum of just under 30 per cent is also highly significant. Only half of the losses of R linalool can be attributed to racemisation from R to S linalool. Other reactions must also play a part, for example conversion to

R and S alpha terpineol or also to nerol [12].

When plotting the percentage change of R linalool of total linalool against storage time, the curve is shown in figure 3. The drop in the R share averages about 15 per cent per annum and is highly significant.

Change in measured values/ storage at 30 °C

Storage tests at higher temperatures accelerate reactions, meaning that results can be obtained faster. Another objective was to verify whether an increase in temperature of 10 °C will result in twice the reaction speed, as is generally assumed. Figure 4 shows the curve of all changes in measured values of R, S and total linalool over a storage period of beers up to 105 days at 30 °C. S linalool increases highly significantly, on averages by about 180 per cent. R linalool drops by about 25 percent, also a highly significant curve.

Various curves for the R share are shown in figure 5. The individual lines run parallel towards the average decrease, i.e. 15.6 per cent in the course of 100 days.

Table 3 is a comparison of changes of R and S linalool and of the R share at storage temperatures of 20 and 30 °C over a storage period of 100 days. The factor indicates the rise in intensity, comparing 20 °C and 30 °C. According to *van't Hoff*, a temperature increase of 10 % will, in many instances, result in a reaction speed that is two to three times higher [14]. In this test arrangement, reactions that are 3.1 to 4 times more intensive seem to be at the upper limit of reasonable reasonable forced ageing temperatures. However, it is not unusual for beers to be subjected to temperatures over 20 °C on their way to consumers.

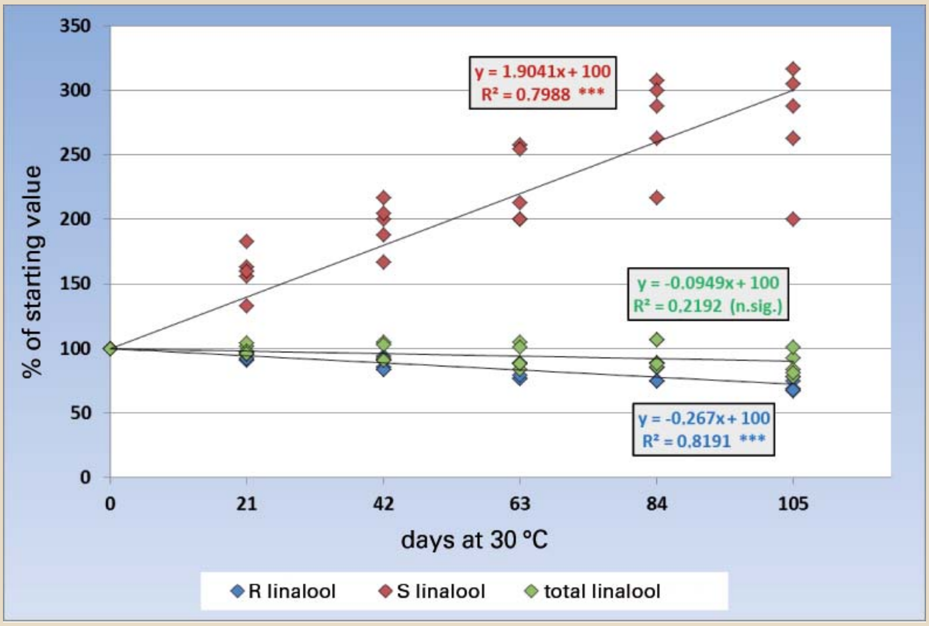

Fig. 4 Curves of R, S and total linalool after storage at 30 °C over a period of 105 days

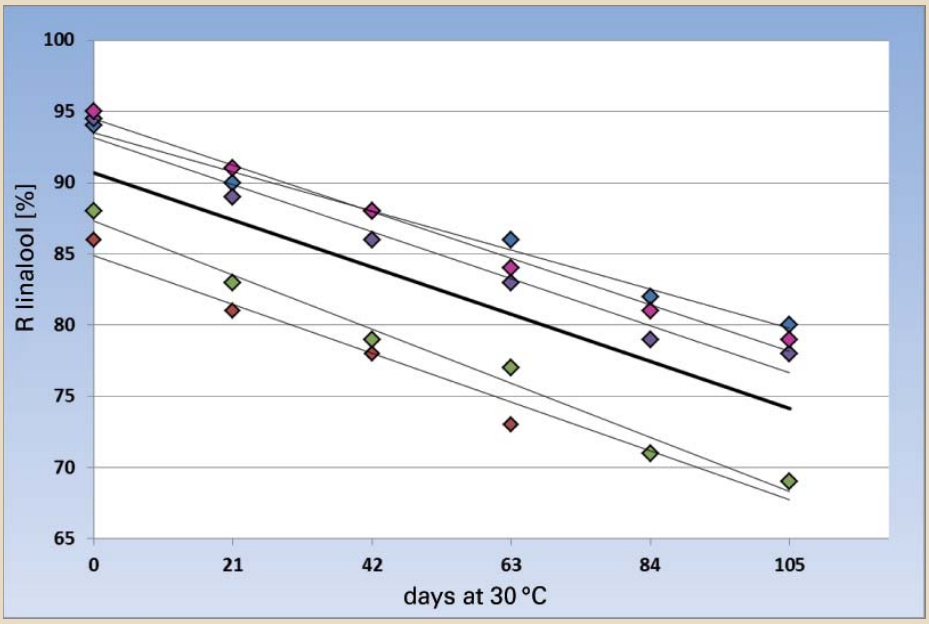

Fig. 5 Curves of R shares as part of total linalool in % rel.; individual series and averaged (thick line)

Other observations

No grave differences between individual hop varieties used and linalool decreases were found to-date. However, this issue will also be researched further.

Two examples can be used to compare late hopped and dry hopped beers. The same varieties were used in series 2 comprising eight late hopped beers and eight beers additionally dry hopped. Average loss of total linalool was 37 per cent (late hopped) and 43 per cent (dry hopped) at 20 °C after 420 days. When comparing two beers each in series 3, eleven per cent (late hopped) and nine per cent (dry hopped) were lost at 30 °C and after 105 days. There is thus no evidence that the behaviour of linalool differs during ageing of late hopped and dry hopped beers.

Summary

When wanting to track the behaviour of linalool during beer ageing, R and S linalool need to be separated at first as the sensory activity of R linalool is several times higher than that of S linalool. Results from extensive storage tests are summarised below:

- S linalool that is less relevant in sensory terms amounts to only about ten per cent of total linalool in fresh beers prepared with fresh pellets; however, it increases constantly in the course of beer staling by about 150 per cent relative in one year at 20 °C.
- In contrast, R linalool decreases by about 30 per cent relative in one year at 20 °C. Only about half of this loss can be attributed to racemisation to S linalool; conversion to nerol or terpineol is also conceivable.
- The R share in per cent of total linalool

COMPARISON OF REACTION INTENSITY DURING STORAGE AT 20 AND 30 °C; ...

... calculation of relative changes after a storage period of 100 days

	20 °C	30 °C	Factor
S linalool	45	180	4
R linalool	–8	–25	3.1
R share	–4.5	–15.6	3.5

Table 3

drops correspondingly from, e.g., 92 to 77 in one year at 20 °C as a result of beer staling.

- An increase in beer storage temperature from 20 to 30 °C resulted in more pronounced increases and decreases of about 3.5 times. Consequently, storage tests should not be conducted at inordinately higher temperatures, just to obtain results faster.
- On the other hand it is not unusual that beers are exposed to temperatures >20 °C on their way to the consumer. The drastically increased reaction velocity of R linalool degradation at 30 °C necessittates to avoid temperatures of more than 20 °C after bottling.
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- Behaviour of linalool does not differ between late hopped and dry hopped beers.
- The hop variety does not seem to play any major role.
- Racemisation of R to S linalool influences the sensory impression of a beer. Intensity of hop aroma inevitably drops during beer staling. ■

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