AN ABSTRACT OF THE THESIS OF

Annette N. Fritsch for the degree of Master of Science in Food Science and Technology, presented on January 4, 2007.

Title: Hop Bittering Compounds and Their Impact on Peak Bitterness on Lager Beer.

Abstract approved:

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Thomas H. Shellhammer

Hop derived bitter compounds, including alpha-acids, reduced and non-reduced iso-alpha-acids, were evaluated for their contribution to peak bitter intensity in lager beer. Alpha-acids are the precursors to the major bittering components in beer (iso-alpha-acids). Typically, alpha-acids do not survive the brewing process, but if a product is dry-hopped, they may solubilize into the finished beer depending on the system pH, temperature and ethanol content. The impact of alpha-acids on the bitterness of lager beer was investigated using a trained panel and a test with a consumer panel. The trained panel evaluated samples with and without alpha-acids to offer initial analysis on aroma and bitterness intensity, and a triangle test comparing an unhopped lager with and without 14 ppm alpha acids (the solubility limit in beer) was presented to over 100 consumers for evaluation. Both panels found no significant difference between the samples. Furthermore, statistical similarity of the samples with and without alpha-acids was validated. This confirmed that alpha acids contribute negligibly to the overall bitterness of lager beer.

Iso-alpha-acids are a hop derived compound formed from thermally induced isomerization of the alpha-acids. When exposed to ultra-violet light (UV), the iso-alpha-acids are degraded forming off-odors and flavors. Therefore, where UV degradation is of concern, it is important to brewers to find an alternative to iso-alpha-acids. The reduced iso-alpha-acids, rho-iso-alpha-acids, hexahydro-iso-alpha-acids, and tetrahydro-iso-alpha-acids, can be used as a substitute for iso-alpha-acids and provide bitterness and UV stability. The reduced iso-alpha-acids offer varying degrees of change to the temporal bitterness qualities of a beer when compared to iso-alpha-acids.

The relative bitterness relationships of reduced to non-reduced iso-alpha-acids were measured using a time-intensity protocol, in which a trained panel evaluated seven concentrations of each compound in an unhopped lager beer. The peak intensities were identified, and a non-linear dose-response curve, called a change-point model, was fit to the data. Three parameters, a, b, and θ, identified the shape of the model. Panelist's replicated well but varied in sensitivity to the compounds and how they rated bitter intensity. Per-panelist and panel-wise equi-bitter equations were constructed from the parameters. Statistical analysis was performed to identify differences in bitter impact. Accordingly, rho was significantly less bitter than iso-alpha-acids, and hexahydro-iso-alpha-acids and tetrahydro-iso-alpha-acids were not different significantly in bitter impact over a range of iso concentration. The predicted equal bitter concentrations for each reduced iso-alpha-acids to iso-alpha-acids were validated by a consumer panel at a single concentration of iso-alpha-acids.

In conclusion, each hop compound researched (alpha-acids, iso-alpha-acids, rho-iso-alpha-acids, hexahydro-iso-alpha-acids, and tetrahydro-iso-alpha-acids) differed in the contribution to peak bitter impact of lager beer. Alpha-acids did not contribute significantly to the bitterness of an unhopped lager as validated by a consumer panel. This is particularly important for brewers that dry-hop their beers. And for those brewers wanting to use reduced iso-alpha-acids to replace iso-alpha-acids as a method for eliminating UV degradation, it is important to understand the peak bitter relationship for each of the compounds. By applying the change-point model, the natural variation among panelists was accommodated and compound differences that were not initially quantifiable were revealed and defined.

Hop Bittering Compounds and Their Impact on Peak Bitterness on Lager Beer

by
Annette N. Fritsch

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APPROVED:

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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.

Annette N. Fritsch, Author

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Dr. Thomas H. Shellhammer assisted in data analysis, collection, experimental design and writing of Chapters 2, 3, and 4. Dr. Alix I. Gitelman assisted in experimental design, data analysis, data interpretation, and writing of Chapters 3 and 4.

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1. THESIS INTRODUCTION

1.1. Hops and brewing

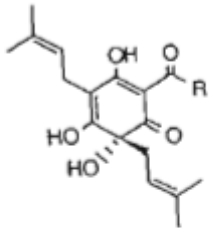
Hops (*Humulus lupulus*) are an essential ingredient for beer production. Besides providing the "spice" to beer, hops are the main source of bitterness, essential for balancing the sweet and acidic qualities of the wort, and have antimicrobial properties which help to extend product shelf-life. They are part of the *Cannabinaceae* family and are a perennial plant native to Europe, Asia and North America. Hops are now cultivated in a variety of locations around the world based on latitude. Dating back to the 12th century, German monks used hops as a beer additive. The Rheinheitsgebot, or German purity law, was established in 1516 and dictated that beer can only be made from water, barley, and hops (later yeast was added). (24, 35).

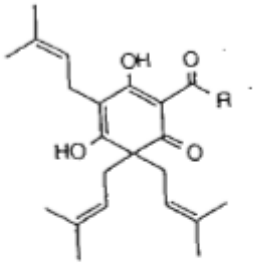
1.2 Hop products

The yellow, spherical lupulin glands of hop cones contain most of the materials important to the brewing process. Of primary concern are the total resins and the essential oils. The total resins are defined as the portion of ground hops which are soluble in methanol or diethyl ether. They contain two fractions, the hard and soft resins, and are typically 15% of the total hop cone weight although newer varieties can be as high as 30%. Soft resins are soluble in hexane and hard resins are not. The soft resins are of principal value to the brewing process and contain alpha-acids, the precursor to the chief bittering components in beer (iso-alpha-acids), and beta-acids,

which provide little addition to the final product (Figure 1). Alpha-acids are typically present from 2-18% weight of hops, and alpha- and beta-acids are virtually non-existent in finished beer that is not dry-hopped (24, 35). They have limited solubility in beer, and the level of solubility is related to the pH and temperature of the beer and the solvating power of ethanol (33).

The hard resins have no particular value to brewing, and may contain oxidized alpha- and beta-acids. The amount of hard resins increase over time as the alpha- and beta-acids degrades. Degradation of alpha-acids is dependant on the storage conditions, including access to air, temperature, and relative humidity (24, 35).





R =	Alpha Acid	Beta Acid
iso-butyl	Humulone	Lupulone
iso-propyl	Cohumulone	Colupulone
sec-butyl	Adhumulone	Adlupulone

FIGURE 1: Alpha- and beta-acid structure (28).

The essential oils compose 0.5-3.0% by weight of hops and provide the hop aroma to beer. Aroma hops are cultivated to emphasize desirable aromas and flavors. Mycerene, humulene and caryophyllene provide 80-90% of the total essential oils, and oxidation products of these hydrocarbons and other hop components comprise the last 10-20%. To increase the “hoppy” characteristics of a beer, aroma hops may

be used to dry hop a finished beer (i.e. adding hops to the finished beer). By passing beer through aroma hops, the essential oils are dissolved into the ethanol resulting in greater "hoppy" aroma and flavor characteristics in the beer (24, 35). A concern of brewers is that by adding hops to the finished product alpha-acids will also dissolve into the beer due to the solvating power of ethanol. Some brewers believe that alpha-acids increase the bitterness of the final product. The role of alpha-acids in bitterness is explored in Chapter 2: Alpha acids do not contribute bitterness to lager beer.

During wort boil, alpha-acids undergo thermally, induced isomerization producing iso-alpha acids (iso) (Figure 2). Iso are the primary contributors to beer bitterness. Iso exists in three forms which reflect the three forms of alpha-acids and include iso-humulone, iso-cohumulone and iso-adhumulone. Photolysis of iso occurs when exposed to ultraviolet light (UV). This is of concern when beer is bottled in clear or green bottles. When photolysis occurs, an isopentenyl radical is released, and this reacts with a thiol radical to form prenyl mercaptans (Figure 3). The prenyl mercaptans cause defect aromas, referred to as light-struck, sun-struck or "skunky" aroma/flavor (24, 35).

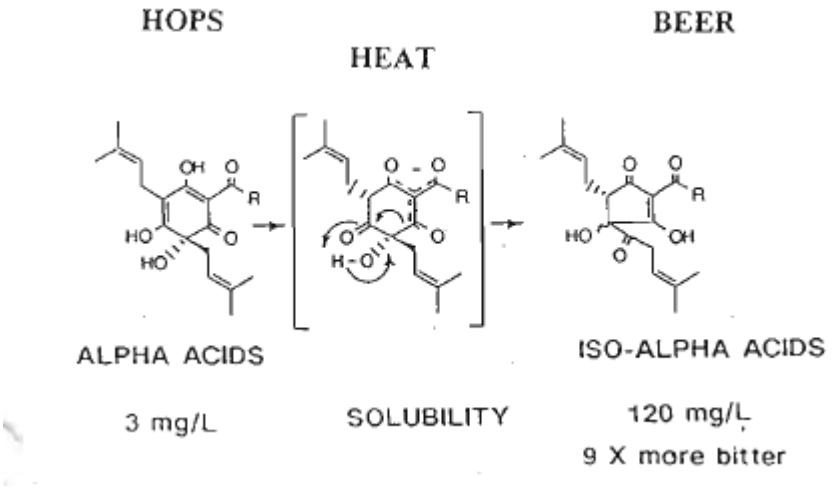


FIGURE 2: Thermally induced isomerization of alpha-acids (28).

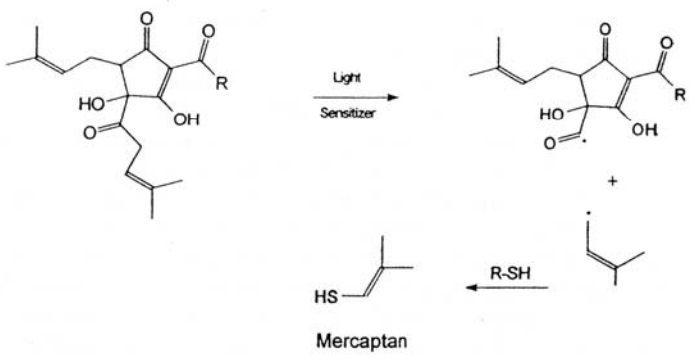


FIGURE 3: Photolysis of iso and formation of prenyl mercaptans (17)

When UV degradation is of concern, an alternative to iso are reduced iso-alpha acids, including rho-iso-alpha-acids (rho), hexahydro-iso-alpha-acids (hexa) and tetrahydro-iso-alpha-acids (tetra) (Figure 4). The production of these compounds was originally aimed at finding a solution for light-struck aroma production and to utilizing hop beta-acids. Both alpha- and beta-acids can be converted into the reduced products. The light stability of these compounds is from a modification of the methylpentenyl group of iso-alpha-acids which stops the release of the isopentenyl

radical (14). Rho, or dihydroiso-alpha-acids, is the reduction of the isohexenoic side chain carbonyl group to an alcohol. This is done by reacting the carbonyl side chain of iso with sodium borohydride (18). Tetra is produced when both the carbon-carbon double bonds, which are usually present in the iso side chain, are saturated, and for hexa, both the isohexenoic acid carbonyl group and the carbon-carbon double bonds are reduced (18)). Tetra and hexa can be formed by converting beta-acids. Beta-acids in presence of hydrogen and lead will produce tetra, and if treated further with sodium borohydride, hexa is produced. Tetra can also be formed by hydrogenating an alpha-acid (17). Utilizing one of the reduced iso-alpha-acids in place of iso can provide functional changes, including increased foam stability, and possible changes in temporal bitterness and sensory quality.

Previous research on the bitter impact of the reduced iso-alpha-acids compared to iso has been performed in various mediums with conflicting results. Initial analyses of the relationships were assessed in a water medium, and the relative bitterness of iso to rho, tetra, and hexa, respectively was determined as 0.67x, 2.03x, and 1.15x (34). Additionally, rho and tetra were evaluated in a commercial lager beer for relative bitterness to iso. For tetra, bitterness was not significantly different from iso but was approximately equal (1.0-1.1x), and rho remained roughly 0.6 times the bitterness of iso (36). Determination of the relationship of each reduced iso-alpha-acid to iso over a range of concentrations was determined in a lager beer medium (see Chapter 4: Relative bitterness of reduced iso-alpha-acids to iso-alpha-acids in lager beer).

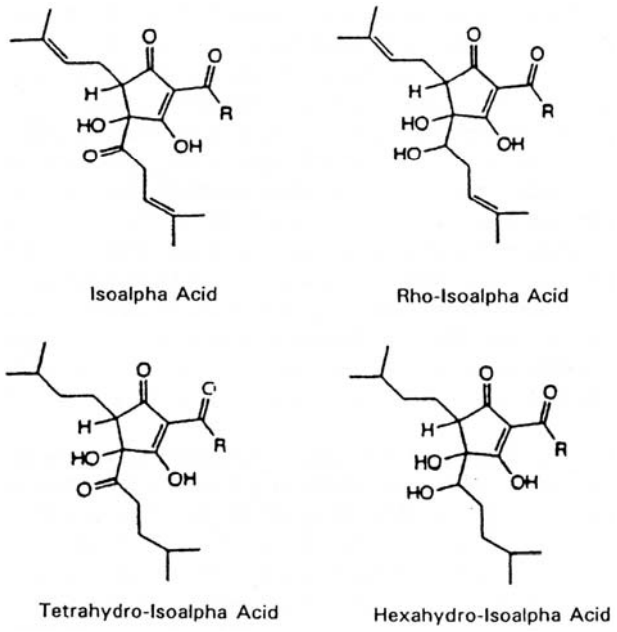


FIGURE 4: Chemical structures of the reduced and non-reduced iso-alpha-acids (17) :

1.3 Challenges in assessing bitter impact

Bitterness is difficult to assess due to a variety of influences on perception including chemical structure, among panelist variation, and medium effects. Compounds that cause bitterness vary widely in chemical structure and, therefore, there is not a consistent mechanism for bitter perception. Additionally, inherent differences among panelists exist and cannot be avoided, including genetic and hormonal differences. Finally, the complexity of the medium determines the degree of perception. For example, some compounds can create synergies or inhibit bitter impact.

1.3.1 Chemical structural effects

The diverse array of chemical structures that cause bitterness poses a problem in understanding bitter perception. Bitter molecules vary widely in compound structure including inorganic cations, small peptides, sugar derivatives, and other chemical structures. The hydrophobic nature of compounds has been directly linked to bitter impact (32). As hydrophobicity increases, the bitterness perceived increases. The compounds may either bind to hydrophobic amino acids on the receptors or penetrate the bilipid layer (32). As hop compounds are reduced, they become more lipophilic in nature which may increase bilipid layer penetration and, therefore, bitterness impact. Research done in the Shellhammer lab looked at the order of HPLC elution time for the reduced and non-reduced iso-alpha-acids and how it related to the degree of compound hydrophobicity. Accordingly, the relative order of hydrophobicity from least to most was rho, iso, hexa and tetra. This suggests that rho would penetrate the bilipid layer least effectively and implies that it would be less bitter. In contrast, tetra would most efficiently enter the bilipid layer signifying that it would be most bitter.

1.3.2 Panelist variation

Individual bitterness perception is decidedly dependant on differences rooted in genetic, age, and hormonal variation. Genetic diversity includes anatomical structure of the tongue, including the number of fungiform papillae and the integrity of the taste buds within the papillae, and inherited traits, such as sensitivities to specific

compounds like those that contain a –N-C≡S group. Perceiving bitterness from compounds that contain a –N-C≡S group, like phenylthiourea (PTC) or 6-n-propylthiouracil (PROP), is an inherited trait, and the inability to taste PROP has been linked to the presence of a homozygous recessive gene. Threshold tests are used to determine if an individual is a "non-taster," have the homozygous recessive gene, or "tasters." An additional category of hypersensitive tasters, called "supertasters," has been identified, and the number of papillae and taste buds on the tongue has been correlated with the taster status (22). Gender is another factor in bitter perception, and women are more frequently PROP supertasters (highly sensitivity to PROP bitterness) (3). Additionally as individual's age, compound dependant sensitivity to bitterness declines. For example, perception of quinine bitterness decreases with age, but sensitivity to urea does not (10).

All of these types of variation make evaluating bitterness unique among the basic tastes because differences among individual panelists cannot be avoided. This has been the case when evaluating hop acids. Wide discrepancies have been observed among panelists evaluating hop compounds; panelists deviated by a factor of 25 when rating iso bitter intensity in water (34).

Multiple types of taste receptors and/or various transduction mechanisms are present in order to accommodate the diverse array of bitter molecules. Taste transduction occurs when a bitter stimuli comes into contact with taste receptor cells. Taste buds contain 75-150 of the receptor cells which are clustered into fungiform, foliate, and circumvallates papillae on the surface of the tongue. Sweet and bitter

perception involve the taste receptors coupled with proteins, including secondary messengers like cyclic AMP or inositol triphosphate which may take one of numerous compound dependant pathways (12). The signals produced from the different receptors are not distinct which results in the inability to discriminate among different molecules (31, 32).

Several researchers have explored the transduction mechanism for iso compounds, in particular, with conflicting results. One study suggested that compounds containing methyl groups, such as iso, denatonium benzoate, sucrose octaacetate, caffeine, and quinine, shared a similar transduction mechanism that contrasted with compounds containing a primary amine, like phenylalanine, urea and tryptophan (11). Yet, psychophysical data from another study addressing the bitterness of isohumulones indicated that iso and thiourea moieties share a common receptor mechanism (15).

1.3.3 Medium effects on the bitter impact of hop compounds

The medium used to test hop bitter compounds has a significant effect on the bitter intensity perceived. Other compounds present in the medium can either create synergies with or inhibit bitter impact. When panelists tasted iso samples between 20 and 30 ppm in a water versus a commercial lager beer medium, the water samples were consistently rated as more bitter (27). This difference was attributed to the complexity of beer. Beer pH affects the degree to which weak acids, like iso, dissociate. As pH increases, the bitter quality was reportedly more harsh and coarse

with increased persistence (4). Additionally, ethanol, glucose, and sodium content affect iso and reduced iso-alpha-acid bitterness. The addition of 2.6% ethanol to commercial lager beer with less than 20 ppm iso resulted in increased bitterness. Similarly, the bitter intensity of rho increased by a factor of 1.3 when the alcohol content was changed from 1 to 7% (27, 36). The addition of 2% glucose, however, suppressed perceived bitterness significantly over a range of iso concentrations (27). Sucrose has also been shown to reduce iso bitterness (19). In contrast, sodium salts, which typically diminish bitterness, have been shown to have no effect and may even enhance iso bitterness(20).

1.4 Intensity Matching Techniques

Various methods have been used by researchers to determine equal-sensation levels for bitter and sweet molecules. A paired-comparison sensory protocol is typical for finding equal-bitter levels for a defined compound at a single concentration. For example, to match intensity of different bitter compounds, a paired-comparison, forced-choice, constant stimulus method has been utilized. In this method, a constant stimulus was defined and test samples were compared to the constant to determine if the test sample was more or less intense. A regression analysis was used to ascertain the concentration at which no more than 50% of the respondents identified the sample as more intense. The identified concentration was considered equi-intense on average across the panel, or in other words, the compound could be added at the determined concentration to create the same level of intensity as the original compound (1, 11).

Breslin and Beauchamp (5) used a variation on the constant stimulus method by performing successive paired-comparisons in order to identify equally intense samples. A reference compound was chosen, and the researchers identified a concentration of medium intensity, one which was neither too extreme nor too weak. Panelists were presented with four pairs of samples; each pair included a test sample, which was a different compound and concentration than the reference, plus the reference sample. They were asked to identify which sample was more bitter. The concentration of the test compound was successively reduced or increased per panel feedback, and the paired-comparisons were again presented to the panel until neither compound was select as more bitter for 55% of the trials. At this point the compounds were considered, on average, to be equivalent in bitterness intensity (5).

The magnitude estimation procedure is another method for equating sensations and has been used to compare sweetener intensities over a range of concentrations. It provides a direct and subjective quantitative measure of the relative sweetness to a compound at a single concentration (6). In this method, a reference sample was defined and assigned an intensity value of 100. Panelists measure the degree of intensity of the test sample in comparison to the reference. For example, if a panelist determined a test sample was twice as bitter as the control, he would assign an intensity of 200 to the sample. Dose response curves for each test sample were fitted to a power function, and a regression analysis was used to determine the relationship between sweetness intensity and concentration. A “potency” curve was created; potency was defined as the number of times sweeter a compound was in comparison

to a concentration of sucrose on a weight basis. From the potency curve, a sweetness equivalence curve was created which defined the sweetness intensity of the test sample in relation to sucrose at defined concentrations (6).

While these methods are useful for evaluating the required concentration of test compounds to create equi-intense sensations to a reference compound at a single, defined concentration, they become somewhat tedious to execute over a broad range of concentrations. In determining equal bitterness for the reduced and non-reduced iso compounds, it was essential to create a method for evaluating relative bitterness over a range of both iso and reduced iso-alpha-acid concentrations. This is addressed in Chapter 3: Using a change point model to evaluate dose-response relationships of hop bittering compounds.

1.5 Research Objectives and Brief Methodology

The goal of this research was to evaluate the contribution of hop products, including alpha-acids, reduced and non-reduced iso-alpha-acids, to bitterness intensity of lager beer. All compounds were evaluated by consumer and trained panels. The trained panel offered qualitative characteristics of beers with alpha-acids and temporal aspects of bitterness for beers with reduced and non-reduced iso-alpha-acids at various concentrations. A triangle test was used with consumers to determine if alpha-acids contributed to bitterness and a paired-comparison was used to validate calculated equal bitterness for the reduced iso-alpha-acids compared to iso.

2. ALPHA ACIDS DO NOT CONTRIBUTE BITTERNESS TO LAGER BEER

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2.1 Abstract

The impact of alpha acids on bitterness intensity of lager beer was investigated using a trained panel and a test with a consumer panel. A trained panel evaluated samples with and without alpha acids to offer initial analysis on aroma and bitterness intensity. Following the trained panel test, a triangle test comparing an unhopped lager with and without 14 ppm alpha acids was presented to over 100 consumers for evaluation. Both panels found no significant difference between the samples. Furthermore, statistical similarity of the control and the 14 ppm alpha acid samples was validated due to the size of the test. This confirmed that alpha acids, at levels as high as the solubility limit in beer, contribute negligibly to the overall bitterness of lager beer.

Key words: alpha acids, bitterness

2.2 Introduction

Alpha acids are part of the total resins found in the lupulin glands of hop cones. They have limited solubility in beer, and the level of solubility is related to the pH and temperature of the beer and the solvent power of ethanol (33). Alpha acids are the precursors of the largest contributor to beer bitterness, iso-alpha acids. During wort boiling, alpha acids are isomerized to produce iso-alpha acids, a reaction considered to be the most important reaction in hop chemistry (35). Because of isomerisation during wort boiling and the limited solubility in beer, there is typically not a significant amount of alpha acids remaining in the final product (22). However

if a beer is dry-hopped, there may be residual alpha acids present in the finished beer because of the solvating power of ethanol. The maximum concentration of alpha acids in beer is roughly 14 ppm (26); Robert Smith, SS. Steiner, personal communication, 9/22/2004), but the bitterness contribution from this level of alpha acids in lager beer is unknown. In this study, we tested the hypothesis that alpha acids do not contribute bitterness to beer.

2.3 Experimental

2.3.1 Beer system and sample preparation.

Two beer systems were used in this analysis. For the first system (Beer system 1), alpha acids (90.9% purity, courtesy of S.S. Steiner) were dosed into 32 oz. bottles of a commercially available lager beer with approximately 8 ppm iso-alpha acids. The alpha acids were initially dissolved at a 0.5% w/v in food-grade ethanol. The solution was added to the beer to create two samples at 14 and 28 ppm concentration. However, their concentrations were not confirmed through analysis. Ethanol was added to the control beer sample to equalize the alcohol content between the test beers and the control. The final alcohol content was approximately 5.27% alcohol by volume.

For the second system (Beer system 2), an unhopped lager beer was brewed in the Oregon State University pilot brewery. The beer was created using pale 2-row malted barley (Great Western Malting Co.). Twenty-five percent of the fermentable extract came from rice syrup solids (California Natural Products). The target starting

and ending gravities were 11.69°P and 3.45°P respectively. This wort was fermented with lager yeast (Wyeast strain 2007) at 14.5°C. After two weeks, PVPP (Polyclar® VT) was added at 35g/hl to the green beer, and the beer was held at 1.7°C for another 7 days. It was filtered through a 1.2 μm nominal filter pad (Cellupore®, 1940SD grade, Gusmer Cellulo Co.) into a sanitized conical fermentor. The beer was analyzed for pH, alcohol, beer color, BU, and specific gravity according to ASBC methods (8). (see Table 1)

TABLE 1: Unhopped Lager Beer Analyses

pH	4.20 at 20°C
Alcohol	5.27% abv 4.20% w/w
Beer Color (A_{430nm}*1.27):	5.27
BU	0.79
Specific Gravity (g/ cm^3)	1.00621
Apparent Extract	1.55°P
Real Extract	3.45°P
Original Gravity	11.60°P
Real Degree of Fermentation	68.98%

Alpha acids were dissolved to 1% w/v in food-grade ethanol , and this solution was added to the unhopped lager beer. The target was an alpha-acids concentration of approximately 14 ppm as determined by high performance liquid chromatography. Analysis was executed according to a modified version of ASBC method Beer–23C based on work by Raumschuh (8, 30). The HPLC analysis ran at a constant temperature of 40°C, a flow rate of 1.4 ml/min, and a 10 μl injection volume. Two

mobile phases were used. The first mobile phase was 100% methanol and the second was 75% water, 24% methanol and 1% phosphoric acid. A gradient elution was applied over a 22 minute analysis cycle beginning with 100% of the second mobile phase for the first 13 minutes. From 13 to 15 minutes, the mobile phases were mixed at a 1:1 ratio. Then, the analysis returned to 100% of the second mobile phase for the remaining 7 minutes. A Discovery C18 column was used for separation and absorbance was measured at 270nm. ASBC International Calibration Extract 2 (ICE-2) for HPLC Analysis of Alpha-Acids was the standard for all measurements.

2.3.2 Sensory Procedures.

A trained bitterness panel and a study using consumers tested the bitterness impact of alpha acids in beer. The trained panel consisted of 11 members who had been trained in bitterness perception in beers. Beer system 1 (commercially available lager beer with and without alpha acids) was utilized for initial evaluation by this panel. Two separate triangle tests on two different days were given to the group comparing the samples with and without additional alpha acids. For each test, the panelists were presented three samples (two the same and one different) in a random fashion and given a paper ballot to record their evaluation. Panelists worked independently and wore nose plugs to eliminate olfactory influence. The panelists evaluated the 14 ppm sample versus the control on the first day, and the 28 ppm sample versus the control on the second day. After completion of the triangle test on the second day, the panelists were asked to remove nose plugs. They received all

three samples (14 ppm, 28 ppm, and the control beer) in a blinded fashion and were asked to describe the differences between the beers.

A test was given to consumers, and the beer from system 2 (unhopped lager beer with and without alpha acids) was used. One hundred and three beer drinking consumers performed a triangle test. Each panelist completed one triangle test, in which they were presented three coded samples. Two samples were the same product, and one was different. Panelists were asked to choose the odd sample. This was a completely randomized block design in which approximately equal numbers of the six possible sample combinations were presented (AAB, ABA, BAA, BBA, BAB, and ABB). Panelists were seated in individual testing booths and were assigned an identification number. The serving order was predetermined by random computer generation and linked to the identification number. Panelists wore nose plugs to eliminate olfactory influence and were instructed to taste each sample presented from left to right and identify the odd sample. Clearly, wearing nose plugs is not a normal or typical habit for beer drinking consumers, but the objective of this test was to evaluate only the alpha acids impact on taste (i.e. bitterness) and not overall flavor. Due to the impurity of the alpha acids sample (90.9% pure), wearing nose plugs was essential for evaluation.

Following the evaluation, panelists were asked demographic questions to confirm age, gender and beer drinking habits. Due to the desirability of the product, historical experience within the sensory laboratory indicated that initial screenings of preference and drinking habits might not yield an accurate account of the true

demographics of the panel. Therefore, the panelists were asked to verify the types of beer they like and consume often. The choices were "domestic (i.e. Budweiser, Coors, Miller, etc.)"," import (i.e. Heineken, Corona, etc.)", "craft beer (i.e. Fat Tire, Mirror Pond, etc.)", "none of these", or "I do not drink beer." Panelists could choose more than one category for this question. To confirm typical consumption frequency, the panelists were asked to identify how often they consume each type of beer (domestic, import or craft). Only one choice could be made for this question.

2.4. Results

Typically, difference testing is used to identify the presence or absence of a significant difference between samples. In this study, the trained panel determined if there was a significant difference between the dosed and undosed samples. The α level was the significance level of the test, which controlled for the Type I error or the probability of finding dissimilarity between the samples when there was no significant difference.

In contrast, the goal of the test with consumers was to test for similarity. A large sample size was used for this test, and the results were analyzed using target α, β, and P_d values. The β value controlled the Type II error, which is finding no significant difference between the samples when there was a significant difference (9). The P_d value identified the confidence limits of the proportion of the population that will distinguish the sample, or the proportion of distinguishers (26). The industry

standard for this value is 0.20 (Mina McDaniel, Oregon State University, personal communication, 6/9/2005).

2.4.1 Trained Panel

There were no significant differences between the commercial beer dosed with 14 ppm alpha acids and the control and between the 28 ppm sample and the control ($p>0.05$). Four panelists out of the eleven chose the correct sample for the 14 ppm test and five chose the sample for the 28 ppm test. To find that the samples were significantly different, 7 panelists were required to choose the odd sample in each case (26).

The 28 ppm sample exceeded the solubility limits for alpha acids in lager beer. The purpose for testing at this level was to determine if the alpha acids at atypically high levels would impart bitterness. The alpha acids concentration produced a slight haze in the sample, but the haze was difficult to observe in the opaque serving cups used for all triangle test samples.

It is important to note that the commercial beer had its own inherent bitterness from approximately 8 ppm iso-alpha acids. Essentially, the trained panel was examining the potential additional bitterness impact by the alpha acids at 14 and 28 ppm. In both cases, there was no additional bitterness imparted by the alpha acids.

When the panel evaluated all samples (14 ppm, 28 ppm, and control) blinded and without nose plugs, they found increased hoppy, piney and perfumey aromas, in

the samples with added alpha acids. There was no consensus on which sample was the most bitter, thereby validating the triangle test results.

2.4.2. Consumer Testing

The test was performed using the unhopped lager beer (system 2). The alpha acids concentration in the test beer was 14.22 ppm ± 0.313 ppm, according to HPLC analysis. Out of 103 panelists, 33 panelists chose the odd sample. With 85% certainty that no more than 20% of the population can detect a difference, the results indicate that there was no significant difference between the samples (α=0.05, β=0.15, P_d=0.20) and, in turn, imply statistical similarity between the two samples. To fail the test, at least 43 panelists were required to choose the correct sample.

Panelists were asked demographic questions to confirm the group's composition according to gender, age, and their beer consumption practices. Eighty-one percent of the panelists were between the ages of 21 and 29, and sixty-five percent of all the participants were male. When the panelists were asked the types of beer they liked and consumed often, they could choose more than one type of beer, but only one frequency identifier could be chosen. Sixty-eight percent of the participants chose craft beer, which is typical of Pacific Northwest beer drinking demographics. This was followed by domestic and import beers, with thirty-one percent and twenty-seven percent respectively. When asked to identify how often each type of beer was consumed, seventy-five percent of the consumers drank domestic beer more than three times a month, and eighty four percent consumed

import and craft beer more than three times a month. All panelists drank beer at least 1-3 times per month, and 72% of panelists drank beer more than once per week irrespective of type.

These segmentations were used to determine if a particular group could significantly distinguish between the samples. These divisions were not analyzed to determine similarity due to the small sample populations; nevertheless, there was no significant difference determined for any of the group segmentations (Figure 5).

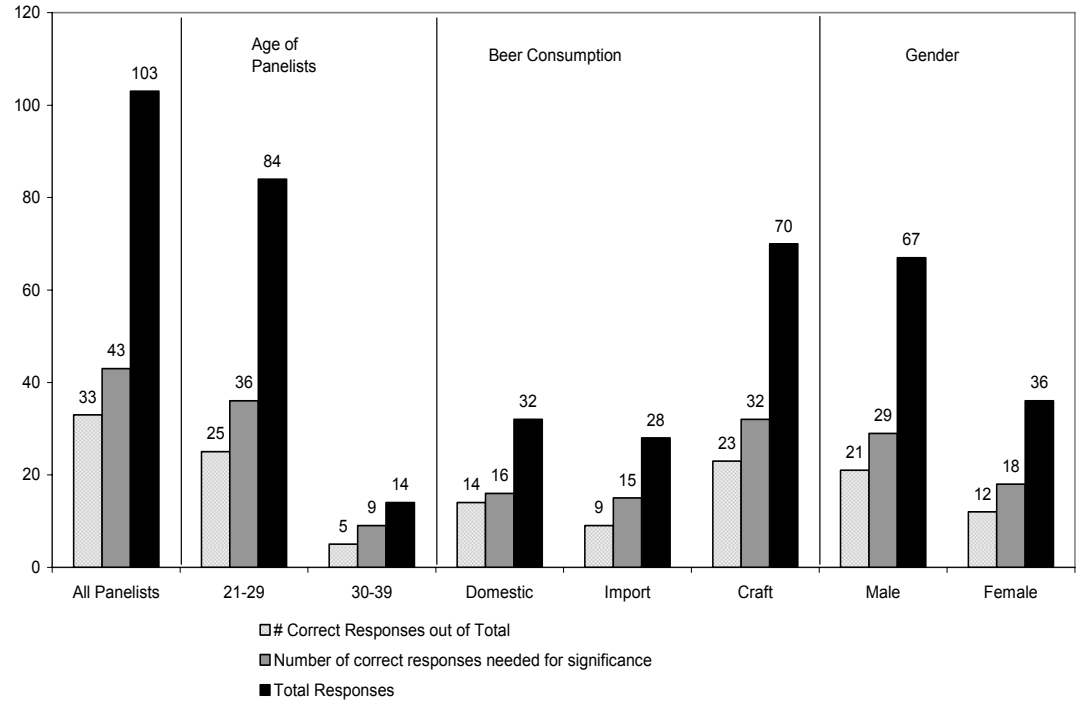

FIGURE 5. Psychographic results broken down by age, beer consumption and gender. Beer consumption was defined according to panelist's responses when asked to identify the types of beers they like and consume most often. Panelists could choose more than one answer.

2.5 Discussion

The purpose of the trained panel was to give direction and initial feedback on the probability of bitterness increasing due to added alpha acids content in a commercial beer medium. The commercially available lager beer contained approximately 8 ppm iso-alpha acids, which contributed perceivable bitterness to the panelists. The triangle test results were in agreement with the hypothesis that alpha acids do not contribute to additional bitterness. The panel did not find a significant difference between either of the samples (14 ppm and the 28ppm) compared to the control. The panel size was small, 11 panelists, and therefore, could not be used to test for similarity between the dosed samples and the control. The hoppy aromas perceived by the panel were not unexpected, since the sample was not exclusively alpha acids, albeit 90.9% pure. Yet, these aromas confirmed the importance of the panelists wearing nose plugs during these triangle tests and indicated the need for nose plugs in the test.

The size of the test and use of an unhopped lager beer produced results that were more powerful than the trained panel results and could be used to determine similarity between the samples. Using nose plugs was essential due to the impurity of the alpha-acids, which imparted aromatic qualities described by the trained panel. But, this created a situation out of context of conventional beer drinking conditions. To reduce the contextual effects, the test was completely randomized and all evaluation session were conducted in an identical environment (21).

The parameters of this portion of the testing were more stringent by defining values for β and P_d. This resulted in a confidence of 85% that no more than 20% of the population could distinguish the difference between the lager beer with and without alpha acids. Therefore, the impact of alpha acids on the bitterness of lager beer is considered negligible. This is important for all brewers, but particularly for those that dry-hop their beers. Since there was not a significant difference between the samples presented to the consumer panel, we can deduce that adding alpha acids to the brewing process, regardless of their origin, do not contribute to beer bitterness.

2.6 Acknowledgements

We would like to thank S.S. Steiner for the donation of the purified alpha acids sample. Additionally, we thank Cindy Lederer and the Oregon State University Sensory Department for assisting in the preparation and performance of the sensory testing.

3. USING A CHANGE POINT MODEL TO EVALUATE DOSE-RESPONSE RELATIONSHIPS OF HOP BITTERING COMPOUNDS

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3.1. Abstract

A trained sensory panel evaluated the bitterness intensity of four hop-derived compounds over a range of concentrations in lager beer according to a time-intensity protocol. A change-point model, defined by three parameters a, b, and θ, was applied to the peak intensity data. Panelist's replicated well but varied in how they scaled the bitterness response and sensitivity to the compounds. Therefore, per panelist and panel-wise equi-bitter equations were constructed from the parameters. Statistical analysis was performed to identify differences in bitter impact, and one compound was found to be significantly less bitter over the concentration range. By adopting the change point model, the variation among panelists was accommodated, and equi-bitter relationships were established over a broad range of compound concentrations.

Keywords: beer, bitterness, dose-response, hop, equal-bitter

3.2 Introduction

The sensory impact resulting from bitter tasting compounds is difficult to assess for many reasons, such as differences among panelists, chemical structures, and perception mechanisms. Bitter perception varies due to genetic diversity among panelists including the number of fungiform papillae on the tongue, the integrity of the taste buds within the papillae, and hormonal differences. These make bitterness unique among the basic tastes and lead to variation among individual panelists that cannot be avoided (2). There are also a wide range of bitter molecules, including inorganic cations, small peptides, sugar derivatives, and other chemical structures that

complicate the understanding of bitterness perception (2). In order to accommodate the assortment of bitter molecules, there are multiple types of taste receptors and/or various transduction mechanisms present (32). However, there is no distinction in the signal produced from the diverse receptors, which may result in the inability to discriminate among dissimilar molecules (31).

To complicate the issue further, the test medium affects the bitter impact and quality; for example, non-bitter compounds may synergize with or inhibit the impact from bitter compounds. For instance, ethanol content, glucose level, and pH influence the bitterness of iso-alpha acids, which is the primary source of hop derived bitterness in beer. The bitterness of iso-alpha acids, at concentrations less than 20 ppm, was enhanced by ethanol, while glucose significantly reduced the perceived bitterness over a range of iso-alpha acid concentrations (27). Beer pH affects the degree of compound dissociation of weak acids, such as iso-alpha acids. As pH increased, the increased dissociation of iso-alpha acids resulted in a bitter quality that was reportedly more harsh and coarse with increased bitter duration (4).

Various methods have been used by researchers to determine equal-sensation levels for bitter and sweet molecules. A paired-comparison sensory protocol is typical for finding equal-bitter levels for a defined compound at a single concentration. For instance, a paired-comparison, forced-choice, constant stimulus method has been used to match intensity of different bitter compounds (1). In this method, a constant stimulus is defined and test samples are compared to the constant to determine if the stimulus is more or less intense. A regression analysis is used to determine at what

concentration 50% of the respondents identified the sample as more intense. The identified concentration is considered equi-intense (11, 15).

Breslin and Beauchamp (5) used a variation on the constant stimulus method by performing successive paired-comparisons in order to identify equally intense samples. A reference compound was chosen, and the researchers identified a concentration of medium intensity, one which was neither too extreme nor too weak. Panelists were presented with four pairs of samples; each pair included a test sample, which was a different compound and concentration than the reference plus the reference sample. They were asked to identify which sample was more bitter. The concentration of the test compound was successively reduced or increased per panel feedback, and the paired-comparisons were again presented to the panel until neither compound was select as more bitter for 55% of the trials. At this point the compounds were considered, on average, to be equivalent in bitterness intensity (5).

The magnitude estimation procedure is another method for equating sensations and has been used to compare sweetener intensities. In this method, a reference sample is defined and assigned a sweetness value of 100. Panelists measure the degree of difference in taste intensity between the test sample and a reference. Researchers used regression analysis to determine relative sweetness of the test samples in comparison to the control (6).

While these methods are useful for evaluating the required concentration of test compounds to create equi-intense sensations to a reference compound at a single, defined concentration, they become somewhat tedious to execute over a broad range

of concentrations. In this study, the goal was to identify an equal bitter relationship that was valid over a large range of concentrations of structurally similar bitter compounds from hops. Large variation among panelists was expected due to differing sensitivities to the bitter molecules as demonstrated previously within our laboratory. To model the relationship over a range of concentrations and to accommodate the expected variation, time-intensity data were collected and a statistical modeling technique called the change point model was applied to the peak intensity values. The goal was to identify differences among compounds despite inherent variation among panelists in compound sensitivity.

3.3 Materials and Methods

3.3.1 Sensory sample preparation.

An unhopped lager beer was prepared in the Oregon State University pilot brewery and used as the medium for all experiments. To accommodate the compositional variation between typical American and European lagers, the beer was prepared as a compromise between the two styles. It was brewed using 75% pale 2-row malted barley (Great Western Malting Co.) and 25% of the fermentable extract from rice syrup solids (California Natural Products). The starting and ending gravities were 11.69°P and 3.45°P respectively. The lager beer contained 5.27% alcohol by volume and 4.20% by weight according to analysis by gas chromatography. Additionally, the beer was low in bitterness impact, less than 1 bitterness unit as defined by the American Society of Brewing Chemists (8).

Four hop compounds were selected for the study. Iso-alpha acids are the main bittering compound in beer, and we used a commercially available product, Isohop®, at 87.5% purity (iso). Three reduced iso compounds were also evaluated. Redihop® and Tetrahop Gold® are commercially available forms of rho-iso-alpha acids (rho) and tetraydro-iso-alpha acids (tetra) at 95.4% and 96.8% purity. Hexahydro-iso-alpha acids (hexa) were provided in an 11.22% solution. All compounds were donated by John I. Haas, Yakima, WA.

Concentrations were selected based on informal analysis with the trained panel in which panelists gave peak intensity feedback on different compound concentrations. The concentrations covered a range of intensities on a sixteen-point sensory scale from just detectable (0-2) to extremely bitter (13-15). To create the samples, the compounds were first diluted to 0.50% in MilliQ water and added to the unhopped lager at high concentrations. These beers were filtered through a 0.45µm, nylon membrane filter (ULTIPOR® N66 Membrane Filter by Pall) to removed insoluble hop materials. The concentrations of the stock solutions were determined by high performance liquid chromatography (HPLC) as 57.2 ppm, 24.0 ppm, 44.2 ppm, and 55.0 ppm for iso, tetra, hexa and rho respectively. Each stock was then diluted with unhopped lager beer to the target concentrations and carbonated at 12 psi, 1°C for a minimum of 48 hours. Final concentrations were evaluated by HPLC. The resulting seven levels for all four compounds are represented in Table 2.

TABLE 2: Compound Concentrations for Sensory Samples

Compound	Level	Concentration (ppm)*	Compound	Level	Concentration (ppm)*
Iso	1	0.0	Hexa	1	0.0
	2	2.5		2	5.0
	3	7.0		3	9.5
	4	11.6		4	15.6
	5	16.1		5	19.5
	6	20.5		6	23.7
	7	26.1		7	27.6
Tetra	1	0.0	Rho	1	0.0
	2	5.6		2	4.7
	3	9.6		3	12.2
	4	13.4		4	18.4
	5	17.8		5	29.6
	6	19.6		6	37.1
	7	24.0		7	45.2

*Averaged values of four separate measurements.

Chromatographic analysis of the concentrations was executed according to a modified version of ASBC method Beer–23C based on work by Raumschuh (7, 29). The HPLC analysis ran at a constant temperature of 40°C and flow rate of 1.4 ml/min with a 10 µl injection volume. Two mobile phases were used. The first mobile phase was 100% methanol and the second was 75% methanol, 24% water and 1% phosphoric acids. A gradient elution was applied over a 22 minute analysis cycle beginning with 100% of the second mobile phase for the first 13 minutes. From 13 to 15 minutes, the mobile phases were mixed at a 1:1 ratio. Then, the analysis returned to 100% of the second mobile phase for the remaining 7 minutes. A Discovery C18 column was used for separation and absorbance was measured at 270nm. ASBC

International Calibration Extracts ICS-I2 (iso), ICS-R1 (rho), ICS-T2 (tetra), and ICS-H1 (hexa) were used as the standard for all measurements.

3.3.2 Sensory Evaluation

Bitterness intensity was evaluated by a thirteen-member trained panel. Nine members had two or more year's previous experience in bitterness evaluation, and four members were new to the panel. A time-intensity sensory protocol was used for evaluation because it would yield a large body of temporal bitterness data. Additionally, it minimized the likelihood of carry over between samples since panelists recorded the bitter intensities until they disappeared or the test had elapsed five minutes.

Prior to testing, the panel participated in three weeks of training. During the first two weeks, the panel trained as a group, and the final week of training was held under the testing conditions, in which panelists were isolated in booths and worked independently. Panelists wore nose plugs during all sessions and rated the intensity of the samples over time. Bitterness intensity was recorded continuously on a sixteen-point sensory scale (0 (None) to 15 (Extreme)).

The experimental design contained blocking by week, compound, and panelist. All four compounds were evaluated within one week. This represented one replication of the experiment in which three were executed. On each day of testing, all levels of one compound were presented in a random order per panelist. Each fifteen milliliter sample was served at approximately 4°C in 2-ounce opaque plastic serving cups with

lids. Prior to beginning the test, a warm-up sample was provided to prepare the palate for the beer medium. The warm-up sample was Level 2 for the compound being tested (iso~2.5 ppm, tetra~5.6 ppm, hexa~4.9 ppm, and rho~ 4.7 ppm). A computer interface, Compusense 5 (version 4.6, Guelph, Ontario, CAN), was used for data collection. To evaluate the test samples, the panelist placed the entire sample in his mouth, pushed the "start" button on the computer screen, and swallowed it completely within 10 seconds. A sixteen-point sensory scale was displayed on the computer screen, and panelists moved the curser up or down to identify the perceived bitterness from the sample. Bitterness intensity was recorded continuously (1 Hz) until zero bitter impact was perceived or 5 minutes had elapsed. Following each sample, the panelists had a forced two minute break during which they rinsed sequentially with a 0.10% pectin solution in spring water and spring water.

3.3.3 Data Analysis

Time intensity data (0-100) were collected. Peak bitterness intensity data were identified and converted to the equivalent 16 point sensory scale values. For example a sensory intensity of 15 was equivalent to an intensity of 100, and an intensity of 50 was equal to a 7.5 on the 16 point scale.

According to Fechner's law, the intensity of a sensation is proportional to the natural logarithm of the concentration (23); therefore, peak intensities were plotted versus the natural logarithm of the concentration for preliminary evaluation of the dose-response relationships. In general, the data displayed a two-phase response with

an area of no increase in bitterness intensity due to concentration and an area in which there was an increased response due to increased concentration (Figure 6). To accommodate for the shape of the data, a change point model was used. This model fit two lines, a flat line and a positively sloped line after a "change point," to each panelist's data per compound. Three values, *a, b*, and θ, were used to identify the shape of the change point model. The *a* value was the bitterness intensity (0-15) of the flat line (Equation 1). This parameter identified the sensory intensity given to all samples that were not significantly different in peak intensity from the base beer. The θ value was the change point, or the point at which the two lines met, and it was the natural logarithm of the concentration above which the panelists were able to significantly distinguish increases in bitter impact according to increases in compound concentration. The *b* value was the slope of the second line and identified the relationship between bitterness intensity and the natural logarithm of the concentration after θ (Equation 2).

$$\text{if } \ln C_{i,j} \le \theta \text{, then } S_{i,j} = a_{i,j} \tag{1}$$

$$\text{if } \ln C_{i,j} > \theta \text{, then } S_{i,j} = b_{i,j} \cdot \left\lfloor \ln C_{i,j} - \theta_{i,j} \right\rfloor + a_{i,j} \tag{2}$$

where C= compound concentration (ppm)

S= sensory intensity (0-15)

i= panelists (1-13)

j= compound (iso, rho, hexa, tetra)

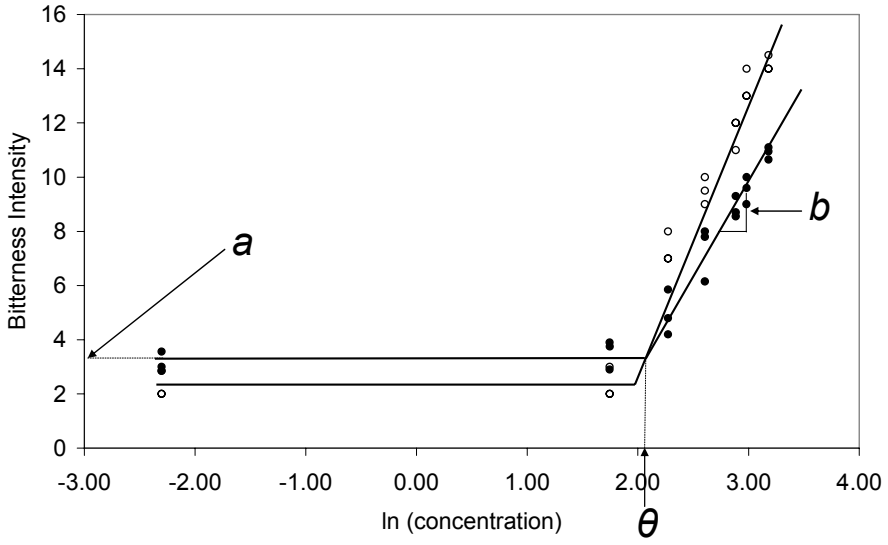

FIGURE 6. Example change-point model for two sets of data. The *a, b,* and θ values are identified.

The parameters, *a*, *b*, and θ, were utilized to create equal-bitter equations between each reduced iso-alpha-acid and iso on a per-panelist basis. By evaluating Equation 2 for two separate compounds using a common sensory intensity (S_{ij}), a per-panelist equi-intensity relationship was produced (Equation 3), which was further simplified (Equation 4). The resulting rearrangement produced new slope and intercept terms for a set of three, per panelist, equal-bitter relationships for each reduced iso-alpha-acid as it related to iso. The slopes ($M_{i,k}$) and intercepts ($I_{i,k}$) from Equation 4 were averaged across *all* panelists to yield three panel-wise equal-bitter relationships.

$$\{b_i \cdot [\ln C_i - \theta_i] + a_i\}_{\text{iso}} = S_i,\text{iso} = S_i,\text{Reduced iso} = \{b_i \cdot [\ln C_i - \theta_i] + a_i\}_{\text{Reduced iso}} \quad (3)$$

$$\ln C_i,\text{Reduced iso} = M_{i,k} \cdot \ln C_i,\text{iso} + I_{i,k} \quad (4)$$

$$\text{where} \quad M_{i,\text{k}} = \frac{b_{i,\text{iso}}}{b_{i,\text{Reduced iso}}}$$

$$I_{i,\text{k}} = \frac{\left(a_{i,\text{iso}} - b_{i,\text{iso}} \cdot \theta_{i,\text{iso}}\right)}{b_{i,\text{Reduced iso}}} + \left(\theta_i - a_i\right)_{\text{Reduced iso}}$$

i= panelists (1-13)

k= Reduced iso (rho, hexa, tetra)

A one-sample t-test was used to determine if the equi-bitter relationships indicated that the reduced iso-alpha-acids were significantly different in peak bitterness impact than iso (α=0.10). The slopes were tested to find if the relationship was significantly different from one, and the intercepts were evaluated against zero. The significance level was determined *a priori* as 0.10, which was less stringent than the typical value of 0.05, but was chosen because we expected large variation in bitter sensitivity across the panel. If either of these relationships were identified as not significant ($p>0.1$), then the two compounds were considered to be equi-bitter over the range of concentrations in our experiment.

Wide compound dependant variation among the panelists was expected; therefore, it was essential to determine if panelists could reliably detect and rate bitterness. This assured that variation in panel-wise results was not due to inadequate training or erratic panelist responses. Each panelist's ability to replicate was evaluated using the a, b, and θ values. The t-statistics for the parameters were compared to a t-multiplier from a 95% family-wise confidence interval with a Bonferroni correction (11 degrees of freedom; 4 compounds and 3 parameters each). If the t-statistic was

greater than the criterion, the panelist was able to reliably measure an effect about the parameter. Additionally, the panelist and compound effects on the *a, b* and θ values were determined by analysis of variance (ANOVA, α=0.10), and equal-bitter relationships per panelist and panel-wise were constructed. All statistical analysis was performed using S-plus 6.1 for Windows Professional Edition (Insightful Corp., 2002).

3.4 Results and Discussion

3.4.1 Panel-wise relationships

The per-panelist slope and intercept terms for each reduced iso-alpha acid to iso relationship were averaged to determine three panel-wise relationships. The equations were back transformed to predict the reduced iso-alpha acid concentration required for equi-bitterness at a defined iso concentration (Equations 5-7; Figure 7). Figure 7 is a graphical display of each reduced iso-alpha acid response compared to a 1:1 relationship with iso, signifying equality to iso. Hexa and tetra did not deviate substantially from the 1:1 iso relationship, but higher concentrations of rho were required.

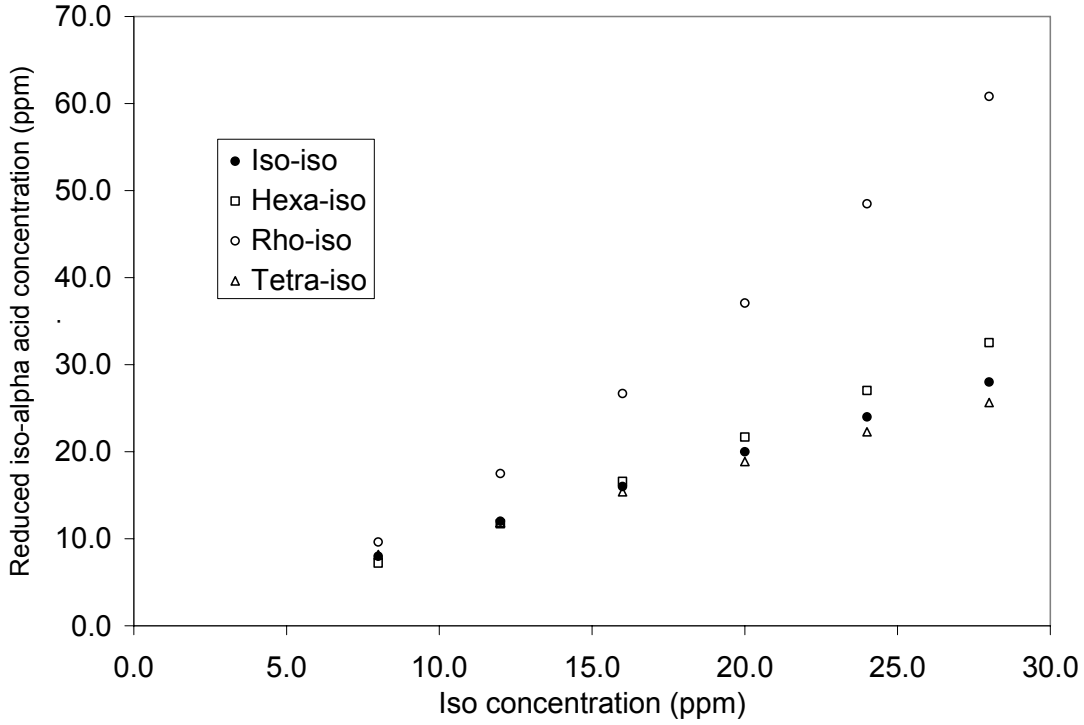

FIGURE 7: Predicted reduced iso-alpha acid concentrations for a defined iso concentration based on the averaged equations.

Hexa-iso: $$C_{Hexa} = e^{(1.204 \cdot \ln C_{Iso} - 0.530)} \quad (5)$$

Rho-iso: $$C_{Rho} = e^{(1.472 \cdot \ln C_{Iso} - 0.797)} \quad (6)$$

Tetra-iso: $$C_{Tetra} = e^{(0.911 \cdot \ln C_{Iso} + 0.209)} \quad (7)$$

Evaluation of the slopes and intercepts revealed differences among the compounds not initially evident through ANOVA of the *b* values, in which there was no significant effect due to compound (p=0.12). Wide variation across the panel was present for the slopes and intercepts (Table 4). Therefore, one-sample t-tests were

performed on each set of slope and intercept data (all panelists) to determine if each reduced iso-alpha acid was different in bitter intensity than iso (α=0.1). Accordingly, the intercepts were not statistically different from zero for all relationships (p-values: hexa = 0.33, tetra= 0.46, rho= 0.27). When comparing the slopes however, neither the hexa nor tetra were different from iso (p-values = 0.25 and 0.33, respectively), but rho was significantly less bitter (p=0.08).

TABLE 3: Panel-wise averages and standard deviations for the slopes and intercepts

Relationship	Slope	Intercept
Iso-hexa	1.204 ± 0.611	-0.630 ± 1.894
Iso-tetra	0.911 ± 0.319	0.209 ± 0.984
Iso-rho	1.472 ± 0.882	-0.797 ± 2.464

3.4.2 **Threshold Determination**.

The usefulness of the change point model for comparing dose-response data was evident in our results. Beyond this, the actual change-point, θ, may yield further information, such as a just noticeable difference threshold. A two phase model, similar to the one shown in this study, was explored by Marin et al. as a way to predict the "take-off threshold" (25). Accordingly, the take-off threshold was defined as the inflection point at which there was a rapid increase in sensory response due to increases in concentration. The inflection point was similar to the change-point, θ, as defined in the present study. Although both studies used a range of compound concentrations, the methodology for data collection was different. The Marin et al.

applied a paired comparison sensory protocol to collect data and had panelists rate the difference in the samples from a blank control. In this study, the time-intensity protocol was applied to gather maximum information about the bitterness profile of each compound in addition to collecting the peak intensity data.

Some might argue that the θ values represented a detection threshold; however, the values for θ were substantially higher than published values for the detection thresholds for these compounds (7-9 ppm). The detection threshold is the minimal amount of the stimulus required for an observer to detect that the stimulus is presented at least 50% of the time (22), and the detection threshold for iso in water ranged from 0.97-1.40 ppm (16). We hypothesize that the θ values represent a difference threshold for the compound in the unhopped lager beer. The difference threshold, or just noticeable difference, is the amount of change in concentration from a constant stimulus that can be reliably identified by an observer (22). Further testing is needed to confirm this hypothesis.

3.4.3 Panelist replication

Replication was measured according to the parameters of the change point model. Replication was defined as a measure of the panelist's ability to reliably evaluate the parameter of the model. The t-statistic for each parameter was compared to the t-multiplier from a 95% family-wise confidence level with a Bonferroni correction. If the t-statistic was greater than the criterion, we determined that the panelist could reliably measure an effect around that variable. In this study, the panelists replicated

well, but there was a large degree of variation among individuals. The most disparity was in the a values, and the θ and b values did not vary in practical significance (see Chapter 4 for more details).

3.5. Conclusion

This study focused on identifying equal-bitter relationships over a wide range of concentrations. Typical methods used, including paired-comparisons and constant stimulus, could not be utilized efficiently because a range of compound concentrations were evaluated. Additionally, panelists vary widely in response to bitter compounds, and in fact, we observed a significant amount of compound specific sensitivities across our panel. In spite of the panelist's individual sensitivities, they were able to replicate well. Due to the large amount of variation among panelist, initial results with univariate ANOVA did not reveal compound differences Upon further analysis with the change point model, variables were created that related each reduced iso-alpha acid response to the iso response. As a result, the variation, which resulted from discrepancies in how panelists used the sensory scale to rate the compounds, was reduced, and compound differences that were not initially quantifiable were revealed and defined.

3.6 Acknowledgements

We would like to thank John I. Haas for supporting this research. Additionally, we thank Cindy Lederer and the Oregon State University Sensory Department for assisting in the preparation and execution of the sensory testing and Jeff Clawson for helping to prepare the unhopped lager and sensory samples.

4. RELATIVE BITTERNESS OF REDUCED ISO-ALPHA-ACIDS TO ISO-ALPHA-ACIDS IN LAGER BEER

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4.1 Abstract

In this study, the relative bitterness of reduced to non-reduced iso-alpha-acids in an unhopped lager were measured using a time-intensity protocol. A trained panel evaluated six concentrations of each compound in an unhopped lager beer. The peak intensities were identified, and a non-linear dose-response curve was fit to the data. Equal bitter relationships were created by evaluating each reduced iso-alpha-acid dose-response relationship to iso. By doing so, variation among panelists in compound sensitivity and use of the sensory scale were accommodated. The parameters of these models were compared statistically using a paired comparison t-test and validated by a consumer panel. Accordingly, rho was less bitter than iso, and hexa and tetra were not different in bitter impact over the range of iso concentration.

Keywords: beer, bitterness, hops, iso-alpha-acids, reduced iso-alpha-acids

4.2 Introduction

Alpha-acids, which are a fraction of the soft resins found in the lupulin glands of hop cones, thermally isomerize to form iso-alpha-acids (iso), the primary source of beer bitterness. Photolysis of iso occurs when exposed to ultraviolet light (UV), and this releases an isopentenyl radical that reacts with a thiyl radical to form prenyl mercaptans causing defect aromas. Therefore, where UV degradation is of concern, it is important to brewers to find an alternative to iso. The reduced iso-alpha-acids, rho-iso-alpha-acids (rho), hexahydro-iso-alpha-acids (hexa) and tetrahydro-iso-alpha-acids (tetra), can be used as a substitute for iso and provide bitterness and UV stability.

The bitter relationships of the reduced iso-alpha-acids to iso have been explored with disagreeing results. Bitterness is difficult to assess for many reasons including variation in compound sensitivity among panelist and the medium's influence on bitter perception. Genetic diversity, such as the number of fungiform papillae on the tongue, the integrity of the taste buds within the papillae, and hormonal differences, leads to differences in the perception of bitter molecules (2). In fact, a deviation by a factor of 25 was observed among panelists when rating iso bitter intensity in water (34). Furthermore, previous research suggests that the ethanol content, glucose level, and pH of the medium influence bitter perception of iso over a range of concentrations. Iso bitterness was enhanced by ethanol, at concentrations less than 20 ppm, while glucose significantly reduced perceived bitterness over a range of iso concentrations (27). The beer pH affects the degree of compound dissociation of weak acids, such as the reduced and non-reduced iso-alpha-acids. Accordingly as a medium becomes increasingly basic, the dissociation of iso rose resulting in bitterness with longer duration and a more harsh and coarse quality (4). More specifically, the equal-bitterness relationships of the reduced iso-alpha-acids to iso have been evaluated in both water and beer mediums with conflicting results. The relative bitterness in a water medium was 0.67x, 2.03x, and 1.15x for rho, tetra and hexa, respectively (34). However when tetra was compared to iso in a commercial lager beer, it was not significantly more bitter but approximately equal in bitterness to iso (36).

In this study, the goal was to identify equal bitter relationships for each reduced iso-alpha-acid to iso over a large range of concentrations in an unhopped lager

beer medium. Large variation among panelists was expected, as demonstrated in previous studies and within our laboratory. To model the relationships and accommodate the expected variation, time-intensity data were collected and a statistical modeling technique, called a change point model, was applied to the peak intensity values. The goals were to identify differences and define the relationships among the compounds across a range of concentrations despite inherent variation among panelists in compound sensitivity.

4.3 Materials and Methods

4.3.1 Sensory sample preparation

An unhopped lager was created in the Oregon State University pilot brewery to be used as the medium for all sensory samples. To accommodate for differences between typical American and European lagers, the final composition of the beer was set to be a compromise between the two styles. It was brewed using 75% pale 2-row malted barley (Great Western Malting Co.) and 25% of the fermentable extract from rice syrup solids (California Natural Products). The starting and ending gravities were 11.69°P and 3.45°P, respectively. Alcohol content was determined as 5.27% by volume and 4.20% by weight according to analysis by gas chromatography. The beer was low in bitterness impact, less than 1 bitterness unit (BU).

The hop compounds were dosed into the base beer at concentrations established according to informal analysis with the trained panel in which panelists gave feedback on perceived bitterness from different concentrations. The

concentrations ranged in intensity on a sixteen-point sensory scale from just detectable (0-2) to extreme bitterness impact (13-15). The iso, tetra and rho samples were commercially available products, Isohop®, Redihop® and Tetrahop Gold® , at 87.5%, 95.4% and 96.8% purity. Hexa was provided in an 11.22% solution. All compounds were donated by John I. Haas.

To create the samples, the compounds were first diluted to 0.50% in MilliQ water and added to the unhopped lager at high concentrations. These beers were filtered through a 0.45µm, nylon membrane filter (ULTIPOR® N66 Membrane Filter by Pall) to removed insoluble material. The stock solution concentrations were determined by high performance liquid chromatography (HPLC) and were 57.2 ppm iso, 24.0 ppm tetra, 44.2 ppm hexa, and 55.0 ppm rho. Each stock was then diluted with unhopped lager to the target concentrations and carbonated at 12 psi in 1°C for a minimum of 48 hours. Final concentrations were evaluated by HPLC. The resulting seven levels are represented in Table 5.

TABLE 4: Compound Concentrations for Sensory Samples

Compound	Level	Concentration (ppm)*	Compound	Level	Concentration (ppm)*
Iso	1	0.0	Hexa	1	0.0
	2	2.5		2	5.0
	3	7.0		3	9.5
	4	11.6		4	15.6
	5	16.1		5	19.5
	6	20.5		6	23.7
	7	26.1		7	27.6
Tetra	1	0.0	Rho	1	0.0
	2	5.6		2	4.7
	3	9.6		3	12.2
	4	13.4		4	18.4
	5	17.8		5	29.6
	6	19.6		6	37.1
	7	24.0		7	45.2

*Averaged values of four separate measurements.

Chromatographic analysis was executed according to a modified version of ASBC method Beer–23C based on work by Raumschuh (7, 29). The HPLC analysis ran at a constant temperature of 40°C and flow rate of 1.4 ml/min with a 10 µl injection volume. Two mobile phases were used. The first mobile phase was 100% methanol, and the second was 75% methanol, 24% water and 1% phosphoric acids. A gradient elution was applied over a 22 minute analysis cycle beginning with 100% of the second mobile phase for the first 13 minutes. From 13 to 15 minutes, the mobile phases were mixed at a 1:1 ratio. Then, the analysis returned to 100% of the second mobile phase for the remaining 7 minutes. A Discovery C18 column was used for separation and absorbance was measured at 270nm. ASBC International Calibration Extracts ICS-I2 (iso), ICS-R1 (rho), ICS-T2 (tetra), and ICS-H1 (hexa) were used as the standard for all measurements.

4.3.2 Relative bitterness determination

Bitterness intensity was evaluated by a thirteen-member trained panel. Nine members had two or more year's previous experience in beer bitterness evaluation, and four members were new to the panel. A time-intensity sensory protocol was used for evaluation because it yielded a large body of temporal bitterness data, and it minimized the likelihood of carry over between samples since panelists recorded the bitter intensities until they disappeared or the test had elapsed for five minutes.

Prior to testing, the panel participated in three weeks of training. During the first two weeks, the panel trained as a group, and the final week of training was held under testing conditions in which panelists were isolated in booths and worked independently. Panelists wore nose plugs during all sessions and rated the intensity of the samples over time. Bitterness intensity was recorded continuously on a sixteen-point sensory scale (0 (None) to 15 (Extreme)).

The experimental design contained blocking by week, compound, and panelist. All four compounds were evaluated within one week, and on each day of testing, all levels of one compound were presented in a random order per panelist. This represented one replication of the experiment in which three were executed. The fifteen milliliter samples were served at approximately 4°C in 2-ounce opaque plastic serving cups with lids. Prior to beginning the test, a warm-up sample was provided to prepare the palate for the beer medium. The warm-up sample was Level 2 for the compound being tested (iso~2.5 ppm, tetra~5.6 ppm, hexa~4.9 ppm, and rho~ 4.7 ppm). A computer interface, Compusense 5 (version 4.6, Guelph, Ontario, CAN), was

used for data collection. To evaluate the test samples, the panelist placed the entire sample in his mouth, pushed the "start" button on the computer screen and completely swallowed it within 10 seconds. A sixteen-point sensory scale was displayed on the computer screen, and panelists moved the curser up or down to identify the perceived bitterness from the sample. The intensity was recorded continuously (1 Hz) until zero bitter impact was perceived or 5 minutes had elapsed. Following each sample, the panelists had a forced two minute break during which they rinsed sequentially with a 0.10% pectin solution in spring water and spring water.

Peak intensities were collected from the time-intensity data (0-100) and used to determine equal bitter relationships for each reduced iso-alpha-acid to iso. Initially, the peak bitterness intensity data were converted to the equivalent 16 point sensory scale values. For example, a sensory intensity of 15 was corresponding to an intensity of 100, and an intensity of 50 was equal to a 7.5 on the 16 point scale. In accordance with Fechner's law, the peak intensities were plotted versus the natural logarithm of the concentration (23). A two phase, change point model was used to accommodate the shape of the data. This model fit two lines, a flat line and a positively sloped line after a "change point," to each panelist's data per compound (Figure 8).

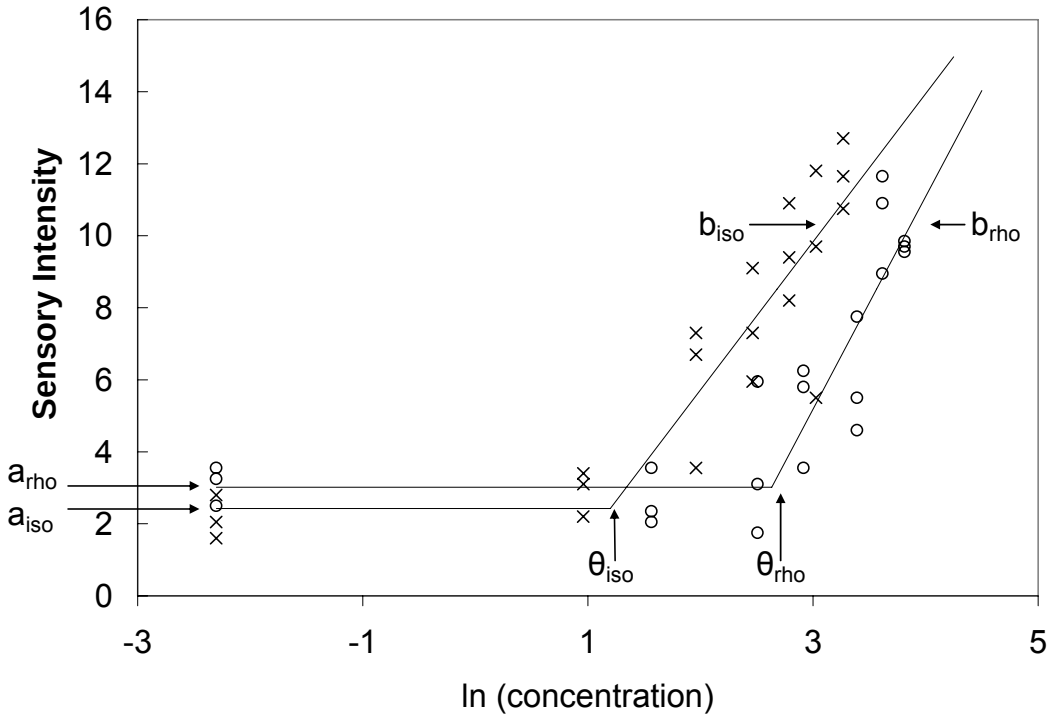

FIGURE 8. Change-point model for one panelist representing iso (×) and rho (○). The *a, b,* and *θ* values are identified for iso and rho, respectively.

Three values, *a, b*, and *θ*, were used to identify the shape of the change point model. The *a* value was the bitterness intensity (0-15) of the flat line (Equation 1). The *θ* value was the change point and was the natural logarithm of the concentration above which the panelists were able to significantly distinguish concentration dependant increases in bitter impact. The *b* value was the slope of the second line and established the rate of increase per changes in concentration above *θ* (Equation 2). These parameters were analyzed by ANOVA (α=0.10) for panelist and compound effects.

$$\text{if } \ln C_{i,j} \le \theta \text{, then } S_{i,j} = a_{i,j} \quad (1)$$

$$\text{if } \ln C_{i,j} > \theta \text{, then } S_{i,j} = b_{i,j} \cdot \left\lfloor \ln C_{i,j} - \theta_{i,j} \right\rfloor + a_{i,j} \quad (2)$$

where C= compound concentration (ppm)

S= sensory intensity (0-15)

i= panelists (1-13)

j= compound (iso, rho, hexa, tetra)

Per-panelist equal-bitter equations for each reduced iso-alpha-acid to iso were created by utilizing the parameters, a, b, and θ (Equation 3) and were averaged across *all* panelists to yield three panel-wise equal-bitter relationships. One-sample t-tests, on the slope and intercept terms, were applied to ascertain if the reduced iso-alpha-acids were significantly different in bitterness intensity to iso at equal concentrations (α=0.10) (13).

$$\ln C_{i,\text{Reduced iso}} = M_{i,k} \cdot \ln C_{i,IAA} + I_{i,k} \quad (3)$$

$$\text{where } M_{i,\text{k}} = \frac{b_{i,iso}}{b_{i,\text{Reduced iso}}}$$

$$I_{i,\text{k}} = \frac{\left(a_{i,\text{iso}} - b_{i,\text{iso}} \cdot \theta_{i,\text{iso}}\right)}{b_{i,\text{Reduced iso}}} + \left(\theta_i - a_i\right)_{\text{Reduced iso}}$$

i= panelists (1-13)

k= Reduced iso (rho, hexa, tetra)

In order to increase confidence in the prediction equations, the panelist's ability to replicate were evaluated by utilizing the t-statistics for the a, b, and θ parameters. These were evaluated against a t-multiplier from a 95% family-wise confidence level with a Bonferroni correction. If the t-statistic was greater than the criterion, the panelist was able to reliably measure an effect about the parameter.

4.3.3 Consumer Validation

After modeling the relative-bitterness relationships, predicted equal-bitter concentrations were assessed by a consumer panel for differences in peak bitter intensity. A two-sample forced choice, sensory protocol was utilized. Panelists were presented three pairs of samples, each a reduced iso-alpha-acid and an iso sample at the predicted equal-bitter concentrations. Panelists were asked to identify which sample was more bitter. The target concentrations of the reduced iso-alpha-acids were determined for equivalent bitterness to 10 ppm iso (10.0 ppm tetra, 9.4 ppm hexa, and 13.3 ppm rho), and the final concentrations of the samples, according to HPLC analysis, were 10.1 ppm iso, 13.4 ppm tetra, 9.1 ppm hexa, and 13.7 ppm rho. The tetra sample was slightly higher than the target value but was within the calculated confidence interval for equal-bitterness. A computer interface and Compusense 5 (Compusense 5.0, version 4.6, Guelph, Ontario, CAN) was used for data collection.

Samples were randomized within and between the pairs. Therefore, all possible pairs and sample orders were presented more than once throughout the study. The one-ounce beer samples were served in 2-ounce opaque plastic serving cups with

lids at approximately 4.4°C. Panelists were seated in individual testing booths and were assigned an identification number that was linked to a predetermined serving presentation. Panelists wore nose plugs to eliminate olfactory influence. A warm-up sample of approximately 6 ppm iso was provided to the panelists. Between each sample pair, there was a 2 minute forced wait during which the panelists rinsed with spring water. Following the test, demographic data were collected to confirm the panelist's age, gender, and beer consumption patterns. Due to the desirability of the product, historical experience within the sensory laboratory indicated that initial screenings of preference and drinking habits might not yield an accurate account of the true demographics of the panel. Therefore, it was essential to validate the demographic data following the testing session. A paired comparison t-test (α=0.10) was used to analyze the consumer data. All statistical analysis was performed using S-plus 6.1 for Windows Professional Edition (Insightful Corp., 2002).

4.4 Results and Discussion

4.4.1 ANOVA of change-point parameters

Analysis of variance was applied to the parameters of the change-point model to determine if there were panelist or compound effects present. The incidence of compound by panelist interactions could not be tested by ANOVA because the parameters, *a, b,* and θ, were a composite of all three replications. As a result, the *a, b,* and θ values were not replicated leading to a lack in sufficient degrees of freedom to test the interactions. However, the interactions were evaluated graphically in

interaction plots. The *a* values yielded significant panelist and compound effects (p=0 and 0.02, respectively), but neither panelist nor compound significantly impacted the θ values (p=0.32 and 0.72 respectively). Additionally, panelist had a significant effect on the *b* values, but compound did not (p=0.01 and 0.12, respectively). Averages and standard deviations for each parameter are in Table 5.

The panelist effect on the *a* value resulted from differences in how the intensity scale was used when evaluating the samples and from dissimilarities in compound sensitivity. The *a* values over all compounds ranged from 0.4 to 6.0 bitterness intensity (on a 16 point scale) with an average of 2.8 and a standard deviation of 1.2 units. However, the relative effect attributed to the compounds was not pragmatically different from that caused by the differences among individual panelist. The narrowest interval of *a* values was for rho, in which the minimum intensity was 0.5, and the maximum intensity was 4.3. The widest variation was for iso and ranged from 0.8 to 6.0 units.

Panelists who were inconsistent for one compound in rating increased bitter intensity per changes in concentration (*b* value) were not necessarily erratic for the other compounds. The *b* values ranged from 1.4 to 13.0 across all compounds. As seen with the *a* values, panelists used the scale differently to rate bitterness. This occurrence affected the *b* values so that as concentration increased the rate of change in perceived bitterness (within panelist) was dependant on how broadly the panelist used the sensory scale.

There was an absence of a compound effect on the *b* value, which was unexpected since previous research suggested that the compounds were dissimilar in concentration dependant bitterness (34, 36). The average *b* values were 6.0, 5.7, 4.9, and 6.9 rate of change in concentration dependant bitterness for hexa, iso, rho and tetra, respectively. The narrowest range of *b* values was for iso (3.9 to 8.9) suggesting that the panelists, as a group, perceived changes in iso concentration similarly when compared to the other compounds. The range of each reduced iso-alpha acids was more than twice the range for iso.

TABLE 5: Panel-wise averages and standard deviations for the *a, b, and θ* values

Compound	*a* (Sensory intensity)	*b* ($\frac{\Delta \text{ Sensory intensity}}{\Delta \ln(\text{concentration})}$)	*Θ* ln(concentration)
Iso	3.1 ± 1.3	5.8 ± 1.7	2.1 ± 0.4
Hexa	2.9 ± 1.3	6.0 ± 3.0	2.1 ± 0.5
Tetra	2.5 ± 1.1	6.9 ± 2.8	2.0 ± 0.4
Rho	2.6 ± 1.0	4.9 ± 2.5	2.2 ± 0.72

Panelist by compound interactions were observed graphically in interaction plots, and the diversity among panelists in compound sensitivity could be observed by constructing the dose-response relationship above *θ*. Figure 9 represents four separate panelist responses to iso and rho, and the concentrations for a sensory intensity of 8.5 are identified. At a sensory intensity of 8.5, over 75% of the panel required more rho than iso to create equal intensity, and on average, panelists required 1.4 times the amount of rho than iso. In the figure, two of the four panelists required concentrations

of rho far above the solubility limit (~50 ppm). These panelists were less sensitivity to rho than the other two panelists who required less concentration.

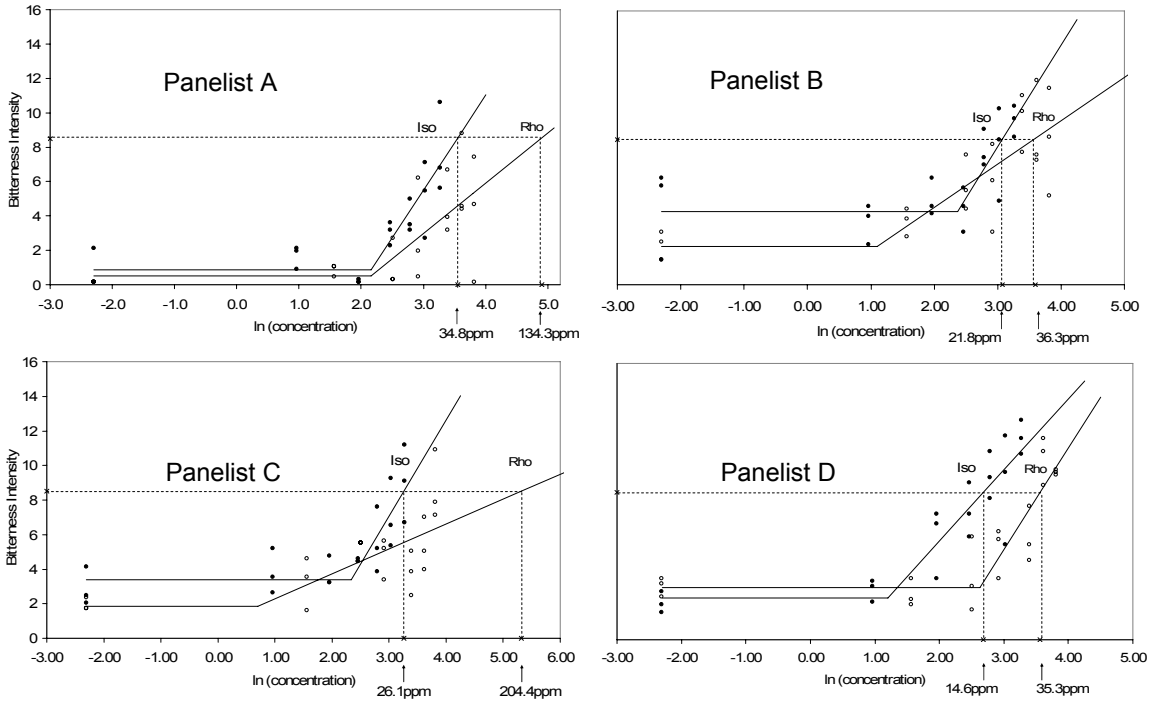

FIGURE 9: Variation in response to increases in concentration for iso (●) and rho (○) from four different panelists. Concentrations indicated reflect predicted requirements for a sensory intensity of 8.5 for both compounds.

Relative sensitivity was determined within panelist by comparing the *b* values of the reduced compounds to iso. Panelists with larger *b* values comparatively were more sensitive to the compound since less concentration change was required for an increased bitter response. The majority of panelists, nearly 70%, were less sensitive to rho than to iso ($b_{rho}<b_{iso}$), and this was also true for hexa. However, approximately 70% of panelists were more sensitive to tetra than iso ($b_{tetra}>b_{iso}$). Notably, panelists were not consistent in sensitivity to the compounds, for example panelists' insensitive

to rho were not necessarily insensitive to tetra, which affirmed the presence of compound by panelist interaction.

4.4.2 Panel-wise relationships

The per-panelist slope and intercept terms for each reduced iso-alpha-acid to iso relationships were averaged to determine three panel-wise relationships. The equations were back transformed to predict the reduced iso-alpha-acid concentration required for equi-bitterness at a defined iso concentration (Equations 5-7; Figure 10). Figure 2 is a graphical display of each reduced iso-alpha-acid response compared to a 1:1 relationship with iso, signifying equality to iso. Hexa and tetra did not deviate substantially from the 1:1 iso relationship, but higher concentrations of rho were required for equal bitterness to iso.

Hexa-iso: $$C_{Hexa} = e^{(1.204 \cdot \ln C_{Iso} - 0.530)} \quad (5)$$

Rho-iso: $$C_{Rho} = e^{(1.472 \cdot \ln C_{Iso} - 0.797)} \quad (6)$$

Tetra-iso: $$C_{Tetra} = e^{(0.911 \cdot \ln C_{Iso} + 0.209)} \quad (7)$$

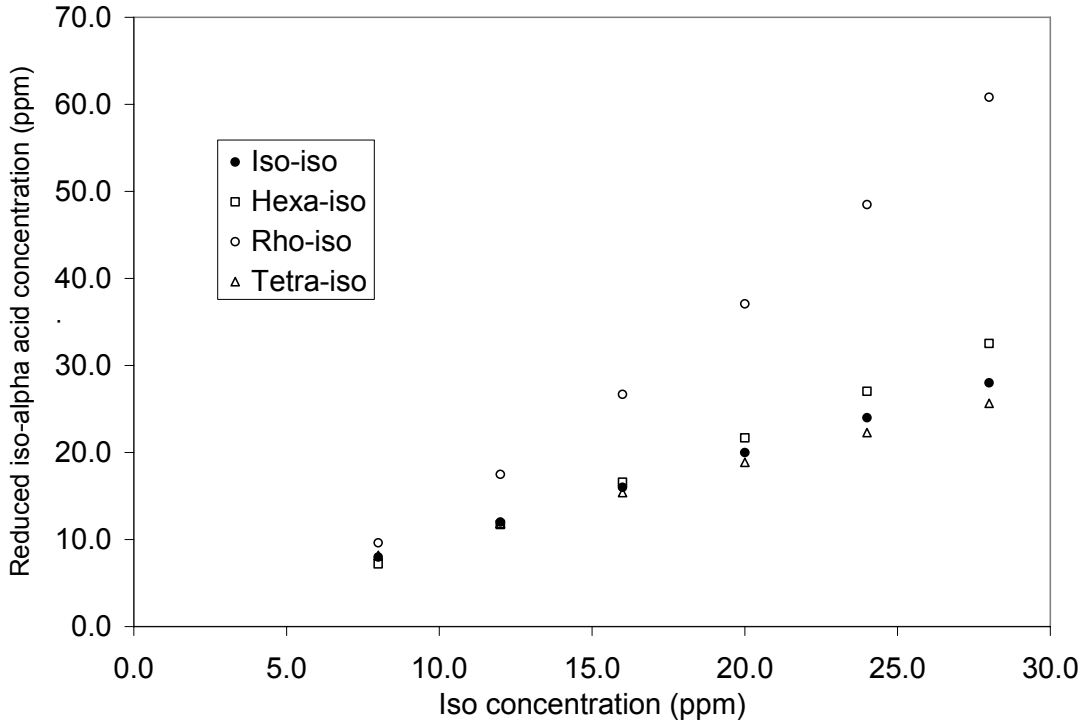

FIGURE 10: Predicted reduced iso-alpha acid concentrations for a defined iso concentration based on the averaged equations.

One-sample t-tests were performed on each set of slope and intercept data all panelists) to determine if each reduced iso-alpha-acid was different in bitter intensity than iso (α=0.10). Accordingly, the intercepts were not statistically different from zero for all relationships (p-values: hexa = 0.33, tetra= 0.46, rho= 0.27). When comparing the slopes however, neither the hexa nor tetra were different from iso (p-values = 0.25 and 0.33, respectively), but rho was significantly less bitter (p=0.08).

Predicted equal bitter equations, according to previous research, were medium dependant, and our results agreed with analysis utilizing a commercial lager for the tetra and rho relationships to iso. Research by Weiss et al. (36) indicated that tetra was approximately equal in bitterness to iso 1.0-1.1, and rho was 0.6 times the

bitterness of iso in a commercial lager beer. Our study created log-linear relationships for each reduced iso-alpha-acid to iso. Therefore, to compare these findings to the previous research, equal-bitter levels for each reduced iso-alpha-acids were calculated for 8 and 15 ppm of iso. In agreement with the earlier findings, tetra was approximately equal in bitterness to iso, and rho was less bitter by a factor of 0.83 times and 0.62 times 8 and 15 ppm iso, respectively. Equal bitter analysis of hexa in a beer medium is not documented. However, when evaluated in water, hexa was 1.15 ± 0.18 times more bitter than iso (34), which collaborates with the results in this study.

4.4.3 Panelist replication

Replication was measured according to the parameters of the change point model. Replication was defined as a measure of the panelist's ability to reliably evaluate the parameter of the model. The t-statistic for each parameter was compared to the t-multiplier from a 95% family-wise confidence level with a Bonferroni correction. If the t-statistic was greater than the criterion, we determined that the panelist could reliably measure an effect around that variable.

The a value was the most variable of the parameters, which was not unexpected. Lower concentrations may have been below the panelist's threshold for evaluating changes in bitterness, and each panelist was expected to have different sensitivities to the compounds. Of the 52 a parameters (13 panelist's × 4 compounds), 25% of the t-statistics were below the defined criterion. In these instances, the panelist's were not able to replicate well or reliably measure concentration dependant bitterness below θ. The 75% of panelists that were above the determined criterion

demonstrated reliable bitterness measurement for concentrations less than θ in spite of not being able to identify intensity changes from the lower concentrations of a particular compound and the unhopped lager beer. When examined by compound, the least variable *a* value was for iso with more than 92% above the criterion. The reduced iso-alpha acids were less reliably measured and had 23%, 31% and 39% below the criterion for hexa, rho and tetra, respectively. This was not surprising since the panel had the most experience with iso, encompassing personal beer consumption and panel experience.

The concentration at which panelists began to detect a change in bitter intensity compared to the unhopped lager was the θ value. These values were the most reliable of the three parameters with more than 93% of panelists above the criterion. The most variable θ was for rho, in which 3 of the 13 panelist's t-statistics were below the criterion. The average overall θ value was 8.1 with a range of 1.8 to 23.1 ppm, and the average θ values per compound were 7.8, 8.1, 9.1 and 7.5 ppm for hexa, iso, rho and tetra, respectively.

For concentrations greater than θ, the panelist's ability to identify differences in bitter impact due to changing concentration was measured by the *b* value.. Inconsistency around the *b* values was minimal with only five of the fifty-two parameters (9.6%) found to be less than the criterion. In these instances, the panelists were unable to dependably measure the changes in bitter intensity due to adjustments in concentration. Iso and hexa were the most unreliable across the *b* values, in which 2 of the 13 panelists had difficulty replicating. All panelists could reliably detect

concentration dependant bitterness for tetra levels above θ. Therefore, panel-wise there was greater consistency in identifying tetra than the other three compounds. We hypothesize that there was a qualitative aspect to tetra's bitterness that increased concentration dependant bitterness recognition. The panelists who were inconsistent for one compound were not necessarily erratic for the other compounds.

Overall, panelists were able to reliably measure the effects around the a*, b,* and θ values. However, there were significant differences in sensitivity across the compounds, and panelists varied in which compounds they were sensitive. This is determining the equal bitter equations per-panelist and then panel-wise yielded a more accurate account of the variation across the panel.

In fact, the predicted reduced iso-alpha-acid concentrations per iso concentrations differed widely among panelists. Figure 11 is box plots of the predicted concentration across the panel for each reduced iso-alpha-acid at the iso concentrations used originally in the sensory study. Panel-wise the concentrations predicted were more closely defined for tetra, identified by a narrow interquartile range, and further implies that there may be a qualitative aspect to tetra which made it more easily identifiable. The widest interquartile range was for rho representing a higher degree of variation in sensitivity to this compound.

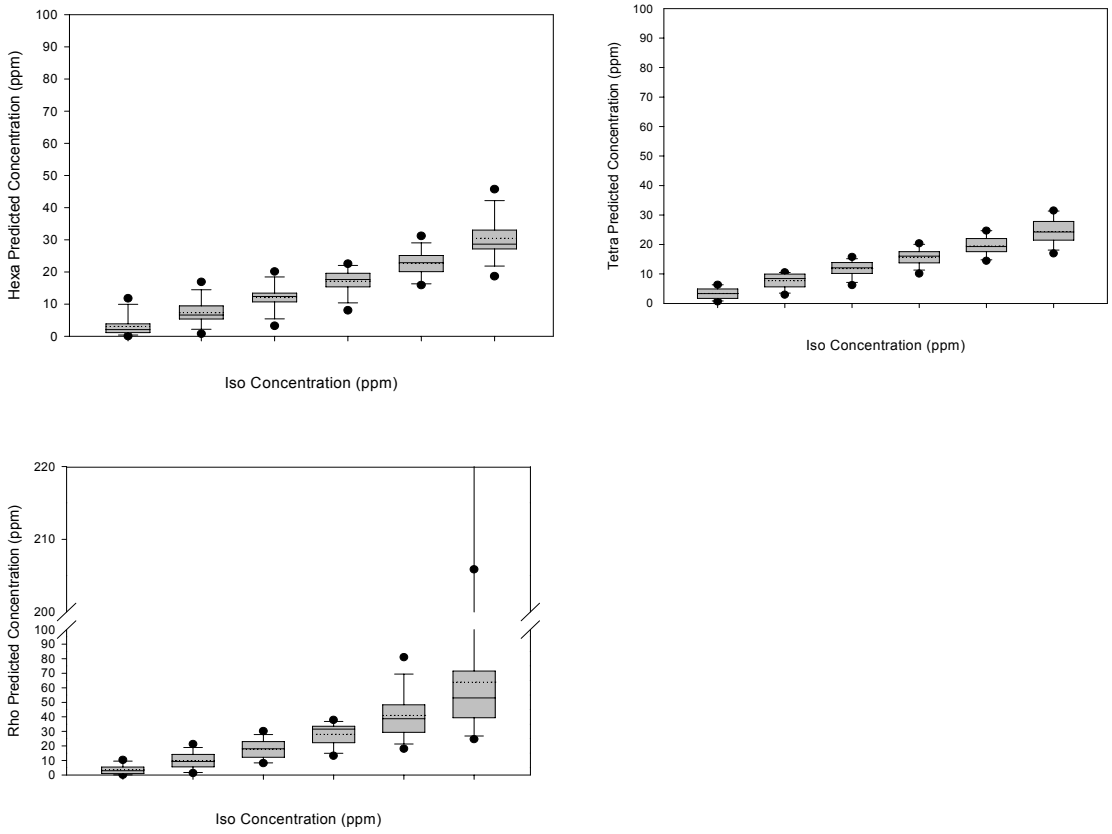

FIGURE 11: Box plot of predicted concentrations of reduced iso-alpha acid concentrations (n=13). The bottom and top of each box, interquartile range, designate the 25th and 75th percentiles, respectively, and the error bars designate the 10th and 90th. A solid line within the box is the median, and the dotted line is the average. All outliers are outside of the box and represented by a dot.

4.4.4 Consumer work

The change point model and relative-bitterness equations were created by a mathematical exercise, and therefore, it was essential to validate the panel-wise prediction equations with a larger consumer panel. One hundred and three beer drinking consumers (>1 consumed per month) participated in the study, with approximately 60% of the participants between 21 and 29 years of age and the

majority male (~63%). Most panelists (96%) consumed beer once or more weekly, and 71% percent of panelists drank craft beer and 45% consumed domestic beer more than once a week (non-exclusive).

Panelists evaluated each set of bitter-equivalent concentrations and identified the sample which was more bitter. A similarity test was not used in the analysis because informal examination within our lab indicated that there were qualitative differences among the compounds, and a paired comparison t-test was used to determine if the samples were not statistically different (α=0.05). As a result, there were no significant differences in any of the paired comparisons presented (p=0.06 hexa-iso, p=0.62 tetra-iso, and p=0.14 rho-iso). Therefore, the equal-bitter equations derived from the trained panel data were adequate at predicting reduced iso-alpha-acid concentrations that were not significantly different iso at a defined level.

4.5 Conclusions

High panelist variability in perceiving bitter compounds leads to complications in defining equal bitter equations, especially over a range of concentrations. The change point model, when applied, provided parameters, *a, b,* and θ, that were used to create equal bitter relationships and to identify how well panelists replicated. Accordingly, tetra and hexa were found to be equal in peak bitterness to iso, and rho was significantly less bitter. This was confirmed by using a consumer panel to identify if significant differences existed between the compounds at predicted levels, and there were no significant differences. Qualitative differences may exist, and further analysis

of the bitter character would be required to determine if tetra has a distinct profile that makes it more identifiable to panelists.

4.6 Acknowledgements

We would like to thank John I. Haas for supporting this research. Additionally, we thank Cindy Lederer and the Oregon State University Sensory Department for assisting in the preparation and execution of the sensory testing and Jeff Clawson for helping to prepare the unhopped lager and sensory samples.

5. CONCLUSIONS

Brewers are concerned with bitterness since it is essential for balancing beer flavor, including the sweet and acidic qualities of malt. Bitterness is sourced from several components in beer, but the primary bittering compounds are from hops. Evaluating bitterness impact from compounds is difficult due to natural variation among panelists caused by genetic, hormonal and age differences. Additionally, the medium may create synergies with or inhibit the bitterness impact. In these studies, an unhopped lager was used to determine the bitterness impact of several hop compounds, including alpha-acids, iso-alpha-acids and the reduced iso-alpha acids, rho-iso-alpha-acids, hexahydro-iso-alpha-acids and tetrahydro-iso-alpha-acids.

Alpha-acids are the precursors to the major bittering components in beer, iso-alpha-acids. Typically, they are not present in finished beer, but when a beer is dry-hopped alpha-acids may dissolve into the product due to the solvating power of ethanol. Brewers who dry-hop have observed increased bitterness after the process. We used a consumer panel to determine if beers with and without alpha-acids were statistically similar, and we found that any bitterness increase perceived from dry-hopping was not a result of added alpha-acids.

Iso-alpha-acids are naturally formed during the boil by thermally induced isomerization of alpha-acids. These compounds are susceptible to UV light degradation which results in off-odors and flavors. When UV degradation is of concern, reduced iso-alpha-acids can be used to replace the bitterness of iso-alpha-acids. There has been conflicting evidence in regards to the relative bitterness of each

of the reduced iso-alpha-acids compared to iso-alpha-acids, and panelists vary widely in sensitivity to these compounds. We evaluated the peak bitterness of a range of concentrations for all the reduced and non-reduced iso-alpha acids, and the dose-response curves were modeled with a change-point model to accommodate these sensitivities. The parameters from the change-point model were used to determine equal-bitter relationships within panelist and across the panel. As a result, tetrahydro-iso-alpha-acids and hexahydro-iso-alpha-acids were determined to be approximately equal in bitterness to iso-alpha-acids. Rho-iso-alpha-acids were less bitter. The prediction equations were used to calculate concentrations for equal bitterness for a defined iso-alpha-acids concentration, and the predictions were validated by a consumer panel at an iso-alpha-acid concentration of 10 ppm.

Determining bitterness contribution potential of each of these components is essential for brewers so that they can forecast the bitterness levels of the final product. Various factors contribute to the final impact resulting from compounds, including the medium, and panelists vary widely in sensitivity to the compounds. This results in each person having a different experience when evaluating the bitterness impact of a beer. Using a consumer panel to validate results was essential for these tests, and as a result, we were able to determine the bitterness impact of alpha-acids and reduced and non-reduced iso-alpha-acids in an unhopped lager beer.

6. FUTURE RESEARCH

Future research on bitterness and hop acids includes the following:

- The medium for evaluation is a determining factor for bitterness perception. In these studies, an unhopped lager beer that was a compromise between a European and an American style lager beer was used, but depending on the content of a beer, like the amount of glucose and ethanol, the perceived impact of bitterness would change. This is a possible area for future research. Determining the degree of effect of different variables, including acidity, salt, sugar, etc., a brewer may be able to more accurately predict the amount of bitterness resulting from the addition of a reduced or non-reduced iso-alpha-acid.
- Another area to be researched is the qualitative aspects of each of the reduced and non-reduced iso-alpha-acids. The trained panel could most reliably resolve changes in concentration dependant bitterness for tetra. This may indicate that tetra has a qualitative characteristic that increases a panelist's ability to detect changes.
- Perhaps, since hydrophobicity is directly linked to bitter impact, the fact that tetra is the most hydrophobic of the compounds may lead to it being more easily identifiable.

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