Hop aroma compounds: transfer rates into beer and beer aging

AGING BEHAVIOR | During experimental trials conducted to assess the reproducibility of the 2 hl pilot brewery at Hopfenveredlung St. Johann GmbH, it was determined that a number of hop aroma compounds were transferred from hops to beer. Subsequently, the behavior of these compounds in the finished beer over the course of aging was investigated [1].

THE TRANSFER RATES of aroma compounds from hops to beer, especially in conjunction with dry hopping techniques, have been discussed in several scientific publications [2, 3, 4, 5]. However, they have not yielded generally applicable findings. The reasons for this are primarily due to the following:

The transfer rate or yield for a single aroma compound is based upon analysis results in both the hops and the beer brewed with those hops. The sources of error for these analyses as well as any conclusions drawn from them are frequently underestimated. Values provided for the transfer rates of the compounds in question must be interpreted as having a considerable range of variation, which can be as high as ± 25 percent.

The transfer rates for individual aroma compounds are influenced by a number of variables, e.g. the hop variety, the type of product, the degree of compression during pelletization (density), movement in the tank, the dosing system, the temperature of the beer and the duration of exposure.

While numerous papers have been published on the transfer of hop aroma compounds to beer during dry hopping, there is little data on the transfer rates during late hopping, either at the end of the boil or in the whirlpool, since so many modern wort boiling systems exist, and they vary greatly in their levels of evaporation. This makes sweeping statements about transfer rates for late-hopped wort in the brewhouse impossible.

Late hopping in the brewhouse simply cannot come even close to imparting sensorially perceptible amounts of monoterpenes and sesquiterpenes to the finished beer due to their low solubility. Only the compounds belonging to the oxygen fraction, which are soluble in wort and beer, such as terpene alcohols (e.g. linalool), carboxylic acid esters (e.g. isobutyl isobutyrate), ketones (e.g. 2-undecanone) and epoxides of the sesquiterpenes, are capable of surviving in the wort kettle and/or whirlpool while the wort is still hot, during primary fermentation due to "stripping" by CO_2 bubbles and during filtration.

Another question that has yet to be thoroughly answered concerns the hop aroma

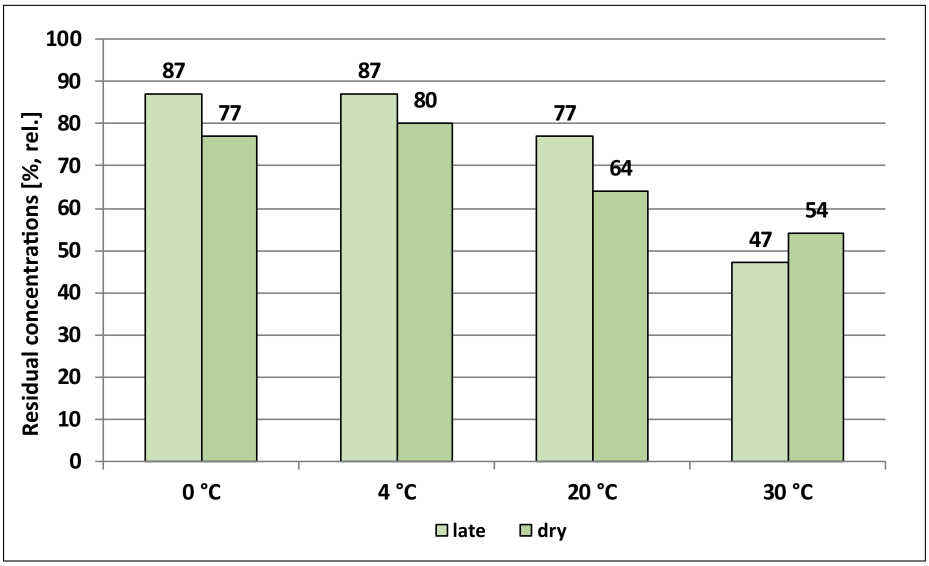

Fig. 1 Percentages of the total monoterpenes remaining in the beers after storage relative to the quantities initially present prior to storage. The beers were stored for 470 days (late-hopped and dry-hopped) and are presented according to their respective storage temperatures

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THE CONCENTRATIONS OF AROMA COMPOUNDS IN THE HOPS, THE QUANTITIES ADDED TO THE LATE-HOPPED AND THE DRY-HOPPED BEERS, THE QUANTITIES IN THE BEERS AND THE TRANSFER RATES

Hop aroma compounds	Amounts in hops [mg/100 g]	Quantity added [µg/l]		Quantities in beer [µg/l]		Transfer rates [% rel.]		
		Late	Dry	Late	Dry	Late	Dry	Ø late & dry
β-myrcene	13	390	195	4.1	5.6	1	< 1	1
β-caryophyllene	8	240	120	1.2	1.1	< 1	–	<1
α-humulene	8	240	120	5.1	4.4	2	–	2
β-selinene	58	1740	870	10.5	30.1	< 1	2	1
α-selinene	44	1320	660	3.0	9.8	< 1	1	< 1
Isobutyl isobutyrate	4	120	60	48.2	98.1	40	84	55
2-methylbutylpropanoate	4	120	60	6.0	13.1	5	12	7
3-methylbutyl-2-methylpropanoate	7	210	105	40.4	87.4	19	45	28
2-methylbutyl-2-methylpropanoate	46	1380	690	352	897	26	79	43
Linalool	1	33.0	16.5	20.8	34.4	63	82	70
Cubenol	2	66	33	4.9	8.6	7	11	9
α-eudesmol	5	150	75	12.8	35.2	9	30	16
α-cadinol	2	60	30	10.9	26.5	18	52	29

Table 1

compounds dissolved in the beer and their stability over the aging process. The data on this topic in the literature are contradictory.

At least there is some clarity regarding linalool [6]. Losses in total linalool were found to amount to approximately 15 percent per year relative to the initial concentration at a storage temperature of 20 °C. Higher losses of R-linalool on the order of 30 percent per year are offset by a corresponding increase in S-linalool.

In the research trials described here, two beers were analyzed: one was late-hopped while the other was late-hopped and dry hopped. Both beers were stored for 470 days at 0 °C, 4 °C, 20 °C and 30 °C. Since previous trials conducted with a series of three batches of beer had already been proven to be highly reproducible, it seemed reasonable to reduce the sample size to one batch of beer each.

The methodology of the reproducibility trials

In the first part of the reproducibility trials, the methodology of beer production in the 2 hl brewery in St. Johann was described in detail [7]. The principal characteristics of the beer were that it was brewed exclusively from malt, was bottom-fermented and late hopped at a rate of 300 g of Hüll Melon hop pellets per hl. This amounted to 2.4 ml of hop oil per hl. The batches were split after primary fermentation. One half was dry-hopped with 150 g of Hüll Melon per hl (1.2 ml oil/hl). After primary fermentation, the green beer was transferred to the maturation tank, where it was dry-hopped at a rate of 150 g of hops per hl. There was no stirring or agitation in the maturation tank, and the beer spent eight days at 14 °C and 14 days at 0 °C in direct contact with the hops.

Determining the transfer rates of hop aroma compounds

The hop addition at the start of the boil amounted to approximately 62 g of HHS pellets per hl. Any contribution of aroma compounds to the beer from hops at this stage was assumed to be negligible, since it has been demonstrated in corresponding trials in the pilot brewery that moderate hop additions at the beginning of the boil do not impart aroma compounds to the wort beyond, for instance, linalool at a concentration of 2 µg/l.

The aroma compounds present in the Hüll Melon hops used for the aroma additions (late in the brewhouse = late, and dry hopping = dry) were analyzed using gas chromatography. The quantity of a particular hop compound dosed into the beer through a pellet addition can be calculated according to equation 1.

Equation 1

$$\text{Aroma compound added}\left[\frac{\mu g}{l}\right] = \text{hop addition}\left[\frac{g}{l}\right] \times \text{concentration of aroma compound}\left[\frac{\mu g}{g}\right]$$

Equation 2

$$TR_{late}\,[\%] = \frac{\text{concentration in beer}\left[\frac{\mu g}{l}\right]}{\text{quantity added to wort}\left[\frac{\mu g}{l}\right]} * 100\ \%$$

Equation 3

$$TR_{dry\ only}\,[\%] = \frac{\text{concentration in beer (late+ dry)}\left[\frac{\mu g}{l}\right] - \text{concentration in beer (late)}\left[\frac{\mu g}{l}\right]}{\text{quantity added through dry hopping}\left[\frac{\mu g}{l}\right]} * 100\ \%$$

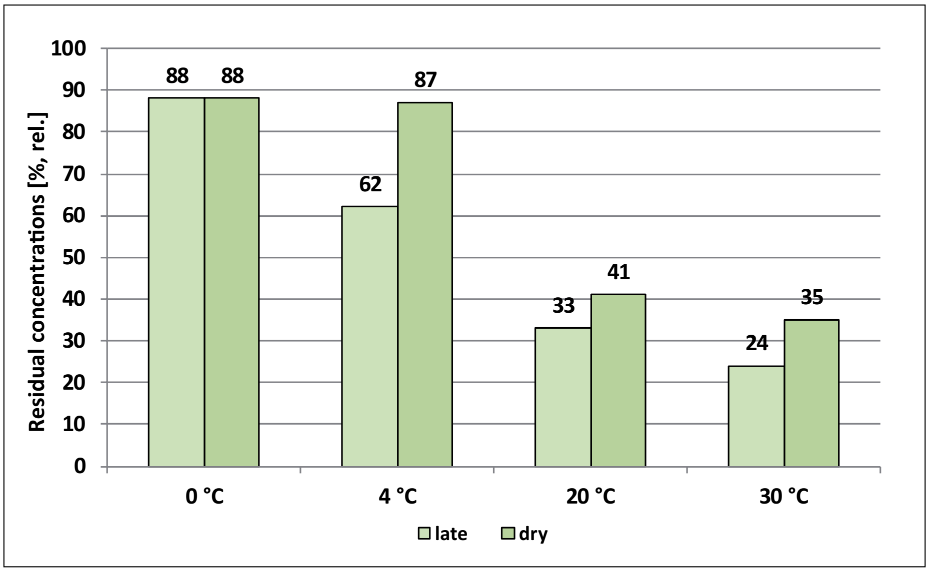

Fig. 2 Percentages of ten relevant hop esters remaining in the beers after storage relative to the quantities initially present prior to storage. The beers were stored for 470 days (late-hopped and dry-hopped) and are presented according to their respective storage temperatures

MONOTERPENES – LATE-HOPPED BEERS [µG/L]

	Fresh	Storage period 470 d			
		0 °C	4 °C	20 °C	30 °C
β-myrcene	4.7	4.4	4.3	4.0	2.5
Limonene	1.3	1.1	1.2	1.0	0.9
cis-ocimene	0.1	0.1	0.1	< 0.1	< 0.1
trans-ocimene	0.8	0.4	0.4	0.3	0.2
γ-terpinene	0.1	0.1	0.1	0.1	0.1
Sum	**7.0**	**6.1**	**6.1**	**5.4**	**3.8**

Table 2

The transfer rate (TR) or yield of a compound in beer through a late hop addition is generally calculated according to equation 2.

Thus, for beers hopped late in the brewhouse, determining the amount of a substance and calculating the hop addition are straightforward. To determine the approximate transfer rate solely attributable to dry hopping, the value obtained from the analysis of the late-hopped beer must be subtracted from that of the beer that was also dry-hopped. This value must then be divided by the quantity used exclusively for dry hopping (equation 3).

$$TR_{late+dry}\,[\%] = \frac{\textit{concentration in beer (late + dry)}\left[\frac{\mu g}{l}\right]}{\textit{quantity added (late + dry)}\left[\frac{\mu g}{l}\right]} * 100\ \%$$

Equation 4

The transfer rates for the late-hopped and dry-hopped beers can then be calculated (equation 4) in their entirety.

It was possible to ascertain the transfer rates in the recent trials conducted with three batches of beer per series, which therefore increases the reliability of the data compared to the single batches brewed for the present trials.

Aging behavior of the beers

A late-hopped beer and a beer that was both late-hopped and dry-hopped were stored for 470 days at the following temperatures: 0, 4, 20 and 30 °C. They were subsequently analyzed only at the end of the storage period. In addition to the absolute concentration of the volatile aroma substances present, the changes in the relative quantities of the substance groups, such as monoterpenes, monoterpene alcohols and carboxylic acid esters, were also calculated.

Transfer rates (TR)

Table 1 shows the concentrations of individual aroma compounds in the hops (Hüll Melon) in mg/100 g, the quantities of these compounds added to the late-hopped and dry-hopped beers as well as the quantities measured in the beers after storage. The transfer rates of the late hopped beer and the dry-hopped beer (minus the late-hopped quantity) as well as the transfer rates for the combination of late hopping and dry hopping are also provided. The results can be summarized as follows:

The transfer rates of myrcene, β-caryophyllene, humulene and the two selinenes are within 1 to 2 percent.

Of the four esters, three exhibited transfer rates of 19 to 40 percent in the late-hopped beers. Only that of 2-methylbutylpropanoate was lower, at five percent. Calculated separately, dry hopping evinced a transfer rate of 45 to 83 percent for the three esters. The transfer rate for 2-methylbutylpropanoate was twice as high, at 12 percent.

The transfer rates for linalool were 63 percent (late) and 82 percent (dry), but it remains unclear from these trials whether glycosidically bound linalool can be released into wort or beer.

The sesquiterpene alcohols exhibited transfer rates ranging from seven to 18 percent (late) and from eleven to 52 percent (dry).

The two epoxides analyzed in these trials were readily detectable in the late-hopped beers, with transfer rates of 27 and 70 percent. However, the quantities of the epoxides did not increase in the dry-hopped beers. There is no explanation for this.

Monoterpene alcohols

One aspect of the monoterpene alcohols also deserves mention. Aside from linalool, only geraniol was able to be detected in the hops but not nerol, β-citronellol or α-terpineol. However, no geraniol was found in the beers. By contrast, relevant amounts of α-terpineol (5 and 10 µg/l) and β-citronellol (14 and 21 µg/l) were measurable. According to Takoi [8], geraniol can be transformed to β-citronellol and linalool to α-terpineol through the action of yeast enzymes – a plausible explanation for their detection in the beer but not in the hops. In another publication, Takoi [9] discussed the behavior of monoterpene alcohols in more detail. He was able to demonstrate the following changes over the course of beer production from the hopped wort to the finished beer:

Linalool decreased by approximately 50 percent from wort to beer.

The geraniol content dropped by around 90 percent. Trace amounts of β-citronellol were measured in the hops and therefore also in the hopped wort, but the concentration increased markedly during fermentation. This can be explained chiefly by the formation of β-citronellol from geraniol.

Similarly, α-terpineol was only detectable in trace amounts in the hops and thus in the wort, but its concentration increased during fermentation, which Takoi attributes to the conversion from linalool.

Nerol is the least interesting representative of the monoterpene alcohols. It was present only in trace amounts in hops and underwent no relevant changes during fermentation.

Since the degradation rates of linalool and geraniol were by no means identical in all trials, one can assume that the enzyme activity of the yeast employed in the production process was not always comparable.

Aging of volatile hop aroma compounds in beer

Monoterpenes

The tables distinguish between late-hopped beers and those which were both late-hopped and dry-hopped. Table 2 provides the data on monoterpenes in the late-hopped beers, while the data for late-hopped and dry-hopped beers can be found in table 3.

Fig. 1 depicts the percentages of total monoterpenes remaining in the beers after 470 days of storage relative to the quantities initially present prior to storage according to equation 5:

$$\textit{Residual amount}\ [\%\ rel.] = \frac{\textit{concentration after storage}\ \left[\frac{\mu g}{l}\right]}{\textit{concentration prior to storage}\ \left[\frac{\mu g}{l}\right]} * 100\ \%$$

Equation 5

No significant difference between the two beers can be observed. In every case, the values were well below their respective sensory thresholds, which are on the order of 100 µg/l [6].

MONOTERPENES – LATE AND DRY-HOPPED BEERS [MG/L]

	Fresh	Storage period 470 d			
		0 °C	4 °C	20 °C	30 °C
β-myrcene	5.1	4.3	4.5	3.3	2.5
Limonene	2.2	1.7	1.7	1.5	1.1
cis-Ocimene	0.2	0.1	0.1	0.1	< 0.1
trans-Ocimene	1.3	0.7	0.8	0.8	0.6
γ-terpinene	0.2	0.1	0.1	0.1	< 0.1
Sum	**9.0**	**6.9**	**7.2**	**5.8**	**4.2**

Table 3

Carboxylic acid esters

The most important carboxylic acid esters derived from hops are provided in table 4 (late) and table 5 (late + dry). Losses were moderate at 0 °C over the 470 days but increased at 4 °C. Storage at 20 and 30 °C resulted in a significant reduction in the relevant hop esters. Isobutyl isobutyrate proved to be somewhat more stable than the others.

Fig. 2 presents the averaged values for the ten most important hop esters remaining in the beers after storage relative to the quantities initially present prior to storage. The graph illustrates the difference between the late-hopped and the dry-hopped beers. The aroma compounds in the late-hopped beer were present over the entire course of primary fermentation, during which the yeast was able to alter the compounds to a certain extent, e.g. transesterification of these substances into ethyl esters. Dry hopping was not carried out until after primary fermentation which could be the reason behind fewer losses. However, this should be studied in more detail.

Two things can be deduced from this:

The sensory thresholds of the esters are reported in the range of 5 to 100 µg/l [2, 10]. In the late-hopped beers, the analysis results for some of these esters were already well above these threshold values, and this was even more pronounced in the dry-hopped beers.

Aging the beers for approximately 15.5 months caused a significant reduction in the esters at 20 °C, which was even more striking at 30 °C. Some of the concentrations likely declined below their respective

CARBOXYLIC ACID ESTERS DERIVED FROM HOPS – LATE-HOPPED BEERS [MG/L]

	Fresh	Storage period 470 d			
		0 °C	4 °C	20 °C	30 °C
Isobutyl isobutyrate	49.8	51.9	42.0	31.6	21.1
Butyl isobutyrate	1.2	1.5	1.2	0.8	0.5
2-methylbutylpropanoate	6.5	7.6	5.6	5.7	4.8
3-methylbutyl 2-methylpropanoate	32.5	28.7	20.7	10.0	7.8
2-methylbutyl 2-methylpropanoate	392	331	227	108	81
Methyl 4-methylhexanoate	8.2	8.3	7.6	5.5	4.0
Ethyl 4-methylnonanoate	0.3	0.5	0.5	0.5	0.2
2-methylbutyl 3-methylbutanoate	8.1	7.9	5.1	2.6	0.9
2-methylbutyl 2-methylbutanoate	6.1	5.9	4.0	2.7	0.8
Ethyl 4-methyloctanoate	1.9	2.4	2.1	1.6	0.3
Sum	**507**	**446**	**316**	**169**	**121**

Table 4

CARBOXYLIC ACID ESTERS DERIVED FROM HOPS – LATE AND DRY-HOPPED BEERS [MG/L]

	Fresh	Storage period 470 d			
		0 °C	4 °C	20 °C	30 °C
Isobutyl isobutyrate	152	146	155	152	117
Butyl isobutyrate	4.2	4.4	4,8	3.0	2.4
2-methylbutylpropanoate	12.3	8.7	7.1	4.1	5.9
3-methylbutyl 2-methylpropanoate	108	94.5	96.1	35.6	34.3
2-methylbutyl 2-methylpropanoate	1109	945	915	337	319
Methyl 4-methylhexanoate	19.5	28.7	24.5	15.7	13.2
Ethyl 4-methylnonanoate	0.7	1.7	2,7	1.5	0.9
2-methylbutyl 3-methylbutanoate	25.9	28.7	29.4	18.4	6.7
2-methylbutyl 2-methylbutanoate	18.9	21.2	22.5	19.4	6.8
Ethyl 4-methyloctanoate	3.8	7.1	7.9	4.4	0.7
Sum	**1454**	**1286**	**1265**	**591**	**507**

Table 5

MONOTERPENE ALCOHOLS – LATE-HOPPED BEERS [MG/L]

	Fresh	Storage period 470 d			
		0 °C	4 °C	20 °C	30 °C
Linalool	17.1	17.8	16.4	16.9	8.2
α-terpineol	5.7	5.8	4.7	9.2	14.5
β-citronellol	13.7	14.2	13.4	12.0	6.8
Sum	**36.5**	**37.8**	**36.5**	**38.1**	**29.5**

Table 6

MONOTERPENE ALCOHOLS – LATE AND DRY-HOPPED BEERS [MG/L]

	Fresh	Storage period 470 d			
		0 °C	4 °C	20 °C	30 °C
Linalool	33.5	31.5	33.5	31.7	15.7
α-Terpineol	9.5	8.9	13.9	22.2	37.0
b-Citronellol	22.8	24.7	29.1	23.5	13.1
Sum	**65.8**	**65.1**	**76.5**	**77.4**	**65.8**

Table 7

sensory thresholds. Thus, a perceptible change in the sensory profile of beers within their shelf life is conceivable.

Monoterpene alcohols

The noteworthy findings of Takoi [8, 9] have already been mentioned in the section on transfer rates above. They describe the transformations of linalool to α-terpineol and geraniol to β-citronellol, which explains why geraniol was undetectable in the beers in this series, although significant amounts of geraniol were found in the Hüll Melon hops (2 mg/100 g). The analysis of the beers in the trial was therefore limited to linalool, α-terpineol and β-citronellol.

Tables 6 and 7 list these three monoterpene alcohols in the late-hopped beers and the beers that were late-hopped and dry-hopped, respectively. Linalool appears relatively stable even up to 20 °C. Only at 30 °C was a loss detectable, amounting to about half of its original quantity. The loss of β-citronellol over time was similar: stable up to 20 °C, losses at 30 °C. α-terpineol was only stable at low temperatures; at 20 °C, the concentration rose significantly, while at 30 °C, the quantity increased several times (late: 1.6x; dry: 3.9x). This increase in α-terpineol is explicable through the loss of linalool which would corroborate Qian's findings [11].

In addition, the linalool was separated into its stereoisomers R-linalool and S-linalool using gas chromatography. The results are presented in table 8, including the percentage of S-linalool in the total quantity of linalool. This transformation may have an impact on the aroma of the beer, as R-linalool is many times more potent in its sensory impact than S-linalool. A shift from R-linalool to S-linalool is thus associated with a significant reduction in the influence of linalool on the sensory characteristics of the beer. This would not be perceptible at 4 °C but becomes evident at higher temperatures.

Summary

In the first part of the study, the level of reproducibility for beer production in the 2 hl pilot brewery was determined [7]. In the results presented here, the data were assessed with reference to two technological parameters. The transfer rates of aroma compounds from hop pellets into a bottom-fermented beer was determined for late-hopped and dry-hopped beers. Since triple batches could be evaluated in each case, the transfer rates determined in these trials are considered to be relatively reliable within this production system. The transfer rates of the hop compounds (in percent relative to the original quantities) can be categorized as follows:

- Myrcene, β-caryophyllene and humulene: < 1 %;
- α-selinene + β-selinene: < 1 % (late), 1–2 % (dry);
- esters: 20–40 % (late), 40–80 % (dry);
- linalool: 60 % (late), 80 % (dry);
- sesquiterpene alcohols: 8 % (late), 10–50 % (dry).

Additionally, a batch of late-hopped beer and one of dry-hopped beer were each aged for 470 days at 0, 4, 20 and 30 °C. Afterwards, the most relevant hop aroma compounds were measured in the beers with the following results: the monoterpenes were relatively stable during cold storage (0 and

4 °C) but were reduced by about 25 percent of their original concentration at 20 °C, and about 50 percent at 30 °C. The quantity of carboxylic acid esters derived from hops drops by about ten percent at 0 °C. Relative losses increase to 60 to 70 percent at 20 °C and rise to over 70 percent at 30 °C. Only at 30 °C, did the total concentration of linalool wane significantly. α-Terpineol increased in a parallel manner. Citronellol underwent a noticeable decline only at 30 °C. Racemization of R-linalool, which is much more sensorially potent, to S-linalool was particularly evident at 20 and 30 °C.

A significant difference in the aging behavior of hop aroma compounds in late-hopped and dry-hopped beers could not be demonstrated. However, based upon the fact that the degradation rates of all aroma compounds are first-order reactions, it is clear that the composition of beer must undergo change within a standard minimum shelf life to such a degree that a perceptible shift in the sensory profile of the beer is inevitable. ■

R-LINALOOL AND S-LINALOOL CONCENTRATIONS – LATE AND DRY-HOPPED BEERS [MG/L]; ...

... the portion of S-linalool in percent relative to the total quantity of linalool

		Fresh		4 °C		20 °C		30 °C	
		Late	Dry	Late	Dry	Late	Dry	Late	Dry
R-linalool	µg/l	14	27	12	25	10	19	5	9
S-linalool	µg/l	2	4	3	6	4	9	5	8
Total linalool	µg/l	16	31	15	31	14	28	10	17
Percentage of S-linalool	% rel.	13	13	20	19	29	32	50	47

Table 8

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