

## Isomerization and Degradation Kinetics of Hop (*Humulus lupulus*) Acids in a Model Wort-Boiling System

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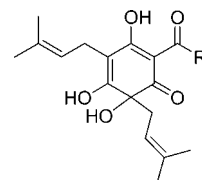
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The rate of isomerization of alpha acids to iso-alpha acids (the compounds contributing bitter taste to beer) was determined across a range of temperatures (90–130 °C) to characterize the rate at which iso-alpha acids are formed during kettle boiling. Multiple 12 mL stainless steel vessels were utilized to heat samples (alpha acids in a pH 5.2 buffered aqueous solution) at given temperatures, for varying lengths of time. Concentrations of alpha acids and iso-alpha acids 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  $k_1 = (7.9 \times 10^{11}) e^{(-11858/T)}$  for the isomerization reaction of alpha acids to iso-alpha acids, and  $k_2 = (4.1 \times 10^{12}) e^{(-12994/T)}$  for the subsequent loss of iso-alpha 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-alpha acids to degradation products were pronounced for cases in which boiling was continued beyond two half-lives of alpha-acid concentration.

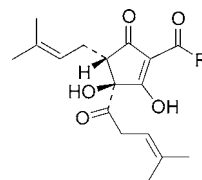
**KEYWORDS:** *Humulus lupulus*; hop bitter acid; humulone; isohumulone; alpha acid; iso-alpha acid; wort-boiling; isomerization; kinetics; utilization; beer; bitterness

### 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 primarily the alpha acids found within the lupulin glands of the hop cone (**Figure 1**). These compounds have little to no bittering value in their natural form (1, 2), but upon heating, an isomerization reaction takes place which converts the alpha acids into bitter-tasting iso-alpha acids (**Figure 2**). 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 not only allows more precise control over the bitterness achieved in finished beer, but also enables 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) can all be simply and accurately calculated once reaction kinetics are known.



**Figure 1.** Alpha acid structure. R denotes side chain of either  $-\text{CH}_2\text{CH}(\text{CH}_3)_2$  (humulone),  $-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$  (adhumulone), or  $-\text{CH}(\text{CH}_3)_2$  (cohumulone).



**Figure 2.** Iso-alpha acid structure. Side chains identical to those of **Figure 1**.

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 set of boiling conditions and deals only with the starting and ending points of the process. This efficiency, termed utilization, is defined in eq 1.

$$\text{utilization} = \left( \frac{\text{iso-alphas in finished beer}}{\text{total alphas added in hop addition}} \right) (100\%) \quad (1)$$

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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-alpha 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 (the insoluble precipitate that forms during kettle boiling, resulting mainly from protein coagulation), 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 utilization beginning at the most basic level – the kinetics of alpha acid isomerization as a function of 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 alpha 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.

## 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-alpha acids and alpha acids. A comprehensive set of data defining reaction order, rate constants, and activation energy has not been found in the published literature.

Askew (3) examined heating of an aqueous solution containing alpha acids within a Pyrex flask, and quantified both alpha acids and iso-alpha acids by spectrophotometric methods. Reaction kinetics were determined to be first order for alpha 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 alpha acids did not lead to appearance of iso-compounds, but later... losses of alpha 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 alpha acid loss. Minor losses (5–9%) were detected for both aqueous solutions and aqueous solutions with added (performed) trub. Losses for wort systems, however, were approximately 35%.

McMurrugh et al. (4) conducted wort-boiling experiments in both a model system (glass reaction vessel with a reflux condenser) and a high-gravity wort heated in a pressurized kettle. The model system consisted of a  $\text{KH}_2\text{PO}_4$  buffer with initial concentrations of approximately 300–330 ppm alpha 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. 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-alpha acids formed was found to be present in the hot break. Utilization was found to be higher for isochumulone (27%) as compared to isohumulone and iso-alpha-acidumulone (18%).

Laufer and Brenner (5) 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-alpha-acidumulone, then isochumulone, and lowest for isohumulone.

The research presented here sought to build upon the work of previous researchers, and comprehensively determine the kinetics of isomerization of hop bitter acids. The model system eliminated as many interfering factors as possible, leaving only alpha acids in a pH-buffered aqueous solution. Losses to factors such as trub were therefore minimized or eliminated. The temperature range exceeded that of previous studies and provided sufficient data to adequately calculate the rate constants and activation energies of the isomerization reaction. The HPLC method employed to quantify alpha acids and iso-alpha acids is a significant advancement over spectrophotometric methods of the past, and this allowed accurate measurement of hop acids at each sample point throughout the boil.

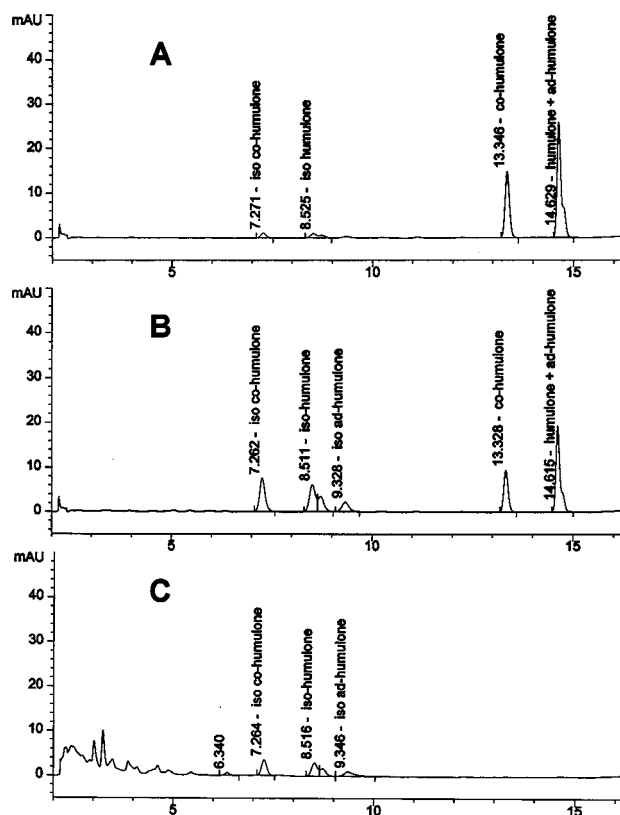
## MATERIALS AND METHODS

**Reagents.** Purified alpha acid extract (Alphahop) was provided by John I. Haas (Yakima, Washington) and was certified to contain 84.7% alpha acids, 3.1% iso-alpha acids, and 1.7% beta-acids. Standardized samples of alpha acids (ICE-2) and iso-alpha acids (ICS-11) were purchased from the American Society of Brewing Chemists (St. Paul, MN). Methanol was HPLC grade. Water for HPLC and all other solutions was filtered and deionized (Millipore Milli-Q). Remaining chemicals were purchased from VWR International.

**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 × 4.6 mm × 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.

**HPLC Conditions.** The HPLC method was based upon that given by Raumschuh et al. (6), with slight modifications to mobile phase composition, and inclusion of a gradient elution. Analysis time per sample was 22 min. Flow rate was set to 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%  $\text{H}_2\text{O}$ , and 1% phosphoric acid (85%). A gradient elution was used, consisting of 0–8 min, 100% B; 13–15 min, 50% B; 18–22 min, 100% B, with percentages linearly ramped between the given time frames. The gradient elution, which increased the percentage of methanol in the mobile phase after 8 min, shortened analysis times by decreasing the polarity of the mobile phase and thereby encouraging faster elution of the alpha acids. Peak area was automatically integrated using Chemstation software. The UV detector was set to a wavelength of 270 nm, which corresponds to an absorbance maximum for iso-alpha acids. The absorbance peak for alpha acids, however, is closer to 325 nm. It was proven experimentally that alpha acid concentration varied linearly with absorbance at 270 nm (a wavelength that corresponds to a relatively flat region of the alpha acid absorbance spectrum, although not a peak), and so quantification for all compounds was made using measured absorbance at 270 nm to simplify the analysis. An external standard was used (ASBC ICE-2) for each set of samples, with response factors calculated separately for each peak. Chromatograms from early, middle, and late time points in the heating process are shown in **Figure 3**.

**Sample Preparation and Heating Conditions.** The alpha 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 alpha acids. This level of alpha acid concentration was chosen so as to remain just below the published (and experimentally verified) solubility limit at the chosen pH value (7), and to fall within the range of industrially relevant world hopping rates. A 0.01 M acetate



**Figure 3.** HPLC chromatograms showing time points early in the boil, predominantly alpha acids (A); midway through boiling, both alpha and iso-alpha acids (B); and late in the boil, iso-alpha acids and degradation products (C).

buffer ( $pK_a$  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 alpha 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). The 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 sample 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 min. 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 micrometer filters (Millipore HAWP01300) prior to HPLC analysis. Sample preparation order, filtration order, and injection order were all randomized, to minimize any potential order effects.

## RESULTS AND DISCUSSION

The rate of conversion of alpha acids to iso-alpha acids was highly dependent on temperature (Figure 4). For typical 100 °C boiling conditions, 77% of alpha acids were isomerized within 120 min. Temperatures of 130 °C isomerized 100% of alpha acids within 30 min of heating. A 90 min boil at 100 °C corresponded to a final iso-alpha acid concentration equal to 60% of the starting alpha acid concentration. The time required to achieve an equivalent extent of isomerization was strongly affected by temperature (Table 1).

**Table 1.** Comparison of Boiling Time Required To Achieve a Concentration of Iso-alpha Acids Equal to 60% of the Starting Concentration of Alpha Acids, in a Model System

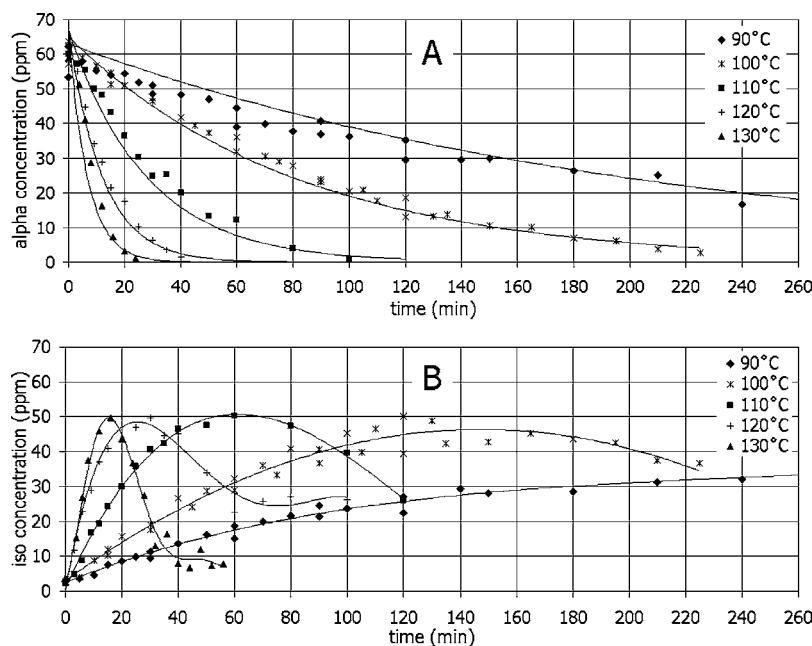
temp (°C)	time required for 60% iso production	
	time (min)	degr. products
90	207	9.4%
100	90	10.8%
110	40	12.3%
120	18	13.4%
130	8	14.0%

Although initial concentrations of 80 ppm were desired (based upon weight of alpha 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 alpha acids to vessel walls and to filter material. It was experimentally determined that 3% of alpha 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 alpha acids added (by weight) and the average measured starting concentration of 64.4 ppm. Alpha acids, being sparingly soluble at room temperature, and surface active, demonstrated a significant affinity to cling to any glassware, stainless steel vessels, or filters with which the solution came into contact. The solubility of alpha acids increases significantly with increasing temperature (7), and so the losses observed would have taken place during sample preparation at room temperature, and not during heating. The more polar iso-alpha acids are much more soluble than their nonisomerized precursors, and so it was assumed they would not have exhibited the phenomenon of leaving solution and clinging to vessel walls.

It is interesting to note that the appearance of iso-alpha acids did not have a one-to-one relationship with the loss of alpha acids, especially for early time points, indicating a loss of iso-alpha acids to degradation products. This phenomenon has been seen by other researchers as well (3, 8). For extended heating times, losses of iso-alpha acids became increasingly significant. In general, degradation became dominant beyond two half-lives of alpha 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 the difference between lost alpha acids and produced iso-alpha acids was equal to the quantity of degradation products produced.

The data are consistent with a reaction scheme in which alpha acids are converted to iso-alpha acids and are then converted into degradation products (Scheme 1). Reaction rate can be expressed using the equation  $v = kc^\alpha$ , where  $v$  is the reaction velocity (ppm per minute),  $k$  is the rate constant ( $\text{minute}^{-1}$ ),  $c$  is the reactant concentration (ppm), and  $\alpha$  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 alpha acid loss, using the relationships given in eqs 2–4, for reaction order of zero, one,



**Figure 4.** Alpha acid (A) and iso-alpha acid concentrations (B) versus heating time in a pH 5.2 acetate buffer.

or two (respectively), linearity was best obtained for first-order kinetics (that is, when  $\ln(c/c_{\text{initial}})$  was plotted versus time).

$$\text{zero order: } c = c_{\text{initial}} - kt \quad (2)$$

$$\text{first order: } \ln\left(\frac{c}{c_{\text{initial}}}\right) = -kt \quad (3)$$

$$\text{second order: } \frac{1}{c} = \frac{1}{c_{\text{initial}}} + kt \quad (4)$$

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 starting concentration of alpha 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 alpha acids would cling to the vessel walls, evade quantification in the HPLC analysis at room temperature, and yet remain reactive in the isomerization reaction that occurred at elevated temperature. This unquantified, yet reactive, portion of alpha acids (stuck to the vessel walls) 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 (3, 4).

For the half-life method, eqs 5–7 apply (9), with  $c$  equal to the concentration of alpha acids at time  $t$ ,  $c_{\text{initial}}$  equal to starting concentration, and  $k$  being the rate constant.

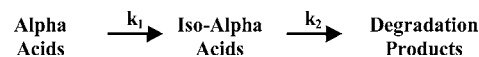
$$\text{zero order: } k = \frac{c_{\text{initial}}}{(2)t_{1/2}} \quad (5)$$

$$\text{first order: } k = \frac{\ln(2)}{t_{1/2}} \quad (6)$$

$$\text{second order: } k = \frac{1}{(c_{\text{initial}})t_{1/2}} \quad (7)$$

Rate constants ( $k$ 's) were found by both the half-life method and by the least-squares fit analysis. In the half-life method,

#### Scheme 1. Fate of Alpha Acids during Heating



**Table 2.** 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	0.00574
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

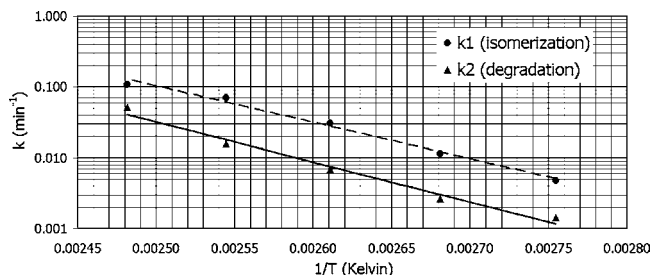
the time required for alpha acid concentration to drop to one-half of its initial value was found using a smoothed line fit (second order polynomial) to the experimental data. The “least squares” values were found using a solver tool in a commercially available spreadsheet package (Microsoft Excel), 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 data, occurred for reaction order equal to one. Half-lives and rate constants, found directly from the experimental data, are given in **Table 2**, for reaction order equal to one.

The kinetics of the degradation of iso-alpha acids to the uncharacterized degradation products were similarly analyzed, assuming that the differences between lost alpha acids and produced iso-alpha 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 of insufficient size to allow accurate quantification.

Assuming the degradation reaction to be first order, a second set of rate constants (the  $k_2$ 's in **Scheme 1**) 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 (alpha acid concentration, iso-alpha acid concentration, and degradation

**Table 3.** 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

**Figure 5.** Isomerization rate constants as a function of temperature.

products) to the experimental data, for each temperature (Table 3). Note that since  $k_1$  and  $k_2$  were both optimized simultaneously in the degradation analysis, new values for  $k_1$  were calculated, which differ slightly from those given in Table 2 (which optimized  $k_I$  alone, based on isomerization only, with no regard to degradation).

Rate constants for the isomerization reaction were plotted versus temperature, and the relationship was characterized. The Arrhenius equation (eq 8) relates the rate constant  $k$ , the activation energy  $E_a$ , and temperature  $T$  (kelvin). Note that  $A$  is the preexponential factor, and  $R$  is the universal gas constant.

$$k = Ae^{(-E_a/RT)} \quad (8)$$

Plotting  $\log(k)$  versus  $1/T$  (as in Figure 5) yields the activation energy,  $E_a$ , as the slope of the straight line fit to the data (9, 10). The fitted line gives the experimentally determined estimate of reaction rate as a function of temperature, and the relationship is given in eq 9, with  $k$  in units of  $\text{min}^{-1}$  and  $T$  in kelvin.

The rate constants for the degradation were also determined, and the temperature dependence was defined by the same method (Figure 5, eq 10). The activation energies were calculated as 98.6 kJ per mole for the isomerization of alpha acids to iso-alpha acids, and 108.0 kJ per mole for the subsequent degradation. The rate of isomerization was faster than that of degradation for all temperatures investigated. Examination of the activation energies, however, showed that the higher activation energy for degradation corresponded to a steeper sloped line for degradation than for isomerization (Figure 5), the implication being that rate of degradation changes with temperature to a greater degree than does the rate of isomerization. For example, a 10 °C increase in temperature would result in rate of isomerization increasing by 229%, while rate of degradation would increase by 248%. Extrapolating the best-fit lines in Figure 5 (which gives questionable accuracy, but illustrates the concept) would show that at temperatures in excess of 417 °C, rate of degradation would actually be faster than rate of isomerization.

$$k_1 = (7.9 \times 10^{11}) e^{(-11858/T)} \quad (9)$$

$$k_2 = (4.1 \times 10^{12}) e^{(-12994/T)} \quad (10)$$

From the chromatographic data, rate constants for isomerization of cohumulone to iso-cohumulone, separate from hu-

**Table 4.** Isomerization Rate Constants for Cohumulone

temp (°C)	$k_{\text{overall}}$	$k_{\text{cohumulone}}$
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

mulone plus adhumulone, were calculated. The HPLC method revealed only one coeluting peak for humulone and adhumulone, even though isohumulone was well separated from isoadhumulone, and so the two were analyzed as a single entity. The two sets of isomerization rate constants (overall, and cohumulone to isocohumulone) are given in Table 4. Statistical analysis showed no significant difference between the two sets of constants. A multiple linear regression model for  $\log(k_i)$  that included both explanatory variables ( $1/T$  as one explanatory variable to account for temperature, and isocohumulone as an indicator or dummy variable) did not show isocohumulone to be a significant explanatory variable ( $p$ -value = 0.815,  $R^2$  = 0.8897, dof = 7) after accounting for temperature (11). Isomerization of cohumulone to isocohumulone proceeded at a rate equivalent to that of humulone and adhumulone. The selective preference for isocohumulone in other studies on utilization (5, 12) is therefore due to some factor affecting 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-alpha acids is zero, the concentration of iso-alpha acids ( $c_{\text{iso}}$ ) and degradation products ( $c_{\text{degradation}}$ ) can be determined at any time  $t$  by using eqs 11 and 12 (9).

$$c_{\text{iso}} = \frac{k_1 c_{\text{alpha,initial}}}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t}) \quad (11)$$

$$c_{\text{degradation}} = c_{\text{alpha,initial}} + \frac{c_{\text{alpha,initial}}}{k_2 - k_1} (k_2 e^{-k_1 t} - k_1 e^{-k_2 t}) \quad (12)$$

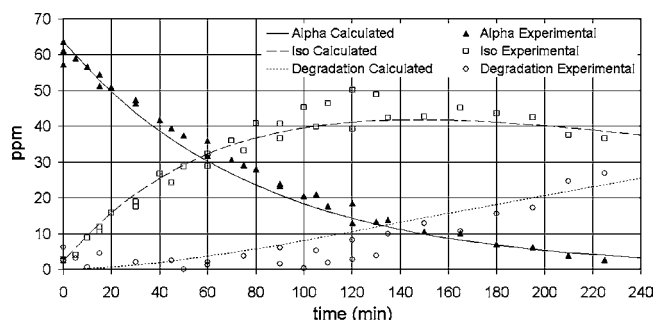
For conditions in which the rate constants change with a changing temperature profile, the concentrations of iso-alpha acids formed during kettle boiling can be calculated using eqs 13–15, which define the differential change in alpha acid, iso-alpha acid, and degradation product concentrations with respect to time.

$$\frac{d(c_{\text{alpha}})}{dt} = -k_1 c_{\text{alpha}} \quad (13)$$

$$\frac{d(c_{\text{iso}})}{dt} = k_1 c_{\text{alpha}} - k_2 c_{\text{iso}} \quad (14)$$

$$\frac{d(c_{\text{degradation}})}{dt} = k_2 c_{\text{iso}} \quad (15)$$

Discrete data, as a function of time, can be calculated by defining a small time step,  $\Delta t$ , and calculating the corresponding change in concentration for each time step (Figure 6). Various computer software packages can be used to determine concentration of iso-alpha 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 1 min, for the temperature



**Figure 6.** Measured and calculated concentrations of alpha acids, iso-alpha acids, and degradation products at 100 °C, pH 5.2.

region investigated here). This, of course, does not account for losses of bitter acids to trub, or other interfering factors.

In conclusion, the experimental data were consistent with a reaction order of one, for the isomerization of alpha acids to iso-alpha acids. This agrees with previously published work, which examined isomerization kinetics in both model systems and actual wort boiling (3, 4, 8). The reaction rate  $k_1$  is a function of temperature (eq 9), with corresponding activation energy of 98.6 kJ per mole.

Extended boiling times (beyond two half-lives of alpha acid concentration) showed significant degradation of iso-alpha acids to uncharacterized degradation products. Although degradation products could not be directly quantified, their concentrations were inferred from the difference between starting alpha acid concentration, and the sum of alpha acid and iso-alpha 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-alpha acids to the degradation products. The reaction rate  $k_2$  is a function of temperature (eq 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-alpha acids at any given time, accounting for both the amount of iso-alpha acids produced by isomerization and the amount of iso-alpha acids lost to degradation, as described above.

The results obtained indicate that the rate of isomerization roughly doubled for every 10 °C increase in temperature (average change was 229% per 10 °C increase). Isomerization of cohumulone to isocohumulone proceeded at a rate equivalent to that of humulone and adhumulone. High temperatures quickly led to degradation products, as evidenced in the dramatic decrease of iso-alpha acid concentration beyond 18 min of heating at 130 °C. While the rate of isomerization slowed at temperatures below 100 °C, substantial amounts of iso-alpha 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.

Beer presents the brewing researcher with a complex matrix of chemical compounds, and study of a model system is often necessary to secure reliable data pertaining to a single particular area of research. While the application of the results achieved herein may be restricted, such study is undoubtedly necessary to break down the complex process of utilization into its subcomponent parts and begin a complete understanding of the entire process. The model employed in this research allowed

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

#### ACKNOWLEDGMENT

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