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Reproducibility Trials in a Research Brewery and Effects on the Evaluation of Hop Substances in Beer

Part 3: Transfer Rates of Aroma Compounds from Hops to Beer and their Ageing Behaviour

The reproducibility aspect of fresh and moderately aged late and dry hopped beers was previously reported in the papers part 1 [1] and part 2 [2] respectively. The present paper (part 3) focuses on the transfer of several compounds from hops to beer and their behaviour during beer ageing at different temperatures for 470 days. Transfer rates were found to be different for the examined substance groups and whether the hops were applied in the brewhouse (late) or during maturation (dry hopped): mono- and sesquiterpene hydrocarbons < 1 % in late hopped beers, up to 2 % in dry hopped beers; esters: 20 to 40 % in late hopped beers, 40 – 80 % in dry hopped beers; linalool: 60 % in late hopped beers, 80 % in dry hopped beers; sesquiterpene alcohols: 7 – 18 % in late hopped beers, 10 – 50 % in dry hopped beers. A late and a dry hopped beer were aged for 470 days at 0 °C, 4 °C, 20 °C and 30 °C and analysed for several fermentation by-products, ageing carbonyls and relevant hop aroma compounds. The concentration of acetates and ethyl esters decreased significantly at 20 °C and 30 °C, higher alcohols showed good stability. The concentrations of all Strecker degradation products and furfural started to increase at 4 °C, with pronounced increases at 20 °C and 30 °C. Monoterpenes proved to be relatively stable when stored at low temperatures (0 and 4 °C); however, at 20 °C and 30 °C, lost approximately 25 % and 50 % of their initial concentrations. The hop-derived carboxylic acid esters in the beers decreased by ca. 10 % at 0 °C. The relative losses increased dramatically to 60 to 70 % at 20 °C, and to more than 70 % at 30 °C. Only at 30 °C there were significant losses of total linalool. By contrast, the concentration of α-terpineol increased. Similar to linalool, a significant decrease of the β-citronellol concentration was observed at 30 °C. The racemization of the flavour-active R-linalool to the more inactive S-linalool was particularly evident at the higher temperatures.

Descriptors: reproducibility, transfer rates, hop aroma compounds, beer ageing, ageing aldehydes

1 Introduction

In order to gain insight into the reproducibility of the beer production process of our pilot brewery a comparative study including a series of brewing trials was carried out and extensive analytical data on the produced beers was collected. The reproducibility aspect of fresh and moderately aged late and dry hopped beers was previously reported in the papers part 1 [1] and part 2 [2] respectively.

In part 1, a good level of reproducibility of the brewing process was reported based on the evaluations of fresh beers from the brewing trials conducted on a two-hectolitre scale [1]. Late hopped beers and the same beers with additional dry hopping were analysed for relevant hop derived compounds (bittering substances, polyphenols and hop oil volatiles) fermentation by-products and carbonyl compounds associated with beer ageing. For all of these attributes, it was demonstrated that the standard deviations for the three batches were either below or equal to those resulting from the analyses performed in triplicate. Therefore it was concluded that the design of the research brewery is well suitable for examining the impact of hopping parameters on the resulting beers.

For the trials in part 2, the beers were stored for a moderate duration of 240 days at 0 °C and –24 °C [2]. The primary goal of this

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investigation was to establish whether the earlier observed level of reproducibility also applies to aged samples. Furthermore, there was great interest in determining whether storing beer under sub-zero conditions would have any impact on the compounds in beer (in particular hop aroma substances). In summary, the reproducibility of the beers stored at 0 °C showed no differences compared to the fresh beers. With the exception of the carbonyl compounds associated with ageing, all volatile aroma compounds showed high stability over the storage period at 0 °C. However, the deep-frozen beers exhibited significant variations compared to the beers stored at 0 °C, which could be attributed to precipitation and/or adsorption. Contrary to popular opinion the freezing of beer samples is not an optimum conservation in any case.

The present paper (part 3) focuses on the transfer of several compounds from hops to beer. In addition, targeted trials were carried out to monitor the impact of beer ageing on these compounds, on fermentation by-products and on carbonyl compounds (aldehydes) generally associated with beer ageing. Accurate investigation of this matter is possible due to the good reproducibility demonstrated for the experimental trials as described above.

Although there have been a number of publications dealing with the transfer rates of aroma compounds from hops to beer, particularly within the field of dry hopping, none have allowed any general conclusions to be drawn. The reasons for this are attributable to the following factors:

- The transfer rate or yield of an individual aroma compound is based on its analysis in hops and beer. The sources of error for both analyses are often underestimated. A relative range of variation of up to ± 25 % should be applied when expressing the transfer rate [3]. As Biendl points out, an inter-laboratory comparison of the measurement of hop aroma compounds in beer has yielded sobering results [4].
- The yields of individual aroma compounds such as those obtained from dry hopping are influenced by many variables, including the hop variety utilized, the type of hop product, the degree of pellet compression, the intensity of circulation inside the tank, the hop dosing system, the temperature of the medium (beer) and the contact time [5–9].
- Despite the fact that the transfer of aroma compounds to beer by means of dry hopping has been addressed in numerous publications, information on yields for late hop additions to the wort, either at the end of the boil or into the whirlpool, is less common. Ultimately this is due to the fact that there are at present many different boiling systems on the market, which vary greatly in their degree of evaporation during wort boiling. The major goal of modern wort boiling is to achieve a sufficient evaporation of DMS and minimal energy consumption. Of course, this efficiency is also accompanied by a similar efficacy in eliminating volatile hop aroma compounds [5, 10, 11].
- A late hop addition cannot result in high levels of monoterpene and sesquiterpene hydrocarbons in beer as they have low solubility in it and, as a consequence, these compounds are present in concentrations far below or close to their flavour threshold. The soluble compounds from the oxygen fraction however have a greater chance of surviving the subsequent stages of beer production (e.g. CO_2 stripping during fermentation, the filtration process) even when the wort is held at high temperatures for an extended period in the wort kettle and/or in the whirlpool. Groups of compounds belonging to the oxygen fraction include terpene alcohols (e.g. linalool), carboxylic acid esters (e.g. isobutyl isobutyrate), ketones (e.g. 2-undecanone) and oxygenated sesquiterpenes (e.g. humulene epoxides and humulenol).

The full spectrum of research findings regarding the transfer rates of hop aroma compounds reported in the literature is not exhaustively addressed here, but can be found in the references [12–14]. Since most of this research was conducted on the analysis of samples from single batches, rather than analysis of batches performed in triplicate, we have included the transfer rates in our reproducibility trials for three beers that were only late hopped and three beers that were additionally dry hopped. The transfer rates between those measured in beers that were late hopped on the hot side versus those that were dry hopped on the cold side are differentiated.

One aspect, which needs to be addressed is the (in)stability of hop aroma compounds in beer throughout the ageing process since literature data on this specific topic is rather fragmentary and contradictory.

For example, when looking at linalool as a marker compound for hoppy aroma in beer, several studies point to a considerable [15, 19] or moderate decrease [16, 21] of the linalool level, whereas other studies reported no significant changes [3, 18, 22, 23] or even an increase in its level upon beer storage [20, 23]. Interestingly, *Kaltner* et al. [18] and *Forster* et al. [22] reported the change in enantiomeric distribution of linalool during beer ageing. It was found that the S-enantiomer of linalool increases at the expense of the R-enantiomer and, given the significant higher flavour activity of the R-enantiomer, the racemization impacts the sensory profile of the beer.

Data on the behaviour of the full spectrum of hop oil derived compounds during beer ageing is rather scarce, however, significant losses of mono- and sesquiterpene hydrocarbons have been reported for instance by Forster and *Gahr* [3] and *Peacock* and *Deinzer* [16] whereas moderate to significant decreases in the content of hop oil derived esters and the oxygenated sesquiterpenoid hop oil fraction were found in beer ageing studies performed by Forster et al. [3, 22] and *Van Opstaele* et al. [23, 26]. The observed decreases in the level of hop oil derived volatiles could be ascribed to chemical degradation because of reaction with oxygen and acid hydrolysis, migration into the packaging and adsorption on bottle cap liners [16, 17, 22].

Taken together, literature data on the behaviour of individual hop aroma components is rather scarce and contradictory, which could be ascribed to many parameters which possibly impact the results, such as the beer matrix, storage times and temperatures applied in the ageing studies, type of crown cork liner used, ….

In the study described in this paper, a late hopped beer and the same late hopped beer with an additional dry hopping were stored for 470 days at 0 °C, 4 °C, 20 °C and 30 °C. The beers were analysed for several hop aroma compounds as well as for a number

of fermentation by-products and carbonyls (aldehydes) associated with beer ageing. Since the previous trials were conducted with three batches of wort, and it has been demonstrated that this process exhibited a decent level of reproducibility [1, 2], it was deemed acceptable to analyse single beers in this series of trials.

2 Materials and methods

The systematic procedure for producing beer on the two-hectolitre pilot system at the St. Johann brewery was described in detail in part 1 of the reproducibility trials [1]. The trials involved brewing a bottom-fermented, all-malt beer; the beer was late hopped with Huell Melon pellets at a rate of 300 g/hl. This corresponds to a dosage of 2.4 ml of hop oil per hectolitre. After primary fermentation, the batches were divided, with one half receiving a dry hop addition of 150 g/hl of Huell Melon (1.2 ml oil/hl). Details regarding the analysis of the fermentation by-products, ageing carbonyls and hop aroma compounds can be found in references [24–27]. The standard deviation obtained from the three-fold analysis of a particular compound was compared to the standard deviations for the three batches brewed for the trials. For those of a comparable order of magnitude, no additional variation due to the three batches was determined which indeed was true for the majority of the results.

2.1 Measuring the transfer rates of aroma compounds from hops to beer

The contribution made by a hop addition at the beginning of the boil (approx. 62 g of Herkules (HKS) pellets) to the aroma compounds in beer is considered negligible. This has been confirmed in previous trials carried out on the pilot system indicating that the aroma transfer is minimal, e.g. less than 7 µg/l for linalool, when relatively high quantities of hops are added at the beginning of the boil [28].

The aroma compounds contributed by the additions of Huell Melon (late hopping and dry-hopping) were analysed according to MEBAK [29].

The quantity of an aroma compound resulting from a hop addition is calculated as follows:

$$Dosage\ (Quantity)\ \left[\frac{\mu g}{l}\right] = addition\ \left[\frac{g}{l}\right] \times concentration\ of\ a\ compound\ \left[\frac{\mu g}{g}\right]$$

The transfer rate (TR) or yield of a compound in beer brought about by a late hop addition can be calculated as follows:

$$TR_{\ late}\ [\%] = \frac{value\ in\ beer,\ late\ hopped\ \left[\frac{\mu g}{l}\right]}{dosage\ (quantity)\ added\ to\ wort\ \left[\frac{\mu g}{l}\right]} * 100\%$$

Moreover, the transfer rate attributable to late and dry hopping, expressed as a percentage, can be calculated likewise:

$$TR_{\ late+dry}\ [\%] = \frac{value\ in\ beer,\ late + dry\ hopped\ \left[\frac{\mu g}{l}\right]}{quantity\ added\ via\ late\ + dry\ hopping\ \left[\frac{\mu g}{l}\right]} * 100\%$$

There was no exclusively dry hopped beer. In order to measure the transfer rate brought about by dry hopping only, the value obtained through analysis of the late hopped beer can be subtracted from the value obtained through analysis of the late and dry hopped beer:

$$TR_{\ dry}\ [\%] = \frac{value\ in\ beer,\ late + dry\ hopped\ \left[\frac{\mu g}{l}\right] - \ value\ in\ beer,\ late\ hopped\ \left[\frac{\mu g}{l}\right]}{quantity\ added\ via\ dry\ hopping\ \left[\frac{\mu g}{l}\right]} * 100\%$$

In the present trials, the transfer rates were determined from the analysis of the fresh batches performed in triplicate, which increases the reliability of the results compared to those obtained from single batches.

2.2 Ageing of the beers

In part 2, we reported that moderate ageing of beers with a strong

Table 1 Content of hop oil volatiles in hops and late and dry hopped beers, calculated dosage levels and transfer rates

Hop volatiles	Content [mg/100g]	Dosages [µg/l]		Value in beer [µg/l]		Transfer rate [% rel.]		
		late	dry	late	dry	late	dry	av. late & dry
β-myrcene	13	390	195	4.1	5.6	1.1	0.8	1
β-caryophyllene	8	240	120	1.2	1.1	0.5	-	<1
α-humulene	8	240	120	5.1	4.4	2.1	-	2
β-selinene	58	1740	870	10.5	30.1	0.6	2.3	1
α-selinene	44	1320	660	3.0	9.8	0.2	1.0	<1
isobutyl isobutyrate	4	120	60	48.2	98.1	40.2	83.5	55
2-methylbutyl propanoate	4	120	60	6.0	13.1	5.0	11.8	7
3-methylbutyl 2-methylpropanoate	7	210	105	40.4	87.4	19.2	44.8	28
2-methylbutyl 2-methylpropanoate	46	1380	690	352	897	25.5	78.9	43
linalool	1	33.0	16.5	20.8	34.4	63.0	82.4	70
cubenol	2	66	33	4.9	8.6	7.4	11.2	9
α-eudesmol	5	150	75	12.8	35.2	8.5	30.0	16
α-cadinol	2	60	30	10.9	26.5	18.2	52.0	29

hop aroma profile over a period of 240 days at 0 °C had a very low impact on the level of volatile fermentation by-products and hop aroma compounds. The only exceptions were the ageing-related compounds, in particular Strecker aldehydes of which the levels increased slightly (but significantly) during the trial period.

Since the high level of reproducibility of the produced beers had already been established, the brews were limited to a late hopped and late and additionally dry hopped beer. Both beers were stored for 470 days at 0 °C, 4 °C, 20 °C and 30 °C. The maximum variation in temperature was ± 1 °C. All the beers were analysed at the end of the storage period.

All the aged samples were analysed at the same time. Along with the absolute concentrations of volatile aroma compounds, the changes in the total amounts of various chemical compound groups such as monoterpene hydrocarbons, monoterpene alcohols and carboxylic acid esters were calculated in this study. The sesquiterpene hydrocarbons and sesquiterpenoids in the stored samples could not be reliably measured at the time of analysis.

Table 2 **Fermentation by-products; values reported as the mean of the values in late and dry hopped beers [mg/l]**

	fresh	Storage time 470 d			
		0 °C	4 °C	20 °C	30 °C
n-propanol	12.4	10.2	9.8	11.2	10.0
isobutanol	8.4	8.2	9.4	9.0	8.1
3-methylbutanol	36.3	38.3	40.3	39.6	39.5
2-methylbutanol	9.2	9.8	9.3	10.1	10.0
ethyl acetate	17.4	15.2	15.7	13.9	11.9
isobutyl acetate	0.024	0.022	0.022	0.015	0.013
isoamyl acetate	0.833	0.823	0.829	0.640	0.603
phenylethyl acetate	0.231	0.181	0.180	0.149	0.143
ethyl butanoate	0.072	0.065	0.068	0.059	0.057
ethyl hexanoate	0.107	0.049	0.049	0.037	0.020
ethyl octanoate	0.193	0.169	0.174	0.147	0.121
ethyl decanoate	0.047	0.049	0.049	0.034	0.020
Sum	**85.2**	**83.1**	**85.9**	**84.9**	**80.5**

3 Results

3.1 Transfer rates (TR)

Table 1 contains the following data:

- Concentration of hop compounds (Huell Melon) in mg/100 g
- Dosage of each compound as a late hop addition and a dry hop addition in µg/l
- Analysis results for the late hopped and dry hopped beers in µg/l
- The transfer rates (TR), calculated according to the formula in 2.1:
  - for the late hopped beers (TR late)
  - for dry hopping (TR dry)
  - and finally the average TR from both additions (TR late + dry)

The results can be summarized as follows:

- The transfer rates for the selected hydrocarbons are extremely low (β-myrcene, β-caryophyllene, α-humulene and both selinenes: 2 % max).
- Of the four esters analysed in these trials, TR for three of them ranged from 19 to 40 % in the late hopped beers; only the TR of 2-methylbutyl propanoate was remarkably lower (5 %). Dry hopping doubled the yield of the ester content (TR ranging from 45 to 83 %).
- The TR of linalool was 63 % (late hopped beers) and 82 % (dry hopped beers). In these trials, it did not emerge whether glycosidically-bound linalool in the hops could be released in wort or beer as described by *Kollmansberger* [30].
- The TR of sesquiterpene alcohols ranged from 7 to 18 % (late hopped beers) and from 11 to 52 % (dry hopped beers).

An interesting aspect regarding the monoterpene alcohols should be stated here. Next to linalool, Huell Melon hops also contain significant levels of geraniol (2 mg/100 g) while compounds such as nerol, β-citronellol and α-terpineol are absent. However, despite its presence in hops, geraniol could not be detected in the produced beers, but relevant concentrations of α-terpineol (5 and 10 µg/l, respectively) and β-citronellol (14 and 21 µg/l, respectively) were measured. According to *Takoi* et al. [31, 32] yeast enzymes are able to convert geraniol into β-citronellol and linalool into α-terpineol which could possibly explain the detection of these compounds in the produced beers. In summary, Takoi [32] was able to track the following changes throughout the beer production process:

- From wort to beer, the linalool content dropped by approximately 50 %.
- Geraniol was reduced by approximately 90 %.
- β-Citronellol was present in trace amounts in hops and therefore also in this quantity in hopped wort. However, the concentration rose greatly over the course of fermentation, which can be primarily attributed to the formation from geraniol.
- α-Terpineol was absent in the hops and consequently, it was only detected in trace amounts in the wort. The concentration also increased during fermentation, which was ascribed by Takoi to the conversion of linalool.
- Nerol was only present in trace amounts in hops and exhibited no relevant changes during fermentation.

Since the degree of changes affecting linalool and geraniol did not reflect those reported in the literature and were not identical to those observed in earlier trials [3], it can be presumed that the enzyme activity of the brewing yeast was not comparable.

Table 3 Aldehydes in late and dry hopped beers [µg/l]

	fresh		Storage time 470 d							
			0 °C		4 °C		20 °C		30 °C	
	late	dry	late	dry	late	dry	late	dry	late	dry
2-methylpropanal	6.2	8.4	6.6	23.9	11.9	77.5	63.6	179	96.9	225
2-methylbutanal	1.5	0.7	2.9	1.4	3.0	1.9	4.2	4.5	15.7	16.1
3-methylbutanal	4.6	1.5	8.9	5.0	7.0	5.3	9.0	7.1	18.1	18.0
methional	4.0	2.5	3.9	2.2	7.4	5.8	10.9	10.5	19.7	18.8
phenylacetaldehyde	7.9	3.6	8.5	4.0	17.6	13.5	24.6	18.3	31.4	28.4
hexanal	0.08	0.07	0.08	0.07	0.07	0.07	0.09	0.18	1.0	0.9
furfural	6.3	8.1	6.7	8.3	11.6	11.9	17.7	108	741	828
Sum	**30.6**	**24.9**	**37.6**	**44.9**	**58.7**	**116**	**130**	**327**	**924**	**1135**

3.2 Ageing behaviour of volatile aroma compounds in beer

3.2.1 Fermentation by-products

Table 2 displays the concentrations of fermentation by-products in the fresh and stored experimental beers. No significant differences were observed between the concentrations of the volatiles in the fresh late and dry hopped beers on the one hand and aged late and dry hopped beers at the respective temperatures, on the other, and therefore the mean values are reported in the table. In general it is concluded that the concentration of the higher alcohols after prolonged storage is highly comparable to the levels found in the fresh beer, even at relatively high storages temperatures of 20 °C and 30 °C. However, the concentration of the acetates (e.g. ethyl acetate, isoamyl acetate and phenylethyl acetate) and ethyl esters significantly decreased, in particular when the samples are stored at higher temperatures (by 31 % at 20 °C and 43 % at 30 °C). These findings are in accordance with data reported by *Lustig* [33] who found that within the groups of fermentation by-products, there was no relevant change to the alcohols in beer during the ageing process while the concentrations of several esters decreased. Interestingly, our data show highly comparable concentrations for all the fermentation by-products stored at 0 °C and 4 °C.

3.2.2 Aldehydes associated with ageing

In fresh beers, the aldehyde content served to confirm that the bottling process was uniform with low oxygen uptake [1]. This research was not actually intended to evaluate the behaviour of carbonyl compounds in beer during the ageing process. However, since the beers were already analysed in a fresh condition, it made sense to extend the analysis to the aged samples as well. In particular, it was of interest to establish whether differences could be observed between late hopped and dry hopped beers. Table 3 lists seven relevant compounds found in fresh and aged samples. The standard deviations of the analyses are similar to those published in part 1 and 2, and therefore not listed. The very low values for trans-2-nonenal (< 0.02 µg/L) are not mentioned. They show a tendency to lower levels in aged samples for which currently no explanation exists. Only 2-methylpropanal, a compound from the Strecker degradation process, exhibited a significant difference between the late hopped and the additionally dry hopped beers. Possible explanations are discussed in the following.

Table 4 Strecker aldehydes and their precursors [21]

Precursor	Strecker aldehyde
valine	2-methylpropanal
isoleucine	2-methylbutanal
leucine	3-methylbutanal
phenylalanine	phenylacetaldehyde
methionine	methional

Table 5 Amino acid content [mg/100 g] in 5 hop varieties

	Huell Melon	Saphir	Hers-brucker	Herkules	Amarillo
valine	21.5	17.9	21.1	16.9	24.5
alanine	141	142	139	131	109
arginine	245	197	78.2	136	187
asparagine	1218	1057	789	972	1171
glutamine	136	72.0	52.6	73.0	75.0
glutamic acid	77.8	43.8	29.5	42.3	43.4
glycine	11.1	10.6	12.6	8.0	8.8
isoleucine	5.0	4.1	4.5	4.3	5.3
methionine	0.5	0.2	0.4	0.8	0.8
proline	50.3	101	114	58.2	42.3
serine	30.2	27.0	30.7	18.6	19.5
threonine	47.3	35.9	37.1	28.3	40.2
tryptophan	30.1	35.0	25.4	28.5	40.8
tyrosine	38.4	8.9	11.5	13.9	15.0
aspartic acid	35.2	23.4	19.6	20.8	18.4
leucine	3.4	2.3	2.8	2.4	2.7
phenylalanine	25.7	20.1	19.9	17.6	16.7
histidine	87.4	57.4	42.8	47.1	63.2
lysine	24.8	11.0	10.2	12.2	15.6
Sum	**2229**	**1866**	**1440**	**1632**	**1899**

Additional oxygen introduced via the dry hop addition could potentially be responsible for the increase in the concentration of

2-methylpropanal, but this was eliminated as a potential source on the basis of regular oxygen measurements. The theory that dry hopping imparts additional reductive capacity to beer could not be confirmed based on the findings in these trials. It would have been more likely that lower values for ageing aldehydes were to be expected in dry hopped beers.

According to [24, 34], the precursors of the Strecker aldehydes are the corresponding amino acids, as shown in the comparison in table 4. *Wietstock* et al. [35] demonstrated that a general relationship exists between amino acids as the precursors in beer and the formation of Strecker aldehydes. Consequently, recommendations are made to avoid excessive levels of free amino nitrogen (FAN) in beer. If the hops contain relevant amounts of these amino acids, these are dosed accordingly when dry hopping them. If dry hopping takes place after primary fermentation, as was the case in these trials, it is questionable whether the yeast can still metabolize these compounds. If not, these compounds could serve as precursors for ageing aldehydes.

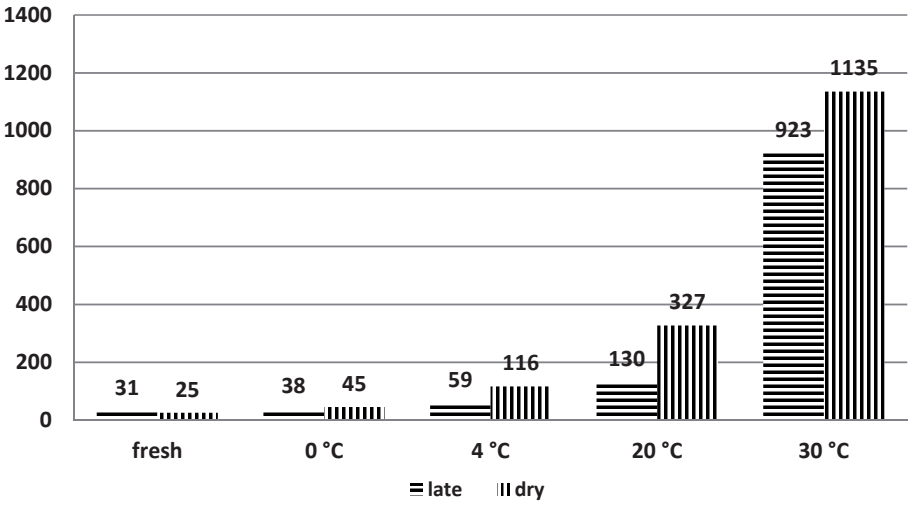

Fig. 1 **Total aldehyde content in late hopped beers and dry hopped beers at different storage temperatures [µg/l]**

As early as 1966, *Kloos* [36] shared finding regarding more than 19 amino acids in hops, including those considered to be precursors of Strecker aldehydes. Using the modest analytical methods available at that time, it was concluded that amino acids make up approximately 40 % of the total nitrogen content in hops. Of these, asparagine is the predominant compound. Since more recent studies on the content of amino acids in hops were not available, five hop samples (crop 2017) and two beer samples were analysed for amino acids using an HPLC MS/MS method [37] in our study.

Table 5 shows the content of 19 amino acids measured in the five hop samples, including the Huell Melon variety. The individual amounts as well as the sums of amino acids vary among the hop samples within a moderate range, the sum differing from 1.44 to 2.23 % by weight. Assuming an average protein content of 15 to 20 % by weight in hops [38], the amino acid fraction comes to approximately 10 %. This corresponds to the proportions present in malt containing approximately 10 % protein and 0.7 to 0.8 % FAN [39].

With a dry hopping rate of 150 g/hl Huell Melon 33 mg/l of amino acids are added. The amounts of amino acids, which can serve as precursors in the hops, vary from 0.5 mg/100 g (methionine) to 26 mg/100 g (phenylalanine). The values for the relevant amino acids in two beers (late and additionally dry hopped) show no significant differences (Table 6) and lie minimum two orders of magnitude above the calculated dry hopping additions. Hence, it is questionable whether the amino acids in hops can really contribute to the formation of Strecker aldehydes when they are introduced in the process by dry hopping. This aspect is definitely worthy of further exploration.

Wietstock et al. [35] also indicated that iron (Fe) and copper (Cu) can promote the oxidative degradation of amino acids via their catalysing effect in the formation of hydroxyl radicals. Hops contain approximately 100 mg/kg Fe [5] and may contain up to 1000 mg/kg of Cu (maximum residue level EU). So with dry hopping substantial quantities of these metal ions are dosed into the lagering beer. On the other hand considerable amounts of bitter substances especially alpha acids are dosed into the beer and are said to be able to defang these metal ions by chelation. Whether metal ions get solubilized in beer during dry hopping and to what extent they can contribute to beer ageing has not been studied yet.

The oxidative degradation of iso-α-acids is also involved in the formation of carbonyl compounds as beer ages. In particular,

Table 6 **Amino acid content in the hops used, beers and dry hopping addition rate**

	Beer [mg/l]		Addition dry [mg/l]	Content hops [mg/100 g]
	late	late & dry		
valine	65.5	62.0	0.323	21.5
isoleucine	18.1	15.8	0.075	5.0
methionine	3.2	3.3	0.008	0.5
leucine	39.2	28.9	0.051	3.4
phenylalanine	71.4	59.1	0.386	25.7
Sum	**197.4**	**169.0**	**0.8**	**56.1**

Table 7 **Monoterpene hydrocarbons in late hopped beers [µg/l]**

	fresh	Storage time 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
terpinolene	0.1	0.1	0.1	0.1	0.1
Sum	**7.0**	**6.1**	**6.1**	**5.4**	**3.8**

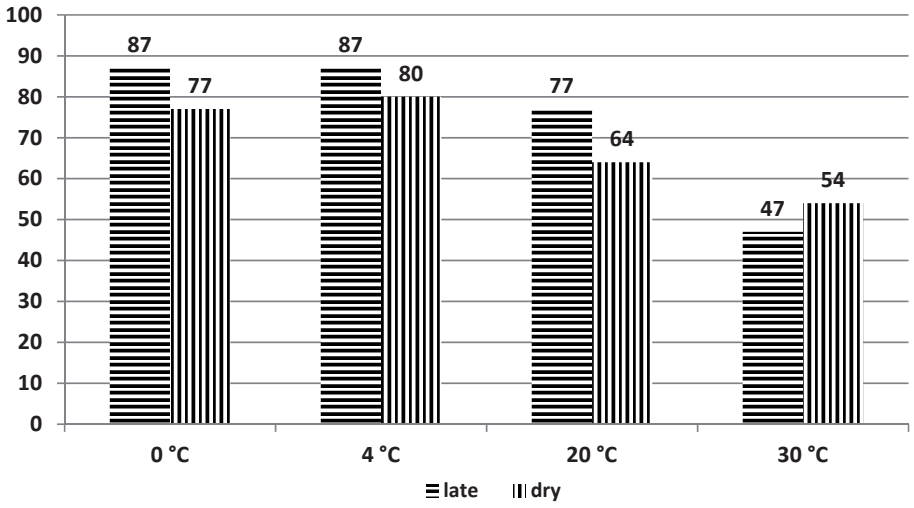

Fig. 2 **The remainder of total monoterpenes in % (relative to the initial concentration) measured in samples aged for 470 days, classified by temperature and divided into late hopped and dry hopped beers**

trans-iso-α-acids significantly decrease during ageing [40]. *De Clippeleer* [25] affirms an active role of iso-α-acids in the formation of 2-methylpropanal. A pathway has been postulated for the formation of 2-methylpropanal from allo-iso-n-humulone under alkaline and to a lesser extent even under slightly acidic conditions. But this does not explain the differences between late and additionally dry hopped beers as no considerable amounts of iso-α-acids are dosed via dry hopping. Whether other bitter acids (alpha and beta-acids) or their oxidation products (e.g. humulinones, hulupones, humulinic acids etc.) [34] as precursors are involved in the formation of 2-methylpropanal is not yet investigated.

Taking into account the discussed possibilities a plausible explanation for the significant higher values for 2-methylpropanal in dry hopped beers couldn´t be found.

The values for furfural in the beers seem to be plausible besides the one in the late hopped beer that has been stored at 20 °C, which looks far too low. The difference between the two beers stored at 30 °C is not significant when taking into account the standard deviations (741 ± 64 ppb for late vs. 830 ± 82 ppb for dry hopped).

Temperatures during ageing above 20 °C lead to a considerable increase of staling aldehydes. The extreme effects of temperature are reflected in figure 1, which depicts the total quantity of seven aldehydes associated with ageing. The value of 20 °C, late hopped has been excluded from the figure because of the questionable value for furfural.

3.2.3 Hop aroma compounds

Monoterpene hydrocarbons

The hop aroma compounds are differentiated in the tables according to their presence in late and dry hopped beers. Table 7 lists the data for the monoterpenes in late hopped beers, while table 8 lists the corresponding data for dry hopped beers. The losses brought about by ageing are lower than those previously published [3]. This can be attributed to the effect of the scavenger crown caps (Pelliconi) used in these trials. Cold storage for 15 months lowered the monoterpene content by less than 20 % relative to the initial concentration. For storage temperatures of 20 °C and 30 °C, the losses increased to around 30 % and 50 %, respectively.

Figure 2 illustrates the remaining amount of the total monoterpenes (relative to the initial concentrations) in beer measured after storage for 470 days. A significant difference between both beers cannot be derived from the data. In all cases, the values are well below those of the corresponding flavour thresholds [5, 41, 42], e.g. β-myrcene 30 – 1000 ppb [43].

Carboxylic acid esters

Takoi et al. [44, 45] have emphasized the importance of hop esters recently. They traced the three key carboxylic acid esters during fermentation of the wort and on through to the finished beer. A

Table 8 **Monoterpene hydrocarbons in late and additionally dry hopped beers [µg/l]**

	fresh	Storage time 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
terpinolene	0.2	0.1	0.1	0.1	< 0.1
Sum	**9.0**	**6.9**	**7.2**	**5.8**	**4.2**

Table 9 **Hop-derived ester compounds in late hopped beers [µg/l]**

	fresh	Storage time 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-methylbutyl propanoate	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**

decrease in esters of 40–50 % was offset by the formation of ethyl esters, which have a much lower sensory (aroma) threshold than the methyl esters. Whether a further decrease in methyl esters correlates with the formation of ethyl esters during the ageing of beer can only be surmised.

Carboxylic acid esters originating from hops and detected in the beers in this study are provided in table 9 (late hopped beers) and table 10 (dry hopped beers). Clearly, the total ester content of the dry hopped beers is about 3 times higher than in the late hopped beers. The losses were quite moderate for beers stored at 0 °C and 4 °C for 470 days but increased significantly starting at 20 °C and led to a considerable reduction of the relevant hop esters especially at 30 °C.

The mean values (expressed in % relative to the initial concentrations) for the remaining concentrations of the hop esters after 470 days storage at the respective temperatures are depicted in figure 3. A clear difference between the late hopped and the dry hopped beer samples was observed in that the decrease in the ester concentration is less pronounced in the dry hopped beers. This observation should be examined in more detail.

Two conclusions can be derived from the data:

- The sensory threshold of the esters is usually cited in a range of 5–100 µg/l [5, 42, 44, 45]. The analysis values of some of the esters in the late hopped beers already exceeded these limits, while the values for the dry hopped beers were even more pronounced. Thus, next to monoterpene alcohols, hop oil esters contribute undoubtedly to the characteristic aroma of late and dry hopped beers.
- Ageing the beers for almost 16 months at 20 °C and especially at a temperature of 30 °C lead to a significant decrease of the ester contents. The values for some of the esters are even found to be below the sensory threshold. This means that a change in the flavour profile of the beer is to be expected before the beer reaches the end of its shelf life.

Table 10 Hop-derived ester compounds in late and additionally dry hopped beers [µg/l]

	fresh	Storage time 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-methylbutyl propanoate	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-methylenehexanoate	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**

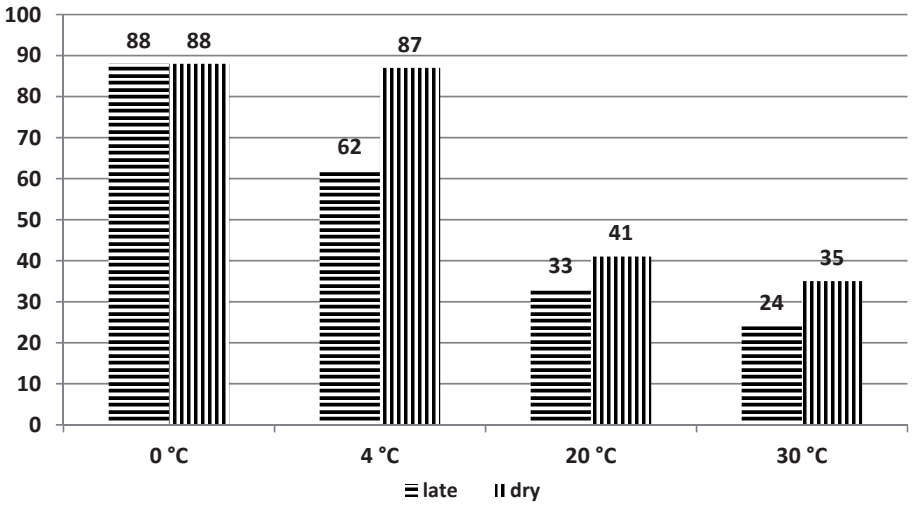

Fig. 3 The remainder in % (relative to the initial concentration) of the total quantities of the ten most relevant hop esters

Table 11 Monoterpene Alcohols in late hopped beers [µg/l]

	fresh	Storage time 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
citronellol	13.7	14.2	13.4	12.0	6.8
Sum	**36.5**	**37.8**	**36.5**	**38.1**	**29.5**

Table 12 Monoterpene Alcohols in late and additionally dry hopped beers [µg/l]

	fresh	Storage time 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
citronellol	22.8	24.7	29.1	23.5	13.1
Sum	**65.8**	**65.1**	**76.5**	**77.4**	**65.8**

Monoterpene alcohols

The important results reported by Takoi [31, 32] regarding the conversion of linalool to α-terpineol and geraniol to β-citronellol have already been discussed in section 3.1 (transfer rates). This explains why geraniol was not detected in the beer brewed in these trials, despite the fact that relevant quantities of the compound were found in the Huell Melon hop variety (2 mg/100 g). The evaluation of the beers was therefore limited to linalool, α-terpineol and β-citronellol.

The three monoterpene alcohols, found in the late hopped and dry hopped beers, are listed in tables 11 and 12. According to the data, linalool appears to be relatively stable, even at 20 °C. Starting at 30 °C, a loss of approximately half of the content is observed. β-Citronellol exhibits similar behaviour (stable up to 20 °C, losses at 30 °C). α-Terpineol shows a different pattern: good stability is observed at low temperatures, however, when stored at 20 °C, the content of α-terpineol increases significantly. This increase is even more pronounced at a storage temperature of 30 °C, at which point the content is several times the initial value (late hopped beers: 1.6 fold; dry hopped beers: 3.9 fold). This increase in α-terpineol can be explained by the loss of linalool, confirming the findings of *Qian* [46], who made similar observations.

Table 13 **Values of R- and S-Linalool in µg/l in late and dry hopped beer; S-ratio in % rel. of S- vs. R+S-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
S-ratio	% rel.	13	13	20	19	29	32	50	47

Linalool was also separated into its stereoisomers R-linalool and S-linalool using gas chromatography [21]. The results of the analysis, including the proportion of S-linalool in relation to total linalool, expressed in percent, are given in table 13. Clearly, the enantiomeric distribution of linalool (13 % S-linalool, 87 % R-linalool in the fresh beers) changed significantly during the storage of the beers and was most pronounced at higher temperatures (50 % S-linalool, 50 % R-linalool after 470 days at 30 °C). The degree of conversion was highly comparable at each temperature for the late and dry hopped beers. The conversion of R-linalool into S-linalool undoubtedly affects the sensory aroma characteristics of the beer, as the sensory impact of R-linalool is considerably higher than that of S-linalool.

4 Summary

In the first part of this study, the level of reproducibility of beer brewed on a two-hectolitre pilot system was determined. This reproducibility was subsequently confirmed in the second part of the study by moderately ageing (240 days, 0 °C) the beers brewed on this system. Of the aldehydes associated with ageing, only several Strecker degradation products exhibited an increase as the beers in the trial were aged. In this third and final part, data collected regarding the transfer rate of hop aroma compounds from pellets to beer was evaluated for late hopped beers and late hopped beers which were additionally dry hopped.

The transfer rates are expressed in % of the quantities added and were grouped as follows:

- mono- and sesquiterpene hydrocarbons: < 1 % in late hopped beers, up to 2 % in dry hopped beers.
- esters: 20 to 40 % in late hopped beers, 40–80 % in dry hopped beers.
- linalool: 60 % in late hopped beers, 80 % in dry hopped beers
- sesquiterpene alcohols: 7–18 % in late hopped beers, 10–50 % in dry hopped beers.

Furthermore a late hopped and a dry hopped beer were aged for 470 days at 0 °C, 4 °C, 20 °C and 30 °C and analysed for several fermentation by-products, ageing carbonyls and relevant hop aroma compounds yielding the following results:

- Of the fermentation by-products analysed, the concentration of acetates and ethyl esters decreased significantly compared to the concentration in the fresh beer at 20 °C and 30 °C, whereas the higher alcohols showed good stability.
- The concentrations of all Strecker degradation products and furfural started to increase at 4 °C, with pronounced increases at 20 °C and 30 °C. The fatty acid degradation products remained relatively stable.
- In the dry hopped beers, the concentration of 2-methylpropanal increased by many times more than that found in late hopped beers. There is no straightforward explanation for this. Whether the amino acids found in hops and introduced to the beer through dry hopping can actually act as precursors for Strecker aldehydes must be examined more closely. An initial analysis of the amino acids in hops actually showed values that are too low to serve as an explanation for this phenomenon. Furfural exhibits this tendency, but to a lesser extent.
- The monoterpenes proved to be relatively stable when stored at low temperatures (0 and 4 °C); however, at 20 °C and 30 °C, they lost approximately 25 % and 50 % of their initial concentrations, respectively.
- The hop-derived carboxylic acid esters in the beers decreased by about 10 % at 0 °C. The relative losses compared to the initial concentrations increased dramatically to 60 to 70 % at 20 °C, and to more than 70 % at 30 °C.
- Only at 30 °C the total linalool content suffered significant losses. By contrast, the concentration of α-terpineol increased. Similar to linalool, a significant decrease of the β-citronellol concentration was observed at 30 °C.
- The racemization of the flavour-active R-linalool to the more inactive S-linalool was particularly evident at 20 °C and 30 °C.

A significant difference in the ageing behaviour of late hopped and dry hopped beers, except for particular hop derived esters could not be determined in these trials. Nonetheless, it is clear – given that the degradation rate of all aroma compounds occurs as a first-order reaction – the composition of the beer does change throughout the shelf life of the product, so that consequences with regard to the sensory profile of the beer remain inevitable.

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