S. Hanke, V. Ditz, M. Herrmann, W. Back, T. Becker and M. Krottenthaler

Influence of Ethyl Acetate, Isoamyl Acetate and Linalool on off-flavour Perception in Beer

Beer flavour is a very complex flavour created by interaction off several hundred aroma compounds. Obvious off-flavours which can be detected by the consumer decrease the drinkability and acceptance of a beverage. The image of a brand can be damaged. It is known that undesired flavours, like stale flavour, can be masked by positive ones like certain hop flavours. Therefore it is of interest to find out if other off-flavours can also be masked by positive aroma compounds. Positive flavour impressions are e.g. linalool and some esters among others. Whether these compounds can suppress the perception of dimethyl sulphide (DMS), diacetyl and isovaleric acid in beer is unknown until today. In this study the influence of ethyl acetate, isoamyl acetate and linalool on flavour thresholds of DMS, isovaleric acid and diacetyl was investigated. The obtained results show that linalool decreases the perceived intensity of off-flavours at low concentrations but increases at higher concentrations. Esters also showed suppressing and synergistic effects. The flavour composition is an important factor for flavour perception and must be taken into account when comparing flavour thresholds of flavour compounds determined in different beers types.

Descriptors: off-flavour perception, dimethyl sulfide, diacetyl, esters, flavour threshold, linalool

1 Introduction

The perceived beer flavour is the result of interactions of several hundred aroma compounds. The flavour profile can be influenced by raw materials, hopping technology, brewing technology, yeast strain, fermentation, mashing regime and all downstream processes like filtration, stabilisation and bottling [4, 27]. The drinkability of beer is influenced by the flavour profile and especially by off-flavour perception [34, 39].

When a single compound is added into water the flavour which arises from the compound is not altered by the solvent (water) or other compounds. Only the pure flavour of the added single compound is perceived. If several compounds are added to water different flavour effects like weaker or stronger impressions in the mixture can occur [5]. Synergistic and antagonistic effects are possible and are a common phenomenon in heterogeneous mixtures [53]. Synergistic effects are well known in mixtures [5]. The perception of flavour compounds also depends on non-volatile substances present in the matrix [36, 41, 48, 52]. For flavour perception also the vapour phase of an aroma compound is of importance [6, 41]. It is known that a concentration of 5 % glucose increases the headspace concentration of diacetyl [6]. Ethanol increases the fruity flavour of cider [6]. Combinatory effects were first described at the beginning of the 1960ies for the oxidation flavour in milk [10, 33]. This oxidation flavour could be perceived although all relevant compounds were below their individual threshold levels [10]. Later, similar effects could also be observed for esters [45], aldehydes [14] and water pollutants [46]. Combinatory effects usually occur between compounds of the same chemical class [14]. According to *Lillard et al.* [33], these effects are relevant for equivalent mixtures of compounds with similar thresholds. Otherwise, a dependence of the total amount of compounds in a solution seems to be important for the threshold of the mixture [54]. Because of these complex interactions, a prediction of the resulting food/beverage flavour is difficult. Flavour thresholds differ in dependence of the solvent or food matrix (eg. water or beer) [8, 61]. *Sega et al.* assumed the existence of additive effects but could not prove them [52]. *Engan* found such effects between higher alcohols [11]. Recently published papers by *Herrmann et al.* [23] and *Saison et al.* [49] could show additive effects for stale flavour compounds and *Culleré et al.* found such effects for wine aroma compounds [9]. A contribution of flavour compounds at subthreshold levels was shown by *Palamand et al.* [42].

Esters are responsible for the fruity character of beer and have great impact on beer flavour [60]. They are generated during fermentation through esterification of fatty acids and ethanol. The biochemical process is described in detail by *Boulton and Quain* [7]. Ester formation depends on fermentation temperature, aeration, yeast cells during pitching, original gravity, sugar composition, yeast strain and geometry of fermentation vessel [7, 8, 21, 58, 60]. It was shown by *Stewart et al.* that higher maltose levels (adjusted with very high maltose syrup) in a high gravity wort lead to lower ester concentration after fermentation and dilution compared to traditional brewed high gravity beers [55–57].

Diacetyl is a well-known off-flavour in beer. It causes a buttery flavour and has a threshold of 0.1 mg/L in lager beers [4]. Diacteyl is formed during fermentation by oxidative decarboxylation of 2-acetolactate, which is an intermediate in the valine biosynthesis of

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Tables and figures see Appendix

yeast [27]. Diacetyl is reduced by yeast to acetoine and 2,3-butandiol. The formation and reduction mechanism is also described by Boulton and Quain [7]. This reduction mechanism depends on yeast vitality, yeast concentration, yeast strain, temperature and pH-value [4, 32]. The Diacetyl flavour occurs if yeast viability, vitality or the concentration of nutrients in wort are deficient [4]. Microbial infections with lactic acid bacteria (*Pediococcus* and *Lactobacillus spp.*) increase diacetyl levels as well [3].

DMS is derived from the thermal cleavage of S-methylmethionine (SMM/DMS-P) during kilning and wort boiling. Insufficient evaporation during wort boiling can result in high levels of this compound which causes a cabbage-like off-flavour in beer. During the brewing process DMS can be influenced during wort boiling [2, 4, 24–26] and at lower rates also during fermentation [32, 47]. DMS is also a by-product of microbial infection by *Enterobacteriaceae* [2].

Linalool is an indicator substance for hoppy flavour. It correlates significantly with the perceived hoppy flavour in beer [12, 13, 15, 16, 18, 28, 50]. Otherwise linalool is the only hop derived compound which exceeds its single threshold in traditionally hopped beers with a perceivable hop aroma. Therefore linalool contributes directly to the beer flavour [19, 28, 44]. Because every hop variety results in a unique perceived flavour profile, linalool is not only an important contributor to hop aroma but also a suitable indicator. To achieve high levels of linalool, late hop additions are necessary [18].

Fresh hops contain 0.8–3 % free fatty acids. They can increase during unfavourable storage conditions up to 20 % [40]. 2-methylpropionic acid and isovaleric acid (3-methylbutyric acid) which are developing during storage impart an intense cheesy flavour. These fatty acids are cleavage products of α- and β-acids [43]. This cheesy flavour will occur in beer when aged hops is used for late additions or dry hopping. A usage of aged hops for early hopping (begin of wort boiling) showed no correlation between cheesy flavour and isovaleric acid [16] because of the evaporation of these unwanted compounds [62]. Another origin of isovaleric acid is autolysis of yeast. Autolysis occurs when yeast is stored under unfavourable conditions and goes along with an excretion of fatty acids, fatty acids ethyl esters and enzymes, like proteinase A [1].

2 Material and methods

To investigate the influence of added flavour compounds flavour thresholds were determined in beer. For this purpose commercial German lager beer with no distinct hoppy or stale flavour was chosen as reference beer. The analytical data of key flavour compounds of this beer is shown in table 1. All compounds were purchased at Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany) in highest available purity.

For sensory testing a stock solution of the compounds was prepared in absolute ethanol (Mallinckrodt Baker, Deventer, The Netherlands). This stock solution was diluted subsequently in absolute ethanol to the desired concentration. 1 mL of this solution was added to 500 mL reference beer resulting in the desired concentrations of flavours. If two flavour compounds were added 1 ml of ethanolic off-flavour solution and 1 ml of ethanolic flavour compound (e.g. linalool) were added. After addition bottles were re-capsulated and the beer was homogenised by gentle shaking. The procedure of spiking the beers was done 4 hours prior to tasting. Flavour threshold determination and statistical evaluation of the compounds in beer was done in duplicate according to EBC method 13.9 [59] as a triangle test with one treated (off-flavour) and two untreated samples. Tasters were advised to find the treated sample. With two aroma compounds (the trials where the beer matrix was changed) the untreated sample was also spiked with the positive aroma compound (eg. linalool).

Samples were coded with three digit random numbers and presented in dark glasses at room temperature. The 15–20 tasters (predominantly male) were between 20 and 45 years and trained with special focus on beer.

3 Results and Discussion

DMS is an off-flavour which can be influenced during malting [51] and wort boiling [4, 24–26]. The threshold found for DMS (129 µg/L] proved to be slightly higher compared with previous reported values which are in the range 50–100 µg/L [4, 35]. Linalool showed at concentrations near threshold, 7 µg/l present in beer and 15 µg/L added, suppressive effects. The threshold of linalool was reported elsewhere by the authors with 27 µg/L [19]. At higher concentrations the suppression of DMS is reduced and at the highest addition level the DMS flavour perception was increased (see Fig. 1). A similar effect of linalool was seen for isovaleric acid and diacetyl as shown in table 2. The presented threshold for diacetyl is conform with previous published values [35, 37]. This shows that a moderate hoppy flavour can help to suppress unwanted off-flavours, an effect known for stale flavour in beer [17, 31]. However a more intense hoppy flavour can lead to unwanted aroma effects by lowering flavour thresholds of the tested off-flavour compounds. To produce a moderate hoppy flavour the last hop dosage should be calculated correctly to avoid an unwanted overdosing. The correct transfer rates of linalool should be known for the individual process. Transfer rates for linalool and other hop aroma compounds have been reported previously [18, 22]. They depend on the brew house technology and hop variety [16, 29, 30, 38]. At higher linalool levels a cabbage-like or butterscotch flavour can be detected. For the practical brewer this implicates that for beers with high linalool levels diacteyl reduction should be more extensive. Also the wort boiling should be supervised to guarantee DMS levels well below threshold. The same effect of linalool was seen for isovaleric acid which means that particular for hoppy beers hop quality (fresh, non-oxidized hop products) and yeast quality (avoidance of autolysis) are of special interest.

Esters are at least partly responsible for the fruity flavour of beer. High levels of esters can result in unwanted solvent like flavour impressions [21]. To achieve higher levels of esters higher fermentation temperatures and other factors have to be considered. The two investigated esters have different effects on DMS threshold. At lower additions isoamyl acetate shows no effect on the threshold of DMS. Higher isoamyl acetate levels resulted in higher suppression of DMS. The banana and apple-like flavour [35] of isoamyl acetate covers the DMS flavour and increases the DMS threshold. Ethyl acetate decreases the threshold of DMS at all investigated concentrations. For DMS it is of importance which ester is dominating the flavour profile. Isoamyl acetate is known to have higher impact on beer flavour than ethyl acetate [35]. Especially for high-gravity brewing the influence of esters on beer flavour is of special importance [56]. During high-gravity fermentation ester formation by yeast is predominately higher compared to normal fermentation. After dilution to normal gravities high gravity beers show increased ester levels [57, 58].

Perception of diacetyl is also increased when ethyl acetate is added. Otherwise the buttery flavour of diacetyl is suppressed by isoamyl acetate. On the one hand higher fermentation temperatures result in higher ester formation through yeast. These higher temperatures increase the maximum of diacetyl during main fermentation but also accelerate diacetyl reduction [4]. In these beers diacteyl should not be a problem because of the accelerated reduction and the higher levels of isoamyl acetate. Higher temperatures lead to higher levels of ethyl acetate which increase diacetyl perception but the threshold increasing properties of isoamyl acetate might cover the decreasing properties of ethyl acetate. Not investigated in this context was a combined effect of a mixture of ethyl acetate and isoamyl acetate. However this assumption yet needs to be confirmed.

The flavour threshold of isovaleric acid (325 µg/L) which was obtained in this study is much lower than those reported previously. *Harrison* reported a minimum concentration of 1 ppm for perception of isovaleric acid [20]. Later *Meilgaard* reported a threshold level of 1500 µg/l in an US beer [35, 36]. This indicates that the beer type influences the perception of flavour compounds as confirmed in this study. At lower concentrations the flavour of isovaleric acid is (stronger) by an (addition) of ethyl acetate of 4 mg/L. An addition of 7 mg/L ethyl acetate increased the threshold and suppressed the flavour perception. Isoamyl acetate suppressed the flavour of isovaleric acid at higher concentrations as well. A moderate increase in isoamyl acetate resulted in a higher threshold compared with the other results. This could be due to unknown suppressing effects of not determined beer flavour compounds.

The average concentration of ethyl acetate in bottom fermented beers is in the range 5–20 mg/L (average 10 mg/L) [4]. The tested concentrations are at the upper limit for bottom fermented beers. Because of the difference in fermentation technology the reported range for top fermented beers is 10–50 mg/L (average 30 mg/L) [4]. For isoamyl acetate a range of 0.5–2 mg/L (average 0.7 mg/L) is reported, top fermenting beers show levels up to 8 mg/l [4]. Within the normal concentration range of these esters these flavour effects change the perceived flavour by increasing off-flavours. The authors could show that the flavour profile is important for flavour perception. Differences in flavour thresholds between different publications are probably due to different beers matrices with different flavour profiles. Because of suppressing or increasing effects between flavour compounds the measurement of single off-flavour compounds does not reflect the perceived final flavour. Wort boiling and green beer maturation should be adjusted to the special beer type.

4 Conclusion

Beer flavour is the result of interaction between several hundred aroma compounds, positive and negative. A shift in the balance between these can result in a changed flavour perception. The presented single flavour thresholds of diacetyl, dimethylsulfide and isovaleric acid in commercial German lager beer differ from previous published results. The flavour thresholds of off-flavour compounds were also determined in a changed beer matrix. Higher fermentation temperatures and higher original gravity of wort increase ester levels of beer and result in a more pronounced fruitiness of the final beverage. In this case esters dominate the beer flavour and can suppress negative flavours like the cheesy impressions of isovaleric acid or the buttery flavour of diacetyl. On the other hand it is shown that higher levels of ethyl acetate are able to increase off-flavour perception.

A hoppy flavour is known to cover unwanted aroma compounds to a certain extent. Moderate levels of linalool showed suppressive effects on off-flavours, the perception was decreased. In contrast linalool at higher levels increased off-flavour perception. Differences between the presented single thresholds and literature values are probably due to different reference beers which differ in their composition. As shown, the flavour composition is very important for off-flavour detection. This must be considered when highly aromatic beers with a strong hoppy flavour shall be produced to avoid the occurrence of off-flavours.

The knowledge of such effects is important to produce a high quality product with constant flavour properties. The control opportunities for off-flavours should also consider interactions between different flavour compounds.

Acknowledgement

The authors want to thank the Barth Haas Grant for the financial support of this project.

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Received 17 May, 2010, accepted 05 August, 2010

Appendix

Table 1 Flavour composition of the reference beer

compound	concentration in reference beer
Ethyl acetate	12 mg/L
Isoamyl acetate	0.8 mg/L
Geraniol	16 µg/L
Linalool	7 µg/L
Isovaleric acid	164 µg/L
Diacetyl	75 µg/L
DMS	80 µg/L

Table 2 Flavour thresholds of DMS, diacteyl and isovaleric acid

	flavour threshold [µg/L] of		
	isovaleric acid	DMS	diacetyl
no addition	325	129	80
+ 4 mg/L Ethyl acetate	259	120	68
+7 mg/L Ethyl acetate	422	115	76
+0,25 mg/L Isoamyl acetate	851	124	92
+0,5 mg/L Isoamyl acetate	364	137	82
+0,75 mg/L Isoamyl acetate	422	150	96
+15 µg/L Linalool	388	176	87
+30 µg/L Linalool	377	142	84
+60 µg/L Linalool	310	107	82

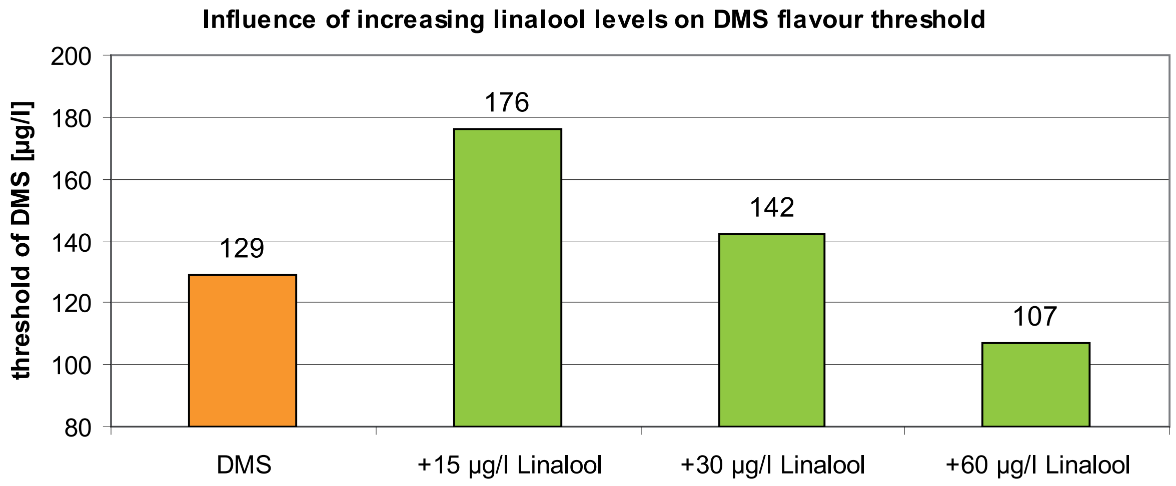

Fig. 1 Influence of increasing linalool levels on DMS flavour perception (7 µg/L Linalool in reference beer without addition)