AN ABSTRACT OF THE THESIS OF

Mark G. Malowicki for the degree of Master of Science in Food Science and Technology presented on September 13, 2004.

Title: Hop Bitter Acid Isomerization and Degradation Kinetics in a Model Wort-Boiling System

Redacted for privacy

Abstract approved: _____

Thomas H. Shellhammer

Solubility limits of alpha-acids (humulones found within the hop cone of the *Humulus lupulus* plant) were experimentally measured in a pH buffered, aqueous solution, and found to be limited to approximately 90 ppm at pH 5.2. The rate of isomerization of alpha acids to iso-alpha acids was then determined across a range of temperatures (90 to 130° C) in order to characterize the rate at which iso-alpha-acids are formed during kettle boiling. Concentrations of humulones and iso-humulones were quantified by high pressure liquid chromatography (HPLC). The isomerization reaction was found to be first order, with reaction rate varying as a function of temperature. Rate constants were experimentally determined as $k_1 = (7.9x10^{11})e^{\frac{-11858}{T}}$ for the isomerization reaction of alpha-acids to iso-alpha-acids, and $k_2 = (4.1x10^{12})e^{\frac{-12994}{T}}$ for the subsequent degradation of iso-alpha-acids to uncharacterized degradation products. Activation energy was calculated as 98.6 kJ per mole for isomerization, and 108.0 kJ per mole for degradation.

Losses of iso-humulones to degradation products were pronounced for cases in which boiling was continued beyond two half-lives of alpha concentration. Several factors known to affect hop utilization were then investigated for potential impact on rate of isomerization. Of the factors tested (glucose at 10° Plato, maltose at 10° Plato, calcium at 100 ppm, and pH ranging from 4.8 to 6.0) none were shown to affect the rate of production of iso-alpha acids. While pH had a marked effect on the concentrations of alpha acids as measured, the differences may be attributed to solubility issues (as the solubility limit was approached and exceeded with decreasing pH) and did not appear to affect the rate of iso-alpha production.

Hop Bitter Acid Isomerization and Degradation Kinetics in a Model Wort-Boiling System

by

Mark G. Malowicki

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented September 13, 2004
Commencement June 2005

Master of Science thesis of Mark G. Malowicki presented on September 13, 2004

APPROVED:

Redacted for privacy

Major Professor, representing Food Science and Technology

Redacted for privacy

Head of the Department of Food Science and Technology

Redacted for privacy

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.

Redacted for privacy

Mark G. Malowicki, Author

ACKNOWLEDGMENTS

Many thanks to Tom Shellhammer, my advisor and mentor, for his tireless support and guidance. Throughout my time at Oregon State, he has not only been a constant source of scientific expertise and enthusiastic encouragement, but also a friend. None of my work would have been possible without his help. I would also like to thank the other members of my committee for their comments and suggestions.

Thanks also to my fellow graduate students within the Brewing Lab, as well as all of my friends here at Oregon State, for their friendship and camaraderie. It will be with great fondness that I remember slices of cheese, two dollar pints, and the (all-too-infrequent) mountain bike rides at McDonald Dunn. Thanks to all of the faculty, staff, and students within the Food Science Department, for making each day enjoyable.

Thanks to my parents and family for their eternal love and support, and to Sarah for everything.

Thanks also to water, *Hordeum vulgare*, *Saccharomyces cerevisiae*, and of course *Humulus lupulus*.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF TABLES

Table Page

Hop Bitter Acid Isomerization and Degradation Kinetics in a Model Wort-Boiling System

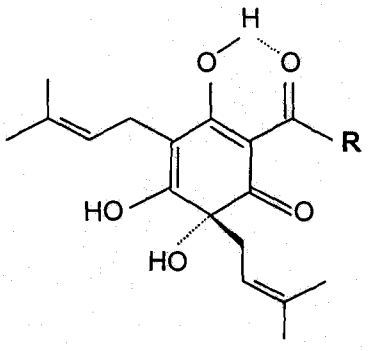
1. INTRODUCTION

1.1. Beer Just Wouldn't be Beer Without the Hops

Throughout the ages, brewers have explored a variety of spices and other ingredients, in hopes of achieving a more balanced, drinkable, and enjoyable beverage. The most successful synergy was reached when the cones of the hop vine (*Humulus lupulus*) were added to the boiling kettle, to contribute bitterness to the brew, and balance the sweetness of malted barley. The practice of adding hops to a lengthy kettle boil also brought with it the unexpected side benefit of decreased microbial spoilage, a factor of prime importance when brewers began shipping beer over significant distances. Hops endure to this day as an essential ingredient used in the brewing process, and are indeed the only herb or spice used to flavor beer in commercial scales. [14, 21]

1.2. Hop Bitter Acids

The ultimate source from which bitterness arises is the small yellow gland (the lupulin gland) tucked within the cone of the hop vine, which contains resins and essential oils. The essential oils are rich in volatile aromatic compounds prized by brewers (myrcene, farnesene, humulene, and caryophyllene). The resins are defined as those compounds soluble in methanol or diethyl ether, and are



R	α-acid
$-CH_2CH(CH_3)_2$	Humulone
$-CH(CH_3)_2$	Cohumulone
$-CH(CH_3)CH_2CH_3$	Adhumulone

FIGURE 1.1. Alpha-acid structure

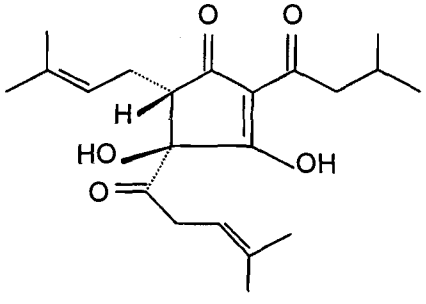


FIGURE 1.2. Iso-alpha-acid structure

composed of both α-acids and β-acids. The α-acids are of prime importance to the brewer due to their bittering potential. β-acids have no recognized brewing value, save that oxidized β-acids are known to be bitter. Resins typically account for 15% of the hop cone by weight, and essential oils approximately 0.5%. [7, 12, 16]

The bitter compounds of interest are known as humulones (a.k.a. α-acids), and are complex enolic acids with a six carbon ring structure (Figure 1.1). The amount of humulones present in the hop cone varies widely by variety and cultiva-

tion conditions, but typically ranges between 2 and 14%. [21, 12] Three analogs of the alpha-acid exist — humulone, ad-humulone, and co-humulone, depending on the side chain present at the R location in Figure 1.1. (Side chains are isovaleryl, 2-methyl butyryl, and isobutyryl, respectively [8].) While these α-acids have little to no bitterness in their natural form [16, 7], they undergo an isomerization reaction when heated, in order to become the bitter compounds desired by brewers and beer drinkers alike.

The products of isomerization are the iso-humulones, or iso-α-acids, as shown in Figure 1.2. Side chains at the R location are identical to those given in Figure 1.1. The isomerization reaction brought about by wort boiling converts α-acids into the more bitter, and more soluble iso-α-acids, and (for typical brewing recipes) requires between one and two hours of kettle boiling in order to achieve proper bitterness levels. The isomerization reaction consists of a ring opening between carbons 5 and 6, and a reattachment forming a five-membered ring having an additional carbon in the side chain. [8] The mechanism of isomerization for humulone, as given by Hough [7], is shown in Figure 1.3. Note the formation of both cis and trans forms of iso-humulone shown in the figure.

1.3. Pertinent Literature

1.3.1. General Information on Hops

Several published articles provide thorough accounts of the history, composition, and usage of hops. Moir [14] provides an excellent overview of hops that includes the history of their first recorded use in brewing prior to 859 A.D., a breakdown of their chemical composition and significance, and also comments

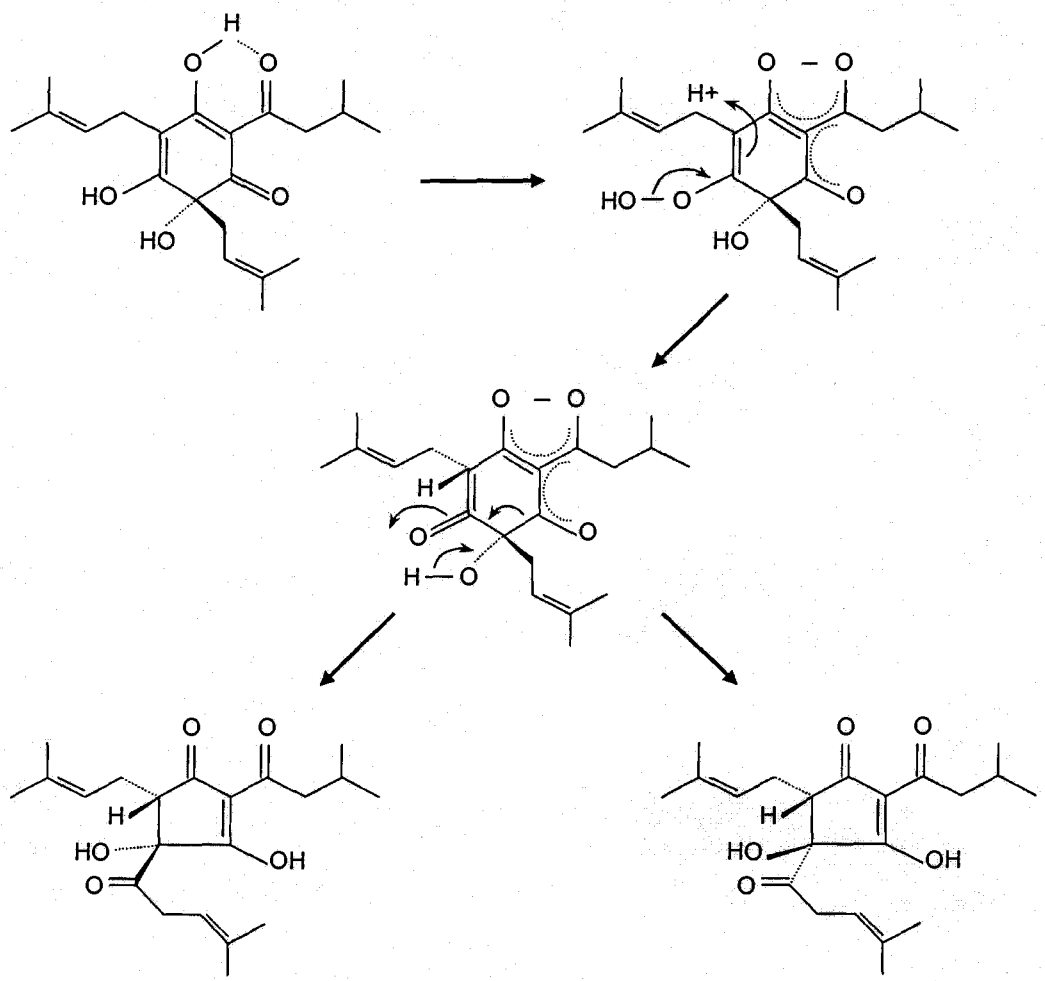


FIGURE 1.3. Mechanism of alpha-acid isomerization

on current trends and issues. Verzele [21] also covers the relevant historical high points, adding a detailed description of the chemical structures for α-acids and many compounds derived therefrom. Peacock [16] concisely covers the fundamentals of hop chemistry relevant to the brewing process.

1.3.2. Hops and Wort Boiling

Wort Boiling is the most energy-intensive portion of the brewing process, accounting for between 30 and 50% of the energy required to brew and package beer. [1, 19] Irwin [9] conducted an economic analysis on the cost of maintaining a kettle boil, and the cost of hop additions, to determine where the combined cost curve would reach a minimum value. The research showed that a 90 min. boil time was the optimum value, with very little cost penalty for variations within the 70 to 110 min. range of kettle boil durations. Significant cost penalties would occur for boil times of less than 60 min, due to the low level of isomerization achieved in a short boil, and the increased cost of hops needed to reach an equivalent bitterness level. The concentrations of α-acids and iso-α-acids appeared to be consistent with first order kinetics; however no kinetic analysis was performed by the researchers.

The study of hop bitter acid isomerization during wort-boiling shows very little published literature. The few relevant articles available either examine isomerization only at atmospheric boiling conditions (typically 100° C), or use (the now outdated) spectrophotometric techniques to quantify alpha acid isomerization. The spectrophotometric method developed by Rigby [17] was in widespread use prior to the development of HPLC techniques, but is much more prone to error

in measurement due to overlapping spectra of α-acids, iso-α-acids, and other wort constituents that also show absorbance in the ultra-violet region. Nonetheless, previous research does exist that show the kinetics of isomerization to be first order.

Askew [2] examined heating of an aqueous solution containing α-acids within a Pyrex flask, and quantified both humulones and iso-humulones by spectrophotometric methods. Reaction kinetics were determined to be first order for α-acid loss during heating in a glucose solution at 80° C, over a range of pH from 4 to 7. It was also found, in experiments carried out at both 78 and 97° C, that "early losses of α-acids did not lead to appearance of iso-compounds, but later losses of α-acids could be equated fairly well with increases in iso-compounds," and that first order kinetics might "not be valid back to time zero." The lack of fit of the data at early time points is seen several times in the literature [15, 13], and the author believes the discrepancy can be traced to solubility issues (refer to APPENDIX A).

McMurrough et al. [13] conducted wort boiling experiments in both a model system (glass reaction vessel with a reflux condenser) and in high-gravity wort heated in a pressurized kettle. The model system consisted of a KH_2PO_4 buffer with initial concentrations of approximately 300 to 330 ppm α-acids, and was continuously stirred during heating. The losses of alpha acids were found to follow first order reaction kinetics, and half-lives were specified for various boiling conditions. While the concentrations of bitter acids were quantified using HPLC, starting concentrations of α-acids were quite high, at nearly thirty times the solubility limit [20] for the pH 4.0 experimental runs. Comparisons between the model system and actual wort showed a significant decrease in utilization when

actual wort was used (49% utilization for model system, 24% for wort). Utilization was shown to decrease with increasing wort gravity, and a large portion (51%) of the iso-α-acids formed were found to be present in the hot break. Utilization was found to be higher for iso-cohumulone (27%) compared to iso-humulone and iso-adhumulone (18%).

For the boiling conditions used within the research presented here, samples were heated in sealed tubes at temperatures exceeding 100° C, and so the contents of the stainless steel tube would have been under pressure. Studies by Fischer [5, 4] in the high pressure treatment of beer have shown that significant levels of α-acid isomerization are not detected until pressures of 700 MPa are reached. The pressures reached within the sample tubes are therefore far, far below a level that would cause any significant amount of isomerization to have occured due to pressurization.

1.3.3. Losses of Bitter Compounds

Laufer & Brenner [10] traced losses of bitter acids during wort boiling and throughout the process to finished beer, finding 38% loss to trub (the insoluble precipitate that forms during kettle boiling, resulting mainly from protein coagulation), 35% to spent hops, and 10% to yeast and covers. Utilization was further broken down and examined for each of the iso-α-acid compounds, and was highest for iso-adhumulone, then iso-cohumulone, and lowest for iso-humulone.

Askew [2] also examined the effect of various conditions on rate of α-acid loss. Minor loss (5-9%) was detected for both aqueous solutions and aqueous solutions with added (pre-formed) trub. Losses for wort systems, however, showed

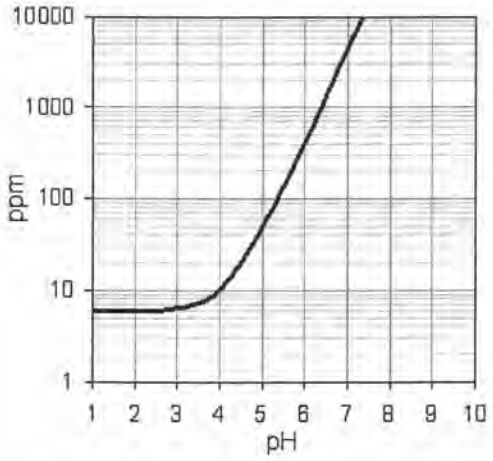


FIGURE 1.4. Aqueous solubility of alpha-acids at 25°C

losses of 35%. It can be concluded that trub, and specifically the formation of trub, leads to greatly increased losses of bitter acids.

Laws et al. [11] cite values for losses of bitter acids to yeast during fermentation ranging from 5 to 17%. Alpha-acids still present in the fermenting beer were especially removed by the "dirty yeast head". Almost all (93%) of the lost bitter acids could be recovered, mainly from the yeast head and deposited yeast.

1.3.4. Alpha-Acid Solubility

The fact that α-acids are sparingly soluble in wort (and aqueous solutions) is sometimes recognized, rarely quantified, and often overlooked altogether. In 1955 Spetsig [20] pointed out that the only available published data on solubility were based upon visual observation of haze formation, and that "more refined methods have not been tried." Spetsig then experimentally determined the dissociation constants, solubility products, and intrinsic solubilities of humulone, and

also lupulone. From this, solubility limits as a function of pH were calculated (Figure 1.4) [20] . The strong dependence of α-acid solubility on pH is obvious from the graph, and it is startling to note how low the limit drops for pH values of 4.0 and less (6 ppm at 25° C). Spetsig's data stands uncontested, and remains quoted as textbook values [7].

1.4. Utilization vs. Isomerization

As is true of all processes mankind seeks to control on this earth, an efficiency of less than 100% is achieved in the production of bitter compounds (iso-α-acids) during wort boiling. The practical brewer speaks of the term *Utilization*, which is an all-encompassing measure of the overall process efficiency, beginning with the hop addition to the kettle, and ending in finished, packaged beer. Utilization is defined as in Equation 1.1.

$$Utilization = \frac{iso\ alphas\ in\ finished\ beer}{total\ alpha\ acids\ added} \times 100\% \tag{1.1}$$

Many factors enter into the efficiency with which iso-α-acids are produced, including the fraction of α-acids extracted from the hop product used, rate at which the isomerization reaction proceeds, losses to precipitates formed during boiling, losses to vessel walls and piping, losses to yeast and sediment during fermentation, and losses to filter material. Typical values for overall utilization range from 10 to 40% [7]. Indeed, a brewery must know the precise utilization of the particular setup, in order to consistently achieve target bitterness levels. The individual contributions of each subcomponent affecting the utilization equation, however, have not been fully defined.

Of prime importance to the research contained herein is the distinction between *utilization* and *isomerization*. Utilization is a characterization of the overall process efficiency, a type of correction factor that allows calculation of required hopping rate without a requirement for scientific understanding of all factors affecting efficiency. *Isomerization*, on the other hand, refers specifically to the chemical reaction in which α-acids are converted to iso-α-acids. The rate at which isomerization occurs is perhaps the most significant subcomponent of the utilization equation, and a major limiting factor that (in part) determines the required length of kettle boiling.

It is surprising, therefore, that the exact kinetics of the isomerization reaction have not yet been completely characterized as a function of time, temperature, and perhaps other factors. Imagine that the hot wort exiting the kettle after boiling encounters an unexpected lag time prior to entering the heat exchanger — increased isomerization will be observed, but how much? At what rate does isomerization occur in a kettle that is not yet boiling, at a lowered pre-heating temperature? Would a pressurized kettle, allowing higher temperature boiling, benefit production of bitter compounds? If so, to what degree? Precise understanding of isomerization kinetics would allow improved accuracy in hopping rate calculations to achieve target bitterness. Furthermore, understanding of the kinetics is essential if novel boiling regimes (short duration, high temperature) are to be explored for potential energy savings.

1.5. Research Objectives

The goal of this research was to begin to dissect the Utilization equation, and to define the factors affecting utilization beginning at its most basic level — the isomerization of α-acids to iso-α-acids as a function of time and temperature, in the absence of any other interfering factors. The kinetics of isomerization were defined using a model wort-boiling system consisting of purified α-acids in a pH buffered aqueous solution. Effects of varying temperature and pH were quantified, and a predictive model was constructed based upon the experimentally determined rate constants.

Once the reaction kinetics were defined in a simple model system, a few chosen parameters were included, individually, to check for potential impact on isomerization rate. Factors known to affect *utilization* (based on experiential knowledge) and thought to affect *isomerization* were examined, including the addition of sugars, the addition of calcium, and variation in pH.

1.6. Brief Overview of Methodology

A model system was chosen, in order to allow examination of isomerization alone, in the absence of any other competing factors. "Wort Boiling" was conducted in small, stainless steel tubes sealed at both ends, each having a fluid capacity of approximately 12 ml (Figure 1.5). This allowed heating at a range of temperatures, including temperatures exceeding atmospheric boiling conditions, and also eliminated losses to evaporization. The model system consisted of purified α-acid extracts in aqueous solution. An acetate buffer (0.01M) was used to maintain a typical wort pH of 5.20, or to vary pH as specified. Purified α-acid

extract was added at a level that fell within real-world hopping rates, yet was low enough to not exceed the solubility limit, and high enough to allow a one log reduction in concentration without loss of ability to quantify by HPLC. 80 ppm of humulones was used for the temperature experiments, while 60 ppm was used for the pH experiments. Samples were heated using a temperature controlled oil bath, then removed and quenched in an ice-water bath at given time points. Concentrations of α-acids and iso-α-acids were quantified using high pressure liquid chromatography (HPLC). Complete details of the materials and methods used are given within the manuscript chapters contained herein (refer to pages 17 and 35).

FIGURE 1.5. Stainless steel tubes used for sample heating

2. HOP ISOMERIZATION AND DEGRADATION KINETICS IN A MODEL WORT BOILING SYSTEM

Paper to be Submitted to the Journal Agricultural and Food Chemistry

by

Mark G. Malowicki and Thomas H. Shellhammer

Department of Food Science and Technology

Oregon State University, Corvallis, Oregon 97330-6602

2.1. Abstract

The rate of isomerization of α-acids to iso-α-acids was determined across a range of temperatures (90 to 130° C) in order to characterize the rate at which iso-α-acids are formed during kettle boiling. Multiple 12 ml stainless steel vessels were utilized to heat samples (α-acids in an pH 5.2 buffered aqueous solution) at given temperatures, for varying lengths of time. Concentrations of humulones and iso-humulones were quantified by high pressure liquid chromatography (HPLC). The isomerization reaction was found to be first order, with reaction rate varying as a function of temperature. Rate constants were experimentally determined to be $k_1 = 7.9x10^{11}e^{\frac{-11858}{T}}$ for the isomerization reaction of α-acids to iso-α-acids, and $k_2 = 4.1x10^{12}e^{\frac{-12994}{T}}$ for the subsequent degradation of iso-α-acids to uncharacterized degradation products. Activation energy was experimentally determined to be 98.6 kJ per mole for isomerization, and 108.0 kJ per mole for degradation. Losses of iso-humulones to degradation products were pronounced for cases in which boiling was continued beyond two half-lives of α-acid concentration.

key words: degradation, hops, isomerization, utilization, wort boiling

2.2. Introduction

Hops have long been used in the brewing process, primarily for their contribution of bitterness, which lends a more balanced and satiating palate to finished beer. The source of bitterness is the α-acids (or humulones) found within the lupulin glands of the hop cone. These compounds have little to no bittering value in their natural form [16, 7], but upon heating, an isomerization reaction takes place which converts the humulones into bitter-tasting iso-humulones (or iso-α-acids). Indeed, this isomerization reaction is one of the main reasons for boiling wort, and the time required for the reaction to take place is one of the main factors determining the duration of kettle boil portion of the brewing process. The published literature, however, has not completely characterized the kinetics of isomerization or its temperature dependency. Knowledge of the reaction kinetics would not only allow more precise control over the bitterness achieved in finished beer, but would also enable accurate prediction of changes in bitterness levels with altered boiling conditions or process deviations. Adjustments for variations in boiling temperature (such as between sea level and high altitude brewing), compensation for unexpected events in the brewery (such as unexpected lag times prior to cooling), and even exploration of novel boiling regimes (high temperature, short duration boiling) could all be simply and accurately calculated once reaction kinetics are known.

The practical brewer, by virtue of experience, is well aware of the overall efficiency regarding the hopping process (from hop addition to finished beer) in his or her particular brewery. This typically concerns a defined, specific set of boiling

conditions, and deals only with the starting and ending points of the process. This efficiency, termed utilization, is defined in Equation (2.1).

$$Utilization = \frac{iso\ alpha\ acids\ in\ finished\ beer}{total\ alpha\ acids\ added} \times 100\% \tag{2.1}$$

Obviously, knowledge of utilization values for a specific brewery is of prime importance in calculating accurate hopping rates to achieve target bitterness levels. Utilization is all-encompassing. It is a big picture quantification of the entire process, taking into account all factors affecting final bitterness level — not only the quantity of iso-α-acids that are actually produced by isomerization, but also the loss of these bitter compounds to various processes and conditions such as losses to trub, yeast, filters, degradation products, vessel and piping walls, etc.

This research seeks to break down the utilization equation into its various subcomponent parts, and define the factors affecting the utilization equation beginning at the most basic level — the kinetics of the isomerization of α-acids to iso-α-acids as a function time and temperature, in the absence of any other interfering factors. As such, the rate of isomerization was the topic of interest, which is a subcomponent part of (and not equivalent to) utilization. A model system was chosen for initial work, consisting of a purified α-acid extract in a pH buffered aqueous solution. This allowed characterization of the isomerization alone, without confounding factors such as removal of compounds by trub formation, effect of sugars present, or other factors.

2.3. Pertinent Literature

There has been limited published data regarding the determination of reaction order, typically for boiling at 100° C. Of these, few studies have examined

varying temperatures, sometimes containing incomplete data or using imprecise spectrophotometric quantification of iso-α-acids and α-acids. A comprehensive set of data defining reaction order, rate constants, and activation energy has not been found in the published literature.

Askew [2] examined heating of an aqueous solution containing α-acids within a Pyrex flask, and quantified both α-acids and iso-α-acids by spectrophotometric methods. Reaction kinetics were determined to be first order for α-acid loss during heating in a glucose solution at 80° C, over a range of pH from 4 to 7. It was also found, in experiments carried out at both 78 and 97° C, that "early losses of α-acids did not lead to appearance of iso-compounds, but later losses of α-acids could be equated fairly well with increases in iso-compounds," and that first order kinetics might "not be valid back to time zero." Askew also examined the effect of various conditions on rate of α-acid loss. Minor loss (5-9%) was detected for both aqueous solutions and aqueous solutions with added (pre-formed) trub. Losses for wort systems, however, were approximately 35%.

McMurrough et al. [13] conducted wort boiling experiments in both a model system (glass reaction vessel with a reflux condenser) and in high-gravity wort heated in a pressurized kettle. The model system consisted of a KH_2PO_4 buffer with initial concentrations of approximately 300 to 330 ppm α-acids, and was continuously stirred during heating. The losses of α-acids were found to follow first order reaction kinetics, and half-lives were specified for various boiling conditions. Comparisons between the model system and actual wort showed a significant decrease in utilization when actual wort was used (49% utilization for model system, 24% for wort). Utilization was shown to decrease with increasing wort gravity, and a large portion (51%) of the iso-α-acids formed were found to be present

in the hot break. Utilization was found to be higher for iso-cohumulone (27%) compared to iso-humulone and iso-adhumulone (18%).

Laufer & Brenner [10] also traced losses of bitter acids during wort boiling and throughout the process to finished beer, finding 38% loss to trub, 35% to spent hops, and 10% to yeast and covers. Utilization was highest for iso-adhumulone, then iso-cohumulone, and lowest for iso-humulone.

2.4. Experimental

2.4.1. Reagents

Purified α-acid extract (Alphahop) was provided by John I. Haas (Yakima, Washington), and was certified to contain 84.7% α-acids, 3.1% iso-α-acids, and 1.7% β-acids. Standardized samples of α-acids (ICE-2) and iso-α-acids (ICS-I1) were purchased from the American Society of Brewing Chemists (St. Paul, Minnesota). Methanol was HPLC grade. Water for HPLC and all other solutions was filtered and de-ionized (Millipore Milli-Q). Remaining chemicals were purchased from VWR International.

2.4.2. Instruments

A programmable heated circulator, volume 13 L with a 1000 W heater, was used to maintain desired oil bath temperatures. The HPLC system was a Hewlett Packard 1090 series II/L with a photodiode array detector, connected to a personal computer with ChemStation software. The octadecyl, reversed-phase column (Supelco Discovery C18, 250 mm x 4.6 mm x 5 μm) was maintained

at 40° C. A Beckman Coulter pH meter, model Φ360, with a Beckman Coulter Futura gel-filled and temperature compensated electrode, was used for titration of buffer solutions.

2.4.3. HPLC Conditions

The UV detector was set to measure absorbance at 270 nm. Flow rate was 1.4 ml per minute, and injection volume was 10 μl. Mobile phase A consisted of 100% Methanol, while mobile phase B contained 75% Methanol, 24% H_2O, and 1% Phosphoric Acid (85%). A gradient elution was used, consisting of 0-8 minutes : 100% B, 13-15 minutes: 50% B, 18-22 minutes : 100% B, with percentages linearly ramped between the given timeframes. Peak area was automatically integrated using Chemstation software.

2.4.4. Sample Preparation and Heating Conditions

The α-acid extract was dissolved into 95% ethanol to allow accurate dosing, such that the final solution for boiling contained 1.0% ethanol by volume and 80 mg/L of α-acids. This level of α-acid concentration was chosen so as to remain just below the published (and experimentally verified) solubility limit at the chosen pH value [20, 8], and to fall within the range of industrially-relevant world hopping rates. A 0.01M acetate buffer (pKa 4.76) was prepared to maintain a wort-representative pH of 5.20 for all experimental runs. The prepared solution (2.5 ml of the α-acid extract plus ethanol solution, made up to 250 ml with pH buffer) was divided among the 16 stainless steel vessels. Each vessel was constructed of 12.7 mm outside diameter stainless steel tube of 1.25 mm wall

thickness and 15 cm length, capped with stainless steel Swagelok fittings (SS-810-C). Volume of each vessel was approximately 12 ml. The tubes were filled to zero headspace, sealed, and then submerged into a temperature controlled oil bath at time zero. The oil bath was filled with Chevron RPM Gear Oil, SAE 90. Initial oil temperature of the bath was adjusted upward to compensate for the sensible heat absorbed by the filled tubes, which were at room temperature prior to immersion. Initial temperature was calculated using simple energy balance equations. In each case, temperature stabilized to the desired value within 3 minutes. Thereafter, at specific time points throughout the heating process, tubes were sequentially removed and quenched in an ice-water bath to halt the isomerization reaction. Samples were filtered through mixed cellulose ester, hydrophilic, 0.45 micron filters (Millipore HAWP01300) prior to HPLC analysis. Sample preparation order, filtration order, and injection order were all randomized, to minimize any potential order effects.

2.5. Results & Discussion

The rate of conversion of α-acids to iso-α-acids was highly dependent on temperature (Figure 2.1). For typical 100° C boiling conditions, 77% of α-acids were isomerized within 120 minutes. Temperatures of 130° C isomerized 100% of α-acids within 30 minutes of heating. Although initial concentrations of 80 ppm were desired (based upon weight of α-acid extract added to the solution), the measured starting concentrations were consistently lower, averaging 64.4 ppm. The difference was only partially attributed to losses of humulones to vessel walls and to filter material. It was experimentally determined that 3% of α-acids present

were lost directly to the walls of the stainless steel tubes used for heating, and an additional 2% were lost during filtering prior to HPLC injection. Additional losses were most likely attributable to the glassware with which the solution came into contact during preparation, prior to being placed in the stainless steel tubes. Indeed, if each item of the four items of glassware actually used accounted for 3% loss, total loss throughout the process would have been 17%, which would have accounted for the discrepancy between the 80 ppm of α-acids added (by weight) and the average measured starting concentration of 64.4 ppm. Humulones, being sparingly soluble, demonstrated a significant affinity to cling to any glassware, stainless steel vessels, or filters with which the solution came into contact.

It is interesting to note that the appearance of iso-α-acids did not have a one-to-one relationship with the loss of α-acids, especially for early time points, a phenomenon seen by other researchers as well [2, 15]. Furthermore, for extended heating times, losses of iso-α-acids became increasingly significant. In general, degradation became dominant beyond two half-lives of α-acid concentration. Degradation products were not quantified in the HPLC method, as these compounds showed decreased absorbance in the UV range, and corresponded to small, broad, overlapping peaks early in the chromatogram. Their total concentration was inferred by assuming that the difference between lost humulones and produced iso-humulones was equal to the quantity of degradation products produced.

The data are consistent with a reaction scheme in which α-acids are converted to iso-α-acids, and are then converted into degradation products (Figure 2.2). Reaction rate can be expressed using the equation $\nu = k \times c^{\alpha}$, where ν is the reaction velocity (ppm per minute), k is the rate constant (minute^{-1}), c is

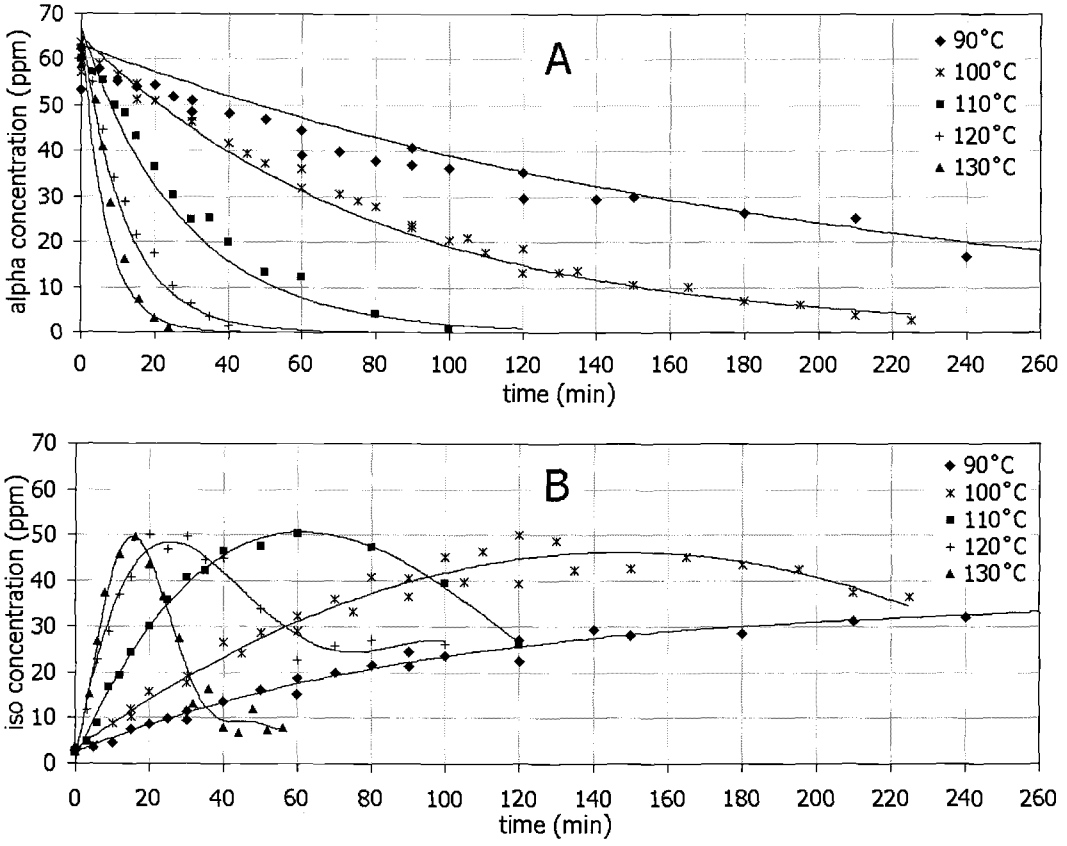


FIGURE 2.1. Alpha-acid and iso-alpha-acid concentrations versus heating time, pH 5.2 buffer

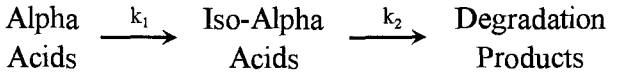


FIGURE 2.2. Fate of alpha-acids during heating

the reactant concentration (ppm), and α is the reaction order (no units). Once reaction order was known, rate constants (as a function of temperature) were experimentally determined, fully defining the reaction kinetics. The progress of the reaction could then be predicted, as a function of time and temperature.

Examining graphical plots of the α-acid loss, using the relationships given in Equations (2.2, 2.3, 2.4), for reaction order of zero, one, or two (respectively), linearity was best obtained for first order kinetics (that is, when $ln(\frac{c}{c_{initial}})$ was plotted versus time).

$$Zero\ Order \qquad c = c_{initial} - kt \tag{2.2}$$

$$First\ Order \qquad ln\left(\frac{c}{c_{initial}}\right) = -kt \tag{2.3}$$

$$Second\ Order \qquad \frac{1}{c} = \frac{1}{c_{initial}} + kt \tag{2.4}$$

The best agreement was obtained with the experimental data when starting concentrations were assumed to be equal to the concentrations of the solution prior to its distribution among the stainless steel tubes. As described above, this concentration of α-acids consistently measured slightly higher than the same solution measured after merely coming into contact with the stainless tube. These data imply that some portion of the α-acids would cling to the vessel walls, evade quantification in the HPLC analysis at room temperature, and yet remain reactive in the isomerization reaction which occurred at elevated temperature. This unquantified, yet reactive, portion of α-acids would account for the increased lack of fit for initial time points, which was seen in this work and in the work of other researchers [2, 13].

TABLE 2.1. Experimentally determined rate constants for isomerization

Temp (°C)	$t_{1/2}$ (min)	k_1 (half-life)	k_1 (least sqrs)
90	120.7	0.00574	0.00547
100	59.5	0.01166	0.01110
110	22.0	0.03150	0.02989
120	9.4	0.07353	0.07504
130	6.9	0.10058	0.13000

For the half-life method, Equations (2.5, 2.6, 2.7) apply [3], with c equal to the concentration of α-acids at time t, $c_{initial}$ equal to starting concentration, and k being the rate constant.

$$Zero\ Order \qquad k = \frac{c_{initial}}{2(t_{\frac{1}{2}})} \tag{2.5}$$

$$First\ Order \qquad k = \frac{ln(2)}{t_{\frac{1}{2}}} \tag{2.6}$$

$$Second\ Order \qquad k = \frac{1}{c_{initial}(t_{\frac{1}{2}})} \tag{2.7}$$

Rate constants (k's) were found by both the half-life method, and by least squares fit analysis. In the half life method, the time required for α-acid concentration to drop to half of its initial value was found using a smoothed line fit (2^{nd} order polynomial) to the experimental data. The "least squares" values were found using a solver tool in a commercially available spreadsheet package (Microsoft Excel), which was set to minimize the sum of squared errors (squared difference between experimental data and calculated values) by modifying k. (The solver tool utilized a generalized reduced gradient nonlinear optimization code.) Best agreement between the two sets of k's, and between the k's and the actual

TABLE 2.2. Rate constants for isomerization and degradation

Temp (°C)	k_1	k_2
90	0.00478	0.00144
100	0.01141	0.00263
110	0.03078	0.00680
120	0.07045	0.01568
130	0.10945	0.05096

data, occurred for reaction order equal to one. Half-lives and rate constants, found directly from the experimental data, are given in Table (2.1), for reaction order equal to one.

The kinetics of the degradation of iso-α-acids to the uncharacterized degradation products were similarly analyzed, assuming that the differences between lost α-acids and produced iso-α-acids were equal to the quantity of degradation products produced. Degradation products were not quantified in this analysis, as the compounds showed decreased UV absorbance characteristics. At higher temperatures and longer times (as in the 130° C case), very small peaks were observed in the early region of the chromatogram for the later data points, but were insufficient to allow quantification. (See chromatograms in APPENDIX C.)

Assuming the degradation reaction to be first order, a second set of rate constants (the k_2's in Figure 2.2) were calculated. A least squares fit methodology was used to find the values for all k_1's and k_2's that would best fit the three curves (α-acid concentration, iso-α-acid concentration, and degradation products) to the experimental data, for each temperature (Table 2.2).

Rate constants for the isomerization reaction were plotted versus temperature, and the relationship characterized. The Arrhenius equation (Equation 2.8)

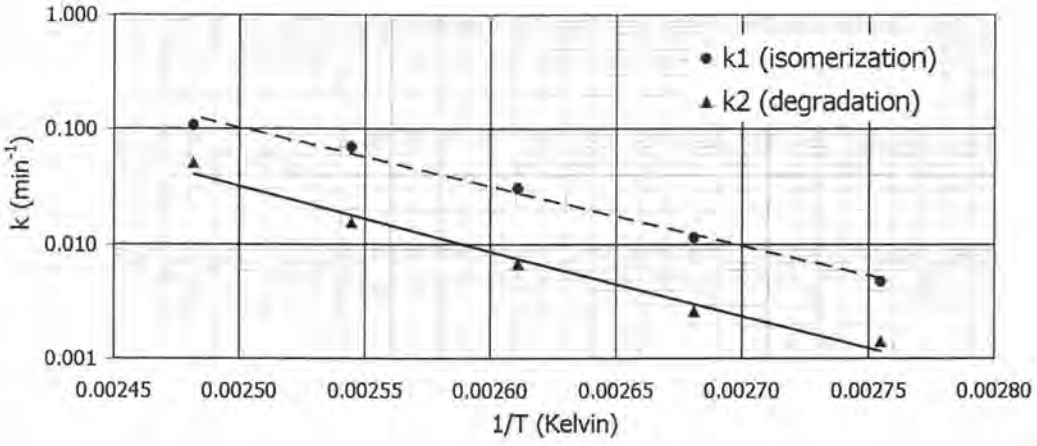


FIGURE 2.3. Isomerization rate constants as a function of temperature

relates the rate constant k, the activation energy E_a, and temperature, T (Kelvin). Note that A is the pre-exponential factor, and R is the universal gas constant.

$$k = Ae^{(\frac{-E_a}{RT})} \tag{2.8}$$

Plotting $\log(k)$ versus $1/T$ (as in Figure 2.3) yields the activation energy, E_a, as the slope of the straight line fit to the data [3, 6]. The fitted line gives the experimentally determined estimate of reaction rate as a function of temperature, and the relationship is given in Equation 2.9, with k in units of minutes^{-1} and T in Kelvin.

The rate constants for the degradation were also determined, and the temperature dependence defined by the same method (Figure 2.3, Equation 2.10). The activation energies were calculated as 98.6 kJ per mole for the isomerization of humulones to iso-humulones, and 108 kJ per mole for the subsequent degradation.

$$k = (7.9 \times 10^{11})e^{(\frac{-11858}{T})} \tag{2.9}$$

TABLE 2.3. Isomerization rate constants for cohumulone

Temp (°C)	$k_{overall}$	$k_{cohumulone}$
90	0.00478	0.00526
100	0.01141	0.01131
110	0.03078	0.03060
120	0.07045	0.07776
130	0.10945	0.10521

$$k = (4.1 \times 10^{12})e^{(\frac{-12994}{T})} \tag{2.10}$$

From the chromatographic data, rate constants for isomerization of cohumulone to iso-cohumulone, separate from humulone plus adhumulone, were calculated. The HPLC method revealed only one co-eluting peak for humulone and adhumulone, even though iso-humulone was well separated from iso-adhumulone, and so the two were analyzed as a single entity. The two sets of isomerization rate constants (overall, and cohumulone to iso-cohumulone) are given in Table 2.3. Statistical analysis showed no significant difference between the sets of constants. A multiple linear regression model for $log(k_1)$ that included both explanatory variables ($1/T$, and *iso-cohumulone* as an indicator or dummy variable) did not show iso-cohumulone to be a significant explanatory variable (p-value = 0.815) after accounting for temperature [18]. Isomerization of cohumulone to iso-cohumulone proceeds at a rate equivalent with that of humulone and adhumulone. The selective preference for iso-cohumulone in other studies on utilizatoin [10, 9] is therefore due to some factor affecting overall utilization, and not due to an increased rate of isomerization.

For conditions in which temperature remains constant over time, and the initial concentration of iso-α-acids is zero, the concentration of iso-α-acids (c_{iso})

and degradation products ($c_{degradation}$) can be determined at any time t by using Equations 2.11 and 2.12. [3]

$$c_{iso} = \frac{k_1(c_{alpha,initial})}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \tag{2.11}$$

$$c_{degradation} = c_{alpha,initial} + \frac{c_{alpha,initial}}{k_2 - k_1}(k_2 e^{-k_1 t} - k_1 e^{-k_2 t}) \tag{2.12}$$

For conditions in which the rate constants change with a changing temperature profile, the concentrations of iso-humulones formed during kettle boiling can be calculated using Equations 2.13, 2.14, 2.15, which define the differential change in α-acid, iso-α-acid, and degradation product concentrations with respect to time.

$$\frac{d(c_{alpha})}{dt} = -k_1 c_{alpha} \tag{2.13}$$

$$\frac{d(c_{iso})}{dt} = k_1 c_{alpha} - k_2 c_{iso} \tag{2.14}$$

$$\frac{d(c_{degradation})}{dt} = k_2 c_{iso} \tag{2.15}$$

Discrete data, as a function of time, can be calculated by defining a small time step, Δt, and calculating the corresponding change in concentration for each time step. A graph of the actual data compared with the calculated values for the 100°C case is given in Figure 2.4. Various computer software packages can be used to determine concentration of iso-α-acids throughout the course of heating, given specific temperature conditions and known starting concentrations. Accurate results are obtained for time steps that are sufficiently small (say one minute, for the temperature region investigated here). This, of course, does not account for losses of bitter acids to trub, or other interfering factors.

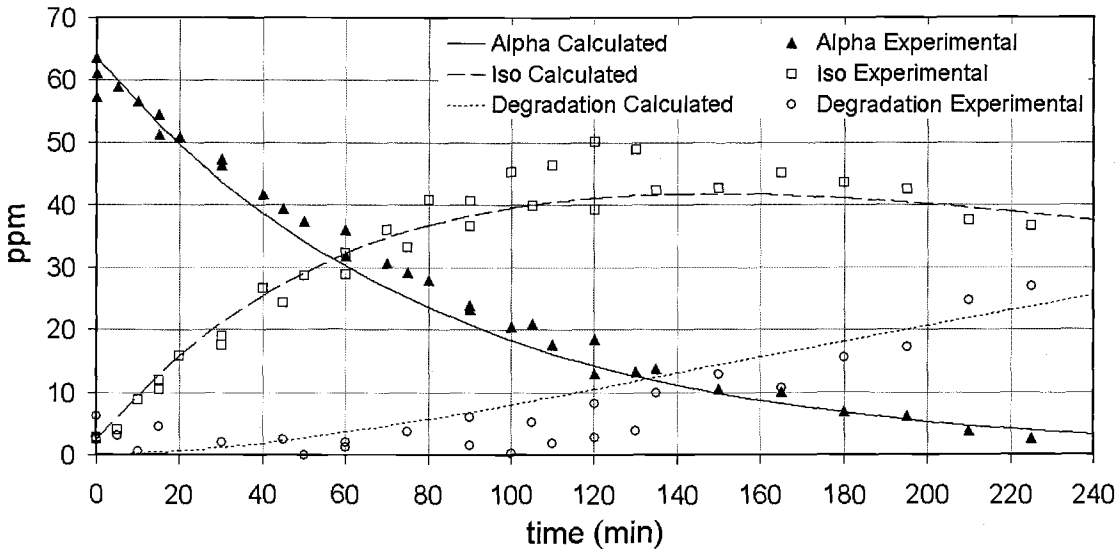


FIGURE 2.4. Measured and calculated concentrations of alpha-acids, iso-alpha-acids, and degradation products at 100°C

2.6. Conclusions

The experimental data were consistent with a reaction order of one, for the isomerization of α-acids to iso-α-acids. This agrees with previously published work which examined isomerization kinetics in both model systems and actual wort boiling [2, 13, 15]. The reaction rate k_1 is a function of temperature as defined in Equation 2.9, with corresponding activation energy of 98.6 kJ per mole.

Extended boiling times (beyond two half-lives of α-acid concentration) showed significant degradation of iso-α-acids to uncharacterized degradation products. Although degradation products could not be directly quantified, their concentrations were inferred from the difference between starting α-acid concentration, and the sum of α-acid and iso-α-acids present at each data point. Assuming the degradation reaction had an order of one, rate constants were also determined

for the conversion of iso-α-acids to the degradation products. The reaction rate k_2 is a function of temperature, as defined in Equation 2.10, with a corresponding activation energy of 108.0 kJ per mole.

The two rate constants can be used to calculate the concentration of iso-α-acids at any given time, accounting for both the amount of iso-α-acids produced by isomerization, and the amount of iso-α-acids lost to degradation, as described above.

The results obtained indicate that the rate of isomerization roughly doubled for every ten degree Celsius increase in temperature (average change was 223% per ten degree increase). Isomerization of cohumulone to iso-cohumulone proceeded at a rate equivalent with that of humulone and adhumulone. High temperatures quickly led to degradation products, as evidenced in the dramatic decrease of iso-α-acid concentration beyond 18 minutes of heating at 130° C. While the rate of isomerization slowed at temperatures below 100° C, substantial amounts of iso-α-acids were still produced at 90° C. This is significant if hot wort is held at temperatures just below boiling after the kettle boil is completed, while in the whirlpool, or awaiting transfer to a heat exchanger.

While this research allows calculation of the net amount of iso-α-acids produced during heating, the model is yet incomplete in terms of utilization. The rate of loss to trub formation (and other interfering factors) are required to fully define the concentrations of iso-α-acids in a real-world wort boiling system. Nonetheless, the knowledge of kinetics of isomerization allows estimations to be made regarding how bitter acid concentrations would be affected by altered boiling conditions.

2.7. Acknowledgment

The authors wish to acknowledge John I. Haas, Inc. for their generous donation of the α-acid extract used in this study.

3. FACTORS AFFECTING HOP ISOMERIZATION KINETICS IN A MODEL WORT BOILING SYSTEM

Paper to be Submitted to the Journal of the American Society of Brewing Chemists

by

Mark G. Malowicki and Thomas H. Shellhammer

Department of Food Science and Technology

Oregon State University, Corvallis, Oregon 97330-6602

3.1. Abstract

Various factors were explored to determine their impact on the rate of isomerization of α-acids to iso-α-acids during kettle boiling. A model wort boiling system was used, employing multiple 12ml stainless steel vessels to heat samples (α-acids in an aqueous, pH-buffered solution, with other compounds included as specified) at 100° C, for 140 minutes. Concentrations of humulones and isohumulones were quantified at discrete time points, using high pressure liquid chromatography (HPLC). Of the factors tested (glucose, maltose, calcium, and pH ranging from 4.8 to 6.0) none were shown to affect the rate of production of iso-α-acids. While pH had a marked effect on the concentrations of α-acids as measured, the differences may be attributed to solubility issues (as the solubility limit was approached and exceeded) and did not appear to affect the rate of iso-α production.

key words: hops, isomerization, pH, utilization, wort boiling

3.2. Introduction

The cones of the hop vine (*Humulus lupulus*) have long been used in the brewing process, primarily for the contribution of bitterness, which lends a more balanced and satiating palate to finished beer. The bitterness originates from compounds known as α-acids (or humulones), which are found within the lupulin glands of the hop cone. These compounds have little to no bittering value in their natural form [16, 7], but upon heating, an isomerization reaction converts the humulones into bitter-tasting iso-humulones (or iso-α-acids). Indeed, this isomerization is one of the prime objectives of wort boiling, and the time required for the reaction is one of the main factors determining the duration of the kettle boil. A previous study by the present authors sought to characterize the kinetics of isomerization at its most basic level - as a function of time and temperature only, in an aqueous, pH buffered solution, and in the absence of any interfering factors. The distinction should be noted between the terms *utilization* and *isomerization* – utilization is an overall efficiency describing the percentage of bitter compounds occuring in beer relative to the amount of α-acids added to the kettle. Utilization is all-encompassing, taking into account not only the amount of isomerization achieved, but also all losses encountered throughout the brewing process. Isomerization refers only to the chemical conversion of humulones to iso-humulones. The previous work of the authors showed isomerization kinetics in an aqueous system to be first order, with pronounced degradation of iso-humulones occurred beyond two half-lives of α-acid concentration. While the kinetics of isomerization are thereby defined as a function of temperature within a model system, any brewer will testify that many other factors significantly affect the utilization of hops in

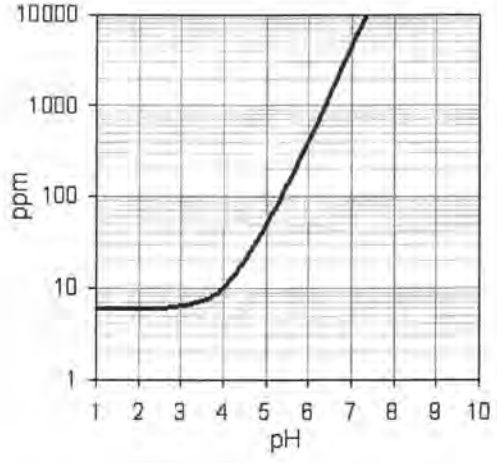


FIGURE 3.1. Aqueous solubility of alpha-acids at room temperature

the brewhouse. The objective of this research was to investigate several of the factors thought to affect isomerization rate, and quantify the potential impact within a model wort boiling system.

3.3. Pertinent Literature

Spetsig [20] determined the solubility limits of α-acids by experimentally finding the dissociation constant and intrinsic solubility, which allowed calculation of solubility as a function of pH. The present authors experimentally verified the solubility limits within a pH 5.2 acetate buffer system, with good agreement. The solubility limit of α-acids varies widely with pH, and Spetsig's curve for humulone (at 25° C) is recreated in Figure 3.1. The importance of solubility and the enormous variation with changes in pH are both often overlooked in discussions of hop utilization, and even in quantification of α-acids present.

Askew [2] examined heating of an aqueous solution containing α-acids within a Pyrex flask, and quantified both α-acids and iso-α-acids by spectrophotometric methods. While it is stated that isomerization is affected by pH, the level of noise in the data is significant (most likely due to the spectrophotometric method of quantification) and does not show a statistically significant difference between pH values. The discrepancies between α-acid concentrations that are attributed to the effect of pH on isomerization often mirror the difference in the solubility limit of α-acid between the given pH values. Furthermore, many of the experiments were carried out at 80° C (duration of 25 min.), a temperature at which the isomerization reaction would be significantly slowed (approximately four times as slow as at 100° C, according to research by the present authors).

McMurrough *et al* [13] conducted wort boiling experiments in both a model system (glass reaction vessel with a reflux condenser) and in high-gravity wort heated in a pressurized kettle. The model system consisted of a KH_2PO_4 buffer, with pH values ranging from 4.0 to 7.0, with initial concentrations of approximately 300 to 330 ppm α-acids, continuously stirred during heating. The data presented in support of increased iso-α-acid yield with increasing pH are actually values for increase in iso-α-acids divided by loss of α-acids. Given the 305-330 ppm initial concentrations of α-acids, solubility limits are potentially skewing the data. Recall that the solubility limits according to Spetsig [20] are 10 ppm and 45 ppm at pH 4.0 and 5.0 (respectively) at room temperature, and 70 ppm and 200 ppm for pH 4.0 and 5.0 at 100° C.

3.4. Experimental

3.4.1. Reagents

Purified α-acid extract (Alphahop) was provided by John I. Haas (Yakima, Washington), and was certified to contain 84.7% α-acids, 3.1% iso-α-acids, and 1.7% beta acids. Standardized samples of α-acids (ICE-2) and iso-α-acids (ICS-I1) were purchased from the American Society of Brewing Chemists (St. Paul, Minnesota). Glucose and Maltose were purchased from Sigma Aldrich and EM Science, respectively. Methanol was HPLC grade. Water for HPLC and all other solutions was filtered and de-ionized (Millipore Milli-Q). Remaining chemicals were purchased from VWR International.

3.4.2. Instruments

A programmable heated circulator, volume 13 L with a 1000 W heater, was used to maintain desired oil bath temperatures. The HPLC system was a Hewlett Packard 1090 series II/L with a photodiode array detector, connected to a PC with ChemStation software. The octadecyl, reversed-phase column (Supelco Discovery C18, 250 mm x 4.6 mm x 5 μm) was maintained at 40° C. A Beckman Coulter pH meter, model Φ360, with a Beckman Coulter Futura gel-filled and temperature compensated electrode, was used for titration of buffer solutions.

3.4.3. HPLC Conditions

The UV detector was set to measure absorbance at 270 nm. Flow rate was 1.4 ml per minute, and injection volume was 10 μl. Mobile phase A consisted of

100% Methanol, while mobile phase B contained 75% Methanol, 24% H_2O, and 1% Phosphoric Acid (85%). A gradient elution was used, consisting of 0-8 minutes : 100% B, 13-15 minutes: 50% B, 18-22 minutes : 100% B, with percentages linearly ramped between the given time frames. Peak area was automatically integrated using Chemstation software.

3.4.4. Sample Preparation and Heating Conditions

The α-acid extract was dissolved into 95% ethanol to allow accurate dosing, such that the final solution for boiling contained 1.0% ethanol by volume and 60mg/L α-acids. This level of α-acid concentration was chosen in hopes of maintaining conditions just below the solubility limit for pH values of 5.2 and higher, and to fall within the range of industrially relevant hopping rates. A 0.01M acetate buffer (pKa 4.76) was prepared to maintain the desired pH values (consisting of 4.8, 5.2, 5.6, and 6.0). Consequently, some insolubility would be expected at the experimentally tested value of pH 4.8. The prepared solution (2.5ml of the α-acid extract plus ethanol solution, made up to 250ml with pH buffer) was divided among the 16 stainless steel vessels. Each vessel was constructed of 12.7 mm outside diameter stainless steel tubes of 1.25 mm wall thickness and 15 cm length, capped with stainless steel Swagelok fittings (SS-810-C). Volume of each vessel was approximately 12ml. The tubes were filled to zero headspace, sealed, and then submerged into a temperature controlled oil bath at time zero. The oil bath was filled with Chevron RPM Gear Oil, SAE 90. Initial oil temperature of the bath was adjusted upward to compensate for the sensible heat absorbed by the filled tubes, which were at room temperature prior to immersion. Initial temperature

was calculated using simple energy balance equations. In each case, temperature stabilized at the desired value of 100° C within 3 minutes. Thereafter, at specific time points throughout the heating process, tubes were sequentially removed and quenched in an ice-water bath to halt the reaction. Samples were filtered through mixed cellulose ester, hydrophilic, 0.45 micron filters (Millipore HAWP01300) prior to HPLC analysis. Sample preparation order, filtration order, and injection order were all randomized, to minimize any potential order effects.

3.5. Results & Discussion

The influence of pH was first investigated, by conducting the experiment for pH values 4.8, 5.2, 5.6, and 6.0. Each pH run was replicated once, with experimental order of all runs randomized. The measured concentrations of α-acids and iso-α-acids across a 140 minute boil are as displayed graphically in Figure 3.2. It is interesting to note that while the α-acid concentrations (graph A) showed slight differences across the boil for the pH 4.8 and 6.0 cases, the level of iso-alpha concentrations (graph B) was nearly identical for all pH levels (4.8, 5.2, 5.6, and 6.0). A possible mechanism to explain the discrepancy involves the low solubility of α-acids at room temperature, where quantification took place, versus the increased solubility at elevated temperatures, at which the isomerization reaction occurred. The α-acids would have been in solution while the reaction proceeded, but when the samples were cooled, would be driven out of solution and cling to either vessel walls or filter material. The measured concentration would therefore be lower than the actual concentration at reaction temperature. Differences within iso-alpha concentration fell within the level of noise in the

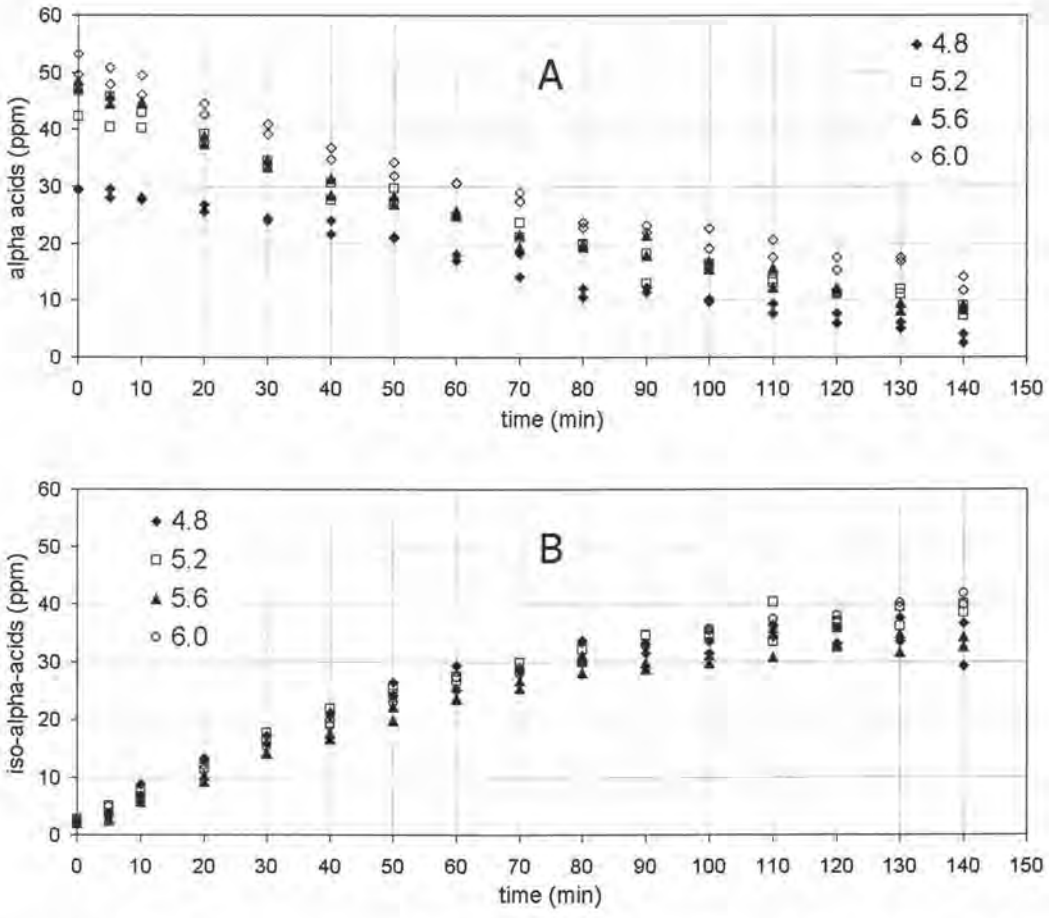


FIGURE 3.2. Concentrations of alpha-acids (A) and iso-alpha-acids (B) during boiling at varying pH values and 100° C

data. A multiple linear regression model for iso-α concentration that included both explanatory variables (time and pH) did not show pH to be a significant explanatory variable (p-value = 0.785) after accounting for time [18]. The dataset did not show a significant effect of pH on rate of iso-α-acids produced, for the pH values tested in this study.

The experience-based knowledge that hop utilization decreases with increasing wort strength led to trials that included the presence of sugars in the

experimental runs. In separate cases, maltose and glucose were added to the baseline system (which consisted of only α-acids in a pH 5.2 acetate buffer), both at a concentration of ten percent by weight (10° Plato, for a specific gravity of 1.040). The results are shown in Figure 3.3 (graph A shows measured α-acid concentration, and graph B shows iso-α-acid concentration normalized by initial α concentration. In comparing the baseline (purified α-acid extracts heated in a pH 5.2 buffered aqueous solution at 100° C) to the samples heated with the addition of glucose or maltose, it is seen that the level of noise in the data is greater than any differences that could be attributed to the effect of glucose or maltose on isomerization rate. Two linear regression models were fit to the data: a full model including time, glucose, and maltose as explanatory variables, and a reduced model including time as the only explanatory variable. A lack of fit F-test comparing the two models yielded a p-value of 0.413 for the hypothesis that the glucose and maltose terms are significant, thereby indicating a lack of effect of these two compounds on isomerization rate.

The presence of divalent cations such as calcium has been shown to affect the rate of isomerization, even in aqueous solutions [13]. An experimental run was conducted in which calcium was added at 100 ppm. The data is included with the sugar datasets shown in Figure 3.3. A multiple linear regression model for iso-humulone concentration that included both explanatory variables (time and calcium) did not show calcium to be a significant explanatory variable (p-value = 0.818) after accounting for time. This data suggest that calcium did not have an effect on the rate of isomerization in an aqueous system at pH 5.2.

All of the data presented here was obtained using a model system and not actual wort. Proteins were not included in the model system, and no precipitates

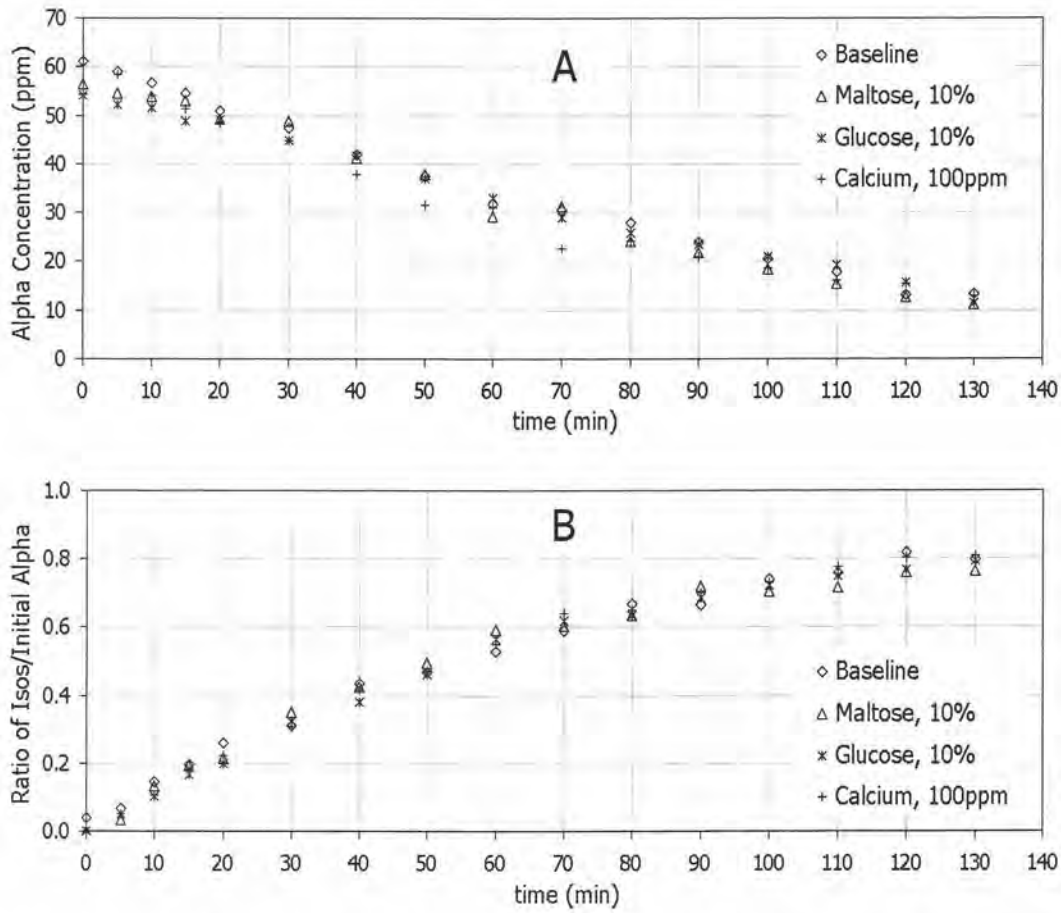


FIGURE 3.3. Effect of sugars and calcium on rate of isomerization (A: alpha-acids, B: ratio of iso-alpha-acids to initial alpha concentration)

or trub were formed during boiling. Previous research has shown losses of hop bitter acids to trub to be significant, accounting for 30 to 38% of the lost bitter acids [2, 10, 11]. It is speculated by the present authors that the losses to trub would better explain the differences in utilization that are attributed to pH, wort strength, and divalent cation effects, since rate of isomerization does not appear to be affected. Increased wort strength would generally correlate well with an increase in protein content at the start of boiling, and increased hot break formation. With increased levels of protein present to strip hop bitter acids from the wort, decreased utilization would be observed. Specific gravity of the wort would correlate well with protein content, but the presence of dissolved sugars would not be the driving factor affecting utilization. Similarly, divalent cations such as calcium would encourage protein precipitation, thereby increasing the stripping effect. Calcium also encourages the flocculation of yeast during fermentation, which is another source of iso-α-acid loss. Furthermore, pH could very well affect utilization due to protein solubility and trub formation issues.

3.6. Conclusions

None of the factors examined in this research (glucose at 10° Plato, maltose at 10° Plato, calcium at 100 ppm, and pH ranging from 4.8 to 6.0) were shown to affect the rate of production of iso-α-acids. While hop utilization is known to decrease with increasing wort strength, the presence of sugars was proven not to be the cause of either lost bitter acids or a change in isomerization rate. Although calcium did not show an effect in the model system, it is likely that an effect would be seen in actual wort, due to the enhancement of trub formation.

pH did not affect the rate at which iso-α-acids were produced during heating, but measured concentrations of α-acids did vary as a function of pH despite equivalent initial concentrations for each case. The chosen level of 60 ppm α-acids is indeed above the solubility limit for pH values below 5.0, and this most likely accounts for the low concentrations in the pH 4.8 case. It is interesting that although the α-acid concentrations measured low for pH 4.8, the rate of iso-alpha production matched the other datasets, implying that the same starting concentrations of α-acids were present in each case. It is likely that the α-acids would cling to the vessel walls at room temperature when solubility issues predominate, and thus evade quantification, while remaining soluble and reactive at the elevated temperatures of boiling conditions. The absence of trub formation brings into question whether the lack of a pH effect on isomerization rate would apply to true wort boiling systems, in which stripping of bitter acids by trub would be considerable.

4. CONCLUSIONS

4.1. Alpha-Acid Solubility

The solubility limit of α-acids in a pH 5.2 buffer was experimentally verified to match that predicted by Spetsig [20], at approximately 90 ppm. The solubility is strongly dependent on pH, with pH values below 4.0 allowing only 6 ppm of humulones in solution. The significance of the low solubility of humulones appears to be often overlooked. Indeed, the experiments within this thesis that attempted to quantify the impact of pH on isomerization rate showed that at pH values below the solubility limit (the pH 4.8 case with 60 ppm concentration), α-acids could apparently cling to vessel walls, and remain reactive, although escaping quantification.

4.2. Kinetics of Isomerization

The rate of isomerization of α-acids to iso-α-acids was characterized for a model wort boiling system. The isomerization reaction is highly dependent on temperature, with reaction rate roughly doubling for every increase of 10° C (average change was 223% increase for every 10° C increase in temperature). The isomerization reaction was found to be first order, with a rate constant defined as $k_1 = (7.9x10^{11})e^{\frac{-11858}{T}}$. Activation energy for the isomerization reaction was calculated as 98.6 kJ per mole.

Significant degradation of iso-α-acids was seen for extended boiling times. The reaction appeared to follow a scheme as detailed in Figure 4.1. Beyond two half-lives of α-acid concentration, significant amounts of iso-α-acids were lost to

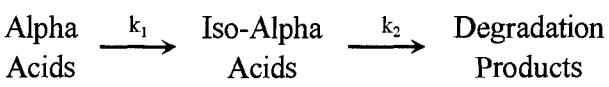


FIGURE 4.1. Fate of alpha-acids during heating

uncharacterized degradation products. The HPLC method did not allow direct quantification of the degradation products, however their concentration could be inferred from the missing amount of humulone plus iso-humulone. Assuming the degradation reaction to be first order yielded a rate constant for degradation of $k_2 = (4.1x10^{12})e^{\frac{-12994}{T}}$. Activation energy for the degradation of iso-α-acids was calculated as 108.0 kJ per mole.

For typical boiling conditions at 100° C, a kettle boil of approximately 120 minutes corresponds to approximately two half-lives of α-acid concentration. Beyond this point, loss of iso-α-acids to degradation increase greatly. One must wonder if this was a driving factor in the evolution of the widespread standard boil times of 90 to 120 minutes.

4.3. Factors Affecting Isomerization Kinetics

Of the factors examined (glucose at 10° Plato, maltose at 10° Plato, calcium at 100 ppm, and pH ranging from 4.8 to 6.0), none were shown to affect the rate of production of iso-α-acids in a model wort boiling system. While hop utilization is known to decrease with increasing wort strength, the presence of sugars was proven not to be the cause of either lost bitter acids or a change in isomerization rate. Although calcium did not show an effect in the model system, it is likely that an effect would be seen in actual wort, due to the formation of trub.

4.4. The Effect of pH on Isomerization Kinetics

pH did not affect the rate at which iso-α-acids were produced during heating. However, measured concentrations of α-acids were seen to vary as a function of pH, despite equivalent initial concentrations for each case. While the α-acid concentrations measured low for the experiments in which the solubility limit was exceeded (the pH 4.8 case), the rate of iso-alpha production matched the other datasets, implying that the same starting concentrations of α-acids were present in each case. Experiments showed that α-acids have a marked tendency to cling to vessel walls, and the isomerization data implied that the α-acids have the capability of thereby evading quantification while remaining reactive inside the vessel. The absence of trub formation in the aqueous solution brings into question whether the lack of a pH effect on isomerization rate would apply to true wort boiling systems, in which stripping of bitter acids by trub would be considerable.

4.5. Future Work

The characterization of the kinetics of isomerization conquers a large hurdle in the understanding of factors affecting hop utilization, but there is much work yet to be done. Questions that remain unanswered include:

- What is the efficiency with which α-acids are extracted from the hop product used (pellets, whole hops, or other)?
- At what rate are α-acids lost to vessel walls, piping, or even spent hops?
- Are α-acids, or iso-α-acids, or both lost to trub formation?

- What are the driving factors causing bitter acids to be removed with trub? At what rate does this stripping occur?
- What is the rate of removal of bitter acids during fermentation, and what factors affect these losses?
- Why does iso-cohumulone show a higher utilization than the other bitter acids, given that all appear to isomerize at the same rate?

The ultimate goal would be to have a complete model of utilization. The model would first account for the entire temperature history experienced by hop bitter acids during the brewing process, and quantify the overall amount of isomerization achieved. The model would then account for all factors affecting losses of bitter acids, such as trub formation due to wort strength, fermentation losses, etc. Such a model would allow brewers to approach the process armed with a predictive tool which would remove the crude estimations and brewing trials used today.

4.6. Concluding Remarks

As research continues into the realm of new boiling regimes, there is much work to do in terms of expanding the scientific knowledge of isomerization and utilization. The brewing community readily accepts utilization values of 30%, whereas in most any other field a process efficiency of only 30% would be cause for alarm. Only by breaking down the utilization equation into its subcomponent parts, then defining and evaluating the relevant variables for each component, can the production of bitterness in beer be better understood. With understanding

would come the ability to predictively model the amount of bitterness produced, allowing brewers to better achieve desired bitterness levels in the face of changing conditions.

BIBLIOGRAPHY

[1] Andrews, J., and Axcell, B. C., Wort Boiling – Evaporating the Myths of the Past, *Tech. Q. Master Brew. Assoc. Am.*, 40(4):249-254, 2003.

[2] Askew, H. O., Changes in Hop Alpha Acids Concentrations on Heating in Aqueous Solutions and Unhopped Worts. *J. Inst. Brew.*, 70:503-514, 1964.

[3] Connors, K. A., **The Study of Reaction Rates in Solution**, VCH Publishers Inc., New York, NY, pp. 17-77, 1990.

[4] Fischer, S., Höhn, G., and Meyer-Pittroff, R., Influence of Hydrostatic High Pressure on the Filterability of Beer, *Tech. Q. Master Brew. Assoc. Am.*, 37(4):515-518, 2000.

[5] Fischer, S., Russ, W., and Meyer-Pittroff, R., High Pressure Advantages for Brewery Processes, *Trends in High Pressure Bioscience and Biotechnology*, 397-404, 2002.

[6] Fogler, H. S., **Elements of Chemical Reaction Engineering**, 3^rd Ed., Prentice Hall Inc., Upper Saddle River, NJ, pp.68-75, 1999.

[7] Hough, J. S., Briggs, D. E., Stevens, R., and Young, T. W., **Malting and Brewing Science: Vol II, Hopped Wort and Beer**, 2^{nd} Ed., Chapman and Hall, 1982.

[8] Hysert, D. W., Hop Products and Hop Quality Evaluation, presentation given at Oregon State University, 6 May 2003.

[9] Irwin, A. J., Murray, C. R., and Thompson, D. J., An Investigation of the Relationships Between Hopping Rate, Time of Boil, and Individual Alpha-Acid Utilization, *J. Am. Soc. Brew. Chem.* 43(3):145-152.

[10] Laufer, S., and Brenner, M. W., Some Practical Considerations on the Fate of Hop Resins in Brewing, *Proc.* 69^th *Anniv. Conv. Am. Soc. Brew. Chem.* 18-28, 1956.

[11] Laws, D. R., McGuinness, J. D., and Rennie, H., The Losses of Bitter Substances During Fermentation, *J. Inst. Brew.* 78:314-321, 1972.

[12] Lewis, M. J., and Young, T. W., **Brewing**, 2^{nd} Ed., Kluwer Academic / Plenum Publishers, 2001.

[13] McMurrough, I., Cleary, K., and Murray, F., Applications of High-Performance Liquid Chromatography in the Control of Bitterness, *J. Am. Soc. Brew. Chem.* 44:101-108, 1986.

[14] Moir, M., Hops – A Millenium Review, Journal of the American Society of Brewing Chemists, 58(4):131-146, 2000.

[15] Mostek, J., Marek, M., and Cepicka, J., Kinetics of the Isomerization of Hop Bitter Acids During Wort Boiling, *Brauwissenschaft*, 31(2):29-39, 1978.

[16] Peacock, V., Fundamentals of Hop Chemistry, *Technical Quarterly of the MBAA*, 35(1):4-8, 1998.

[17] Rigby, F. L., and Bars, A., The Determination of Iso-Compounds and Alpha-Acids in Wort, *Proc. Am. Soc. Brew. Chem.*, 46-50, 1961.

[18] S-PLUS (version 6.1), ©(2002), Insightful Corporation, Seattle, WA http://www.insightful.com

[19] Schwill-Miedaner, A., Wort Boiling Today – Are There Alternatives?, *Brauwelt Intl.*, 142(1):42-46, 2003.

[20] Spetsig, L. O., Electrolytic Constants and Solubilities of Humilinic Acid, Humulone, and Lupulone, *Acta Chem. Scand.* 9:1421-1424, 1955.

[21] Verzele, M., 100 Years of Hop Chemistry and its Relevance to Brewing, *Journal of the Institute of Brewing*, 92:32-48, 1986.

APPENDICES

APPENDIX A. Alpha-Acid Solubility in Aqueous Solutions

In the preparation of aqueous α-acid solutions, it was noticed that a haze would form above certain concentrations. For the range of concentrations seen in Figure A-1, a milky-white haze is readily apparent for those concentrations containing 80 ppm or more of alpha acids. The concentrations of the pH 5.2 buffered aqueous solutions shown in the figure are 10, 20, 30, 40, 50, 60, 80, 100, 200, and 400 ppm, from left to right. A search of the literature for data quantifying hop bitter acid solubility revealed that texts and papers alike referred only to the Spetsig article of 1955 [20]. In an attempt to ensure that experiments carried out for the purposes of this research were performed with α-acid concentrations below the solubility limit, an experiment was devised to experimentally measure the limit in the pH 5.2 model system.

While the visual observance of haze indicates when the solubility limit is surpassed, a more precise method than the human eye was desired. Since it was known that the prepared solutions contained purified α-acid extracts, without other compounds that would interfere by absorbing at wavelengths in the UV range, a spectrophotometric method was chosen. It was theorized that if solutions of increasing concentration were prepared ranging above and below the solubility limit, and then filtered to remove the haze, the solubility limit would be observed as the highest attainable value of concentration measured. That is, for lower concentrations, absorbance would increase linearly as concentration increased, but once the solubility limit was crossed, insoluble material would be removed by filtering and the absorbance would then remain constant with increasing concentration. The expected results are depicted graphically in Figure A-2.

FIGURE A-1. Increasing alpha-acid concentrations, pH 5.2

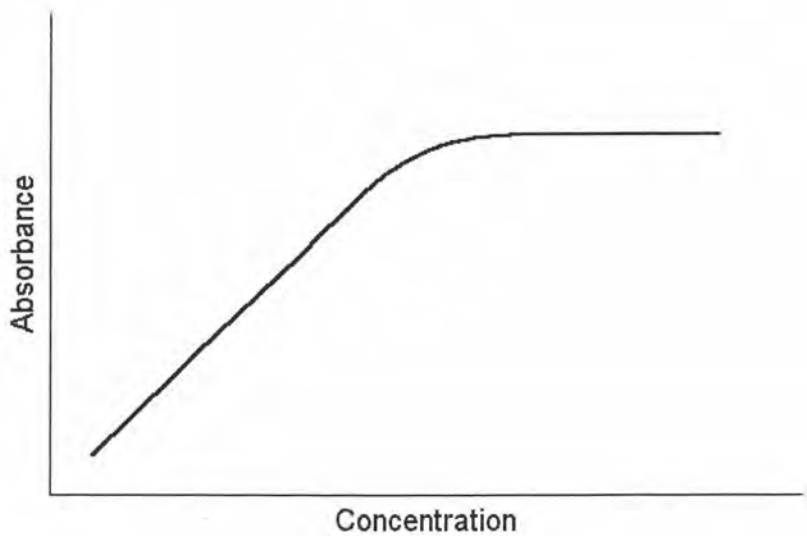


FIGURE A-2. Expected absorbance for increasing alpha-acid concentration

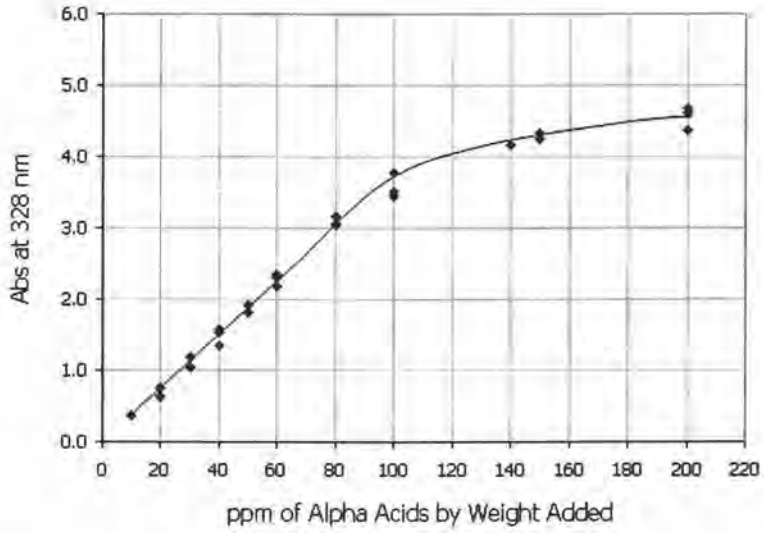


FIGURE A-3. Measured absorbance of alpha-acids in pH 5.2 buffer

The solutions for the solubility experiment were prepared identically to the samples used for the wort boiling experiments (sections 2.4 & 3.4). A stock solution of α-acids was prepared in ethanol, such that the final concentration of ethanol in the sample of highest concentration would be 1%. Solutions of lesser concentration were adjusted such that all samples contained 1% ethanol, and varying levels of α-acids. The pH buffer consisted of an acetate buffer (0.01M) titrated to a pH of 5.20. Each solution was then filtered through a mixed cellulose ester, hydrophilic, 0.45 micron filter (Millipore HAWP01300) prior to measurement of ultra-violet absorbance (Shimadzu UV-1700). Absorbance peaks occurred at 328nm, with peak values corresponding to the values given in Figure A-3. Note that samples were diluted to maintain absorbance values below 1.0, with values in the graph having been corrected for the dilution factor.

Although the curve did not completely plateau, there was a distinct knee in the curve and break from linearity for prepared concentrations of approximately

90 ppm and higher. This implied a solubility limit of approximately 90 ppm for α-acids in aqueous solution at pH 5.2. This shows reasonable agreement with the data of Spetsig [20], which specify 70 to 80 ppm. Additional experiments to verify solubility at other pH values were not carried out, due to time constraints. Based upon this data, a starting concentration of 80 ppm was chosen for the wort boiling experiments in which rate constants were examined as a function of temperature.

For the set of experiments examining effect of pH on isomerization rate, pH ranged from 4.8 to 6.0. Given the decreased solubility of the lower pH values, a starting concentration of 60 ppm was chosen for all experiments in the pH dataset. As seen in Section 3.5, this concentration was optimistic, and did indeed result in solubility issues at the lower pH values.

APPENDIX B. Removal of Alpha Acids by Filter Material

When in the course of the solubility experiments it was noticed that filtering the α-acid solutions removed haze, the question arose as to whether filtration removed bitter acids from solution even when the solubility limit was not exceeded. An experiment was devised to quantify the amount removed by filtration, and to compare the use of different filter media for sample preparation in the lab.

A solution of 100 ppm α-acids in pH 5.2 buffer, 2% ethanol, was prepared to ensure saturation of α-acids and formation of a visible haze. Three types of filters were examined:

- 0.45μm PVDF, hydrophilic, Pall Gellman
- 0.2μm inorganic, aluminum, Whatman Anotop
- 0.45μm mixed cellulose ester, hydrophilic, Millipore HAWP Nitrocellulose

The solution was filtered once, twice, and three times, with a measurement of UV absorbance taken after each filtration. That is, the solution was filtered once, the absorbance measured, then this same solution was passed through a second filter, the absorbance measured, etc.

The UV absorbance spectrum (190 to 500 nm) for each case is shown graphically in Figures B-1, B-2, and B-3. For each case, the line of greatest absorbance corresponds to the solution filtered one time, the next lower line corresponds to two total filtrations, and the lowest absorbance corresponds to three total filtrations. The 0.45μm hydrophilic PVDF (polyvinylidine fluoride) membrane filters removed a significant amount of α-acids with each step (Figure B-1), averaging

a removal of 23% of α-acids with each filtration. While the inorganic filters averaged a similar removal rate of 24%, the initial filtration apparently removed a larger quantity of humulones (Figure B-2) in comparison to the other filter media examined. The nitrocellulose filters showed the lowest rate of removal (Figure B-3), with an average of 7% of α-acids removed. The nitrocellulose filters were therefore chosen as a suitable media for sample prep in the solubility experiments as well as in the isomerization kinetics experiments.

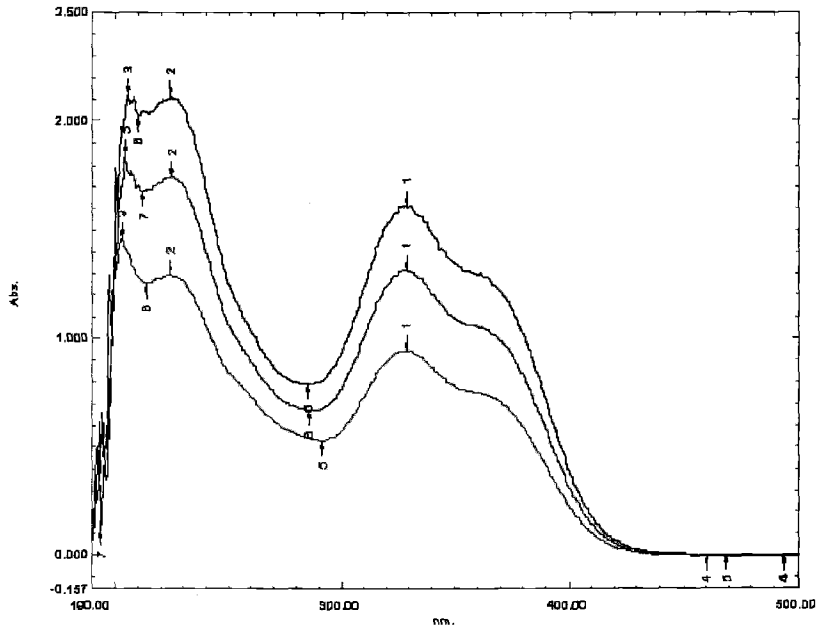


FIGURE B-1. Sequential filtrations with 0.45μm PVDF filters

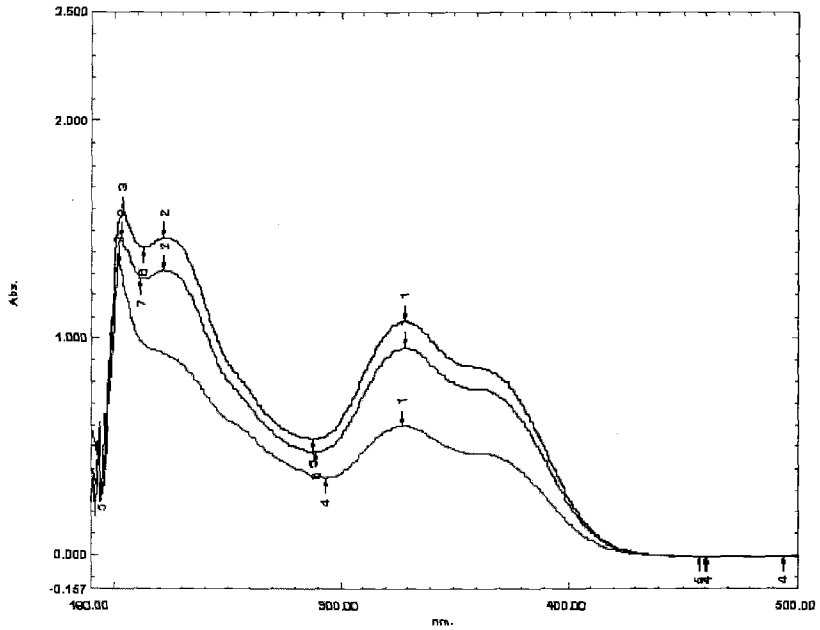


FIGURE B-2. Sequential filtration with 0.2μm inorganic filters

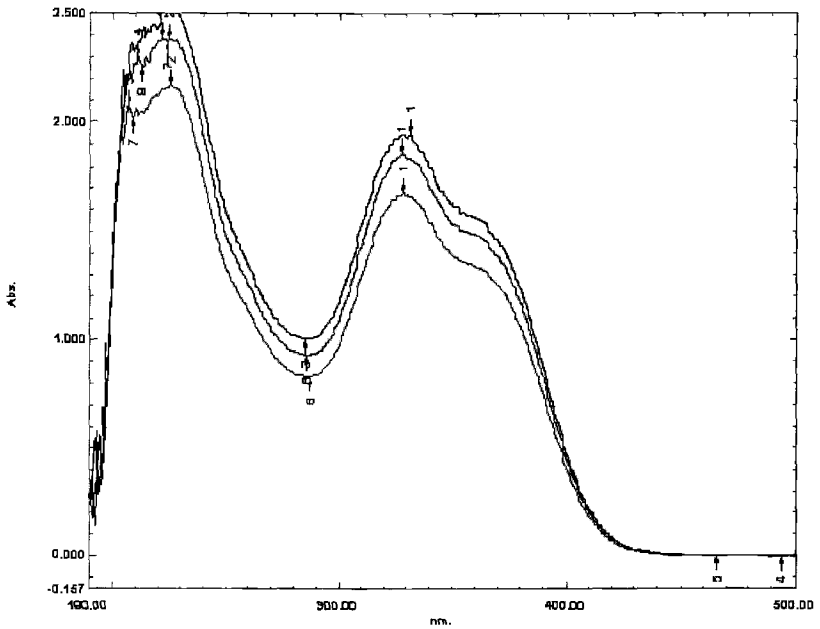


FIGURE B-3. Sequential filtration with 0.45μm nitrocellulose filters

APPENDIX C. HPLC Chromatograms

The following chromatograms show early, middle, and late sample analyses in the heating process (130° C, pH 5.2 dataset). Note appearance of small peaks early in the chromatogram (degradation products) for the late case (Figure C-3). HPLC conditions were as specified in the Materials & Methods sections, with detector set to 270 nm. Note that for the α-acids, humulone and ad-humulone show a co-eluting peak, while for the iso-α-acids, iso-humulone shows a split peak due to cis-iso-humulone and trans-iso-humulone having slightly different retention times (other analogs show co-eluting peaks for cis and trans isomers).

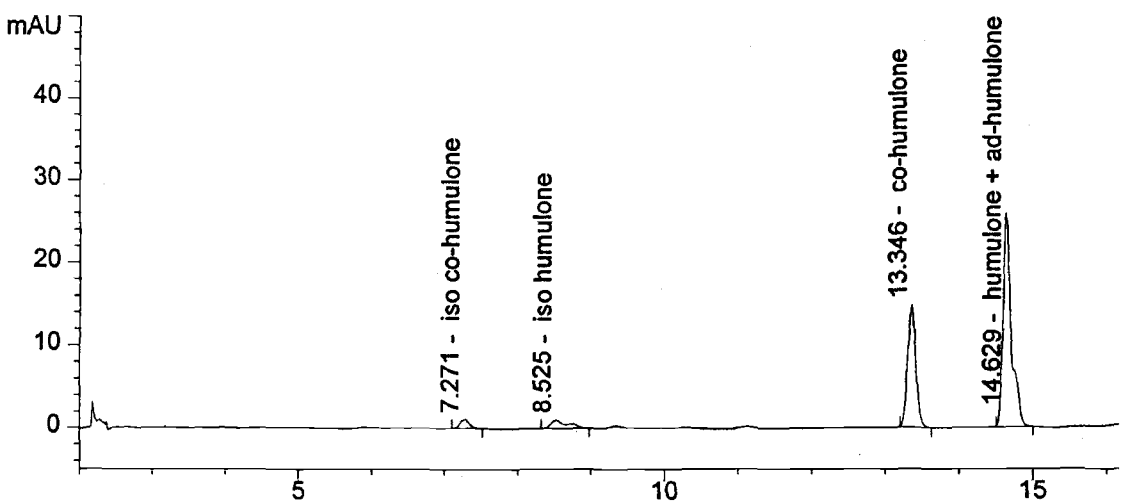


FIGURE C-1. Chromatogram: early in the boil

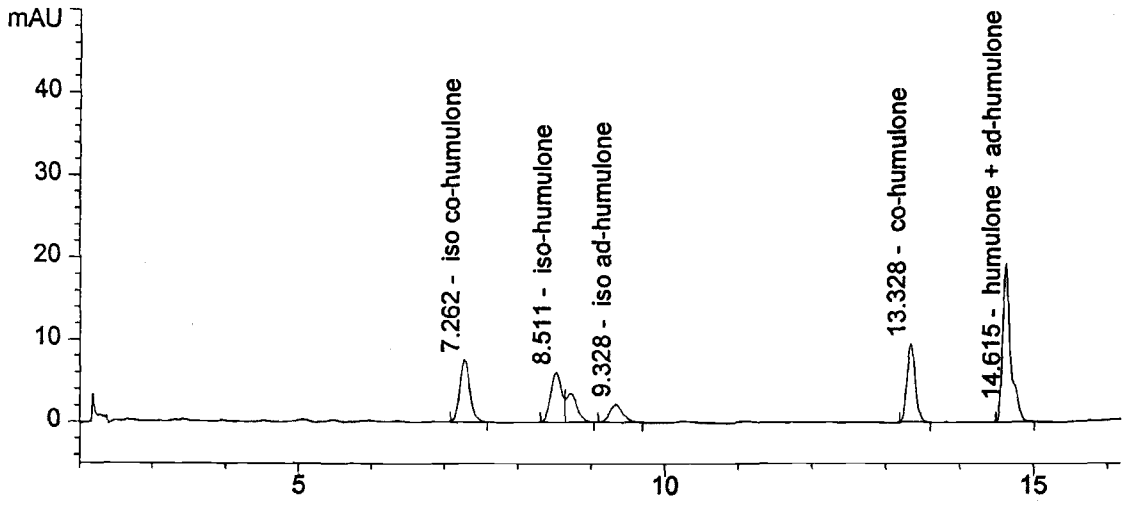


FIGURE C-2. Chromatogram: midpoint in the boil

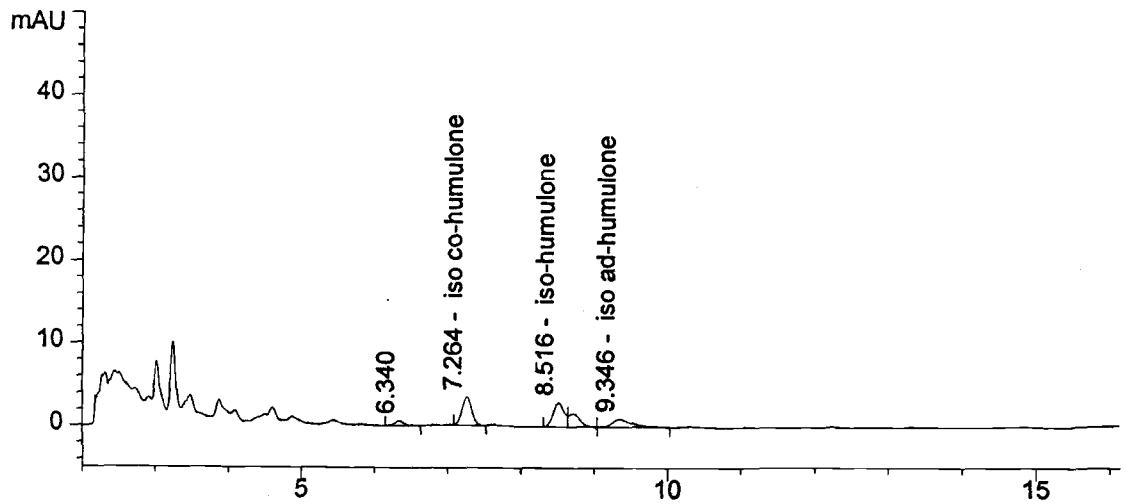


FIGURE C-3. Chromatogram: late in the boil

APPENDIX D. Data from Temperature Wort Boiling Experiments

		Iso-Alpha-Acids (ppm)				Alpha-Acids (ppm)		
	Time (min)	Iso-Cohumulone	Iso-Humulone	Iso-Adhumulone	Total Iso	Cohumulone	Humulone + Adhumulone	Total Alphas
70deg C, pH 5.2	0	0.00	0.00	0.00	0.00	21.72	44.09	65.80
	5	0.00	0.00	0.00	0.00	21.61	43.27	64.88
	10	0.00	0.00	0.00	0.00	20.96	41.93	62.89
	15	0.00	0.00	0.00	0.00	21.30	42.39	63.69
	20	1.41	1.47	0.00	2.87	20.92	41.62	62.54
	30	1.47	1.58	0.00	3.05	20.40	39.61	60.01
	40	1.61	1.79	0.00	3.40	19.99	39.23	59.22
	50	1.74	1.89	0.00	3.63	20.28	39.71	59.99
	60	1.79	1.94	0.00	3.73	19.84	37.75	57.59
	70	2.09	2.25	0.00	4.33	17.88	33.18	51.06
	80	2.07	2.34	0.00	4.42	19.18	37.76	56.94
	90	2.15	2.39	0.00	4.54	19.34	37.47	56.80
	100	2.22	2.45	0.00	4.67	19.05	36.13	55.19
	120	2.43	2.68	0.00	5.11	19.25	36.91	56.16
	140	3.16	3.44	0.00	6.60	16.54	30.24	46.79
	160	2.95	4.66	0.00	7.60	18.20	34.82	53.01
80deg C, pH 5.2	0	1.23	1.25	0.00	2.48	20.76	40.34	61.10
	5	1.31	1.32	0.00	2.63	20.23	38.90	59.13
	10	1.52	2.50	0.00	4.02	20.20	39.12	59.32
	15	1.65	2.65	0.00	4.30	20.12	39.09	59.21
	20	1.92	3.04	0.00	4.95	20.14	40.22	60.36
	30	2.22	3.55	1.04	6.80	19.87	39.22	59.09
	40	2.59	4.25	1.22	8.05	19.33	37.84	57.18
	50	2.78	4.22	1.22	8.22	19.00	37.69	56.69
	60	3.21	4.88	1.45	9.54	19.14	39.25	58.40
	70	3.63	5.60	1.69	10.92	18.14	36.08	54.22
	80	3.88	5.90	1.75	11.54	18.28	36.96	55.24
	100	4.60	7.14	2.09	13.84	17.56	35.18	52.74
	120	4.98	7.33	2.15	14.46	16.50	32.99	49.50
	140	5.50	8.13	2.39	16.02	16.31	32.97	49.28
	150	6.29	9.64	2.91	18.83	16.71	33.77	50.48
	160	6.71	10.54	3.07	20.33	14.97	28.46	43.43
90deg C, pH 5.2	0	1.12	1.79	0.00	2.91	19.50	40.56	60.06
	5	1.39	2.24	0.00	3.63	19.48	38.49	57.97
	10	1.85	2.86	0.00	4.71	18.54	36.79	55.33
	15	2.53	3.87	1.15	7.55	17.99	36.01	54.00
	20	2.82	4.50	1.28	8.60	17.86	36.52	54.38
	25	3.34	5.05	1.42	9.80	17.20	34.59	51.79
	30	3.81	5.74	1.74	11.28	16.95	34.08	51.03
	40	4.64	6.96	2.07	13.67	16.03	32.30	48.33
	50	5.65	8.22	2.31	16.18	15.24	31.86	47.10
	60	6.23	9.69	2.78	18.69	13.23	25.77	39.00
	70	6.94	10.16	2.91	20.01	13.40	26.52	39.92
	80	7.45	11.06	3.17	21.68	12.81	24.93	37.73
	90	8.55	12.41	3.54	24.50	12.32	24.53	36.86
	100	8.37	11.89	3.43	23.69	12.17	24.07	36.24
	120	9.61	13.69	3.81	27.11	10.13	19.32	29.46
	140	10.40	14.73	4.20	29.33	9.89	19.57	29.46

Data from Temperature Wort Boiling (cont.)

		Iso-Alpha-Acids (ppm)				Alpha-Acids (ppm)		
	Time (min)	Iso-Cohumulone	Iso-Humulone	Iso-Adhumulone	Total Iso	Cohumulone	Humulone + Adhumulone	Total Alphas
90deg C, pH 5.2 (2nd Run)	0	1.06	1.75	0.00	2.81	20.04	42.15	62.18
	0	1.05	1.70	0.00	2.75	18.75	34.50	53.25
	30	3.30	4.80	1.42	9.52	16.83	31.74	48.57
	60	5.31	7.61	2.23	15.14	15.03	29.55	44.59
	90	7.40	10.78	3.12	21.29	13.34	27.41	40.75
	120	8.24	11.05	3.15	22.44	11.45	23.68	35.12
	150	9.81	14.32	4.01	28.15	10.02	19.92	29.94
	180	10.48	14.02	3.95	28.45	8.54	17.86	26.41
	210	11.36	15.56	4.39	31.32	7.80	17.19	24.99
	240	11.69	15.85	4.50	32.04	5.73	10.93	16.66
	270	12.12	15.99	4.53	32.64	5.12	10.32	15.44
	300	13.17	18.03	5.07	36.28	4.78	9.46	14.24
	360	14.07	20.06	5.38	39.51	4.70	9.54	14.24
	390	13.46	18.17	5.05	36.68	3.21	6.67	9.89
	420	14.19	19.75	5.36	39.31	3.23	5.96	9.19
	450	14.20	19.41	5.30	38.92	2.50	4.81	7.31
	480	15.30	22.13	5.80	43.22	2.39	4.71	7.10
100deg C, pH 5.2	0	1.17	1.26	0.00	2.43	20.23	40.94	61.17
	5	1.51	2.47	0.00	3.98	19.59	39.35	58.94
	10	3.10	4.42	1.27	8.78	18.47	38.15	56.62
	15	4.14	6.05	1.79	11.98	17.67	36.87	54.54
	20	5.56	7.93	2.31	15.80	16.55	34.37	50.92
	30	6.53	9.63	2.81	18.98	15.51	31.88	47.39
	40	9.18	13.61	3.86	26.66	13.47	28.32	41.79
	50	10.14	14.45	4.07	28.66	11.97	25.42	37.39
	60	11.43	16.31	4.52	32.27	10.32	21.49	31.80
	70	12.72	18.22	5.04	35.98	9.73	20.79	30.52
	80	14.28	20.89	5.71	40.88	8.83	19.03	27.86
	90	14.55	20.58	5.52	40.64	7.52	16.39	23.91
	100	15.87	23.20	6.22	45.29	6.45	14.05	20.49
	110	16.36	23.77	6.31	46.44	5.67	12.09	17.76
	120	17.56	25.84	6.78	50.18	4.34	8.73	13.07
	130	17.48	24.89	6.49	48.86	4.33	9.05	13.38
100deg C, pH 5.2, 2nd Run	0	1.04	1.70	0.00	2.74	20.65	42.96	63.61
	0	1.03	1.64	0.00	2.66	19.81	37.42	57.23
	15	3.64	5.16	1.54	10.35	17.35	33.94	51.29
	30	6.17	8.85	2.52	17.53	15.33	31.15	46.47
	45	8.60	12.11	3.44	24.15	12.75	26.61	39.36
	60	10.27	14.54	4.04	28.85	11.31	24.69	36.00
	75	11.96	16.71	4.59	33.25	9.26	19.86	29.12
	90	13.08	18.50	5.07	36.65	7.43	15.85	23.28
	105	14.33	20.11	5.46	39.90	6.56	14.34	20.90
	120	14.22	19.67	5.42	39.31	5.38	13.09	18.48
	135	15.16	21.49	5.78	42.42	4.38	9.38	13.76
	150	15.32	21.61	5.80	42.72	3.42	7.07	10.49
	165	16.08	23.01	6.14	45.23	3.18	6.96	10.13
	180	15.43	22.35	5.88	43.66	2.21	4.74	6.95
	195	15.32	21.48	5.79	42.59	1.94	4.22	6.17
	210	13.38	19.12	5.09	37.59	1.22	2.61	3.83
	225	13.17	18.32	5.10	36.59	0.95	1.72	2.67

Data from Temperature Wort Boiling (cont.)

		Iso-Alpha-Acids (ppm)				Alpha-Acids (ppm)		
	Time (min)	Iso-Cohumulone	Iso-Humulone	Iso-Adhumulone	Total Iso	Cohumulone	Humulone+ Adhumulone	Total Alphas
110deg C, pH 5.2	0	1.16	1.19	0.00	2.35	20.08	39.97	60.05
	3	1.94	2.93	0.00	4.87	19.37	37.85	57.23
	6	2.98	4.52	1.31	8.81	18.44	37.02	55.46
	9	5.84	8.55	2.46	16.85	16.31	33.67	49.98
	12	6.82	9.70	2.81	19.32	15.52	32.75	48.27
	15	8.50	12.36	3.46	24.32	13.87	29.34	43.21
	20	10.64	15.06	4.18	29.88	11.58	24.90	36.47
	25	12.55	18.21	4.98	35.74	9.78	20.60	30.38
	30	14.29	20.78	5.63	40.70	7.96	16.86	24.82
	35	14.90	21.63	5.77	42.31	7.86	17.44	25.30
	40	16.50	23.72	6.28	46.50	6.23	13.85	20.08
	50	16.93	24.25	6.36	47.54	4.18	9.08	13.26
	60	17.81	25.92	6.66	50.40	3.89	8.41	12.30
	80	16.77	24.25	6.30	47.32	1.22	2.74	3.96
	100	13.87	20.21	5.50	39.57	0.00	0.88	0.88
	120	8.83	12.99	4.25	26.07	0.00	0.00	0.00
120deg C, pH 5.2	0	1.15	1.76	0.00	2.91	20.70	42.64	63.34
	3	4.06	5.90	1.77	11.72	17.94	37.08	55.02
	6	8.04	11.44	3.30	22.78	14.19	30.43	44.63
	9	10.38	14.44	4.05	28.88	10.90	23.29	34.19
	12	13.15	18.81	5.07	37.04	9.06	19.89	28.95
	15	14.74	20.68	5.53	40.95	6.69	14.72	21.41
	20	17.37	25.92	6.75	50.03	5.17	12.41	17.57
	25	16.93	23.83	6.21	46.97	3.19	7.10	10.29
	30	17.54	25.59	6.64	49.77	1.97	4.41	6.38
	35	16.17	22.61	5.88	44.65	1.24	2.30	3.54
	40	16.15	22.88	6.04	45.07	0.59	0.96	1.56
	50	12.00	17.09	4.87	33.96	0.00	0.00	0.00
	60	8.00	10.97	3.69	22.66	0.00	0.00	0.00
	70	8.92	12.74	4.10	25.77	0.00	0.00	0.00
	80	9.31	12.98	4.80	27.09	0.00	0.00	0.00
	100	8.72	12.29	5.28	26.29	0.00	0.00	0.00
130deg C, pH 5.2, 2nd Run	0	1.41	2.28	0.00	3.68	19.33	39.46	58.79
	0	1.07	1.74	0.00	2.81	20.10	40.67	60.77
	4	5.39	7.66	2.23	15.28	16.55	34.77	51.32
	6	9.66	13.41	3.71	26.78	12.47	28.71	41.17
	8	13.46	18.90	5.08	37.44	8.46	20.30	28.76
	12	16.38	23.45	6.06	45.89	4.49	11.68	16.17
	16	17.47	25.78	6.47	49.72	1.96	5.47	7.43
	20	15.27	22.55	5.68	43.50	0.85	2.40	3.25
	24	12.69	18.99	4.92	36.60	1.14	0.00	1.14
	28	9.29	14.10	4.00	27.39	0.00	0.00	0.00
	32	4.20	6.33	2.45	12.97	0.00	0.00	0.00
	36	5.42	8.22	2.74	16.38	0.00	0.00	0.00
	40	2.40	3.65	1.95	8.00	0.00	0.00	0.00
	44	1.94	2.99	1.70	6.62	0.00	0.00	0.00
	48	3.82	6.04	2.18	12.04	0.00	0.00	0.00
	52	2.30	3.50	1.43	7.23	0.00	0.00	0.00
	56	2.13	3.39	2.15	7.68	0.00	0.00	0.00

APPENDIX E. Data from pH Wort Boiling Experiments

		Iso-Alpha-Acids (ppm)				Alpha-Acids (ppm)		
	Time (min)	Iso-Cohumulone	Iso-Humulone	Iso-Adhumulone	Total Iso	Cohumulone	Humulone+ Adhumulone	Total Alphas
pH 4.8, 100deg C	0	0.91	1.41	0.00	2.32	11.87	17.61	29.48
	5	1.40	2.33	0.00	3.73	11.92	17.54	29.46
	10	2.91	4.55	1.29	8.75	11.01	16.52	27.53
	20	4.54	6.61	1.96	13.11	10.34	16.38	26.73
	30	5.98	8.55	2.55	17.08	9.33	15.11	24.45
	40	7.51	10.64	3.00	21.15	8.87	15.12	23.99
	50	8.43	12.36	3.46	24.25	7.63	13.33	20.95
	60	10.15	14.91	4.15	29.21	6.33	11.78	18.11
	70	10.20	14.15	4.07	28.41	6.08	12.22	18.30
	80	11.69	17.11	4.76	33.55	3.92	6.60	10.52
	90	11.60	16.69	4.65	32.93	4.29	7.98	12.27
	100	12.04	16.64	4.75	33.43	3.58	6.61	10.19
	110	12.75	18.34	5.03	36.13	3.24	6.17	9.41
	120	12.59	18.18	4.98	35.75	2.65	5.08	7.74
	130	13.21	19.29	5.19	37.69	2.23	3.94	6.16
	140	12.61	18.81	5.20	36.62	1.52	2.54	4.06
pH 4.8, 100deg C (2nd Run)	0	0.98	1.60	0.00	2.58	11.30	18.00	29.29
	5	1.42	2.15	0.00	3.57	11.13	16.78	27.91
	10	2.23	3.41	1.09	6.73	10.86	17.03	27.89
	20	4.41	6.23	2.08	12.72	9.73	15.80	25.53
	30	5.69	7.68	2.43	15.80	8.94	15.04	23.98
	40	7.33	10.14	3.11	20.58	7.64	13.99	21.63
	50	9.77	12.76	3.87	26.40	7.57	13.63	21.20
	60	9.19	12.07	3.75	25.01	5.74	11.38	17.11
	70	10.49	13.96	4.04	28.49	5.01	9.20	14.21
	80	10.99	14.55	4.14	29.69	4.35	7.73	12.08
	90	11.46	15.63	4.41	31.49	3.92	7.56	11.48
	100	11.52	15.52	4.39	31.43	3.19	6.54	9.73
	110	12.32	16.99	5.03	34.35	2.67	5.00	7.67
	120	11.75	16.60	4.73	33.09	2.13	3.86	5.99
	130	12.13	16.69	4.68	33.50	1.82	3.30	5.12
	140	10.70	14.36	4.10	29.17	0.82	1.80	2.62
pH 5.2, 100deg C	0	1.07	1.68	0.00	2.75	14.68	27.60	42.28
	5	1.40	2.34	0.00	3.75	14.29	26.15	40.44
	10	2.50	3.58	1.15	7.23	13.89	26.26	40.16
	20	4.05	5.87	1.78	11.70	12.89	26.33	39.22
	30	5.85	8.22	2.47	16.53	11.57	22.94	34.52
	40	7.57	10.98	3.25	21.79	9.43	18.13	27.56
	50	8.54	12.26	3.57	24.37	9.36	20.28	29.65
	60	9.76	13.31	3.72	26.79	8.09	16.62	24.71
	70	10.88	14.71	4.17	29.76	7.50	16.12	23.62
	80	11.62	15.98	4.43	32.04	6.49	13.53	20.01
	90	12.35	17.33	4.75	34.43	5.70	12.61	18.30
	100	12.66	17.43	4.70	34.79	4.63	11.44	16.06
	110	14.35	20.61	5.49	40.45	4.09	9.54	13.64
	120	13.41	18.69	4.96	37.05	3.37	7.96	11.33
	130	14.44	19.76	5.31	39.52	3.55	7.47	11.02
	140	14.60	20.16	5.24	40.00	2.66	6.29	8.96

Data from pH Wort Boiling(cont.)

		Iso-Alpha-Acids (ppm)				Alpha-Acids (ppm)		
	Time (min)	Iso-Cohumulone	Iso-Humulone	Iso-Adhumulone	Total Iso	Cohumulone	Humulone+ Adhumulone	Total Alphas
pH 5.2, 100deg C (2nd Run)	0	1.01	1.38	0.00	2.38	14.78	31.97	46.75
	5	1.32	1.82	1.98	5.12	14.24	31.47	45.71
	10	2.40	3.06	2.07	7.53	13.66	29.38	43.04
	20	4.25	5.85	1.54	11.64	12.00	25.80	37.80
	30	6.13	8.28	3.17	17.59	10.91	22.85	33.77
	40	6.94	9.49	2.84	19.26	9.77	20.96	30.73
	50	9.20	12.74	3.35	25.30	9.03	18.21	27.24
	60	9.34	14.54	3.49	27.37	8.20	16.56	24.76
	70	10.46	14.00	4.01	28.48	6.91	14.21	21.12
	80	11.00	14.73	4.10	29.84	6.15	13.66	19.81
	90	12.86	16.38	5.37	34.61	4.47	8.49	12.96
	100	12.38	16.54	5.63	34.55	4.97	11.35	16.32
	110	12.26	16.44	4.72	33.42	4.27	8.78	13.05
	120	13.26	17.75	4.87	35.88	3.42	7.72	11.15
	130	13.20	17.87	5.05	36.13	3.46	8.49	11.95
	140	13.98	19.16	5.47	38.61	2.38	4.98	7.36
pH 5.6, 100deg C	0	0.93	1.81	0.00	2.74	14.06	33.54	47.60
	5	1.42	1.54	0.00	2.96	15.33	29.54	44.87
	10	2.08	3.19	1.12	6.39	12.93	30.75	43.67
	20	3.71	5.12	1.45	10.28	11.68	25.15	36.82
	30	5.28	7.00	1.98	14.27	10.47	22.34	32.81
	40	6.39	8.68	2.56	17.64	9.66	21.15	30.82
	50	7.22	9.74	2.86	19.81	8.50	17.89	26.39
	60	8.49	11.75	3.43	23.67	7.85	16.60	24.45
	70	9.57	13.78	3.30	26.66	6.62	14.58	21.19
	80	9.69	18.17	3.31	31.17	5.96	13.59	19.55
	90	11.00	14.71	4.03	29.75	5.29	12.18	17.46
	100	11.10	15.74	4.18	31.02	5.03	11.49	16.52
	110	11.61	20.01	4.14	35.76	4.53	10.79	15.32
	120	12.41	16.65	4.06	33.12	3.71	8.18	11.89
	130	13.04	16.98	4.87	34.89	3.04	4.99	8.03
	140	12.73	16.98	4.69	34.39	2.66	6.28	8.94
pH 5.6, 100deg C (2nd Run)	0	0.94	1.13	0.00	2.06	14.66	31.70	46.36
	5	1.11	1.34	0.00	2.45	13.70	29.83	43.53
	10	1.90	2.18	1.73	5.81	14.17	29.75	43.93
	20	3.03	4.97	1.27	9.27	11.78	26.28	38.06
	30	4.90	7.46	1.61	13.97	10.81	23.33	34.14
	40	6.13	8.47	2.05	16.64	8.87	19.06	27.94
	50	8.29	11.35	2.57	22.21	9.09	18.67	27.76
	60	8.32	12.27	2.89	23.49	7.94	17.23	25.17
	70	9.13	13.10	3.08	25.31	6.53	12.54	19.06
	80	11.26	13.62	3.14	28.02	5.88	13.22	19.10
	90	9.76	13.87	5.14	28.77	7.20	13.88	21.08
	100	10.56	15.69	3.51	29.76	4.85	10.42	15.27
	110	11.52	15.97	3.35	30.84	3.97	8.12	12.09
	120	11.58	16.60	4.40	32.58	3.70	7.81	11.51
	130	11.08	16.21	4.27	31.56	2.87	6.42	9.30
	140	11.60	17.25	3.77	32.62	2.71	5.47	8.17

Data from pH Wort Boiling(cont.)

		Iso-Alpha-Acids (ppm)				Alpha-Acids (ppm)		
	Time (min)	Iso-Cohumulone	Iso-Humulone	Iso-Adhumulone	Total Iso	Cohumulone	Humulone+ Adhumulone	Total Alphas
pH 6.0, 100deg C	0	1.02	1.55	0.00	2.57	16.62	36.51	53.13
	5	1.53	2.53	0.83	4.90	16.03	34.72	50.74
	10	2.16	3.35	1.26	6.77	15.45	33.91	49.36
	20	3.97	6.00	1.97	11.94	13.81	30.71	44.52
	30	5.19	7.67	2.41	15.26	12.76	28.08	40.84
	40	6.38	9.88	2.58	18.83	11.93	24.95	36.88
	50	8.08	12.37	3.30	23.74	10.75	23.56	34.31
	60	9.29	13.93	3.93	27.14	9.28	21.25	30.52
	70	10.08	14.63	4.23	28.94	8.85	20.05	28.89
	80	11.33	17.20	4.73	33.27	7.47	16.24	23.71
	90	11.40	17.15	4.41	32.96	7.40	15.70	23.10
	100	12.38	18.29	4.96	35.63	6.72	16.00	22.72
	110	12.81	19.03	5.55	37.39	6.04	14.56	20.60
	120	13.26	19.61	5.22	38.09	5.34	12.22	17.56
	130	13.90	20.83	5.50	40.24	5.01	11.81	16.82
	140	14.53	21.78	5.57	41.87	4.21	9.90	14.11
pH 6.0, 100deg C (2nd Run)	0	1.05	1.37	0.00	2.42	15.49	34.04	49.53
	5	1.43	2.12	0.00	3.55	14.92	32.95	47.87
	10	2.22	3.06	0.96	6.24	14.36	31.66	46.02
	20	3.91	5.46	1.85	11.22	13.06	29.55	42.61
	30	5.45	7.51	2.30	15.26	12.12	27.08	39.20
	40	6.81	9.14	2.99	18.95	10.58	24.22	34.80
	50	8.10	11.49	3.29	22.88	9.54	22.28	31.82
	60	9.00	12.47	3.50	24.97	9.26	21.44	30.70
	70	10.04	13.82	4.11	27.97	8.19	19.32	27.50
	80	10.93	15.30	4.13	30.36	7.09	15.70	22.79
	90	11.62	16.44	4.51	32.57	6.59	15.43	22.02
	100	12.57	17.81	4.93	35.31	5.55	13.54	19.09
	110	13.03	18.33	4.98	36.34	5.26	12.35	17.61
	120	13.13	18.57	5.08	36.77	4.70	10.69	15.39
	130	14.74	19.40	5.17	39.30	5.32	12.28	17.60
	140	14.34	20.16	5.37	39.88	3.34	8.43	11.77

APPENDIX F. Data from Sugars & Calcium Wort Boiling

		Iso-Alpha-Acids (ppm)				Alpha-Acids (ppm)		
	Time (min)	Iso-Cohumulone	Iso-Humulone	Iso-Adhumulone	Total Iso	Cohumulone	Humulone+ Adhumulone	Total Alphas
10% Glucose (100deg C, pH 5.2)	0	1.23	2.02	0.00	3.25	19.05	35.18	54.23
	5	2.00	3.11	0.96	6.07	18.53	33.64	52.17
	10	2.94	4.49	1.33	8.77	18.08	33.45	51.52
	20	4.21	6.19	1.86	12.25	16.98	31.87	48.85
	30	4.94	7.10	2.10	14.14	16.63	32.53	49.16
	40	7.32	10.47	2.98	20.77	14.71	30.02	44.74
	50	8.49	11.94	3.43	23.86	13.66	28.05	41.71
	60	10.00	14.23	4.01	28.24	11.95	25.09	37.03
	70	11.87	17.18	4.86	33.92	10.49	22.41	32.90
	80	12.94	18.71	5.21	36.87	9.36	19.52	28.88
	90	13.58	18.90	5.23	37.71	8.02	17.74	25.76
	100	14.54	20.32	5.60	40.46	7.31	16.10	23.41
	110	15.29	21.31	5.84	42.45	6.52	14.45	20.97
	120	15.83	22.12	5.93	43.88	6.01	13.30	19.31
	130	16.12	22.68	6.07	44.88	4.92	10.81	15.72
	140	16.57	23.62	6.21	46.41	3.81	7.54	11.35
10% Maltose (100deg C, pH 5.2)	0	1.28	1.29	0.00	2.57	20.00	36.08	56.08
	5	1.70	2.81	0.00	4.52	19.27	34.50	53.77
	10	3.45	5.15	1.55	10.14	18.00	34.41	52.41
	20	4.49	6.76	2.07	13.32	17.73	34.21	51.94
	30	5.20	7.57	2.18	14.96	16.48	31.39	47.88
	40	7.97	11.33	3.35	22.66	16.29	31.72	48.01
	50	9.14	13.28	3.74	26.17	13.05	26.41	39.45
	60	10.85	15.23	4.29	30.37	11.62	24.72	36.35
	70	12.35	18.33	5.13	35.81	9.36	18.62	27.98
	80	12.76	18.12	5.06	35.95	9.70	20.35	30.05
	90	13.67	19.51	5.46	38.64	7.43	15.57	22.99
	100	14.92	21.70	5.93	42.55	6.64	13.99	20.63
	110	15.07	21.22	5.89	42.18	5.58	11.44	17.02
	120	14.99	21.25	5.83	42.07	4.44	9.50	13.94
	130	15.84	22.41	5.97	44.23	3.88	7.61	11.49
	140	15.99	22.61	6.04	44.64	3.36	7.00	10.36
100 ppm Calcium (100deg C, pH 5.2)	0	1.16	1.86	0.00	3.02	19.03	35.35	54.38
	5	1.87	3.12	0.94	5.93	19.31	38.98	58.28
	10	3.06	4.73	1.43	9.22	18.00	36.07	54.07
	20	4.56	7.03	2.05	13.64	16.96	34.38	51.34
	30	5.54	8.19	2.37	16.10	15.80	32.60	48.40
	40	7.34	10.54	3.18	21.06	14.59	30.19	44.78
	50	9.33	14.00	3.92	27.25	12.69	25.15	37.85
	60	10.71	15.51	4.34	30.56	10.48	21.02	31.49
	70	12.30	17.90	4.95	35.14	10.20	20.89	31.09
	80	14.04	20.77	5.58	40.39	7.87	14.77	22.63
	90	14.55	20.71	5.56	40.82	7.86	16.30	24.15
	100	15.62	22.32	5.93	43.88	6.91	14.06	20.97
	110	16.22	23.26	6.18	45.66	6.18	12.39	18.57
	120	17.17	24.64	6.49	48.30	5.27	10.70	15.97
	130	17.63	25.67	6.63	49.93	4.94	10.55	15.50
	140	17.81	25.71	6.67	50.19	4.26	8.68	12.94