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Humulus Lupulus: Hop Alpha-acids Isomerization – A Review

We dedicate this paper to our colleague, Dr. Koen Goiris, who passed away much too early on June 24th, 2017.

This paper gives an extensive overview of the research performed in relation to hop α-acids isomerization. The objective is to present a summary on developments and findings which can serve as a background for future work regarding more efficient use of humulones and isohumulones. To this end, first, an overview of the research which has been performed in relation to the α-acids isomerization mechanism and kinetics is provided. Following, the influence of common parameters, such as temperature, pH, the concentration of humulones, metal ion catalysts, etc., on α-acids isomerization is presented. In this review, structures and mechanisms that are presented correspond to original data found in literature and were, consequently, not adapted to the newly proposed structure of α-acids and iso-α-acids (According to *Urban* et al. [1]).

Descriptors: hops, α-acids, isomerization, kinetics, iso-α-acids, influence of parameters, utilization

1 Hops

1.1 Botanical overview

Hops (*Humulus lupulus* L.), a perennial, dioecious climbing plant of the hemp family *Cannabinaceae* [2], belong to the order of Urticales, which also includes the nettle family. The genus Humulus consists of two species, *Humulus lupulus* L. and *Humulus japonicus* Sieb. and Zucc. It is worth pointing out that Japanese hop has no brewing value and is grown only as an ornamental plant [3]. Although the hop plant is dioecious (i.e. there are separate male and female plants), only the female plants are cultivated since they bear the inflorescences which, due to their shape, are called hop cones. The hop cone consists of egg-shaped, yellowish-green bracts and seed-bearing bracteoles attached to a central axis or string. At the base of the bracteoles, the lupulin glands and the seed develop as the hop ripens. This beaker-like glands contain lupulin, a yellow, sticky powder consisting of secondary metabolites, secreted by the hop plant. The gland is covered by a membrane to prevent the contents escaping. The main brewing principles of hops, the resins and essential oils are synthesized and accumulated in these lupulin glands [4]. The lupulin content of the hop cone can reach up to 32 % (w/w) [5].

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1.2 Chemical composition of hops

The hop cone contains several metabolites, comprising many different groups of organic compounds (see Table 1). The multiplicity of the chemical structures of secondary metabolites is enormous and their amount depends on the hop variety and many associated variables [6, 7].

1.3 Hop resins

Goldstein et al. [8], showed that hops contain 20-25 % of water-soluble constituents which dissolve directly in the boiling wort. This fraction includes carbohydrates, amino acids, proteins, polyphenols, and inorganic salts. However, most of the brewing value of the hops is found in the resins and essential oils, which are only slightly soluble in water. Hop resins are a complex mixture of so-called soft resins (mainly hop acids) and hard resins (mainly oxidation

Table 1 Average chemical composition of dried hop cones [4]

Constituent	Amount % (w/w)
Total resins	15–30
Essential oils	0.5–3 (v/w)
Polyphenols (tannins)	4
Monosaccharides	2
Protein	15
Cellulose	43
Moisture	10
Ash	8
Pectins	2
Amino acids	0.1
Lipids	Traces-25

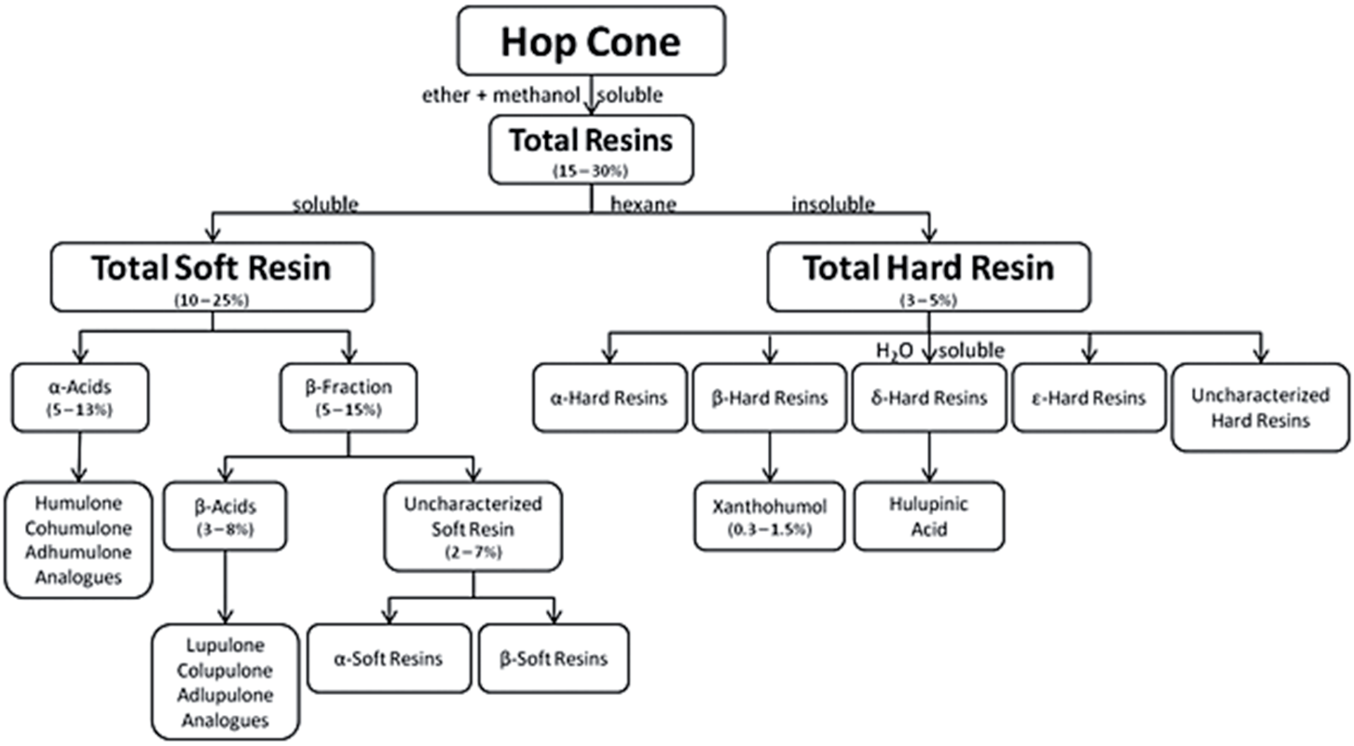
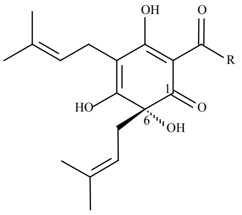
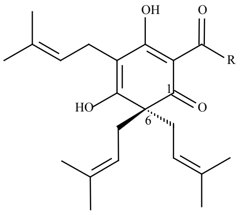


Fig. 1 **Classification and nomenclature of hop resins [4]**

Table 2 Analogues of α- and β-acids [10]

	alpha acids					**beta acids**		
								
Acyl side chain (R)	Name	Formula	m.p (°C)	[α]D^{26}	pKa	Name	Formula	m.p. (°C)
-CO CH_2 $CH(CH_3)_2$								
isovaleryl	humulone	$C_{21}H_{30}O_5$	64.5	−211	5.5	lupulone	$C_{26}H_{38}O_4$	92
-CO $CH(CH_3)_2$								
isobutyryl	cohumulone	$C_{20}H_{28}O_5$	oil	−208.5	4.7	colupulone	$C_{25}H_{36}O_4$	93–94
-$COCH(CH_3)CH_2CH_3$								
2-methyl butyryl	adhumulone	$C_{21}H_{30}O_5$	oil	−187	5.7	adlupulone	$C_{26}H_{38}O_4$	82–83
-$COCH_2CH_3$								
propionyl	posthumulone	$C_{19}H_{26}O_5$	oil	–	–	–	$C_{24}H_{34}O_4$	101
-$COCH_2CH_2CH(CH_3)_2$								
4-methyl pentanoyl	prehumulone	$C_{22}H_{32}O_5$	oil	−172	–	–	$C_{27}H_{40}O_4$	91
-$COCH_2CH_2CH(CH_3)CH_2CH_3$								
4-methyl hexanoyl	–	–	–	–	–	–	$C_{28}H_{43}O_4$	91

[α]D^{26}: specific rotation of chemical compound

products of hop acids). Soft resins are soluble in hexane, while hard resins are insoluble. The combined soft and hard resins form the total resins (see Fig. 1), which are soluble in cold methanol and diethyl ether [7]. The properties of hop resins are connected with their main components, which are cyclic β,β'-triketones [9]. This fraction of 'soft resins' contains important classes of hop constituents, i.e. hop α-acids (humulones) and β-acids (lupulones) (for structures, see Table 2). β-di- and β-tri-carbonyl compounds

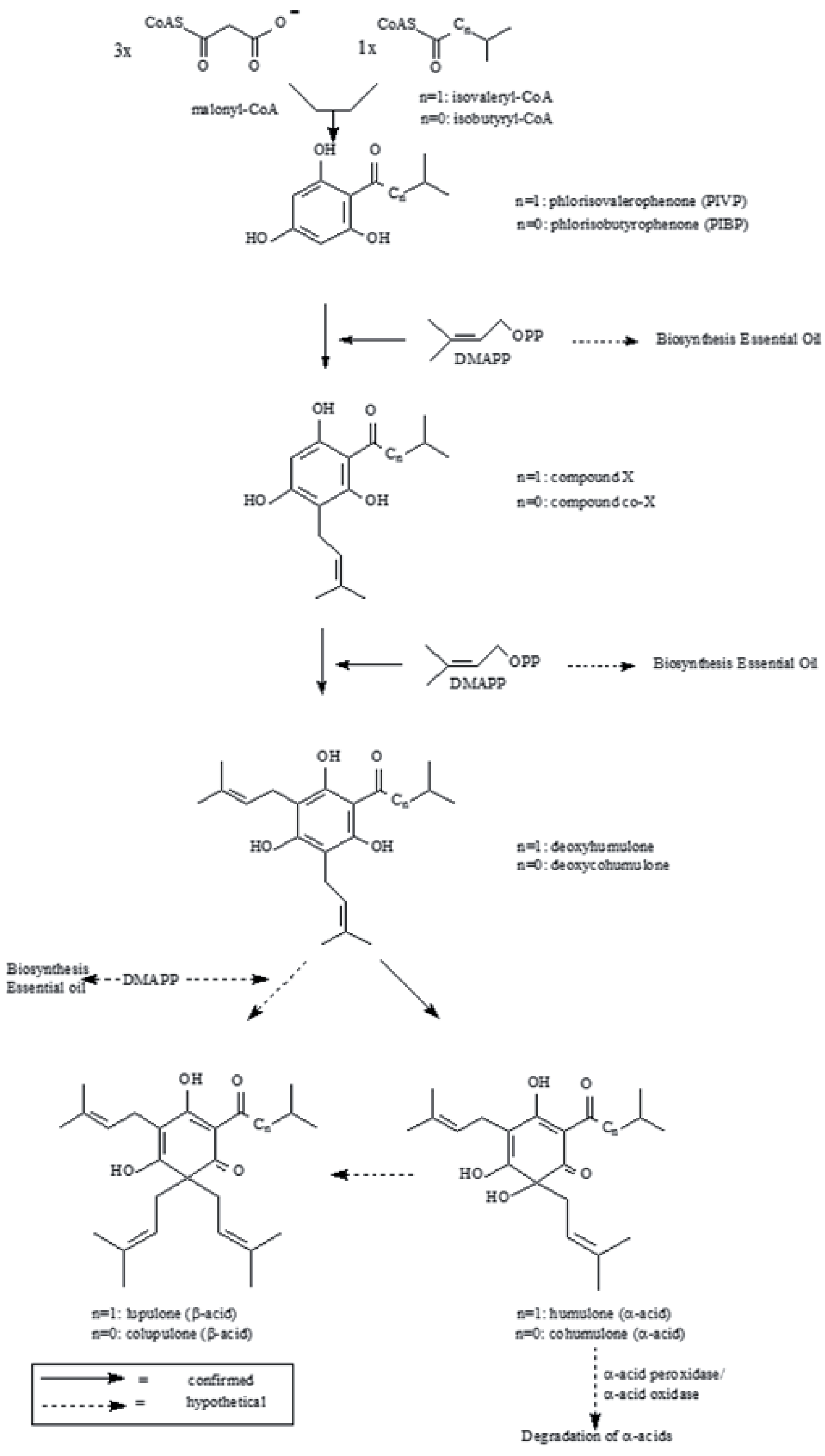


Fig. 2 **Schematic representation of the biosynthesis of bitter acids in the hop plant [11]**

are acids and this function provides the acidity of the hop resins. Furthermore, the β-triketo system is responsible for the salt-forming and chelating properties [2]. They are termed acids, because they have substantial proton-donating tendencies.

Both types of hop acids, i.e. α-acids and β-acids, can be connected via a photochemical pathway. Upon irradiation (λ = 350–366 nm) of the β-acids in methanol, an isoprenyl side chain at C-6 is eliminated, thereby giving rise to the 6-deoxy-α-acids, which are the biochemical precursors of both α-acids and β-acids. Further oxidation in the presence of lead (II) acetate, followed by acid treatment, renders α-acids [6].

The biosynthesis of hop acids includes three major types of reactions. The first reaction is similar to the initial step in the biosynthesis of plant flavonoids, i.e. the successive condensation of three molecules of malonyl-CoA to isobutyryl-CoA or isovaleryl-CoA, leading to the formation of phloroglucinol intermediates [11, 12], (see Fig. 2). Further, two dimethylallyl pyrophosphate moieties act as precursors for the prenyl substituents thereby producing deoxyhumulones which are then converted into humulones by reaction with molecular oxygen [13].

pKa values of humulones and lupulones have been determined several times by different research groups using varying methods based on potentiometry, spectrophotometry and solvent partition potentiometry, but the values reported vary widely. For example, values of 4.0–5.5 have been reported for humulone, whereas those reported for lupulone range from 5.5–7.8 [14, 15]. Although pKa measurements should be made in dilute aqueous solution, the pKa's of the hop resins were estimated in aqueous methanol solutions because of their limited solubility, giving pKa values for humulone, 5.5, cohumulone, 4.7, and adhumulone, 5.7. *Simpson and Smith* [16], found equilibrium pKa values in aqueous solutions for the most acidic functions: humulone, 5.0, colupulone, 6.1, *trans*-isohumulone, 3.1 and *trans*-humulinic acid, 2.7.

In 1955, *Spetsig* [17] measured the solubilities of humulone, lupulone and *trans*-humulinic acid (Fig. 3). As expected, the solubility of each compound increases with temperature and increasing pH.

2 Alpha-acids

α-Acids or humulones are the principal brewing constituents of hops (2–19 % w/w in dried hop cones). Six analogues of the α-acids have been characterized (see Table 2). Quantitatively, the most important are humulone, cohumulone and adhumulone (side chains at the R location are isovaleryl, isobutyryl, and 2-methyl butyryl, respectively). The relative amount of adhumulone within the α-acids is fairly constant between varieties (about 15 %), while the relative amounts of humulone and cohumulone are strongly variety-dependent (in general between 20–50 %) [18]. The relative amount of cohumulone, better known as the cohumulone ratio, has been used as a criterion to characterize hop varieties [19]. A high cohumulone content has been associated with poor hop quality [20]. Humulone was the first analogue of the α-acid fraction that was isolated in a crystalline

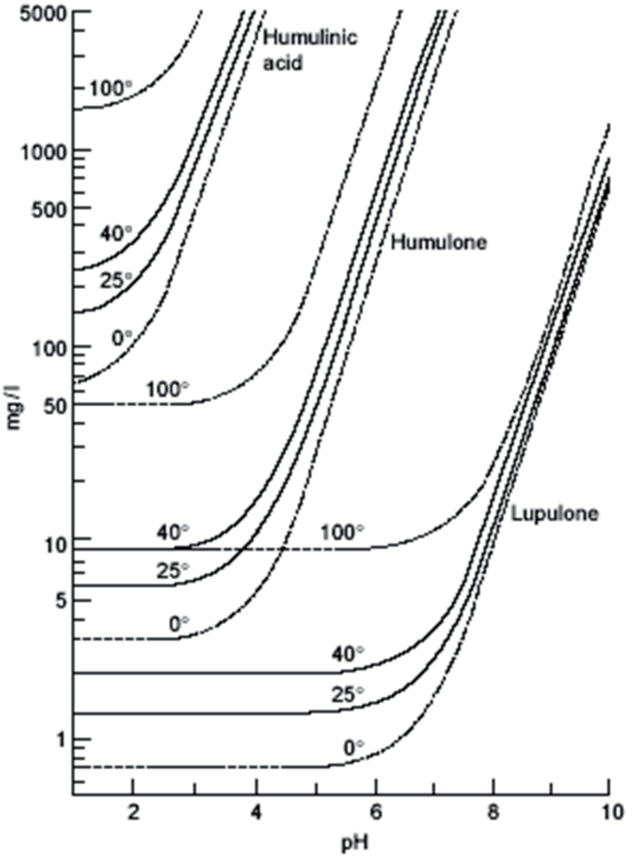


Fig. 3 Solubility diagram [17]

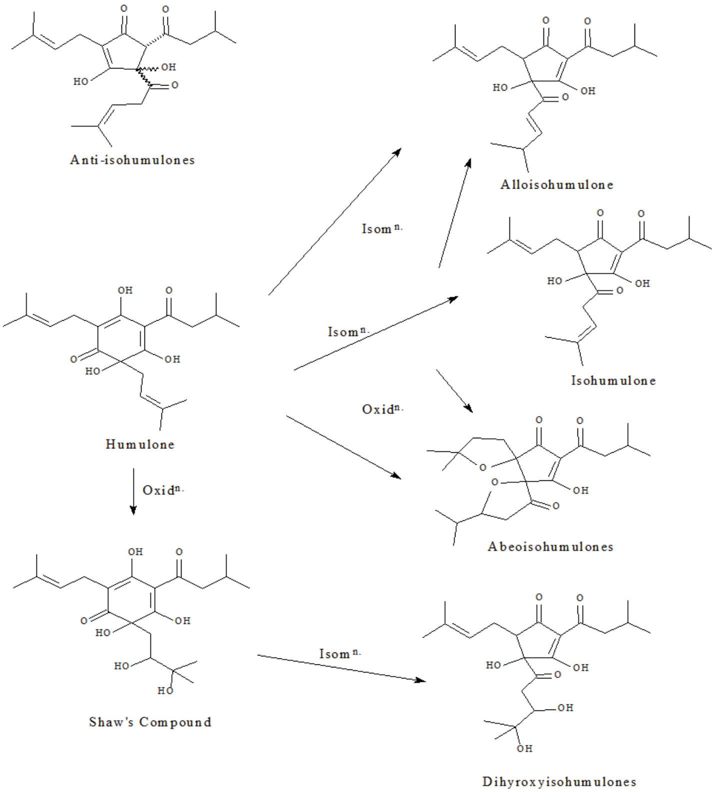


Fig. 4 Changes in humulone during wort boiling [23]

state. In 1970, *De Keukeleire* and *Verzele* [21] established the structure of naturally occurring (-)-humulone, including the enolization pattern and the absolute configuration. Chemical transformation and chiroptical studies demonstrated that (-)-humulone has the R configuration at C-6 [2]. However, more recent findings by Urban et al. [1] point to an S-configuration (see further).

In both humulone and lupulone, carbon atom-6 in the ring is sp^3 hybridized; the other atoms in the ring are sp^2 hybridized. In humulone, C-6 is substituted by four different groups rendering humulone optically active [10]. However, in adlupulone there is a chiral centre at C-2 of the 2-methylbutyryl-side chain, so natural adlupulone should be optically active. It follows that natural adhumulone has two chiral centers, so four enantiomers are possible.

Separation of the α-acids from other soft resins can be performed through their ability to form lead salts which are insoluble in methanol [2]. Humulones occur in beer in concentrations up to 4 mg/L. They do not give a bitter taste, not even in amounts of 100 mg/L. In 2007, the research group of *Shellhammer* [22] confirmed that α-acids, at levels as high as their solubility limit in beer, contribute negligibly to the overall bitterness of lager beer. Therefore, in traditional brewing practice, hops are added during the wort boiling stage to convert α-acids into strongly bitter tasting isomeric forms, known as iso-α-acids or isohumulones. However, the poor solubility of α-acids in the wort medium (approx. 60 mg/L at pH 5 and 100 °C) is a major problem limiting their conversion to iso-α-acids. In addition to the isohumulones (iso-α-acids), alloiso-α-acids are formed during wort boiling, with a yield of only 4 % [23]. Also the anti-isohumulones are formed upon boiling of humulone, however, the yield is at least 50 times lower than this of isohumulones [2]. Some of the humulone changes during boiling are presented in figure 4. It is clear that even more α-acids derivatives occur as a result of degradation of both α-acids and iso-α-acids, principally by oxidation.

Next to being the precursors of beer bitterness, hop α-acids improve foam stability, suppress gushing of beer and contribute to its bacteriological stability [2, 24]. Hops contain both anti-gushing factors and promoting factors. These factors differ widely, depending on the hop variety [25]. It is generally known that the higher the hopping rate, the smaller the gushing potential of beer. Investigations of the use of hop products on microbiological stability of beer showed that α-acids have the best anti-microbiological effect against the growth of strains of *Lactobacillus* and *Pediococcus* followed by hexahydro-, tetrahydro-, rho- and iso-α-acids [26]. According to *Simpson* and *Smith* [16] at pH > 4.7, humulone is a more potent bacteriostat against lactic acid bacteria, than *trans*-isohumulone. The mechanism of hop resistance in bacteria, based on research of *Sakamoto and Konings* [27], is illustrated in figure 5.

According to *Sakamoto* and *Konings* [27], hop compounds act as ionophores that exchange protons for cellular divalent cations. In a hop-sensitive cell, hop compounds (Hop-H) enter the cell and dissociate into hop anions and protons due to the higher internal pH. Next, hop anions combine with divalent cations such as Mn^{2+} and diffuse out of the cell. The ionophoric action together with the diffusion of the hop – metal complex results in an electroneutral exchange of cations. However, release of protons from hop compounds decreases the intracellular pH and results in a disruption of the transmembrane proton gradient and the proton motive force

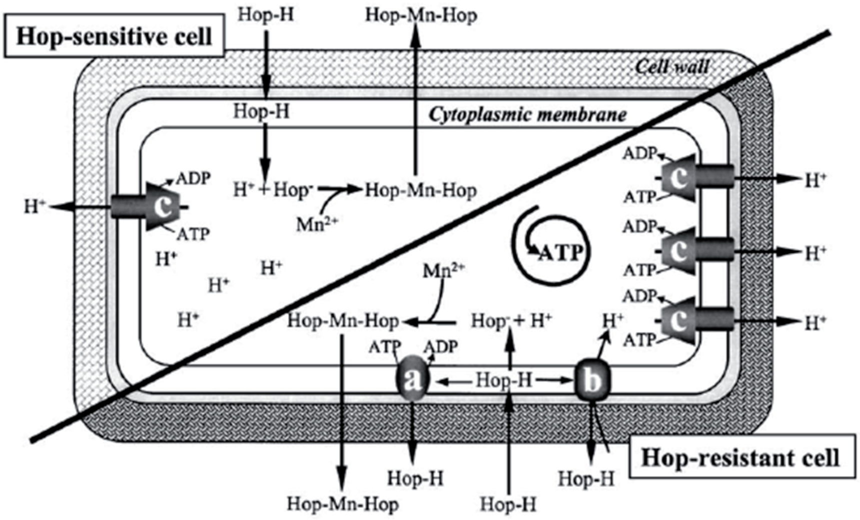


Fig. 5 Mechanism of hop resistance [27]

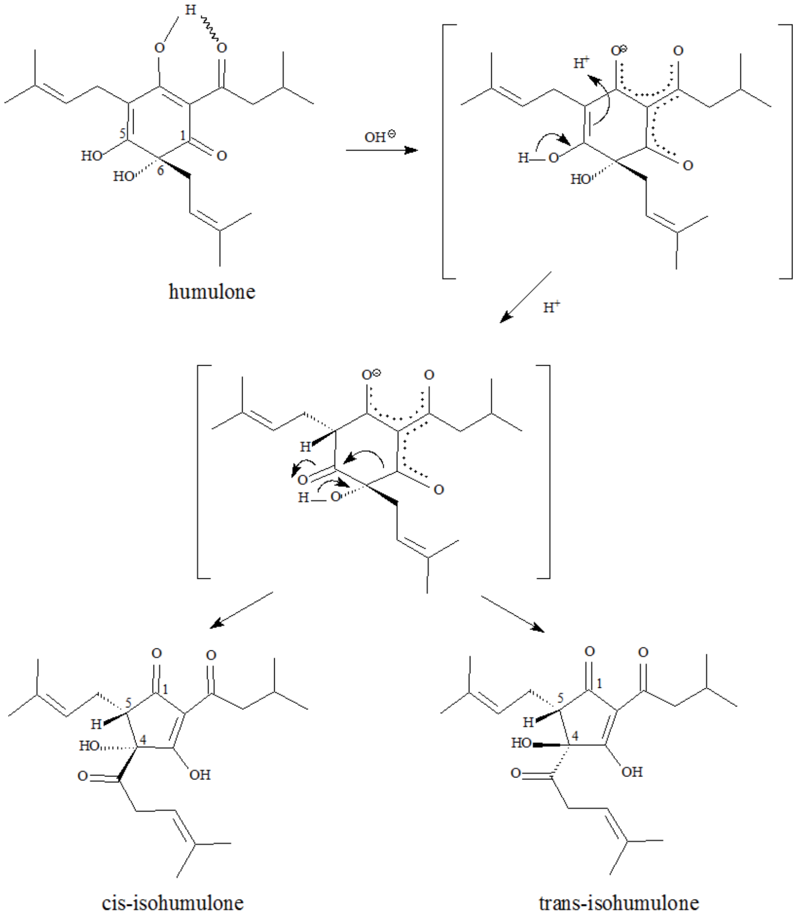


Fig. 6 The mechanism of α-acids isomerization into iso-α-acids [47]

(PMF). Consequently, uptake of nutrients, which is driven by the proton motive force, will be decreased. In hop-resistant cells, hop compounds can be removed from the cytoplasmic membrane by HorA (a) [28] and probably also by a PMF-dependent transporter (b) [29]. Furthermore, over-expressed H$^+$-ATPase increases the excretion of protons, released from the hop compounds (c) [30]. More ATP is generated in hop-resistant cells than in hop-sensitive cells [31]. Galactosylated glycerol teichoic acid in the cell wall [32] and a changed lipid composition of the cytoplasmic membrane of beer spoilage lactic acid bacteria may further increase the barrier to hop compounds.

3 Isomerization of alpha acids

3.1 Introduction

α-Acids isomerization represents the key reaction of hops in brewing, because iso-α-acids are largely responsible for the very characteristic beer bitterness [2, 33, 34, 35]. However, using conventional hopping, too low isomerization yields at the end of the boil (at maximum 50–60 %) and much too low final α-acids utilizations are obtained in the beer (35–40 %) (% Hop utilisation =Amount of iso-α-acids in beer/Amount of α-acids in hops used (x 100 %)). Under brewing conditions, a major problem associated with the isomerization reaction is the poor solubility of the α-acids in the wort medium, slowing down their extraction from the added hops and thus limiting their conversion. As shown in figure 6, isomerization requires the anionic forms of α-acids (pKa 5–5.5), which are however partially protonated at the wort pH. Outside the brewery, increased isomerization yields can be obtained under alkaline reaction conditions. Furthermore, the isomerization reaction can be catalysed by divalent ions, such as magnesium or calcium, resulting in iso-α-acids formed free from degradation products, like humulinic acids.

3.2 Reaction mechanism

Thermal isomerization of α-acids to iso-α-acids occurs via an acyloin-type ring contraction. The isomerization reaction has been known since the late 1920's [36] and has been studied by different researchers since then [37, 38, 39, 40, 41, 42, 43, 44, 45, 46], The mechanism of the isomerization of humulone (see Fig. 6) was revealed in 1971 by *De Keukeleire and Verzele* [47]. The reaction involves the mono-anion presence of humulone (pKa 5.5). Via ketonisation, the two 3-methyl-2-butenyl side chains are situated in *trans* position. Consequently, an acyloin entity containing a tertiary alcohol function is formed. As a result another α-ketol system and new chiral centre is created. Thus each α-acid gives rise to two epimeric iso-α-acids, distinguished as *trans*-iso-α-acids and *cis*-iso-α-acids depending on the spatial arrangement of the tertiary alcohol function at C-4 and the prenyl side chain at C-5. Terms *trans* and *cis* indicate that these groups point to opposite faces and to the same face of the five-membered ring, respectively. Consequently, 6 major iso-α-acids (*trans*-isohumulone and *cis*-isohumulone, *trans*-isocohumulone and *cis*-isocohumulone, *trans*-isoadhumulone and *cis*-isoadhumulone) are present in beer, resulting from the conversion of the 3 major α-acids, humulone, cohumulone and adhumulone, respectively. Most recently Urban et al. [1] determined the absolute configuration of *trans*- and *cis*-iso-α-acids using X-ray crystallography. In this study, the configuration of (-)-humulone was determined as (6S), thus the conserved stereocentre during isomerization is C4, resulting in the stereochemical difference between *cis*- and *trans*-iso-α-acids at C5 [1, 48].

The two isomers of isohumulone are not readily converted into each other but, since the isomerization of humulone is reversible, they can be inter-converted via humulone. When isohumulone is heated in wort, in a buffer solution of pH 4.5, or in 0.1N disodium carbonate, 10–15 % of humulone is formed. The same percentage of humulone is found when isohumulone is shaken in a two phase system of isooctane and aqueous buffer solution of pH 5.0 [10]. Furthermore, *Ting* et al. [49] observed that for thermal isomerization using refluxing in ethanol in the absence of alkali metal hydroxides, alkali metal carbonates, or multivalent metal ion catalysts, air, and light, *cis*-isomers slowly reverse to humulones and *trans*-isomers produce unknown compounds.

The ratio of the stereoisomers of the iso-α-acids depends on the reaction conditions. In the wort medium (pH 5.0–5.5), it is normally 68:32 in favour of the *cis*-compounds [50]. However, when considering the evolution of the T/C-ratio as a function of boiling time, it is interesting to notice that more *trans*-iso-α-acids than *cis*-iso-α-acids are formed at the beginning of boiling. The higher T/C-ratio observed at the beginning of boiling is connected with the lower activation energy for formation of *trans*-isomers. On the other hand, the reaction is thermodynamically controlled due to the higher stability (less free energy) of the *cis*-isomers, since the two vicinal side chains at C-4 and C-5 are in the *trans* configuration [2, 34, 51].

3.3 Kinetic study

Knowledge of α-acids utilization is of prime importance for each brewery in calculating accurate hopping rates to aim at precise bitterness levels Detailed knowledge of the isomerization kinetics should allow more precise control of iso-α-acids levels achieved in finished beer. As early as in the 60's *Spetsig* found that α-acids isomerization follows the pattern of a first order reaction [39]. At about the same time, also *Askew* [52] found the reaction kinetics for α-acids 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. [53] stated that during the first 10 min of hop boiling, the rate of the isomerization of α-acids varied with the concentration and time and did not follow the pattern of first order reaction kinetics. However, in the subsequent 10–120 min of boiling, α-acids isomerization did occur as a first order reaction. *McMurrough* et al. [54] 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. [54] 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 the highest, suggesting a faster conversion of cohumulone than n- and ad-humulone [46, 55, 56, 57]. In 2005, the group of *Shellhammer* [43] confirmed the reaction kinetics of iso-α-acids formation to be of first order. However, in contrast to McMurrough et al. [54], these authors claim that the isomerization of cohumulone to isocohumulone proceeds at a similar rate as the conversion of humulone and adhumulone to their corresponding iso-α-acids. *Malowicki* and Shellhammer [43] were the first to report on essential kinetics parameters of iso-α-acids formation, i.e. the rate constant and activation energy amounting to $(7.9 \text{ x}10^{11})\ e^{(-11858/T)}\ min^{-1}$ and 98.6 $kJmol^{-1}$, respectively.

More recently, *Jaskula* et al. [45] also demonstrated that iso-α-acids formation follows first order kinetics. In the selected temperature range (80 °C–100 °C), the fastest formation was observed for isocohumulone, followed by isoadhumulone, and isohumulone, which is related to the pKa value of the corresponding α-acids (pKa: cohumulon: 5.0; humulon: 5.5; adhumulon: 5.7) and to the inductive effect of the characteristic side chain at C-2. The rate limiting step in isomerization is the ionization of the β-triketo system in the α-acids (see Fig. 6), and electron density within this β-triketo system is influenced by the nature of the acyl group at C-2 of the α-acid analogue. Furthermore, within each type of iso-α-acid, the rate constant for the formation of the *trans*-isomer was always significantly lower than the rate constant for the formation of the *cis*-isomer. This is related to the higher required activation energies and in particular the higher pre-exponential factors as found for the formation of *cis*-isomers (for more details see [45]).

Authors determined a free energy of activation of total iso-α-acids formation of 96–97 kJ mol^{-1}. This result is close to the value of 98.6 kJ mol^{-1} as reported by Malowicki and Shellhammer [43]. Further, Jaskula et al. [45] demonstrated that the free energies of activation for formation of the different iso-α-acid analogues, i.e. isocohumulone, isohumulone, and isoadhumulone, respectively, appear to be very similar. This can be ascribed to the fact that within the transition state, the same bonds (a similar enthalpy of activation will lead to a similar free energy of activation(Ea)) are being broken and formed during acyloin ring contraction, regardless of the nature of the α-acid being converted. 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.

Interestingly, different free energies of activation were noticed for individual iso-α-acids in that free energies of activation related to the formation of *trans*-isomers are, on average, about 9 $kJmol^{-1}$ lower than free energies of activation related to the formation of *cis*-isomers. Basically, the reaction mechanism for the formation of *trans*- and *cis*-iso-α-acids, respectively, is the same. Just like the overall free energy change of a reaction ($\Delta G°$), the free energy of activation (ΔEa) is composed of an enthalpy factor (ΔHa) and an entropy factor (ΔSa). 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 in respect of formation of transition states are usually considered relatively small [58]. A possible explanation of the higher activation energies involved in *cis*-iso-α-acids formation may lie in the conformational

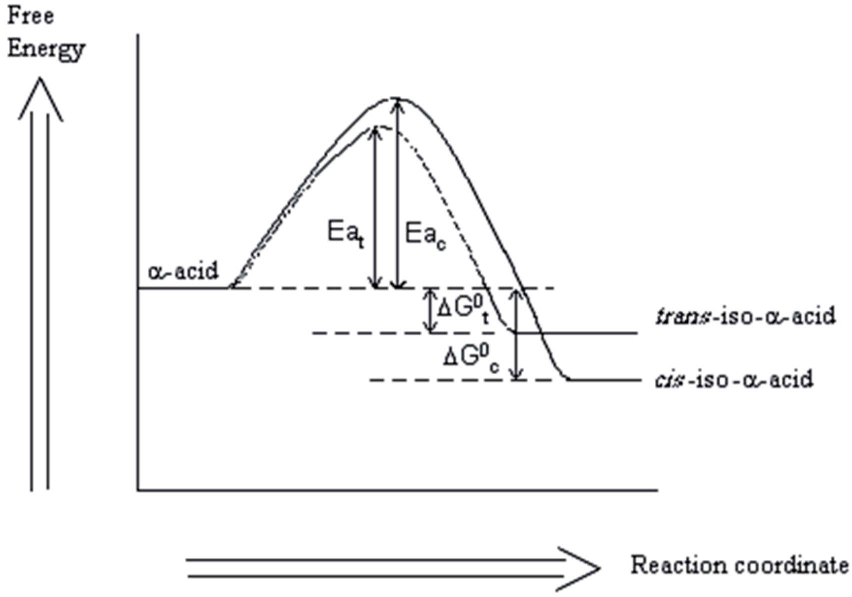


Fig. 7 Proposed free energy diagram for the formation of *trans*- and *cis*-iso-α-acids from α-acids ($\Delta G°_t$: overall free energy change related to formation of *trans*-iso-α-acids; $\Delta G°_c$: overall free energy change related to formation of *cis*-iso-α-acids; Ea_t: free energy of activation related to formation of *trans*-iso-α-acids; Ea_c: free energy of activation related to formation of *cis*-iso-α-acids) [45]

differentiation of the keto form of the alpha 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 downwards 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 upwards (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 (approx. 103 kJ mol^{-1}), when compared with the other *cis*-iso-α-acids (96–97 kJ mol^{-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 its 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 unfavourable situation when the *cis*-isomer is being formed. Based on our kinetic study, a free energy diagram for the formation of *trans*- and *cis*-iso-α-acids from hop α-acids has been proposed for the first time [45]. Whilst confirming the basic scheme of α-acids isomerization (see Fig. 6), this study has revealed new and more detailed insight from both the kinetic and the thermodynamic point of view into the underlying mechanism of the key reaction of hops in brewing. The lower free energy of activation associated with the formation of *trans*-compounds vs. *cis*-compounds is indicated in figure 7. Furthermore, the proposed scheme is compatible with the higher relative stability of *cis*-iso-α-acids vs. *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 a lower energy conformations with a resultant increase in entropy, compared to *trans*-iso-α-acids [59]. Thus, according to the thermodynamic equation $\Delta G° = \Delta H° - T\Delta S°$, *cis*-iso-α-acids formation (lower free energy of an end product) from α-acids is energetically favoured compared to *trans*-iso-α-acids formation (higher free energy of an end product).

Furthermore, the same authors [46] showed that both α-acids conversion and iso-α-acids formation follow first order kinetics when pilot scale wort boiling is applied. However, it was noticed that the rate constants for α-acids conversion are always higher than the rate constants for iso-α-acids formation, suggesting generation of by-products during wort boiling, besides iso-α-acids formation. Moreover, this study demonstrated that the rate constants for the formation of *cis*-iso-α-acids are always higher than the rate constants for the formation of *trans*-isomers, and that the reaction is mainly thermodynamically controlled (more formation of *cis*-isomers than *trans*-isomers after 60 minutes of wort boiling).

3.4 Influence of parameters

The isomerization process in the kettle is influenced by temperature, vigour and length of boil, pH of the wort, specific gravity, hopping rate, type of hop product(s) used, and kettle design [7, 41, 43, 44, 45, 46, 60, 61]. The α-acids isomerization occurs spontaneously at elevated temperature between 70 and 120 °C. However, very low isomerisation yields are obtained when short boiling is applied, the same holds true for the low temperature [43]. Furthermore, high gravity brewing results in a significantly lower isomerization rate [7]. On the other hand, the isomerization reaction can be catalysed by divalent ions, such as magnesium or calcium, resulting in very high reaction yields. Magnesium (II) ions for instance catalyse the reaction yielding 90 % isomerization within 10 min at 70 °C and a *cis*/*trans* ratio of 55/45 [2]. The increase in isomerization rate caused by such catalysis is due to the formation of the intermediate di-anion. Most recently, the research group of *De Vos* [62] tested the influence of organic bases on α-acids conversion. The results revealed that the addition of proline, the most active component, to an α-acids containing feed in a solvent-free system at 105 °C, results in a yield of iso-α-acids above 90 % after 6 h. Also, application of biometallic gold-based catalysts has been explored for the solvent-free production of iso-α-acids aiming for an enhanced rate of the reaction [63].

Unfortunately, α-acids can be transformed during wort boiling to other products than iso-α-acids, like anti-iso-α-acids [1] and both α-acids and iso-α-acids can be subject to oxidative degradation (for instance formation of oxidation products like abeo-iso-α-acids derived from iso-α-acids [2, 28].

Moreover, a significant part of the hop acids and beer bitter acids is removed with the hot trub during wort clarification and also during fermentation/maturation and final beer filtration [7, 64]. In consequence, only up to 40 % of added α-acids is found in beer in the form of iso-α-acids. Utilization of hop α-acids during brewing was studied by several authors [54, 56, 57, 60, 65, 66, 67, 68]. In conventional brewing, major losses of bitter substances occur when the hops are boiled in the copper [69]. Losses of bitter acids during fermentation attracted less attention. *Laws* et al., [64] found that losses of the α-acids still present in the wort after boiling occur mainly during fermentation, whereas the losses of iso-α-acids during fermentation are smaller than the losses of α-acids. The losses of the bitter substances (α-acids and iso-α-acids) occurred mainly on the dirty head and on the top yeast. Only small losses were associated with suspended yeast. As it was expected, greater losses were observed as the gravity of the wort increased. Furthermore, greater losses of hop substances during fermentation were correlated with a higher nitrogen content of the wort. In 2009, Jaskula [46] observed that more iso-α-acids than α-acids can be lost with the hot trub in the whirlpool, whereas significantly more α-acids than iso-α-acids are adsorbed by yeast/cold trub and further lost during maturation and beer filtration. Furthermore, differences in the losses of individual α-acids and iso-α-acids were observed. Especially losses of isohumulones and isoadhumulones were pronounced and greater amounts of *trans*-isomers were lost than *cis*-isomers [46].

In their study on the behaviour of α-acids and iso-α-acids, *Howard* and *Slater* [70] suggested that the rates at which α-acids are extracted from hops and subsequently isomerized are not responsible for their inefficient use, but that their utilization is strongly impaired due to precipitation by proteins. Furthermore, it was found that α-acids precipitate more easily than their isomers and within each acid group (α-acids or iso-α-acids), the ad-compound precipitates most easily and the co-compound the least easily. In 1972, *Rigby* [20] showed that cohumulone was utilized more efficiently than humulone. In 1984, *Ono* et al. [55] showed that during wort boiling the relative amount of isocohumulone formed is significantly higher than that of isohumulone plus isoadhumulone, because cohumulone is more completely isomerised to isocohumulone, whereas the relative amount of isocohumulone lost during fermentation is lower than that of isohumulone plus isoadhumulone. Therefore, cohumulone is utilised to a significantly higher degree than humulone plus adhumulone in the brewing process. In the work of *Irwin* et al. [60] we can find that cohumulone is better utilized than humulone or adhumulone probably due to higher losses of humulone and adhumulone in the kettle and of isohumulone and isoadhumulone in the fermenter. Furthermore, Jacobsen et al. [56] showed that cohumulone is utilized/isomerized most, regardless of the used hop variety or brewery where the research took place. Moreover, during fermentation and maturation, no further relative changes in the ratio of isocohumulone to the other iso-α-acids were observed so that the iso-α-acids profiles of the wort and the derived beer were identical. This finding is, however, in disagreement with earlier published data where it was stated that during fermentation, isohumulone and isoadhumulone decrease at a similar rate, whereas isocohumulone decreases at a slower rate and to a lesser degree [55, 60].

In 1986, McMurrough et al. [54] compared the α-acids isomerization in wort boiling experiments at laboratory scale (500 mL) and industrial scale (900 hL). The authors found a significant difference in the isomerization yields, 49 % isomerization in the laboratory scale boiling system and only 24 % isomerization yield in the industrial brew. The differences in the isomerization were ascribed to the higher pH of the wort in the laboratory system (pH = 5.5) compared with that of the industrial scale wort (pH = 4.8). Furthermore, it was shown that the isomerization yield decreases with increasing wort gravity and approx. 50 % of the formed iso-α-acids appeared to be present in the hot trub. Isomerization yield of cohumulone was found to be higher than this of humulone and adhumulone.

Hughes and *Marinowa* [57] showed that during wort boiling using different hop products, the relative proportion of isocohumulone is at a maximum after 30 min of wort boiling, suggesting that cohumulone is isomerized at a faster rate than humulone or adhumulone. However, the reason for this difference in isomerisation rate was unclear (more rapid dissolution of cohumulone or faster isomerization?). According to *Briggs* et al. [10], it could be explained through the ionisation difference between cohumulone (pKa 4.7) and humulone (pKa 5.5) in the wort medium. Furthermore, the study by Hughes and Marinowa [57] on the utilization of individual iso-α-acids during beer production showed that the greatest losses post boiling occur during fermentation, and that there is a change in the relative proportion of isocohumulones throughout beer production, especially during fermentation.

Besides studies on hop α-acids conversion and behaviour in brewing, α-acids isomerization has been the subject of several chemical investigations under a variety of experimental conditions, aiming at efficient off-line production of beer bittering principles. *Carson* [71] investigated humulone rearrangement in methanol. It was found that humulone rearrangement occurs only in an excess of alkali beyond that needed for the neutralization of the humulone. No reaction occurred when a methanolic solution of the sodium salt of humulone was refluxed for six hours. However, a solution of the sodium or potassium salts of humulone in methanol containing sodium hydroxide, potassium hydroxide, or sodium methylate, when refluxed three to six hours, resulted in “oils” in practically quantitative yields and with equivalent molecular weights. Askew [52] examined heating of α-acids in several media (distilled water and aqueous solutions of glucose, (hydrolyzed) proteins, tannins, and sodium carbonate, respectively), at various temperatures, pH values and reaction times. The higher the heating temperature, the more α-acids were converted and very fast conversion was observed at high pH (11.4), even at relatively low reaction temperatures (48–58 °C). As reported by *Köller* [72, 73], heating of humulone in an aqueous alcoholic solution under an inert atmosphere and in the presence of catalytic amounts of a divalent cation (Mg^{2+}, Ca^{2+}, Cd^{2+}, Mn^{2+}, and Ni^{2+}, respectively), leads to the formation of isohumulone in a nearly quantitative yield (close to 95 %). On the other hand, Mg^{2+} can be added directly to hops in the form of MgO and when this mixture is further heated for a period up to 2 weeks at 40 to 50 °C, iso-α-acids yields up to 95 % are found [74]. In this way, pre-isomerized hop pellets are now produced. Several alkali metal salts and/or alkaline earth metal salts have been used, e.g. K_2CO_3 and $MgCl_2$ solutions, by other investigators [75, 76, 77, 78]. Furthermore, in 1994 [79], an alkaline earth metal salt ($MgSO_4$) and an alkali metal salt (K_2CO_3) were combined into one process to speed up the α-acids isomerization. *Hildebrand* et al., [80] studied the isomerization reaction in the solid state, as a function of heating temperature (70 °C to 100 °C) and time (10–30 min), using magnesium, calcium, nickel, cobalt, zinc or cadmium as potential catalysts. It was shown that the use of metals results in the formation of the corresponding metal salt complexes of isohumulone, without any evidence of degradation. In particular, when the magnesium and calcium salts are isomerized, the yield of isohumulone is virtually 100 % under relatively mild conditions (e.g. heating at 70 °C for 20 min). *Lance* et al. [81] further examined the effect of heat (1 hour at 110 °C) on a range of solid metal salts of humulone. It was found that some humulone salts, e.g. those of calcium, magnesium, cobalt (II) and nickel (II) undergo isomerization to the corresponding salts of isohumulone without any significant degradation. Other metal salts undergo only partial isomerization without apparent degradation, and some lead to both isohumulone and uncharacterized degradation products. The humulone salts of aluminium, chromium (II), copper (II), lead (II) and mercury (II) show no formation of isohumulone. More recently, *Paul* [82] reported on the accelerating effect of Al_2O_3 on α-acids isomerization. Also Jaskula et al. [83] demonstrated that addition of Al^{3+} or Fe^{3+} salts to α-acids containing wort medium, results in a more efficient isomerization reaction, compared to the presence of Mg^{2+}. Furthermore, *Mertens* et al. [84] reported on the use of alkaline earth-based aluminates as heterogeneous catalysts for the isomerization of α-acids. $BaAl_2O_4$ was the most active catalyst reported. With a molar ratio of α-acids to $Ba^{2+}$of 10:1, iso-α-acid yields of 90 % were found after 18 h at 90 °C [85].

Verzele [69] studied the influence of the wort pH on α-acids isomerization. With increasing pH (pH range 5.0–5.6), an increase in the isomerization yield was found. Furthermore, addition of calcium or magnesium silicate to wort increased the isomerization rate and, consequently, the levels of iso-α-acids in the boiled wort. The positive influence of an increased pH on the isomerization yield was further confirmed by McMurrough et al. [54].

Most recently, *Gabel* et al. [86] studied the formation of isohumulones in aqueous hop solutions at very low temperatures. The authors found that isomerization of α-acids can occur at 10 °C, however, after a prolonged exposure time up to 600 h.

Finally, in addition to the above described thermal isomerization of α-acids, a totally different type of isomerization, i.e. photo-isomerization, resulting in only *trans*-isohumulones has been described by several authors [40, 87, 88, 89].

4 Conclusions

This review provides a description of the knowledge on the chemistry of hop α-acids and on the complex chemistry behind the mechanism involved in the formation of iso-α-acids, i.e. isomerization of α-acids. A lot of research on α-acids isomerization has been conducted in the past, however, new aspects continue to be revealed.

The production of hops worldwide exceeds 100,000 mt and is almost exclusively utilised by the brewing industry. The value of hops is highly attributed to its bittering, aromatizing and preservative components. However, efficiency of the use and/or transformation of these metabolites during the brewing process is rather low. In this respect, further research on hop chemistry remains of major importance, and the authors hope that this review paper will help scientists to find their way and discover new things in the fascinating field of hop chemistry.

5 References

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