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A Kinetic Study on the Isomerization of Hop α-Acids

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In this article, a detailed study on hop α-acid isomerization kinetics is presented. Because of the complex wort matrix and interfering interactions occurring during real wort boiling (i.e., trub formation and α-acids/iso-α-acids complexation), this investigation on α-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-α-acids were determined. It was found that iso-α-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-α-acids being approximately 9 kJmol^{-1} lower.

KEYWORDS: Hop α-acids; *trans*-/*cis*-iso-α-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 α-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 α-acids to the bitter tasting iso-α-acids via an acyloin-type ring contraction (**Figure 1**). Conversion of each α-acid results in two epimeric iso-α-acids, distinguished as a *trans*- and *cis*-iso-α-acid. Consequently, six major iso-α-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 α-acids into iso-α-acids is invariably low (at most 50−60%) and also subject to variations, even from brew to brew. At the origin of the poor α-acid isomerization are the limited solubility of α-acids in wort, incomplete isomerization during the boil, and depletion of α-acids and iso-α-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 α-acids upon addition of hops all have important influences on the α-acid isomerization yield and final utilization (*3–10*).

Final overall α-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-α-acids postwort boiling, i.e., further losses during wort clarification, fermentation, maturation, and beer filtration.

Knowledge of α-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-α-acid levels achieved in finished beer. However, published literature has only modestly characterized the kinetics of α-acid isomerization into their corresponding iso-α-acids.

In 1964, Spetsig (*13*) found that α-acid isomerization follows the pattern of a first-order reaction. At about the same time, Askew (*3*) also found the reaction kinetics for α-acid conversion to be of first-order by heating α-acids in aqueous solution and further quantifying both α-acids and iso-α-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 α-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, α-acid isomerization did occur as a first-order reaction. McMurrough

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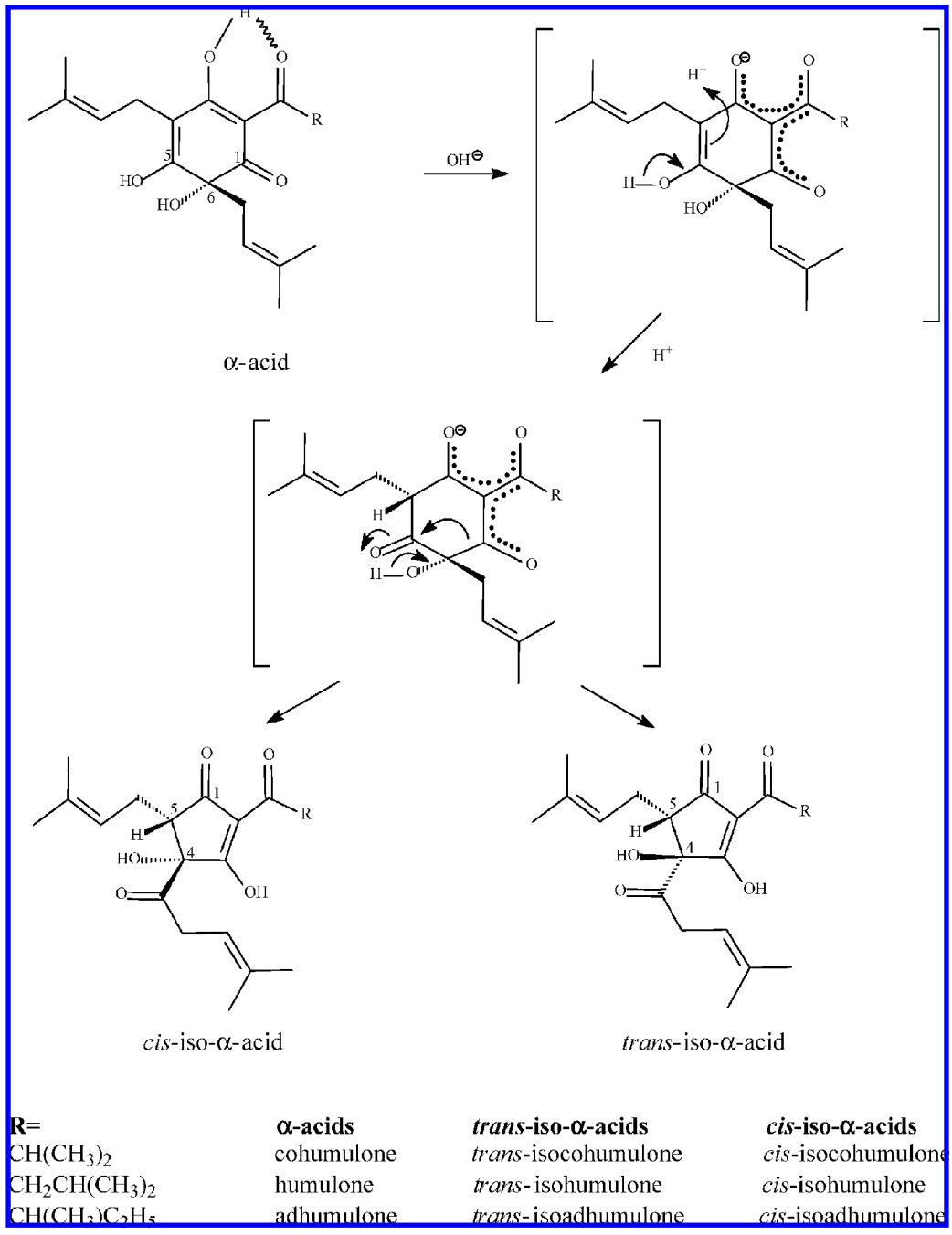

R=	α-acids	*trans*-iso-α-acids	*cis*-iso-α-acids
$CH(CH_3)_2$	cohumulone	*trans*-isocohumulone	*cis*-isocohumulone
$CH_2CH(CH_3)_2$	humulone	*trans*-isohumulone	*cis*-isohumulone
$CH(CH_3)C_2H_5$	adhumulone	*trans*-isoadhumulone	*cis*-isoadhumulone

Figure 1. The mechanism of α-acid isomerization into iso-α-acids (*12*).

et al. (*15*) demonstrated that decreases in α-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 α-acids during boiling with hop extract were always greater than the corresponding increases in iso-α-acids throughout the boiling period. When looking at the individual α-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-α-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-α-acids. Malowicki and Shellhammer (*9*) were the first to report on essential kinetic parameters of iso-α-acid formation, i.e., the rate constant and activation energy amounting to $(7.9 \times 10^{11})\ e^{(-11858/T)}$ 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-α-acids, including individual *trans*- and *cis*-iso-α-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 α-acids/iso-α-acids due to trub formation, as observed in wort boiling.

MATERIALS AND METHODS

Determination of Rate Constant and Activation Energy of Iso-α-acid Formation. *Boiling Experiments 1 and 2.* An all-glass round-

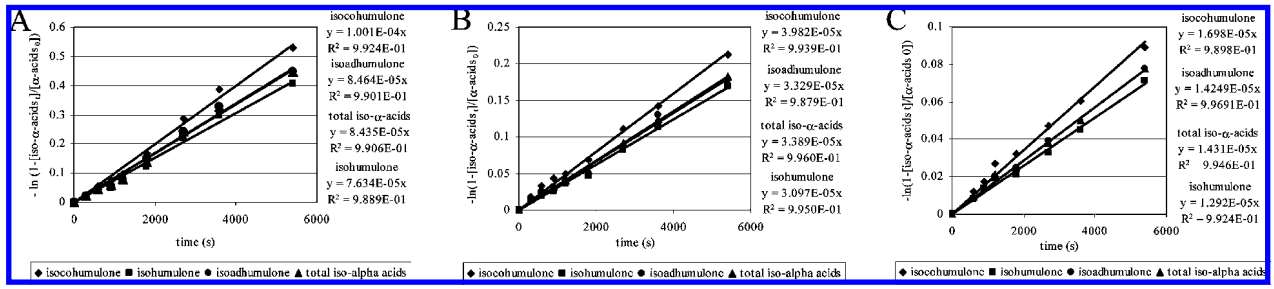

Figure 2. First-order linearization for total iso-α-acids, isocohumulone, isohumulone, and isoadhumulone upon heating of hop α-acids in buffer system at 100 °C (**A**), 90 °C (**B**), and 80 °C (**C**); experiment 1.

Table 1. Influence of Heating Temperature on the Rate Constant k (s^{-1}) of Iso-α-Acid Formation: Experiment 1 and Experiment 2[a]

temperature	100 °C		90 °C		80 °C	
compound[b]	experiment 1	experiment 2	experiment 1	experiment 2	experiment 1	experiment 2
total iso-α-acids	0.84×10^{-4}	0.82×10^{-4}	0.34×10^{-4}	0.36×10^{-4}	0.14×10^{-4}	0.14×10^{-4}
isocohumulone	1.00×10^{-4}	1.00×10^{-4}	0.40×10^{-4}	0.43×10^{-4}	0.17×10^{-4}	0.18×10^{-4}
isohumulone	0.76×10^{-4}	0.73×10^{-4}	0.31×10^{-4}	0.33×10^{-4}	0.13×10^{-4}	0.13×10^{-4}
isoadhumulone	0.85×10^{-4}	0.80×10^{-4}	0.33×10^{-4}	0.38×10^{-4}	0.14×10^{-4}	0.14×10^{-4}
trans-isocohumulone	0.34×10^{-4}	0.38×10^{-4}	0.14×10^{-4}	0.15×10^{-4}	0.07×10^{-4}	0.07×10^{-4}
cis-isocohumulone	0.65×10^{-4}	0.66×10^{-4}	0.26×10^{-4}	0.28×10^{-4}	0.10×10^{-4}	0.10×10^{-4}
trans-isohumulone	0.23×10^{-4}	0.22×10^{-4}	0.09×10^{-4}	0.10×10^{-4}	0.05×10^{-4}	0.04×10^{-4}
cis-isohumulone	0.53×10^{-4}	0.51×10^{-4}	0.22×10^{-4}	0.24×10^{-4}	0.09×10^{-4}	0.09×10^{-4}
trans-isoadhumulone	0.28×10^{-4}	0.28×10^{-4}	0.11×10^{-4}	0.12×10^{-4}	0.05×10^{-4}	0.05×10^{-4}
cis-isoadhumulone	0.55×10^{-4}	0.54×10^{-4}	0.22×10^{-4}	0.28×10^{-4}	0.09×10^{-4}	0.10×10^{-4}

[a] Calculation of rate constants was based on common equations and procedures as published by Tinoco et al. (*22*). [b] Compound identification based on reference preparations of α-acids and iso-α-acids, respectively (see Materials and Methods).

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 wort 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) was added.

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 H_3PO_4 (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/H_3PO_4 (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 $mL.min^{-1}$. Temperature: ambient temperature. UV-detection: 270 nm (iso-α-acids) and 314 nm (α-acids). A dicyclohexylamine (DCHA)-iso-α-acids ICS-I1 complex (66.5% (w/w) 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) 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*/*cis* 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.

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

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.

$$d[\text{iso-}\alpha\text{-acids}_t]/dt = k[\alpha\text{-acids}_t] \quad \text{(I)}$$

In eq I, [iso-α-acids $_t$] is the concentration (mol/L) of iso-α-acids at reaction time t, [α-acids $_t$] is the concentration (mol/L) of α-acids at reaction time t, and k is the rate constant (s^{-1}).

Following integration, eq II is derived from eq I:

$$\ln(1-[\text{iso-}\alpha\text{-acids}_t]/[\alpha\text{-acids}_0]) = -kt \quad \text{(II)}$$

where [iso-α-acids $_t$] is the concentration (mol/L) of iso-α-acids at reaction time t, [α-acids $_0$] is the concentration (mol/L) of α-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(1-[iso-α-acids$_t$]/[α-acids$_0$]) versus t, when linear, proves that the reaction follows first-order kinetics.

The kinetic data on α-acid isomerization obtained via experiment 1 are shown in **Figure 2**. Apparently, at each heating temperature (80 °C, 90 °C, and 100 °C), iso-α-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-α-acids, isocohumulone, isohumulone, and isoadhumulone are further shown in **Table 1**.

In addition, **Table 1** contains the rate constants of individual iso-α-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 α-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-α-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-α-acid, i.e., isohumulone, is always the lowest. Obviously, depending on the iso-α-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-α-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 α-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, α-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-α-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

Table 2. Total Isomerization Yield (%) and Isomerization Yield of Individual α-Acids (%) as a Function of Heating Time at Different Temperatures: Experiment 1

Heating Temperature 80 °C

	time (min)							
isomerization yield (%)	5	10	15	20	30	45	60	90
total iso-α-acids	0.0	0.9	1.5	2.1	2.5	3.7	4.9	7.4
cohumulone	0.0	1.2	1.7	2.6	3.1	4.6	5.9	8.5
humulone	0.0	0.8	1.4	1.9	2.1	3.2	4.4	6.9
adhumulone	0.0	0.9	1.3	2.0	2.4	3.8	4.8	7.5

Heating Temperature 90 °C

	time (min)							
isomerization yield (%)	5	10	15	20	30	45	60	90
total iso-α-acids	1.5	2.5	3.1	3.9	5.2	8.7	11.7	16.7
cohumulone	1.9	3.3	4.2	4.8	6.6	10.5	13.3	19.1
humulone	1.3	2.0	2.5	3.5	4.6	7.9	10.8	15.6
adhumulone	1.5	2.5	3.1	3.7	4.7	8.2	12.2	16.3

Heating Temperature 100 °C

	time (min)							
isomerization yield (%)	5	10	15	20	30	45	60	90
total iso-α-acids	2.2	4.4	5.7	7.7	13.0	21.4	27.9	36.0
cohumulone	2.7	5.3	7.7	9.2	15.4	24.9	32.0	41.0
humulone	2.1	3.9	4.8	6.9	11.5	19.6	25.7	33.3
adhumulone	1.8	4.3	5.3	7.6	13.7	21.4	28.0	36.0

Table 3. T/C-ratio (%)[a] as a Function of Reaction Time and Temperature: Experiment 1

	time (min)							
temperature (°C)	5	10	15	20	30	45	60	90
80		153.1	111.6	89.7	84.1	70.7	53.9	57.6
90	100.0	77.5	75.6	55.4	55.4	49.3	49.1	46.7
100	60.8	54.1	61.8	54.9	47.3	47.3	47.1	46.3

[a] T/C (%) = (*trans*-isocohumulone+*trans*-isohumulone)/(*cis*-isocohumulone+*cis*-isohumulone) × 100%.

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 α-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:

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

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

Equation III can also be expressed as follows:

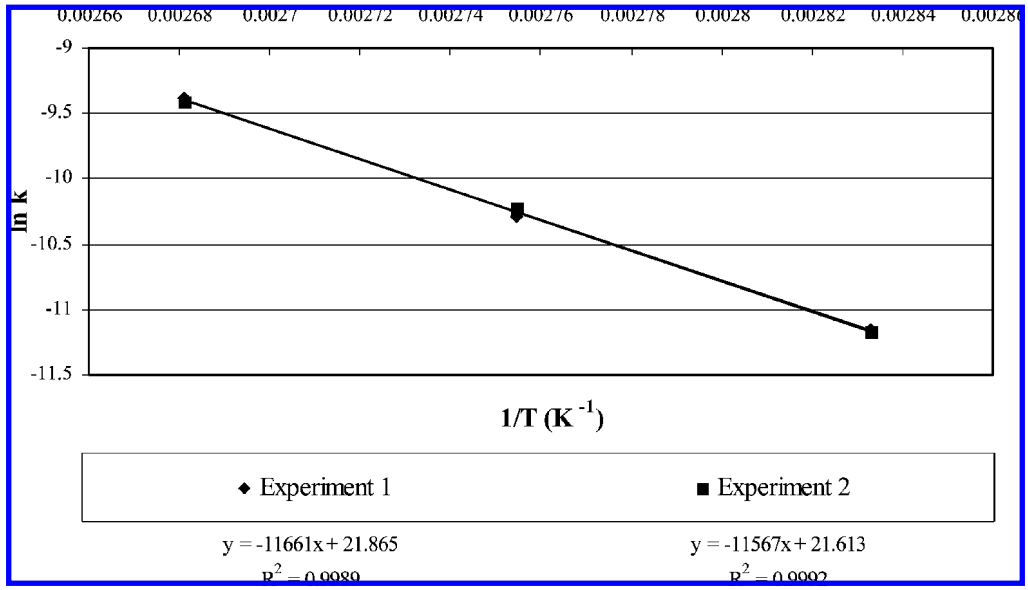

Figure 3. Arrhenius behavior of formation of total iso-α-acids from α-acids; experiment 1 and experiment 2.

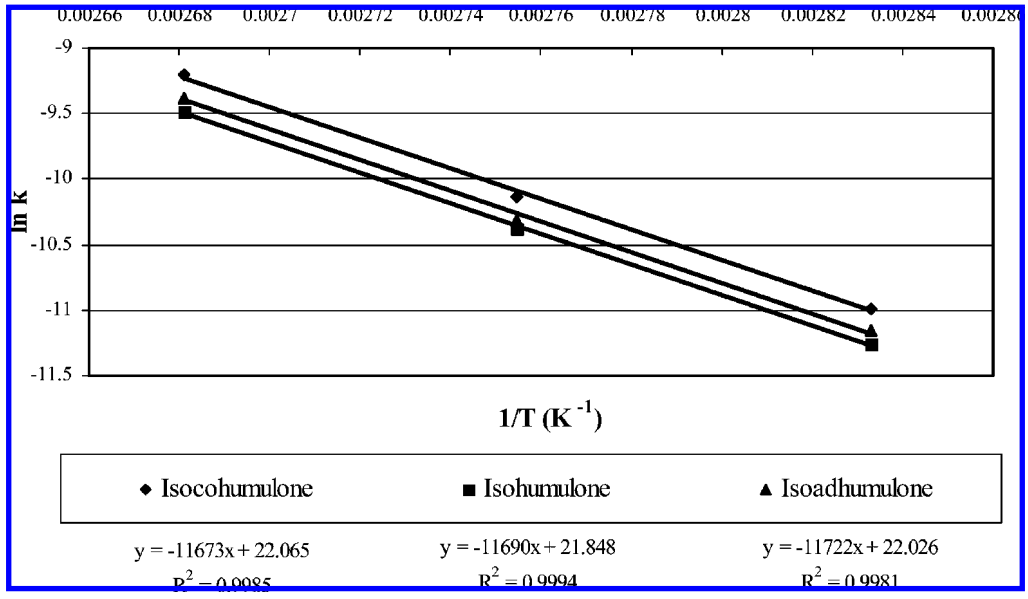

Figure 4. Arrhenius behavior of formation of isocohumulone, isohumulone, and isoadhumulone; experiment 1.

$$\ln k = -E_a/(RT) + \ln A \qquad \text{(IV)}$$

A 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 α-acid isomerization in our kinetic experiments follows Arrhenius behavior. This holds true for both total iso-α-acid formation (**Figure 3**), formation of isocohumulone, isohumulone, and isoadhumulone (**Figure 4**), and formation of individual *trans*- and *cis*-iso-α-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-α-acids amounts to 96−97 kJmol^{-1}. This result is in agreement with the activation energy of iso-α-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 isoadhumulone 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 α-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 α-acid appears to be negligible.

However, with respect to the formation of *trans*- and *cis*-iso-α-acids, a difference in activation energy was always noted, regardless of the iso-α-acid analogue being formed (see **Table 4**). Activation energies associated with the formation of *trans*-

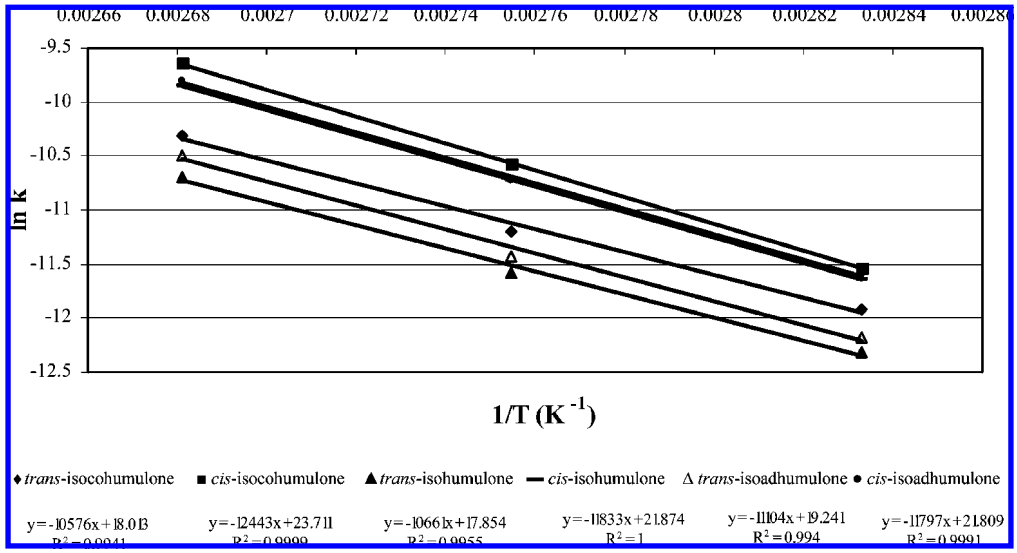

Figure 5. Arrhenius behavior of formation of *trans*-isocohumulone, *cis*-isocohumulone, *trans*-isohumulone, *cis*-isohumulone, *trans*-isoadhumulone, and *cis*-isoadhumulone; experiment 1.

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

	experiment 1		experiment 2	
	Arrhenius equation	E_a (kJmol^{-1})	Arrhenius equation	E_a (kJmol^{-1})
total iso-α-acids[a]	$3.13 \times 10^9\ e^{-11661/T}$	97.0	$2.43 \times 10^9\ e^{-11567/T}$	96.2
isocohumulone	$3.83 \times 10^9\ e^{-11673/T}$	97.1	$2.45 \times 10^9\ e^{-11499/T}$	95.6
isohumulone	$3.08 \times 10^9\ e^{-11690/T}$	97.2	$1.77 \times 10^9\ e^{-11492/T}$	95.5
isoadhumulone	$3.68 \times 10^9\ e^{-11722/T}$	97.5	$1.81 \times 10^9\ e^{-11456/T}$	95.3
trans-isocohumulone	$0.07 \times 10^9\ e^{-10576/T}$	87.9	$0.15 \times 10^9\ e^{-10847/T}$	90.2
cis-isocohumulone	$19.8 \times 10^9\ e^{-12443/T}$	103.5	$16.6 \times 10^9\ e^{-12363/T}$	102.8
trans-isohumulone	$0.06 \times 10^9\ e^{-10661/T}$	88.6	$0.09 \times 10^9\ e^{-10838/T}$	90.1
cis-isohumulone	$3.16 \times 10^9\ e^{-11833/T}$	98.4	$1.31 \times 10^9\ e^{-11505/T}$	95.7
trans-isoadhumulone	$0.23 \times 10^9\ e^{-11104/T}$	92.3	$0.23 \times 10^9\ e^{-11102/T}$	92.3
cis-isoadhumulone	$2.96 \times 10^9\ e^{-11797/T}$	98.1	$1.03 \times 10^9\ e^{-11386/T}$	94.7

[a] According to Malowicki and Shellhammer (*9*), the rate constant and activation energy for total iso-α-acids amount to $(7.9 \times 10^{11})\ e^{(-11858/T)}$ min $^{-1}$ and 98.6 kJmol^{-1}, respectively.

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 ($\Delta G°$), the free energy of activation (ΔE_a) is composed of an enthalpy factor (ΔH_a) and an entropy factor (ΔS_a). $\Delta E_a = \Delta H_a - T\Delta S_a$. 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 (formation of *cis*-iso-α-acids) should be stronger than in the conformer in which the carbonyl is pointing upward (formation of *trans*-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 pK_a 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

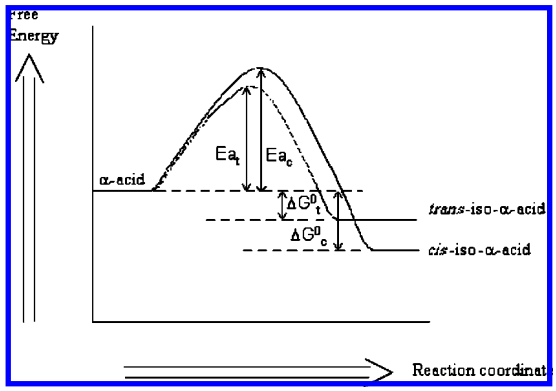

Figure 6. Proposed free energy diagram for the formation of *trans*- and *cis*-iso-α-acids from α-acids ($\Delta G\,^\circ_t$, overall free energy change related to the formation of *trans*-iso-α-acids; $\Delta G\,^\circ_c$, overall free energy change related to the formation of *cis*-iso-α-acids; Ea_t, free energy of activation related to the formation of *trans*-iso-α-acids; Ea_c, free energy of activation related to the formation of *cis*-iso-α-acids).

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 form 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 pK_a value of cohumulone versus the pK_a value of the other α-acid analogues (pK_a cohumulone, 4.7; pK_a humulone, 5.5; pK_a adhumulone, 5.7; (*23*)). The lower pK_a 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 $\Delta G\,^\circ = \Delta H^\circ - T\Delta S^\circ$, *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.

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