AN ABSTRACT OF THE THESIS OF

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Abstract approved:

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This work set out to examine the methodologies of dry hopping, compare different hop materials, and look at the extraction behavior of different types of hop compounds. This work consists of two discrete studies, where the first study informed the design of the second.

The first study measured the concentrations of hop aroma compounds extracted from Cascade hops during dry hopping using a model beer system devoid of malt, yeast aromas, and hops. Cascade hops pelletized by four different processors yielded different particle size distributions and pellet densities. These pellets were dosed into a degassed medium (water, 6% v/v ethanol, pH 4.2) and the hop aroma extraction was measured periodically over a one week period. Solid phase micro-extraction (SPME) followed by gas chromatography (GC-FID) was used to analyze the levels of aroma compounds in the extraction medium. Variation in the hop pellet physical properties did not significantly impact the extraction rate of hop volatiles such as linalool, geraniol, limonene and myrcene with one exception. One treatment showed an increased absolute concentration of geraniol. Separately, dry hop aroma extraction was measured over a short time (1 day) at room temperature in an unhopped beer using small-scale (1L), stirred vessels. Irrespective of the hop form (whole or pellet), the concentrations of hydrocarbon terpenes peaked between 3 and

6 hours and subsequently declined, while the concentrations of terpene alcohols continued to increase throughout the 24 hour dry hop extraction. The rate of hop aroma extraction did not appear to be significantly influenced by hop pellet properties and occurred rather rapidly regardless of the hop form.

The second study examined the extraction of hop aroma compounds during a pilot brewery scale (~4hL) dry hop treatment. Dry hop treatments consisted of whole cone hops and pellet hops (Cascade cultivar, 2011 harvest) which were dosed into cylindroconical vessels which were either stirred with a pump or left quiescent. Samples were taken for GC-FID and HPLC analysis as well as sensory evaluation at various time points between 30 minutes and 12 days. Polyphenol and alpha acid extraction was highest in a stirred system dosed with pellets. Hop aroma compound extraction was also the highest in the stirred system utilizing pellet hops. The sensory panel rated the stirred pellet samples as having the highest hop aroma, bitterness, and astringency. The results showed that hop flavor from dry hopping can be readily achieved with much shorter contact time than the current 4-12 day industry practice.

# A Study of Factors Affecting the Extraction of Flavor When Dry Hopping Beer

by
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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes the release of my thesis to any reader upon their request.

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# Table of Contents

# List of Figures

# List of Tables

# A Study of Factors Affecting the Extraction of Flavor When Dry Hopping Beer

## Chapter 1. Dry Hopping: A Review of Goals, Processes, and Outcomes

(to be submitted to the Journal of the Institute of Brewing)

### 1.1 Introduction

Hops are used in the brewing process to add flavor and microbial stability to beer. Hops added on the hot side of the brewing process (typically during the kettle boil) primarily add bitterness and a small amount of aroma. In contrast, dry hopping is the practice of using hops late in the brewing process with an emphasis on adding aroma and flavor (without undue bitterness) to beer. Dry hopping is performed on the "cold-side" of the brewing process, anytime after boiled wort has traversed a heat exchanger and cooled to fermentation temperatures or lower. A strict definition is nearly impossible given the breadth of practices used by brewers today. Dry hopping is experiencing a revival in the United States and the United Kingdom, and even traditional continental brewers that historically eschewed dry hopping are experimenting with it. Most US craft brewers have at least one and usually multiple styles utilizing the dry hop method, and the explosive growth of the craft brewing industry indicates that consumers have an interest in these types of uniquely hoppy beers.

The science of brewing and hopping beers and ales has a rich, innovative history. 21$^{st}$ century brewers have an arsenal of tools, techniques, and shared experiences to draw upon, and over a thousand years of experimentation as a foundation. The first recorded use of hops directly associated with brewing is the often cited statues of Corbie dating to 822, where the Abbot Adalhard decreed that ten percent of all (wild) hops gathered should be delivered as tithe to the porter "to make beer thereof" *(1)*. Hopping began in earnest in Bavaria and the low countries of present day Netherland during the 12$^{th}$ century *(1,2)*. Hopping was initially resisted in

England, where a distinction was maintained between unhopped brews, simply called ale, and hopped brew *(2,3)* which was referred to as beer (bière) or "Flemish Ale". Initially imported to England or brewed domestically by Dutch and German immigrants *(2)*, hopped beer made significant headway in the marketplace in the early 15th century *(2)*. Hopping ales became more and more popular, and by the 16th century most ale included some level of kettle hopping, but a distinction was still maintained between lightly hopped ales and the more heavily hopped beers.

It is not clear from historical texts whether hop usage was exclusive to the boil kettle or whether there was always some measure of dry hopping. It is likewise not entirely known precisely when the practice of dry hopping was adopted in England. We do know that 18th century brewers realized that hop dosage affected microbial stability *(4)*, and early 19th century brewers adopted hopping rate guidelines based on the time of year that the beer was brewed and/or the climate of its intended destination *(5)*. Dry hopping at this time was achieved by adding a plug of dried whole leaf hops to a cask prior to sealing the bung. The practice was limited to certain styles of beer (published records do not exist of any 19th century brewers dry hopping a porter or stout), but it persisted at some level until present day. In the United States, the practice was inconsistent and functionally extinct after brewing industry consolidation following Prohibition. The revival of the practice was certainly due in some part to a minority of consumers who rejected the overwhelming commercial presence of lightly hopped lagers being produced during the latter half of the 20th century in the US. Many of these consumers were part of the growing home brewing movement that began when it was legalized by President Carter in 1978 and gathered steam in the 1980's, particularly after the publication of Charlie Papazian's book, "The Joy of Homebrewing" in 1984. These brewers had a desire to resurrect traditional English ale and other old world recipes; in the United States many of these

homebrewers later became professional craft brewers or vocal supporters thereof. Dry hopping processes fit naturally with the palate, respect for old world tradition, and culture of innovation widespread among American craft brewers and their patrons.

## 1.2 Dry Hopping Goals

The primary goal of a dry hopping regime is to extract flavor and aroma compounds from the hops, solubilize them in the beer matrix, and do this with a minimal impact on colloidal and oxidative stability. There are key differences between a dry hop extraction and a kettle extraction. When considering beer as a solvent for hop compounds one must consider the low temperatures and the aqueous/ethanol/$CO_2$ composition of beer relative to the composition and temperature of wort in the kettle.

Beer flavor is an amalgam of taste, aroma, and mouthfeel. Dry hopping imparts aroma, but may also impact taste and mouthfeel. The hop aroma components are almost entirely terpene oils, sulfur compounds, or derivatives thereof. These aroma compounds are not exclusive to the hop plant, but the hop plant remains the brewer's primary beer aroma source because of the large overall variety of aroma compounds, their desirable ratio based on consumer flavor expectations, and brewing tradition. There are other hop-derived components that may affect beer flavor: bittering acids, polyphenols, and esters. Of these, only α and β bittering acids are found exclusively in hops.

Flavor consistency is expected by consumers and therefore is of utmost importance to brewers of all sizes. Furthermore, the contributions to flavor perception are estimated to be 80-90% olfaction and 10-12% basic taste. With that in mind, it is worth noting that the concentrations of terpene oils and other aroma compounds in hops can fluctuate dramatically from year to year, even in the same

cultivar on the same farm *(6–8)*. The average daily temperature, precipitation, and even the type of pesticides and fungicides used during the growing season can have a profound effect on aroma. The hop industry is beginning to address aroma consistency through maturity and harvest studies, but it will likely remain an issue for the foreseeable future. One possible approach to maintaining flavor consistency is expanding the library of oil profiles (used in the past to identify hop cultivars) with year to year data and incorporating those data in recipe formulations to enable augmentation or substitution.

## 1.3 Hop Components Which Affect Flavor

### 1.3.1 Hop Essential Oils

Dried hops cones (containing 9-11% moisture) typically contain 0.5%-3% oil by mass *(9)*. The oil is primarily made up of a class of compounds referred to as terpenes. Terpenes are classified by how many carbon atoms they posses, in units of five. Monoterpenes are the prototypal molecule; they are made of two $C_5$ isoprene subunits. Sesquiterpenes are created by the addition of another isoprene subunit to a terpene, creating a $C_{15}$ molecule.

Hop oil is generated in during flowering, and the synthesis occurs primarily in the trichomes of the hop plant. Trichomes are specialized glandular plant organs which exist in nearly every plant which produces essential oil; in hops they are located on the surface of the bracts near the strig (central stem) of the hop cone. They are composed of secretory cells containing specialized plastids which surround a lipophillic cavity that fills with secreted compounds as they are produced *(10)*. The oils are produced from isopentenyl pyrophosphate via the ubiquitous terpene pathway. The monoterpene myrcene is produced in the young cones immediately, and is typically the largest constituent of the essential oil (as much as 70% by

volume). As the cone ages, oxygenated terpenes (terpenoids) are formed, followed by the synthesis of sesquiterpenes *(7,10)*. Humulene and Caryophyllene are the dominant sesquiterpenes and are also the second and third largest constituent of the overall oil.

The remaining fractions of the essential oil contain a vast number of terpenes and terpenoids in small amounts. Exact numbers vary, but studies have put the number of compounds in the essential oil just under 500 *(11,12)*. While that may seem like an imposing number, only a fraction of those compounds are important to beer aroma: the established number of compounds which have been directly associated with hop aroma in beer is around 21-25 *(13,14)* although the actual number is likely to grow as work in that area continues.

A great deal of work was done in the early 1990s to categorize the odor-active compounds and assess their importance to hop aroma in beer *(13,15)*. Nickerson and Van Engel proposed the use of the "Hop Aroma Unit" to quantify a given hop's potential to impart hop aroma through late kettle additions or dry hopping. They built a basic list of odor active compounds and ascribed them characteristics such as spicy, floral, citrus, piney, etc.; it was assumed that more compounds would be isolated through gas chromatography/olfactory (GC-O) work and added to the list. Though the motivations for creating the Hop Aroma Unit have not been obviated, work on expanding the list has stalled and it remains much as it began despite ongoing interest. Table 1 shows compounds either present in hop oil *(7,13)* or derived from hop oil *(7,16)* which have a significant impact on beer aroma.

Table 1. Important Aroma Compounds Derived From Hops

| Spicy/Herbal | Floral/Fruity | Citrus/Pine |
|---|---|---|
| Humulene | Linalool | Limonene |
| Humulene Epoxide I | Geraniol | Citral |
| Humulene Epoxide II | Geranyl Acetate[b] | Farnesene |
| Humulene Epoxide III | Geranyl Isobutyrate | α-Pinene |
| Humulenol II | Citranellol[b] | Citranellal |
| Humulol | β-Ionone | Linalool |
| Caryophyllene Oxide | Nerol | Ethyl 4-methylpentanoate |
| Myrcene | γ-nonalactone[b] | Ethyl butyrate |
| Eudesmol | 4mmP [a] | |
| Farnesol | Ethyl 4-methylpentanoate | |
| Ethyl cinnamate[b] | β-damascenone | |

[a] 4-mercapto-4-methyl-pentan-2-one, [b] Found in beer only

The impact of hop essential oil aroma is greater than the sum of its parts. Many aroma compounds which are derivatives of hop essential oil are found in finished beer but not in freshly extracted hop oil. Terpenoids undergo biotransformation when exposed to yeast metabolism *(17)* and the moderately acidic

pH (3.9-4.3) of beer systems can encourage hydrolysis reactions, such as the conversion of humulene epoxide to humulenol *(18)*.

Noble hop aroma, such as is typically present in continental hop varieties, differs from British or American hop aroma. Noble hop aroma is characterized as a full nose of spice, cedar, black pepper, with floral notes *(19–22)*. It has a notable absence of the fruity, warm citrus tones American hops are well known for. Noble hop aroma is associated with German and Czech hop varieties like Hallertauer Hersbrucker and Saaz *(7)*. This spicy hop aroma is often attributed to the presence of oxygenated derivatives of the sesquiterpenes humulene and caryophyllene as well as farnescene, in the case of the Saaz variety (and conspicuously lower concentrations of other aroma compounds).

Both humulene and caryophyllene have multiple epoxide configurations *(23)*, and each one produces a slightly different aroma *(7,19)*. Furthermore, each epoxide can undergo numerous hydrolysis reactions, again each one producing a slightly different aroma *(19)*, but they are characterized as moderately spicey or citrussy but mostly woody and cedary. Yang et al indentified 17 humulene epoxide hydrolysates in beer. The most intense aroma descriptors these compounds were given by sensory panelists were cedar, spicy, lime, lemon, and pineapple. One must bear in mind that these compounds often exist below their sensory threshold in finished beer *(22)*. Furthermore, studies have been mixed when attempting to correlate hop oil epoxide fractions and spicy aroma *(22,24,25)*.

Table 2 shows a list of hop aroma compounds considered important to the classic profile of noble hop aroma. This list is not considered exhaustive, but it includes some of the important hydrolysis products discussed above.

Table 2. Noble Hop Aroma: Humulene and Caryophyllene Epoxides and Hydrolysates

| Compound | Aroma descriptor | References |
|---|---|---|
| Humulene Epoxide I | Hay | *(7)* |
| Humulene Epoxide II | Cedar, Moldy, Sage-brush (weak) | *(7,19)* |
| Humulene Epoxide III | Cedar | *(7)* |
| Humulenol II | Lime, Cedar, Pineapple, Sage-brush | *(7,19)* |
| Humulol | Hay | *(7)* |
| Caryophyllene Oxide | Methanol, Musty, Floral, Cedar | *(7)* |
| 1,5,5,8-tetramethyl-8-bicyclo[8.1.0]undecene-2,9-diol | Lime, lemon, cedar | *(19)* |

There is mounting evidence that the pepper aroma which often accompanies noble hop aroma and is sometimes present in New Zealand cultivars may be attributable to a bicyclic sesquiterpenoid called rotundone or a very similar structure (Figure 1). Early studies looking at spicy aromas using GC-O found that oxygenated sesquiterpenes were predominantly responsible for spicy aroma, although identification of exact compounds proved difficult *(16,24)*. Oxygenated sesquiterpenes are difficult to extract via steam distillation, so characterizing the large polar aroma compounds in hops has not occurred until recently. The work by Wood and others which finally characterized rotundone found that it has a very low sensory threshold (8 ng/L in water) and a very strong pepper aroma. It has recently been identified as the single most important black pepper aroma compound in Shiraz wines *(26)*. It is present in grapes and many spices which produce essential oil, such as black pepper, white pepper, majoram, rosemary, and geranium *(26,27)*. Rotundone survives fermentation intact *(26)*. More work is needed in this area, as several compounds with similar empirical formulae ($C_{15}H_{22}O$, $C_{15}H_{24}O$, $C_{15}H_{26}O$) and similar sensory characteristics remain unresolved in the sesquiterpenoid fraction of hop oil *(7,16,24)*.

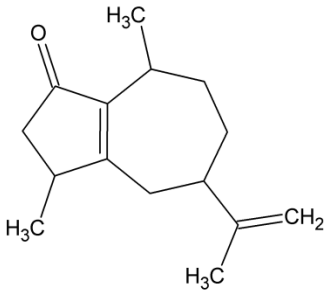

Figure 1. Rotundone

## 1.3.2 Sulfur Compounds in Hops

Although terpenes are the most well known and recognizable flavor and aroma compounds in hops, recent work has uncovered the sizable impact that thiol and thioester compounds can also have. Thiols can impart an onion, garlic, cheesy, fruity, grapefruit, tropical fruit, or currant-like aroma *(28,29)*. Some of these can be considered faults, while others complement and enhance terpene hop aroma. Similar to terpenes, when hops are added at the beginning of a vigorous 60 minute

boil, volatile sulfur compounds are undetectable in the finished beer *(28)*. Hop-derived sulfur compounds that remain in beer are introduced via late-hopping or dry-hopping. Of the compounds that generally survive beer maturation, S-methyl-2-methylthiobutanoate (SMMB) and 4-mercapto-4-methylpentan-2-one (4MMP, Figure 2) are potent odorants *(28,29)*. 3-mercapto-hexanol has also been found to contribute significant aroma in beer; it is present in unhopped beer at low levels and at higher concentrations in hopped beer *(29,30)*. 4MMP has been identified as one of the more potent contributors of fruity (sometimes described as black currant or "ribes") and floral aroma in some hop cultivars *(30)*. All of these compounds are soluble in beer and could be expected to be present at some level from both from kettle hopping and dry hopping.

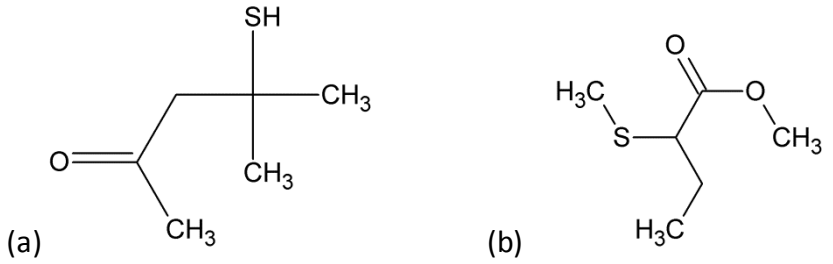


Figure 2. Thiol Odorants: 4MMP (a), SMMB (b)

Interestingly, the production of 4MMP and possibly other thiols is thought to be blocked by the presence of copper. This is not entirely unexpected as evidence exists in the wine and distilling industries of the role of copper at reducing sulfur aroma. European cultivars which were grown using fungicides which contained copper sulfate showed low or no levels of this compound, while hops grown without copper sulfate in the U.S., New Zealand, and Australia all showed a fairly high level *(30)*. Previous to Kishimoto's work showing the correlation between copper in the hop field and 4MMP, it had been shown that adding granular copper dramatically reduced the presence of currant-like and onion aromas in beer *(31)*.

The hop oils humulene and caryophyllene can also form episulfides instead of epoxides, particularly if the hops were grown on farms which sprayed sulfur to control powdery mildew *(31)*. 1,2-epithiohumulene exhibits a musty, cardboard-like aroma *(31)* but limited work has been done to see if these compounds are typically present at a high enough level to impart any significant aroma in contemporary beers.

Table 3. Hop Aroma from Sulfur Compounds

| Compound | Aroma descriptor | References |
|---|---|---|
| 4MMP | Black Currant, Passion Fruit, Onion | *(28,29,32)* |
| SMMB | Truffle-like, Fruity, Cheesy, Sweat | *(28,29,32)* |
| 3-mercapto-hexanol | Black Currant, Grapefruit, Burnt | *(28,32)* |
| Dimethyl Trisulfide | Onion | *(28,29)* |
| Dimethyl Disulfide | Cheesy | *(28)* |
| 2-mercaptoethyl acetate | Broth, Roasted | *(29)* |

### 1.3.3 Hop α and β acids

There has been speculation that dry hopping qualitatively increases the perceived bitterness of beer. While α and β acids have an extremely low solubility in beer, there is the possibility of limited extraction during dry hopping. However, the

cold-side beer matrix would not be conducive to the isomerization of α-acids, so any α-acids extracted would stay in their original, un-isomerized form. Work has been performed to test whether non-isomerized α-acids contribute to perceived bitterness in beer; it was shown that even in fairly high quantities (28ppm) α-acids did not increase perceived bitterness *(33)*.

Although dry hopping is extremely unlikely to lead to an increase in iso-alpha-acids because of the low temperatures, there may be extraction of humulinones if they are present in the hop material used. Humulinones form via spontaneous peroxidation of alpha acids and they are chemically very similar to iso-alpha-acids; the only difference is the addition of a hydroxyl group.

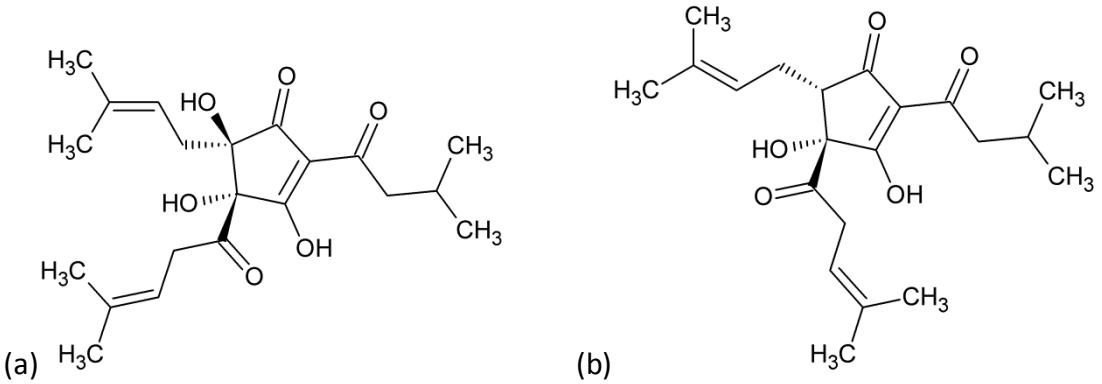


Figure 3. Humulinone (a) and iso-Humulone (b)

Humulinones are a strong acid (pka 2.8) and will be ionized in a beer medium, making them more water soluble than both alpha acids and iso-alpha acids *(34)*. They have been found in both pellets and kiln-dried whole cone hops *(35)*. Humulinones have a reported bitterness that is roughly 0.4 times that of iso-alpha-acids *(36)*, but that work was carried out before modern sensory methodology was developed and it has never been replicated. Since humulinones absorb UV light in a similar manner to iso-alpha-acids (strongly at 270 nm), their presence could lead to an overestimation

of iso-alpha-acids or BUs when performing spectrophotometric analysis on dry-hopped beer.

Regarding the impact of β acids on beer bitterness, the brewing science community is somewhat divided. β-acids are even more insoluble than α-acids, and the amount remaining in beer is negligible or even undetectable. If β-acids undergo oxidation during storage, they become more soluble, but their bitterness impact in the oxidized state is debated and in need of thorough investigation.

### 1.3.4 Polyphenols

Polyphenols are among the water soluble compounds extracted during dry hopping which contribute flavor to beer. As a polyphenol class, proanthocyanidins are present in large quantities and are important to beer flavor; the monomers include catechin, epicatechin, gallo catechin, and epigallocatechin *(37)*. These compounds are capable of forming dimers, trimers, and larger polymers when they undergo oxidation *(37,38)*. Polyphenols specifically have been shown to increase the perceived bitterness of beer both by themselves and synergistically with iso-alpha acids *(38–40)*. Polyphenols can also change the bitterness to be both more “harsh” and give the perceived bitterness a lingering quality *(39,40)*. Given that alpha acids have been shown to not increase perceived bitterness, it is certain that bitterness increases from dry hopping are actually derived from some combination of humulinones and polyphenols.

Apart from bitterness impact, polyphenols impart an astringent character to beer. This sensation is usually described as a drying feeling caused by the precipitation of polyphenol/protein complexes *(41)*. It is typically considered a part of mouthfeel rather than flavor, and is not necessarily considered a negative characteristic unless it dominates or overly distracts from other flavor components. The amount of astringency imparted by polyphenols changes based on overall

polymerization; the higher the degree of polymerization and molecular weight the more astringent character is perceived *(38)*. This phenomenon is accompanied by a decrease in overall bitterness *(38)*.

In addition to their direct impact on flavor, it's also possible that polyphenols may exhibit a fining effect on hop acids and terpenes in a manner similar to yeast cell membranes. Research on the interaction of catechins and epigallocatechins with cell membranes, micelles, and lipid-soluble molecules showed that these polyphenols can directly adsorb small hydrophobic compounds *(42)*.

### 1.3.5 Glycosides

β-Glycosides are another potential aroma source extracted during dry hopping. Glycoconjugation appears to be an important mechanism that allows the transport and continued synthesis of volatile aroma compounds *in situ*, especially when synthesizing against an increasing concentration gradient *(43,44)*. It also allows a plant to produce and store volatile molecules in a soluble and inactive state until such a time as they are needed (e.g. invasion by an insect predator or attractant for pollination). In a summary of 150 plant species, the ratio of glycosidically bound aroma compound to its free volatile form varied from 2:1 to 5:1 *(43)*. Comprehensive data has not yet been gathered on the precise ratios of glycosides in hops, nor has an exhaustive list of the aglycones been compiled. When examining just the water soluble portion of hop solids, glycosides are present in concentrations up to 25% of the total mass *(45)*, and given their solubility they would certainly be extracted during dry hopping.

Glycosides are present not only in the lupulin glands but in the cones and surrounding vegetative tissue as well *(44,45)*. When a $CO_2$ extraction is performed on hops, the remaining spent material has a potentially high glycoside content. This is a potential flavor source from what has been traditionally considered a waste stream.

A variety of glycosides have been found in hops, including linalyl glycoside (linalool) and geranyl glycoside (geraniol).

Studies which have examined the effect of aging in beer have implicated glycosides as the main avenue for positive aroma generation post-bottling when there is no yeast activity present. One such investigation found that in a commercial Belgian dark beer, β-damascenone levels rose from 8 ng/L in a fresh sample to 210 ng/L in an aged sample *(46)*. A fresh sample of the same beer rose from 8 ng/L up to 79 ng/L when an exogenous β-glucosidase enzyme was added to liberate glycosidically bound β-damascenone *(46)*.

Because these glycoconjugations are β-glycosides, they are resistant to hydrolysis by α-glycocidases (such as malt or yeast amylase). While some *Saccharomyces cerevisiae* strains show limited hydrolysis potential, and a few even showed evidence of true 1,4-β-glucosidase activity, the vast majority of *S. cerevisiae* strains which have been screened showed no ability to hydrolyze β-glucosides *(47)*. Commercial preparations of 1,4-β-glucosidase are available, usually purified from a fungus such as *Aspergillus niger*. Many bacteria and non-*saccharomyces* yeasts show 1,4-β-glucosidase activity, including some commonly used *Brettanomyces* strains.

Given that *S. cerevisiae* strains show limited 1,4-β-glucosidase activity but glycoside hydrolysis is known to occur in beer, it is suspected that acid hydrolysis may be occurring. Acid hydrolysis of glycosides has been shown to occur starting around pH 4.4 *(43)*, and reaction speed increases as the pH drops. Beer pH is typically between 4-4.2, so acid hydrolysis could occur, albeit slowly *(48)*. The kinetics of acid hydrolysis favor some glycoconjugations over others; tertiary alcohols hydrolyze more easily than primary alcohols, for instance *(49)*. In light of this, one could expect acid hydrolysis to change the aroma profile of a beer as some terpenoid concentrations increase more rapidly than others. Given that these terpenoid

compounds (and others, such as β-citronellol) have extremely low odor thresholds and also exhibit an additive or synergistic effect when present together *(17)*, only a minimal amount of hydrolysis would need to occur to potentially have a large impact on aroma.

### 1.3.6 Biotransformed Hop Compounds

Dry hopping is often performed prior to filtration or centrifugation and sometimes while active fermentation is still occurring. When this is the case, it is reasonable to assume that there is viable yeast present which are capable of metabolizing various hop-derived components. Biotransformation of hop compounds can have a dramatic effect on dry hop flavor *(17,50)*. Generally speaking, only terpenoids were shown to undergo biotransformation; there exists no published evidence of the transformation myrcene, humulene, or caryophyllene. While these hydrocarbon terpenes do not undergo biotransformation, they are affected by yeast in much the same way hop acids are; hydrophobic yeast cell membranes can act as a fining agent and remove them from solution.

Work by Takoi et al showed that geraniol is transformed by yeast into β-citronellol rapidly during the initial 2-4 days of primary fermentation *(17)*. This transformation did not accompany a 1:1 decrease in geraniol, and it is believed that the hydrolysis of geranyl glycoside is likely responsible for supplementing geraniol concentrations. Other work showed the transformation of geraniol to β-citronellol is also accompanied by the production of geranyl acetate and citronellyl acetate *(51)*. King and Dickinson proposed a scheme which showed biotransformation of geraniol and nerol by *S. cerevisiae* with 4 possible outcomes: citronellol, linalool, α-terpineol, and terpin hydrate *(52)*.

The study of the biotransformation of hop compounds is relatively new, and it is likely that the next several years will bring about a rapid increase in knowledge in

this area. It is already apparent, however, that interactions among yeast and hop compounds during dry hopping can have a profound influence. If a brewer is seeking to replicate a hop aroma "as-is", it would be beneficial to dry hop after removing the yeast biomass from the system. That being said, many of the products of biotransformation are considered positive contributors to beer aroma, and they may be desired in the finished product, especially when making a bottle conditioned beer.

## 1.4 Dry Hopping Practices

### 1.4.1 Hop Materials used in Dry Hopping

Dry hopping can be achieved with a number of hop products. The simplest and still often used material is dried whole cone hops. Typically whole cone hops are put into a polymer mesh bag prior to adding them to beer in order to make their removal easier. In the UK, whole cone hops are usually compressed into cylindrical cakes called "plugs" (sometimes referred to as type-100 pellets) which are about an inch in diameter. These plugs can be added directly to casks.

Pelleted hops are widely used to dry hop in the US. The majority of pellet hops are type-90. A small number of breweries have also experimented with using type-45 pellets. Even though the majority of the research done on the pelleting process has been focused on retention of α-acids, the pelleting process is known to have some impact on the overall hop aroma via both oxidation and the evaporative loss of terpenes. While some oxidation is unavoidable and may even be desirable *(20,22,53)*, pelleting above 50°C (125°F) is associated with excessive essential oil loss *(53)*.

Research on the use of hop oil extracts instead of hops to impart dry hop flavor began in the early 1980's *(54,55)*, primarily in the United Kingdom. Hop oil products consisting of essential oils extracted from hop material with liquid or supercritical $CO_2$ have been commercially available for about 25 years. These

products can consist of the entire essential oil content of a hop cultivar or specific fractions thereof. If a hop oil product is intended to reflect or replace late kettle hopping, the hydrocarbon fraction will either be reduced or entirely eliminated in order to reproduce what would occur during the normal brewing process. A hop oil product designed to replace dry hopping would retain the hydrocarbon fraction *(56)*. These products are generally not used by American craft brewers but they are used increasingly by brewers in the United Kingdom *(54,56)*. On its own, hop oil is not very soluble and needs to be mixed with a solvent (typically ethanol) or made into an emulsion prior to dosing into beer. When used by British brewers to substitute for dry hops, these extracts were typically injected inline as beer was transferred from a fermenter to a cold conditioning vessel or en route to packaging *(54)*.

### 1.4.2 Techniques

When reviewing the literature for studies which incorporated a dry hopping treatment, the methods used in contemporary research are quite straightforward: add hop pellets to the bright tank or lagering tank for a period of a week to three weeks *(6,21)*. This treatment seems to be the most common picture when people think of dry hopping but it is likely not the case in contemporary, working breweries. Because there is very little published literature covering techniques outside of research and pilot breweries, we decided that asking the brewers directly would be the most accurate way to ascertain how dry hopping is currently being performed. Nine breweries (8 from the United States, 1 from the United Kingdom) agreed to share their procedures with us. These survey questions can be seen in Appendix C.

What we learned from our survey of was the lack of a common approach to dry hopping execution among different breweries. No two breweries dry hopped their beers in exactly the same fashion, and there is even some lack of agreement as to what exactly dry hopping is. Most of the confusion arises from the blurry line

between late hopping and dry hopping. For the purposes of this review, we define dry hopping as any hop additions that occur on the cold side of brewing. Late hopping occurs prior to heat exchange and is either done very late in the kettle boil (just before kettle knock out) or on the way to the heat exchanger via a hop back (or Grant). Under that definition, hopping done in-line or in a hop back after heat exchange could be called dry hopping, even though many brewers would include this in the category of late hopping. We find this distinction important because the chemistry of extraction is heavily influenced by the temperature of the wort or beer, especially as it relates to the potential for added bitterness. To add further distinction, any dry hopping performed when there is significant amounts of yeast present will result in biotransformation and an aroma profile that is markedly different than dry hopping without yeast.

Having provided that distinction; there are many techniques being used by breweries around the United States and the United Kingdom. The most common is a hop addition added to a cylindroconical vessel (CCV) which is either filled already (thus free of $O_2$) or about to be purged with $CO_2$ and filled. If adding hops to a filled vessel, yeast which has settled into the cone of the CCV is typically dumped prior to the hop addition. An important factor for many brewers when deciding which method to use is how to deal with dissolved oxygen (DO) which is inevitably introduced whenever hops are added. Because of their greater surface area resulting from the multitude of crevices inherent to their anatomy, DO is a larger issue with whole cones than intact pellets, but both hop materials would introduce some amount of oxygen to the beer. One approach is to add the hops to an empty CCV and purge the tank with $CO_2$. A similar method involves using an airlocked port on a CCV which can be independently purged. Other brewers approach this problem by dry

hopping with yeast present, assuming that most if not all of the oxygen will be metabolized by the yeast before it can significantly oxidize their beer.

Table 2 shows a summary of dry hopping methods from our interviews with brewers. It is important to note that all of the breweries that consented to be interviewed would be considered Regional Breweries (15,000-6,000,000 barrels/year) as defined by the Brewers Association (Boulder, CO).

Table 4. Dry Hopping Techniques Currently in Use

| Vessel | Length | Yeast Present[a] | Hop Product |
|---|---|---|---|
| Cylindroconical | 3-7 days | No | Pellets or Cones |
| Cylindroconical | 18-21 days | Yes | Cones |
| Bright Beer Tank | 4-7 days | No | Pellets |
| Ancillary Vessel[b] | 1-3 days | No | Cones |
| In-line[c] | Minutes | No | Hop Oil Emulsion |
| Keg | 3-? days[d] | No | Bagged Cones |
| Cask | 3-? days[d] | Yes | Cones |

[a]If yes, dry hopping is performed either during primary fermentation or in a cask with unfiltered beer. If no, some small amount yeast is still assumed present in all cases except post filtration.

[b]This indicates an adjoining vessel connected to filled CCV via hose or piping. Continuous pumping moves beer between the vessels.

[c]This method is performed en route to filtration.

[d]Dry hopping continues until the beer is completely consumed.

Regardless of whether pellets or whole hops are used, brewers often used a method to stir or "rouse" the hops, believing it would lead to increased aroma extraction. A common technique is the injection of $CO_2$ from the bottom port of the CCV cone to lift any hop material that had settled to the bottom. However, if the $CO_2$ is allowed to then escape the vessel, this technique may have the unintended effect of "scrubbing" some of the more hydrophobic aroma compounds from the beer resulting in a loss of aroma. Some brewers are experimenting with pumping beer through an external vessel which contains trapped hop material. This technique has the benefit of eliminating the potential $CO_2$ scrubbing effect while minimizing oxygen introduction. Dry hopping in this manner can present a process problem especially if pellets are used; vegetative hop particles are entrained throughout the entire system resulting in a suspension that is very difficult to clarify using sedimentation or filtration. However, this problem is easily overcome using a centrifuge.

It is interesting to note that hops (especially whole cone) used for dry hopping are not devoid of brewing value after their oil has been extracted during the dry hopping process. They retain most of their starting α-acid content and still have bittering potential. None of the brewers we surveyed reused hops following dry hopping.

There is tremendous variation in how brewers choose to evaluate the effect dry hopping has on their beers. Roughly half of the survey respondents reported that sensory evaluation of dry hop aroma is included as part of their brewery's normal sensory QA. Less than 20% of the respondents had performed laboratory analysis of beer flavor (via gas chromatography) as it related to flavor alterations brought about by dry hopping. When performing sensory analysis, it was common for brewers to have a ranked category simply called "dry-hop" or "dry-hop character". This contrasts with sensory analyses in published literature which consistently separates dry hop

character into its constituent aroma categories. When brewers performed in-depth sensory work on their dry hopped beers (as opposed to regular QA), it was reported that the main goal was to figure out how long the beers would retain their dry hop aroma post-packaging. In the opinion of the brewers who had performed such work, dry hop aroma declines rapidly within three weeks of bottling even in relatively good storage conditions. This agrees with work performed by Peacock et al., who found that after 18 days of storage 80% of the hydrocarbon terpenes had disappeared from a bottled model beer *(57)*.

We asked brewers whether they felt dry hopping had a positive or negative effect on the shelf life of their beers. The responses were evenly split. Some brewers thought that dry hop aroma helped cover the flavor effects of oxidative spoilage, which agrees with published literature *(21)*. Other brewers thought that despite their efforts at controlling the influx of DO, dry hopping had a deleterious effect on the shelf life of their beer.

We also asked the brewers about the impact of dry hop additions on their brewery from a business perspective. About half of the brewers reported they charged slightly more for their dry hopped beers to assist with materials cost. Dry hop additions were reported in the range of ½ lb/barrel (227g/117L) to 3 lbs/barrel (1360g/117L). There was consensus that although dry hopping tended to add little in the way of direct cost associated with the hops themselves, dry hopped beers tended to be higher gravity and therefore carry a higher materials cost from the malt bill.

We asked the brewers to report whether supply chain shortages or costs had ever affected their dry hopping regimes and again, the responses were evenly split between “yes” and “no”. Interestingly, all of the brewers who answered to the negative added the caveat “not yet”, as though the issue had loomed before. The brewers who had dealt with supply chain shortage indicated that instead of limiting

production of the beers produced using a dry hopping regime, they switched hop varieties and continued production. There were comments that this was a source of frustration, as reproducing an expected flavor with a new hop variety was a very difficult task.

Lastly, we asked brewers why they dry hopped their beers. On this subject there was a great deal more agreement among the brewers. To paraphrase the common answer, they responded, “We dry hop to add hop oil character to the beer that is otherwise impossible to achieve with kettle hop additions.” Specifically, brewers said that dry hopping introduces a “bright citrus and floral aroma” that contrasts with late hopping aroma (which was described as “fruity”).

## 1.5 Assessing Dry Hop Aroma

### 1.5.1 Instrumental Analysis

Keeping relative solubilities in mind, the aroma imparted by dry hopping should somewhat reflect the essential oil composition of the hop or hops used. Dry hopped beers contain unmodified essential oil directly from hops added during the dry hopping extraction. They also contain the thermal degradation products of the essential oil that survived the boiling process as well as yeast-transformed hop compounds *(17)*. Most instrumental analysis focuses on quantifying terpenes in beer that are also present in unaltered hop essential oil.

There are several techniques available to assess dry hop aroma. Since all of the compounds of interest are volatile, gas chromatography (GC) is the preferred instrument for compound identification and quantification. Typically the GC will be coupled to a flame ionization detector (FID) for quantification and a mass spectrometer (MS) for identification. If analyzing non-volatile contributions from dry

hopping, high performance liquid chromatography (HPLC) is the commonly used instrument.

If analyzing hops directly, steam distillation or $CO_2$ extraction are the most common means of extracting the essential oil from raw hops. Subsequently, the hop oil can be directly injected into a GC for quantification. If analyzing beer, a method must be employed to extract the aroma compounds from the beer matrix prior to GC analysis. Three methods have been employed to extract aroma compounds from beer: liquid-liquid extraction, solid-phase micro-extraction (SPME), and stir bar sorptive extraction (SBSE). Liquid-liquid extraction is gradually falling into disuse both because it requires a large volume of solvents and is more variable and labor intensive than the other two methods. SPME is a flexible methodology that requires relatively little sample preparation. SPME is generally used to sample the headspace of a sample, and a silica fiber coated with various sorptive materials adsorbs volatile aroma compounds for GC analysis. Changing the sorptive material on the SPME fiber allows fine-tuning of volatile/fiber interaction and enables analysis of a wide variety of volatile compounds *(21,58,59)*. SBSE is a relatively new method that is in most ways similar to SPME; the sorptive materials are identical but are attached to a submerged stir bar instead of a retractable fiber. Like SPME, SBSE is widely used to evaluate terpene aroma in wines and foods, and has been used successfully to examine beer aroma *(32)*. Both methods produce accurate and consistent results if the methodology is optimized for the aroma fraction of interest. For instance, SPME of dry hop aroma consisting mainly of terpenes benefits greatly from an addition of a salt (typically NaCl or $K_2CO_3$) to enhance the volatility of those compounds *(59)*.

Instrumental analysis of dry hop aroma using the above methods will provide precise quantification of volatiles. This allows aroma profiling which is both time- and dose-dependent. It also allows observation of changes in the aroma profile which

may be related to yeast metabolism or oxidation – in a closed system the disappearance of a given compound should correlate to an increase in another, although the resulting metabolites or degradation products may not be observable using the same assay.

### 1.5.2 Sensory Analysis

Sensory analysis of beer is typically performed at all breweries regardless of whether it is accompanied by instrumental analysis. In the case of hop aroma, we saw that it is common industry practice to lump dry hop character into one category. Depending on the hop cultivar used, it can be useful to add additional categories such as citrus, pine, or fruity. Sensory analysis as it occurs in industry is used for one of two things: recipe development or product consistency (the latter being predominant).

In the case of assessing dry hop aroma for consistency, the ASBC has published several applicable tests. The triangle test and the duo-trio test can be used to determine if there is a significant difference between two samples *(60)*. When assessing dry hop aroma as a part of recipe development or an aging study, descriptive analysis and ranking tests can be used to evaluate multiple beers or time points *(60)*.

## 1.6 Stability of Dry Hop Aroma

The aging of beer and its hop derived aroma is a very important consideration. Brewers design their recipes around the flavor they experience when tasting fresh beers and it is usually assumed that the consumer will have a similar experience. Unfortunately, beer often arrives in the consumer's hands at a much later date than desired by the brewing industry, and it is not always refrigerated properly in transit

or during storage. It is useful, then, to understand how aging (combined time and temperature) can impact the aroma of beer.

In aging studies conducted on dry-hopped beers, the levels of monoterpenoids such as linalool and geraniol have either been relatively stable or even slightly increased over time *(21,46,57)*. Terpenes and sesquiterpenes such as myrcene and humulene, however, have shown a gradual decline *(21,57)*. In sensory studies, the loss of sesquiterpenes have been associated with the loss of the spiciness or "noble" character of a dry hopped beer, exposing more floral or ester character *(57)*. However, and of great interest to the brewer, the stable terpenoid fraction has been shown to completely mask the flavor and aroma of staling aldehydes *(21)*, acting as an aroma preservative from a sensory perspective. This indicates that if a brewery's dry hopping process does not otherwise introduce staling components to its beer (namely oxygen), dry hopping could have a positive effect on shelf life.

In addition to simply hiding the aroma of staling aldehydes, non-volatile compounds extracted during dry hopping can increase the reducing power of beer, capturing reactive oxygen species (ROS) and slowing down oxidation cascades such as lipid oxidation and the Fenton reaction *(61,62)*. The presence of metal ions (namely iron and copper in beer systems) is often implicated in the generation of ROS and hop-derived polyphenols can slow this process down both by chelating the metal or capturing electrons from the ROS before it otherwise causes damage *(62)*. In addition to polyphenols, hop acids (both α and β) are excellent antioxidants and free radical scavengers *(61)*. Hops added during the kettle boil would provide very little α and β acids to finished beer, but dry hopping may extract enough hop acids to have a considerable effect. While the aroma characteristics would still change over time, increased reducing power guards against rapid oxidation and classical staling, especially in situations where beer is allowed to reach warmer temperatures.

### 1.6.1 Packaging and its potential ability to scalp dry-hop flavor

The hydrophobic nature of dry hop aroma compounds makes them vulnerable to adsorption and absorption by hydrophobic polymers such as polyethylene, polyvinylchloride and polyester. This phenomenon, called scalping in the food packaging industry, occurs when polymers with similar chemical properties to volatile aromas are used to packaging food and/or beverages. The most common occurrence of this in beer packaging is with the polymeric cap liners for the metal crowns of glass bottles, which have been shown to scalp aroma compounds *(57,63)*. Research into food contact polymer formulation and aroma scalping has historically focused on the juice industry, especially the sorption of limonene by orange juice containers.

The extent to which this occurs depends both on the particular type of polymer and the type and concentration of aromatic chemicals present. Peacock and Dienzer's work showed that extensive scalping occurs when using a crown liner formed from polyvinylchloride (PVC) *(57)*. Hydrocarbon terpenes such as myrcene and humulene were found to have completely migrated into the crown liner when retail beers were examined. Terpenoids such as linalool and citral have also been shown to migrate into lining material, but at a much slower rate than hydrocarbons *(57,63,64)*.

The rate at which migration occurs depends on several factors: the concentration of the compound, the boiling point of the compound, the concentration of similar compounds (termed copermeants), the polarity and/or hydrophobicity of the polymer, and the structure of the polymer (crystalline or amorphous, depending on its glass transition temperature) *(63)*. If the formulation of the liner is such that it is in a glassy state during storage, the penetration of volatiles is significantly reduced *(63)*.

When aromatic compounds dissolve in the liner material, they act as a plasticizer thereby increasing the permeability of the entire structure to a broad

array of compounds including oxygen *(64)*. This has been shown to be especially true for LDPE and it is assumed that it occurs with other polymers. This could result in a "snowballing" effect when beer is aged, both reducing hop aroma in the beer while reducing the oxygen barrier properties of the package and in turn increasing the oxidative damage to beer flavor. This makes the case for using a liner material that resists aroma scalping even more compelling.

The exact formulation of most crown liners is a carefully guarded secret. Liners which were developed to scavenge oxygen from the headspace are still being improved upon and reformulated on a regular basis. Historically, liners for crowns and aluminum cans have variously contained PVC, polyethylene, polypropylene, low density polyethylene, and polyethylene terephthalate , all of which are capable of significant aroma scalping *(63)*. New and promising materials are currently being examined, and ethylene vinyl alcohol is one of the new copolymers shown to reduce scalping *(63)*. Proprietary formulations also include using high barrier nylon resin plus polymers having unsaturated bonds which have the capacity to scavenge oxygen thereby producing a polymeric system that can scavenging oxygen while at the same time reducing flavor scalping.

## 1.7 Conclusion

Dry hopping adds aroma to beer, primarily via the addition of hop oil containing terpenes and terpenoids. Some terpenes, especially the sequiterpene humulene, can undergo oxidation and hydrolysis reactions which result in a wide variety of aroma compounds not originally present in hop lupulin. Aroma contribution from thiol compounds can also be very important to the overall aroma of some hop varietals.

Dry hopping results in the extraction of more than just hop oil. Hops contain a large amount of glycosidically bound terpenes which are very soluble, and in some beer systems these may hydrolyze and contribute to hop aroma. More work is needed to characterize the hop aroma contribution from glycosides. Hop acids and polyphenols would also be extracted during dry hopping and they may affect both the flavor and shelf life of the beer.

A survey of brewers found that there is a great deal of variety in dry hopping techniques. While widely available, American regional breweries eschew the use of advanced hop products such as $CO_2$ extracts and continue to use whole cone and pellet hops to dry hop their beers.

# The Effect of Pellet Processing and Exposure Time on Dry Hop Aroma Extraction

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# Chapter 2 - The Effect of Pellet Processing and Exposure Time on Dry Hop Aroma Extraction

## 2.1 Introduction

During the beer manufacturing process hops are traditionally added prior to fermentation during a vigorous boil. Because of volatilization during boiling, thermal degradation, and biological transformation via yeast *(17)*, hop aromas present in finished beer that has been traditionally hopped often do not resemble the aroma profile of the original whole hop cone. These transformations do not occur appreciably during dry hopping as it is performed in most American craft breweries because the yeast is either dormant due to low temperatures or absent due to centrifugation. The thermodynamics of dry hopping are very different from traditional hopping in that dry hopping is usually carried out at 1 to 6°C and there is often little or no agitation of the beer. Thus there is little stripping effect and the oils coming from the hops are retained to a large degree in the finished beer. Because of its volatility, the hydrocarbon fraction of hop essential oil is not typically found in beer that has been hopped using traditional techniques of adding hops to the boil, yet it can be found in appreciable amounts in finished beer when it has been dry hopped.

Dry hopping results in beers with intense hoppy aroma profiles. Traditional hopping followed by dry hopping produces beers that contain both the thermal degradation products of the essential oil that survived the boiling process and yeast-transformed hop compounds as well as the unaltered essential oils coming directly from hops added during the dry hopping process.

The hops used by American brewers for dry hopping generally fall into two categories: whole hops or pelletized hops. The former category refers to whole, intact hop cones that have been dried and baled without any further processing. The latter category involves taking whole cones, milling them in a hammer mill to

produce a pulverized/powdered hop grist and then extruding the powder through a pelleting die to produce a compact pellet. This results in a hop product that has a much higher bulk density than the former whole cone and a powderized grist that disperses easily upon addition to hot wort. Dispersability in cold, unagitated beer can be affected by the pellet properties, particularly the pellet density. Most of the previous work published on the effect of the pelletizing process on hops has focused on the conservation of α-acids *(53,65)*. With a commercial interest in dry hopping, retention of hop aroma compounds during processing is gaining interest by brewers and hop processors.

Pellet density is partially a function of the die size and speed of extrusion during the pelleting process, which also correlates to heat produced during pellet formation *(53,65)*. All else being equal, less dense pellets should experience less heat during formation, which could result in conserved essential oils and fewer oxidation products. It is recognized as good manufacturing practice to maintain the pelleting temperature between 38°C (100°F) and 50°C (125°F). Operating in this range ensures that the lupulin glands remain liquid but inordinate losses of α-acids and essential oils do not occur *(53)*. In other manufacturing processes employing a pelleting process (such as pharmaceuticals), the density of the pellet affects its speed of dissolution. It can then be assumed that hop pellet density affects the speed at which the pellet hydrates and disintegrates in a liquid medium.

The studies presented herein examine how hop oil extraction during dry hopping can be affected by physical properties of the hop material. The first part of this investigation was designed to test the impact of the pellet characteristics on aroma compound extraction rate. Particle size distribution and pellet density were identified as the dominant characteristics that could impact the rate of extraction. Particle size distribution of the hop material varies greatly among pellet

manufacturers and is largely determined by the milling process. Smaller particles present more surface area per unit volume of hops potentially resulting in a greater degree of solvent interaction.

The second part of this study was designed to examine the extraction rate of aroma compounds during the initial 24 hour period of dry hopping. Most commercial dry hopping regimes last anywhere from 3 days to 1 week with some brewers dry hopping for up to one month, but it was unknown whether that timeline represents the optimal extraction time for hop aroma compounds or whether it is simply a brewing tradition.

## 2.2 Materials and Methods

### 2.2.1 Dry Hop Materials

The week-long extraction study utilized pelletized Cascade hops harvested in 2009 and whole hops harvested in 2010. Three separate lots of pelletized hops each from four different manufactures were obtained and stored at -23 °C until used. The short term extractions utilized Cascade whole hops and pellets harvested in 2010 from the same hop farm.

Dry hopping was carried out in a model beer system consisting of acidified, filtered water (94%) and ethanol (6%). The solution was buffered at pH 4.2 with sodium citrate/citric acid (0.0116 M). The water was degassed by boiling and then cooled prior to blending with ethanol and acid. The solution was dispersed in 18 L aliquots into modified Cornelius kegs and cooled to 1 °C prior to dry hop dosing.

The short term aroma extraction study was conducted using smaller scale bench top equipment. Each sample was extracted in a 0.5 liter sealed, brown glass bottle that had been flushed with nitrogen. The extractions were performed using both the model beer solvent and unhopped beer brewed specifically for this study.

The unhopped beer was brewed using 98% pale 2-row malt and 2% acidulated malt. Alpha acids (from $CO_2$ extract) were added at the beginning of a 60 minute boil at a concentration of 12 ppm. Original gravity was 1.0442 (11° Plato) and final apparent gravity was 1.0047 (1.03 ° Plato) after fermentation with an ale yeast at 18 °C.

Standard curves of hop aroma compounds were prepared using analytical grade chemicals (Sigma-Aldrich Corp, St. Louis, MO). Direct oil injections were dissolved in hexane, which was redistilled prior to use.

### 2.2.2 Dry Hop Method

The week-long dry hopping experiments were carried out by adding 23.2 grams (1/3$^{rd}$ pound/barrel or 127 g/hL) of hop pellets to a chilled model solution in a sealed stainless steel keg that was flushed with $CO_2$. An equal mass of whole hops was placed into a mesh bag and kept submerged about 6 cm from the bottom via an inert stainless steel weight. Following the addition of the hops, the keg's headspace was flushed with $CO_2$ three times to ensure little to no oxygen remained, and the headspace pressure was reduced to ambient pressure. There was no agitation of the systems during the dry hopping trial. Samples (20 mL) were removed via a shortened dip tube after 1 day, 4 days, and 7 days. The shortened dip tube reached to the middle of the keg and allowed a drawn sample that contained no visible vegetative hop matter. Each of the 16 hop treatments was used once during this study. Thus, the replication of the hop treatment was dealt with by using 3 independent Cascade hop samples from each of the 4 suppliers, plus one single, whole hop sample.

For the short term extractions, dry hopping was also performed at a dose of 1/3$^{rd}$ lb. per barrel (127g/hL). The extractions were performed at room temperature (20°C). After hop dosing, the headspace of each bottle was flushed with nitrogen to limit oxidation and then sealed. The bottles were agitated using a shaker table so that diffusion from the hop particles to the medium would be maximized.

Extractions were sampled at 30 minutes after dosing and at various intervals over 24 hours. After sampling, the extraction bottle's contents were discarded, thus each sampling point can be considered an individual treatment.

### 2.2.3 Pellet Characteristics

Pellet density was measured using a bench top micrometer (Mitutoyo Corp, Model: SDV-6"A,) and an analytical balance (Sartorius, Model: R16OD, Goettingen, Germany). Each measurement included 10 randomly chosen pellets. Hop pellets were treated as a cylinder for purposes of calculating volume. Where needed, the ends of the pellets were straightened with a razor to create uniform cylinders.

Particle size was measured using a five sieve system utilizing U.S. standard sieve sizes: 2.36mm, 1.20mm, 0.59mm, 0.25mm, and 0.15mm (Dual Manufacturing, Chicago, IL). Samples were prepared by first dispersing pelletized hops in 20°C water then drying the particulate matter overnight on a screen. This method was preferable to disintegrating the pellets manually or via crushing under a rolling pin as it prevented any further milling effect from occurring during sample preparation. The dried sample was then placed in the sieve system and shaken via a mechanical shaker for five minutes. Retained portions from each sieve were weighed and recorded. Percent retained (as a percent of total mass) was calculated, as well as an aggregate weighted mean diameter. The weighted mean diameter was calculated as per the ASBC standard method for malt grist analysis *(60)*.

### 2.2.4 Solid Phase Micro-Extraction

Hop oils transferred to beer or model beer solution via dry hopping were measured using a headspace solid phase micro-extraction (SPME) technique. 10 mL of sample was loaded into a 40 mL amber glass vial with a Teflon-lined silicon septum which was placed in a 45 °C circulating water bath. A 2 cm tri-phase fiber, consisting of polydimethylsiloxane, carboxen, and divinylbenzene (PDMS/CB/DVB) with a 50/30

µm coating thickness was inserted in the headspace above the solution in the glass vial and volatiles were allowed to adsorb to the fibers during a 60 minute extraction period. During the extraction, the sample was stirred by a glass-coated magnetic stir bar at 500 RPM. 4-octanol was added as an internal standard during SPME sample preparation at a final concentration of 1 ppm for long-term extractions and 0.5 ppm for short term extractions.

Short term extraction samples were also dosed with 2g NaCl. Because of the nature of the extraction (shaker table agitation), the short term extraction samples included an additional filtration step using a 0.45 micron cellulose syringe filter. A side-by-side comparison was done to ensure that the syringe filter did not remove significant amounts of aroma compounds. Samples were prepared and analyzed within one hour of being drawn.

### 2.2.5 Gas Chromatography

Volatiles adsorbed to the SPME fiber were identified and quanitified using gas chromatography (GC) analysis via a Hewlett Packard 5890 with a flame ionization detector (FID). Detector temperature was 250 °C. The column was a Supelcowax 10, 30m x 0.25mm x 0.5µm (Supelco, Bellfonte, PA). Carrier gas was nitrogen with a flow rate of 1 mL/minute (splitless mode for SPME, 1:50 split ratio for oil direct injections). Desorption of volatiles from the SPME fiber was performed at 250°C for 10 minutes. Oven temperature started at 50°C, and underwent the following temperature ramp: 50°C for 1 minute then at 4°C/min to 90°C, hold for 5 min, 5°C/min until 185°C, hold for 6.5 minutes, 3°C/min until 230°C and hold for 10 minutes. SPME injections and oil direct injections utilized the same temperature program, but all SPME injections were conducted manually whereas direct injections of oil samples were performed

using an auto sampler to minimize injection volume variation. The oil analysis followed the standard ASBC method *(60)*.

Essential oil content of each pellet type was measured via steam distillation, which was carried out according to the ASBC standard method *(60)*. Distilled oil volume was recorded and a portion was retained and stored at 4.5°C for further analysis.

## 2.3 Results

### 2.3.1 Pellet Density

Pellet process treatments had a significant effect on pellet density (Figure 4).

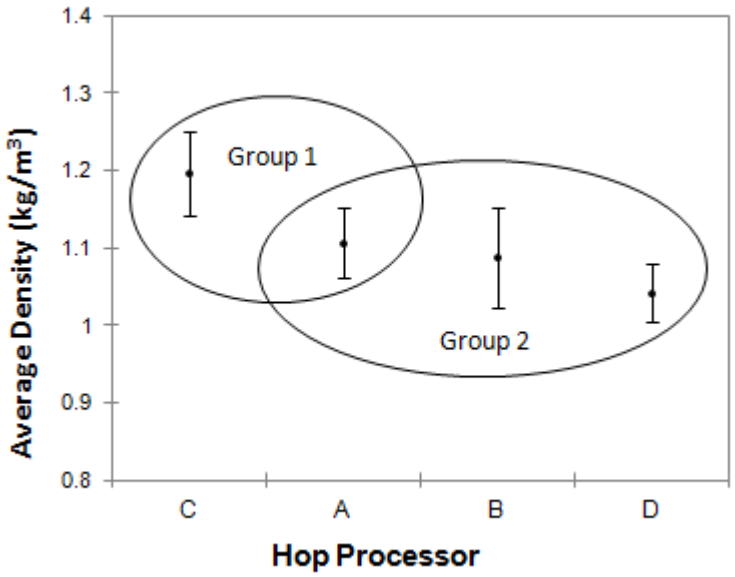


Figure 4. Hop Processor's Pellet Density. N=3, mean values ± one standard deviation. Means within the same group are not significantly different at α = 0.05.

Group 1 (Pellet C and Pellet A) were not significantly different from each other, likewise group 2 (Pellets A, B, C) were not significantly different from one another (Tukey's HSD test, α=0.05).

### 2.3.2 Pellet Particle Size

The hop grist particle size varied significantly from producer to producer. Analysis of variance of the hop pellet particle size data showed that Pellet D's particle size distribution was significantly larger than distributions from Pellet C (P=0.031), Pellet A (P=0.013), and Pellet B (P=0.0025). Pellet C was significantly larger than Pellet B (P=0.0037). Pellet A did not significantly differ from Pellet B or C. The aggregate weighted mean diameters for each pellet type are shown in Table 5.

Table 5. Weighted Mean Diameter of 4 types of hop pellets.

| **Aggregate Weighted Mean Diameter** | |
|---|---|
| **Hop Processor** | **Mean Diameter** |
| Pellet D | 1.72 mm |
| Pellet C | 1.37 mm |
| Pellet A | 1.09 mm |
| Pellet B | 0.95 mm |

There was a lot of unsorted information above the largest bin (2.36mm) that remained unresolved for the two treatments with the largest particle sizes (Pellets D and C), so their aggregate mean particle diameter could potentially be slightly higher.

### 2.3.3 Long Term Dry Hop Aroma Extraction

GC chromatograms were obtained for each sample (3 per treatment, 3 time points). Figure 5 shows the average concentration of linalool at days 1, 4, and 7. Figure 6 shows those same time points for the compounds myrcene. Surprisingly, extraction data did not show an increase in compound concentration over the time periods examined; in all cases the day 7 concentrations were either near the same level as day one (within standard deviation) or had fallen slightly. Final concentrations did not significantly differ between treatments, with the exception of geraniol.

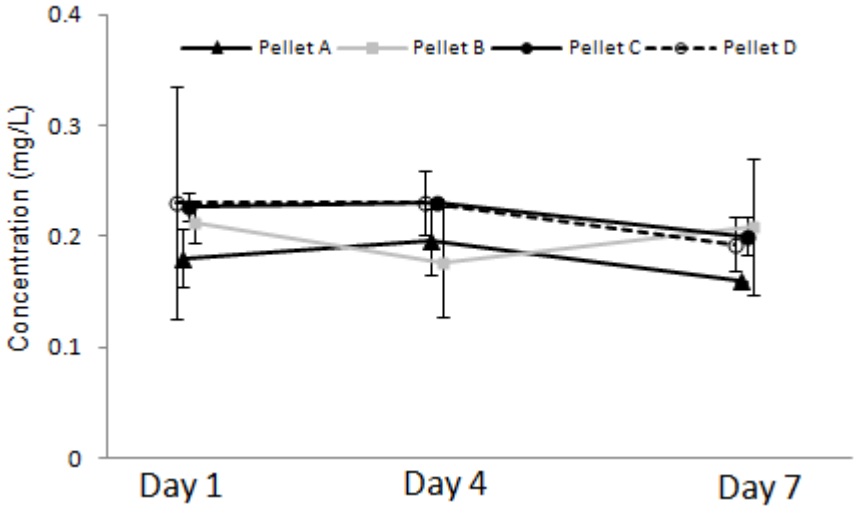


Figure 5. Average linalool concentration at Days 1, 4, and 7.

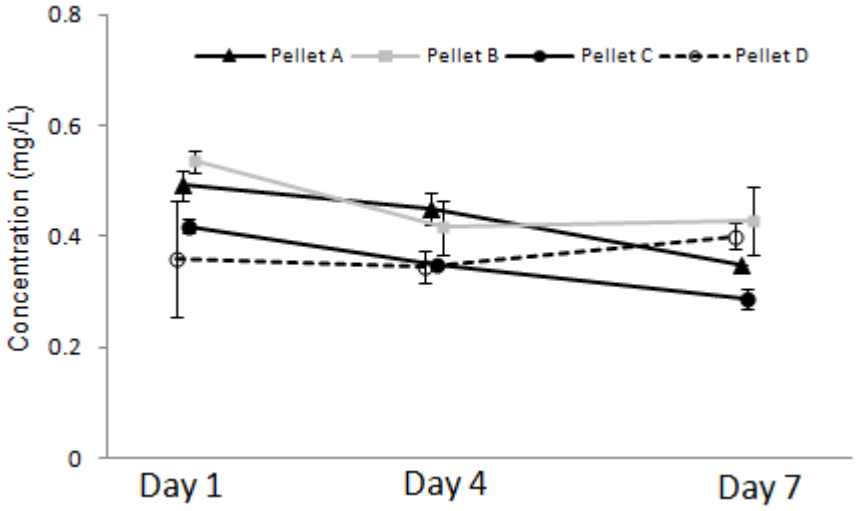


Figure 6. Average myrcene concentration at Days 1, 4, and 7.

## 2.3.4 Short Term Dry Hop Aroma Extraction

The results from GC analysis of the short term, agitated aroma extraction showed that hydrocarbon compounds are fully extracted in as little as 4 hours. The overall trend for hydrocarbon compounds is a rapid increase in concentration followed by a decline during which the rate of decline flattens out. In contrast, the terpene alcohols appear to extract rapidly at first and then either remain static, or increase very slowly over the extraction period. Figures 7-8 show the concentrations for aroma compounds from 30 minutes out to 24 hours. We also examined these short term samples at the end of 24 hours on an HPLC, looking at hop acids (data not shown). We found significant extraction of both alpha acids and oxidized alpha acids. While we did not directly measure them, the HPLC data also suggested significant extraction of polyphenols.

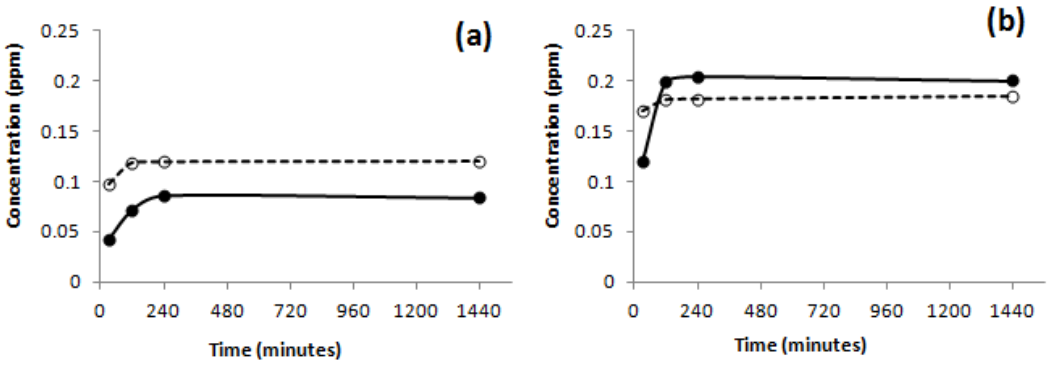


Figure 7. Myrcene (a) and humulene (b) concentrations during a 24 hour dry hop treatment with pellets (--o--) or whole cone hops (—●—).

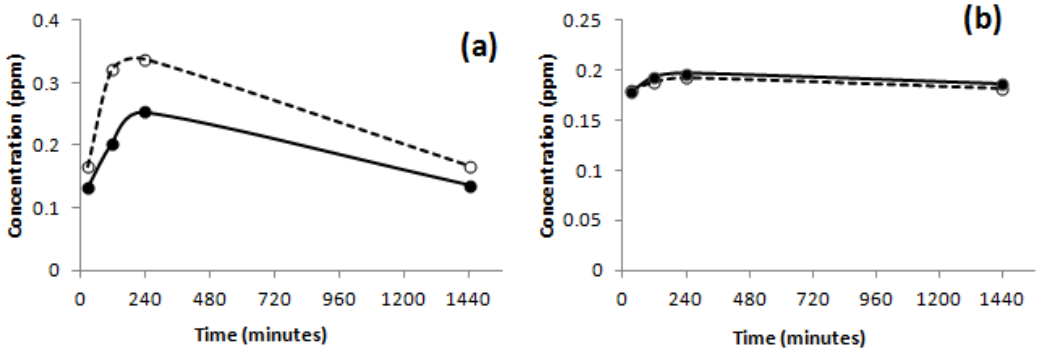


Figure 8. Linalool (a) and geraniol (b) concentrations during a 24 hour dry hop treatment with pellets (--o--) or whole cone hops (—●—).

## 2.4 Discussion

### 2.4.1 Week-Long Extractions

Early, bench-top observations of pellet dispersals revealed that in all cases pellets disintegrated in cold water in less than thirty minutes; on a dry hop timescale of 24 hours to one week the dissolution time would be irrelevant. Thus, the differences in pellet density did not affect disintegration rates. The pattern of dispersal, however, varied greatly among pellet types with some pellets dispersing and then coalescing on the bottom of the vessel and others forming one layer near the surface of the medium and another on the bottom of the tank. This behavior is assumed to be related to pellet density and particle size. While this pattern of dispersal may affect extraction in the short term, no effect was seen during the longer intervals tested in this work.

Each of the four suppliers produced pellets with different densities which were apparent to the eye. The pellet density mirrored the physical inspection of the pellets with the densest pellets possessing a reflective sheen associated with exposure to excessive heat during processing *(53)*.

The pellet particle size data also reflected what a hand inspection revealed; Pellet B pellets were powdery when broken apart, whereas the Pellet D pellets most closely resembled ground whole hops and had recognizable hop cone bracteoles. There was a loose correlation (data not shown) between particle size and the tendency for particles to stay in suspension near or on the surface or settle out on the bottom of the tank, with the smallest particles tending to settle out. While this behavior is interesting and may have some brewing process ramifications during tank cleanout or whirlpooling, no treatment effect was seen on aroma compound extraction rate in the present study. This is likely because extraction occurred outside of the timeframe we observed in the week-long extraction study.

Headspace sampling of hop aroma volatiles via solid phase micro-extraction was selected for this work because of its relative simplicity and reproducibility when dealing with hydrophobic, volatile analytes. It allowed immediate analysis of samples taken directly from the dry hop tanks with no further modification, and has been previously used in similar systems with great success *(21,58)*. While SPME proved to be effective here, other methods of analysis (such as stir bar sorptive extraction) should not be overlooked and could easily be adapted to the same system.

Typical extraction curves in food applications (such as aqueous extraction of tea leaves) have a positive slope indicating an increase in compound concentration over time with an exponential rise to an equilibrium concentration. It was expected that the dry hop extraction data would follow this pattern. The fact that these data instead showed no positive trend with time indicates that the extraction may have been complete by the time the first samples were analyzed.

Analysis of variance showed that there were significant differences in the physical properties among the pellet treatments examined. However, these differences did not significantly affect the extraction rate of the terpene and terpenoid compounds between day one and day seven. These data indicate that the extraction of aroma compounds may occur much faster than the typical commercial dry hopping regime of several days to several weeks; terpenes may reach their solubility threshold in a matter of hours instead of days. These data were the impetus for the short term extraction experiments.

While our study was designed to examine rate of extraction, the final concentrations themselves deserve attention. The final concentrations of linalool, myrcene, and limonene were not grossly different among treatments with one exception. Pellet D showed a treatment effect with respect to geraniol concentrations (data not shown); the final geraniol concentration from Pellet D was

significantly higher ($p<0.001$) than the other treatments. Geraniol contributes a floral and ester note to the aroma of beer *(13)*.

The oils present in the hop pellets was examined first distilling the oils from the pellets in an aqueous boil using standard methods *(60)* followed by chromatographic separation and analysis. The hop oil analysis showed that the pelleting process tended to reduce overall myrcene levels and increase levels of oxidation products. This agrees with a large body of previous work *(53,65,66)*. In particular, Pellet C samples showed high levels of oxidation products (humulene oxide and caryophyllene oxide). Pellet C samples also had the greatest density, and although this study did not attempt to correlate these data, it is possible the more intense pelleting process (as inferred by the highest density) had a direct effect on oxidation levels of the oils in these pellets.

When looking at the oil data across all treatments, there was sufficient variability in the replicates within each processor that there appeared to be little difference among the pellet treatments beyond the oxidation products for the Pellet C samples. The Pellet C samples had greater variation than the other three producers. While the single sample of whole hops had no measure of sample variation, it was highest in myrcene and very low in humulene epoxide and caryophylene oxide (oxidation markers).

### 2.4.2 Short Term Extractions

As expected based on the data from the long term extraction, the extraction of hop aroma compounds occurred much faster than the interval of days or weeks typically used in commercial breweries. These data displayed peak concentrations typically occurring around 300 minutes. Bearing in mind that these extractions occurred at 23°C and were continually stirred, this is still much faster than we initially expected. If the extractions occurred at the more typical temperature of 1-4°C, peak

concentration would take longer to achieve but would still probably occur in less than 3 days. Note that the work by reserachers at the Technical University of Munich in Weihenstephan (discussed below) had hop aroma peak intensity during bench top dry hopping experiments occurring at approximately 3 days during a stirred dry hop extraction at 1°C.

Following their peak concentrations, the terpene alcohols (linalool and geraniol) and hydrocarbons (myrcene, humulene, and limonene) exhibited dichotomous behavior. The terpene hydrocarbons were unstable in both the beer matrix and the model system and declined in concentration (Figure 7). The terpene alcohols were stable and either maintained their peak concentrations in the beer matrix (Figure 8) or continued to slowly increase in the model system (data not shown). Similar results were found by Krottenthaler et al. *(67)*. They observed no change in linalool and geraniol concentration over a 1 week extraction. Their hydrocarbon data was slightly different with a longer time required to rise to maximum at day 3 and then a subsequent decline; this time difference can be explained by a lower extraction temperature (0°C) as compared to that used in the studies presented herein (23°C). While they found a dose-response effect, they were equally surprised to see no change in polar compound concentrations with time.

Regarding the form of the hop material, pellet dosing resulted in a larger concentration of extracted compounds relative to whole cone hops for all samples taken at the initial 30 minute time point. There are a couple of hop pellet characteristics that may account for this. Firstly, the hop material in pellets has a greater overall surface area relative to whole cones because of their smaller milled particles. Secondly, the lupulin glands, which contain hop essential oils, have been crushed and distributed throughout the vegetative matter during the milling and pelleting processes. Both of these factors expose more essential oil for extraction

immediately upon the pellet's dissolution. However, this initial jump in concentration did not always result in a higher concentration after 24 hours of extraction.

# Chapter 3 – Brewery Scale Dry Hopping: Aroma, Hop Acids, and Polyphenols (to be submitted to the Journal of the ASBC)

## 3.1 Introduction

Our previous work showed that traditional dry hopping regimes (one week, unstirred) are likely not optimal in terms of aroma extraction or sensory characteristics. Week-long dry hopping treatments may be much longer than what is needed for full extraction of hop essential oil compounds, and the extra exposure time represents a processing cost that may be superfluous. In addition, after reaching peak concentration some hop essential oil compounds may actually decline during the dry hopping process; our earlier work in small-scale, stirred systems showed that the hydrocarbon terpenes (such as myrcene and humulene) gradually declined in concentration after peaking early on.

This study utilized the data from our previous work to design an experiment that evaluated dry hopping on a scale that better represents production brewing. This work was also designed to use agitation within cylindroconical vessels to determine if mixing impacts the rate and extent of hop aroma extraction. The cold extraction temperature was chosen to keep the beer at its maximal density throughout the experiments and reduce convective currents in the non-stirred treatment during the extraction process.

This work provided insight into designing an optimal dry hopping schedule and process with extraction of essential oil characteristics foremost in mind. It also shed light on the extraction of non-aroma compounds and their potential flavor impact on a dry hopped beer. In particular, HPLC measurement of hop acids and spectrophotometric quantification of polyphenols were used to correlate bitterness and nonvolatile compound concentration to aroma concentration during the dry-hopping process.

## 3.2 Materials and Methods

### 3.2.1 Beer Production

The beer matrix consisted of pale ale brewed specifically for this project. The malt bill was 100% Great Western Malting pale ale malt (2.9 SRM). The wort was dosed in the kettle with α-acid extract at a concentration of 30 ppm, which resulted in a final concentration of 21 ppm iso-α-acid. The wort was pitched with Wyeast 1056 American Ale yeast. Original gravity was 12°P, final gravity was 2.4°P (5.1% ABV), and the pH was 4.22. A total of six separate brews were carried out, fermented separately under identical conditions, and blended together for the dry hop trials. The beers were not force carbonated; however they retained whatever $CO_2$ was produced during fermentation. In order to remove all yeast prior to dry hopping, the beer was filtered bright using a pad filtration system (Pall HS 2000, Kreuznach, Germany).

### 3.2.2 Dry Hopping Protocol

Cascade hop pellets (crop year 2011) and whole hops were used for all experiments in this study. The hops were grown on the Gayle Goschie hop farm in Silverton Oregon, and pelleted by Indie Hops LLC. We worked closely with Indie Hops to ensure that the pellets and whole hops were from the same raw material bale to eliminate any regional or harvest time discrepancies. Pellets and whole cone samples were received in vacuum packed polymer bags and stored at -23°C until use.

The dry hop treatments were conducted in 3.5 hL (3 bbl.) stainless steel CCVs. 340 liters (90 gallons) of beer were used for each treatment. Pellets were dosed directly into two separate 3 bbl CCVs at a dose of 1 lb. per barrel (386 g/hL). Before dosing hops into the CCV's, whole hops were placed into polymer bags along with several stainless steel pipe fittings to keep the bag at the bottom of the CCV during the dry hop extraction. In all cases, the hop pellets or bagged whole hops were submerged in a small amount of brew house water to purge oxygen from the void spaces in the hop material. The hops were added to the CCV via a large, sealable entry port at the top of the vessel. Following the hop addition the CCV headspace was flushed thoroughly with $CO_2$. One of the CCVs was attached to an external centrifugal pump (Figure 9). The pump inlet pulled from a port 60 cm from the base of the CCV (the entire CCV was 165 cm high) and the discharge was plumbed to the lowermost port at the bottom of the cone. The pump ran at a constant 1000 rpm, which equated to a flow rate of 45.7 liters per minute (12 gallons per minute) or an entire tank volume in roughly 8 minutes. This forced convection created a constant but gentle stirring effect. A sampling apparatus was designed from which samples could be pulled without opening the entire vessel and allowing oxygen ingress. The apparatus sampled vertically via a stainless steel tube approximately 1/3$^{rd}$ of the way down from the top of the liquid level (55 cm from the top).

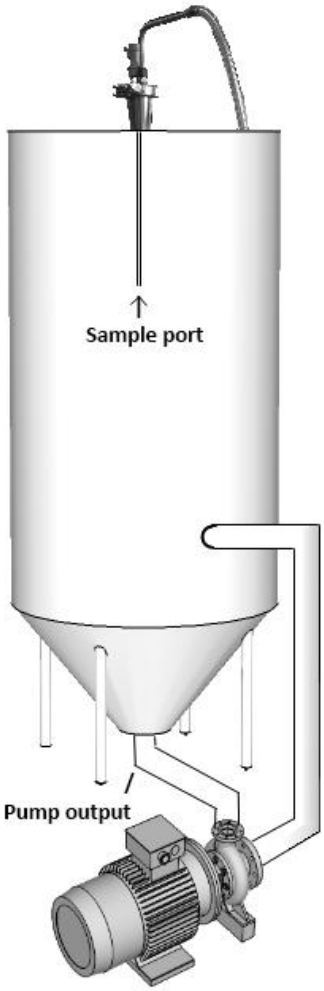


Figure 9. CCV and pump

### 3.2.3 Sampling Protocol

Treatments were sampled at 0.5 hours, 2 hours, 4 hours, 6 hours, 24 hours, 4 days, 7 days, and 12 days. The samples were filtered through 10 layers of cheesecloth to remove visible hop particles. Samples for instrumental analysis were then centrifuged and prepared immediately, while samples for sensory analysis were placed into 330 mL brown glass bottles, flushed with nitrogen, capped with $O_2$ scavenging lids, and promptly frozen at -23°C.

### 3.2.4 Sensory Evaluation

The sensory panel consisted of 11 individuals, 9 male and 2 female. Of those 11 individuals, 6 had extensive previous experience and training. The panel was trained for 3 sessions prior to evaluating samples. The training introduced the panelists to the aroma types and intensities expected during testing, and it also included the use of external standards to identify and scale aroma, bitterness and astringency. Whole cone treatments were not included in the sensory panel; the panel examined beers dosed with pellets (stirred and unstirred) and sampled at 6 hours, 24 hours, 4 days, and 12 days for a total of 8 samples per session.

The finalized ballots contained the following categories: overall aroma intensity, herbal/tea-like, citrus/fruity, pine/resin, bitterness intensity, bitterness duration, and astringency. The herbal/tea-like category included a component we identified as having a “powdered instant ice tea” aroma. Testing consisted of 4 sessions during which every sample was presented once to each panelist in a randomized order unique to that panelist. Panelists were asked to scale each characteristic using a 0-15 point scale. Between samples, panelists rinsed their mouth sequentially with a carbonated, pectin rinse solution followed by filtered spring water. The pectin rinse consisted of 0.1% pectin (93.4% polygalacturonic acid, of

which 9.4% was methoxylated) in deionized water. The rinse was carbonated at 30 psi and 2°C until saturated.

### 3.2.5 Instrumental Evaluation

Hop aroma extraction was quantified using headspace solid phase micro-extraction (SPME) and GC-FID analysis, while non-volatile extraction was measured with HPLC and spectrophotometry.

SPME was performed using a 10 mL sample that was filtered using a 0.45 micron cellulose syringe filter. An internal standard, 4-octanol, was added to the beer sample during SPME sample preparation at a final concentration of 0.5 ppm. Salt, 2g NaCl (Sigma-Aldrich Corp, St. Louis MO), was added to each sample to enhance aroma compound volatility *(59)*. The sample was loaded into a 40 mL amber glass vial with a Teflon-lined silicon septum and placed in a 30°C water bath. A 2 cm tri-phase fiber (Supelco, Bellefonte, PA), consisting of polydimethylsiloxane, carboxen, and divinylbenzene (PDMS/CB/DVB) with a 50/30 µm coating thickness was inserted in the headspace above the solution in the glass vial and volatiles were allowed to adsorb to the fiber during a 60 minute extraction period. During the extraction, the sample was stirred by a glass-coated magnetic stir bar at 500 RPM. The SPME procedure was carried out on each sample immediately after being drawn from the CCV.

Aroma compounds adsorbed to the SPME fiber were identified and quantified using a Hewlett Packard 5890 gas chromatograph with a flame ionization detector (FID). Detector temperature was 250 °C. The column was a Supelcowax 10, 30m x 0.25mm x 0.5µm (Supelco, Bellfonte, PA). Carrier gas was nitrogen with a flow rate of 1 mL/minute (splitless mode). Desorption of volatiles from the SPME fiber was performed at 250°C for 10 minutes. Oven temperature started at 50°C, and underwent the following temperature ramp: 50°C for 1 minute then at 4°C/min to

90°C, hold for 5 min, 5°C/min until 185°C, hold for 6.5 minutes, 3°C/min until 230°C and hold for 10 minutes. SPME injections were conducted manually. External standard curves were prepared using analytical grade compounds obtained from Sigma Aldrich (Sigma-Aldrich Corp, St. Louis, MO).

Hop oil (both total oil and essential oil characterization) measurements were performed as per the ASBC standard methods Hops-13 and Hops-17 *(60)*. The GC profile for essential oil characterization was as follows: 60°C for 1 minute, followed by a 3°C/min ramp until 175°C, then hold for 10 minutes. 3°C/min ramp until 230°C, then hold for 10 minutes.

Hop acids were measured using an Agilent 1200 HPLC. Sample preparation and measurement was performed as per the ASBC method for measuring iso-alpha acids in beer *(60)*, with the minor difference of 7 µL injection volume instead of the prescribed 20 µL.

Polyphenol extraction was quantified using the ASBC method Beer-35 *(60)*. This assay uses ferric oxidation of polyphenols to induce a color shift from yellow to deep red. The color change and the resulting changes in absorbance at wavelength 600 nm were measured using a Shimadzu UV-1700 spectrometer.

## 3.3 Results

### 3.3.1 Sensory Evaluation

The sensory panel rated the beers significantly different in every category (ANOVA p-value <0.0001 for all categories). A Tukey's Honestly Significant Difference (HSD) test showed that in general the mean of the panelist's responses for the unstirred samples grouped together. Tables 6-12 show the results of the Tukey's HSD tests ($\alpha$=0.05). The extraction regime (stirred vs. quiescent) resulted in statistically

significant differences in the sensory characteristics of the beer throughout the dry hopping process.

Table 6. Overall aroma intensity

| Sample | Overall Intensity (panelist mean value) | Groups | | |
|---|---|---|---|---|
| Stirred, 4 days | 9.432 | A | | |
| Stirred, 12 days | 9.227 | A | | |
| Stirred, 24 hours | 8.591 | A | B | |
| Stirred, 6 hours | 7.614 | | B | |
| Passive, 4 days | 5.750 | | | C |
| Passive, 6 hours | 5.545 | | | C |
| Passive, 12 days | 5.205 | | | C |
| Passive, 24 hours | 5.023 | | | C |

Table 7. Hop aroma category: herbal/tea-like

| Sample | Herbal/Tea-like (panelist mean value) | Groups | | | |
|---|---|---|---|---|---|
| Stirred, 12 days | 5.386 | A | | | |
| Stirred, 4 days | 5.273 | A | B | | |
| Stirred, 24 hours | 5.091 | A | B | C | |
| Stirred, 6 hours | 4.545 | A | B | C | D |
| Passive, 4 days | 3.773 | | B | C | D |
| Passive, 6 hours | 3.614 | | | C | D |
| Passive, 12 days | 3.545 | | | C | D |
| Passive, 24 hours | 3.227 | | | | D |

Table 8. Hop aroma category: citrus/fruity

| Sample | Citrus/Fruity (panelist mean value) | Groups | | |
|---|---|---|---|---|
| Stirred, 4 days | 6.795 | A | | |
| Stirred, 12 days | 6.409 | A | | |
| Stirred, 24 hours | 5.886 | A | | |
| Stirred, 6 hours | 5.341 | A | B | |
| Passive, 4 days | 4.000 | | B | C |
| Passive, 6 hours | 3.932 | | B | C |
| Passive, 24 hours | 3.750 | | | C |
| Passive, 12 days | 3.614 | | | C |

Table 9. Hop aroma category: pine/resin

| Sample | Pine/Resin (panelist mean value) | Groups | | | |
|---|---|---|---|---|---|
| Stirred, 12 days | 6.273 | A | | | |
| Stirred, 4 days | 6.068 | A | | | |
| Stirred, 24 hours | 5.659 | A | B | | |
| Stirred, 6 hours | 4.295 | | B | C | |
| Passive, 6 hours | 2.795 | | | C | D |
| Passive, 24 hours | 2.545 | | | | D |
| Passive, 12 days | 2.500 | | | | D |
| Passive, 4 days | 2.455 | | | | D |

Table 10. Bitterness Intensity

| Sample | Bitterness Intensity (panelist mean value) | Groups | | | |
|---|---|---|---|---|---|
| Stirred, 12 days | 9.932 | A | | | |
| Stirred, 4 days | 9.773 | A | | | |
| Stirred, 24 hours | 8.568 | A | B | | |
| Stirred, 6 hours | 7.614 | | B | C | |
| Passive, 24 hours | 6.250 | | | C | D |
| Passive, 4 days | 6.136 | | | | D |
| Passive, 12 days | 5.932 | | | | D |
| Passive, 6 hours | 5.659 | | | | D |

Table 11. Bitterness Duration

| Sample | Bitterness Duration (panelist mean value) | Groups | | |
|---|---|---|---|---|
| Stirred, 12 days | 8.068 | A | | |
| Stirred, 4 days | 8.068 | A | | |
| Stirred, 24 hours | 7.045 | A | B | |
| Stirred, 6 hours | 6.386 | | B | C |
| Passive, 4 days | 5.523 | | B | C |
| Passive, 24 hours | 5.295 | | | C |
| Passive, 12 days | 5.250 | | | C |
| Passive, 6 hours | 4.773 | | | C |

Table 12. Astringency

| Category | Astringency (panelist mean value) | Groups | | |
|---|---|---|---|---|
| Stirred, 12 days | 5.773 | A | | |
| Stirred, 4 days | 5.636 | A | | |
| Stirred, 24 hours | 5.273 | A | | |
| Stirred, 6 hours | 4.864 | A | B | |
| Passive, 4 days | 3.568 | | B | C |
| Passive, 24 hours | 3.545 | | B | C |
| Passive, 12 days | 3.545 | | B | C |
| Passive, 6 hours | 3.227 | | | C |

### 3.3.1 Instrumental Evaluation

Headspace sampling using SPME/GC-FID analysis showed that the pellet treatments extracted to a higher final concentration despite having lower total oil content. The whole cone hops used in this study had 1.85 mL of oil per 100g while the pellets contained 1.47 mL oil per 100g (presumably due to evaporative losses during pelleting since they were of the same raw starting material). The stirred treatments extracted both faster and to a higher final concentration relative to the passive treatments of the same hop material. Figures 10 and 11 show the extractions of myrcene and linalool.

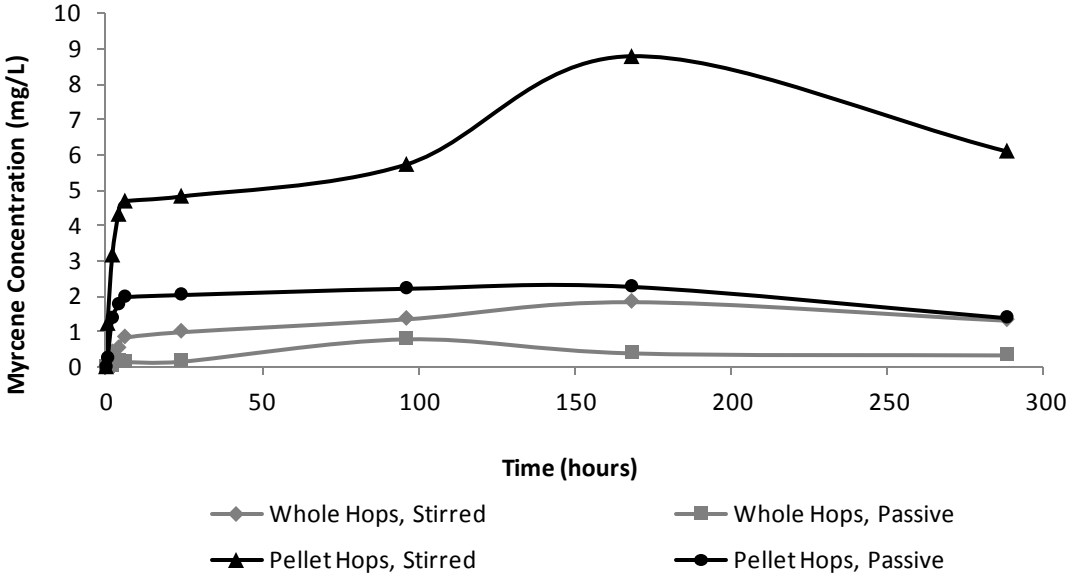


Figure 10. Extraction of Myrcene during a 12 day dry hop treatment.

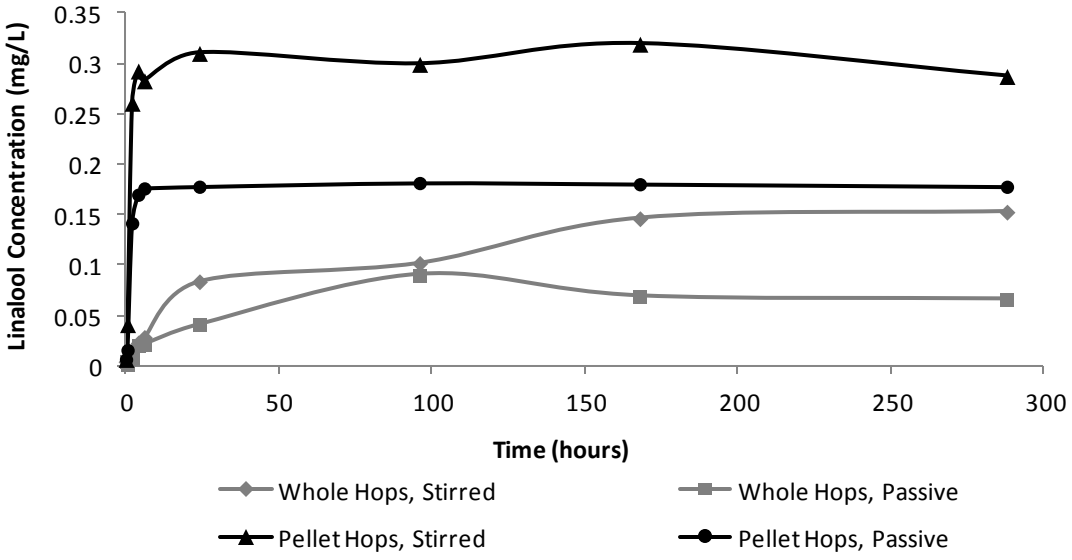


Figure 11. Extraction of Linalool during a 12 day dry hop treatment.

Extraction concentrations, expressed as a percentage of what was available in the total hop oil (as measured by steam distillation/GC) added to the beer, were calculated and compared. The pellet treatments displayed dramatic extractions of geraniol (over 90%) within only 6 hours of dry hopping. Tables 13 (whole cone hops) and 14 (pellet hops) show those relative values.

Table 13. Extraction percentages (ratio of total oil in whole cones to concentration in beer) for **whole cone** hop treatments

| Compound | 6 Hours, Passive | 6 Hours, Stirred | Peak, Passive | Peak, Stirred | Final, Passive | Final, Stirred |
|---|---|---|---|---|---|---|
| **Myrcene** | 0.5% | 2.6% | 2.4% | 5.6% | 1.0% | 4.0% |
| **Limonene** | 40.7% | 41.8% | 42.7% | 52.2% | 17% | 30.8% |
| **Linalool** | 6.8% | 9.0% | 28.7% | 48.5% | 21% | 48.5% |
| **Humulene** | 0.5% | 1.1% | 0.94% | 0.9% | 0.15% | 0.64% |
| **Geraniol** | 70.3% | 57.3% | 70.3% | 119% | 50.3% | 91.0% |

Table 14. Extraction percentages (ratio of total oil in pellets to concentration in beer) for **pellet** hop treatments

| Compound | 6 Hours, Passive | 6 Hours, Stirred | Peak, Passive | Peak, Stirred | Final, Passive | Final, Stirred |
|---|---|---|---|---|---|---|
| **Myrcene** | 8.4% | 19.9% | 9.7% | 37.2% | 5.9% | 25.8% |
| **Limonene** | 43.1% | 69.1% | 43.1% | 82.4% | 39.5% | 75.2% |
| **Linalool** | 55.6% | 89.2% | 57.2% | 100.7% | 56.1% | 90.6% |
| **Humulene** | 1.3% | 8.3% | 1.3% | 17.1% | 1.2% | 14.2% |
| **Geraniol** | 90.8% | 105.2% | 97.1% | 117.9% | 97.1% | 102.9% |

Extraction of nonvolatile constituents behaved similarly to many of the aromatic compound extractions. Figure 12 shows the extraction of hop-derived polyphenols. Values shown are for hop derived polyphenols only; the beer contained an additional 170 mg/L of malt derived polyphenols (e.g. the stirred pellet treatment contained a total of ~320 mg/L polyphenols).

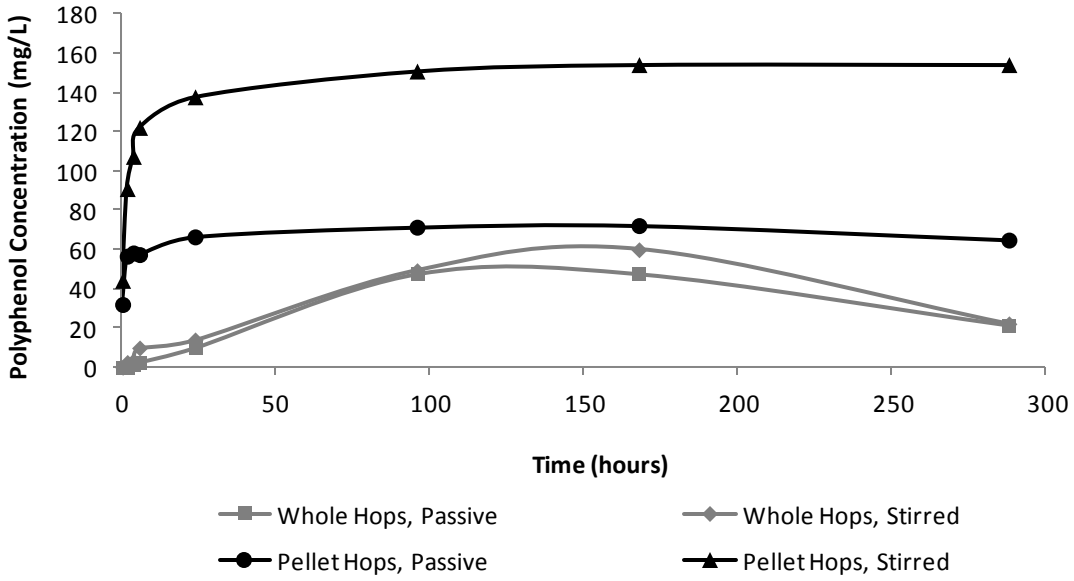


Figure 12. Extraction of hop-derived polyphenols during a 12 day dry hopping treatment.

HPLC analysis of the base beer showed that the extraction of α-acids was rapid at first, particularly for the pellet hops (Figure 13). The iso-α-acid concentration did not change much during the experiment for the whole hop treatments, remaining constant at about 21 mg/L (data not shown). However, iso-α-acids in the pellet hop treatment began at 20.3 mg/L and fell to 18.5 ppm in the passive CCV and to 14 ppm in the stirred CCV. Oxidized α-acids (presumably humulinones) were rapidly extracted

at a low level (about 2 ppm) in the whole cone treatments, and at a moderate level in the pellet treatments (3.3 and 5.1 ppm for passive and stirred, respectively). In all cases they reached maximal concentrations after 24 hours, and once extracted their levels remained static during the remainder of the treatment (data not shown).

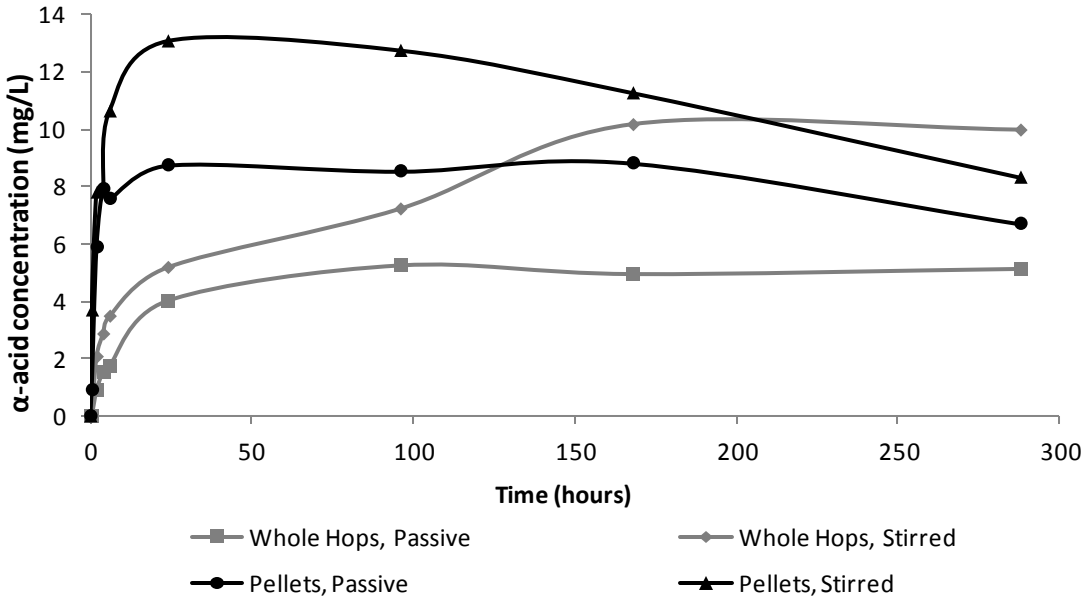


Figure 13. Extraction of α-acids during a 12 day dry hopping treatment.

## 3.4 Discussion

The data from the sensory panel indicated a significant dry hopping regime effect. The stirred treatment yielded more overall aroma intensity, bitterness, and astringency relative to the unstirred treatment. Interestingly, bitterness intensity and duration increased with extraction time as well as between the dry hopping regimes. Given that iso-α-acid levels in the both treatments declined over time, it is clear that the bitterness was coming from an additional source. A correlation test showed that bitterness intensity correlated to polyphenol content (Pearson's correlation

coefficient =0.985, p-value = 0.015). The polyphenol extraction is hypothesized to contribute to bitterness in this experiment. This hypothesis is supported by the volume of literature that identifies the bitterness properties of polyphenols *(37–40)*. These data also show that polyphenol content also correlated to astringency (Pearson's correlation coefficient = 0.973, p-value = 0.027) as well as overall aroma intensity (Pearson's correlation coefficient = 0.987, p-value = <0.0001). It assumed the correlation of polyphenol concentration with overall aroma intensity is not due to properties of the polyphenols but the concomitant extraction of aroma compounds along with the polyphenols. Given this relationship, it is possible that a total polyphenol assay could be used as a tracking indicator of aroma extraction since most breweries are equipped with a spectrophotometer and the assay is relatively quick and inexpensive.

The results describing the actual extraction relative to maximal extraction potential in each treatment (tables 13 and 14) show that terpenoid compounds were readily extractable and very soluble in the beer matrix. Linalool reached 100% extraction in the stirred pellet treatment and geraniol appears to have reached 119% in the stirred whole cone treatment. Since we don't believe any geraniol was synthesized during the experiment, it is reasonable to assume there is a secondary source of geraniol in the hop material. We suspect this may be due to the presence of geranyl glycoside, which can acid hydrolyze and has been shown to be present hops and beer *(17,43,45)*.

As expected, α-acid concentration did not correlate to increased bitterness even though significant extraction occurred. A previous study from our lab has shown that alpha acid concentrations as high as 28 mg/L in beer were not detected as being bitter by beer drinking consumers as well as a trained panel *(33)*.

It is not clear why iso-α-acid levels dropped in the pellet treatments. The ability of yeast cell membranes to act as a fining agent and adsorb iso-α-acid is well documented, but this beer contained no yeast cells, having undergone pad filtration (approximate particle size cutoff: 3-4 microns) prior to dry hopping. It's possible that polyphenols could have acted in a similar manner, since it has been shown that the polyphenols which are found in hops can absorb lipid-soluble compounds *(40,42)*.

While the sensory data showed that the stirred pellet samples had the most aroma and the instrumental data showed that they also had the highest terpene extraction, no correlation was found between overall aroma intensity and an individual aroma compound. Thus, tracking overall hop aroma to a single hop oil constituent would not provide a marker for estimating total dry hop aroma intensity.

Comparing the hop materials to each other, the pellet treatments showed both more rapid and higher overall extraction than whole hops. This result was not unexpected given the physical disintegration that occurs to hops during the pelleting process. With respect to the hydrocarbon terpenes, the passive pellet treatment and the stirred whole hop treatment ended up very close to each other in overall extraction. Polyphenol extraction was dramatically higher with the pellet treatments. Since polyphenols increase both bitterness and astringency depending on their degree of polymerization *(40)*, it could be a balancing act of getting the desired amount of dry hop aroma into a beer against the level of polyphenol-derived bitterness. It's also possible that in addition to polyphenol contribution, some degree of bitterness in the stirred pellet samples results from the presence of oxidized α-acids; they are known to be bitter (reportedly about half as bitter as iso-α-acid) and the 5 ppm present in that treatment would be sufficient to have a perceptible effect *(36)*.

Another goal of this work was to closely examine the initial hours of dry hopping to see whether shorter dry hopping times can be used while still extracting an acceptable amount of hop aroma. It is apparent from these data that whole cone hops benefit from longer contact time, but pellet hops were nearly fully-extracted after 24 hours. From a sensory perspective, the stirred 24 hour, 4 day, and 12 day samples always grouped together when looking at the Tukey's test results (tables 6-12). This makes a strong case both for shorter contact times when using pellets and for incorporating some method of stirring if faster production or turnaround time is a goal. While stirring did benefit the whole cone hop treatments it was much less pronounced than in the pellet treatment. When examining the unstirred pellet treatment (see table 6), the difference between the mean overall hop aroma intensity at 6 hours (5.5) compared to the peak score (5.7 at 4 days) is very small and not statistically significant. Since most brewers are currently dry hopping with unstirred pellets, this could indicate that a reduction in contact time would be negligible in terms of aroma difference but beneficial from a processing and production perspective.

It should be noted that the dry hop treatments which used pellets were very difficult to filter. There was a great deal of vegetative manner homogenized throughout the entire CCV, and when we attempted to filter the finished beer it rapidly blinded the filter pads and halted the process. This problem could be easily be circumvented by using a centrifuge instead of a filtration system. Indeed, most American craft breweries do not filter their beer and consider a centrifuge standard equipment; thus, a centrifugation step would not be unreasonable to expect in commercial practice. That being said, the sheer amount of polyphenolic matter solubilized during these treatments indicates that a chill haze is likely to occur in spite of centrifugation unless further processing steps (PVPP, etc.) are taken to prevent it.

The added polyphenol load in the final beer may also benefit the oxidative stability of the beer given the antioxidative nature of hop polyphenols *(37,61)*.

## 3.5 Conclusion

This work compared the efficacy two dry hopping methods and the flavor potential of two dry hopping materials in a four treatment matrix. We found that dry hopping with pelletized hops resulted in more rapid extraction and greater final amounts of hop aromatic compounds compared to dry hopping with whole cone hops, but their use also resulted in higher total polyphenols. Likewise, a stirred system resulted in higher overall aroma compound extraction (even when the unstrirred system has a very long contact time) but at the cost of higher polyphenol concentrations. In all cases, significant levels of α-acids were extracted but these compounds did not correlate with increases in beer bitterness. In light of their previously discussed ability to scavenge radicals, quench ROS, and negligible sensory impact, their addition can only have positive effect on beer shelf life. The addition of polyphenols cannot be overlooked. Given their potential benefits (antioxidant, enhanced bitterness, metal ion chelation) and potential detriments (bitterness, astringency, possible oxidizing agent), it must be left up to the brewer to consider whether or not having higher total polyphenols is acceptable.

## Chapter 4 – Future Work

These studies fill a void in the literature as well as giving brewers some practical data to consider when designing a dry hop protocol for their beers. They also, however, raise some interesting questions. When considering the terpene levels in the dry hopping vessels, these data show it's common for the hydrocarbon variety to decline in concentration after reaching a peak value. Considering that this is occurring in a stainless steel vessel before the beer reaches packaging, aroma scalping by a polymer is not a possibility. Where, then, are these compounds going? Previous work on packaging has implicated oxidation as a possible agent when the terpene loss cannot be explained by scalping but it's also possible they are adsorbing to polyphenol complexes. Work to determine the fate of the hydrocarbon fraction of dry hop aroma would be helpful in order to find ways to preserve that aroma both before and during packaging.

There has been an interest in finding ways to slow or reduce oxidation reactions in beer at various phases in the brewing process. Polyphenols from dry hopping may influence these reactions both during dry hopping and after the beer has been packaged. An investigation into this possibility could have implications for brewers looking for ways to reduce oxidation.

These studies also showed that it is possible for the terpenoid levels to rise above what would be expected when looking at the total oils from steam distillation. A large body of work implicates glycoside hydrolysis for this phenomenon, but as of yet no one has directly tracked levels of hop glycosides prior to hopping and quantified their hydrolysis during fermentation or beer aging. The possibility of flavor from glycoside hydrolysis has been a subject of interest for over a decade, and more work in this area would be a welcome addition to the field.

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# APPENDICES

# Appendix A. Hop Aroma in beer: SPME and GC analysis (a methodology)

This assay was developed to examine hop aroma (terpenes, terpenoids, esters) in beer via sampling the headspace volatiles with solid-phase micro-extraction. The SPME takes approximately 70 minutes including sample preparation, followed by a 66.5 minute GC profile.

**Reagents**

(a) NaCl, USP or higher grade.

(b) Internal standard, such as 4-octanol. 4-octanol elutes approximately in the middle of the GC profile given below, did not co-elute with any hop compounds, and provided excellent run-to-run consistency. Any appropriate internal standard could be substituted.

**Apparatus**

(a) Volumetric flask(s), 100 mL (for creating standards)

(b) Amber vials, 40 mL. Vials must have a lid with pierceable septa.

(c) Water bath with a heating element or other apparatus capable of maintaining the amber vials at 25-40°C. This method performs the SPME at 30°C, but you may want the freedom to change temperatures if you're trying to examine an ester fraction more closely.

(d) Manual sampling SPME fiber holder (notched gray type). Referred to as "plunger" below.

(e) SPME fiber assembly. Tri-phase SPME fiber (2 cm, 50/30µm DVB/Carboxen/PDMS). I used Supelco part # 57348-U.

(f) Gas Chromatograph with a polar column such as Carbowax 20M. We use a (Length x I.D.) 30 m x 0.25 mm fused silica capillary Supelcowax 10 column (Supelco) with a 0.5 µm film thickness. The GC used was an Agilent 5890 with an FID detector.

**Operating Conditions for Chromatography**

Carrier gas was nitrogen with a flow rate of 1 mL/minute (splitless mode). Detector temperature was 250°C. Injection and desorption of volatiles from the SPME fiber was performed with an inlet temperature of 250°C for 10 minutes. Oven temperature started at 50°C, and underwent the following temperature ramp: 50°C for 1 minute then ramped at 4°C/min until 90°C, held for 5 minutes, then ramped at 5°C/min until 185°C, then held for 6.5 minutes, 3°C/min until 230°C and hold for 10 minutes. SPME injections were conducted manually.

**Method**

If examining a packaged beer, degas via sonication or beaker transfer. If the sample is direct from a fermentation or conditioning vessel, centrifuge prior to sample preparation to remove hop particles.

Weigh 2g of NaCl and transfer to 40mL amber vial. This value was arrived at by referencing similar SPME studies done on wine aroma, and then fine tuning via trial and error. Add 10mL beer sample. Add internal standard solution. The internal standard should be formulated such that (a) it is completely dissolved in ethanol (b) the final concentration when added to the amber vial is less than the largest beer aroma component and more than the smallest aroma component. For my work with terpene dry hop aroma a final concentration of 0.5 ppm proved satisfactory. Adding too much internal standard can result in suppressed analyte adsorption due to fiber and headspace competition.

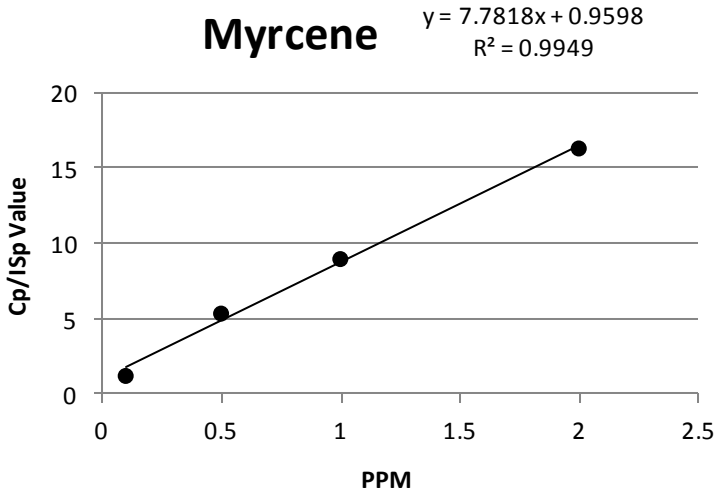
Once the beer sample and all reagents are in the vial, add a glass (or other inert material which will not adsorb aroma compounds) stir bar and close the cap tightly. Heat vial to 30°C via the water bath or whatever heating apparatus you're using. Allow 15 minutes for the vial to come to equilibrium while stirring (I stir at 500 RPM), and then pierce the septum with the SPME plunger. Fully expose the fiber for one hour. If using a new fiber, follow conditioning instructions. If the fiber has been sitting unused and potentially exposed for longer than 2 hours, the fiber should be cleaned via desorption in the GC inlet at 250°C for 10 minutes prior to use.

After SPME is complete, desorb the fiber in the GC inlet as shown in the GC profile given above. For quantification of aroma compounds, external standards may be used at different concentrations to plot a linear "standard curve". If a real data point falls outside your external standard concentrations, it's not disastrous; FID detectors have a very large linear range (generally seven orders of magnitude) so your extrapolation is likely very accurate. That being said, it is good practice to "bookend" your analyte concentrations by running external standard concentrations both above the maximum and below the minimum analyte concentrations. Multiple external standards must be combined in the same vial during SPME in order to accurately reflect the conditions of the beer sample headspace with respect to volatility and fiber competition.

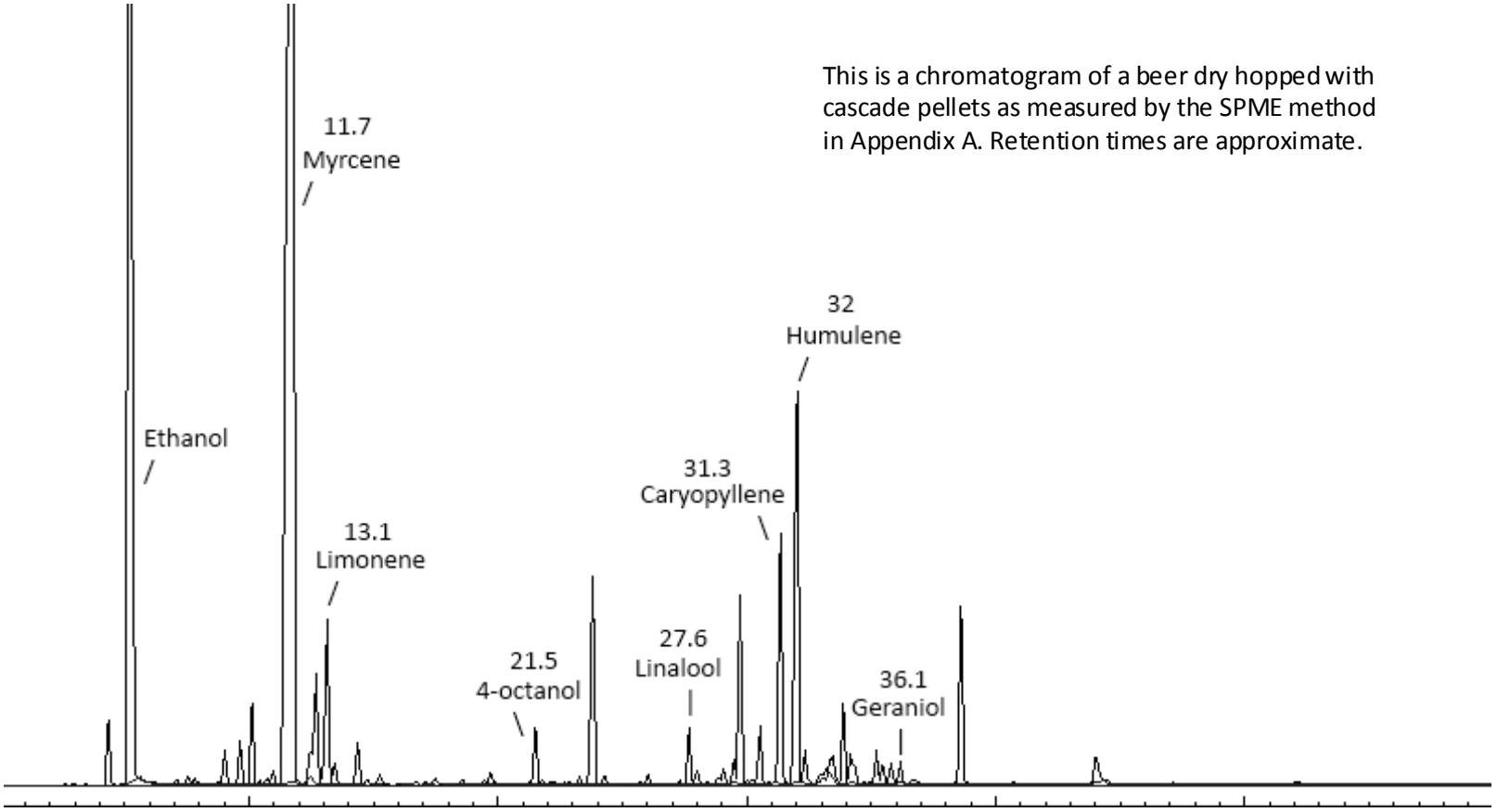
GC run-to-run variation is corrected for via normalization with the internal standard. Calculation of analyte concentration is done using the best-fit linear equation produced by the external standard peak areas. Below is an example of an external standard curve for Myrcene.

Notes: Cp = Compound peak area, ISp = Internal standard peak area. This ratio is calculated to correct for normal run-to-run variation since the ISp always represents a known concentration. Plot the concentration in ppm versus the Cp/ISp value.

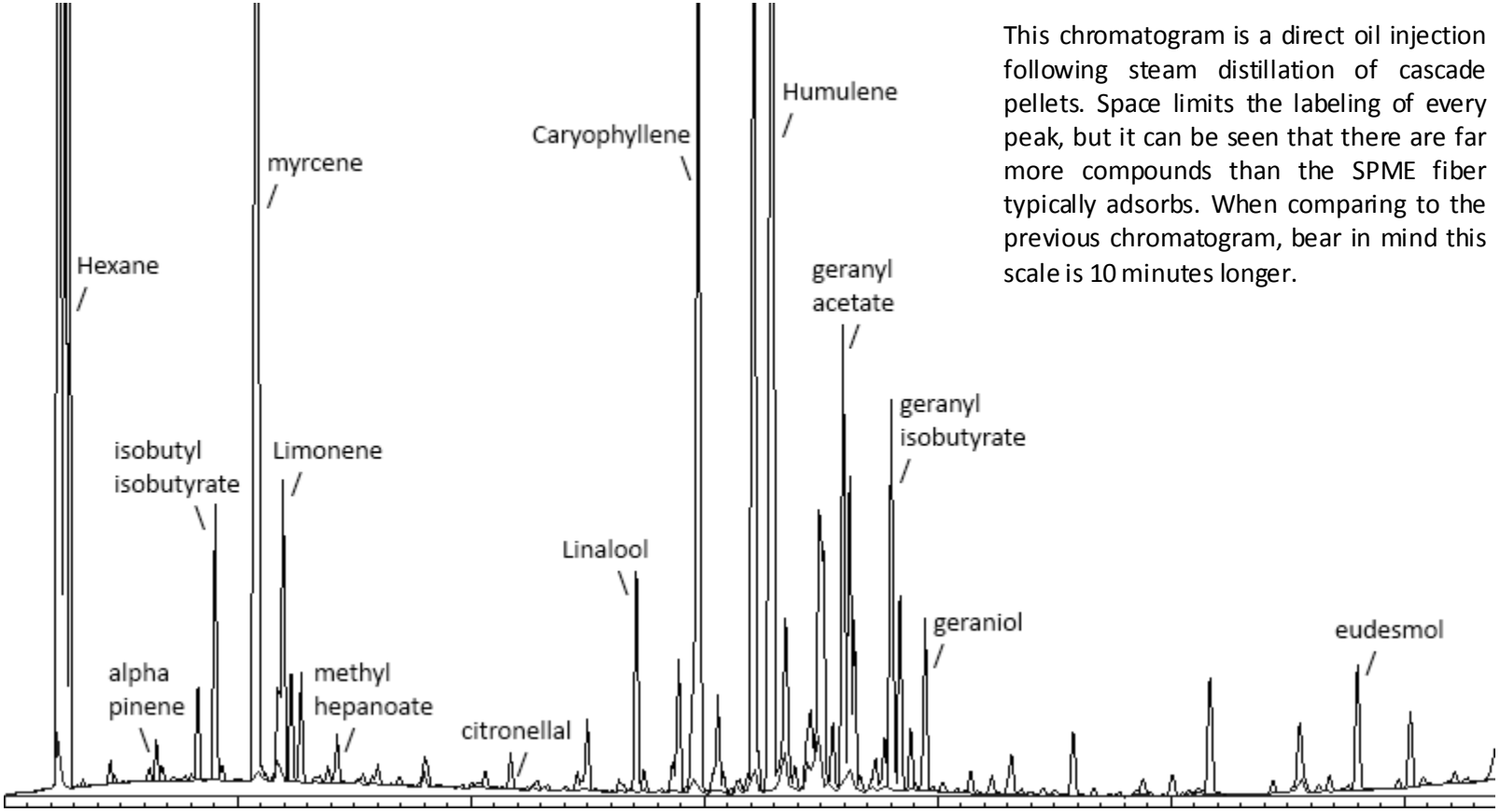
| | Myrcene | | 4-octanol |
|---|---|---|---|
| **(ppm)** | Avg. Area | Cp/ISp | Avg. Area |
| 0.1 | 604.2 | 1.219 | 495.57 |
| 0.5 | 1897 | 5.355 | 354.25 |
| 1 | 2818.7 | 8.971 | 314.2 |
| 2 | 3587.9 | 16.31 | 220 |



Appendix B. Representative GC Chromatograms




76




This chromatogram is a direct oil injection following steam distillation of cascade pellets. Space limits the labeling of every peak, but it can be seen that there are far more compounds than the SPME fiber typically adsorbs. When comparing to the previous chromatogram, bear in mind this scale is 10 minutes longer.

## Appendix C. Industry Dry Hopping Survey Questions

Dry Hop Process

1) At what point during the brewing process is dry hopping conducted?

1.1) Is there any yeast present at this time?

2) Describe your dry hopping procedure.

3) What temperature is dry hopping performed at?

4) What kind of hop material is utilized (if more than one, please indicate)?

5) How long is the beer exposed to the hop material?

6) Is the vessel (or system) agitated or passive? That is to say, is there any pumping or active movement during dry hopping( skip if you covered this in question 2)?

7) How is the hop material separated from the beer once the desired exposure is reached?

7.1) Is the dry hop material ever reused for bittering?

8) If you distribute beer in kegs (or casks), do you ever add dry hops to a keg (or cask) and distribute it so?

Other Questions

9) Why do you dry hop your beers? What is your desired outcome?

10) Do you have a KPI or other system in place to benchmark consistent dry hopping?

10.1) Is the concentration of any particular hop compound tracked during or after the process (in your lab or by a 3rd party QA lab)?

11) Does your brewery perform sensory evaluation of dry hop aroma as part of QA?

11.1) If yes, how much variation is allowable?

12) Do you feel that dry hopping extends/shortens/has no effect on the shelf life of your product?

13) Do you charge more for dry hopped beers (are the higher costs associated with dry hopping passed along)?

14) Has an inadequate hop supply or unavailability ever forced a change in your dry hopping procedures?