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On the Behavior of the Important Hop Odorant 4-Mercapto-4-methylpentan-2-one (4MMP) during Dry Hopping and during Storage of Dry Hopped Beer

The transfer of the potent, black currant-like smelling hop odorant 4-mercapto-4-methylpentan-2-one (4MMP) from Eureka hops into a Pilsener style beer was monitored during static dry hopping. Quantitation of 4MMP, also known as 4MSP (4-methyl-4-sulfanylpentan-2-one), was accomplished by using a previously developed approach based on a stable isotope dilution assay, selective isolation of thiols by mercurated agarose gel, and analysis by GC×GC-TOFMS. Major transfer of 4MMP happened during days 1 and 2 of dry hopping, whereas only little further increase was observed between days 2 and 8. The concentration data were well in line with the sensory evaluation of the dry hopped beers and suggested a hop contact time of 2 days during dry hopping for a both efficient and economic transfer of 4MMP from hops into beer. The same quantitation approach was also applied to assess the stability of 4MMP during storage of the dry hopped beers. Storage resulted in severe losses of 4MMP after 3 months and a further decrease in 4MMP between months 3 and 6 of storage. Losses during storage were significantly higher at 20 °C than at 5 °C.

Descriptors: 4-mercapto-4-methylpentan-2-one, 4MMP, 4-methyl-4-sulfanylpentan-2-one, 4MSP, dry hopping, transfer rate

1 Introduction

The aroma of beer is basically determined by fermentation by-products such as higher alcohols and esters as well as some substances transferred from malt such as (*E*)-β-damascenone and 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one [14]. Volatiles from hops added at the beginning of wort boiling are almost completely evaporated and are not recovered in the finished wort in odor-active amounts [8]. To effectively transfer hop odorants into beer, an addition of hops later in the process is essential [5]. This can either be done by late hopping or by dry hopping. In late hopping, hop addition takes place at the end of wort boiling or immediately after wort boiling to the whirlpool. In dry hopping, hops are added to the green beer after fermentation and before conditioning, leading to a more intense and characteristic hoppy aroma in the final product [11]. Dry hopping has become a commonly applied procedure in recent years, particularly in craft breweries. However, the aroma of dry hopped beers is not very stable thus clearly limiting their shelf life [16]. It has been demonstrated that the key odorant responsible for the hoppy note in late hopped beers brewed with traditional hop varieties is citrusy and floral smelling (3*R*)-linalool [4]. However, further compounds may contribute to the overall olfactory properties of dry hopped beers. Among these are geranium-like smelling myrcene [15] and, depending on the hop variety employed, rosy smelling geraniol and black currant-like smelling 4-mercapto-4-methylpentan-2-one (4MMP) [7, 9, 17]. 4MMP, also known by its IUPAC name 4-methyl-4-sulfanylpentan-2-one (4MSP), is outstanding due to its very low odor threshold, thus even amounts of 4MMP in the ng/L range may significantly influence the aroma of beer [17].

We recently developed an analytical approach for the sensitive quantitation of 4MMP based on a stable isotope dilution assay using 4-mercapto-4-(^{13}C)methyl(1,3,5-$^{13}C_3$)pentan-2-one as internal standard, selective isolation of thiols by a mercurated agarose gel, and analysis by GC×GC-TOFMS [12]. Application of this approach to 53 hop samples of different varieties revealed 4MMP concentrations between < 1 and 114 µg/kg. Extraordinarily high 4MMP concentrations were detected in some US varieties such as Citra, Eureka, Simcoe, and Apollo, whereas the compound was neither found in English varieties such as East Kent Golding and Fuggle nor in traditional German varieties such as Hallertau and Hersbrucker (Fig. 1).

The aim of the present study was to apply the previously developed quantitation approach to investigate the transfer of 4MMP from

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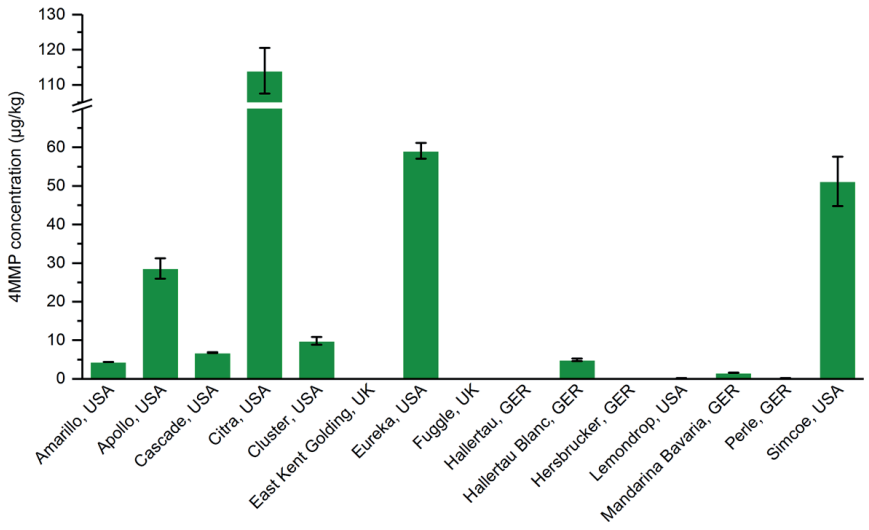

Fig. 1 4MMP concentrations in hops of different varieties

hops into beer during static dry hopping and its behavior during storage of dry hopped beer.

2 Materials and methods

2.1 Beers

The base beer for the dry hopping experiments was an industrially produced Pilsener style beer brewed with 4MMP-free hops. The beer was centrifuged but unfiltered. The beer showed the following analytical parameters: 11.6 % original gravity, 35 IBU, 5.0 vol% EtOH, pH 4.5. Dry hopping was performed in parallel in four cylindroconical vessels (10 hL) at –1 °C. To each vessel, base beer (9 hL) from one batch was added. Subsequently, pellets type 90 of US-grown Eureka hops, harvest 2016, were added from the top of the vessels in amounts of 250 g/hL. No agitation was applied. After 24 h contact time, the hop sediment was removed from the bottom of vessel 1. Likewise, the hop sediments from vessels 2, 3, and 4 were removed after 2, 4, and 8 days. Afterwards, the contents of each vessel were divided into halves and one part was filtered (5 hL/h) using diatomaceous earth (100 g/hL) as filter aid. In the same way, a sample of the base beer was filtered. Filtered and unfiltered beers were bottled after removal of air from the bottles by evacuation and purging with carbon dioxide. Bottles were closed with crown corks having oxygen scavenging properties. Dry hopped beers for the storage trials were produced using the same approach, but with another batch of base beer. Hop contact time was 4 days.

2.2 Chemicals

4MMP was purchased from Alfa Aesar (Karlsruhe, Germany). ($^{13}C_4$)-4MMP was synthesized from (1,3-$^{13}C_2$)acetone (Sigma-Aldrich, Taufkirchen, Germany) as detailed in [6]. Mercurated agarose gel was prepared from Affi-Gel 10 (Bio-Rad, Munich, Germany) [2]. Dithiothreitol was purchased from Sigma-Aldrich. Dichloromethane and diethyl ether were freshly distilled through a column (120 cm × 5 cm) packed with Raschig rings.

2.3 Sensory ranking tests

Orthonasal ranking tests were conducted according to ISO 8587:2006 [10]. Beers (20 mL) were presented in cylindrical ground neck glasses (7 cm height, 3.5 cm i.d.) with lids labeled with random 3-digit codes. In each test, panelists were given a set of five beer samples consisting of the dry hopped beers with 1, 2, 4, and 8 days of hop contact and the base beer without dry hopping in random order and asked to rank them according to the intensity of the black currant-like note. A solution of 4MMP in water (0.4 ng/L) served as odor reference. Each panelist assigned the beer with the weakest perceived black currant aroma a ranking score of 1, the beer with the second weakest perceived black currant aroma a ranking score of 2 and so on, until finally the beer with the highest perceived black currant aroma received a ranking score of 5. The scores of 17 panelists were averaged.

2.4 4MMP quantitation

4MMP quantitation in the Eureka hop sample was performed as detailed in [12]. Beer was degassed by filtration through a folded filter and a sample of the filtrate (1.5 L) was spiked with ($^{13}C_4$)-4MMP (50 ng) dissolved in dichloromethane (100 µL). After stirring (30 min), the beer was subjected to solvent extraction with diethyl ether (total volume 2400 mL). The combined organic phases were dried over anhydrous sodium sulfate and subsequently applied onto mercurated agarose gel (2 g) in a cooled (10 °C) glass column (1 cm i.d.). After the gel had been rinsed with dichloromethane (100 ml), trapped thiols were eluted by a solution of dithiothreitol (7.7 mg) in dichloromethane (50 mL). The thiol fraction was purified by solvent-assisted flavor evaporation (SAFE) [3] at 40 °C and

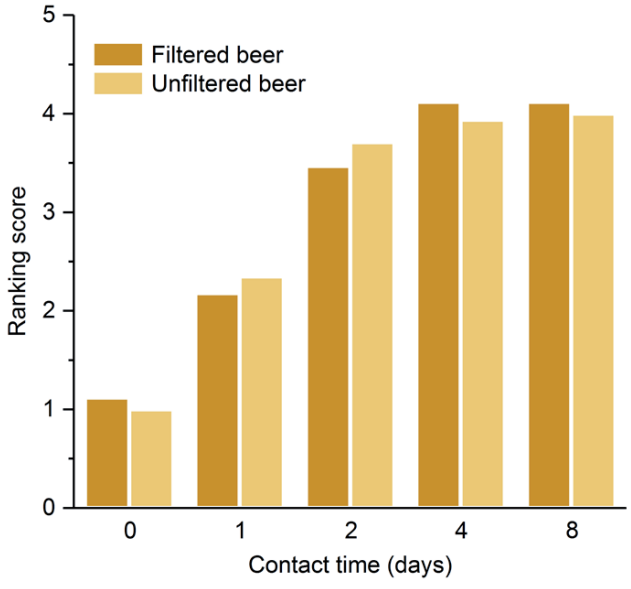

Fig. 2 Ranking of beers according to the intensity of the 4MMP note (RSDs were 0–31%)

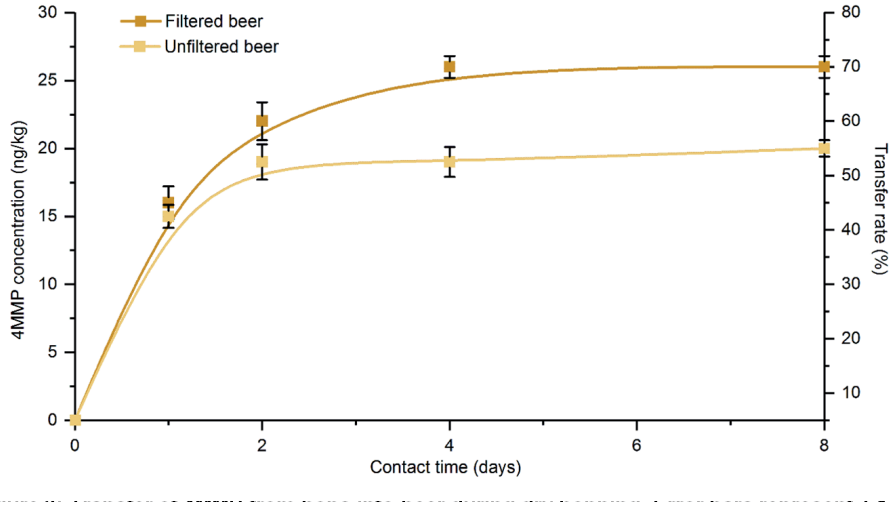

Fig. 3 **Transfer of 4MMP from hops into beer during dry hopping. Error bars represent ± 1 SD of triplicate analyses**

concentrated to 100 µL by using a Vigreux column (50 cm × 1 cm) and a Bemelmans microdistillation device [1]. Analysis of an aliquot (1 µL) of this solution by GC×GC-TOFMS and calculation of the 4MMP concentration from the peak volumes of the 4MMP peak and the ($^{13}C_4$)-4MMP peak by using the extracted ions m/z 132 for 4MMP and m/z 136 for ($^{13}C_4$)-4MMP were done as detailed in [12]. All analyses were carried out in triplicates.

3 Results and discussion

3.1 Sensory evaluation of beers

Ranking tests were separately applied to the filtered and to the unfiltered beers. Differences between both kinds of beers, however, were marginal. In both cases, the base beer was clearly ranked as the beer with the least intense 4MMP note. Sensory scores showed an increase of the 4MMP note during days 1 and 2 of dry hopping, whereas only little further effect was observed between days 2 and 8 (Fig. 2).

3.2 Transfer of 4MMP from hops into beer during static dry hopping

Quantitation of 4MMP confirmed its absence in the filtered and unfiltered base beers. In agreement with the sensory data, 4MMP increased during days 1 and 2 of hop contact time and leveled out between days 4 and 8 (Fig. 3). In all dry hopped beers, 4MMP concentrations were well above its odor threshold value in beer reported to be ~1 ng/L [13, 17]. Surprisingly, 4MMP concentrations were consistently higher in the filtered beers than in the unfiltered beers. Repetition of the entire set of dry hopping experiments (data not shown) revealed the same effect. A potential explanation could be the release of 4MMP from a precursor during filtration, however, no efforts were undertaken to substantiate this assumption.

Using the 4MMP concentration in the hop pellets used for dry-hopping (14.6 ± 0.4 µg/kg) and the hopping rate (250 g/hL), the 4MMP concentrations in the beers were converted into transfer rates:

Transfer rate = 4MMP concentration in beer / (14.6 µg/kg × 250 g/hL)

Final transfer rates after 8 days of hop contact were 71 % for the filtered beer and 55 % for the unfiltered beer. However, the major transfer (43 % and 41 %) already occurred during the first 24 hours and transfer rates reached 60 % and 53 % after only 2 days of hop contact. In combination with the results of the sensory experiments, data suggested a contact time of 2 days as the most reasonable

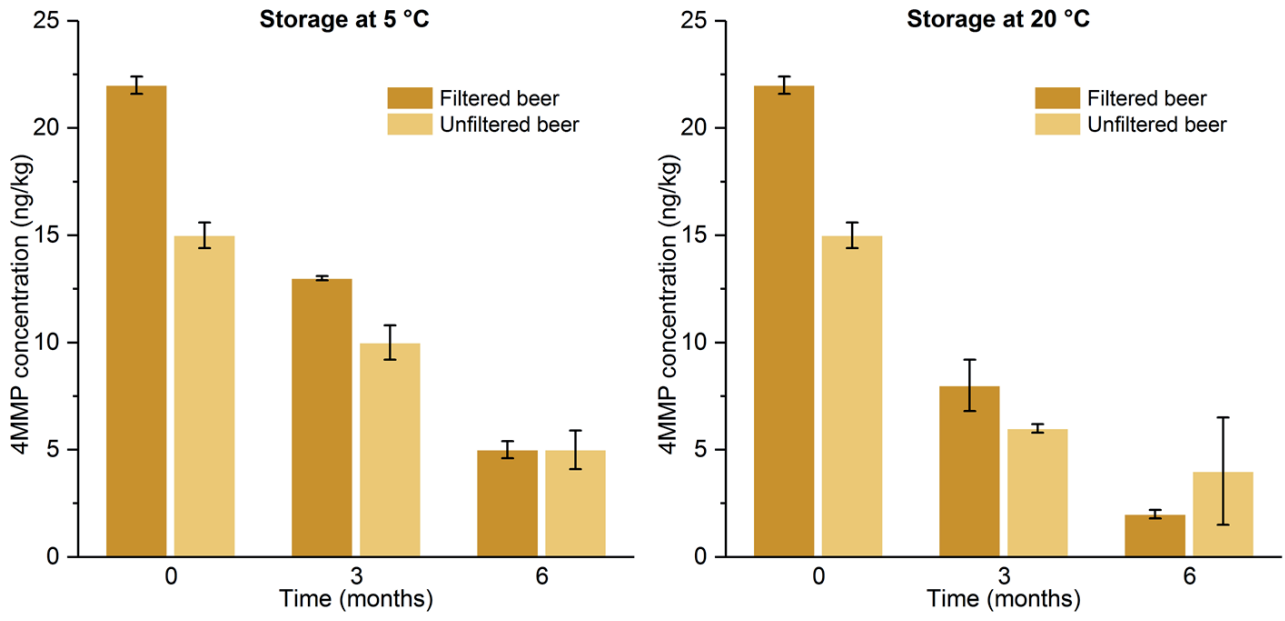

Fig. 4 **Changes in 4MMP concentrations during storage of dry hopped beers at 5 °C (left) and 20 °C (right). Error bars represent ± 1 SD of triplicate analyses**

compromise between short time and effective transfer of 4MMP during dry hopping.

3.3 Changes in 4MMP concentrations during storage of dry hopped beers

A filtered and an unfiltered dry hopped beer were bottled and stored at 5 °C and 20 °C. The 4MMP concentration in the beers was determined before storage, after 3 months of storage, and finally after 6 months of storage. Results revealed a clear decrease during storage at both temperatures, however, the effect was stronger at the higher storage temperature (Fig. 4). After 3 months of storage at 5 °C, the 4MMP concentrations had dropped to 59 % (filtered beer) and 67 % (unfiltered beer), whereas at 20 °C only 36 % (filtered beer) and 40 % (unfiltered beer) were left in the stored beers. A further decline was observed when the beers were stored for additional 3 months. Potential causes of the 4MMP decline include its oxidation into disulfides, its addition to electron-deficient species such as α,β-unsaturated carbonyl compounds, and its migration into the crown cork liner material [18].

4 Conclusion

The study revealed that the major transfer of black currant-like smelling 4MMP from hops into beer occurred during days 1 and 2 of static dry hopping, whereas only a small further increase was observed between days 2 and 8. These findings were in agreement with sensory evaluations and suggested a hop contact time of 2 days during static dry hopping as most reasonable for an appropriate 4MMP transfer. Storage of dry hopped beers resulted in a clear decrease of 4MMP. The drop in 4MMP concentration was significantly higher at 20 °C than at 5 °C. Beers dry hopped with 4MMP rich hops should therefore preferably be consumed as fresh as possible and low temperatures should generally be employed during storage.

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