Fermentation temperature impacts polyfunctional thiol biotransformation in beer

Hop-forward beers such as IPAs potentially draw their fruity and sometimes exotic tropical aroma profile in part from yeast biotransformation of hop compounds. This study examined how yeast strain and fermentation temperature influence the interplay of thiol release and fermentation ester production and ultimately the flavor of these types of beers.

The biotransformations and aromatic release of polyfunctional thiols (PFTs) such as 3-sulfanylhexan-1-ol (3SH) and 4-methyl-4-sulfanylpentan-2-one (4SMP) by yeast during alcoholic fermentation are known to impart exotic tropical flavors such as passionfruit, guava, and black currant - flavors in demand by beer consumers today. However, these compounds are difficult to manage by brewers due to the very low levels (parts per trillion) found in beer, along with the limited understanding of how raw materials and processing parameters can affect their biochemical formation and sensory perception. We hypothesized that yeast strain and fermentation temperature are key parameters impacting thiol release from bound precursors during beer production. We conducted pilot scale (1.4 hL) brewing trials using a pale ale recipe with fermentation being carried out at three temperatures (15 °C, 22 °C, and 30 °C) and examined the performance of five commercial yeast strains (LalBrew BRY-97™, LalBrew Nottingham™, LalBrew Verdant IPA™, LalBrew London™, and LalBrew Diamond™). Wort was prepared from an all-pilsner malt and hopped solely with Cascade hops at a rate of 1.2g/L at the start of kettle boil and 3.0 g/L in the whirlpool. We refrained from dry-hopping these beers to minimize the impact of endogenous PFTs in Cascade hops on the experimental beers while focusing intently on the odorless, bound precursors that are found in relative abundance in this hop variety. Beers were chemically analyzed for 31 compounds including free thiols, thiol precursors, esters, and other aroma-related compounds (hop terpenes, C13-isoprenoids, and dimethyl sulfide). Additionally, sensory descriptive analysis was performed ortho- and retro-nasally on the beers using the Check-all-that-apply method (N = 22 panelists). Chemistry results showed a strong correlation between increasing fermentation temperature and free thiol formation. 3SH increased 33 – 54 % from 15 – 22 °C and 45 – 72 % from 15 – 30 °C ranging from 24 – 81 ng/L (at 15 °C) to 55 – 162 ng/L (at 30 °C) across yeast strains. 4SMP also showed an 11 – 41 % increase from 15°C to 22°C fermentations but remained similar at 30 °C, ranging from 6 – 12 ng/L. The LalBrew Diamond™ lager yeast

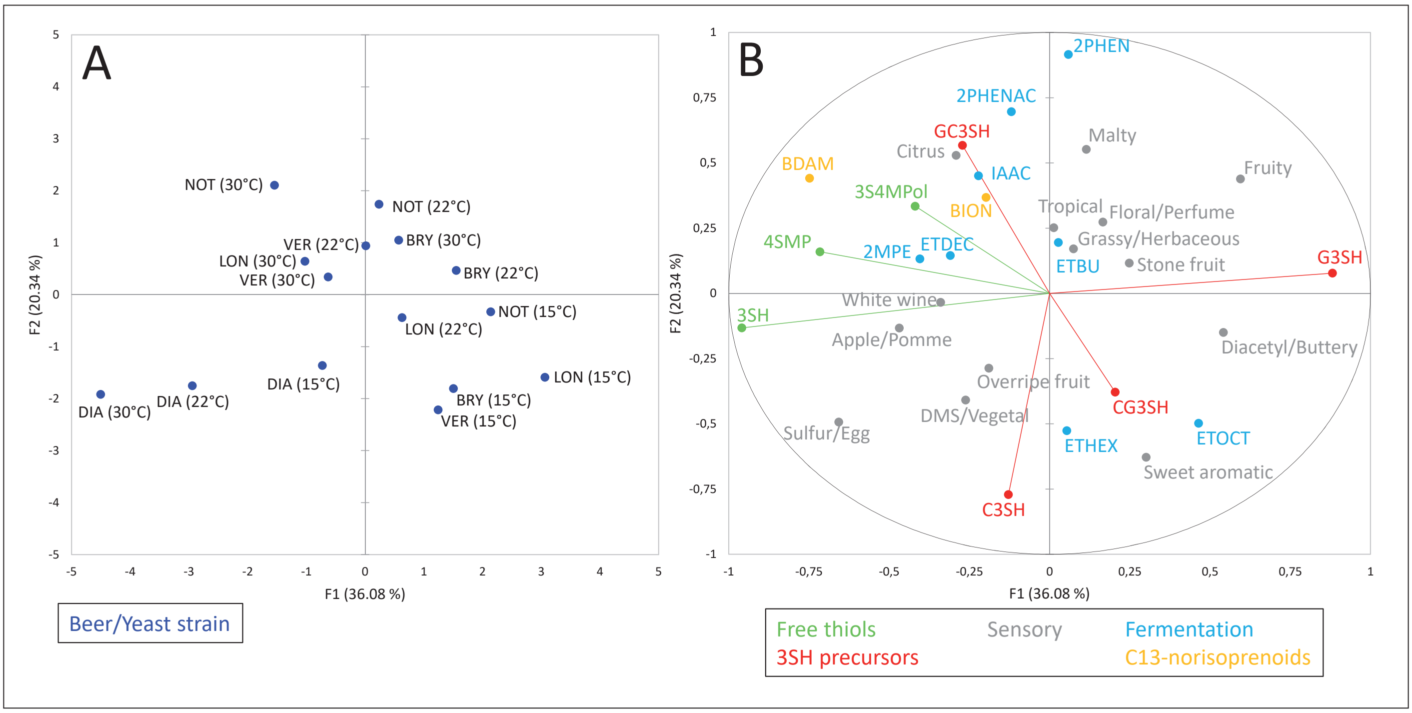

Fig. 1 **Multi-Factorial Analysis of brewed beers, sensory analysis, and chemical analysis. Objects observed (i.e. beers/yeast strains in dark blue dots, plot A) that are positioned closer to each other are more similar to each other based on their respective sensory and chemical results (plot B). The x-axis of plot B is influenced by increasing free thiols (green dots) towards the left, with decreasing free thiols towards the right. The y-axis of plot B is influenced by the increasing concentrations of fermentation-related aromatic compounds towards the top, along with increasing frequencies of positive fruity, citrus, malty descriptors. It is observed that lower temperature 15 °C ale beers are located in the lower right quadrant (plot A) showcasing lower fruity/tropical character and lower free thiol concentrations. Warmer temperature 22 °C and 30 °C ale beers are positioned in the upper 2 quadrants, showing increasing free thiols concentrations, aromatic compound concentrations, and fruity/tropical sensory character; more so for 30 °C beers in the upper left quadrant. All LalBrew® Diamond™ lager beers are positioned solely in the lower left quadrant as all 15 °C, 22 °C, and 30 °C beers have the most 3SH free thiol levels, with warmer temperatures bringing forth Sulfur/Egg character**

produced the highest overall concentrations of both thiols while the LalBrew Nottingham™ and London™ ale strains showed the greatest changes with temperature. In contrast, LalBrew Verdant IPA™ and BRY-97™ showed less change in thiol concentrations with temperature. Sensory results indicated that free thiols alone did not necessarily provide the most fruity, citrus, and tropical-flavored beers. For instance, the LalBrew Diamond™ strain produced over 2x higher levels of 3SH and 4SMP than the other strains but had the lowest perceived fruity-tropical character. This was likely due to lower amounts of other aromatic fermentation compounds in addition to an accompanying noticeable sulfur/egg character. Therefore, interaction of other aromatic compounds along with PFTs appears to play an important role in tropical flavor expression. This can be seen in how the LalBrew Nottingham™, LalBrew Verdant IPA™, and LalBrew London™ strains produced elevated thiols levels at 20 – 30 °C along with higher levels of 2-phenylethyl acetate, isoamyl acetate, 2-phenylethanol (fermentation-related compounds), β-ionone and β-damascenone (C13-norisoprenoids), and α-terpineol (hop-related compound), all while correlating with higher perceived citrus and tropical aroma than the other beers. In summary, combining measured thiol and sensory results show that the yeasts fell into 3 generalized categories: temperature-sensitive ale yeasts (LalBrew Nottingham™ and London™), temperature-stable ale yeasts (LalBrew Verdant IPA™ and BRY-97™), and the LalBrew Diamond™ lager yeast. Based on category, brewers can select yeasts for their desired purposes. The temperature-sensitive yeasts can be used to create greater and more unique flavors by increasing temperature. Meanwhile, temperature-stable yeasts may not provide the greatest range of aroma changes with higher temperatures but can more stably produce their unique flavor signatures. This study reveals how the biotransformation of PFTs in beer can be impacted by fermentation temperature and yeast strain, and how other aromatic compounds may synergize with PFTs to create the unique fruity and tropical aromas sought by consumers. Furthermore, these findings can help brewers create strategies to produce more flavors using practical and affordable solutions.

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