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Bitter is better

A review on the knowledge about bitterness in beer

Bitter is, or should be, a descriptive term for beers. What makes the beer bitter in the first place is the use of hops. The range of bitter units in beers used to be between 20 and 50 IBU (International Bitterness Units). Nowadays there is a very clear trend towards mild beers with bitter units between 10 – 25 and sometimes even lower. Originally used as a preservative in beer, newest findings show us what hop-derived substances offer in regard to health benefits. So there is hope that a new triumphant success of hops might go along with a new increase of bitterness in beer. Bitterness is a topic which is very important for all brewers and being a question of taste it can be discussed to everyone's taste. This article is to give a review on the knowledge about bitterness in beer and the challenges in sensory and analytical evaluation of bitterness.
The bitter taste reception is different from the reception of the other four taste qualities, which are sour, sweet, salty and umami, because evolution told us to be alert to bitter foods and rightly so since it can be equated with dietary danger. Rancid fats, hydrolysed proteins, plant-derived alkaloids, and toxins generally have an unpleasant bitter taste. Also microbial fermentation often results in bitter tasting compounds (1). But bitter flavours also contribute to the palatability and digestibility of food and beverages. Beer is not the only consumer good with the tendency of decreasing bitterness. Responding to taste-driven consumer demand of less bitter food, the food industry generally tries to remove bitter compounds like phenolic compounds, flavonoids, isoflavones, terpenes, and tannins from foods destined for human consumption. Because of such efforts, current food supply is less bitter than it might otherwise be (2, 3).

Descriptors: bitterness, taste perception, iso-alpha acids, bitter units, sensory

How does bitter taste perception work?

Bitter taste can be caused by more than 1000 different molecules of totally different structures. In this context, we are rather depauperate because we are not able to distinguish the differences in bitter tastes, though this is a biological advantage, because this way we are able to detect the bitter taste rapidly which saves us from poisoning (4).

For the taste reception we use our taste papillae, which contain the taste buds, which in turn contain hundreds of cells. Approx. 100 of those cells act as receptor cells, that actually give the signals of tasting one of the five taste qualities. At the apex of the taste bud, microvillar processes protrude through a small opening, the taste pore, into the oral milieu. The sense of taste has the role of a dietary watchdog, because each taste quality is related to physiological processes and will use different ways for signal transduction in form of electrical impulses (depolarisation). The bitter substances themselves do not cause the bitter taste, but activate the receptor molecules which are on the receptor cells' exterior. Those bitter receptor molecules are called T2R. 25 different T2R molecules have been identified so far (5, 6, 7). Each T2R receptor can respond to various bitter compounds. Neurotransmissions send the information through a complicated network of neurone fibres to the cerebral nerves. Most taste receptors cells respond to several taste qualities. Recent investigations describe the role of TRPM5 (transient receptor potential cation channel, subfamily M, member 5) in taste transduction. It plays a key role in the perception of sweet, umami and bitter tastes. It is a highly temperature-sensitive heat activated channel, leading to enhanced sweetness perception at high temperatures and "thermal taste", a phenomenon whereby heating or cooling of the tongue evoke sensations of taste in the absence of taste stimuli (8).

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Figures see Appendix

Recently an older variation of one of the T2R receptor genes was identified in various African regions, which is responsible for a relative insensitivity to bitter toxic cyanides. Its existence correlates with specific malaria resistance genes spread in the same regions, indicating that this insensitivity can save people from malaria infections (9). Figure 1 shows the bitter transduction pathway.

We have an inherent preference for sweet and umami and an inherent dislike for bitter and sour. The diet and our taste experiences during infancy are very likely to influence later preferences. In this context the breast milk is of particular importance. It contains sugars and glutamic acid (responsible for the umami taste) and other flavour compounds that will have an influence on later dietary preferences (11). A comparative study with newborn and older infants concluded that there has to be an early developmental change in bitter taste perception since the newborns did not reject bitter food whereas the older infants did (12). Still a preference for the bitter taste in coffee or beer has to be learned in adulthood. So far it is not known if individual perceptions correlate with dietary habits and body weight.

The bitterness perception is also related to age, as with increasing age the sensitivity will decrease; also the gender plays a certain role, as women were found to be in general more sensitive to primary taste stimuli (13, 14). The individual perceptions may diverge considerably. Subjects who are highly sensitive to one bitter compound can still be quite insensitive to another and salts of saccharin, which are intensively sweet, can have a bitter taste to other individuals, without being correlated to the taste perception of PROP (6-n-propylthiouracil) (15, 16). Depending on taste responsiveness the population is split into PROP (6-n-propylthiouracil, a bitter tasting substance) nontasters, normaltasters, and supertasters. PROP-tasters are very sensitive to bitter taste; they often dislike moderate bitter food as e. g. cabbage, spinach or beer (17).

How about bitter compound thresholds?

Bitter compounds, including extremely toxic bitter poisons, are detected by humans in micromolar amounts, although there is no

direct relation between toxicity and bitter taste thresholds. A threshold is the concentration of the respective compound that elicits a detectable sensory change. There are several methods for the determination of taste thresholds (e.g. EBC 13.9). The calculated threshold for a substance is the average threshold value which is supposed to be a Gaussian variable and which represents the actual threshold for 68% of the population (± standard deviation). A threshold determination strongly depends on the composition (pH value, alcohol content etc.) of the respective medium (mostly tap water), which explains the sometimes palpable differences in threshold values for one substance that can be found in different publications. Detection thresholds for bitter taste can be in the range of 25 µmol/L (for quinine). In contrast, the detection threshold for sucrose is on the order of 10 000 µmol/L (18, 19). The most bitter substance is 4-H-quinolizinium-7-olate. It is generated by a specific Maillard reaction and has a taste threshold of 0.25 µmol/kg (20)! A compound is referred to being "taste active" if the concentration of the compound in beer/ water etc. exceeds the threshold concentration (21). A evaluation of bitter tasting compounds can hence be carried out by calculating the Taste Activity values. For example, the taste threshold of iso-alpha acids is indicated with 5 mg/l. For a beer with 25 mg/l iso-alpha-acid content the Taste Activity value for iso-alpha acids in this beer is 5. It is obvious that this only roughly indicates the actual taste contribution for a substance, since all interactions with other taste qualities or possible synergetic and masking effects are totally neglected. It is also possible that substances which are present in concentrations below their threshold concentration may elicit a taste if they act additive in sharing the same taste perception mechanism.

Which compounds in beer can elicit a bitter taste?

Taste-perception-related research often focuses on substances as caffeine, denatonium benzoate, quinine, naringin etc. but seldom hop bitter acids or other bitter compounds present in beer are investigated.

There are various compounds present in beer that may taste bitter. Predominantly of course the iso-alpha-acid, or their reduced or hydrogenated forms. Furthermore there are some malt-based amino acids that exhibit a bitter taste like L-tyrosine, L-tryptophan, L-leucine, L-threonine, L-phenylalanine and other L-amino acids which are often associated with both bitter and sweet taste impressions (see fig. 2). Newer findings support the hypotheses that these amino acids share the same peripheral bitter taste mechanisms (19, 22). Likewise dipeptides and cyclic diketopiperazines are likely to give a bitter taste (23). Also Maillard reactions with the amino acid proline can produce bitter tasting substances, though it is not known if their concentration in beer can exceed the threshold limits (24, 25). Furthermore the brewing water may have an influence as $MgSO_4$ can give an unpleasant bitterness to beer, while $CaCO_3$ and $CaCl_2$ may increase and even improve the perceived bitterness in beer (26, 27). Potassium can give a bitter taste depending on its occurring salt form (28). Other studies have shown an influence on the bitter taste by sodium salts and sucrose (29).

Phenolic compounds derived from both hops and malt can give a bitter or astringent taste (30). Whereas lower-molecular-weight phenolic compounds (see fig. 3), such as catechin, epicatechin, quercetin etc. tend to taste bitter, higher-molecular-weight polymers are more likely to taste astringent (26, 31). Some results also indicate that the addition of e. g. catechin or the increase of the pH may enhance bitterness intensity (32), whereas the bitterness of phenolics can be reduced by sucrose and is substantially enhanced by ethanol (31). Certain health benefits may cause increasing contents of polyphenols in beer, food resp in the future. Plantbased phenols, flavonoids, isoflavones, terpenes, and glucosinolates are reported to have antioxidant and anticarcinogenic properties and a wide spectrum of tumor-blocking activities (34, 35, 36). Specific hop constituents account for bioactivities as sedative, antistress, soporific, estrogenicity, treatment of complaints related to the menopause and anticancer properties (e.g. xanthohumol) (37). Hops may contribute up to about one third of the total polyphenols in beer. The taste thresholds for xanthohumol and isoxanthohumol were recently determined and were found to be 0.9 mg/l for xanthohumol, having an astringent taste and 0.5 mg/l for isoxanthohumol, tasting bitter (38) (see fig. 4). But contents of xanthohumol in beers exceeding 0.9 mg/l are rather unlikely unless xanthohumol-enriched products are used. The addition of gallotannin and xanthohumol also proved to influence the bitterness in beer positively (finer, more harmonic) (39, 40). It was also shown that the addition of flavonol glycosides and prenylated hop flavonoids to beer influenced the mouthfeel and improved flavour stability (41).

One of the bitter tasting substances produced during fermentation is tyrosol. The taste threshold is indicated with 20 mg/l and is likely to be exceeded in top-fermented beers (42). Also trihydroxy fatty acids that can be present in beer may exhibit a bitter and astringent taste (43).

The perceived bitterness of binary bitter compound mixtures proved to be an additive function, synergetic effects have not been detected for bitter substances so far, and are reported to be rather unlikely (44).

What do we know about the bitterness perception of iso-alpha acids?

We know that iso-alpha acids are intensely bitter, even more than quinine (approx. 14 µmol/l) (45). By saying "Iso-alpha acids" we encounter a complicated issue, since there are 6 different isomers and homologues of Isohumulones (*cis/trans*-isohumulone, *cis/trans*-isocohumulone and *cis/trans*-isoadhumulone) (see fig. 6). They differ only in the nature of the saturated acyl side chain and the absolute configuration of one of the chiral centres but still they have different sensory properties. *Hughes* found that bitterness intensity increases with a higher dose of isohumulones but the intensity increase is very dependent on the individual bitterness perception. The bitterness intensity proved to be in the following order: *cis*-isohumulone > *trans*-isohumulone ≈ *cis*-isocohumulone > *trans*-isocohumulone. The *cis* component is significantly more bitter than the *trans* counterpart, and isohumulones are more bitter than isocohumulones (46, 47, 48). Those studies did not find any evidence indicating that isocohumulones have neither a poorer bitterness quality nor a harsher more lingering bitterness, which is often stated.

In addition to the iso-alpha acids, derived from traditionally brewed beer, a lot of breweries use purified hop bitter acids, as e. g. tetrahydro-iso-alpha acids, rho(dihydro)-iso-alpha acids and hexahydro-iso-alpha acids (see fig. 7). One should guess that the different sensory properties of the individual iso-alpha acids also apply for modified iso-alpha acids. But scientific data on this subject is very rare. Due to their chemical modification by hydrogenation and/or reduction those acids differ in their structures and the production process. Rho-iso-alpha acids can be present in 12 different structures since for each of the 6 iso-alpha acids, the creation of a new chiral centre forms two epimeric molecules. This also applies for hexahydro-iso-alpha acids, although complexity can be controlled by reaction conditions (49). The relative bitterness of the general

compounds from least bitter to most bitter is reported to be: rho-iso-alpha acids, iso-alpha-aicds, hexahydro-iso-alpha acids and tetrahydro-iso-alpha acids. The corresponding bitterness factors, relative to the iso-alpha acids indicated with 1.0 are reported to be 0.7 – 0.8 for rho-iso-alpha acids, 1.1 for hexahydro-iso-alpha acids, and 1.0 – 1.7 for tetrahydro-iso-alpha acids (50, 51, 52, 53). The wide range of the factor for the bitterness of tetrahydro-iso-alpha acids can be attributed to different bitterness perceptions, a bitterness saturation effect in higher concentrations and a dependency on the beer composition (19). Considering the different sensory properties of each iso-alpha-acid structure it is questionable how reliable bitterness factors actually can be. Using these factors one has to note all the above mentioned possible interactions of bittering compounds and the bitter perception deficiencies. The rather unknown class of anti-iso-alpha acids are reported to be twice as bitter as the isohumulones, and could therefore be capable to exceed the bitterness of the tetrahydro-iso-alpha acids (50).

Using the TI (Time Intensity) method the bitter taste caused by tetrahydro-iso-alpha acids proved to have a prolonged after taste, whereas the aftertaste of dihydro-iso-alpha acids was shown to be shorter compared to iso-alpha acids (54). In contrast to these findings are the results of a psychophysical experiment on the question whether isohumulones share a common receptor mechanism with other bitter compounds, and whether parotid saliva flow affects perception of their bitterness. These findings suggest that iso-alpha acids and tetrahydro-iso-alpha acids may share a common receptor mechanism with other bitter compounds since iso-alpha acids and tetrahydro-iso-alpha acids displayed a unique dome-shaped Time Intensity profile. The bitterness of the two compounds took longer to develop, but it lasted as long as for other bitter stimuli. No significant correlation was found between saliva flow and maximum intensity or total duration of bitterness (55).

The isomeric ratio depends on the hop variety and the production process. The content of humulone and cohumulone is quite specific for every hop variety, and will also result in different ratios and therefore different bitterness in the beer. In the case of hop products prepared by magnesium-mediated, solvent-free isomerisation of CO_2 hop extract, the ratio of *cis*- to *trans*- isomers is approximately 80:20. But beers bittered with traditional hop products will have a *cis*- to *trans*- ratio around 68:32. As the bitterness of the stereoisomers and congeners of hop bitter acids differ, precise control of beer bitterness via measurements of each iso-alpha-acid is desirable but not feasible for every brewery. Figure 5 shows a surface plot for perceived bitterness as it relates cohumulone content to hop processing conditions (*cis*- to *trans*- ratio) (56).

In regard to the properties of oxidative decomposition *trans*-iso-alpha acids are more prone to oxidation in ageing beer than *cis*-iso-alpha acids, and the corresponding *trans/cis* iso-alpha acids ratio is useful to monitor and evaluate bitterness deterioration. In contrast with conventional iso-alpha acids, tetrahydro-iso-alpha acids proved to be extremely resistant to oxidative decomposition (57).

The perceived bitterness of iso-alpha acids is also influenced by adaptation. In general, duration, maximum intensity, and time to maximum intensity of bitterness increase upon repeated beer ingestion, and perceived bitterness intensity increases with concentration of iso-alpha acids and sample size (58). A further influence is caused by the ethanol content in the beer. An increased bitterness intensity due to a higher ethanol content was shown for reduced and hydrogenated hop bitter acids. In case of identical amounts of tetrahydro-iso-alpha acids an increase from 1% to 7% (v/v) alcohol content proved to enhance the perceived bitterness by about 35% and in case of identical amounts of dihydro-iso-alpha acids the perceived bitterness even increased by about 130% (53).

Obviously there are inconsistent views on the bittering power of the individual iso-alpha acids, which may also be attributed to differences in purity of the compounds that have been used in those taste trials. Purification of iso-alpha acids is particularly difficult and reported results should always be interpreted with great caution (45).

Summarizing other psychophysical studies it was found that there might be a correlation between the PROP and iso-alpha acids sensitivity. Supertasters rated Pilsner Urquell significantly more bitter than medium tasters and supertasters reported consuming significantly less beer than nontasters when drinking beer on a regular basis (59). The relationship between bitterness intensity and the content of iso-alpha acids using partial least-square regression proved to have a correlation value of 0.92 exclusively working with normal tasters (60). It is also given the statement that PROP supertasters may to some degree be protected against alcoholism by their dislike for bitter substances (59). By a comparison of persons with low and high beer consumption it was concluded, that the intake of beer is not a major determinant of taste responses to the bitterness of isohumulone (61).

There is some very interesting data on the taste perception of iso-alpha acids and it clearly shows that with every new result a lot of new questions arise.

What are suitable analytical methods to reflect sensory evaluations of hop bitter acids in beer?

Bitterness in beer is quantified using International Bitter Units (IBU). The big challenge has always been, and still is, to find an analytical method to correctly reflect our sensory evaluation on bitterness, despite the deficiencies of sensory bitterness evaluation itself. It is obvious, that we are facing a very complex issue.

The IBU method measures the light absorption at 275 nm by an iso-octance extract of an acidified beer sample which is multiplied by the empirical factor 50. Since this analysis is unspecific also compounds unrelated to bitterness can be detected, whereas other substances that contribute to bitterness are not detected. The factor 50 is based on the assumption that 70% of the absorbance reading is caused by iso-alpha acids and the non-iso-alpha acids to the remainder. Therefore the bitter units determination is easy to carry out, but it does not always really tell if a beer is bitter or not. For instance a misleadingly high IBU value is obtained when deteriorated hops are used though the resulting beer will taste far less bitter than measured. Brewing with pure iso-alpha acids, the factor has to be changed and still the use of different factors (see Table 1) is a compromise and specific analysis (HPLC) should be preferred.

The discrepancy is obvious and has been investigated twenty years ago, trying to establish correlations between sensory bitterness

Table 1 Absorptivity and bittering factors of pure bittering agents in Isooctane at 275 nm (62)

Pure acid	E 1cm 1% (Absorptivity)	Factors
Iso-alpha acids	285	70.2
Tetrahydro-iso-alpha acids	275	72.7
Hexahydro-iso-alpha acids	245	96.1
Dihydro (rho)-iso-alpha acids	276	72.5

evaluation and the measurement of IBU values (63).

HPLC Analysis is the appropriate way to determine the exact content of iso-alpha acids and its isomers and homologues, suitable HPLC applications have already been published (64, 65). But bitterness measurement should also include all contents of other bitter tasting compounds. Provided that we know all substances in beer that elicit a bitter taste we would at least be able to quantify the total amount of bitter tasting substances. The related bitterness intensity would still be subject to personal perception in context to individual taste thresholds and possible interactions with other compounds or taste qualities.

Of course for beers with a lower bitterness the control of the concentration of the bitter acid content has to be more precise as for a beer with a higher bitter acid content. With the trend towards weakly bittered beers this is one of today's challenges in bitterness control. Figure 8 illustrates the "Just Noticeable Difference (JND) concept of Fechner. With the decreasing concentration of the hop bitter acids, the ability of humans to distinguish between similar concentrations increases until the detection threshold is reached. Therefore a lower bitterness level in mainstream beers will need a greater control over bitterness (66).

There are other promising approaches for the measurement of bitterness with a better reliability. For instance the use of multichannel taste sensors („electric tongue") consisting of eight electrodes made of lipid/polymer membranes. The sensor output (S), the change of membrane potential caused by adsorption, corresponding to aftertaste (C), and the ratio C/S are measured. This method shows a very good correlation with human gustatory sensation (67). A similar approach is the adsorption and desorption of beer and coffee on a lipid membrane simulating the bitter reception of the tongue. The measurement of bitter intensities and durations showed good correlation to sensory experiments (68, 69).

Conclusion

The bitter taste perception has only recently started to be understood more clearly but there are still a lot of open questions to be answered since so many different structures are involved in the bitter taste. Predominantly bitter in beer (still) are the hop bitter acids, but other bitter substances such as amino acids, dipeptides, Maillard products, polyphenols and salts in beer can also contribute to the bitter taste. There is no coherent data on taste interaction with other taste qualities nor evidences for additive, synergetic, suppression or masking effects, though synergetic effects are reported to be rather unlikely in context of bitter taste perception.

There are very promising approaches to understand bitterness perception elicited by isohumulones but by far not enough. We know that the different isomers and homologues, and also the stereoisomers, have different bitter properties and that the contents of homologues, isomers and stereosiomers depend on the hop variety, the hop processing, the hop product and the brewing process. Since all homologues, isomers and stereoisomers exhibit different stabilities in beer in regard to deterioration, bitterness in beer is a continuously changing attribute; a true nightmare for the quality assurance, furthermore all other factors contributing to flavour stability being unaccounted in this context. In terms of purified and modified hop bitter acids as tetrahydro-iso-alpha acids, bitterness factors are helping to determine the bitterness influence but can only be an approximation.

The determination of bitter units is a routine analysis and very helpful with corresponding experience. Advanced hopping with purified hop bitter acids requires a different understanding and adapted empirical factors are only a compromise in regard to their IBU measurements. Bitterness control with HPLC analysis is far more effective though a major financial burden for a brewery. It holds the possibility to determine all isomers, stereoisomers and homologues of iso-alpha acids. But other non-hop derived bittering substances in beer should be identified and included as well. The lower the bitterness levels get, the more important is a precise measurement of bitterness intensity in beer.

It is shown that bitterness in beer is a very complex issue and has to be seen from a bifocal perspective, the psychophysical perspective with ongoing findings about taste perception and taste interactions on one side and on the other side suitable analytical means to determine bitterness in relation to individual sensory perceptions. The non-linear relationship between bitterness intensity and concentration of active bittering compounds, together with yet poorly understood sensory interactions of bitterness with other beer taste qualities are further challenges to be first understood and then included in bitterness determinations. As soon as we understand how hop-derived bitter acids and all other bitter tasting substances in beer elicit sensory bitterness, we will more precisely know how to create a detection system that is related to the way in which we really do taste, because flavour control is and will increasingly be of utmost importance to the brewing industry.

This review shows that interdisciplinary research projects are in demand to adequately approach the complexity of bitter taste. Maybe evolution will bless us in the course of future generations with sophisticated bitter perception in order to differentiate bitter tastes and bitter intensities more precisely in order to ease future sensory research.

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Appendix

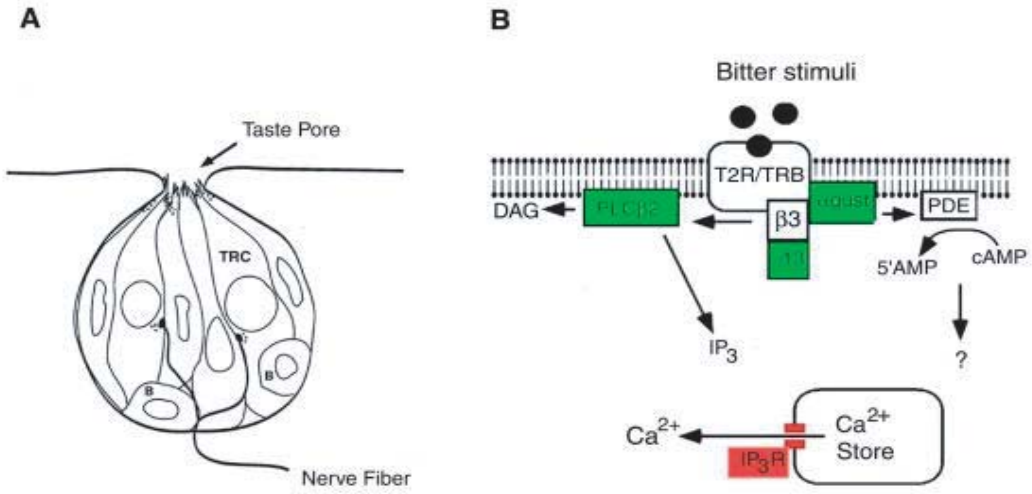

Fig. 1 **Rodent taste bud and important components of the bitter transduction pathway. (A) A typical taste bud consists of 50 100 taste receptor cells (TRCs) that extend from the basal lamina to the taste pore. Taste stimuli interact with taste receptors on the apical membrane, while nerve fibers form chemical synapses with the basolateral membrane. Basal cells along the margin of the taste bud are proliferative cells that give rise to taste receptor cells. (B) Bitter stimuli interact with T2R/TRB receptors located on the apical membrane. These receptors couple to a heterotrimeric G protein consisting of α-gustducin, β3, and γ13. Alpha gustducin activates phosphodiesterase (PDE), causing decreases in intracellular cAMP, while β3γ13 activates phospholipase C β2 (PLCβ2) to produce the second messengers inositol 1,4,5 trisphosphate (IP_3) and diacylglycerol (DAG). The IP_3 binds to receptors located on smooth endoplasmic reticulum, causing a release of Ca_2+ into the cytosol (10).**

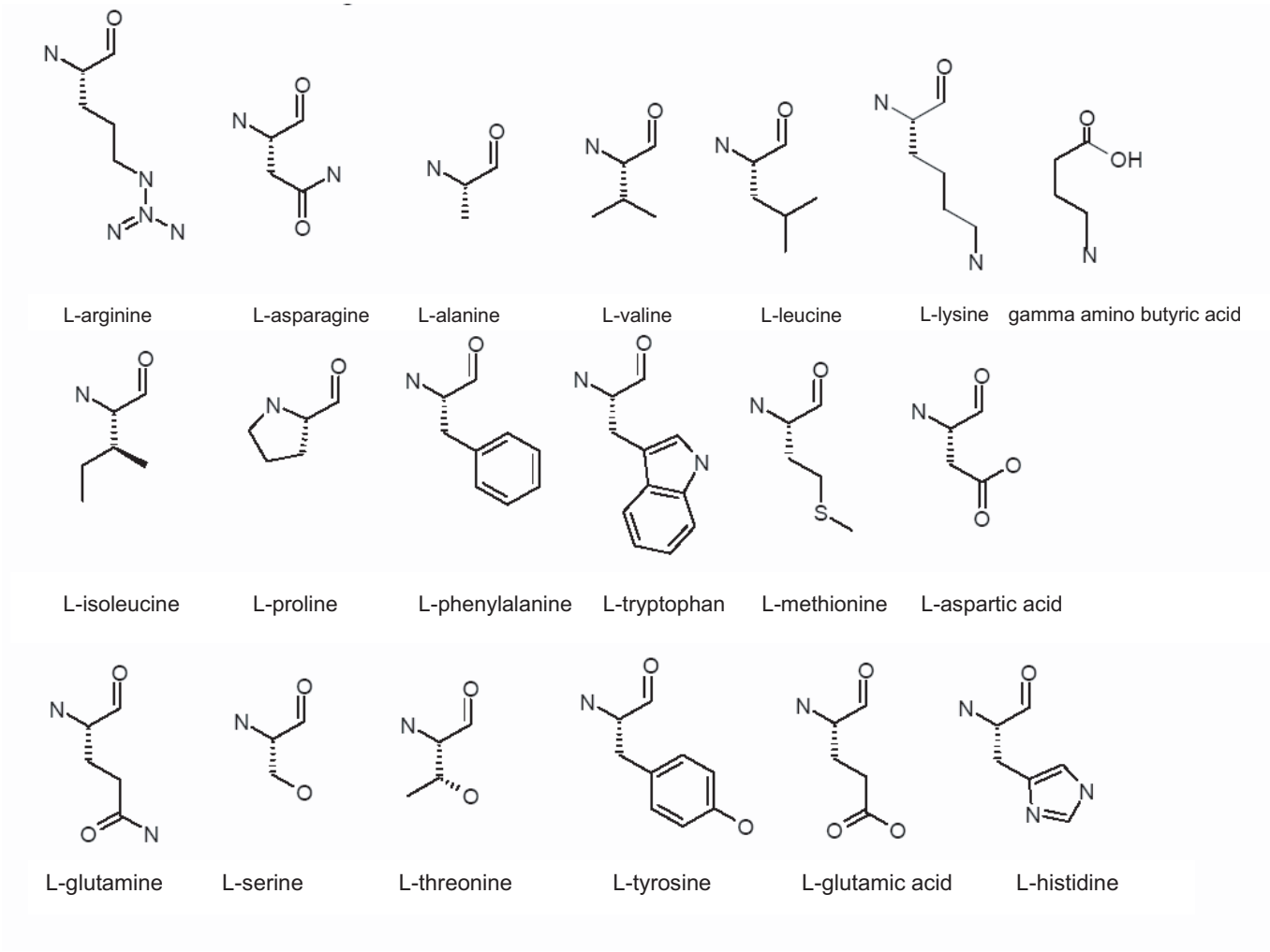

Fig 2 Structures of L-amino acids

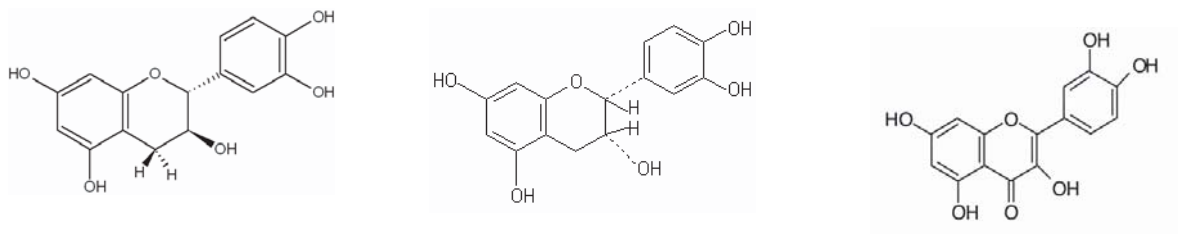

Fig. 3 structures catechin, epicatechin and quercetin

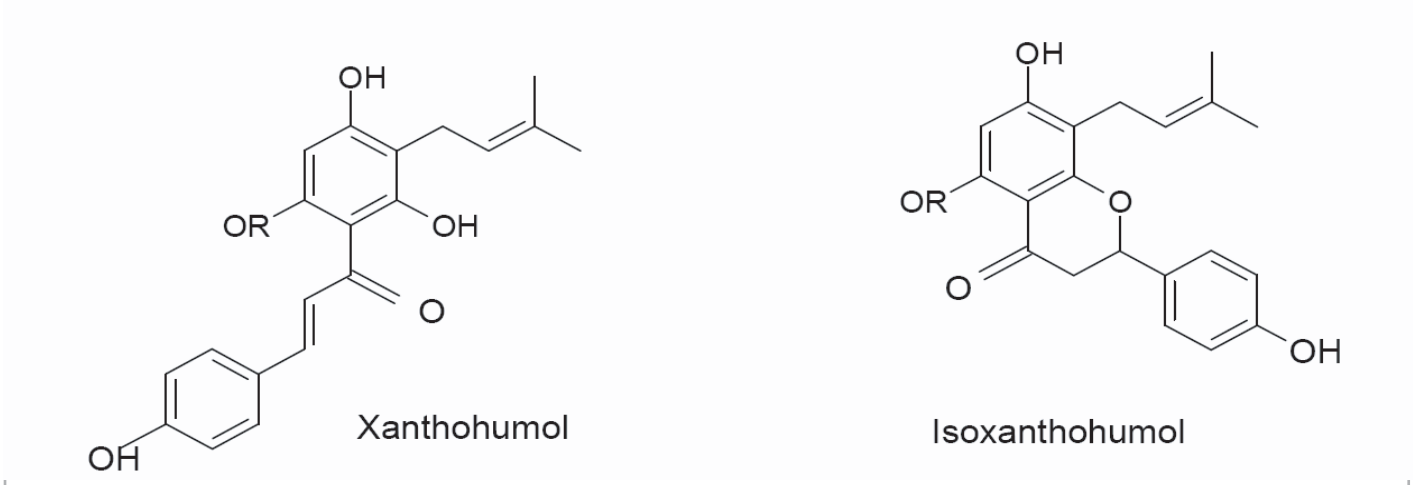

Fig. 4 structures of xanthohumol and isoxanthohumol

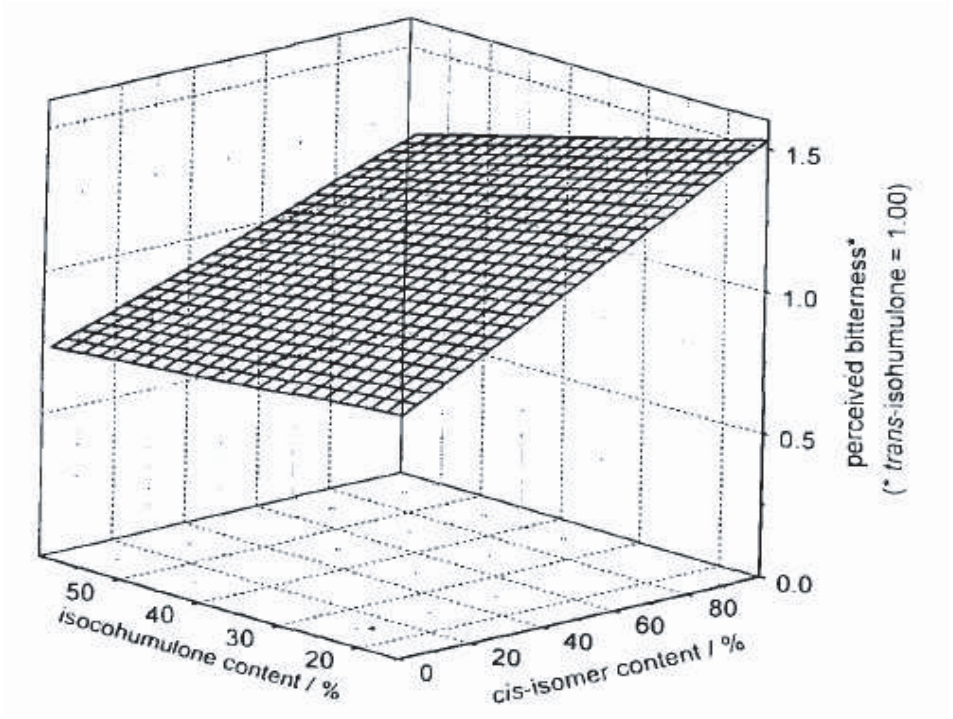

Fig. 5 surface plot for perceived bitterness as it relates cohumulone content to hop processing conditions (cis- to trans- ratio) (56)

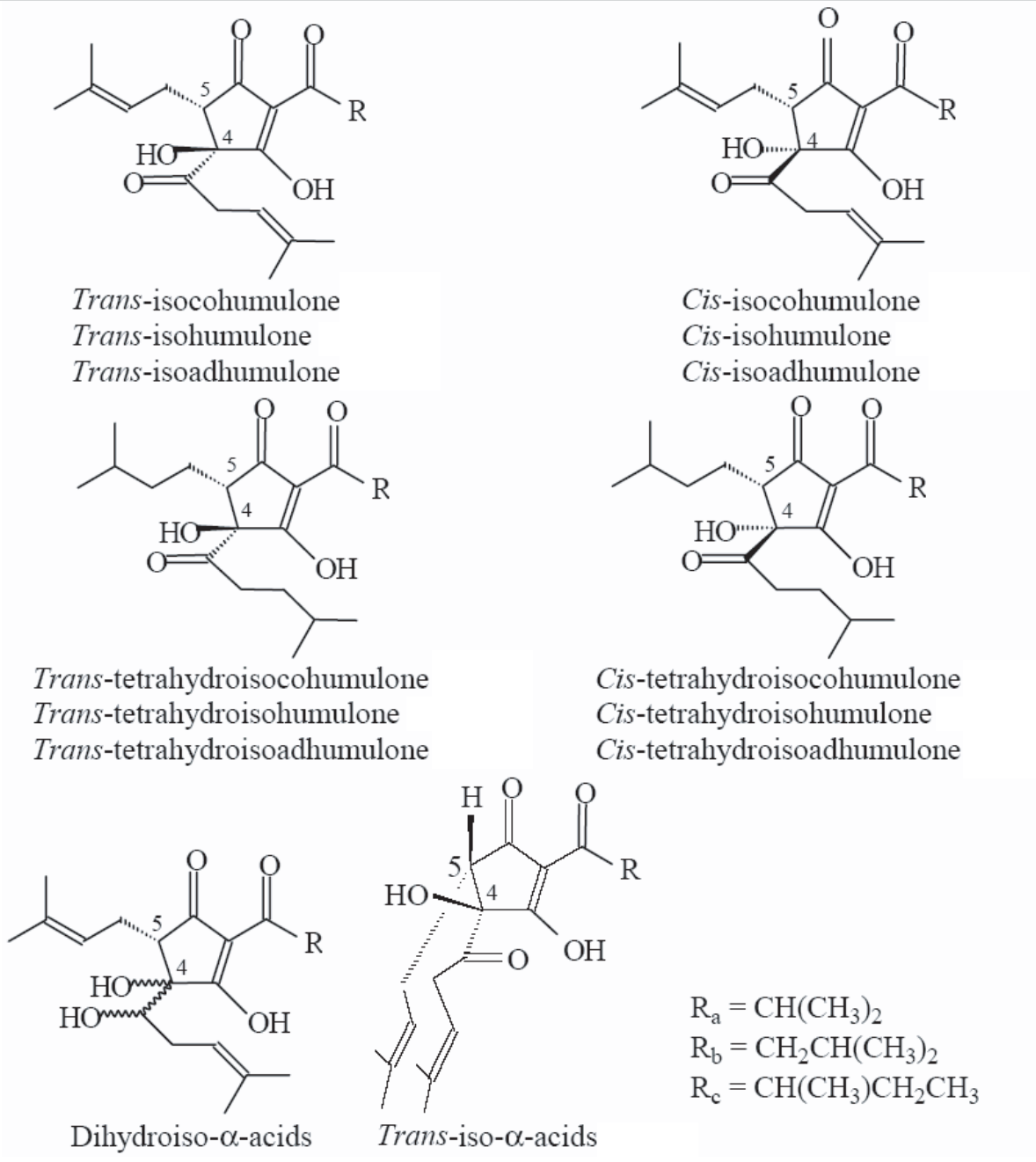

Fig. 6 structures of iso-alpha-, dihydro-iso-alpha- and tetrahydro-iso-alpha acids (57)

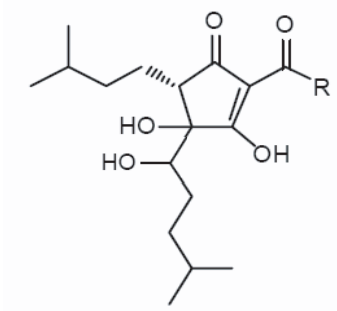

Fig. 7 structure of hexahydro-iso-alpha acids

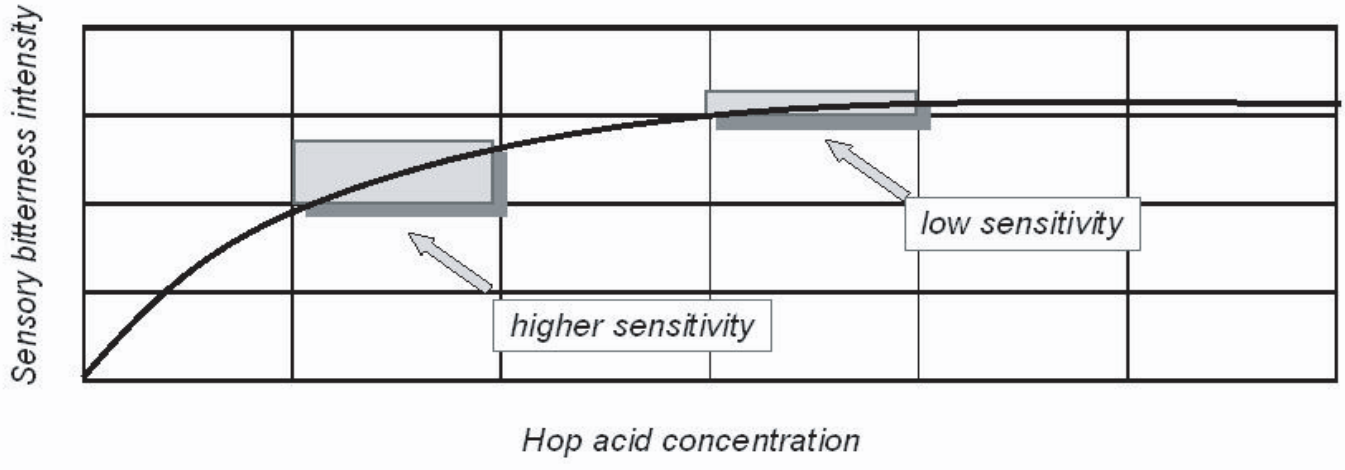

Fig 8 **Illustration of the Just Noticeable Difference (JND) concept of Fechner. With the decreasing concentration of the hop bitter acids, the ability of humans to distinguish between similar concentrations increases until the detection threshold is reached (66)**