AN ABSTRACT OF THE THESIS OF

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Abstract approved:

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Thomas H. Shellhammer

It is generally recognized within the brewing industry that hop aroma and flavor in beer changes as beer ages post-packaging. Lager beer staling has been the primary focus of research investigations, with dry-hopped beers receiving limited attention. In the interest of exploring dry-hopped beer staling, this study investigated the impact of dissolved oxygen on the sensory and hop volatile profiles of dry-hopped beers during storage. Dry-hopped beer, which was prepared with low dissolved oxygen throughout its production, was dosed with oxygen in a controlled fashion to create beers with a variety of dissolved oxygen levels ranging from low (40-70 ppb), moderate (70-100 ppb), medium (100-150), to high (>150 ppb) and stored under chilled (3°C) and accelerated (30°C) storage conditions. A sensory panel comprised of 17 participants was used to evaluate the impact of staling on beer after two weeks of accelerated storage. Projective Mapping (Napping®) was used to establish unique Euclidian configurations for each panelist, and ultra-flash profiling helped to enrich the product map with sensory descriptors. Sensory results very clearly identified storage temperature, used as a proxy for aging duration, as having the greatest effect on aromatic changes during storage. DO concentration was also observed to have a lesser, but significant, impact at both high and low storage temperatures after only two

weeks of aging. Higher storage temperature and dissolved oxygen concentrations resulted in a loss of tropical, citrus and hoppy characteristics and the production of malty, dried fruit and cardboard aromas. Analytically, haze and color increased with increasing DO, while bitterness units (BUs) decreased. Hop derived monoterpenes were not significantly affected by treatment temperature or dissolved oxygen, suggesting stale character expression from alternate sources (lipid oxidation, and/or Strecker aldehydes).

Evaluating the Impact of Dissolved Oxygen and Aging on Dry-Hopped Aroma Stability in Beer

by
Bradley M. Barnette

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APPROVED:

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Major Professor, representing Food Science and Technology

_____

Head of the Department of Food Science and Technology

_____

Dean of the Graduate School

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

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Bradley M. Barnette, Author

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CHAPTER 1 – LITERATURE REVIEW

1. Brewing Process

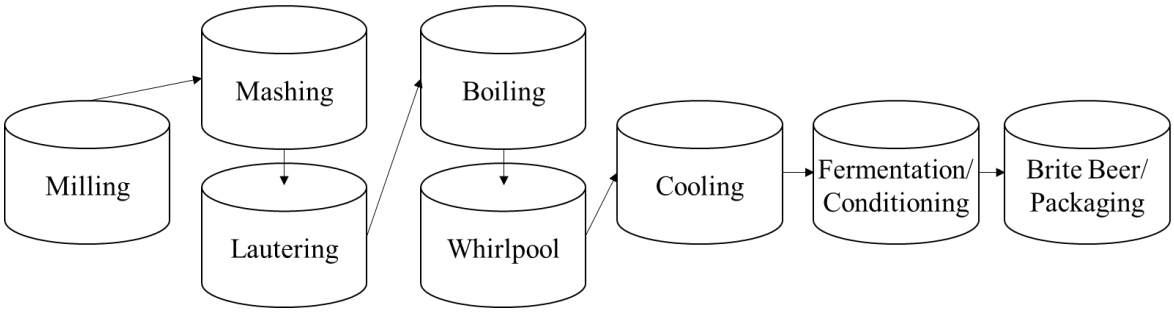

Figure 1. Brewing process overview

Brewing is a process of transforming four raw ingredients into a finished alcoholic beverage. To begin, malted barley is milled into grist to expose the starch-rich endosperm during the process of mashing. Mashing is the step in which hot water is added to the grist, and enzymatic hydrolysis of the starch forms the sweet liquid known as wort. Wort separation from the spent grist occurs in the lautering vessel. The liquid wort is then diverted to the boil kettle where it is boiled to sterilize the product. Hops are typically added in the boil, and isomerization of alpha acids present in hops occurs to form the bitter iso-alpha acids. Evaporation concentrates the wort, and both flavor and color develop over the course of the boil. Boiled wort is then passed through the whirlpool vessel to separate solids, and hop material from the liquid, and is cooled to fermentation temperature. Yeast is pitched into the fermentation vessel that has been filled with cool wort. After fermentation completes, the beer is separated from the yeast and undergoes conditioning to further stabilize the product. Following a clarification step, the now

brite beer is moved to a tank for carbonation, blending, and final packaging. The final step is for the packaged beer to be distributed and enjoyed by consumers.

2. Hops

2.1 Hops and their Brewing Value

The cultivated hop (*Humulus lupulus* L.) plant is a perennial, dioecious, climbing vine and is one of two genera in the Cannabaceae family (the other being *Cannabis*) [1]. Hops have a variety of uses, the most familiar being the use of mature female flowers, or "cones," in brewing [1]. Hops are commonly known for imparting a bitter quality, and microbial stability to the beers in which they are incorporated, and a large body of work has been compiled around the non-volatile chemistry of hops [2-6]. While bitterness is an important contribution to beer, recent trends in the industry suggest that the "hoppy" aroma provided by hops has gained popularity. Aroma intensity is dependent on brewing practice, and dosing level during production [7]. For instance, late-hop addition refers to a hop addition at the end of boil or in the whirlpool where the hops are subject to a temperature treatment, without unnecessary evaporation of many valuable aroma compounds [5]. Flavor results from late-hopping versus dry-hopping regimes are different and often in craft brewing, extreme hopping additions in total of about 500–800 g/hL can be applied [5]. Hop essential oil is the primary carrier for a majority of the constituents related to hop aroma [8]. Oil generally contains monoterpenes, sesquiterpenes, oxygenated terpenoids, and sulfur-containing compounds [5, 8]. While difficult to evaluate, it has been determined that interactions between different hop compounds can have additive, synergistic, or even masking effects regarding sensory perception [5].

2.2 Kettle Hopping

During beer production, wort extracted from the grain mash is transported to the boil kettle for further processing. Target temperatures of the kettle are approximately 100°C, and subsequently produce a vigorous rolling boil inside the vessel [9]. Over the course of 60-90 minutes, the hot wort undergoes several chemical and physical changes: sterilization, concentration by evaporation, volatilization of undesirable aroma compound precursors, and when hops are added, extraction and isomerization of bitter hop acids [9]. Hop additions in the kettle are primarily targeted for adding bitterness to the final beer [9]. Extended boiling time yields more efficient extraction and utilization of the hop bitter acids [9]. While long boiling times result in greater production of isomerized alpha acids it also results in volatilization of many compounds that contribute to the hoppy aroma in beer [9]. In fact, evaporation rate is a key performance index for wort boiling [9].

2.3 Late Hopping

Late hopping techniques offer brewers the ability to combat losses of desirable aroma compounds during wort boiling. A whirlpool vessel is commonly found in production brewhouses, and, as the name implies, it produces a swirling action via a tangential inlet as the wort is pumped to the vessel from the kettle [10]. Temperature, and contact time are both less than that experienced during the kettle boil. Other techniques include use of a hop jack, which is an open vessel that is filled with hops and hot wort from the whirlpool is dispersed over the top of the hop material [9]. As with the whirlpool vessel, temperature and contact time are reduced in comparison to the kettle additions. Late hop additions offer better aroma compound extraction and retention with lower bitter hop acid extraction and isomerization compared to a kettle addition.

2.4 Dry-hopping

Dry-hopping is a technique used by brewers to impart delicate flavor and aroma characteristics to beer. Different from kettle hop additions, which occur during wort production, dry-hopping relies on the addition of hop material following primary fermentation and is a cold extraction of hops in beer [10]. Dry-hopping is typically carried out at the very end of fermentation or post-fermentation. Carbon dioxide generation by yeast during primary fermentation makes aroma hop additions during this time less desirable. The potential for volatile stripping is at a high point during this part of beer production. Dry-hopping is not standardized across the industry, and it is common for brewers to experiment with methods that best suits their brewing practices. The duration of hops extraction, temperature of extraction and degree of mechanical action will play a factor in the final flavor of the beer [10]. A point of concern with dry-hopping is the inclusion of "stowaway" oxygen in the hops during their addition to a fermentor or bright beer tank. The method of addition should be such that oxygen ingress is reduced as much as possible. Work done by Wolfe et al. provides an in depth review of pilot scale optimization for dry-hopping extraction techniques [11]. This work serves as a strong preliminary review, while additional investigation is required to evaluate the impact of temperature, dosage rate, and dissolved oxygen presence during the dry-hopping process.

3. Beer Aging

3.1 Dissolved Oxygen and Beer Staling

Beer flavor stability is an ongoing challenge facing brewers for all types and styles of beer [12]. Achieving low oxygen concentration throughout the brewing process has become a primary focus for brewers operating larger, packaging breweries [12]. Access to reliable and durable instrumentation has made it easier for breweries of all sizes to track oxygen concentrations in the cellar and post-packaging. The development of optochemical or

luminescent dissolved oxygen sensors in portable devices has relieved the strain and inconsistency from former membrane-based devices. Oxygen has a relatively high solubility in water, with a value of 8.9 mg/L at 20°C, 1 atm [12]. As temperature increases, or dissolved solids concentration increases, oxygen becomes less soluble [12]. Based on these criteria, beer is at its most vulnerable state between completion of fermentation, and final packaging.

Oxygen in its ground state (O_2) is not the primary concern when evaluating its potential to react in the beer matrix [13]. Generation of reactive oxygen species poses the greatest risk to flavor (in)stability [14]. Transition metal ions are attributed to the generation of these reactive oxygen species [14-18]. In particular, the negative impact of copper and iron has been extensively explored, specifically in the beer matrix [14-17]. Manganese has also been found to elevate staling through the generation of reactive oxygen species [19]. The presence of ferrous iron (Fe2+) in beer, oxygen can capture an electron and form the superoxide anion (O2-) Fe3+ [17]. It is believed that Cu+/Cu2+ and Fe2+/Fe3+ ions are part of a mixed function oxidation system in which polyphenols, sugars, iso-alpha acids, and alcohols might act as electron donors [20]. The superoxide anion can also be reduced by Fe2+ or Cu+ to the peroxide anion O_2^{2-} [17]. In beer, this anion is readily protonated to hydrogen peroxide (H_2O_2). Hydroxyl radicals (OH^-) can then be produced from H_2O_2 or the superoxide anion O^{2-} by metal-induced reactions, such as the Fenton and the Haber–Weiss reaction (Figures 1 & 2) [17].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\cdot}OH + OH^-$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe + O^{2-} + 2H^+$$

$$net: 2H_2O_2 \xrightarrow{Fe} {}^{\cdot}OH + OH^- + O_2^- + 2H^+$$

Figure 2. Fenton reaction ((adapted from Kaneda et al. (1992) [20])

$$Cu^{2+} + O_2^- \rightarrow Cu^+ + O_2$$

$$Cu^+ + H_2O_2 \rightarrow Cu^{2+} + {}^{\cdot}OH + OH^-$$

$$net: O_2^- + H_2O_2 \xrightarrow{Cu} O_2 + {}^{\cdot}OH + OH^-$$

Figure 3. Haber-Weiss reaction (adapted from Kaneda et al. (1992) [20])

3.2 Additional Potential Pro-Oxidant Sources

Raw materials for beer production have been shown to have pro-oxidant potential when evaluated for their contribution to the beer matrix. In a study of pale malts carried out by Woffenden et al., the pro-oxidant activity of the malt extracts has been partially attributed to Maillard reactions and the associated Maillard-derived flavor compounds [21]. More highly kilned malts provide a source of Maillard-derived flavor compounds and will vary in composition depending on the kilning process carried out by the maltster. Use of higher kilned specialty malts may shorten beer flavor shelf life. Additionally, other hypothesized sources of pro-oxidant activity are flavonoids, and proanthocyanidins [21]. Proanthocyanidins and flavonols also have the potential to act in the formation of reactive oxygen species, thus acting in a pro-oxidant manner [22]. Redox cycling of phenolics can be catalyzed by cations such as iron and copper to result in ROS that are capable of altering lipids, proteins, enzymes, and other biological molecules [22-23].

4. Anti-Oxidants in Beer

4.1 Sulfur Dioxide

Sulfur dioxide is a very powerful antioxidant that is found in beer. Origins of sulfur dioxide include yeast metabolism, addition of raw materials that contain sulfur dioxide as a processing aid/preservative, or from finings agents added to beer [24]. Sulfur dioxide can delay the staling of beer by scavenging ROSs and acting as an antioxidant or forming hydroxysulfonates and stepwise disulfonates with carbonyls through bisulfite ions (HSO^{3-}) [25]. Kaneda et al. suggested that the optimal SO_2 content of beer to be 8–9 mg/L [26]. Work performed by Bushnell et al. has shown that sulfur dioxide in low concentrations appears to be acting more as an antioxidant than a carbonyl binder [27]. Additionally, the results of the study suggest that low sulfur dioxide (below 10 mg/L) has a limited ability to slow beer aging as compared to storage at low temperatures [27]. United States federal regulations require label declarations for beer containing SO_2 concentrations above 10 mg/L [25].

4.2 Hop Components

Certain components of hops (*Humulus lupulus*) have been reported to have antioxidant potential in beer. Alpha acids and iso-alpha acids have been shown to interact with transition metal ions, thus reducing the ions ability to further catalyze reactions such as Fenton and Haber-Weiss [28]. While not directly able to scavenge highly reactive hydroxyl radicals, the ability to lessen the impact of iron makes alpha acids and iso-alpha acids an important factor in increased flavor stability [28]. Further research is needed to understand the role of hop acids on additional metal ions such as copper and manganese, as these ions are also able to contribute to the Fenton and Haber-Weiss reaction schemes.

4.3 Yeast

To ferment wort produced into finished beer, yeast (*Saccharomyces cerevisiae, or Saccharomyces pastorianus*) are used to convert glucose, fructose, maltose, and maltotriose into ATP, carbon dioxide and ethanol. Yeast rely on oxygen during the start of fermentation to

produce sterols and unsaturated fatty acids needed to support the cell membranes as they grow and replicate [9]. The requirement for oxygen during early phase fermentation demonstrates the oxygen scavenging ability of yeast in the beer matrix. When utilizing bottle conditioning as a production technique, priming sugar and yeast are added to finished, fermented beer [9]. In contrast with bright beer where yeast has been removed by filtration or centrifugation, bottle conditioned beer exhibits more antioxidant potential and reducing power based on the inclusion of yeast in the packaged product [29-30].

5. Accelerated Aging

Accelerated aging protocols are a necessary tool to rapidly assess the impact of extended storage. Often, it is difficult to completely evaluate a product by means of natural aging prior to its release to the public. Storage at 20°C, as evaluated by François et al., over a course of 9 months is a common timeframe [31]. The goal of accelerated aging at higher temperatures is to simulate extended duration of normal/typical beer storage, within a reasonable, condensed timeframe. It should be noted that the selected storage temperature will have an impact on the sensory characteristics expressed. Work by Kaneda et al. (1995) reveals that lager beer aging temperatures of 25°C results in expression of more caramel-like characteristics, compared to aging temperatures of 30°C or 37°C which yield cardboard notes [32]. The degree to which the temperature is elevated should be considered to ensure the impact of the sample treatment is still captured. Across brewing literature, a variety of different approaches to accelerated aging models have been proposed. Example studies performed by François et al. and Heuberger et al. have utilized temperatures between 37 and 40°C for accelerated aging to achieve end of shelf life examples of the product [31, 33].

5.1 Arrhenius Model (Q10)

The Arrhenius model is frequently used to relate rate of a chemical reaction to a change in temperature in a variety of food systems. The simplified model known as Q10, takes the Arrhenius model and uses a ratio of reaction rates when the temperature is changed by a fixed increase or decrease in temperature of 10°C.

$$Q_{10} = \frac{reaction\ rate_{T+10°C}}{reaction\ rate_{T}}$$

Figure 4. Q_{10} reaction equation

This type of model is frequently used to help determine shelf life length for products, by providing a value for the factor of change in reaction rate based on the temperature increase. Demonstrated by Bamforth et.al, Q10 values ranging from 2-3 have been suggested for accelerated aging trials in a beer model [12]. Thus, assuming a Q10 of 3, storing beer at 30°C for 2 weeks is equivalent to 6 weeks at 20°C, 18 weeks at 10°C or 54 weeks at 1°C.

6. Sensory Evaluation

6.1 Descriptive Analysis

Descriptive Analysis (DA) is a staple methodology in sensory evaluation. One method consists of panelist training whereby the panel develops a consensus language for the perceived attributes in the product, and multiple data collection sessions involving intensity-based scaling for each of the agreed upon attributes [34]. Panelist are qualified based on their ability to discriminate among the samples that are being tested, and requirements will vary depending on the design of the study [34]. In contrast to the Projective Mapping (Napping®) methodology, which will be described next, DA can provide feedback regarding intensity differences for multiple descriptors among the products being evaluated. Projective Mapping (Napping®) alone

is not able to produce these differences. While DA is a staple methodology, it is quite time intensive to establish and maintain a panel, especially if panels are not successive. Projective Mapping (Napping®) has the appeal of utilizing a single session to generate the unique map configuration for a panelist, while still providing a high level of discrimination between samples.

6.2 Projective Mapping/Napping®

Rapid sensory evaluation has gained popularity for its ability to collect types of product data in a quick and cost-effective manner. Projective Mapping (or Napping®) is one technique used to accomplish rapid sensory evaluation. Panelists are provided with a two-dimensional space such as a piece of poster board, or a digital representation of a poster board on which they will generate Euclidean coordinates for each sample [35]. Samples are all provided simultaneously and are blind coded. Panelists are instructed to place samples perceived as similar in close proximity to one another, and dissimilar samples are to be placed apart from one another. The simultaneous processing of all these configurations provides a graphical display of the products in which two products are near if they were perceived similar by the whole panel of subjects, each panelist having used and weighted their own criteria according to their own way [35]. While effective at describing differences between samples, it should be noted that Projective Mapping (Napping®) alone is unable to provide scalar data to quantify the differences in attributes associated with the samples [35-37]. By pairing Projective Mapping (Napping®) with a method using free-text comments, such as ultra-flash profiling, panelists can enrich their Euclidean configurations of each sample based on unique criteria.

6.3 Ultra-Flash Profiling

Ultra-flash profiling is a descriptive method which provides quick access to relative sensory positioning of a set of products [38]. This method requires that panelists describe the simultaneously presented samples based on their own criteria, and assign free-text comments to

each of the samples [36]. In contrast to traditional Quantitative Descriptive Analysis, panelists are not asked to score the attributes that they assign. When paired with Projective Mapping (Napping®), the panelists perform a more holistic sensory task.

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CHAPTER 2 – EVALUATING THE IMPACT OF DISSOLVED OXYGEN AND AGING ON DRY-HOPPED AROMA STABILITY IN BEER

Bradley M. Barnette, and Thomas H. Shellhammer

Keywords: hops, beer, stability, staling, dissolved oxygen

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Introduction

Flavor deterioration in packaged beer is an ongoing challenge faced by brewers. Numerous reactions have been related to flavor deterioration, but oxidation has been identified as one of the most important [1]. It is widely agreed that maintaining the lowest possible dissolved oxygen (DO) in packaged beer is critical for reducing the chemical reactions responsible for oxidation and general flavor deterioration. A large body of work has been carried out on the flavor stability of lager beer, and it provides a good foundational understanding for beer oxidation [1-5]. However, there is little published research on the effects of oxygen and hop aroma stability.

Beer flavor and aroma are comprised of hundreds of chemical compounds that all interact to form a perceived response to the consumer. As beer ages, its fresh aroma diminish and are replaced with cardboard, sweet, and caramel aromas [6]. Increased concentrations of (E)-2-nonenal have been attributed to an elevated sensory perception of cardboard flavor, and is typically attributed to aged beer aroma [6]. Regarding analytical markers of flavor degradation, it has been shown that aldehyde markers (such as furfural, 2-methylpropanal, 2-methylbutanal, 3-methylbutanal) correlate with increased sample age [4]. The formation of aldehydes, such as hexanal, from lipid oxidation also correlates with increased sample age, but not to the degree

expressed by the aforementioned aldehyde markers [1, 4]. Introduction of oxygen into the production stream is considered a major contributing factor to general beer flavor and aroma deterioration. While oxygen in its ground state is not particularly reactive, upon conversion into a reactive oxygen species, ROS, (such as a hydroxyl radical) it can have a significant impact on flavor [7-8]. Transition metal ions are attributed to the generation of these reactive oxygen species [1, 9-12]. The negative impact of copper and iron has been extensively explored, specifically in beer [1, 9-11]. Manganese has also been found to elevate staling through the generation of reactive oxygen species [13]. Ground state oxygen present in the beer matrix is converted to a reactive oxygen species via the Fenton, and Haber-Weiss reactions [1, 14]. In lager beer, it has been suggested that DO levels should be limited to ≤ 50 ug/l (ppb) in finished, packaged beer to reduce negative effects on flavor as well as haze stability and thus obtain maximum flavor shelf life [15]. However, dry-hopped beers pose a challenge to brewers because there is a higher potential for DO to enter the product stream during cold-side processing, principally via hop additions. This conversion to ROS is catalyzed by iron and recent research by Porter et al., showed that significant quantities of metal ions (including iron) can arise from dry-hopping [16]. Taken in combination, these findings indicate that the dry-hopping process can drastically impact flavor stability in packaged beer.

Using a sensory-directed approach, the goal of this investigation was to obtain an understanding of how dissolved oxygen impacts dry-hopped beer flavor stability and establish a baseline chemical analysis for fresh and aged samples. Understanding the degree to which dissolved oxygen affects dry-hopped beer chemistry will help brewers improve beer quality in terms of hop aroma stability and offer insight to the magnitude of its impact.

Experimental

Oxygen Dosing Process:

Dry-hopped base beer for the study was a commercial brand of pale ale that was procured from a local craft brewery at the time of packaging. Half-barrel stainless steel kegs filled with the beer were transferred to Oregon State University and immediately analyzed for starting dissolved oxygen (DO) content using an Orbisphere 3100 (Hach, CO). During transfer to 1/6-barrel vessels, the beer was dosed with a blend of sterile filtered air and carbon dioxide using an in-line infusing vessel (Pall Corporation, NY) to achieve four different levels of dissolved oxygen: low (40-70 ppb), moderate (70-100 ppb), medium (100-150), and high (>150 ppb). Replicate gas infusion treatments were carried out to yield 9 beers: 2 reps of low, moderate, medium, and high, plus an untreated base beer. Following transfer into 1/6-barrel vessels, the total dissolved oxygen content was measured and this value was treated as the packaged beer DO concentration. An un-dosed base beer served as a control. One set of 1/6-barrel kegs were stored cold (3°C) and another at 30°C for accelerated aging (Figure 5). Upon reaching 14 days of aging, all samples were moved into cold storage (3°C) until they were evaluated.

Sensory Analysis:

Two panels consisting of 10, and 7 panelists respectively, were recruited. Inclusion criteria for the panel was as follows: panelists were required to have experience evaluating beer aroma, and previous experience on a descriptive panel. Panelists were trained to perform ultra-flash profiling and Directed Projective Mapping (or Napping ®) [17-18]. A room with neutral stimulus was selected for panel execution, and conditions were held consistent across the three training sessions, and final data collection session. Panels were held at two locations, and conditions were replicated at each location. The panel consisted of 10 individuals from Oregon State University, and 7 individuals from a local craft brewery. Separate group sessions were held

for each panel, as panelist availability was varied between the two locations. Panel composition was as follows: 10 males, 7 females, age range: 22-56. To create sensory standards for training, the same brand of beer was forced oxidized at 30°C prior to commencing the study, and freshly packaged product was obtained on the day of the panel. These samples served as an end-point or minimum/maximum staling examples. The "control" (un-dosed, control beer), and the abused control (dosed with 440 μg/L DO and stored at 30 °C) were provided as external standards during the panel evaluation of the aged product. The panel utilized ultra-flash profiling to develop a large pool of descriptors during three, one-hour training sessions [19]. At the end of the third training session, panelists were able to group similar terms, and achieve a selection of 11 descriptors that best represented the observed differences. All 11 descriptors were retained, and no terms were removed. Preliminary evaluations of the beer treatments revealed a range of sweetness. While aroma was the primary focus of the evaluation, panelists felt that inclusion of sample sweetness would provide additional insight. To accomplish this, the x-axis of the projective mapping space was anchored with perceived sweetness (low to high) and this led to the selection of a Directed Projective Mapping approach. The directed Projective Mapping ballots were generated using Compusense Cloud (Compusense Inc., Canada). Panelists were presented with a poster board (22" h x 28" l) so they could carry out spatial placement of the samples prior to using the digital ballot. All eight samples were presented simultaneously, with three-digit random codes associated to each sample. Black plastic cups were used to minimize bias based on sample appearance. General randomization was utilized for the samples due to the inherent shuffling of the cups during evaluation. Replicates for each DO treatment were represented by the low and high temperature samples, respectively. Once spatial placement was complete, the panelists were asked to enrich their spatial maps by assigning terms from the

agreed upon lexicon to each of the samples. Outputs for the directed projective mapping included X and Y coordinates for each sample, and a frequency table outlining terms used for each sample (Table 1). Data were analyzed using XLSTAT (Addingsoft Co., NY). Multiple Factor Analysis (MFA) was used to analyze the X and Y coordinate data for each of the treatments, and Correspondence Analysis (CA) was used to evaluate the frequency of response of the terms assigned by the panelists.

Analytical Analysis:

Reagents and Standards

Ferric ammonium citrate (green) was purchased from Fisher Chemicals. Ethylenediamine-tetraacetic acid disodium salt, dihydrate (EDTA), medium viscosity carboxymethyl-cellulose, and octyl alcohol were obtained from Sigma-Aldrich Chemical Co (St. Louis, MO) [20]. HPLC grade methanol was obtained from VWR International, BDH analytical (West Chester, PA, USA). Hydrochloric acid, 2,2,4-trimethylpentane, phosphoric acid, and ammonium hydroxide obtained from Avantor performance materials (Center Valley, PA) [20]. DCHA-Iso ICS-I3 was obtained from ASBC. DCHA humulinone standard was produced [21] and pure standards were obtained through Robert Smith at S.S. Steiner, Inc [20].

Hop Acids Analysis

The concentrations of hop acids in each beer sample were analyzed using a modified version of ASBC method Beer-23E [29]. The conditions were modified as follows: a 2.6 um EVO C-18 100 A LC column 100 × 4.6 mm^2 column was used (Phenomenex, Torrance, CA) at 40 °C measuring absorbance at 275 nm for the isohumulones and humulinones [20]. Beers were degassed prior to being loaded into sample vials and 7 μL of each beer sample was injected into a mobile phase containing 10% reagent water (reagent A) and 90% (75% MeOH, 24% H2O, 1%

H3PO4) (reagent C) [20]. The instrument maintained a flow rate of 1.6 mL/min. A mobile phase gradient program was employed with the following parameters: ten minutes following sample injection, the mobile phase was shifted to 100% (100% MeOH) (reagent B), and at 14 min post injection, was switched back to 10% reagent A and 90% reagent C.

Basic Beer Chemistry Analysis

Total polyphenols were measured spectrophotometrically, adhering to EBC Analytica method (9.11) [28]. Bitterness units were measured according to ASBC methods of analysis Beer 23A. Spectrophotometric analysis for both total polyphenols and bitterness units were carried out using a Shimadzu PharmaSpec UV-1700 spectrophotometer, Shimadzu Corporation (Columbia, MD).[20] Ethanol, real extract, color, haze (S25/S0 and S90/S0), and pH were analyzed using an Anton-Paar DMA-4500 Alcolyzer with supporting pH module (Anton Paar USA, Ashland, VA). Metal ions (Fe, Cu, Zn, and Mg) were evaluated at a separate Oregon State University lab utilizing an Agilent 5110 ICP-OES. Sulfur Dioxide (SO_2) was measured by an external lab, in accordance to ASBC- Beer-21A- *p*-rosaniline method [29].

GC-MS Analysis

Hop aroma in beer were measured using Headspace-Solid Phase Micro Extraction (HS-SPME) was performed on the treatment beers using a 1 cm 24-gauge divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) Stableflex fiber with 30/50 mm coating thickness (Supelco, Bellefonte, PA) [29-30]. An 8mL sample of each was placed into a 20-mL screw top amber vial with 3 g sodium chloride. The compound 4-octanol (911 μg/L) was used as an internal standard and added to each vial. A Multipurpose auto sampler (MPS2; Gerstel, Mülheim, Germany) was used for pre-incubation, stirring, extraction, and injection. Samples were preincubated for 15 min at 30°C and adsorbed by piercing the vial septa and exposing the fiber to the headspace for 45 min with agitation. After adsorption, the fiber was

desorbed into the GC sample inlet (splitless mode, 250°C) for 10 min. The analytical column was a 30m x 250 mm x 0.25 mm Zebron ZB-1MS (Phenomenex, Torrance, CA, U.S.A.) and ultrapure helium was used as the carrier gas (at constant pressure, 11 psi). The following temperature program was used: 50°C hold for 1 min, 50–250°C (5°C/min) hold for 11 min and 250°C hold for 5 min. The auxiliary line and mass spectrometer were operated at, 280 and 180°C, respectively. The mass spectrometer was operated using electron-impact mode at 70 eV and in full scan mode set up to detect ions with a mass-to-charge ratio (m/z) of 30–350. The 3-point calibration curves (40,100, and 200 μg/L) were created for all target analytes. Calibration curves were made in a model beer solution (5% v/v ethanol) and were prepared using the methodology previously described. Target analytes were quantified using the following ions for each analyte: m/z 55 (4-octanol), m/z 59 (a-terpineol), m/z 69 (b-farnesene, geraniol, nerol, methyl geranate, geranial, and geranyl acetate), m/z 71 (terpinen-4-ol and linalool), and m/z 93 (b-Myrcene, b-caryophyllene, and a-humulene).

Results and Discussion

Accelerated Aging Protocol:

Natural aging studies of beer are typically carried out in an environment of 20 °C, for a duration that is determined by sensory evaluation of the product at set intervals during the aging process. Preliminary work by François et al. on beer polyphenols during aging used a 9 month at 20°C natural aging protocol [22]. For the purposes of this study, higher temperature storage was chosen to act as a proxy for extended aging at room and/or refrigerated temperatures. Previous anecdotal evaluations of aged beer suggest that the temperature proxy treatment had a similar impact on sample perception. Other beer studies have utilized between 37 and 40°C for accelerated aging to achieve end of shelf life examples of the product [22-23]. With this in mind,

and in order to focus attention on the dissolved oxygen treatments, a slightly lower temperature of 30°C was selected for the high temperature storage. The remainder of the protocol remains in line with what was used by Heuberger et al; 14 day total storage time, and a cold storage temperature as a control (4°C used by Heuberger et al, 3°C used in this study) [23]. For context, work performed by Li et al. demonstrates a good comparison of natural and forced aging equivalency times [2]. Their findings suggest that one day storage at 50°C was equivalent to four weeks of storage at room temperature, and 60°C was equivalent to eight weeks of room temperature storage (defined to be between 20-30°C, as measured in the storage room during the study) [2]. These temperature trials differ from those in this study in that aging was carried out using a water bath to maintain temperature by Li et al., where as an incubation fridge was used to house the kegs in this study. An additional study supports that beer samples stored at 40°C for four days was able to predict natural aging at three to five months based on sensory and the sum of analytical aging indicators [24].

Sensory:

Multiple Factor Analysis (MFA) was performed using the X and Y coordinates corresponding to the spatial placement of each treatment in the Projective Mapping (Napping®) session. The data from the two panels was labeled separately, and a combined MFA was run to evaluate the presence of any panel effect that would hinder combining the data (Figure 6). The lack of clustering based on panel location provides evidence that the data is suitable for combining. The X-axis was anchored with low perceived sweetness on the left and high on the right. With this iteration of directed Projective Mapping (Napping®), positive dimension F1 is to be interpreted as higher perceived sweetness, and negative dimension F1 is interpreted as less perceived sweetness. All high temperature stored samples grouped in the high perceived sweetness region, and all low temperature stored samples ground in the low perceived sweetness

region. Two distinct groupings were apparent based on storage temperature of the samples (Figure 7). Dimension F1, while directly related to perceived sample sweetness, corresponds well to the storage temperature, and dimension F2 corresponds to the level of dissolved oxygen added. Together, dimensions F1 and F2 are responsible 50.3% of the variance observed (F1-32.6%, F2-17.7%). Separation of samples based on dissolved oxygen content was consistent for the low and high dosed samples in both storage temperatures. Moderate and medium dosage levels varied between the two treatment temperatures, suggesting that in low temperature storage, medium DO mapped more closely with high DO, while in high temperature storage, moderate DO mapped more closely with high DO.

The sensory descriptor frequency table (Table 1) illustrates the distribution of terms assigned to each sample by the panelists. Descriptors that exhibited low utilization by the panel (less than 15% of the highest frequency term, overall) were removed from further analysis. The correspondence analysis on the frequency data, Figure 8, shows the correlation between treatment, and frequency of the terms used to describe that treatment. Like the MFA, dimension F1 corresponds to the treatment storage temperature, and dimension F2 corresponds to the level of dissolved oxygen in the beer. Dimensions F1 and F2 account for 80% of the variability (F1-69.5%, F2-10.5%). Within the low temperature treatment group, the effect of DO was observed as trend from the lower right quadrant to the upper right quadrant and suggest a transition from fresh pineapple/dank-cannabis/hoppy/citrus aromas, to more tea/herbal spicy aroma. The aromatic changes were still within the realm of hoppy aroma. The low DO and high DO samples map closely in Figure 8, suggesting that panelists assigned similar descriptors to these samples. Likewise, moderate and medium DO level samples map closely together. In contrast to the low temperature samples, those stored at higher temperature, and thus representing extended aging,

saw a loss of hoppy aroma and the production of malt-derived aroma. A trend from lower left quadrant to upper left quadrant exists where the beers moved from stone fruit/DMS-corn chip to dried fruit/malty/wet cardboard with increasing amounts of dissolved oxygen.

Analytical:

At the end of 2 weeks storage, all samples were moved to 3°C storage and chemical analyses were carried out over the following weeks. Basic beer chemistry including total polyphenols, bitterness units (BU), and HPLC for hop acid concentrations were all carried out within 24 hours of the conclusion of aging. Final keg dissolved oxygen content, and final headspace GC-MS analysis was prepared and executed 8 weeks following the completion of the accelerated aging protocol, based on instrument availability. Careful attention to the sampling method was crucial to minimize any additional DO pickup during sample collection. Results from the Anton Paar DMA-4500 Alcolyzer (Table 2) show that there was minimal change observed across the treatments. The following parameters expressed a slightly increasing trend with increasing dissolved oxygen content (Figure 9): haze H25/H0 (R^2= 0.39), haze H90/H0 (R^2= 0.35), and color (R^2= 0.53). Additional non-volatile analysis (Figure 9) show that bitterness units (BUs) have a slightly negative trend as dissolved oxygen concentration increased (R^2 = 0.42). While not tracked over the course of this study, the initial concentration of SO_2 was 0.8 mg/L. Metal ion content was as follows: Cu- 60 µg/L, Fe-35 µg/L, Mn- 120 µg/L, and Zn- 10 µg/L. Manganese content aligned with findings by Porter et al, with commercial examples of a dry-hopped pale ale containing 150 µg/L [16]. DO concentration at the beginning of the study ranged from 34.8 µg/L (control) to 440.1 µg/L (extreme) (Table 3) and dropped markedly during storage. When evaluated 8 weeks following the conclusion of the accelerated aging, the kegs measured between 14.3-21.4 µg/L dissolved oxygen with one exception, the extreme treatment at 49.0 µg/L (Table 3). These findings are profound, in that samples containing DO greater than

200 μg/L had similar final DO to samples starting with less than 50 μg/L. It is hypothesized that the DO was consumed through various oxidation reactions, including Strecker aldehyde generation, lipid oxidation, or thiol oxidation. This demonstrates how imperative it is to measure dissolved oxygen during production and immediately at packaging.

Despite the significant changes in sensory characteristics of the various treatments, there were no significant differences among the hop-related compounds (Table 4) when evaluated within treatment temperature, nor within dissolved oxygen concentration treatment group as evaluated by two-way analysis of variance. These findings suggest that the sensory changes of decreased hoppy, fruity, and citrus character may be more related the production of compounds not related to the hop monoterpenes present in the beer, rather than a decrease in concentration of hop derived compounds. Stale character may be related to a variety of oxidation products from lipids, or Strecker aldehydes. Aroma interaction is a phenomenon that has been well documented in wine and beer [25-26]. Combinations of certain aroma compounds can yield an enhanced perception of one or more sensory characteristics, compared to individual aroma compounds alone. Likewise, flavor masking can lead to the disappearance of desirable aromas in the product [27]. Flavor masking by Strecker aldehydes or lipid oxidation products provides a viable explanation for the disappearance of hop aroma sensorially, with retained analytical concentrations of hop monoterpenes.

Conclusions

Sensory results clearly indicated that accelerated storage conditions (i.e. temperature at 30°C versus 3°C) had a greater effect than DO levels on hoppy aromatic characteristics during storage. All high temperature stored samples exhibited high perceived sweetness, and all low temperature stored samples exhibited low perceived sweetness. The impact of dissolved oxygen

concentration was also observed at both high and low storage temperatures after only two weeks of aging. Analytically, haze H25/H0, haze H90/H0, and color expressed a slightly increasing trend with increasing dissolved oxygen content, while bitterness units (BUs) expressed a negative trend. Sensory descriptor frequency suggests that as a sample aged, there was a noticeable decrease in hoppy, fruity, and citrus character, and consequently, a greater expression of malty, dried fruit, and cardboard characters. Based on the headspace SPME-GC-MS data, there were no significant correlations between the hop-related monoterpenes and perceived aged character in the treatment beers. While not exhaustive in scope, the lack of change in hop-derived monoterpenes in this study suggests that a combination of other volatile compounds, such as Strecker aldehydes, or lipid oxidation compounds, are responsible for the stale character experienced by panelists.

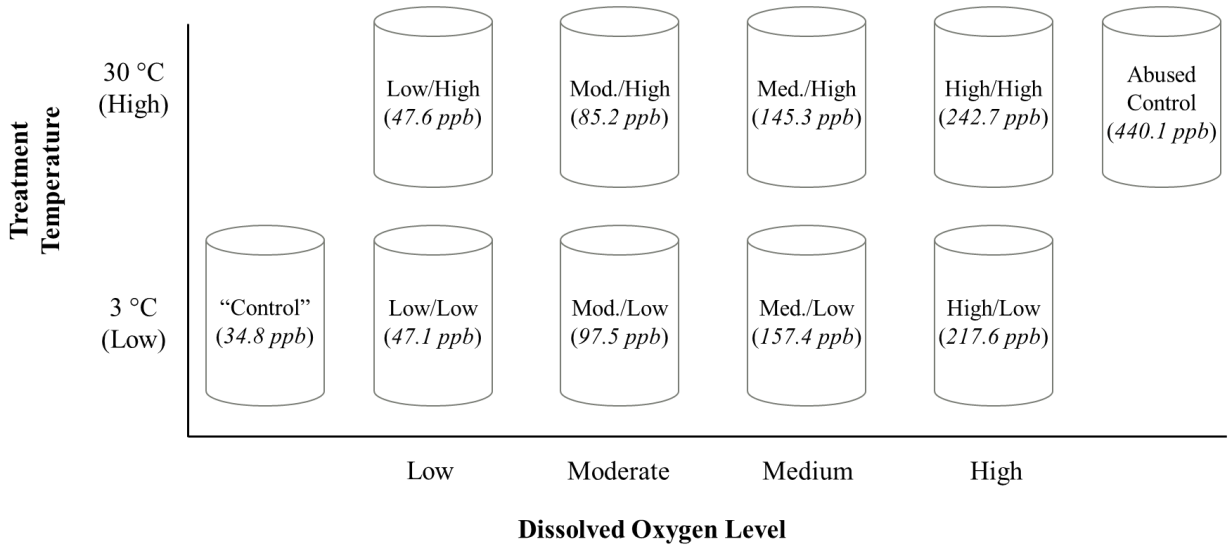

Figure 5. Experimental design for oxygen and storage temperature

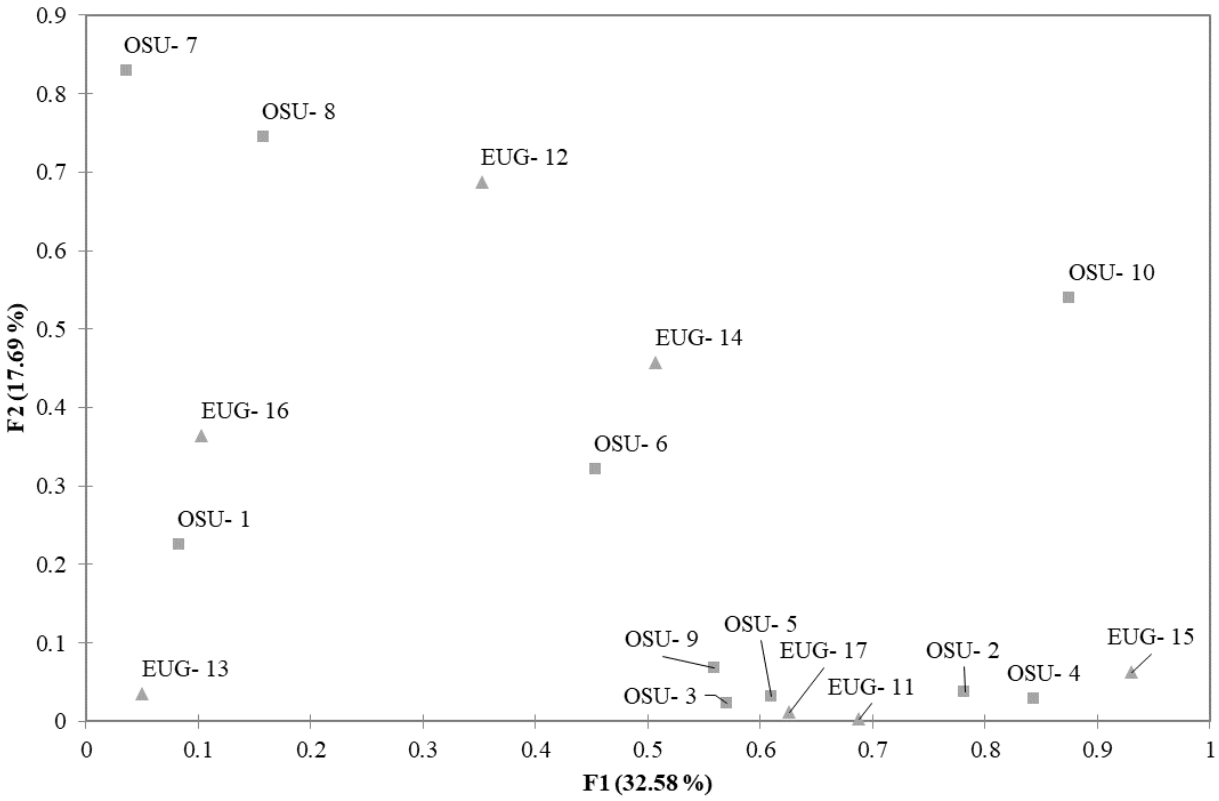

Figure 6. Multiple Factor Analysis (MFA) of replications

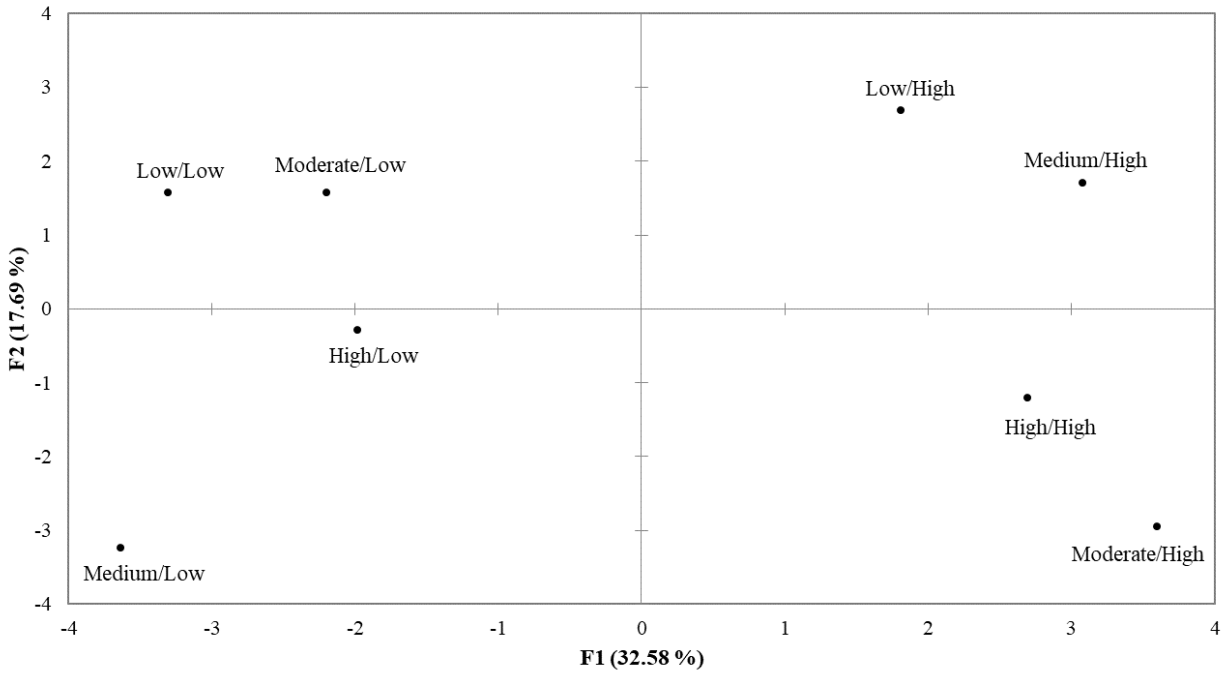

Figure 7. Multiple Factor Analysis (MFA) for X and Y Coordinates of each treatment presented as DO level/Storage Temperature

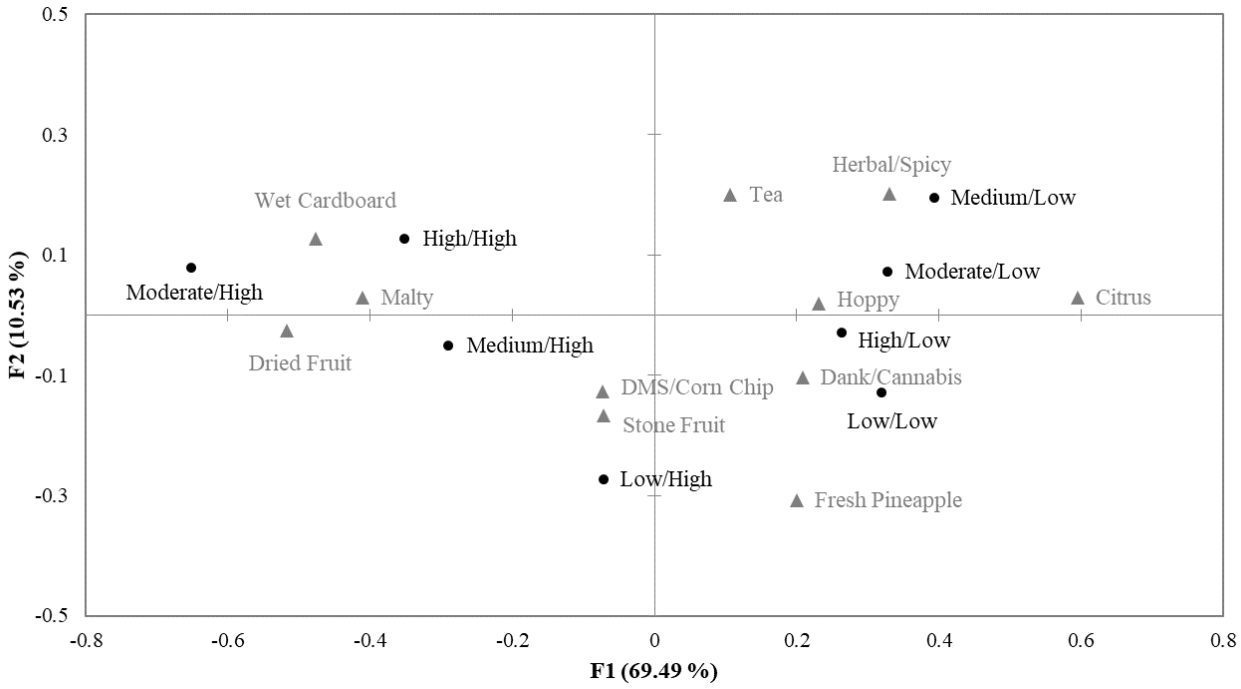

Figure 8. Correspondence Analysis (CA) for terms assigned to each treatment presented as DO level/Storage Temperature

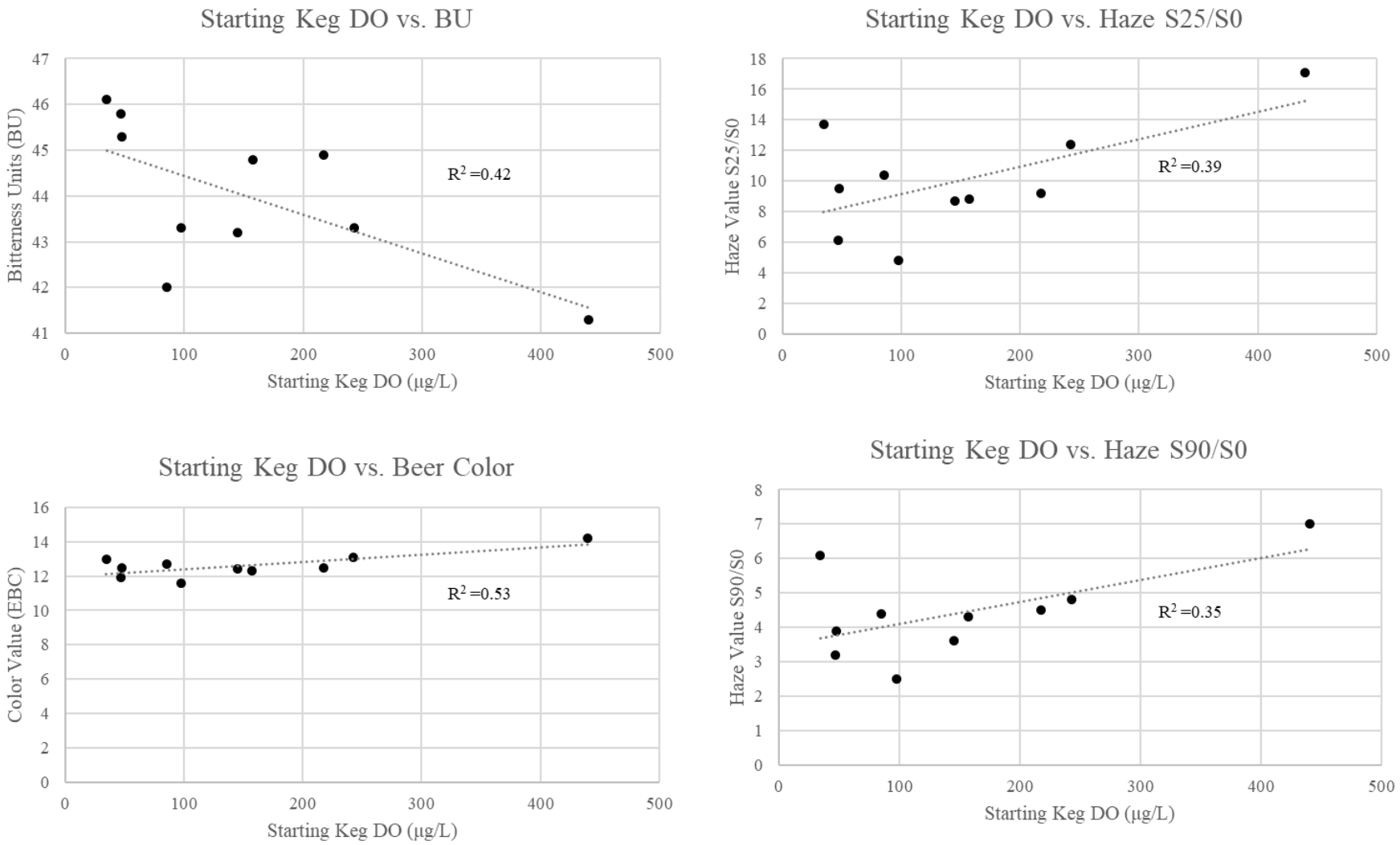

Figure 9. Non-volatile beer chemistry and starting keg DO trend evaluation

Table 1- Frequency table of panelist-assigned descriptors for each sample treatment

(note: descriptors in grey were removed due to <15% utilization by panel compared to highest frequency term overall)

Descriptors	Low/ Low	Moderate/ Low	Medium/ Low	High/ Low	Low/ High	Moderate/ High	Medium/ High	High/ High	Total
Hoppy	10	10	10	7	6	4	8	5	60
Dried Fruit	3	4	3	6	9	14	10	10	59
Malty	4	6	4	5	7	13	10	8	57
Herbal/Spicy	8	11	11	8	4	4	3	6	55
Citrus	6	9	11	8	6	0	3	1	44
Fresh Pineapple	9	3	6	6	8	3	5	2	42
Wet Cardboard	3	2	5	2	5	10	6	7	40
Dank/Cannabis	6	7	3	7	5	2	3	5	38
Stone Fruit	5	4	2	3	5	4	3	4	30
Tea	4	2	5	4	1	3	3	3	25
DMS/ Corn Chip	1	4	2	2	4	3	3	1	20
Acetaldhyde	0	0	1	0	1	1	0	1	4
Bitter	0	0	1	1	0	0	1	1	4
Clean Bitter	1	1	0	0	1	0	0	0	3
Medium Sweet	1	0	0	0	0	1	0	1	3
Sweet	0	1	0	0	1	0	1	0	3
Lingering Bitter	0	0	1	1	0	0	0	0	2
Medium Harsh Bitter	0	0	0	0	0	1	0	1	2
Not Sweet	0	0	1	1	0	0	0	0	2
Harsh Bitterness	0	0	0	0	1	0	0	0	1

Table 2- Anton Paar DMA-4500 Alcolyzer data for initial sample, and aged treatment samples

(Treatment is presented as follows: DO level/storage temperature)

Treatment	**Ethanol**	**Real extract**	**Original extract**	**Color Value**	**Haze Value S25/S0**	**Haze Value S90/S0**	**pH Value**
	%v/v	*%w/w*	*°Plato*	*EBC*	*EBC*	*EBC*	-
Initial Product	5.6	5.0	13.3	11.2	8.4	3.7	4.3
"Control"	5.4	4.9	13.0	13.0	13.7	6.1	4.2
Low/Low	5.3	4.9	12.9	11.9	6.1	3.2	4.1
Moderate/Low	5.4	4.9	13.1	11.6	4.8	2.5	4.1
Medium/Low	5.4	4.9	13.0	12.3	8.8	4.3	4.1
High/Low	5.4	4.9	13.0	12.5	9.2	4.5	4.2
Low/High	5.4	4.8	12.9	12.5	9.5	3.9	4.1
Moderate/High	5.4	5.0	13.1	12.7	10.4	4.4	4.2
Medium/High	5.4	4.9	13.1	12.4	8.7	3.6	4.2
High/High	5.3	5.0	13.0	13.1	12.4	4.8	4.2
Abused Control	5.4	4.9	13.1	14.2	17.1	7.0	4.2

Table 3- Analytical values for initial received sample and aged treatment samples

(Treatment is presented as follows: Dissolved Oxygen (DO) level/storage temperature)

Treatment	**Total Polyphenols**	**Iso-Alpha Acids (HPLC)**	**Humulinone (HPLC)**	**BU (ASBC)**	**Initial* and/or Dosed Keg DO**	**Final Keg DO**
	mg/L	*mg/L*	*mg/L*	-	*mg/L*	*mg/L*
Initial Product	225	46.1	5.3	43.4	34.8*	-
"Control"	262	40.7	5.2	46.1	34.8	21.4
Low/Low	258	41.2	5.0	45.8	47.1	19.6
Moderate/Low	274	39.1	5.0	43.3	97.5	16.5
Medium/Low	275	39.7	5.0	44.8	157.4	16.7
High/Low	273	39.3	4.7	44.9	217.6	17.6
Low/High	279	38.9	4.3	45.3	47.6	14.3
Moderate/High	266	37.7	4.3	42.0	85.2	17.1
Medium/High	278	41.0	4.6	43.2	145.3	14.4
High/High	253	38.6	4.7	43.3	242.7	14.6
Abused Control	260	39.3	4.5	41.3	440.1	49.0

*Denotes that value is the initial received keg DO reading

Table 4- HS-SPME-GC-MS data for treatment samples (all results in μg/L unless otherwise noted)

	3 °C Storage Temperature					***30 °C Storage Temperature***				
	Control	**Low**	**Moderate**	**Medium**	**High**	**Low**	**Moderate**	**Medium**	**High**	**Abused Control**
Beta Myrcene	22.4	20.9	19.4	20.6	20.4	31.8	20.3	17.2	20.4	18.3
Linalool	202.1	185.5	198.8	196.4	193.8	190.5	229.5	179.7	178.4	218.6
a-terpineol	41.0	45.5	41.6	40.8	41.3	43.4	52.4	38.4	39.6	43.5
Nerol	28.5	28.6	28.2	28.3	28.4	27.7	30.3	27.3	27.2	28.1
Geraniol	45.0	47.7	44.9	45.1	45.4	42.4	53.6	39.9	41.3	42.5
Geranial-citral	22.4	23.1	22.4	22.6	22.3	22.4	23.2	22.4	22.5	22.5
Geranic Acid	26.9	28.5	27.1	26.4	27.2	28.2	31.1	25.0	24.8	27.6
Geraniol Acetate	21.3	21.3	21.2	21.2	21.0	21.2	21.2	21.1	20.9	21.0
Alpha Humulene	24.2	24.3	24.3	24.3	24.3	24.3	24.3	24.2	24.2	24.3

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