Contributions to the quality control of two crops of economic importance : hops and yerba mate

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chapter 2

Hops and Beer

Erica G. Wilson and Robert Verpoorte

2.1 Hops

Hop plants (*Humulus lupulus*) have been used popularly since ancient times. References to the growth of hops as garden plants for their flowers and shoots which were eaten in the same way as asparagus can be traced back to century X AD (Lewis et al., 1993).

Fig. 2-1 Humulus lupulus L. (Cannabaceae)

Humulus is a Latin name of uncertain origin; it may have come from the Low German word "humela" for hop and lupulus literally means a "small wolf." This is in reference to the plant's habit of climbing over and smothering trees. *Humulus lupulus* was once called "willow-wolf" due to "its propensity for climbing on willows" (Charters, 2006).

The term hops refers to the strobiles or "cones" of female individuals of the dioecious species *Humulus lupulus*, L (Cannabaceae), a species that was domesticated more than 1000 years ago (Behre, 1999) and is native to the Eurasian region. Native lineages have also been detected in temperate zones of North America (Hampton *et al.*, 2002) and cultivars were successfully introduced into many other parts of the world including S. America, S. Africa, Australia and New Zealand. According to the most recent International Code of Botanical Nomenclature (ICBN; St. Louis Code) adopted by the Sixteenth International Botanical Congress in 2000, the currently accepted name for the family is Cannabaceae (Greuter *et al.*, 2000) a family that comprises only two genera, *Humulus* L and *Cannabis* L. The *Humulus* genus itself arguably comprises three species, *Humulus lupulus* L., *Humulus japonicus* Sieb. & Zucc Japanese hop, endemic to East Asia and which does not produce bitter acids or xanthumol, and a less cited autochthonous Chinese species, *Humulus yunnanensis* Hu (Small, 1978; Neve, 1991; Zheng-yi *et al.*, 1994; Stevens *et al.*, 2000; GRIN, 2011). Some confusion has appeared as a consequence of the existence of hop plants native to N. America that readily interbred with the hops introduced by the original European settlers. Early settlers to N. America could pick native wild hops in the woods around their settlements. Dutch settlers, however, chose to import dried hops from their homeland, while English settlers imported cuttings from England. Moreover, in 1629 the Massachusetts Company began growing hops commercially. Thus, the names *H. americanus* Nutt, *H. neomexicanus* Rydb. *H. volubilis* Salisb., *H. vulgaris* Gilib., *Lupulus communis* Gaertn., and *L. humulus* Mill. that are reported in N. America, are not species but most likely synonyms for various *H. lupulus* varieties (Small, 1980; Farnsworth *et al.*, 2007).

Nowadays, all cultivated hops are varieties of *H. lupulus*. It is estimated that there are over 100 different cultivars of hops that have been bred to produce increasing amounts of bitter acids (up to 20% in weight) or volatile oils according to their purpose (Neve, 1991).

2.1.2 Botanical description

Hop is an herbaceous perennial plant, which produces annual vines from an over-wintering rootstock. In the spring and early summer the vines grow rapidly, reaching their maximum height of 5-7 m by the beginning of summer when in response to the shortening of the day length, they stop growing.

In the northern hemisphere, hop strobiles ("cones") are typically harvested in late August or September, from the perennial vine (Neve, 1991). The cones are immediately dried after harvest by forced hot air, and are often pressed into dense cylindrical pellets, ca. 5–8 mm in diameter and up to 25 mm long. Palletisation greatly reduces the overall surface area, consequently reducing the rate of chemical oxidation/degradation, and also providing a more compact product for shipping. Typical microscopic characteristics of powdered hop cones include numerous large yellow glands unique to the species ("lupulin glands"), abundant bracteole and leaf fragments, as well as sparsely distributed glandular and covering trichomes (Youngken, 1950; Jackson et al.1968; Neve, 1991;Yamada et al., 1998).

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Fig. 2-2: Hop cones (strobiles) A) Before harvesting; B) Lupulin glands in the strobiles.

2.1.3 Industrial production of hops

As mentioned above, Humulus lupulus is a perennial that regrows each spring from the rhizomes of an underground rootstock. In the wild it spreads through underground rhizomes and by seed. Small (1978) separated H. lupulus into five taxonomic varieties based primarily on leaf characteristics, including pubescence, leaf hairs, leaf lobes and number of glands. European-type hops are less pubescent, have fewer glands, and have heart-shaped leaves with few lobes. Brewers have long identified traditional European hops as milder and more aromatic than those from N. America. Haunold et al. (1993) reported that native N. American hops had "deeply lobed leaves with five or seven lobes predominating." Small also observed the heavily pubescent and deeply lobed leaves of the native American hop varieties also noted by early English hop breeders for their high resin content, describing them as rich with glands and "buttery" to touch. In commercial hop production, hop vines are trained to climb strings suspended from 25-foot or higher trellises. The vines clasp the strings with strong hooked hairs and twine clockwise (in the N. hemisphere) to the top of the trellis. Lateral arms develop at the nodes, producing flowers at their terminal buds. At harvest the vines are cut down and the cones stripped off, but the crown remains in the ground as the source for the next years' growth. Crowns can remain productive for years and are generally only removed when a hop yard is either removed from production or replaced with a new variety. Hops require long summer days to flower and produce good cone yields. Only the cones of the female plants are used by the brewing industry. Mature cones are covered with tiny glands that hold the sticky yellow resin containing the compounds used by the brewing industry. Cones range in size from approximately 1 to 10 centimetres in length with papery green bracts and bracteoles. The resin glands coat the bracteoles and seeds of the cones (Burgess, 1964).

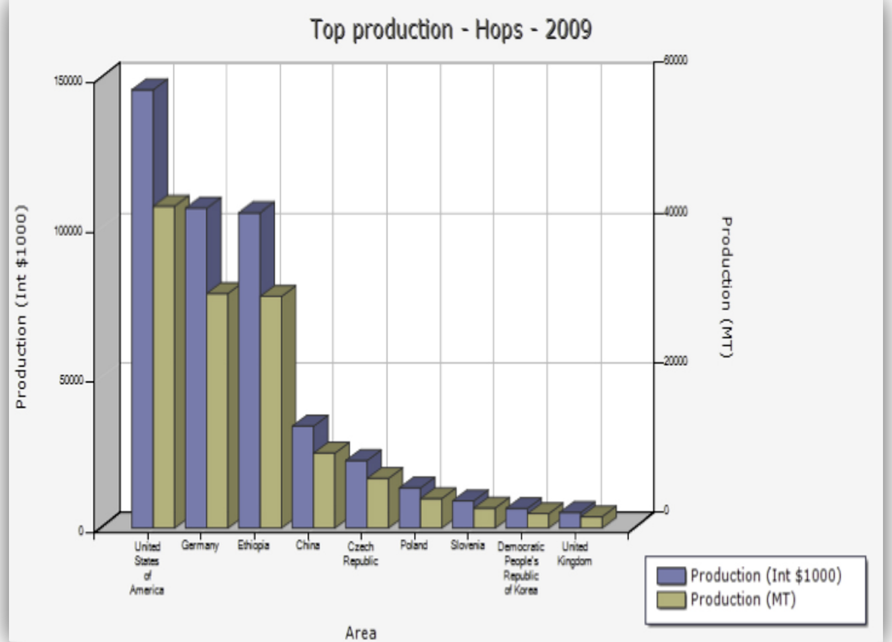


Fig. 2-3: Total hop production by country (http://faostat.fao.org/site/339/default.aspx)

Hops are grown in several parts of the world, restricted by its daylight amount requirement. As mentioned before it is a very latitude sensitive plant and a major factor concerning its economic performance as a commercial crop, is the length of daylight hours, which is the flowering period. In order to flower successfully the hop plant must, at a certain stage of its growth (usually expressed as a minimum height) be exposed to specific hours of daylight (and darkness, which must be continuous). A cultivated hop of high quality requires a mean value of solar irradiation of 1800-2000 hours per year including 1300-1500 hours during the vegetative period; the ideal latitudes for growing crops are 35-51° N and 34-43° S with an annual temperature of 5-21°C (FAO- Ecoport). Hop plants are grown near George, South Africa and Kashmir, India, at 34°, but artificial light is necessary for successful commercial production of most varieties. The best hop crops are obtained at maximum latitude of 52°. In the northern hemisphere, wild hops occur, even further north, however, and have been sighted up to latitudes of 66°. In the Southern hemisphere, the most southern point where hops can grow is New Zealand and south of Argentina (latitude 42°).

In total, hops are cultivated in about 30 countries. World production amounted to 141,307 tonnes in 2009 (FAOSTAT). The total surface coverage of hops crops was estimated to be of 83,283 ha by the FAO in 2009 and the highest producing countries of the world were USA, Germany and Ethiopia (FAOSTAT). In 2009, USA produced 30.2 %, Germany 22.2%, and Ethiopia 21.9 % of the total world production. Way behind these are China with a production that represents a 7.1% and Czech Republic approx. a 4.7 % of world production, accounting between these 5 countries for 86.1% of the world production (Fig. 2-2).

The data provided by other sources, such as IHGC (International Hop Growers Convention) and the European Union do not mention the prominent contribution of Ethiopia to the world production. In fact, Ethiopia is not even mentioned among African hop-growing countries, and no explanation is provided. Ethiopians refer to another plant, *Rhamnus prinoides*, (known popularly as Gesho) as hops and use it as a bittering agent in beer. This could not be confused for hop production in the FAOSTAT database, so it is certainly a fact that should be clarified.

2.2 Chemistry of hop cones and hop-derived compounds

Female hop cones contain glandular structures (lupulin glands) that secrete lupulin powder, rich in secondary metabolites. Over 1000 compounds have been identified in hop mature cones. These can be classified as volatile oils, polyphenols and (resinous) bitter acids (Table 2-1).

In terms of economic importance however, volatile oils and bitter acids (identified in the tables as α- and β- acids) constitute the most important group.

2.2.1 Hop resins

The resin content of the cultivated hops used nowadays for brewing is close to 30%. This resin is constituted by a number of identified compounds, including α- and β-bitter acids and minor derivatives of these such as desoxy-α-acids. It also contains most of the hop cone fats and waxes (approx. 5% of the total weight) (Roberts *et al.*, 2006).

Compound(s)	Amount (percentage)
α –acids	2-17
β - acids	2-10
Amino acids	0.1
Ash/salts	10
Cellulose/lignin	40-50
Monosaccharides	2
Oils and fatty acids	1-5
Pectins	2
Polyphenols and tannins	3-6
Proteins	15
Volatile oil	0.5-3 (v/m)
Water	8-12

Table 2-1 Chemical composition of air-dried hops (from Benitez, 1997).

The proportion of different components in the lupulinic resin (total resin) is associated to its quality and the European Brewery Convention (EBC) and The American Society of Brewing Chemists (ASBC) proposed the following system of nomenclature based on the fractions which can be obtained according to the solvent used: hard resin (portion of the total resin that is insoluble in hexane) and soft resin (portion of the total resin that is soluble in hexane). This difference in solubility is due to their chemical composition. However, both hard and soft resin fractions are soluble in cold methanol and diethyl ether, providing a way to distinguish them from hops waxes that are not soluble in cold methanol. These waxes consist of long-chain alcohols, acids, esters and hydrocarbons (Verhagen, 2010).

The soft resin fraction consists basically, of an analogous series of closely related homologues, α- and β- bitter acids while the hard resin fraction is composed of a mainly undefined mixture of oxidation products of the soft resins. This fraction is greater in hops that have received improper post-harvest treatment or inadequate storage conditions.

The major compounds present in hard and soft resins can be observed above in Table 2-2.

Hard Resins	Soft Resins
Xanthohumol Iso-xanthohumol Flavone	α-Acids:cohumulone, humulone, adhumulone, prehumulone, and posthumulone.
	β-Acids: colupulone, lupulone, adlupulone, prelupulone, postlupulone.

Table 2-2. Composition of hop resin

2.2.2 Bitter acids

The major components of soft hop resin are the bitter acids, which are distinguished as α-acids or humulones and β-acids or lupulones (see fig. 2-4), being, respectively, di or tri-prenylated phloroglucinol derivatives.

In 1888, Hayduck classified the hop bitter compounds in two fractions according to their behaviour in presence of the lead ion, namely, a lead precipitable α-acids fraction and compounds that remained in the supernatant, the β-acids. Wieland and Wollmer assigned the structure of these compounds in 1925-1926. Further on, between 1950 and 1975, Rigby in USA and Howard in UK, showed that α- and β-acids were a mixture of homologues and analogues belonging to two series comprising, in fact, three constituents differing in the nature of their side chain, derived from the hydrophobic amino acids, leucine, valine and isoleucine. The original structure of α-acids was proposed by Wieland in 1926, and confirmed by their synthesis by Riedl in the 1950s. This proposed structure did not differ substantially from that confirmed finally by De Keukeleire and Verzele in 1970.

Verzele and De Keukeleire had started working on hops in 1945 in the University of Gent, Belgium, and became one of the major contributors to the knowledge and understanding of hop chemistry. Among many other things, they not only clarified the structure of hop bittering compounds, but also their enolization pattern and the absolute configuration of naturally occurring (-)-humulone, their oxidation and reduction reactions as well as isomerisation during wort-boiling (Enari, 1995, Moir, 2000).

In 1979, Verzele verified the primary structure of humulone with its 100-MHz proton nuclear magnetic resonance spectrum, in which all the signals were unambiguously assigned (Moir, 2000). These are humulone (1) / lupulone (6), cohumulone (2) / colupulone (7) and adhumulone (3) / adlupulone (8) as can be observed in Fig. 2-4.

The β-acids (lupulone, colupulone and adlupulone) differ structurally from α-acids (humulone, cohumulone and adhumulone) by the presence of one more prenyl group, as a result of which they are slightly more lipophilic. The resinous bitter acids are unique to hops and have not been found in any other species in the world, to date (Verhagen, 2010). In addition to the two series of normal, co- and ad-homologues, some minor bitter acids can be found, i.e., posthumulone / postlupulone (5,10), prehumulone / prelupulone (4,9) and adprehumulone. In the lupulinic glands, these acids are present in a free, totally protonated state. In this state, they are highly insoluble in water but can be extracted with methanol, though more selectively with hexane, toluene, dichloromethane or diethyl ether. If isolated, α-acids have a yellow, pasty aspect while β-acids are less yellow and almost crystalline. However when slightly heated they become more liquid, though β-acids require more heat to liquify. These compounds are termed as acids because of their proton-donor characteristics, and dissolve freely in alkaline solutions, α- acids having much lower pKa values (Simpson, 1993).

The pK_a value for α-acids has a pH value close to 4.7, while β-acids only reach 50% dissociation at pH 5.7. The practical consequence for beer-brewing is that at the typical pH of beer (between 3.8 and 4.5) the α-acids will be quite soluble, so that a large amount of unisomerised α-acids will pass from the boiling wort into the beer, while a very low proportion of the β-acids will solubilise. Thus, while average beer contains several parts per million (ppm) of α-acids, it never contains more than 1 ppm of β-acids (Ono *et al.*, 1985). Another characteristic of these compounds is their proclivity to chelate a wide range of metal cations due to their β, β′-triketone moiety.

As regards UV absorbance, their structure contains chromophores that absorb strongly in the 310-340 nm region with an ε c.a 104, ensuring their detection in solution at low concentrations (Hughes P, 1996).

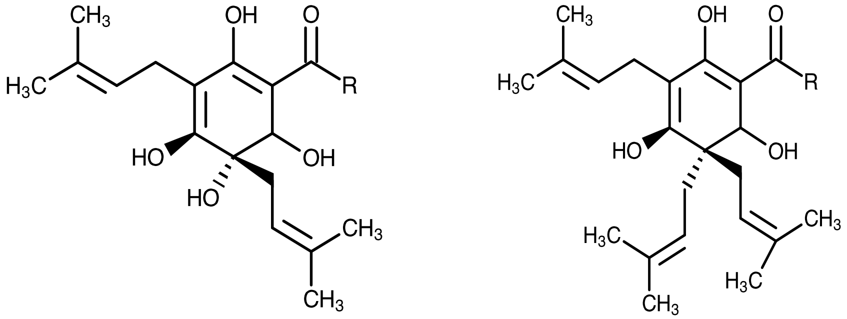
An intriguing feature of the hop acids is their exceptionally high content, accounting for up to 25% or even more of the dry weight of the hop cones. The relative proportions of the individual constituents depend strongly on the hop variety and, for a given variety, on the conditions of growing.

Hop bitter acids are very sensitive to oxidation, forming a mixture of ill-defined products that are soluble in diethyl ether, but no longer in hexane (hard resins). Deterioration of hops as a function of time is accompanied by development of a strong odour that is generally not welcomed by brewers.

To prevent this, hops are rapidly dried after harvesting, pelleted, and stored in airtight bags, preferably at low temperatures. Today, apart from the use of pellets, hop extracts are also often added to confer bitterness to beer. The advantages of doing this is that these extracts have higher concentration of brewing principles, increased stability, improved uniformity, and easier handling (Combes, 1998).

2.2.3 α- Acids

These acids are the most important constituents of hops. The acidic, salt-forming, and chelating properties of α-acids reside in their β-triketo system. As mentioned above, the absolute structure of humulone (1) was established by De Keukeleire and Verzele in 1970, using a combination of chemical, spectrometric, and chiroptical techniques (De Keukeleire *et al.*, 1970). It appears that only the R- configuration at C-6 within a fully enolized β-triketo system is formed stereo- selectively in Nature (Verzele *et al.*, 1991).



α- acids (humulones)

β- acids (lupulones)

1	(n)	R: $CH_2CH(CH_3)_2$	6
2	co	R: $CH(CH_3)_2$	7
3	ad	R: $CH(CH_3)CH_2CH_3$	8
4	pre	R: $CH_2CH_2CH(CH_3)_2$	9
5	post	R: CH_2CH_3	10

Fig. 2-4 Structures of hop bitter acids (Adapted from De Keukeleire, 2000)

Determination of the composition of the α-acids mixture is important, as high levels of cohumulone are often associated with low hop quality, although there is much controversy around this topic (Meilgaard, 1960). The major component of the mixture of α-acids is humulone (1). While the relative amounts of humulone and cohumulone (2) are variety-dependent (20-50%), adhumulone (3) constitutes invariably *ca.* 15% of the mixture.

As mentioned above, for research purposes, α-acids can be precipitated from a hop acid mixture (e.g., a liquid carbon dioxide extract) by adding lead (II) acetate, thus forming yellow-colour lead salts that can be stored for years without deterioration. The exact structure of the salts is unknown, but the tertiary alcohol function must be involved, as the β-acids cannot form such salts. Humulone (1) can be isolated from the mixture of α-acids by complexation with 1,2-phenylenediamine followed by repeated crystallization. Rigby and Behune did a great amount of preparative work using counter-current distribution, obtaining all humulones (Rigby *et al.*, 1952, 1953). Later on, Hermans-Lokkerbol *et al.* (1994) published an efficient CPC- based method that allowed the isolation of the three α-acids from a hop extract.

Hops α- acids	Percentage (%)
Humulone	35-70
Cohumulone	20-65
Adhumulone	10-15
Prehumulone	1-10
Posthumulone	1-3

Table 2-3 Composition of the hops α acids (Haunold, 1993)

While regarded as the principal “bitter acids” from hops, they perhaps paradoxically, do not have a bitter taste, even at concentrations of 100 mg/ml (Neve, 1991).

2.2.4 β – Acids

The β-acids are less acidic than α-acids, colupulone having a pKa of 6.1 (Simpson, 1993) as compared to that of cohumulone that is 5.4 (Simpson *et al.*, 1994). This is because an extra prenyl side-chain replaces the tertiary alcohol function at C-6. In contrast to α-acids, for each β-acid, two different enolisation patterns prevail (Fig 2-5). However, β-acids are mostly drawn in the predominant conjugated dienolic form, which corresponds to that of the α-acids.

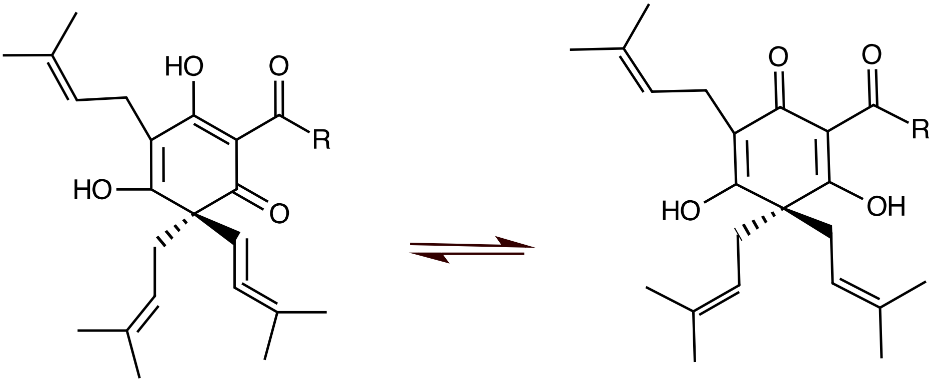


Fig. 2-5: Enolisation patterns of β-acids.

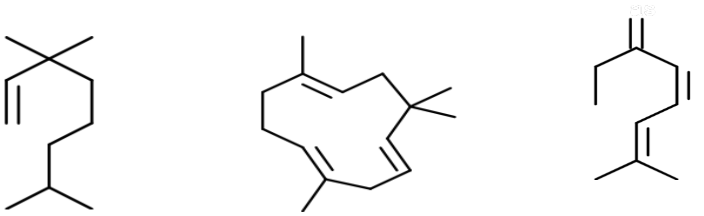
Most hop varieties contain approximately equal levels of lupulone (6) and colupulone (7) (*ca.* 20-55%), but less variable levels of adlupulone (8) (10-15%). Prelupulone (9) and postlupulone (10) are only present in trace quantities (Neve, 1991).

β-acids are extremely sensitive to oxidation, which is initiated by air (auto-oxidation), thus giving rise to a number of oxidized compounds and derivatives. A particular oxidative reaction leads to formation of the highly stable hulupones. All β-acids have been obtained as pure compounds by Hermans-Lokkerbol et al. (1997) from a CO2 hop extract by modification of their previously published method for α-acids.

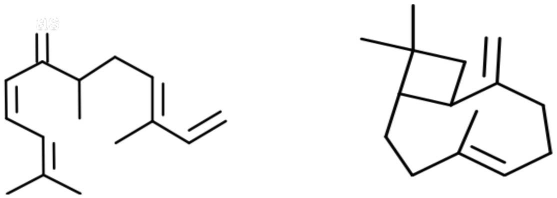
2.2.5 Essential oils

Hops can contain between 0.3 and 1.0% of essential oil. Apart from volatile constituents such as simple oxidised alkanes, hundreds of terpenoid components including monoterpenes and sesquiterpenes have been identified (Chadwick et al., 2006).

In 1994, Moir reported that more than 300 compounds belonging to seven different groups of compounds had been identified in hop essential oil (see Table 2-4).



Linalool *Humulene* *β-myrcene*



Farnesene *β-Caryophyllene*

Fig. 2-6. Main constituents of hop essential oil (hydrocarbons)

Group	Approximate number of identified compounds
Hydrocarbons	60
Aldehydes/ketones	60
Esters	70
Acids	10
Alcohols	60
Oxygen heterocyclics	30
Sulfur compounds	30

Table 2-4 Type and number of compounds identified in hop essential oil (Moir M, 1994).

Eri et al. (2000) detected 286 distinct chemical entities in the chromatogram of the volatile oil, more than 100 of which were not identified. The primary volatile constituents in all cultivars are hydrocarbons, constituting around 40 to 80% of the total oil content (Verhagen, 2010). Within this type of compound, the monoterpene myrcene, (30-50%) and the sesquiterpenes humulene (15-25%) and β- caryophyllene are the most dominant. Other major constituents in beer are oxygenated compounds that represent between 20 and 50% of the total essential oils. They consist mainly in esters derived from straight and branched alcohols and acids, Fig. 2-6 (Verhagen, 2010).

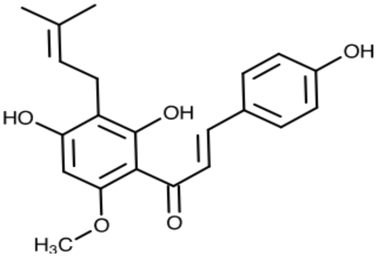
Other authors (Malizia et al. 1999; Eri et al., 2000) though coinciding with the predominance of hydrocarbon compounds, have found higher quantities of myrcene (30-50%), humulone (15-25%), β-caryophyllene and farnesene, which together were shown to comprise between 57% and 82% of the volatile oil, depending on the cultivar and the method of detection.

2.2.6 Polyphenols

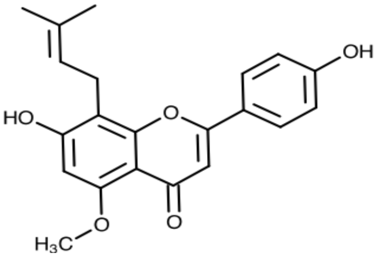
Dried hop cones have been found to contain between 4 and 14% of polyphenols (Stevens et al., 1998; De Keukeleire et al.; 1999, Taylor et al., 2003). These belong to different classes, including a number of prenylated, geranylated, oxidised and/or cyclised chalcones, of which 30 have been isolated to date (Mizobuchi et al., 1994; Milligan et al., 1999; Zuurbier et al., 1998; Stevens et al., 1998, 1999a, 2000). The chalcone xanthohumol (XH) is the most abundant prenylated flavonoid in fresh and properly preserved hops, present at a concentration of ca. 0.01–0.5% (Hermans-Lokkerbol et al., 1997; Stevens et al., 1999b). Together with desmethylxanthohumol, another prenylated flavonoid, they are precursors of the isomeric flavanones isoxanthohumol and 8-prenylnaringenin, respectively.

Xanthohumol and 8-prenylnaringenin, specifically, have been intensely studied in recent years due to interesting bioactivities that have been attributed to them, including prevention of osteoporosis, breast cancer and a beneficial activity in diabetes. In the case of 8-prenylnaringenin, in vivo studies have shown it to be a potent phytoestrogen (Milligan et al., 1999, 2000; Bowe et al., 2006). However it is present in very small amounts since the plant does not synthesize it, as mentioned above, but is the product of the slow spontaneous isomerisation of desmethylxanthohumol (De Keukeleire, 2003).

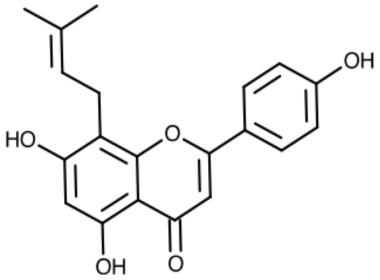
Interesting medicinal properties have also been reported for isoxanthohumol such as anti-cancer activities, but at a much lower level than that observed for xanthohumol (Stevens et al., 1998; Gerhauser et al., 2002a, 2002b).



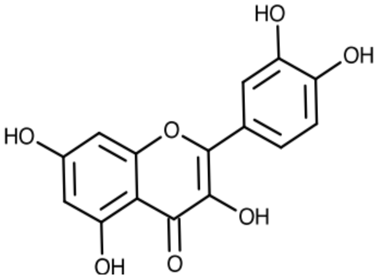
1. Xanthohumol (XH)



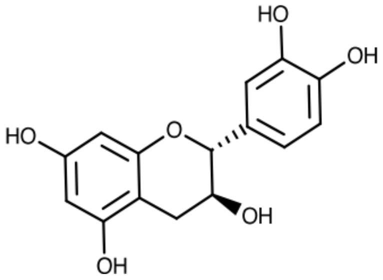
2. Isoxanthohumol



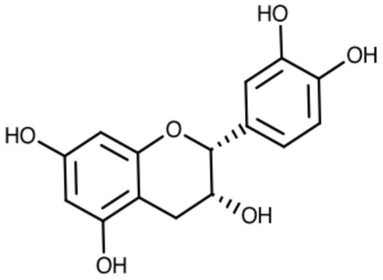
3. 8-prenylnaringenin (PN)



4.Quercetin



5. Catechin



6. Epicatechin

Fig. 2-7. Polyphenols in hops: Prenylated flavones: 1-3; flavonols: 4; procyanidins: 5-6.

Hops are also rich in flavonol aglycones (kaempferol, quercetin), flavonol glycosides (quercitrin, rutin) (Sägesser et al., 1996) and procyanidins (catechin, epicatechin). These procyanidins are present in relatively large amounts and as monomers contribute to the stability of hops and later beer due to their antioxidant properties. However, when they polymerise as dimers or more still as oligomers (3-8+) they can bind to proteins and precipitate, and are thus haze inducing (Roberts et al., 2006). Other procyanidins that are present are gallocatechin and epigallocatechin (Gorissen et al., 1968).

2.3 Hops and Beer

Beer is a fermented aqueous drink based on starch and flavoured by hops (Moir, 1994). This simple definition encompasses the four essential ingredients, which are necessarily used in the brewing of beer: barley malt, brewing water, hops and yeast.

Beer however, was not always made with hops. The first references of this drink date back to ancient times, where in Egypt for example it was made by the spontaneous fermentation of breadcrumbs steeped in the very sweet juice of dates. This drink, known as 'Zithum', was very popular. During the Gallo-Roman period beer was named 'cervoise' (hence the Spanish 'cerveza') in honour of Ceres, the Roman goddess of agriculture. The origin of the word 'beer' is not well established, but it is closely related to the Latin word 'bibere' (to drink) (Arnold, 2005). There are authors who hold that these drinks cannot be considered to be beer, and consider that it was really of European origin (Nelson, 2005).

Between the 6th and 7th centuries, most brewing in northern Europe was monopolised by monks and it was one of these Benedictine monks, Saint Arnold, who apparently introduced the use of hops as a flavouring agent in beer, replacing other herbs that had been used up to that moment. It has to be borne in mind that hops themselves have an ancient tradition of medicinal uses, for example, there are references to its use for its anti-inflammatory, antiseptic, antidiuretic, aphrodisiac, hypnotic, sedative, and stomachic properties (Gessner et al., 1974; Asano et al., 1976; Van Hellemont, 1993; Stevens et al., 2000; Brunetton, 2003; Ebadi, 2006; Duke, 2007; Van Cleemput et al., 2009). Since then, hops are the only flavouring agent added to beer, though this is of course, an understatement, since hops have many other functions in beer as will be explained later.

The real onset of modern brewing occurred in the second half of the 19th century. After Louis Pasteur had published the secrets of fermentation in his book "Etudes sur la biere" (1876), a new yeast strain was developed which had the peculiarity of fermenting at low temperature (ca. 5°C) and precipitating to the bottom of the fermenter at the end of the fermentation. The name Pilsner beer was coined for this new drink after the Czechoslovakian city Pilsen, where it was first introduced. Soon a number of brewing schools were founded, particularly in Germany (Munich, Berlin) and Belgium (Ghent, Leuven), and the brewing business started a very flourishing period as the bottom-fermentation of beers turned out to be readily amenable to large-scale production.

Briefly, the body of the beer is provided by barley, more specifically barley malt, and, in general, a few hundred grams are used for one litre of beer. The malt may be partly substituted by starch-rich adjuncts, such as rice, corn or wheat. When a slurry of barley malt and brewing water (called 'mash') is heated at a temperature around 60°C, the malt enzymes, mainly amylases but also proteases, degrade starch and proteins, leading to a mixture of sugars and peptides or amino acids. For that purpose, barley must be subjected, prior to mashing, to a controlled germination, during which these enzymes are formed in the barley grain Such germinated barley is known as barley malt. The starch to sugar conversion is stopped by heating. At this stage and after filtration, the sugar solution or 'wort' in brewers' jargon is transferred to the brewing kettle, where it is boiled for at least one hour with the After cooling and removal of spent hops, the liquid, known as 'hopped wort' is pumped to the fermentation vessels and yeast is added under aeration for growth. During the anaerobic phase yeast cells convert sugars to ethanol and carbon dioxide. Depending on the temperature during fermentation and the nature of yeast collection at the end of the fermentation period, beers are distinguished as being produced by 'bottom fermentation' or 'top fermentation'. Yeast strains, appropriate for bottom-fermented beers (Saccharomyces carlsbergensis), are active below 5°C and they settle to the bottom of the fermenter after production of about 5% ethanol. Conversely, yeasts, typical for the production of top-fermented beers (Saccharomyces cerevisiae), operate at ambient temperature and resist higher concentrations of ethanol, up to 12%. When the activity stops, the yeast cells collect to the top as dense foam. Figure 2-8 shows the basic scheme of beer-brewing.

2.3.1 Bitter α-acids and iso-α-acids in beer

Hop α-acids as such occur in beer in concentrations up to 4 mg/L. They improve foam stability, suppress gushing, and contribute to the preservation of beer. Their main contribution to beer, however, is via isomerisation during the boiling of wort with hops, thereby forming the extremely bitter iso-α-acids (Verzele, 1991).

Nowadays, industrial brewers do not add hop cones but rather one or more types of processed hop products that are standardized and can guarantee better reproducibility of taste and aroma. They can also be stored during longer periods of time.

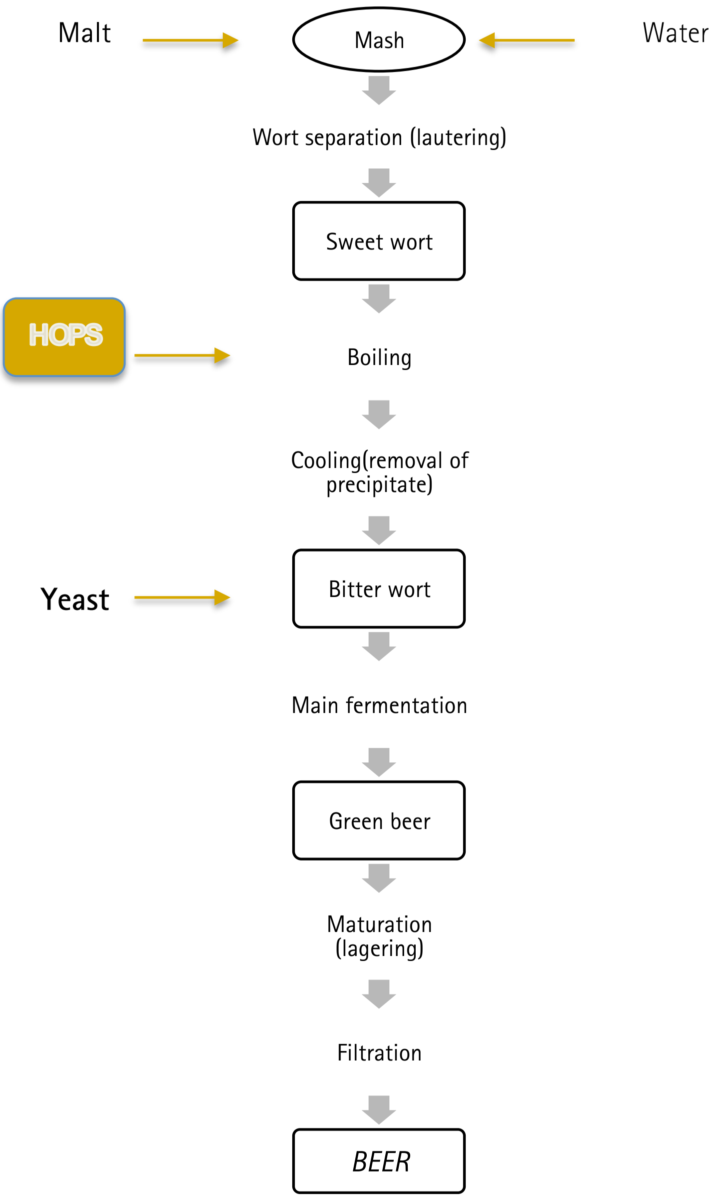


Fig. 2-8 Main steps in the beer-brewing process (Adapted from Verhagen, L.- Beer Flavour, 2010)

2.3.2.1 Hops products

Hops are harvested and immediately dried in order to preserve the bitter acid content, since these compounds are relatively unstable. The drying process is critical as it is necessary to reduce the water content to approximately 10% but the temperature and time to which hops are exposed is critical to avoid the degradation of the bitter acids and essential oil contents. Dry hops are then compressed into bales to exclude oxygen as far as possible and stored below 5°c (verhagen, 2010).

Hops can be used as such or are further processed to improve storage and handling. These processes include their conversion to pellets, extracts with solvents, CO2 or ethanol, each of which produce different hop preparations.

Hop pellets: Whole dried hops are milled to a fine powder and compressed to form pellets that are approximately 10 x 6 mm and packed normally packed in laminated polythene/metallised polyester foils within cartons either as 'hard' packs under vacuum or as 'soft' packs under inert gas (N_2 and /or CO_2) at atmospheric pressure. The advantage of this is that they are easier to store and better preserved. However they have to be kept at low temperature (0 – 5 °C) for use within 2- 3 years or at -20°C for 5 years according to the manufacturer.

The pellets produced as explained above have a content of 2 - 5% α-acids. Hop pellets with a higher content of α-acids can be obtained by previously freezing the cones to -30 or -40°C in order to harden the resin-rich lupulin glands which are then separated from the rest of the cone by special sieving machines (Verhagen, 2010). These are then milled and compressed to form pellets that contain between 4 - 20% α-acids. The advantage is that they have a much lower amount of polyphenols and other undesirable products.

CO_2 extract: is an extract of hops produced by extraction of hop pellets using carbon dioxide under liquid or supercritical conditions. This practically non-polar inert solvent allows the selective extraction of the hydrophobic non-polar α-and β- acids and hop essential oil while polar polyphenols, including tannins and xanthohumol and derivatives, and also heavy metals and nitrates are not extracted. Most pesticides and herbicides can also be removed during extraction. The product thus obtained is a thick, greenish-yellow paste that can be fluidified with heating and contains, according to the variety of hops, between 35 - 50% α-acids. It is exceptionally stable if stored at low temperatures (between 4 and even 8 years according to the manufacturer at 0 - 5°C).

Ethanol extract: In this case, hops are extracted with boiling 90% ethanol. After filtration, the ethanol is removed by reduced pressure evaporation yielding a two – phase system that allows the separation of the more polar hydrophilic compounds such as tannins and other polyphenols from the bitter acids and essential oil. Due to the high temperatures that are used for extraction, a proportion of the bitter acids are isomerised to iso-α-acids. Despite this, the remaining extract has a high content of α-acids, approximately 50% according to the variety, whereas, heavy metals, nitrites and to a certain extent, pesticides and herbicides are removed. Prenyl flavonoids, such as xanthohumol are present in this ethanol extract while they are not present in the CO_2 extracts. These extracts are also stable products and if properly preserved can last up to 4 years (stored < 10 °C).

Most industrial beer manufacturers add hops pellets or CO_2 extracts to beer during the wort-boiling process.

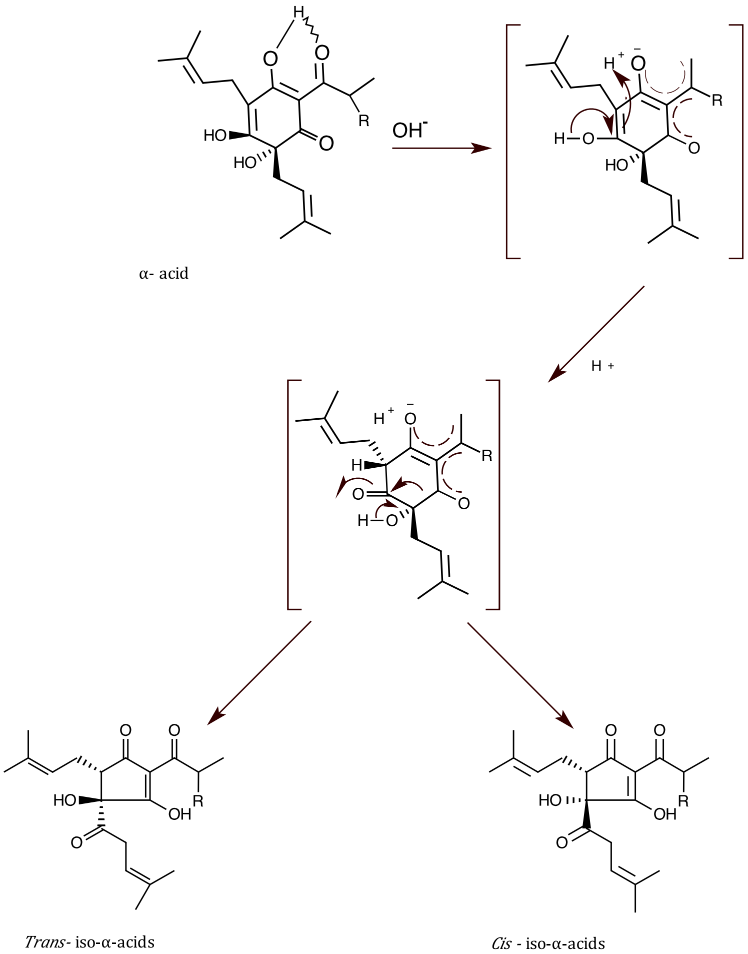
2.3.3 Iso-α-acids in beer

When hops are added to beer in the wort boiling stage of beer production, the α-acids which are themselves not bitter even at concentrations of 100 mg/l (100ppm) thermally isomerise into intensely bitter compounds known as iso-α-acids.

This reaction occurs in the relatively acid medium in the aqueous wort medium (pH≈5.0-5.5) and the iso-α-acids are formed after an intra molecular rearrangement of α-acids, resulting in two series of five membered-ring compounds, the *trans-* iso-α-acids and the *cis-* iso-α-acids (Fig.2-9). In these conditions, the isomerisation results in a ratio of approximately 68:32 of cis / trans iso-α-acids.

These compounds are relatively acid (pKa approximately 3) and are thus much more soluble in water than the α-acids.

Since the rate of conversion of the α-acids into beer is only 30-35%, the hop industry has developed diverse pre-isomerised products, which, as shown in Fig.2-10, are added to the brewing process after wort- boiling. These isomerized hop extracts are prepared either by storage at 50°C of hop pellets with magnesium oxide or by isomerisation of CO2 extracts with potassium hydroxide. In the first case, dull yellowish pellets are obtained that contain between 2 - 20 % of iso-α-acids, apart from β-acids (>92% of α-acids present in the starting material are isomerised). Extracts with higher concentrations of iso-α-acids are obtained in the second case and between 20-30% of the potassium salt of the iso-α-acids can be found in the thick syrup-like alkaline aqueous solution.



R=	α-acids	*cis*/*trans*-iso-α-acids
$-CH(CH_3)_2$	cohumulone	isohumulone
$-CH_2CH(CH_3)_2$	humulone	isocohumulone
$-CH(CH_3)CH_2CH_3$	adhumulone	isoadhumulone

Fig. 2-9 Isomerisation of humulones to produce cis- and trans-iso-α-acids in the wort medium. (Adapted from Jaskula et al., 2008)

Iso-α-acids survive wort boiling to a large extent although it is assumed that numerous oxidised derivatives are produced, albeit to a lesser extent. Other structural variants of these acids can be detected in low quantities - particularly allo-iso-α-acids and anti- iso-α-acids (Hughes, 1996).

The *cis*-isomer is more stable (half-life >> 5 years) (De Keukeleire, 2000) than the *trans*-isomers (half-life of ca. 1 year) owing, perhaps to the lower steric hindrance between the two large vicinal side chains (Verzele *et al.* 1991). This naturally alters the *cis* : *trans* ratio and has significant consequences with respect to taste and flavour stability. Thus, it appears interesting to have the highest content of *cis*-isohumulones possible in the mixture of isohumulones. Intelmann et al. (2009b) revealed the mechanism of the reactions that *trans*- and not *cis*- iso-α-acids undergo, leading to their instability.

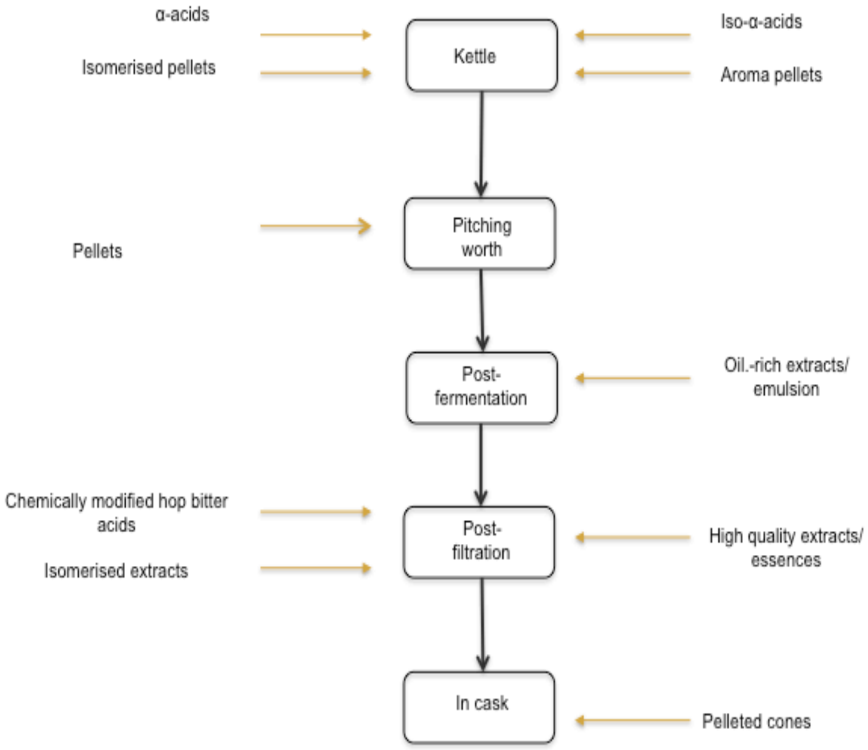


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Fig. 2-10 Typical points of addition for hop products (Adapted from Hughes, 1996)

The total concentration of iso-α-acids in beers is typically in the range of 5 - 50 ppm. It has been proved that these compounds are essential for the formation of a stable foam head on beer, thereby serving as complexing vehicles for ions that are vital for the fortification of the structures surrounding the bubbles. The traditional model for beer foam structure is that of Asano *et al.* (1976) who proposed that enhancement of foam stability results from ionic attractions between negatively charged iso-α-acids and positively charged ammonium ions on the foam polypeptides. The most accepted model nowadays, however, indicates that the binding forces that stabilise foam are not ionic but rather involve the formation of metal-complexes (Simpson et al, 1994). The synergetic effects between iso-α-acids and metal ions, which lead to dramatic increases in beer foam stability, have been demonstrated by Roberts (1975), who showed that in unhopped beer most metal cations (1.0 x10^{-4} mol dm^{-3}) have little effect or even reduce foam stability. However, in the presence of a concentration of 1.0 x10^{-4} mol dm^{-3} iso-α-acid, the foam stability of beer is markedly increased by different cations such as Fe, Ni, Co or Cu. Further evidence of this can be found in the study done by Blanco *et al.* (2006) who reported that if the cations, Ca(II), Ni(II) or Fe(III) are present in beer, complexes between the iso-α-acids and the cations cross-link the foam polypeptides in the bubble wall, consequently stabilizing it. In practice, a minimum of 20 ppm of iso-α-acids is necessary to keep a stable foam head (De Keukeleire *et al.* 1992).

Several studies have been performed to determine the role of iso-α-acids in beer quality. Its bitterness has been observed by a study conducted by BRI (Brewing Research International) in psychophysical experiments. It was demonstrated that there were large differences in the bitterness of the individual compounds and it was concluded that the *cis*-component is significantly bitterer than its *trans*-counterpart, and that isohumulone is bitterer than isocohumulone (Hughes, 1996).

Observations about their antibacterial properties showed that iso-α-acids could act as ionophores that have antibacterial activity. The study of the impact of metal cations on the efficacy of *trans*-isohumulone as an antibacterial agent showed inhibition activity of gram-positive bacteria due to the exchange of H^+ for cellular cations such as Mn^{2+} (Simpson, 1992). In this study it was also reported that the addition of β-cyclodextrin to a solution of *trans*-iso-α-acids decreased their antimicrobial properties.

2.3.3 Iso-α-acids and aging of beer

Aside from these positive contributions, iso-α-acids have been proved to be key ingredients in the development of disagreeable flavours during storage, which have a direct incidence in the determination of its shelf life.

As beer ages, and depending on its storage conditions, it undergoes a number of changes. Most of these changes are associated to deterioration in sensory attributes, especially in its taste or aroma. These changes as well as the appearance of opacity shorten the shelf life of beer and have thus a great economic relevance for the beer industry. Most of these factors have been accounted for: opacity, for example, appears in beer that has a high protein and tannin content. A stale, cardboard-like flavour (Kamimura *et al.*, 1992) is allegedly due to the formation of (E)-2-nonenal, which evolves in stored beer. This compound, together with volatile carbonyls were thought to be the product of oxidation of intermediates of unsaturated fatty acids that occur in malted barley and hop lipids (De Keukeleire, 1999).

Three degradation reactions have been attributed, however, exclusively to iso-α-acids, two of which affect both *cis*- and *trans*- isomers: photooxidation and autoxidation and another only *trans*-isomers: proton catalysed cyclisation.

Photooxidation. All iso-α-acids are unstable in presence of oxygen. This instability is increased in presence of light, since it leads to a reaction of photoxidation that results in the appearance of the so-called '*lightstruck flavour*' or LSF, a disagreeable taste described as "*skunky*" due to its thiol-like after-taste. The taste was attributed to the formation of 3-methylbut-2-ene-1-thiol (MBT) (Kuroiwa *et al.,* 1961a) who first demonstrated the appearance of LSF in model systems, composed of riboflavin, iso-α-acids, and a sulfur source (cysteine), when irradiated with visible light (350–500 nm) and demostrated the reaction in beer, proposing a possible mechanism for the reaction, with riboflavin (readily available in beer) as a photosensitizing agent (Kuroiw*a et al.*, 1961 b). In 1978, Gunst *et al.* published further details of this reaction. Discussions on the effects on the taste and its relation to concentration followed (Irwin *et al.*, 1993; Goldstein *et al.*, 1993; Templar *et al,* 1995; Hughes, 1997,1999) but the involvement of iso-α-acids and the mechanism of this reaction are generally accepted. As can be seen below in Fig. 2-11, the reaction is initiated by the absorption of light by the acyloin group composed of the tertiary alcohol function at C(4) and the carbonyl group of the side chain at C(4) present in all iso-α-acids (a) that suffers bond cleavage by a Norrish Type I reaction, leading to a ketyl-acyl radical pair.

Subsequent fast decarbonylation results in the formation of a 3-methylbut-2-enyl radical, which can be trapped by a thiyl radical to give MBT(c) and dihydrohumulinic acid (b)(Heyerick *et al.*, 2003, 2005). The LSF is, however, much more evident when beer is exposed to higher wavelength energy (in the blue region) that is not absorbed by the acyloin group, confirming the role of a photosensitizer such as riboflavin (vitamin B2) which does absorb energy in this wavelength and can thus intervene in the formation of radicals that are most likely involved in the initial step in the decomposition to a 3-methylbut-2-enyl radical, a key intermediate in the pathway leading to the lightstruck flavour in beer (Huvaere *et al.*,2004,2006). Both *cis/trans*-iso-α-acids suffer this type of photooxidation.

Production of this thiol, with a flavour threshold of less than 1 ppb, causes a disagreeable off flavour in beer (Kuroiwa *et al.*, 1963; Gunst *et al.* 1978; Templar *et al.*, 1995, Hughes, 1999). The mechanism for this reaction can be observed in Fig. 2-11.

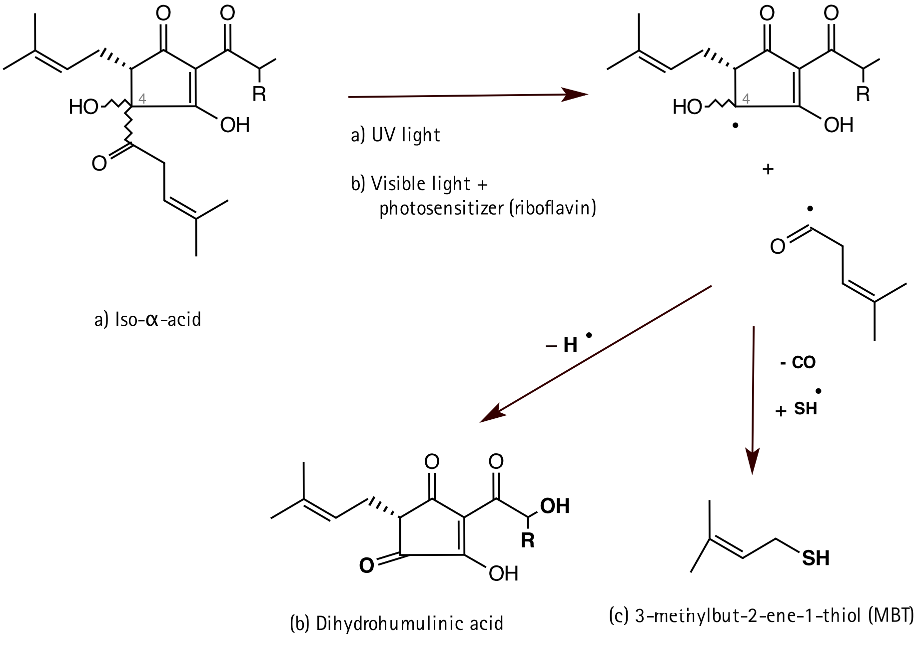


Fig. 2-11 Formation of "light struck" flavour of beer by exposure to light of iso-α-acids (Adapted from Van Cleemput 2009)

The light-sensitive chromophore in the isohumulones is the acyloin group composed of the tertiary alcohol function at C(4) and the carbonyl group of the side chain at C(4). Activation with UV light causes bond cleavage by a Norrish Type I reaction, leading to a ketylacyl radical pair. Subsequent loss of carbon monoxide from the acyl radical and recombination of the resulting fragment with a thiol radical yield 3-methylbut-2-ene-1-thiol (b), known as 'skunky thiol' or MBT and dehydrohumulinic acid(c) (Fig. 2-8) (Heyerick *et al.*, 2005, Huvaere *et al.*, 2006). The reaction is initiated by riboflavin (vitamin B_2) as a photosensitizer, since iso-α-acids do not absorb light in the visible region.

Autoxidation. Iso-α-acids easily decompose due to autooxidation reactions which occur in contact with oxygen. In beer brewing, they survive the wort-boiling step to a large extent although a number of oxidised products can be detected in low quantities.

These products were thought to be produced in a stepwise transformation to allo-iso-α-acids, hydrated allo-iso-α-acids, acetylhumulinic acids and finally humulinic acids (Verzele et al., 1991). Traces of humulinic acid in beer appeared to be a confirmation of this (Verzele et al., 1973). The stability of iso-α-acids in beer during storage, protected from the light, was then studied. Hughes et al. (1997) analysed commercial lager kept at 250C and 400C during 3 months observing a decrease of the concentration of iso-α-acids only at the higher temperature and particularly of the *trans*-isomers. Later studies, performed on selected high quality top-fermented and lager beer kept in different conditions and periods of storage yielded slightly different results. In this case, De Cooman et al. (2000) observed that while the concentration of *cis*-iso-α-acids decreased less than 10% after 15 months, the decrease of *trans*-iso-α-acids concentration was pronounced. This difference was more dramatic in the case of higher temperatures where the trans/cis ratio was observed to decrease from 47 to 11% in 12 months at 25 °C and from 48 to 17% in just 10 days at 60 °C. They also compared the behaviour of beer that contained tetrahydro-iso-α-acid derivatives -in which the C(4) side-chain is reduced- finding that these showed a good stability throughout the same storage times and temperature. As an explanation to this, the authors hypothesized that this difference in stability was associated to a greater propensity of *trans*-iso-α-acid to autoxidation due to the *cis*-configuration of the side-chains at C(4) and C(5), which created a region of electrondensity, allowing thus the ready initiation of the oxidation reactions (Fig. 2-12).

Studies published recently, however, reported the identification of previously unidentified compounds that result from the autoxidation of iso-α-acids to produce *cis*- and *trans*-hydroxy-peroxyalloisocohumulone, hydroxy-peroxyalloisohumulone, hydroxyperoxyalloisoadhumulone and *cis / trans*-hydroxy-alloisocohumulone, *cis/trans* -hydroxy-alloisohumulone and cis/trans - hydroxy-alloisoadhumulone (Intelmann et al., 2010). Quantitative determination of these oxidation products showed that both cis/trans iso-α-acids were equally sensitive.

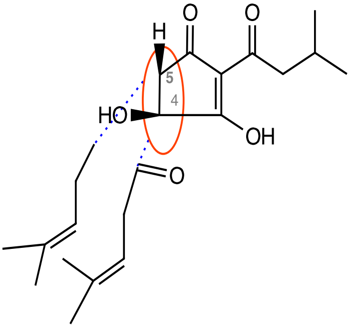
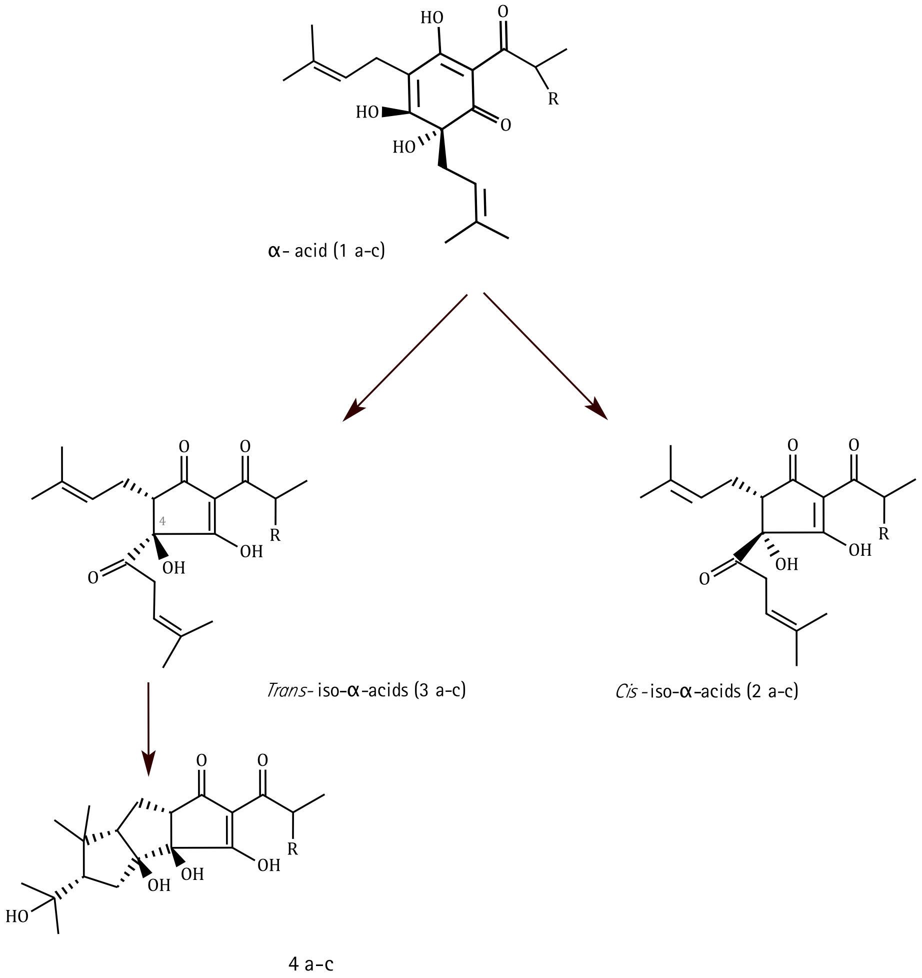


Fig. 2-12: Trans-iso-α- acid C4-OH configuration results in cis - configuration of C4 and C5 side -chain (De Cooman et al., 2000)

Proton-catalysed cyclisation. The two reactions described above could not account, thus, for the difference in stability exhibited by *cis*- and *trans*- iso-α-acids during beer storage in light-protected cans or bottles, reflected in a decrease of bitterness and the appearance of a harsh, lingering bitter after-taste. Intelmann et al. (2009a) published results obtained with model experiments followed by LC-MS/MS and NMR analysis, including the measurement of residual dipolar couplings (RDCs) in gel-based alignment media as well as a novel broadband and B(1)-field-compensated incredible natural abundance double-quantum transfer experiment (INADEQUATE) pulse sequence, which allowed the the identification of a series of previously unknown *trans*-specific iso-α-acid transformation products: tricyclocohumol, tricyclocohumene, isotricyclocohumene, tetracyclocohumol, and epitetracyclocohumol. Among these previously unreported molecules, tricyclocohumol, tricyclohumol and tricycloadhumol were found as the quantitatively predominant reaction products generated from *trans*- iso-α-acids both in isolated iso-α-acids and beer. proposing as a mechanism the formation of a proton-catalysed carbon/carbon bond between the carbonyl atom C(1') of the isohexenoyl moiety and the alkene carbon C(2'') of the isoprenyl moiety of the *trans*-iso-α-acids (Fig.2-13) (Intelmann et al., 2009 b).

In further studies, the researchers confirmed the reaction to be oxygen-independent and *trans*-specific, proposing as a mechanism the formation of a proton-catalysed carbon/carbon bond between the carbonyl atom C(1') of the isohexenoyl moiety and the alkene carbon C(2'') of the isoprenyl moiety of the *trans*-iso-α-acids (Fig.2-13) (Intelmann et al., 2009 b).



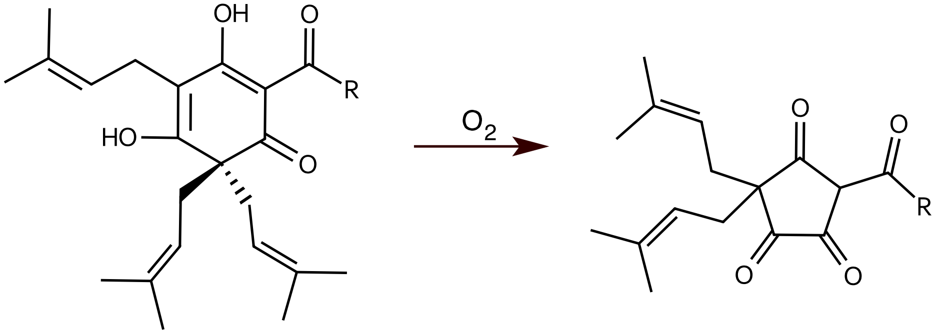
R (a)= $CH(CH_3)_2$ (a)
R (b)= $CH_2CH(CH_3)_2$ (b)
R (c)= $CH(CH_3)CH_2CH_3$ (c)

Fig. 2-13 Thermal isomerisation of the α-acids cohumulone (1a), humulone (1b), and adhumulone (1c) to give the diastereomeric cis- and trans-isocohumulone (2a and 3a), isohumulone (2b and 3b), and isoadhumulone (2c and 3c) and proton-catalyzed degradation of the trans isomers 3a-c to give the tricyclic products tricyclocohumol (4a), tricyclohumol (4b), and tricycloadhumol (4c), respectively.

The effect of all these compounds on beer taste was recently evaluated by the same group of researchers through a sensomics-based profiling of beer stored in different conditions. They determined that the most abundant products of degradation were the tri- and tetracyclic compounds derived from the proton-catalysed cyclisation of *trans*-iso-α-acids, which provided a harsh, lingering bitter taste (Intelmann et al., 2011). This reaction was shown to be dependent on the pH and temperature, occurring at much higher rates at low pH values and higher temperature. It was thus clear that while *cis*-iso-α-acid degradation in beer could be minimised by storage in light and in air-tight cans or bottles, the degradation of *trans*-iso-α-acids could be delayed by controlling the pH of beer during its brewing or storing at low temperatures, but could not be avoided.

The β-acids, on the other hand react quite differently to α-acids. Most importantly, they do not undergo isomerisation because they lack the tertiary alcohol function on C6. They do, however, suffer a series of oxidations that are often present in beer (Verzele et al., 1991). β-acids have a function as a foam stabilizer, suppressing the gushing of the beer and contributing to bacteriological stability. In 1991, Rybacek reported that β-acids are less efficient than α-acid in terms of quantity of bitterness, but produce " a gentle and harmonious bitterness".

Therefore, more than 80% of all hop-derived components in beer are iso-α-acids. They account for the typical bitter taste of beer and apart from the foam enhancing/lacing activities associated to their tensioactive properties, they protect beer against microorganisms (Simpson et al. 1992, Blanco et al., 2007).



β- acids

Hulupone

Fig. 2-12 Oxidation of β- acids to hulupones

2.4 Contribution of individual iso-α-acids to beer quality

Bitterness is one of the most important quality attributes of beer, contributing undoubtedly to its typical taste. As was discussed previously, the bitterness of beer is produced exclusively by iso-α-acids which are produced during the wort-boiling process from the α-bitter acids of hops added at that point of the brewery process, or directly further on in the process as pre-isomerised products. The concentration of these iso-α-acids can range between 5 and 50 mg/L (Verhagen, 2010).

Another important attribute of beer is foam. While foam stabilisation is undoubtedly due to the presence of foam-active polypeptides, iso-α-acids are known to enhance foam stability. Other features of foam, such as the lacing and cling that is characteristic of so many beers, will only occur if iso-α-acids are present (Hughes, 2000).

Thus, the importance of iso-α-acids in general in the bitterness and foam stability of beer has been fully established (Bamforth, 2000; Briggs *et al.*, 2004). Individual iso-α-acids, while similar, exhibit some differences in their chemical behaviour, a fact that was suspected might lead to differences in their organoleptic characteristics or other parameters such as their foam lacing or stabilising properties among others. In the case of foam, tests with the individual iso-α-acids demonstrated that larger quantities of isocohumulones (on a molar basis) were required to stabilise model foams than their less polar isohumulone and isoadhumulone counterparts. There appeared to be, however, little difference in the relative abilities of either of the *cis*- and *trans*- pairs to stabilise model foams (Hughes, 2000).

In other aspects, such as bitterness and stability, differences in the behaviour of the *cis*- and *trans*- series were evident, very probably based on their different hydrophobicity, acidity, stability and certain complexation capacity.

Hydrophobicity: this term refers to the affinity that a compound has with water and is usually inversely related to its lipophilic nature or affinity with non-polar solvents. All iso-α-acids are known to be hydrophobic, but it is important to have some type of measurement of their relative hydrophobicity. Hughes estimated this by association of the compounds with their relative retention volume in a reverse phase HPLC system and by comparison of their octan-1-ol/phosphate buffer partition coefficients (Table 2-5).

As can be observed, *cis*-iso-α-acids are less hydrophobic or more lipophilic than their *trans*- counterparts in general and also within each pair of isomers. In the case of hydrophobicity, especially, there is research that supports a direct relationship between this characteristic and bitterness (Gardner, 1978,1979).

Table 2-5: Measure of hydrophobicity for iso-α-acids

	Partition coefficient[a]	Capacity factor (k´)[b]
Trans –iso-α-acids	1.99	
Cis-iso-α-acids	2.23	
Cis-isocohumulone		2.93
Trans-		3.14
Cis-isohumulone		3.51
Trans-isohumulone		4.02
Cis- adhumulone		5.46
Trans-adhumulone		5.83

a) Octan-1-ol /aqueous phosphate buffer (pH= 5.0); b) C18 NovaPak 100 x 8 mm; mobile phase: methanol: water: o-phosphoric acid (30:70: 0.025)(v/v); flow: 2.0ml/min

Acidity: Iso-α-acids are considered as such due to their appreciable proton-donor properties. In this case, no major differences were found between the isomer pairs, but is expected that there is some difference in acidity, though it was not possible to find any reference for pKa values of all isomers. Simpson (1993) obtained an approximate value of pKa= 3.1 for *trans*-isohumulone. The work carried out by us later, in which isomers were separated using pH-refining zone CPC would indicate that there is some, albeit, slight difference in acidity of the isomers and that the order is: *trans*-isocohumulone > *cis*-isocohumulone > *trans*-isohumulone> *cis*-isohumulone> *trans*-iso-adhumulone > *cis*-isoadhumulone.

Bitterness: As flavour and stability of the flavour constitute a major quality issue in beer, a great deal of research has been done in order to establish the role of each iso-α-acid. There was a sort of consensus that isohumulone was bitterer, establishing a preference for humulone-poor hop products, but there was no real science to back this idea. In order to shed some light on this issue, Hughes (2000) carried out a psychophysical test with four of the six major iso-α-acids that he isolated – the *cis*- and *trans*- isomers of isocohumulone and isohumulone obtained using a preparative HPLC method (Hughes, 1996). Tests could not be carried out with *trans* /*cis*-iso-adhumulone owing to the difficulty in collecting sufficient amounts of pure compounds. Solutions of the pure isomers in sodium phosphate buffer and ethanol were used and the results allowed them to conclude that in these conditions, the bitterest compound was *cis*-isohumulone, while *trans*-isocohumulone was the least bitter, i.e. *cis*-isohumulone > *trans*-isohumulone ≈ *cis*-isocohumulone > *trans*-isocohumulone. In view of the confirmation of notable differences between the different iso-α-acid isomers, in most aspects related to the quality and stability of beer, it was necessary to carry out further research on all aspects related to these qualities.

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- REFERENCES

- **Arnold JP** (2005). [1911]- Origin and History of Beer and Brewing: From Prehistoric Times to the Beginning of Brewing Science and Technology. Cleveland, Ohio: Beer Books.
- **Asano K, Hashimoto N** (1976). Contribution of hops bitter substances to head formation of beer. Report of the Research Laboratories of Kirin Brewery Company Ltd: Vol. 19 (pp. 9–16).
- **Behre KE** (1999). The history of beer additives in Europe - a review. Veget Hist Archeobot 8:35–48.
- **Benitez JL, Forster A, De Keukeleire D, Moir M, Sharpe FR, Verhagen LC, Westwood KT** (1997). Hops and Hop Products, Verlag Hans Carl, Nuremberg, Germany.
- **Bamforth CW** (2000). Brewing and brewing research: past, present and future. J Sci Food Agric, 80: 1371–1378
- **Blanco CA, Rojas A, Caballero PA, Ronda F, Gómez M, Caballero** (2006). A better control of beer properties by predicting acidity of hop iso-α-acids. Trends Food Sci Technol, 17, 373-377.
- **Bowe J, Li XF, Kinsey-Jones J, Heyerick A, Brain S, Milligan S, O'Byrne K** (2006). The hop phytoestrogen, 8-prenylnaringenin, reverses the ovariectomy-induced rise in skin temperature in an animal model of menopausal hot flushes. J Endocrinol, 191(2): 399–405.
- **Briggs DE, Boulton CA, Brookes PA, Stevens R.** (2004). Brewing: Science and Practice; Woodhead Publishing: Cambridge UK (ISBN 1855734907)
- **Bruneton J** (1999). Pharmacognosy, Phytochemistry, Medicinal Plants; Lavoisier Publishing: Paris, 1999; pp 455-456.
- **Burgess AH** (1964). Hops: Botany, Cultivation, and Utilization. Interscience Publishers Inc. New York.
- **Chadwick RL, Pauli GF, Farnsworth NR** (2006). The pharmacognosy of Humulus lupulus L. (hops) with an emphasis on estrogenic properties. Phytomedicine. 13(1-2): 119–131
- **Charters ML** (2006). Latin and Greek Meanings and Derivations. In: calflora –California Plant Names.

- **Combes RP** (1998). The Brewer, 29–35.
- **De Cooman L, Aerts G, Overmeire H, De Keukeleire D (2000).** Alterations of the profiles of iso-α-acids during beer ageing marked instability of *trans*-iso-α-acids and implications for beer bitterness consistency in relation to tetrahydroiso-α-acids. J Inst Brew, 106, 169–178.
- **De Keukeleire D, Verzele M** (1970). The structure and absolute configuration of (-) humulone. Tetrahedron 26:385-393.
- **De Keukeleire D, Verzele M** (1971). The absolute configuration of the isohumulones and the humulinic acids. Tetrahedron, 27, 4939–4945
- **De Keukeleire D, Vindevogel J, Szücs R, Sandra P** (1992). The history and analytical chemistry of beer bitter acids. TrAC, 11, 8., 275, 280
- **De Keukeleire D, De Cooman L, Rong H** (1999). Functional properties of hop polyphenols. In Gross, et al, Eds. Plant polyphenols 2: chemistry, biology, pharmacology, ecology. New York, Kluwer Academics/Plenum Publishers-pp. 739–760
- **De Keukeleire D** (2000). Fundamentals of Beer and Hop Chemistry. Química Nova, 23(1)
- **De Keukeleire D** (2003). Beer, hops and health benefits. Scan Brew Rev, 60(2): 10-17.
- **Duke JA** (2007). Handbook of Medicinal Herbs; CRC Press: Boca Raton, FL, pp 233-234.
- **Ebadi M** (2006). Pharmacodynamic Basis of Herbal Medicine; CRC Press: Boca Raton, FL, p 96.
- **Enari TM 1995). One Hundred Years of Brewing Research. J Inst Brew.- Centennial Edition.**
- **Eri S, Khoo BK, Lech J, Hartman TG** (2000). Direct thermal desorption-gas chromatography and gas chromatography- mass spectrometry profiling of hop (Humulus lupulus L.) essential oils in support of varietal characterization. J Agric Food Chem 48:1140–1149.
- **FAO-ECOPORT**: http://ecoport.org/perl/ecoport15.pl?searchType=entityDisplay&entityId=6779
- FAOSTAT (2008): http://faostat.fao.org/site/339/default.aspx
- **Gardner RJ** (1978). Lipophilicity and bitter taste. J Pharm Pharmacol, 30: 531–536.
- **Gardner RJ** (1979). Lipophillicity and Perception of Bitterness. Chem Senses 4 (4): 275-286
- **Gerhauser C, Alt A, Heiss E, Gamal-Eldeen A, Klimo K, Knauft J, Neumann I, Scherf HR, Frank N, Bartsch H & Becker H** (2002a). Cancer chemopreventive activity of Xanthohumol, a natural product derived from hop. Mol Cancer Ther 1: 959–969.
- **Gerhauser C, Alt AP, Klimo K, Knauft J, Frank N, Becker H** (2002b). Isolation and potential cancer chemopreventive activities of phenolic compounds of beer. Phytochem Rev, I, 369-377.
- **Goldstein H, Rader S, Murakami AA** (1993). Determination of 3-methyl-2-butene-1-thiol in beer. J Am Soc Brew Chem, 51, 70–74.
- **Gorissen H, Bellink C, Vancraenenbroeck R, Lontie R** (1968). Separation and identification of (+)-gallocatechine in hops. Archives Internationales de Physiologie et de Biochimie 76, 932–934.

- **GRIN** (2011)-USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network - (GRIN) [Online Database]. National Germplasm Resources Laboratory, Beltsville, Maryland. .
URL: http://www.ars-grin.gov/cgi-bin/npgs/html/stdlit.pl?Syst%20Bot (22 April 2011).
- **Greuter W, Mcneill J, Barrie FR, Burdet H-M, Demoulin V, Filgueiras TS, Nicolson DH, Silva PC, Skog JE, Trehane P, Turland NJ, Hawksworth DL** (2000). Regnum Vegetabile Vol. 138.International Code of Botanical Nomenclature (St. Louis Code) adopted by the Sixteenth International Botanical Congress St Louis, Missouri; July–August 1999; Königstein: Koeltz Scientific Books.
- **Gunst F, Verzele M** (1978). On the sunstruck flavour of beer. J Inst Brew, 84, 291-292.
- **Hermans-Lokkerbol ACJ, Verpoorte R** (1994). Preparative separation and isolation of three α- bitter acids from hop, Humulus lupulus L., by centrifugal partition chromatography. J Chromatogr A, 664 (1), 45-53.
- **Hermans-Lokkerbol ACJ, Hoek AC, Verpoorte R** (1997). Preparative separation of bitter acids from hop extracts by centrifugal partition chromatography. J Chromatogr A, 771, (1-2) 71-79
- **Hampton R, Nickerson G, Whitney P, Haunold A** (2002). Comparative chemical attributes of native North American hop, Humulus lupulus var. lupuloides E. Small. Phytochemistry, 61:855–862.
- **Haunold A, Nickerson GB, Gampert U, Whitney PA, Hampton RO** (1993). Agronomic and quality characteristics of native North American hops. J Am Soc Brew Chem, 51, 133–137.
- **Heyerick A, Zhao Y, Sandra P, Huvaere K, Roelens F, De Keukeliere D** (2003). Photolysis of hop-derived *trans*-iso-α-acids and *trans*-tetrahydroiso-α-acids: product identification in relation to the lightstruck flavour of beer. Photochem Photobiol Sci, 2, 306–314
- **Heyerick A, Huvaere K, De Keukeleire D, Forbes MDE** (2005). Fate of flavins in sensitized photodegradation of isohumulones and reduced derivatives: studies on formation of radicals via EPR combined with detailed product analyses. Photochem Photobiol Sci, 4, 412–419
- **Huvaere K, Olsen K, Andersen ML, Skibsted LH, Heyerick A, De Keukeleire D** (2004). Riboflavin-sensitized photooxidation of isohumulones and derivatives. Photochem Photobiol Sci, 2004,3, 337–340.
- **Huvaere K, Andersen ML, Storme M, Van Bocxlaer J, Skibsted LH, De Keukeleire D** (2006). Flavin-induced photodecomposition of sulfur-containing amino acids is decisive in the formation of beer lightstruck flavor. Photochem Photobiol Sci-5, 961–969.
- **Hughes PS** (1996a). Behaviour of iso-α-acids and their chemically modified variants, Brewing Research International, BN 107084/Eur.Brew.Conv.Beer Foam Sub-Group Meet, 74-91

.

- **Hughes PS** (1996b). Preparative regime for the purification of bitter acids derived from hops (Humulus lupulus, L.). J Chromatogr A, 731, 327-330.
- **Hughes PS, Menneer ID, Walters MT, Marinova G** (1997). Proceedings of the 26th. Congress of the European Brewery Convention, Maastrictht, The Netherlands. (Oxford, England: Oxford University Press, 1997, p.231).
- **Hughes P** (1999). The lightstruck flavour problem. Cerevisia 24(2), 21–25.
- Hughes PS (2000). The Significance of Iso-α-Acids for Beer Quality Cambridge Prize Paper. J Inst Brew, W6, 5
- **IHGC (International Hop Growers Convention):** http://www.hmelj- giz.si/ihgc/doc/IHGC%20hop%20supply%202001-2006.pdf
- **Intelmann D, Kummerlöwe G, Haseleu G, Desmer N, Schulze K, Fröhlich R, Frank O, Luy B, Hofmann T** (2009a). Structures of storage-induced transformation products of the beer's bitter principle, revealed by sophisticated NMR and LC/MS techniques. Chem Eur J, 15, 13047–13058.
- **Intelmann D, Demmer O, Desmer N, Hofmann T** (2009 b). 18O stable isotope labeling, quantitative model experiments, and molecular dynamics simulation studies on the *trans*-specific degradation of the bitter tasting iso- α-acids of beer. J Agric Food Chem, 57 (22) 11014–11023.
- **Intelmann D, Hofmann T** (2010). On the Auto-oxidation of Bitter-Tasting Iso-α-Acids in Beer. J Agric Food Chem, 58 (8), pp 5059–5067.
- **Jaskula B, Kafarski P, Aerts G, De Cooman L** (2008). A Kinetic Study on the Isomerization of Hop r-Acids. J Agric Food Chem, 56, 6408–6415.
- **Kamimura M, Kaneda H (1992).** Off-flavors in Beer. In "Off- flavors in Foods and Beverages", Ed. Charalambous, G., Elsevier, Amsterdam, The Netherlands, pp. 433-472.
- **Kuroiwa Y, Hashimoto H** (1961a) Studies on hops with reference to their role in the evolution of sunstruck flavor of beer. Rep Res Lab Kirin Brew Co Ltd, 4, 35–40.
- **Kuroiwa Y, Hashimoto N** (1961b), Composition of sunst ruck flavor substance and mechanism of its evolution. Proc Am Soc Brew Chem, 28–36.
- **Kuroiwa Y, Hashimoto N, Hashimoto H, Kokubo E, Nakaganaf K** (1963) Actors essential for the evolution of sunstruck flavour. Proc Am Soc Brew Chem, 181-193
- **Meilgaard** M (1960). Hop Analysis, Cohumulone Factor and The Bitterness of Beer: Review and Critical Evaluation. J Inst Brew, 66 (1) 35-50
- **Malizia RA, Molli JS, Cardell DA, Grau RJA** (1999). Essential oil of hop cones (Humulus lupulus L.). J Essent Oil Res, 11, 13–15.
- **Milligan SR, Kalita JC, Heyerick A, Rong H, De Cooman L, De Keukeleire D** (1999). Identification of a potent phytoestrogen in hops (Humulus lupulus L.) and beer. J Clin Endocrinol Metab, 84:2249–2252.
- **Milligan SJ, Kalita V, Pocock A, Heyerick L, De Cooman H, Rong, De Keukeleire** (2002). Oestrogenic activity of 8-prenylnaringenin. Reproduction, 123, 235–242.

- **Mizobuchi S, Sato Y** (1994). A new flavanone with antifungal activity isolated from hops. Agric Biol Chem, 48:2771–2775.
- **Moir M** (1994). Hop Aromatic Compounds. In European Brewery Convention Monograph XXII - EBC-Symposium on Hops, Zoeterwoude, The Netherlands, May/June 1994, Verlag Hans Carl, Nuremberg, Germany, 1994, pp. 165-180.
- **Nelson M** (2005). The barbarian's beverage: a history of beer in ancient Europe. London: Routledge. p. 6
- **Ono M, Kakudo Y, Yamamoto Y, Nagani K, Kumada J** (1985). Simultaneous analysisof hop bittering components by High Performance Liquid Chromatography and its application to the practical brewing. J Am Soc Brew Chem, 43; 136-144.
- **Riedl W** (1952). Constitution and synthesis of hop bitter principles DL- humulone and lupulone and some analogues. Chem Ber 85:692- 710.
- **Riedl W** (1954). Bitter principles of hops. VI. The synthesis of lupulone analogues with varied acyl groups. Liebigs Ann Chem, 585:38-42.
- **Rigby FL, Bethune JL** (1952). Countercurrent distributionof hop constituents. Proc Am Soc Brew Chem, pp. 98-105.
- **Rigby FL, Bethune JL** (1953). Countercurrent distributionof hop constituents. The isolation and properties of cohumulone. Proc Am Soc Brew Chem, pp. 119-129.
- **Roberts RT** (1975). Some aspects of the stability of beer foam, SCI Symposium, Brunel University, SCI monograph (pp. 257–271). London: Academic Press.
- **Roberts RT, Wilson RJH** (2006). "Hops" in Handbook of Brewing, edited by Priest F.G & Stewart G. (2006) CRC- Francis and Taylor- USA.
- **Rybacek V** (1991). Hop Production, Developments in Crop Science 16, Elsevier, Amsterdam.
- **Sägesser M, Deinzer M** (1996). HPLC-ion spray-tandem mass spectrometry of flavonol glycosides in hops. J Am Soc Brew Chem, 54, 129–134
- **Simpson WJ, Smith ARW** (1992). Factors affecting antibacterial activity of hop compounds and their derivatives. J Appl Bact, 7:325-334,
- **Simpson WJ** (1993). Ionization Behaviour of Hop Compounds and Hop-Derived Compounds. J Inst Brew, 99, 317-326.
- **Simpson WJ, Hughes PS** (1993). Cooperative binding of potassium ions to *trans*-isohumulone in the presence of divalent and trivalent cations. Bioorg Med Chem Let, 3, 769-772.
- **Simpson WJ, Hughes PS** (1994). Stabilization of foams by hop-derived bitter acids. Cerevisiae Biotechnol 19(3), 39–44.
- **Small E** (1978). A numerical and nomenclatural analysis of morpho-geographical taxa of Humulus. Syst Bot 3(1): 37-76.
- **Stevens JF, Ivancic M, Hsu V, Deinzer M** (1997). Prenylflavonoids from Humulus lupulus. Phytochemistry, 44:1575–1585.

- **Stevens JF, Miranda CL, Buhler DR, Deinzer ML** (1998). Chemistry and biology of hop flavonoids. J Am Soc Brew Chem: 56:136–145.
- **Stevens JF, Taylor AW, Clawson JE, Deinzer ML** (1999a). Fate of xanthohumol and related prenylflavonoids from hops to beer. J Agric Food Chem; 47:2421–2428.
- **Stevens JF, Taylor AW, Deinzer ML** (1999b). Quantitative analysis of xanthohumol and related prenylflavonoids in hops and beer by liquid chromatography-tandem mass spectrometry. J Chromatogr A; 832:97–107.
- **Stevens JF, Taylor AW, Nickerson GB, Ivancic M, Henning J, Haunold A, Deinzer ML** (2000). Prenylflavonoid variation in Humulus lupulus: distribution and taxonomic significance of xanthogalenol and 4′-Omethylxanthohumol. Phytochemistry, 53:759–775.
- **Templar J, Arrigan K, Simpson JW** (1995). Formation, measurement and significance of lightstruck flavor in beer. A review. Brewers Dig, 70(5), 18-25.
- **Taylor AW, Barofsky E, Kennedy JA.** (2003). Hop (Humulus lupulus L.) proanthocyanidins characterized by mass spectrometry, acid catalysis, and gel permeation chromatography. J Agric Food Chem, 51, 4101–4110.
- **Van Cleemput M, Cattoor K, De Bosscher K, Haegeman G, De Keukeleire D, Heyerick A** (2009). Hop (Humulus lupulus)-Derived Bitter Acids as Multipotent Bioactive Compounds. J Nat Prod, 72, 1220–1230.
- **Van Hellemont J** (1993). Fytotherapeutisch Compendium; Bohn Stafleu van Loghum: Houten, The Netherlands; pp 302-305.
- **Verhagen LC** (2010). Beer Flavor. In Comprehensive Natural Products II Chemistry and Biology; Mander, L, Lui HW- Eds.; Elsevier: Oxford,2010; 3- 967-997.
- **Verzele M, Boven M** (1971). The isomerization mechanism of Humulone. Bull Soc Chim Belg, 80, 677-682.
- Verzele M (1979). The chemistry of hops. Brewing Science, Vol. 1. J. R. A. Pollock, ed. Academic Press, London.
- **Verzele, M, De Keukeleire D** (1991). Chemistry and Analysis of Hop and Beer Bitter Acids; Elsevier: Amsterdam.
- **Wieland H, Martz E, Hoek H** (1925). Chemical nature of the hop resin acids. Chem Ber, 58B: 2012-2017.
- **Yamada M, Suzuki T, Oho E, Matsushima H** (1998). Color scanning electron microscopy of peltate glandular trichomes of fresh developing hops (Humulus lupulus L.). J Electron Microscopy 47:539–542
- **Youngken, HW** (1950). Textbook of Pharmacognosy. Blakiston Company; Philadelphia: Humulus.
- **Zheng-yi W, Raven PH et al., Eds.** (1994)–. Flora of China (English edition) (F China Eng)).
- **Zuurbier KW, Leser J, Berger T, Hofte AJ, Schroder G, Verpoorte R** (1998). 4-Hydroxy-2- pyrone formation by chalcone and stilbene synthase with nonphysiological substrates. Phytochemistry, 49:1945–1951.