J. Titze, V. Ilberg, F. Jacob and H. Parlar

A comparison of the EBC 9.8 method with a common applied HPLC analysis for Determining Bitterness in Beer

Descriptors: Bitter substances, iso-α-acids, analysis, sample preparation, HPLC, EBC 9.8, EBC 7.8

1 Introduction

1.1 Current situation

In addition to alcohol content, original gravity and extract, bitter substances represent one of the basic criteria for evaluating beer quality. The most important bitter substances in beer are the iso-α-acids [3].

Two methods for determining bitter substances in beer have become widely adopted as the methods of choice:

1. European Brewery Convention, EBC 9.8 – Bitterness of Beer (IM) [1], (cf. Mitteleuropäischen Brautechnischen Analysenkommission e. V. MEBAK 2.18.1 [2]),
2. HPLC analysis according to EBC 7.8 – Iso-α-, α- and ß-acids in Hop and Isomerized Hop Extracts by HPLC [1].

EBC 9.8 is the "wet chemistry" method for determining EBC bitter units (BU). This method measures the sum of the bittering substances by means of extraction with acidic iso-octane followed by photometric measurement at 275 nm. This analysis method measures α-acids, primarily iso-α-acids.

The second method as to HPLC analysis is currently not considered a commonly used standardized method for analysing bitter substances (iso-α-, α- and ß-acids) in beer, since it is based on EBC 7.8, which uses high performance liquid chromatography (HPLC). For the method as applied herein, only the section of EBC 7.8 pertaining to the procedures for the HPLC analysis was consulted. After separation of the iso-α-acids by HPLC, the individual components were measured at 270 nm. In contrast to EBC 9.8, the HPLC analysis determines individual bitter substances rather than the sum of all bitter components.

1.2 Description of the problem

Over the past few years, collaborative trials have been conducted for the purpose of assessing the reliability of the two methods described above. As part of this assessment, different beers were tested in each of the participating laboratories.

Sample preparation procedures for the HPLC analysis have not yet been standardized. Therefore, laboratories have employed a variety of preparation methods prior to conducting this analysis; however, the procedures for the HPLC analysis are described in EBC 7.8. Up to now, the collaborative test results have yielded unusually high values for the comparability R according to DIN/ISO 5725.

Authors: Dipl.-Ing. Jean Titze, Dr. Vladimir Ilberg, Dr. Fritz Jacob and Prof. Dr. Dr. Harun Parlar, Research Centre Weihenstephan for Brewing and Food Quality, Alte Akademie 3, D-85354 Freising, Germany; e-mail: j.titze@wzw.tum.de

The concept of comparability R is understood as the value below which the absolute difference between two results of the same analysis on the same materials under different conditions (e. g. in two different laboratories) can vary and still be within a defined probability, generally 95 %.

The results clearly underscore the inaccuracy between these two methods for determining bitter substances.

Data from the research conducted by Rigby and Bars show that at the wavelengths given in each of the analysis methods, not only is absorption by iso-α-acids present, but by α-acids as well [4]. The spectra for both pure iso-α-acids and pure α-acids are shown in figure 1.1 below.

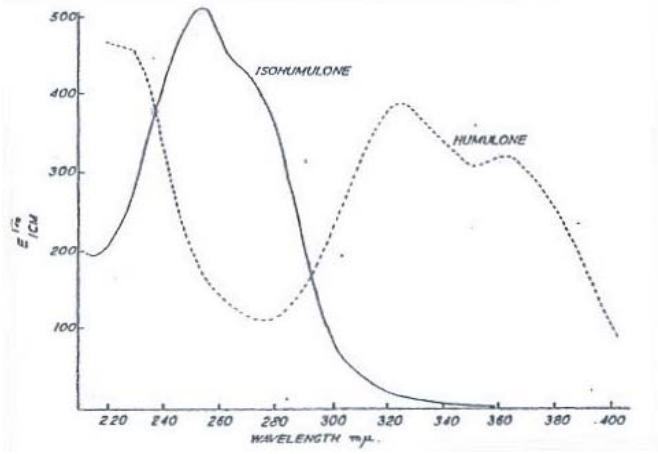

Fig. 1.1 The spectra for α-acids (humulone) und iso-α-acids (isohumulone) in alkaline methanol [4]

At wavelengths between 270 nm and 275 nm, a distinct minimum in the absorption curve of the α-acids is recognizable. Nevertheless, additional absorption by α-acids contributing to that of the iso-α-acids presumably occurs, given that the absorption by α-acids cannot be completely eliminated in either analysis.

1.3 Purpose of the investigation

The difficulties encountered in reconciling these two methods served as impetus for an investigation into this problem. Not only was each method to be more closely examined, but a comparison of the two was to be carried out.

The primary focus of this investigation was to analyse standardized beer samples with defined concentrations of iso-α-acids.

2 Investigation

2.1 Procedures

The investigation was conducted in two parts:

1. In the first part of the analysis, ten beers were tested by five different laboratories as part of collaborative trials. The beer samples were filtered and tested for iso-α-acid content and bitter units. At this point, the comparability was determined not only among the participating laboratories but also within each one.
2. In the next step of the analysis, an additional 100 beers were tested. The primary objective in selecting samples was to incorporate an extremely diverse set of beers with respect to the concentration of their bitter substances; for example, a standard iso-α-acid solution was added in increasing amounts to an unhopped beer to create a concentration series. The analyses were conducted by two independent laboratories. Furthermore, a strongly hopped beer was diluted with increasing amounts of distilled water to create a dilution series. All of the test samples possessed a beer matrix. Moreover, a broad range of beer styles was tested as well, including lager, non-alcoholic beer, ale and Bavarian-style wheat beer. The primary purpose of this part of the investigation was to compare the two analysis methods with one another.

2.2 Collaborative trials

In order to verify results previously obtained from collaborative testing, ten different beers were analysed by five independent laboratories.

A list of the beers which were analysed in the collaborative trials appears in table 2.1.

Table 2.1 Beers analysed in the collaborative trials on 5 June 2005

No.	Type of Beer
1	Lager
2	Dark strong beer
3	Dark lager
4	Filtered wheat beer
5	Pilsener
6	Premium Pilsener
7	Low alcohol beer
8	Dark export
9	Specialty beer
10	Lager

2.2.1 Sample preparation in the collaborative trials

Since EBC 7.8 was not developed to determine the amount of iso-α-acids in a beer matrix, the procedures contain no information regarding sample preparation for beer prior to the analysis. Various methods of sample preparation (e. g. ultrasound or manual agitation in order to degas the beer) and processing (solid-liquid and liquid-liquid extraction) are performed. Often, the sample to be analysed is directly injected into the HPLC without any preparation.

In order to guarantee the best possible comparability R determined among different laboratories, the following procedures for preparation of the samples were developed by the Research Centre Weihenstephan.

After the beer to be tested has reached room temperature, the bottle should be opened, and:

- an aliquot of the beer should be placed in an ultrasound bath for approximately 7 minutes or until no further effervescence is visible, then
- wait until any foam which might be present has collapsed; the glass may need to be tilted in order to return any residual foam adhering to the glass back into the beer, and
- the sample should then be directly injected into the HPLC without any further preparation (without a solid phase extraction).

The standard DCHA-ISO ICS-I2 (Labor Veritas, Switzerland) should be used to calibrate the HPLC as outlined in EBC 7.8. If possible, a C18-column should be utilized as well.

In order to prevent any loss of bitter substances, special care was taken to ensure that the beer foam had completely collapsed. If foam remained on the glass, it was rinsed back into the beer by tilting the glass. Neither the sample nor the standard should be filtered, because bitter substances can remain behind in the filter.

2.2.2 Results of the collaborative trials

The results of the collaborative trials for EBC 9.8 and HPLC analysis are listed in tables 2.2 and 2.3.

Table 2.2 The collaborative trial results from EBC 9.8; bitter units are given in BU

No.	Lab. 1	Lab. 2	Lab. 3	Lab. 4	Lab. 5
1	21.6	21.7	21	21	21.2
2	23	22.9	22	22	21.9
3	21.8	20.5	21	20	20.9
4	12.8	12.4	14	12	12.7
5	32.1	32.5	31	31	31.8
6	29.2	29.5	31	30	31.3
7	27.2	24.4	27	25	27.1
8	19.7	18.9	19	18	18.5
9	19.1	18.7	19	18	18.9
10	18.5	17.3	19	18	17.9

Table 2.3 The collaborative trial results from HPLC analysis; iso-α-acids are given in mg/l

No.	Lab. 1	Lab. 2	Lab. 3	Lab. 4	Lab. 5
1	22.49	23.71	29.43	26.2	23.10
2	17.51	21.08	21.39	21	16.90
3	18.93	19.74	23.17	22	19.20
4	9.48	10.39	10.33	11.6	9.90
5	35.53	37.39	37.94	41.6	37.40
6	33.75	36.16	39.97	38.4	36.00
7	26.75	28.06	30.90	32.3	27.60
8	14.52	17.19	17.00	17.1	14.60
9	17.36	17.61	20.97	20.2	17.80
10	16.67	18.80	22.71	19.7	17.50

Figures 2.1 and 2.2 show a graphic representation of the values from tables 2.2 and 2.3. As can be seen in figure 2.1, EBC 9.8 yielded consistent results in all five laboratories; however, the results depicted in figure 2.2 obtained form HPLC analysis are markedly less consistent. The Grubbs' test for identifying outliers was conducted for the results from both analyses. As can be seen, no outliers were present.

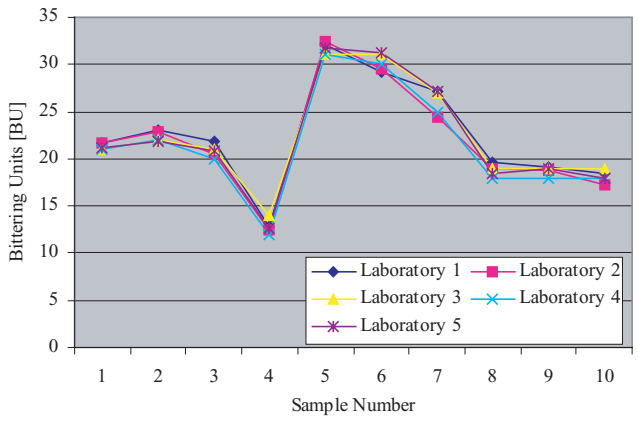

Fig. 2.1 Results from five different laboratories for EBC 9.8, bitterness in beer

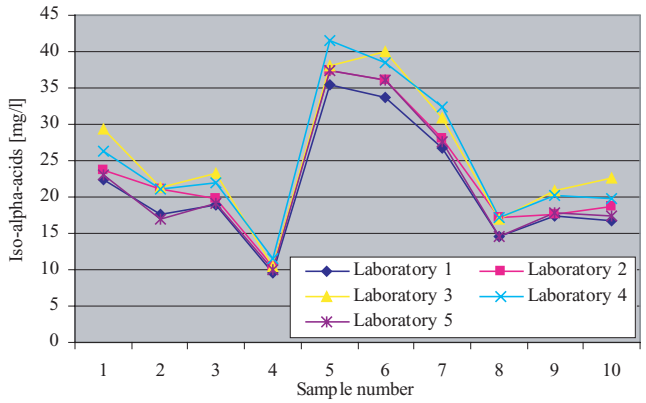

Fig. 2.2 Results from five different laboratories for HPLC analysis, iso-α-acids in beer

The mean values and the standard deviation were calculated for the results from each laboratory for bitterness and iso-α-acids. Tables 2.4 and 2.5 show the linear equations and the coefficient of determination R^2 for the results from each laboratory plotted against the mean values of their analysis results.

Table 2.4 Linear equations and R^2 after plotting the bitter units from each laboratory against the mean values

	linear equation	R^2
Lab. 1	f(x) = 1.0157 x	0.9880
Lab. 2	f(x) = 0.9910 x	0.9832
Lab. 3	f(x) = 1.0103 x	0.9861
Lab. 4	f(x) = 0.9747 x	0.9959
Lab. 5	f(x) = 1.0082 x	0.9934

The values in tables 2.4 and 2.5 confirm that both methods produce comparable results given that the preparation of the samples is consistent.

Table 2.5 Linear equations and R^2 after plotting the iso-α acids from each laboratory against the mean values

	linear equation	R^2
Lab. 1	f(x) = 0.9155 x	0.9974
Lab. 2	f(x) = 0.9825 x	0.9888
Lab. 3	f(x) = 1.0795 x	0.9706
Lab. 4	f(x) = 1.1284 x	0.9948
Lab. 5	f(x) = 0.9508 x	0.9905

In order to establish which of the two methods yields better comparability, a precise statistical analysis needed to be carried out.

First, the mean and the standard deviation for each of the individual samples measured using both analysis methods were calculated. Tables 2.6 and 2.7 show the sample mean $\bar{x}$, the standard deviation s and the mean deviation a calculated for the population mean μ. Additionally, table 2.7 includes the maximum values for the mean deviation amax, as stipulated in EBC 9.8. With the exception of sample no. 4, all of the results lie within the stipulated limits.

Conducting an error analysis on the data in table 2.6 through comparing a_{max} according to EBC 7.8 would be irrelevant due to the legal provision, which was developed for the regulation of hop and isomerized hop products. Were the stipulated limits nevertheless to be used as criteria for the sake of comparison, none of the results from the collaborative trials would fall within these limits.

If the comparability for all the samples from both analysis methods are calculated according to DIN/ISO 5725 and used, in turn, to calculate the mean of the computed values for the comparability, this results in $R = 1.94$ for EBC 9.8 and $R = 5.62$ for HPLC analysis.

Table 2.6 Sample mean, mean deviation, standard deviation and confidence intervals of the beer samples from the collaborative trials for HPLC analysis

No.	Mean	a	s	Confidence Interval
1	24.99	± 3.54	2.85	μ1 = 24.99 ± 3.54
2	19.57	± 2.71	2.18	μ2 = 19.57 ± 2.71
3	20.60	± 2.34	1.88	μ3 = 20.60 ± 2.34
4	10.34	± 0.98	0.79	μ4 = 10.34 ± 0.98
5	37.97	± 2.77	2.23	μ5 = 37.97 ± 2.77
6	36.85	± 2.99	2.41	μ6 = 36.85 ± 2.99
7	29.13	± 2.92	2.35	μ7 = 29.13 ± 2.92
8	16.08	± 1.73	1.40	μ8 = 16.08 ± 1.73
9	18.80	± 2.06	1.66	μ9 = 18.80 ± 2.06
10	19.08	± 2.90	2.33	μ10 = 19.08 ± 2.90

In calculating the sample mean $\bar{x}$, it should be noted that the values given in tables 2.6 and 2.7 represent only an estimation of the true value for μ. According to the test results, there is a 95 % probability that the true value μ lies within the calculated confidence intervals. Using the confidence intervals, the accuracy of each analysis method can be evaluated.

In order to allow for a reasonable amount of mathematical certainty, the absolute difference between each analysis result and the mean was calculated (i.e. five values per sample and analysis method). For each analysis method, the new population sample was checked for a normal distribution. Through the application of the χ^2–distribution (P = 95 %), the confidence limits for the standard deviations σ from EBC 9.8 and σ from HPLC analysis were calculated:

Table 2.7 Sample mean, mean deviation, standard deviation, confidence intervals and maximum mean deviation of the beer samples from the collaborative trials for EBC 9.8

No.	Mean	a	s	Confidence Interval	amax
1	21.30	± 0.41	0.33	μ1 = 21.30 ± 0.41	± 1.57
2	22.36	± 0.67	0.54	μ2 = 22.36 ± 0.67	± 1.66
3	20.84	± 0.83	0.67	μ3 = 20.83 ± 0.83	± 1.53
4	12.78	± 0.93	0.75	μ4 = 12.78 ± 0.93	± 0.80
5	31.68	± 0.83	0.67	μ5 = 31.68 ± 0.83	± 2.50
6	30.20	± 1.14	0.92	μ6 = 30.20 ± 1.14	± 2.37
7	26.14	± 1.66	1.33	μ7 = 26.14 ± 1.66	± 2.00
8	18.82	± 0.78	0.63	μ8 = 18.82 ± 0.78	± 1.34
9	18.74	± 0.55	0.44	μ9 = 18.74 ± 0.55	± 1.34
10	18.14	± 0.80	0.64	μ10 = 18.14 ± 0.80	± 1.28

σ_{EBC} 9.8: 0.2886 to 0.4206,

σ_{HPLC}: 0.8226 to 1.227.

Additionally, the variance ratio F (according to Roland Fischer) was calculated from both values for the variance [5]:

$$F = \frac{s^2_{EBC9.8}}{s^2_{HPLC}} = 4.61 \qquad (2.1).$$

If the upper and lower limits are computed with a statistical probability of 95 % as depicted below:

$F_{lower} = 0.25$ and $F_{upper} = 4.03$,

then value for F lies outside of these limits.

Through statistical analysis, it is possible to prove that the two analysis methods (EBC 9.8 and HPLC analysis) as performed in the collaborative trials, differ significantly with respect to their accuracy.

2.3 Comparative test

The second part of this investigation involved not only conventional beer samples but also several concentration and dilution series. Increasing the number of samples to more than 100 enabled the subsequent evaluation to be carried out through the application of the central limit theorem.

2.3.1 Materials and preparation of the samples

In order to conduct a comparison of both methods, one for measuring the iso-α-acids in beer, the other for bitterness, a concentration series was created on the basis of unhopped beer with an iso-α-acid standard. For this purpose, first wort was fermented using bakers' yeast to produce an unhopped beer.

The results from the analysis of the wort and the beer are summarized in table 2.8.

Reagents:

- unhopped beer (see table 2.8)
- 250 mg dicyclohexamylamine-iso-α-acid complex (DCHA). Iso-α-acid 64.3 %. laboratory Veritas, Switzerland.
- non-denatured ethyl alcohol (96 % v/v)

Table 2.8 Analysis results for the wort and the unhopped beer

	Wort	Beer
Apparent Extract (%w/w)	10.40	1.50
Apparent Extract (%w/v)	10.83	1.51
Specific gravity	1.04168	1.00585
Alcohol (%w/w)		3.28
Alcohol (%v/v)		4.17
Original gravity (%w/w)		10.05
Original gravity (%w/v)		10.43
pH	5.07	4.02

The beer was centrifuged at 3300 rpm for 15 minutes in order to clarify it. Subsequently, it was filtered twice through a paper filter and then manually shaken in order to degas it.

246.5 mg of the DCHA powder was weighed out using a precision balance and dissolved in 100 ml of ethanol with the aid of a magnetic stirrer. This is the method by which the standard solution containing 158.5 mg of iso-α-acids in 100 ml of ethanol was created. This standard solution was pipetted into the unhopped beer in 250 ml volumetric flasks, increasing the concentration at intervals of 3.17 mg of iso-α-acid solution per litre, which is equal to a volume of 500 µl. The iso-α-acid solution was added to the unhopped beer with an adjustable 5000 µl Eppendorf pipette.

To ensure consistency among all the samples containing different concentrations of the standard solution, the alcohol content of each sample was corrected to the maximum, which was 8.5 ml, using the 96 % (v/v) ethanol solution.

Table 2.9 shows the number of each sample, the volume of standard solution added to each one, as well as their respective iso-α-acid content and concentrations.

Table 2.9 Concentration series

No.	Standard added [ml]	Ethanol added [ml]	Iso-α-acid content [mg]	Conc. of iso-α-acids [mg/l]
0	0.0	8.5	0.00	0.00
1	0.5	8.0	0.79	3.17
2	1.0	7.5	1.58	6.34
3	1.5	7.0	2.38	9.51
4	2.0	6.5	3.17	12.68
5	2.5	6.0	3.96	15.85
6	3..0	5.5	4.75	19.02
7	3.5	5.0	5.55	22.19
8	4.0	4.5	6.34	25.36
9	4.5	4.0	7.13	28.53
10	5.0	3.5	7.92	31.70
11	5.5	3.0	8.72	34.87
12	6.0	2.5	9.51	38.04
13	6.5	2.0	10.30	41.21
14	7.0	1.5	11.09	44.38
15	7.5	1.0	11.89	47.55
16	8.0	0.5	12.68	50.72
17	8.5	0.0	13.47	53.89

2.3.2 Analysis methods

After mixing the samples, an aliquot was placed in HPLC vials and analysed. A triple determination was carried out in laboratories 1 and 5 according to the HPLC method according to EBC 7.8.

The standard solution for calibrating the HPLC was also a DCHA complex from laboratory Veritas, Switzerland.

The rest of each sample was analysed in laboratories 1 and 5 according to EBC 9.8.

2.3.3 Results of the comparative test

Table 2.10 shows the results for EBC 9.8 from laboratories 1 and 5.

Table 2.10 Results from the analysis of bitterness in beer

No.	Lab. 1 [BU]	Lab. 5 [BU]
0	3.3	2.59
1	5.3	4.83
2	7.1	6.97
3	9.7	9.36
4	11.8	11.77
5	14	14.16
6	16.3	16.28
7	18.5	18.86
8	18.6	19.07
9	20.6	22.04
10	22.9	24.85
11	25.2	27.03
12	26.6	29.28
13	29.5	31.04
14	31.5	32.54
15	36.2	35.90
16	38.6	37.82
17	39.3	40.02

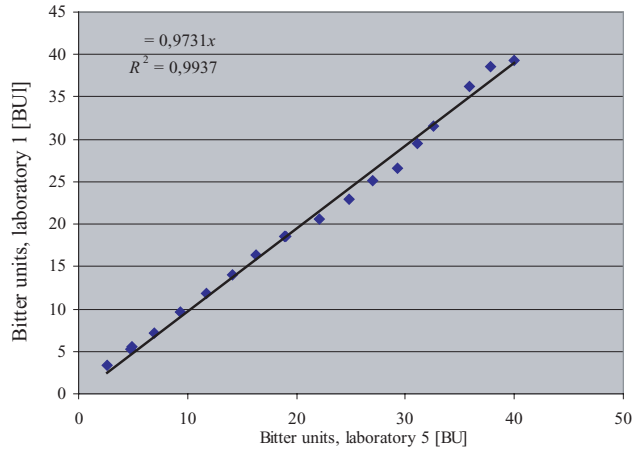

Fig. 2.3 Comparison of the measurements of bitter units from laboratories 1 and 5

A comparison of the values from both laboratories is depicted in figure 2.3. As can be seen here as well, these methods exhibit an acceptable level of comparability between the two laboratories, which is similar to the results obtained from the collaborative trials.

Table 2.11 summarizes the results from the HPLC analysis performed in laboratory 5 and the iso-α-acid concentrations calculated from table 2.9.

Table 2.11 Results for iso-α-acids [mg/l], measured and calculated

No.	Lab. 5 Iso-α-acids [mg/l]	Calculated concentration Iso-α-acids [mg/l]
0	0.00	0.00
1	3.00	3.17
2	5.80	6.34
3	8.90	9.51
4	12.10	12.68
5	15.10	15.85
6	18.60	19.02
7	21.60	22.19
8	25.80	25.36
9	28.50	28.53
10	32.50	31.70
11	35.70	34.87
12	38.00	38.04
13	41.40	41.21
14	45.10	44.38
15	47.30	47.55
16	50.40	50.72
17	54.60	53.89

A comparison of the measured versus the calculated data yields a coefficient of determination of $R^2 = 0.9991$ and a slope of 0.9962.

Figure 2.4 shows the differences between the results from EBC 9.8 and HPLC analysis plotted against the calculated iso-α-acid concentrations from table 2.11. From the graph, it can be seen that as the concentration increases, the disparity between the measurements also increases, because in an ideal case (i.e. no difference between the two methods) a horizontal line at zero would be the result.

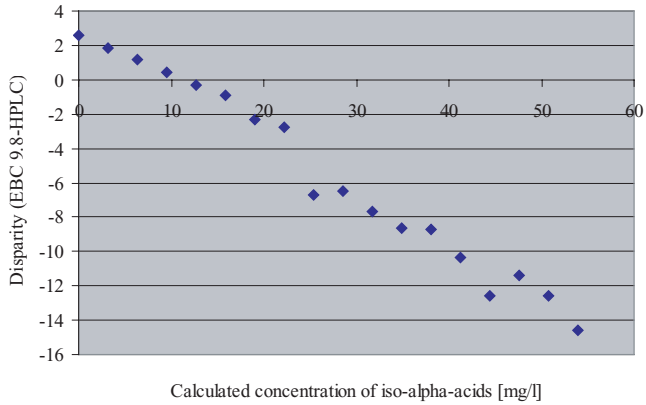

Fig. 2.4 Differences in the data from both EBC 9.8 and HPLC analysis relative to the calculated iso-α-acid concentration

2.4 Discussion of the Results

The results from section 2.2.2 raise the question of whether the accuracy as well as the absolute values of both methods in the collaborative trials are significantly different.

After completion of the second test series, data were obtained for all the samples from each analysis method. 110 data sets (x, y) can be generated from the results of 110 test samples. These data sets

can then be arranged to form so-called blocks (see table 2.12), paired observations and bound or paired test samples.

Does this, therefore, guarantee that the null hypothesis H_0, which in both methods is **not** significantly different, is within the 5 % level? If the null hypothesis were valid, then the mean of n pair differences $(\sum d)/n = \bar{d}$ would be equal to zero ($\mu_d = 0$). The alternative hypothesis would then be $\mu_d \neq 0$.

Table 2.12 Paired test samples

No.	x	y	d (x-y)	d²
1	21.200	23.100	1.900	3.610
2	21.900	16.900	-5.000	25.000
3	20.900	19.200	-1.700	2.890
4	12.700	9.900	-2.800	7.840
5	31.800	37.400	5.600	31.360
6	31.300	36.000	4.700	22.090
7	27.100	27.600	0.500	0.250
8	18.500	14.600	-3.900	15.210
9	18.900	17.800	-1.100	1.210
…	…	…	…	…
110	21.95	21.95	-0.050	0.003
n = 110	-	-	∑d = -98.216	∑d² = 2414.433

First, the distribution of the paired samples was tested for normal μ_d distribution, then adjusted to fit a Gaussian curve. Figure 2.5 shows that the mathematical approximation using a Gaussian curve and a coefficient of determination $R^2 = 0.98$ is valid.

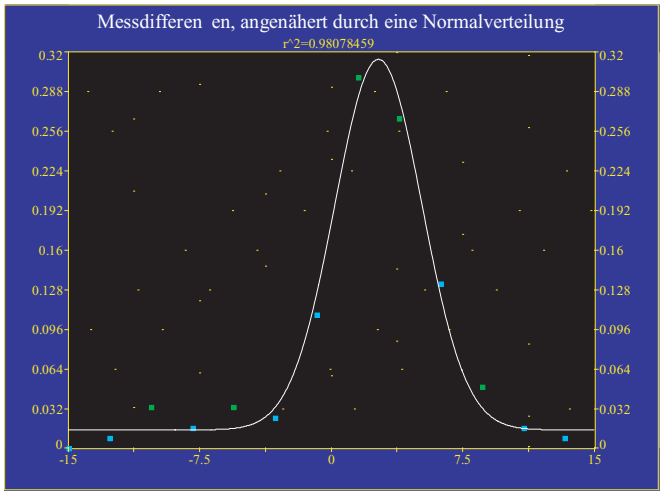

Fig. 2.5 Distribution of the differences in the analysis results adjusted to fit a Gaussian curve

With this test, independent and approximated Gaussian differences with variances s^2_x, s^2_y, s^2_{x-y}, which are independent of the number of the data set, are assumed. When $\hat{t} \geq t_{v;}$ with the degree of freedom of $v = n - 1$, the null hypothesis is not supported at the 100α-% level, whereupon the two-tailed test applies [5]:

$$\hat{t} = \frac{\left|\frac{d}{n}\right|}{\sqrt{\frac{d^2 - \frac{(d)^2}{n}}{n(n-1)}}} = \frac{|\bar{d}|}{s_{\bar{d}}} \qquad (2.2).$$

The confidence interval of 95 % for the true mean of the difference μ_d is determined by:

$$\bar{d} \pm t_{n-1;0,05} \cdot s_{\bar{d}}$$

($s_{\bar{d}}$ is the denominator in formula 2.2).

The results from the test samples yield:

$$\hat{t} = \frac{\left|\frac{-98.216}{110}\right|}{\sqrt{\frac{2414.433 - \frac{(-98.216)^2}{110}}{110(110-1)}}} = \frac{0.89}{0.44} = 2.02$$

with $v = 110 - 1 = 99$,

$$\hat{t} = 2.02 > 1.96 \approx t_{\infty;0.05}$$

Therefore, H_0 must be rejected at the 5 % level. It should be noted that $\bar{d} = 0.89$ is positive, and the 95 % confidence interval:

$0.03 \leq \mu d \leq 1.76$,

i. e. μ_d never reaches zero ($P < 0.05$). In figure 2.5 it is clear that the mean is not at zero.

This proves that the absolute values from each of these methods differ significantly from one another.

3 Summary

Due to the considerable disparity between the results obtained from the two analytical methods for determining bitter substances in beer, coupled with the requisite comparability *R* of their respective techniques, the goal of this investigation was to compare EBC 9.8, the analysis method for determining bitterness in beer, with the HPLC analysis according to EBC 7.8, the method for measuring the amount of iso-α-acids in beer. To this end, collaborative trials as well as a number of comparative tests were conducted, and the results from both methods were contrasted and statistically evaluated.

A collaborative test with five participating laboratories revealed that EBC 9.8, with a mean *R* of 1.94, produces much more consistent results than HPLC analysis with *R* of 5.62. Additionally, all the results from EBC 9.8, with one exception, fell within the limits stipulated by the EBC. It was possible to demonstrate that the level of accuracy for both methods was significantly different. However, uniform sample preparation proved to be beneficial for conducting a more accurate comparison of the results from the various laboratories.

The results of the comparative tests, by means of a concentration series with an iso-α-acid standard added to unhopped beer, have

shown that the detection of iso-α-acids in a beer matrix using HPLC is quite accurate. However, it should be noted that these values were obtained through calibration with the same iso-α-acid standard which was used to create the concentration series. If the values obtained from the concentration series via HPLC analysis according to EBC 7.8 are compared with those from EBC 9.8, a difference in the results of the two methods becomes apparent. As the concentration of iso-α-acids in the beer increases, EBC 9.8 yields lower values than the HPLC analysis for identical samples.

The findings of the collaborative trials, as well as of the comparative tests, conclusively show a significant difference between the two methods in terms of the absolute values obtained from each one. For this reason, the results obtained from both methods were compared on the basis of 110 analysed test samples.

There were sufficient data to prove that the results from EBC 9.8, used for the determination of the bitterness in beer, and HPLC analysis, as used to determine the iso-α-acids in beer, differ significantly. These results show the necessity for the optimization of the HPLC analysis as a method for measuring iso-α-acids in beer. A contribution to the latter may be the standardized sample preparation procedure as has been developed by the Research Centre Weihenstephan for Brewing and Food Quality.

4 References

1 EBC Analysis Commitee (Hrsg.): Analytika – EBC. Nürnberg: Hans Carl Getränke-Fachverlag, 1998

2 Miedaner, H. (Hrsg.): Brautechnische Analysenmethoden, Band II. 4. Auflage. Freising: MEBAK, 2002

3 Narziss, L.; Schuster, K.; Weinfurtner, F.: Die Bierbrauerei, Zweiter Band. Die Technologie der Würze-bereitung. 7. Auflage. Stuttgart: Enke, 1992

4 Rigby, F. L.; Bars, A.: The Determination of Iso-Compounds and Alpha-Acids in Wort. ASBC Proceedings (**1955**), pp. 46-50

5 Sachs, L.: Statistische Methoden – Planung und Auswertung. 7. Auflage. Berlin: Springer, 1993

Received 10. 11. 2005, accepted 09. 12. 2005

Acknowledgement

We would like to thank all of the laboratories which took part in this investigation.

Abbreviations

BU	Bitter Units
DCHA	Dicyclohexamyl-trans-iso-α-acid-complex
EBC	European Brewery Convention
HPLC	High Performance Liquid Chromatography
ICS	International Calibration Standard
MEBAK	Mitteleuropäische brautechnische Analysen-kommission

List of Symbols

Greek Alphabet

μ	(true) population mean
σ	(true) population standard deviation
σ^2	(true) population variance
ν	degree of freedom

Latin Alphabet

a	mean deviation
d	difference between two comparable measurements
$\bar{d}$	mean of the differences
F	variance ratio according to Roland Fischer
f(x)	function
H0	null hypothesis
n	number of samples
P	probability
r	repeatability
R	comparability
$\bar{R}$	mean comparability
R^2	coefficient of determination (= mean squared deviation)
s	sample standard deviation
s^2	sample variance
$t_{\nu,}$	Student's t with a given degree of freedom and probability
$\hat{t}$	test criterion of the t-distribution
x	variable
$\bar{x}$	sample mean
x, y	data set