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The Influence of Yeast Strains and Hop Varieties on the Aroma of Beer

Hops and yeast are two crucial factors when it comes to beer flavor. Depending on the stage hops are added during the brewing process, they can have a high impact on the final beer flavor. Over 400 flavor active compounds in hop oil have been identified so far. Moreover, there is also a range of complex biochemical reactions catalyzed by fermentation that can largely contribute to the hoppy aroma in beer. Biotransformation of mono- and sesquiterpene hydrocarbons as well as monoterpene alcohols, the effect of enzymes on hop aglycones and the release of odor-active thiols are the main biochemical reactions that have been proven to affect the hop aroma in beer. The influence of two yeast (California Ale Yeast and German Ale/Kolsch Yeast) and two hop varieties (Cascade and Hallertau Mittelrüh) on the final aroma profile was analyzed especially on the content of thiols 3-mercaptohexan-1-ol, 3-mercaptohexanacetat, and 4-mercapto-4-methylpentan-2-one terpenes and esters in beer. All the beers with dry hopping treatment that were analyzed had higher concentrations of 3-mercaptohexan-1-ol and 4-mercapto-4-methylpentan-2-one than the aroma threshold. The concentration of 4-mercapto-4-methylpentan-2-one is mainly dependent on the hop variety chosen and not on the yeast. The results show differences in the concentration of geraniol and citronellol when using two different top fermenting yeast strains.

Descriptors: biotransformation, hop aroma, hops, yeast, thiols

1 Introduction

Beer is one of the most complex beverages in the world and the different combinations of ingredients will lead to an endless range of flavor impressions. More than 250 different hop varieties, around 40 types of malt and over 200 different yeast strains exist to be used in the brewing process [1].

The contribution of yeast and hops on their own to the overall beer flavor was subject to many studies over the last few decades. However, studies on how these two ingredients affect each other in terms of flavor development are scarce. The mono- and sesquiterpene hydrocarbons like β -caryophyllene, β -myrcene and α -humulene as well as monoterpene alcohols like linalool, geraniol, β -citronellol, etc. in hop oil have been proposed to be the main contributors to hop aroma in beer [2, 3]. Kaltner et al. [4, 5], proposed linalool to be the key aroma compound for the intensity and quality of hoppy aroma in beer.

King and Dickinson [6, 7] were some of the first researchers to investigate a possible biotransformation of monoterpene alcohols by

Saccharomyces cerevisiae. They could find proof for the reduction of geraniol to citronellol, the translocation of geraniol and nerol to linalool and the cyclization of nerol and linalool to α -terpineol. Studies from Takoi et al. [8, 9] confirmed these findings and stated that the citronellol levels increased during fermentation while geraniol levels decreased. As the rapid decline of geraniol in the first days of fermentation did not correspond with the continuous increase of citronellol, Takoi et al. [9] suggested that citronellol is not only reduced from geraniol, but also glycosidically-bound citronellol is hydrolyzed by yeast.

The release of glycosidically bound aroma compounds present in the vegetative hop material and their contribution to the hoppy aroma in beer has been reported in various studies [10–13]. Biendl et al. [10] and Kollmannsberger et al. [13] investigated the glycoside content of a lager beer hopped with ethanol hop extract and pellets. They found that acid and enzymatic hydrolysis of glycoside fractions led to the release of a whole series of compounds, including linalool and geraniol. Kollmannsberger et al. [13] concluded that an addition of hop cones, pellets or ethanol extracts in wort or beer leads to an increase of hop-derived glycosidically bound aroma precursor. The impact of those glycosides on the beer flavor however, depends on their concentration and the degree of hydrolysis, which is necessary to create flavours. The number of hydrolyzed glycosides also depends on the selected yeast strain.

The origin of thiols in beers is complex, partially elucidated and depends on several parameters such as the type of raw ingredients (malts, hops especially) and the brewing process (from mashing step to post-fermentation operations) [14]. The hop derived thiols 3-mercaptohexan-1-ol (3MH), 3-mercaptohexanacetat (3MHA) and 4-mercapto-4-methylpentan-2-one (4MMP) have not been sub-

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Table 1 Specifications of the hop pellets measured by the provider

| | Hallertau Mittelfrüh | Cascade US |
|----------------------------------|-------------------------|------------|
| Alpha-Acids (% w/w) | 4.7 | 5.6 |
| Oil Content (mL/100g) | 0.95 | 1.10 |
| Myrcene (mg/100g) | 234 | 375 |
| β -Caryophyllene (mg/100g) | 62 | 65 |
| α -Humulene (mg/100g) | 216 | 176 |
| Farnesene (mg/100g) | 15 | 41 |
| Linalool (mg/100g) | 7 | 5 |
| Geraniol (mg/100g) | 2 | 17 |
| Sum of Monoterpene (mg/100g) | 238 | 384 |
| Sum of Sesquiterpene (mg/100g) | 322 | 325 |

jected to many studies in the past years, although it is suggested that their contribution to hoppy aroma in beer is important. Hops represent a significant source of thiol precursors either as free or bound forms [14]. A low flavor threshold (below 80–90 ng/L) and a fruity, cassis and passion fruit-like aroma is characteristic for volatile sulphur compounds [15]. Because of the very low odor threshold, thiols can possibly influence the final flavor of hoppy beers. 3MH, 3MHA and 4MMP are well known for contributing to wine flavors and have been identified in Sauvignon Blanc and other wines [16, 17]. The enzymatic release of aromatic, grape-derived thiols by *Saccharomyces cerevisiae* has been reported by *Swiegers et al.* [18]. Recent studies by *Gros et al.* [19, 20] focused on hop derived thiols and their enzymatic release. *Gros et al.* [19] analyzed the odorant polyfunctional thiols from cysteine conjugates and described Cascade to have the highest bound 3-sulphanylhexan-1-ol (grapefruit-like) potential, while both Tomahawk and Nelson Sauvin are to be important sources of bound 3-methyl-2-butene-1-thiol, 3-sulphanylpentan-1-ol and 4-sulphanyl-4-methylpentan-2-one. These authors also found cysteine conjugates in CO₂ extract. *Tran et al.* [21] described the evolution of minor thiols through beer aging.

The aim of this work was to investigate the influence of two different yeast strains on the overall hop aroma in finished beers. It was focused on the most important monoterpene alcohols linalool, geraniol, β -citronellol, nerol and α -terpineol as well as on the thiols 4MMP, 3MH and 3MHA. 4MMP, 3MH and 3MHA were chosen because of their very low flavor threshold, which means

that these thiols can significantly affect the hoppy aroma in beer even in low concentrations. This work is supported by previous studies on biotransformation of hop derived monoterpene alcohols, release of glycosidically bound precursor and the enzymatic release of hop derived thiols by *Saccharomyces cerevisiae* and should give a guideline on how different yeast strains can intensify hoppy aroma in beer.

2 Materials and Methods

2.1 Hops and hop products

Isohop[®] extract (Barth-Haas Group) with a concentration of 30% was used to adjust bitterness in the beers. Pellets type 90 from the varieties Hallertau Mittelfrüh (Germany) and Cascade (USA) from the 2015 harvest (Barth-Haas Group) were used for dry hopping treatments. Before use, the hop products were stored at -5°C in vacuum-sealed packaging. Table 1 shows the specifications of the hop pellets measured by the provider.

2.2 Yeast

Two top fermenting yeast strains from White Labs (San Diego, USA) were used. Both WLP001 California Ale Yeast and WLP029 German Ale/Kolsch Yeast were in liquid form with a cell count of at least 2.5 billion cells per mL yeast suspension.

2.3 Pilot brewing

Beers were produced in a 100 L microbrewery (Barth-Haas Group, UK) with the same brewing protocol. For the production of all pilot beers, 13 kg of Pale Ale malt, generously donated by Muntions PLC, was brewed in 50 L of water, according to the following mashing program: 100 min at 67°C . The wort was then filtered through the lauter tun. After sparging, 75 L of wort with a density of 11.1 °P was obtained. The wort was boiled for 90 min under atmospheric conditions, and the final density was adjusted to 12 °P by addition of water. Isohop[®] extract 30% was added after wort cooling for all beers to achieve a bitterness of 20 IBUs.

To evaluate the influence of the yeast and hops, the cooled wort was split into four fermenting vessels with 12 L each. Two fermenting vessels were pitched with WLP001 California Ale Yeast and two with

Table 2 Mean values of the sum of esters, the content of the six main esters and sum of esters ($\mu\text{g/L}$) with the highest concentration of the beers brewed with Cascade or Mittelfrüh and with WLP001 or WLP029. Lowercase letters indicate significant differences between the beers according to the t-tests.

| ($\mu\text{g/L}$) | WLP001+CA | WLP001+HHA | WLP029+CA | WLP029+HHA |
|-----------------------|----------------------------------|----------------------------------|-----------------------------------|-----------------------------------|
| Sum of terpenes | 808.5 \pm 34.97 ^a | 385.4 \pm 37.50 ^b | 693.1 \pm 53.92 ^c | 384.4 \pm 15.60 ^d |
| 2-Phenylethyl acetate | 121.0 \pm 18.63 ^a | 162.0 \pm 24.36 ^b | 756.7 \pm 46.65 ^c | 816.0 \pm 35.62 ^c |
| Ethyl butyrate | 94.6 \pm 14.28 ^a | 112.0 \pm 18.55 ^a | 130.2 \pm 11.73 ^a | 131.5 \pm 19.97 ^a |
| Ethyl hexanoate | 180.5 \pm 12.79 ^a | 192.0 \pm 15.72 ^a | 208.0 \pm 24.04 ^a | 179.8 \pm 24.09 ^a |
| Ethyl lactate | 1204.6 \pm 25.95 ^a | 1251.1 \pm 49.95 ^a | 1150.9 \pm 39.12 ^b | 1179.1 \pm 62.46 ^b |
| Ethyl octanoate | 202.4 \pm 91.42 ^a | 200.5 \pm 43.43 ^a | 153.3 \pm 13.08 ^a | 130.6 \pm 10.77 ^b |
| Isoamyl acetate | 662.4 \pm 133.08 ^a | 779.7 \pm 149.69 ^a | 2697.3 \pm 821.52 ^b | 2814.3 \pm 804.11 ^b |
| Sum of esters | 2609.4 \pm 221.50 ^a | 2826.1 \pm 249.35 ^a | 5492.9 \pm 1347.90 ^b | 5681.4 \pm 1217.17 ^b |

Table 3 Barth Haas tasting scheme

| | Nuance | Intensity 1–10 |
|----------------|---|-------------------|
| Floral | Elderflower, camomile blossom, lily of the valley, jasmine, apple blossom, rose, geranium, carnation, lily, lilac, lavender | |
| Citrus | Grapefruit, orange, lime, lemon, bergamot, lemon grass, ginger, tangerine | |
| Sweet Fruits | Banana, watermelon, honeydew melon, peach, apricot, passion fruit, lychee, dried fruit, plum, pineapple, cherry, kiwi, mango, guava | |
| Green Fruits | Pear, quince, apple, gooseberry, white wine grapes | |
| Red Berries | Cassis, blueberry, raspberry, blackberry, strawberry, red currant, black currant, wild strawberry, cranberry | |
| Cream Caramel | Butter, chocolate, yoghurt, honey, cream, caramel, toffee, coffee, vanilla, tonka | |
| Woody Aromatic | Tobacco, cognac, barrique, leather, woodruff, incense, myrrh, resin, earth, cedar, pine | |
| Menthol | Mint, balm, camphor, menthol, wine yeast | |
| Herbal | Lovage, marjoram, tarragon, dill, parsley, basil, fennel, coriander, rosemary, thyme, green tea, black tea, mate tea, sage | |
| Spicy | Pepper, chilli, curry, juniper, aniseed, nutmeg, liquorice, clove, gingerbread, fennel seeds, cinnamon, coriander seed | |
| Green | Green grassy, fresh cut grass, hay, tomato leaves, green pepper, nettle, cucumber | |
| Vegetal | Celery stalk, celery root, leek, onion, artichoke, garlic, wild garlic | |

WLP029 German Ale Yeast. A 40 mL pack of yeast was added to each vessel. According to the supplier, the cell count was at least 2.5 billion cells per mL. Pitching and fermentation were carried out at 20–22 °C. Once the wort reached 4°P (around 3 days of fermentation), dry hopping was applied to each beer with a single hop variety. One of the vessels pitched with WLP001 California Ale Yeast was dry hopped with 43.6 g Cascade US (CA) and the other with 50.5 g Hallertau Mittelfrüh (HHA). The same was done with the vessels pitched with WLP029 German Ale Yeast. This hop dosage equals 4 mL hop oil per hL wort. Afterwards, when fermentation reached 2°P the temperature was reduced to 4–5 °C for 5 days. After fermentation, the hops were separated from the beer by sedimentation. Trials were replicated seven times in total. A total of 6 beer were brewed, two control beers, one with WLP001 (WLP001 CTRL) and one with WLP029 (WLP029 CTRL), two beers brewed with Cascade (WLP001+CA and WLP029+CA) and two beers brewed with Hallertau Mittelfrüh (WLP001+HHA and WLP029+HHA).

Beers were bottled immediately and stored under cold conditions (<5 °C) until further analysis. Control beers were brewed the same way, without the hop addition during fermentation. No filtration was done. Shipping of the samples was done under cold conditions and within two days.

2.4 Analysis of terpenes and esters

Terpenes and esters were analyzed by the company Nyseos, Montpellier, by GC-MS/MS and partial stable isotope dilution assay (method not published). The terpenes analyzed were α -terpineol,

cis-rose oxide, trans-rose oxide, citronellol, geraniol, linalool and nerol. The esters analyzed were 2-phenylethanol, 2-phenylethyl acetate, butyl acetate, ethyl 2-methyl butyrate, ethyl 3-hydroxybutyrate, ethyl butyrate, ethyl cinnamate, ethyl decanoate, ethyl hexanoate, ethyl isobutyrate, ethyl isovalerate, ethyl lactate, ethyl laurate, ethyl octanoate, hexyl acetate, isoamyl acetate, isobutyl acetate, methyl 2-methylbutyrate, methyl isovalerate, octyl acetate, and propyl acetate.

2.5 Analysis of 3-mercaptohexan-1-ol and 3-mercaptohexanacetat

The beers were analyzed by the company Nyseos, Montpellier, by LC-MS/MS to quantify thiols and thiol precursors according to published method [22]. Briefly, CO₂ gas was removed by filtering through glass wool, and the corresponding liquid (1 mL) was derivatized and subsequently analyzed by nanoLC-MS/MS. Quantification was performed by Stable Isotope Dilution Assay (SIDA) for the different analysis to ensure the best accuracy and reliability. The disulfides were analyzed with the same method after the transformation of disulfides into free thiols.

2.6 Analysis of 4-mercapto-4-methylpentan-2-one

The beers were analyzed by the company Nyseos, Montpellier, by LC-MS/MS to quantify thiols and thiol precursors according to published method [22, 23]. Briefly, CO₂ gas was first removed by filtering through glass wool and the corresponding liquid was aliquoted. Derivatization, standardization and analyses were done by means of by GC-MS/MS (Varian; GC3800; MS4000).

2.7 Sensory evaluation

For all sensory tests, 50 mL beer samples were served in 250 mL clear glasses. Every taster obtained an own beer sample. Prior to evaluation, all samples were allowed to equilibrate to room temperature (approximately 20 °C) for approximately 10 min to maximize aroma and to minimize temperature changes during evaluation.

The beers were tasted using a scheme especially developed by the Barth-Haas Group for the sensory profiling of hop aroma in beer. The scheme contains 12 descriptors: floral, citrus, sweet fruits, green fruits, red berries, cream caramel, woody, menthol, herbal, spicy, green/grassy and vegetal. The intensity for each descriptor is rated on a scale from 1 (no hop aroma) to 10 (intense hop aroma) (see Table 3). The evaluation of these descriptors describes the aroma as well as the gustatory perception. Sensory evaluation was done by a trained internal tasting panel consisting of 21 tasters.

2.8 Statistical Analysis

Statistical analysis was performed by one-way ANOVA to test for differences within the beers. Additionally, unpaired two-way t-tests were applied to determine differences between the single beers. The software used was Microsoft Excel 2010 (Microsoft, Seattle, WA, USA). Statistical significance was set at $p < 0.05$. Different letters following numbers indicate significant differences between the samples.

3 Results and Discussion

3.1 3MH concentration

3MH is found in US and European varieties and is described in beer as a grapefruit and passion fruit aroma. Odor threshold in beer is at around 55 ng/L [22]. 3MH was found to be present in the beers brewed with Cascade hops and in the beers made with Hallertau Mittelfrüh hops as well as in the control beers made with no hop pellet addition. As figure 1 shows, beers made with a dry hop addition had a concentration of 3MH above the flavor threshold. In general, the beers brewed with Cascade hops had a higher concentration of 3MH than the beers brewed with Hallertau Mittelfrüh. Roland et al. [22] also found Cascade to have the highest amount of 3MH conjugates from all the hop varieties analyzed. There is a significant difference in the amount of 3MH in the beers that were made with Cascade hops and with the yeast strains WLP001 and WLP029. For the beers brewed with Cascade, the yeast WLP001 releases significantly higher amount of 3MH than WLP029. The results show that 3MH levels are more dependent on the hop variety than on the yeast strain.

Moreover, 3MH was also found in the control beers made with no hop pellets, which proves

that 3MH is brought into beer by hops and by malt. These results agree with previous data of Roland et al. [24] who defined barley and malt as a source of 3MH.

3.2 3MHA concentration

3MHA was studied in literature [18] and was proven to be synthesized from 3MH by the action of yeast. Kishimoto et al. [25] suggested that selecting yeast strains with a higher capacity for bioconversion of 3MH into 3MHA is a useful strategy to increase the aroma impact of beer. 3MHA is described in beer, as a black currant and passion fruit aroma and has an aroma threshold in beer of 4 ng/L [14]. In this study, 3MHA was not detected in the analyzed beers which strongly suggests that the hops used did not contain significant levels of 3MHA or 3MHA precursors.

3.3 4MMP concentration

4MMP is a very volatile thiol, described to have a blackcurrant, box tree, and catty aroma [14]. Studies [25–27] have shown, that 4MMP largely contributes to the overall hop aroma intensity in finished beer. The odor threshold for 4MMP in beer is very low at around 1.5 ng/L [14]. Figure 2 shows the concentrations of 4MMP in beers made with Cascade, Hallertau Mittelfrüh and the control beers. 4MMP was not found in the control beers but was found in all beers made with Cascade as well as some of the beers made with Hallertau Mittelfrüh. However, the average value of the beers brewed with Hallertau Mittelfrüh is below threshold whereas the concentrations in the beers brewed with Cascade are much higher (mean values between 6.53 and 7.80 ng/L). These results agree with those from Kishimoto et al. [27] who analyzed 17 hops cultivars from different growing regions in the U.S., Australia, New Zealand, Germany, UK and Czech and only observed 4MMP in hop cultivars from Australia, U.S. and New Zealand. Reglitz and Steinhaus [28] also found the highest concentrations of 4MMP associated with U.S. varieties. These authors analyzed 53 varieties among them

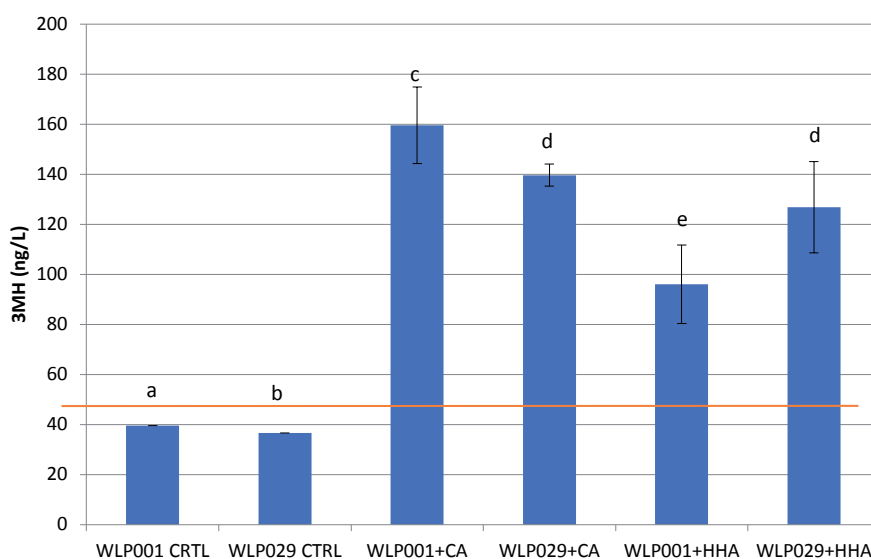


Fig. 1 Mean values of the concentration of 3-mercaptohexan-1-ol content (ng/L) \pm standard deviation in beers made yeast WLP001 or WLP029 and hop variety Cascade (CA) or Hallertau Mittelfrüh (HHA) and the control beers (CTRL). $n=7$. Lowercase letters indicate significant differences between the beers. Line indicate the odor threshold in beer

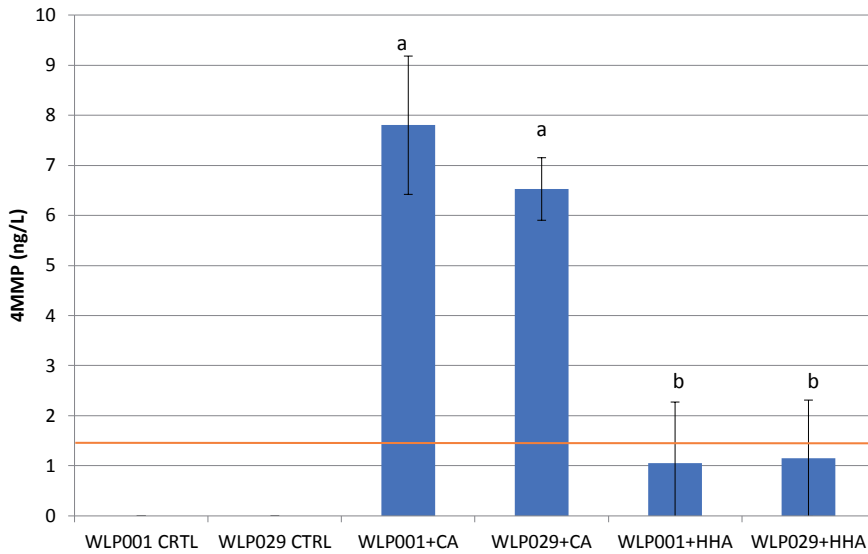


Fig. 2 Mean values of the concentration of 4-mercapto-4-methylpentan-2-one content (ng/L) \pm standard deviation in beers made with yeast WLP001 or WLP029 and hop variety Cascade (CA) or Hallertau Mittelfrüh (HHA) and control beers (CTRL). $n=7$. Lowercase letters indicate significant differences between the beers according to the t-tests

Cascade from the US and Cascade from Germany and the US Cascade had 3 times more 4MMP than the German hops.

The results show no significant difference in the 4MMP concentration for the beers WLP001+Cascade and WLP029+Cascade and for the beers WLP001+HHA and WLP029+HHA. According to these results it can be said that the amount of 4MMP released depends more on the hop variety than it does on the yeast strain. 4MMP was only found in hopped beers, which means that 4MMP, compared to 3MH, is either only brought into beer by hops and not by malt or is evaporated or removed during the brewing process.

3.4 Monoterpene alcohols

Linalool is one of the most important aroma compounds for hoppy aroma in beer and it is agreed to be a key aroma compound to measure intensity and quality of hoppy aroma [29–33]. Linalool is found in every hop variety at various levels.

Figure 3 shows the mean values for each analyzed terpene distinguished by the different combinations of yeast strain and hop variety. Concentration of cis-rose oxide and trans-rose oxide are not shown as the first had a constant concentration for all beers analyzed (1.25 $\mu\text{g/L}$) and the latter was not detected in any beer. It is clear to see that the beers made with Cascade hops have much higher levels of geraniol and citronellol, whereas the beers made with Hallertau Mittelfrüh hops show higher levels of linalool. The concentration of the terpene α -terpineol does not have a significant difference between the beers. The levels for nerol are higher in beers made with

the hop variety Cascade but they are also higher in the beers made with yeast WLP001 than in beers made with WLP029. Linalool content for beers made with yeast WLP001 and Cascade ranges from 210 $\mu\text{g/L}$ to 238 $\mu\text{g/L}$ and for the beers with yeast WLP029 and Cascade the range is from 209 $\mu\text{g/L}$ to 217 $\mu\text{g/L}$. In this case, different yeast strains do not have a significant influence on the linalool levels of the beer. On the opposite, there is a significant difference of the yeast used for the terpenes geraniol and citronellol. The beers made with yeast WLP001 have higher levels of geraniol and citronellol. The higher levels of geraniol can be derived from hydrolyzation of geranyl acetate as described by Forster et al. [34] or it could mean that yeast WLP001 can release more glycosidically bound geraniol precursors. Further, it seems that the levels of geraniol correspond with the levels of citronellol, which matches the finding of King and Dickinson [7].

The sum of terpenes, shown in table 2, is defined here as the sum of the concentrations of α -terpineol, cis-rose oxide, citronellol, geraniol, linalool and nerol. Beers made with Cascade show almost twice as much terpenes (693–808 $\mu\text{g/L}$) as beers made with Hallertau Mittelfrüh (384–385 $\mu\text{g/L}$). For the hop variety Hallertau Mittelfrüh and both yeast strains, no significant differences in the levels of the analyzed terpenes were found.

Beers made with Cascade and yeast WLP001 show a higher level of terpenes than beers made with Cascade and yeast WLP029. This leads to the suggestion that yeast WLP001 is able to produce or release more terpenes than yeast WLP029. However, this is not

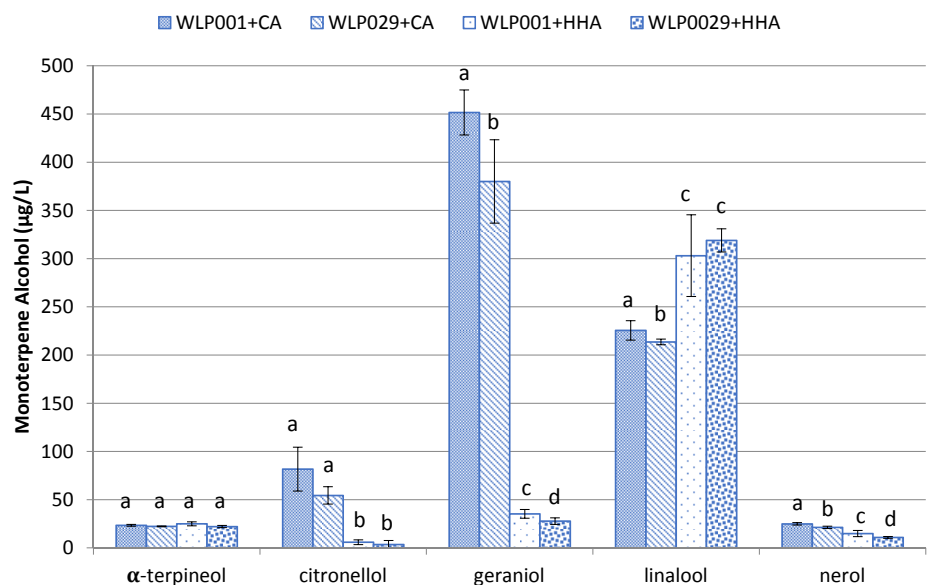


Fig. 3 Mean values of the concentration of monoterpene alcohols concentration ($\mu\text{g/L}$) \pm standard deviation in the beers brewed with Cascade (CA) or Hallertau Mittelfrüh (HHA) and yeast WLP001 or WLP029. $n=7$. Lowercase letters indicate significant differences between the beers according to the t-tests

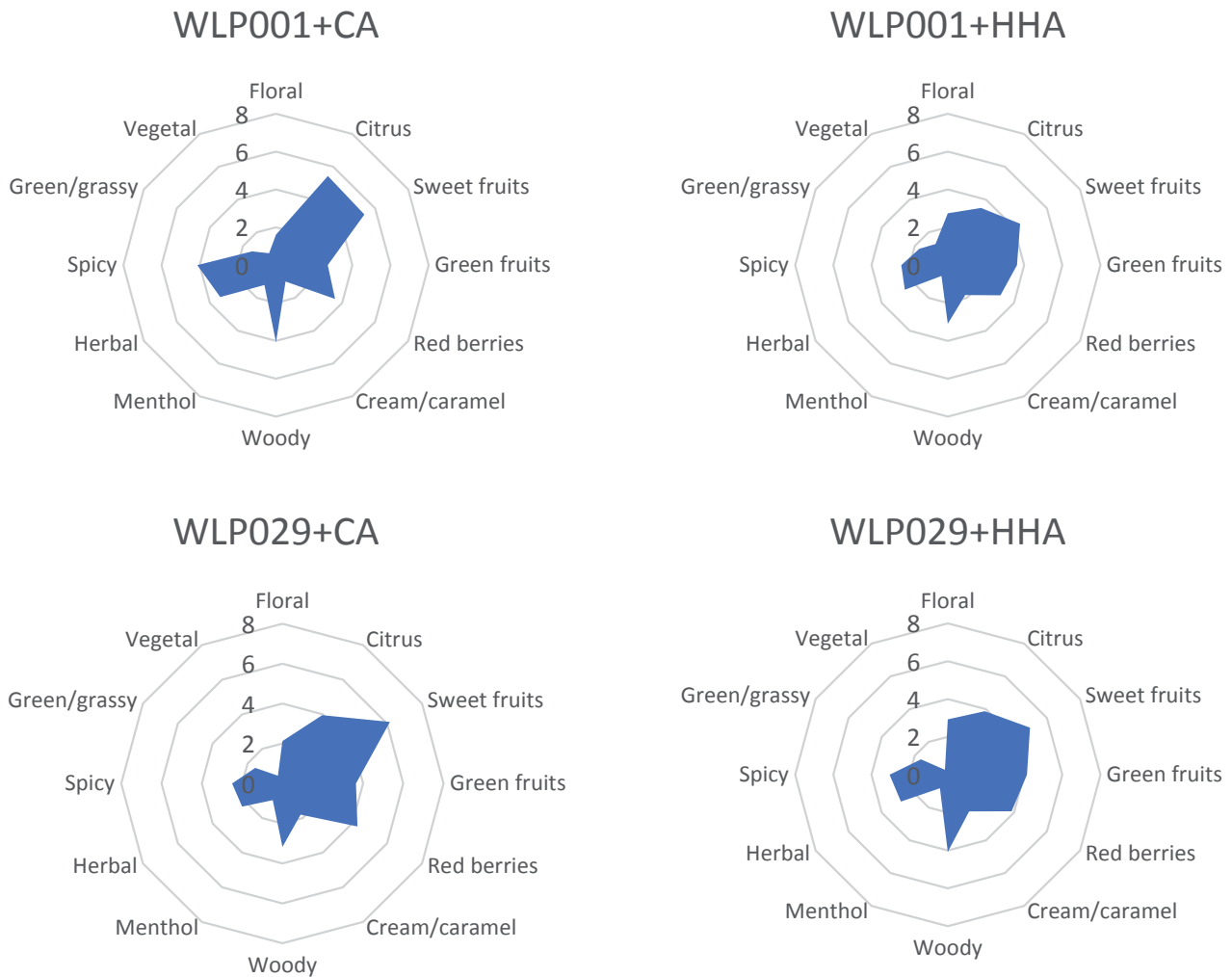


Fig. 4 Sensory profile according to the Barth-Haas Group Tasting Scheme of the beers brewed with WLP001 or WLP029 and with the hop variety Cascade or Hallertau Mittelfrüh. Intensity from 1–10

seen in the beers brewed with Hallertau Mittelfrüh wick could mean that this hop variety does not contain any precursors.

3.5 Esters

Table 2 shows the content of the seven main esters found in all the beers. From the analyzed esters, the following compounds were not detected in any sample: butyl acetate, ethyl isovalerate, ethyl laurate, hexyl acetate, and octyl acetate. The three main esters were 2-phenylethanol, ethyl lactate, and isoamyl acetate. The sum of esters is found in table 2. Opposite to the results of the terpenes, the sum of esters is more dependent on the yeast used. Beers brewed with WLP029 show higher values than beers brewed with WLP001. However, there is not a significant influence on the content of esters due to the hop variety.

3.6 Sensory Profiling

All beers were sensory evaluated by a trained panel. Figure 1 shows the sensory profiles, for the beers made with the combinations of hop varieties and yeast strains used in these trials. This tasting scheme evaluates the intensity of 12 descriptors, floral, citrus, sweet fruits, green fruits, red berries, cream caramel, woody, menthol, herbal, spicy, green/grassy and vegetal on a scale from 1 to 10.

The evaluation of these descriptors describes the aroma as well as the gustatory perception.

The beers made with yeast WLP001 and Cascade show the highest levels for the descriptors “citrus” and “sweet fruits”. “Spicy”, “woody” and “red berries” scored average. Beers made with WLP001 and Hallertau Mittelfrüh score very low on most characters, only “sweet fruits”, “citrus” and “green fruits” scored average.

Beers made with yeast WLP029 and hop Cascade show relatively high scores for “red berries” and “sweet fruits”, average scores for “green fruits”, “citrus” and “woody”. The beers made with WLP029 and Hallertau Mittelfrüh hops show the highest scores for “sweet fruits”, “red berries” and “woody”, average scores for “citrus” and “green fruits”. It is suggested that the characters “sweet fruits” and “red berries” mainly depend on esters produced by the yeast.

To compare the influence of the yeast on the hop aroma of the final beer, the sensory profile of beers WLP001 + Cascade and WLP029 + Cascade were compared to the results of the terpene concentration. “Citrus” character is higher in WLP001+CA beers, whereas “sweet fruits”, “green fruits” and “red berries” is higher in WLP029+CA beers. The higher score for “citrus” in beer WLP001+CA corresponds to the higher concentrations of geraniol

and citronellol after fermentation in those beers. On the other side, the total concentration of esters in the beers made with yeast WLP029 could explain the high sensory intensity of “sweet fruits”.

The analytical results of the sum of terpenes and ester shown in table 2 can be compared with the sensory results shown in figure 4. The higher levels of 4MMP in the beers made with Cascade hops also show higher intensities for “red berries” in the sensory profiling. However, the results show a higher influence of the hop variety than of the yeast on the levels of 4MMP. Higher levels of linalool in beers made with HHA also match the sensory results where the same beers scored higher on “floral” than the beers made with Cascade. For beers made with Cascade hops, the higher contents of geraniol and citronellol match the sensory results for “citrus” aroma.

4 Conclusion

In the last two decades, several studies have been conducted on the influence of yeast on various hop derived odor active compounds. The influence of two yeast and two hop varieties on the final aroma profile was investigated, especially on the content of thiols 3MH, 3MHA and 4MMP and on the content of terpenes and esters. The results proved that hops are a main source of 3MH although malt also delivers this thiol into beer. The concentration of 4MMP was mainly dependent on the hop variety. Beers brewed with Cascade showed a concentration high above the odor threshold whereas beers brewed with Hallertau Mittelfrüh or unhopped beers had lower concentrations or no 4MMP was detected.

The results suggest that the yeast WLP001 could have a higher ability to release monoterpene alcohols and that the hop variety Hallertau Mittelfrüh has no terpene precursors. The higher levels of geraniol can be derived from hydrolyzation of geranyl acetate as described by Forster et al. [34] or it could mean that yeast WLP001 can release more glycosidically bound geraniol precursors. The beer with higher levels of geraniol and citronellol brewed with Cascade were also described as more citrusy in aroma and taste. In this study, we were not able to prove that one of the chosen yeast strains releases significantly more thiols than the other. However, with their large contribution to hoppy aroma in beer, hop-derived thiols and the influence of fermentation on their behavior is going to be another important step to reveal the whole picture of hop aroma in beer.

The beers made with yeast WLP001 have higher levels of geraniol and citronellol. The higher levels of geraniol can be derived from hydrolyzation of geranyl acetate as described by Forster et al. [34] or it could mean that yeast WLP001 can release more glycosidically bound geraniol precursors. Further, it seems that the levels of geraniol correspond with the levels of citronellol, which matches the finding of King and Dickinson [7].

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