C. Almaguer, M. Gastl, E. K. Arendt and Th. Becker

Contributions of Hop Hard Resins to Beer Quality

The brewing value of hops (*Humulus lupulus*) is primarily attributed to the flavor- and bitter-active compounds found in the resins. These resins are synthesized and accumulated in the lupulin glands of the female hop cones. Early work on the fractionation of hop resins, based on the solubility of resins in various organic solvents, classified them into soft resins and hard resins. The hard resin is mainly composed by oxidation products insoluble in hexane. Hitherto, research has primarily focused on studying the impact on beer properties of the major hop bitter acids (α- and β-acids) present in the soft resin. Therefore, little information is available on the functionality of the hard resin and for years it has been considered of no brewing value. Further the exact role of the hard resins in the brewing process is not well understood. For these reasons, a detailed study of the hard resins contributions to beer quality was performed. Brewing trials were conducted in which hop pellets were replaced with resin rich extracts. Thereby it was possible to establish the brewing potential of the hop hard resin. There is evidence to suggest that further degradation products were formed upon vigorous boiling of the hard resin. It is believed that upon extended storage periods these ultimate oxidation products are also formed. From the analytical data it was established that the hard resins positively contribute to the foam stabilizing properties. Furthermore, in the different sensory evaluations of the fresh beers, the hard resins proved to impact all analyzed attributes. With increasing hard resin concentrations, higher bitter intensities were recorded. The perceived bitterness of the hard resin beers suggested that the hard resins have a high bittering potential. Finally, at high hard resin concentrations a pleasant bitterness was produced and the overall flavor stability of the beers was high.

Descriptors: hops, hard resins, soft resins, bitterness, flavor stability

1 Introduction

Hops (*Humulus lupulus*) determine the typical taste in beer and are responsible for the fine bitterness. In addition to the foam enhancing properties [13, 15, 36], the comfortable bitterness and refreshing hoppy aroma delivered by the hops, it was shown, as early as 1888, that hops also contribute to the microbial stability of beer [18]. It is in the lupulin glands of the female hop cone that the main brewing principles, the resins and the essential oils, are synthesized and accumulated [39]. The characteristic resins of hops include many substances; Hayduck originally separated the hop resins into α-, β-, and γ- fractions on the basis of their solubility in different solvents and their ability to form a precipitate with lead acetate [18]. Over the years, the hop resin nomenclature constantly changed and already in 1897 the term γ-resin became obsolete and ever since this fraction has been more commonly referred to as hard resins [9]. In 1957, the situation was clarified by joint proposals of the European Brewery Convention (EBC) and the American Society of Brewing Chemists (ASBC) [1]. It was then revised in 1969 by the Nomenclature Sub-committee of the Hops Liaison Committee [12]. The total resin is defined as the fraction soluble in ether and cold methanol, whereas the hard resin is the portion of the total resin insoluble in low-boiling paraffinic hydrocarbons [10]. Several resin separation methods were developed throughout the years, but all seemed to be flawed and further proved the difficulty of achieving a total purification of the resins [40]. Currently, the method most commonly used for fractionation of hop resins is a modified version of Wöllmer's protocol [2]. To date, it is well recognized that the total soft resin is soluble in hexane and that the bulk of the brewing and bittering value of the hop is found in this fraction. The soft resin is mainly a mixture of α-acids, β-acids, and uncharacterized soft resins. The α-acids are by far the most important constituent of the hop resins. Upon addition of hops to the wort kettle, the α-acids are extracted and thermally isomerized during the boiling process into the more soluble and bitter iso-α-acids. By definition the hard resin is soluble in methanol and diethyl ether but insoluble in hexane [10]. It is generally accepted that the hard resins arise

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Table 1 Overview of hopping regimes for experimental beers

Beer	Hopping regime	Amount dosed [mg/L]
Beer UH	Control – Unhopped beer	–
Beer T90	Reference – T90 hop pellets of cv. H. Taurus (17 % α-acids)	500
Beer SH	Reference – CO_2-spent hop material cv. H. Taurus	500
Beer HR(50)	50 ppm Hard resin extract from cv. H. Taurus	50
Beer HR(100)	100 ppm Hard resin extract from cv. H. Taurus	100
Beer HR(200)	200 ppm Hard resin extract from cv. H. Taurus	200
Beer SR(50)	50 ppm Soft resin extract from cv. H. Taurus	50
Beer SR(100)	100 ppm Soft resin extract from cv. H. Taurus	100
Beer SR(200)	200 ppm Soft resin extract from cv. H. Taurus	200

by oxidation of the soft resins, however, it is not well defined nor proven conclusively what constitutes the hard resins.

In 1952, *Walker* et al found a water soluble portion of the hard resin which also had a bitter character. Walker termed this portion of the hard resin the δ-resin [44]. Several years later, *Bausch* et al conducted research on the δ-resin of the total hard resin, in their studies, they were able to quantify the δ-resin in fresh green hops [7]. Also, *Schild* and *Raum* found hard resins in hops at the earliest stage of development [34], therefore it is necessary to differentiate between the native hard resin of hops and that which arises by autoxidation of the soft resin during kilning and storage [39]. Unfortunately, hitherto, no distinction has been made between the deterioration or oxidation products and the natural hard resin constituents of fresh hops [33]. However, given that the soft resins are prone to oxidation this would result in a challenging task.

As hops age during storage, the percentage of soft resin falls while that of the hard resin increases [11]. In 1964, *Ashurst* et al inconclusively addressed the question of what constituents are responsible for the bittering ability of stored hops when the α-acids have been transformed into other substances. At that point, it had been known for many years that the resins of the hop undergo several changes during storage: the α- and β-acids become oxidized, the products still being analytically classed as soft resins, while further oxidation results in gradual transformation to hard resins. Thus, the α- and β-acids decrease continually during storage while the amount of uncharacterized soft resin increases at first and then decreases as the hard resin steadily increases [4, 25]. While some authors [11, 29] automatically considered oxidized soft resins to be hard resins, others [4, 19, 25, 33] considered the uncharacterized soft resins in old hops to be the intermediate deterioration products of the α- or β-acids which, upon further oxidation, will ultimately turn into hard resins. It is believed that these intermediate deterioration products have brewing value, but the exact role of oxidation products, be it oxidized soft resins or hard resins, in the brewing process is not fully understood [25, 33].

Xanthohumol is the most abundant prenylated chalcone present in the hop and naturally found in the hard resins. Xanthohumol was first isolated from hops by *Power*, *Tutin*, and *Rogerson* in 1913 [31]. In the last decade, on account of the wide range of potential health benefits, xanthohumol has been the source of numerous investigations [16, 26, 37]. Although xanthohumol is the major compound in hop hard resins, trace amounts are found in beer as it is lost in significant quantities in the conventional brewing process. During the brewing process, the thermal isomerization of chalcones into flavones takes place and xanthohumol is cyclized to isoxanthohumol. Attempts to increase the amount of xanthohumol present in the finished beer have been made by several brewing scientists. To achieve this, brewing trials using xanthohumol enriched extracts were carried out [8, 27, 38, 48]. Although, to date, xanthohumol is the only hard resin compound known to have some brewing value, these studies fail to investigate the additive effect of all the oxidation products present in the hard resin. Other studies [24, 32, 46] have evaluated the beer quality of experimental brews produced using aged hops. This experimental setup is not representative and does not allow the proper evaluation of the contribution of hop hard resins to beer quality. Even if all the hard resin constituents are present and the hard resin content in aged hops is considerably higher than that in fresh hops, a significant amount of soft resins is to be found in aged hops. Therefore, the quality of the final product has to be attributed to the complex mixture of deteriorated soft and hard resins present in the old hops. Few studies [25, 33] have been done where a hard resin enriched extract is used to bitter the wort. However, these experiments were inconclusive and failed to provide a detailed explanation of the impact of these oxidation products on beer quality.

Several studies have considered the effect of hop hard resins on beer quality in particular taste, yet the results remain somewhat conflicting. In view of the conflicting data in the literature on the contributions of hop hard resins to beer, the present study was conducted to establish the bittering potential of the hop hard resins. The purpose of this study is to provide a better understanding of how hard resins impact beer properties in particular taste, bitterness, and flavor stability. In this study, beer bitterness derived from the hard resins is discussed both from the analytical and from the sensory point of view. Overall flavor deterioration of the experimental brews upon aging is evaluated through sensory assessment. Further attention is given to the taste and bitterness stability with beer aging; to achieve this, sensory evaluations of the force aged and cold aged beer samples were carried out. The data obtained from this study could help better understand the extent to which hard resins contribute to beer quality.

2 Materials and methods

2.1 Preparative extraction of hop-derived resin rich extracts

All hop products were generously donated by the Barth-Haas Group (Nuremberg, Germany). Prior to extraction, the hop products were stored at –20 °C in vacuum-sealed packaging. It was necessary to modify the Wöllmer fractionation protocol [2] to produce the hop-derived resin rich extracts. A hard resin (HR) rich extract was isolated from cultivar (cv.) Hallertauer Taurus (H. Taurus) spent hop (SH) material (i.e. hop powder remaining from the supercritical CO_2 extraction). To produce the soft resin (SR) enriched extract, hop pellets type 90 (T90) of the same variety were used. The hop products used in all experiments were produced from a single batch of cv. H. Taurus CO_2 spent hop powder or hop pellets T90. The resin rich extracts were flushed with N_2 and kept at –20 °C in amber glass bottles prior to use.

2.2 Brewing experiments on a 10 L scale

Brewing was performed using the micro scale equipment (10 L) in the Institute of Brewing and Beverage Technology, Technische Universität München, Germany. Lager "*Vorderwürze*" (first wort) with an original gravity of 17.7 °P was taken from the local brewery. The first wort was stored chilled at 0 °C until boiling. The wort was transferred to the kettle and using brewing water the base wort was diluted to "*Pfannevollwürze*" (kettle-full-wort) concentration of approximately 11.5 °P. The wort was brought up to boil and boiled for 60 minutes at atmospheric pressure. All hop treatments were single additions (i.e. full hopping) of the hop products applied at the onset of wort boiling.

In these brewing trials, nine brews were produced; unhopped lager wort was used as a control (Beer UH). Table 1 presents an overview of the hopping regimes used for the different brews produced in these trials. Two references were brewed, one was prepared using hop pellets T90 and the other using CO_2 spent hop material. For the pellet treatment, hop pellets T90 from cv. H. Taurus (17 % α-acids) were added at a target bitterness concentration of 25 bitterness units (BU). Based on previous results and assuming a final α-acids utilization of 30 %, 500 mg/L of the pellets were needed. For the beer produced using spent hop pellets, the same amount (500 mg/L) of hop material was dosed as for the T90 pellet beer.

A series of experimental beers was produced using an ethanolic solution of hard resin rich extract from cv. H. Taurus. In separate brews, the extract was added in increasing amounts (50 ppm, 100 ppm, and 200 ppm) when the wort began to boil. Using an ethanolic solution of soft resin rich hop extract from cv. H. Taurus the same series was carried out. This was done to evaluate beer bitterness and flavor stability conferred by the different concentrations of the resin rich extract. Further, the impact of the resin extracts on the beer quality parameters was assessed.

All worts were boiled with similar evaporations of approximately 5 %. To separate the hot break, the boiled worts were allowed to settle for 20 minutes. Following whirlpool, the produced worts were cooled and pitched with approximately 240 mL of unhopped bottom fermenting yeast (*Saccharomyces pastorianus* Weihenstephan 34/70, cell count: 4.97 x 10^8 cells/mL) to achieve a pitching rate

Table 2 Average values (standard deviations in parentheses; n = 3) of the standard analyses of finished beers[a]

Parameter	Beer UH	Beer T90	Beer SH	Beer HR(50)	Beer HR(100)	Beer HR(200)	Beer SR(50)	Beer SR(100)	Beer SR(200)
Original gravity (% w/w)	10.89 (0.08)	10.80 (0.43)	10.73 (0.11)	11.41 (0.08)	10.82 (0.30)	11.05 (0.11)	11.10 (0.07)	11.24 (0.08)	11.23 (0.03)
Original gravity (% w/v)	11.35 (0.09)	11.25 (0.47)	11.17 (0.12)	11.91 (0.08)	11.27 (0.33)	11.52 (0.11)	11.57 (0.08)	11.73 (0.09)	11.72 (0.04)
Alcohol content (Vol%)	4.30 (0.03)	4.33 (0.27)	4.14 (0.05)	4.51 (0.03)	4.18 (0.18)	4.50 (0.04)	4.29 (0.02)	4.62 (0.04)	4.19 (0.00)
Residual extract (% w/w)	4.33 (0.04)	4.19 (0.04)	4.41 (0.04)	4.55 (0.04)	4.45 (0.04)	4.18 (0.05)	4.58 (0.04)	4.20 (0.03)	4.87 (0.04)
Apparent extract (% w/w)	2.76 (0.03)	2.60 (0.06)	2.90 (0.03)	2.91 (0.03)	2.92 (0.03)	2.54 (0.03)	3.02 (0.04)	2.52 (0.03)	3.34 (0.03)
Real attenuation (%)	61.6 (0.1)	62.6 (1.2)	60.9 (1.0)	61.6 (0.1)	60.3 (0.9)	63.5 (0.0)	60.2 (0.1)	64.0 (0.0)	58.1 (0.2)
Apparent attenuation (%)	74.7 (0.1)	75.9 (1.5)	73.8 (1.1)	74.5 (0.1)	73.0 (1.1)	77.0 (0.1)	72.8 (0.1)	77.6 (0.0)	70.3 (0.2)
pH	4.75 (0.03)	4.50 (0.04)	4.58 (0.03)	4.69 (0.02)	4.62 (0.06)	4.64 (0.04)	4.74 (0.06)	4.60 (0.00)	4.68 (0.01)
Beer color [EBC]	5.3 (0.7)	5.5 (0.6)	5.6 (0.8)	5.7 (0.7)	5.3 (0.6)	6.1 (0.7)	5.5 (0.7)	5.4 (1.1)	5.4 (0.4)
Bitterness units [EBC]	0 (0.0)	25 (0.4)	5 (0.2)	6 (0.0)	8 (0.1)	14 (0.1)	10 (0.2)	19 (0.3)	34 (0.9)
Total polyphenols[b] [mg/L]	135 (1)	164 (1)	125 (7)	137 (4)	165 (8)	159 (4)	129 (0)	123 (1)	141 (0)
Foam stability, NIBEM [s]	41 (3)	290 (1)	131 (4)	185 (1)	209 (4)	249 (4)	226 (1)	271 (1)	319 (3)
Foam cling [%]	0 (0.0)	48 (0.6)	0 (0.0)	0 (0.0)	37 (6.9)	41 (1.7)	32 (3.0)	46 (0.8)	49 (1.8)

[a] Hopping regimes are described in Table 1.
[b] Corrected to 12 °P extract.

Table 3 Concentrations [mg/L] of Hop Compounds in Finished Beers[a] as Determined by HPLC

Compound	Beer UH	Beer T90	Beer SH	Beer HR(50)	Beer HR(100)	Beer HR(200)	Beer SR(50)	Beer SR(100)	Beer SR(200)
Iso-α-acids [mg/L]	–	27.34	1.15	1.55	2.77	4.82	9.64	19.02	35.22
α-acids [mg/L]	–	0.90	–	0.16	0.20	0.40	1.30	2.00	4.70
Isoxanthohumol [mg/L]	–	0.98	1.42	1.91	2.97	4.53	–	–	0.27
Xanthohumol [mg/L]	–	0.02	0.02	0.03	0.06	0.07	–	–	0.01

[a] Hopping regimes are described in Table 1

of 12 x 10^6 cells/mL. The fermentations were performed in 10 L cylindro-conical vessels at 12 °C for approximately one week, until the original gravity dropped to about 3.5 °P. After fermentation, excess yeast was removed and the beers were transferred to sterile 10 L Cornelius kegs and lagered for 3 days at 16 °C. Maturation of the beers was conducted over a 10 day period at 0 °C. The conditioned beers were sterile filtered (one sheet filtration) into 10 L Cornelius kegs. The bright beers were then manually bottled into 0.33 L brown glass bottles.

Throughout this paper, brews will be referred to by a combined letter and number system: the first set of letters indicates the hop product added and, when required, the number in parenthesis is the amount (in ppm) of resin rich extract dosed (e.g. Beer HR(50) = hard resin and an extract addition of 50 ppm).

2.3 Aging of beer samples

The accelerated aging of the bottled beer samples was performed according to Eichhorn's protocol [14]. The beer samples were force aged in the dark following a two-stage method. First, the samples were pretreated by shaking for 24 hours at room temperature. This was followed by a 4 day incubation period at 40 °C in a thermostatically controlled chamber.

In addition to the forced aging of beers, all experimental beers were allowed to naturally age over a 12 month period. The fresh samples were not exposed to extreme conditions; all beer samples were stored in a dark cold room at approximately 0 °C.

2.4 Beer Standard Analyses

To characterize the produced beers, standard beer analyses were performed according to Mitteleuropäische Brautechnische Analysenkommision (MEBAK) methods [28]. All analyses were performed in triplicate. Original gravity and alcohol content in the beers were measured using an Anton Paar Alcolyzer (Anton Paar, Graz, Austria) following MEBAK method 2.10 [28]. The pH of the finished beers was also measured. The beer color was determined using a spectrophotometer according to MEBAK method 2.13.2 [28]. The bitterness units in the final beers were measured as described in MEBAK method 2.18.1 [28]. The total polyphenol content was determined following MEBAK method 2.17.1 [28]. The foam stability of the experimental beers was evaluated using the NIBEM Foam Stability Tester according to MEBAK method 2.19.2 [28]. The foam cling adherence was measured according to the manufacturer's procedure using the NIBEM Cling Meter. To obtain reproducible results all samples were analyzed on the same day.

2.5 HPLC analyses of hop compounds

Hop compounds were determined by high-performance liquid chromatography (HPLC) coupled with a diode array detector (DAD). The α-acids and iso-α-acids were measured by HPLC-DAD according to EBC standard method 7.8 [43] for HPLC analysis of hop components in beer. The xanthohumol and isoxanthohumol content was determined following the method presented by *Wunderlich* et al [48].

2.6 Sensory evaluation of beers

The produced beers were evaluated by a trained panel consisting of twelve professional panelists. All panelists have been trained with previous sensory evaluations regarding beer bitterness, as well as a broad variety of other sensory studies. In previous beer tastings, the chosen panelists were able to adequately replicate the beer characterization. In this study, the sensory evaluation of the brewed beers was carried out in triplicate in three independent tasting sessions.

For the sensory profiling of the beers, all experimental beers were evaluated according to the Deutsche Landwirtschaftsgesellschaft (DLG; known as "The German Agricultural Society") approved scheme (MEBAK method 2.34 [28]), where different sensory analyses were carried out. The beers were evaluated in the fresh, the force aged, and a naturally cold aged state for the following attributes: Smell (S), Taste (T), Mouthfeel (M), Carbonation (C), and Bitterness (B). From the scores of all five attributes, the Weighted Mean was calculated ((2S + 2T + M + C + 2B)/8).

To characterize the bitterness of the produced beers in their fresh state, the panelists were asked to rate the bitter intensity. The bitter intensity of the beers was evaluated according to *Kaltner* on an 11-point sensory scale ranging from just detectable (0 -2) to extreme (9 -10). Additionally, the panelists estimated the perceived BU content of the experimental beers. The panelists were also presented with time-intensity curves, these represent the different bitter profiles, and were asked to select the one that best describes the perceived bitterness profile in the trial beer [21].

To evaluate the flavor stability, all experimental beers were assessed for staling compounds in the fresh and the aged (forced and cold) state. The staling degree of three flavor relevant parameters: Smell, Taste, and Bitterness was scored according to *Eichhorn* on a 4-point scale. In the 4-point sensory scale, 1 = fresh; 2 = weakly aged; 3 = strongly aged; and 4 = very strongly aged. From the Smell, Taste, and Bitterness scores, the Weighted Mean was calculated

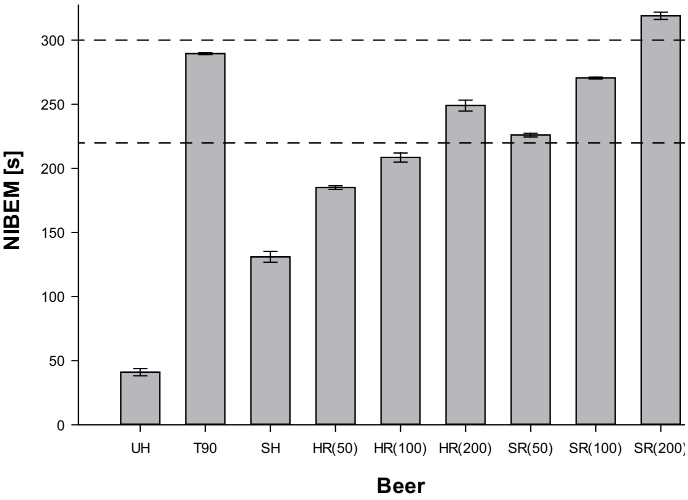


Fig. 1 **Comparison of the foam stability as assessed by the NIBEM method. Shown are the mean head retention times [s] (n = 3) for the experimental beers. According to MEBAK standards, a good foam stability (for a beer with an extract of 11.0 - 15.9 °P) is a head retention time between 220 - 300 seconds [28]**

((2S + 2T + B)/5). In addition, the panelist was asked to grade the acceptance in terms of aging on a 0 - 100 % scale, where 100 % = fresh; 50 % = aging is detectable; 0 %= undrinkable [14].

3 Results and Discussion

3.1 Beer standard analyses of the experimental 10 L brews

To characterize and verify that the obtained experimental beers are similar and, thus, comparable, standard beer analyses were carried out. The detailed quantitative data for the experimental beers are summarized in Table 2. Based on the data on alcohol content, residual extract, attenuation, pH, and color it can be concluded that the produced beers are comparable. Although the same original first wort was diluted to produce all brews, there are minor inconsistencies in the resulting real attenuation values. This discrepancy could influence the resulting taste profile of the beers.

The relatively high total polyphenol content in the control beer (Beer UH) is unaccounted for. A possible explanation is that the polyphenols present arise from the malt used and due to the absence of hop products, these were not properly removed. For Beer SH, brewed with CO_2 spent hop material, the total polyphenol content is unexpectedly low. The slightly higher total polyphenol content in the beers treated with the hard resin enriched extract can be attributed to the high amount of vegetative hop material present in this extract. Conversely, the comparable soft resin beers (i.e. brewed with the equivalent amount of extract), has a lower total polyphenol content. This can be related to the pure nature of the soft resin extract. In the Wöllmer fractionation process, most polyphenols were removed from the hop pellet material to produce the soft resin enriched extract. The impact of the total polyphenol content on the sensory profile of the trial beers was not evaluated. It is not possible to dismiss the potential synergistic effect of the polyphenols on the taste profile. However since no beer was brewed exclusively with a polyphenol rich extract it is not possible to make direct comparisons.

The bitterness units value of Beer T90 proved that the assumed final α-acid utilization of 30 % during wort boiling was accurate. When the BU values of Beer T90 and the brews treated with the soft resins are compared to the iso-α-acid concentrations shown in Table 3, it can be observed that there is almost a direct relationship between these values. This effect was not replicated by the hard resin brews. Furthermore, the sum of the concentrations of the four hop compounds determined by HPLC is lower than the quantified BU content. Thus suggesting that in Beer SH and the hard resin treated beers there are other unknown chemical substances present which are not detectable by the HPLC but that absorb light at 275 nm.

Further, it must be borne in mind that not all hop substances are identified by the BU analysis. Although the method has its limitations and it is nonspecific, it is far more specific for iso-α-acids than it is for other hop substances. The oxidation products of the α- and β-acids formed during hop storage, as well as the hard resins, are more polar and not extracted out of beer as well as iso-α-acids. However, it is known that to a lesser extent, the oxidation products and other interfering substances contribute to analytical BUs [30].

3.2 Foam evaluation of the produced beers

Hop acids not only contribute to beer bitterness, they also play an essential role to achieve foam formation and stability. Foam is an important quality factor in the beer production process, for this reason, in addition to the standard analyses, foam measurements (NIBEM) of the experimental beers were taken and compared. The foam suppressor-enhancer properties of the hop acids have long been known. In 1976, the first observations into the role of the iso-α-acids of hops in the structure of beer foam were documented by *Fly* and *Chicoye* [15]. Following their studies, numerous researchers [13, 20, 22, 23, 35, 36, 45] have looked into the effect of different hop compounds on the foam properties. It has been known for

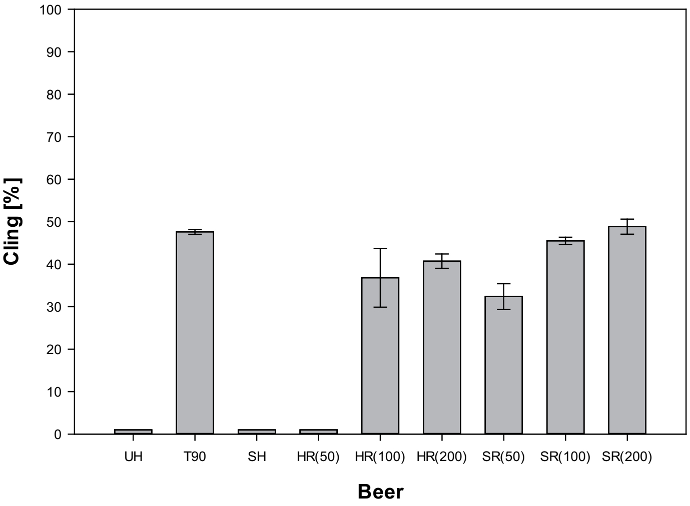


Fig. 2 **Comparison of the foam cling adherence [%] for the finished beers, as determined by the NIBEM Cling Meter. Shown are the mean values of three independent experiments**

Table 4 **Mean values (standard deviations in parentheses; n = 3) of the sensory evaluation of the experimental fresh beers, according to the DLG**

Attribute	Beer UH	Beer T90	Beer SH	Beer HR(50)	Beer HR(100)	Beer HR(200)	Beer SR(50)	Beer SR(100)	Beer SR(200)
Smell	3.9 (0.7)	4.3 (0.3)	4.3 (0.3)	3.7 (0.4)	3.4 (0.7)	3.9 (0.4)	4.1 (0.4)	4.1 (0.4)	4.2 (0.4)
Taste	3.8 (0.5)	4.1 (0.2)	4.1 (0.2)	3.9 (0.5)	3.6 (0.5)	4.2 (0.4)	3.9 (0.5)	3.9 (0.6)	4.1 (0.4)
Mouthfeel	3.8 (0.5)	4.3 (0.4)	4.3 (0.4)	4.0 (0.0)	4.0 (0.0)	4.3 (0.3)	4.1 (0.2)	3.8 (0.4)	4.1 (0.2)
Carbonation	3.8 (0.4)	4.3 (0.4)	4.0 (0.0)	4.1 (0.2)	3.8 (0.3)	4.0 (0.3)	3.9 (0.2)	4.0 (0.0)	4.0 (0.0)
Bitterness	3.7 (0.4)	4.2 (0.3)	3.9 (0.2)	4.0 (0.0)	3.9 (0.2)	4.1 (0.4)	3.9 (0.2)	4.0 (0.5)	3.7 (0.6)
Weighted mean (2S + 2T + M + C + 2B)/8	**3.8** (0.5)	**4.2** (0.3)	**4.1** (0.2)	**3.9** (0.3)	**3.7** (0.4)	**4.1** (0.4)	**4.0** (0.3)	**4.0** (0.4)	**4.0** (0.4)

a number of years that the bitter iso-α-acids, derived from the α-acids of hops, are the major contributor to the improvement in beer foam stability [36]. It was further observed by *Diffor* et al that from the iso-α-acid analogs, isohumulone provides a more stable foam than isocohumulone [13].

Other natural but minor hop components such as isoadprehumulone, dihydroisohumulone, and tetrahydroisohumulone have been suggested to promote foam stability and lacing [36, 47]. All compounds mentioned are some analog form of the iso-α-acids, derived from the α-acids and, thus, from the soft resin. While most of the α-acid derivatives have been studied, the role of the hard resin and their possible effect on foam quality has not yet been assessed. Figure 1 shows the effect of each hop fraction on foam stability. It can be seen that as the hopping level increases the foam stability improves. Compared to the unhopped beer, the hop hard resins improved the foam stability of the produced experimental beers in a concentration-dependent manner.

At the tested concentrations (50, 100, and 200 ppm) it was not found for the resins to suppress rather than enhance the foam. When compared to the control, for all tested resin concentrations the foam quality substantially improved. According to MEBAK standards, a good foam stability for a “Vollbier” (full beer; 11.0 - 15.9 °P) is a head retention time between 220 - 300 seconds [28]. While all soft resin beers yielded a good foam, of the hard resin treated beers, only Beer HR(200) (highest hard resin addition) was able to promote a stable beer foam. The head retention time of Beer HR(200) was 23 seconds longer than that of Beer SR(50). This phenomenon can be explained by the chemical nature of the hard resin enriched extract. It has been established that hypdrophobicity is the key factor in determining the effectiveness of a hop compound to stabilize beer foam [47]. The hard resin as a whole is more polar than the soft resin, however there are non-polar compounds found in the hard resin (i.e. xanthohumol). Even if these compounds are more polar than the α-acids, these are still considered to be to some extent hydrophobic. Therefore, xanthohumol and other unknown hard resin compounds could promote the foam stabilizing effect. Although the iso-α-acid content of Beer SR(50) is almost twice that of Beer HR(200), the foam stability of Beer HR(200) was better. This can be explained by the interaction of the non-polar hard resin compounds and the iso-α-acids. These hydrophobic compounds have the tendency to leave the beer and concentrate in the foam, thereby increasing the foam stability. This observation demonstrates that hop hard resins are also of importance in determining the beer foam quality. Furthermore, it proves that other than the iso-α-acid derivatives, there are components present in the hop resins with foam enhancing properties. In these brewing trails, from the hop treated beers, Beer SR(200) had the best foam and Beer SH had the poorest.

As beer is being consumed, the texture of the foam changes from being liquid to almost solid, this solid or dry foam can adhere to the glass surface. The ability of the foam to adhere to the glass wall is known as foam “cling” or “lacing” [5]. The NIBEM Cling Meter quantitatively calculates the area of the glass covered by foam by driving a scanning head down into a rotating Haffmanns standard beer glass. From the proportion of the area covered by residual beer foam and the total scanned area, the cling value is calculated and expressed in foam coverage percentage [17]. To date, there are no defined standards for cling measurements, further it may be supposed that the perception of what constitutes a good lacing is a subjective matter [47], however this foam quality aspect cannot be disregarded.

The results of the foam cling adherence of the experimental beers are shown diagrammatically in Figure 2. As seen in the results, hops not only help improve the beer foam stability but also the foam lacing. The unhopped control and the brew treated with CO_2 spent hop material produced no lace. This phenomenon could already be anticipated from the foam stability results. As seen before, the head retention times for both beers were rather poor and did not reach the MEBAK standards. The foam head of both beers dwindled pretty rapidly even while pouring the beers and, thus, prevented adhesion to take place. This behavior was also observed for Beer HR(50), however with increasing hard resin concentrations, better foams were produced and consequently higher cling coverage. A steady increase in the cling coverage area was observed across the range of hop soft resin concentrations. As seen in Table 2, the cling coverage areas were of 48 % and 49 % for Beer T90 and Beer SR(200), respectively. The similarity in the cling values is unaccounted for, particularly since the determined iso-α-acid content and the measured head retention time for Beer SR(200) were significantly higher than both recorded values for Beer T90. This observation

Table 5 **Sensory mean results (standard deviations in parentheses; n = 3) of final beers in their fresh, force aged[a], and cold aged[b] state, according to the DLG**

	Attribute	Smell		Taste		Mouthfeel		Carbonation		Bitterness		Weighted mean (2S + 2T + M + C + 2B)/8	
Beer UH	Fresh	3.9	(0.7)	3.8	(0.5)	3.8	(0.5)	3.8	(0.4)	3.7	(0.4)	3.8	(0.5)
	Force aged	3.5	(0.4)	3.1	(0.2)	3.7	(0.4)	3.6	(0.4)	3.6	(0.4)	3.4	(0.4)
	Cold aged	4.3	(0.3)	4.2	(0.3)	4.1	(0.2)	4.4	(0.2)	3.9	(0.5)	4.1	(0.3)
Beer T90	Fresh	4.3	(0.3)	4.1	(0.2)	4.3	(0.4)	4.3	(0.4)	4.2	(0.3)	4.2	(0.3)
	Force aged	3.3	(0.6)	3.2	(0.3)	4.0	(0.0)	3.8	(0.3)	3.7	(0.5)	3.5	(0.4)
	Cold aged	4.1	(0.2)	4.3	(0.3)	4.3	(0.3)	4.3	(0.3)	4.2	(0.4)	4.2	(0.3)
Beer SH	Fresh	4.3	(0.3)	4.1	(0.2)	4.3	(0.4)	4.0	(0.0)	3.9	(0.2)	4.1	(0.2)
	Force aged	3.3	(0.5)	3.2	(0.4)	3.9	(0.2)	4.0	(0.0)	3.7	(0.4)	3.5	(0.4)
	Cold aged	4.0	(0.0)	4.3	(0.3)	4.3	(0.3)	4.3	(0.3)	3.9	(0.2)	4.1	(0.2)
Beer HR(50)	Fresh	3.7	(0.4)	3.9	(0.5)	4.0	(0.0)	4.1	(0.2)	4.0	(0.0)	3.9	(0.3)
	Force aged	3.3	(0.3)	3.3	(0.4)	4.1	(0.5)	3.8	(0.3)	3.8	(0.3)	3.6	(0.3)
	Cold aged	3.8	(0.3)	3.8	(0.3)	4.0	(0.0)	4.3	(0.3)	3.8	(0.3)	3.9	(0.2)
Beer HR(100)	Fresh	3.4	(0.7)	3.6	(0.5)	4.0	(0.0)	3.8	(0.3)	3.9	(0.2)	3.7	(0.4)
	Force aged	3.5	(0.5)	3.3	(0.4)	4.0	(0.0)	3.8	(0.3)	3.6	(0.5)	3.6	(0.4)
	Cold aged	3.2	(0.3)	3.2	(0.3)	4.0	(0.0)	4.0	(0.0)	3.8	(0.4)	3.5	(0.2)
Beer HR(200)	Fresh	3.9	(0.4)	4.2	(0.4)	4.3	(0.3)	4.0	(0.3)	4.1	(0.4)	4.1	(0.4)
	Force aged	3.4	(0.4)	3.5	(0.5)	4.0	(0.4)	3.9	(0.5)	3.8	(0.3)	3.7	(0.4)
	Cold aged	3.9	(0.2)	4.0	(0.3)	4.1	(0.2)	4.0	(0.0)	3.9	(0.2)	4.0	(0.2)
Beer SR(50)	Fresh	4.1	(0.4)	3.9	(0.5)	4.1	(0.2)	3.9	(0.2)	3.9	(0.2)	4.0	(0.3)
	Force aged	3.6	(0.5)	3.3	(0.4)	4.0	(0.0)	3.9	(0.2)	3.8	(0.4)	3.7	(0.4)
	Cold aged	3.5	(0.4)	3.9	(0.2)	4.1	(0.2)	4.1	(0.2)	4.0	(0.0)	3.9	(0.2)
Beer SR(100)	Fresh	4.1	(0.4)	3.9	(0.6)	3.8	(0.4)	4.0	(0.0)	4.0	(0.5)	4.0	(0.4)
	Force aged	3.5	(0.5)	3.1	(0.2)	4.0	(0.0)	4.0	(0.0)	3.5	(0.4)	3.5	(0.3)
	Cold aged	4.1	(0.4)	4.2	(0.3)	4.0	(0.0)	4.1	(0.2)	4.2	(0.3)	4.1	(0.2)
Beer SR(200)	Fresh	4.2	(0.4)	4.1	(0.4)	4.1	(0.2)	4.0	(0.0)	3.7	(0.6)	4.0	(0.4)
	Force aged	3.6	(0.4)	3.7	(0.4)	4.0	(0.1)	3.9	(0.2)	3.7	(0.5)	3.7	(0.4)
	Cold aged	3.9	(0.2)	3.8	(0.6)	4.2	(0.3)	4.2	(0.3)	3.7	(0.6)	3.9	(0.4)

[a] Force aged – beers stored for 4 days in the dark at 40 °C

[b] Cold aged – beers stored for 12 months in the dark at approximately 0 °C

suggests the possibility of having substances present in the soft resin enriched extract which hinder foam adhesion.

3.3. Sensory evaluation of the experimental beers

All experimental beers were evaluated in the fresh, the force aged, and a naturally cold aged state according to the DLG approved scheme [28]. The results for the fresh beers assessed for Smell, Taste, Mouthfeel, Carbonation, and Bitterness, are shown in Table 4. According to the results in Table 4, Beer T90 scored the highest on the DLG scheme, while Beer HR (100) had the lowest mark. Based on these results, taste contributions by the hard resin could already be perceived by the panelists at concentrations as low as 50 ppm (Beer HR(50)). When the Taste results of Beer HR(50) and Beer UH are compared, the beer brewed with the hard resin enriched extract scored slightly higher than the unhopped beer. It was at higher resin concentrations where the taste contributions were no longer negligible. Another important attribute and the focus of this study are the Bitterness results. These show DLG scores for Beer UH and Beer HR(50) at 3.7 and 4.0, respectively. These results indicate that the hard resins not only enhance the beer taste but also improve the bitter quality of the produced beers.

In general, for the brews treated with hard resins, the Taste and Bitterness scores improved at high concentrations. Beer HR(100) was the exception scoring the lowest overall DLG mark of 3.7. Another important observation is that the three beers treated with the hard resin rich extract scored well for the Mouthfeel attribute. At the highest dosed hard resin concentration, Beer HR(200), the Mouthfeel mark (4.3) was the same as the reference beer brewed with hop pellets. The scores for the soft resin beers were fairly constant and all three beers scored an overall DLG mark of 4.0.

In Table 5, the Smell, Taste, Mouthfeel, Carbonation, and Bitterness DLG results for all experimental beers evaluated in the fresh, the force aged, and a naturally cold aged state are shown. The scores for all force aged beers show a similar trend as the fresh beers. For all beers, the Smell and Taste scores decreased while the Bitterness scores were only slightly affected. For most beers, the Mouthfeel and Carbonation marks remained constant.

The DLG results for the beers that were naturally cold aged during a 12 month period are mixed. In some cases the results were slightly higher than the fresh beer scores. The results suggest that when the beers are stored cold and not exposed to extreme conditions, the beer profile is similar to that of the fresh beers. The overall score of the reference (Beer T90) in the cold aged state is similar to that in the fresh state. However, the Smell mark decreased from 4.3 to 4.1, this can be due to the degradation of hop compounds and, simultaneous, formation of staling aroma compounds. The overall DLG score for the force aged resin treated beers decreased by 0.3 - 0.5 points. The recorded values of the naturally cold aged beers decreased by no more than 0.2 points.

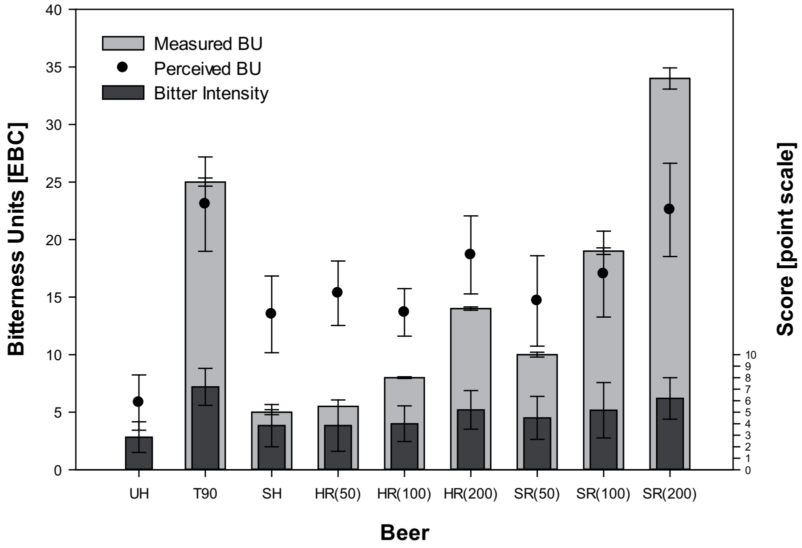


Fig. 3 **Comparison of the sensory (mean of the estimated perceived BU) and analytical (measured BU) bitterness characterization of the resulting experimental beers in their fresh state. The light gray columns show the mean results (n = 3) of the analytically measured bitterness units. The black dots represent the mean values of the perceived bitterness units (n = 3) as estimated by the trained panel (n = 12). The bitter intensity was evaluated according to Kaltner on an 11-point sensory scale (shown on the right y axis) ranging from just detectable (0 - 2) to extreme (9 - 10) [21]. The dark gray columns show the average values (n = 3) of the bitter intensity as evaluated by the panelists**

3.4 Bitterness characterization of fresh beers

The results obtained from the DLG tasting sessions are not enough to fully characterize the bitterness of the produced beers. For this reason, in addition to the common DLG tastings, a hop specific scheme was selected. The panelists were asked to evaluate the bitter intensity of the experimental beers in their fresh state. The bitter intensity was rated according to Kaltner on an 11-point sensory scale [21]. According to the obtained results (see Figure 3), with increasing hard resin concentrations, higher bitter intensities were recorded. The same behavior was perceived for the soft resin treated beers (Beer SR(50) and Beer SR(100)).

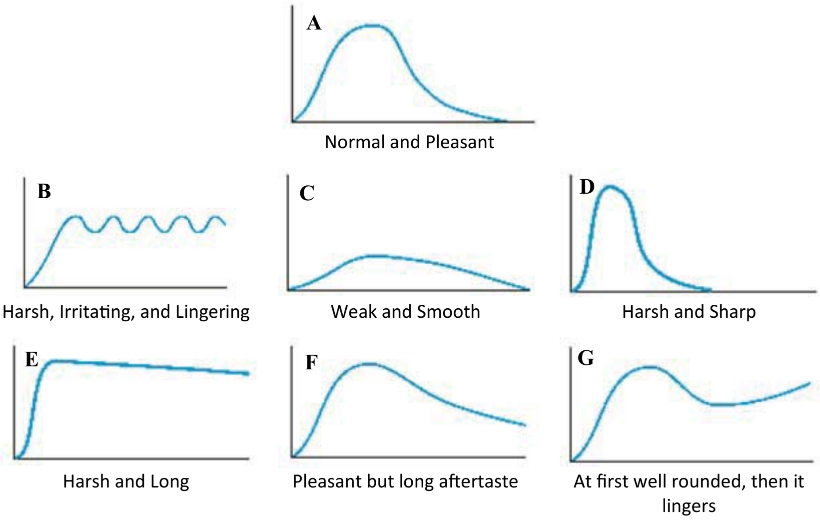
In order to couple and compare the sensory and analytical bitterness characterization of the resulting beers, the panelists were asked to estimate the perceived BU content of the experimental beers. The analytical data (i.e. spectrophotometrically measured BUs) and the sensory data (i.e. perceived bitterness units) of the produced beers are compared in Figure 3. Except for Beer T90, Beer SR(100), and Beer SR(200), the perceived bitterness of the resulting beers was higher than the measured one.

For the resin rich beers, the perceived bitterness was about 4 units higher than the measured BU content. For the hard resin beers this confirms that, indeed, a large portion of this fraction is not quantified by the BU method. It further proves that the hard resins have a high bittering potential; even if, hitherto, it is not possible to analytically quantify it. As previously mentioned, the complex nature and composition of the soft resin enriched extract is unknown. However, it is believed that it is composed by more than just α-acids and, thus, the delivered bitterness has to be caused by other substances besides the iso-α-acids. Therefore, the inconsistency between both values is to be expected. Although most bittering substances are found in the soft resin, a small amount is not detected at 275 nm.

Temporal bitterness characteristics of the experimental beers were also evaluated. To achieve this, the panelists were presented with seven bitter time-intensity curves (Figure 4 A - G), each representing a different bitter profile. For each beer, the panelist selected the curve that best describes the perceived bitterness profile. Usually, the desired bitter profile is a bell-shaped one (Figure 4 A), which depicts a balanced, normal, and pleasant bitterness with no extreme lingering. There was a split opinion for the bitter profile of Beer T90, 50% of the panelists perceived a harsh, irritating, and lingering bitterness (Figure 4 B) whereas the other half indicated that the bitterness was at first well rounded but then it lingered (Figure 4 G). The perceived bitterness of the beer produced with CO_2 spent hop material, as well as the beers brewed with the hard resin enriched extract, was considered by almost 90 % of the panelists to be mild, weak, and smooth (Figure 4 C). The bitter profiles assigned to the soft resin beers varied as the concentration increased. The produced bitterness of Beer SR(50), brewed with the lowest soft resin concentration, was considered by 50 % of the panelists to be normal and pleasant (Figure 4 A), the remaining 50 % rated it as smooth (Figure 4 C). At addition rates of 100 ppm of soft resin it was possible to produce a normal and pleasant bitterness (Figure 4 A). However, when 200 ppm of soft resin were added the bitterness was judged by 90 % of the panelists to be harsh and long lasting (Figure 4 E).

3.5 Flavor stability assessment of experimental beers

To assess the flavor stability, all experimental beers were evaluated for staling compounds in the fresh and the aged (forced and cold) state. No oxidized flavor was perceived in any of the fresh beer samples, a score of 1 was assigned for all attributes. The results of the sensory evaluation of the force aged and cold aged beers samples are shown in Table 6. A weak to moderate aged flavor was noted in the force aged beers. After a year of cold storage, the beers were able to preserve most of their character, no extreme



Normal and Pleasant

Harsh, Irritating, and Lingering

Weak and Smooth

Harsh and Sharp

Harsh and Long

Pleasant but long aftertaste

At first well rounded, then it lingers

Fig. 4 Time-intensity curves depicting different bitter profiles according to Kaltner [21]

staling was detected, and the scores are comparable to those of the beers in their fresh state. The cold aged beers were not exposed to extreme conditions, therefore it is possible that no intense oxidation took place and it could explain the similarity to the fresh beers. Upon aging (forced and cold) the hard resin beers showed a higher Bitterness stability than the soft resin beers. At high hard resin concentrations (100 and 200 ppm) the overall aging score is relatively low. Thus, suggesting that large amounts of this resin can protect the beers from staling and afford a better flavor stability.

To fully evaluate the flavor stability, the panelist graded the acceptance in terms of aging on a 0 - 100 % scale, where 100 % = fresh; 50 % = aging is detectable; 0 %= undrinkable [14]. All experimental beers were 100 % accepted in their fresh state. Figure 5 diagrammatically compares the acceptance of the force and cold aged beers. Except for Beer HR(100) all beers were better accepted in their cold aged state. All experimental beers scored above 50 %, for most beers only minor staling was detectable. Moreover, compared to the hard resin beers, Beer SH had a comparably low acceptance level. Thereby indicating that the hard resins, purified from the spent hops, possess better brewing properties and have a higher brewing potential than the untreated spent hop material.

3.6 Taste contributions of hop hard resins to beer

As early as 1888, *Hayduck* was the first to identified the γ-resin [9] to be insoluble in light petroleum. Thereupon, the hard resin was regarded by Hayduck of no brewing value [18]. Several studies have considered the impact of hard resins on beer quality, and yet, hitherto a comprehensive understanding of the hard resins does not exist. To add to this confusion, not only is very little understood regarding the composition of the hard resins, but also much of the existing data is conflicting.

Notwithstanding Hayduck's breakthrough in hop chemistry, some of his conclusions can be refuted. First, it can be argued that the separation method of the α-, β-, and γ-resin on the basis of their solubility in organic solvents [18] is flawed. In 1911, *Tartar* and *Bradley* published a comparative study of the methods for the determination of hard and soft resins in the hop [40]. In their review it was concluded that in all the methods devised for the determination of hop resins, the separation of the soft resins from the hard resin is dependent upon the ready solubility of the former in petrolic ether, the latter being practically insoluble in this solvent. It was found that in some instances, the hard resin fraction of the hops was simply too high. This inconsistency is believed to be due to the presence of soft resins left from incomplete extraction with petrolic ether [40]. From the comparison of the different methods of analysis, it was essentially found that a great variety of results had been obtained by various chemists. According to Tartar and *Pilkington*, since much of the data was found to be conflicting, this would lead one to suspect that some of the analytical methods used were inaccurate; and as a consequence, in many instances, erroneous results had been obtained [42]. Based on this information, there is a high possibility that in Hayduck's experiments the hard resins were not properly separated and found in solution with the α- or β-resin. Thus, what Hayduck classified as γ-resins may, in fact, be a mix of hop wax and other hop constituents and not hard resins *per se*.

To further invalidate Hayduck's statement that the γ-resin is tasteless; this feature can be attributed to the kilning practices of the time. It is well known that hops are inevitably deteriorated to some extent during kilning. Already in 1898, it was shown by *Remy* that a considerable portion of the soft resin present in the fresh hop is converted into hard resin during the kiln-drying process [6]. In 1907, *Stockberger* stated that at the time, the average grower would frequently contrive to dry the hops at a temperature so high that a considerable quantity of valuable soft bitter resin was oxidized and converted into the hard, worthless form [41]. From these findings, it can be assumed that the temperatures were so high that extreme oxidation of the hop resins took place. In such way suggesting that the hard resins later found in the dried hops by Hayduck were actually further oxidation products of the hard resins. As mentioned previously, based on the obtained data, these extremely oxidized products can indeed be classified as tasteless. To further substantiate this, evidence was found in research done almost half a century later, that the xanthohumol content of fresh green hops (0.86 % dry weight) dramatically reduced (to 0.31 % dry weight) during kilning, but the, expected, corresponding increase in the isoxanthohumol content was not observed [3]. In another study, it was observed that the δ-resin content of the hop cones also decreased during drying [7]. Both studies suggest that further uncharacterized oxidation products arise from the hard resins during kilning. Further, it may be concluded that what Hayduck believed to be fresh hops would currently be considered extremely aged hops. Thus, no adequate comparison can be established between Hayduck's γ-resin and the current version of hard resins. Finally, Hayduck does not mention the amount of γ-resin used in his trials. As mentioned previously, it was at hard resin amounts above 50 ppm that bitterness was perceptible (see Figure 3). From the results obtained from the brewing trails in this study, it can be concluded that contrary to Hayduck's finding, the hard resins are, indeed, capable of imparting bitterness to wort.

In 1958, 60 years after Hayduck's findings, *Rigby* looked into the bittering potential of the hard resins. In this study, pilot plant brews were carried out using, as the only hop adjunct, the hard

Table 6 **Mean aging scores[a] (standard deviations in parentheses; n = 3) of the sensory evaluation of flavor stability of experimental beers in their force aged[b] and cold aged[c] state**

	Attribute	Smell		Taste		Bitterness		Weighted mean (2S + 2T + B)/5	
Beer UH	Force aged	1.6	(0.5)	1.7	(0.7)	1.2	(0.3)	1.5	(0.5)
	Cold aged	1.0	(0.0)	1.0	(0.0)	1.1	(0.2)	1.0	(0.0)
Beer T90	Force aged	1.3	(0.4)	1.8	(0.7)	1.4	(0.2)	1.6	(0.5)
	Cold aged	1.0	(0.0)	1.0	(0.0)	1.1	(0.2)	1.0	(0.0)
Beer SH	Force aged	2.1	(0.7)	2.0	(0.8)	1.6	(0.8)	2.0	(0.8)
	Cold aged	1.0	(0.0)	1.0	(0.0)	1.0	(0.0)	1.0	(0.0)
Beer HR(50)	Force aged	1.7	(0.4)	1.8	(0.3)	1.4	(0.2)	1.7	(0.3)
	Cold aged	1.4	(0.5)	1.6	(0.8)	1.4	(0.8)	1.5	(0.7)
Beer HR(100)	Force aged	1.3	(0.4)	1.3	(0.4)	1.3	(0.4)	1.3	(0.4)
	Cold aged	1.2	(0.3)	1.1	(0.2)	1.3	(0.4)	1.2	(0.3)
Beer HR(200)	Force aged	1.7	(0.4)	1.6	(0.4)	1.3	(0.4)	1.6	(0.4)
	Cold aged	1.3	(0.4)	1.3	(0.4)	1.2	(0.4)	1.2	(0.4)
Beer SR(50)	Force aged	1.4	(0.4)	1.5	(0.3)	1.4	(0.7)	1.5	(0.4)
	Cold aged	1.4	(0.2)	1.4	(0.7)	1.3	(0.4)	1.4	(0.4)
Beer SR(100)	Force aged	1.7	(0.5)	1.8	(0.6)	1.8	(0.5)	1.7	(0.6)
	Cold aged	1.0	(0.0)	1.2	(0.3)	1.0	(0.0)	1.1	(0.1)
Beer SR(200)	Force aged	1.5	(0.5)	1.6	(0.5)	1.7	(0.7)	1.6	(0.5)
	Cold aged	1.3	(0.4)	1.3	(0.3)	1.3	(0.3)	1.3	(0.3)

[a] Aging scores, according to Eichhorn [14]: 1 = fresh; 2 = weakly aged; 3 = strongly aged; and 4 = very strongly aged

[b] Force aged – beers stored for 4 days in the dark at 40 °C

[c] Cold aged – beers stored for 12 months in the dark at approximately 0 °C

resins from fresh hops. From the taste test evaluation of the beer it was determined that the hard resins had a modifying effect on flavor although not one of bitterness [33]. These results not only disagree with the findings from the experimental beers brewed in this study, they are also a contradiction to *Walker*, *Zakomorny*, and *Blakebrough* observations. In 1952, few years before Rigby's study, Walker et al found a water soluble portion of the hard resin which also had a bitter character [44]. Therefore, Rigby's results would seem to be a contradiction to the findings of Walker et al. The δ-resin is part of the hard resin, thus, brewing trials using hard resins as the wort bittering material can be expected to be to some extent bitter. However, in Rigby's report the dosed amount of hard resins is not mentioned, consequently it is not possible to compare his results with the ones obtained in the present study. Furthermore, if the amount used by Rigby can be considered negligible, then no significant taste contribution can be expected.

In a later study, comparable to the present one, *Laws* added hard resins to wort at a concentration rate of 200 ppm. Contrary to the sensory evaluation results from the experimental brews produced in the present study, Laws' beer was considered by the taste panel to be sweet [25]. A number of factors ranging from the wort composition to the brewing regime, the yeast used, and finally the original state of the hops can account for this. It is stated that the hard resins were extracted from old Bullion hops. It is highly probable that a larger part of the hard resins had undergone further oxidation, as a result the unelucidated and overoxidized products were no longer bitter. Another possible source for discrepancy is that in this study, for screening purposes, the brewing trials were carried out on a 10 L scale. It is known that with small scale fermentations it is often not possible to replicate the flavor profiles produced by large scale brewing. Since all current publications do not mention the brewing scale, it is not possible to confirm that this is the reason for the inconsistencies in the results. The results obtained in the present study disagree with most of the previous research. However, due to the omission of information in the other studies, it is impossible to determine the source of discrepancy. Based on the results from the brewing trails in this study, it can be concluded that it is, in fact, possible to produce a perfectly acceptable beer with pure hard resins as the only bittering source.

4 Conclusions

As hops age during storage, the percentage of soft resin falls while that of the hard resin increases. It is generally accepted that the hard resins have no brewing value. However, the contribution of the oxidation products to beer quality is not fully understood. In this study, brewing trials were conducted to investigate the additive effect, on beer quality, of all the oxidation products present in the hard resin. The obtained results help provide a better understanding of the impact of hard resins to beer properties in particular taste, bitterness, and flavor stability.

The contributions of hop hard resins to beer quality were evaluated in 10 L brewing trials. According to the results from the standard beer analyses, nine comparable experimental beers were produced. Based solely on the analytical data it can be concluded that high

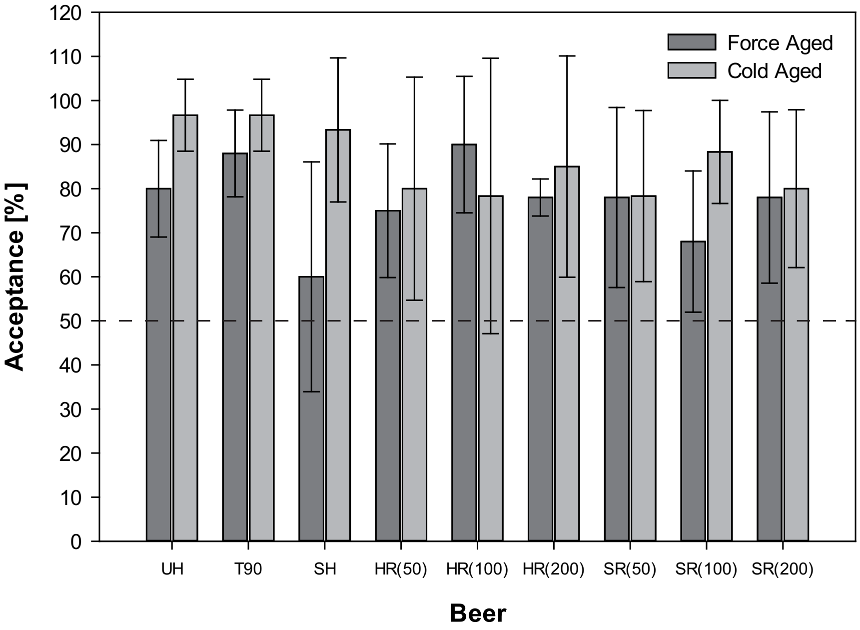


Fig. 5 **Comparison of the flavor stability of the force aged (beers stored for 4 days in the dark at 40 °C) and cold aged (stored for 12 months in the dark at 0 °C) state. Shown are the mean acceptance values (n = 3) in terms of aging on a 0 - 100 % scale, where 100 % = fresh; 50 % = aging is detectable; 0 %= undrinkable [14]**

quality beers can be brewed using exclusively hard resins to bitter worts. With increasing hard resin concentrations, better foams were produced and consequently higher cling coverage. At additions above 50 ppm of hard resin, this fraction positively contributes to the foam stabilizing effect as well as to the chemical properties.

The taste contributions of the hard resins were assessed using different schemes. The DLG results of the fresh beers indicate that the hard resins have a positive impact on all attributes. Taste contributions delivered by the hard resin were perceptible by the panelists at concentrations as low as 50 ppm. Further, the results indicate that the hard resins improve the bitter quality of the produced beers as well as the Mouthfeel of the beers. The DLG Smell and Taste scores for the force aged beers decreased while the Bitterness scores were only slightly affected. The DLG results for the beers that were naturally cold aged during a 12 month period were similar to that of the fresh beers.

In further characterization of the produced bitterness it was observed that with increasing hard resin concentrations, higher bitter intensities were recorded. The perceived bitterness of the hard resin beers was about 4 units higher than the measured BUs. This confirms that although a large portion of this fraction is not quantified by the BU method, the hard resins have a bittering potential. This can be attributed to the limitations of the method, to further complicate matters, the trial brews in this study are non standard beers. As a result the BU analysis results less suitable than usual. Temporal bitterness characterization of the experimental beers treated with hard resins was considered to be weak and smooth.

A weak staling flavor was noted in the aged (forced and cold) beers. Upon aging, the hard resin beers showed a better Bitterness stability than the soft resin beers. At high hard resin concentrations the overall aging score was relatively low. These results indicate that high amounts of this resin can protect the beers from staling and afford a better flavor stability. Finally, for most beers only minor staling was detectable and the acceptance of all experimental beers was above 50 %. Based on the sensory assessment of the experimental brews, it can be concluded that to some extent, the hard resins contribute to the taste properties of the produced beers. Further, the results indicate that the hard resins, purified from the spent hops, possess better brewing properties and have a higher brewing value than the untreated spent hop material. The results lead to the suggestion that further treatment of the worthless spent hop material yields a valuable extract high in hard resins with potential brewing applications.

Hitherto, limited research has been conducted on the hard resin fraction as a whole. As of recent, much attention has been given to the hard resin prenylated chalcone, xanthohumol. It is not possible to make generalizations on the hard resin contributions to beer based solely on the available data for xanthohumol. Given the lack of information and chemical data available, it is challenging to conclusively address the contributions of hard resins to beer quality. The present study is a starting point which provides some insight into the impact of the hard resins on beer.

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