Addendum

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What are Auxiliary Bitter Compounds in Hops and how do they Affect the Quality of Bitterness in Beer?

The term "auxiliary bitter compounds" in hops refers to all bitter compounds in the hop resin which are transferred to the beer and are not iso-α-acids. The majority of these substances are considered desirable from a sensory perspective. The ratio of the non-specific EBC bittering units (spectrophotometric method) to the specific iso-α-acids (HPLC method) serves as an indicator for the amount of auxiliary bitter compounds in beer. The evaluation of various existing data demonstrates: adding larger amounts of aroma hops over several additions not only influences the aroma but also serves to improve the harmony of the beer bitterness.

Descriptors: hops, bitter substances, beer bitterness

1 Preface

Bitterness in beer has been researched extensively and there exist many publications on it. As early as in the 1960s, the evaluation of hops concentrated on alpha acid content, which fueled the breeding of high-alpha hops. At the same time, many brewers often concluded that iso-alpha acids were not only the major, but even the singularly most important beer bitter components. Nowadays, beer bitterness is again considered in a more differentiated manner. **The following article will not introduce new findings, but intends to present one aspect of bitter compounds in the context of already existing results that were not analysed in sufficient depth before.**

2 Introduction

The essential bitter compounds in hops are the α-acids. They undergo isomerization during wort boiling. Depending on the duration of the boil and the type of hop product employed, approximately 45 % of the α-acids are converted to readily soluble iso-α-acids. The latter are primarily responsible for the bitterness in beer. Many brewers consider iso-α-acids to be the only relevant bitter compounds in beer, and therefore, they do not attribute any value to other bitter compounds introduced through the addition of hops.

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However, aside from α-acids, hops also contain numerous other compounds, such as β-acids. Opinions differ regarding their contribution to bitterness in beer. Since they are soluble in wort only to a limited degree, do not undergo isomerization and are present in trace amounts in beer, their sensory contribution is frequently questioned.

Furthermore, reports vary for other bitter compounds detectable in hops and beer with regard to their impact on beer flavor. In a manual on hops from 2014, the term "auxiliary hop bitter substances" was introduced and explained [1, pp 225–230]. In this overview, the correlation between auxiliary bitter compounds and their influence on the quality of beer bitterness is explained in more detail and complemented by already existing works.

3 Defining auxiliary bitter compounds in hops

In recent years, a variety of terms have emerged for the bitter compounds found in beer which are not iso-α-acids. These include "non-iso-alpha-acid bitter substances" (or compounds) or "accompanying bitter substances". The term "auxiliary bitter compounds" will be used in subsequent passages to refer to all of the substances in beer which are not characterized as iso-α-acids. Based on this definition, unisomerized α-acids are considered auxiliary bitter compounds.

It has been demonstrated that α-acids and β-acids in hops are unstable in the presence of the oxygen in air, which causes an increase in the quantity of auxiliary bitter compounds in hops and subsequently in beer. Aging compounds created through oxidation are not addressed here. All conclusions and research results are based on "fresh" hops. The term "fresh" is explained in [1, pp.152] and can be simplified as follows: after harvesting, hops are stored in a normal atmosphere (with exposure to air) for a period of one to up to eight months before being processed to a product (extract, pellets) which is protected against exposure to oxygen. If the hops are stored at a low temperature (< 5 °C) prior to processing, the losses in quality are tolerable and consequently the product is considered “fresh”.

3.1 Analysis of bitter compounds in hops

For a long time, the only analysis method available for the separation or fractionation of hop resins was the method published by *Wöllmer* in 1916 [2], in which the total resins were measured as a methanol-soluble group of components. This was then separated into a soft resin fraction (“positive”, soluble in hexane) and hard resin fraction (“negative”) which increases as a result of oxidative aging reactions. A clear understanding of the composition of hard resins has existed since the publication of *Dresel’s* work [1, p. 224; 3]. Hard resins in fresh hops consist primarily of prenyl flavonoids, e.g. xanthohumol. The accumulation of degradation products derived from bitter compounds only occurs in hops which have been subjected to aging reactions involving oxidation.

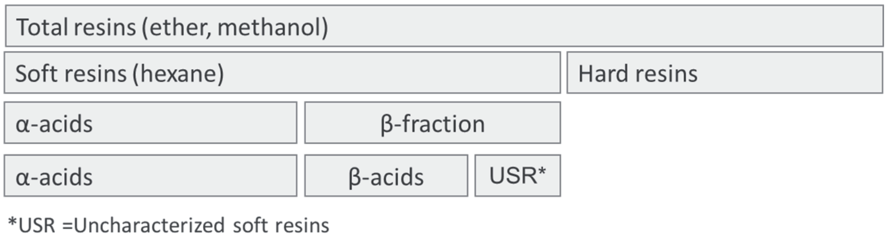

Fig. 1 Resin fractionation according to Wöllmer, extended version

Until the 1960s, α-acids could only be analyzed gravimetrically. This entailed the precipitation of the methanol-soluble α-acids with lead acetate to form an insoluble lead humulate (salt). An aliquot of lead humulate was collected in a glass frit and weighed – an elaborate procedure that was susceptible to error. The introduction of the conductometric method, still in use today, greatly simplified the analysis of α-acids. The measurement principle is likewise based on the precipitation of α-acids in hops. A hop extract is prepared using methanol and the α-acids are precipitated with a lead acetate solution. The excess lead acetate causes an increase in conductivity, from which the content of α-acids can be determined as described in the analysis method [e.g. EBC 7.4 or 7.5]. Today automatic measuring devices exist.

Soft resins can be divided into α-acids and other soft resins, also known as the β-fraction. Even during Wöllmer’s time, the contribution of the β-fraction to the bitterness in beer was recognized. Calculations were developed including the “Wöllmer bitter value” (α-acids plus the β-fraction ÷ 9) or the “bactericidal power” (α-acids plus the β-fraction ÷ 3). In addition to the conductometric determination of α-acids, further fractionation of the total resins became possible with the spectrophotometric analysis of β-acids [ASBC Method Hops 6], resulting in yet another fraction, the “uncharacterized soft resins”. The individual resin fractions are shown in figure 1.

A disadvantage of this “separation” or fractionation of hop resins is necessary for combining several analysis methods, which include:

- gravimetric resin fractionation according to Wöllmer requiring multiple steps and measurements based on differences in weight;
- earlier, α-acid determination was performed with gravimetric methods, which have now been replaced by conductometric, spectrophotometric or HPLC methods;
- β-acid determination by means of spectrophotometry or HPLC.

Gravimetric methods are rarely carried out today due to their highly complex nature and their susceptibility to error. It is also not possible to automate the measurement and evaluation steps. However, despite inherent difficulties, gravimetry did yield valuable contributions in the past before alternative methods were developed [4–8].

The current method of choice is HPLC. Earlier, HPLC was limited to the determination of α-acids and β-acids [EBC 7.7], but has since been utilized for the separation of numerous compounds [e.g. 9].

Important findings were reported by the work group headed by *T. Hofmann* (TU München – Weihenstephan) regarding bitter compounds in hops [10–12]. A summary of these findings can be also found in [1, p. 225]. A great number of compounds have been found in beer which can be grouped as follows:

- compounds present in hops, for example, deoxyhumulone;
- compounds with a polyphenolic character such as xanthohumol, which is converted to isoxanthohumol similar to the isomerization of alpha acids during wort boiling;
- compounds which are formed in conjunction with α-acids, for example humulinones [13, 14];
- compounds formed from β-acids during wort boiling such as hulupones, hulupinic acid or hydroxytricyclolupulones.

Along with these compounds, unisomerized α-acids may also be present, depending on when the hops are added in the brewing process. With a hop addition at the beginning of the boil, only traces of α-acids are detectable in beer, with the concentration increasing to several mg/l for the corresponding additions later in the boiling process.

The majority of these compounds were described as pleasantly bitter by *Haseleu* [9,10]. Although the concentrations of these compounds are often lower than the respective sensory thresholds in beer, additive and synergistic effects occur, contributing to the overall sensory perception.

Despite the fact that HPLC is a very suitable method for determining auxiliary hop bitter compounds in beer, only a few research institutes [9, 11, 14] and laboratories in the hop industry [13] possess the resources to perform this complex analysis. One, therefore, wonders whether other tools are available to assist brewers in estimating the quantity of the auxiliary bitter compounds present in their beer.

3.2 Measuring the quantity of auxiliary bitter compounds from hops

As strongly hopped beers continue to rise in popularity, it is clear that auxiliary bitter compounds will play an increasingly greater role in the view on the quality of beer bitterness.

This presents two challenges:

- defining a simple method, which can serve as an indicator for the general quantity of non-iso-alpha acid bitter compounds in beer;
- finding a figure in hops which corresponds to this indicator in beer.

Two methods are available for the determination of bitterness in beer. The specific method described in EBC 9.47 utilizes HPLC to determine the concentration of iso-α-acids in a sample. The other is a non-specific spectrophotometric method which measures the absorption of all substances dissolved in iso-octane at a wavelength of 275 nm (EBC 9.8). This includes α-acids and other bitter compounds in addition to iso-α-acids. The ratio of the value obtained for bittering units (IBU) for unspecific compounds to the concentration of specific iso-α-acids (measured by means of HPLC) represents the relative quantity of auxiliary bitter compounds. The greater the difference between the unspecific bittering units and the specific iso-α-acids is, the more the other bitter compounds contribute to measured value for IBU. This principle is depicted in figure 2. As the ratio of IBU : iso-α-acids increases, so does the quantity of auxiliary bitter compounds.

It should be noted that the ratio of IBU to iso-α-acids is not an absolute measure but rather serves as a reference for the relative contribution made by the auxiliary hop bitter compounds to the corresponding level of bitterness. This relationship is shown in table 1. Different IBU values were measured in three beers. Although all three beers contained 20 mg/l iso-alpha-acids, each yielded a different IBU to IAA ratio: 1.0, 1.25 and 1.5. This difference in IBU corresponds to 0, 5 and 10 bittering units, respectively, which are not attributable to iso-alpha acids. The relative contribution of this non-iso-alpha IBU as a percentage can be expressed as 0, 20 and 33 %. This facilitates an understanding of the term "non-iso-α IBU".

Multiple batches of wort were brewed for a number of research trials. The results from one of the series in the trials are plotted on the graph in figure 3. In the graph, the ratios of IBU to iso-α-acids were plotted against the values obtained for non-iso-α-acid bitter compounds which include α-acids, hulupones and humulinones. The results show a significant relationship and a strong correlation confirming the initial assumption. The ratio of IBU to iso-α-acids represents a viable indicator for the quantity of auxiliary bitter compounds present in beer.

A further relationship can be found in the following empirical observation. Depending on the kind of hop product and the hop variety, beers are produced containing varying ratios of IBU to iso-α-acids [1, p. 226]. The ratios of IBU to iso-α-acids according to the kind of hop product and the respective range of variation are provided in figure 4. If iso-extract is used exclusively, the value is below 1, which is an indication that the factor of 50 for pure iso-α-acids is too low for the IBU calculation in the equation IBU = $E_{275\ nm}$ x 50. Extract from high alpha hops yielded values around 1.0, while the values measured in beers produced with high alpha pellets are calculated to be somewhat higher (approx. 1.03). A value of around 1.1 was measured for pellets of the hop variety Perle. Additions at a rate of 100 g/hl with traditional aroma varieties (e.g. Tettnang,

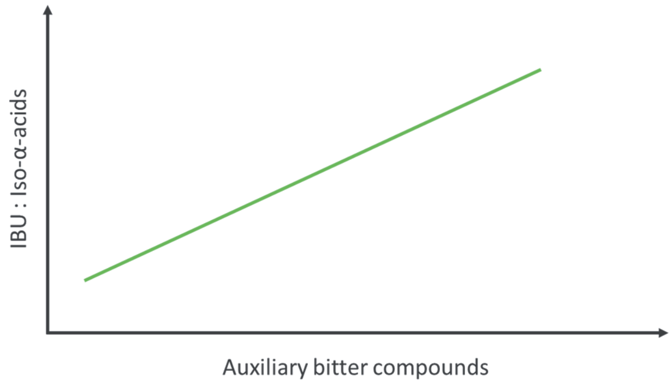

Fig. 2 Relationship between the concentration of auxiliary bitter compounds as a function of the ratio of IBU : iso-α-acids

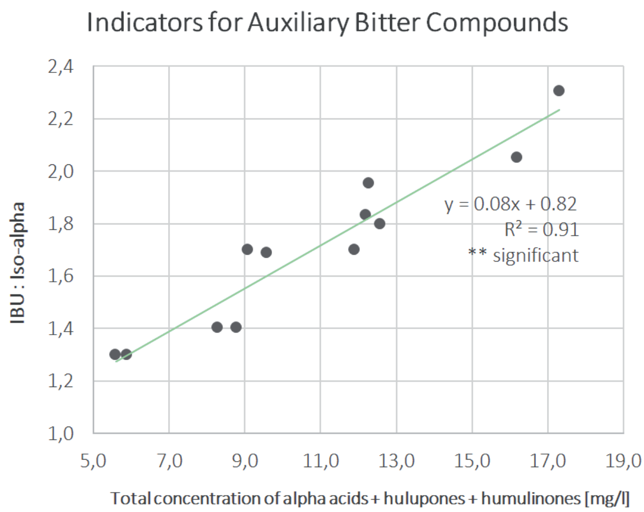

Fig. 3 IBU to iso-α-acids as a function of the concentration of α-acids, humulinones and hulupones in 12 beers

Spalt, Hallertau Mfr.) resulted in values near 1.3. If the hopping rate is increased to 500 g/hl, for example, the IBU to iso-α-acids values are closer to 2.0 can be achieved. Delaying the hop addition until the end of the boil brought the ratio to above 2.0 [15]. The more complex the aroma hop additions are, the greater the amount of auxiliary hop bitter compounds is.

Since Haseleu [10,11] reported that many bitter compounds are derived from β-acids, it was only logical to look for a relationship between the β-acid content in hops and the IBU to iso-α ratio. This was done as part of the brewing trials with single hop varieties conducted by the CMA [16] in 2005. A total of 16 beers were produced at that time. Although the test results had been available

Table 1 Bittering units (BU), iso-α-acid content, IBU to iso-α, IBU minus iso-α = non-iso-α-bittering units and relative proportion of BU to total IBU in three different beers

IBU	iso-α	IBU : iso-α	non-iso-α BU	relative proportion (%)
20	20	1	0	0
25	20	1.25	5	20
30	20	1.33	10	33

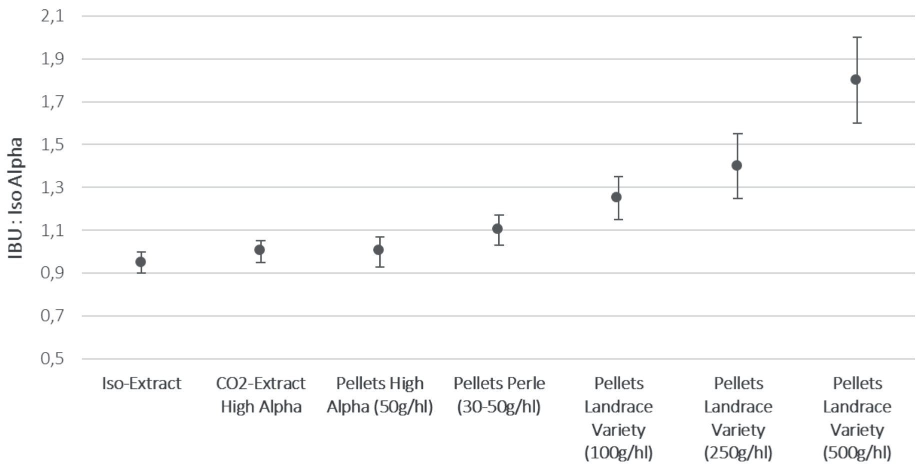

Fig. 4 **Ranges of IBU to iso-α-acid ratios for different hop products**

for a long time, they were not evaluated until the publication of the manual [1] nor checked for their context.

Figure 5 shows a graph of the ratio of IBU to iso-α-acids measured in the beers plotted against the ratios of β-acids to α-acids in the hops. A significant correlation was observed between hops and auxiliary hop bitter compounds in beer. For this reason, including the ratio of β-acids to α-acids in information brochures describing individual hop varieties is helpful [1, p. 146, 17].

3.3 Sensory analysis results for auxiliary bitter compounds in hops

The sensory attributes of single hop batches brewed by the CMA [16,18] were evaluated according to a rating system developed by the organization. Members of a trained tasting panel scored the beer samples for harmony and quality of the bitterness on a scale of 1 to 10. Figure 6 shows the sensory evaluation results for the ratio of bittering units to iso-α-acids. A high degree of correlation was observed. The harmonious aspect of bitterness increased with the quantity of auxiliary hop bitter compounds. The relationship between the quality of bitterness and the β to α-acid ratio is explained by the IBU to iso-α-acid ratio in beer and the ratio of β-acids to α-acids in hops (Figure 7). Also this context was not mentioned when published in 2006.

Additional sensory results from brewing trials are cited as follows: reference [19] lists five pairs of values as shown in figure 8. The results indicate that a significant relationship exists between the quality of bitterness expressed in scores of 1 to 10 and the ratio of IBU to iso-α-acids. In reference [20], three light beers and three low-alcohol beers are described. The relationship between the quality of the bitterness scored according to the DLG system on a scale of 1 to 5 and the ratio of IBU to iso-α-acids shown in figure 9 also exhibits a high correlation.

Yet another correlation [21] is presented in figures 10 and 11. In both series of trials, in which low-alcohol beers were produced, it was evident that the quality of the bitterness (scores ranging from 1 to 10) increased with higher ratios of IBU to iso-α-acids.

If the results from figures 8, 10 and 11 are combined (three separate series of trials, 17 beers in total), a strong correlation between the quality of the bitterness and the ratio of IBU to iso- α-acids (Figure 12) is evident.

In essence, these practical brewing trials confirm the fundamentals established by Haseleu. If undamaged by oxygen, auxiliary bitter compounds from fresh hops round out the bitterness in beer.

Parallel to these findings, the works of Dresel are interesting, too [3, 9]. Dresel was able to identify as well as give a sensory description of a multitude of components, which are hidden behind the term „hard resin". These are mainly polyphenolic substances, i.e. prenyl flavonoids like xanthohumol and numerous related substances, but not typical bitter compounds. Only in an oxidative aging oxidized

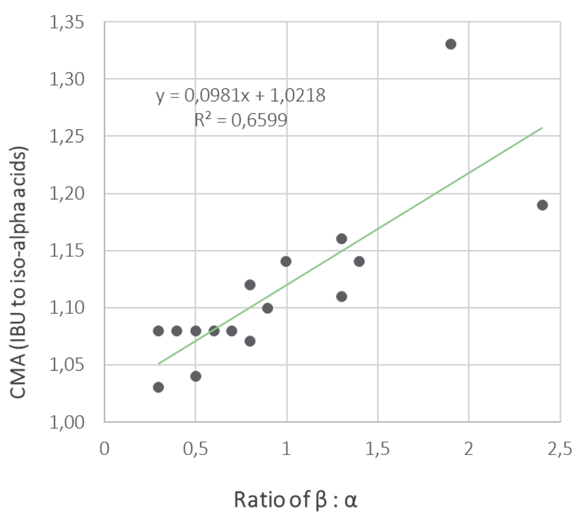

Fig. 5 **IBU to iso-α-acid ratios in beer plotted against the ratio of β-acids to α-acids in the hops (16 single-hop beers)**

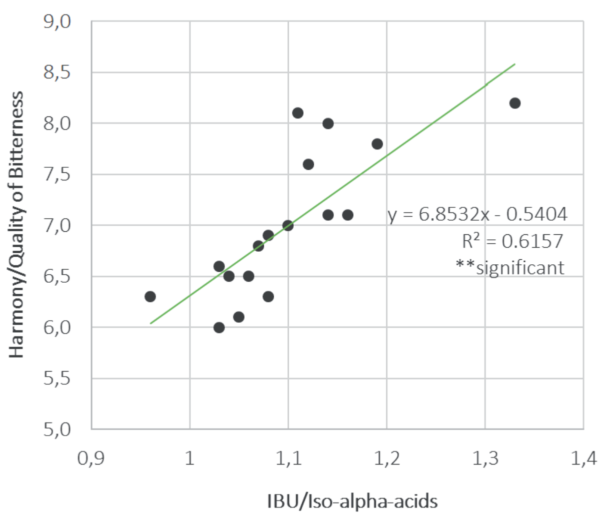

Fig. 6 **Quality of the bitterness in 16 single-hop beers plotted against the ratio of IBU to iso-α-acids**

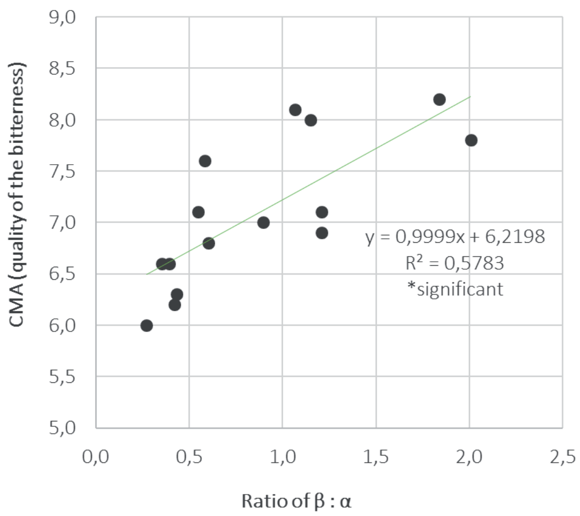

Fig. 7 **Quality of the bitterness of 16 single-hops beers plotted against the ratio of β-acids to α-acids in the hop varieties**

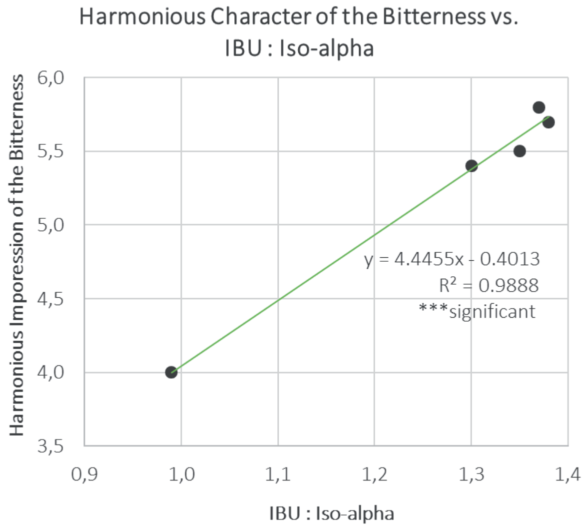

Fig. 8 **Harmonious quality impression of the bitterness of five beers plotted against the ratio of IBU to iso-α-acids**

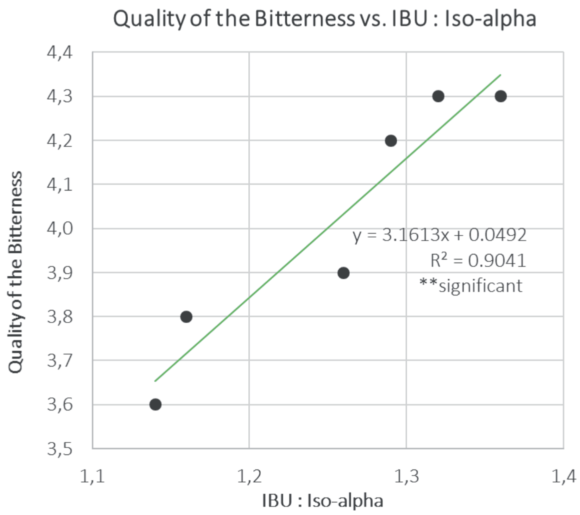

Fig. 9 **Quality of the bitterness in three light beers and three low-alcohol beers plotted against the ratio of IBU to iso-α-acids**

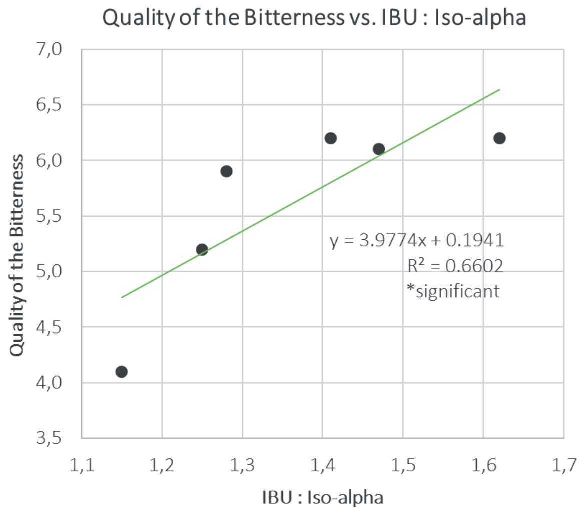

Fig. 10 **Quality of the bitterness in six low-alcohol beers plotted against the ratio of IBU to iso-α-acids (data from the first series)**

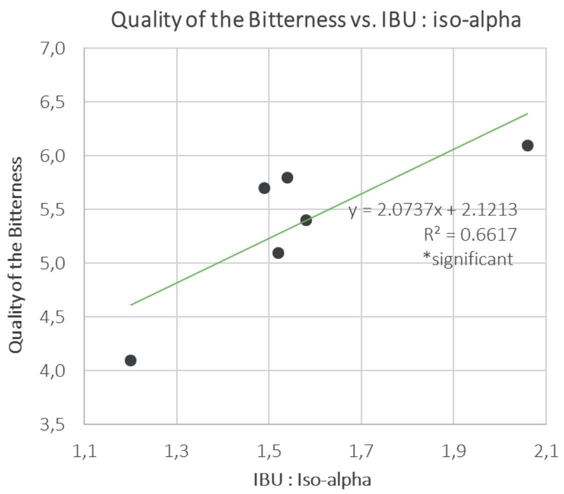

Fig. 11 **Quality of the bitterness in six low-alcohol beers plotted against the ratio of IBU to iso-α acids (data from the second series)**

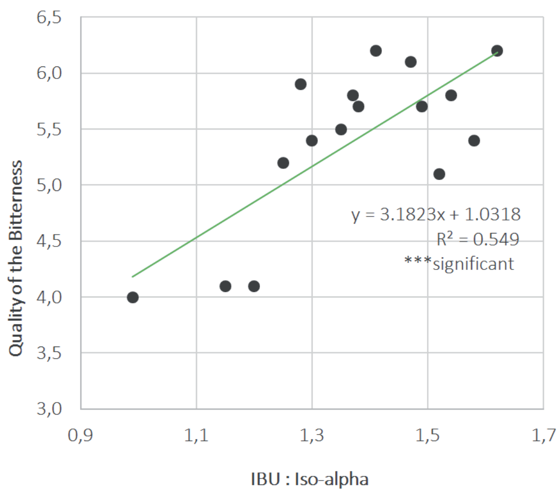

Fig. 12 **Combined results for all beers in figures 8, 10 and 11: a total of 17 beers**

bitter substances develop, as is explained in [1, pp 224]. According to Dresel, the polyphenolic substances of a "fresh" hard resin contribute positively to the taste in the form of a mild bitterness.

3.4 The impact of brewing procedures

As discussed above, several factors influence the amount of auxiliary hop bitter compounds in beer:

- Using hops with a higher β : α ratio leads to higher concentrations of auxiliary bitter compounds in beer. This is primarily true of aroma hops. Along with traditional landrace aroma hop varieties such as Saaz, Spalt, Tettnang, Hallertau Mfr. and Hersbrucker with β : α ratios of 1.3 to 2.4, Saphir also exhibits a high value of 1.9, while those for Hallertau Tradition, Spalt Select, Opal and Smaragd range from 0.8 to 1.0.
- Contrary to aroma hops, high alpha varieties are utilized at considerably lower rates (20 to 50 g/hl) and the β to α ratio is only 0.4. Thus, the potential of high alpha varieties contributing relevant amounts of auxiliary bitter compounds is limited.
- Short boiling times increase the ratio of IBU to iso-α-acids. Many of the auxiliary bitter compounds are extracted from the hops during this time and therefore shorter boiling times are sufficient. Fewer iso-α-acids are formed in the same period which enhances the impact of auxiliary bitter compounds. However, the qualitative, positive effect is countered by an economic aspect, namely poorer utilization of the α-acids.
- The practice of dry hopping also transfers bitter compounds to the beer without the formation of iso-α-acids. In beers exclusively dry hopped at a rate of 500 g of hops per hl of beer, bitterness values of up to 28 IBU were measured with iso-α-acid concentrations of slightly more than 1 mg/l.
- Specifically, late hop additions with a high potential for auxiliary bitter compounds, such as the case with most aroma hops, have a direct effect on the non-iso-α-acid compounds in beer [15].

4 Summary

The term auxiliary bitter compounds in hops refers to all bitter compounds in the hop resin which are transferred to the beer and are not iso-α-acids. This includes all such components found in fresh hops and excludes those formed through oxidative aging reactions. There are numerous auxiliary bitter compounds found in hops that are either present in a directly soluble form in hops or are formed from α-acids and β-acids during the wort boiling process.

The majority of these substances are considered desirable from a sensory perspective. They reduce a lingering character of bitterness and make a positive contribution to the quality and harmony of the bitterness in beer. The ratio of the non-specific EBC bittering units (spectrophotometric method) to the specific iso-α-acids (HPLC method) serves as an indicator for the amount of auxiliary bitter compounds in beer. This ratio is equal to 1 in beers brewed with only one hop addition of high-alpha hops at the beginning of the boil, in which case the bittering units are equivalent to the iso-α-acid content.

By contrast, beers brewed with a complex hopping regimen, e.g. with several additions of aroma hops, exhibit significantly higher levels of bittering units than iso-α-acids. Here, the values for IBU to iso-α-acids have been measured as even higher than 2. In this situation, the iso-α-acids are present in comparable quantities to the non-iso-alpha bittering units. Thus, auxiliary bitter compounds make up a sizable portion of the bittering units.

The results from the brewing trials described previously show that the bitterness of beers with higher ratios of IBU to iso-α-acids is less harsh and lingering, while the overall impression is more balanced and pleasant.

Adding larger amounts of aroma hops over several additions not only influences the aroma but also serves to improve the harmony of the beer bitterness.

5 Literature

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