A Kinetic Study on the Degradation of Iso-alpha-Acids

Yarong Huang [1+], and Thomas Becker [1]

[1] Technical University Munich, Chair of Brewing and Beverage Technology

Weihenstephaner Steig 20, 85354 Freising, Germany

Abstract. The kinetics of the degradation of iso-alpha-acids has been studied 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 aim of our present study was to investigate the free energy of activation and the reaction rate constant (k) of the degradation of iso-alpha-acids; a first-order kinetic process. The relative proportion of isocohumulone stays almost constant. We developed a kinetic model for the irreversible consecutive reaction; alpha-acids are isomerized into iso-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. That fit the experimental data and allows the determination of all relevant reaction rates constant. This kinetic model is useful to calculate time to added hops into boiling vessel.

Keywords: Humulus lupulus, Hop bitter acid, Iso-alpha-acids, Alpha-acids, Kinetics, Activation energy, Degradation, pH value

1. Introduction

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 (1). 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 isomerization 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 acid and other undesirable compounds. Some researchers (2-5) 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 (6). Up to now the reaction kinetic of the degradation of iso-alpha-acids has been just studied based on assumptions. The reaction rates were calculated using mathematic modeling without experimental data.

This research seeks to discover the kinetics of degradation of iso-alpha-acids as a function of temperature and pH-value. A purified iso-alpha-acids extract in different pH buffered aqueous solution 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.

2. Materials and Methods

2.1. Instruments

A 22 ml covered glass test tube with closure 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 auto-sampler, a quaternary pump, thermostat and

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[+] Corresponding author. Tel.: + 498161713264; fax: +498161713883.
E-mail address: yarong.huang@wzw.tum.de

an Agilent 1200 series diode array detector (DAD). Peak area was automatically integrated using Agilent Chemstation software.

2.2. Sample Preparation and Heating Condition

Prior to the boiling experiments, phosphate buffer solution was made with monohydrogen phosphate, dihydrogen phosphate or hydrochloric acid to attain the desired pH value. The initial iso-alpha-acids concentration was 100 mg/l. 20 ml sample was placed in each 22 ml glass test tube. 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 to 0 °C in an ice water bath. Boiling time was set to 360 min.

3. Results and Discussion

3.1. Effect of temperatures and pH value on Degradation of Iso-alpha-acids

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. Iso-alpha-acids concentrations in % vs time for pH values 4.5, 5.5, and 6.5 with different temperatures are plotted. PH value also has a strong influence on the degradation of iso-alpha-acids, especially at lower temperatures. At 100 °C and pH 4.5 the recovery rate of iso-alpha-acids was double that at 100 °C 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 from 1.6% nearly to 0%. Raising the temperatures has more influence on degradation of iso-alpha-acids than raising pH value.

3.2. 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. Eq. 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.

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

Eq. 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) vs. time. Typical plots of -ln(recovery rate of iso-alpha-acids) vs time are shown in Fig. 1. 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. It is clear that the correlation coefficients of -ln(recovery of iso- alpha-acids) vs. time are a little better than of concentration vs. time, especially at low temperature (90, 100 and 110 °C). To determine which reaction order (zero-order and first-order) should be used, we used the software Matlab to define the reaction order. Eq. 2 can be seen by taking the logarithm on the both sides of the Eq. 1. lnv and lnCiso express a linear function with intercept *lnk* and slope *n*.

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

Eq. 2

Tab. 1: Reaction rate constants and reaction orders for degradation of iso-alpha-acids

Tem.(°C)	pH 4.5		pH 5.2		pH 5.5		pH 6.5	
90	0.0002±0.00002	1.23	0.0003±0	1.20	0.0005±0.0001	1.30	0.0015±0.0001	1.26
100	0.0009±0.000012	0.91	0.0012±0.000065	0.98	0.0015±0.000015	0.93	0.003±0.00015	0.89
110	0.0022±0.00011	1.01	0.0023±0	0.97	0.0027±0.0003	0.87	0.0046±0.0002	0.94
120	0.0057±0.00005	0.99	0.0063±0.00022	1.03	0.0066±0.00036	0.99	0.0081±0.00011	0.94
130	0.0107±0.0004	1.12	0.0091±0.00023	1.01	0.0137±0.0011	1.08	0.0173±0.00058	0.96

The orders of reaction were calculated as approximately 1 in all of the experiments (Tab. 1). 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.
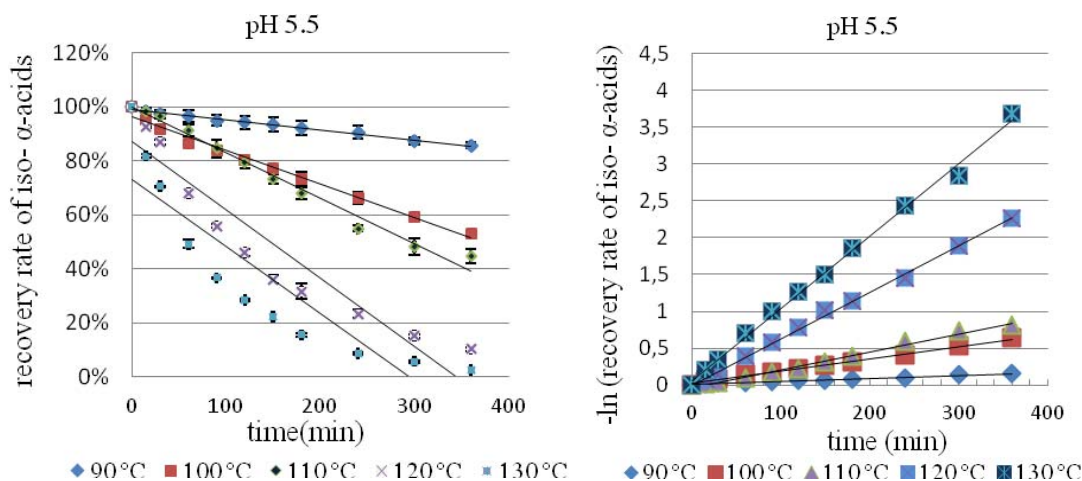

Fig. 1: (iso-alpha-acids) in % vs. time (left) and ln(iso-alpha-acids)in % vs. time (right) for pH 5.

A plot of ln k vs 1/T is shown in Fig. 2, which follows the Arrhenius equation.

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

Eq. 3

Take the *ln* of both sides

$$\ln k = -E_a/(RT) + \ln A$$

Eq. 4

The experimentally determined Arrhenius equations and activation energies derived for total iso-alpha-acids with different pH values are summarized in Tab. 2. 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 by about 20 kJ/mol.
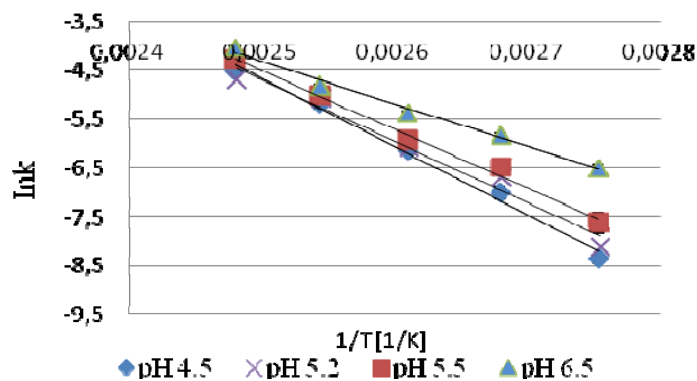

Fig. 2: Arrhenius behavior of degradation of total iso- alpha-acids

Clarke (1) 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 at a high pH value. Fig. 3 shows that the regression straight lines of total iso-alpha-acids, 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%.

Tab. 2: Free energies of activation for degradation of total iso-alpha-acids, isocohumulone isohumulone and isoadhumulone with different pH values

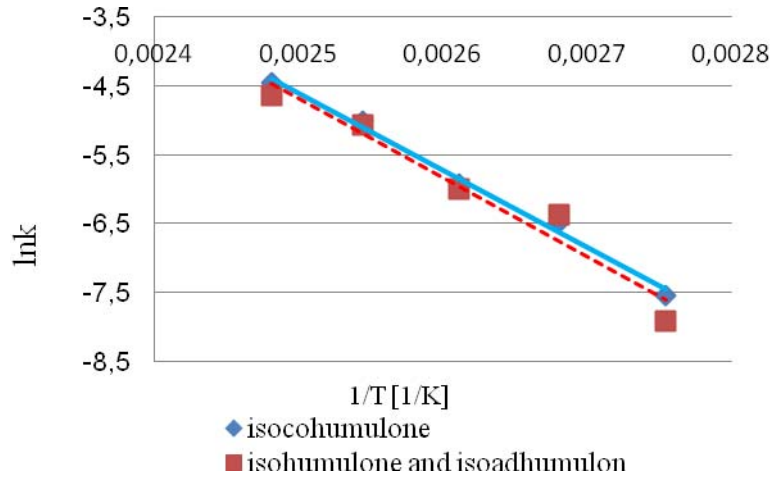| Reaction energies Ea (kJ/mol)	4.5	5.2	5.5	6.5
total iso-alpha-acids	116.16±2.85	103.32±1.34	100.90±5.2	71.80±1.63
isocohumulone	117.1±2.92	103.7±2.32	101.6±4.8	73.8±2.0
isohumulone and isoadhumulone	115.8.±2.78	102.3±1.21	98.1±4.5	70.5±1.6

Fig. 3: Arrhenius behavior of degradation of isocohumulone, isohumulone, isoadhumulone with pH 5.5

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 (Eq. 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 Eq. 8, which came from Eqs. (5-7). 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. If the rate constant of each step has been found, the integration of the kinetic Eqs. can also be determined.

Scheme 1: irreversible consecutive reaction of alpha-acids

$$alpha-acids \xrightarrow{k_1} iso-alpha-acids \xrightarrow{k_2} humulinic-acids$$
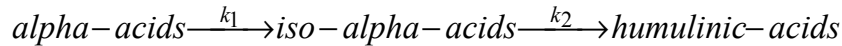
For the particular case of a two-step reaction (Scheme 1) starting with C_0 (alpha-acids), the solution can be expressed in the following form:

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

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

$$(C_{alpha-acids}) = (C_{alpha-acids})_0 e^{-k_1 t} \qquad \text{Eq. 7}$$

$$(C_{iso-alpha-acids}) = (C_{alpha-acids})_0 \left(\frac{k_1}{k_2 - k_1}\right)\left(e^{-k_1 t} - e^{-k_2 t}\right) \qquad \text{Eq. 8}$$
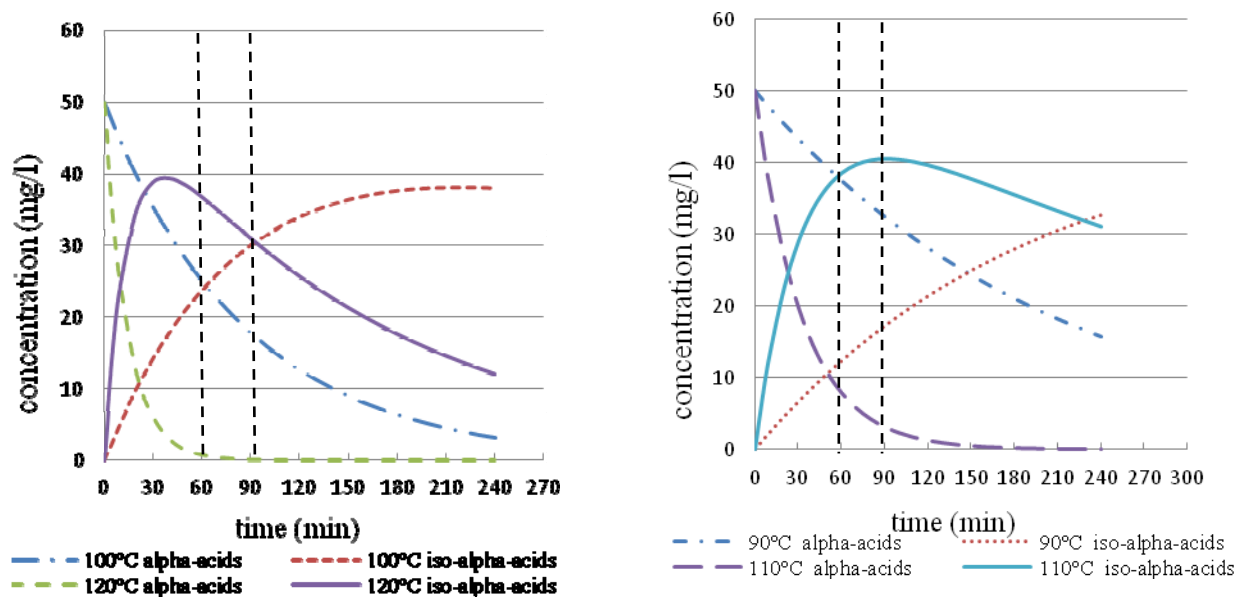
Fig. 4: Concentration of alpha-acids and iso- alpha-acids during the wort boiling according to Eqs. 7 and 8

The rate constants for isomerization of alpha-acids were calculated by *Schellhammer et al.* (6). Reaction rate k_1 are about 10 times greater the k_2. By using the Software Matlab the concentration of alpha-acids and iso-alpha-acids can in time series be predicted (Fig. 9).

4. 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 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 brewhaus, to save the energy and improve the hops utilization.

5. Acknowledgements

6. References

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