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How alcohol content in dry-hopped beer affects final beer composition – a model study

The technique of dry hopping is used to produce many different beer styles with various alcohol contents. Information about the alcohol-dependent behaviour of hop components after dosing is crucial to control the resulting flavour and ensure consistent beer quality. This systematic pilot-scale study was therefore performed using standardised procedures by only varying the alcohol content in the beer samples in four steps from 0.5 % to 10.5 % alcohol by volume (ABV). A commercially available alcohol-free wheat beer was used as a base beer to adjust the alcohol concentrations while keeping the hop dosing rate consistent at 250 g/hl using Type 90 Pellets of the variety Solero. After a semi-static contact time of 14 days, the following attributes were analysed: hop-derived bitter and aroma compounds, polyphenol content, nitrates, foam stability and pH value. The conclusions for the non-volatile attributes are as follows: iso-alpha acids, humulinones, polyphenols and foam stability remained unchanged with varying ABV. In contrast, increasing the alcohol content improved the transfer and solubility of hydrophobic alpha and beta acids as well as xanthohumol, resulting in higher concentrations. Increasing the alcohol also caused lower quantities of nitrates to be transferred. Foam stability was negatively affected when more ethanol was added, but this drop in stability was compensated by more foam-positive alpha acids introduced. The beer pH showed very little increase and was hardly influenced by the ABV. For the hop-derived volatile substances, the conclusions are as follows: The terpene alcohols displayed very good solubility with the ABV having hardly any impact. The transfer of hop esters was only somewhat ABV-dependant and the presence of alcohol above 0.5 % further increased the solubility to finally reach a certain plateau. Ketone concentration did depend on the ABV. Mono- and sesquiterpenes are most clearly influenced by the alcohol content and the highest concentrations were reached at the highest ethanol addition.

Descriptors: dry hopping, alcohol content, transfer rates, solubility behaviour of hop bitter and aroma components, polyphenols, nitrates, foam stability, pH value

1 Introduction

The main goal of dry hopping is to flavour beer by transferring hop volatiles on the cold side of beer production. For certain beer styles, dry hopping is a characteristic and essential part of the recipe but is also found to be highly suitable for beers with no or (very) low alcohol content. Non-alcoholic beers (NAB) in particular can lack body and/or aroma and, depending on the dealcoholisation technique used, may also possess an unwelcome residual sweetness [1–3]. In this case, dry hopping is one option to improve the overall flavour – a solution which is also in compliance with the German Purity Law [4, 5]. The rapidly increasing market for alcohol-free and alcohol-reduced beers is a consequence of today's more responsible beer consumers and customer demand addressed to the brewing industry. Breweries across the globe are extending their portfolios accordingly [6] and have set ambitious goals for the next few years [7–10]. As a result, even typically dry-hopped beer styles such as India Pale Ales (IPA) are now available as alcohol-free beers. It is important to point out that different countries around the globe have varying definitions of non-alcoholic beer, mostly ranging from ≤ 0.005 % to ≤ 0.5 % ABV [1]. In a few countries such as Italy or Canada, NAB is defined as having an ABV above 0.5 % or is declared differently, for example as "extra-light beer". In this study, NAB was defined on a basis of ≤ 0.50 % ABV.

Changing the focus to beers with high(er) ABVs, dry-hopped styles such as Belgian Style Triple or Imperial IPA, in some cases with 10.0 % ABV and above have been established on the market for many years. They indicate a boundary of the alcohol range of beer, not only as speciality beers [11]. Hence, dry hopping is a technique used for many beer categories, and also when developing new products, in any ABV range of existing beer styles. It is therefore crucial to understand the effect of alcohol on the volatile and non-volatile hop components after dry hopping. Interactions in the beer matrix might also occur in a varying combination of both, alcohol content and dry hopping.

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Studies have been conducted by *Haslbeck* et al. for example, who performed dry hopping trials using various varieties and hop dosing rates, and three different ABVs at two different temperatures [12]. An excerpt from the results shows that the proportion of monoterpenes such as β-myrcene among the hop volatiles in dry-hopped beers increased significantly after increasing the ethanol content. Transfer rates were also determined for various hop-derived aroma components.

Holbrook performed trials on an un-hopped IPA using two different temperatures, three different hop dosing rates, two types of hop products and two ABVs of 7.5 % and 9.5 % [13]. Again, the main focus was on the key hop aroma substances: linalool, β-myrcene, β-caryophyllene and α-humulene. As a result, hop dosing rate and temperature were assessed as having more impact on the extraction of aroma substances than the ABV or hop product. However, the ABV difference in the tested beers was only 2.0 % ABV and the alcohol may have had less impact due to the small range between tested beers.

Huismann et al. also investigated the extraction of essential oil and various terpene compounds by varying temperature, hop addition, alcohol content and exposure time in a recombined beer matrix [14]. An ABV of 3.0 %, 6.5 % and 10.0 % ABV was chosen. As the first trials at 6.5 % ABV did not confirm their hypothesis, more extensive trials comparing static and stirred dry hopping were carried out. Only stirring the samples resulted in a (slight) increase in the majority of hop-derived aroma compounds when more hops were added and at a higher ABV.

Ethanol is an effective solvent to dissolve the hydrophobic constituents of hops. This benefit highlights the long-term use of ethanol as an excellent extraction solvent in today's commercial hop processing. The result of this process is a complex, unique and highly purified hop extract that contains all the essential bitter and aroma substances that are relevant for beer production [15, 16]. Compared to other common hop extraction methods, this ethanolic "Total Resin Extract" comes closest to the bitter substance composition of the origin hops, also with regard to the resulting taste in the beer [17]. Finally, ethanol is also a suitable solvent for extracting xanthohumol: a prenylated flavonoid from hops that has gained a lot of interest in recent decades because of its health benefits together with the very minor impact it has on a beer's bitter taste [18–21].

Finally, ethanol concentration in beer can have a significant effect on the sensory perception of various volatile and non-volatile substances in beer. *Clark* et al. investigated the physico-chemical effects of ethanol, carbonation and hop acids and their influence on flavour perception in beer, in particular their effects on aroma release [22]. Hop-derived aroma compounds were not examined in this study, but in-vivo experiments showed that ethanol increased the release of all tested aroma substances.

Ford et al. investigated the effect of different ethanol concentrations on 101 consumers, asking for taste preferences and perception of sensory attributes [23]. Differences in sweetness, fullness (body of beer) and alcohol warming sensation were observed when comparing beers with 0 and 5 % ABV. Potentially, interactions with other beer components such as hop acids and CO_2 might also have influenced the increase in warming sensation.

Peltz et al. investigated the effects of ethanol on orthonasal detection and the thresholds of 10 hop-derived aroma compounds in a Pale Ale with 5 and 10 % ABV [24]. Although the solubility of certain aroma compounds in the liquid was increased at higher ABVs, the corresponding threshold remained mostly unchanged, except for geraniol, linalool and β-damascenone.

It is not possible to predict the flavour and overall impression of beers at different alcohol contents, but there is no doubt that alcohol influences the sensory perception of beer. Information about the ABV-dependent behaviour is an important key to understand the interactions of hop-derived compounds added to different beer styles and dry hopping recipes. The aim of this study was to investigate the transfer of hop constituents and their solubility behaviour after dry hopping beer of different ABV. To our knowledge, no systematic test has yet been carried out and this model study provides information on the effects of ethanol concentration on pH, foam and in particular the volatile and non-volatile hop-derived substance groups after dry hopping.

2 Material and Methods

2.1 Hop pellets (Solero)

Table 1 provides an overview of the hop pellets utilised in this study. Except for nitrates, the analyses were performed according to the most recent methods of the European Brewery Convention (EBC).

The new aroma variety Solero was released in 2019 and is known

Table 1 Characterisation of the hop pellets used

	Method [25]	Type 90 pellets
Variety		Solero (crop 2020)
Lead conductance value	EBC 7.5	10.1 %
Alpha acids	EBC 7.7*	8.8 %
Beta acids	EBC 7.7*	6.1 %
Humulinones	EBC 7.7*	0.2 %
Xanthohumol	EBC 7.15*	0.8 %
Polyphenols	EBC 7.14	5.7 %
Total oil content	EBC 7.10	1.1 ml /100 g
β-Myrcene	EBC 7.12*	56.0 % rel.
β-Caryophyllene	EBC 7.12*	4.8 % rel.
α-Humulene	EBC 7.12*	6.4 % rel.
Farnesene	EBC 7.12*	< 1.0 % rel.
Linalool	EBC 7.12**	0.7 % rel.
Geraniol	EBC 7.12**	0.4 % rel.
Nitrate	HHV*** 18a (internal method)	548 mg /100 g

* the most recent international standards or pure substances were used for the calibration

** also based on EBC 7.12

*** Hallertauer Hopfenveredelungsgesellschaft mbH

Table 2 Characterisation of base beer

	Method [27]	Base beer
Original gravity	MEBAK 2.9.6.3	4.99 % w/w
Alcohol	MEBAK 2.9.6.3	0.39 % vol
Extract, real	MEBAK 2.9.6.3	4.38 % w/w
Degree of fermentation, real	MEBAK 2.9.6.3	12.3 %
Foam stability (NIBEM)	MEBAK 2.18.2	259 s
pH value	MEBAK 2.12	4.51
Yeast cell number	MEBAK 10.11.4.4	< 15000/ml

for its tropical flavour. Solero is typically used as a late hop aroma variety but is increasingly used in dry hopping to produce distinctive hoppy beers. This flavour is mainly attributed to hop esters, particularly isobutyl isobutyrate. Compared to traditional aroma hop varieties from Germany, Solero contains about 3 to 5 times more of this flavourful ester, observed from internal water extractions according to *Schmidt* et al. [26]. The total amount of esters of the used pellets is above 600 µg/l in this aqueous extract, which is about 4 times more in comparison to Hallertauer Tradition for example. In both varieties isobutyl isobutyrate is about one third of the total amount detected. This single aroma compound can contribute to an intense fruity sensory impression in hop cones, as well as in the resulting beer. Containing about 10 % total bittering substances, this aroma variety represents a mid-range composition of bitter acids.

2.2 Base beer and alcohol adjustment

The base beer was produced in a German brewery and commercially purchased. The original gravity of the used wheat beer was 12.7° Plato prior to dealcoholisation via thermal evaporation. A wheat beer was chosen as a suitable top-fermented unfiltered beer style and, as usual, this type of beer was not dry hopped. Analyses of the base beer used for each individual trial are shown in table 2. The applied methods refer to the latest version of the Central European Commission for Brewing Analysis (MEBAK).

Based on the base beer analyses, the quantity of ethanol to be added was calculated to reach an ABV of 0.5 %, 3.5 %, 7.0 % and 10.5 %. According to the ABV set in this way, the samples are designated as "N" (no), "L" (low), "M" (mid) and "H" (high) in the following. In this study, "alcohol-free" was defined as ≤ 0.50 % ABV. By using four different volumes of ethanol dosed to a consistent volume of 15 litres base beer, samples with low alcohol had an almost unchanged total volume, whereas the samples containing 10.5 % ABV had an overall volume of little more than 17 litres. A commercially purchased ethanol with a purity of 96 % (pharma grade) was used to adjust the ABV. This ethanol was added to the empty 20-litre NC kegs, after which the hop pellets (see 2.3) and the base beer were directly added. To minimize the oxygen concentration in the KEG, the headspace was flushed with CO_2. Afterwards the keg was directly closed and lagering pressure in the headspace was continuously connected through the CO_2 valve. For each prepared ABV, the addition of the corresponding amount to base beer was replicated four times: this gave one control sample without any hop pellets but alcohol-adjusted ("N0", "L0", "M0" and "H0") and three samples including pellets at dry hopping ("N1-3", "L1-3", "M1-3", "H1-3"). In addition, one base beer without any adjustments at all (neither hops nor ethanol) was kept as a blank control throughout the process ("base beer"). This sample was also used to calculate the transfer rates of non-volatile components. The whole set-up resulted in a total of 17 individual trials, shown in table 3.

All beers shown in table 3 were analysed according to the methods listed in 2.4 and 2.5. It is important to note that the analysed yeast cell count of < 15 000 yeast cells/ml is below the detection limit of the applied method using a Thoma hemocytometer. Adsorption of hop components on yeast cells is therefore negligible (if at all) and is therefore excluded for the following conclusions.

Table 3 Trial set-up including hop and ethanol dosage (if any)

Samples	ABV (%)	Dry hopping (g) per trial beer	Dry hopping (g/hl) volume adjusted	Corresponding oil dosage (ml/hl)
Base beer	0.4			
N0	0.5			
N1-N3 average	0.5	37.5	242	2.66
L0	3.5			
L1-L3 average	3.5	37.5	234	2.58
M0	7.0			
M1-M3 average	7.0	37.5	226	2.48
H0	10.5			
H1-H3 average	10.5	37.5	217	2.39

Table 4 Overview of applied methods

Methods	
MEBAK 2.9.6.3 [27]	Alcohol [% vol.], Extract [% w/w], Original gravity [% w/w], Degree of fermentation [%]
MEBAK 2.13 [27]	pH value
MEBAK 2.18.2 [27]	Foam stability acc. to NIBEM-T Meter [s]
MEBAK 10.11.4.4 [27]	Yeast cell number
EBC 9.8 [25]	Bittering units [IBU]
EBC 9.11 [25]	Polyphenols [mg/l]
EBC 9.50* [25]	Alpha acids, Iso-alpha acids, Humulinones, Beta acids**, Xanthohumol** [mg/l]
HHV*** 18b (internal method)	Nitrate [mg/l]
EBC 9.49* [25]	Aroma substances [µg/l]

* the most recent international standards or pure substances were used for calibration

** analysis based on method EBC 9.50

*** Hallertauer Hopfenveredelungsgesellschaft mbH

Table 5 Mean values of non-volatile hop-derived substances

Samples	IBU	IAA (mg/l)	AA (mg/l)	BA (mg/l)	HUM (mg/l)	PP (mg/l)	XN (mg/l)	NO_3 (mg/l)	pH	Foam (s)
Base beer	9.4	6.5	0.5	n.d.	0.2	121	0.05	12	4.51	259
N0									4.53	209
N1-N3	21.2	7.4	13.2	n.d.	2.2	160	1.5	25	4.60	362
L0									4.56	196
L1-L3	34.2	7.0	35.2	3.1	3.1	167	3.7	25	4.63	359
M0									4.62	171
M1-M3	37.8	6.9	46.1	4.6	3.2	159	3.8	19	4.67	351
H0									4.65	168
H1-H3	38.5	6.6	62.0	8.4	3.2	164	4.3	18	4.70	336

2.3 Dry hopping (semi-dynamic)

Each dry hop addition was performed using an identical dosage of 37.5 g Type 90 Pellets of the Solero variety and the same batch. As beer volumes differed slightly after alcohol adjustment (see 2.2. and Table 3), the resulting dry hop amount was 242 g/hl pellets for the 0.5 % ABV beers (N-samples) and decreased to 217 g/hl for the 10.5 % ABV beers (H-samples). These dry hop additions correspond to an oil-based dosage of 2.39 to 2.66 ml/hl pure hop oil. Dry hopping was performed in triplicate for each ABV-adjusted range. The pellets were added loosely into 20 litre NC kegs. Two weeks of contact time between the hops and beer was chosen. The beer was stored at a consistent temperature of 5 °C. During this period, the kegs were inverted twice a week to simulate movement of the beer in the tank and to improve the extraction of the hop components into the beer. The kegs were equipped with shortened extractor tubes, so that after dry hopping, the slightly hazy beer was sampled and analysed without cold break sediments and without the vast majority of hop particles.

2.4 Beer analyses

Table 4 shows the methods employed for the analysis of chosen beer attributes and hop components before and after dry hopping.

All analyses were performed within two weeks of bottling at the accredited central lab of Research Center Weihenstephan for Brewing and Food Quality, TU Munich, Freising and the central lab of Hallertauer Hopfenveredelungsgesellschaft m.b.H. (HHV), Mainburg, Germany. For the nitrate determination, the beer is degassed, and the nitrate content is then measured directly with a reflectometer (Merck Reflectoquant System “RQ Flex20”).

2.5 Anti-microbial actions and verification

In order to eliminate an impact such as foreign fermentation due to microbial contamination (especially in the N- and L-beer samples), Nagardo© glycolipids (a natural preservative) were added to all of the 17 single trial beers. The product was provided by LANXESS Deutschland GmbH [28]. Microbial analyses (microscopy and enrichment in NBB agar according to MEBAK III 10.5 [27] of all samples showed no growth of any foreign yeast or microorganisms.

3 Results and discussion

The individual results of the 17 beers are listed in the appendix in the tables overview A and overview B. Observations and conclusions are made on the basis of the corresponding mean values and all transfer rates are calculated after subtracting the original measured values of the base beer. The transfer rates for the non-volatile hop components were calculated taking into account the varying “input” from the pellets (see g/hl hop pellets added, Table 3). With regard to these calculated transfer rates, it should be noted that lower dry hop quantities in beers with higher ABVs may have led to slightly better utilisations of certain substances. This ultimately might have resulted in a little increased transfer the more ethanol added. As microbial analyses did not give any findings, the observed effects result from chemical substance transfers only. Adsorptions on yeast cells can also be excluded (see 2.2).

3.1 Bitter substances and bitter units

3.1.1 Bitter substances

Based on the concentration of 6.5 mg/l iso-alpha acids (IAA) of the blank sample compared to the ethanol-adjusted samples, the content of IAA varied between + 0.1 and + 1.1 mg/l as shown in table 5.

Surprisingly, no loss of IAA was detected after dry hopping, although a certain decrease was expected from published research [29, 30]. The highest deviation of 1.1 mg/l in the N-samples compared to the base beer is still within the analytical tolerance and no sig-

Table 6 Transfer rate of introduced non-volatile hop components

Samples	AA (% transfer)	BA (% transfer)	HUM (% transfer)	PP (% transfer)	XN (% transfer)	NO_3 (% transfer)
N1-N3	6.4	0	41.4	28.3	7.5	101
L1-L3	17.5	2.2	61.2	34.2	19.5	99
M1-M3	23.2	3.4	67.3	29.3	20.8	59
H1-H3	32.3	6.4	69.2	35.1	24.7	53

nificant changes were found. Therefore, in this study IAA can be assessed as unchanged after dry hopping and as being unaffected by alcohol concentration.

In contrast, all other analysed hop bitter components increased after dry hopping and in some cases, also depending on the alcohol. Subtracting the base beer, the alpha acids (AA) rose from 12.7 mg/l for the N-beers and peaked at 61.5 mg/l in case of the H-beers. Undoubtedly, the solubility of hydrophobic AA in beer is influenced by ethanol. Higher ABVs of the beers resulted in significantly more AA that were extracted from hop pellets and found in the final beer. The corresponding transfer rates were 6.4 % for the lowest and 32.3 % for the highest alcohol content (Table 6).

Compared to IAA, the AA have a sensory impact of 10 %, which theoretically resulted in an increased sensory bitterness of more than 6 bitter units for the H-samples. A change in sensory characteristics, which presumably can be perceived in the finished beer.

In addition, hydrophilic humulinones (HUM) also have a remarkable sensory impact after dry hopping. These were detected as having increased 2.0 mg/l at the low ABV level and 3.0 mg/l for the L-, M-, H-beers, based on a low transfer rate of only 41.4 to 69.2 % (Table 6). Higher transfer rates were reported in recent studies due to the polarity of HUM and hence excellent solubility in beers after dry hopped [31, 32]. Although transfer rates are on a lower level in general, the varying content of alcohol in the L-, M- and H-samples did not increase the concentration significantly. Only the N-samples that contained almost no alcohol might have shown less extraction of HUM. However, this assumption is based on the very low concentrations of HUM in general and it can be concluded that HUM are not affected by increasing the beers' alcohol contents.

In this study, hydrophobic beta acids (BA) were also analysed by HPLC analysis according to EBC 9.50. Even at an ABV of 3.5 % there was a clear improvement in the solubility, which resulted in a concentration of 3.1 mg/l (Table 5). At higher ABVs, this value was further increased by up to 8.4 mg/l in the case of the H-samples. Concentrations at this level are very rarely found in regular beers because nonpolar BA are generally regarded as insoluble in beer [33]. However, a recent study about NEIPAs detected an average BA concentration of 5.0 mg/l (ranging from 1.0 to 14.0 mg/l), which resulted from high late and dry hopping rates. There was a proven interaction between haze-forming protein-polyphenols and BA, resulting in more BA detected in these beers [34]. The H-samples' highest concentration of 8.4 mg/l found in our study might be a result of both, the use of a hazy wheat beer combined with the high ABV. In any case, transfer rates remained at a low level below 6.4 % (Table 6).

3.1.2 Bitter units

Finally, and as a consequence of dry hopping, bitter units (BU) increased after dry hopping within the range from 11.8 to 29.1, resulting in 38.5 BU for the H-beers (Table 5). The BU method primarily covers the determination of IAA at the used wavelength, but the hop components mentioned above also have a specific adsorption at 275 nm [25]. Since the IAAs are constant, any increase above the originally measured BU of 9.4 of the base beer can be attributed to the additional concentrations primarily of AA but also dissolved HUM and BA.

3.2 Other hop-derived components

3.2.1 Polyphenols

The total polyphenol (PP) content of 121 mg/l analysed in the base beer increased after dry hopping. Considering all ABV groups, the increase was in the range of 38 to 46 mg/l (Table 5). For the N-samples with the highest dry hopping, an increase of 39 mg/l of additional PP was observed and 43 mg/l for the lowest dry hopping rate in the H-samples. More hops resulted in lower PP transfer rates, ranging from 28.3 % to 35.1 % (Table 6). In view of an analytical tolerance for the applied method that is even above 20 mg/l, the variation is not significant for any of the ABV-grouped samples, and so alcohol content did not change the total PP transfer at all.

NOTE: An additional study of these beers was performed, where the single components of the total polyphenol spectrum of these beers were investigated by HPLC-MS. A separate publication will refer to these findings.

3.2.2 Xanthohumol

In the base beer, xanthohumol (XN) was hardly detectable and was found at a concentration of less than 0.05 mg/l (Table 5). Therefore, any increase observed is exclusively attributed to substance transfer from pellet to beer after dry hopping. It is well known that XN is hardly soluble in water and ethanol is an ideal solvent [35]. Even the lowest amount of alcohol in the N-samples resulted in an extraction of 1.5 mg/l of XN into beer, with a plateau of about 3.8 mg/l for L- and M-samples. The highest concentration of 4.3 mg/l was detected in the H-samples, which is about one quarter (24.7 %) of the XN introduced by dry hopping as shown in table 6. When comparing the highest XN concentration found in XN-rich Stout and Porter-style beers (up to 1.2 mg/l) or beers brewed with XN-enriched products (up to 3.3 mg/l), the value in the H-samples is still higher [36]. NEIPAs were recently found to have similar concentrations of up to 3.5 mg/l, but according to Maye et. al. this value was achieved with the help of a polyphenol-protein haze carrier for nonpolar substances [34]. A general comparison of our H-samples with NEIPAs shows that the latter are supposed to have a lower alcohol range of typically 6.3 to 7.5 % ABV [37]. This ABV range corresponds rather well to the M-grouped samples with a XN concentration of 3.8 mg/l detected in our study, and so a similar and very comparable level has been achieved. As more alcohol resulted in even more dissolved XN, it appears that both effects (ethanol and haze-protein career) would be beneficial for increasing XN concentrations in dry-hopped beer.

3.2.3 Nitrates

The nitrate content of the base beer was 12 mg/l. After dry hopping, a complete transfer of the nitrate introduced by dry hopping (+ 13 mg/l) was observed for the N- and L-samples (Table 6), resulting in a total concentration of 25 mg/l. The increase in the M- and H-samples was about half as much, i.e. only + 6 and + 7 mg/l, which

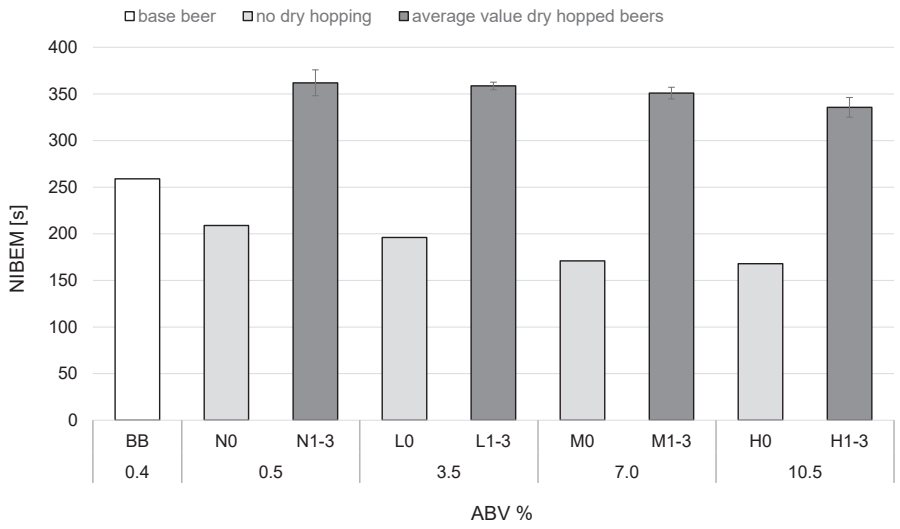

Fig. 1 Foam stability according to NIBEM

corresponds to a transfer rate of 59 % and 53 % respectively. Nitrate is not an organic compound but a salt. Therefore, nitrates are less soluble in more nonpolar solvents, which is the case the more ethanol was added to the base beer. Consequently, there needs to be a greater focus on nitrate input for the production of dry-hopped NABs, especially if regulations or limits for nitrate levels in beer are set by the authorities.

3.3. Further observations of non-volatile attributes

3.3.1 pH value

The pH value of the base beer was 4.51. For the ABV-grouped 0-samples, the pH values increased continuously from 4.53 to 4.65, i.e. approximately an increase of 0.01 per % ethanol added. On top of this, the actual dry hopping of 217 to 242 g/hl caused an increase in the pH of 0.07 to 0.05. Both ethanol and dry hopping resulted in higher beer pH values, with more ethanol added; in total + 0.17 from N0 to H1-H3-samples. It is known that for every 100 g of pellets added by dry hopping, there is an increase in pH of about 0.03 to 0.036 [30, 31]. For the 217 to 242 g/hl hop pellets added in this study, the pH value after dry hopping and ABV adjustment behaved exactly as expected and confirms recent findings.

3.3.2 Foam stability

In most cases, alcohol reduces foam stability [38] but contradictory statements can also be found, depending on the applied method [39–41]. We have observed a negative effect of ethanol on the foam stability according to NIBEM, as shown in figure 1.

The first drop from base beer to all other non-dry hopped 0-samples is most probably due to the handling and preparation of the trial beers. Looking at the 0-samples, there's a constant decrease in foam stability from N0 to H0, which is caused by the increasing quantities of ethanol added. On average, every percent of added ethanol reduced the foam stability for approximately 4 seconds. In contrast, the dry-hopped samples N1-3, L1-3 and M1-3 are rather consistent with a foam stability of about 350 seconds. A minor drop can be observed for the H-samples only. For the latter, the drop does not seem to be fully compensated by the foam-positive alpha acids introduced by dry hopping [42], which is obviously the case for L- and M-samples, and to a minor extent for the N-samples. If foam stability is a concern, all results measured in the dry-hopped beers are still assessed to be at a very good level [40]. Therefore, dry hopping with pellets or purified downstream products primarily containing extracted alpha acids [42], will positively contribute to foam stability at any ABV range of beer.

3.4 Hop-related aroma compounds

Hop-aroma substances were measured according to Schmidt et al. [25, 43]. Individual results of the hop-related aroma compounds are shown in table overview B. Table 7 shows the averaged values including standard deviation of each ABV-group.

A relative analytical error of ± 10 % can be assumed for the method used. In most cases, the individual results varied within or close

Table 7 Mean values of hop-derived aroma compounds in µg/l

	Ketones (µg/l)			Esters (µg/l)				Terpene alcohols (µg/l)			Mono- and sesquiterpenes (µg/l)			
Samples	2-Undecanone	2-Dodecanone	2-Decanone	Isobutyl isobutyrate	3-Methylbutyl isobutyrate	2-Methylbutyl isobutyrate	Geranyl acetate	Geraniol	Linalool	α-Terpineol	β-Myrcene	β-Caryophyllene	α-Humulene	β-Limonene
Base beer	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
N1-3	14.7	n.d.	11.0	166.3	16.1	160.1	60.4	193.4	195.3	19.7	708	5.6	7.6	8.6
SD	1.5	–	1.2	2.7	0.5	4.9	4.7	4.4	0.5	0.5	125	0.3	0.4	0.8
L1-3	30.0	19.6	14.6	238.5	26.4	261.2	115.9	246.8	213.0	30.1	13718	51.3	55.9	85.4
SD	2.3	1.0	0.8	4.7	1.2	11.1	3.3	29.6	5.5	0.4	1667	1.7	2.4	7.4
M1-3	28.3	18.9	13.8	238.3	27.3	266.0	122.2	250.0	204.0	22.9	13974	60.7	77.2	95.4
SD	2.5	1.3	1.0	17.4	2.8	35.4	8.6	4.4	5.3	0.8	4907	8.1	8.7	25.2
H1-3	44.2	33.6	17.8	237.5	28.0	277.0	130.9	198.4	214.5	22.7	13502	86.8	102.8	103.1
SD	2.4	2.0	1.0	0.7	1.1	10.8	7.1	14.4	2.2	0.7	1069	1.7	2.4	11.8

to this tolerance with the exception of the M-samples of isobutyl isobutyrate and 2-methylbutyl isobutyrate, the L- and H-samples of geraniol, the M-samples of β-limonene and additionally β-myrcene in case of all grouped samples. In particular, the huge range of β-myrcene detected in the M-samples makes it rather difficult to make clear conclusions, but L- and H-samples indicate a similar level of β-myrcene as expected in the M-samples.

3.4.1 Ketones

At an absolute level below 50 µg/l, the two ketones 2-undecanone and 2-dodecanone were found to have the highest values in the H-samples, although 2-dodecanone could only be detected at an alcohol concentration of 3.5 % and above (Table 7). For 2-dodecanone, both the L- and M-samples had almost identical concentrations around 19.0 µg/l and increased to 33.6 µg/l in the H-samples. The concentration of 2-undecanone rose to 14.7 µg/l for the N-samples. This concentration was roughly doubled for the ABVs of 3.5 and 7.0 %. A similar increase was observed at an ABV of 10.5 %, resulting in a concentration of 44.2 µg/l. Reported threshold in beer is 7 µg/l and therefore 2-undecanone could contribute to the final sensory properties [44].

In contrast to the two specified ketones, 2-decanone seems to be extracted in a similar range for ABVs up to 7.0 %, ranging between 11.0 and 14.6 µg/l. Only an ABV of 10.5 % again increased the solubility slightly to a maximum of 17.8 µg/l.

In almost all cases, the concentration of ketones increased with higher ABVs. Especially given the fact that slightly fewer hops were added with increasing ethanol content, it can be concluded that ketones show an alcohol-dependant solubility.

3.4.2 Esters

The hop-derived esters, geranyl acetate, isobutyl isobutyrate, 3-methylbutyl isobutyrate and 2-methylbutyl isobutyrate were examined. All four compounds were clearly transferred after dry hopping from hops to beer. Already 46 to 70 % of the highest amount detected within any ABV group were found in the corresponding N-samples. The best extraction at only 0.5 % ABV was found for isobutyl isobutyrate (70 %), followed by 3- and 2-methylbutyl isobutyrate (58 %) and 46 % of the corresponding maximum concentration detected in the case of geranyl acetate. This first initial leap after dry hopping demonstrates the excellent solubility of hop-derived esters, which can be beneficial for the aroma in dry-hopped NABs. At 3.5 % ABV and above, all components reached a certain plateau (and the corresponding maximum concentration) and was scarcely dependent on alcohol at all. Only concentrations of 2-methylbutyl isobutyrate and geranyl acetate indicate a marginal additional increase when the ABV was higher. It must be noted that 3-methylbutyl isobutyrate behaved as mentioned above but at a level below 30 µg/l, whereas isobutyl isobutyrate and 2-methylbutyl isobutyrate reached maximum levels of around 238.5 and 277.0 µg/l respectively, geranyl acetate in the mid-range of all other esters. With the exception of 3-methylbutyl isobutyrate, all other concentrations were much higher than the reported thresholds and therefore clearly contribute to the sensory properties of beer [3, 45, 46]. Isobutyl isobutyrate, in particular, is known to produce an intense fruity flavour in beer, and the high concentration introduced by the Solero hop variety can be beneficial when fruitiness in beer is desired.

3.4.3 Terpene alcohols

The terpene alcohols, geraniol and linalool, are known to have good solubility in beer even at low alcohol concentrations and this can be clearly demonstrated when comparing the N-samples with the base beer (Table 7). After dry hopping, concentrations of almost 200 µg/l were reached in the N-samples. In all other ABV-adjusted samples, concentrations above 200 µg/l were detected and at more or less constant plateaus with the exception of the H-samples of geraniol. The latter might be caused by a sampling error, as all other values indicate the maximum concentration was reached at 3.5 % ABV and above. As there is only a very small difference between N- and all other grouped samples, it can be concluded that ethanol additionally improves the solubility of most important terpene alcohols, but only to a certain extent. At the levels measured, both geraniol and linalool contribute to the overall flavour even in a NAB (reported thresholds are 36 µg/l and 5, 27, 80 µg/l respectively [12]), and this can become even more intense at higher ABVs.

The additionally analysed terpene alcohol α-terpineol was detected in concentrations of 19.7 to 30.1 µg/l (N- and L-sample respectively). Concentrations within this range were found for the M- and H- samples. However, it cannot be assumed that higher ABVs negatively influence the solubility of α-terpineol and variations between all grouped samples are rather small (≤ 10.4 µg/l). An error in sampling might explain the highest concentration detected in the L-samples. In any case, all measured α-terpineol concentrations are far below the reported threshold of 330 µg/l [33], and so there is no expected contribution to sensory properties.

3.4.4 Mono- and sesquiterpenes

β-myrcene, β-caryophyllene, α-humulene and α-limonene were examined within the group of mono- and sesquiterpenes. Alcohol-dependent behaviour can be observed for all components and results in higher concentrations the more ethanol added, with the exception of β-myrcene. The latter was clearly solved at an ABV of just 0.5 %, but exceptionally high concentrations have been detected in all other samples with an ABV of 3.5 % and above. It should be noted that outliers were measured within the M-samples. However, once again the average reached a similar plateau to that observed in all other L-, M- and H-samples. The general high level of almost 14.000 µg/l can be explained by dosing hop pellets into a closed system and the use of a finished beer with very few yeast cells. There were no losses due to adsorption on yeast cells [47], which is usually the case when removing yeast and/or cold trub under regular production conditions. Both issues resulted in virtually no losses of nonpolar mono- and sesquiterpenes and explain the exceptional high levels, in particular for β-myrcene which is the major aroma substance in the hop's essential oils (Table 1). Compared to the other shown mono- and sesquiterpenes, the level of β-myrcene is too high to still find a significant upwards trend in correlation with ABV.

At concentrations below 10 µg/l, β-caryophyllene, α-humulene and α-limonene were barely soluble at an ABV of 0.5 % but concentrations clearly increased at 3.5 % (in two cases by approx. a factor of 10) and continued upwards with increasing ABVs. Hence, their peaks were observed in all corresponding H-samples with more than half of these values already found in the L-samples. For α-humulene and β-caryophyllene, 54 to 59 % of the maximum value was already found in the L-samples, for α-limonene the corresponding value was 83 %. For the latter, more ethanol did not significantly increase the levels reached at 3.5 % ABV.

Table 7 clearly demonstrates that ethanol is beneficial for the solubility of mono- and sesquiterpenes. If dry hopping is applied for beers containing alcohol, these compounds will be transferred in higher concentrations and might ultimately contribute to the herbal, resinous and hop-spicy aroma of beer, however, a minor sensory impact can be expected in the case of NABs.

4 Conclusions

This study demonstrated the analytical impact of ABV on dry-hopped beers. A variation in the alcohol content (0.5 to 10.5 % ABV) resulted in different solubilities of hop-derived volatile and non-volatile substances. This information is crucial to understand substance transfer and to achieve consistent beer quality, also with regard to sensory aspects.

The main bittering compounds in dry-hopped beer (iso-alpha acids and humulinones) remained unchanged, whereas concentrations of the hydrophobic bitter substances, alpha and beta acids as well as xanthohumol were significantly higher the more ethanol was added. The alpha acids increased by almost 5 mg/l for each % of additional alcohol and an increase of just under 1 mg/l was observed for the beta acids. ABV had zero to little effect on polyphenol content, foam stability and pH value. Due to the complete nitrate transfer, which is especially initiated by dry hopping at lower ABV, special attention must be paid to possible legal restrictions.

The ABV-dependant behaviour of the analysed groups of hop-derived aroma substances has been characterized and has shown significantly higher ABV-dependant concentrations for mono- and sesquiterpenes as well as ketones, if the beers contained increasing alcohol contents. This proportional shift has to be considered when producing beers with regular or high ABVs. The terpene alcohols displayed hardly any ABV dependency and in particular, geraniol and linalool achieved about 77 to 91 % of their maximum concentration already at an ABV of 0.5 %. The concentrations of the already highly soluble esters could be further increased by adding more ethanol, and three of the four compounds investigated also reached concentrations above their thresholds.

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Appendix

Table overview A Analytical values of non-volatile hop-derived compounds

Samples	pH	Foam (s)	BU	IAA (mg/l)	AA (mg/l)	BA (mg/l)	HUM (mg/l)	PP (mg/l)	XN (mg/l)	NO_3 (mg/l)
Base beer		259	9.4	6.5	0.5	n.d.	0.2	121	< 0.05	12
N0	4.53	209								
N1	4.59	353	21.2	7.2	13.3	n.d.	2.2	170	1.5	26
N2	4.61	355	20.5	7.3	12.2	n.d.	2.2	153	1.5	25
N3	4.59	378	21.8	7.6	14.2	n.d.	2.2	157	1.5	25
L0	4.56	196								
L1	4.62	361	34.3	6.6	37.4	3.5	2.9	170	3.6	25
L2	4.63	354	33.1	7.1	36.8	3.1	2.9	164	3.6	24
L3	4.63	361	35.1	7.2	34.2	2.8	3.4	166	3.9	25
M0	4.62	171								
M1	4.67	353	34.6	6.6	41.7	4.4	3.3	154	3.3	20
M2	4.67	344	34.5	7.1	41.1	3.7	3.0	159	3.3	19
M3	4.67	356	44.2	7.0	55.5	5.8	3.4	163	4.8	19
H0	4.65	168								
H1	4.71	334	41.6	6.3	59.6	6.6	3.3	171	4.8	19
H2	4.70	347	37.6	6.5	55.8	5.7	3.2	176	4.3	18
H3	4.70	326	36.2	7.0	70.5	13.0	3.1	146	3.9	18

Table overview B Analytical values of hop-derived aroma compounds in µg/l

	Ketones (µg/l)			Esters (µg/l)				Terpene alcohols (µg/l)			Mono- and sesquiterpenes (µg/l)			
Samples	2-Undecanone	2-Dodecanone	2-Decanone	Isobutyl isobutyrate	3-Methylbutyl isobutyrate	2-Methylbutyl isobutyrate	Geranyl acetate	Geraniol	Linalool	α-Terpineol	β-Myrcene	β-Caryophyllene	α-Humulene	β-Limonene
Base beer	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
N1	13.7	n.d.	10.1	163.2	15.7	155,0	56.6	197.9	195.3	19.4	730	5.7	7.8	8.4
N2	13.6	n.d.	10.2	165.9	15.8	158.6	57.6	187.4	196.0	19.3	545	5.1	7.1	7.7
N3	16.9	n.d.	12.7	169.8	16.9	166.7	67.0	194.8	194.7	20.5	851	5.9	8.0	9.6
L1	29.8	19.4	14.8	239.7	25.0	255.9	111.3	211.0	205.4	30.0	11791	50.2	53.0	82.2
L2	32.9	20.9	15.5	232.3	26.3	251.0	119.0	246.1	215.4	30.6	13506	53.7	58.8	78.4
L3	27.3	18.4	13.6	243.6	28.0	276.6	117.4	283.4	218.3	29.6	15859	50.1	56.0	95.6
M1	27.6	18.3	13.5	224.5	26.5	247.0	116.3	256.1	197.8	23.5	12549	56.4	71.2	100.3
M2	25.7	17.7	12.7	227.6	24.3	235.3	116.0	245.7	203.5	21.7	8805	53.7	70.9	62.4
M3	31.6	20.8	15.2	262.8	31.0	315.6	134.4	248.2	210.8	23.5	20569	72.0	89.4	123.5
H1	44.3	32.9	18.3	237.5	29.0	286.7	125.1	186.8	211.4	23.3	13952	88.3	105.2	110.4
H2	47.0	36.3	18.6	238.4	28.6	282.3	140.9	189.7	215.6	23.2	14528	87.6	103.8	112.4
H3	41.2	31.5	16.4	236.6	26.5	261.9	126.6	218.6	216.5	21.7	12027	84.4	99.5	86.5