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Isohumulones – a Review

Isohumulones, an isomerisation product from hop *(humulus lupulus sp.)* derived humulones are the main bittering substance in beer. They contribute to more than 85 % of the allover bitterness of beer [65]. Hops and its constituents as well as its application in the brewery is subject of investigations for more than a century already. Hops as an ingredient in beer has been used since the early Middle Ages. The objective back than was to save beer from microbiological spoilage and to use the hops as a flavouring ingredient [6]. Today we know that hops and its constituents also improve foam stability and contributes to the flavour stability of beer in various ways [71]. This paper gives a short overview about the most important literature which was published throughout the last decades.

Descriptors: hops, humulones, isohumulones, isomerisation, beer flavor, beer quality, bitterness

1 Isomerisation of humulones into isohumulones

Among other constituents hops contains about 2–17 % humulones [3]. The chemical structure of humulone, also called α-acids, as it was postulated by *Verzele* [98] is shown in figure 1. A similar structure was already postulated in the 1920s by *Wieland* [104]. This structure was modified by *Cook and Harris* [11] and *Carson* [9]. Five different homologues exist of the humulones which differ in their side chain at the C2 position as shown in figure 2 [98]. It has to be mentioned that only three of them, humulone, cohumulone and adhumulone are present in hops in major concentrations [55, 66, 105]. By thermal treatment during wort boiling the six-member-ring structure changes into a five-member-ring structure as shown in figure 3 [96]. But also without a thermal impact an isomerisation reaction can occur although this reaction is very slow and the resulting amount of isohumulones very small [17]. The isomerisation of humulones into the more soluble isohumulones is obviously the most important reaction in hop chemistry [96]. Already *Spetsig* could show that two isohumulones are formed from each humulone [86] called cis- and trans-isohumulone [60]. This observation could get approved in following years. It could be shown that there are two chiral centres at the C4 and C5 atom [87]. Due to this asymmetric carbon two stereoisomers appear called cis- and trans-isohumulone. This stereoisomerity leads to a change in hydrophobic properties which results in a different behavior in context with bitterness, foam stability and antibacterial properties [33].

2 Utilisation rate

The overall utilisation rate of isohumulones in finished beer is quite small. The yield of isohumulones in finished beer is usually not more than 30 % of the initially dosed humulones [70]. This low yield is affected by numerous factors. One of the main reasons is the poor solubility of the unisomerised humulones, which has been determined by *Kolbach* [51] and Spetsig [86]. The different homologues show different utilisation rates. A high co-humulone content always results in a higher overall utilisation rate [42, 65, 80]. Solubility of α-acid can be improved by an increased pH-value which may result in an accelerated isomerisation reaction though the isomerisation reaction itself is not affected by changes in pH [64]. Additionally boiling time, boiling temperature, original gravity, type of hop product and hop variety used influence the yield of iso-α acids [22, 63–64, 73]. Prolonged boiling time, a higher temperature and a higher concentration of α-acids in the dosed product result in higher utilisation rates because of a more extensive extraction as shown by *Hertel* [19, 26–27]. Higher original gravity and higher concentrations of α-acids in wort result in lower yields [63]. Another factor is the content of divalent cations and their salts in the reaction medium. Some of them, for example calcium and magnesium salts were found to promote isomerisation of α-acids into iso-α acids to a higher extent [59]. The use of pre-isomerised hop products like iso-kettle-extract or iso-pellets is a good possibility to achieve higher yields in the cooled wort. Another way to improve utilisation rates is a pre-isomerisation of the used hop product just before dosing it into the boiling wort. However previous work indicates a decrease in bitter quality of the finished beer with an increasing yield of isohumulones [46]. In recent years also alternative ways to isomerise isohumulones were presented such as photochemical isomerisation [99], which results in only trans-isohumulones or catalytic isomerisation with divalent metal ions or cerium prior to dosage of the hop product to the boiling wort [53] to increase utilisation rate. Until now it was not possible to find economically viable and in an industrial-scale

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Figures see Appendix

feasible solutions to implement such technologies in breweries. Losses during fermentation and filtration are further reasons for a poor recovery rate of isohumulones in finished beers [30, 61, 66]. These losses are due to adhesion on yeast cells and filter aids. Also carbon dioxide as primary yeast metabolite beside ethanol removes hop derived compounds from the fermenting medium into the krausen [61]

3 Analysis of the isohumulones

The easiest way to determine the amount of bittering substances is the photometrical measurement at a specific wavelength of 270 nm [35, 49, 68, 75, 79]. Nevertheless the results of this analysis often do not correlate with the sensory estimated bitterness of beer. The main reason is a large amount of non-bitter substances in beer which also can absorb light at a wavelength of 270 nm [10]. Modern analysis of isohumulones usually is based on chromatographic methods. While in the past a complete separation of all homologues and stereoisomers was difficult to obtain, nowadays powerful analytical devices are available to solve this problem [43]. In routine analysis often direct injection of the sample is state of the art [95, 97]. However the integration of the resulting peaks often presents a problem. To increase peak sharpness various extraction methods are known. The most popular sample pretreatment methods include solid phase extraction and liquid liquid extraction methods which both have deficiencies [43] in recovery rate or complexity of sample preparation. Therefore accuracy or convenience decide over the choice of method.

4 Post fermentation hopping

The possibility to purify isohumulones from a reaction medium resulted in the development of so called downstream hop products or post fermentation hop products [52]. These products are aqueous solutions of pure isohumulones or reduced isohumulones and are dosed into the beerstream just before filtration in order to increase bitterness without losses during wort boiling and fermentation [30, 61, 92]. They are produced by a catalytic hydrogenation of the purified humulones and subsequent isomerisation [24, 88]. Another way is a catalytic hydrogenation of isohumulones and also by oxidation and hydrogenation of purified lupulones [5, 92]. Their structure is shown in figure 4. Depending on the degree of hydrogenation they are called rho-, tetra- and hexa-isohumulones. Each step of hydrogenation creates a further chiral center so that there can exist 4 stereoisomers of the rho-isohumulones, 8 of the tetra-isohumulones and 16 stereoisomers of the hexa-isohumulones [8, 23, 74, 95]. The use of reduced isohumulones leads to an increase in bitterness and foam stability and a decrease or change in lightstruck flavor, but allover bitterness impression can be changed [13, 15, 21, 38, 93, 103].

5 Bitterness of isohumulones

Isohumulones contribute to more than 85 % of the allover beer bitterness in fresh beers [65]. Because of lower hydrophobicity co-isohumulones appear more bitter than the other homologues [31]. No significant differences could be shown according to isohumulone and ad-isohumulone. Previously published work shows that a mixture of cis- and trans-isohumulones tastes more bitter in beer than trans-isohumulones alone [1]. It is also known that pure cis-isohumulones are more bitter than solely trans-isohumulones [18, 34, 79, 98, 103]. However in usual wort boiling regimes, the ratio of cis- : trans-isohumulones can not be changed significantly without varying wort boiling conditions [62]. The bitterness of reduced isohumulones is described as follows: rho-isohumulones 0.7–0.8, tetra-isohumulones 1.0–1.7 and hexa-isohumulones 1.1 compared with isohumulones indicated with 1.0. The bitter taste of the rho-isohumulones seems to be shorter, the aftertaste of the hexa-isohumulones prolonged in comparison to isohumulones [32]. It also was shown that pure isohumulones create a sharp bitterness which is softened by the addition of a small amout of unspecific bitter substances [1, 16, 50, 54].

6 Foam stability

It is known, that isohumulones promote foam and lacing because of their hydrophobic properties [85]. Various homologues and stereoisomers have different foam stabilizing potential. This behavior depends on the strength of the hydrophobic character. It is known that trans-isohumulones are enriched in foam more than cis-isohumulones because of a lower hydrophobic character [31] and thereby exhibits a higher foam stabilizing character. This is also a reason for higher losses of trans-isohumulones during fermentation and maturation. It also was published, that some minor constituents, for example adpre-isohumulone stabilise foam more effectively than other homologues of the isohumulones [105]. Furthermore reduced isohumulones are more effective in terms of foam enhancement than isohumulones. In this context rho-isohumulones are the least hydrophobic, tetra-isohumulones the most hydrophobic derivatives of the isohumulones. Therefore the foam stabilizing properties of tetra-isohumulones are the best in beer [14, 18].

7 Antibacterial properties

The beginning of the use of hops in brewing is believed to date back to the 11th or 12th century. It probably took place in different regions of Belgium and Germany. The growing popularity of hops is believed to be due to the bacteriostatical properties of the α- and iso-α-acids [25, 44]. Thus beers brewed with hops were more stable regarding infections and negative health-effects occurred less often in these days if hops was used [6]. Nowadays it is known that several hop compounds inhibit the growth of gram-positive bacteria [78, 83] and some of them also the growth of fungi [69]. Most of the typical beer spoilage bacteria are gram-positive [2]. Today also most mechanisms are known [4, 96, 100]. The antibacterial activity of isohumulones increases with decreasing pH-value and content of monovalent cations [84]. The inhibitory effect of isohumulones is greater than those of humulones and other compounds [84]. It is not known yet if there is a difference in antibacterial activity of different homologues and stereoisomers. But it is known that a small number of bacteria strains, mostly Lactobacillus brevis species, show a tolerance mechanism against hop compounds and thereby can cause problems in brewing by causing microbiological spoilage [90].

8 Lightstruck flavor

One of the problems caused by isohumulones is the appearance of the so called "lightstruck flavor". Especially beers filled into colourless or green-colored bottles can get a skunky aroma after light-irradiation [89]. This phenomenon was detected already in 1875 [48]. Nowadays it is known that the formation of 3-methyl-but-2-ene-1-thiol (MBT), a pungent off-flavour with a flavour threshold of only about 4 ng per litre [56–57] is induced by a photochemical reaction of the isohumulones together with riboflavin as a sensitizer and a suitable sulphur-containing compound [37]. Also a direct UV-irradiation of isohumulones can lead to radical precursors of MBT [48]. It could be shown that the blue part of the visible light (350–500 nm) is the most efficient in generating lightstruck flavour [57]. Beer in brown glass bottles is preserved because brown glass cuts off light below 500 nm. If the use of colourless or green-colored bottles is desired, lightstruck flavor can be avoided by the use of reduced isohumulones. But already traces of residual non-reduced isohumulones cause a lightstruck-flavour in beer [18, 29]. A similar effect was shown after UV-irradiation of trans-tetra-isohumulone. Flavor active compounds with higher flavor thresholds are formed, but not MBT [28].

9 Co-isohumulone

The role of co-isohumulones was discussed controversially for a long time. Schönberger summarised as following [80]: With increasing pH value more of the isohumulones are dissociated. This can lead to an unpleasant and harsh bitterness in beer [7, 67]. Rigby's research [77] which compared beers with a different amount of isohumulones, was misinterpreted in saying co-isohumulones create a harsher and more intense bitterness. Newer research could not find a negative effect of co-iso-humulone to bitter quality [58, 72, 82, 101]. Furthermore, co-isohumulone has a higher yield due to its higher polarity and thus better solubility [80]. Nevertheless until now many brewers still favour a low iso-co-humulone content in finished beer.

10 Degradation of isohumulones

Isohumulones are only an intermediate product on the reaction pathway to the humulinic acid [41, 81, 94]. Recent research showed that about 25 % of the dosed humulones to the boiling wort could not be detected at the end of boiling [20]. During every production step isohumulones are degraded. Previous work revealed losses by degradation accounting for up to 35 % under inappropriate boiling conditions [45]. The most common reaction mechanisms therefore are alkaline and acidic hydrolysis mechanisms and oxidative degradation [36, 63, 76]. Especially trans-isohumulones are quite instable and easily degrade [12, 33]. There also exist some trans-specific degradation reactions [39, 62, 102], but also inspecific mechanisms [40]. Some of the resulting degradation products such as the anti-isohumulones are known well [91]. Most of them do not or only a little contribute to bitterness. Only a few of them are described being more bitter than isohumulones [98]. Only recently it was shown that a small amount of degradation products may help to create a fine bitterness [1, 16, 54]. In higher concentrations they are responsible for a harsh and long-lasting unpleasant bitterness[47, 54].

11 Conclusion

Isohumulones derived from humulones are a unique ingredient in beer. Bitterness of beer is mainly influenced by the amount of isohumulones. The utilisation rate in the finished beer is usually not more than about 30 %. Although utilisation can be increased for example by increasing the pH-value, applicable procedures in brewing have never been published. A common way to improve the yield is the use of pre-isomerised hop products or a pre-treatment of hops before dosage to the boiling wort. Nevertheless, both cen lead to sensory differences in the beer. Nowadays routine analysis of isohumulones usually is done by photometrical measurement or by HPLC-DAD. Various homologues and stereoisomers of the isohumulones have different behavior according to their bitter quality and intensity. The cis : trans ratio, which only can be measured by HPLC, can be used as an indicator for the sensory bitter quality. The past few decades many breweries started using downstream products. The dosage of these products just before filtration minimises the losses in the brewing process. The use of reduced isohumulones also increases bitter intensity and foam stability as well as it reduces lightstruck flavour. Other important characteristics of isohumulones are the ability to increase the foam stability of beer as well as the ability to prevent beer from microbiological deterioration. However, in interaction with UV-irradiation lightstruck-flavour, a skunky dislikeable aroma can occur. The role of co-isohumulone was discussed controversially. Newer research could show that there is no negative flavour impact related to a high co-humulone content in hops. Isohumulones are quite instable. Reactions to unspecific degradation products will inevitable occur during the production of beer and during storage. It has to be considered that a high amount of degradation products in beer negatively influences the bitter quality and bitterness stability.

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12 References

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Appendix

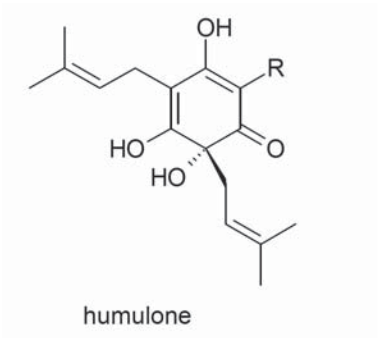

Fig. 1 Structure of the humulones

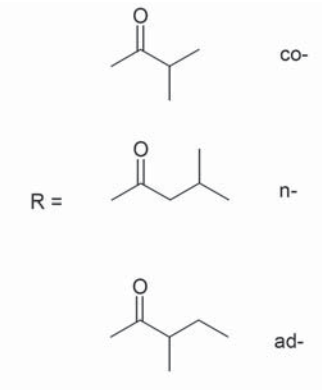

Fig. 2 The three main homologues of the humulone

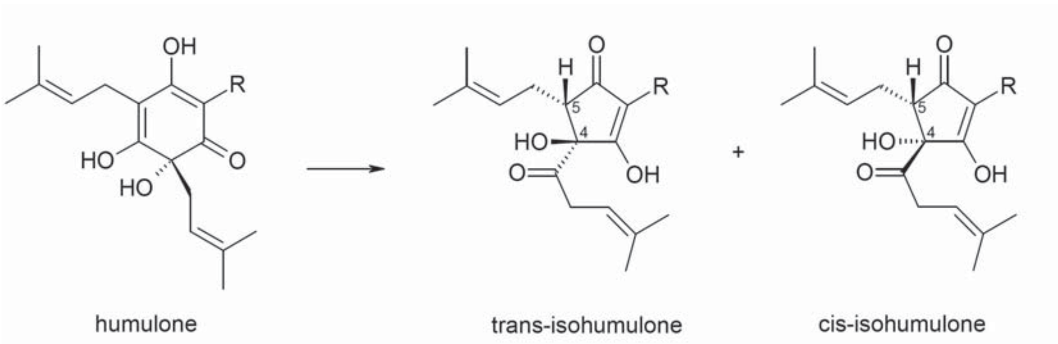

Fig. 3 The isomerisation reaction from the humulones to the isohumulones

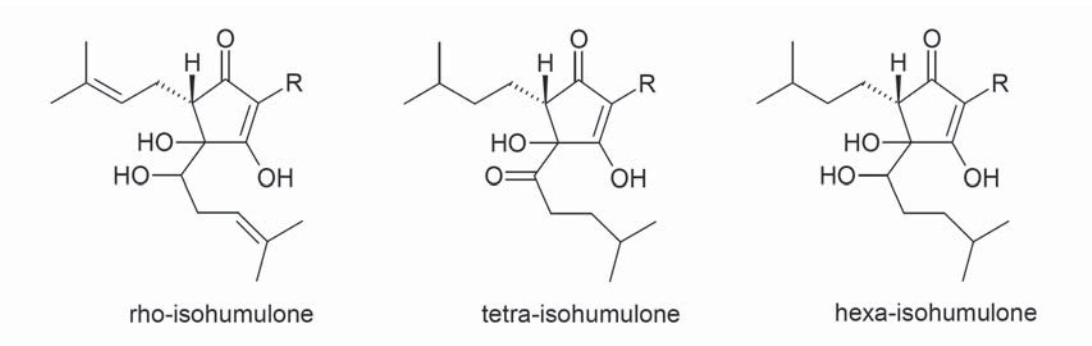

Fig. 4 Structure of the reduced isohumulones