Sensory and Analytical Characterisation of Reduced, Isomerised Hop Extracts and Their Influence and Use in Beer

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ABSTRACT

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The use of reduced, isomerised hop extracts became very popular during the last decade in terms of achieving both better foam and light stability. Today the most common products are those with tetra-hydro-iso-α-acids (THIA) or rho-iso-α-acids (RHIA). There is still not clarity concerning the taste properties of these products in comparison with iso-α-acid Bitter Units (BU). The experiments conducted showed that the bitter conversion factors determined in previous work (1.6 for THIA and 0.7 for RHIA) represented only perceived bitterness when presented in tap water. In the test beers a bitter conversion factor of 1.0-1.1 for THIA was determined and the factor of 0.7 for RHIA in beer was confirmed. Photometric methods led to considerable deviations when used for the determination of THIA and RHIA, whereas the HPLC method, in combination with either Solid Phase Extraction (SPE) or Liquid Liquid Extraction (LLE), led to very good results. RHIA was found to have a foam stabilising effect and it was also shown that the foam stabilising effect of THIA in unhopped beer was higher than in hopped beer. The quality of bitterness decreased with an increase of THIA and RHIA. A dependency on the alcohol content in regard to the bitter intensity was ascertained.

Key words: Bitter conversion factor, foam stability, HPLC methods, RHIA, sensory analyses, THIA.

INTRODUCTION

Hops with their different compounds contribute in an important manner to both the taste and to the sensory characteristics of beer. In this paper, in co-operation with Simon H. Steiner Hopfen GmbH, a rho-iso-α-acid product and a tetra-hydro-iso-α-acid product were investigated as to their sensory and analytical properties in beer[5,8,11,20,21]. Technological aspects such as yield and dosage as well as analytical aspects (sample preparation and the determination of reduced and isomerised compounds) were considered[2,9,13]. The principal aims of this research were to determine the taste contribution of these hop products with varying dosage, by extensive sensory analyses and to examine the influence of the matrix.

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MATERIALS AND METHODS

The abbreviations of the rho-iso-α-acid product and the tetra-hydro-iso-α-acid product are referred to as RHIA and THIA respectively. Both are aqueous solutions of the potassium salts of the respective iso-α-acids, which are produced from a CO_2 extract[5]. A DCHA-(Dicyclohexylamine)-iso-α-standard in water was used. [Editor's note (added 5 May 2003): Because of toxicity DCHA is an analytical standard only and not a substance for sensory evaluation.]

The matrices employed were beers brewed in the pilot plant of the institute, a commercially available lager and a freeze dried lager beer. To characterize the beers, original gravity, content of alcohol and investigations on foam stability were conducted.

For determination of the iso-α-acid content, spectrometric analyses (Rigby and Bars) cp. MEBAK[16] and Bitter Units (BU), cp. MEBAK[15] were performed. RHIA and THIA were separated after solid phase extraction (SPE)[18] and Liquid-Liquid-Extraction (LLE)[3] on a C-18 RP column using a HP-HPLC system and quantified at 235/295/270 nm. In a validation study a variation coefficient of 2.6% for the combination of SPE and HPLC was determined, and for the HPLC itself a variation coefficient[10] of 0.9% was found. For the determination of foam stability the NIBEM Foam Stability Tester was used[17]. Within the scope of sensory analysis triangular tests for the threshold determination (i.e., threshold of added substances), descriptive analyses and evaluation tests were performed[4].

TABLE I: Sample codes of the test beers produced.

Sample code	Amount added (mg/L)	Yield %
T10	17 THIA	60
T15	25 THIA	60
T20	33 THIA	60
R10	13 RHIA	80
R15	19 RHIA	80
R20	25 RHIA	80

TABLE II: Content of iso-α-acids in reduced hop products[14].

Product	Content of iso-α-acids by Steiner-UV-photometric method (%)	Iso-α-acids by HPLC-analysis (%) Steiner/Institute
THIA	10.7	10.0/9.3
RHIA	37.1	25.9/25.1

Beers produced in the pilot plant

First wort was diluted to 'pfannevollwürze' i.e., kettle-full-wort concentration, then boiled, fermented using hop free yeast, aged, filtered and bottled. Half of the wort was fermented without hop addition. This non hopped beer was hopped after filtration, but before bottling, with the following amounts of isomerised, reduced hop products: 10 mg/L, 15 mg/L, and 20 mg/L assuming a yield of 60% for THIA and 80% for RHIA.

The other half of the wort was hopped with CO_2 extract during the boil. This wort was also fermented with hop free yeast and treated in the same way as the non hopped beer (Table I).

Commercial lager. For the sensory and analytical investigations, a commercial lager was used. Amounts of 5/10/15/20/25/35 mg/L of the THIA or RHIA, assuming 60% and 80% yield respectively, were added to the commercial lager to determine the effect on both foam stability and sensory acceptance. These spiked samples were analysed by HPLC[13].

Freeze dried commercial beers. A commercial lager was freeze-dried and 15 mg/L of the reduced product THIA and RHIA, assuming a yield of 60 and 80% respectively, was added to the beer powder, which was then dissolved in alcoholic aqueous solutions of 1,3,5 and 7% (v/v) ethanol. These samples were then bottled using carbonated distilled water.

RESULTS

Analysis of the reduced hop products

For the characterisation of the hop products RHIA and THIA, sensory analyses were performed before adding them to the beers. For the determination of the content of iso-α-acid in the reduced hop products both spectral photometric analyses and HPLC analyses were performed (Table II and Table III).

The taste thresholds of RHIA and THIA in several media, and the intensity of bitterness using the same concentrations, were determined (Table IV).

The taste threshold of THIA in tap water (German hardness grade 19.7) with a content of 1.6 mg/L was 3.7 times lower than the taste threshold of RHIA, and 2.8 times lower than the threshold of the DCHA-iso-α-standard (Table IV). The threshold in unhopped beer was 8.5 mg/L which was 5.3 times the value of that compound in tap water. This was attributed to the masking effect of beer.

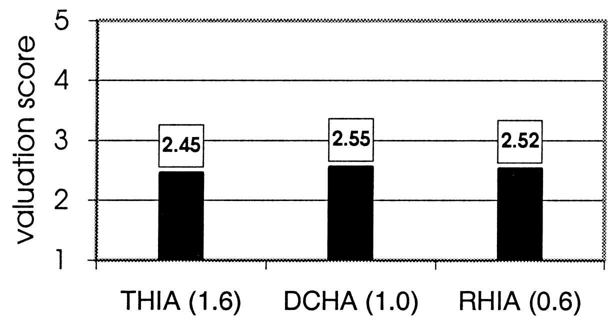

FIG. 1. Taste intensity of bitterness of THIA, RHIA, and DCHA-iso-α-acid-standard in water referring to the conversion bitter factors found in the literature.

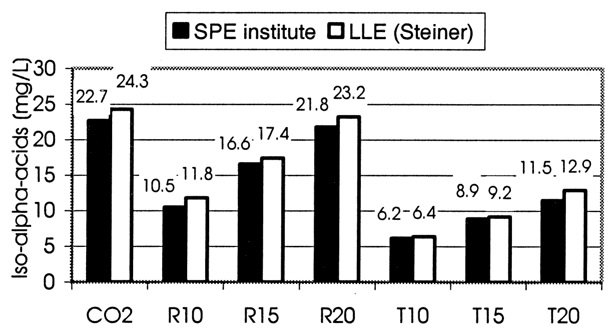

FIG. 2. Content of iso-α-acids of the test beers by HPLC with SPE or LLE.

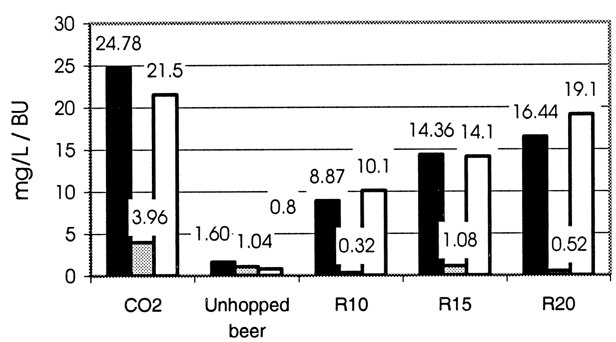

FIG. 3. Content of (black bar) iso-α-acids, (grey bar) α-acids and (white bar) bitter units (BU) of the test RHIA beers by photometric methods.

TABLE III: Content of co-, N-, and adhumulones on reduced hop products[14].

Product	Co-[1] %	N-[2] %	Ad-[3] % -
THIA	43.6	47.3	9.1
RHIA	27.5	62.6	9.9

[1] Cohumulone.
[2] Humulone.
[3] Adhumulone.

TABLE IV: **B**est **E**stimated **T**hresholds (BET) of the reduced hop products and DCHA-iso-α-acids (dicyclohexylamine)-standard in various media.

Substance/Medium	BET (mg/L)
THIA/tap water	1.61
THIA/unhopped beer	8.50
RHIA/tap water	6.03
RHIA/commercial beer	11.22
DCHA-standard/ tap water	4.54

TABLE V: Comparison of the THIA beers with different conversion factors.

Sample	CO_2	T10	T15	T20
Analytical bitterness (%)	100	26	38	53
Sensory bitterness (%) (1.0 as conversion factor)	100	31	36	55
Sensory bitterness (%) (1.6 as conversion factor)	100	20	23	34

The threshold value of 11.22 mg/L of RHIA in commercial lager was only 1.8 times higher than the threshold in tap water. Based on the THIA value in unhopped beer one might have expected a higher value, since the determination was performed using hopped lager. It is possible that the difference in taste of RHIA, compared to that of normal iso-α-acids, was easier to distinguish.

The conversion bitter factors (0.6/0.8 for RHIA, 1.9/1.6 for THIA) found in the literature[8,20] which describe the correlation between sensory bitterness and analytical content of iso-α-acids, were verified by means of evaluation testing. The hop products were dissolved in tap water at concentrations of 6.3 mg/L THIA, 10.0 mg/L DCHA-iso-α-acids and 16.6 mg/L RHIA to obtain the same sensory bitterness. The assessors were requested to judge the intensity of bitterness (Fig. 1).

The samples were evaluated to have almost the same bitterness (2.45, 2.52, and 2.55 on an intensity scale of 1-5). Together with the judgment of bitter intensity of THIA and RHIA, the conversion bitter factors 1.6 for THIA and 0.7 for RHIA were confirmed, in reference to the DCHA-iso-α-standard in tap water.

To evaluate response sensitivity, THIA and RHIA were dissolved in tap water at a higher concentration of 12.5 mg/L. Considering the taste thresholds the calculated Taste Activity Values[19] (TAV = concentration in sample / threshold concentration) were 7.8 for THIA and 2.1 for RHIA and the TAV's would be expected to be 3.79. But the value of the tasted bitterness of THIA was found to be

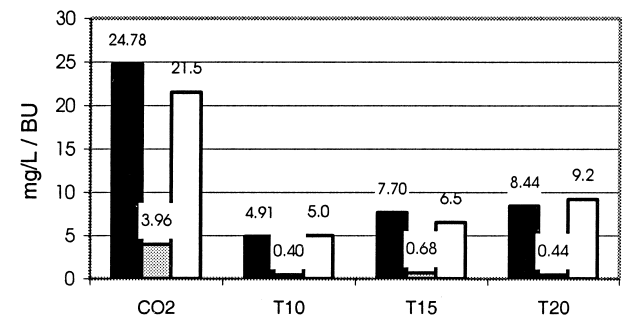

FIG. 4. Content of (black bar) iso-α-acids, (grey bar) α-acids and (white bar) bitter units (BU) of the test THIA beers by photometric methods.

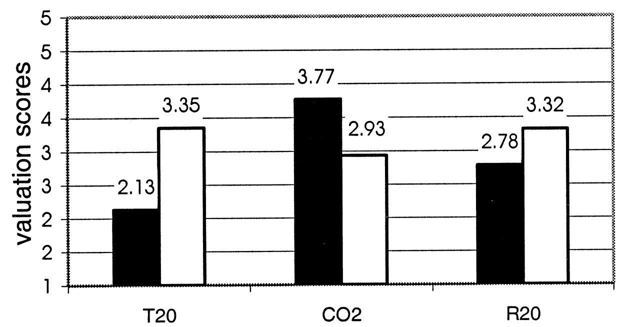

FIG. 7. (■) Intensity and (□) quality of bitterness of the test beers.

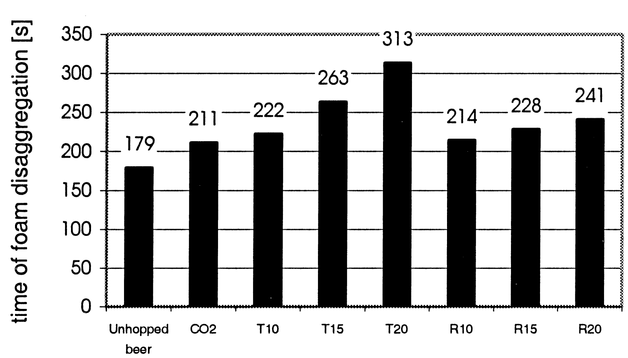

FIG. 5. Nibem foam stability measurements of the test beers.

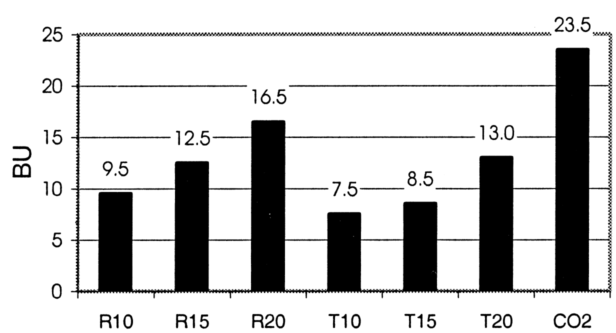

FIG. 8. Averaged estimated BU of the test beers.

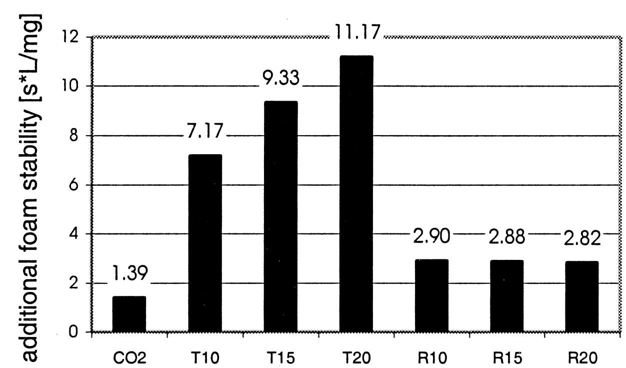

FIG. 6. Additional foam stability per mg/L THIA and RHIA in the unhopped test beer.

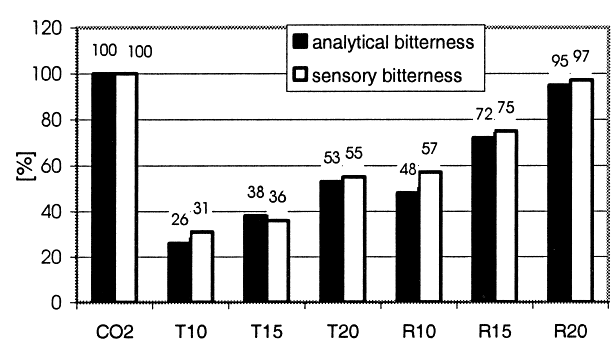

FIG. 9. Comparison of analytical (HPLC) and sensory bitterness of the test beers using conversion factors of 0.6 for RHIA and 1.0 for THIA.

only 2.3 times higher than the value of RHIA. This demonstrates that at higher concentrations, a saturation of the perceived bitterness was reached.

Analyses of the test beers

After sample preparation performed using SPE and LLE, the content of iso-α-acids of the test beer and the yield of the products were determined by HPLC (Fig. 2).

In addition to the HPLC analyses, photometric methods were employed. To rank the results the absorption maxima of THIA (235nm) and RHIA (295 nm) were determined (Fig. 3 and Fig. 4).

The results of the HPLC determinations of the beer, hopped with CO_2 extract and that with RHIA, coincided with the desired amount of iso-α-acids. This indicated that an assumed yield of 30% for the CO_2 extract and 80% for the RHIA product was achieved. For the beers hopped with THIA, a yield of only 37% was ascertained, by the method of Rigby & Bars[16] (only 30% compared to the expected 60 % yield). This was probably due to the mode of hopping. For technical reasons, the reduced hop products could not be dosed continuously to the beer flow, and were put into the containers before the beer was added.

The foam stability of the test beers was measured using the NIBEM Foam Stability Tester[17]. To obtain reproducible results all samples were measured on the same day.

Both products, THIA and RHIA, noticeably increased the foam stability (Fig. 5). The addition of THIA induced a stronger foam stability than RHIA. This confirmed the foam stabilising effect of THIA described in the literature[1,7]. The stabilising effect of RHIA (per added amount in mg/L) is higher than that of iso-α-acids per added amount in beer hopped with CO_2 extract (cp. Fig. 6). The increase of the stabilising effect of RHIA seemed to be independent of the total amount of RHIA in the beer, whereas the stabilising effect of THIA was significantly higher per added amount in mg/L. It was shown that the stabilising effect of THIA increased with the increase in the total amount of THIA.

For determination of bitter intensity and bitter quality of the test beers and to grade the test beers in the DLG (Deutsche Landwirtschaftsgesellschaft) Scheme[14], various sensory analyses were carried out[4]. Bitter intensity and bitter quality were evaluated and the assessors estimated the number of BU in addition to creating a ranking list[10,12] (Fig. 7).

The estimated bitterness ranking by the assessors matched well with the analytical data (Fig. 8). The test beer "T20" was judged as the least bitter beer, followed by the beer "R20". The beer hopped with CO_2 extract was attributed to have the highest bitter intensity by the assessors. The judgment of the bitter quality, and the ranking list, indicated a tendency for beers of lower bitter content to be preferred and they were consequently rated higher.

To compare the analytical data obtained by HPLC with the sensory data, the analytical content of iso-α-acids (22.7 mg/L) and the sensory data (24 BU) of the beer hopped with CO_2 extract was equated with 100% (Fig. 9). Including the conversion bitter factors found in litera-

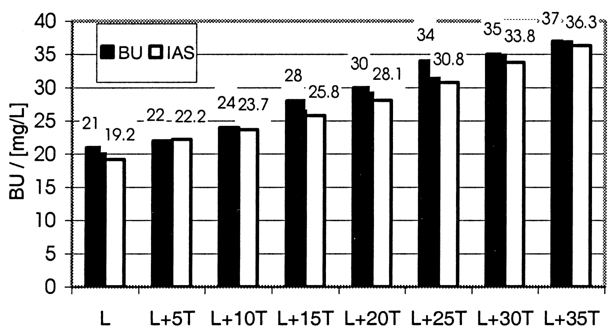

FIG. 10. Determination of iso-α-acids by means of spectrometry and BU in a commercial lager (L) with addition of 5/10/15/20/25/30/35 mg/L THIA.

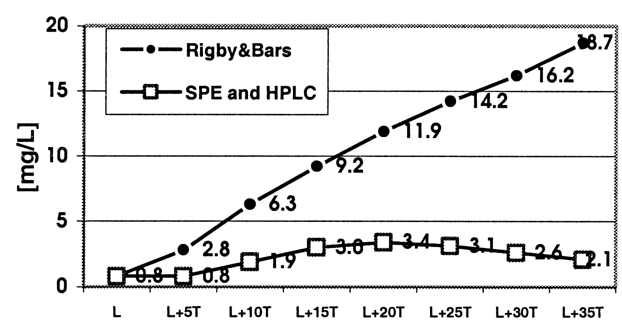

FIG. 12. Deviation of recorded iso-α-acids subject to the added amount of THIA (5/10/15/2025/30/35 mg/L) to a lager beer (L) using as analytical methods Rigby & Bars and SPE and HPLC.

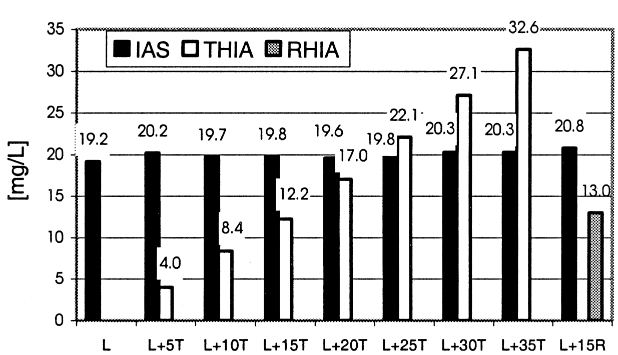

FIG. 11. Determination of (black bar) iso-α-acids, (white bar) THIA and (grey bar) RHIA by HPLC in combination with SPE.

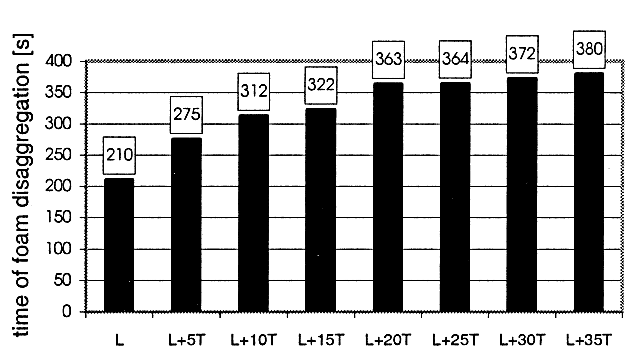

FIG. 13. Time of foam disaggregation of a commercial lager (L) with added amounts of THIA.

ture[8,20] for THIA a difference of 20% between analytical and sensory bitterness for the beer "T20" was evident[6,10] (Table V).

The different contents of iso-α-acids led to various estimations of BUs in the test beers. The estimated BUs of the assessors were averaged. For the comparison with the analytical data the conversion bitter factors found in literature were applied[8,20]. The factor of 0.6 for RHIA was confirmed, whereas for THIA the factor was found to be 1.1-1.0. This lower factor, as mentioned previously, could be attributed to the masking effect of beer.

Analyses of commercial beers

After adding the reduced and isomerised hop products to the test beers, the BU and the content of iso-α-acids were determined by both photometric and HPLC analyses (Fig. 10 and Fig. 11).

Fig. 12 displays the difference of the expected and the measured content of iso-α-acids in beer subject to the applied analytical method.

The analytical results showed a continuous increase in the measured BU and the iso-α-acids. In contrast to the HPLC method (Fig. 11) the photometric methods (Fig. 10) are unable to distinguish between iso-α-acids and reduced iso-α-acids. Due to the different absorption maximum of THIA the results of the HPLC measurement of the sum of iso-α-acids were not obtained. Up to 35 mg/L THIA were added to a commercial lager (considering a yield of 60%) with circa 20 mg/L iso-α-acids. Instead of an expected amount of 55 mg/L in beer (with added 35 mg/L of THIA) only 36.3 mg/L was measured using the photometric method (Fig. 10). Concerning the measured BU (Fig. 10) one has to consider the calculation method of the BU equation[15].

$$\text{Bitter Units (BU)} = \text{measured extinction} * 50$$

The factor 50 was empirically determined and based on the assumption that iso-α-acids contribute about 70 % to the absorbance reading and the non iso-α-acids to the remainder[15]. Working with reduced hop products and spectrometric methods other empirical factors should be used[5,22].

The HPLC method in combination with either SPE or LLE appeared to be the most suitable method for the determination of iso-α-acids. The deviation between expected iso-α-acids and measured iso-α-acids was between 0-3.4 mg/L.

Foam stability was measured using the NIBEM foam stability tester. The correlation between the added amounts of THIA and increasing foam stability are shown in Fig. 13.

The results showed a parabolic run. Higher amounts of THIA resulted in smaller influences on foam stability. By adding 35 mg/L of THIA a saturation effect was noted. Fig. 14 shows the increasing rate of foam stability per added amount of THIA in mg/L. Here the foam stability is not trending upwards, which shows that the foam stabilising effect of THIA is higher in unhopped beer.

The samples with added THIA were judged by evaluation testing[4] and the DLG Scheme[14]. In contrast to the parabolic form of increase in foam stability, the intensity of bitterness increased consistently. The quality of bitterness decreased in value with increasing amounts of THIA and RHIA. Also the results of the DLG tasting indicated that the overall impression of the beers with THIA and RHIA were judged inferior when adding more than 5 mg/L THIA or 15 mg/L RHIA. In context with the analyses of foam stability, hopping with THIA in amounts of 5 mg/L was positive for foam and did not cause any adverse effects to sensory parameters.

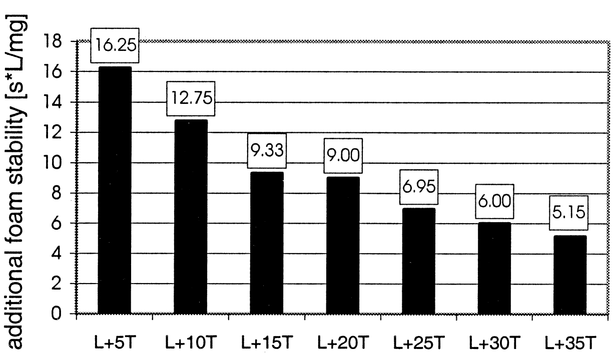

FIG. 14. Additional foam stability per mg/L THIA in a commercial lager (L).

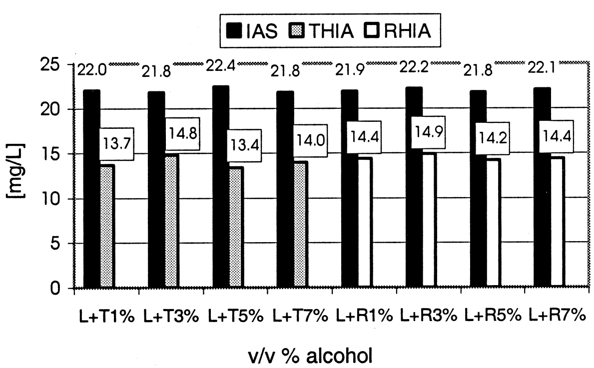

FIG. 15. (black bar) Iso-α-acid content in freeze dried commercial lager (L) spiked with (grey bar) THIA and (white bar) RHIA and diluted to different alcohol concentrations (1/3/5/7 (v/v) % ethanol).

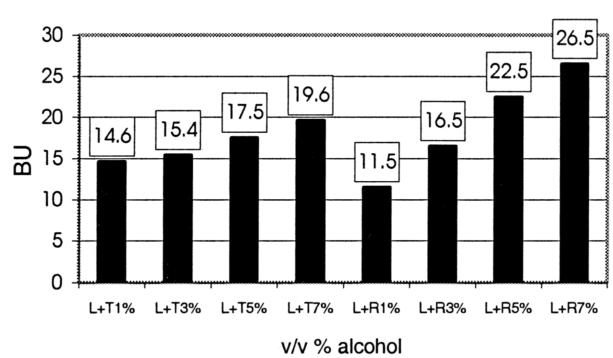

FIG. 16. Averaged estimated BU of a freeze dried lager (L) with THIA (T) and RHIA (R) diluted to different alcohol concentrations (1/3/5/7 (v/v) % ethanol).

Analysis of freeze dried commercial beers of different alcohol contents

To investigate the influence of alcohol content in regard to rate of yield and the sensory bitter impression of THIA and RHIA, a freeze dried commercial lager was treated as described in the Materials and Methods. The content of iso-α-acids was analysed by means of HPLC in combination with SPE and this value proved to be constant and independent of alcohol content (Fig. 15).

In addition to the evaluation of the bitter quality, which proved to be independent of the alcohol content, the assessors were asked to estimate the BU of the samples.

The sensory analyses (Fig. 16) showed that the bitterness was perceived as more intense with the increase in alcohol content and the BUs were estimated to be higher with increasing alcohol content. With an estimated 19.6 BU, the beer with an alcohol content of 7% (v/v) was judged 35% higher than the one with 1% (v/v) alcohol content.

The bitter quality seemed to be independent of the alcohol content. Similar results were obtained with the freeze dried beers containing RHIA. In contrast to the THIA beers, the bitter impression in the RHIA beers proved to be even more intense with the increase in alcohol content. The beer with an alcohol content of 7% (v/v) was estimated 130% higher than the one with 1% (v/v) alcohol content. As shown in the threshold determination of RHIA, it appeared that the RHIA in commercial beer had a stronger bitterness related to the matrix. Further investigations will need to be performed to confirm this.

CONCLUSIONS

The taste thresholds of RHIA and DCHA-iso-α-acids-standard in tap water, THIA in tap water and in unhopped beer, as well as RHIA in a hopped commercial lager, were determined.

The perceived sensory bitterness for the products at equal concentrations in regard to the conversion bitter factors found in the literature[8,20] were investigated. The results confirm the conversion factors of 1.6 for THIA and 0.7 for RHIA in tap water. For THIA a saturation effect in reference to the perceived bitterness for higher concentrations was shown.

In the test beers a conversion bitter factor for THIA of 1.0-1.1 and for RHIA of 0.7 proved appropriate. In consideration of these latter factors, the results of the HPLC analyses corresponded well with the perceived sensory bitterness.

Photometric methods such as Rigby and Bars[15] and the determination of BU[16] proved to be unsuitable for determining the content of reduced iso-α-acids in beer. When determining the BU caused by reduced hop products other empirical factors should be used[5,22]. Whereas SPE and LLE in combination with HPLC led to very good results.

The foam stabilising effect of THIA was confirmed. It was shown that the increase of foam stability is higher in unhopped beer than in hopped beer. Furthermore, a saturation effect appeared with higher concentrations of THIA.

The bitter quality was judged lower and more intense as the amount of THIA and RHIA increased. The perceived intensity of bitterness of the freeze-dried commercial lager increased notably with higher alcohol content, but the same amount of THIA or RHIA.

Concerning the sensory parameters, in regard to alcohol content in beer, additions of THIA up to 5 mg/L for foam stabilising effect and 10 mg/L of RHIA were justifiable. To achieve a worthy hop flavour the total hopping should be attuned to aroma hop and conventional hop products. The exclusive use of reduced hop products to produce light stable beer was justifiable considering the sensory characteristics at different concentrations, especially since international beer brands often have lower BU ranges.

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