AN ABSTRACT OF THE THESIS OF

Ellen Jane Parkin for the degree of Master of Science in Food Science and Technology presented on July 11, 2014.

Title: The Influence of Polyphenols and Humulinones on Bitterness in Dry-Hopped Beer

Abstract approved:

Thomas H. Shellhammer

This research sought to determine the origin of bitterness as a result of dry-hopping. An unhopped ale was dry-hopped and examined in the controlled dry-hop experiment and commercial beers were examined for chemical changes from dry-hopping in the commercially dry-hopped beer survey. This thesis work suggested specific bitter hop components that contribute to the bitterness of dry-hopped beer.

The controlled dry-hopping experiment set out to determine specific sensory properties of dry-hopped beer. Using quantitative descriptive analysis, UV-spectroscopy, and liquid chromotography, the perceived bitterness intensity, aroma intensity, and hop component concentrations were determined. An unhopped ale (13° Plato Original Gravity, 2.53° Plato Final Gravity, 5.1 pH) was employed as the base and control for the study. Chinook pellets (13% alpha acids, 3.4% beta acids) were dry-hopped into the beer at 4g/L and 16g/L and examined after several exposure times: 6, 24, and 72 hours. Samples were then taken from each of the beers and analyzed for concentrations of hop acids, polyphenols, and Bitterness Units (BU). A trained sensory panel rated the samples for bitterness intensity and aroma intensity on two separate categorical scales (0-9) over 6 testing sessions. Chemical analysis

indicated an increase in BU, polyphenols (mg/L), and humulinones (mg/L) from dry-hopping. The dry-hopped samples were also found to have significantly higher perceived bitterness intensity and aroma intensity compared to the unhopped control. Correlations between the quantitative data and the sensory data were determined. Overall, perceived bitterness intensity and aroma intensity for these dry-hopped samples could be predicted by polyphenols, humulinones, and the BU measurement.

The commercially dry-hopped beer survey examined pre- and post-dry-hopped samples of commercial beers for chemical changes. The aim of this study was to determine the specific non-volatile hop compounds coming from dry-hopping. Commercial examples from breweries in the Pacific Northwest were examined for differences in BU, polyphenols, iso-alpha-acids (IAA), humulinones, hulupones, and alpha acids. Consistent with findings from the first study, polyphenols and humulinones increased as a result of dry-hopping. A notable finding indicated total IAA decreased with dry-hopping. Contributions to the BU measurement were predicted with multiple linear regressions, indicating humulinone concentrations as a major contributor to BU from dry-hopping. Further research is needed to determine predictions for the BU measurement using concentrations of IAA, humulinones, hulupones, and polyphenols.

These studies indicate dry-hopping contributed bitterness to beers. The addition of humulinones, hulupones and polyphenols as a result of dry-hopping further suggested these compounds as the bittering components. With the knowledge of the

bitter compounds added as a result of dry-hopped, the brewing industry can better understand the measured and perceived bitterness of their beers.

The Influence of Polyphenols and Humulinones on Bitterness in Dry-Hopped Beer

by
Ellen Jane Parkin

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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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Cheers!

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DEDICATION

I dedicate this thesis to my mother, Sarah, who always told me to follow my passion.
I love you to the moon and back.

THE INFLUENCE OF POLYPHENOLS AND HUMULINONES ON BITTERNESS IN DRY-HOPPED BEER

CHAPTER 1: BITTERNESS CONTRIBUTED BY HOP COMPOUNDS DURING DRY-HOPPING

1.1 Introduction

Although the origin of boiling hops in the process of brewing beer is unknown, they have played a vital role in the brewing industry originally as a microbial inhibitor and today as an addition of bitterness, aroma, and flavor (4,27,48). The hop plant *Humulus lupulus* L. grows between the latitudes of 35 and 55° in the Northern and Southern hemispheres (4). Hop cones develop from the flowers of the female plants and are harvested in late summer. The hop cones are dried and used whole or processed into pellets or extract. The lupulin glands found inside the hop cones are comprised mainly of alpha acids, beta acids, and essential oils – key components for beer (4). Hops are a major agricultural product specifically grown for the brewing industry, adding bitterness, aroma, flavor and microbial stability to the beer (4).

Prior to brewing, grains are germinated to initiate enzymatic activation, then kilned to halt the grains' seed growth. Brewing consists of three main steps: mashing, sparging, and boiling. Mashing and sparging steep the grains and then separate the sugars from the malt, respectively. Boiling adds a depth of flavor and kills microbes. Hops are traditionally added at the beginning of the boil for bittering and later in the boil, known as late-hopping, to increase the hop aroma in the beer. However, recent interest in the craft brewing industry to maximizing hop aroma led to the

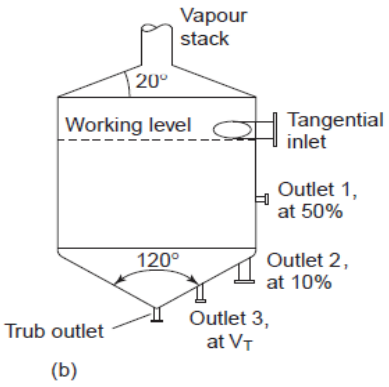


Figure 1. Diagram of a whirlpool. (4)

understanding that late-hopped beers contain much less aromatic oils than previously suspected (21). Different techniques for further addition of hops at various stages of brewing are heavily used throughout the brewing industry in attempts to incorporate more hop aroma. Several techniques are additions in the whirlpool or a hop back (Figures 1 and 2 (4)) as the hot wort is transferred into a fermenter, thereby decreasing the exposure time for the hops with the boiling wort and thus the rate of volatilization of the hop oils.

Another technique, dry-hopping, allows for hops to be added to the finished beer without heat input. This is traditionally accomplished by dropping hops into the fermentation tank from the top hatch. Finding ways to add extra hop aroma to the beer has led to many new and innovative dry-hopping methods such as hop cannons and torpedoes. Hop cannons use pressurized CO_2 to force hop pellets from a sanitized vessel up through the gas arm into the top of the fermentation tank (Figure 3 (44)); torpedoes' specific design allows beer to be pushed through a bed of hops and extract significant hop aroma and flavor. These

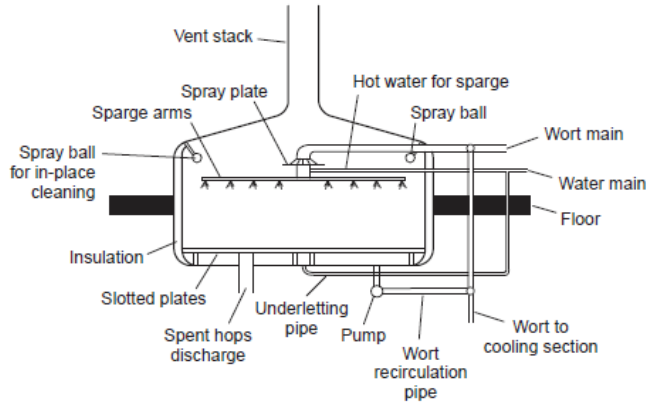


Figure 2. Diagram of a hop back. (4)

methods decrease the potential for dissolved oxygen uptake, which can cause spoilage by oxidation. All of these post-boil techniques are used to exploit the many facets of the hop including the essential oils that add a depth of flavor and aroma to the beer.

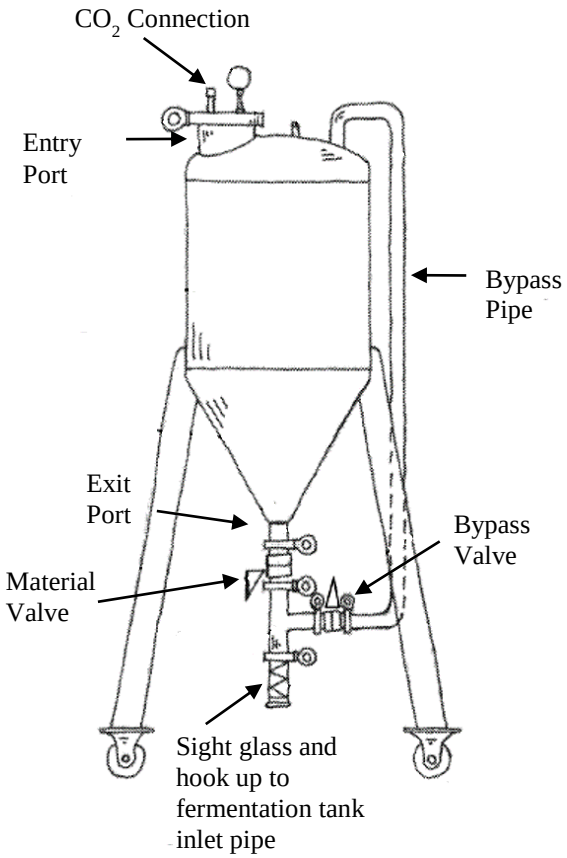


Figure 3. Diagram of a hop cannon. (46)

After boiling, wort is cooled to a proper temperature (15-22°C for ales, 6-12°C for lagers), transferred to a fermentation vessel and yeast is added. In the industry, the fermentation vessel is commonly a closed stainless steel conical tank or an open topped vessel - historically to promote wild fermentation. Yeast, most commonly *Saccharomyces cerevisiae*, is added to begin the fermentation of ales (top fermenting) and lagers (bottom fermenting). The yeast digest the fermentable sugars in the wort, producing predominately ethanol and carbon dioxide, leading to the final product, beer.

This review examines the hop components contributed through the technique of dry-hopping and the perceived bitterness attributes in beer. Discussion will include

a review of major bittering components in beer and hop components that potentially contribute bitterness from dry-hopping.

1.1.1 Hop contributions during the boil

Before discussing dry-hopping and its potential bitterness, an understanding of the main bittering components of beer is necessary. A thermal isomerization of alpha acids occurs in the boil, producing iso-alpha-acids (IAA), regarded as the major bittering component in beer (4,27,43,48). Other hop compounds such as humulinones, hulupones, and polyphenols significantly influence the perceived bitterness and flavor development (13,27,34,35,43). The essential oils of hops are a major contributor of aroma and are more prevalent in dry-hopped beers than in kettle-hopped beers, as the exposure to heat during the additions in the boil volatilizes the oils (21,27,43,48).

1.1.2 Dry-hopping

Dry-hopping – the technique of incorporating additional hops to fermenting or fermented beer at room or cooler temperatures – is commonly used in the brewing industry to contribute further aroma and flavoring to the beer (27). From this addition, different hop constituents are extracted into the beer such as polyphenols, alpha acids, humulinones, hulupones, and essential oils (13,21,24); beta acids are not incorporated due to their lack of water and beer solubility (43). These compounds incite the sensory perception of bitterness (18,19,31,36), as well as other aromatic characteristics. The incorporation of aromatic oils is the primary goal of dry-hopping. With additional hop

material incorporated into the fermenting or fermented beer, the oils – otherwise lost due to evaporation in the early kettle hop additions – are extracted (21).

Dry-hopping contributes aroma to beer, but it may also increase perceived bitterness (35,43,54). Given that dry-hopping does not include thermal isomerization of the alpha acids, other hop components may be the source of increased bitterness in dry-hopped beers. From previous research, Fritsch and Shellhammer (2007) identified alpha acids as a non-bitter constituent of beer (14). One study conducted by Wolfe, et al. (2013) focused on flavor contributed by dry-hopping. Using a trained sensory panel, the study examined the intensities of hop aromas along with bitterness intensity and bitterness duration. This study found that perceived bitterness were generally rated higher (on a 0 to 15 categorical scale) for beers subject to longer dry-hop regimes (e.g. 6 hours compared to 12 days). Instrumental analysis indicated that the beers in the Wolfe study contained higher levels of polyphenols and humulinones, which were thus hypothesized to be a contributing factor to bitterness from dry-hopping (54).

1.2 Bitterness

The perception of bitterness has an evolutionary benefit as the taste denotes the ingestion of a potentially poisonous or dangerous substance (8). Bitterness is perceived through interaction of a bitter compound with the TAS2R receptors on taste buds embedded over the entire tongue (8,25). As depicted in Figure 5, the taste receptor cells (TRCs) cluster together in groups of 5 to 100 cells (7,39). The assembly of TRCs is known as a taste bud and can be found on several different papillae across

the tongue including the circumvallate, foliate, and fungiform (7). Each TRC is genetically coded to express certain bitter receptors. When a bitter compound interacts with G-protein-coupled receptors encoded with TAS2R genes, the reaction initiates an increase in calcium ions within the cell and generates a response to the brain, signaling danger (7,25).

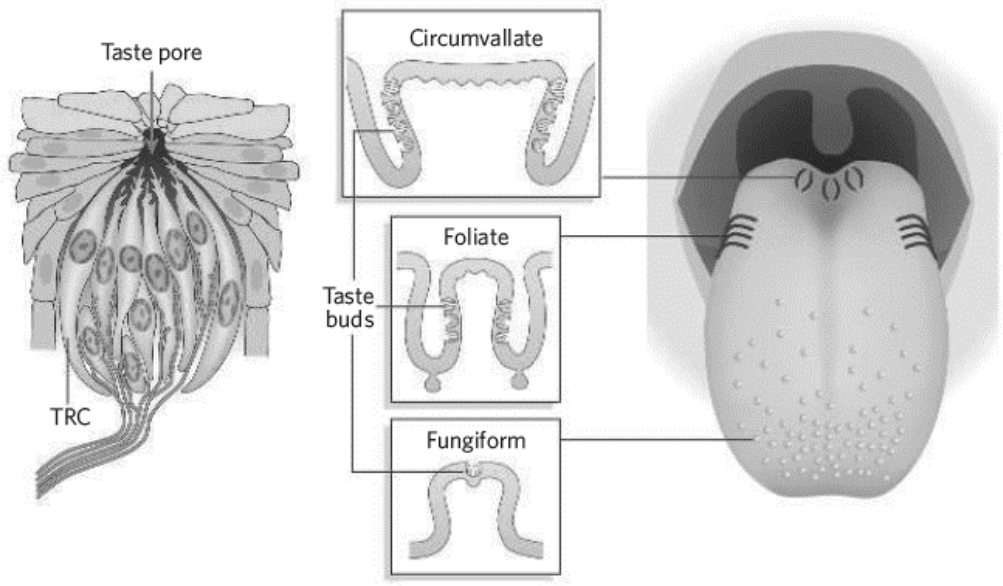


Figure 4. Illustration of taste receptors found on the papillae of the tongue. (7)

1.3 Bitterness Measurements

The bitterness from hop components such as IAA, humulinones, hulupones, and polyphenols can be measured several ways, noted in the American Society of Brewing Chemists' Method of Analysis (2). A common practice known as Bitterness Units (BU) uses an isooctane extraction and spectrophotometry (ASBC Beer-23A) to quantify the chemical components contributing to bitterness. This measurement

accounts for tannins from the malt and hops as well as IAA and other hop constituents that influence perceived bitterness. Other methods of bitterness measurement are solid-phase extraction of IAA or direct injection using HPLC (ASBC Beer-23C and 23E, respectively) to provide chromatographic separation and identification of the concentration of IAA in the beer. Bitterness can also be measured through sensory evaluation, commonly with categorical intensity scaling. The use of a categorical or line scale allows sensory panelists to denote their perception of bitterness with numbers (23,25,37,43).

1.4 Bitterness from Hops

Isomerized alpha-acids and other components of the hop cone, such as humulinones, hulupones and polyphenols, activate TAS2R bitter receptors in the oral cavity (23,25,43). Reduced iso-alpha-acids (RIAA) also contribute a perceived sensory bitterness similar to IAA (15,16). RIAA have yet to be examined for activation of TAS2R bitter receptors.

As described by Intelmann, et al. and Meyerhof, et al., there are several receptors specifically responsible for the bitter perception of hop compounds: hTAS2R1, hTAS2R14, and hTAS2R40 (25,38). In several studies, different compounds activated each receptor with some compounds activating multiple receptors (25,38,46). Research conducted by Intelmann et al. observed that all transformations of the IAA (trans- and cis- conformations of isocohumulone, isohumulone, and isoadhumulone) activated both hTAS2R1 and hTAS2R14,

indicating that the receptors interacted with similarly structured compounds (25). Yet, this did not indicate the receptors had similar structural motifs as sterically different compounds also activated said two receptors (25,46).

1.4.1 Iso-alpha-acid interactions with bitter receptors

As the main bittering component from kettle-hopped beers, IAA interact with bitter receptors in the oral mucosa as beer is being consumed. The varying conformations of IAA allow it to interact with different TAS2R receptors on the tongue. hTAS2R1, hTAS2R14, and hTAS2R40 are all activated by hop compounds and can be activated simultaneously, as noted by Intelmann, et al. (25). Intelmann, et al. further examined the receptor hTAS2R1 with a combined mixture of known IAA to determine activation levels. The results suggested an additive effect of the combination of either *trans*-isocohumulone and *trans*-isohumulone or *trans*-isoadhumulone and *cis*-isoadhumulone on the perception of bitterness (25). Nothing was mentioned on the possible additive effect of other hop compounds that activated different bitter receptors. For example, no interactions were tested between the polyphenol 8-prenylnaringenin, identified only to activate hTAS2R14, when found in combination with any of the untransformed hop acids, which did not activate hTAS2R14, but specifically activate hTAS2R1 and hTAS2R40. The interaction of polyphenols and other bitter hop compounds in the mucosal cavity with multiple bitter receptors may lead to increased bitterness perception. This activation has yet to be examined.

It should be noted that the receptors' activation was dependent on the concentration of the bitter compounds; yet, the bitterness perception of different compounds at the same concentration provided differing perceived intensities due to differing human sensitivities and detection thresholds (25). Therefore, there was a difference between the human perception and the cell-based assays contrary to previous studies that used differing bitter compounds to elicit a response from hTAS2R16 receptor (6). This led to the investigation of bittering compounds interacting with the salivary proteins to cause different perceptions in the oral cavity conducted by Intelmann, et. al (25). The results of the study suggested an association of the bitter compounds with proline-rich salivary proteins or the oral epithelium, which explained the observed difference between human perception and in vitro cell-culture assays (25).

1.4.2 Reduced iso-alpha-acids

Reduced IAA are used in the industry as a means to prevent the "skunky/lightstruck" defect aroma in beer and promote foam stability. This defect is caused by IAA photolysis and subsequent formation of 3-methylbut-2-ene-1-thiol (15,16,27). IAA can be reduced by sodium borohydride, hydrogenated, or both to create rho-isohumulones, tetrahydro-isohumulones, or hexahydro-isohumulones, respectively, as depicted in Figure 6 (27). These reduced IAA do not degrade when exposed to UV light (4,16), and therefore are used for light- and foam-stability in

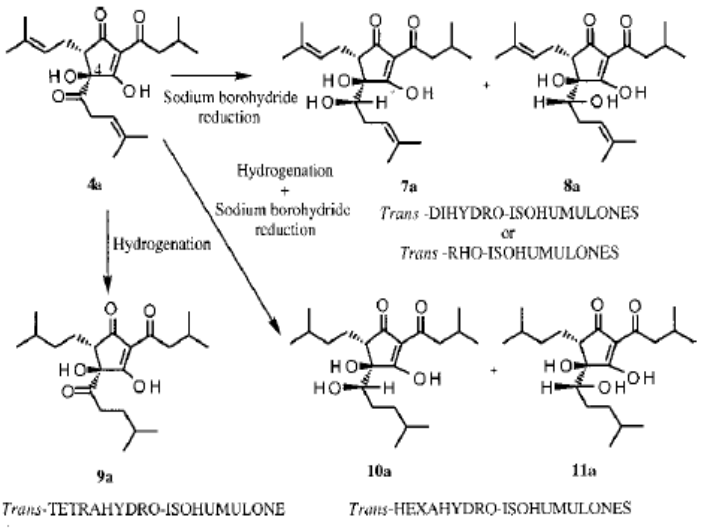
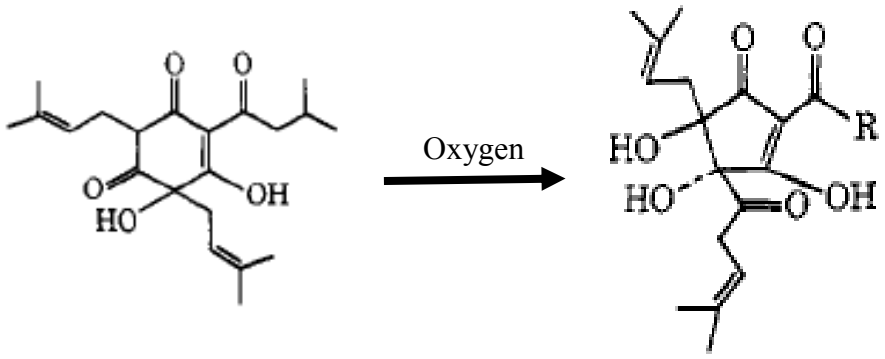


Figure 5. Production of reduced isohumulones. (28)

beers (48,52). These compounds also add to the bitterness of the beer (15,16,51). One study that used an untrained consumer panel to compare commercial beers that incorporated IAA and reduced IAA with a rank-rating test found that IAA and reduced IAA both contributed to bitterness in beer (51). Fritsch & Shellhammer (2008 & 2009) investigated bitterness and quality of IAA and reduced IAA and found they vary in both bitterness intensity and quality. Rhohydro-IAA was described as smooth and more vegetative; tetrahydro-IAA and hexahydro-IAA described as harsh, medicinal, and metallic (15,16). With IAA as the major bitterness contributor, reduced IAA still play a role in beer bitterness (15,16,48,51,52)

1.4.3 Oxidized hop acids

Oxidation of alpha and beta acids leads to the formation of humulinones and hulupones, respectively (Figure 7 (42)). Although their existence in beer is known, understanding of their contribution as bitter components is lacking. Kowaka and Kokubo (1977) suggested the use of a new method of bitterness measurement,



Humulone Humulinone

Figure 7. Oxidation of alpha acids. (40)

indicating the use of the constitution of beer bitterness. Kowaka and Kokubo included BU and IAA measurements in this idea, but also mentioned humulinones and hulupones as bitter substances (29). Oxidation occurs during hop aging and storage as well as during the boil (42). These hop oxidation products add to the flavor of beer, although research is inconclusive on the bitterness from humulinones and hulupones. Stevens and Wright focused specifically on hulupones and found them at extremely low levels – 0.5% of the original hulupones found in the hop cone. Hulupone bitterness was noted as comparable to isohumulone (50). Palamand and Aldenoff noted bitterness from humulinones as well as hulupones and other degradation products of beta acids. Hulupones were specifically denoted as being 50% as bitter as IAA (42). Peacock noted there was evidence of hulupones being twice as bitter as IAA and the humulinones as less bitter than IAA (43). Haseleu, et al. quantified and identified bitter tasting beta acids in beer, including hulupones. This examination observed that different beer styles had varying concentrations of the beta acid transformations, including high levels of cohulupone in commercially available bitter

beers (22). Krofta, et al. noted sensory bitterness from a hulupone addition to beer. The bitterness of hulupones in this study were approximated as 35-40% that of IAA (30). Another study by Intelmann, et al. indicated low hulupone concentrations in beer (0.02-0.07µmol/L); humulinones were more prevalent at 1 µmol/L however the alpha acids were noted as more likely to convert to IAA than to their oxidized product in the boil (26). The effects of humulinones and hulupones are still under investigation. This knowledge will help the industry in developing a better understanding of beer quality, bitterness, and flavor.

1.4.4 Hop polyphenols

Polyphenols, hop acids, and oils are the main hop components added to beer from dry-hopping. These compounds provide bitterness and potential astringency to the beer. Other food and beverage products such as wine, apple cider, olive oil, and tea also contain astringent and bitter compounds. Astringency is noted as the drying, puckering or roughing sensations in the mouth as proline-rich proteins precipitate from the saliva (3,17,33). The sensory bitterness of these products is mainly contributed by their high phenolic content (1,5,20,32,40,41). Studies indicated polyphenols from hops contributed sensory bitterness to beer (35,46). Although research is lacking on polyphenol contributions to dry-hopped beer, these studies suggest polyphenols as a bitter contribution to dry-hopped beer.

Research conducted by Roland, et al. and Soares, et al. focused on activation of TAS2R bitter receptors by polyphenols (46,49). Roland, et al. mapped the structure of

some bitterness receptors for certain polyphenols – flavonoids and isoflavonoids – with some success. It was found that certain ligand substitution patterns of flavonoids and isoflavonoids activated the receptors more often than those flavonoids and isoflavonoids with similar backbones. This was measured by calcium ion release of *in vitro* bitter receptor cells dosed with different phenolic compounds at 500μM concentrations (46). Soares, et al. identified receptors activated by polyphenols, including TAS2R1, TSR2R14, and TAS2R40 (49) – receptors known to be activated by hop compounds, noted by Intelmann, et al. (25). Intelmann, et al.'s research focused specifically on hop compounds, including 3 hop polyphenols; these compounds were found to activate hTAS2R1, hTAS2R14, and hTAS2R40 (25). As the polyphenols activate the same bitter receptors, the combination of polyphenols and hop acids may have an additive effect thereby increasing bitterness perception.

Studies in wine systems demonstrate the enhancement of bitterness by ethanol, a known astringent compound. Varying levels of ethanol in solutions of dealcoholized wine with different phenolic catechin concentrations showed increased bitterness with increased ethanol (11,12,28). With these findings, the polyphenol additions in dry-hopped beers may contribute bitterness in higher alcohol beers.

Yeast can also have an effect on polyphenol perception. When dry-hopping in a fermenter containing yeast, the yeast can interact with the polyphenols by van der Waals bonds (47) and precipitate out of solution when the yeast flocculate (i.e. clump together and fall to the bottom of the fermenting vessel). By dry-hopping with yeast

still in the fermentation tank, bitterness from the polyphenols will be less detectable as decreased polyphenols will be in the beer solution.

The interactions of polyphenols and other bitter compounds from hops was examined by McLaughlin, et. al (35). Using varying concentrations of IAA and polyphenols extracted from hops, the compounds were dosed into a light commercial lager and examined for chemical and sensory differences. Bitterness and astringency were examined as 2 of the many attributes. McLaughlin, et. al, found that 10ppm IAA and 100 to 200ppm of polyphenols incited increased astringency and bitterness compared to the samples lacking IAA or polyphenols (35). These findings indicated that polyphenols have an additive effect on beer bitterness. It should be noted that from the panel ratings of astringency, perceived astringency was not concentration dependent (35). This may be due to polyphenol interactions with bitter TAS2R taste receptors or their interaction with salivary proteins. With an increase in salivary protein-bitter compound interactions, there may be an increase in the interaction of other bitter compounds (e.g. IAA, humulinones, and hulupones) with the TAS2R receptors (25,43,46,49). The lack of research in beer of bitter and astringent reactions may be due to low levels of astringency perceived in beers.

1.5 Conclusions

IAA are a main source of bitterness in beer. Reduced IAA can also contribute bitterness as well as light and foam stability. Due to lack of heat treatment during dry-hopping, further bitterness may accumulate through the addition of non-IAA

compounds such as polyphenols and oxidized hop acids. Inconclusive results from humulinones and hulupones describe the compounds as bitter, but with no agreement on the degree of bitterness compared to IAA. Their addition during dry-hopping may cause an increase in bitterness. More extensive research on polyphenols described the compounds as both bitter and astringent, interacting with both TAS2R receptors and salivary proteins. When in the presence of higher levels of polyphenols, salivary proteins are precipitated from the oral mucosa allowing increased amounts of bitter compounds to interact with more TAS2R receptors. As bitter compounds are also noted to interact with salivary proteins, this interaction could represent the common industry descriptor "harsh" bitterness and lead to the conclusion that polyphenols are a source of bitterness from dry-hopping. However, their contributions from dry-hopping are lower than the levels used to examine their bitterness in beer (35,53).

Overall, further research is necessary to investigate numerous factors that affect the perception of bitterness. Discussion should focus on the addition of hop material during dry-hopping and its subsequent taste and flavor contributions. Additional research is also needed on the activation of bitter taste receptors by reduced isomerized hop compounds, humulinones, hulupones, and hop polyphenols. This research will allow for a greater understanding of human perception of beer and beer bitterness and lead to the ability to predict sensory bitterness from the hop compounds found in beer.

CHAPTER 2: TOWARDS UNDERSTANDING THE BITTERNESS OF DRY-HOPPED BEER

(to be submitted to the Journal of the American Society of Brewing Chemists)

2.1 Abstract

The impact on beer bitterness of hop-derived compounds resulting from dry-hopping was investigated using a controlled dry-hop experiment and a commercial dry-hop survey. The controlled dry-hop experiment utilized a trained sensory panel to quantify increases in bitterness caused by dry-hopping an unhopped ale at different dosing rates (0-16g/L) and exposure times (0-72 hours). The Bitterness Unit (BU) and a range of hop components were measured in the dry-hopped beer to determine which specific bitter hop components may have been responsible for dry-hopping bitterness. The commercial survey examined 15 different beers, pre- and post-dry-hopped, from Pacific Northwest breweries. Multiple linear regression was used to predict bitterness and the BU values based on the beer chemistry. While iso-alpha acids were the main contributor to beer bitterness, humulinones (oxidized alpha acids) and polyphenols were also potentially significant contributors to bitterness, particularly in heavily hopped beer. The increase in beer bitterness as a result of dry-hopping was attributed to humulinone extraction and, in some cases, polyphenol extraction. Humulinones were the more dominant bitter contributors from dry-hopping compared to polyphenols. The commercial survey noted a decrease in the total iso-alpha-acid concentrations as a result of dry-hopping in a majority of the samples tested. This is

the first published evidence that dry-hopping may lead to a decrease in iso-alpha-acids in commercial beer.

2.2 Introduction

Dry-hopping is the technique of adding hops during or after fermentation for the purpose of incorporating aroma and flavor from hops beyond that achieved in the boil. While dry-hopping is meant to increase hop aroma in beer, brewers often describe increases in bitterness following this treatment. Given that the process occurs on the cold side of the brewing process, thermal isomerization does not lead to the formation of iso-alpha-acids (IAA) in the added hops. Thus any additional bitterness must come from other sources. Various hop-derived compounds can be extracted from the hops during the dry-hopping process, such as hop oils, acids and polyphenols. Alpha acids do not contribute to the bitterness of beer at the levels one normally finds them in beer (14). Due to their insufficient solubility, beta acids are not incorporated into the beer during boiling or during dry-hopping (43). However, the oxidation products of beta acids and alpha acids, hulupones and humulinones, respectively, may be extracted into the finished beer. Other water-soluble components, such as polyphenols are extracted as well. Humulinones and hulupones are bitter as are some polyphenols (29,35,42,43). For instance, McLaughin, et. al noted that hop polyphenols at concentrations of 100 and 200 ppm – levels previously noted in beer – enhanced the perceived bitterness intensity and astringency of the dosed beers (35). An increase in

bitterness and astringency was also noted in other high polyphenol beverages (e.g. wine, coffee, tea) (9,40,45).

Beer bitterness is measured instrumentally using the International Bitterness Units (BU) assay (2). This technique measures the chemical compounds contributing to bitterness in beer. In most beer, the BU is mainly comprised of IAA but also includes other undetermined bitter compounds. In the brewing process, IAAs are formed from the thermal isomerization of alpha acids and these are the main contributors to the bitterness of beer (43). But in dry-hopped beer, formation of IAA does not occur and the BU increase likely includes other bitter compounds that may be more important to the bitterness increase in dry-hopped beer. The study presented herein identified non-IAA contributors to beer bitterness that are extracted during dry-hopping and gauged their relative impact on the BU measurement and perceived bitterness in dry-hopped beer.

2.3 Materials and Methods

2.3.1 Reagents and Materials

Chinook pellet hops (13% alpha acids, 3.4% beta acids) were donated from Yakima Chief, Inc. (Yakima, WA). All reagents were ACS grade and purchased from Sigma Aldrich (Sigma Aldrich Corporation, St. Louis, MO) and Fisher Scientific (Fisher Scientific International, Inc., Hampton, NH).

2.3.2 Beer Produced for Dry-Hopping

An unhopped pale ale was prepared using the Oregon State University pilot brewery. The malt base consisted of 98.5% of pale ale malt (Great Western Malting Company) and 1.5% acidulated malt (Weyermann Specialty Malting Company). A single temperature infusion mash (68 °C) was used to prepare a 13°P (pH 5.1) wort that was fermented at 18°C with an ale yeast (strain 1056, Wyeast Laboratories, Inc., Odell, OR). The finished beer, with an apparent gravity of 2.53° Plato and 5.2% ABV, was filtered (Sietz HS 2000, Pall Corporation, Germany) prior to dry-hopping.

10 L aliquots of unhopped ale were dry-hopped with Chinook pellets. Hop bags were created using synthetic cheesecloth (Plyban, Dairy Connection, Madison, WI), cut to a length of 57 cm, width of 12 cm, and folded in half lengthwise to form a long hop bag. To minimize flavor contributions from the bags, they were left overnight in samples of the base beer. The hop bags were washed, filled with a specified amount of hops, and heat-sealed to secure the hops within the bags. For each treatment, a bag was placed in a sanitized, CO_2-flushed 20L stainless steel keg, sealed and purged with CO_2 to remove any entrained air in the hop bag. The desired quantity of beer was added to the keg and headspace was purged with CO_2. The dry-hop extractions were carried out without agitation at 18°C for 6, 24, or 72 hours.

At the allotted time, the entire volume of dry-hopped beer was pushed through a stainless steel cartridge (Pall SealKleen Filter Housing, Cortland, NY) that was packed with synthetic cheesecloth into a clean, sanitized, and CO2-purged keg. Filtration ensured adequate mixing of the beer and removed hop particles thereby stopping the

dry-hopping process. The filtered beer was carbonated to 2.8 volumes of CO2 and held at 1°C prior to evaluation.

2.3.3 Commercial Beer Sample Collection

Twelve commercial breweries located in the Pacific Northwest of the United States of America donated 15 commercial beer brands (> 1 L) to the OSU Brewing Science lab. For each brand, samples of the beer were examined pre- and post-dry-hopping. All pre-dry-hopped samples were fermenting or fully fermented when the samples were collected. Some contained visible yeast, so all samples were filtered through a 0.45µm syringe filter before instrumental analyses.

2.3.5 Analytical Procedures

Alpha acids, beta acids, IAA, humulinones and hulupones were analyzed via HPLC according to ASBC methods of analysis (2). With a method adapted from Donley (10), the liquid chromatography analysis (Agilent 1200 Series, Agilent Technologies, Santa Clara, CA) was performed using a C-18 column (Kinetex C-18, Phenomenex) and measuring absorbance at 270 nm. A 7 µL injection was performed using a gradient mobile phase with a flow rate of 1.2 mL/minute through a column heated to 40°C. At 12 minutes, the mobile phase changed from 10% A (100% H_2O) and 90% C (75% MeOH, 24.5% H_2O, 0.5% H_3PO_4) to 100% B (100% MeOH) and at 14 minutes it switched back to 10% A and 90% C. Each sample eluted for 16 minutes. ASBC International Calibration Extract 3 (ICE-3) for HPLC Analysis of Alpha Acids and Beta Acids and the International Calibration Standards (ICS-I3) iso-alpha-acid

standard were used to quantify alpha, beta and iso-alpha acids concentrations, respectively. The humulinone and hulupulone standards were prepared internally by the OSU Brewing Science lab. (Note, the preparation of the humulinone and hulupulone standards is being published in a manuscript by Victor Algazzali that is simultaneously being reviewed by the JASBC.) Bitterness units and polyphenols were measured according to ASBC analytical methods (2) using a PharmaSpec UV-1700 spectrophotometer (Shimadzu Corporation, Columbia, MD).

2.3.6 Sensory Analysis

A panel of 11 participants (7M, 4F) with previous experience on an Oregon State University bitterness panel rated the bitterness intensity using a ten-point scale (0 to 9). The panelists were trained on bitterness scaling using equi-bitter concentrations of quinine, caffeine, and IAA dosed in water and beer. For bitterness evaluation, 30 ml samples of each treatment were presented in 60 ml black, plastic sample cups (Sysco Corporation, Houston, TX) with lids. Samples were blind coded with a 3-digit number and presented in a randomized order for each panelist. Six independent replicate sensory evaluations were performed on each treatment. An online survey software (Qualtrics, LLC, Provo, UT) was used for ballot entries and data collection. The randomization sequence within Qualtrics was monitored for each panelist to ensure uniform and consistent randomization of individual treatments across all panelists. For sensory evaluation of aroma intensity a separate ballot was prepared using the same ten-point scale and randomized through Qualtrics. A separate set of 30 ml samples

were presented to panelists in 150 ml glassware (5 oz. juice glasses, Libbey Glass, Toledo, OH) topped with lids.

2.3.7 Statistical Analysis

Chemical analyses were conducted in duplicate or triplicate while sensory evaluation was carried out with 6 independent replications. ANOVA, multiple linear regression, and correlation analyses were performed using XLStat (Addisoft, Coppell, TX). Multiple linear regression model selection was determined using a "best model" approach with a criterion of maximizing adjusted R^2.

2.4 Results and Discussion

2.4.1 Controlled dry-hopping experiment

Table 1. The bitter components concentrations during the dry-hop extraction in an unhopped beer.

Hop Conc. (g/L)	Extraction time (hours)	BU[a]	Polyphenols[a] (mg/L)	Total IAA[b] (mg/L)	Alpha Acids[b] (mg/L)	Humulinones[b] (mg/L)
0	0	4.5±0.7	111±5.1	1.7±0.03	0.0±0.0	0.0±0.0
4	6	7.0±0.9	128±2.4	1.7±0.01	0.0±0.0	1.0±0.01
16	6	20.0±1.1	177±3.0	1.9±0.05	11.5±0.2	6.8±0.01
4	24	19.0±3.4	193±8.7	1.8±0.07	11.2±0.0	4.6±0.07
16	24	12.5±1.9	156±2.4	1.7±0.01	7.5±0.0	2.7±0.05
4	72	14.0±1.1	185±1.6	1.7±0.01	8.7±0.3	3.3±0.20
16	72	13.0±1.3	211±2.8	1.7±0.02	5.6±0.0	3.6±0.30

[a]Mean of 3 repeated measurements ± 1 standard deviation.
[b]Mean of 2 repeated measurements ± 1 standard deviation.

The bitterness increase resulting from dry-hopping beer was quantitatively characterized by changes in BU, IAA, polyphenols, and humulinones (Table 1). No beta acids or hulupones were found in the dry-hopped beer samples. Beta acids, as noted previously, are insoluble in beer (43) and thus one does not expect to find them migrating from hops into beer. The oxidation products of beta acids, hulupones, are considerably more soluble in beer and theoretically could be found in finished beer. However there is published evidence that hulupones may only be found in low concentrations even in heavily hopped beers (50). Dry-hopping increased the BU measurement between by 2.5 to 15.5 units, which was practically significant given that the unhopped control was only 4.5 BU. Polyphenols, alpha acids, and humulinones significantly increased in the dry-hop treatments compared to the control while the

total IAA remained unchanged. Since no thermal isomerization occurs during dry-hopping one would not expect to see the formation of IAA as a result of this hopping technique.

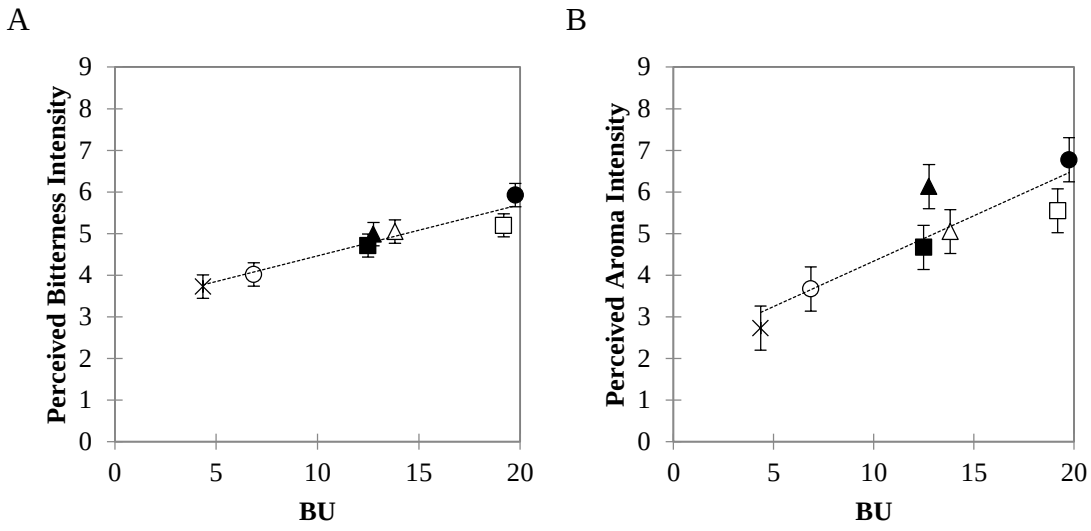


Figure 8. Bitterness units (BU) predict (A) perceived bitterness intensity and (B) perceived aroma intensity for the controlled dry-hopped beers. The dry-hop samples are represented by control 0g/L-72 hours (X), 4g/L-6 hours (○), 16g/L-6 hours (●), 4g/L-24 hours (□), 16g/L-24 hours (■), 4g/L-72 hours (△), 16g/L (▲). Error bars represent ± 1 standard deviation.

An unexpected outcome of the dry-hopping trial was the degree to which the extracted components did not entirely correspond to extraction time and hop dosing concentration. In general the concentration of the extracted hop components increased with dose and time; however, the highest and second highest levels were found in the 16 g/L-6 hour extraction and the 4 g/L-24 hour extraction, respectively. Both of these time points yielded much higher levels of extraction than the longest time point (72 hours) for either hop dosing level. It is unclear to what extent hop sample inhomogeneity or extraction procedure variation contributed to this result.

Nonetheless, the relative amounts of extracted hop components were consistent for all time points in relation to their BU values (Table 1). Although the dry-hopping times and hop dosing levels did not yield extractable concentrations as expected, they did result in beers that represented a broad range of samples that differed significantly in bitterness and aroma intensities. Dry-hopping resulted in significant increases in hop aroma and bitterness (P <0.001). Furthermore, the changes in sensory bitterness intensity were significantly correlated (P = 0.001) to the changes in the BU measurements (Figure 8A). Interestingly, the changes in hop aroma intensity were also significantly correlated with increases in BU (Figure 8B) but to a slightly lesser degree (P = 0.007). While the BU does not measure hop aroma, it is apparent in this experiment that the extraction of volatiles that lead to perceivable hop aroma also correlated with the extraction of nonvolatile bitter components, which contribute to the BU value. Thus, the BU value served as an indicator variable for the extent of hop extraction for both volatile and non-volatile components.

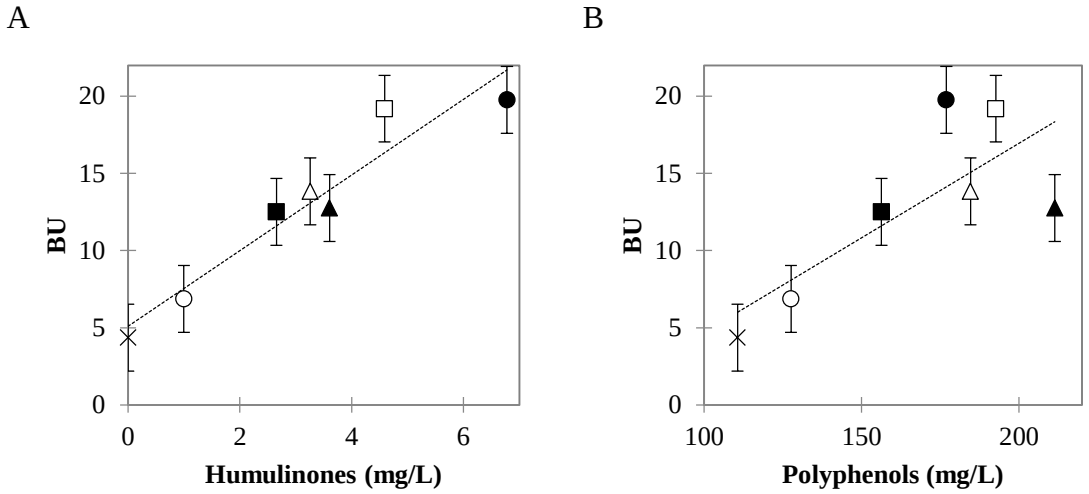


Figure 9. The relationship of (A) humulinones and (B) polyphenol concentration on bitterness units (BU) for the dry-hop samples: control 0g/L-72 hours (X), 4g/L-6 hours (○), 16g/L-6 hours (●), 4g/L-24 hours (□), 16g/L-24 hours (■), 4g/L-72 hours (△), 16g/L (▲). Error bars represent ± 1 standard deviation.

The main goal of this experiment was to identify which extracted hop components were the source of the increased bitterness as a result of dry-hopping. Given that the IAA did not increase, and despite the fact that IAAs are regarded as the chief source of bitterness in beer, these compounds were not the source of dry-hop bitterness in this study. The alpha acid concentration was highly correlated with the BU measurement (P <0.001). However, it was unlikely that the alpha acids could be a source of BU increase because at the concentrations found in beer they were not perceived as bitter (14). Humulinones and some polyphenols have been described as imparting bitterness (35,42,43). Thus their increase as a result of dry hopping may be associated with the increase in BU from dry-hopping (Figure 9). There was a strong correlation between humulinone concentration and BU (P = 0.001) (Figure 9A) as well as perceived bitterness (P <0.0001) (Figure 10A). This is strong evidence that

humulinones may be one source of dry-hop bitterness. The same relationship with polyphenols (Figures 9B & 10B) existed but the correlations were not as strong (*P* = 0.042 & 0.037, respectively).

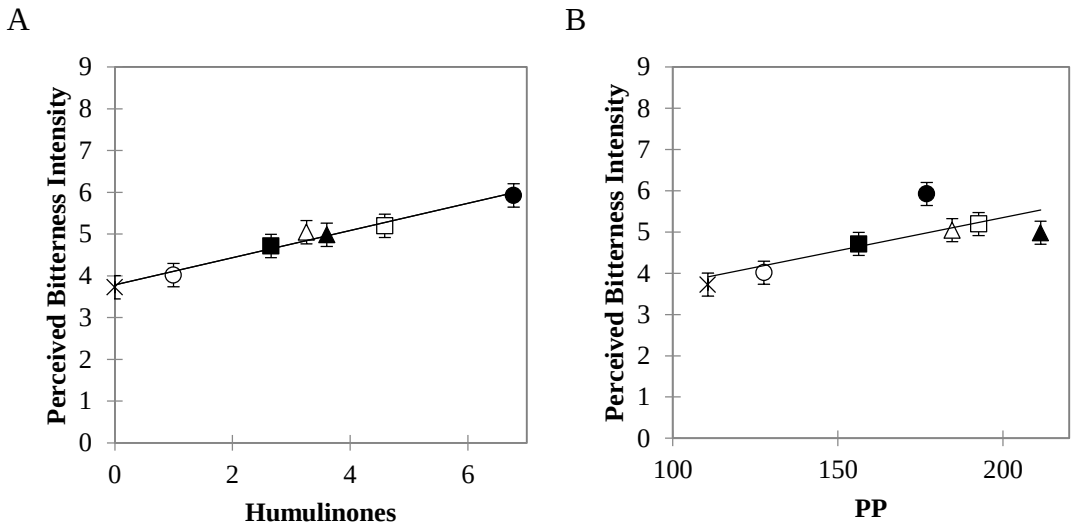


Figure 10. Increases in perceived bitterness intensity are linearly associated with increases in (A) humulinones and (B) polyphenols. The dry-hop samples are represented by control 0g/L-72 hours (X), 4g/L-6 hours (○), 16g/L-6 hours (●), 4g/L-24 hours (□), 16g/L-24 hours (■), 4g/L-72 hours (△), 16g/L (▲). Error bars represent ± 1 standard deviation.

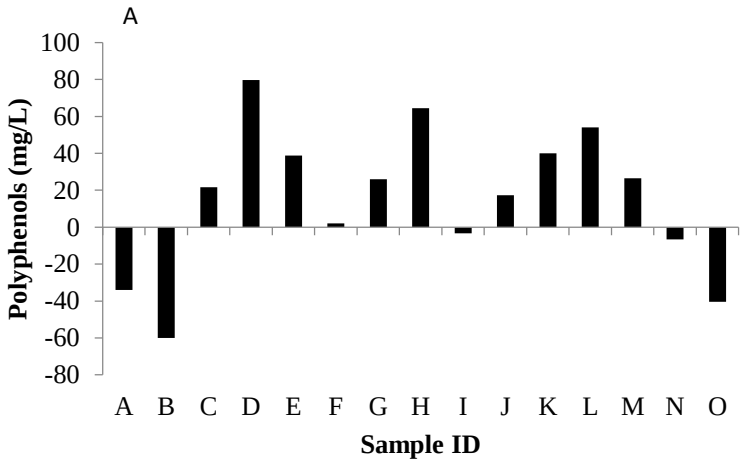
Table 2. A summary of the significant multiple linear regression coefficients for predicting bitterness and aroma in the controlled dry-hopped samples.

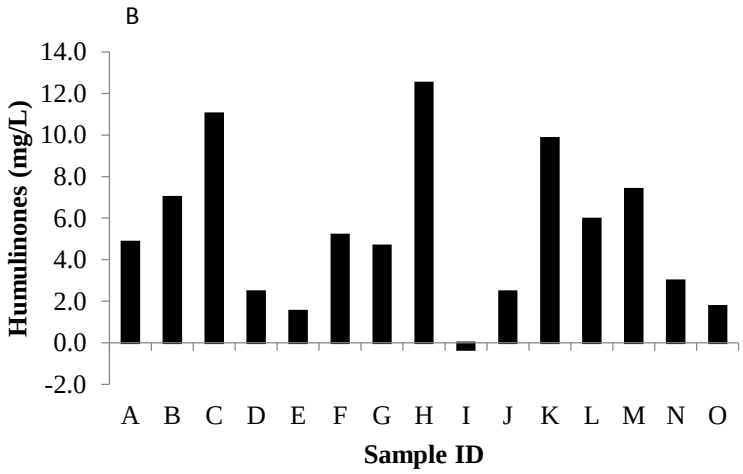
		Independent Predictor Variables			
		Intercept	Polyphenols	Humulinones	R^2
Dependent Response Variables	Bitterness Units	2.372	0.021	2.195	0.929
	Bitterness Intensity	3.495	0.002	0.300	0.985
	Aroma Intensity	1.213	0.015	0.417	0.968

A multiple linear regression statistical analysis was used to examine the relationship between the extracted hop components and measured BU, perceived bitterness intensity, and perceived aroma intensity. Using a “best model” approach,

humulinones and polyphenols were used to predict their importance as contributors to all three dependent response variables (Table 2). Total IAA and alpha acid concentrations were not expected to contribute bitterness in this experiment due to unchanged concentrations and non-bitter contributions at low levels, respectively. Consquently, IAA and alpha acids were intentionally excluded in the models. Taking into consideration the concentration ranges of the humulinones (0 – 7 ppm) and polyphenols (111 – 211 ppm) along with the magnitude of the regression coefficients in Table 2, we estimate that the humulinones had up to a 7 - 10 times greater influence than polyphenols on dry-hop BU and sensory bitterness. That is, an increase of 100 mg/L of polyphenols was predicted to increase the BU value by 2.2 and sensory bitterness by 0.2 (on a 10 point scale) while an increase of 7 mg/L humulinone was predicted to result in a BU increase of 15 and a sensory bitterness increase of 2.2. While both humulinones and polyphenols may contribute significantly and linearly to the BU and sensory bitterness it is the former that is potentially responsible for the majority of the increase.

2.4.2 Commercially dry-hopped beer survey





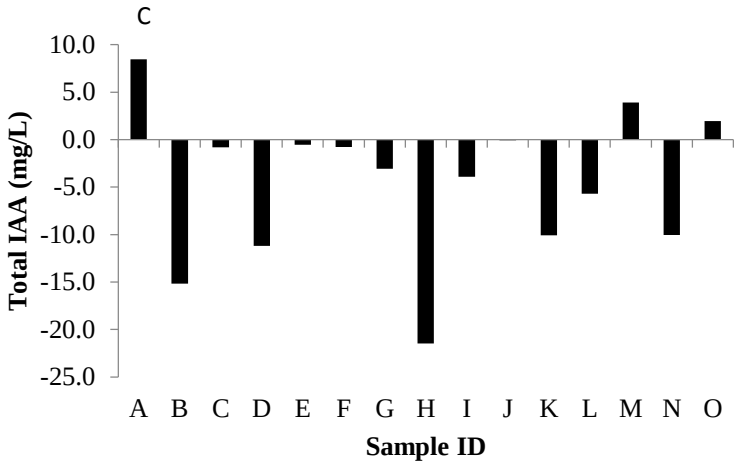


Figure 11. The change in (A) polyphenol, (B) humulinones and (C) IAA concentrations as a result of dry-hopping. Each bar represents the difference between post- and pre-dry-hopping.

Pre- and post-dry-hopped samples of 15 commercially produced beers were compared by examining differences in their bitter components' chemistry. Similar to the observations made in the controlled dry-hop study, the BU increase as a result of dry-hopping was mainly driven by increases in polyphenol and humulinone concentrations (Figure 11). Polyphenols on average increased by 15 mg/L, ranging from 2 to 80 mg/L as a result of dry-hopping with the exception of 5 samples that decreased, ranging from a 3 to 60 mg/L decrease (Figure 11A). Humulinones increased after dry-hopping in nearly all samples by an average of 5.3 mg/L with a range of 1.5 to 12.5 mg/L. There was one exception where there was essentially no change in humulinones concentration (Figure 11B). In contrast to the polyphenols and humulinones, total IAA on average decreased by 4.5 mg/L, ranging from a decrease of 0.5 to 21.5 mg/L with the exception of 3 samples that increased with a range of 2.0 to

8.5 mg/L (Figure 11C). The phenomenon of dry-hopping resulting in decreased levels of IAA in the finished beer has been previously observed dry-hopping on a pilot (3 hL) scale by our lab (53). The mechanism(s) by which IAA are removed from beer during dry-hopping is unknown. It should be noted that the changes in IAA did not correlate with the changes in the BU for most sample sets, which suggested that changes in IAA concentration may not significantly influence the bitterness resulting from dry-hopping. Contrary to the controlled dry-hop study, the BU and alpha acids did not consistently increase in the commercial beer samples; therefore, some samples displayed positive increases while others displayed reductions in both BU and alpha acids. Hulupone levels also lacked consistent trends, furthermore they were found in the smallest concentrations of all measured hop components, ranging from 0.0 to 8.0 mg/L with an average concentration of 3.4 mg/L. The presence of only very low concentrations of hulupones was consistent with previous research that found hulupones were present at low concentrations in beer (50). By comparison, the humulinone concentrations in the commercial beers were considerably higher and ranged from 3.0 to 24.3 mg/L with an average concentration of 11.0 mg/L.

Table 3. A summary of the significant multiple linear regression coefficients for predicting bitterness (BU) in pre- and post-dry-hopped beers.

		Independent Predictor Variables					
		Intercept	Polyphenols	Total IAA	Humulinones	Alpha Acids	R^2
Dependent Response Variables	Pre-Dry-Hop BU	8.303	0.046	0.785	2.000	-0.821	0.857
	Post-Dry-Hop BU	12.012	0.032	0.855	1.201	-0.787	0.766
	Delta BU (Post – Pre Dry-Hop)	2.541	0.000	0.073	0.594	0.000	0.315

Multiple linear regression was used to estimate chemical contributors to BU measurements for the pre- and post-dry-hopped commercial beers, separately. The same approach was used to identify chemical changes that were responsible for the change in BU as a result of dry-hopping (Table 3). When looking at the BU of the beer before or after dry-hopping, both models suggested that polyphenols, total IAA, humulinones, and alpha acids were predictors of the BU measurement, both with a relatively robust R^2. As expected, IAA was a major contributor to the BU value and contributed approximately 2 and 5 times the magnitudes of the BU than did humulinones and polyphenols concentrations, respectively. When comparing the bitter contributions of humulinones and polyphenols, humulinones predicted the BU with a magnitude of 2.3 and 2.4 times higher than polyphenols for the pre- and post-dry-hop BU models, respectively. The negative alpha acid contributions in the models were likely an artifact of the multiple regression analysis trying to achieve the best linear response using the selected independent predictor variables (the bitter hop components). While in general there was a positive but poor correlation between alpha acids concentration and the BU, the regression procedure yielded a negative coefficient for this particular compound in an effort to achieve the best linear fit in the entire model. One should keep in mind that at the levels observed in this study alpha acids contribute negligibly to bitterness (14). Using the pre-dry-hop regression model, that with the highest R^2, to predict a beer's BU using either the pre- or post-dry-hop data yielded an acceptable prediction of the BU (Figure 12). Most of the post-dry-hop BU values were reasonably well predicted by the pre-dry-hop model, however four

beers had a notably higher predicted BU than actual BU. These four samples had exceptionally high polyphenol concentrations, all higher than 300 mg/L and one as high as 370 mg/L. These extreme values may have led the model to predict a considerably higher BU value than actually observed.

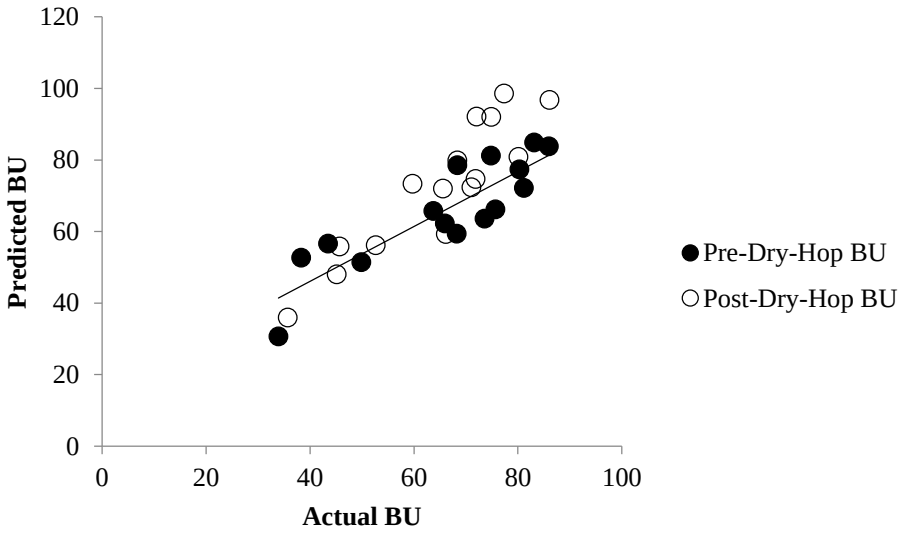


Figure 12. Predicted BU vs. actual BU values using the pre-dry-hop multiple linear regression model. The trendline indicates the slope of the pre-dry-hop BU values.

2.5 Conclusions

This study demonstrated that dry-hopping beer results in an increase in bitterness that can be verified both sensorially and chemically. In a controlled experiment, where dry-hopping took place in an unhopped base beer, the increase in sensory bitterness was heavily correlated with increases in both humulinone and polyphenol concentrations but not with iso-alpha-acid or alpha acids concentrations. When surveying commercial dry-hopped beers, the increase in the BU as a result of

dry-hopping was attributable only to increases in humulinone concentration. For these heavily hopped beers, which had on average 65 BU, 41 mg/L iso-alpha-acids, and 260 mg/L polyphenols, the main source of bitterness was the iso-alpha-acids. Yet, it was evident that bitter components in addition to iso-alpha-acids contributed substantially to bitterness, namely humulinones and polyphenols. The main source of humulinones was from dry-hopping. In contrast to the unhopped beer experiment, the impact of polyphenol extraction during dry-hopping was not as substantial because the levels of polyphenols in the pre-dry-hopped beer were already so high (averaging 247 mg/L). Finally, the total iso-alpha acids concentrations decreased post-dry-hopping in the commercial samples, however the mechanism responsible for this reduction remains unclear.

CHAPTER 3: CONCLUSIONS

The aim of this research was determine why dry-hopping contributes bitterness to beer. Little research has been conducted on the bitterness contributed from dry-hopping. Therefore, this research sought to understand the bitter hop compounds added as a result of dry-hopping.

The controlled dry-hopping experiment investigated bitterness additions from controlled dry-hopping of an unhopped ale by chemical and sensory analysis. Although no trends were noted in regards to the changes in dosage or time exposure among the dry-hopped samples, there was a significant increase in measured BU and perceived bitterness between the unhopped ale (control) and dry-hopped samples (treatment). It is likely that the increase in the bitter components polyphenols and humulinones is associated with the increase in bitterness, both instrumentally and perceived, was noted. Alpha acids also increased and correlate with the increase in BU and perceived bitterness intensity. However, previous research noted alpha acids did not contribute bitterness to the beer (14). This correlation might not be a causation of bitterness. No hulupones were found in the beer, consistent with previous research indicating hulupones were only noted at low concentrations in heavily hopped beers (50). Overall, bitterness was detected from dry-hopping an unhopped ale and increases in humulinones and polyphenols were likely the cause of the bitterness increase in the controlled dry-hopping beers.

The commercially dry-hopped beer survey observed analytical trends in pre- and post-dry-hopped commercial beer samples. Similar to the findings in the first

study, polyphenols and humulinones were noted to increase in a majority of the samples. These findings were supported by the multiple linear regressions and correlation tests that indicated total IAA and humulinones were major bittering predictors to the BU measurements, particularly in the post-dry-hop model. Polyphenols had a lesser contribution from dry-hopping, but were a predictor for the BU measurement. Although IAA are major bitterness contributors to beer, total IAA concentrations decreased in many of the samples post-dry-hopping. This phenomenon might be caused by the hops added during dry-hopping extracting compounds like IAA. The decrease in IAA was noted in previous research of the Shellhammer lab, occurring at low levels (53). Contrary to the observations in controlled dry-hopping experiment, BU and alpha acids did not increase with dry-hopping, possibly due to the lack of control in the second study.

Thus far, bitterness has been described as a combination of BU and IAA (29). IAA is the major bittering component in beer, yet they are not the only bitter compound found in hops. The addition of humulinones and polyphenols during dry-hopping increased bitterness in the final beer. Further, multiple linear regressions suggested humulinones and total IAA were the main contributors and polyphenols as a lesser contributor to bitterness in beer, although the magnitudes for each contributor was not fully developed. The understanding of bitterness needs to encompass not only IAA, but also polyphenols and humulinones.

FUTURE WORK

The understanding of dry-hopped bitterness would allow for brewers to predict bitterness from the compounds added to their beer and provide another level of quality assurance.

To further the understanding of dry-hopped bitterness, the magnitudes of the BU predictors should be determined. The work done in this thesis described IAA, humulinones, and polyphenols as the predictors of BU values. However, further controlled studies could determine more precisely the influence of each predictor. This could be carried out by creating a base beer and dosing the beer with varying levels of IAA, humulinones, and polyphenols. Using the chemical and sensory analysis from these beers, magnitudes of the BU predictors could be established.

The commercially dry-hopped beer survey was a one-time experiment conducted in uncontrolled conditions. The study should be repeated, using several replicates for each beer and a controlled manner of sample collection. This would allow for a more predictable understanding of the bitterness achieved from dry-hopping in a commercial setting.

Other potential bitter contributors should also be investigated. Although humulinones, polyphenols, and IAA are heavily prevalent in dry-hopped beer, other hop components are added during dry-hopping such as the essential oils. The oil has anecdotally been noted as bitter, but it's presence in hops and therefore beer occurs in low quantities and may be imperceptible. This could be examined by measuring the oil contents of commercially dry-hopped beers and dosing an unhopped ale at similar

rates. Comparative measurements of BU and perceived bitterness would determine the oils' bitterness contribution to beer.

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APPENDIX

Table 4. Type III SS ANOVA Summary for the Bitterness Intensity Ratings

Source	DF	Sum of squares	Mean squares	F	Pr > F
Panelist	10	59.215	5.921	2.272	**0.013**
Sample	6	214.591	35.765	13.725	< **0.0001**
Rep	5	16.163	3.233	1.241	0.289

Table 5. Type III SS ANOVA Summary for the Aroma Intensity Ratings

Source	DF	Sum of squares	Mean squares	F	Pr > F
Panelist	10	66.059	6.606	3.190	**0.001**
Sample	6	688.785	114.797	55.440	< **0.0001**
Rep	5	0.583	0.117	0.056	0.998

Table 6. Summary of ANOVA Interactions for Bitterness Intensity Ratings (Type III SS). Values in bold represent probabilities less than 0.05.

Source	DF	Sum of squares	Mean squares	F	Pr > F
Panelist	10	57.929	5.793	2.251	**0.015**
Sample	6	215.433	35.905	13.953	< **0.0001**
Replicate	5	17.534	3.507	1.363	0.239
Panelist*Sample	60	244.668	4.078	1.585	**0.007**
Panelist*Replicate	50	61.263	1.225	0.476	0.999
Sample*Replicate	30	62.119	2.071	0.805	0.758

Table 7. Summary of ANOVA Interactions for Aroma Intensity Ratings (Type III SS). Values in bold represent probabilities less than 0.05.

Source	DF	Sum of squares	Mean squares	F	Pr > F
Panelist	10	61.212	6.121	3.492	**0.000**
Sample	6	681.880	113.647	64.838	**< 0.0001**
Replicate	5	2.510	0.502	0.286	0.920
Panelist*Sample	60	262.536	4.376	2.496	**< 0.0001**
Panelist*Replicate	50	69.449	1.389	0.792	0.839
Sample*Replicate	30	42.741	1.425	0.813	0.747

Table 8. Summary of the correlation matrix p-values for the controlled dry-hopped samples.*

Variables	PP	Total IAA	Humulinones	Alphas	BU	Bitterness Intensity	Aroma Intensity
PP	0	0.706	0.056	0.052	0.042	0.037	0.010
Total IAA	0.706	0	0.047	0.178	0.095	0.085	0.178
Humulinones	0.056	0.047	0	0.005	0.001	< 0.0001	0.001
Alphas	0.052	0.178	0.005	0	0.000	0.002	0.019
BU	0.042	0.095	0.001	0.000	0	0.001	0.007
Bitterness Intensity	0.037	0.085	< 0.0001	0.002	0.001	0	0.001
Aroma Intensity	0.010	0.178	0.001	0.019	0.007	0.001	0

*The values with shading represent probabilities less than 0.05.

Table 9. A summary of the contributions to bitterness of the pre- and post-dry-hop commercial samples.*

Sample	BU	PP (mg/L)	Total IAA (mg/L)	Humulinones (mg/L)	Hulupones (mg/L)	Alphas (mg/L)
Beer A Pre-Dry-Hop	76.0±4.3	270±22.5	40.9±1.0	8.1±0.5	1.5±0.1	3.5±0.1
Beer A Post-Dry-Hop	80.0±1.1	236±3.8	49.4±1.2	13.0±0.0	1.7±0.1	3.6±0.8
Beer B Pre-Dry-Hop	80.5±1.7	289±9.5	47.0±0.6	9.4±0.2	2.5±0.3	0.0±0.0
Beer B Post-Dry-Hop	72.0±1.8	229±6.9	31.9±0.4	16.4±0.4	3.0±0.2	2.4±0.1
Beer C Pre-Dry-Hop	81.0±1.6	290±6.2	34.8±0.9	11.6±0.5	2.6±0.3	0.0±0.0
Beer C Post-Dry-Hop	75.0±0.6	311±4.1	34.0±0.6	22.7±0.4	3.1±0.1	3.2±0.1
Beer D Pre-Dry-Hop	43.5±2.5	160±5.0	49.7±0.3	3.7±0.3	5.7±0.1	6.7±0.0
Beer D Post-Dry-Hop	46.0±0.8	240±8.6	38.5±2.7	6.2±0.2	4.9±0.7	7.4±1.2
Beer E Pre-Dry-Hop	50.0±2.4	174±1.3	40.6±0.1	4.3±0.1	3.8±0.1	6.4±1.0
Beer E Post-Dry-Hop	53.0±1.7	212±3.9	40.0±1.9	5.8±0.2	3.2±0.0	6.1±0.3
Beer F Pre-Dry-Hop	64.0±0.5	241±0.0	55.2±4.2	7.8±0.2	6.7±0.6	15.3±0.3
Beer F Post-Dry-Hop	65.5±.3	243±4.9	54.5±0.5	13.0±0.4	7.8±0.5	19.8±3.5
Beer G Pre-Dry-Hop	73.5±4.0	316±2.5	45.4±0.2	7.8±0.1	3.9±0.2	12.9±0.1
Beer G Post-Dry-Hop	71.0±2.7	342±4.0	42.4±4.2	12.5±3.4	3.6±0.5	12.2±1.9
Beer H Pre-Dry-Hop	86.0±0.2	200±6.8	78.3±4.6	10.0±0.2	8.0±0.0	18.3±0.1
Beer H Post-Dry-Hop	86.0±2.2	265±7.5	56.9±0.2	22.4±0.1	7.9±0.4	16.1±1.6
Beer I Pre-Dry-Hop	68.0±1.9	267±2.5	46.1±0.8	7.0±0.0	5.5±0.0	13.9±0.5
Beer I Post-Dry-Hop	66.0±2.1	264±1.7	42.2±5.0	6.7±0.6	5.1±0.1	9.2±0.6
Beer J Pre-Dry-Hop	34.0±0.7	83±3.0	20.6±2.5	3.0±0.1	0.0±0.0	4.6±1.8
Beer J Post-Dry-Hop	36.0±0.7	101±4.8	20.6±0.2	5.5±0.9	0.0±0.0	5.0±0.2
Beer K Pre-Dry-Hop	83.0±0.5	301±7.9	46.7±0.0	14.5±0.2	1.8±0.2	3.5±0.7
Beer K Post-Dry-Hop	77.5±3.7	341±12.2	36.6±0.8	24.3±0.3	2.5±0.1	3.3±0.4
Beer L Pre-Dry-Hop	75.0±1.4	314±2.3	43.3±0.7	14.2±0.3	1.8±0.1	4.7±0.2
Beer L Post-Dry-Hop	72.0±3.0	369±10.0	37.6±0.2	20.1±0.4	2.1±0.1	3.5±0.7
Beer M Pre-Dry-Hop	66.0±1.5	185±1.1	48.4±2.4	4.9±0.3	1.6±0.1	2.9±0.5
Beer M Post-Dry-Hop	60.0±1.4	212±4.5	52.3±0.6	12.3±1.0	2.7±0.0	12.6±0.7
Beer N Pre-Dry-Hop	68.5±0.5	303±4.1	49.8±5.3	16.1±0.2	3.9±0.2	18.1±1.9
Beer N Post-Dry-Hop	68.5±1.6	296±0.1	39.7±2.2	19.1±0.9	3.3±0.1	13.8±0.2
Beer O Pre-Dry-Hop	38.5±0.9	310±1.2	29.6±1.1	3.5±0.2	1.2±0.1	0.0±0.0
Beer O Post-Dry-Hop	45.0±1.7	270±1.9	31.6±0.2	5.2±0.3	2.2±0.1	9.6±0.0

*values represent the average of triplicate samples ± standard error.

Table 10. Type III Sum of Squares output for the pre-dry-hop multiple linear regression.

Source	DF	Sum of squares	Mean squares	F	Pr > F
PP	1	81.662	81.662	0.887	0.368
Total IAA	1	768.013	768.013	8.342	**0.016**
Humulinones	1	506.968	506.968	5.506	**0.041**
Hulupones	0	0.000			
Alphas	1	232.844	232.844	2.529	0.143

Table 11. Type III Sum of Squares output for the post-dry-hop multiple linear regression.

Source	DF	Sum of squares	Mean squares	F	Pr > F
PP	1	39.369	39.369	0.958	0.351
Total IAA	1	481.007	481.007	11.709	**0.007**
Humulinones	1	555.787	555.787	13.530	**0.004**
Hulupones	0	0.000			
Alphas	1	132.509	132.509	3.226	0.103

Table 12. A summary of the correlation matrix for the chemical data of the commercial dry-hopped beer samples.*

Variables	BU	PP	Total IAA	Humulinones	Hulupones	Alphas
BU	0	0.001	0.002	0.000	0.135	0.545
PP	0.001	0	0.628	0.001	0.800	0.953
Total IAA	0.002	0.628	0	0.488	< 0.0001	0.000
Humulinones	< 0.0001	0.001	0.488	0	0.534	0.617
Hulupones	0.135	0.800	< 0.0001	0.534	0	< 0.0001
Alphas	0.545	0.953	0.000	0.617	< 0.0001	0

*The values with shading represent the probabilities less than 0.05.

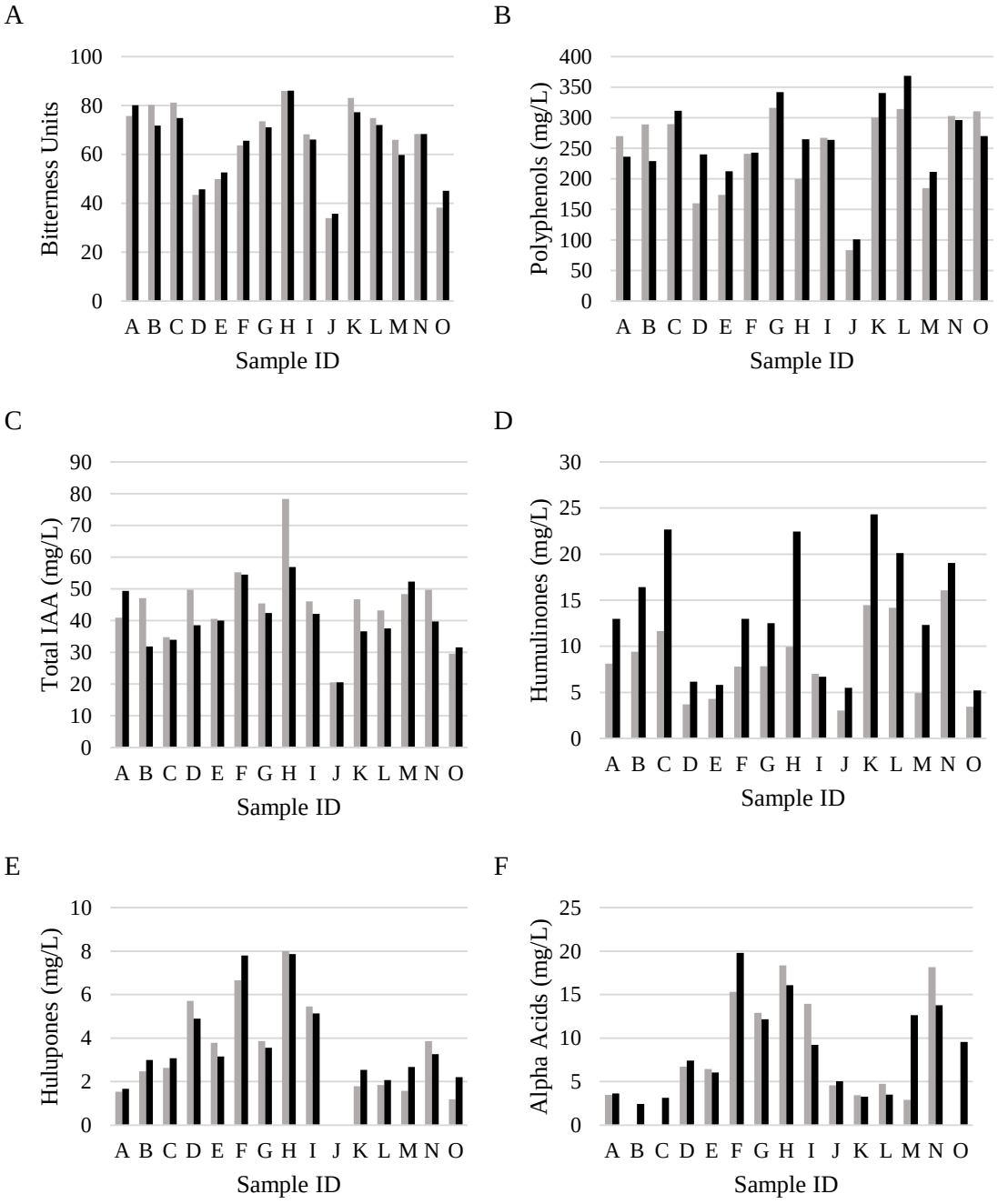

Figure 13. The impact of dry-hopping for pre-dry-hop (gray) and post-dry-hop (black) on (A) bitterness units, (B) polyphenols (mg/L), (C) total IAA (mg/L), (D) humulinones (mg/L), (E) hulupones (mg/L), and (F) alpha acids (mg/L).

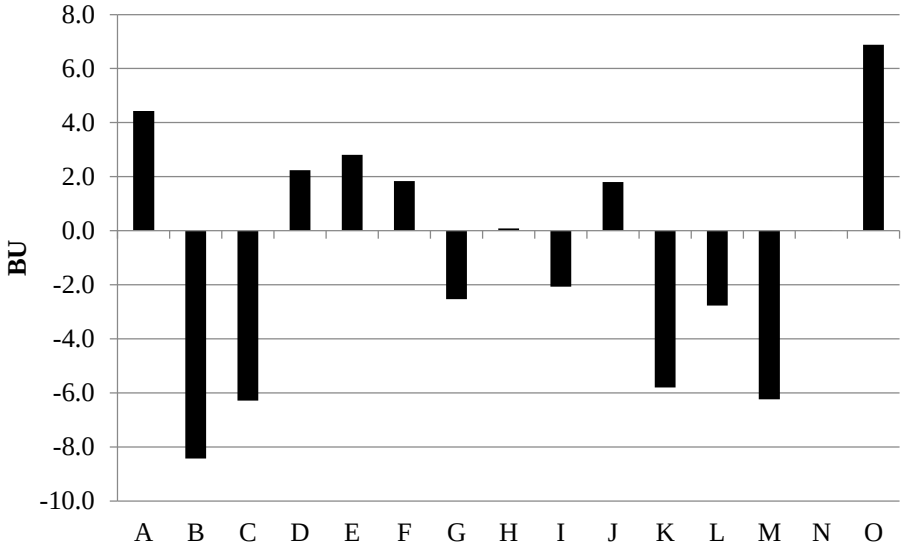

Figure 14. The change in **BU** as a result of dry-hopping. Each value represents the difference (post minus pre) of BU in the pre- and post-samples.

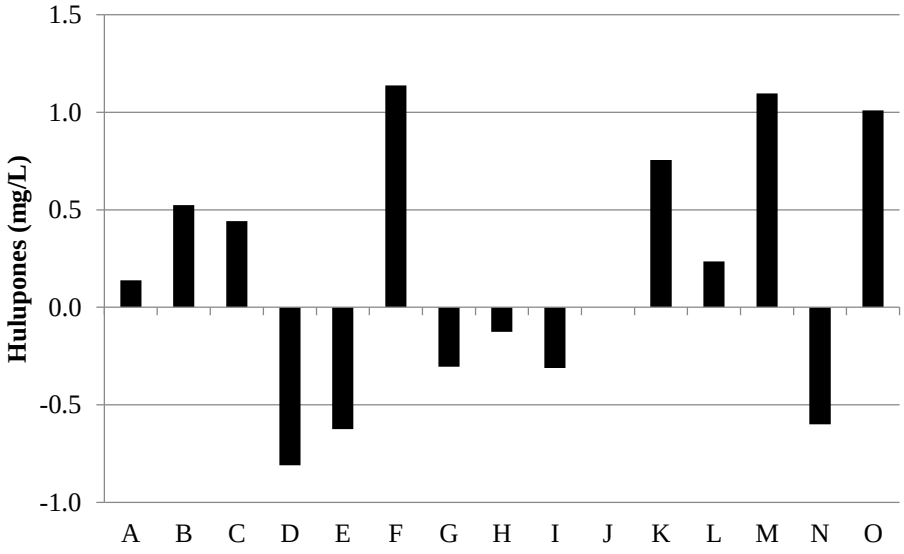

Figure 15. The change in **hulupones (mg/L)** as a result of dry-hopping. Each value represents the difference (post minus pre) of hulupones (mg/L) in the pre- and post-samples.

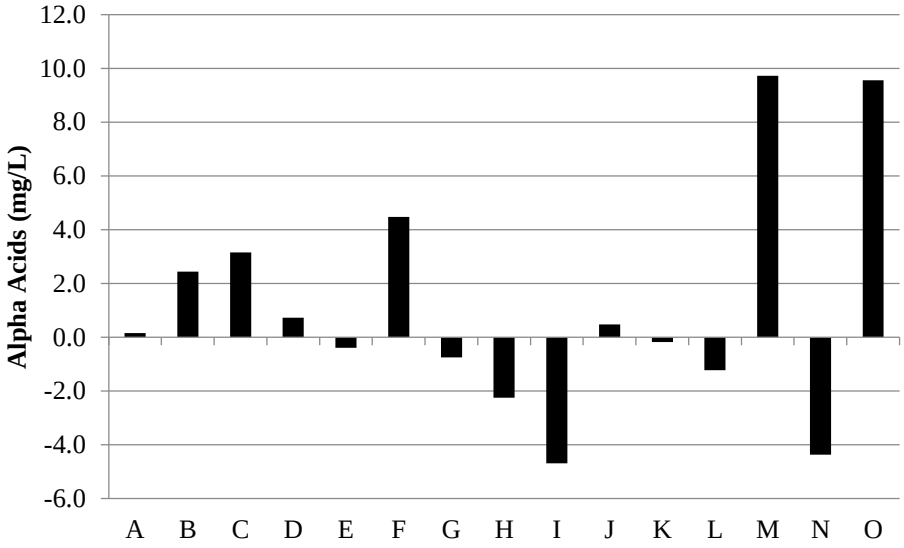

Figure 16. The change in **alpha acids (mg/L)** as a result of dry-hopping. Each value represents the difference (post minus pre) of alpha acids (mg/L) in the pre- and post-samples.