Journal of the American Society of Brewing Chemists

The Science of Beer

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ujbc20

Brief Insight into the Underestimated Role of Hop Amylases on Beer Aroma Profiles

Pierre-Yves Werrie, Sylvie Deckers & Marie-Laure Fauconnier

To cite this article: Pierre-Yves Werrie, Sylvie Deckers & Marie-Laure Fauconnier (2021): Brief Insight into the Underestimated Role of Hop Amylases on Beer Aroma Profiles, Journal of the American Society of Brewing Chemists, DOI: 10.1080/03610470.2021.1937453

To link to this article: https://doi.org/10.1080/03610470.2021.1937453

JOURNAL OF THE AMERICAN SOCIETY OF BREWING CHEMISTS
https://doi.org/10.1080/03610470.2021.1937453

Brief Insight into the Underestimated Role of Hop Amylases on Beer Aroma Profiles

Pierre-Yves Werrie, Sylvie Deckers and Marie-Laure Fauconnier

Laboratory of Chemistry of Natural Molecules (ULg), University of Liège, Belgium

ABSTRACT

The current trend in craft breweries is to carry out heavy dry-hopping by increasing the hopping rate. This practice sometimes leads to uncontrolled and aberrant aroma profile production. The aim of this work was to determine whether part of the enzymatic content of hop (α-amylase and β-amylase) could impact yeast metabolism, resulting in aroma profile modification during secondary fermentation. In this research, spectrophotometric methods were used to assess the amylase activity within hop. Moreover, liquid chromatographic methods (HPLC-ELSD) showed modification of the beer sugar profile by production of glucose and maltose as well as by the degradation of a higher degree of polymerization sugar by hop enzymes. Furthermore, gas chromatographic techniques (GC-ECD/FID) were used to assess yeast metabolism using vicinal diketones (diacetyl/pentanedione) as a marker of the secondary fermentation. Finally, a principal component analysis (PCA) of the yeast main aromas (esters, higher alcohols, and aldehydes) demonstrated the significance of this yeast-hop interaction on the beer's aroma profile.

KEYWORDS

Beer aroma profile; dry-hopping; hop enzymes; vicinal diketones; yeast physiology

Introduction

Dry-hopping techniques are usually defined as hop cold extraction in beer during or just after primary fermentation. Dry-hopping performances depend on various parameters including time, temperature, hopping rate, hop variety (harvest date and location) or dispersion method (static or dynamic). These parameters have been extensively studied in order to understand the extraction rate of hop aromatic compounds as well as their fate in the final beer.[1–5] Nevertheless the hop oil content considered as a predictive standard seems not to be directly linked to the overall hop aroma intensity of a beer.[6] Such findings imply that the volatile compounds extraction of the hop oils during dry-hopping alone fail to explain the resultant flavor profile and that other phenomena must occur concomitantly.

Yeast can be metabolically active during dry-hopping and part of the aroma profile modification results from this activity. Indeed, yeast has been demonstrated to interact with hop and beer components by biotransformation of hop aroma,[7,8] glycosyl liberation[9,10] and release of polyfunctional thiols.[11]

The presence of a trace amount of dextrin hydrolyzing enzyme in hop has recently been demonstrated. This activity led to a significant sugar profile modification throughout dry-hopping.[12] Although this "diastatic" activity has long been known,[13,14] its effects during dry-hopping are just being understood. The so-called freshening power of hop (FPH) can indeed result in high yeast activity leading to CO_2 and ethanol production.[15,16] Furthermore, the presence of yeast during dry-hopping affects the beer by consuming dissolved oxygen, and some authors suggest that changes in the aroma profile may occur thanks to its fermentation process.[17]

Beside the many factors controlling flavor release, the dry-hopping technique simultaneously triggers other interactions between yeast and beer components, making it even harder to predict the resulting beer aroma profile. The aim of this work is to demonstrate the impact of hop enzymatic activity on yeast fermentation metabolism and its consequences for the beer aroma profile. In this study, specific amylase activity was assayed, (non-)fermentable sugar content was monitored during the dry-hopping process as well as yeast main fermentation compounds (vicinal diketones, esters, alcohols, and aldehydes).

Experimental

Amylase assay

The quantification method was used by adapting the two amylase assay kits Betamyl-3®and Ceralpha® from Megazyme© (Megazyme International, Bray, Ireland). First, 0.5 ± 0.01 g of homogenized Strisselspalt hop (whole hop, 2017 harvest, obtained from Comptoir agricole hops (Brumath, France)) powder was mixed with 5 mL of Tris/HCl buffer pH 8 (1 M), disodium ethylenediaminetetraacetic acid (Na_2EDTA, 20 mM) and sodium azide (NaN_3, 0.02% w/v) for 1 h on ice (at 4 °C) to allow enzyme extraction with short vortexing (10 s) every 10 min. The samples were then centrifuged (5,000 g, 10 min)

CONTACT Pierre-Yves Werrie Pierre-Yves.Werrie@uliege.be

and filtrated on a 0.45 µm nylon syringe filter. In order to assess the amylase activity, the filtrates were incubated after dilution in a buffer with their respective substrate (p-nitrophenyl-α-D-maltoheptaoside (BPNPG7) and p-nitrophenyl-β-D-maltotrioside (PNPβ G3) at 40 °C for 1,000 min. The nitrophenyl liberated by the glucosidase was then determined by reading absorbance at 400 nm using a Ultrospec 7000 spectrophotometer thermostatted at 25 °C.

Laboratory dry-hopping design

Small scale dry-hopping tests were performed in triplicate with six different modalities over a 14-day period in a water bath at 17 °C. Indeed, a sample of beer was combined either with yeast (20×10^6 cells/mL) or with sodium azide (NaN_3 0.02%) to prevent microbial development. The first two modalities isolated the impact of the hop alone with concentrations of 5 g/L and 25 g/L. Two other modalities combined both hop and yeast to evaluate the effect of their interaction. The last two modalities (beer and beer + yeast) were blank modalities ensuring that change could not take place without hop, yeast, or the interaction of the two. The beer, a traditional Belgian ale (abbey style beer) produced with top fermented yeast, was analyzed by Anton-Paar (Anton Paar DMA 4500 Density Meter/Alcolyzer Plus) to ensure identical characteristics (alcohol content and density) before the dry-hopping.

Determination of carbohydrates by high performance liquid chromatography (HPLC) with evaporative light scattering detector (ELSD) in dry-hopped beer

The official ASBC wort-22 method recommends the use of an ELSD, which allows the use of a gradient of elution and the separation of sugars with a higher degree of polymerization as also demonstrated by Floridi et al.[18] The apparatus was an Agilent 1200 series equipped with an ELSD detector and drift tube temperature of 40 °C. The column used for this analysis was an NH_2 Spherisorb from Waters with dimensions of 250 mm x 4,6 mm x 5 µm. For each analysis, the run lasted for 35 min with an eluent flow rate of 1 mL/min. During the first 10 min, the eluent was composed of 75% acetonitrile in water, before decreasing to 50% over a 15 min period. A plateau of 5 min at this concentration finished the run. Calibration curves were established at concentrations from 0.2 – 1 g/L and 1 – 10 g/L with fructose >99% (CAS 57-48-7), glucose >99% (CAS 50-99-7) and maltose monohydrate >99% (CAS 6363-53-7) purchased from Sigma-Aldrich.

Determination of volatile organic compounds (VOC) by gas chromatography (GC) in dry-hopped beer

The apparatus was a Perkin Elmer AutoSystem Gas Chromatograph equipped with Perkin Elmer Headspace Sampler HS40. The column was a CP-WAX 52CB 50 m × 0.32 mm × 1.2 µm. The samples were thermostatted for 20 min at 70 °C before being injected into the column. The temperature program started at 50 °C, was held for 2 min then increased to 80 °C at 3 °C/min, with a final increase to 140 °C at 15 °C/min. Two types of detector (temperature at 150 °C) were connected to this apparatus, a Flame Ionisation Detector (FID) for esters and higher alcohol analyses, and an Electron Capture Detector (ECD) for vicinal diketone analysis. The following standards were purchased from Sigma-Aldrich: isoamyl-acetate >95% (CAS 123-92-2), ethyl-acetate >99% (CAS 141-78-6), isoamyl alcohols <98% (CAS 123-51-3), isobutanol >99% (CAS 78-83-1), propanol >99% (CAS 67-63-0), ethyl caprylate >98% (CAS 106-32-1), ethyl caproate >99% (CAS 123-66-0), acetaldehydes >99% (CAS 75-07-0), 2,3-butanedione >97% (CAS 431-03-8) and 2,3-pentanedione >99% (CAS 600-14-6).

Statistical analysis of the results

The results were gathered on Microsoft Excel, and the graph as well as the principal component analysis (PCA) were generated on R studio v 3.5.1. Moreover, ANOVA three-way type II statistical analysis was performed with time (1,2,3,4,7,14 days), hop (0,5,25 g/L) and yeast (presence/absence) as factors. Residual analysis was performed to test for the assumptions. Normality was assessed using the Shapiro-Wilk's test and homogeneity of variances was assessed by Levene's test. Two-way interactions analyses were performed using residuals from the three-way ANOVA and statistical significance accepted at a Bonferroni-adjusted alpha level. Statistically significant simple main effects were followed by multiple pairwise comparisons to determine which group means were different.

Results and discussion

Beer characterisation

Beer analysis before dry-hopping revealed similar parameters for the three replicates of the beer sample (Table 1).

Hop α and β-amylase activity

The α and β-amylase activities were measured in hop (shown on Table 2). The results were similar to, though slightly higher than those found with other related varieties such as Hersbrücker, as presented in the cultivar-based screening of Kirkpatrick and Shellhammer.[16] Other starch degrading enzymes such as limit dextrinase and amyloglucosidase may also be present in hop crude extract but were not analyzed in this study. Furthermore, the presence of amylase inhibitors has been acknowledged in both hop[19]

Table 1. Beer analysis before dry-hopping.

Samples	Alcohol (ABV%)	Real extract (°P)	Apparent extract (°P)	Original extract (°P)	pH
Repetition 1	6.67	3.75	1.37	13.81	4.10
Repetition 2	6.56	3.91	1.57	13.81	3.98
Repetition 3	6.61	3.73	1.37	13.78	3.98

Table 2. Mean amylase activity of Strisselspalt used for the dry-hopping ($n = 3$) and genetically close related variety* from similar protocol work.[16]

Hop variety	α-Amylase (U/g)	β-Amylase (U/g)
Strisselspalt whole hop	0.13 ± 0.01	0.25 ± 0.03
Saazer*	0.12	0.21
Hersbrucker*	0.10	0.19
Hallertau*	0.08	0.17

and beer materials. These two last remarks have to be kept in mind and may prove useful in the discussion of the results.

Sugar profile of the dry-hopped beer

Regarding the beer sugar profile over time, the superposed HPLC signals after 14 days of dry-hopping with hop, yeast or both, shown in Figure 1, reveal major modification in fermentable carbohydrates namely fructose, glucose and maltose. Furthermore, concerning the higher degree of polymerization sugars, maltotriose and maltopentaose present a sharp decrease. Their relative retention time compared with Floridi et al.[18] displayed on Table 3, suggests that it could be maltotriose and maltopentaose. No analytical standard being commercially available, the rest of the chromatogram was tentatively identified on the basis of the relative retention time. Owing to the method and column specificity, it can only consist of carbohydrates. Finally, these data highlight on the substrate selectivity of hop enzyme for low degree of polymerization sugars. In the presence of hop, these higher degrees of polymerization sugars are degraded by hop enzyme into simple sugars such as fructose, glucose, and maltose. In the presence of yeast, only higher degree of polymerization sugars are present. In the presence of hop and yeast, the higher degree of polymerization sugars are enzymatically degraded into glucose, fructose and maltose, which are directly consumed by the yeast.

Furthermore, the heatmap displayed in Figure 2, representing the six experiment modalities for each compound area after 1 and 14 days, demonstrates the impact of the interaction between hop and yeast. Indeed, the yeast metabolization of the enzyme product (fermentable carbohydrates glucose and maltose) removes the retro-inhibition usually encountered in enzymatic reactions. This phenomenon leads to the same attenuation of the enzyme main substrate presented earlier (maltotriose and maltopentaose) with either 5 g/L or 25 g/L of hop. The first modality represents the common brewery concentration of hop during dry-hopping. The small decay of maltotriose in the yeast alone modality informs us on yeast's ability to partially metabolize this sugar but is not in any way comparable to the modalities containing hops.

The calibration carried out for the fermentable sugar glucose and maltose allow their content during dry-hopping to be quantified exactly. The results shown in Figure 3 reveal two noticeable tendencies. On the one hand, the modalities with hop produce up to 4.5 g/L of sugar (for 25 g/L hop modality). On the other hand, modalities containing both hop and yeast decline to almost zero after a first rise indicating their total metabolization by yeast during dry-hopping. It is noteworthy to highlight the higher variation in maltose content for beer with 25 g/L of hop. The enzymatic transfer and activity may therefore greatly vary with the environmental conditions and base beer. Statistical significance of the treatments (time, hop and yeast) on glucose content was performed on squared root

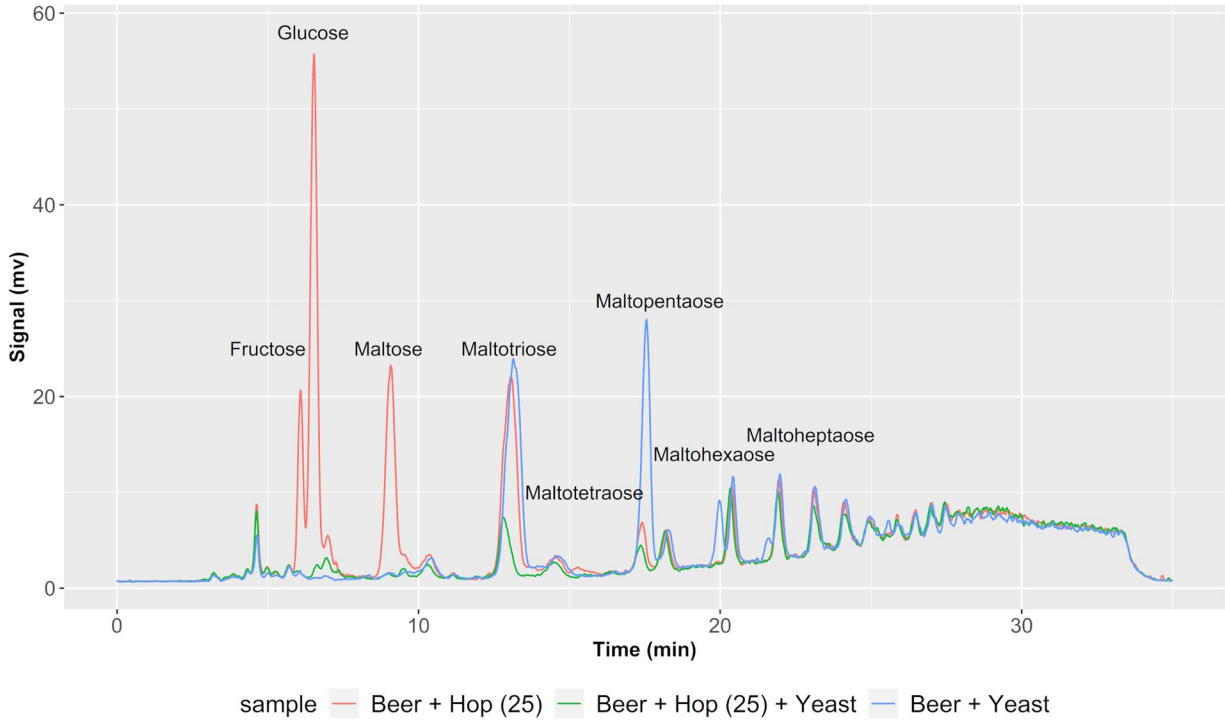

Figure 1. HPLC-ELSD beer sugar chromatogram after 14 days of dry-hopping. The red line represents beer in the presence of hop, the green line represents beer in the presence of hop and yeast and the blue line represents beer in the presence of yeast. (Color figure available online.)

Table 3. No reference standard being available for polymerization degree from 3 to 7, the peaks were tentatively identified (*) based on relative retention times available from Floridi et al.[18]

Carbohydrates	Retention time (min)	Relative retention time (RTT)	Theoretical RRT [32]
fructose	6.00	1.0	1.0
glucose	6.54	1.1	1.2
maltose	9.10	1.5	1.7
maltotriose*	12.79	2.1	2.0
maltotetraose*	14.49	2.4	2.6
maltopentaose*	17.37	2.9	3.0
maltohexaose*	20.34	3.4	3.2
maltoheptaose*	21.98	3.7	3.4

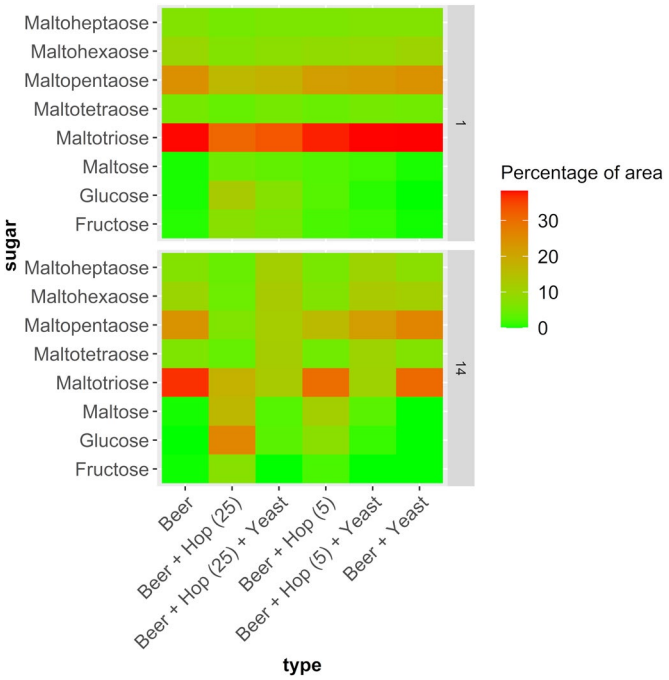

Figure 2. Heatmap of sugar area percentage after one day (on the top) and fourteen days (on the bottom) of dry-hopping.

transformed data. Due to significant three-way interaction between factors ($F(10, 72)= 2.44$, $P=0.015$) analyses were divided in two-way ANOVA in the presence and in the absence of yeast. In the presence of yeast, pairwise comparison shows significant differences only at day 1 (between hop 25, hop 5 and no hop). This implies that the liberated glucose was rapidly metabolized during the entire dry-hopping period. In the absence of yeast, the hop 25 yield gave significantly more glucose than hop 5 and no hop during the 14 days. The hop 5 impact was significant only at the end of the experiment. This analysis implies that the quantity of hop or enzymatic content impact more drastically the product than enzyme reaction time. Regarding the maltose content, similar statistical analyses were performed resulting in significant three-way interaction ($F(10,72)=4.1$, $P= <0.001$). In the presence of yeast, pairwise comparison shows significant difference only at day 1 between hop 25 and hop 0. As for glucose, maltose liberated by enzymatic activity is rapidly metabolized by the yeast. In the absence of yeast, the maltose concentration results are similar to glucose with a significant mean difference between hop 25 and hop 5 and no hop, at each time. Moreover, the difference between hop 5 and hop 0 was statistically significant from days 3 to 14. The results suggest that hop amylase activity produces significantly more maltose than glucose.

Aroma profile

Vicinal diketone production characterizes yeast's physiological activity in a nitrogen exhausted environment. Its production originates from endogenous amino acid production by yeast, the rate being impacted by other environmental parameters such as pH, temperature, etc.[20,21] Diketone production during dry-hopping has already been observed when stirring pellets.[22] The increase observed in Figure 4 after three days for modalities containing both hop and yeast further demonstrates that the sugar, liberated by both the hop and its enzymes, leads to secondary fermentation by the yeast, resulting in the production of vicinal diketones. With the sensory threshold of diacetyl being as low as 0.1 ppm, this may potentially alter the product's aroma profile. Regarding the ANOVA results for diacetyl, there was a statistically significant three-way interaction between time, hop, and yeast ($F(10, 70)= 2.99$, $P=0.003$). Following similar decomposition to sugar concentration, analyses were divided into the presence and absence of yeast. In the absence of yeast, there was no significant difference between modalities, which is obvious diacetyl production being conditioned by yeast metabolism. In the presence of yeast, diacetyl content significantly increased up to 227 ppb after 3 days. Moreover, pairwise comparison showed a significant difference between hop 25 and hop 5 and no hop, each time except after day 14. This decrease to a non-significant difference after 14 days implies that the lagering period may mitigate the hop creep. Finally, even a smaller hop concentration (5 g/L) yielded significantly higher diacetyl content than no hop from days 3 to 7.

Pentanedione statistical analysis also yielded significant three-way interaction ($F(10,69)=5.01$, $P<0.001$). In the presence of yeast, content significantly increased up to 189 ppb after 2 days. Moreover, pairwise comparison showed a significant difference between hop 25 and hop 5 or hop 0 except for day 14. The smaller hop concentration (5 g/L) also resulted in higher pentanedione content from days 3 to 7, as for diacetyl. The similar results further corroborated the reactivation of yeast metabolism through sugar production by hop amylase. In all of the previous analyses, every single interaction was highly significant implying that the factor cannot be interpreted separately and that the evolution with time is not the same for different treatments.

Concerning the other main fermentation aroma produced by yeast (alcohols, esters, and aldehydes), the concentration variation during dry-hopping is displayed in Table 4. Odor perception threshold values vary considerably and ranges are commonly used. The respective thresholds and aroma impressions are summarized as follows: diacetyl (100–200[21,23] µg L^{-1}), pentanedione (900–100[24] µg L^{-1}), isoamyl

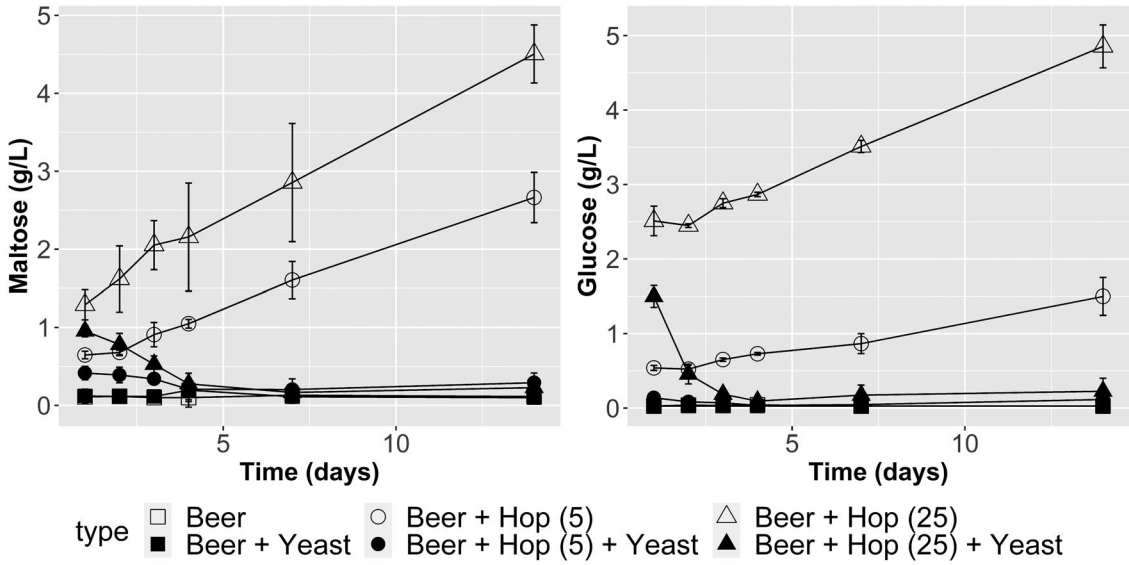

Figure 3. Fermentable sugars (maltose on the left and glucose on the right) concentration during dry-hopping . Beer alone (□), Beer with yeast (■), Beer with 5 g/L hop (○), Beer with 5 g/L hop and yeast (●), Beer with 25 g/L hop (△), Beer with 25 g/L hop and yeast (▲)

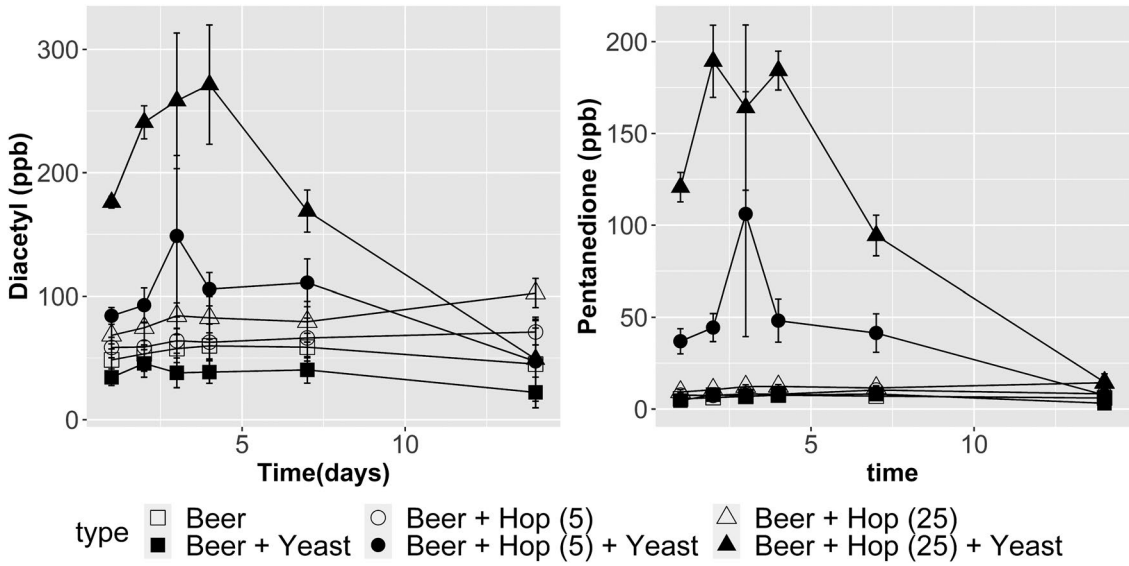

Figure 4. Vicinal diketone (diacetyl on the left and pentanedione on the right) production during dry-hopping. Beer alone (□), Beer with yeast (■), Beer with 5 g/L hop (○), Beer with 5 g/L hop and yeast (●), Beer with 25 g/L hop (△), Beer with 25 g/L hop and yeast (▲).

acetate (1.2–2,[25] 0.6–1.2[26] mg L^{-1}, banana), ethyl acetate (25–30,[25] 20–30,[26] 33[27] mg L^{-1}, solvent), propanol (600,[25], 700,[28], 800[27] mg L^{-1}, alcohol/sweet), isobutanol (100[25], 200 [29] mg L^{-1}, solvent) , isoamyl alcohols (50–65,[25] 70[29] mg L^{-1}, banana/alcoholic), ethyl caproate (0.2–0.23,[25], 0.21[29,30] mg L^{-1}, apple/fruity), ethyl caprylate (0.9–1.0,[25] 0.9[29,30] mg L^{-1}, apple/aniseed), acetaldehyde (25,[25] 0.11[31] mg L^{-1}, green leaves/fruity).

Hardly any general trend could be found when analyzing them alone, except for the higher acetaldehyde content of modalities containing yeast. Nevertheless, as some authors suggest regarding odor perceptions, changes in the concentration of a blend, even below the threshold value, can affect the overall perception.[32,33] It is therefore relevant to analyze the global variation of the beer aroma profile using a multivariate statistical analysis such as the principal component analysis (PCA).

Indeed, the PCA displayed in Figure 5 is a linear transformation of the many variables measured for each sample in order to create uncorrelated principal components maximizing the variance. The total percentage of explained variance for this analysis being 73.9% (Figure 5b). As can be observed, blank modalities remain in the center, whereas hop-yeast samples differentiate following the first component and hop alone following the second. The volatile contribution to each component is represented on the plot of variable contribution to component (Figure 5a). The first component therefore mainly reflects changes in ester while the second reflects changes in alcohol and diketone concentration. The hop-yeast modalities are well separated

Table 4. Volatile organic compound (VOC) quantification of the main aroma compounds produced by yeast throughout the dry-hopping following treatment and time.

		Diacetyl (μg L^{-1})	Pentanedione (μg L^{-1})	Acetaldehyde (mg L^{-1})	Ethyl acetate (mg L^{-1})	Propanol (mg L^{-1})	Isobutanol (mg L^{-1})	Isoamyl acetate (mg L^{-1})	Isoamyl alcohol (mg L^{-1})	Ethyl caproate (mg L^{-1})	Ethyl caprylate (mg L^{-1})
Aroma threshold		100–200[21,23]	900–100[23]	0.11–25[25,31]	20–33[25–27]	600–800[25,27,28]	100–200[25,29]	0.6–2[25,26]	50–70[25,29]	0.2–0.23[25,29,30]	0.9–1.0[25,29,30]
Beer	1	48.4 ± 13.1	5.5 ± 0.9	2.2 ± 0.4	33.8 ± 6.7	22.5 ± 1.3	23.5 ± 2.5	3.1 ± 0.7	132.7 ± 13	0.19 ± 0.04	0.81 ± 0.37
	2	53.3 ± 10.8	6.2 ± 1.7	2.2 ± 0.4	34.3 ± 9	22.7 ± 2	23.8 ± 4.5	3.1 ± 0.9	134.3 ± 22.7	0.17 ± 0.05	0.63 ± 0.53
	3	57.6 ± 11.3	6.9 ± 1.4	2.3 ± 0.3	35.3 ± 6.8	22.8 ± 2.4	23.7 ± 4.1	3.3 ± 0.5	144.8 ± 14.7	0.21 ± 0.01	0.8 ± 0.31
	4	59.8 ± 10.6	7.6 ± 1.2	2.3 ± 0.3	35.6 ± 6.1	22.6 ± 1.3	23.5 ± 2.7	3.2 ± 0.5	132.2 ± 14.7	0.18 ± 0.02	0.61 ± 0.3
	7	58.8 ± 11.3	7 ± 1.9	3.4 ± 1.7	28.2 ± 13.2	20 ± 3.1	20.8 ± 4.8	2.4 ± 1.3	117.6 ± 25.8	0.11 ± 0.06	0.38 ± 0.37
	14	45 ± 35.3	6 ± 2.9	2.4 ± 1.6	33.2 ± 1.3	22.2 ± 0.7	23 ± 2.3	2.7 ± 0.1	129.5 ± 9.1	0.13 ± 0.02	0.27 ± 0.14
Beer + Yeast	1	34.4 ± 6.8	4.6 ± 1.9	17.3 ± 7.1	35.2 ± 8.9	22.5 ± 1.8	23.4 ± 3.6	3.2 ± 0.9	130.7 ± 18.8	0.21 ± 0.04	0.9 ± 0.22
	2	45.6 ± 11.2	7.8 ± 0.9	35.2 ± 7.5	37.9 ± 7.2	23.7 ± 2.7	24.3 ± 4	3.4 ± 0.6	136.2 ± 23.5	0.22 ± 0.04	0.86 ± 0.35
	3	37.9 ± 12	7 ± 0.9	40.8 ± 3.2	37.7 ± 7.4	23.2 ± 2.1	23.5 ± 3.2	3.3 ± 0.6	130.5 ± 18.5	0.19 ± 0.04	0.61 ± 0.39
	4	38.6 ± 9.1	7.6 ± 0.8	48.8 ± 9.6	37.5 ± 6.1	22.9 ± 1.8	23.4 ± 4	3.3 ± 0.6	130.8 ± 20.9	0.2 ± 0.03	0.78 ± 0.23
	7	40.4 ± 10.7	8.2 ± 1.2	59.6 ± 12	38.5 ± 5.9	23.5 ± 1.1	23.8 ± 2.4	3.2 ± 0.5	131.7 ± 13.6	0.18 ± 0.04	0.48 ± 0.29
	14	22.1 ± 12.5	3.1 ± 2.5	50.1 ± 2.4	34.5 ± 1.9	22.9 ± 1.5	23 ± 1.3	2.5 ± 0.1	126.2 ± 6.2	0.15 ± 0.03	0.27 ± 0.12
Beer + Hop (5)	1	58.5 ± 8.3	7.6 ± 1.2	3.2 ± 1.1	33 ± 12.9	24.2 ± 3.7	23.3 ± 4.1	2.8 ± 1.1	132.1 ± 24.1	0.17 ± 0.06	0.67 ± 0.34
	2	58.9 ± 19.7	7.4 ± 2.9	3.1 ± 1.2	33.2 ± 9.1	23.9 ± 3.4	22.3 ± 5.2	2.7 ± 0.8	128.9 ± 27.2	0.15 ± 0.05	0.48 ± 0.43
	3	64 ± 10.1	8.3 ± 0.8	3.4 ± 0.7	32.2 ± 7.4	24.1 ± 2.3	23.2 ± 3.5	2.5 ± 0.6	130.2 ± 19.8	0.15 ± 0.04	0.6 ± 0.31
	4	62.7 ± 14.8	8 ± 2.6	3.3 ± 1.2	34.7 ± 10.6	23.6 ± 4.8	22.4 ± 5.4	2.6 ± 0.8	126.3 ± 33.4	0.14 ± 0.05	0.43 ± 0.37
	7	66.2 ± 16.1	10.3 ± 0.4	8.1 ± 7.9	27.8 ± 10.4	24.1 ± 1.7	22.2 ± 3.7	1.9 ± 0.8	124.8 ± 17.7	0.1 ± 0.06	0.32 ± 0.28
	14	71 ± 10.4	8.3 ± 0.5	3.3 ± 2.4	30.7 ± 3.9	24.6 ± 3.3	22.7 ± 2.5	1.6 ± 0	126.8 ± 21.2	0.12 ± 0.04	0.25 ± 0.16
Beer + Hop (25)	1	68.4 ± 11	9.3 ± 1.3	4.8 ± 2.8	30.3 ± 10.4	30 ± 3	22.2 ± 2.9	1.8 ± 0.6	125 ± 17	0.12 ± 0.04	0.42 ± 0.17
	2	74.6 ± 14	10.5 ± 1	5.2 ± 3.3	33.7 ± 7.9	30.6 ± 1.8	22.4 ± 2.3	1.7 ± 0.3	126.4 ± 14.3	0.13 ± 0.05	0.3 ± 0.24
	3	84.2 ± 10.5	12.2 ± 1	6.2 ± 2.9	29.1 ± 6.7	29.4 ± 2.4	21.3 ± 3.6	1.3 ± 0.2	118.2 ± 19.5	0.11 ± 0.04	0.31 ± 0.14
	4	82.5 ± 17.3	12.3 ± 1	6.1 ± 3.6	32 ± 6.5	30.8 ± 2.5	21.8 ± 3.6	1.4 ± 0.2	122.8 ± 21.8	0.11 ± 0.04	0.23 ± 0.18
	7	79.4 ± 16.5	11.5 ± 1.2	6.2 ± 2.6	21 ± 15.5	29.1 ± 2.4	19.8 ± 3.7	0.7 ± 0.5	112.9 ± 21.3	0.07 ± 0.06	0.16 ± 0.12
	14	102.5 ± 11.7	14.3 ± 2.7	7.5 ± 5.6	30.6 ± 3.2	38.3 ± 0.5	24.5 ± 0.3	0.5 ± 0	136.1 ± 4.1	0.11 ± 0.04	0.13 ± 0.08
Beer + Hop (5) + Yeast	1	84.2 ± 6.8	36.9 ± 6.8	31.1 ± 5.7	32.7 ± 10.7	24 ± 2.7	22 ± 3.8	2.7 ± 0.9	123.2 ± 21	0.19 ± 0.06	0.65 ± 0.2
	2	92.8 ± 13.8	44.4 ± 7.6	37 ± 2.2	37.5 ± 8.2	25.9 ± 2.7	23.3 ± 3.9	3 ± 0.6	129 ± 23	0.21 ± 0.05	0.58 ± 0.21
	3	124.3 ± 62.6	82 ± 63	34.7 ± 0.7	35.8 ± 6.9	31.2 ± 9.7	23.3 ± 2.8	2.3 ± 0.4	126.7 ± 13	0.18 ± 0.03	0.35 ± 0.07
	4	105.7 ± 13.4	48.1 ± 11.7	40.9 ± 7.6	37.8 ± 5.7	28.5 ± 1.7	24.6 ± 2.9	2.8 ± 0.4	133.8 ± 17	0.21 ± 0.04	0.54 ± 0.14
	7	110.8 ± 19.2	41.4 ± 10.5	28.5 ± 14.6	38.2 ± 4.1	30.4 ± 2.4	24.7 ± 1	2.5 ± 0.3	129.7 ± 3.1	0.19 ± 0.04	0.33 ± 0.16
	14	47.2 ± 27.1	7.6 ± 2.9	12.8 ± 5.9	38.9 ± 5.3	33.5 ± 3.2	27.7 ± 1.2	1.8 ± 0.1	142.4 ± 11.6	0.17 ± 0.05	0.18 ± 0.06
Beer + Hop (25) + Yeast	1	176.1 ± 4.8	120.7 ± 8	23.5 ± 4.6	32.1 ± 9.1	33.5 ± 2.8	23 ± 3.2	1.8 ± 0.4	126.5 ± 16.8	0.14 ± 0.04	0.38 ± 0.1
	2	240.9 ± 13.4	189.3 ± 19.6	32.8 ± 14.3	35 ± 9.5	37.8 ± 3.9	24.2 ± 4.5	1.7 ± 0.3	130.7 ± 24	0.16 ± 0.06	0.34 ± 0.12
	3	227.7 ± 103.9	142.9 ± 81.7	38.3 ± 20.7	37.1 ± 8	35.7 ± 6.6	24.2 ± 2.3	2.1 ± 1.1	130.8 ± 16.5	0.17 ± 0.08	0.35 ± 0.31
	4	271.4 ± 48.4	184.3 ± 10.6	22.9 ± 14.7	36.7 ± 8.2	43.5 ± 4	26.1 ± 3.1	1.5 ± 0.3	137.8 ± 18.7	0.18 ± 0.05	0.33 ± 0.07
	7	168.9 ± 17.1	94.4 ± 11.1	7.9 ± 2.8	36.3 ± 4.6	46 ± 3.7	25.5 ± 1.4	1.1 ± 0.1	129.6 ± 7.9	0.16 ± 0.04	0.2 ± 0.08
	14	49.1 ± 34.1	14.6 ± 4.6	16.2 ± 5.9	37.8 ± 4.2	48.4 ± 4.5	28.2 ± 1.8	0.6 ± 0.1	137.6 ± 12.4	0.15 ± 0.03	0.13 ± 0.03

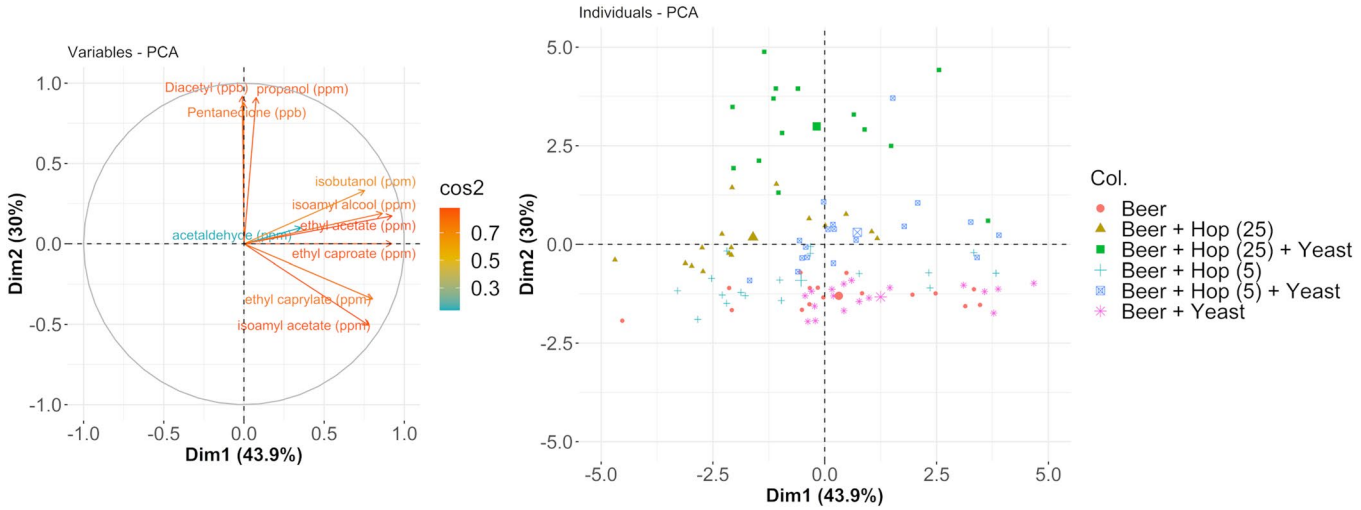

Figure 5. First two dimensions of correlation matrix principal component analysis (PCA) of yeast aroma products where (a) plot of variable contribution to component represents by arrows length. (b) plot of individuals colored by treatment were dot size represent the quality of representation for a given observation. (Color figure available online.)

following this second component, demonstrating beer aroma profile modification generated by their interaction. Treatments containing both yeast and hop result in a higher content in esters than hop alone. This result suggests that as for vicinal diketones, yeast sugar metabolization may result in other aroma production. It is also noteworthy to consider that esterase contained in hop may explain this phenomenon. Indeed, in the first draft of the hop genome presented by Natsume et al.[19] many sequences (35) correspond to esterase. Nevertheless, this does not imply that these sequences are expressed or that the enzyme is active in this specific condition and on those specific substrates.

Conclusion

This work highlights the potential contribution of hop enzymatic content to the beer aroma profile during dry-hopping through yeast metabolization of the sugars produced. Indeed, α and β-amylase activity were assessed for fermentable sugar production. Furthermore, the metabolization of carbohydrates by yeast in a nitrogen deprived environment led to vicinal diketone formation (both diacetyl and pentanedione) above their threshold value, after three days of dry-hopping. Lastly, the reactivation of the yeast's metabolic activity led to a change in the aromatic profile of the beer as demonstrated by the PCA.

However numerous parameters are to be considered for this potential contribution depending on the dry-hopping method, which many brewers perform differently. Previous works have demonstrated the effect of hop variety.[16] Temperature of dry-hopping may also be crucial for enzyme and yeast activity. Moreover, yeast concentration and physiological state are determinant to convert the fermentable sugars.

Nevertheless, this research reflects that even a degree of enzyme activity can lead to drastic changes, especially in the field of flavor. Indeed, due to the synergy and antagonism between these volatile compounds, the slightest variation in concentration leads to perception modification.

In order to ensure consistent dry-hopped beer production, numerous actions can be undertaken to mitigate the hop creep and avoid the alterations previously developed. They will therefore be discussed and their limitations will be developed.

The first one being to avoid the presence of yeast during dry-hopping, either by pasteurization, filtration, or centrifugation. Pasteurization may also inactivate the enzymes sensitive to temperature. However, yeast presence may be critical for beer ageing,[34] biotransformation[7,8, 35,36] and release of bound volatiles by its β-lyase activity.[5, 37]

The second one is the inactivation of these enzymes following hop kilning at higher temperature. Classical kilning temperature is around 50 °C but enzymatic activity reduction by ~1.2, ~1.6, and ~2.6 times was observed for temperature from 60 °C to 80 °C. However, this may slightly impact hop oil content and constitution depending on the variety.[38] Other works suggest a great loss of hop oil content at higher kilning temperatures.[39] Although as stated, overall hop aroma intensity of a beer does not solely depend on the hop oil.[6]

The last one being to dry-hop beer at a lower temperature. However, transfer rate of volatile compounds may also be impacted. The concentration of monoterpenes such as β-myrcene in dry-hopped beers are higher in a warmer extraction.[40]

Enzymatic content and other non-volatiles transfer during dry-hopping should therefore be considered as it may significantly impact beer aroma and stability.[38] Enzymatic content should be acknowledged in hop quality for the dry-hopping process. Brewers must bear in mind its potential influence on the aroma in presence of yeast by reactivation of its metabolism as a result of the enzyme activity. The effect of temperature, duration of dry-hopping, and the

presence of yeast should also be considered in regard to this freshening power of hops.

Acknowledgments

The authors would like to acknowledge the technical support of Franck Michels, Thomas Bertrand as well as Danny Trisman.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Pierre-Yves Werrie http://orcid.org/0000-0002-5767-0887

Literature cited

[1] Forster, A.; Gahr, A. On the Fate of Certain Hop Substances during Dry Hopping. *BrewingScience* **2013**, *66*, 93–103.
[2] Wolfe, P. H. A Study of Factors Affecting the Extraction of Flavor When Dry Hopping Beer. MSc Thesis, Oregon State University, **2012**.
[3] Wolfe, P.; Qian, M. C.; Shellhammer, T. H. The Effect of Pellet Processing and Exposure Time on Dry Hop Aroma Extraction. In *Proceedings of the ACS Symposium Series*, American Chemical Society, **2012**, 1104, 203–215.
[4] Bailey, B.; Schönberger, C.; Drexler, G.; Gahr, A.; Newman, R.; Pöschl, M.; Geiger, E. The Influence of Hop Harvest Date on Hop Aroma in Dry-Hopped Beers. *Tech. Q. Master Brew. Assoc. Am* **2009**, *46*, 1–7. DOI: 10.1094/ TQ-46-2-0409-01.
[5] Lafontaine, S.; Varnum, S.; Roland, A.; Delpech, S.; Dagan, L.; Vollmer, D.; Kishimoto, T.; Shellhammer, T. Impact of Harvest Maturity on the Aroma Characteristics and Chemistry of Cascade Hops Used for Dry-Hopping. *Food Chem.* **2019**, *278*, 228–239. DOI: 10.1016/j.foodchem.2018.10.148.
[6] Vollmer, D. M.; Shellhammer, T. H. Influence of Hop Oil Content and Composition on Hop Aroma Intensity in Dry-Hopped Beer. *J. Am. Soc. Brew. Chem.* **2016**, *74*, 242–249. DOI: 10.1094/ASBCJ-2016-4123-01.
[7] King, A. J.; Dickinson, J. R. Biotransformation of Hop Aroma Terpenoids by Ale and Lager Yeasts. *FEMS Yeast Res.* **2003**, *3*, 53–62. DOI: 10.1016/S1567-1356(02)00141-1.
[8] King, A.; Dickinson, J. R. Biotransformation of Monoterpene Alcohols by *Saccharomyces cerevisiae, Torulaspora delbrueckii* and *Kluyveromyces lactis*. *Yeast* **2000**, *16*, 499–506. DOI: 10.1002/(SICI)1097-0061(200004)16:6<499::AID-YEA548>3.0.CO;2-E.
[9] Daenen, L.; Saison, D.; Sterckx, F.; Delvaux, F. R.; Verachtert, H.; Derdelinckx, G. Screening and Evaluation of the Glucoside Hydrolase Activity in *Saccharomyces* and *Brettanomyces* Brewing Yeasts. *J. Appl. Microbiol.* **2008**, *104*, 478–488. DOI: 10.1111/j.1365-2672.2007.03566.x.
[10] Sharp, D. C.; Steensels, J.; Shellhammer, T. H. The Effect of Hopping Regime, Cultivar and β-Glucosidase Activity on Monoterpene Alcohol Concentrations in Wort and Beer. *J. Inst. Brew.* **2017**, *123*, 185–191. DOI: 10.1002/jib.418.
[11] Gros, J.; Tran, T. T. H.; Collin, S. Enzymatic Release of Odourant Polyfunctional Thiols from Cysteine Conjugates in Hop. *J. Inst. Brew.* **2013**, *119*, 221–227. DOI: 10.1002/jib.80.
[12] Kirkpatrick, K. R.; Shellhammer, T. H. Evidence of Dextrin Hydrolyzing Enzymes in Cascade Hops (*Humulus lupulus*). *J. Agric. Food Chem.* **2018**, *66*, 9121–9126. DOI: 10.1021/acs.jafc.8b03563.
[13] Janicki, J.; Kotasthane, W. V.; Parker, A.; Walker, T. K. The Diastatic Activity of Hops, Together with a Note on Maltase in Hops. *J. Inst. Brew.* **1941**, *47*, 24–36. DOI: 10.1002/j.2050-0416.1941.tb06070.x.
[14] Brown, H. T.; Morris, G. H. A Contribution to the Chemistry and Physiology of Foliage-Leaves. *Ann. Bot.* **1893**, *7*, 271–289.
[15] Kirkendall, J. A.; Mitchell, C. A.; Chadwick, L. R. The Freshening Power of Centennial Hops. *J. Am. Soc. Brew. Chem.* **2018**, *76*, 178–184. DOI: 10.1080/03610470.2018.1469081.
[16] Kirkpatrick, K. R.; Shellhammer, T. H. A Cultivar-Based Screening of Hops for Dextrin Degrading Enzymatic Potential. *J. Am. Soc. Brew. Chem.* **2018**, *76*, 247–256. DOI: 10.1080/03610470.2018.1546091.
[17] Oladokun, O.; James, S.; Cowley, T.; Smart, K.; Hort, J.; Cook, D. Dry-Hopping: The Effects of Temperature and Hop Variety on the Bittering Profiles and Properties of Resultant Beers. *Brew. Sci.* **2017**, *70*, 187–196.
[18] Floridi, S.; Miniati, E.; Montanari, L.; Fantozzi, P. Carbohydrate Determination in Wort and Beer by HPLC-ELSD. *Monatsschrift Für Brauwissenshaft* **2001**, *54*, 209–215.
[19] Natsume, S.; Takagi, H.; Shiraishi, A.; Murata, J.; Toyonaga, H.; Patzak, J.; Takagi, M.; Yaegashi, H.; Uemura, A.; Mitsuoka, C.; et al. The Draft Genome of Hop (*Humulus lupulus*), an Essence for Brewing. *Plant Cell Physiol.* **2015**, *56*, 428–441. DOI: 10.1093/pcp/pcu169.
[20] Dzialo, M. C.; Park, R.; Steensels, J.; Lievens, B.; Verstrepen, K. J. Physiology, Ecology and Industrial Applications of Aroma Formation in Yeast. *FEMS Microbiol. Rev.* **2017**, *41*, 95–128.
[21] Krogerus, K.; Gibson, B. R. 125th Anniversary Review: Diacetyl and Its Control during Brewery Fermentation. *J. Inst. Brew.* **2013**, *119*, 86–97.
[22] Baillo, A. J.; Ley, A.; Brigham, M. Dry Hopping and Stirring Pellets Increases Vicinal Diketones and Lowers Apparent Extract. Presented at the ASBC Annual Meeting, Fort Myers, FL, June 4-7 **2017**.
[23] Wainwright, T. Diacetyl—a Review: Part I—Analytical and Biochemical Considerations: Part I—Brewing Experience. *J. Inst. Brew.* **1973**, *79*, 451–470. DOI: 10.1002/j.2050-0416.1973.tb03567.x.
[24] Leça, J. M.; Pereira, A. C.; Vieira, A. C.; Reis, M. S.; Marques, J. C. Optimal Design of Experiments Applied to Headspace Solid Phase Microextraction for the Quantification of Vicinal Diketones in Beer through Gas Chromatography-Mass Spectrometric Detection. *Anal. Chim. Acta* **2015**, *887*, 101–110.
[25] Pires, E. J.; Teixeira, J. A.; Brányik, T.; Vicente, A. A. Yeast: The Soul of Beer's Aroma—a Review of Flavour-Active Esters and Higher Alcohols Produced by the Brewing Yeast. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 1937–1949. DOI: 10.1007/s00253-013-5470-0.
[26] Verstrepen, K. J.; Derdelinckx, G.; Dufour, J. P.; Winderickx, J.; Thevelein, J. M.; Pretorius, I. S.; Delvaux, F. R. Flavor-Active Esters: Adding Fruitiness to Beer. *J. Biosci. Bioeng.* **2003**, *96*, 110–118. DOI: 10.1016/S1389-1723(03)90112-5.
[27] Renger, R. S.; Hateren, S. H. v.; Luyben, K. C. A. M. The Formation of Esters and Higher Alcohols during Brewery Fermentation: The Effect of Carbon Dioxide Pressure. *J. Inst. Brew.* **1992**, *98*, 509–513. DOI: 10.1002/j.2050-0416.1992.tb01137.x.
[28] Hughes, P. S.; Baxter, E. D. **2001**. An Overview of the Malting and Brewing Processes. In *Beer: Quality, Safety and Nutritional Aspects*. Cambridge, UK: The Royal Society of Chemistry; pp 1–13.
[29] Olaniran, A. O.; Hiralal, L.; Mokoena, M. P.; Pillay, B. Flavour-Active Volatile Compounds in Beer: production, Regulation and Control. *J. Inst. Brew.* **2017**, *123*, 13–23. DOI: 10.1002/jib.389.
[30] Meilgaard, M. C. Flavor Chemistry in Beer: Part I: Flavor Interaction between Principal Volatiles. *Tech. Q. Master Brew. Assoc. Am.* **1975**, *12*, 107–117.
[31] Saison, D.; De Schutter, D. P.; Uyttenhove, B.; Delvaux, F.; Delvaux, F. R. Contribution of Staling Compounds to the Aged Flavour of Lager Beer by Studying Their Flavour Thresholds. *Food Chem.* **2009**, *114*, 1206–1215. DOI: 10.1016/j.foodchem.2008.10.078.

[32] Vanderhaegen, B.; Neven, H.; Coghe, S.; Verstrepen, K. J.; Verachtert, H.; Derdelinckx, G. Evolution of Chemical and Sensory Properties during Aging of Top-Fermented Beer. *J. Agric. Food Chem.* **2003**, *51*, 6782–6790. DOI: 10.1021/jf034631z.

[33] Genva, M.; Kemene, T. K.; Deleu, M.; Lins, L.; Fauconnier, M. L. Is it Possible to Predict the Odor of a Molecule on the Basis of Its Structure? *IJMS.* **2019**, *20*, 3018. DOI: 10.3390/ijms20123018.

[34] Saison, D.; De Schutter, D. P.; Delvaux, F.; Delvaux, F. R. Improved Flavor Stability by Aging Beer in the Presence of Yeast. *J. Am. Soc. Brew. Chem.* **2011**, *69*, 50–56. DOI: 10.1094/ASBCJ-2011-0127-01.

[35] Peacock, V. E.; Deinzer, M. L.; Likens, S. T.; Nickerson, G. B.; McGill, L. A. Floral Hop Aroma in Beer. *J. Agric. Food Chem.* **1981**, *29*, 1265–1269. DOI: 10.1021/jf00108a041.

[36] Takoi, K.; Koie, K.; Itoga, Y.; Katayama, Y.; Shimase, M.; Nakayama, Y.; Watari, J. Biotransformation of Hop-Derived Monoterpene Alcohols by Lager Yeast and Their Contribution to the Flavor of Hopped Beer. *J. Agric. Food Chem.* **2010**, *58*, 5050–5058. DOI: 10.1021/jf1000524.

[37] Abe, H.; Ishizuka, Y.; Inui, T. Behavior of Thiols Present in Hop during Brewing Process under Various Brewing Conditions. *Brewing Summit*, San Diego, CA, August 12–15, **2018**.

[38] Lafontaine, S. R.; Shellhammer, T. H. Investigating the Factors Impacting Aroma, Flavor, and Stability in Dry-Hopped Beers. *Tech. Q. Master Brew. Assoc. Am.* **2019**, *56*, 13–23.

[39] Rybka, A.; Krofta, K.; Heřmánek, P.; Honzík, I.; Pokorný, J. Effect of Drying Temperature on the Content and Composition of Hop Oils. *Plant Soil Environ.* **2018**, *64*, 512–516.

[40] Haslbeck, K.; Minkenberg, D.; Coelhan, M. Investigations into the Transfer Rate of Volatile Compounds in Dry Hopping Using an Octanol-Water Partition Coefficient Model. *J. Am. Soc. Brew. Chem.* **2018**, *76*, 169–177. DOI: 10.1080/03610470.2018.1483701.