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RESEARCH ARTICLE

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Studies on the odorant concentrations and their time dependencies during dry-hopping of alcohol-free beer

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Abstract

Both the market for alcohol-free beer and the number of craft beer breweries increased in the past years. Thus, dry-hopping as a possibility to compensate/mask aroma deficiencies of alcohol-free beers is gaining more and more interest. To better understand the transfer of odorants from hops into alcohol-free beer during dry-hopping, their concentrations were monitored over a period of 7 days in a laboratory-scale experiment simulating the dry-hopping process. Thereby, a main transfer occurred during the first 2-3 days. However, the relevance of the transferred odorants to the overall aroma can only be evaluated by taking their sensory properties into consideration. Therefore, orthonasal odour thresholds and sensorial dose-response relationships were investigated in an alcohol-free beer matrix. Sensory tests showed a nearly linear increase in the odour intensities of different esters, whereas further typical hop odorants, such as linalool, geraniol, and myrcene, showed a multistage increase. Overall, the present study clearly corroborated that dry-hopping of alcohol-free beers can be optimised regarding the hop dosage and the contact time, depending on the desired flavour of the final product.

KEYWORDS
alcohol-free beer, aroma transfer, contact time, dose-response relationship, dry-hopping

1 | INTRODUCTION

Hops as beer ingredient offer different possibilities to modify the aroma of the final beer. Therefore, many studies focused on the key odorants of different hop varieties, to evaluate their potential for the brewing industry. By applying the molecular sensory science concept, linalool, myrcene, 3-methylbutanoic acid, and geraniol were identified as important aroma-active compounds in hop varieties.[1-3] For some hops, variety-specific odorants were identified like ethyl 2-methylbutanaote in Huell Melon or 4-mercapto-4-methylpentan-2-one in US varieties like Cascade, Citra, and Eureka.[4] Especially by late- or dry-hopping, higher concentrations of these hop odorants can be achieved in the final product. So that the transfer of hop odorants is not affected by evaporation during wort boiling, a more intense hoppy aroma can be achieved by hop addition into the whirlpool or in the cold area. While, among others, 3-methyl-1-butanol, 2-phenylethanol, 2-methoxy-4-vinylphenol, and 2- and 3-methylbutanoic acid were identified as important key odorants in different beer types,[5,6] hop key odorants like linalool, geraniol, or 4-mercapto-4-methylpentan-2-one were shown as highly important for the typical aroma of dry-hopped beers.[4,7-9] Also, different esters like ethyl 3-methylbutanoate and ethyl methylpropanoate were demonstrated to be present in concentrations above their odour thresholds in regular and alcohol-free dry-hopped beers.[7,10,11]

In the past years, the market for alcohol-free beers strongly increased. But the industry is still facing some challenges in regard to alcohol-free beers. On the one side, thermally dealcoholised beers are lacking in the typical aroma and in the full body elicited by regular beers. On the other side, the sweeter taste along a wort-like odour is an issue of alcohol-free beers produced by stopped fermentation.[12] With a growing number of craft beer breweries, dry-hopping also gained more importance for low alcohol and alcohol-free beers as an opportunity to increase the popularity and acceptance of these beer types. While studies on dry-hopping of alcoholic beers[7,13-15] and of low alcohol beers[16] have already shown a clear improvement of beer attributes like full body and bitter quality, in parallel with the compensation of aroma deficiencies due to the lower alcohol content, limited data are available for the transfer of odorants into alcohol-free beer. In a very recent study, it has been shown that dry-hopping can be a possibility to mask aroma deficits of alcohol-free beers, and therefore, increase its consumer's acceptance. Thereby, especially linalool, geraniol, myrcene, and ethyl esters of hop-derived monocarboxylic acids were identified as important contributors to the aroma of alcohol-free beer after dry-hopping.[10]

Recent studies on aroma-active compounds in beer established odour thresholds only in an alcoholic beer matrix or in model solutions.[17-19] For alcohol-free beer, Piornos et al. published orthonasal and retronasal odour thresholds of typical beer odorants like 3-methyl-1-butanol, 2-methoxy-4-vinylphenol, or 2-phenylethanol in a model alcohol-free beer, also considering different calculation methods. Thereby, a comparison of the retronasal thresholds in alcohol-free beer to data in alcoholic beer showed lower thresholds in alcohol-free beer on the one side, but most of the time higher thresholds compared to those in water on the other side.[20]

Perpète and Collin studied the retention of odorants in dependency of the alcohol and sugar contents, with a higher retention of aldehydes in solutions with a higher ethanol content. In addition, a 'salting out' effect in solutions with higher sugar content, as it is likely in alcohol-free beers after stopped fermentation, was shown.[21]

Beside the effects of alcohol or sugar contents on the release of odorants, and thus, on the respective odour thresholds, also chirality can have an influence on the sensory perception of odorants. It is well established that enantiomers can clearly vary in their sensory properties. Beside differences in their odour qualities, like the well-known example of (*S*)-carvon ('caraway-like') and (*R*)-carvon ('spearmint-like'), also big differences in their odour thresholds are well-known.[22] For hops, the enantiomeric distribution of linalool is well examined,[8,23] whereas enantiomeric ratios of other chiral hop odorants have not been published yet.

Thus, the aims of the present study were first the evaluation of the time dependency of the transfer of odorants during dry-hopping into alcohol-free beer. Second, sensory experiments like the determination of odour thresholds and dose-response relationships were performed in alcohol-free beer to get an insight into changes of sensory properties of different odorants depending on their respective concentrations. In addition, the enantiomeric ratios were determined in hops and alcohol-free beer for all sensorial investigated odorants, as their odour characteristics can be very different.

2 | EXPERIMENTAL

2.1 | Beer samples

Wort and beer samples for the determination of the enantiomeric ratios of chiral odorants were provided by the Chair of Brewing and Beverage Technology (Technical University of Munich, Freising, Germany). Samples were produced via stopped fermentation and dry-hopped on a vibration table for one week with hop pellets, equivalent to 1.5 mL of hop oil/hL, as described very recently.[10] For all other experiments, different alcohol-free beers were purchased at local supermarkets.

2.2 | Hop pellets

Hop pellets type 90 of Hallertauer Mandarina Bavaria (HMB), harvest year 2015, were provided by Hopsteiner (Mainburg, Germany). The pellets were stored under vacuum at −20 °C prior to use, ensuring the aroma quality. Identification and quantitation of the odorants have been performed in a previous study.[1]

2.3 | Chemicals

The following odorants were obtained commercially: linalool and (*R*)-linalool (Fluka, Neu-Ulm, Germany); ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl methylpropanoate, geraniol, methyl 2-methylbutanoate, (*S*)-methyl 2-methylbutanoate, and myrcene (Sigma-Aldrich, Taufkirchen, Germany); and propyl 2-methylbutanoate (TCI, Eschborn, Germany).

The following chemicals were purchased from commercial sources: liquid nitrogen (Linde, Munich, Germany) and diethyl ether, ethanol, and anhydrous sodium sulphate (Merck, Darmstadt, Germany).

2.4 | Stable isotopically labelled internal standards

[$^2H_6$]-Myrcene (Santa Cruz Biotechnology, Dallas, TX) was obtained commercially.

[$^2H_5$]-Ethyl 2-methylbutanoate, [$^2H_9$]-ethyl 3-methylbutanoate, [$^2H_5$]-ethyl methylpropanoate, [$^2H_3$]-methyl 2-methylbutanoate, and [$^2H_3$]-propyl 2-methylbutanoate were synthesized by esterification of the respective labelled carboxylic acid with the respective alcohol.[24] [$^2H_2$]-Geraniol[25] and [$^2H_{2-3}$]-linalool[23] were synthesized as recently described.

2.5 | Laboratory-scale dry-hopping of alcohol-free beer

Seven portions of hops (150 mg each) were filled into tea bags and hung into an amber glass bottle (1 L). Alcohol-free beer (700 mL) was added and stirred at room temperature for 7 days. Each 24 hours, hops (1 bag) and beer (100 mL) were removed from the bottle and the odorant concentrations were determined. The experiment was conducted in two technical duplicates.

2.6 | Quantitation by stable isotope dilution analysis (SIDA)

The isolation and quantitation of the volatiles in alcohol-free beer were performed as recently reported.[10]

2.7 | Two-dimensional high-resolution heart-cut gas chromatography-mass spectrometry (HRGC/HRGC-MS)

For quantitation of geraniol, linalool, and myrcene, HRGC/HRGC-MS was performed as recently described.[10] For the determination of the enantiomeric ratios of ethyl 2-methylbutanoate, linalool, methyl 2-methylbutanoate, and propyl 2-methylbutanoate, a chiral capillary column (BGB-175 or BGB-176; both 30 m × 0.25 mm i.d., 0.25 μm film thickness; BGB Analytik, Boeckten, Switzerland) was used in the second GC.

2.8 | Solid phase microextraction-comprehensive high-resolution gas chromatography-time-of-flight mass spectrometry (SPME-HRGCxHRGC-TOF-MS)

For quantitation of several esters, the SPME-HRGCxHRGC-TOF-MS approach was applied following the recently published procedure.[10]

2.9 | High-resolution gas chromatography-olfactometry (HRGC-O) and odour thresholds in air

Determination of odour thresholds in air was performed following a formerly published protocol.[26] For this, a recently described HRGC-O system[1] equipped with a chiral BGB-176 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; BGB Analytik) and (*E*)-2-decenal as the internal standard with an odour threshold of 2.7 ng/L air[27] were used.

2.10 | Odour thresholds in water and in alcohol-free beer

Orthonasal odour thresholds were determined via three-alternative forced-choice tests as recently described,[28] using either water or alcohol-free beer as the matrices.

2.11 | Sensorial dose-response relationships of odorants in alcohol-free beer

A solution of each odorant was prepared in ethanol and added separately to alcohol-free beer. Thereby, concentrations in beer were approximately 100 μg/L for all esters, 4800 μg/L for geraniol and linalool, and 3200 μg/L for myrcene, leading to concentrations about 50-100 times higher than previously determined in dry-hopped alcohol-free beer.[10] This stock solution was diluted with alcohol-free beer stepwise 1 + 2 (v + v). Seven to 10 of these dilution steps were prepared, depending on the applied concentration range starting always with the lowest concentration below the known odour threshold in alcohol-free beer and ending with an odorant-specific chosen maximum concentration. These solutions were presented in increasing concentrations to a trained sensory panel consisting of at least 10 weekly trained persons. Assessors were asked to rate the intensity of the odorant in the solutions on a line scale (15 cm), as described by the German Agricultural Society (DLG, Deutsche Landwirtschafts-Gesellschaft).[29] Starting and end point of the scale were represented by reference solutions. For the starting point, alcohol-free beer without the addition of an odorant was used; for the end point, a solution of the maximal concentration of the odorant, that was used in the sensory test, was presented. For data processing, the ratings of each assessor were standardised to values between 0 and 10.

3 | RESULTS AND DISCUSSION

3.1 | Evaluation of a suitable matrix for analytical and sensory experiments

For the laboratory-scale dry-hopping experiments and sensory tests, first, the concentrations of the target odorants were determined in 6 different commercially purchased alcohol-free beers. The beer with the lowest concentrations of these analytes was chosen as the matrix for the following experiments. Thereby, beer **F** showed the lowest concentrations for all analytes, which were also below the respective odour thresholds in water (except for linalool, with only a slightly higher concentration and ethyl 2-methylbutanoate with < LOQ that was slightly above the respective odour threshold; Table 1).

3.2 | Time dependency of the transfer of odorants during dry-hopping

A laboratory-scale experiment was chosen to evaluate the time dependency of the odorant transfer from hops into alcohol-free beer during dry-hopping. Every 24 hours, the concentrations of eight different odorants (ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl methylpropanoate, geraniol, linalool, methyl 2-methylbutanoate, myrcene, and propyl 2-methylbutanoate) were determined, which have already been identified as important odorants in hops and dry-hopped alcohol-free beer.[1,10]

For geraniol, linalool, and myrcene, the maximal transfer rates were reached after 2-3 days (Figure 1). Afterwards, a decline in concentrations was observed. But while the concentrations of geraniol and linalool reached a stable level after one week (geraniol around 100 μg/L; linalool around 35 μg/L), for myrcene a constant decrease was noticeable. The latter might be explained

TABLE 1 Concentrations of selected odorants in different commercially purchased alcohol-free beers. Odour thresholds in water are given for comparison

Odorant	Concentrations (μg/L)						Odour threshold in water[a] (μg/L)
	Beer A	Beer B	Beer C	Beer D	Beer E	Beer F	
linalool	72.8	7.34	3.13	2.11	1.77	1.20	0.58
myrcene	21.1	0.80	0.14	0.13	0.17	0.10	1.2
geraniol	6.90	0.89	0.90	0.89	0.20	0.03	1.1
ethyl methylpropanoate	0.790	0.393	0.023	0.098	0.753	< 0.012	0.089
propyl 2-methylbutanoate	0.297	<0.051[b]	<0.051[b]	<0.051[b]	<0.051[b]	<0.051[b]	0.15
ethyl 3-methylbutanoate	0.149	0.173	0.013	0.066	0.166	< 0.007	0.023
methyl 2-methylbutanoate	0.149	0.038	<0.029	<0.029	<0.029	< 0.029	0.048
ethyl 2-methylbutanoate	0.068	0.071	<0.011[b]	0.023	0.085	<0.011[b]	0.008

[a]Data from an in-house database obtained via three-alternative forced-choice tests.
[b]Limit of quantitation (LOQ) was determined based on a signal-to-noise ratio of 10.

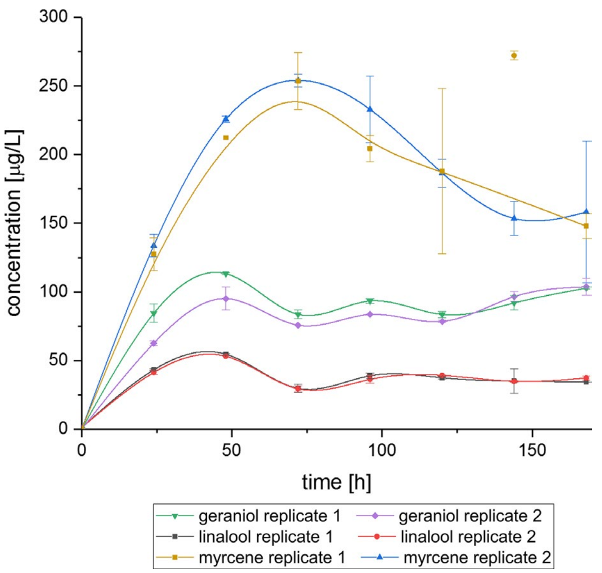

FIGURE 1 Time-dependent transfer of geraniol, linalool, and myrcene during dry-hopping of alcohol-free beer. [Colour figure can be viewed at wileyonlinelibrary.com]

by adsorption processes to yeast cells or crown caps during storage.[30,31] A quite similar course of the curve for linalool and geraniol was already shown for dry-hopped alcoholic beer in different production scales.[32] In a semi-industrial scale, a maximal transfer of linalool and geraniol in the first 24 hours and a slight decline of the concentrations in the following days were reported.[32] An increase of geraniol concentration at the end of the dry-hopping period can be attributed to the use of the hop variety Mandarina Bavaria, that has been shown to have high contents of geranyl acetate, acting as geraniol precursor.[10] In a laboratory-scale experiment, also a decline in the myrcene concentration was observed between days 1 and 7.[32] In contrast, Wolfe et al. showed almost constant concentrations of linalool and myrcene during a dry-hopping period of 7 days.[33]

For all analysed esters, transfer rates up to 91%, referred to the concentrations in hops, were obtained during the first 2-3 days (Figure 2). After days 3-4, an additional formation of ethyl and methyl esters started (Figure 2A-D). This phenomenon of a hop-induced ester formation has been recently described.[11] As the reaction type has not yet been completely clarified, it cannot finally be explained, which external factors caused the differences between the two replicates. Nevertheless, it was observed that a higher formation rate of methyl esters seems to correlate with a lower rate of ethyl esters (except for propyl 2-methylbutanoate with a maximal transfer rate of 60% within the first 24 hours, followed by a constant decrease of its concentration; Figure 2E). In a very recent study, it has been reported that the propyl ester is not affected by the hop-induced ester formation.[11]

As it has already been shown, that the aroma transfer during dry-hopping, especially of myrcene and linalool, is highly dependent on the batch size,[32] it is noteworthy, that in the actual study, the concentrations of all odorants were at the same level as it was the case for dry-hopped alcohol-free beers, that have been analysed in a previous study using the same hop pellets.[10] Thus, the results of the laboratory-scale experiment should be comparable to a bigger research scale approach. Nevertheless, parameters like the headspace volume in the dry-hopping tank or the way of assuring a distribution of the hops (by circulation, vibration table, manual turn-over, HopGun®, etc) had an influence on the odorant transfer,[32] while pellet properties like density and particle size did not have a significant influence on the mass transfer.[33]

3.3 | Enantiomeric ratio of chiral aroma compounds in different alcohol-free beers

Since decades, it is well-known that enantiomers can clearly differ in their odour qualities and odour thresholds.[22] Therefore, the ratio of (*R*)- and (*S*)-enantiomers of ethyl 2-methylbutanoate, linalool, methyl 2-methylbutanoate, and propyl 2-methylbutanoate

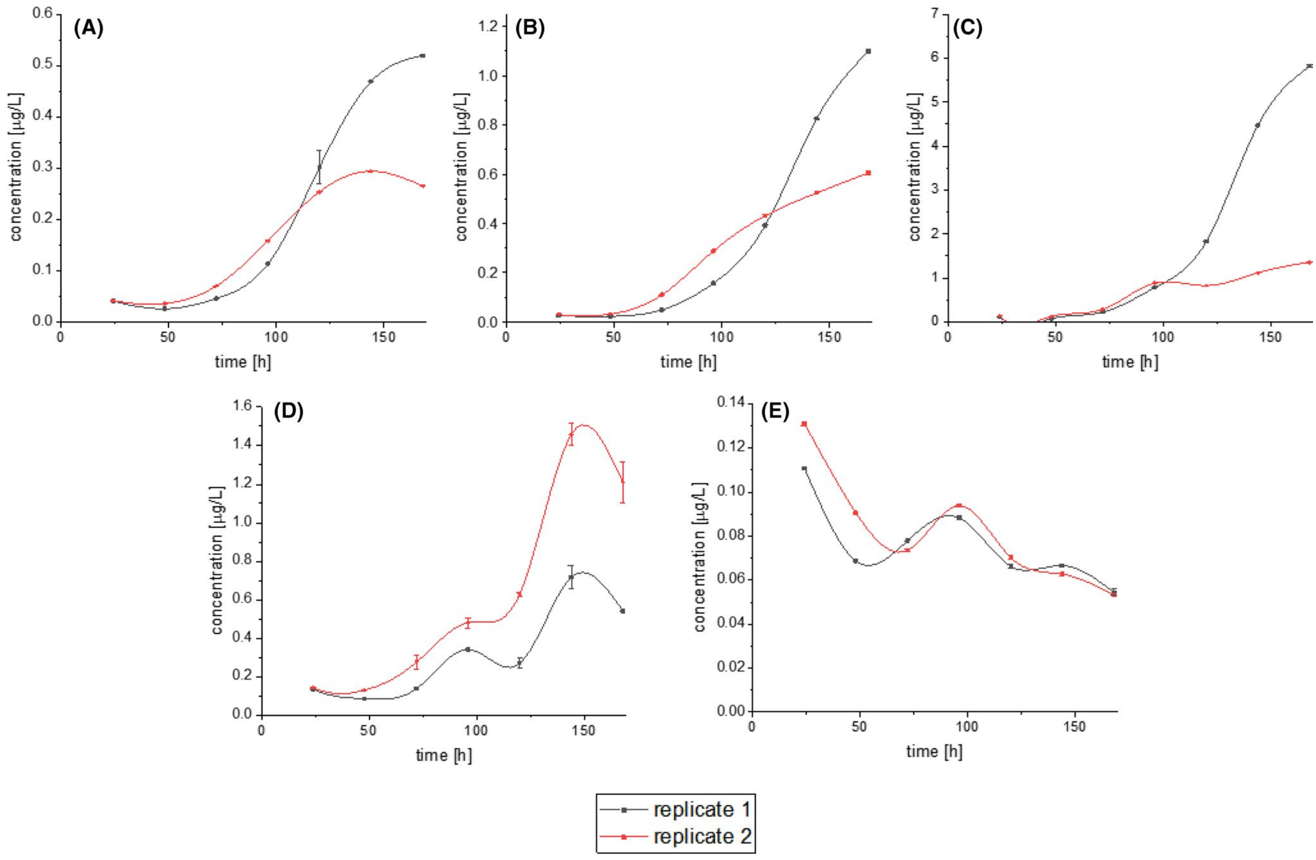

FIGURE 2 Time-dependent transfer of ethyl 2-methylbutanoate (A), ethyl 3-methylbutanoate (B), ethyl methylpropanoate (C), methyl 2-methylbutanoate (D), and propyl 2-methylbutanoate (E) during dry-hopping of alcohol-free beer. [Colour figure can be viewed at wileyonlinelibrary.com]

were determined in different hop varieties, wort, and beer samples (Figure 3; Table 2). For the following sensory experiments, either the quantitatively predominant enantiomer in the dry-hopped samples (for linalool and methyl 2-methylbutanoate) or the racemate (for ethyl 2-methylbutanoate, which was nearly present in a racemic distribution in dry-hopped beers (beers 3 and 4; Table 2), and for propyl 2-methylbutanoate, due to the lack of enantiopure (*S*)-propyl 2-methylbutanoate) were used.

By comparing the enantiomeric ratios in the analysed wort and beer samples after stopped fermentation, the influence of the fermentation can be evaluated. While for methyl 2-methylbutanoate, no changes were observed, propyl 2-methylbutanoate changed from 100% of the (*S*)-enantiomer in wort to a racemic mixture after fermentation (beers 1 and 2; Table 2). Also, ethyl 2-methylbutanoate showed mainly the (*S*)-enantiomer in wort, while the (*R*)-enantiomer was predominant after fermentation. These data were of interest due to the fact that in a recent study, nearly 100% of (*S*)-ethyl 2-methylbutanoate was reported in different types of beer.[34] For linalool, only minor changes occurred during fermentation (Table 2). For fully fermented beers that can be dealcoholised by different physical methods (e.g. heat treatment, nanofiltration, or dialysis), the changes in the enantiomeric ratios of odorants could also be influenced, but the performed experiments clearly showed the influence of fermentation on the enantiomeric distribution.

In all hop samples, only or mainly the (*S*)-enantiomer of the different esters were present. Consequently, the dry-hopping process (beers 3 and 4; Table 2) led to a clear shift in the enantiomeric ratios in favour of the (*S*)-enantiomer of ethyl 2-methylbutanoate and propyl 2-methylbutanoate in the beer samples. In contrast, (*R*)-linalool (ratio not determined in the actual study) was already known as the predominating enantiomer in hops.[8] Accordingly, 92% and 90% of (*R*)-linalool were determined in dry-hopped beers (beers 3 and 4; Table 2) compared to only 75% and 70% in the corresponding non-hopped beers (beers 1 and 2; Table 2), which was in accordance with earlier studies on hopped beer.[8,23]

3.4 | Sensory experiments I: Odour thresholds in air and in alcohol-free beer

To evaluate the orthonasal odour perception of important aroma-active compounds in dry-hopped alcohol-free beer, odour thresholds in an 'analyte-free' matrix (beer F) were determined. Based on the enantiomeric ratios in different beer samples, for linalool ((*R*)/(*S*) = 92/8 and 90/10) the (*R*)-enantiomer, for methyl 2-methylbutanoate ((*R*)/(*S*) = 100/0) the (*S*)-enantiomer, and for ethyl 2-methylbutanoate ((*R*)/(*S*) = 57/43) the racemate were used (beers 3 and 4, Table 2). Both enantiomers of ethyl 2-methylbutanote showed an

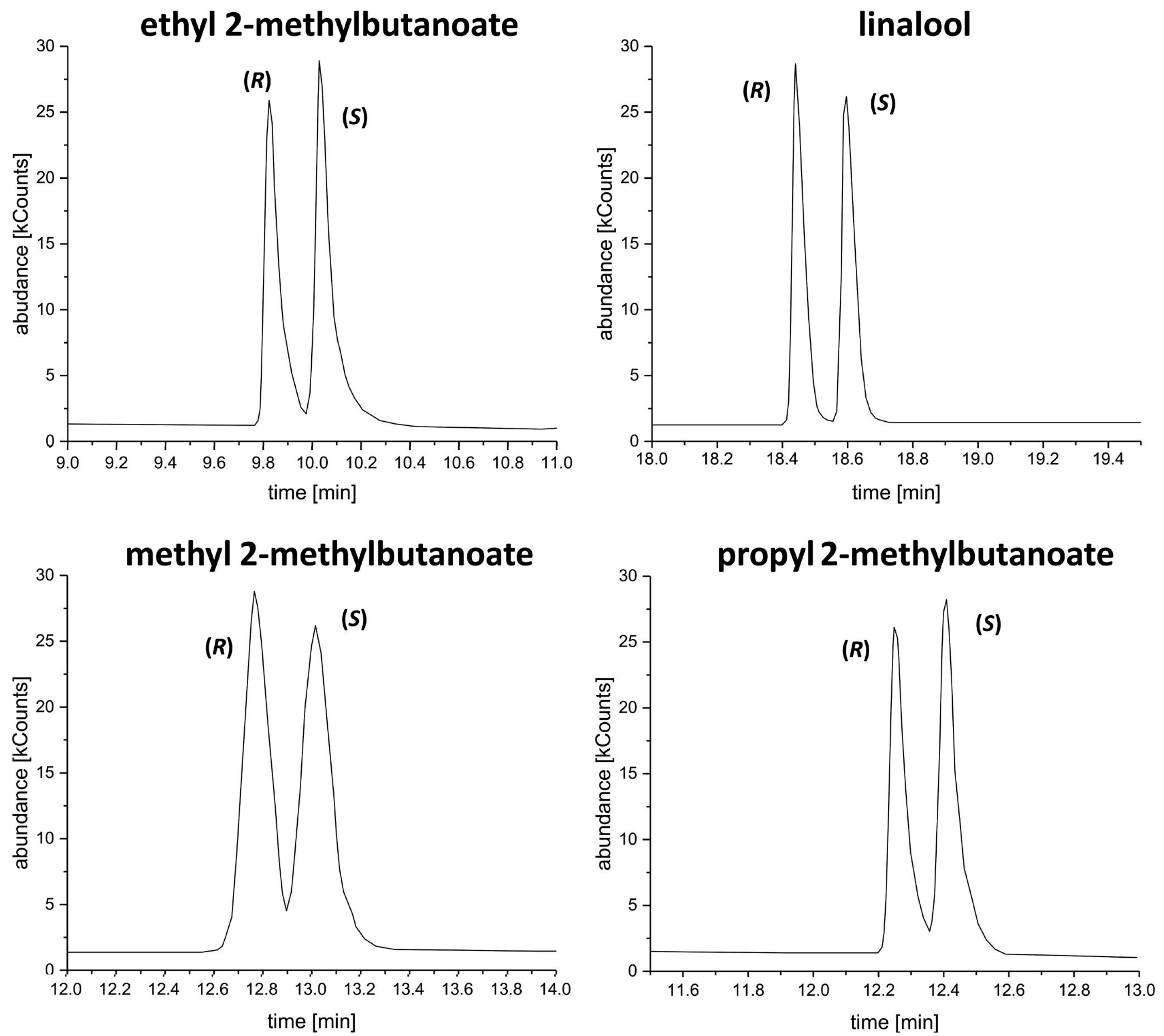

FIGURE 3 Separation of reference racemates (ethyl 2-methylbutanoate, linalool, methyl 2-methylbutanoate, and propyl 2-methylbutanoate) on a chiral GC capillary column

odour threshold in air of 1.27 ng/L air and the same odour quality. Thus, it can be assumed that they are likely showing the same sensory properties also in other matrices, which has also been shown in a previous study.[34]

For propyl 2-methylbutanoate, no enantiopure reference was available. To assess the differences of the two enantiomers, the odour thresholds in air of each enantiomer were determined after GC separation. Thereby, (*S*)-propyl 2-methylbutanoate showed an odour threshold of 6.08 ng/L air, whereas the threshold of (*R*)-propyl 2-methylbutanoate was >1215 ng/L air. Due to this high discrepancy, the sensory experiments were performed with the racemate (consequently, for threshold determination, only the half of the applied amount was considered), as there should be no sensory influence of the (*R*)-enantiomer.

As shown in Table 3, odour thresholds in alcohol-free beer were 4 (ethyl methylpropanoate: 0.34 μg/L in alcohol-free beer vs 0.089 μg/L in water) to 65 ((*S*)-propyl 2-methylbutanoate: 1.3 μg/L in alcohol-free beer vs 0.02 μg/L in water) times higher compared to the respective odour thresholds in water. Also in a recent study, Piornos et al. reported that orthonasal odour thresholds of aroma compounds in an alcohol-free model beer were most of the time higher than in water.[20] The present data clearly corroborated the influence of the beer matrix, and of the sensory complexity in general, on the perception of different odorants.

3.5 | Sensory experiments II: Dose-response relationships

It is also well accepted that the odour quality of aroma-active compounds can change in dependency of the respective concentrations.

TABLE 2 Enantiomeric ratios of chiral odorants in hop varieties, wort, and beers after stopped fermentation

	Enantiomeric ratios (%)							
	ethyl 2-methylbutanoate		linalool		methyl 2-methylbutanoate		propyl 2-methylbutanoate	
Sample	(*R*)	(*S*)	(*R*)	(*S*)	(*R*)	(*S*)	(*R*)	(*S*)
HMB[a]	7	93	nq	nq	0	100	0	100
HCA[b]	3	97	nq	nq	0	100	0	100
HHA[c]	1	99	nq	nq	2	98	0	100
Wort 1	0	100	89	11	0	100	0	100
Wort 2	13	87	64	36	0	100	0	100
Beer 1[d]	70	30	75	25	0	100	49	51
Beer 2[e]	72	28	70	30	0	100	50	50
Beer 3[f]	43	57	92	8	0	100	0	100
Beer 4[g]	43	57	90	10	0	100	23	77
Sensory tests[h]	racemate		(*R*)		(*S*)		racemate	

[a]Hallertauer Mandarina Bavaria.
[b]Hallertauer Cascade.
[c]Hallertauer Mittelfrüh.
[d]Beer 1 = top-fermented beer after stopped fermentation.
[e]Beer 2 = bottom-fermented beer after stopped fermentation.
[f]Beer 3 = beer 1, dry-hopped with Hallertauer Mandarina Bavaria.
[g]Beer 4 = beer 2, dry-hopped with Hallertauer Mandarina Bavaria.
[h] Selected odorant(s) for the sensory experiments.
nq = not quantitated.

TABLE 3 Comparison of odour thresholds in water and in alcohol-free beer

	Odour thresholds (μg/L)	
Odorant	In water[a]	In alcohol-free beer
myrcene	1.2	76
geraniol	1.1	19
(*R*)-linalool	0.58	4.7
ethyl methylpropanoate	0.089	0.34
(*S*)-methyl 2-methylbutanoate	0.048	2.5
ethyl 3-methylbutanoate	0.023	0.24
(*S*)-propyl 2-methylbutanoate	0.020	1.3
ethyl 2-methylbutanoate	0.014	0.24

[a]Data from an in-house database obtained via three-alternative forced-choice tests.

Thus, sensorial dose-response experiments were performed to evaluate (a) such possible changes in the alcohol-free beer matrix and (b) the possible sensorial impact of these odorants to the overall aroma of dry-hopped alcohol-free beers in dependency on their concentrations, which might affect not only the odour intensity but also the odour quality.

Myrcene, present at maximal concentrations of approximately 250 μg/L in the transfer experiments before declining to about 150 μg/L, showed a plateau in the sensory curve between 40 and 120 μg/L (Figure 4A). Intensities > 0 below the respective odour threshold can be explained by single panellists, whose personal threshold was lower than the average threshold. A relevant increase in odour intensity started at about 120 μg/L, and thus, a higher hop dosage can definitively lead to a strengthened odour perception. The panellists described the odour quality in alcohol-free beer in this concentration range as 'floral, green', higher concentrations led to a typical 'hoppy, geranium-like' odour, enabling not only an intensified aroma, but also the possibility to get a clearly hoppy odour note by a higher hop dosage during dry-hopping.

Also for (*R*)-linalool, a plateau in the sensory dose-response relationship was determined for concentrations at around 100 μg/L of alcohol-free beer (Figure 4A). The concentrations in the actual transfer experiments were between 40 and 50 μg/L, which led to a 'citrus-like, fruity' aroma. In higher concentrations (>500 μg/L), the odour quality changed to an unpleasant 'soapy' impression. Thus, the content of linalool (known as a key odorant in alcoholic dry-hopped beer[8]) of the used hop variety must be considered when choosing the hop dosage for dry-hopping, as a maximal transfer is already reached after 2-3 days and should not exceed a certain concentration to avoid an undesired aroma note (Figure 4A).

Geraniol showed a quite flat increase in its odour intensity, except for the concentration range between 60 and 170 μg/L. As the dry-hopped beer samples showed amounts of about 100 μg/L, a relevant increase in its odour intensity can be reached by the use of hop varieties either high in geraniol or in its precursor geranyl acetate or by the increase of the hop dosage. The odour was described as 'floral, rose-like' at concentrations around 100 μg/L (Figure 4A).

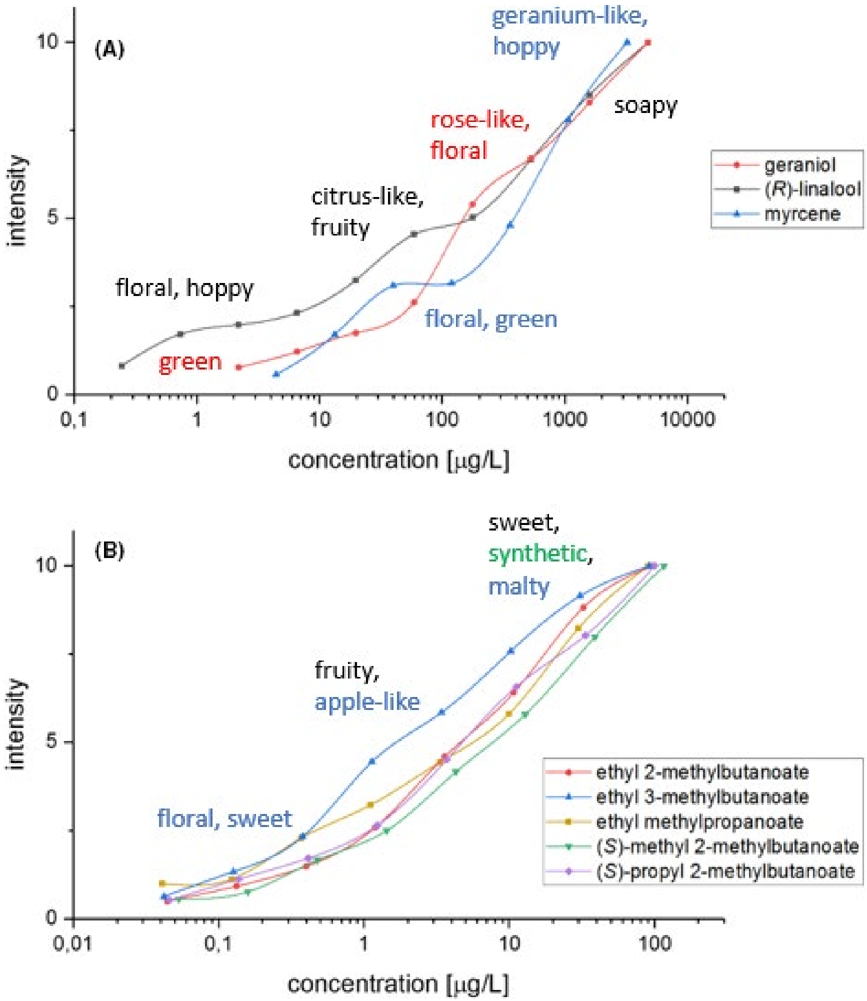

FIGURE 4 Sensorial dose-response relationship of geraniol, (*R*)-linalool, and myrcene (A) and of ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl methylpropanoate, (*S*)-methyl 2-methylbutanoate, and (*S*)-propyl 2-methylbutanoate (B; black odour descriptors related to all esters, coloured odour descriptors are related to the specific esters) in alcohol-free beer. [Colour figure can be viewed at wileyonlinelibrary.com]

All analysed esters showed a nearly linear increase in regard to odour perception with increasing concentrations, without any plateau formation (Figure 4B). The odour quality was described as 'fruity' at lower concentrations and changed to 'sweet' notes at higher concentrations (100 µg/L). Thus, also for the esters, a too high amount must be avoided, but is normally not reached by common practices. Since the major amount of these esters in alcohol-free beer is caused by the hop-induced ester formation[11] and not by the transfer during dry-hopping, a higher hop dosage does not necessarily lead to an increase in the odour intensity.

On the one side, these dose-response relationships clearly proved the opportunities of dry-hopping to influence and vary both the odour quality and intensity of alcohol-free beers, which can help to increase their acceptance by consumers. On the other side, detailed knowledge is necessary to avoid too high concentrations of certain odorants, which can finally lead to an aroma disliked by the consumers, as shown for linalool and the esters.

4 | CONCLUSION

In summary, this study gives a deeper insight into the sensorial impact of hop odorants on the aroma of alcohol-free beer. It is known that transfer rates during dry-hopping of alcohol-free beer do not differ too much from those of alcoholic beer.[10] But since the aroma profiles of alcohol-free beers strongly differ from those of regular beers,[10] depending on various production and dealcoholisation processes, the sensory influence of the transferred hop odorants can be totally different in alcohol-free beers. By knowing the sensory properties of important key odorants of hops in the alcohol-free beer matrix, the dry-hopping process can be optimised to reach and modify desired aroma characteristics in the final product, and to specifically mask undesired odour properties of alcohol-free beers. Sensory data like odour thresholds and dose-response relationships in alcohol-free beer can help to decide about the amount of hop dosage used for dry-hopping, depending on the desired intensities of aroma qualities like 'citrus-like' or 'fruity'. The results confirmed that a higher hop dosage is not always the method of choice to obtain, for example a typical citrus-like odour note in alcohol-free beers, as the odour quality of (*R*)-linalool can change to an undesired 'soapy' odor attribute in higher concentrations. The present study was the first on the time dependency of the aroma transfer during dry-hopping of alcohol-free beer, while this issue has already been examined in alcoholic beer.[32,33] It was shown that a longer contact time does not necessarily increase the transfer of linalool, myrcene, or geraniol into beer, but can be used if a higher concentration of 'fruity' esters is desired. Using this knowledge, the dry-hopping process can also be optimised in regard to the contact time to receive, for example, a more pronounced hoppy aroma in alcohol-free beers, which can lead to a higher consumer's acceptance of this beer type.

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CONFLICTS OF INTERESTS

The authors declare no competing interests.

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