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The Behaviour of the Stereoisomers of Linalool During Beer Ageing

There have always been beers with a hop aroma, especially in the Pilsner sector. Linalool with its floral character is often cited as an indicator substance for the corresponding sensory impression [1–4]. The rapid development of the craft brewing scene originating in the USA, not least through the re-discovery of dry hopping, has led to the appearance of numerous hoppy beers on the market. This trend includes not only the practically mandatorily dry hopped pale ales and India pale ales, but now also pilsners, wheat beers and other beer styles.

Descriptors: Hop aroma in beer, aroma stability, linalool enantiomers, beer ageing

1 Tasks

In general the flavour stability of such full-bodied, often high-alcohol and hoppy beers is usually better than standard low hopped pale lager beers. However, hop aroma is subject to change during the course of beer aging too. Since the success and the increasing request of craft beers have led to greater distribution distances and associated longer times before consumption, the demand is becoming ever stronger for stability of the hop aroma in beer. To date there is no extensive knowledge on this topic.

In the first part of our study we limited ourselves to the behaviour of linalool as a key aroma substance during the aging of beer. Linalool as a monoterpenoid can be assigned to the aroma spectrum of hops (hop oil) as shown in figure 1.

The linalool content of hops is variety dependent and varies generally between 4 and 12 mg/100 g [1]. The transfer rates of linalool with a late hop addition in the brew kettle or in the whirlpool fluctuate greatly between 20 % and 60 % in the bottled beer with reference to the dosed quantity [1–5]. Dry hopping produces transfer rates of 40 % to almost 100 % [6, 7]. In this way, depending on the dosed quantity, with late hopping between 20 and 100 µg/l linalool in the beer can be expected, whereas dry hopped beers have a linalool content of up to 500 µg/l [8].

An important factor here is that linalool consists of two stereoisomers, the R- and S-forms, as shown in figure 2. The sensory intensity of R-linalool, with a rough threshold value of 10 to 20 µg/l beer, is much greater than that of S-linalool at about 200 µg/l. *Fritsch* et al. reported odour threshold values of 2.2 µg/l for R-linalool and 170 µg/l for S-linalool [17]. In non-aged hops the portion of R-linalool is about 90 %, which is reflected in the beer produced. Aged or oxidized hops are characterized by more S-linalool and less R-linalool, which again is reflected in the beer and ultimately manifested by a lower sensory perception [1, 2].

Although the use of mainly pellets and a boiling point of linalool at approx. 200 °C the relevant literature provides very contradictory information about the behaviour of linalool during beer aging. For example, *Tressl* et al. [9] reported a practically total loss (96 %) during a very moderate beer aging of 8 months at 4 °C. In contrast

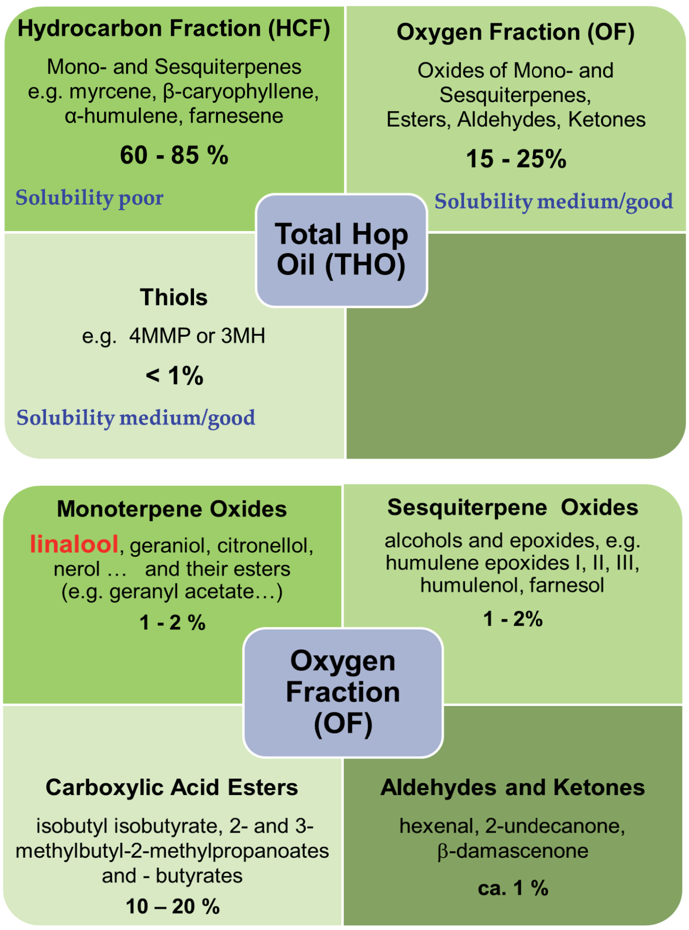

Fig. 1 Overview of the aroma substances in hop oil; figures in % of total oil content

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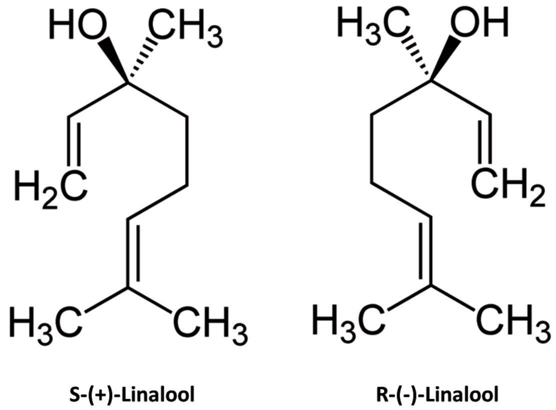

Fig. 2 The chemical structure of the two stereoisomers R-linalool and S-linalool

Peacock et al. [10] established a linalool reduction of 11 % after beer storage of only 57 days at 20 °C. *Kaltner* et al. [11] on the other hand were not able to detect any loss of linalool after beer aging of 1 year at 20 °C. However, the aging did cause a moderate shift from R-linalool to S-linalool (from 72 % to 64 % R-linalool). Our working group reported a linalool increase of about 21 % with beer aging of 110 days at 20 °C [7]. In contrast to these values [7] that were obtained with SPME GC-MS the beers of this study were analysed via GC FID after solid phase extraction. In two other projects linalool concentration was monitored based on forced aging. An aging day is a sequence of 24 hours at 60 °C and 24 hours at 0 °C. After 4 such aging days there was a linalool reduction of 58 % in one case [12], and an increase of 25 % in the other case [13]. Doubt may be justified that such forced aging can simulate practical conditions of storing at 20 to 25 °C.

Qian [14] recently reported of linalool conversion during beer aging. Alongside racemization from R-linalool to S-linalool he determined conversion to S-α-terpineol and R-α-terpineol as well as to nerol. Similar to [11] a drop in pH favoured the conversion of R-linalool into S-linalool.

The statements about the behaviour of linalool during beer aging do not coincide, which may be due, among other things, to the fact that generally too few beer samples were examined. Reference to one or two beers and one or two samples results more in a snapshot from which no general conclusions can be drawn. An examination without separation of the two enantiomers also seems rather pointless.

For this reason the aging behaviour of linalool was investigated in this study on the basis of several beers and numerous samples at storage temperatures of 3 °C, 20 °C and 30 °C. Different beer styles, hop varieties and hopping technologies like late and dry hopping were evaluated.

2 Material and Methods

2.1 Hops used for the test beers

Apart from series 8, the hops used to produce a hop aroma were dosed as pellets type 90. Only in series 8 hop oil of the Taurus variety was used - the product of vacuum distillation employing a short-path evaporator and thin-film evaporator [1].

Below is a list of abbreviations used in the following for the hop varieties:

- 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 from Hallertau and Yakima
- CM = Comet from Hallertau and Yakima

The hops originate from the Hallertau unless otherwise specified.

2.2 Common characteristics of all beers

All of the beers tested, except for series 9, originate from trial beers produced also for other purposes in the 2-hl St. Johann Research Brewery. They are generally bottom fermenting lager beers hopped with different varieties and quantities. They all had the following characteristics in common:

- 100 % Pilsner malt; in series 5 and 7: 10 % Carahell®
- Infusion mashing method
- Internal boiler, 70-minute boiling time
- Hopping at begin of boil (BoB), middle of boil (MoB); late hopping was split into 50 % at end of boil (EoB) and 50 % in the whirlpool (WP)
- Main fermentation with yeast W34 at 8 °C, approx. 1 week
- Maturation at 14 °C, 1 week
- Storage at 0 °C, 2 weeks
- Diatomaceous earth and membrane filtration
- Bottled in bottles with double pre-evacuation using scavenger crown caps

The dry hopping is most easily done with the pre-addition of pellets in the maturation tank upon change of tank after the main fermentation. This gives a contact time of 1 week at 14 °C and 2 weeks at 0 °C.

2.3 Different test series

Series 1: Series as per [7]; late hopped control beer (HT, HA) with four additionally dry hopped beers (MB, HN, HC and PA); 5 beers.

Series 2: Series as per [15]; comparison of one variety each late (EoB and WP) and additional dry hopped beers (HC, MB, HN, PA, HA, SR as well as CA from USA and the Hallertau); 16 beers, 8 late and 8 additionally dry hopped beers.

Series 3: Comparison of dry hopped beers before and after filtration; varieties HC, MB; 2 beers.

Series 4: Due to problems during storage the values of series 4 were not included.

Series 5: 2 alcohol-reduced light beers; both late hopped with SR, 2 beers.

Series 6: 6 beers without hopping at BoB; hopping at MoB + EoB with HT and MB, only at EoB with HT and SR and only dry hopped with TU and HC; 6 beers.

Series 7: Comparison of two varieties late hopped and additionally dry hopped; PA and Huell breeding line 89/002/025; 4 beers.

Series 8: Beer with TU hop oil; addition only at EoB, as well as EoB + dry hopped; 3 beers.

Series 9: Extraordinary beers, 3 top and 1 bottom fermented, all dry hopped, as per [8]:

- Pale 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.
- "All In": bottom fermented Festbier, late hopped with HA/HE/SR/SP, dry hopped with HC/PA/MB/HN.

Table 1 shows the temperatures and storage days before sampling for each series. With three different temperatures and 42 beers, 107 samples were taken with measured values for R-linalool and S-linalool after the corresponding storage times. In addition there were the 42 sets of initial data.

2.4 Analysis of R-linalool and S-linalool

In contrast to the values of [7] that were obtained with SPME GC-MS the beers of this study were analysed via GC FID after solid phase extraction.

The aroma substances from wort and beer are enriched via solid phase extraction and then separated via GC. This brings about

Table 1 Test series each with number of beers, storage temperatures and storage days as well as number of measured values

Series	Beers	Temperature / °C	Storage time /ays	Measurements
1	5	20	700	5
2	16	20	420	16
3	2	3	550	2
4	0	–	–	–
5	2	3	440, 530	4
6	6	20	79, 140	12
7	4	20	28, 56, 84, 112	16
		30	21, 42, 63, 84, 105	20
8	3	20	28, 105, 112	9
		30	21, 42, 63, 84, 105	15
9	4	20	56, 84	8
Sum	**42**			**107**

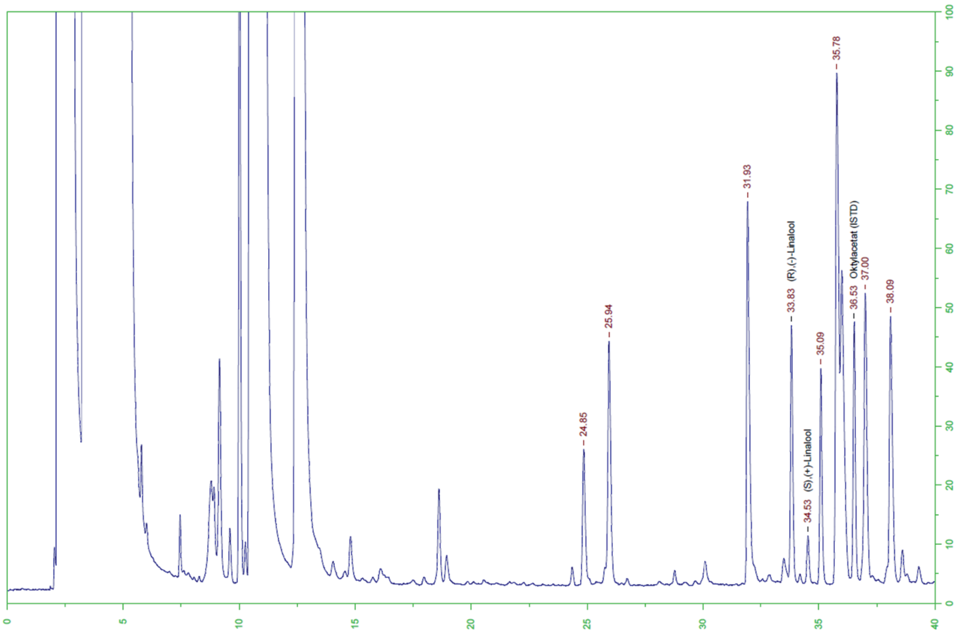

Fig. 3 Gas chromatogram of a beer sample with R-linalool and S-linalool as well as octyl acetate as internal standard

the chiral separation of the linalool. Using FID the R-enantiomer and S-enantiomer of linalool are detected and quantified, whereby the stability of the calibration is checked and guaranteed over long periods of time.

The solid phase extraction is done using the ENV+ column method (200 mg/3 ml volume), activated with ethanol and conditioned with HCl. Octyl acetate (Sigma-Aldrich O5500, purity > 99 %) serves as an internal standard. A mixture of R- and S-linalool is used as calibration standard (Sigma-Aldrich 62139, purity > 95 %). The relationship of the peak areas in the GC chromatogram correlate with the concentrations of the two enantiomers.

For the gas chromatographic separation a M&N separation column, HYDRODEX β TBDAc, 50 m x 0.25 mm is used, the oven temperature is programmed for 65 °C to 190 °C. Detection is done on an FID at 220 °C. Figure 3 shows a gas chromatogram of a beer sample.

The stability of a calibration plays an outstanding role with such long storage intervals (up to 700 days). Calibrations with different concentrations of a standard solution were performed twice a year. Their statistical evaluations showed relative standard deviations of about 1.5 %. Additionally a beer stored at 0 °C with known concentration was introduced regularly into the analysis program to prevent unrecognized deviations. Since all the beers were strongly hopped, the absolute contents were at a comparatively high level. A threefold determination of a beer with about 300 µg/l linalool offered the following data for the ranges (r95): R-linalool 15 µg/l and S-linalool 2 µg/l.

2.5 Data Evaluation

The data evaluation is based on an ageing index which specifies the amount of linalool (in % rel. of the initial value in fresh beers) present after a certain beer ageing time and which is calculated according to the following formula 1:

$$\text{Ageing Index} = \frac{\text{value after storage} * 100\%}{\text{value prior storage}}$$

Here it can be a matter of increase or decrease. The coefficient of variation (CV) of the ageing index lies between ± 3 and ± 5 % for R-linalool and between ± 4 and ± 8 % for S-linalool because of the lower absolute values.

The R-linalool ratio is calculated as follows (formula 2):

$$\text{R} - \text{Linalool ratio} = \frac{\text{R} - \text{Linalool} * 100\%}{\text{total Linalool}}$$

The standard deviation is calculated here with ± 3 %.

For the reason of a clear illustration of the values in the figures a display of each value together with its error bars is omitted. To give an idea about the significance of the ageing indices including error considerations an example is shown in table 2. This example provides confidence intervals for the ageing indices of R-linalool (58–66 %), S-linalool (198–284 %) and total linalool (73–85 %). The relatively high CI of S-linalool originates in the low values prior storage.

3 Results and Discussion

3.1 Absolute linalool values of the beers

Individual values for R-linalool and S-linalool and the resulting total linalool were omitted due to data volume. Table 3 specifies the absolute linalool values in each series in the range of fluctuation (values in µg/l) in the fresh beers. The R-linalool varies between 37 µg/l (series 3) and 666 µg/l (series 6) and the flavour threshold is consistently exceeded at least by the R-linalool which is given in the literature as 2 to 80 µg/l [1]. In our trained panel in St. Johann consisting of up to 18 panellists it is in a range of 10 to 20 µg/l depending on the beer type.

Table 2 Ageing Indices, mean values and confidence interval (CI 95 %) of R-, S- and total linalool of 6 beers (series 2) after a storage of 420 days at 20 °C in %

	Ageing indices (420 d/20 °C) of		
	R-Linalool	**S-Linalool**	**Total Linalool**
Beer 1	64	217	77
Beer 2	66	275	83
Beer 3	59	173	74
Beer 4	55	244	69
Beer 5	62	286	81
Beer 6	66	254	86
MEAN	**62**	**241**	**79**
CI	**4**	**43**	**6**

Table 3 R-linalool and S-linalool in µg/l as well as the R-linalool ratio in the fresh beers of the separate series in their ranges of fluctuation

Series	R-Linalool	S-Linalool	R-Ratio
1	45–123	4–9	92–94
2	44–112	4–13	90–92
3	37–45	3–7	87–93
5	72–175	6–10	92–95
6	50–666	5–35	91–95
7	36–342	6–20	86–94
8	66–254	5–16	93–94
9	119–264	10–21	92–93

Furthermore the variations of the R-linalool ratio (see formula 2) of the fresh beers are also listed in table 2. On an average of all the beers the R-linalool ratio is over 90 % with fluctuations between 86 % and 95 %. This high level speaks for the use of fresh hops or properly stored hops and the pellets produced thereof [11].

3.2 Ageing indices

3.2.1 Cold storage at 3°C

Four beers of series 3 and 5 were stored more than 17 months (> 500 days) at 3 °C. Even at that cold temperature changes in the linalool contents were observed. While S-linalool decreased moderately (definitely less than during a storage at higher temperatures), R-linalool showed an inconsistent behaviour.

Whereas in series 3 there was a moderate R-linalool loss of 19 %, the R-linalool increased slightly by 8 % in the beers of series 5. Due to the lack of more data a statistically backed conclusion cannot be drawn.

3.2.2 Storage at 20 °C

Average ageing indices of the individual series are plotted versus the storage time. Figure 4 shows the relative values for the total linalool versus a storage time of up to 140 days. The relative error deviations were calculated with ± 5 %. Losses of up to 12 % compare with increases of up 18 %. However, on average no change could be determined.

By adding the measured values of series 1 and 2 with storages of 420 and 700 days, as shown in figure 5, a clearer picture resulted.

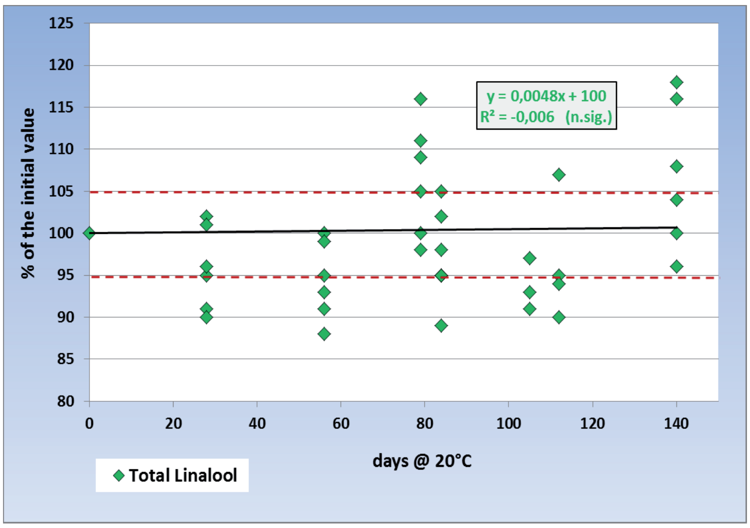

Fig. 4 Average ageing indices of total linalool in 6 series after storage of 140 days at 20 °C; values in % of the initial value

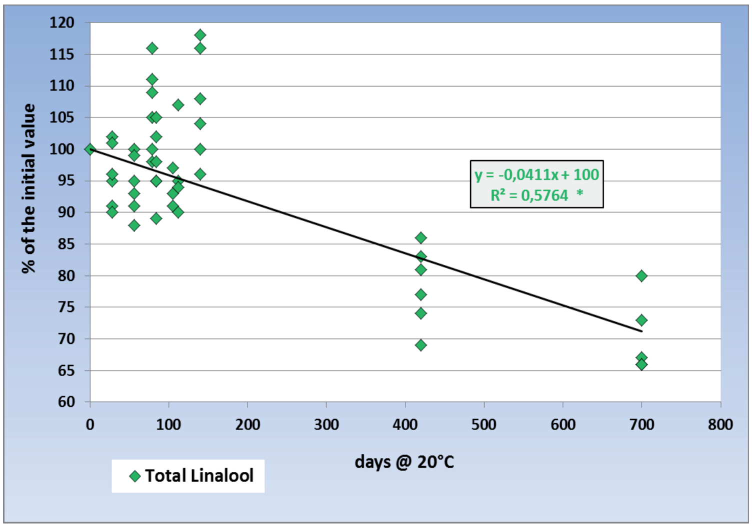

Fig. 5 **Trend as in figure 4 but with storage of up to 700 days**

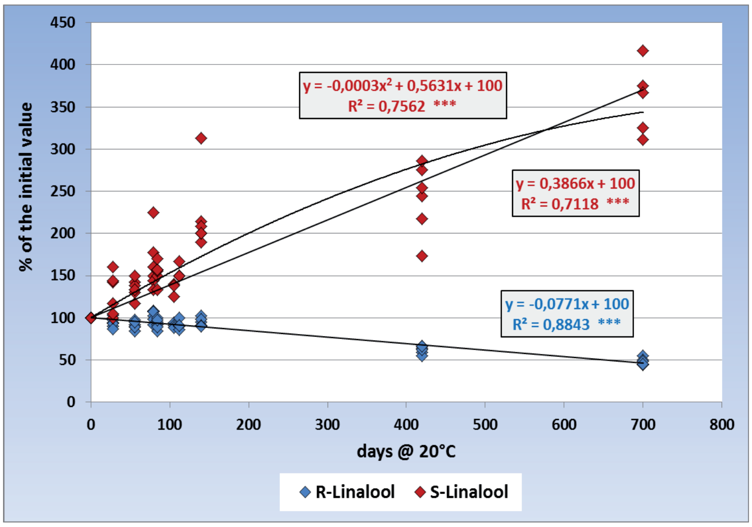

Fig. 6 **Changes of R-linalool and S-linalool determined over 6 series after storage of up to 700 days at 20 °C; values in % of the initial values**

There is an average loss of about 15 % per year. It is therefore obvious that linalool is not a chemically stable substance in beer. The changes show a clear direction only after a longer time period of more than 200 days.

However, it is more informative to consider R-linalool and S-linalool separately, as shown in figure 6. The decrease of R-linalool as well as the increase in S-linalool is highly significant, if a polynomial trend is used for S-linalool even with a higher coefficient of determination (R^2 = 0.7562 compared to 0.7118 for the linear trend) and is valid only in the examined time sector.

There is a calculated average annual increase of S-linalool of about 1.5-fold. The annual decrease in R-linalool on the other hand is just on 30 %. So the losses of R-linalool can only be half explained by the racemization from R-linalool to S-linalool. There must still be other reactions as established by Qian [14], for example, with a conversion to S-α-terpineol and R-α-terpineol as well as to nerol.

The percent of R-linalool of the total linalool plotted against the storage time, as shown in figure 7, decreases. All the individual data of the series follow a parallel trend to the determined regression

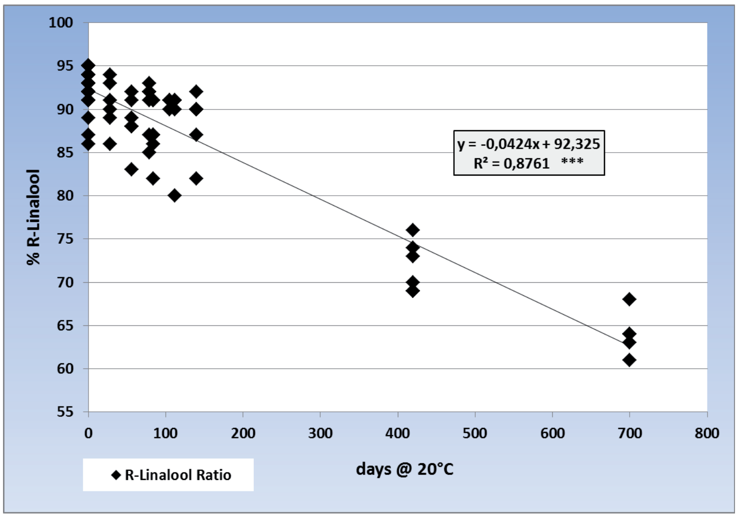

Fig. 7 **Decrease of R-linalool as part of the total linalool; values in %**

lines as will be explained later. The highly significant decrease in R-linalool is on average about 15 % per year.

3.2.3 Storage at 30 °C

Storing the beers at a higher temperature has the advantage that results are available more readily. “Already in the past century it has been found, that in most cases the reaction speed rises with increasing temperatures. According to van´t Hoff the reaction speed doubles to triplicates incrementing the temperature by 10 °C.” [18]. It was intended to see whether the ageing reactions of linalool follow this rule. On the other hand, a temperature load of more than 25 °C is by no means unrealistic for a beer on its way to the consumer.

Figure 8 shows the characteristic of all the average ageing indices of R-linalool, S-linalool and total linalool versus the beer storage time of up to 105 days at 30 °C. A clear picture is given:

- S-linalool increases with high significance both with a linear trend (R^2 = 0.7988) as well as with a polynomial one (R^2 = 0.8412) at an average value of about 180 % in 100 days.
- Approx. 25 % of R-linalool are lost in 100 days. The linear trend is highly significant with an R^2 value of 0.8191.

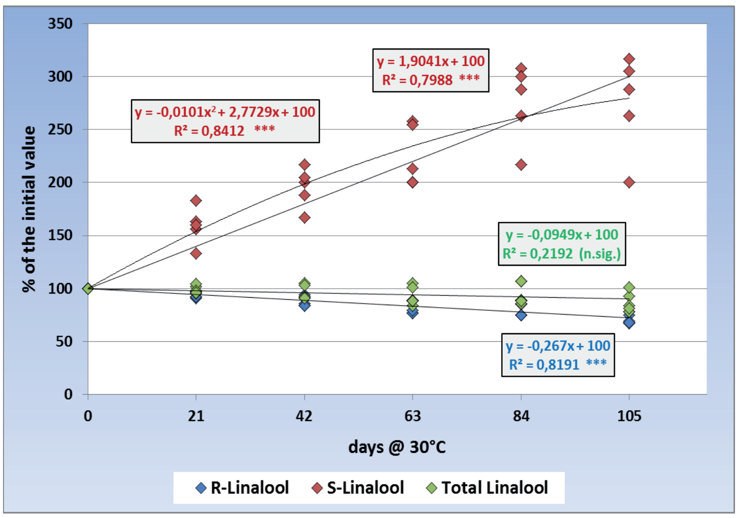

Fig. 8 **Average ageing indices of R-linalool, S-linalool and total linalool for a storage time of 105 days at 30 °C**

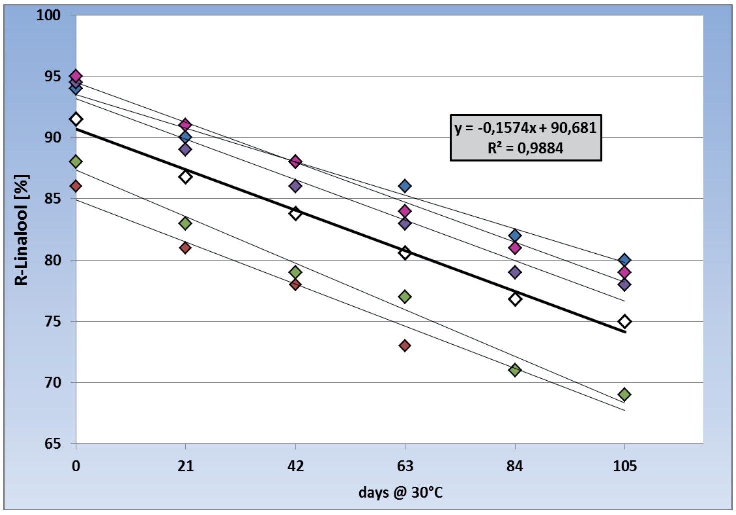

Fig. 9 Decrease of R-linalool as part of the total linalool in %; displayed as separate series and average (thick line)

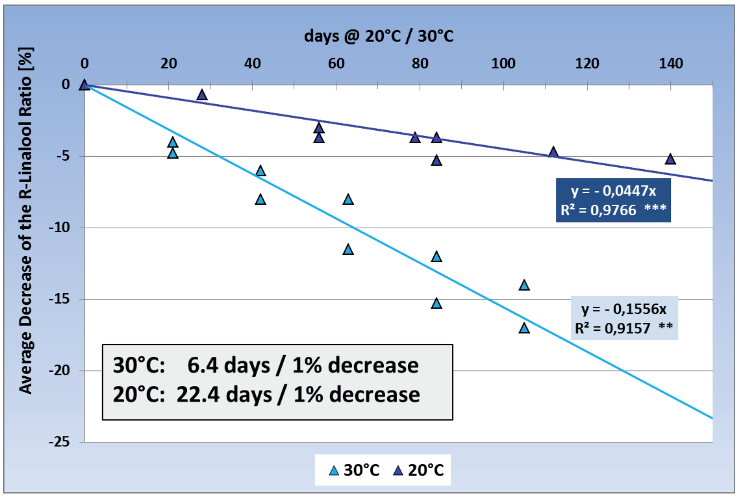

Fig. 10 Comparison of all measured decreases in R-linalool at 20 °C and 30 °C

Figure 9 shows the separate decrease for R-linalool. The average of R-linalool-ratio at a given time follows a high significant trend (thick line), which results in 15 % losses in 100 days.

A direct comparison of a storage at 20 °C and 30 °C is possible as follows: The loss of R-linalool is calculated by subtracting the values of the storage sample from the initial values:

Loss of R-linalool = R-linalool prior to storage minus R-linalool after storage

This gives a picture as shown in figure 10. The decrease in R-linalool at 30 °C is 15.6 % in 100 days, 3.5 times faster than at 20 °C with a value of 4.5 %. This is more than the van´t Hoff´s rule, which indicates that there are limits forcing the storage temperature. Storage tests at temperatures exceeding 30 °C do not appear to be reasonable.

3.2.4 Increase in R-linalool

An increase in R-linalool was observed in two series. In series 5 there was an increase of 19 % with storage of 420 days at 3 °C. Series 6 shows an increase of 8 % after 79 days at 20 °C. In both cases these increases are higher than the CV of ± 5 %. It is therefore conceivable that in some cases there is a short phase of increase in R-linalool during beer aging. However, this plays no great role in the brewing practice.

3.2.5 Comparison of late hopped and dry hopped beers

Two examples are taken. In series 2 the same varieties were used in each case with the 8 late and 8 dry hopped beers. The average loss of the total linalool after 420 days at 20 °C was 37 % (late hopped) and 43 % (dry hopped). A comparison of 2 beers each in series 7 after 105 days at 30 °C showed losses of 11 % (late hopped) and 9 % (dry hopped).

Therefore there are no signs that linalool behaves differently during the aging of late hopped and dry hopped beers.

4 Summary

The results of the comprehensive tests on the behaviour of linalool during beer aging can be summarized as follows:

- Since it might be known that linalool consists of two stereoisomers with totally different sensory activity (R- many times more active than S-linalool) most publications do no not refer to this fact. For future studies it is recommended to analytically separate R-linalool and S-linalool.
- S-linalool is present only as about 10 % of the total linalool in fresh beer brewed with fresh pellets, but increases steadily as the beer ages.
- In contrast are the decreases in R-linalool due to a racemization to S-linalool, which however cannot be held responsible for all losses.
- A minor increase in R-linalool at the beginning of storage has been observed only in two cases.
- However, the conversion of R-linalool into S-linalool definitely has a sensory effect, because it is accompanied by a loss of aroma intensity.
- This is manifested in the decrease of R-linalool as a ratio of the total linalool in the course of beer aging, for example from 92 % to 77 % in one year at 20 °C.
- The sum of R-linalool and S-linalool (= total linalool) does not show an homogeneous development at the beginning of beer aging, which might well be the reason for many contradictory results in the literature.

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