

# From Wort to Beer: The Evolution of Hoppy Aroma of Single Hop Beers produced by Early Kettle Hopping, Late Kettle Hopping and Dry Hopping

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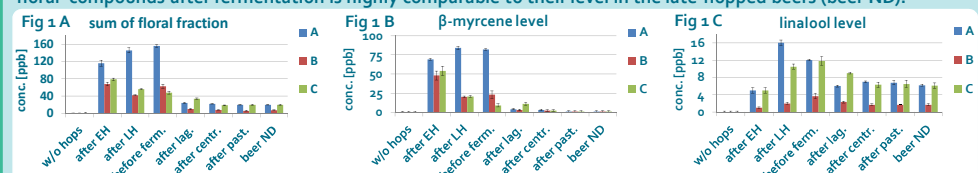
**INTRODUCTION** Knowledge of the impact of early kettle, late kettle and dry hopping on the final flavour sensation of beers is rather fragmentary. To gain insights into the way hoppy aroma develops, samples were taken at different stages along the brewing process of single-hop beers and analyzed using HS-SPME GC-MS, thereby aiming at accurate determination of the full spectrum of hop oil-derived volatiles. This study pinpoints analytical and sensory changes induced by the boiling and fermentation process, as well as the influence of dry hopping.

**Methods** All beers were prepared on a semi-industrial scale (40 hl) using single-hopping-technology with particular hop varieties (Var. A, B, C and D). Wort and beer samples were taken at different time points (see Table 1) and immediately stored at -20°C until analysis. Semi-quantitative determinations of hop oil-derived constituents were performed using HS-SPME in combination with GC-MS operating in both full scan and selected ion-monitoring mode. The sensomics heatmap was calculated using the heatmap function of GENE-E (Version 3.0.12). Therefore, z-scores were calculated and the dendrogram was constructed by means of Kendall's Tau agglomerative linkage algorithm. Comparative and descriptive sensory analyses were performed to assess flavour impressions according to DIN 10954 (1997) und DIN 10964 (1996).

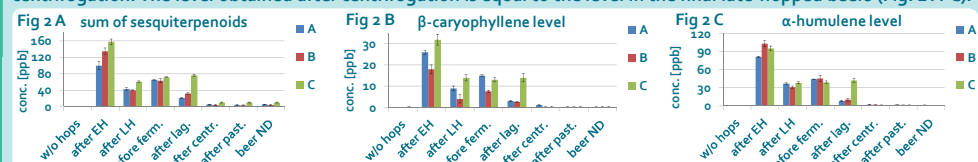
Table 1: wort and beer sample codes and time points of sampling	
abbreviation	time point of sampling
w/o hops	after 15' boiling - just before early hopping
after EH	after 75' boiling - just before late hopping
after LH	after 5' cooling
before ferm.	after 10' cooling - just before fermentation
after lag.	after lagering - just before centrifugation
after centr.	after centrifugation
after past.	after pasteurisation
beer ND	bottled beer without dry hopping;
beer DH	bottled beer with additional dry hopping

## Monitoring of Hop Volatiles along the Brewing Process

The level of the most volatile fraction of hop essential oil ('floral fraction'), containing highly flavour-active monoterpene hydrocarbons (e.g.  $\beta$ -myrcene), monoterpene alcohols (e.g. linalool), characteristic esters and ketones, reaches maximal values just before fermentation and significantly decreases as a result of the fermentation step (Fig. 1 A). The level of monoterpene hydrocarbons (e.g.  $\beta$ -myrcene (Fig. 1 B)) dramatically decreases upon fermentation while the level of other compounds such as linalool (Fig. 1 C) decreases to a lower extent. The level of 'floral' compounds after fermentation is highly comparable to their level in the late-hopped beers (beer ND).



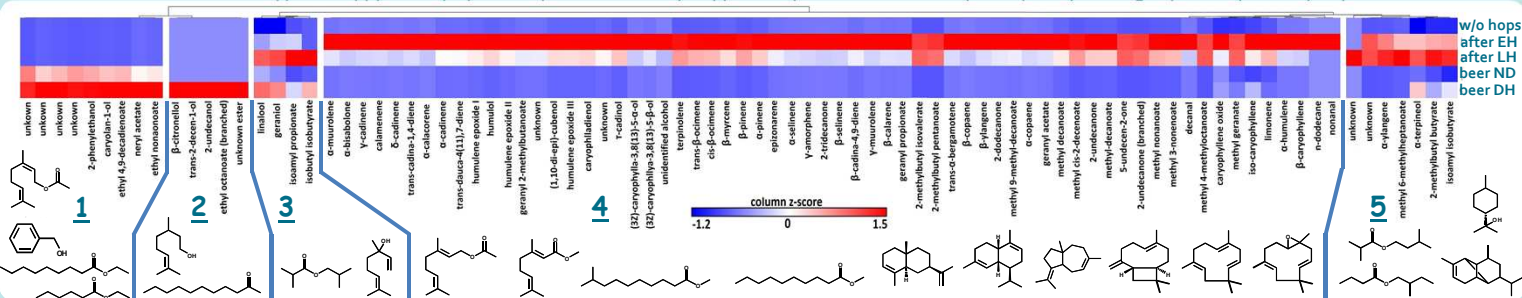
The sesquiterpenoid hop oil fraction contains both sesquiterpene hydrocarbons as well as oxygenated sesquiterpenoids and has been associated with the so-called 'noble' or 'spicy' character of hoppy aroma. The level of this particular class of hop oil constituents decreases significantly upon fermentation of the brews made with varieties A and B (Fig. 2 A). However, the most crucial step leading to the loss of almost the entire sesquiterpenoid fraction is centrifugation. The level obtained after centrifugation is equal to the level in the final late-hopped beers (Fig. 2 A-C).



## Cluster Analysis

Cluster analysis (Var. D) revealed significant differences in the volatile composition of wort samples taken before fermentation and beer samples.

In total 5 clusters (clusters 1 - 5) that represent different compound classes can be distinguished. Cluster 5 consists of branched esters and the terpenoids  $\alpha$ -terpineol and  $\alpha$ -ylangene. High concentrations of these compounds are more characteristic for the wort samples taken after late-hopping. Cluster 4 contains by far most of the volatile compounds thereby reflecting the original hop essential oil composition. This cluster consists of characteristic estery compounds, sesquiterpene hydrocarbons and oxygenated  $\alpha$ -humulene and  $\beta$ -caryophyllene derivatives. High concentrations of these compounds are characteristic for the wort samples when compared with the late- and dry-hopped beers derived from the same brew. Cluster 3 contains the monoterpene alcohols geraniol and linalool as well as 2 branched esters (isoamyl propionate and isobutyl isobutyrate). Clearly, these compounds are present in higher concentrations in the late-hopped wort and in the dry-hopped beer, but not in the late-hopped beer without the additional dry-hopping step. Cluster 1 and 2 contain compounds that are characteristic exclusively for the beer samples. Besides some unknown compounds, Cluster 1 contains transformation products of terpenoids whereas the compound spectrum of cluster 2 is more diverse (esters, alcohols and  $\beta$ -citronellol). Sensory characteristics of the unhopped wort were described as 'typical wort aroma'. Early kettle and late kettle hopped worts expressed a 'hoppy' and 'herbal' aroma, which was slightly more pronounced in the late-hopped wort. The final beers showed a typical 'hoppy' and 'spicy' flavour, whereas the dry hopped sample was dominated by floral, fruity and slightly citrusy sensory impressions.



## CONCLUSION

The level and composition of the floral and sesquiterpenoid hop oil fraction significantly changes along the brewing process. Fermentation and centrifugation were identified as the crucial process steps. As shown via cluster analysis, methyl esters, branched esters, terpenoids as well as oxidized derivatives of  $\alpha$ -humulene and  $\beta$ -caryophyllene are characteristic for early and late kettle hopped wort. Early and late hopping show an impact on absolute concentrations but not on the composition of hop-derived volatiles. Highest concentrations of geraniol and linalool are found in the late hopped wort. Although dry-hopping has a predominantly quantitative influence on the volatiles, high concentrations of compounds like  $\beta$ -citronellol are characteristic for dry hopping. Clearly, this study proves that dry hopping also boosts individual volatiles which might be linked to the characteristic fruity and citrusy aroma impressions.

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