C. Zufall, K. Wackerbauer and C. Brandt

The Influence of Hop Products on Beer Flavour Stability

The use of reduced iso-alpha-acids in brewing has become more widespread in past years, although their characteristics during beer ageing are not completely known. During our investigations, important differences in ageing characteristics were detected not only between the categories of reduced and non-reduced hop extracts, but also within the group of beers containing reduced hop products. Forced ageing in the absence of light had the strongest impact on beers hopped with CO2-extract, followed by iso-alpha-extracts. Rho, Tetra and Hexa showed a significantly better stability with the tendency to improve from Rho- over Tetra- to Hexahydro-isohumulone. Under light exposure, besides showing lightstruck flavour, beers hopped with CO2-extract were the first to show cardboardy oxidation aroma, while beers containing only reduced hop products were virtually unaffected. Extended periods of light exposure, however, led to the formation of methyl-furfuryl-disulphide (MFDS) off-flavour, also in light-stable beers, as previously reported [1]. The findings from sensory analysis could be confirmed by instrumental analysis of chemiluminescence behaviour. The results indicate clearly that the choice of hop products has a decisive influence on beer flavour stability.

Descriptors: flavour stability, light-stable beer, reduced iso-alpha-acids, sensory analysis, flavour descriptors

1 Introduction

The use of reduced iso-alpha-acids in brewing has become more widespread in past years, both to achieve full light-stability and also as additional ingredients in beers brewed with conventional hop products. The partial or complete replacement of conventional hop products represents an important modification of beer characteristics in itself, but furthermore, beer ageing behaviour is most likely affected by this measure.

As is commonly known, so-called "light-stable" formulations for beer are only focused on the avoidance of the formation of prenyl mercaptan (3-methyl-2-buten-1-thiol) in beer by the influence of light. The main purpose is to make it possible to bottle the product in transparent glass. There is no protection against any other alterations of the beer's flavour during ageing under the influence of light [2].

In general, hop bitter acids share a chemical structure which makes them function as redox systems and thus, it is obvious that they are able to play a role in beer ageing reactions. In this context, Hashimoto and Kuroiwa [3] showed in 1975 that isohumulone could be oxidatively degraded in model solutions. The magnitude of the sensory impact of this oxidation, however, remained unclear. Our earlier studies showed that ageing characteristics of beers hopped with reduced iso-alpha-acids contrast significantly from those of conventional beers. Some specific well-known stale flavours do not differ between beer types in terms of the flavour impression perceived. However, it was possible to detect flavour notes in light-stable beers which cannot be found in conventional products at all [1]. This paper deals with the latest findings about stale flavour intensity and characteristics of beers brewed with the most important types of reduced hop extracts compared within this group and against conventional hop products.

Authors: PD Dr.-Ing. Carsten Zufall, Cervecería Polar, C. A., Centro Tecnológico Polar, 2da. Avenida de Los Cortijos de Lourdes, Caracas 1071-A, Venezuela, carsten.zufall@empresas-polar.com; Univ.-Prof. em. Dr.-Ing. K. Wackenbauer, cand.-Ing. C. Brandt, Berlin University of Technology, Institute for Biotechnology, Seestr. 13, 13353 D-Berlin, Germany

Tables and figures see Appendix

2 Materials and Methods

Samples of hop products have been provided by two different commercial suppliers. Sensory evaluations of staling beers were carried out by an expert taste panel specifically educated for this purpose. Flavour stability characteristics have been assessed instrumentally by chemiluminescence analysis (CLA) of radical reactions.

Sample preparation

Ageing of the bottled beer samples in the dark took place at a temperature of 28 °C. Light exposure took place in a chilled environment (6 °C) using a 500 W halogen lamp (7,700 lm) placed at a distance of 120 cm to the samples bottled in flint (transparent) glass bottles. Reflective aluminium foil behind the samples provided for even illumination from the back side.

Sensory analysis

A specialized taste panel for aged flavour consisting of twenty-five expert tasters has been formed. Training of the panellists was carried out using both naturally and force-aged beers as well as chemical flavour descriptors developed for this purpose.

The sensory evaluations were carried out in accordance with MEBAK methods [4], but in the case of freshness evaluation with an intensity scale ranging from 0 to 10 in series 3 and 4. Conclusions from sensory analysis were corroborated with Fisher's statistical significance test. According to the trial design, the corresponding value from the applicable table (F_T) needs to be smaller than the value calculated from the data set (F_C) to prove significance of the difference observed [5].

Chemiluminescence analysis

Chemiluminescence measurement is based on the extremely sensitive determination of photons which are always being formed during radical reactions. The chemiluminescence detector itself consists of a measuring chamber which is completely shielded against outside light. Beer samples are introduced into the chamber where they are heated up to a defined temperature. The extremely small quantities of light (around 10^{-15} W) are measured using a photo electron multiplier. The result of a measurement is a graphical representation of light intensity (photon counts) over the duration of the radical reaction (typically a couple of hours). This is equivalent to a diagram of the intensity of radical reactions over time.

A typical chemiluminescence graph starts at a relatively low reaction rate after the initial warm-up phase. After a certain time, a strong increase of the signal can be noticed. This is when the radicals have consumed most of the reducing substances or radical quenchers in the beer. After reaching a maximum, the radical reaction rate decreases again indicating the depletion of oxidizable substances in the sample. To compare different graphs, the so-called „lag-time" until the signal increases can be used. However, we found it to be more precise to measure the time until the maximum signal occurs. The longer it takes to reach this maximum of light intensity, the more resistant is the sample against oxidative radical reactions and thus the beer's flavour stability is considered better.

10 ml of the beer sample, after being degassed by supersonication, were placed in a sample dish and its chemiluminescence was measured at 60 °C for up to 20 hours. Beer was stored at 0 °C before being analysed. To record the radiated photons of the sample a CLA-2100 instrument from Tohoku Electronic Industrial Co. Ltd. (Rifu, Japan) was used.

3 Results and Discussion

For the trials, single pilot-scale brews were produced with different commercial hop products from two different suppliers, namely CO_2-extract, isohumulone extract, and Rho-, Tetra- as well as hexahydro-isohumulone extracts. The resulting beers were bottled and subjected to an ageing at 28 °C over a period of eight weeks. Alternatively, light exposure of the beers was carried out during up to 192 hours.

The main focus of the work was sensory analysis. However, an extensive cross-check by chemiluminescence measurement has been made to verify the results obtained during the tastings.

Taste panel results

In the first series of trials, light exposure experiments were carried out in clear glass bottles with the main objective of confirming the light stability of the reduced hop products. As can be seen from Table 1, the reduced isohumulone products gave no lightstruck or "skunky" flavour in beer at all, not even after 48 hours of exposure to the light source. For the two non-reduced isohumulone-hopped beers (produced with CO_2 extract and Iso extract, respectively), lightstruck flavour appeared after a certain time of light exposure, as expected.

However, it was rather unexpected to see that beers produced with isohumulone instead of CO_2 extract seem to have a slightly better resistance against lightstruck flavour. The beer hopped conventionally with CO_2 extract showed "skunky" flavour after 16 hours of exposure, while at the same time, there was none detected in the Iso extract beer. Then, after 24 hours under the influence of light, lightstruck flavour was also present in the latter beer.

Ageing of the same beers from series 1 at 28 °C, protected from the influence of light gave two different results (see Fig. 1). In terms of a general freshness character, there was virtually no difference during two weeks of ageing. Only after four weeks at 28 °C, a decrease in the perception of freshness of the beers brewed with CO_2 extract, Rho and Tetra was observed, while the Hexa beer showed a tendency to maintain its freshness slightly better. Then again, the quality of bitterness decreased in the case of the CO_2 extract beer, showing harsh, clinging and adstringent notes, while the downstream beers remained unchanged. Series 1 has shown an advantage of reduced hop product over conventional ones.

For the sake of completeness, it has to be mentioned that beer brewed with CO_2 extract had a more pronounced tendency to form cardboard flavour, while beers produced with downstream hop products developed more bread-like notes in the first place, according to previously published results [1]. However, for the general freshness evaluation, these slight differences were not considered.

In the following two series of trials the main objective was to elucidate the impact of light energy on the formation of stale flavour in beers produced with downstream hop products. Series 2 is a comparison of commercial Tetra and Hexa products from two different suppliers. Figure 2 shows that during the first 24 hours of light exposure, all hop products performed equally well. Only after 48 and 96 hours, some differences became visible. Mainly the two Tetra beers deteriorated somewhat in their freshness ratings, but also one of the Hexa beers suffered a decrease in quality. The tendency, however, seems to be that Tetra is slightly more susceptible to ageing than Hexa. In the evaluation of bitterness quality, there were no significant differences between the individual beers. Methyl-furfuryl-disulphide (MFDS) flavour was detected sensorically in all of the beers beginning after 24 hours of light exposure, as previously described (1). However, the component has not been quantified by chromatography.

Based on the findings from series 2, the following series 3 was set up as a broader comparison including Iso extract and crystalline

Rho and Hexa products from supplier 2, besides a standard Hexa extract. Crystalline products contain around 90 % by weight of the respective reduced bittering acid in a purified form and require dissolving prior to their use in the brewery. During an extended period of light exposure of up to 192 hours (eight days) in clear glass bottles, no significant change in bitterness quality was observed in any of the beers. Although the evaluation of the Iso beer was more difficult due to the formation of lightstruck flavour, the tasting panel succeeded in focusing on the sensory indicators for ageing. Again, MFDS flavour was detected in all of the reduced hop extract beers exposed to light.

As can be seen from Figure 3, in comparison, the Iso extract beer deteriorated more rapidly than its Rho and Hexa counterparts. Up to 48 hours of light impact, both Hexa products were most stable and superior to Rho and Iso. Fisher's test results in $F_C > 4{,}74$, proving that the differences are significant. This advantage of Hexa is lost after 96 and 192 hours, and it is after 192 hours that the standard Hexa product deteriorates in freshness compared to the crystalline type ($F_C > 4{,}74$). Again the overall conclusion can be that beers produced with reduced hop products are less susceptible to staling than those made with non-reduced products.

When amber glass bottles were used in the light exposure experiments, the somewhat better protection led to slightly better notes for freshness of the beers in general. However, the formation of MFDS flavour could not be avoided. Already after 12 hours, Iso beer is characterized by a lower freshness than the reduced varieties (significant with $F_C > 4{,}74$). Over the course of the experiment, both crystalline Rho and Hexa are very stable and the crystalline Hexa beer maintains its freshness best until the end of the trial (significant with $F_C > 4{,}74$). In quality of bitterness, a slight variation could be observed in the case of the beer containing standard Hexa extract, which was evaluated slightly worse than all other beers from after 48 hours on, while there was no change whatsoever in any of the beers made with the other hop products (see Fig. 4).

Ageing of the same beers from series 3 in darkness at 28 °C showed no difference at all after four weeks ($F_C < 4{,}74$). However, after a period of eight weeks, a sequence of freshness ratings (significant with $F_C > 4{,}74$) could be observed, with the Iso beer being more deteriorated than the two crystalline products and the standard Hexa product beer being most stable (see Fig. 5). Beer flavour stability increased thus from Iso to Rho and finally Hexa, which also reconfirms the findings from the previous series that reduced hop products provide for a better beer flavour stability than their non-reduced counterparts.

Series 4 was carried out in search of a reconfirmation of the findings for ageing of differently hopped beers in darkness. Rho, Tetra and Hexa extracts, all from supplier 1, were employed. Additionally, malt from low-LOX barley was included in the trials besides the standard malt used in the previous series. All resulting beers were aged at 28 °C for an extended period of up to 17 weeks and evaluated by out expert taste panel.

Figure 6 shows that the freshness ratings increased from Rho over Tetra through to Hexa products in each of the different ageing steps. In contrast to that, we could not find any difference in bitterness quality of Hexa- and Tetra-hopped beers. However, Rho was clearly inferior to both aforementioned hop products for the characteristic discussed (significant with $F_C > 3{,}29$). It is noteworthy that the use of low-LOX barley malt had no significant influence on flavour stability in our trials ($F_C < 3{,}29$).

Results from instrumental chemiluminescence analysis

Chemiluminescence measurements were carried out on three different batches of base beer. However, it is not possible to compare the figures between batches quantitatively because the levels of sulphite in the batches were never exactly the same and it is well-known that chemiluminescence measurement is very easily influenced by these reducing substances. Qualitatively, all base beer batches gave the same results, that means the sequence of beer stability (given by chemiluminescence analysis lag times) was identical. The data presented here has thus been taken from one single series of trials representative for the entire set of data.

The findings from sensory analysis and the tendencies observed could be confirmed by chemiluminescence measurement. The shortest "lag times", actually the times required to reach the turning point of light abundance, were observed in the case of beers hopped with CO_2 extract, followed by Iso, Rho and Tetra products. Beers with Hexa gave the longest lag times, corresponding to the best flavour stability rating (see Tab. 2). First of all, this indicates a decisive advantage of downstream hop extracts over CO_2 extract, as well as an advantage of reduced over non-reduced hop products. Furthermore, these findings, together with the well-coinciding results from sensory analysis, help establish a sequence of hop products according to their influence on beer stability.

4 Conclusions

The results of our study indicate that the choice of hop products has a decisive influence on flavour stability of the final beer. Beers produced from the same base but with different hop products show a distinctive behaviour during ageing and under the influence of light. We have demonstrated that both in the absence of light as under light exposure, reduced isohumulone products are superior to conventional or non-reduced ones in terms of flavour stability. Generally speaking, reduced hop products cannot avoid the formation of stale flavour completely. However, the beers brewed without conventional hops are significantly more resistant. Furthermore, we detected a strong tendency towards improved beer stability with a higher degree of reduction, i. e. from Rho- over Tetra- to Hexa-products.

Results from sensory analysis coincided well with the data obtained by chemiluminescence measurement. Furthermore, the previously reported [1] formation of a "roasted" flavour in light-exposed beer due to the formation of methyl-furfuryl-mercaptan (MFDS) was confirmed. Additionally, there was no noticeable difference between beers brewed with standard barley malt and those made with a low-LOX variety.

There are indications in literature that staling under the influence of light is strongly promoted by compounds acting as activators such as riboflavin [6]. Similar effects can be expected from the various "impurities" contained in conventional hop products. This would explain the improvement in light-mediated flavour stability from beers brewed with CO_2-extract to those made with reduced isohumulones. It would also explain the slight advantage of beer hopped with Iso extract against beer produced conventionally with CO_2 extract in terms of the occurrence of lightstruck flavour. However, also the ageing process in the dark is most probably promoted by redox systems introduced through conventional hop products. The sheer quantity of different redox systems seems thus to be an important factor in beer staling, which as a consequence leads to the conclusion that eliminating as many redox systems as possible should enhance flavour stability.

Beyond doubt, the chemical reduction of isohumulone molecules has one well-known stabilizing effect: The formation of 3-methyl-2-buten-1-thiol under the influence of light energy is suppressed. With this in mind, our results clearly indicate that reduced isohumulone molecules are also much more stable against oxidation, both with and without light energy involved. From the point of view of chemical structures, reduction of isohumulones is equivalent to a removal of double bonds from the molecule. As double bonds are well-known targets and starting points of oxidative reactions, it can be assumed that their elimination should stabilize the molecule against oxidation or make its participation in redox reactions more difficult.

It can be concluded that there seem to be two big groups of compounds from hop products influencing beer flavour stability, namely "impurities" in conventional products such as aroma compounds and redox systems, and of course the isohumulone molecule itself, which can apparently be stabilized by different degrees of chemical reduction. For the sake of completeness, it must be stressed that the complete replacement of conventional hop products, such as pellets or CO_2-extracts by downstream products such as iso-, Rho-, Tetra- or Hexahydro-isohumulones leads to an entirely different beer character.

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Some of the results have been presented at the EBC Congress in Venice, May 2007 [7].

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Appendix

Table 1 **Series 1, results of sensory analysis after light exposure of beer packaged in clear glass and containing different hop products**

Time of light exposure [h]	CO_2 extract	Iso	Rho	Tetra	Hexa (9H : 1T)
4	0	0	0	0	0
8	0	0	0	0	0
16	**X**	0	0	0	0
24	**X**	**X**	0	0	0
32	**X**	**X**	0	0	0
48	**X**	**X**	0	0	0

0: Absence of lightstruck flavour
X: Presence of lightstruck flavour
All hop products in this series were from supplier 1.

Table 2 **Chemiluminescence analysis lag times (time required to reach turning point of light abundance)**

Hop product	Perceived bitter units	SO_2 [mg/l]	Max. CL counts	Lag time [min]
CO_2 extract	17	4.3	36,647	**17**
Iso	17	4.5	29,100	**38**
Rho	17	4.5	37,167	**43**
Tetra	16	4.5	25,999	**49**
Hexa	17	4.4	27,293	**53**

All hop products were from supplier 1.

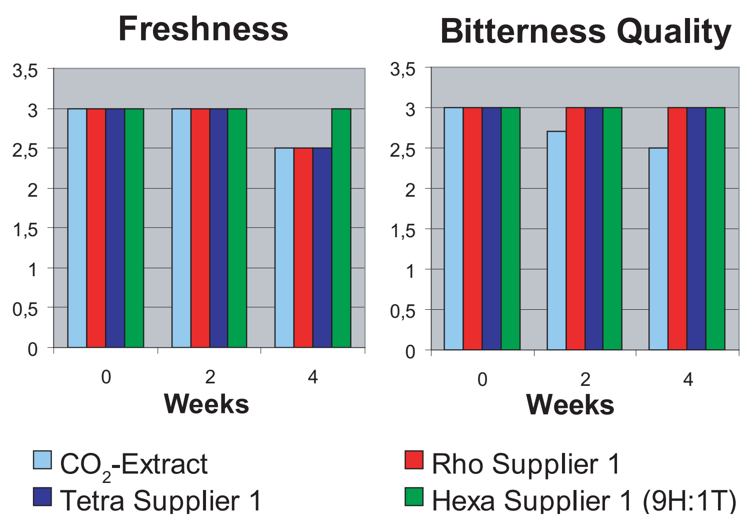

Fig. 1 **Series 1, results of ageing at 28 °C**

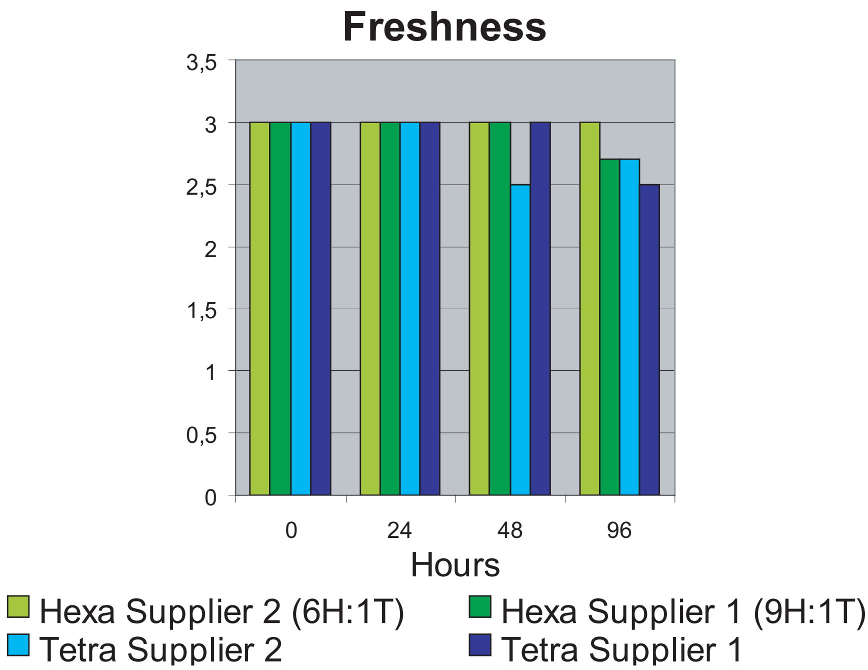

Fig. 2 **Series 2, results of light exposure. No differences were observed in Bitterness Quality**

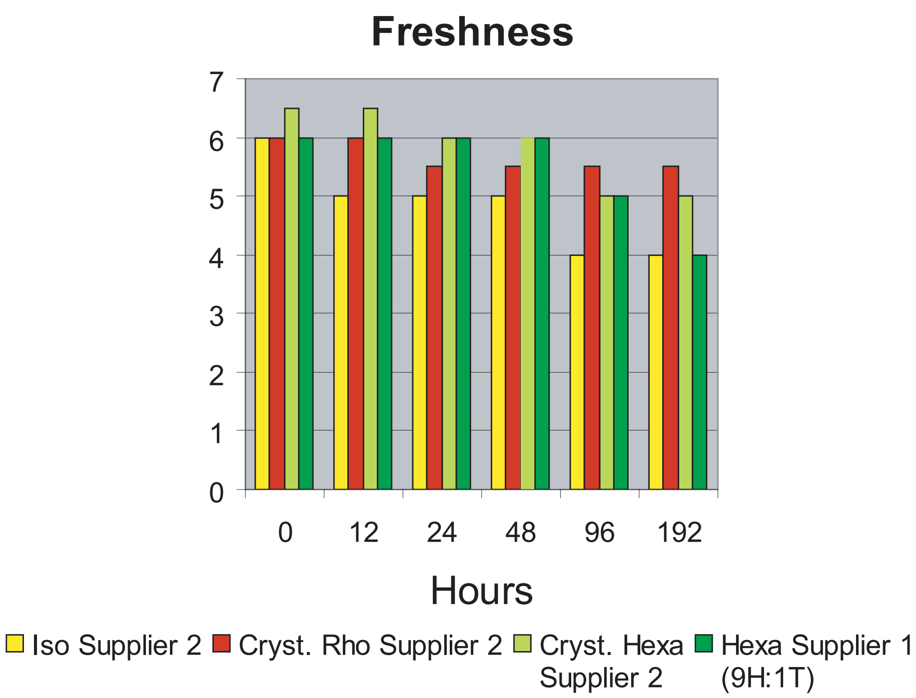

Fig. 3 **Series 3, results of light exposure in clear glass bottles. No differences were observed in Bitterness Quality**

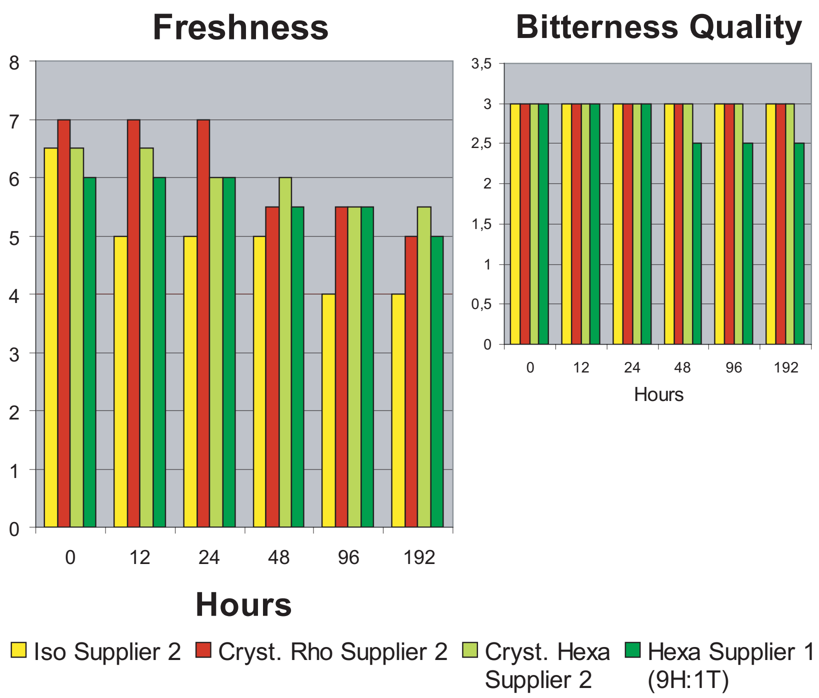

Fig. 4 **Series 3, results of light exposure in amber glass bottles**

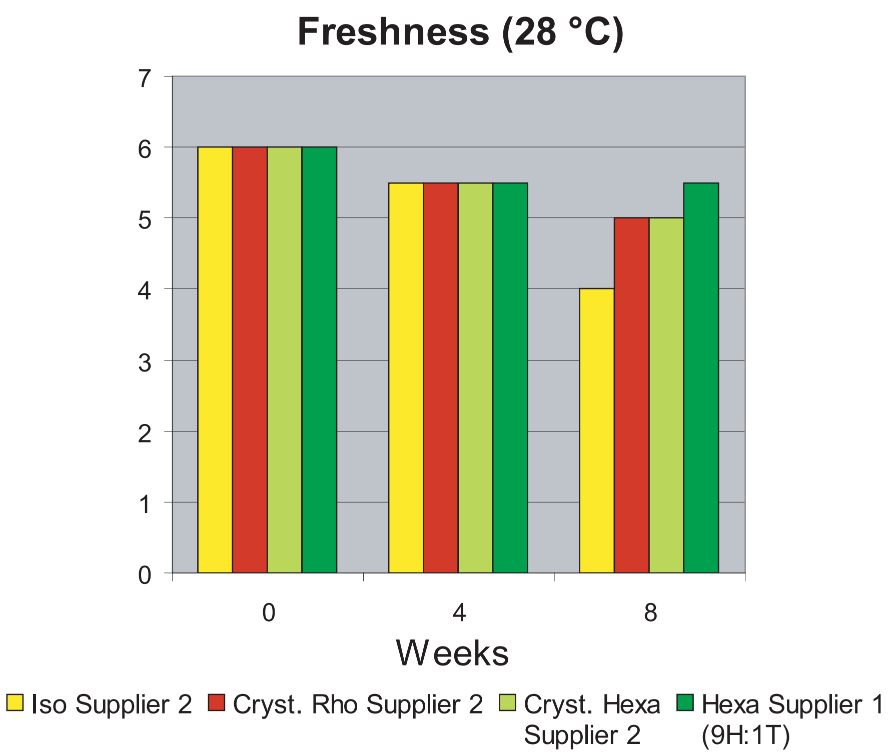

Fig. 5 **Series 3, results of ageing at 28 °C**

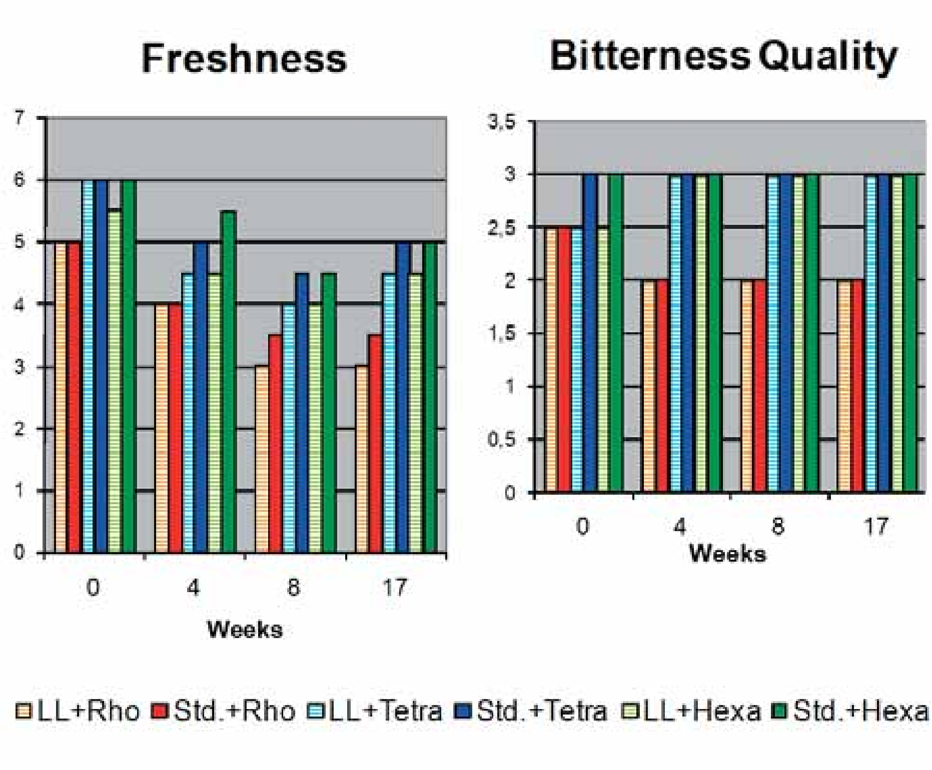

Fig. 6 Series 4, results of ageing at 28 °C, all hop products from supplier 1

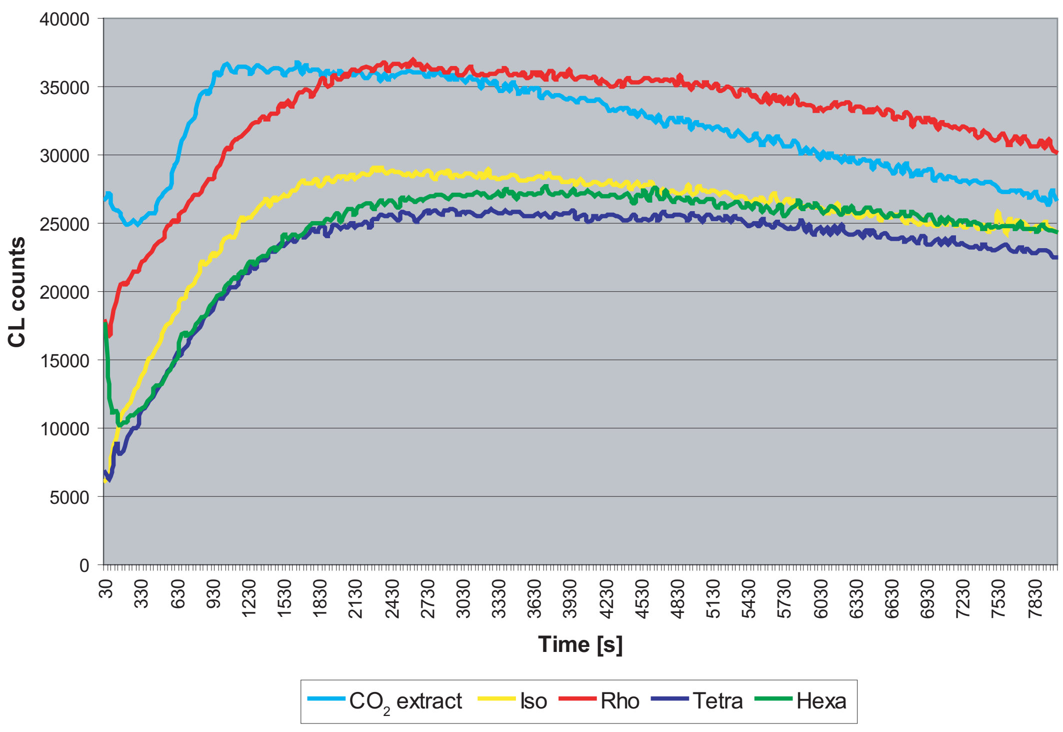

Fig. 7 Chemiluminescence analysis results from the same base beer, but with different hop products