Kinetic Modeling of Hop Acids during Wort Boiling

Yarong Huang, Johannes Tippmann, and Thomas Becker

Abstract—The thermal degradation of iso-alpha-acids was investigated at four pH levels between 4.5 and 6.5 at different boiling temperatures (90-130 °C) during the wort boiling time (0-360 min). The free energy of activation and the reaction rate constant (k) of the degradation of iso-alpha-acids were calculated. Raising pH value from 4.5 to 5.5 and from 5.5 to 6.5 decreased the reaction energy about 20 kJ/mol. The relative proportion of isocohumulone stays almost constant. We developed a kinetic model for the irreversible consecutive reation; iso-alpha-acids are isomerized form of alpha-acids by the application of heat in solution and at the same time iso-alpha-acids are degraded to humulinic acids and other compounds. This kinetic model is useful to calculate time to added hops into boiling kettle.

Index Terms—Activation energy, alpha-acids, degradation, iso-alpha-acids, kinetics, pH value.

I. INTRODUCTION

Hops (humulus lupulus) are an important ingredient in beer brewing and have been used for centuries for the purpose of contributing a clean bitter taste to beer. The value of hops for beer brewing is determined by the alpha-acids, essential hop oils and polyphenols. Alpha-acids are the quantitatively predominating class of polyketides accounting for up to 10% of hops dry matter and comprising of at least six congeners differing in the alkanoyl side chain. Whereas cohumulone, n-humulone, and adhumulone account for more than 98% of the total amount of alpha-acids, the minor congeners, namely, posthumulone, prehumulone, and adprehumulone, reach a relative quantity of less than 2% [1]. Iso-alpha-acids are isomerized forms of the alpha-acids (humulones) from the hops. The main transformation products account for up to 70% of the amount of alpha-acids initially added during wort boiling [2]. The iso-alpha-acids are mostly responsible for the characteristic, fine bitter taste and the foam properties of beer. The degree of bitterness imparted by hops depends on the degree of isomerizes of the insoluble alpha-acids during the wort boiling.

A thorough study of any chemical reaction must therefore include structure and kinetic investigations. It is known that the iso-alpha-acids are hydrolyzed or converted to humulinic acids and other undesirable compounds (Fig. 1). Some researchers have already investigated the kinetic of alpha-acids isomerization and found that the appearance of iso-alpha-acids did not equal the loss of alpha-acids, especially at the beginning of the wort boiling. For extended heating times, losses of iso-alpha-acids becomes increasingly significant [3]. Up to now the reaction kinetic of the degradation of iso-alpha-acids has been just studied basing on the assumptions. The reaction rates were calculated using mathematic modeling without experimental data.

This research seeks to discover the kinetics of degradation on iso-alpha-acids as a function of temperature and pH value. A purified iso-alpha-acids extract in different pH buffered aqueous solutions was used. The influence of some reaction parameters, such as temperature and pH value on the first-order rate constants involved in the kinetic model, has been investigated.



Fig. 1. Hops acid structures and chemical significance [4].

II. PERTINENT LITERATURE

Quantitative studies and model experiments have revealed a huge influence of the stereochemistry on the reactivity of the iso-alpha-acids and demonstrated a rapid degradation of trans-iso-alpha-acids, whereas the corresponding cis-isomers have been found to be comparatively stable. Recent molecular studies led to the discovery of harsh and lingering bitter tasting tricyclohumols, tricyclohumenes, isotricyclohumenes, tetracyclohumols, and epitetracyclohumols as the major transformation products of trans-iso-alpha-acids in model experiments as well as in authentic beer samples [5].

Some researchers have published data regarding the isomerization of alpha-acids during wort boiling with a first-order reaction. *Mostek* et al. [6] stated that during 120 min boiling time the rate of the isomerization of alpha-acids follows the first-order reaction except for the first 10 min. Knowledge of degradation of iso-alpha-acids during the wort boiling is relatively limited.

Schellhammer *et al.* [3] conducted the wort boiling experiments in a model wort boiling system and heated the

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purified alpha-acid extract solution in an oil bath with a programmed heated circulator. The acetate buffer (pKa 4.76) maintains the pH value for all experiments in a wort-representative pH 5.20. Schellhammer examined the losses of alpha-acids during wort-boiling across a range of temperatures from 90 °C to 130 °C for varying lengths of time and analyzed the kinetics of the isomerization of alpha-acids to iso-alpha-acids and similarly analyzed the degradation of iso-alpha-acids to the degradation products. The differences between the lost alpha-acids and produced iso-alpha-acids were assumed equal to the quantity of degradation produced. The rate constant and activation energy for the total iso-alpha-acid amounted to $(7.9 \cdot 1011)e^{(-11858/T)} \text{ min}^{-1} \text{ and } 98.6 \text{ kJmol}^{-1}.$

Recently Jaskula *et al.* [7] have examined heating of an aqueous buffer solution, which contained commercial nonisomerized hops extract, from 80 $^{\circ}$ C to 100 $^{\circ}$ C. They did the experiment twice and became totally different the rate constants.

Kappler *et al.* [8] have found that when iso-alpha-acids were boiled at a low pH value degradation velocity was faster than at a high pH value. After 90 min atmospheric wort boiling the cis-trans rate showed a slight increase from 4.5 to 4.7.

In our work, we investigated the reaction order of the degradation of iso-alpha-acids to the degradation products at different temperatures (90-130 °C) and pH values during the wort-boiling. The free energy of activation and reaction rate constant (k) of the degradation of iso-alpha-acids were investigated by measuring the concentration of total iso-alpha-acids, isocohumulone, isohumulone and isoadhumulone.

III. MATERIALS AND METHODS

A. Chemicals and Materials

Iso-Extract (30% w/v aqueous solution of iso-alpha-acids solution) was provided by Hopsteiner (Mainburg, Germany). Iso-Extract was certified to contain 30% iso-alpha-acids, < 0.6% alpha-acids and < 0.2% β -acids. Standardized samples of dicyclohexylamine (DCHA)-iso-alpha-acids ICS-I2 complex (66.5% (w/w) iso-alpha-acids were purchased from Labor Veritas, Zürich, Switzerland).

Di-distilled water used for HPLC was purified by means of a Milli-Q Gradient A 10 system (Millipore, Billerica, MA, USA). Methanol (HPLC LiChrosolv, gradient quality), phosphoric acids (85%) monohydrogenphosphat, dihydrogenphosphat and hydrochloric acid obtained from Merck (Darmstadt, Germany).

B. Instruments

A 22 ml covered glass test tube with cover was heated in an oil bath with temperature controller and stirrer. An inoLab pH 730 precision pH meter was used for titration of buffer solutions. All HPLC analyses was performed on an Agilent 1100 series system with an autosampler, a quarternary pump, thermosat and an Agilent 1200 series diode array detector (DAD).

C. HPLC Analysis of iso-alpha-acids

High-performance liquid chromatography (HPLC) is a

widely used method for the analysis of alpha-acids, iso-alpha-acids and their degradation products. The injection volume was set to 10 µl. A column temperature was set at 35 ℃. The UV detector was set to a wavelength of 270 nm, providing the maximum absorption. Mobile phase A consisted of 100% methanol, while mobile phase B contained 75% methanol, 24% di-desitilled water and 1% phosphoric acid (85%). For analysis of iso-alpha-acids 100% mobile phase B was used, consisting of 0-17 min, 17-25 min 65% B and 25-60 min 100% B. For analysis of iso-alpha-acids a compact 10-min-method was developed and only 100% mobile phase B was used. After 10 samples in this way, standardized sample (DCHA-iso-alpha-acids) was measured with the long method. Degradation of DCHA-iso-alpha-acids and iso-alpha-acids can also occurred during the analysis but a very small percentage. Peak area was automatically integrated using Agilent Chemstation software.

D. Sample Preparation and Heating Condition

Prior to the boiling experiments, phosphate buffer solution was made with monohydrogenphosphat, dihydrogenphosphat or hydrochloric acid to attain the desired pH value: 4.5, 5.2, 5.5, and 6.5, respectively. Iso-alpha-acids extract was added into the buffer solutions. The initial iso-alpha-acids concentration was 100 mg/l. 20 ml sample was placed in each 22 ml glass test tube. When the oil bath achieved the required temperature (90 to 130 $^{\circ}$ C), the glass test tubes were put into it. The temperature-sensor was inserted into the test tube and measured the temperature inside. When the temperature in the test tubes achieved the required temperature the timing began. In this case, temperature in the glass test tube increased to the desired value within 5 min. After a planned time interval, the test tube was taken out and cooled immediately in 0 % cold water. Boiling time was set to 360 min. The tests for given temperature and pH value were repeated at least three times. Before HPLC analysis, the samples were filtered with a 13 mm syring filter (0.45 µm PTFE) (Alltech Associates, Deerfield, IL, USA) and analysed by quantitive HPLC as described below.

Procedure in this work, we used Matlab to determine reaction order and calculate reaction rates. Using differential methods, the following procedure is developed.

% The experimental concentration-time data are loaded;

load time.m

load concentration.m

% 0.1 min time instant within experiment time;

x=min(time):0.1:max(time);

% Interpolation of the concentration at each time point;

y=interpl(time, concentration, *x*, `pchip`);

% For any arbitrary time instant, get the slopes of the curves;

v=diff(y)./diff(x); a=length (y)-1; logc=log(y(1:a));

% The logc-log(-*v*) are plotted;

nk=polyfit(logc, log(-*v*), 1);

% Get the slope and intercept of the plotted curve;

n=nk(1);

 $k = \exp(nk(2));$

IV. RESULTS AND DISCUSSION

A. Effect of Temperatures and pH Value on Degradation of iso-alpha-acids

To determine the relationship between pH value, temperature, and degradation of iso-alpha-acids, experiments were carried out at five temperatures (90, 100, 110, 120, and 130 ℃) and at pH values in the range of 4.5-6.5.The degradation of iso-alpha-acids was strongly dependent on temperature. It is a well-known fact that raising the temperature increases the reaction rate. Typical plots of iso-alpha-acids concentration in % vs. time for pH values 4.5, 5.5, and 6.5 with temperature (100 and 120, 110, and 130 $^{\circ}$ C) are shown in Fig. 2 and Fig. 3. As is seen, at 100 °C and pH value 4.5 64% of iso-alpha-acids were recovered after a 360 min of boiling, but at 130 °C only 2.3%. pH value also has a strong influence on the degradation of iso-alpha-acids, especially at lower temperatures (90, 100, and 110 °C). At 100 ℃ and pH 4.5 the recovery rate of iso-alpha-acids was double that at 100 ℃ pH 6.5 after 360 min boiling. But for higher temperatures (120 and 130 °C) the influence of pH decreased significantly. At 130 °C almost no changes were observed. At 130 °C and pH 6.5 the recovery rate of iso-alpha-acids sank to 1.6% nearly to 0%. Finally, it can be seen from Fig. 2 that raising the temperatures has more influence on degradation of iso-alpha-acids than rising pH value.



Fig. 2. Recovery rate of iso-alpha-acids after boiling with different pH value at 100 $\,$ C and 120 $\,$ C.

B. Determination of Reaction Order

To explore the reaction order, that the detailed sequential fluctuation of concentration of iso-alpha-acids is analyzed at the time intervals. (1) is a general representation of the reaction rate, k represents the rate constant (min⁻¹), c is the reactant concentration (mg/l), n is the reaction order (no units) and v is the reaction velocity (mg/l·min), negative for reactants (iso-alpha-acids) and positive for degradation products.

$$v = -\frac{d(C_{iso})}{dt} = kC_{iso}^{n}$$
(1)

A zero-order reaction yields a linear plot of concentration vs. time. A first-order reaction yields a linear plot of

-ln(recovery rate of iso- alpha-acids) or log(recovery rate of iso- alpha-acids) vs. time. The two plots have been done in Fig. 4 and Fig. 5. Typical plots of -ln(recovery rate of iso- alpha-acids) vs. time are shown in Figs. 5-7. The rate constant k is independent of the concentrations of iso- alpha-acids but depends upon environmental factors, such as the temperature and pH value. Comparison between zero-order and first-order Plots has been made. It is clear that the correlation coefficients of -ln(recovery of iso- alpha-acids) vs. time are a marginal better than of concentration vs. time, especially at low temperature (90, 100 and 110 $^{\circ}$ C).





Fig. 3. Recovery rate of iso-alpha-acids after boiling with different pH value at 110 $\,$ C and 130 $\,$ C



Fig. 4. (iso-alpha-acids) in % vs. time with different temperatures for pH 5.5.

To determine which reaction order (zero-order and first-order) should be used, we used the software Matlab to define the reaction order (2) can be seen by taking the logarithm on the both sides of the (1). Inv and InCiso express

a linear function with intercept lnk and slope n.

$$\ln v = \ln(-\frac{d(C_{iso})}{dt}) = \ln k + n \ln C_{iso}$$
⁽²⁾



Fig. 5. ln(iso-α-acids)in % vs. time with different temperatures for pH 5.5



Fig. 6. ln(iso-alpha-acids) in % vs. time with different temperatures for pH 6.5



Fig. 7. ln(iso-alpha-acids) in % vs. time with different temperatures for pH 4.5.

The reaction rate constants and order of reactions are presented in Table I. The orders of reaction were calculated as approximately 1 (between 0.87 and 1.30) in all of the experiments. Therefore, it is possible to consider that the degradation of iso-alpha-acids were first-order reactions. The reaction rate constants k can be calculated direct with this program or using the slope of the linear regression line. Both values are very similar. Other authors, in the previously published work, used the slope of the linear regression. To compare the results of other authors in this paper, the reaction rate constants k were from the slope of the linear regression line in Fig 5-7.

It is a well-known fact that raising the temperature increases the reaction rate. Arrhenius equation is an equation that represents the dependence of the rate constant k of a reaction on the absolute temperature T. (Laidler 1996)

A plot of ln k vs. 1/T is shown in Fig. 8, which illustrates the degradation of iso-alpha-acids follows the Arrhenius equation.

$$\frac{d\ln k}{dT} = \frac{E_a}{RT^2} \quad ; \quad k = Ae^{-E_a/(RT)}$$
(3)

Take the natural log of both sides

$$\ln k = -E_a / (RT) + \ln A \tag{4}$$

 $\ln k$ and 1/T express a linear function with intercept $\ln A$ and slope -Ea/R.

k is a first-order kinetic constant that changes with temperature, as given by the Arrhenius equation.Ea is the free energy of activation (kJmol⁻¹) in alkaline solution.

R is the universal gas constant (8.314 J K^{-1} mol⁻¹)

T is the absolute temperature (K)

A is the pre-exponential factor (\min^{-1})

The Slop of line $(-E_a/R)$ allows calculation of the activation energy (E_a). And the pre-exponential factor A can

be calculated with the intercept of the line.

The experimentally determined Arrhenius equations and activation energies derived therefrom for total iso-alpha-acids with different pH values, are summarized in Table I. The results obtained indicated that raising pH value from 4.5 to 5.5 and from 5.5 to 6.5 decreased the reaction energy fast 20 kJ/mol. *Clarke* noted that the iso-alpha-acids can be hydrolyzed or converted to humulinic acid with aqueous alkali. In iso-alpha-acids, splitting of the isohexenoyl side chain at C₄ prefers to at a high pH value.

 TABLE I:
 FREE ENERGIES OF ACTIVATION FOR DEGRADATION OF TOTAL

 ISO-ALPHA-ACIDS, ISOCOHUMULONE ISOHUMULONE AND ISOADHUMULONE
 WITH DIFFERENT PH VALUES

Reaction energies (Ea) (kJ/mol)	4.5	5.2	5.5	6.5
total iso-alpha-acids	116.2±2.8	104.4 ±0.14	100.9±5.2	71.8±1.6
isocohumulone	117.1±2.9	103.7±0.15	101.6±4.8	73.8±2.0
isohumulone and isoadhumulone	115.8.±2.8	102.9±0.12	98.1±4.5	70.5±1.6

Fig. 8 shows that the regression straight lines of

isocohumulone, isohumulone and isoadhumulone are almost the same. Arrhenius equation and reaction energies of degradation of total iso-alpha-acids, isocohumulone, isohumulone and isoadhumulone are nearly identical. Degradation of isocohumulone and the sum of isohumulone and isoadhumulone proceeded very similarly to the degradation of total iso-alpha-acids. The acyl side chain at C2 of iso-alpha-acids has no effect on splitting the isohexanoyl side chain at C4. The relative proportion of isocohumulone is around 30%.



Fig. 8. Arrhenius behavior of degradation of total iso- α -acids (up) and of isocohumulone, isohumulone, isoadhumulone with pH 5.5(down).

The concentration of iso-alpha-acids during the wort boiling depended on the isomerization rate constants of alpha-acids and degradation rate constants of iso-alpha-acids. The concentration of alpha-acids decreased exponentially as a function of time (5). The initial concentration of iso-alpha-acids is zero. For this consecutive reaction, the concentration of iso-alpha-acids can be determined at time by using (8), that came from (5-7) [9].

Iso-alpha-acids are an isomerized form gained

iso-alpha-acids by the application of heat in solution. At the same time, iso-alpha-acids are degraded into humulinic acids and other compounds. This reaction is an irreversible consecutive reaction. Each reaction step is first order. When the rate constants of each step was found, the integration of the kinetic Eqs. could also be determined.

Scheme 1: irreversible consecutive reaction of alpha-acids

$$alpha - acids \xrightarrow{k_1} iso - alpha - acids \xrightarrow{k_2} humulinic - acids$$

For the particular case of a two-step reaction (Scheme 1) starting with C_0 (alpha-acids), the solution can be expressed in the following form.

$$\frac{d(C_{alpha-acids})}{dt} = -k_1 (C_{alpha-acids})_0$$
(5)

$$\frac{d(C_{iso-alpha-acids})}{dt} = k_1 (C_{alpha-acids})_0 - k_2 (C_{iso-alpha-acids})$$
(6)

$$(C_{alpha-acids}) = (C_{alpha-acids})_0 e^{-k_i t}$$
(7)

$$(C_{iso-alpha-acids}) = (C_{alpha-acids})_0 \left(\frac{k_1}{k_2 - k_1}\right) \left(e^{-k_1 t} - e^{-k_2 t}\right)$$
(8)

By using the Software Matlab the concentration of alpha-acids and iso-alpha-acids can be predicted in time series (Fig. 9). If the initial concentration of alpha-acids is 50 mg/l, after a normal boiling time of 60 min, at 100 °C, the concentration of iso-alpha-acids was calculated to be 23.79 mg/l in the solution. After 90 min, i. e. normal boiling time and half dwell time in a whirlpool, the concentration was calculated to be 30.01 mg/l. The maximum concentration was reached after 200 min. Raising the boiling temperature decreased the time to reach the maximum concentration of iso-alpha-acids significantly. At 110 °C 90 min, at 120 °C 70 min und at 130 °C only 20 min.



Fig. 9. Concentration of alpha-acids and iso- alpha-acids duing the wort at 100 $\,^\circ\!\! C$ and 120 $\,^\circ\!\! C.$



Fig. 10. Concentration of alpha-acids and iso- alpha-acids duing the wort at 90 $\,$ C and 110 $\,$ C.

V. CONCLUSION

In this paper, the kinetics of degradation of iso-alpha-acids has been studied through boiling experiments in an aqueous buffer model system as a function of time, temperature and pH value. The free energy of activation and the reaction rate constant (k) of the degradation of total and individual iso-alpha-acids were calculated at four pH levels. The determination of the reactions order was run by a program compiled in Matlab R2007a language, which is a powerful software for numerical calculation. By raising the pH value of the reaction mixture increased the degradation of iso-alpha-acids. By raising the boiling temperature decreased the influence of pH value on the degradation of iso-alpha-acids significantly. Together with the kinetic rate of isomerization of alpha-acids, we developed a kinetic modeling for the irreversible consecutive reaction. This knowledge can be used for pre-isomerization of hops in the brewhouse, to save the energy and improved the hops utilization.

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