A Kinetic Study on the Isomerization of Hop \( \alpha \)-Acids

BARBARA JASKULA,*,†,‡ PAWEL KAFARSKI,§ GUIDO AERTS,†,‡ AND LUC DE COOMAN†,‡

Laboratory of Enzyme, Fermentation and Brewing Technology, KaHo St.-Lieven, Gebroeders Desmetstraat 1, B-9000 Gent, Belgium, Leuven Food Science and Nutrition Research Centre (LFeRCe), Department M2S, Katholieke Universiteit Leuven, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium, and Wrocław University of Technology, Institute of Organic Chemistry, Biochemistry and Biotechnology, Wybrzeże Wyspianskiego 27, 50-370 Wrocław, Poland

In this article, a detailed study on hop \( \alpha \)-acid isomerization kinetics is presented. Because of the complex wort matrix and interfering interactions occurring during real wort boiling (i.e., trub formation and \( \alpha \)-acids/iso-\( \alpha \)-acids complexation), this investigation on \( \alpha \)-acid isomerization kinetics was performed in aqueous buffer solution as a function of time (0–90 min) and heating temperature (80–100 °C). Rate constants and activation energies for the formation of individual iso-\( \alpha \)-acids were determined. It was found that iso-\( \alpha \)-acid formation follows first-order kinetics and Arrhenius behavior. Differences in activation energies for the formation of trans- and cis-isomers were noticed, the activation energy for the formation of trans-iso-\( \alpha \)-acids being approximately 9 kJmol\(^{-1}\) lower.

KEYWORDS: Hop \( \alpha \)-acids; trans/cis-iso-\( \alpha \)-acids; isomerization yield; kinetics; rate constant; activation energy

INTRODUCTION

Hops (Humulus lupulus L.) have long been used in brewing as they are a natural preservative, and part of the early use of hops in beer was to preserve it. Hops have been used for centuries to flavor the beer and are considered, along with water, malt, and yeast, to be an essential ingredient. Nowadays, it is recognized that hops are implicated in the main quality features of beer, i.e., taste and aroma, foam, color, and final product stability (1, 2).

Hop \( \alpha \)-acids and the essential hop oils are at the origin of key flavor attributes of beer, namely, typical beer bitterness and hoppy aroma. With respect to conventional hopping, a most important chemical conversion occurs during wort boiling, namely, the thermal isomerization of the hop \( \alpha \)-acids to the bitter tasting iso-\( \alpha \)-acids via an acyloin-type ring contraction (Figure 1). Conversion of each \( \alpha \)-acid results in two epimeric iso-\( \alpha \)-acids, distinguished as a trans- and cis-iso-\( \alpha \)-acid. Consequently, six major iso-\( \alpha \)-acids are present in beer, i.e., the trans- and cis-isomers of isocohumulone, isohumulone, and isoadhumulone.

Unfortunately, upon wort boiling, the isomerization yield of \( \alpha \)-acids into iso-\( \alpha \)-acids is invariably low (at most 50–60%) and also subject to variations, even from brew to brew. At the origin of the poor \( \alpha \)-acid isomerization are the limited solubility of \( \alpha \)-acids in wort, incomplete isomerization during the boil, and depletion of \( \alpha \)-acids and iso-\( \alpha \)-acids because of adsorption on the trub being formed. Furthermore, factors such as pH, wort gravity, hopping rate, hop product(s) used, presence of divalent cations, duration and temperature of the boil, and the degree of dispersal of the \( \alpha \)-acids upon addition of hops all have important influences on the \( \alpha \)-acid isomerization yield and final utilization (3–10).

Final overall \( \alpha \)-acid utilization is related to the beer, and this value is still significantly lower than the isomerization yield, amounting to only 30–40% or even as low as 10–20% (11). This is due to further losses of iso-\( \alpha \)-acids postwort boiling, i.e., further losses during wort clarification, fermentation, maturation, and beer filtration.

Knowledge of \( \alpha \)-acid utilization is of prime importance for each brewery in calculating accurate hopping rates to aim at precise bitterness levels, and, in particular, detailed knowledge of the isomerization kinetics should allow more precise control of iso-\( \alpha \)-acid levels achieved in finished beer. However, published literature has only modestly characterized the kinetics of \( \alpha \)-acid isomerization into their corresponding iso-\( \alpha \)-acids.

In 1964, Spetsig (13) found that \( \alpha \)-acid isomerization follows the pattern of a first-order reaction. At about the same time, Askew (3) also found the reaction kinetics for \( \alpha \)-acid conversion to be of first-order by heating \( \alpha \)-acids in aqueous solution and further quantifying both \( \alpha \)-acids and iso-\( \alpha \)-acids using spectrophotometric methods. Later on, Moštek et al. (14) stated that during the first 10 min of hop boiling, the rate of the isomerization of \( \alpha \)-acids varied with concentration and time and did not follow the pattern of first-order reaction kinetics. However, in the subsequent 10–120 min of boiling, \( \alpha \)-acid isomerization did occur as a first-order reaction. McMurrough

* Corresponding author. Phone: +32-(0)9-265 86 13. Fax: +32-(0)9-265 87 24. E-mail: barbara.jaskula@kahosl.be.
† KaHo St.-Lieven.
‡ Katholieke Universiteit Leuven.
§ Wrocław University of Technology.
et al. (15) demonstrated that decreases in $\alpha$-acids upon boiling follow first-order reaction kinetics and that the rate is influenced by temperature, pH, and the concentration of divalent cations. Furthermore, it was observed by McMurrough et al. (15) that decreases in $\alpha$-acids during boiling with hop extract were always greater than the corresponding increases in iso-$\alpha$-acids throughout the boiling period. When looking at the individual $\alpha$-acids, the isomerization yield of cohumulone was always highest, suggesting a faster conversion of cohumulone than n- and ad-humulone (16–18).

Recently, Malowicki and Shellhammer (9) confirmed the reaction kinetics of iso-$\alpha$-acid formation to be of first-order. However, in contrast to previous work (16–18), these authors claim that the isomerization of cohumulone to isocohumulone proceeds at a rate similar to that of the conversion of humulone and adhumulone to their corresponding iso-$\alpha$-acids. Malowicki and Shellhammer (9) were the first to report on essential kinetic parameters of iso-$\alpha$-acid formation, i.e., the rate constant and activation energy amounting to $(7.9 \times 10^{11}) e^{(-11856/T)} \text{ min}^{-1}$ and 98.6 kJmol$^{-1}$, respectively.

In this work, we aimed at the reliable determination of basic kinetic parameters (rate constants and activation energies) related to the formation of iso-$\alpha$-acids, including individual trans- and cis-iso-$\alpha$-acids. To perform the isomerization reactions, a model buffer system was selected in order to allow characterization of the isomerization as such, i.e., without interference of the reaction by the removal of $\alpha$-acids/iso-$\alpha$-acids due to trub formation, as observed in wort boiling.

**MATERIALS AND METHODS**

Determination of Rate Constant and Activation Energy of Iso-$\alpha$-acid Formation. *Boiling Experiments 1 and 2.* An all-glass round-
bottom flask (500 mL) with 3 necks was used in two separate laboratory scale boiling experiments (250 mL scale). The first neck was used for online measurement of the temperature, the second neck was fitted to a reflux condenser to prevent evaporation, and the third opening was closed with a rubber septum for sampling as a function of time using a syringe. Boiling experiments were performed in aqueous buffer solution (0.1 M), prepared with 3.3-dimethylglutaric acid and NaOH (sodium hydroxide) to maintain a c molar representative pH of 5.20. The flask containing buffer solution (250 mL) was heated in a laboratory stirrer/hot basket and continuously homogenized during the experiment with a magnetic stir bar. When the buffer achieved the required temperature (80, 90, or 100 °C), 2 mL of an ethanolic solution of commercial nonisomerized hop extract (International Calibration Extract ICE 2 containing 49.39% α-acids (w/w) and 24.94% β-acids (w/w); Labor Veritas, Zürich, Switzerland) with known composition was used as external standards.

Initial α-acids levels were 60 mg/L. At selected time intervals (5, 10, 15, 20, 30, 45, 60, and 90 min), heated samples (5 mL) were taken using a syringe and cooled immediately in liquid nitrogen (−196 °C). Frozen samples were kept at −24 °C until further extraction and HPLC analysis.

Extraction of α-Acids and Iso-α-Acids from the Aqueous Buffer System. α-Acids and iso-α-acids were isolated from the boiled buffer samples by liquid–liquid extraction, on the basis of the modified IOB method 9.16 (19) by Jaskula et al. (20). Buffer sample (5 mL) was acidified with H3PO4 (0.5 mL; 12 M; Merck, Darmstadt, Germany) and partitioned in a separating funnel with 10 mL iso-octane (Acros Organics, Belgium). After phase separation, the iso-octane layer (10 mL) was collected. This extraction with 10 mL iso-octane was performed three times, and the collected iso-octane fractions were concentrated to dryness under reduced pressure (30 °C). The residue was redissolved in 1 mL ethanol/H3PO4 (99.75/0.25; v/v). Before HPLC analysis, the extract was filtered through a 13 mm syringe filter (0.20 µm PTFE) (Alltech Associates, Deerfield, IL, USA).

HPLC Analysis of α-Acids and Iso-α-Acids. HPLC separations were performed on a Merck Hitachi Liquid Chromatograph (Merck, Darmstadt, Germany), consisting of a programmable HPLC pump L-7100 with a quaternary low pressure gradient system, a diode array detector L-7450A, an interface module D-7000, a solvent degasser L-7612, an autosampler L-7200 (sample loop: 100 µL), a Compaq Deskpro 2000 (software: Merck HPLC-System-Manager Software D-7000 Rev.2.1), and an Alltima C18 5 µm column (150 mm × 4.6 mm i.d., Alltech Associates, Deerfield, IL, USA).

Chromatographic conditions were as described by De Cooman et al. (21). Eluent A: milli-Q water adjusted to pH 2.80 with phosphoric acid (85%, Merck, Darmstadt, Germany). Eluent B: HPLC-grade acetonitrile (Acros Organics, Belgium). Isocratic elution was using 52% (v/v) B and 48% (v/v) A. Analysis time: 50 min. Flow rate: 1.8 mL/min. Temperature: ambient temperature. UV-detection: 270 nm (iso-α-acids) and 314 nm (iso-α-acids; Labor Veritas, Zürich, Switzerland) with known composition was used as external standard for quantification of iso-α-acids. The International Calibration Extract ICE 2 (49.39% (w/w) iso-α-acids, 24.94% (w/w) β-acids; Labor Veritas, Zürich, Switzerland) with known composition was applied for the quantification of α-acids. The trans iso-α-acids ratio (T/C-ratio) was based on the measured concentrations by HPLC of trans- and cis-isocohumulone, and trans- and cis-isohumulone.

\[
T/C(\%) = \frac{[\text{trans-isocohumulone}]}{[\text{ cis-isocohumulone}]} \times 100%
\]

RESULTS AND DISCUSSION

α-Acid isomerization kinetics was investigated in a simple model system (3,3-dimethylglutaric acid/NaOH buffer solution; 0.1 M; pH 5.20). Hop α-acids were added to heated buffer solution in the form of nonisomerized hop extract, predissolved in ethanol. Two independent series of heating experiments were performed in order to determine rate constants and activation energies. Iso-α-acid formation was measured by HPLC as a function of heating time and temperature (for more experimental details, see Materials and Methods).

According to previously published work (9, 13, 14), iso-α-acid formation from hop α-acids follows first-order kinetics. Thus, as shown by eq I, the rate of iso-α-acid formation upon heating of the reaction mixture is proportional to the instantaneous concentration of α-acids at any time during the reaction.

\[
\text{rate} = k \cdot [\alpha\text{-acids}]
\]
In eq I, \([\text{iso-}\alpha\text{-acids}_t]/[\text{o-}\alpha\text{-acids}_0] = -kt\) (II)

where \([\text{iso-}\alpha\text{-acids}_t]\) is the concentration (mol/L) of iso-\(\alpha\)-acids at reaction time \(t\), \([\text{o-}\alpha\text{-acids}_0]\) is the concentration (mol/L) of \(\alpha\)-acids at reaction time \(t = 0\) (s), \(k\) is the rate constant \((s^{-1})\), and \(t\) is the reaction time (s).

A graph of \(-\ln([\text{iso-}\alpha\text{-acids}_t]/[\text{o-}\alpha\text{-acids}_0])\) versus \(t\), when linear, proves that the reaction follows first-order kinetics.

The kinetic data on \(\alpha\)-acid isomerization obtained via experiment 1 are shown in Figure 2. Apparently, at each heating temperature (80 °C, 90 °C, and 100 °C), iso-\(\alpha\)-acid formation follows first-order kinetics, and the higher the applied heating temperature, the higher the rate constant of the reaction \((k)\). Rate constants corresponding to the formation of total iso-\(\alpha\)-acids, isoohumulone, isohumulone, and isoadhumulone are further shown in Table 1.

In addition, Table 1 contains the rate constants of individual iso-\(\alpha\)-acids, i.e., the trans- and cis-isomers, as a function of the applied heating temperature for both experiments 1 and 2. For determination of the rate constants of each pair of trans- and cis-isomers derived from the same \(\alpha\)-acid, kinetics of parallel reactions was applied (22).

In agreement with previous work on wort boiling at a pilot scale (Jaskula et al., unpublished results), it can be seen that the rate constants for the formation of cis-isomers are always higher than the rate constants for the formation of trans-isomers. Especially at the highest heating temperatures (100 and 90 °C), the difference in rate constant between cis- and trans-iso-\(\alpha\)-acids is very pronounced. This is related to the higher required activation energies and higher pre-exponential factors as found for the formation of cis-isomers (see further). The rate constant for the formation of the major iso-\(\alpha\)-acid, i.e., isohumulone, is always the lowest. Obviously, depending on the iso-\(\alpha\)-acid produced, there are clear differences in rate constants, which contradicts the previous work by Malowicki and Shellhammer (9).

The observed differences in rate constants, as a function of heating temperature and nature of the iso-\(\alpha\)-acid (see Table 1), are further reflected in clear differences in isomerization yields, as can be derived from Table 2. Clearly, in order to obtain relatively satisfying isomerization yields, a sufficiently high heating temperature is required, which is related to the value of the free energy of activation of the isomerization reaction (approximately 97 kJmol\(^{-1}\); see further). When looking at the efficiency of the reaction for the individual \(\alpha\)-acids, the isomerization yield of humulone is the lowest at each time interval and at each applied heating temperature. On the basis of kinetic data (rate constants) and calculated isomerization yields, \(\alpha\)-acid conversion occurs more efficiently for cohumulone, followed by adhumulone, and humulone.

Also in agreement with the kinetic data (see Table 1) are the iso-\(\alpha\)-acids T/C-ratios measured in the course of the heating period (Table 3). The lower the heating temperature, the higher the T/C-ratios, i.e., the more trans-isomers and the less cis-isomers are proportionally formed. This is due to the lower free energy of activation of trans-isomers versus cis-isomers (see further).

Moreover, when considering the evolution of the T/C-ratio as a function of heating time, it is interesting to notice the highest T/C-ratios after short heating periods, in particular at the lowest heating temperature (80 °C). As heating continues, T/C-ratios become lower. The higher T/C-ratios observed at the lower heating temperatures (80 and 90 °C) are connected with the lower free energies of activation for the formation of trans-isomers. However, the reaction is thermodynamically controlled because of the higher stability (less free energy) of the cis-isomers, and therefore, the T/C-ratio declines as a function of reaction time.

As \(\alpha\)-acid isomerization kinetics was monitored at different temperatures, rate constants were related to the heating temperatures in order to find out whether the reaction follows Arrhenius behavior. According to the general Arrhenius eq III, the relationship between the rate constant of the reaction and the absolute temperature is as follows:

\[
k = A e^{-E_a/(RT)}
\]

(III)

where \(k\) is the rate constant \((s^{-1})\), \(A\) is the pre-exponential factor \((s^{-1})\), \(E_a\) is the free energy of activation \((\text{kJmol}^{-1})\), \(R\) is the gas constant \((8.314 \text{ J K}^{-1} \text{ mol}^{-1})\), and \(T\) is the absolute temperature (K).

Equation III can also be expressed as follows:

\[
\ln k = \ln A - \frac{E_a}{RT}
\]
\[ \ln k = \frac{-E_a}{RT} + \ln A \]  

An plot of \( \ln k \) versus the reciprocal of the absolute temperature \( 1/T \) should be straight provided that the reaction follows Arrhenius behavior. The slope of the line \( (-E_a/R) \) allows calculation of the activation energy \( (E_a) \), whereas from the intercept of the graph with the ordinate \( (\ln k) \), the value of the pre-exponential factor can be derived.

As obvious from the correlation coefficients, Figures 3, 4, and 5 clearly demonstrate that \( \alpha \)-acid isomerization in our kinetic experiments follows Arrhenius behavior. This holds true for both total iso-\( \alpha \)-acid formation (Figure 3), formation of isocohumulone, isohumulone, and isoahumulone (Figure 4), and formation of individual trans- and cis-iso-\( \alpha \)-acids (Figure 5).

The experimentally determined Arrhenius equations and activation energies derived thereof are summarized in Table 4. Clearly, when comparing the results of both independent experiments 1 and 2, reproducible data have been obtained.

The activation energy related to the formation of total iso-\( \alpha \)-acids amounts to 96–97 kJmol\(^{-1}\). This result is in agreement with the activation energy of iso-\( \alpha \)-acid formation as measured by others (98.6 kJmol\(^{-1}\); 9). Furthermore, as apparent from Table 4, activation energies related to the formation of isocohumulone, isohumulone, and isoahumulone are nearly identical. This can be ascribed to the fact that within the transition state, the same bonds are being broken and formed during acyloin ring contraction, regardless of the nature of the \( \alpha \)-acid being converted. A similar enthalpy of activation will lead to a similar free energy of activation \( (E_a) \). Consequently, the influence on the activation energy of the hydrocarbon residue in the acyl side chain at C-2 of the \( \alpha \)-acid appears to be negligible.

However, with respect to the formation of trans- and cis-iso-\( \alpha \)-acids, a difference in activation energy was always noted, regardless of the iso-\( \alpha \)-acid analogue being formed (see Table 4). Activation energies associated with the formation of trans-
iso-α-acids were on average approximately 9 kJmol⁻¹ lower than activation energies measured in relation to their cis-counterparts.

Just like the overall free energy change of a reaction (ΔG°), the free energy of activation (ΔE_a) is composed of an enthalpy factor (ΔH_a) and an entropy factor (ΔS_a). ΔE_a = ΔH_a - TΔS_a. The enthalpy of activation (ΔH_a) is the difference in bond energies between the reactant(s) and the transition state; the entropy of activation (ΔS_a) is the difference in entropy between the reactant(s) and the transition state.

For the moment, it is not clear whether the higher activation energy associated with the formation of cis-compounds is due to a higher change in enthalpy of activation and/or entropy of activation, although changes in entropy with respect to the formation of transition states are usually considered relatively small (22).

A possible explanation of the higher activation energies involved in cis-iso-α-acid formation may lie in the conformational differentiation of the keto form of the α-acids in the transition state, whereby the intramolecular (very weak) hydrogen bond between the tertiary alcohol at C-6 and the carbonyl at C-5 guides the outcome. Indeed, in intermediary states, the hydrogen bond in the conformer in which the carbonyl is pointing downward at a syn-position with the hydroxyl at C-6 guides the outcome. Indeed, in intermediary states, the hydrogen bond in the conformer in which the carbonyl is pointing upward (formation of trans-iso-α-acids) should be stronger than in the conformer in which the carbonyl is pointing upward (formation of cis-iso-α-acids). As a result, the hydrogen of the hydroxyl group at C-6 should be more readily released in the conformer that leads to the trans-iso-α-acids, and consequently, activation energies for the formation of trans-isomers are lower than those of the cis-iso-α-acids.

In particular, the free energy of activation associated with the formation of cis-isocohumulone is relatively high (approximately 103 kJmol⁻¹), when compared with the other cis-iso-α-acids (96–97 kJmol⁻¹). This observation may be ascribed to the lower pKₐ value of cohumulone, causing more easy formation of the anion in the β-triketo system, thus a higher electron density within this system, which in turn may induce a slightly higher, partial negative charge on the oxygen of the carbonyl at C-5 in the transition state, resulting in an energetically unfavorable situation when the cis-isomer is being formed.

Notwithstanding, the lower free energy of activation associated with the formation of trans-iso-α-acids, rate constants of trans-iso-α-acid formation are significantly lower than rate constants of cis-iso-α-acid formation (Table 1). At first sight, this seems to be contradictory when looking at the Arrhenius eq III, in that normally a reaction with a lower free energy of activation should occur faster than a reaction with a higher one. However, further inspection of Table 4 reveals that the explanation of this apparent contradiction lies in the differences of the pre-exponential factors related to trans- and cis-iso-α-acid formation, respectively. cis-Isomer formation always

Table 4. Arrhenius Equation and Free Energies of Activation for Total Iso-α-acid and Individual Iso-α-acid Formation

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Total iso-α-acids</th>
<th>cis- and trans-iso-α-acids</th>
<th>cis-iso-α-acids</th>
<th>trans-iso-α-acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arhenius equation</td>
<td>E_a (kJmol⁻¹)</td>
<td>Arhenius equation</td>
<td>E_a (kJmol⁻¹)</td>
</tr>
<tr>
<td>experiment 1</td>
<td></td>
<td></td>
<td>experiment 2</td>
<td></td>
</tr>
<tr>
<td>cis-iso-humulone</td>
<td>3.13 × 10⁸ e⁻¹¹⁶⁶⁰/T</td>
<td>97.0</td>
<td>2.43 × 10⁸ e⁻¹¹⁵⁶⁰/T</td>
<td>96.2</td>
</tr>
<tr>
<td>isocohumulone</td>
<td>3.83 × 10⁸ e⁻¹¹⁶⁷³/T</td>
<td>97.1</td>
<td>2.45 × 10⁸ e⁻¹¹⁴⁹⁹/T</td>
<td>95.6</td>
</tr>
<tr>
<td>isohumulone</td>
<td>3.08 × 10⁸ e⁻¹¹⁶⁹⁰/T</td>
<td>97.2</td>
<td>1.77 × 10⁸ e⁻¹¹⁴⁹⁰/T</td>
<td>95.5</td>
</tr>
<tr>
<td>isoadhumulone</td>
<td>3.68 × 10⁸ e⁻¹¹⁷²²/T</td>
<td>97.5</td>
<td>1.81 × 10⁸ e⁻¹¹⁵⁴⁶/T</td>
<td>95.3</td>
</tr>
<tr>
<td>trans-isocohumulone</td>
<td>0.07 × 10⁸ e⁻¹⁰⁵⁷⁶/T</td>
<td>87.9</td>
<td>0.15 × 10⁸ e⁻¹⁰⁸⁴⁷/T</td>
<td>90.2</td>
</tr>
<tr>
<td>cis-iso-humulone</td>
<td>19.8 × 10⁸ e⁻¹²⁴⁴⁰/T</td>
<td>103.5</td>
<td>16.6 × 10⁸ e⁻¹²²⁵⁵/T</td>
<td>102.8</td>
</tr>
<tr>
<td>isocohumulone</td>
<td>0.06 × 10⁸ e⁻¹⁰⁶⁶¹/T</td>
<td>88.6</td>
<td>0.09 × 10⁸ e⁻¹⁰³⁶⁸/T</td>
<td>90.1</td>
</tr>
<tr>
<td>isohumulone</td>
<td>3.16 × 10⁸ e⁻¹¹⁸³³/T</td>
<td>98.4</td>
<td>1.31 × 10⁸ e⁻¹¹⁵⁵⁶/T</td>
<td>95.7</td>
</tr>
<tr>
<td>trans-isohumulone</td>
<td>0.23 × 10⁸ e⁻¹¹¹⁰⁴/T</td>
<td>92.3</td>
<td>0.23 × 10⁸ e⁻¹¹¹⁰⁷/T</td>
<td>92.3</td>
</tr>
<tr>
<td>cis-isoadhumulone</td>
<td>2.96 × 10⁸ e⁻¹¹⁷⁹⁷/T</td>
<td>98.1</td>
<td>1.03 × 10⁸ e⁻¹¹³⁸⁶/T</td>
<td>94.7</td>
</tr>
</tbody>
</table>

*According to Malowicki and Shellhammer (9), the rate constant and activation energy for total iso-α-acids amount to (7.9 × 10¹⁳ e⁻¹¹⁸⁵⁷/T) min⁻¹ and 98.6 kJmol⁻¹, respectively.
Involves higher pre-exponential factors, which explains the higher rate constants, despite the higher activation energies required to attain their transition state. The higher pre-exponential factor related to cis-iso-α-acids may suggest a higher probability of the cis-iso-α-acid transition state to end up in the finished product (a cis-iso-α-acid), which indeed has a lower free energy, i.e., a higher stability from the thermodynamic point of view than its trans-counterpart.

Finally, it can be seen from Table 4 that the pre-exponential factor associated with isocohumulone formation is higher than that of isohumulone and isoadhumulone formation. As mentioned previously, the rate constant of isocohumulone formation is always significantly higher than the rate constants of the formation of the other analogues. Because the activation energy related to isocohumulone formation is not different from the other activation energies (Table 4), its higher rate constant can be associated with the higher pre-exponential factor, which in turn may be related to the lower pKₐ value of cohumulone versus the pKₐ value of the other α-acid analogues (pKₐ cohumulone, 4.7; pKₐ humulone, 5.5; pKₐ adhumulone, 5.7; (23)). The lower pKₐ of cohumulone will lead to more efficient formation of the required stabilized anion in the β-triketo system of the transition state, thus resulting in a higher probability of the reaction taking place and, ultimately, higher isomerization yields.

On the basis of the data collected in the Arrhenius experiments 1 and 2, a free energy diagram for the conversion of hop α-acids into trans- and cis-iso-α-acids is proposed (Figure 6).

The proposed scheme is compatible with the higher relative stability of cis-iso-α-acids versus trans-iso-α-acids. In cis-iso-α-acids, the bulky side chains at C-4 and C-5 are actually in the trans-configuration, leading to more low energy conformations with a resultant increase in entropy, compared to that of trans-iso-α-acids (24). Thus, according to the thermodynamic equation ΔG° = ΔH° - TΔS°, cis-iso-α-acid formation (lower free energy of end product) from α-acids is energetically favored compared to trans-iso-α-acid formation (higher free energy of end product). The higher stability of cis-isomer vs trans-isomer is expressed by the thermodynamic equilibrium of approximately 80:20, in favor of cis-iso-α-acids. Also indicated in Figure 6 is the lower free energy of activation associated with the formation of trans-compounds versus cis-compounds.

In summary, the above scheme explains why at the onset of a heating experiment trans-iso-α-acid formation is prevailing, whereas as heating proceeds and equilibrium is being attained, cis-iso-α-acids are becoming the dominant ones because cis-iso-α-acids are thermodynamically more stable. In accordance with this proposed free energy diagram and thermodynamic control of α-acids isomerization, partial conversion, i.e., reverse isomerization, of trans-iso-α-acids via α-acids into cis-iso-α-acids has been demonstrated by us in both heated buffer systems and boiling wort, starting from pure trans-iso-α-acids (data not shown). Furthermore, trans-cis interconversion was also reported previously by Verzele and De Keukeleire (25).

In conclusion, the ionization of the β-triketo system in the α-acids is considered as the rate-limiting step of the isomerization reaction. Electron density within this β-triketo system will be influenced by the nature of the acyl group at C-2 of the α-acid analogue. While conforming the known basic mechanism of hop α-acid isomerization, the kinetic study carried out in this work, in particular results on activation energies of individual trans- and cis-iso-α-acids, reveals new and more detailed insight into the mechanism of the key reaction of hops in brewing.

**ABBREVIATIONS USED**

HPLC, high performance liquid chromatography; T/C-ratio, (trans-isocohumulone + trans-isohumulone)/(cis-isocohumulone + cis-isohumulone) × 100%; PTFE, polytetrafluoroethylene.

**LITERATURE CITED**

Hop α-Acid Isomerization Kinetics


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