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The Role of Hops in Flavour Stability – Some Aspects

What is the role of hops in beer flavour stability? A lot of hop constituents show beneficial properties in terms of supporting the flavour stability of beer. (E.g. alpha acids as well as various polyphenolic compounds have high antioxidant activities). The role of Iso-alpha acids in this context is discussed controversially but also other bitter acids may contribute positively to the flavour stability of beer. In addition to these non-volatile hop compounds hop oil compounds can also play an important role in regard to flavour stability. Therefore different hop products will have different impacts on flavour stability in beer. This paper gives a short overview of today's knowledge of the role of hops in flavour stability in beer.

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Descriptors: Flavour stability, stale flavours, hop bitter acids, hop polyphenols, ROS formation, hop aroma

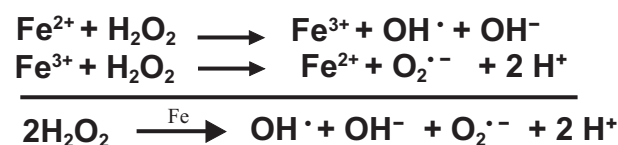
1 Introduction

Flavour stability is a very important part of the current brewing research. Flavour stability is the wish of the brewer that the consumer, wherever he is, may experience exactly the same fresh flavour of beer as it was just before it was bottled. It would be more correct and maybe also more satisfying to investigate flavour instability, as it is clear that with a liquid as complex as beer, flavour stability is an illusion. *McFarlane* reported in 1968 "What we know and more importantly what we don't know about the defects in beer quality which involve oxidative changes, can bear repeating—it is well to remind ourselves of the many gaps in our knowledge of the oxidation reactions accompanying the aging of beer" [10].

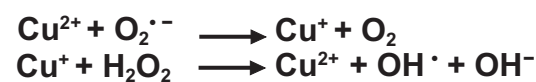
Since the beginning of flavour stability research, oxygen is the main suspect in this regard. A multitude of parameters influence the flavour stability of beer. The sensory effects are a declined bitterness quality and changing intensity, the development of a sweet taste and the appearance of a cardboard flavour, caramel, ribes and toffee like flavours [11]. For these changes, mainly the formation of stale flavours, volatile aldehydes are made responsible. There are many possible reactions that have been discussed that can cause these changes including oxidation, Strecker degradation, aldol condensation, furanic ether formation, degradation of hop bitter acids, Maillard reactions and hydrolysis of esters. Especially

for the cardboard flavour the trans-2-nonenal and its formation by lipid oxidation was intensively investigated, as its flavour threshold is very low. Newer research activities focus on different compounds such as methional, 3-methylbutanal, 2-furfuryl ethylether, beta-damascenone and acetaldehyde etc. as stale flavour indicators [9, 11, 13, 15]. However it is still not completely clear how important these often measured volatile compounds are in a sensory context. The flavour stability is also very beer-type dependent and pale lager beers are regarded as more susceptible than other beer types. The skilled combination of realistic storing parameters with a wise combination of analytical and sensory measurements determines the value of the research results.

Fenton's Reaction



Haber-Weiss Reaction



Formation of lipid radicals by metals



M = Transition Metal (Fe, Cu, Mn)

LH = unsaturated fatty acids

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Fig. 1 Reactions leading to the formation of reactive oxygen species (ROS) (2)

2 Important methods to judge flavour stability

For the measurement of reducing, antiradical and antioxidant activity or potential, various methods are being used. Every method yields different results, e.g.:

- Reducing capacity (2,6-dichlorophenol indophenol, DCPI).
- Reducing activity by the use of stable free radical DPPH (1,1-dipyridyl-2-picryl hydrazyl) for slow reducing substances (not SO₂).
- Determination of endogenous antiradical capacity (EAC) of worts and beers (lag-time value) for the impact of SO₂ and oxygen charge.
- The Oxygen Radical Averting Capacity (ORAC) assay reflects the capacity for scavenging peroxy radicals by hydrogen atom transfer.
- The Hydroxyl Radical Averting Capacity (HORAC) assay reflects antioxidants for their hydroxyl radical preventing capacity. The HORAC and ORAC assay measure two different but equally important aspects of antioxidant properties, i.e. radical chain breaking and prevention of radical formation [17].

3 ROS formation and metal chelation

Reactive oxygen species are a result of various reactions. Oxygen itself in its ground state is slow to react. Reactions involving oxygen are thought to be catalyzed by transition metals. In the presence of a metal catalyst (Fe²⁺ or Cu⁺), oxygen can capture an electron to form superoxide anion (O₂⁻). Upon protonation, superoxide forms the perhydroxyl radical (OOH•). Superoxide also undergoes reduction to form peroxide anion (O₂²⁻). Peroxide ion can in turn become protonated to form hydrogen peroxide (H₂O₂). Furthermore, iron can catalyze the generation of hydroxyl (OH•) and peroxy radicals (OOH•) from H₂O₂ via the Haber-Weiss and Fenton reactions [2].

To be effective as a catalyst in radical reactions, transition metals must be present in their free or ionic forms. Various substances act as indirect anti-oxidants by binding and effectively reducing concentrations of divalent transition metals from solution.

4 The role of hop polyphenols and hop bitter acids in ROS formation

Both malt and hop polyphenols are considered to have an impact on flavour stability due to their antioxidant activity. The term polyphenols describes various molecules and simply refers to the presence of phenol units in the compounds. The concentration of polyphenols in beer can range from 50 mg/l to over 300 mg/l. Depending on beer type, up to 80% are malt derived, the remainder from hops. The concentration of alpha acids can range up to 25 mg/l, depending on hopping regime. The amount of iso-alpha acids can be somewhere between 5–100 mg/l.

Flavones readily chelate metal ions. Flavan-3-ols behave as antioxidants via scavenging of free radicals, chelation of transition metals and mediation and inhibition of enzymes. By releasing

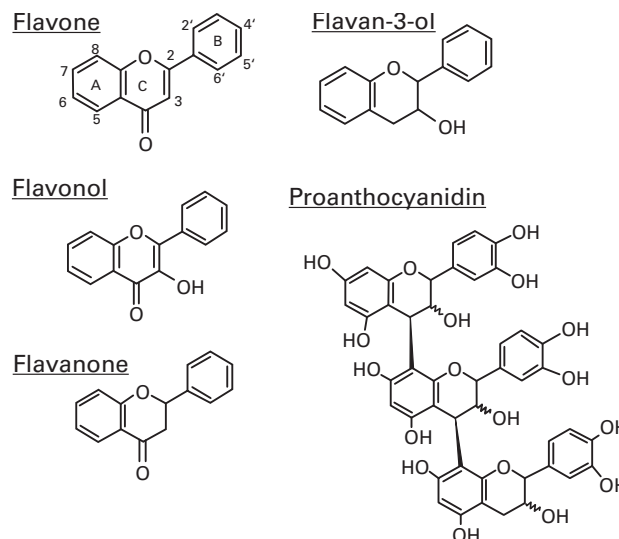


Fig. 2 Flavonoid phenolic and proanthocyanidin (condensed tannin) structures [2]

electrons flavan-3-ols receives a radical character and forms oligomers. Antioxidant activity increases from monomer to trimer then decreases from trimer to tetramer. *Ting* already found in 2008 that alpha acids form stable phenoxyl radicals that act directly as antioxidants, alpha acids may also suppress the initiation through chelating functionality toward transition metals (such as Fe²⁺ and Cu⁺) that may circumvent the formation of ROS [14]. This was confirmed by *Wietstock* especially for the alpha acids [19]. Also they found that alpha and beta acids reduced the formation of radicals measured via ESR. A different study that investigated a range of different hop products found that the peroxy radical scavenging capacities of prenylated flavonoids, especially that of Xanthohumol were considerably higher [16].

5 Prooxidant behaviour

Flavan-3-ols, proanthocyanidins and flavonols may also be pro-oxidant by promoting ROS formation due to the presence of a gallic acid moiety in these molecules, as they bind with Fe³⁺ to form ROS scavenging complexes. When the ratio Fe³⁺ : gallic acid is more than two the complex becomes pro-oxidant by forming H₂O₂

Table 1 sensory evaluation of flavour stability according to different regimes. Sensory ageing scores (0: completely fresh to 8: very strongly aged, undrinkable)

hopping regime for beer	time of addition	fresh	30d	60d
with pellets	boil start	0	2,60	3,7
with CO ₂	boil start	0	2,90	3,8
no hops		0	3,20	4,9
only hop oil	after filtration	0	2,90	4,2
only polar hop oil fraction	after filtration	0	2,10	3,2
only floral hop oil fraction		0	2,50	4
only spicy hop oil fraction		0	2,70	3,8
only dry hopping	lager tank	0	2,20	3,9
only late hopping	boil end	0	1,70	2,7

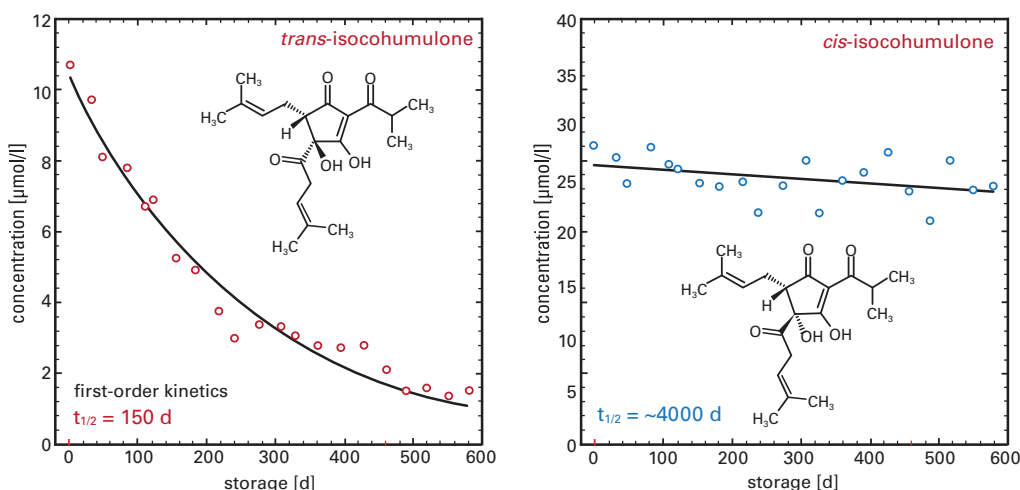


Fig. 3 Degradation of iso-alpha acids upon storage at 28 °C [7]

derived hydroxyl radicals. Proanthocyanidins may also reduce Cu^{2+} to Cu^{+1} , with Cu^{+1} capable of auto-oxidation to form more ROS. Prodelphinidins (3'4'5'trihydroxyflavans) with a more meaningful concentration in beer than gallic acid may react similar. However literature with clear findings in this regard is scarce.

The flavonols also display pro-oxidant activity due to their propensity to form quinines. Some ESR measurements also indicate that iso-alpha acids may act as prooxidants [2].

6 The role of alpha acids and iso-alpha acids degradation in flavor stability

In 1978 Williams and Wagner dedicated a paper to the contribution of hop bitter substances to beer stale flavour mechanisms [20]. Since a work by Sandra and Verzele found that the amount of acetic, 2-methyl propionic (isobutyric), 2-methyl butyric, and 3-methyl butyric (isovaleric) acid was higher in hopped beers than in unhopped beers it was concluded that these could be oxidation products of side chains of the alpha or beta acids [12]. This was also shown by Williams and Wagner with the exception of 2-methyl butyric acid, however they also found that in sensory testing these esters had no impact on the sensory stale flavour as the overall hoppy impact was masking the stale flavours. It was proposed by Araki to use the ratio of trans-isohumulones : cis-isohumulones as a staling indicator as this ratio was proven to correlate with stale flavour intensity of beer [1]. A recent study of de Clipeleer *et al.* further investigated the role of iso-alpha acids in stale flavour formation. Working with purified trans-iso-alpha acids and cis-iso-alpha acids they even report an increase in trans-iso-alpha acids and propose a reisoimerization via alpha acids. They further state that the aldehyde formation as a function of forced ageing was irrespective of the mode of bittering [3]. Hofmann determined that approx. 75 % of the trans-iso-alpha acids but only 15 % of the cis-iso-alpha acids are degraded within the first 12 months of beer storage. In this regard neither oxygen nor light proved to be of major importance but the temperature and the pH value are. They also identified several degradation products (tricyclohumole, tricyclohumene, isotricyclohumene, tetracyclohumole and Epitetracyclohumole) and determined their thresholds. These molecules seem to make up the biggest part of the loss in bitterness [8]. It is also obvious that the concentration of the co-version increases more than that of the n-homologues.

7 Light stability

When talking about flavour stability it is almost impossible not to discuss light stability. For whatever reason, beers in clear or green glass bottles are preferred over brown bottles, and thus light stability is of importance for a lot of brewers. We know that 3-MBT is easily formed from isohumulones under light exposure in presence of a sulfur source and has an extremely low threshold (1 ng/l). Hop suppliers sell light stable hop products such

as solutions of rho-iso-alpha acids, tetrahydro-iso-alpha acids and hexahydro-iso-alpha acids. However de Keukeleire showed that the photodecomposition by visible light occurs via one-electron oxidation of the beta-tricarbonyl chromophore and is common to isohumulones and reduced isohumulone, though the resulting products are not 3-MBT, and possibly have higher flavor thresholds, so they can add to sulfury and cardboard flavours [4]. In actual brewing trials Zufall found that in the absence of light and under light exposure reduced isohumulone products were superior to non-reduced in terms of flavour stability, but admitted that they do not avoid the formation of stale flavor. He also reported a strong tendency towards improved beer stability with a higher degree of reduction. In these "light stable beers" they identified a roasted flavour untypical for normal lagers and attributed to methyl-furfurylmercaptan. He also characterized aged light stable beers to have bready, sweet and caramel flavours where as conventional beers rather showed cardboard or leather flavours [21].

8 Hop Aroma

Findings on the importance of hop aroma in beer on flavour stability are scarce, especially regarding the influence of a hop intensive aroma e.g. dry hopped beers. A hop aroma can be achieved by various ways: hop additions during boiling (kettle hop aroma, late hop aroma) or hop addition during lagering (dry hop aroma). Depending on the amount of hops, totally different hop characters are achieved in the final beer. Every type of hop aroma will have different effects on flavour stability. Also the quality (freshness) of the hops used is of major importance especially in the case of hop pellets. One study, that was investigating the influence of the time of harvest of the hops on flavor stability, came to the conclusion that later picked hops may positively influence flavor stability if the "real" storing conditions are moderate (cool and dark) [5]. We also know from the interaction of aroma compounds in beer that masking effects of aroma compounds are likely to cover emerging stale flavours, but only to a certain degree. Linalool was shown to be very stable in final beer, even to increase with aging in dry hopped beers [6]. The sesquiterpenes were found to be less stable in beers and to decrease to a certain extent, possibly due to oxidation and hydrolysis. There is some evidence that the use of hop oil products goes along with lower levels of stale flavor components than conventional hopping [18]. Here we encounter discrepancies between analytical and sensory stale flavour measurements. While

late hopped beer had a normal pattern of measurable stale flavours, in sensory evaluation they proved to be superior. Still especially in this field more work is needed to answer the following questions:

- How stable are hop aroma components in beer and what is their sensory importance?
- Which interaction of hop aroma compounds with other beer aroma compounds take place?
- Which interactions of hop aroma compounds with stale flavor compounds take place?
- Is dry hopping an effective tool to enhance flavour stability and if so by which mechanisms?

9 Conclusion

If we look at the brewing process from the perspective of enhancing the capabilities of hops to increase the flavour stability in beer what would be the theoretical consequences?

The use of hop polyphenol extracts e. g. spent hops, could be somewhat advantageous in the boil, or even in the mash. However the results of the anti-oxidative potential of polyphenols are contradictory. To benefit from the potential of hop prenyl-flavonoids, their addition should be done in the final beer to avoid isomerization or adsorption. To benefit from the chelating effects of alpha acids an incremental hop dosage should be done during boiling, at the end of boil or in the whirlpool with pellets or pure alpha-acids. A stable hop bitterness can theoretically be obtained with isomerized hop products with a high cis-isomer content. For this iso-pellets, isomerized extract or pure iso-alpha acid can be used. For improved light stability, brown glass is maybe more effective than the use of light-stable hop products but may be in contrast to consumer preferences. A perceivable hop aroma can mask the sensory development of stale flavours for a certain amount of time. However the intensity and quality of hop aroma and especially of a dry hop aroma is changing over time. An intense hop aroma can be obtained with hop pellets, oil rich extracts or hop oil products. Linalool can be chosen as a marker to monitor the development of hop aroma. For both late hopping and dry hopping fresh hops, picked rather later than earlier, should be used.

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