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Review Article

Review: Impact of Dry Hopping on Beer Flavor Stability

Oberholster A* and Bradley M. Titus

Department of Viticulture and Enology, University of California, USA

***Corresponding author**

Anita Oberholster, Department of Viticulture and Enology, University of California, Davis, One Shields Avenue, Davis, CA, 95616, USA, Tel: 1-530-754-4866; Fax: 1-530-752-0382; Email: aoberholster@ucdavis.edu

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Abstract

Hops are the flowers of the perennial plant *Humuluslupulus* and are used primarily as a flavoring and bittering agent in beer. They have also been shown to increase the flavor stability of beer over time. They can be added during different stages of the brewing process. Hops added at the beginning of the boil are deemed bittering hops as they primarily contribute to the bitterness of the finished beer. During the boiling process the majority of their flavor active volatile oils are evaporated. Hops added in the middle or end of the boil are called late hop additions. These hops contribute some bitterness due to isomerization of α-acids (AA) during the boil but are mainly used to deliver hop flavor and aroma into the beer. Hops can also be added immediately after the boil in the whirlpool to impart hoppy aroma. A practice that has gained popularity is dry-hopping. Dry-hopping is the addition of hops to fermented, conditioned beer. Polyphenols and other compounds are known to be extracted during dry hopping and have been shown to contribute to beer bitterness. It is widely accepted that polyphenols increase the reductive potential of beer. They act to delay the degradation of beer over time as well as the production of chemical off-flavors and undesirable sensory attributes. Aged beer is characterized largely by staling attributes such as cardboard and a decrease in bitterness. Many of the compounds associated with beer staling exist in sub-threshold levels in beer. It is the synergistic effects of many compounds in aggregate that create some of the undesirable characteristics of an aged beer. The causes and mechanisms behind beer staling are not well understood and more investigations are needed.

ABBREVIATIONS

IAA: *Iso*-α-Acids; BMT: 3-Methyl-2-Butene-Thiol; BA: β-Acids; NMR: Nuclear Magnetic Resonance; t-2-N: *trans*-2-Nonenal; FRAP: Ferric-Reducing Ability Power; RP-HPLC: Reverse Phase High Performance Liquid Chromatography; SPE: Solid Phase Extraction; MS/MS: Tandem Mass Spectrometry; SPME: Headspace Solid Phase Microextraction; GC-MS: Gas Chromatography Mass Spectrometry

INTRODUCTION

Hops, the flower of the female perennial *Humulus lupulus*, have not always been one of the main ingredients in beer. It wasn't until the 12th century that we have evidence of hops replacing herbs like mugwort, dandelions and heather as a flavoring agent in beer [1]. Prior to their use in beer, hops were recognized primarily for their medicinal and preservative purposes. They have both antibacterial and sedative qualities as well as high concentrations of phenolic compounds that can act as antioxidants and radical scavengers [2-5]. In addition to their medicinal qualities hops contribute enormously to the flavor and bitterness of beer.

Hop composition and flavor

There are over 80 varieties of hops sold commercially and no two varieties are quite the same. Chemical and sensory analyses of hops have provided evidence for key distinctions of varieties. Aberl and Coelhan performed headspace trap GC/MS and showed some of the key differences between many European varieties [6]. Free terpenoids and thiols were investigated by Kankolongo et al., to elucidate the differences between dual-purpose hops [7]. Differences have been shown in sensory experiments as well by descriptive analysis and free-choice profiling [8,9].

The flavor and bitterness from hops comes from oils and resins packed into its lupulin glands [10]. Efficient extraction of these chemicals relies on the lupulin glands being ruptured by chemical and mechanical means. Most hops grown are processed into pellets to effectively expose these flavor active chemicals. When added into the boil during brewing many groups of chemical compounds are extracted over time. Terpenes, terpenoids, oxygenated sesquiterpenes, polyphenols, sulfur compounds and glycosylated species all contribute to hop-derived aroma [11]. Bitterness in beer is mainly contributed by the isomerized form of compounds called α-acids (AA) which are found in the resin glands of hops (Figure 1) [10]. These compounds are isomerized

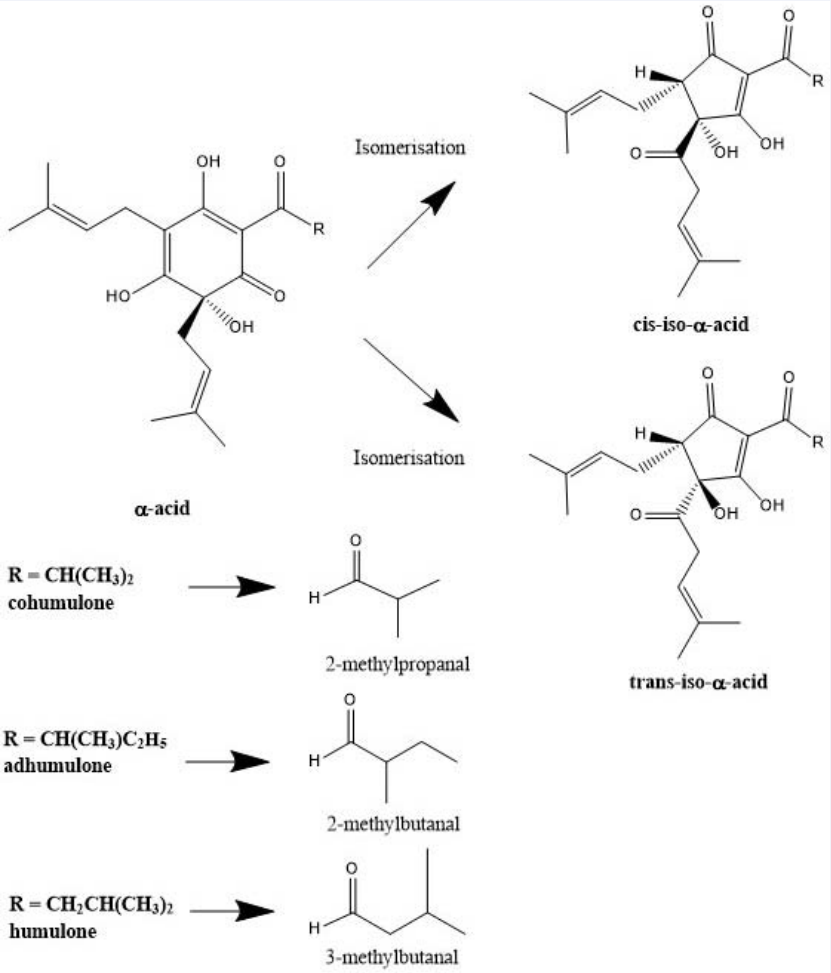

Figure 1 Adapted schematic of the hypothetical degradation products of iso-humulones in beer following deacylation of the side chain at C2 [64].

during the boil to form *iso*-α-acids (IAA) during the brewing process. There are a total of six IAA in beer: *cis* and *trans* of iso-humulone, iso-cohumulone, and iso-adhumulone - with the *cis* isomers being more bitter than the *trans* [12]. A conventionally hopped wort (or unfermented beer) in which only hop pellets or whole flowers are added typically has a ratio of *cis* to *trans* acids of about 70:30 [13,14]. In addition to its higher bitterness the *cis* isomer is also more thermodynamically stable and degrades much slower than the *trans* [15]. This phenomenon makes the ratio of *trans*- to *cis*-isohumulones a metric for beer flavor stability [14]. The IAA in beer can degrade by multiple pathways. Acid-catalyzed degradation, oxidation and photo-oxidation are the most relevant pathways with regards to beer [16]. The proposed mechanism for acid-catalyzed degradation elucidates the relative instability of the *trans*-IAA over the *cis*-IAA due to an increased distance of the reacting carbocation and double bond of the isopentenoyl side chain in the reaction [17,18]. Aside from this pathway the well understood flavin sensitized photo-oxidation of IAAs is another source of degradation. This pathway produces the notorious 3-methyl-2-butene-thiol (MBT) that imparts a characteristic skunky or "light struck" aroma [19,20]. Autoxidation can also degrade IAAs by hydrogen abstraction in the presence of molecular oxygen [21]. This is a prime reason to minimize total packaged oxygen in beer production. Much less is known about the β-acids (BA) in hops. They are thought to contribute in some way, albeit minor, to perceived bitterness. Sensory and chemical analyses have shown that proposed BA oxidation products are bitter in a beer matrix and have relatively low taste recognition thresholds [22]. The proposed mechanism of formation occurs at ambient temperatures in beer and is accelerated in the presence of oxygen and a reducing agent such as glucose or ascorbic acid [23]. The resulting compound, n-hulupone, is water soluble. Identification and quantification of the BA oxidation products in beer has been done by HPLC [24]. Oxidation of BA can occur during wort boiling and can yield bitter compounds [22]. In addition to hop acids, hop-derived polyphenols have also been shown to contribute to perceived bitterness [25].

Timing of hop addition

All of the above mentioned hop-derived compounds are not only introduced during the boil. At any time during the brewing process that hops are added these compounds will be extracted to some degree. A practice that has gained popularity over the past 150 years is dry-hopping. Dry-hopping is the addition of hops to fermented, conditioned beer. This is markedly different to other

methods of hop additions. The most traditional method of hop delivery takes place during the boil and can be thought of as a hot water extraction. Hops added at the beginning of the boil are deemed bittering hops as all of their volatile oils are evaporated during the boil. Hops added in the middle or end of the boil are called late hop additions. These hops contribute some bitterness due to isomerization but are mainly used to deliver hop flavor and aroma into the beer. Hops can also be added immediately after the boil in the whirlpool to impart hoppy aroma. The flavor contribution to beer by late hopping is different to that of dry hopping. The chemical compositions of beers hopped by these methods have been investigated by NMR metabolomics [26]. Since no isomerization takes place during dry hopping, any increase in bitterness contributed by IAA is arguably negligible. This does not mean that dry hopping does not impart bitterness. Polyphenols and other compounds are known to be extracted during dry hopping and have been shown to contribute to beer bitterness [25,27]. This said, bitterness contribution is not at all the primary intent of dry hopping. Dry hopping is performed to increase the "hoppy" aroma of the beer and has seen resurgence in popularity thanks in part to the growth of the craft beer industry in the United States as well as other countries. Since dry hopping is done at relatively low temperatures, the thermal degradation and volatilization of flavor compounds are significantly reduced. This allows for a higher concentration of these compounds in the finished product.

Beer aging

Polyphenols, in particularflavan-3-ols, flavonols and phenolic glycosides, are known antioxidants and antiradicals [28]. Beer aging is largely driven by lipid oxidation, Maillard reactions, Strecker degradation and radical-mediated oxidation [29,30]. It is widely accepted that polyphenols have an effect on the reductive potential of beer. [25,30-34]. They act to retard the degradation of beer over time and the production of chemical off-flavors and undesirable sensory attributes. Aged beer is characterized largely by staling attributes such as cardboard and a decrease in bitterness which may in part be due to the instability of iso-humulones over time or possibly due to the masking effects of sweet flavors [35]. Over time both sweet aroma and taste increase as the concentration of ethyl esters and Maillard Reaction product increase [36-38]. As beer ages there is a fast increase in ribes/black currant character followed by a steady decline. Volatile sulfur compounds have been shown to contribute to this character in beer [39,40]. Lastly, due to lipid oxidation, long chain aldehydes such as trans-2-nonenal (t-2-N) are formed and increase the papery/wet cardboard character of beer [37]. Of the long chain aldehydes, t-2-N is widely considered to contribute the most to this characteristic [41].

Phenolics in beer

Polyphenols, along with other compounds, are extracted during dry hopping at a relatively fast rate. It has been demonstrated that about 80-90% of the polyphenols extracted during dry hopping are extracted within the first 12 hours [11]. This was determined by investigating the ferric-reducing ability power (FRAP) of polyphenols in beer during the dry hopping process. FRAP relies on the phenomenon that polyphenols have the ability to reduce ferric iron. As the demand for beers with more hop flavor increases brewers will rely on the dry hopping process to help deliver this. Dry hopped beers have more hop-derived polyphenols than those that are not dry hopped. Some beers can get almost 50% of the total polyphenolics in the finished beer from hops. This is much more than the average lager where the hops contribute less than 20% [42]. The polyphenols extracted during dry hopping represent a complex matrix of hydroxybenzoic acids, hydroxycinnamic acids, flavonols, proanthocyanidins, prenylchalcones and stilbenes (Figure 2) [43]. The flavan-3-ols have received a lot of attention due to their multifaceted influence on beer quality. Both flavan-3-ol monomers and oligomers play a role in flavor, colloidal and foam stability [44]. They are known to possess both anti-radical and antioxidant capabilities [45-49] and have been shown to have positive effects on the stability of both foods and beverages [49]. Flavan-3-ol monomers complex with proline-rich proteins in beer to form what is known as chill haze. The mechanisms for this process were originally suggested by Siebert and Lynn [50]. A complex network of cross linking occurs between the polyphenols and proline residues from hordein protein from barley [51]. This interaction is pH dependent and uses the many hydroxyl groups on the polyphenols to create bridges from hydrogen bonding. Beer pH and alcohol content is ideal for polyphenol-protein haze formation [52]. The resulting haze is known to appear when the beer is cooled and is common at refrigerator temperatures; thus the name chill haze. Dry hopping is often done after filtration, the latter process removing left-over yeast and particulate matter that can precipitate hop flavor compounds and contribute to flavor modification by biotransformation. The dry hopping process can cause the development of chill haze due to the polyphenols extracted into the beer. This and the fact that some polyphenols, notably gallic acid, have been shown to act as pro-oxidants demonstrate the dichotomous role of polyphenols in beer [31]. They aid in flavor stability but have the potential to diminish colloidal stability and even increase oxidation in the beer. Beer chill haze has no effect on beer flavor but it is visually displeasing and many breweries go to great lengths to prevent it. Phenolics and specifically flavan-3-ols have been shown to increase the perceived bitterness and "harsh flavor" in beer often associated with astringency [27,53,54]. Dozens of phenolic compounds have been identified and quantified in beer. Beer has been found to be the main source of dietary hydroxybenzoic acids by a European Prospective Investigation into Cancer and Nutrition study [55]. New polyphenolic compounds are still being discovered in beer by advanced analytical methods [56]. The most complete list of phenolics in beer, including various methodologies, was assembled by Callemein and Collin [43]. The most accepted quantitative method for chemical analysis of hop, barley and beer phenolic species is reverse phase high performance liquid chromatography (RP-HPLC) [43]. Both the American Society of Brewing Chemists (ASBC) and the European Brewers Convention (EBC) have standard RP-HPLC methods for measuring phenolics in beer. However, many groups have attempted to quantify polyphenols in beer by RP-HPLC analysis and the reported recoveries after solid phase extraction (SPE) are unacceptably low for some phenolic compounds [57,58]. An alternative method of quantifying polyphenols is by tandem mass spectrometry (MS/MS) after chromatographic separation [56]. The sensitivity of MS/MS allows for the omission of a concentration step by SPE, resulting in increased recoveries.

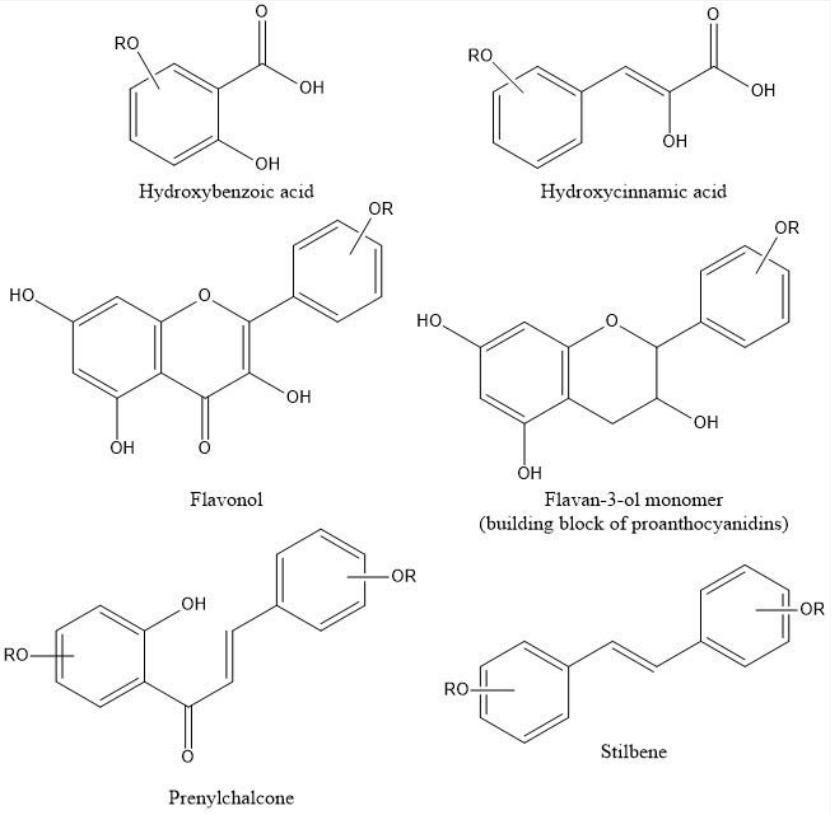

Figure 2 Global definitions of the main polyphenols in beer [43].

Chemical staling markers in beer

The production of degradative aldehydes has been investigated as potential stability markers for beer. Malfliet et al., investigated markers for flavor instability in lager beers and concluded that in addition to the T/C ratio, aldehydes, specifically furfural, hexanal, 2-methylpropanal, 2-methylbutanal and 3-methylbutanal, are adequate markers for flavor stability [30]. Aldehydes in beer are produced from many sources including Strecker degradation, Maillard reactions, as well as lipid oxidation and they have been shown by Saison et al., to increase during forced aging of beer [59]. GC-MS analysis of aldehydes in beer can be problematic due to matrix effects and low reproducibility even with derivatization to improve sensitivity. A common derivatization agent used in aldehyde analysis is O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA). Moreover, many of the compounds associated with beer staling exist in sub-threshold levels in beer. It is the synergistic effects of many compounds in aggregate that create some of the undesirable characteristics of an aged beer [60]. Oxidative aldehydes can originate from many sources as described above, but not all the major pathways of formation in beer have been confirmed. Although hops may act to retard aldehyde formation, they may also contribute to their production. Iso-humulones have been shown to degrade into 2-methylpropanal, 2-methylbutanal and 3-methylbutanal in model solutions (Figure 1) [61].

Arguably the most important degradative aldehyde found in beer is *trans*-2-nonenal (t-2-N). This notorious oxidation product of linoleic acid has an extremely low flavor threshold in beer. It can be detected at levels as low as 0.035 µg/L in beer and delivers a distinct wet cardboard flavor [62]. Although t-2-N has been found to deliver the largest contribution to beer staling, it cannot be looked at in isolation. Furfural and hexanal have been investigated alongside t-2-N as potential major contributors to beer staling [63]. More recently, an investigation into the causes of the increase in various aldehydes during aging found a link between thiazolidine compounds and aldehyde production in a model system [64]. The sources, causes and mechanisms behind beer staling are far from wholly understood and more work is needed.

In summary, as the demand for hoppy beers increases, brewers will rely on dry hopping to help deliver this. More than 50% of the polyphenolics in dry-hopped beer can come from the hops. Polyphenolics, and more specifically the flavan-3-ols, can have a multifaceted influence on beer quality as they play an important role in flavor, colloidal and foam stability. Different markers for flavor instability have been identified and the T/C ratio in combination with aldehydes, in particular furfural, hexanal, 2-methylpropanal, 2-methylbutanal, 3-methylbutanal and trans-2-nonenal, have been determined as adequate markers for flavor stability. Further study is needed to improve current methodology in determining specifically aldehyde content as well as to understand the mechanisms and causes of beer staling.

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